EUROMAR

EUROMAR 2011

Magnetic Resonance Conference

33. Discussion Meeting of the MR Spectroscopy Division of the GDCh

8. European Federation of EPR Groups Meeting

21.-25. August 2011
Frankfurt am Main
Germany

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Internationaler wissenschaftlicher Fachverlag

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Magnetic Resonance Conference

21 – 25 August 2011, Frankfurt am Main, Germany

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WELCOME TO THE EUROMAR 2011 IN FRANKFURT

We are extremely grateful and proud to announce that more than 800 scientists will participate in this major European conference. The EUROMAR emerged 2004 from the AMPERE congress, the European Experimental NMR conference (EENC) and the UK RSC NMR Discussion Group meeting and covers the whole field and bandwidth of magnetic resonance spectroscopy. Presentations cover all aspects of magnetic resonance spectroscopy, ranging from methodological and technical advancements in NMR, DNP, MRI and EPR to new areas of application in material and life sciences, physics, chemistry and biology. In the afternoon, during poster sessions, many new results will be presented and discussed. Moreover, all major companies will present their new products and achievements to the scientific community.

This year the meeting will be joint by the 33rd Discussion Meeting of the Magnetic Resonance Spectroscopy Division of the Gesellschaft Deutscher Chemiker (GDCh), which commonly takes place in September. Traditionally in the annual meeting of the German Magnetic Resonance Spectroscopy Division, the Ernst Awards for exceptional papers of young scientists will be presented in a dedicated plenary session, followed by short talks of the price winners. We are in particular proud that Richard Ernst himself will hand over the awards! Additionally, the EUROMAR 2011 will be accompanied by the European Federation of EPR Groups Meeting, a triennial joint meeting of all European EPR Groups and Societies. For this reason the EUROMAR is also the main meeting of the International EPR Society (IES). The Ulderico Segre Prize for an outstanding doctoral thesis in the field of theoretical or computational modeling applied to magnetic resonance will be awarded to two young scientists: one working in the field of NMR and one in EPR. I dearly hope that this 'crossing of borders' also takes place within the conference between the various fields of magnetic resonance. This is as well the spirit of the Sunday afternoon tutorial "Crossing the Borders: From Liquid and Solid State NMR to DNP and EPR".

Sponsorship by a number of scientific institutions are gratefully acknowledged: EMAR, DFG, Goethe University (Freunde und Förderer), BMRZ, CEF and GDCh all contributed to the conference, allowing us to keep the registration fees for students very low and to give numerous travel grants to young scientists from countries around the whole world. Many companies support the conference by their attendance and by sponsoring social activities, as stated in the program.

Organizing such a huge event is a big challenge and task; fortunately the Scientific Committee was very helpful to propose and select the invited speakers and, even more difficult, the other half of the speakers - out of numerous very good abstracts! I am very thankful to the tutorial speakers, who accepted to prepare students to be able to get the most from conference talks, especially in other disciplines. Finally, our entire Center for Biological Magnetic Resonance (BMRZ), with six research groups and about 100 persons (the blue shirts), has been and will be involved within this week to get everything smoothly done. Nevertheless, the success of the conference has to come from you: I wish all of us stimulating talks with new and unpublished results, challenging questions and interesting scientific discussions at the posters.

Thomas Prisner

(Chair of EUROMAR 2011 Conference)

GENERAL INFORMATION

BADGES

All participants are kindly asked to visibly wear their badges on Campus throughout the duration of the conference.

LOCATIONS OF SESSIONS

All sessions will take place at Goethe University, Frankfurt am Main, Campus Westend in the Lecture Hall Center.
The plenary sessions: ground floor in lecture halls 1 and 2 (red).
The parallel sessions: first and second floor in lecture halls 3 (yellow), 4 (orange) and 5 (pink).
The poster session: in foyers on first, second and third floors as well as in Casino building in ground floor in hall 823.

CONFERENCE OFFICE

The conference office and registration desk, ground floor of Lecture Hall Center, will be open at 10:00 on Sunday 21st August for registration, and daily from 08:00 to 19:00 during the conference.

SPEAKERS

Speakers are asked to bring their presentation to the lecture halls 15 minutes before the session starts. Stewards are available to assist in transferring presentations or setting-up of personal computers. Please also familiarize yourself with audiovisual system.

POSTERS

CATEGORIES

All poster abstracts uploaded prior to the according deadline of 20th July 2011 are listed in the poster abstract section, sorted by category:

Bs Biosolids
Cs Catalysis and Surface
Ct Computation and Theory
Hs Hyperfine Spectroscopy
Im Imaging
Cv In Cell and In Vivo Studies
Ls Liquid State NMR Methods
Mp Materials and Polymers
Me Membrane Proteins
Mb Metabolomics
Md Methodological Developments in EPR
Mi Molecular Magnets and Inorganic Materials
Na Nucleic Acids
Ps Paramagnetic Systems
Rc Radical Chemistry
Rd Relaxation and Dynamics
Se Sensitivity Enhancement
Sm Small Molecules
Ss Solid State NMR Methods
Sp Solid State Physics
So Soluble Proteins
Td Transport and Diffusion
Ot Other Topics

SESSIONS

There are four formal poster sessions from Monday to Thursday from 14:30 – 16:00. Authors are asked to present their posters according to the following schedule (modulo-4):

Poster session One: Monday, MOD4 (poster Nr.) = 0 (e.g. 200, 204, 208,...)
Poster session Two: Tuesday, MOD4 (poster Nr.) = 1 (e.g. 201, 205, 209,...)
Poster session Three: Wednesday, MOD4 (poster Nr.) = 2 (e.g. 202, 206, 210,...)
Poster session Four: Thursday, MOD4 (poster Nr.) = 3 (e.g. 203, 207, 211,...)

SET-UP AND REMOVAL

Posters should be on display for the duration of the conference. The authors are asked to put the posters on the boards by 14:00 on Monday, 22nd August latest and to remove them by 19:00 on Thursday, 25th August. Posters will be discarded after the deadline. The poster boards are numbered in the same way as the poster abstracts in this book (see the author index).

SPECIAL SESSIONS

The special sessions will be held daily from 13:00 to 14:30. EUROMAR Board, Ampere Board, EUROMAR 2012 Scientific Committee and EMAR Committee will meet in the Casino building on first floor in room number 1.802 (special invitation only). International EPR (ESR) Society General Assembly, EFEPR Meeting, FGMR General Assembly and Industrial Lunch will be held in the Lecture Hall Center on third floor in lecture hall 5.

VENDOR ACTIVITIES

The main sponsors, Bruker and Agilent Technologies, have organized their users meetings on Sunday in the Lecture Hall Center. Both companies also organize hospitality suites in the Casino building next to their exhibition areas on Monday and Tuesday at 19:00, respectively. Agilent Technologies holds in addition a lunch time seminar on Tuesday at noon in the Lecture Hall Center lecture hall 5.

REFRESHMENTS AND MEALS

Lunch boxes will be distributed daily from 13:00 to 14:00 in the Casino building.
Refreshments, coffee and tea will be available at several points in Lecture Hall Center, as well as in the Casino building

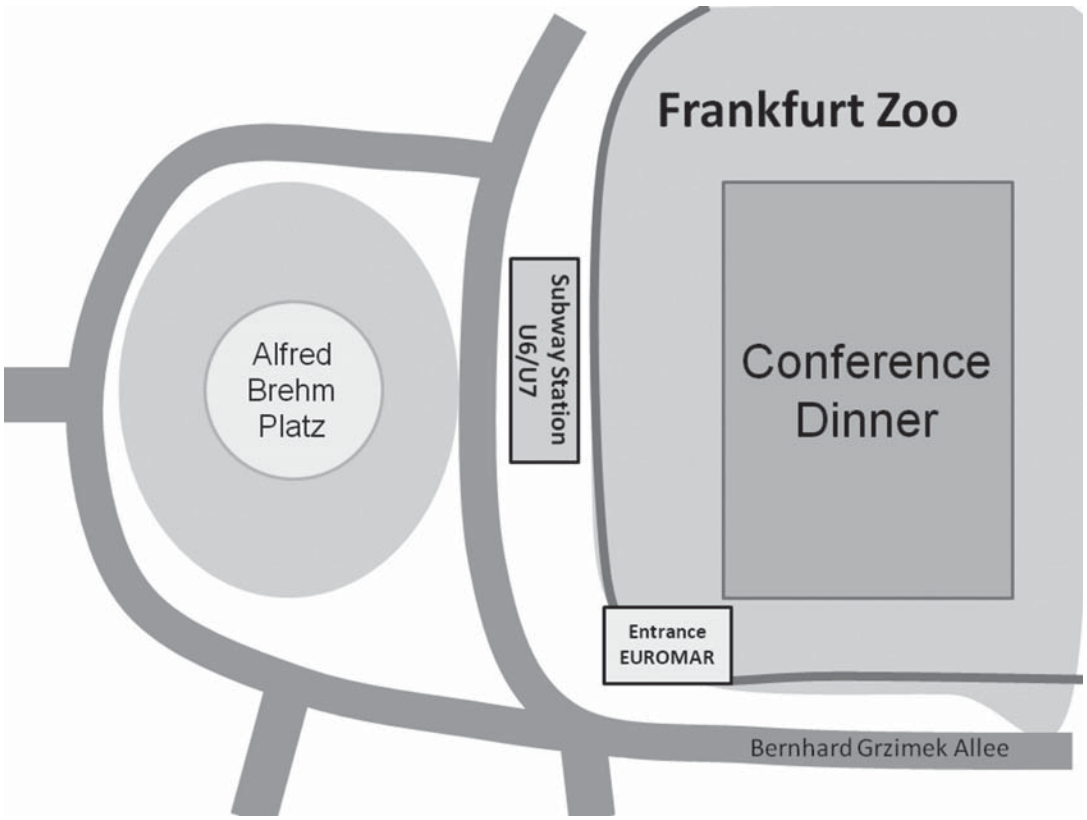
SOCIAL EVENTS

WELCOME MIXER

Join us to the welcome party and get an impression of the famous Octoberfest.
Sunday 21st August at 19:30-23:00, at the oval Cafeteria in “IG Farben Building” on Campus Westend.

CONFERENCE DINNER

Thursday 25th August at 20:00, at Zoo-Palais, Bernhard-Grzimek-Allee 1, Frankfurt am Main. The easiest way to the Zoo-Palais from Campus Westend is via subway: take U1, U2, U3 or U8 (direction Südbahnhof), to Hauptwache. Change to U6 (direction Frankfurt Ost) or U7 (direction Enkheim) and exit at Zoo.



PUBLIC TRANSPORTATION

All EUROMAR 2011 attendees receive a free public transportation ticket being valid on regional trains, subway, trans and buses from 21st – 26th August.

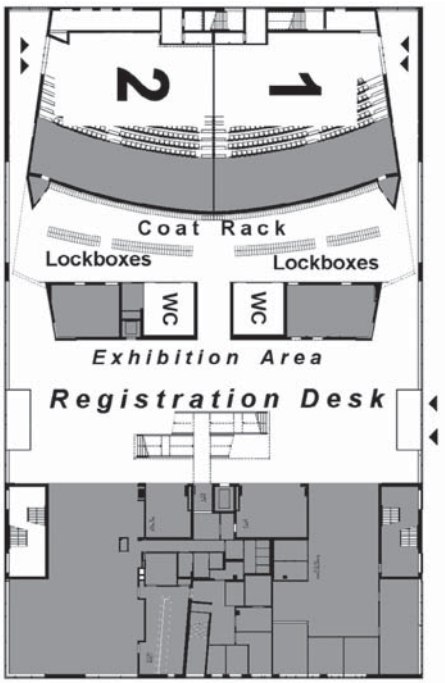
INTERNET

WiFi is available during the conference in the whole area of Campus Westend. Due to internet security, individual access codes (username and password) have to be used. Access codes are available at the conference office. Moreover, a computer pool in the Casino will allow free access to the web.

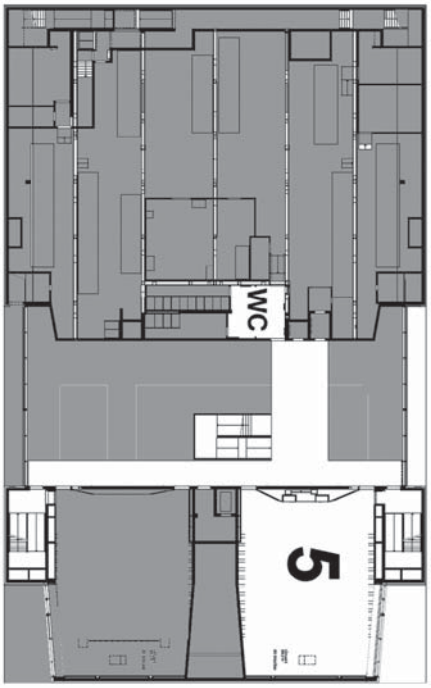
Hörsaalgebäude / Lecture Hall

Plenary Sessions 1, 2 **Parallel Sessions 3, 4, 5**

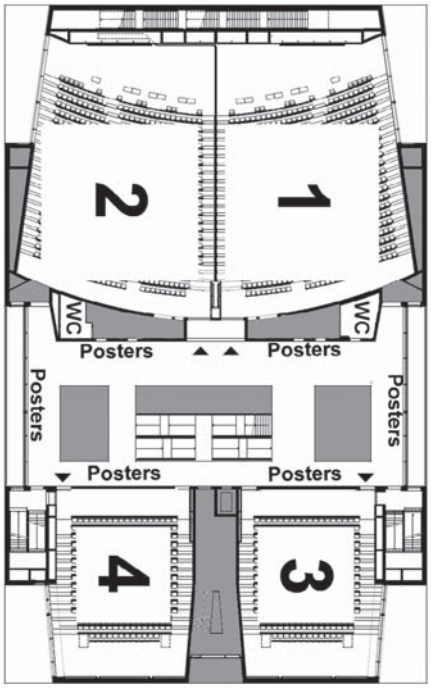
Ground Floor



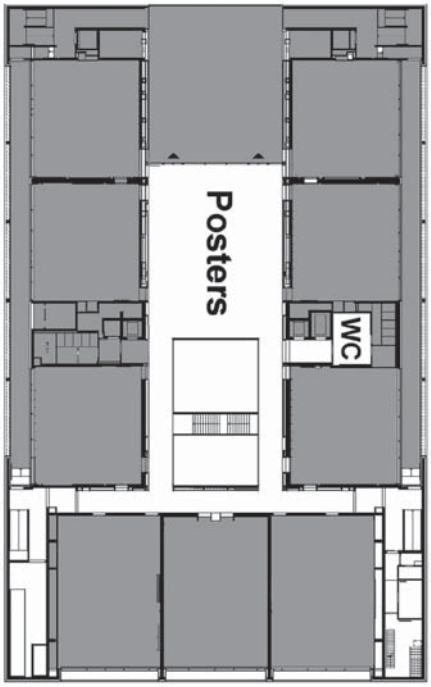
2nd Floor



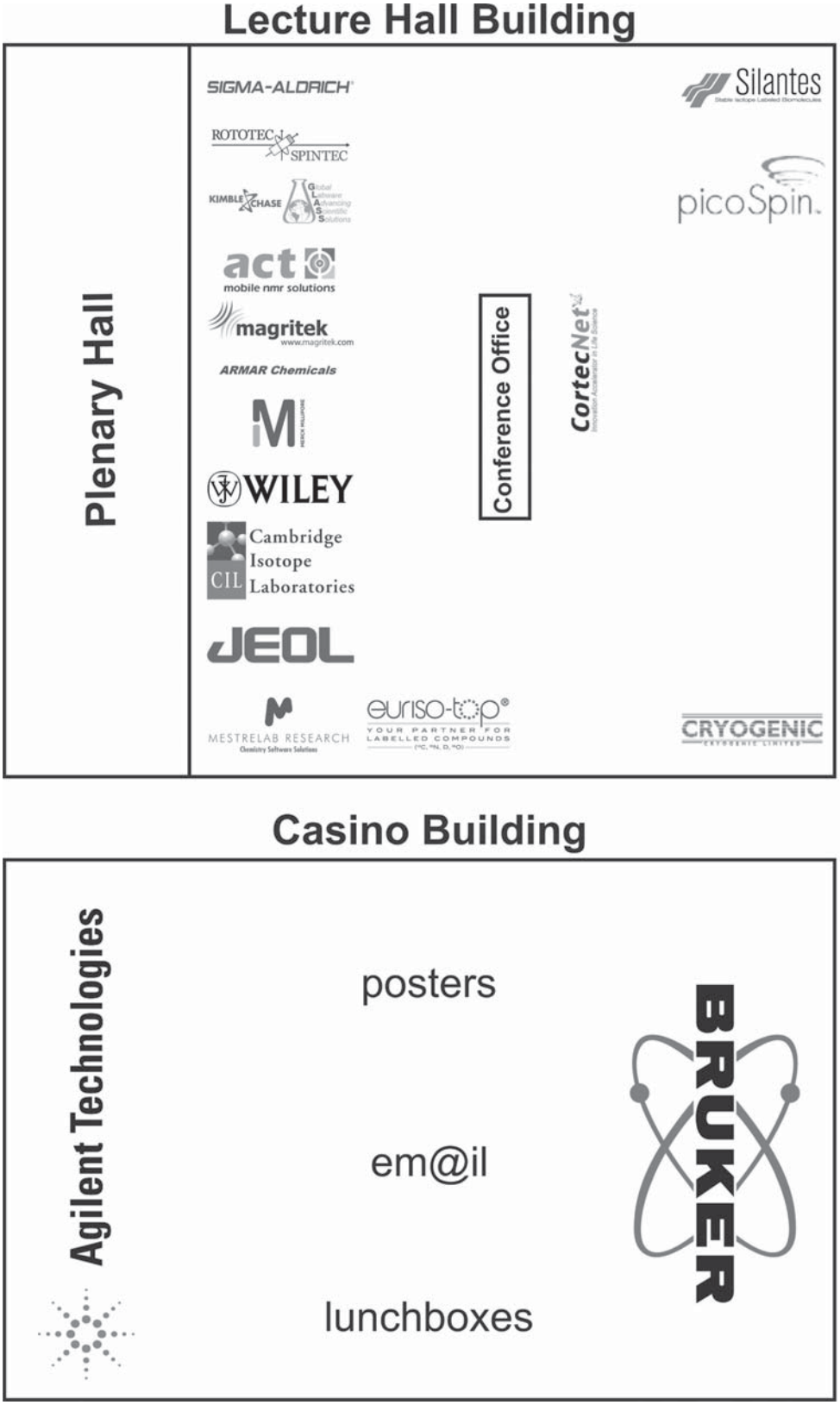
1st Floor



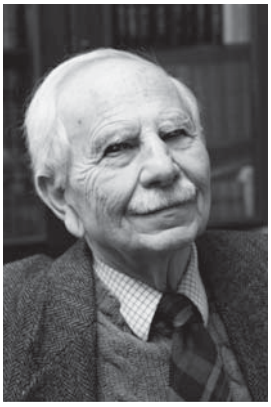
3rd Floor



SPONSORS / EXHIBITORS



In Memory of Anatole Abragam



Anatole Abragam has been born in Moscow on December 15, 1914. He left Russia for France at the age of ten. He graduated in Sciences in 1936, and after an interruption due to World War II he resumed studies at Ecole Supérieure d'Electricité, from which he graduated in 1947. He joined the same year the newly founded Commissariat à L'Energie Atomique (in short CEA, the French Atomic Energy Commission) and he made his entire career at the Centre of Saclay, successively as Physicist, Chief of Section, Chief of Service, Chief of Department and Director of the Physics of CEA. He became Professor of Nuclear Magnetism at the prestigious Collège de France in 1960 and lectured there until his retirement in 1985.

Through his scientific achievements and his lectures and books, Anatole Abragam has become very early, and continued to be in the following and up to now, a leading and prestigious figure in the theory of EPR and NMR, not counting some excursions in other physical domains. It is his career that we have endeavoured to present at the occasion of his 90th birthday, through a brief description of some of his most important scientific contributions.

Initially in the Group of Mathematical Physics, it is during a 2-year visit at the Clarendon Laboratory in Oxford, Great Britain from 1948 to 1950 that he started working on the theory of EPR, essentially in collaboration with Maurice Pryce. He developed with him the theory of the Spin Hamiltonian (1949), which brought about an enormous conceptual simplification in the theoretical description and understanding of localized paramagnetic ions in non-conducting solids. The following year he developed the theory of core polarization, a major theoretical success, which made it possible to explain the anomalous hyperfine structures and was Anatole Abragam's first step towards fame.

Jumping from Oxford (England) to Cambridge (Massachusetts, USA) in 1952, he developed with Robert Pound the complete theory of perturbed angular correlations in a cascade of two radiations emitted in a nuclear radioactive decay, produced in condensed matter by static or variable electric and magnetic fields. This work was the seed of the formalism of relaxation theory developed a few years later at Saclay.

This development was triggered by the work of Ionel Solomon at Harvard in 1955, who generalized the Overhauser effect to dipolar interactions and discovered cross-relaxation. Abragam's formalism of relaxation was entirely based on the use of operators and of the density matrix. For most cases, it has become and remains up to this day *the* method of relaxation calculations.

Another major achievement was the invention in 1958, in collaboration with Jean Combrisson and Ionel Solomon, of an earth-field magnetometer of unprecedented sensitivity. It is a MASER oscillating at the proton Larmor frequency in the earth field, based on the Overhauser inversion of the solvent proton polarization by saturating an appropriate hyperfine resonance of dissolved nitroso radicals. This magnetometer is routinely used for geophysical surveys, in particular in connection with oil prospecting, and for the detection of metallic objects underground or under sea, such as sunken ships, submarines, gas pipes, etc.

The validity of the Spin Temperature Concept in the laboratory frame was established beyond doubts by a series of supremely elegant experiments devised by Anatole Abragam and performed in 1957 with Warren Proctor. Equally important has been the role of Abragam in popularizing the slightly earlier invention, by Alfred Redfield, of Spin Temperature in the rotating frame and in showing how the theories for both cases could be expressed in a common conceptual frame.

Barely a few months after the excitement of the Spin Temperature experiments, another invention of fundamental importance was made by Anatole Abragam, that of Dynamic Nuclear Polarization (DNP), initially under the name of Solid Effect, whereby the polarization of nuclear spins can be made nearly equal to unity, either parallel or antiparallel to the external magnetic field, by off-resonance irradiation of paramagnetic centres at low concentration in non-conducting solids. The main objective of this invention was the production of polarized targets for Nuclear and Particle physics experiments. Initially developed in the Saclay laboratory and in parallel in Berkeley by Carson Jeffries, polarized targets became extremely successful, popular and important tools used at the most important accelerator centres around the world. In recent years, polarized targets proved to be indispensable for new important physics, firstly for the experimental study of Time Reversal and Parity Conservation violation in neutron-nucleus interactions, and secondly for the investigation of the completely unexpected spin structure of the nucleons, that is protons and neutrons, still under study and not elucidated yet. The invention of DNP cannot be dissociated from another one devised in collaboration with Jacques Winter: an experimental scheme for producing polarized proton beams, whose interest complements that of polarized targets in nuclear and particle physics.

One of the most brilliant ideas of Anatole Abragam was to combine the concepts of Spin Temperature and of DNP for inventing the principle of production of Nuclear Magnetic Ordering. The idea was to perform in succession a polarization of the nuclear spins by DNP followed by a nuclear adiabatic demagnetization, either in the laboratory frame or in the rotating frame. Increasing the nuclear polarization amounts to lowering the nuclear entropy. The role of the adiabatic demagnetization it is to turn the Zeeman order into dipolar order at constant entropy. At sufficient low entropy, the nuclear spins undergo a phase transition to an ordered state. The interactions are either the full dipolar interactions, in the laboratory frame, of truncated dipolar interactions, in the rotating frame. In this last case, the interactions depend on the orientation of the single-crystal sample in the external magnetic field. Furthermore, it is possible to choose at will the spin temperature to be either positive or negative. This method was used in the Saclay laboratory for over two decades and led to the production in a number of different crystals of a whole series of nuclear spin orders: ferromagnetic, antiferromagnetic and rotating transverse helical structures, whose study was made both through NMR and neutron diffraction.

As an offspring of Abragam's pondering about the possibility of using neutron diffraction for studying nuclear magnetic ordering, he invented the so-called nuclear pseudo magnetism. When a neutron travels through a polarized material, the average spin-dependent interaction between the neutron and the nuclear spins has the same form as a Zeeman interaction for the neutron. The corresponding pseudo-magnetic field is proportional to the nuclear concentration, their polarization and their "pseudo-magnetic moment". The latter has nothing to do with magnetism: it describes the spin-dependent neutron-nucleus interaction originating from strong interactions. Although the analogy with a Zeeman interaction had been found slightly earlier by two soviet physicists, it was Abragam's merit to push the concept to its limits and to devise experimental schemes to investigate it. This was done essentially in the Saclay laboratory. After a verification of the reality of pseudo-magnetic precession and resonance, a systematic campaign of measurements yielded the pseudo-magnetic moments of more than 25 nuclear isotopes, providing the practitioners of neutron scattering with information of fundamental importance.

Last but not least, Anatole Abragam has revolutionized the practice of μSR (Muon Spin Rotation) with the idea of level crossing, an idea which arose during his last course at the Collège de France before his retiring. Physicists then used pulsed beams to implant polarized muons into matter, and followed the time evolution of their polarization, oscillation or damping, through the angular anisotropy of the μ emission. Abragam's idea was to sweep a magnetic field parallel to the initial muon polarization. At those fields where a level crossing takes place in the system of the muon coupled

to other spins, flip-flop processes result in a decrease of the muon polarization, which is monitored. There is a double advantage in this procedure. Firstly, one can use a *continuous* beam rather than a pulsed one, thereby increasing by an enormous factor the counting rate, and as a consequence the sensitivity of the method. Secondly, one can detect not only the resonance frequency of the muon itself, but also the level structure of the spins coupled to the muon, which was a decisive progress that turned μSR into a completely mature spectroscopic method. Both μSR and level crossing had a long history at the time of this discovery, but nobody had had the idea of combining them together.

Anatole Abragam's prestige is not only due to his scientific achievements, but also to his remarkable pedagogical qualities and his prominent role in diffusing an elaborate and theoretically rigorous "wisdom" in Magnetic Resonance. This was done for the benefit of the members of his laboratory through his constant interest in their work, his advice and example. The French community took advantage of his remarkably brilliant and penetrating lectures, at Saclay and later at the Collège de France. The rest of the world was deeply influenced by his classic books. The first of them, *The Principles of Nuclear Magnetism*, published in 1961 by Oxford University Press, was welcome as a major event in scientific literature and became known as "The Bible". Forty-three years later, and after all the important developments that were done in NMR since, it is still considered as the fundamental basic treatise in the field. Among his principal other books, two are purely scientific:

1970: *Electron Paramagnetic Resonance of Transition ions*, with B. Bleaney (Oxford University Press),
1982: *Nuclear Magnetism: Order and Disorder*, with M. Goldman (Oxford University Press),

one is a collection of essays about science and scientists:

1983: *Réflexions d'un Physicien*,(in French) (Hermann),

and three consist of the respective French, English and Russian versions of his memoirs:

1987: *De la physique avant toute chose*, (Odile Jacob),
1989: *Time Reversal*, (Oxford Press),
1991: *Physicist, where have you been ?*, (in Russian) (Nauka).

Anatole Abragam was recognized and honoured in many ways through Prizes and Medals in France and abroad, and through becoming Doctor *Honoris Causa* of various universities and institutes. He has been President of the French Physical Society and Vice-President of the International Union of Pure and Applied Physics (IUPAP). He has been Invited Professor in the universities of Oxford, Harvard, Amsterdam, Yale, Washington, Leiden, etc. He is Member of the Académie des Sciences in France and Foreign Member of the American Academy of Arts and Sciences, of the National Academy of Sciences (USA) and of the Royal Society of London.

Maurice Goldman

Ionel Solomon

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Registration desk: A. Abragam's "Bible" is available at a special EUROMAR rate.

The Russell Varian Prize of the AMPERE Group:

Gareth A. Morris (University of Manchester)

The Raymond Andrew Prize of the AMPERE Group:

Mark Hunter (Victoria University of Wellington)

The MRC Award for Young Scientists by John Wiley & Sons

Ilia Kaminker (Weizmann Institute of Science): "Mn^{2+}-nitroxide W-band DEER as tool to measure nm scale distances in RNA and protein RNA complexes"

Sami Jannin (École Polytechnique Fédérale de Lausanne): "Ultra High Resolution NMR: Sustained Induction Decays of Long-Lived Coherences"

Maria-Teresa Türke (Max Planck Institute for Biophysical Chemistry): "Saturation factor of nitroxide radicals in liquid DNP by pulsed ELDOR experiments at 0.34 T and 3.4 T"

International EPR/ESR Society (IES) Awards

Gold Medal:

Ronald P. Mason (National Institute of Environmental Health Sciences)

Silver Medal – Instrumentation:

Graham Smith (University of St Andrews)

Young Investigator Award:

Enrica Bordignon (ETH Zurich) and

Alexey Silakov (Max-Planck Institute of Bioinorganic Chemistry)

IES Fellows of the Society:

Klaus Möbius (Free University Berlin)

Ernst Award of the Magnetic Resonance Spectroscopy Division of the GDCh

Michael Braun (Technical University Munich) for his article: "Cooperative Pulses", Michael Braun and Steffen Glaser, J.Magn.Reson (2010), 207: 114-123.

Jiři Nováček (Masaryk University) for his article: "5D ^{13}C-detected experiments for backbone assignment of unstructured proteins with a very low signal dispersion", Jiři Nováček, Anna Zawadzka-Kazimierczuk, Veronika Papoušková, Lukáš Žídek, Hanna Šanderová, Libor Krásný, Vladimír Sklenař, J. Biomol. NMR, (2011), 50: 1-11

Robert Hänsel and Ivan Krstić (Goethe University) for their article: "Long Range Distance Measurements on Nucleic Acids in Cells by Pulsed EPR Spectroscopy", Ivan Krstić, Robert Hänsel, Olga Romainczyk, Joachim W. Engels, Volker Dötsch and Thomas F. Prisner, Angew.Chem. Int. Ed. (2011) 50: 5070-5074.

The Ulderico Segre Prize

Loïc Salmon (Université Joseph Fourier, Grenoble)

Hans Moons (University of Antwerp)

for their outstanding contribution to the development of new methodologies in the field of Magnetic Resonance.

PROGRAM

EUROMAR 2011

33RD DISCUSSION MEETING OF THE MR SPECTROSCOPY DIVISION OF THE GDCH

8TH EUROPEAN FEDERATION OF EPR GROUPS MEETING

21-25 AUGUST 2011, FRANKFURT AM MAIN, GERMANY

Sunday 21st August

10:00-17:30	Registration
13:15-17:00	Tutorial Lectures James Keeler: "Coherence order and coherence selection" Malcolm Levitt: "Superoperators and relaxation phenomena" Marina Bennati: "Dynamic nuclear polarization" Edgar Groenen: "Trends in pulsed and high-frequency EPR"
17:30-19:30	Opening & Prize Session Gareth A. Morris: "What's in a name?" Mark Hunter: "Measurement and simulation of the nonlocal dispersion tensor" Ronald P. Mason: "The Fidelity of Spin Trapping with DMPO in Biological Systems"
19:30-23:00	Welcome Mixer

Monday 22nd August

08:30	**Geoffrey Bodenhausen:** "Shuttling and Spinning Samples with Dynamic Nuclear Polarization"		
09:15	**Mei Hong:** "Structure, dynamics, and mechanisms of the influenza M2 protein from solid-state NMR"		
10:00	Coffee		
	Lecture Hall 3	*Lecture Hall 4*	*Lecture Hall 5*
10:50	**P.E. Wright:** "Characterization of Transient Protein Folding and Unfolding Processes by NMR Relaxation Dispersion"	**I.V. Koptyug:** "Recent Advances in MR Imaging of Heterogeneous Catalysis"	**W. Lubitz:** "Multifrequency EPR Studies of Oxygen-Tolerant Hydrogenases"
11:25	**P. Neudecker:** "Solution Structure of a Low-populated Protein Folding Intermediate from NMR Relaxation Dispersion Spectroscopy Rationalizes Aggregation Propensity at Atomic Resolution"	**S. Gloeggler:** "Para-hydrogen Induced Polarization of Amino Acids, Peptides and Deuterium-hydrogen Gas"	**A.M. Bowen:** "Progress and Challenges in Measuring the Orientational Dependence of DEER for Transition Metals in Model Systems and Proteins"
11:50	**B. Brutscher:** "Polarization Enhancement in BEST-TROSY NMR. Application to the Study of Protein Folding Intermediates and Intrinsically Disordered Proteins"	**Y. Hertzberg:** "Tissue Elasticity Measurement Using Acoustic Radiation Force Imaging"	**G. Mathies:** "Continuous-wave EPR at 275 GHz. New Insights into the Iron-binding Sites of Transferrin"
12:15	**J. Christodoulou:** "Co-translational protein folding on the ribosome: Using NMR spectroscopy to provide structure and dynamics of ribosomes and ribosome-nascent chains"	**S. Aime:** "CEST (Chemical Exchange Saturation Transfer) agents for innovative MR-Molecular Imaging investigation"	**D. Goldfarb:** "Nanometer scale distance measurements in biomolecules using Gd^{3+} spin labelling"
12:50	Lunch		
14:30	Poster Session One		
	Lecture Hall 3	*Lecture Hall 4*	*Lecture Hall 5*
16:00	**R. Boelens:** "Structure and Dynamics in Gene Regulation and DNA Repair"	**B. Griffin:** "Dynamic Nuclear Polarization at High Magnetic Fields"	**A. Schnegg:** "FD FT THz EPR on High Spin Transition Metal Ion Clusters"
16:35	**C.B. Post:** "Domain Orientation for Controlling Protein Interactions"	**M. Lelli:** "Surface Enhanced NMR by DNP: Analysis of the Sensitivity Enhancement and Application to a New Class of Porous Materials"	**A. Blank:** "High Sensitivity Pulsed Electron Spin Resonance Spectroscopy with Induction Detection"
17:00	**D. Lee:** "Molecular Recognition Kinetics within Inaccessible Time Window"	**C. Hilty:** "Hyperpolarized NMR of Polypeptides"	**I. Kaminker:** "Mn^{2+} – Nitroxide W-band DEER as Tool to Measure nm Scale Distances in RNA and Protein RNA Complexes"
17:25	**M. Blackledge:** "Towards A Robust Description of Intrinsic Protein Disorder using Nuclear Magnetic Resonance Spectroscopy"	**W. Köckenberger:** "Dissolution Dynamic Nuclear Polarization – Advances in Theory and Experimental Implementation"	**D. Suter:** "EPR with Small Resonators and Small Numbers of Spins"
18:15	**Kurt Wüthrich:** "Exploring the Protein Universe with Biomolecular NMR"		
19:00	Hospitality Suites		

Tuesday 23rd August

08:30	**Anne McDermott:** "Conformational Exchage Processes in the Ion Channel KcsA"		
09:15	**Wayne L. Hubbell:** "Exploring Molecular Flexibility and the Energy Landscape of a Protein with Site-Directed Spin Labeling"		
10:00	Coffee		
	Lecture Hall 3	*Lecture Hall 4*	*Lecture Hall 5*
10:50	**I. Garcia-Rubio:** "Magnetic Resonance Studies on Magnetotactic Bacteria"	**G. Parigi:** "Is There a Sensible Approach to the Inverse Problem of Many Conformations Providing Only Few Average Parameters?"	**D. Gourier:** "What EPR Reveals about the Origin of Life"
11:25	**I. Krstić:** "In-cell Pulsed EPR on Nucleic Acids"	**B. Corzilius:** "High Field Dynamic Nuclear Polarization with High-spin Transition Metal Ions "	**J. van Slageren:** "Quantum Coherence in Molecular Nanomagnets"
11:50	**P. Barraud:** "An Unexpected Zinc-binding Motif Embedded in a dsRBD Revealed a New Class of Regulatory Domain Mediating Nuclear Localization of Dicer "	**M.J. Knight:** "Fast Fold Determination of the 153-residue Protein Superoxide Dismutase by High-Resolution Proton-detected Solid-state MAS NMR "	**G. Mitrikas:** "Pulsed EPR Characterization of Encapsulated Atomic Hydrogen in Octasilsesquioxane Cages"
12:15	**T. Carlomagno:** "Protein Recognition and Functional Mechanisms of Non-coding RNAs "	**J. Kowalewski:** "Field-dependent Paramagnetic Relaxation Enhancement in Solutions of Ni(II): What Happens above the Proton Frequency of 1 GHz?"	**D. Hinderberger:** „EPR Spectroscopy on Serum Albumin"
12:50	Lunch		
14:30	Poster Session Two		
	Lecture Hall 3	*Lecture Hall 4*	*Lecture Hall 5*
16:00	**R.M. Gschwind:** "Enamine and Brønsted Acid Catalysis-Intermediates Trapped by NMR"	**C. Glaubitz:** "Biophysical Insight into Structure and Function of Proteorhodopsin by Solid-state NMR"	**J. Dolinšek:** "NMR of Quasicrystals and Complex Metallic Alloys"
16:35	**A. Jerschow:** "Long Lived Coherent Response Signal Imaging"	**A. Loquet:** "Supramolecular Assemblies Studied by Solid-state NMR: The Structure of the Type Three Secretion System Needle"	**T.E. Exner:** "Towards Quantum Chemical NMR Chemical Shifts of Proteins"
17:00	**B.E. Bode:** "The Heart of Photosynthesis Illuminated by Joining Photo-CIDNP and Quantum Chemistry"	**G. Gröbner:** "Insight into Apoptotic Events in Intact Mitochondria by Solid State NMR"	**D.J. Hirsh:** „ Measuring Long-Range Distances and Exchange Couplings in DNA Using Saturation-Recovery EPR"
17:25	**M. Pons:** "Dynamic Interactions of Proteins and DNA Related to Pathogenicity"	**A. Böckmann:** "Prion structures: a single architecture?"	**J.H. Enemark:** "Determination of the Structure of the Mo(V) Center of Sulfite Oxidase by Variable Frequency Pulsed EPR Spectroscopy, ^{33}S and ^{17}O Labeling, and DFT Calculations"
18:15	**Ilme Schlichting:** "Nanocrystals – Extending Opportunities in Structural Biology"		
19:00	Hospitality Suites		

Wednesday 24th August

08:30	**Klaas Pruessmann:** "Mind the field - Dynamic magnetometry for MRI"		
09:15	**Jürgen Haase:** "NMR at the Highest Magnetic Fields and Pressures – Applications to Quantum Solids"		
10:00	Coffee		
10:30	**Horst Kessler:** "NMR Studies of Proteins and Their Interactions: Dynamics of Folding and Defolding of Helices is Essential for Biological Function"		
11:15	Ernst Awards **Michael Braun:** "Cooperative Pulses" **Jiři Nováček:** "5D 13C-detected experiments for backbone assignment of unstructured proteins with a very low signal dispersion" **Robert Hänsel & Ivan I. Krstić:** "Long Range Distance Measurements on Nucleic Acids in Cells by Pulsed EPR Spectroscopy"		
12:15	**Gunnar Jeschke:** "Membrane Protein Structure and Structural Transitions: An EPR View"		
13:00	Lunch		
14:30	Poster Session Three		
	Lecture Hall 3	*Lecture Hall 4*	*Lecture Hall 5*
16:00	**S. Zinn-Justin:** "Structural Organization of Bacteriophage Head-to-tail Connection, as Characterized by EM, NMR and Bioinformatics"	**F. Neese:** "Theoretical EPR Spectroscopy of Open-Shell Transition Metal Complexes with Strong Spin Orbit Coupling"	**S. Sigurdsson:** "Strategies for Site-Directed Spin Labeling of Nucleic Acids"
16:35	**U. Akbey:** "Spin Gymnastics with Deuterated Proteins: Solid-State NMR & DNP"	**N.C. Nielsen:** "Optimal Rf Pulse, Cross-Polarization, and Multiple-Dimensional Sampling Package for Solid-State on Perdeuterated Proteins"	**B. Guigliarelli:** "Dynamic Disorder Evidenced by SDSL-EPR in a Multienzyme Complex Involved in CO_2 Assimilation by Microalgae"
17:00	**K. Tripsianes:** "Structural Basis for Dimethyl-arginine Recognition by Tudor Domains"	**E.R.H. van Eck:** "Unprecedented ^{27}Al MAS NMR Resolution on Zeolite Single Crystals"	**M.-T. Türke:** "Saturation Factor of Nitroxide Radicals in Liquid DNP by Pulsed ELDOR Experiments at 0.34 T and 3.4 T"
17:25	**A.M. Gronenborn:** "Mannose-binding Lectins – Cyanovirin and Beyond"	**G. Papavissiliou:** "NMR Studies of Novel Strongly Correlated Electron Materials"	**H.-J. Steinhoff:** "Lipid Sensing and Transmembrane Signaling Studied by Site-directed Spin Labeling EPR"
18:15	**Ivano Bertini:** "Lightening from NMR in Life Sciences"		
19:00	Cracker & Drinks		

Thursday 25th August

08:30	**Shimon Vega:** "A closer look at Dynamic Nuclear Polarization"		
09:15	**Robert Bittl:** "Multi-frequency EPR in biophysics and material science"		
10:00	Coffee		
	Lecture Hall 3	*Lecture Hall 4*	*Lecture Hall 5*
10:50	**J. Balbach:** "Exploring Protein Energy Landscapes by NMR"	**B. Reif:** "Solid-State NMR Investigations of Aggregates Formed by Perdeuterated Alzheimer's Disease Ab Peptides"	**O. Schiemann:** "X- and W-Band PELDOR: Conformational States of Model Systems and the Ion Channel MscS"
11:25	**S. Jannin:** "Ultra High Resolution NMR: Sustained Induction Decays of Long-lived Coherences"	**M.R. Hansen:** "A Method for Revealing the Local Packing Organization in Conjugated Semi-crystalline Polymers"	**O.G. Poluektov:** "Biomimetic Hydrogen Production: Multifrequency EPR and DFT Study of Cobaloxime Catalyst"
11:50	**G. Pileio:** "Longtime Storage of Hyperpolarization via Singlet States in High Field"	**M.J.N. Junk:** "Interplay of Order, Disorder, and Dynamics in Polymer-Fullerene Blends for Photovoltaic Applications"	**S. van Doorslaer:** "EPR Analysis of Chromium-sugar Interactions"
12:15	**S. Glaser:** "Robust and Cooperative Control of Spins"	**H. Heise:** "Protein Misfolding, Membrane Interactions and Paramagnetism Studied by Solid State NMR Spectroscopy"	**J.H. Freed:** "ESR Studies of Dynamics and Structure of Proteins and Membranes at ACERT"
12:50	Lunch		
14:30	Poster Session Four		
	Lecture Hall 3	*Lecture Hall 4*	*Lecture Hall 5*
16:00	**A. Ramos:** "Molecular Bases of Gene Regulation by FUSE Binding Proteins"	**L. Frydman:** "Alternatives in the Rapid Acquisition of Multidimensional NMR and MRI Data"	**G. Smith:** "Bringing the NMR Paradigm to EPR"
16:35	**L. Salmon:** "Protein Conformational Dynamics and Weak Complex Formation"	**T. Meersmann:** "Hyperpolarization of spin I > 1/2 Noble Gasses Beyond 10% Spin Polarization for Biomedical MR Applications"	**E. Bordignon:** "Helpful tools for SDSL EPR on membrane proteins: DNP water accessibility, His-tag labeling and high power Q band"
17:00	**E. Kupce:** "Detecting the 'Afterglow' of ^{13}C NMR in Proteins Using Multiple Receivers"	**E.B. Brunner:** "High-Pressure in situ ^{129}Xe NMR spectroscopy of breathing transitions in Metal-Organic Framework (MOF) compounds	**A. Silakov:** "Understanding the Hydrogen-converting Cluster of [FeFe] Hydrogenase"
17:25	**J. Wöhnert:** "The Functional Dynamics of Synthetic Riboswitches"	**A. Lesage:** "Surface Enhanced NMR Spectroscopy by Dynamic Nuclear Polarization"	**K. Möbius:** "Conformational Changes During Primary Photosynthesis as Studied by Orientation Resolving Pulse Dipolar EPR Spectroscopy"
18:15	**Beat H. Meier:** "Amyloids by Solid-State NMR: Structure, Dynamics and Interactions with Small Molecules"		
20:00	**Dinner**		

Plenary Lectures

	Sunday 21st August
19:30	**Gareth A. Morris** **Mark Hunter** **Ronald P. Mason**
	Monday 22nd August
8:30	**Geoffrey Bodenhausen** **Mei Hong**
18:00	**Kurt Wüthrich**
	Tuesday 23rd August
8:30	**Ann McDermott** **Wayne L. Hubbell**
18:00	**Ilme Schlichting**
	Wednesday 24th August
8:30	**Klaas Pruessmann** **Jürgen Haase**
10:30	**Horst Kessler**
	Ernst Awards: **Michael Braun** **Jiři Nováček** **Robert Hänsel & Ivan Krstić**
12:15	**Gunnar Jeschke**
18:00	**Ivano Bertini**
	Thursday 25th August
8:30	**Shimon Vega** **Robert Bittl**
18:00	**Beat Meier**

PL01

What's in a name?

Gareth A. Morris

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INEPT began life as an extension of early 2D ^{1}H-^{13}C correlation experiments[1,2]. These showed surprisingly good sensitivity, because of the twin advantages of polarization transfer from the higher γ protons and the shorter proton T_1. It was then an obvious step to crystallise these advantages in a 1D experiment by removing the evolution period and adding refocusing pulses to make the technique broadband[3]. Two different experiments were performed, one generating antiphase peaks and the other refocused to allow the measurement of decoupled spectra, but only the first of these was published at the time[3], the refocused experiment being developed independently by Burum and Ernst[4]. One of the first applications of INEPT was, at the suggestion of Howard Hill, to enhance ^{15}N signals[5], but the method was rapidly extended to multiplicity determination and to incorporation as a building block in ever more complex sequences for protein structure determination. New uses continue to appear, including applications in high resolution solid state NMR and quantum computing.

The early history of the INEPT pulse sequence will be described, and, time permitting, some more recent developments touched on.

1. A.A. Maudsley, L. Müller and R.R. Ernst, *Chem. Phys. Lett.*, 50, 368 (**1977**)
2. R. Freeman and G.A. Morris, *J. Chem. Soc., Chem. Commun.* 684-686 (**1978**)
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4. D.P. Burum and R.R. Ernst, *J. Magn. Reson.* 39, 533-538 (**1980**)
5. G.A. Morris, *J. Am. Chem. Soc.* 102, 428-429 (**1980**)

PL02

Measurement and simulation of the nonlocal dispersion tensor

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Dispersion describes the phenomenon whereby particles on the same streamline separate during flow. The physics of dispersion is governed by stochastic processes arising from the interplay between advective velocity gradients, molecular diffusion and boundary layer effects[1]. The dispersion tensor, $\mathbf{D}^*$, commonly measured with NMR[2], is a local measurement in the sense that it does not depend on positional relationships and is measured as time asymptotes. For situations where the length- and time-scales on which transport occurs are not much larger than the scale of the fluctuations in the velocity field, a nonlocal description is required[3]. The study of fluid dispersion in porous media is important to a wide range of applications including chromatography, filtration, oil recovery, groundwater flows and catalysis.

Pulsed Gradient Spin Echo (PGSE)-NMR provides a wealth of information about the separation of particles and velocity correlations in porous media. Presented here is a set of NMR pulse sequences and a superposition designed to extract the velocity correlations necessary to calculate the dispersion as a function of displacement and hence the nonlocal dispersion[4]. Experiments performed on porous media will be discussed including further tensors and nonlocal measurements with higher dimensionality. Measurements on rock cores will also be discussed. Numerically, a lattice-Boltzmann generated flow field[5], with a large set of virtual tracer particles is used to calculate the nonlocal dispersion tensor[4] in regimes difficult to access experimentally.

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1. G. Taylor, *Proc. Royal Soc. Lon. B.* 67, 857-869 (**1954**)
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3. Koch, D. L. and Brady, J.F. *J. Fluid Mech.* 180, 387-403 (**1987**)
4. Hunter, M.W. and Callaghan, P.T. *PRL* 99, 210602-1,-4(**2007**), Hunter, M.W. *et. al. Phys. Fluids.* 22 027101 (**2010**),
5. Manz, B. *et. al. AIChE J* 45, 1845-1854 (**1999**)

PL03

The Fidelity of Spin Trapping in Biological Systems

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Unlike direct ESR, the spin trap methodology depends on the absolute fidelity of the spin trap reaction. Two alternative reactions of 5,5-dimethyl-1-pyrroline *N*-oxide (DMPO) leading to radical adduct artifacts have been discovered: inverted spin trapping and the Forrester-Hepburn nucleophilic mechanism. These two alternate pathways to radical adducts are a combination of one-electron oxidation and nucleophilic addition in either order. In biological systems the most serious artifact is due to the Forrester-Hepburn mechanism, which is initiated by the addition of a nucleophile to DMPO. These challenges to spin trapping were first put forward forty years ago, but only recently have approaches been developed to distinguish these artifacts from authentic spin trapping.[1] One approach is kinetic: The quantitative rate of free radical formation is measured by spin trapping and another indirect approach. Since oxygen is Mother Nature's spin trap, we used the rate of its consumption resulting from its reaction with the free radical.[2] Another approach developed by Timmins and coworkers[3,4] uses isotopically labeled spin traps to detect the nucleophilic addition of spin traps to the free radical precursors in preincubation experiments. We have used a related approach where the free radical precursor is isotopically labeled and preincubated with the spin trap to detect the nucleophilic addition.

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4. Silvester, J.A., Wei, X.D., Davies, M.J., and Timmons, G.S., *Redox Report*, 3, 225-231 (**1997**)

PL04

Shuttling and Spinning Samples with Dynamic Nuclear Polarization

Geoffrey Bodenhausen[1,2]

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Dynamic Nuclear Polarization (DNP) at temperatures around 1.5 K can lead to population differences across spin states that may be 10 000 times larger than Boltzmann's law would imply at room temperature. DNP can be followed by rapid 'dissolution', but care must be taken to avoid losses of the polarization through longitudinal relaxation. It will be shown that long-lived states (LLS) can offer a safe haven where polarization can be stored over extensive intervals, after shuttling the sample to a moderate field on the order of 0.1 T. Dissolution DNP can be combined with various 'one-shot' experiments, such as 'on-the-fly detection' of Long-Lived Coherences (LLC), which can provide remarkably narrow line-widths (on the order of 0.01 Hz) for protons in suitable systems.

DNP can also be combined with low-temperature magic angle spinning (LT-MAS) to 'see' signals of organic molecules that can be grafted on the surfaces of porous materials. One can observe dipole-dipole couplings between protons belonging to the organic grafts on the one hand, and silicon-29 on surfaces of silicates on the other. This allows one to check hypotheses about the orientation of organic grafts with respect to surfaces.

This research involves several co-authors from EPF Lausanne, ENS Lyon, ETH Zurich, and ENS Paris.

PL05

Structure, dynamics, and mechanisms of the influenza M2 protein from solid-state NMR

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The influenza M2 protein forms a pH-activated tetrameric proton channel that mediates virus entry into the cell. We present our studies of M2 structure and dynamics using MAS solid-state NMR, which give valuable insights into the molecular basis for the proton channel function. We determined the conformation, dynamics, hydrogen-bonding and proton exchange of the proton-selective residue, His37, as a function of pH. pH-dependent chemical shifts indicate the presence of both τ and π tautomers at high pH for the closed channel, which pack in a CH-π stacked fashion based on backbone – sidechain distances and rotameric conformation. The high-pH neutral rings are immobilized, while the cationic imidazoliums at low pH are dynamic, with bond order parameters that indicate restricted-amplitude two-site reorientations on the sub-microsecond timescale. The energy barrier of this motion was measured through temperature-dependent dipolar couplings and found to be consistent with the functional barrier of proton conduction. His-water proton exchange was directly observed in ^{15}N spectra at physiological pH, and ^{15}N T_2's of the exchange peaks, when analyzed taking into account chemical exchange, ^{1}H decoupling and MAS, gave quantitative estimates of the proton exchange rate. These results show definitively that M2 conducts protons through a His37-mediated shuttle mechanism, while several alternative models, such as water wire and hydrogen-bonded His-His dimer, are ruled out by the data. The antiviral drug, amantadine, inhibits the M2 proton channel function. We measured protein-drug distances by REDOR, drug dynamics by ^{2}H NMR, and protein conformational perturbation through 2D correlation NMR. These results elucidate the pharmacologically relevant binding site in the protein and reveal the inhibition mechanism of this channel.

PL06

Exploring the Protein Universe with Biomolecular NMR

Kurt Wüthrich[1,2], Pedro Serrano[1], Reto Horst[1], Michael Geralt[1], Biswaranjan Mohanty[1], Pawel Stanczak[1], Fred F. Damberger[2], Barbara Christen[2]

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In classical "structural biology", NMR structure determination is focused on previously well-characterized proteins with known biological roles or biomedical interest. In today's post-genomic era, with the availability of the complete DNA sequences of a wide range of organisms, we have additional new opportunities and challenges in "structural genomics". For PSI:Biology 2010-15 (http://www.nigms.nih.gov/Research/FeaturedPrograms/PSI/psi_biology/) the initially formulated goal of structural genomics to provide coverage of large parts of the protein sequence universe with three-dimensional structures has been expanded with a more function-focused strategy. In this context, my research team represents solution NMR in three structural genomics consortia, JCSG (http://www.jcsg.org), JCIMPT-Complexes (http://jcimpt.scripps.edu/) and GPCR-Network (http://gpcr.scripps.edu/), which all use structure determination by X-ray crystallography as the principal technique. This presentation describes our approach for use of solution NMR spectroscopy with soluble and membrane proteins in these crystallography-centered environments. The strategies used should ensure an exciting role for NMR in the longer-term challenge leading from the expanding protein structure universe to new insights into protein functions and chemical biology, by generating data on protein structure, conformational equilibria, dynamics and intermolecular interactions in solution, as will be illustrated with studies of prion proteins.

PL07

Conformational Exchage Processes in the Ion Channel KcsA

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ABSTRACT The plasticity of ion channels is clearly critical to the many essential processes they carry out in all cells. A conformational exchange process in the ion-binding selectivity filter of a simple ion channel, KcsA, will be discussed. This highly conserved region of KcsA exhibits clearly resolved and site specifically assigned NMR chemical shifts. These reporters allowed us to probe site-specific affinities of the ions, and to observe a related slow conformational exchange process. We observe a significant degree of anti-cooperativity in their binding behaviour. Methods for characterizing millisecond and microsecond conformational exchange processes in high resolution SSNMR experiments will also be discussed.

PL08

Exploring Molecular Flexibility and the Energy Landscape of a Protein with Site-Directed Spin Labeling

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Protein function relies on molecular flexibility, particularly for proteins involved in signal transduction, where flexibility gives rise to promiscuity in protein-protein interactions. Remarkably, high flexibility may even characterize interaction domains in the complexes themselves. Discovery in the field of functional protein dynamics requires techniques capable of monitoring backbone and conformational motions in the time range of ps to ms under physiological conditions without restrictions as to molecular size. Site-directed spin labeling (SDSL)-EPR meets these requirements. For example, spectral line shape analysis of a spin labeled protein provides quantitative information on the amplitude of ps-ns backbone dynamics, while pulse saturation recovery (pSR), osmotic perturbation and high pressure perturbation can reveal the presence of conformations in slow exchange on the EPR time scale, including "invisible" states of low population. Exchange rates in the 10 KHz to 1 MHz range can be measured by pSR and pulse electron-electron double resonance (pELDOR). To access slower motions, high pressure jump EPR will allow exchange rates of ≤10 KHz to be measured. With the above strategies, protein states with a life time ps to ms and beyond can be characterized via SDSL/EPR using extremely small samples of systems of any degree of complexity.

PL09

Nanocrystals – extending opportunities in structural biology

Ilme Schlichting

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Crystalline nanoparticles have drawn a lot attention recently. Reasons include technological interests since many of their electrical and thermodynamic properties show strong size dependence (e.g. quantum dots), favorable formulation properties for drug delivery, use as molecular sieves etc. Structures of crystalline nanoparticles can be determined for example by solid state NMR. So far, analysis by X-ray crystallography was limited to powder diffraction due to radiation damage. Crystals cooled to liquid nitrogen temperature tolerate a dose of ~30 MGy (1). It has been predicted that this dose can be increased significantly if the diffraction data is collected using intense femtosecond X-ray pulses that are short enough to have passed the sample before significant electronic rearrangements and atomic displacements occur ("diffraction-before-destruction" (2). The advent of free-electron lasers (FELs) provides femtosecond pulses with a peak brilliance that is about nine orders of magnitude higher than that provided by third generation synchrotron sources. Recently, the Linac Coherent Light Source (LCLS) has become accessible to users, accessing the hard X-ray regime, thereby allowing Ångstrom-resolution studies with femtosecond time resolution. The concept of serial femtosecond crystallography has been demonstrated on photosystem I by injecting a stream of hydrated nanocrystals into the FEL interaction region, collecting stroboscopic diffraction patterns from single, randomly oriented crystals, hit by the femtosecond X-ray pulses (3).

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PL10

Mind the field - Dynamic magnetometry for MRI

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This contribution will discuss the concept of magnetic field monitoring during MRI scans, a recent approach designed to enhance reconstruction results by improving the accuracy of the underlying encoding models. Arrays of miniature NMR probes yield highly sensitive, time-resolved accounts not only of intentional gradient encoding but also of undesired field dynamics.

Field perturbations from 0th spatial order (uniform) to very high spatial orders frequently occur in MRI scans due to hardware imperfections, physiological susceptibility effects, and external sources. It is shown that field monitoring permits incorporating such perturbations in a suitable signal model, enabling high-fidelity reconstruction despite experimental imperfections. Special attention will be given to the sensitivity of NMR field probes and the important distinction between measuring the magnetic field per se and measuring its integral over time, which is required for image reconstruction purposes. Concerning reconstruction on the basis of measured field evolutions, the presentation will cover current algorithms and requirements in terms of computing resources. One important realm of applications of field monitoring per se is the characterization of MR hardware, including measurements of impulse responses of gradient and shim systems as well as the assessment of thermal and mechanical effects, main magnet drifts and siting issues.

Current imaging applications include high-field imaging in the presence of physiological field fluctuations, diffusion-tensor imaging with higher-order image reconstruction to account for eddy current effects, enhancing the geometric fidelity of anatomical scans, and correcting for gradient imperfections in phase-contrast flow measurements.

PL11

NMR at the Highest Magnetic Fields and Pressures - Applications to Quantum Solids

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Nuclear Magnetic Resonance analyzes the interaction of nuclei with an external magnetic field and draws rich conclusions about a material's chemical or electronic structure from electron-nucleus and inter-nuclear interactions. The applied magnetic field, pressure, or temperature also influence the electronic properties or internal degrees of freedom, and structure, which is reflected in corresponding changes of the NMR parameters. This makes NMR a powerful tool in the physics and chemistry of today's functional materials. We will report here on two frontiers of our current research. First, the development of NMR in pulsed high-field magnets that aim at 100 Tesla fields. Here we could show recently, e.g., that one can track the field over about 10 milliseconds with ppm precision above 60 Tesla, demonstrating that shift measurements with ppm resolution are possible. Second, we report on our endeavor to perform high-sensitivity diamond anvil cell Giga-Pascal-NMR. We demonstrate our powerful new approach that uses NMR micro-coils inside the high-pressure region with two examples. We show for simple aluminum metal an unexpected shift as a function of pressure, which let us solve some old riddles. We also show how high pressure pushes up important new details about the electronic physics of high-temperature superconductors.

PL12

NMR Studies of proteins and their interactions: dynamics of folding and defolding of helices is essential for biological function

Horst Kessler[1], Franz Hagn[1,2,3], Stephan Lagleder[1,2], Johannes Buchner[2], Thomas Scheibel[3]

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Protein-protein interactions are preferentially investigated by NMR which allows studying a system under native-like conditions. Molecular recognition often occurs in flexible regions affording mutual adoption of the interaction partners. These flexible regions are not only provided in loops but also (partial) defolding of helices is used for this function.

One prominent example is the tumor suppressor protein p53 whose DNA binding domain mediates the interaction with different proteins like BclxL and Hsp90. Here, the flexibility of the helices enables for binding (recognition of) different shapes to regulate apoptosis.

Helix formation is induced in SDS micells in the small heat shock proteins Hsp12, which functions directly at the membrane and not by interaction with cytosolic proteins.

Kinetic investigation of some antibody fragments CL, VL and in β2 microglobulin identified the initial formation of a helix as essential to prevent dangerous fibril formation of these domains.

The folded C- and N-terminal domains (CTD and NTD) of spidroin, the proteins spider silk is composed of, control formation of the thread. The NMR structure of the CTD and their properties under the different conditions (salt concentration, pH) in the gland and in the duct explains how spidroin can be stored at high concentration but form silk in less than a second on demand. The controlled dimerization of the NTD in the duct yields a multivalent cross linking of the microcrystalline substructures to form the extremely stable silk thread.

PL13

Membrane protein structure and structural transitions: An EPR view

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Combination of site-directed spin labeling with EPR techniques provides information on membrane proteins that are too large for current NMR technology and on protein states that evade crystallization. Long range distance restraints and distance distribution widths from the DEER experiment allow for characterization of disordered parts of the structures. These opportunities have led to a still increasing surge of EPR studies on membrane proteins. Yet, most of the conclusions drawn from the primary data are qualitative or at best semi-quantitative, and it is often unknown whether these data would be consistent with alternative models, too.

These problems arise mainly from uncertainties in accounting for conformational distribution of the spin label[1] and from sparsity of restraints. Here, I try to give an answer to the question what can and what cannot be concluded from such data at the current state of the art.

Examples include 1) folding of major plant light harvesting complex LHCII[2,3] and structure of its N-terminal domain, 2) EPR-restraint supported homology modeling of proline/sodium symporter PutP, and 3) an assessment of reliability and precision of modeling of structural transitions in cases where the structure is known in one endpoint of the transition.

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PL14

Lightening from NMR in Life Sciences

Ivano Bertini

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NMR is a flagship in life sciences. The NMR power in structure determination is well documented. An application towards mechanistic systems biology will be presented.

The contribution of paramagnetic metal ions to “adjust” X-ray structures to the solution state is addressed. Attention will be payed to proteins with two mobile domains and reflection will be devoted to the inverse problem of extracting structures from few parameters of conformationally mobile proteins, again using paramagnetic restraints or other pieces of information. Methodological advancements in solution, solid state and sedimented systems will be discussed.

Metabolomics by NMR may not be a challenging field for NMR but surely is of help in biomedicine projects. Finally, NMR should enter into Information and Communication Technology projects. The future of NMR is still bright as it is an essential part of the ESFRI (European Strategy Forum on Research Infrastructures) infrastructures, INSTRUCT, Openscreen, Eurobioimaging and BBMRI.

PL15

A closer look at Dynamic Nuclear Polarization

Shimon Vega

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The renewed broad interest in DNP in recent years has motivated us to have a closer look at the spin dynamics of electron-nuclear systems that are exposed to microwave irradiation, resulting in large nuclear spin polarizations. Distinctions must be made between liquid-DNP, relying on the Overhauser effect, and the solid effect (SE), cross effect (CE) and thermal mixing (TM) mechanisms in DNP on static and rotating solid samples. In this presentation the action of the different DNP mechanisms providing the nuclear polarizations during static- and magic angle spinning-DNP will be discussed. Special attention will be given to the competition between the SE and CE mechanisms, the extra signal enhancements of TM, DNP-assisted spin diffusion processes[1] and the characteristics of MAS-DNP.

All discussions will be based on quantum descriptions, including the necessary relaxation processes, of the DNP mechanisms and conclusions will be supported by simulations of the eigenstate populations of model spin systems.

Experimental results showing the transition between CE and SE enhancements in static solids as well as MAS-DNP enhancement[2] data will be presented and discussed.

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PL16

Multi-frequency EPR in biophysics and material science

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Multi-frequency EPR has proven a powerful tool in biophysics, in particular for studying bioenergetic processes as photosynthesis. In this talk applications in blue-light photo-reception and biological hydrogen conversion will be discussed, focusing on studies under *in cell* conditions[1,2]. In both cases results from the *in cell* experiments are at variance to results obtained on isolated protein, e.g. a standard protein environment of the catalytically active NiFe site from oxygen tolerant hydrogenases was deduced from the *in cell* EPR, while modifications have been deduced earlier from isolated protein studies and the unusual oxygen tolerance was interpreted as related to such modifications.

The concept of multi-frequency EPR is similarly powerful in material science but so far not as commonly used as in biophysics. As an example, analysis of light-induced defects in thin-film photovoltaic cell materials will be discussed, including S-, X-, Q-, and W-band EPR on stationary paramagnetic defects as well as multi-frequency electrically detected EPR (EDMR) on spin-dependent transport processes in fully processed solar cell devices.

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PL17

Amyloids by Solid-State NMR: Structure, Dynamics and Interactions with Small Molecules.

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The solid-state NMR characterization of amyloids remains a challenge. We will discuss technical advances including optimized polarization-transfer sequences, the application of 3D and 4D spectroscopy, structure determination from experimental restraints in the presence of high ambiguities, protein dynamics, as well as the choice of optimum labeling schemes and sample preparation techniques. The state of the art in the authors' lab on systems like α-synuclein, a-beta, and on peptide-hormones in their amyloid storage form will be discussed in detail.

Amyloids are universally defined by their stainability with Congo red and the resulting green birefringence. Yet, remarkably, the binding mechanism, geometry, and fine structure of the Congo red/amyloid complex remained longtime unknown. We characterized, at atomic resolution, the binding interface between Congo red and amyloid fibrils formed from the prion domain of the fungal HET-s protein. The three-dimensional (3D) structure of the fibril is strongly conserved upon the binding of Congo red. Remarkably, a single point mutation, designed according to the binding information, provides an artificial amyloid structurally indistinguishable from HET-s but not stainable by Congo red. We presently extend our studies to further dyes, markers and drugs to further characterize the pharmacophore of an amyloid.

Parallel Sessions Lectures

Monday 22nd August

	Lecture Hall 3	*Lecture Hall 4*	*Lecture Hall 5*
10:50	Wright	Koptyug	Lubitz
11:25	Neudecker	Gloeggler	Bowen
11:50	Brutscher	Hertzberg	Mathies
12:15	Christodoulou	Aime	Goldfarb
16:00	Boelens	Griffin	Schnegg
16:35	Post	Lelli	Blank
17:00	Lee	Hilty	Kaminker
17:25	Blackledge	Köckenberger	Suter

Tuesday 23rd August

	Lecture Hall 3	*Lecture Hall 4*	*Lecture Hall 5*
10:50	Garcia Rubio	Parigi	Gourier
11:25	Krstić	Corzilius	van Slageren
11:50	Barraud	Knight	Mitrikas
12:15	Carlomagno	Kowalewski	Hinderberger
16:00	Gschwind	Glaubitz	Dolinšek
16:35	Jerschow	Loquet	Exner
17:00	Bode	Gröbner	Hirsh
17:25	Pons	Böckmann	Enemark

Wednesday 24th August

	Lecture Hall 3	*Lecture Hall 4*	*Lecture Hall 5*
16:00	Zinn-Justin	Neese	Sigurdsson
16:35	Akbey	Nielsen	Guigliarelli
17:00	Tripsianes	van Eck	Türke
17:25	Gronenborn	Papavissiliou	Steinhoff

Thursday 25th August

	Lecture Hall 3	*Lecture Hall 4*	*Lecture Hall 5*
10:50	Balbach	Reif	Schiemann
11:25	Jannin	Hansen	Poluektov
11:50	Pileio	Junk	van Doorslaer
12:15	Glaser	Heise	Freed
16:00	Ramos	Frydman	Smith
16:35	Salmon	Meersmann	Bordignon
17:00	Kupce	Brunner	Silakov
17:25	Wöhnert	Lesage	Möbius

Ps100

Characterization of transient protein folding and unfolding processes by NMR relaxation dispersion

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NMR relaxation measurements provide a powerful approach for direct experimental characterization of protein dynamics and protein folding processes on a broad range of time scales, ranging from ps to ms, and yield unique insights into the protein energy landscape. In particular, relaxation dispersion experiments permit quantitative analysis of the kinetics and mechanism of spontaneous protein folding and unfolding events under equilibrium conditions. Dispersion experiments also provide chemical shift data that allow detailed structural characterization of weakly populated folding intermediates. Applications of NMR to study kinetic folding and unfolding pathways of apomyoglobin will be discussed. Native apomyoglobin unfolds on a sequential pathway via two intermediates: an intermediate that involves local unfolding of one helix, and a disordered molten globule intermediate. Analysis of transient state chemical shifts reveals the location and population of residual helical structure in the intermediates and identifies regions that unfold or rearrange into non-native structure during the transition to the molten globule state. The experiments also identify regions of energetic frustration that "crack" during unfolding and impede the refolding process. Relaxation dispersion measurements on acid-denatured states of apomyoglobin provide novel insights into the earliest steps in the refolding process. Folding is seen to proceed along a sequential pathway, although unproductive off-pathway processes are observed. Application of relaxation dispersion methods yields unprecedented insights into the complex protein folding landscape of apomyoglobin.

Ps101

Solution Structure of a Low-Populated Protein Folding Intermediate from NMR Relaxation Dispersion Spectroscopy Rationalizes Aggregation Propensity at Atomic Resolution

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Protein folding intermediates are suspected to play a critical role in amyloid fibrillation commonplace in neurodegenerative disorders. The structural basis by which this occurs has, however, remained largely elusive. Here we present the atomic-resolution three-dimensional solution structure of a 2% populated on-pathway folding intermediate of the A39V/N53P/V55L Fyn SH3 domain, which was determined by reconstructing the backbone chemical shifts and RDCs/RCSAs of the "invisible" intermediate from CPMG relaxation dispersion NMR spectroscopy and using them as experimental input for structure calculations based on chemical shift restrained replica exchange molecular dynamics simulations. Formation of the 5-stranded SH3 fold proceeds via a 4-stranded intermediate that is stabilized by several non-native long-range interactions, most notably an additional inter-strand hydrogen bond and non-native hydrophobic core packing. Critically, the C-terminus is disordered, thereby exposing an aggregation-prone β-strand. Accordingly, mutants lacking the C-terminus and thereby mimicking the intermediate spontaneously form β-sheet-rich fibrillar aggregates with a diameter of several nanometers and an affinity for the dye Congo red. The structure provides a rare atomic-resolution glimpse of interactions stabilizing aggregation-prone intermediates under native conditions and strong experimental evidence for a link between such intermediates and fibrillation.

Ps102

Polarization enhancement in BEST-TROSY NMR. Application to the study of protein folding intermediates and intrinsically disordered proteins

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Experimental sensitivity and spectral resolution remain the major drawbacks for the application of NMR spectroscopy to challenging biomolecular systems. Here we describe an efficient polarization enhancement mechanism in longitudinal-relaxation enhanced fast-pulsing (BEST-TROSY)[1] experiments. By recovering undetectable ^{1}H polarization originating from longitudinal relaxation during the pulse sequence, the steady-state ^{15}N polarization becomes enhanced by up to a factor of ~5 with respect to thermal equilibrium yielding significant sensitivity and/or resolution improvements compared to conventional schemes.[2] We show two different applications where such improvements are of particular importance. The first application concerns the NMR assignment and structural characterization of short-lived protein states. This will be illustrated for the example of β2-microglobulin (B2M), a protein of the immunoglobulin family that converts into amyloid fibrils in patients undergoing long-term dialysis. Using sensitivity-optimized real-time 2D and 3D NMR, we have identified and characterized distinct folding intermediates (monomeric and oligomeric) that are also potential precursors of fibril formation.[3] In a second example, we have investigated a 188-residue fragment of non-structural protein 5A (NS5A) of hepatitis C virus, an intrinsically disordered protein (IDP). The use of BEST-TROSY experiments provided the required spectral resolution for NMR assignment,[4] and titration studies to identify binding modes with SH3 domains.

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Ps103

Co-translational protein folding on the ribosome: Using NMR spectroscopy to provide structure and dynamics of ribosomes and ribosome-nascent chains

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The folding processes of nascent chains are intricately linked to their chain elongation, which occurs in a vectorial manner as the N-terminal part of the nascent chain emerges from the ribosome. The use of NMR spectroscopy on ribosomes and ribosome nascent-chain complexes to provide structural insights of the conformations of protein chains while they are being created on the ribosome will be described as will some of the advances in preparative biochemistry that have made this work possible. This work is allowing us to describe in detail the relationship between biosynthesis and folding and also in our understanding of how molecular chaperones such as the trigger factor, that interact with the nascent chain, affect protein folding.

Ps104

Recent Advances in MR Imaging of Heterogeneous Catalysis

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In heterogeneous catalysis, mass and heat transport processes are important. We used ^{1}H MRI technique to map liquid distributions in a packed catalyst bed reactor with a co-current gas-liquid flow. The results demonstrate that these distributions are significantly different during alkene hydrogenation and under non-reactive conditions. The direct ^{27}Al MRI of Pd/Al_2O_3 catalyst was used for the spatially resolved thermometry of an operating packed bed catalytic reactor. The 2D temperature maps of the catalyst obtained directly in the course of an exothermic catalytic reaction revealed the temperature changes with the variation of the reactant feed and also the temperature gradients within the catalyst at a constant feed. We also utilize parahydrogen-induced polarization produced using various types of solid catalysts for the development of the novel hypersensitive NMR/MRI techniques for heterogeneous catalysis. This approach can also provide hyperpolarized gases and catalyst-free hyperpolarized liquids for a wide range of novel applications of NMR and MRI in, e.g., materials science, chemical engineering and in vivo biomedical research. Applications of this hypersensitive approach to the studies of microfluidic chips and packed bed microreactors will be demonstrated.

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Ps105

Para-hydrogen induced polarization of amino acids, peptides and deuterium-hydrogen gas

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We demonstrate, that traces down to nanomoles of all proteinogenic amino acids as well as some peptides can be hyperpolarized by non-hydrogenative para-hydrogen induced polarization, in specific by the Signal Amplifiaction by Reversible Exchange (SABRE)[1] method and can be detected at low magnetic fields down to 0.25 mT. An outstanding observation is, that depending on the amino acid and the chemical state of the used catalyst, which is necessary for SABRE, hyperpolarized deuterium-hydrogen gas is formed, which can still be detected at 10 kHz proton frequency and 20 kHz deuterium frequency.

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Ps106

Tissue Elasticity Measurement Using Acoustic Radiation Force Imaging

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Magnetic resonance acoustic radiation force imaging (MR-ARFI) is a recently developed technique [1], which utilizes MRI to measure the displacement caused by the ultrasound radiation force. The focused ultrasound (FUS) pulse induces micron-scale static displacement, which is encoded to the MR phase signal by gradients. We have shown how that aberration caused by the skull can be corrected using the MR-ARFI technique, leading to near optimal focus and more reliable and safer brain FUS treatments [2].

Here we show that the temporal behavior on the displacement caused by the radiation force can be utilized for the assessment of the elastic properties of the tissue. Development of accurate tissue elasticity measurement using MR-ARFI sequences can improve small tumors detection and classification (benign, malignant) using clinical MRI system equipped with FUS. The early detection of small tumors, e.g. cancer breast tumors, is critical for the success of the clinical treatment. The small wavelength (~0.5mm) of the ultrasound waves in tissue in combination with high-resolution MRI provides the most accurate (up to 10^{-8}m displacement resolution and spatial resolution of few tens of microns) elasticity measurement tool for noninvasive detection of small tumors *in vivo*.

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Ps107

CEST (Chemical Exchange Saturation Transfer) agents for innovative MR-Molecular Imaging investigation

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Paramagnetic complexes (mostly Gd(III)- and Mn(II)-based chelates) and superparamagnetic particles (primarily based on iron-oxide nanoparticles) have been the two major classes of contrast agents for MRI applications. These species have played an important role in the development of clinical applications of MRI technique. The advent of molecular imaging era prompted the search for new paradigms in the design of MR imaging reporters. MRI-CEST agents[1,2] are frequency-encoding probes. They offer the possibility of tackling novel applications as multiplex detection in cell tracking or as responsive agents to the physico-chemical parameter of the microenvironment. Research in this field has progressed along different paths in order to tackle the issue of the overall safety of the MRI-CEST experiment and the sensitivity/reliability of the CEST detection. The systems developed so far range from small diamagnetic molecules to nanosized paramagnetic liposomes passing through paramagnetic complexes and supramolecular adducts. In an attempt to accelerate the entry of these agents in the clinical practice, it was deemed of interest to consider chemicals already approved for human use and potentially able to generate CEST contrast. On this basis we have undertaken a systematic study of either currently used X-ray agents containing mobile protons (e.g. iopamidol) or the paramagnetic lanthanide analogues of the clinically approved MRI agent Gadoteridol. The *in vivo* results are very encouraging for a future clinical translation of these CEST agents for many Molecular Imaging applications.

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Ps108

Multifrequency EPR Studies of Oxygen-Tolerant Hydrogenases

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The enzyme hydrogenase catalyzes the reversible oxidation of molecular hydrogen via a heterolytic splitting mechanism. It plays an important role in the metabolism of many anaerobic micro-organisms. Its understanding is of great interest for a future hydrogen economy based on modified organisms (bacteria, algae) or bioinspired chemical catalysts.[1]

The active site of the [NiFe] hydrogenase contains a bridged transition metal core and a special ligand environment, including sulfur (cysteinyl) and CN^-/CO ligands. Pulse EPR/ENDOR studies of the various paramagnetic intermediates of the activation, inhibition and catalytic cycle are presented that are instrumental in understanding the electronic structure and function of this enzyme.[2]

Electron transport from the catalytic site to exogenous electron acceptors/donors is performed by a chain of three iron-sulfur centers (2 [4Fe4S], 1 [3Fe4S]). The different states are characterized by multifrequency EPR and Mössbauer spectroscopy. In the important class of oxygen-tolerant [NiFe] hydrogenases a complex spin coupling situation is observed including spin states of the FeS centers and the [NiFe] cluster.[3,4] The redox potentials of all clusters were determined using EPR-monitored titration experiments.[3] For the 4Fe cluster proximal to the active site two reversible one-electron redox transitions were observed in a small potential range. This is related to two extra cysteines in the surrounding of this 4Fe cluster and plays a role in the oxygen tolerance of such enzymes.[3,5]

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Ps109

Progress and challenges in measuring the orientational dependence of DEER for transition metals in model systems and proteins.

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This work presents a complete study, including measurement and analysis, of the orientational selectivity in DEER spectra recorded between two copper(II) centres in the protein homodimer of copper amine oxidase from *Arthrobacter globiformis* as well as model chemical systems with differing inter-copper distances.

The degree to which orientational selectivity affects the shape of the resultant DEER trace differs across the EPR spectrum with regard to the selected pump and probe positions. Therefore in order to observe and interpret significant orientational effects some prior knowledge of the system is required.

Orientationally selective DEER spectra were simulated using home-written software.[1] This program requires an initial structural input; in the case of the copper amine oxidase this was an X-ray structure of the protein. However for the model systems it was necessary to use a combination of Density Functional Theory (DFT) calculations and X-ray structures of chemical precursors to predict likely conformers of the molecular structure. In order to verify the structural model used for the model systems similar Copper-Nitroxide and Nitroxide-Nitroxide compounds were also studied. DFT calculations were also employed to confirm the orientation of the g-matrix with respect to the molecular structure for both the model systems and the protein.

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Ps110

Continuous-wave EPR at 275 GHz. New Insights into the Iron-Binding Sites of Transferrin

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The understanding of the electronic structure of the active sites of metalloproteins can be greatly advanced by performing EPR spectroscopy at microwave frequencies higher than the conventional 9 GHz. Recording high-frequency EPR spectra of metalloproteins is challenging, particularly if relaxation times are short and only continuous-wave (cw) EPR is feasible. The spectra tend to cover large field ranges and due to conformational strain the resonances are very broad. Recently we have acquired the ability to record high-quality cw EPR spectra on 20 nl of 1 mM frozen solution of high-spin Fe^{3+} proteins (S = 5/2) at 275 GHz.[1]

We have applied this acquisition in the study of the iron-binding sites of transferrin. Present-day transferrins consist of two homologous lobes, which each contain a deep cleft capable of strong, but reversible iron binding. They transport iron to cells and act as bacteriostatic agents in a variety of biological fluids. We have recorded cw 275 GHz spectra of two mutants of human serum transferrin in which either of the binding sites is disabled, which has given us new information on the electronic structure of the individual iron-binding sites. This led to reconsideration of the current interpretation of the characteristic 9 GHz spectrum of transferrin and, moreover, to new insight into the iron binding.

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Ps111

Nanometer scale distance measurements in biomolecules using Gd^{3+} spin labelling

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Methods for measuring nanometer scale distances between specific sites in bimolecules (proteins and nucleic acids) and their complexes are essential for analysis of their structure and function. In the last decade pulse EPR techniques, mainly pulse double-electron-electron resonance (DEER), have been shown to be a very effective for measuring distances between two spin labels attached to a bimolecule. DEER is routine for distances up to 5 nm and with some extra effort and favorable conditions distances as high as 8 nm can be accessed. So far such measurements have been applied mostly to biomolecules labeled with nitroxide stable radicals. The measurements are usually carried out at standard X-band frequencies (~9.5 GHz, 0.35 mT). Here we introduce a new family of spin labels that are based on Gd^{3+} for DEER measurements at high frequencies, particularly W-band (95 GHz, ~3.5 T). The benefit such spin label offers is the considerable increase in sensitivity that reduces the amount of the biomolecule needed by more than an order of magnitude. Gd^{3+} has a spin of 7/2 and its unique ERP spectral properties turn it into an excellent spin label for distance measurements at high fields and this will be discussed. Then, examples of Gd^{3+}- Gd^{3+} distance measurements in models compounds will be described followed by a presentation of a few applications distance measurements in peptides, proteins, protein complexes and DNA molecules. The Gd^{3+} is attached to the biomolecule using a chelator that can be covalently attached at specific sites of the molecule, just like nitroxide spin labels. The chemical and physical requirement for the ultimate Gd^{3+} will be discussed.

Ps112

Structure and dynamics in gene regulation and DNA repair

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Various regulatory mechanisms exist to control gene expression in response to changes in the environment. Such control can be during transcription by DNA binding proteins, post-transcriptionally by interference with mRNA or by ribosomal proteins during translation. We study the factors involved in such regulatory processes by high resolution NMR spectroscopy, other biophysical methods and computational modelling. Our studies focus on the following topics: (i) structural studies of gene regulatory and DNA repair proteins, (ii) ribosomal translation initiation factors and (iii) molecular recognition modeling, as of the *E.coli* Lac repressor[1,2], initiaton factor IF2[3,4], complexes of human DNA repair proteins Rad6 and Rad18.[5,6] The examples demonstrate the strength and flexibility of NMR for studying the structure and dynamics of proteins and complexes involved in transcription, translation and DNA repair.

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Ps113

Domain orientation for controlling protein interactions

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ABSTRACT. Many multidomain signaling proteins interact with multiple binding partners, and thus function by exchanging partners spatially and temporally during signaling. Proper selection among alternative binding partners in response to external stimuli is an essential component of control of signaling networks. Protein association/dissociation of these complexes is often tied to domain-domain rearrangements, and a variety of mechanisms have evolved to carefully regulate these binding processes. We discovered an unusual allosteric mechanism whereby tyrosine phosphorylation at a distant site alters domain orientation and triggers the dissociation of Syk tyrosine kinase from the membrane B cell receptor (BCR). Syk associates with the BCR ITAM region via a bi-functional, high-affinity binding of Syk's two tandem SH2 domains to two phosphotyrosine residues of ITAM. Tyrosine phosphorylation in the linker region joining the two SH2 domains releases Syk from the BCR, even though the linker region is far from the binding sites. NMR relaxation and RDCs were used to explore conformation of the tandem SH2 domains of Syk and its phosphorylated forms. We also exploited chemical shift perturbation to individually characterize binding of each SH2 domain. Together, the data support a mechanism for regulating Syk-BCR association that involves a decrease in conformational order of the linker but is not a transition to a fully disordered state. A key feature of the model is loose coupling, as opposed to complete decoupling, of the tandem SH2 domains, so that the domain-domain orientation prevents bi-functional binding and a switch to lower affinity binding. This mechanism would provide a quick response to cellular phosphorylation by imposing a fast off-rate of the receptor. Kinetic control with a fast off-rate would enable a prompt response given that phosphorylation occurs while Syk is engaged at the membrane BCR. The results demonstrate the power of NMR to characterize the dynamic nature of domain organization in modular proteins.

Ps114

Molecular Recognition Kinetics within Inaccessible Time Window

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Molecular recognition is a fundamental aspect in biological processes. As a mechanism for molecular recognition, conformational sampling in the ns to ms time-scale has been proposed for ubiquitin.[1] However, the possible lifetime of these conformations spans four orders of magnitude, ranging from 4 ns to 50 ms and its kinetics was inaccessible due to the limitation of current methodology. Through a novel combination of methods, we have specified this lifetime to values between 2 and 20 ms at 298 K. Furthermore, we discuss how this lifetime of conformational interconversion is connected to the kinetics of complex formation. Consequences on fundamental mechanisms of molecular recognition will be discussed.

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Ps115

Towards A Robust Description of Intrinsic Protein Disorder from NMR

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The development of meaningful descriptions of the behaviour of intrinsically disordered proteins (IDPs) is a key challenge for contemporary structural biology, due to their inherent conformational disorder. Explicit molecular ensembles representing a dynamic equilibrium of rapidly interconverting conformers are gradually becoming established as appropriate descriptors to determine protein conformational disorder. [1] Due to the increase in available degrees of freedom compared to a static picture, the identification of accurate protein conformational ensembles requires the development of robust approaches to determine the significance and uniqueness of any proposed equilibrium.[2] We will present novel approaches to determine local and long-range structural behaviour in IDPs from NMR and small angle scattering data. We will describe the development of new techniques to determine the level of intrinsic structure in IDPs and apply this to describe the pre-recognition state of active sites of viral proteins.[3,4] The development of these tools allow us to study the conformational behaviour of these proteins in their physiological context, resulting in the first description of measles virus nucleocapsid including the disordered domain that mediates the initial steps of transcription and replication. [5]

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Ps116

Dynamic Nuclear Polarization at High Magnetic Fields

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Over the last few years we have developed gyrotron microwave sources that operate at frequencies of 140, 250, 330 and 460 GHz that permit DNP enhanced NMR (DNP/NMR) experiments in magnetic fields of 5-16.4 T (1H NMR frequencies of 211, 380, 500, and 700 MHz, respectively). We review the instrumentation used for these experiments, which include new probe designs and tunable gyrotron sources including results from our initial experiments at 700 MHz/460 GHz. In addition, we discuss two mechanisms that are currently used for DNP experiments in solids at high fields – the solid effect and cross effect -- and the polarizing agents appropriate for each. These include new water soluble mono- and biradicals, the latter with TEMPO moieties locked in orientations for efficient cross effect DNP. In addition, we discuss applications of DNP/NMR that illustrate its utility in enhancing signal-to-noise in MAS NMR spectra of a variety of biological systems such as amyloid proteins whose structures are of considerable scientific interest. Presently, enhancements range from 40-250 depending on experimental variables such as temperature, magnetic field, microwave B1, polarizing agent, etc. Finally, we show that 90 K spectra of cryoprotected protein samples permit observation of many resonances not observed at higher temperatures due to the interference of motion with the 1H decoupling fields. Thus, in addition to higher sensitivity there are other significant advantages to performing low temperature DNP experiments.

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Ps117

Surface Enhanced NMR by DNP: Analysis of the Sensitivity Enhancement and Application to a New Class of Porous Materials

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Dynamic nuclear polarization (DNP) is attracting considerable attention as a general method to enhance the sensitivity of solution and solid-state biomolecular NMR experiments.[1] We recently demonstrated the use of DNP in MAS solid-state NMR at ~100 K to study the surfaces of porous materials, by introducing a radical species by incipient wetting impregnation with aqueous solutions.[2,3] The DNP enhancement involving first the ^{1}H nuclei can be transferred to the surface heteronuclei by cross-polarization, with enhancement factors of the order of 20-40 in the detection of ^{13}C, ^{15}N, or ^{29}Si nuclei. Here we will report results obtained from surface DNP NMR on mesoporous hybrid materials, analyzing both DNP enhancement (ε) and the effective sensitivity enhancement per unit of time compared to NMR experiments performed without DNP on the dry material (Σ) at room and at low temperature, as a function of the radical concentration. The contributions to Σ due to the shortening of the ^{1}H T_1, as well as the partial "quenching" of the surface related to presence of paramagnetic centers will be also discussed. In addition, we will show that the CPMG acquisition protocol can further improve the sensitivity of DNP ^{29}Si SSNMR experiments. Preliminary results from the application of surface DNP NMR to characterize metal-organic framework (MOF) materials are also presented.

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Ps118

Hyperpolarized NMR of Polypeptides

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Dynamic nuclear polarization (DNP) provides a substantial signal enhancement in NMR. When coupled to high-resolution spectroscopy, solid-to-liquid state DNP not only allows the measurement of samples at low concentration, but more importantly in single scans with previously unattainable time resolution.[1] DNP-NMR in these modalities has up to now been most successfully used with small molecules, which exhibit long spin-lattice relaxation times and are soluble to high concentrations. Here, we present initial results showing that folded and unfolded proteins are equally amenable to solid-to-liquid state DNP polarization. Substantial enhancements on the order of 10^2 – 10^3 are readily obtained.[2] Proteins are easy to label uniformly or selectively, by biosynthetic incorporation of ^{13}C, ^{15}N, ^{2}H or ^{1}H isotopes. DNP-NMR of these samples presents itself as a new tool for the analysis of dynamic processes involving polypeptides. With an accessible sub second to second time scale, this technique enables real-time NMR study of protein folding.

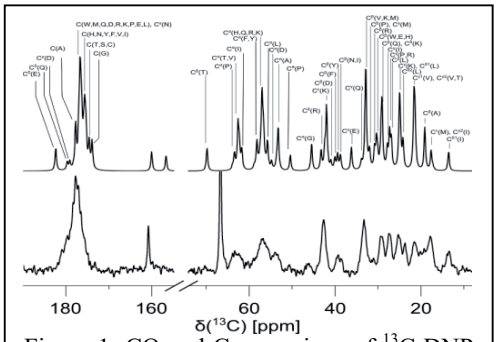

Figure 1: CO and C_{aliph} regions of ^{13}C-DNP polarized liquid state spectrum of L23 polypeptide (bottom) compared with simulated spectrum (top).

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Ps119

Dissolution Dynamic Nuclear Polarization – Advances in Theory and Experimental Implementation

Walter Köckenberger[1*], Anniek van der Drift[1], Ilya Kuprov[2], James Leggett[1], Peter Roberts[1], Sebastian Kemper[1], Alexander Karabanov[1]

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Low temperature dynamic nuclear polarisation in conjunction with a fast melting or dissolution step can be combined to generate liquid state samples with substantially increased spin polarisation[1] that can be used in novel NMR and MRI applications. To understand the physical processes taking place during solid state DNP at low temperature, it is important to develop models and spin dynamics simulations that can provide insight into the complexity of the polarisation build-up process. The simulations can help to understand the parameter dependence and to identify possible improvements that can be made in sample preparation and in the design of the radicals to speed up the polarisation build-up and achive even higher levels of nuclear spin polarisation. An important hurdle to overcome is the exponentially growing dimensionality of the arising quantum mechanical problem with the number of spins included in the model spin system[2]. We have made progress in understanding how the number of interacting spins can be maximised by working with reduced basis sets in DNP simulations. This strategy will be explained in detail and example calculations will be presented.

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Ps120

FD FT THz EPR on High Spin Transition Metal Ion Clusters

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In transition metal ions (TMIs) complexes with multiple unpaired electrons and high electron spins ($S > 1/2$), spin-orbit and spin-spin interactions among the unpaired electrons can lead to zero-field splitting (ZFS) of the magnetic sublevels. ZFS may be employed as a sensitive diagnostic to probe the electronic structure of function determining TMIs in metalloproteins or inorganic catalysts and determines the magnetic properties of single molecule magnets (SMMs). The ideal technique to extract this valuable parameter is electron paramagnetic resonance (EPR). However, difficulties in extracting spin coupling parameters for high spin systems by single frequency EPR arise from the fact that the spin transition energies are oftentimes distributed over a very wide energy range, exceeding the microwave energy of conventional EPR spectrometers. In order to overcome this limitation, we recently set-up a broad-band frequency-domain Fourier-transform (FD FT) THz EPR spectrometer operating from 200 GHz to 1.5 THz and +10 T to – 10 T, which exploits coherent synchrotron radiation in the THz range [1]. Here FD FT THz EPR is presented as a powerful tool to ascertain the spin transition energies in high spin systems exhibiting large ZFS. A description of the FD FT THz EPR set-up is given together with measurements on catalytic TMI clusters and novel SMMs [2, 3].

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Ps121

High Sensitivity Pulsed Electron Spin Resonance Spectroscopy with Induction Detection

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All commercial electron spin resonance (ESR) spectroscopy and imaging systems make use of the so-called “induction” or “Faraday” detection method, which is based on a radio frequency (RF) coil or a microwave (MW) resonator. This detection method is very robust and enables one to acquire complex spectra as well as efficient acquisition of images of inhomogeneous samples. Induction detection, however, suffers from sensitivity problems; its state-of-the-art capabilities do not exceed $\sim 3\times10^8$ spins/$\sqrt{\text{Hz}}$. Here we show that through the use of a new type of surface loop-gap microresonators (with typical inner dimensions of 20 μm), operating at cryogenic temperatures in a field of ~0.5 T, one can improve upon this sensitivity barrier by more than two orders of magnitude and reach spin sensitivities of $\sim 1.5\times10^6$ spins/$\sqrt{\text{Hz}}$ or $\sim 2.5\times10^4$ spins for a reasonable averaging time of one hour. The experimental results conform well to theoretical assessments, which also predict that one can approach single electron spin sensitivity at an increased magnetic field using an even smaller resonator size made of materials with higher conductivity. The applications of this new methodological capability range from the observation of paramagnetic doped fixed biological samples to the inspection of defects in semiconductors, and it may possibly be used as an efficient detection tool for an array of spins used in a future quantum computer.

Ps122

Mn^{2+} - nitroxide W-band DEER as tool to measure nm scale distances in RNA and protein RNA complexes.

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The pulse DEER (Double Electron-Electron Resonance) technique is frequently applied for nanometer scale distances measurements in biomolecules. It's most common application is to measure distances between two nitroxide spin labels attached at specific positions in the macromolecules of interest. Nevertheless, the DEER experiment is not limited to distance measurements between nitroxide spin labels and it has been successfully applied for distance measurements between other types of paramagnetic centers, such as pairs of organic radicals, Cu^{2+} and Gd^{3+} ions and metal ion-nitroxide pairs.

In this work we demonstrate, high field, W-band (95GHz) DEER distance measurements between nitroxide spin-labeled RNA and Mn^{2+} that binds either to the same RNA molecule, or to the RNA helicase DbpA. In the latter it substitutes for the Mg^{2+} in the ATPase active site of the enzyme. Optimal experimental parameters such as the selection of the pump and observer spins, microwave (MW) power, pulse length and frequency separation between the two MW channels are discussed and evaluated.

In many biological systems paramagnetic Mn^{2+} occurs naturally or it can be introduced artificially as a substitute for the diamagnetic Mg^{2+}. Thus our results pave the way for many new applications of DEER in the systems where nitroxide spin labeling is problematic or where introduction of the additional, orthogonal, paramagnetic probe may provide additional information.

Ps123

EPR with Small Resonators and Small Numbers of Spins

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Traditional cavity resonators for microwaves are well suited for ESR studies of large samples. However, for mass-limited samples, these resonators do not provide optimal sensitivity. To improve this situation, we have designed and tested sub-wavelength resonators that concentrate the microwave field in two-dimensional surface coils with diameters of the order of a few micrometers. The reduction of the resonator size results in a corresponding increase in the conversion efficiency of microwave power to microwave field, as well as a large increase in the detection sensitivity. Scaling the resonator size to 50 μm reduces the required microwave power to ~10 mW for pulsed excitation and ~10 μW for cw experiments. The detection sensitivity scales almost linearly with the inverse diameter of the resonator. Since they are open structures, these microresonators can be well combined with other techniques, such as scanning probes or optical detection schemes. Spatial separation of the magnetic and electrical fields provides good compatibility with conducting samples. As a specific application, we measured spin-waves in nanostructured ferromagnetic samples with volumes of $(100\ nm)^3$. Uniform as well as non-uniform volume modes of the spin wave excitation spectrum are identified and found to be in excellent agreement with the results of micromagnetic simulations. This allows the visualization of the spatial distribution of these modes in the magnetic nanostructures.

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Ps124

Magnetic Resonance Studies on Magnetotactic Bacteria

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Magnetotactic bacteria benefit from their ability to form cellular magnetic dipoles that they use as a compass. Single-domain ferromagnetic particles encapsulated in membranes (magnetosomes) are formed in the cells and assembled in one-dimensional arrays that are stabilized by cytoskeletal protein filaments. The alignment of magnetosomes, usually with their easy-axes along the filament, generates a magnetic dipole.

EPR, or rather, Ferromagnetic Resonance, can be used to obtain information about the state of the magnetite particles with respect to the presence of magnetic domains, the size of magnetosomes and also to provide information about the assembly of magnetosomes through the measurement of the magnetic anisotropy.

Our approach, combining measurements at different microwave frequencies and temperatures with spectral simulations provides separate information about magnetocrystalline and shape anisotropy. In this contribution we present how magnetic resonance has been successfully applied to 1) study the process of magnetosome and cellular dipole formation[1], 2) detect magnetofossils in geological sediments[2] and 3) detect oxidation of magnetosomes.

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Ps125

In-cell Pulsed EPR on Nucleic Acids

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Structure and dynamics of nucleic acids (NAs) depend on environmental factors, such as concentration of ions and small molecules, molecular crowding, viscosity and interactions with proteins. Therefore, it is important to investigate if the *in vitro* determined NA structure reflects the intracellular (*in vivo*) conformation. In the present study we used PELDOR spectroscopy [1] on a double spin-labeled 12-base pair DNA duplex, the 14-mer cUUCGg tetraloop hairpin RNA and the 27-mer neomycin-sensing riboswitch to obtain long-range distance constraints on such systems in *Xenopus laevis* oocyte cells [2] and to compare them with *in vitro* measurements [3]. The reduced lifetime of nitroxide radicals under *in vivo* conditions is a major obstacle for such measurements. Our results show that in comparison to free nitroxide, the in-cell reduction kinetic for the spin labels covalently attached to NAs is significantly slower.

We report the first application of pulsed EPR spectroscopy to map the global structure of nucleic acids inside intact cells. No alterations in the measured distances between *in vitro* and in-cell experiments imply the existence of stable overall conformations of the 14-mer hairpin RNA and the 27-mer neomycin-sensing riboswitch, whereas the 12-bp duplex DNA experiences stacking in-cell but retaining the secondary structure.

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Ps126

An Unexpected Zinc-Binding Motif Embedded in a dsRBD Revealed a New Class of Regulatory Domain Mediating Nuclear Localization of Dicer

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RNAse III ribonucleases function in RNA interference pathways by generating short RNAs that act at both post-transcriptional and transcriptional levels[1]. In contrast to human Dicer, *Schizosaccharomyces pombe* Dicer (Dcr1) localizes in the nucleus[2] where it generates siRNA required for heterochromatin formation. Its C-terminal domain, which consist of a predicted double-stranded RNA binding domain fold (dsRBD) followed by a 33 amino-acid motif (refers to as C33), plays a pivotal role in regulating the subcellular localization of Dcr1[2].

We have determined the solution structure of the C-terminal domain of Dcr1. The structure revealed an extended dsRBD fold embedding a unexpected zinc-binding motif that is formed jointly by dsRBD and C33. This unconventional zinc-binding motif is highly conserved among dicers in yeasts. The extension to the canonical dsRBD fold generates a conserved surface for protein-protein interaction that mediates the subcellular localization of Dcr1. Strikingly, although the extended dsRBD of Dcr1 binds to both dsRNA and dsDNA, this property is dispensable for proper functioning of Dcr1 in the RNAi pathway. In contrast, disruption of zinc coordination or mutation in the conserved surface render Dcr1 mainly cytoplasmic and are accompanied by remarkable changes in gene expression and failure to assemble heterochromatin. This raise the attractive possibility that this new class of extended dsRBD might generally function in nucleo-cytoplasmic traffiking and not substrate binding.

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Ps127

Protein recognition and functional mechanisms of non-coding RNAs

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Piwi proteins are germline-specific Argonautes that associate with small RNAs called the Piwi-interacting RNAs (piRNAs), and together they are implicated in transposon silencing. The PAZ domain of Argonautes recognizes the small RNA 3'-end, which in piRNAs is invariably carrying a 2'-O-methyl mark. Structures of PAZ domains in isolation or in complex with RNA/DNA substrates have been solved for eukaryotic Ago PAZ domains or as part of full-length Argonautes from prokaryotic thermophilic organisms. Here, we present the solution structure of the PAZ domain from the mouse Piwi protein, Miwi, in complex with an 8-mer piRNA mimic. Our structure is similar to that of Ago-PAZ, but subtle differences illustrate how the PAZ domain has evolved to accommodate distinct 3'-ends from a variety of RNA substrates and to provide specificity towards the different cognate RNA.

In the second part of the talk we will show the structure of a catalytic RNA forming a lariat product. This 2'-5'branch forming ribozyme undergoes a transterification reaction that shows striking similarity to the first step of both pre-mRNA and group II introns splicing . Thus, understanding the structure-activity relationship of this small ribozyme can help to understand the mechanism of spliceosomal catalysis. Here we present the structure of the ground state of the ribozyme, which shows peculiar dynamic properties. With the help of this information and of an extensive mutational analysis, we propose a structure for the activated, catalytic state of the ribozyme.

Ps128

Is there a sensible approach to the inverse problem of many conformations providing only few average parameters?

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The presence of conformational heterogeneity in multidomain proteins allowing interdomain mobility can be monitored through the observation of pseudocontact shifts (pcs) and self-orientation residual dipolar couplings (rdc) arising from the presence of a paramagnetic ion in one protein domain. The measured pcs and rdc are in fact the average of the pcs and rdc values on the ensemble of the conformations experienced by the protein in a ms time scale or shorter. In the presence of motion, the range of the observed rdc values is reduced, and they collapse to zero in the limit of overall isotropic reorientation. From the averaged pcs and rdc it is impossible to recover the conformations actually experienced by the system. The concept of maximum occurrence for a conformation has thus been introduced as the maximum weight that this conformation can have in any conformational ensemble, and thus whatever the real ensemble of conformations sampled by the protein is. The approach is based on the knowledge of the position, orientation and anisotropy of the magnetic susceptibility tensor from the pcs and rdc values measured for the metal containing domain; the pcs and rdc values measured for the other domain are used to determine the maximum occurrence of a representative pool of protein conformations. Further restraints, like SAXS or PRE data, can be added to the analysis. The different protein conformations can then be ranked according to their maximum occurrence.

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Ps129

High Field Dynamic Nuclear Polarization with High-Spin Transition Metal Ions

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In various biochemical applications dynamic nuclear polarization (DNP) has proven to be able to enhance nuclear polarization in magic-angle-spinning nuclear magnetic resonance (MAS NMR) by factors of ~40 to ~300 even at high magnetic field, therefore allowing NMR experiments which would not be feasible with conventional MAS NMR.

To date almost exclusively organic-radical based polarizing agents are used to provide the high electron spin polarization subsequently transferred to nuclear spins via DNP. However, endogenous electron spins (e.g. Mn(II)-centers) open the possibility of site-specific DNP without need of attaching spin-labels and therefore altering the sample structure (*e.g.* protein folding). The investigation of this site-specific polarization could yield important information which can be used as additional constraints in structure determination.

In this paper we introduce high-spin transition metal and rare earth (Mn(II) and Gd(III)) complexes as novel sources of polarization for solid effect DNP. At 5 T and temperatures close to LN_2 enhancements of ~16 can be obtained under MAS conditions using a Gd(III) complex (Gd-DOTA).[1] By effectively increasing the microwave field strength in a microwave resonator, enhancements of >100 are feasible under static NMR conditions at 80 K. We discuss the influence of zero-field splitting, hyperfine coupling to the metal ion, microwave field strength, sample temperature and ^{1}H-^{1}H spin-diffusion on DNP parameters.

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Ps130

Fast Fold Determination of the 153-residue protein Superoxide Dismutase by High-Resolution Proton-detected Solid-state MAS NMR

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A continuing obstacle to structural studies of solid-state proteins is the difficulty in recording high-resolution proton spectra. The use of perdeuterated proteins with partial amide back-protonation has helped to overcome the problem by diluting the proton dipolar network(1). Using moderate MAS speeds, very good proton resolution can be accomplished using 10-40% back-exchanged amide sites (2,3). We show here that the use of high magnetic fields and ultra-fast MAS (> 50 kHz) lifts the requirement of using partial back-exchange of amide sites for the efficient detection of proton resonances in perdeuterated samples of medium-sized proteins. We have applied proton-detected solid-state NMR to the 153-residue (16 kDa) protein Superoxide dismutase, fully [2H,13C,15N]-labelled and 100% protonated at the exchangeable sites. At1 GHz field and 60 kHz MAS, it is possible to obtain sensitive, high-resolution proton-detected experiments, which facilitate extensive resonance assignment. In the present study, 140 backbone amide resonances were assigned, out of 148 non-proline residues. This notably allows the acquisition of high-sensitivity 1H-1H correlation experiments. Then, using automated signal identification and cross peak assignment as implemented in the ATNOS/CANDID modules of the UNIO solution and solid-state NMR data analysis software suite, we have calculated the fold of SOD using only backbone amide 1H-1H restraints.

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Ps131

Field-dependent paramagnetic relaxation enhancement in solutions of Ni(II): what happens above the proton frequency of 1 GHz?

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The aqueous solutions of Ni(II) salts are a classical object of measurements of field-dependent paramagnetic relaxation enhancement (PRE) of solvent protons. Recently, we reported ^{1}H spin-lattice relaxation time (T_1) measurements on solutions of Ni(II) salt in water and water-glycerol mixtures [1] at magnetic fields up to 21 Tesla. One of the conclusions from that work was that adding glycerol not only changed the solution viscosity, but also resulted in formation of species with effective symmetry lower than octahedral.

Here, we extend that work in two ways. First, we report measurements of proton T_1 at very high magnetic fields, up to 32.9 Tesla. The experiments were performed at the "Laboratoire National des Champs Magnétiques Intenses" (LNCMI) in Grenoble. At these very high fields we observe an increase of the measured T_1 with increasing field, predicted by theory, but not observed in earlier studies. Second, we employ a new protocol for analysis of the data, including both intra- and intermolecular dipolar interactions between the protons and the electron spin in the fitting process [2].

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Ps132

What EPR reveals about the origin of life

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It is generally agreed that life emerged relatively rapidly on Earth in a window between ~ 4-3.5 billion years (Byr) ago, very soon after the solar system formation (4.6 Byr). Also, early Mars experienced Earth-type conditions at that epoch, so that primitive life could also have appeared on this planet. This hypothesis is further supported by the fact that Earth and Mars were heavily bombarded by comets and meteorites during their first 500 million years. A fraction of these meteorites contain up to 4% of organic carbon, including molecules such as amino-acids, amino bases etc…at the ppm level. Organic-rich objects have thus undouptedly rained upon surfaces of both planets at the same time. The insoluble organic matter (IOM) of carbonaceous meteorites –among the most ancient objects of the solar system- offers a unique record of the formation of organic matter in the early solar system. One important question concerns the origin of this IOM, which can be interstellar or synthesized in the protoplanetary disk, or a combination of both. The most ancient traces of life on Earth are found in the form of IOM microstructures fossilized in sedimentary rocks. However their origin, biogenic or abiogenic, is still among the most debated questions. Also, this IOM may sometimes originate from a late contamination by endolithic bacteria that fossilized in the rock. Two important issues are thus to assess the biogenic origin of the carbonaceous matter, and its syngeneity with the host rock. All these IOMs, terrestrial or extraterrestrial, contain paramagnetic defects which can be used as local probes. By using a combination of EPR methods, important information can be obtained about the origin of the organic matter in the solar system, and about the biogenicity and the syngeneity of the carbonaceous matter in the most ancient rocks. These paramagnetic markers can thus be used for the search of extinct life in martian rock samples.

Ps133

Quantum Coherence in Molecular Nanomagnets

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Molecular Nanomagnets (MNMs) are molecular clusters of (transition) metal ions that are bridged by simple ligands that efficiently transmit magnetic interactions. They are encapsulated in an organic ligand shell that shields them from each other and from the environment. Depending on composition and geometry, MNMs can possess high- or low-spin ground states and large or small anisotropies. MNMs have been proposed for data storage (high-spin, high-anisotropy), quantum computing (low-spin), and magnetic cooling (high-spin, low-anisotropy) applications. They are extremely well-suited to the study of quantum mechanical properties of mesoscopic systems, bridging the gap between the quantum and classical worlds.

We have studied quantum coherence in MNMs by pulse EPR and ENDOR. We have shown that quantum coherence times can reach the microsecond regime and are limited by the hyperfine interaction between the electron spin and the nuclear spins of the MNM and its surroundings. We have also been able to quantify the hyperfine interaction, and determine its origin. We will present recent results on lanthanide systems.

Ps134

Pulsed EPR characterization of encapsulated atomic hydrogen in octasilsesquioxane cages

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Hydrogen atoms encapsulated in molecular cages are potential candidates for quantum computing applications. They provide the simplest two-spin system where the 1*s* electron spin, S=1/2, is hyperfine-coupled to the proton nuclear spin, I=1/2, with a large isotropic hyperfine coupling (A =1420.406 MHz for a free atom). It has been found that atomic hydrogen is exceptionally stable in octasilsesquioxane cages even at room temperature [Sasamori et al., Science, 1994, 256, 1691].

Here we present a detailed spin-lattice and spin-spin relaxation study of atomic hydrogen encapsulated in $Si_8O_{12}(OSiMe_2H)_8$ using X-band pulsed EPR spectroscopy. The spin-lattice relaxation times T_1 range between 1.2 s at 20 K and 41.8 μs at room temperature. The temperature dependence of the relaxation rate shows that for $T < 60$ K the spin-lattice relaxation is best described by a Raman process with a Debye temperature of $\theta_D = 134$ K, whereas for $T > 100$ K a thermally activated process with activation energy $E_a = 754$ K (524 cm^{-1}) prevails.

The phase memory time $T_M = 13.9$ μs remains constant between 200 and 300 K and is determined by nuclear spin diffusion; below 200 K, T_M decreases by an order of magnitude and is attributed to dynamic processes like rotation of the methyl groups of the cage organic substituents. The hyperfine couplings of the encapsulated proton and the cage ^{29}Si nuclei are obtained through numerical simulations of field-swept FID-detected EPR spectra and HYSCORE experiments, respectively. The results are discussed in terms of existing phenomenological models based on the spherical harmonic oscillator and compared to those of endohedral fullerenes.

Ps135

EPR Spectroscopy on Serum Albumin

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Serum albumin is a versatile transport protein for various endogenous compounds, e.g. for fatty acids, and drugs in humans (human serum albumin, HSA) and many animals. Albumin is a standard test protein in the life sciences. While mainly extensive crystallographic data on HSA–fatty acid binding exist, relatively few studies since the 1970s have dealt with CW EPR spectroscopy on a simple self-assembled system of albumin with spin-labeled stearic acids (5- and 16-doxyl stearic acid, DSA).

We have recently started a new spectroscopic approach to gain information on the functional structure of (in particular) HSA in solution. Using 5- and 16-DSA as ligands and applying double electron–electron resonance spectroscopy (DEER), the functional protein structure could directly be accessed from the *ligands' point of view* [1]. It was surprisingly found that the distribution of the anchor groups for fatty acids is mainly consistent with the crystallographic data, while the entry points of the fatty acid binding pockets are distributed much more homogeneously and symmetrically on the protein surface than suggested by the crystal structure.

During our studies we found that albumin is a well suited "guinea pig" protein for CW EPR and DEER, which allows studying DEER on metal ion-nitroxide [2] and multispin effects [3]. Furthermore, it is a model protein for studying protein-solvent interactions and their effect on protein solution structure [4] as well as protein-protein interactions.

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Ps136

Enamine and Brønsted Acid Catalysis- Intermediates Trapped by NMR

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In the rapidly expanding field of asymmetric organocatalysis, mechanistic studies and structural investigations on intermediates are scarce compared to new synthetic applications. Thus, e.g. in proline-catalyzed aldol reactions, both origin and prototype for asymmetric aminocatalysis, the central enamine intermediate has never been detected experimentally for years. Similarly, in Brønsted acid catalysts, the understanding of the catalyst-substrate complexes in solution is rather limited despite the crucial role of hydrogen-bonding interactions and proton transfer in organo¬catalysis.

Here, our recent NMR studies about the detection, formation pathway and stabilization trends of enamines in proline-catalyzed aldol reactions will be presented [1]. Also the erosion of aldol selectivity due to proline-catalyzed aldehyde self condensation will be discussed [2]. Furthermore, we could reveal distinct trends for the formation and stability of prolinol and prolinol ether enamines [3] and found surprisingly strong conformational preferences for these enamines [4]. In addition, in the field of Brønsted acid catalysis, recent results about hydrogen bonding versus ion pairing in imine activation are discussed, which reveal the hydrogen bonded systems to be the active species [5].

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Ps137

Long Lived Coherent Response Signal Imaging

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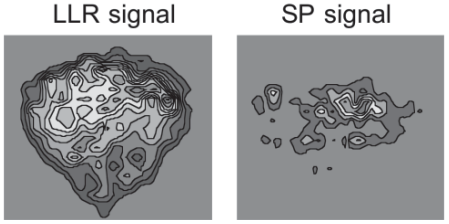
The Long Lived coherent Response (LLR) signal has been previously reported for information storage with liquid crystal and solid sample imaging.[1,2] This signal can be produced by applying a long weak pulse to the thermal equilibrium state. It is believed that this long-lived response originates from the nonlinear combination of a network of spin-spin couplings, molecular motions, and spin-lattice relaxation. The higher spatial resolution provided by its long-living feature and its insensitivity to a static field inhomogeneity makes LLR an ideal tool for bone imaging.



Comparison between LLR and conventional bone image.

We demonstrate here the feasibility of LLR sequence for bone imaging. This long lived signal provides high resolution images and its successful application in rigid tissue systems shows its potential for in vivo imaging bone tissues, tendons, ligaments, and other related tissues, which may contribute to the diagnostics of musculoskeletal disorders.

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Ps138

The heart of photosynthesis illuminated by joining photo-CIDNP and quantum chemistry

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In this contribution we investigate the primary charge separation in the two light-driven electron pumps photosystems I and II. The light-induced electron transfer in these proteins lays the foundation of natural carbon-fixation, the global food chain and the natural conversion of sunlight into chemical energy. Despite decades of intense research, the molecular basis of the high efficiency of the initial charge separation is still sought after.

Here we utilize uniformly ^{15}N labeled isolated reaction centers[1] and demonstrate that photo chemically induced (photo-CIDNP)[2] magic angle spinning (MAS) NMR is a unique tool for studying these systems. A 10,000-fold signal enhancement allows studying the active cofactors at atomic resolution. The chemical shift information refers to the electronic structure in the ground state, whereas the signal amplitude reports on the charge transfer state. Both effects can be simulated based on parameters obtained from thoroughly validated quantum chemistry performed on high-resolution X-ray coordinates.

As a result, we find that the joined forces of both methods identify the electronic structure of the ground and primary charge transfer state at atomic resolution. An outlook regarding selective isotope labeling and measurements of reaction centers in higher organizational units will be presented.

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Ps139

Dynamic Interactions of Proteins and DNA Related to Pathogenicity

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Bacterial nucleoid associated proteins act as global regulators of gene expression facilitating the colonization of hosts. As a consequence, understanding their function will help in the control of pathogenicity. Ler is the master regulator of the LEE pathogenicity island in virulent strains of *Escherichia coli*. We shall present the solution NMR structure of the first complex between the DNA binding domain of Ler (CT-Ler) and a 15-mer DNA duplex. CT-Ler recognizes a preexisting structural pattern in the DNA minor groove. This indirect readout mechanism is also present in the abundant repressor H-NS. This mechanism explains the capacity to regulate a large number of genes by H-NS and the higher specificity of Ler.

Proteins of the Hha/YmoA family bind to H-NS in enterobacteria and enable the selective regulation of genes linked to pathogenicity. Using NMR relaxation dispersion experiments we have shown that the formation of H-NS complexes is controlled by the internal dynamics of YmoA and is preceded by the formation of electrostatically driven encounter complexes. We suggest that this mechanism explains the decoupling of the regulation of pathogenicity-associated from core genes, thus improving the capacity of enterobacteria to incorporate new horizontally acquired genes.

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Ps140

Biophysical Insight into Structure and Function of Proteorhodopsin by Solid-state NMR

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The proteorhodopsin (PR) family consists of hundreds of integral membrane proteins, which occur almost ubiquitously in bacteria near the ocean's surface and show a high level of adaptation to their local environment, especially in terms of light absorption. Proteorhodopsins could fulfill a whole range of potential functions but light-driven proton pumping has been shown to be of particular importance for the green absorbing species. Here, an extensive high-field solid-state NMR study on the active site of green PR, the formation of an H-bond within PR's unusual His-Asp cluster and general structural and dynamic characterization of PR within lipid bilayers will be presented. The data will be complemented by biochemical studies, site-directed mutagenesis and cwDNP experiments. PR also serves as a suitable system to demonstrate new technical developments due to its favorable biochemical properties. Here, recent approaches for faster data acquisition based on selective excitation and paramagnetic relaxation enhancement will be presented. Furthermore, the perspective for cwDNP enhanced MAS-NMR on membrane proteins will be discussed.

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Supramolecular Assemblies Studied by Solid-State NMR: The Structure of the Type Three Secretion System Needle

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Supramolecular assemblies such as biological nanomachines and amyloid fibrils are often challenging for solution NMR or X-ray crystallography due to their molecular weights/insolubility/non-crystallinity. We present new solid-state NMR methods to tackle the structure determination problem of supramolecular assemblies: I) A strategy for simplified and complete NMR assignment based on ^{13}C spin dilution resulting in excellent spectroscopic features (1). The strategy is demonstrated with the assignment of the *S. Typhimurium* Type Three Secretion System Needle. II) A method to study supramolecular interfaces with ^{13}C-^{13}C spectroscopy (2), first illustrated with the determination of the supramolecular arrangement of alpha-synuclein amyloid fibrils.

We applied these methods to study the *Salmonella typhimurium* Type Three Secretion System Needle, a complex nanomachine that allows bacteria to inject virulence factors into host cells (3). Using our ^{13}C spin dilution approach, solid-state NMR spectra of unprecedented quality can be recorded, allowing for the collection of numerous distance restraints as well as to determine the supramolecular assembly of the Needle. Our results combined with cryo-EM data pave the way to a complete atomic-resolution structure of the Type Three Secretion System Needle.

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Ps142

Insight into apoptotic events in intact mitochondria by solid state NMR

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Mitochondrion functions not only as the cells' powerhouse but is also involved in their suicide via apoptosis. However, is still a mystery how tumour cells escape their fate in cancer therapies. Normally, these treatments interfere with the mitochondrial apoptotic pathway; a major regulator in mammalian cells death, where pro- and anti-apoptotic Bcl-2 proteins meet at the mitochondrial membrane and tightly regulate the fate of a cell. But treatment resistant tumor cells often possess mitochondria which are not only enriched in overexpressed pro-survival Bcl-2 membrane protein but also have lipid compositions quite different from the ones found in mitochondria originating from healthy cells. How the molecular activities of the Bcl-2 protein and its interplay with the mitochondrial membrane system are being transferred as physiological consequences at the organelle (mitochondrion) and cellular level is still mysterious. Therefore, we use a biophysical, solid state NMR based approach to obtain insight into the mechanism of Bcl-2 mediated prevention of programmed cell death in mitochondria on a molecular and cellular level. We will provide a molecular, structural description of the Bcl-2 mechanism of action and its interplay with mitochondrial membranes, with focus on oxidized lipids and cardiolipin. By combining this NMR information with HR MAS NMR profiling of Bcl-2 overexpressing tumor cell derived intact mitochondria upon apoptotic stress, we will generate knowledge about the relationship between the protein's conformational activity at the mitochondrial membrane level and the physiological consequences at the mitochondrion level.

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Ps143

Prion structures: a single architecture?

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A subset of neurodegenerative diseases is infectious and related to prions. Solid-state NMR is a promising approach to reveal structural details of prion oligomers at the atomic level, but still needs to overcome lack of resolution and sensitivity. Increase in sensitivity has been achieved during the last years mainly by going to higher fields, by the development of efficient polarization transfer schemes, and by increasing the amount of sample in the rotor. Resolution has equally improved by the use of high fields, but also through optimized sample preparation. These advances allow today to address larger and larger proteins, and it will be demonstrated that the spectral resolution in full-length prion fibrils is indeed sufficient to assign the resonances and to promise atomic-resolution structure determination. Details of the approaches and the resulting spectra of prion proteins ranging from 289 to 685 residues will be discussed, and features and limitations of the applied methods will be highlighted. We also demonstrate that a homogenous picture of prion fibril architecture is not consistent with structural information from NMR, which reveals that the folds of the different domains do differ largely for the three fungal prions studied here, HET-s [1], Ure2p [2] and Sup35 [3].

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NMR of Quasicrystals and Complex Metallic Alloys

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The term "complex metallic alloys" (CMAs) denotes exceptional intermetallic phases with giant unit cells that contain some hundreds up to several thousand atoms. Examples are cubic $NaCd_2$ with 1152 atoms/unit cell, cubic β-Al_3Mg_2 (1168 atoms/u.c.) and the heavy-fermion compound $YbCu_{4.5}$, comprising as many as 7448 atoms in the supercell. CMAs are periodic crystals on the scale of many nanometers, whereas on the atomic scale, they resemble quasicrystals (QCs) that are characterized by crystallographically forbidden symmetries such as 5-, 8-, 10- and 12-fold rotation axes. QCs and CMAs show interesting physical properties, like a "smart" combination of metallic electrical resistivity with insulating thermal conductivity and a change of sign of the thermoelectric power and the Hall coefficient from positive hole-like to negative electron-like with crystallographic direction. Polytetrahedral atomic order found in QCs is also at the origin of an enhanced hydrogen-storage capability. NMR spectroscopy of QCs and CMAs is difficult due to the extreme width of the inhomogeneously broadened spectra that extend over many tens of MHz. We applied the field-sweep and frequency-sweep NMR spectroscopy to the investigations of many QCs and CMAs [1]. The orientation-dependent quadrupole-perturbed ^{27}Al NMR spectra of Al-rich icosahedral and decagonal QCs reveal forbidden symmetries of a 5-fold and 10-fold rotation axis. The ^{27}Al Knight shifts were used to determine the electronic density of states (DOS) at the Fermi energy E_F, which is in QCs and CMAs many times depleted due to the existence of a pseudogap in the DOS at E_F. Slow migration of atoms due to QC-specific atomic diffusion was studied by the NMR relaxation and the self-diffusion.

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Ps145

Towards Quantum Chemical NMR Chemical Shifts of Proteins

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Calculations of accurate NMR chemical shifts of proteins, protein-protein and protein-ligand complexes are highly valuable in many applications like NMR structure evaluation and complex-structure predictions. We will present calculations using our fragment-based quantum chemical method: the adjustable density matrix assembler (ADMA)[1,2]. In such calculations the target system is subdivided into small fragments, for which separate quantum chemical calculations are performed and which are then combined to get an approximation of the macromolecule.

The presented results will show that ^{13}C chemical shifts of reasonable accuracy can be obtained that already provide a powerful measure for structure validation[3]. ^{1}H and even more ^{15}C chemical shifts deviate more strongly from experiment due to the insufficient treatment of solvent effects and conformational averaging. Approaches to overcome these limitations will be outlined.

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Ps146

Measuring Long-Range Distances and Exchange Couplings in DNA Using Saturation-Recovery EPR

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In the last decade, efforts to measure nanometer-scale distances between paramagnetic moieties have focused on pulsed dipolar EPR experiments such as DEER. These experiments measure the effect of a fluctuating dipolar field on the electron spin echo. While considerable progress has been made with this approach, it remains difficult to measure distances when one of the paramagnetic moieties is a metal ion with significant magnetic anisotropy. This is unfortunate, from the biophysicist's perspective, because the endogenous metal centers of many proteins fall into this category. We report here on distance measurements between a nitroxide radical and the magnetically anisotropic dysprosium ion, $S = 5/2$, using saturation-recovery EPR. DNA duplexes, identical except for the distance between EDTA-bound Dy(III) and a spin-label attached via a flexible linker, were prepared in buffered cryoprotectant solution.[1] Low temperature measurements of spin-lattice relaxation enhancement in the nitroxide radical were used in conjunction with molecular modeling and trilateration[2] to arrive at set of structures with a narrow range of Dy(III) - radical distances. The saturation-recovery transients are best fit using the *B*-term of the dipolar Hamiltonian with antiferromagnetic exchange coupling between Dy(III) and the nitroxide radical. The data are consistent with dipole-dipole and scalar exchange couplings at distances as great as ~5.6 nm.

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Ps147

Determination of the Structure of the Mo(V) Center of Sulfite Oxidase by Variable Frequency Pulsed EPR Spectroscopy, ^{33}S and ^{17}O Labeling, and DFT Calculations

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Reduction of human sulfite oxidase (hSO) by ^{33}S-labeled sulfite at low pH produces a sulfur-containing ligand that was initially proposed to be coordinated sulfate (product). However, the large ^{33}S quadrupole coupling constant (~36 MHz) could indicate coordinated sulfite (reactant). Additionally, reduction with ^{17}O-labeled sulfite in $H_2{}^{17}O$ produces signals from weakly and strongly coupled ^{17}O atoms, which have been investigated at ~29 and 95 GHz to extract their respective hyperfine and nuclear quadrupole interactions. Density Functional Theory (DFT) calculations for models of the Mo(V) center (> 250 atoms) unambiguously show that the ^{33}S data require bound sulfite. The weakly coupled ^{17}O signal is due to remote O atoms of the sulfite ligand, and the strongly coupled ^{17}O to the coordinated O atom of the sulfite. The distinctive ^{17}O parameters for different types of ligands should be generally useful for determining the structures of other paramagnetic metal-oxygen systems.

Ps148

Structural organization of bacteriophage head-to-tail connection, as characterized by EM, NMR and bioinformatics

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Building of phage particles requires a complex sequential program of protein-protein and protein-DNA interactions whose complete molecular description is yet to be established. *Caudovirales* phages are composed of a capsid enclosing a double-stranded DNA molecule and a tail. We here focus on the assembly and structural organization of the proteins located at the interface between the capsid and the tail, essential for capsid closing after DNA packaging and opening during infection. Subcomplexes of these proteins were characterized by cryo-EM. Solution structures of the individual proteins were determined by NMR. Their oligomerisation properties and internal dynamics were studied in parallel to functional studies. Docking of the NMR structures into the EM maps pointed to residues involved in protein oligomerisation or participating in connector assembly, and highlighted conformational changes essential for opening of the connector pore at the beginning of viral infection. Despite the great sequence variability of phage proteins, bioinformatics profile-profile comparison methods were efficient in identifying similar mechanisms in several phage subfamilies, in classifying the *Caudovirales* head-to-tail structures and in suggesting new proteins essential for head-to-tail assembly.

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Ps149

Spin Gymnastics with Deuterated Proteins: Solid-State NMR & DNP

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We present solid-state NMR experiments to be used for high sensitivity/resolution spectroscopy of perdeuterated proteins. Protein deuteration has been proven to be very useful to achieve solution-state-like resolution in the solid-state,[1,2] and experiments based on proton detection have been implemented in the solid-state. Despite the progress in the proton detected experiments, there has been a lack of demonstrations for the heteronucleus-detected experiments. As a result, achieving spin-diffusion between heteronuclei and sufficient initial polarization in deuterated systems is a major issue.

Here, we explain our tool-package which we have recently shown to be very successful to be used on perdeuterated proteins, to achieve superior initial magnetization and to sufficiently distribute magnetization between heteronuclei. First, the *double nucleus enhanced recoupling* (DONER) method will be demonstrated,[3,4] which re-introduces the nearly-collapsed spin-diffusion process by the use of both proton and deuterium spins. Second, we will show the quantitative demonstration of the nature of spin-diffusion process by using DONER experiments with theoretical insight. Third, we will present new methods for increasing overall sensitivity by utilizing protons and deuterons, such as; *triple cross-polarization* (TCP).[5] optimal-control based schemes to increase performance,[6] and $^{1}H/^{2}H$ NMR spectroscopy at ultra-fast MAS. Finally, DNP experiments on deuterated systems will be shown.[7]

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Ps150

Structural basis for dimethyl-arginine recognition by Tudor domains

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Arginine methylation has long been observed in RNA binding proteins and histones, and has recently been implicated in gene regulation and other cellular processes. Here, we describe the NMR structures of two prototype Tudor domains (from SMN and SPF30) in complex with symmetric (sDMA) and asymmetric (aDMA) dimethylated arginine. Isotope-filtered NOE experiments and temperature-dependence of NMR data, combined with DFT calculations, were used to characterize the conformation and dynamics of the DMA ligand in the free and bound forms. The structures reveal that the Tudor aromatic cage accommodates the dimethylated guanidino group by a combination of van der Waals contacts and cation-π interactions. ITC data complement the structural findings and provide insight into enthalpic and entropic contributions to achieve low micromolar affinities. Mutational analysis confirms the structural findings on DMA recognition and allows the prediction of *bona fide* dimethyl-arginine readers from a large pool of Tudor domains. Based on the structural analysis we were able to rationally design and modulate the binding affinities of SMN and SPF30 Tudor domains. NMR and ITC data demonstrate that the hydrogen bonding of a tyrosine hydroxyl group potentiates the cation-π interactions, consistent with the observed difference in binding affinities between SMN and SPF30. Our observations on Tudor/DMA interactions have substantial implications for a mechanistic understanding of the assembly of ribonucleoprotein complexes in pre-mRNA splicing, where dimethylated arginine-rich target proteins play a central role.

Ps151

Mannose-binding lectins - Cyanovirin and beyond

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Homologs of the potent HIV-inactivating protein CV-N have been identified in other organisms and we determined solution NMR and X-ray structures for several additional members of this family of lectins. All proteins of the type I family exhibit the same fold and the overall structures resemble that of the founding member of the family, CV-N, albeit with noteworthy differences in loop conformation and detailed local structure. We recently also solved the NMR solution structure of a type III family member in which a LysM domain is inserted between individual repeats of a single CVNH domain. The structure revealed that intact and functionally competent CVNH and LysM domains are present. Carbohydrate specificities for both domains were determined by NMR and it was found that each domain behaves as an isolated unit without any inter-domain communication. Furthermore, live-cell imaging revealed a predominant localization of the protein within the appressorium, the specialized fungal cell for gaining entry into rice tissue. A very different structure was formed for *Oscillatoria Agardhii* Agglutinin (OAA). It binds both Man 9 and α3,α6-mannopentaose tightly and specifically at two binding sites. These structures provide atomic details about the specific protein-sugar contacts in the recognition loops.

Our combined NMR and crystallographic results provide structural insights into the mechanism by which these anti-HIV proteins specifically recognize Man-9. Our results highlight the versatility in lectin-carbohydrate recognition, and may aid in the development of protein-based vaccines or diagnostic and pharmacological reagents in the quest to combat HIV transmission.

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Ps152

Theoretical EPR Spectroscopy of Open-Shell Transition Metal Complexes with Strong Spin Orbit Coupling

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The majority of molecules have orbitally nondegenerate ground states. The molecular electric and magnetic properties are well described by standard models of electronic structure theory, e.g. perturbation or linear response theory. These methods have found very widespread use in the community of quantum chemistry users. The ORCA program developed in our group features many such methods for the calculation of optical and magnetic properties of transition metal complexes. There is, however, a significant class of molecules with orbitally (nearly) degenerate ground states where the established methods do not work. Here, a more careful treatment of the leading relativistic effects is necessary in order to correctly predict their spectroscopic properties and obtain molecular level electronic structure insight. This is not possible on the basis of density functional theory (DFT). Recently efficient methods based on multireference wavefunction theory have been implemented into ORCA that allow such calculations on large molecules. Their use will be demonstrated by a recent study [1] that deals with the electronic structure of the only low-molecular weight catalyst known to be capable of reducing dinitrogen to ammonia.

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Ps153

Optimal Rf Pulse, Cross-Polarization, and Multiple-Dimensional Sampling Package for Solid-State on Perdeuterated Proteins

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Recently it has become very popular to exploit extensive deuteration as a supplement to uniform ^{15}N and ^{13}C labeling of proteins in biological solid-state NMR. The motivation is to a) dilute the ^{1}H spin systems to provide proton-based assignment and distance measurements, b) use ^{2}H as a source of high resolution, e.g., through the use of double-quantum ^{2}H evolution, or c) use ^{2}H as a probe to molecular motion. In all cases it may be of great interest to be able to handle ^{1}H as well as ^{2}H spins through rf irradiation to ensure optimal use of polarization and establishment of structure/dynamics information.

In this presentation, we address three central issues in relation to ^{2}H MAS NMR of proteins, including RESPIRATION (Rotor-Echo Short Pulse IRrAdiaTION) ^{2}H rf pulses, efficient ^{2}H -> ^{13}C cross-polarization schemes, and efficient sampling of multiple-dimensional spectra involving polarization from ^{1}H and ^{2}H spins. The pulse sequences, developed using combinations of average Hamiltonian theory and optimal control, essentially solves the finite rf pulse problems in ^{2}H MAS NMR with fast spinning frequencies and thereby facilitates implementation of advanced pulse sequences involving ^{2}H spins and provides overall sensitivity gains by up to an order of magnitude.[1] A variety of different pulse sequences are demonstrated on differently labelled protein samples.

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Ps154

Unprecedented ^{27}Al MAS NMR resolution on zeolite single crystals.

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The distribution of aluminium atoms within the zeolite silicate lattice is important as it determines the performance of the zeolites as a catalyst. For a microcrystalline powder it can be possible to resolve the various T-sites present in a zeolite with ^{29}Si NMR. For aluminium however, this has remained a challenge as the resonances are intrinsically broadened by the quadrupolar interaction and often overlap strongly. Here we represent experiments on zeolite single crystals would greatly enhance resolution. Due to the size of available single crystals standard probeheads are usually not sensitive enough. To overcome this limitation we have been developing microcoil probe heads in our group for studying nano-liter volume solid samples by matching the coil to sample dimensions.

Initial experiments on a single crystal of natural zeolites mesolite and ferrierite show unprecedented resolution in the aluminium NMR spectrum. Ferrierite has 5 different crystallographic positions where aluminium could be located. Compared to the NMR spectrum of the powder the single crystal spectrum has a much higher resolution. Still, not all of the five sites are resolved and therefore 2D Multiple Quantum Magic Angle Spinning (MQMAS) experiments were performed at multiple fields. DFT calculations of the chemical shift and the quadrupole tensors were done with VASP, based on the known single crystal structure. This allows assignment of the T-sites and hence the distribution of aluminium over them to be determined directly for the first time. Further experiments are underway, aiming to exploit the intrinsic high resolution of single crystal NMR and its application to zeolite type materials.

Ps155

NMR studies of Novel Strongly Correlated Electron Systems

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The study of strong electron correlations in transition metal oxides (TMOs) unveiled a complex world of interweaving properties, concerning their electron spin, charge, and crystal structure. Predominant examples are high temperature superconducting cuprates and hole doped manganites. Competition among different interactions in these systems generates spectacular phenomena, such as the formation of charge and spin stripes, mesoscopic phase separation, and the colossal magnetoresistance (CMR) effect. Despite the intensive research in this field there are still a lot of important open questions. In this lecture we will show how NMR can be used in order to acquire important information about the local structural and magnetic environment and its dynamics in characteristic TMO systems [1-3].

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Ps156

Strategies for Site-Directed Spin Labeling of Nucleic Acids

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Studies of nucleic acids by Electron Paramagnetic Resonance (EPR) spectroscopy require spin-labeled oligomers. Several different site-directed spin labeling (SDSL) methods have been developed for nucleic acids over the years for incorporation of nitroxides at specific sites. These methods have utilized either of two approaches, incorporation of the spin label during chemical synthesis of the nucleic acid or post-synthetic modification with a spin-labeling reagent. Both of these strategies require incorporation of the spin label through chemical reactions. Some of the potential drawbacks associated with these chemical modifications include a labor-intensive preparation of the spin label, incomplete labeling, side-reactions and purification of the labeled oligomer.

To avoid labor-intensive and costly chemical modifications of the biopolymer, we have developed a noncovalent and site-directed spin labeling (NC-SDSL) approach for nucleic acids.[1] The strategy utilizes a spin label that binds site-specifically and noncovalently to abasic sites in nucleic acids. Thus, spin-labeled samples can be prepared by simply mixing the spin-labeling reagent with a solution of the nucleic acid, prepared from commercially available phosphoramidites.

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Ps157

Dynamic Disorder Evidenced by SDSL- EPR in a Multienzyme Complex Involved in CO_2 Assimilation by Microalgae

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CP12 is a small chloroplastic protein that is well known to interact with GAPDH, an essential enzyme involved in CO_2 assimilation by microalgae, and regulates its activity. In the green alga *Chlamydomonas reinhardtii,* CP12 is mainly unstructured, showing properties of Intrinsically Disordered Proteins, and contains four cysteine residues involved in two disulfide bridges (1). In order to analyze the structural transitions associated with the CP12-GAPDH recognition, we have undertaken a site-directed spin-labeling (SDSL) EPR study (2). To overcome the difficulties related to the presence of the functional cysteines, we present new strategies which are based: i) on the design of new spin probes able to be grafted on non-cysteine residues (3) and ii) on a combination of cysteine mutagenesis, SDSL with maleimido-proxyl, EPR spectroscopy, CD and mass spectrometry. Our study shows clearly that although the spin-label keeps a high mobility upon the CP12-GAPDH complex formation, its solvent accessibility is strongly impaired. Taken together, our results demonstrate that CP12 remains highly flexible in the enzyme complex, indicating that this dynamic structural disorder is of physiological significance in the regulation mechanism of an essential environmental process.

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Ps158

Saturation factor of nitroxide radicals in liquid DNP by pulsed ELDOR experiments at 0.34 T and 3.4 T

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Dynamic nuclear polarization (DNP) in aqueous solution is a major topic of current efforts to enhance the sensitivity of high resolution NMR and magnetic resonance imaging.[1]

In recent studies, nitroxide radicals have been favoured as polarizing agents for DNP since they are soluble in water, non-toxic and have been found to account for large DNP enhancements up to 9 T. However, the determination of the saturation factor for this class of polarizers has emerged as one of the major difficulties in rationalizing the observed enhancements in terms of the Overhauser equation since the NMR signal enhancement depends on the saturation level of all EPR lines of the polarizer.

Therefore, the theory for saturation transfer between the hyperfine states of nitroxides has been re-examined.[2] We exemplarily show at 0.34 T and 3.4 T that the effective saturation factor in Overhauser DNP can be directly determined in a pulsed electron-double-resonance (ELDOR) experiment, which measures the intensity of a hyperfine line when pumping a coupled line.[3] The obtained values for ^{15}N-^{2}H-TEMPONE and ^{15}N-Fremy's Salt at different concentrations are rationalized in terms of spin relaxation and are shown to fulfil the Overhauser theory.

A comparison of the two widely used radicals at 0.3 T yields similar maximum DNP enhancements but clearly different power dependence of their saturation behaviour.

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Ps159

Lipid sensing and transmembrane signaling studied by site-directed spin labeling EPR

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Electron paramagnetic resonance (EPR) spectroscopy, site-directed spin labeling (SDSL), and molecular dynamic simulations were combined to study the structure and conformational dynamics of membrane protein complexes. Analysis of the spin label side chain mobility, its solvent accessibility, the polarity of the spin label micro-environment and interspin distances determined by DEER provide information for restraint modeling of protein domains or protein - protein interaction sites and their conformational changes. The presentation reviews our recent results on vinculin tail conformational changes upon binding to actin filaments and lipid membranes. Furthermore, light induced conformational changes of the spin labeled halobacterial phototaxis receptor sensory rhodopsin (pSRII) in complex with the receptor specific transducer (pHtrII) are shown (i) to be uncoupled from the deprotonation of the Schiffbase, and (ii) shift the thermodynamic equilibrium between two states of the first HAMP domain of pHtrII (1-3). EPR analysis of spin labeled pSRII-pHtrII complexes reconstituted into nano-lipoprotein particles or membrane sheets reveals functional clustering of the protein complexes in the membrane sheets.

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Ps160

Exploring protein energy landscapes by NMR

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The energy landscape of a protein predetermines its folding reaction and its function. NMR provides several tools to explore these landscapes at equilibrium and during kinetic experiments at high molecular resolution. (A) The cold shock protein CspB shows a smooth energy landscape, which we could explore with residue resolution in a complete pressure – temperature phase diagram between –13 to 57 °C and 1 to 2200 bar including complete heat and cold denaturation using a ceramic high pressure NMR cell. (B) For the gene-3-protein at the tip of fd phage, we could characterize the infectious state by 2D real time and H/D exchange competition NMR, which is a local minimum at the energy landscape of the protein [2,4]. The global minimum is not infectious. (C) In the case of ankyrin repeat proteins controlling the human cell cycle, the cell uses their ragged protein folding landscape to control their inhibitory function [1]. By employing the concept of conformational selection, a high energy intermediate state becomes accessible upon phosphorylation, which can be characterized at residue resolution. A second phosphorylation is required for subsequent ubiquitination and proteasome degradation. (D) A low-energy folding intermediate of RNase T1 is the target of the chaperone and PPIase SlyD [3], where we could characterize the Michaelis-Menten complex by 2D and 3D real time NMR [5] using the BEST approach for fast data acquisition.

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Ps161

Ultra High Resolution NMR: Sustained Induction Decays of Long-Lived Coherences

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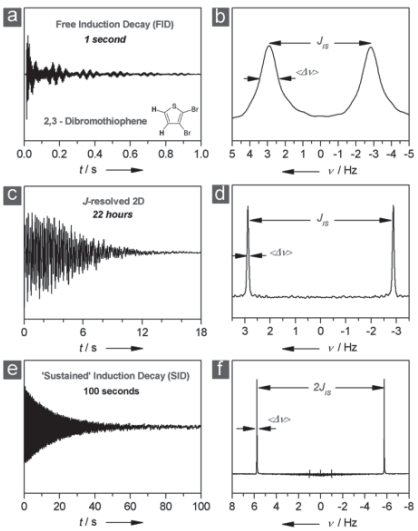
Long-lived coherences (LLC's)[1] in homonuclear pairs of chemically inequivalent spins can be excited and sustained during protracted radio-frequency irradiation periods that alternate with brief windows for signal observation[2]. Fourier transformation of the Sustained Induction Decays (SID's) recorded in a single scan yields NMR spectra with line-widths in the range $10 < \Delta\nu < 100$ mHz, even in moderately inhomogeneous magnetic fields. The resulting doublets, which are reminiscent of *J*-spectra, allow one to determine the sum of scalar and residual dipolar interactions in partly oriented media. The signal intensity can be boosted by several orders of magnitude by 'dissolution' dynamic nuclear polarization (DNP).



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Ps162

Longtime storage of hyperpolarization via singlet states in high field

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Current nuclear spin hyperpolarization techniques are able to enhance the otherwise poor sensitivity of NMR by greatly increasing spin polarization. These techniques, however, are unable to store the hyperpolarized spin order beyond the limits imposed by the longitudinal magnetization decay constant, T_1. Recently, it was shown that the thermal nuclear polarization can be stored for longer than T_1 via the use of long-lived nuclear spin states. No need to explain how great the outcomes from the combination of these two techniques will be for many NMR and MRI applications - *in-vivo,* particularly. To accommodate these interests, successful attempts to demonstrate the feasibility of this combination have already been made and examples of hyperpolarized long-lived states have been published. However, these examples may result impractical in many situations - e.g. *in-vivo* MRI.

In this contribution we show a neat methodology able to create hyperpolarized spin states that live longer than T_1 even in high magnetic fields like, for example, that of a commercial MRI scanner. To achieve this goal and to make the long-term storage of hyperpolarization practical, the use of near-equivalence spin pairs and the ability to access long-lived spin order in those pairs was fundamental. For this purpose, a new technique based on J-coupling-synchronized trains of 180° pulses was developed together with a nontrivial way to perform continuos monitoring of the long-term stored spin order.

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Robust and Cooperative Control of Spins

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Typical NMR and EPR magnetic resonance experiments consist of a series of pulses with well defined tasks. However, due to experimental constrains (e.g. maximum B_1 amplitudes) and imperfections (e.g. B_1 field inhomogeneity) the performance of the individual pulses and of the entire pulse sequence is suboptimal in many applications. A number of new approaches have recently been developed for pulse sequence design based on powerful optimal control methods: individual point-to-point (PP) and universal rotation (UR) pulses can be optimized. Recent applications include both standard experiments (for which a general set of robust "*plug and play*" pulses has been developed) and experiments in toroid probes with highly inhomogeneous B_1 fields[1]. Further improvements can be realized by allowing different pulses of a complex pulse sequence to compensate each other's imperfections in a *cooperative* way[2]. Novel broadband and selective heteronuclear decoupling and saturation sequences[3] are currently also being developed based on optimal control methods.

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Ps164

Solid-State NMR investigations of aggregates formed by perdeuterated Alzheimer's disease Aβ peptides

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Perdeuteration and back-substitution of exchangeable protons in microcrystalline proteins in combination with recrystallization from D_2O containing buffers reduces ^1H, ^1H dipolar interactions such that amide proton line widths on the order of 20 Hz are obtained (2). Aliphatic protons are either accessible via specifically protonated precursors or by using low amounts of H_2O in the bacterial growth medium (1). This labeling scheme is applied to amyloid aggregates like fibrils formed by the Alzheimer's disease ß-amyloid peptide (Aß) (3). Observation and assignment of side chain exchangeable groups like hydroxyl and imidazole protons yields valuable restraints to refine the fibril quarternary structure. In addition, solid-state NMR studies on the structure of drug induced Aβ aggregates are presented, focussing on the interactions between Aβ and the polyphenolic green tea compound epigallocatechin-gallate (EGCG).

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Ps165

A Method for Revealing the Local Packing Organization in Conjugated Semi-Crystalline Polymers

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Low band gap polymers belong to a new class of technologically important materials widely used as semiconductors in organic-based electronics. Common for many of these polymers is their lack of long-range order due their inherent semi-crystalline nature, preventing the access to the local organization on the molecular level from X-ray scattering experiments. In this contribution, a method utilizing solid-state NMR methods and quantum-chemical calculations is presented that provides a route to this kind of information. The method relies on chemical shift information from 1H and 13C and 1H-1H dipolar couplings combined with Nucleus Independent Chemical Shift (NICS) calculations.[1,2] From this combination, it is possible to validate different structural models by constructing these in silico, employing the experimental chemical shifts and 1H-1H dipolar couplings as finger prints. The potential of the approach is demonstrated by two recent examples, namely poly(3-hexylthiophene) (P3HT) and a new donor-acceptor polymer with excellent field-effect properties.[3-5]

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Ps166

Interplay of Order, Disorder, and Dynamics in Polymer–Fullerene Blends for Photovoltaic Applications

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Understanding and controlling the nanoscopic morphology, organization, and interfaces of photovoltaic composite materials are keys toward improving the efficiencies of organic solar cells. Two-dimensional solid-state NMR spectroscopy provides new molecular-level insights on the local structures and dynamics of composite materials, such as the electron donor poly(2,5-bis(3-alkylthiophen-2-yl)thieno[3,2-b]thiophene) (pBTTT) with the fullerene electron acceptor phenyl-C71-butyric acid methyl ester ($PC_{71}BM$). The pristine conjugated polymer pBTTT assembles into large crystalline domains, promoted by π–π interactions of the polymer backbone in one direction and by an interdigitation of the pendant alkyl chains in a second direction.[1] $^{1}H\{^{1}H\}$ double-quantum and $^{13}C\{^{1}H\}$ heteronuclear correlation NMR experiments unambiguously establish co-assembly of pBTTT with the fullerene derivative into a bimolecular crystalline solid, as suggested by recent studies.[2] Such composites, however, exhibit complicated molecular order, disorder, and dynamics, in particular with respect to the aliphatic polymer side chains and the π–π network of the polymer, as elucidated by $^{13}C\{^{1}H\}$ REREDOR and REPT-HDOR experiments. The resulting molecular insights provide new understanding of the structure-function relationships of these technologically promising composite photovoltaic materials.

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Protein misfolding, membrane interactions and paramagnetism studied by solid state NMR spectroscopy

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Amyloid fibrils or proteinaceous aggregates are not easily accessible to high-resolution structure determination, as they are neither crystalline nor soluble. In the recent decade, solid-state NMR spectroscopy has developed into a powerful tool to study even larger complex biological systems (1). Here, we report studies on different amyloidogenic proteins obtained under different fibrillization or seeding conditions. Initial results obtained for the prion protein PrP are compared to different existing structural models.

Secondly, we study the interaction of HIV-1 viral protein (VpU) with a fragment (comprising the transmembrane and cytoplasmic domains) of human the T-cell receptor CD4 (2,3). Individual proteins reconstituted in liposomes have a rigid transmembrane domain and flexible cytoplasmic domains.

Finally, we present results obtained on the paramagnetic heme protein NP7 (4), which can be stabilized in three different electron spin states. Although this protein does not yield resolved liquid-state NMR spectra, it can be studied by MAS NMR spectroscopy either attached to liposomes, or in solution, stabilized in glycerol or sedimented at the rotor walls (5).

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X- and W-Band PELDOR: Conformational States of Model Systems and the Ion Channel MscS

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Pulsed Electron Electron Double Resonance (PELDOR) is an established EPR method for measuring distances in the range of 1.4 to 8nm.[1] We apply this method to ion channels with the aim to unravel their gating mechanisms. Choosing the right sample conditions, we were able to resolve modulations in the PELDOR time traces of the ion channel of small conductans (MscS) in detergent and membranes. This enabled us to resolve conformational states of MscS that can be matched with X-ray structures of the open and closed form. Interestingly, for those mutants that did not show modulations at X-band, we could obtain modulated PELDOR time traces using the W-band HIPER-System.[2] In order to analyse these data, we use a simulation program similar to the one published by the Prisner lab.[3] On organic model systems it will be shown that our program works and that multifrequency PELDOR yields more precise information.

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Biomimetic Hydrogen Production: Multifrequency EPR and DFT Study of Cobaloxime Catalyst

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Solar fuels research aims to mimic photosynthesis and devise integrated systems that can capture, convert, and store solar energy in high-energy molecular bonds. Currently, we are designing both synthetic supramolecular photocatalytic systems as well as Photosystem I - catalyst biohybrids that photochemically produce hydrogen. Further development and improvement of these systems relies on understanding the inherent, fundamental mechanisms for coupling captured photons to fuel generation. To this end, we are applying advanced spectroscopic techniques such as multifrequency pulsed EPR to elucidate important structure-function relationships in our artificial and biochemical complexes. The catalysts of choice for our research are cobaloxime derivatives. The catalytic properties of cobaloximes depend on the local surrounding and on the direct ligands to the central metal ion. The knowledge of the electronic properties is essential for understanding the catalytic activity of the compound. EPR is an excellent tool to achieve this goal. In this work, difluoroboryl cobaloxime $Co(dmgBF_2)_2$ has been investigated in a variety of solvents with multi-frequency EPR spectroscopy at X-band (9 GHz), Q-band (34 GHz), and D-band (130 GHz) microwave frequencies. DFT modeling of the experimental data allows us to distinguish between different stable conformers and validate the structure of the axial ligand(s)-$Co(dmgBF_2)_2$ complexes. This work was supported by the Office of Basic Energy Sciences of the U.S. Department of Energy through Grant DE-AC02-06CH11357.

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EPR Analysis of Chromium-Sugar Interactions

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Chromium, widely used in various forms of industry, has been shown to have toxic and carcinogenic effects in humans and animals. Specifically, the reactive Cr(V) and Cr(IV) intermediates formed during reduction of Cr(VI) to Cr(III) are harmful in many ways, oxidizing biomolecules such as DNA, inducing the formation of reactive oxygen species and disrupting several enzymatic reactions. Although EPR has been extensively used to study the complexation of Cr(V) with different biologically relevant molecules, the vast majority of these experiments consist of room-temperature X-band CW-EPR experiments, where the limited information provided by this technique (*i.e.* isotropic g value, and, in the best case, some resolved isotropic ^{1}H and ^{53}Cr hyperfine couplings) is then used to derive quite complex models of the formed oxo-Cr(V) complexes. It is clear that in many cases, the available data is over-interpreted.

In this work, we study the oxo-Cr(V) complexes formed by reaction of Cr(IV), gluthatione and a number of sugar-type molecules, such as sorbitol and galacturonic acid. A combination of low-temperature X-/Q-band CW-EPR, X-band HYSCORE and pulsed ENDOR experiments are performed and confronted with DFT experiments on different models. These results are then confronted with some of the earlier 'room-temperature EPR'-based models.

Ps171

ESR Studies of Dynamics and Structure of Proteins and Membranes at ACERT

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Currently the study of protein structures by pulse dipolar ESR methods is becoming widely utilized. The most useful methods for such studies will be reviewed and instrumental developments at ACERT to enhance pulse dipolar ESR will be discussed. The state-of-the-art at ACERT will be illustrated with the example of a large protein complex consisting of an assembly of six proteins important in signal transduction in bacterial chemotaxis.

Since proteins at physiological temperatures are engaged in complex dynamical processes, it is valuable to develop ESR methodologies for their study. Instrumentation and methodologies at ACERT for multi-frequency 1 and 2D studies of protein and membrane dynamics over the range of 9 to 240 GHz will be described. Their application will be illustrated with an extensive study, covering 9, 95, 170, and 240 GHz, of the dynamics of spin-labeled T4-Lysozyme in aqueous solution, and 2D-ELDOR studies on membranes.

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Molecular bases of gene regulation by FUSE Binding Proteins

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FUSE Binding Proteins (FBPs) are a family of multi-domain proteins that regulate gene expression at multiple levels. For example FBPs regulate both the synthesis[1] and stability of the *c-myc* mRNA[2] as well as the biogenesis of miRNA effectors[3].

We have examined the molecular interplay between the three members of the FBP family and their protein and Nucleic Acid partners[4,5] and dissected the structural bases of macromolecular recognition. Our results indicate that multiple synergistic interactions are necessary for recognition and have identified key contacts defining specificity. We discuss this work in the functional context of FBP(s)-mediated regulation and we compare it with other findings on multi-domain nucleic acid binding proteins.

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Protein Conformational Dynamics and Weak Complex Formation

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Nuclear magnetic resonance (NMR) spectroscopy is a unique tool to probe protein flexibility at atomic level and at physiologically important timescales, through the analysis of spin relaxation or residual dipolar couplings (RDCs).

Analytical approaches to describe the dynamics of folded proteins using RDCs were developed. The approaches, that allow the quantitative determination of molecular motions, were applied to Ubiquitin and the third CD2AP SH3 domain (SH3C) and compared to accelerated molecular dynamics simulations, leading to a unified description of protein dynamics, both in terms of motional modes and amplitudes and free-energy weighted statistical mechanical ensembles.

Then the ultra-weak complex formed between Ubiquitin and SH3C was studied using spin relaxation, by developing a general approach for the study of the structure, dynamics and kinetics of those complexes, which are very difficult to study using standard techniques.

All those studies provide insights about molecular recognition mechanisms and how conformational flexibility can influence those processes.

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Detecting the 'Afterglow' of ^{13}C NMR in Proteins Using Multiple Receivers

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We show that the weak signal that remains after C-13 detected experiments (the ^{13}C 'afterglow') can still be measured with high sensitivity by proton detection. This is illustrated by the incorporation of two experiments, 2D (HA)CACO and 3D (HA)CA(CO)NNH, into a single pulse sequence that makes use of two receivers in parallel. The measurement time can be further substantially reduced with projection-reconstruction techniques. For instance, both 2D and 3D spectra were recorded on the 54 residue protein GB1 in one single measurement lasting only 15 minutes. High quality data sets for the 143 residue nuclease A inhibitor at $2^{o}C$, (correlation time 17.5 ns) were recorded in 3 hours, illustrating the utility of the method in studies of moderately sized proteins.

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The functional dynamics of synthetic riboswitches

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Riboswitches are novel regulatory RNA-elements which change their conformation upon binding of a small-molecule ligand and thereby regulate gene expression. Thus, structural dynamics is a mandatory prerequisite for their regulatory function. We investigated the functional dynamics of two synthetic riboswitches – one regulated by the aminoglycoside neomycin (1) and the other by tetracycline – using NMR-spectroscopy in solution. Interestingly, both riboswitches show a very different dynamic behavior. The neomycin riboswitch binds its ligands by a conformational selection mechanism which can be directly visualized by NMR. In contrast, the tetracycline riboswitch shows an Mg^{2+}-dependent highly structured ground-state with extensive preorganization of tertiary structure elements. The utilization of different ligand recognition strategies by the synthetic riboswitches resembles related findings for their naturally occurring counterparts.

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Ps176

Alternatives in the Rapid Acquisition of Multidimensional NMR and MRI Data

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We will describe a series of complementary schemes enabling the rapid acquisition of arbitrary multidimensional NMR spectra and/or images (MRI). These methodology can be implemented using conventional NMR/MRI hardware, and have in common the melding of concepts familiar to the NMR spectroscopy and the MRI physics communities –but arguably not to both of them. A number of different protocols will be discussed based on temporal, spatial, Hadamard, multidimensional RF pulsing and compressed-sensing, and their performance will be exemplified and compared for a number of prototypical NMR and MRI acquisitions on chemical, biochemical and clinical systems. The incorporation into these experiments of nuclear hyperpolarization procedures capable of increasing the single-scan sensitivity of liquid state NMR by factors ranging from 10^3-10^6, will also be assessed.

Ps177

Hyperpolarization of spin I > 1/2 noble gasses beyond 10% spin polarization for biomedical MR applications.

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Hyperpolarized (hp) ^{3}He and hp ^{129}Xe (both spin $I = 1/2$) have enabled a wide range of novel MR spectroscopy and magnetic resonance imaging (MRI) applications in chemistry, chemical physics, materials science, and pulmonary diagnostics. The recent development of hp ^{83}Kr MR provides a new pulmonary biomarker that utilizes the nuclear electric quadrupole moment of ^{83}Kr (spin I = 9/2) as a probe for surfaces [1, 2, 3]. Spin exchange optical pumping (SEOP) has recently produced hp ^{83}Kr with 4.4% spin polarization [3] and a 2.2% spin polarization was obtained with the spin I = 3/2 isotope ^{131}Xe [4].

Biomedical MRI applications with these quadrupolar noble gasses would benefit from further increased spin polarization. SEOP typically leads to high spin polarization when dilute concentrations of xenon or krypton in nitrogen and helium are used. However, until now, no method is reported that would allow for concentrating the hp quadrupolar noble gasses without detrimental depolarization during the separation process. We have now solved this conundrum and developed a method that maintains the high level of hyperpolarization generated in our experiments.

$$P = \frac{|\gamma|\hbar B_0}{3k_B T}\left(I+1\right)$$

Eq. 1: Spin polarization P for a general nuclear spin $I \geq 1/2$ at high temperature thermal equilibrium (see ref (4)).

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Ps178

High-Pressure *in situ* ^{129}Xe NMR spectroscopy of breathing transitions in Metal-Organic Framework (MOF) compounds

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^{129}Xe NMR has found numerous applications in biological and surface NMR spectroscopy. Here, we describe the use of high-pressure *in situ* ^{129}Xe NMR spectroscopy to study the so-called gate-pressure effect in Metal-Organic Framework compounds. The novel paramagnetic MOF compound $Ni_2(2,6\text{-ndc})_2(dabco)$ = DUT-8(Ni) {2,6-ndc = 2,6-naphthalenedicarboxylate, dabco = 1,4-diazabicyclo[2.2.2]octane} exhibits interesting adsorption properties [1]. Sorption of molecules such as nitrogen or xenon results in the opening of the pore system, *i.e.*, a pronounced gate-pressure effect accompanied by a large change of the structure and specific volume. To study this effect, we have developed a special apparatus which allows the *in situ* application of high and defined xenon pressures at controlled temperatures to the sample which is located in a pressure-resistant sapphire tube inside the magnet. This apparatus allows the NMR spectroscopic measurement of xenon adsorption/desorption isotherms and isobars in order to characterize the gate-pressure effect [2]. Deeper insight into the interactions between the host DUT-8(Ni) and the guest atom xenon is gained from *ab initio* Molecular Dynamics (MD) simulations. The use of DFT-vdW turned out to be crucial to correctly describe the breathing behavior of DUT-8(Ni), in particular the closed structure.

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Ps179

Surface Enhanced NMR Spectroscopy by Dynamic Nuclear Polarization

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Solid-state NMR is a powerful technique for the characterization of inorganic and hybrid materials, offering the possibility to directly investigate both the bulk, and the surface functionalities. However the concentration of the NMR active nuclei often remains relatively low, which strongly limits the characterization power of solid-state NMR in surface chemistry. We have recently shown how high-field Dynamic Nuclear Polarization (DNP) could be implemented to yield a remarkable increase in the NMR sensitivity of surface organic functionalities in hybrid nanoporous materials (1). The gain in time provided by carbon-13 or silicon-29 DNP NMR spectroscopy (typically on the order of a factor 400) allows the fast acquisition of 2D correlation spectra and therefore the detailed structural characterization of surface bonding patterns and local conformations (2). The latest developments in this field will be presented. In particular, we have recently investigated the feasibility of using organic, non-aqueous solvents for DNP NMR spectroscopy. These new solvents are demonstrated with the first DNP SENS characterization of a water sensitive organometallic complex supported on a hydrophobic surface, as well as with preliminary investigations on other classes of porous materials.

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Ps180

Bringing the NMR paradigm to EPR

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NMR has seen the rapid evolution of complex pulse techniques at ever-increasing fields and frequencies, often with FID detection. EPR has been much slower to follow this lead, largely because of technical reasons associated with the much faster relaxation times relative to NMR, and the very large, often field dependent, inhomogeneous linewidths relative to optimum microwave cavity bandwidths. Nevertheless, modern applications of pulse EPR are proving very persuasive scientifically and there is a strong impetus to improve performance particularly at high frequencies.

This talk will describe the HIPER project, whose aim was to design a frequency scalable, high power, pulsed EPR system operating at 94 GHz. This offers a combination of very high concentration sensitivity, large instantaneous bandwidth (GHz), high spectral resolution, relatively easy sample handling, together with very low (ns) deadtime. It also offers considerable flexibility in specifying pulse sequences with sub-ns time resolution, fast frequency switching with up to 16 different available phases.

Examples will be given showing the advantages of such a system including PELDOR measurements at concentration levels of less than 1 micro-molar and where it is possible to advantageously extract quantitative information associated with the relative orientation and distance and distribution of pairs of (site-directed) spin labels. Demonstrations showing state-of-the art deadtime, DNP applications, and examples where composite pulses can be usefully used will also be shown.

Ps181

Helpful tools for SDSL EPR on membrane proteins: DNP water accessibility, His-tag labeling and high power Q band

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Application of site directed spin labeling EPR to membrane proteins is often difficult due to limitations imposed by fast T2 relaxation and by the amount of protein available.

Short interspin distance can be precisely measured by conventional X-band DEER down to 20 μM spin concentration, as demonstrated with peptides bound to the human transporter associated with antigen processing [1]. Reliable extraction of long distances (6 nm range) from DEER traces on membrane proteins require S/N ratios which can be routinely achieved in 12 hours using a high power Q-band spectrometer. Examples of the DEER performance on the home-made Q-band spectrometer with non selective excitation pulses are shown on the proapoptotic protein Bax and on the ABC transporter MsbA. The possibility to measure directly the nitroxide accessibility towards water molecules via DNP at room temperature on a limited amount of sample is presented as a valuable tool to complement the study of conformational changes of the vitamin B12 importer BtuCD-F [2].

Overcoming invasive spin labeling strategies has immense potential in studying membrane proteins under physiological conditions. A new spin-labeled chemical recognition unit for switchable and concomitantly high affine binding to His-tagged proteins is presented. The spin-labeled unit allows the extraction of structural constraints within the ABC maltose transporter.

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Ps182

Understanding the hydrogen-converting cluster of [FeFe] hydrogenase

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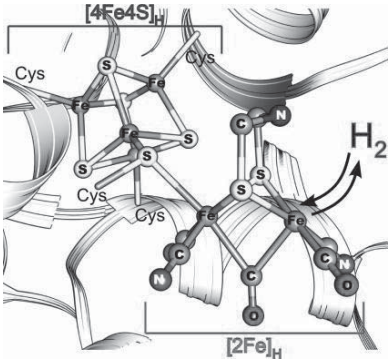

The general function of hydrogenases is to catalyze the reversible splitting of molecular hydrogen. The active site of [FeFe] hydrogenases (the so called H-cluster) has been examined in several EPR active states using advanced pulse EPR techniques. It was found that the electronic structure of the H-cluster is characterized by a rather strong $[2Fe]_H$-$[4Fe4S]_H$ exchange interaction, which induces strong singlet triplet mixing in the $[4Fe4S]_H$ subcluster, leading to large ^{57}Fe HF couplings in the 'cubane'. Moreover, the unpaired spin density in the $[2Fe]_H$ subcluster was found to be largely delocalized.[1,2] One of the most important results of this study, is the identification of a nitrogen in the dithiol bridgehead, which was not resolved in earlier X-ray crystallographic studies. [3] The obtained data from the native system was compared with results of EPR studies of {2Fe2S} and {2Fe3S} model compounds, closely related to the structure of the bi-nuclear subcluster in active as well as the CO inhibited state.[4] The absence of the [4Fe4S] subcluster and variations in the second coordination sphere of the models have a significant effect on the unpaired spin density distribution over the 2Fe core as compared to that of the H-cluster.

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Ps183

Conformational Changes During Primary Photosynthesis as Studied by Orientation Resolving Pulse Dipolar EPR Spectroscopy

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To understand the efficiency of light-induced electron transfer in photosynthesis the electronic and spatial structures of the initial, intermediate and final cofactor states of the reaction center (RC) protein are of particular concern. In bacterial photosynthesis of *Rb. sphaeroides* the primary donor is a dimeric bacteriochlorophyll (P), the primary acceptor a ubiquinone (Q_A). Subtle cofactor-protein interactions provide an essential "fine-tuning" of the electronic structure of the intermediate states to secure high quantum yield. It involves transient *intra*-molecular conformational changes of the redox cofactors and/or modifications of weak *inter*-molecular interactions with the "solvent" matrix. From pulsed 95 GHz high-field EPR, ESE and PELDOR as well as 35 GHz ENDOR experiments on frozen-solution RCs at 90 K we were able to characterize in detail potential conformational changes under light-induced charge separation. In contrast to earlier predictions, our PELDOR and ENDOR data analysis consistently reveal that neither the distance nor the relative orientation of $P^{+\bullet}$ and $Q_A^{-\bullet}$ change significantly under charge separation [1]. A substantial energetic contribution to stabilizing the primary charge-separated radical-pair state, $P^{+\bullet}Q_A^{-\bullet}$, may be expected from nearby unbound water molecules or weakly hydrogen-bonded water interacting with the RC, as is implied by recent X-ray crystallographic data.

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POSTERS

POSTER TOPIC		POSTER NUMBER
BS	BIOSOLIDS	**BS200 – BS215**
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Bs200

Atomic resolution structural features of the Ure2 prion by solid-state NMR

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The yeast prion Ure2 is a two-domain protein composed of a globular functional C-terminal domain (94-354) and a N-terminal domain, essential for prion induction and propagation. We initiated solid-state NMR studies of this protein, and also of fragments thereof, in view of revealing its assembly mode into fibrils.
Ure2 prion fibrils show highly resolved NMR spectra with narrow lines,[1] demonstrating that the proteins have a mostly well-ordered atomic structure. While the spectra of the isolated globular domain show a similar resolution, spectra of the isolated N-terminal domain show mostly broad and featureless lines.
Preliminary studies already indicated that the structure of the globular C-terminal is highly conserved in the protein fibrils when compared to the isolated domain.[1] We recently achieved the *de novo* sequential assignment of the isolated 33 kDa C-terminal domain using an optimized set 3D spectra,[2] which allowed us, considering the few small chemical shift changes (< 0.6 ppm), to confirm on a residue-per-residue basis its structural conservation. Remarkably, the C-terminal domain displays a higher order in the full-length protein fibrils than in the C-terminal crystals. The extensive assignment of the C-terminal domain allows now the assignment of the around fifty sharp resonance signals present exclusively in the full-length protein fibril spectra to the N-terminal domain, with the exciting perspective of their sequential assignment, which should yield first insights at atomic resolution into the structure of the N-terminal prion domain in its natural context.

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Bs201

Investigation of Zn^{2+}-binding sites and its effect on the molecular structure of $A\beta_{42}$ amyloid aggregates using Solid-State NMR.

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Amyloid beta (Aβ) is a 39-43 residue long peptide whose progression from a relatively unstructured monomer to a hairpin structured fibrillar aggregate is associated with the onset of events that lead to Alzheimer's disease. Zn^{2+} is considered to be a major neurochemical factor associated with Aβ aggregation and Alzheimer's disease (AD). Zn^{2+} at low concentrations (<10 μM) reduces Aβ toxicity by destabilizing the highly toxic intermediate species which are formed during aggregation process, and/or by binding to membrane associated Aβ. On the other hand, at higher concentrations (100's of μM) it appears to abet the cytotoxic effects of Aβ. Aβ aggregates are primarily fibrillar in nature, however Zn^{2+} can interact with Aβ on a millisecond time-scale, causing rapid aggregation into non-fibrillar species. These observed morphological differences and the modulations of toxicity are likely due to structural differences at the molecular level. Previous studies on Aβ40 and its truncated fragments have characterized various zinc binding sites in Aβ monomers, but not much is known about the molecular structure of Zn^{2+} containing aggregates of Aβ. Zn^{2+} ions have been indicated to not induce major structural changes in the β-hairpin architecture of Aβ40, however, a clearer picture of this and the observed effects of Zn^{2+} on Aβ aggregation and toxicity are yet to be fully understood. In this work, we have used solid-state NMR to identify the Zn^{2+} binding sites and to characterize the critical structural changes induced by Zn^{2+} binding to the pathologically relevant $A\beta_{42}$ aggregates.

Bs202

Water Dependent Interaction Study of Collagen Protein and Mineral Interface in Bone by Solid – State NMR Spectroscopy

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Bones are amorphous material comprising of inorganic hydroxyapatite, organic proteins and water molecules. Among organic components, collagen is most abundant protein in bone matrix and is responsible for mechanical properties of bone[1-2]. Study of interactions among different components of bone is important in order to understand mechanism responsible for bone formation, biomineralization and for its unique mechanical properties. In this presentation, we present first direct method to study interaction of protein collagen and inorganic surface through water molecules in intact mammalian bones by high resolution solid state NMR (SSNMR) spectroscopy. We performed ^{13}C $\{^{31}P\}$ Rotational Echo Double Resonance[3] (REDOR) SSNMR experiments to measure accurate distances between collagen and phosphorus of inorganic surface. Role of water in this interaction were estimated by measuring the distance between collagen and inorganic phosphorus after removing water molecules from bone matrix and also by reducing the strength of hydrogen bonding network by exchange of water protons with deuterium. Other SSNMR experiments like T_2 measurement of ^{13}C resonances of collagen and ^{1}H chemical shift measurement of collagen gives structural changes in bone matrix. We find that as water molecules from bone matrix are removed, interaction strength of collagen with inorganic surface increases. The study explains the role of water in stabilizing the structural properties of amorphous material like bone[4].

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Bs203

C-terminal domain of human Centrin 2 complex. Crystallinity, structure and local dynamics by solid state NMR.

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The C-terminal domain of a complex of human centrin 2 (C-ter HsCen2/P17-XPC), which is involved in the Nuclear Excision Repair (NER) pathway,[1] was studied by solid-state NMR. Crystallogenesis leads to different μ-crystal motifs, spherulites and plates. The latter present reasonably well-resolved ^{13}C and ^{15}N CPMAS spectra at moderate spinning frequencies. CP-MAS spectra and 2D dipolar through-space correlation experiments have been carried out to assign the spectra, which are compatible with the presence of a dimer in the lattice.[2] Relaxation studies to detect dynamics in a range from ns to ms are under way in order to obtain a better understanding of how centrin 2 recognizes damaged DNA in the NER pathway.

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Bs204

Solid-State NMR of Aβ Protofibrils Implies a β-Sheet Remodelling upon Maturation into Terminal Amyloid Fibrils

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Aβ(1-40) is the major fibril-forming peptide from Alzheimer's disease. Monomeric Aβ is unstructured but adopts a highly ordered β-sheet conformation upon aggregation into amyloid fibrils. These fibrils are the products of a complex formation mechanism that is not well understood. We use solid-state NMR to elucidate the structure of Aβ protofibrils. This analysis is possible because binding of the antibody B10AP prevents the conversion of these metastable intermediates into mature fibrils. A set of 8 peptides with varying labeling schemes was obtained from chemical synthesis. The labels cover 30 residues distributed over the peptide sequence. ^{13}C CP MAS spectra and 2D correlation experiments were recorded for assignment. From the conformation dependent chemical shifts we could identify peptide segments of stable secondary structure and evaluated the backbone structure using TALOS. Based on this data, Aβ protofibrils encompass residues 16-22 and 30-36 in β-sheet conformation. Three structural regions of the protofibrils present random coil-like chemical shifts. One encompasses residues 23-26 and forms an intermediate segment in between the adjacent β-strands. The other regions occur at the termini. Information about peptide dynamics is provided by order parameter measurement derived from of dipolar couplings. We find that protofibrils show high order parameters (>0.8) within the β-strand regions, while the measured *S* values are below 0.8 at the termini. We never observed *S* values below 0.4 that would have indicated very high mobility. Thus, significant structural order exists also within those sequence segments that have chemical shift values corresponding to a random coil.

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Bs205

*μ*MRI and NMR study of regenerated extracellular matrix in bone defects

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Since bone substitutes are increasingly used in orthopaedic interventions a high interest in optimization of the required materials exists. Using a tibial head defect of Wistar rats we investigate the influence of the porosity of biodegradable poly(lactic-co-glycolic acid) (PLGA) scaffolds which provide a macro-porous three-dimensional carrier and thus should support cell migration.

Cylindrical scaffolds with pore sizes of 100-300, 300-500 or 500-710 μm and a diameter of 2.5 mm were implanted. Two or four weeks after implantation, the regenerated extracellular matrix (ECM) was *ex vivo* qualitative and quantitative monitored by μMRI and solid state NMR.

Using μMRI, the implanted PLGA scaffolds are clearly visible and a homogeneous generation of ECM was obvious in the presence of the scaffolds. The regeneration of the collagen moiety was followed by ^{13}C MAS NMR and depended on the pore size of the scaffolds at all time points. The inorganic moiety was investigated by ^{31}P MAS NMR and exhibited this dependence not earlier than four weeks due to the known biomineralization delay. Thus, the amount of hydroxyapatite increases significantly during the last implantation interval and larger amino acid order parameters are indicative of progressed biomineralization. However, a pore size of 300 to 500 μm is most effective as carrier and results in *de novo* regenerated ECM quality close to the native, healthy bone.

Hence untreated PLGA scaffolds support ECM formation in early stages of regeneration and result in a more homogeneous healing process indicating that PLGA scaffolds in combination with multifunctional coatings are a promising "multifunctional construction kit" for bone substitute.

Bs206

Elucidating metal ion-Aβ interaction at a molecular level using solid-state nuclear magnetic resonance studies

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Aβ continues to be an interesting model for investigating different aspects of amyloid aggregation. Fine structural models currently are available for this system. Whilst it is known that metal ions binding to Aβ does play a significant role in the toxicity, morphology, and aggregation kinetics, the nature of this binding or its influence on the molecular structure of Aβ is not clearly established. It is also necessary to investigate the role of various residues in the formation of turns, β–sheets, and salt-bridge in Aβ. We here report on certain aspects of these, one with designed peptides that form shorter fragments of Aβ, with which we have monitored essential interactions giving rise to the basic β–hairpin type structural model. The metal ion-Aβ interaction has been investigated with Zn^{2+} ions bound to aggregated forms of $A\beta_{42}$. We have used solid-state NMR to identify the Zn^{2+} binding sites and to characterise the critical structural changes induced by Zn^{2+} binding to the pathologically relevant $A\beta_{42}$ aggregates. We found that $A\beta_{42}$ adopts a β-hairpin structure when aggregated either in presence, or in absence of Zn^{2+} ions, with significant structural changes in the N-terminal and the loop region connecting the two β-sheets. Zn^{2+} ions bring more order to the side-chains of His13 and His14 present on N-terminal. In the loop region, presence of Zn^{2+} ions breaks an important Asp23-Lys28 salt-bridge by driving these residues to unique, but non salt-bridge forming structural conformations. Despite these significant changes, the β-hairpin structure of $A\beta_{42}$ is retained.

Bs207

Acidic residues in β_2-microglobulin are involved in the binding of fibril stabilizer serum amyloid-P component

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Serum amyloid-P component is a ubiquitous component of amyloid deposits and plays an important role in a broad spectrum of amyloid diseases stabilizing the fibrillar structures and preventing the host's defenses from clearing these deposits. Despite this little is known about the motifs present on the surface of the fibrillar structures present in amyloid deposits that are recognized by serum amyloid-P component. Here we report on solid-state magic angle spinning NMR studies of serum amyloid-P component bound to the fibrillar structures composed of β_2-microglobulin, the protein deposited in patients suffering from dialysis related amyloidosis

To facilitate the assignment 2D homo- and hetero-nuclear correlation spectra have been obtained at 850 MHz from uniformly and selectively and extensively labeled β_2-microglobulin fibrils both at neutral pH in the absence of denaturants and under acidic conditions. The spectra are of sufficient resolution to permit the sequence specific assignment of many of the resonances arising from the β_2-microglobulin fibrils. Comparable spectra have also been obtained for β_2-microglobulin fibrils in the presence of serum amyloid-P component. A detailed comparison of the spectra reveals significant perturbations to chemical shifts assigned to the sidechains of acidic amino acid residues in β_2-microglobulin. These studies corroborate biochemical studies that have highlighted the importance of acidic amino acids in the recognition of amyloid fibrils by serum amyloid-P component.

Bs208

Structure and Dynamics of The Bacterial Cell Wall by Solid-State NMR

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The cell wall is essential for the survival of bacteria. It gives the bacterial cell its shape and protects it against osmotic pressure, while allowing cell growth and division. It is made up of peptidoglycan (PG), a biopolymer forming a multi-gigadalton bag-like structure, and additionally in Gram-positive bacteria, of covalently linked anionic polymers called wall teichoic acids (WTA). TAs are thought to play important roles in ion trafficking, host-cell adhesion, inflammation and immune activation.

In this contribution, we compare the flexibility and the organization of PG from different Gram-positive bacteria using solid-state NMR under magic-angle sample spinning (MAS). Flexibility of the PG network is found to mainly correlate with its reticulation rate. A wide range of dynamics is present in the different polymers of the cell wall. A proper choice of the solid-state NMR sequence using either CP-based or INEPT-based elements can be used to filter rigid or mobile parts of the system. The dynamic range detected using these different filters is discussed on the base of residual dipolar coupling measurements. We also show that ^{31}P solid-state NMR is particularly well adapted to characterize WTAs on isolated cell walls as well as on intact cells. Complexation of the cell wall with divalent ions (Mg^{2+} and Mn^{2+}) was investigated, allowing us to propose a new model for the interaction of divalent ions with both WTAs and carboxyl groups of peptidoglycan[1].

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Bs209

Solid-state NMR on the large membrane protein BamA at critical sensitivity and resolution

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Studying structure and dynamics of large membrane proteins by solid-state NMR (ssNMR) is challenging both in terms of spectroscopic sensitivity and resolution. Here we report on recent progress to address such issues on the integral multi-domain membrane protein BamA, which constitutes the core component of the β-barrel assembly machinery (BAM) in the outer membrane of gram-negative bacteria (see, e.g., Ref.[1]).

We succeeded in reconstituting the 790 residue full-length BamA in lipid bilayers at a high protein-to-lipid ratio, allowing high-resolution ssNMR experiments in a functional environment. To reduce spectral crowding, we furthermore employed specific amino acid labeling. Finally, initial results show the beneficial effect of dynamic nuclear polarization (DNP) and the utility of high levels of protein deuteration. Taken together, these approaches significantly enhance the prospects for analyzing biological macromolecules such as membrane-embedded BamA on the amino-acid specific level.

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Bs210

Eumelanin and pheomelanin: a comparative analysis through solid-state NMR

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Melanins are a class of pigments ubiquitously found in the animal and plant kingdoms [1]. They are associated with a variety of biological functions, including the pigmentation of skin, eyes and hair. The biological functions of melanins are often attributed to their unique chemical properties [2]. However, the exact molecular and supramolecular structure of eumelanin and pheomelanin remains, at present, not completely understood. Solid-state NMR spectroscopy represents a powerful method of investigation, especially for solid samples lacking long-range translational symmetry. Here, we present the results of the investigation of natural samples of black and red melanin extracted from human hair by means of 1D and 2D solid-state NMR techniques. A significantly different degree of local order and mobility between the two samples was revealed by ^{1}H and ^{13}C data obtained at both 400 MHz and 1 GHz. In particular, an investigation of the dynamic properties of the two samples is supported by the quantitative analysis of the ^{1}H lineshape extracted from 2D WISE spectra [3], while a discussion of the structural features relies on ^{13}C data.

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Bs211

Influence of fibrillization conditions on the structural heterogeneity and polymorphism exhibited by Alpha-Synuclein fibrils

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α-Synuclein (AS) fibrils are found in Lewy bodies that are the pathological signature of neurodegenerative diseases such as Parkinson's disease. The aggregation of AS fibrils into amyloid fibrils from monomers is considered the disease-causing toxic mechanism[1]. This protein has been shown to form a variety of polymorphs[2-5] as well as structural heterogeneity.

The morphology of *in-vitro* fibrillized AS is dependent on the fibrillization conditions such as the pH, salt concentration etc[6]. It therefore becomes imperative to understand the driving forces resulting in the polymorphism and the structural transitions exhibited by AS fibrils. In this study, using solid-state NMR, a technique now widely used to investigate fibrillar and other insoluble proteins, we have investigated the spectral and conformational features of AS fibrils fibrillized at different solution conditions. The spectral features indicate a strong dependence of the morphology on the solution conditions confirming the previous study, in addition to the existence of a form that is not known so far.

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Bs212

Segmental Isotope labeling of amyloid fibrils

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Solid State NMR Spectroscopy (ssNMR) involving magic angle spinning (MAS) is capable of delivering high resolution structural information of weakly soluble complex biomolecules including amyloid fibrils and membrane Proteins. With increasing size or proteins containing a highly repetitive hydrophobic amino acid sequence, commonly found in amyloids, spectra of uniformly $^{13}C^{15}N$ labeled samples suffer from high spectral overlap. Several isotope labeling strategies including selective amino acid labeling or position specific labeling using partially isotope enriched carbon sources (*2*-glycerol, *1*-glucose) have been implemented. Nevertheless resolving of highly overlapped spectra and collecting of unambiguously intramolecular distance restrains remains challenging. Segmental isotope labeling by protein-*trans*-splicing has great potential to become an indispensable tool to overcome these problems as well as to analyze domains of large proteins. Exclusively intramolecular distance restrains can be elucidated. This labeling technique is accurately described for soluble proteins though not yet demonstrated for ssNMR. Here we show ssNMR investigations of segmental labeled Het-s (218-289) amyloid fibrils. Het-s molecules consist out of four β Sheets forming a left handed β solenoid. Split Intein fusion proteins containing each two β sheets have been generated and segmental labeled Het-s has been produced by in vitro *trans*- splicing. Chemical shifts obtained from BMRB can directly be assigned to the resonances in 2D ^{13}C-^{13}C spectra.

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Bs213

Influences of the NSAID sulindac sulfide on the fibrillization properties of the Alzheimer's peptide amyloid-beta 40.

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The Alzheimer peptides amyloid-beta 40 and 42 (Aβ40/Aβ42) are the main constituents of amyloid plaques, which cause neurodegeneration in Alzheimer's disease. They are derived from the amyloid precursor protein (APP) by cleavage involving the β- and γ-secretases. It has recently been shown that the nonsteroidal anti-inflammatory drug (NSAID) sulindac sulfide decreases Aβ42 production, and that it binds directly to Aβ42 (1). Furthermore, it has been reported that sulindac sulfide inhibits the formation of fibrillar Aβ, prevents fibril elongation, and destabilizes pre-formed fibrils (2). The focus of this project is to describe the interactions of Aβ40 with the NSAID sulindac sulfide and to elucidate its influence on the fibrillization behaviour of Aβ40. In the presence of sulindac sulfide, aggregation of Aβ40 is enhanced. Solution-state NMR spectroscopy was employed to follow the Aβ aggregation kinetics and interaction dynamics. The structures of the insoluble Aβ40 aggregates containing sulindac sulfide were investigated using MAS solid-state NMR. We find that sulindac sulfide induced aggregates yield well-dispersed spectra and are amenable for a structural analysis with solid-state NMR.

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Bs214

Conformational analysis of steroid hormone molecules in the lipid environment – A solid-state NMR approach

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Solid-state $^{1}H/^{13}C$ cross-polarization/magic angle spinning (CP/MAS) NMR spectroscopy has been applied to two steroid compounds: dehydroepiandrosterone (DHEA) and spironolactone (SPI), to analyze their conformations at the atomic level.[1] In the absence of lipid, the high-resolution ^{13}C CP/MAS NMR signals of DHEA and SPI in a powder form reveal multiple patterns, with splittings of 30–160 Hz, indicating the existence of multiple conformations.[2] In the mimic lipid environment formed by mixing 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC) and 1,2-diheptanoyl-*sn*-glycero-3-phosphocholine (DHPC) in a molar ratio 3:1, the resulting DHEA and SPI spectra revealed mostly *singlet* patterns, suggesting that these steroids undergo a conformational change leading to a specific conformation in the lipid environment. Evidences from chemical shift isotropy and anisotropy analysis indicate that DHEA might adopt conformations subtly different from that seen in solution and in the powder form. In conclusion, we demonstrate by solid-state NMR that the structures of DHEA and SPI may adopt slightly different conformations in different chemical environments.

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Bs215

SOLID STATE NMR OF PROTEINS SEDIMENTED BY ULTRACENTRIFUGATION

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We demonstrate that solid-state (SS) NMR rotors behave as ultracentrifuges, creating a field of force of up a few million g and that, under MAS, a relatively large protein in solution sediments at the rotor walls, and its NMR signals are observable as if the protein were in the solid state.

The proof of principle of this new way of performing NMR spectroscopy is provided, showing examples based on ferritin, a 24-mer of 480 kDa molecular weight in the apo form. Because of the line broadening due to its size, it could be studied in solution only in the perdeuterated form. Apoferritin can be obtained in the microcrystalline state and provides high quality SS NMR spectra (1).

A ferritin solution was sealed in a 4 mm rotor without further manipulation and was spun at different rates: CP signals of immobilized molecules appear from 3 kHz (2). When spinning is stopped, the sample reverts to solution and the SS NMR signal is lost. Sedimented solutes NMR can overcome the size limitations of solution NMR without the need for sample manipulation required by solid state NMR. Large complexes and soluble prefibrillar states are attractive targets for this technique.

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Cs216

Adsorption of Phosphines and Phosphine Oxides on Silica Surfaces

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Understanding the adsorption characteristics of phosphines and phosphine oxides on silica has great importance in many fields of chemistry, including reaction product purification,[1] surface acidity analysis of oxide supports,[2] and characterization of immobilized catalyst systems.[3] Knowing more about the surface/adsorbate interactions and the mobilities of the compounds on the silica surface is of fundamental interest.

To gain deeper insight into these surface interactions, the adsorption of phosphines and phosphine oxides has been extensively studied by ^{31}P solid-state NMR spectroscopy. Significant changes in the chemical shift anisotropy (CSA), the chemical shift, and the residual linewidth provide valuable information regarding the different behavior of the functional groups on the silica support.

Compounds containing multiple phosphine groups, with one being prevented mechanically from interacting with the surface and in this way serving as an intramolecular standard, are employed. They help to attribute the notable reduction of the CSA, when adsorbing phosphines on surfaces, to either partial quaternization of the phosphorus center or to the mobility of the compound on silica.

Here, we will present surprising new insights regarding the strength of the adsorption of phosphines and phosphine oxides on various silicas and their diverse modes of mobility.

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Evaluation of heterogeneous hydrogenation reaction mechanism and NMR imaging of catalytic hydrogenation by using parahydrogen

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Parahydrogen-induced polarization (PHIP) phenomenon has become one of the very important tools for the investigation of homogeneous hydrogenation or hydrogen activation reactions mechanisms. The polarization providing by the using of parahydrogen in the catalytic hydrogenation reaction is orders of magnitude higher than thermal one, therefore due to the significant signal enhancement it is possible to study the fast reactions and identify intermediates of the catalytic reaction even that presented in the low concentration. Obviously, heterogeneous catalysts are much easier to separate from a reaction mixture than the homogeneous ones. Therefore, combination of PHIP with heterogeneous hydrogenation processes appears to be a promising route toward novel approaches for the production of hyperpolarized catalyst-free liquids and gases.[1] Furthermore, PHIP has a potential of becoming a useful tool for studying industrially important heterogeneous catalytic processes such as hydrogenation. PHIP may be observed in an aqueous phase heterogeneous hydrogenation of unsaturated amides and ethers using supported metal catalysts as well as for gas phase or organic-liquid phase hydrogenations. Therefore, the observation of PHIP produced with supported metal catalysts is very important for MRI applications and for the verification of reaction mechanisms of heterogeneous catalytic reactions.

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Cs218

^{31}P Solid State NMR of the immobilized homogenous catalysts

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The Wilkinson's catalyst $RhCl(PPh_3)_3$ and ruthenium complex $RuCl_2(PPh_3)_3$ were immobilized on the surface of functionalized silica materials. The structure of the modified silica surface and the immobilized catalysts complex is determined by a combination of different Solid State NMR methods. The successful modification of the silica surface is confirmed by ^{29}Si CP-MAS NMR experiments, which present the way of binding the organic groups to the surface of the mesopores. ^{31}P-^{31}P J-resolved 2-D MAS NMR experiments are conducted in order to characterize the binding of the immobilized catalyst to the amine groups of the linkers attached to the silica surface. The pure catalyst exhibits a considerable ^{31}P-^{31}P J-coupling, well resolvable in 2-D MAS NMR experiments. This J-coupling is utilized to determine the binding mode of the catalysts to the linkers on the silica surface and the number of triphenylphosphine ligands which are replaced by coordination bonds to the amine groups. From the absence of any resolvable ^{31}P-^{31}P J-coupling in off magic angle spinning experiments, as well as slow spinning MAS experiments, it is concluded, that two triphenylphosphine ligands are replaced and that the catalyst is bonded to the silica surface via two linker molecules(1).

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Radioliticaly generated paramagnetic centers in molecular sieves with adsorbed carbon monoxide.

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Free radicals are very often important intermediates in many processes of heterogeneous catalysis[1,2]. However, in real catalytic system they are very reactive and short-lived, thus difficult to study. We generated free radicals in zeolites exposed earlier to small molecular adsorbates by γ-irradiation at liquid nitrogen temperature.

The combination of EPR measurements with quantum chemical computation has been applied in order to identify the radical species and define their geometry and reactivity. The EPR spectrum of H-ZSM-5/^{13}CO sample γ-irradiated at 77 K and recorded at 300K shows two signals: anisotropic doublet **A**: with g_x=2.0005, g_y=2.0007, g_z=1.9991, A_x=30,4 mT, A_y=27,5 mT, A_z=25,9 mT and isotropic doublet **B**: with g_{iso}=2.0002, A_{iso}=21,3 mT. DFT calculations showed couple of stabilization sites for $^{\bullet+}$CO radical cation in ZSM-5 framework. It turned out that calculated values of hyperfine splittings for **A** doublet are close to the experimental value equal 27,7 mT for two different radical centers: [≡Si-O-Al≡]$^{\bullet}$CO and [≡Si-O-Si≡]$^{\bullet+}$CO. The second doublet **B** (A_{iso}= 21,3 mT) had not been observed earlier. The preliminiary DFT calculations showed that this signal could be assigned to the [≡Si-O]$^{+\bullet}$CO center. We observed the EPR spectra of $^{+\bullet}$CO radicals also in other zeolites like X, LTA and MOR. The analysis of experimental results and DFT calculations is in progress.

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Cs220

Selective inhibitor studies of CAI and CAII proteins by NMR and molecular modelling

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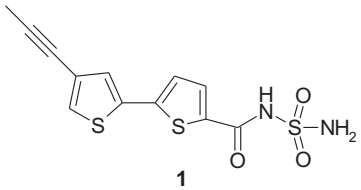
Carbonic anhydrases (CA) are proteins that are well-suited to serve as models in many types of studies in biophysics, bioanalysis, the physical-organic chemistry of inhibitor design, and medical chemistry. In vivo, these enzymes catalyze the hydration of CO_2 and dehydration of bicarbonate.

Sulfonamides are well known inhibitors of CA. In our studies we used NMR and molecular modelling approaches to investigate the reason for the differences in the inhibition activities of compound **1** to CAI (0.63 μM) and CAII (83.4 μM).



1

The t1ρ experiments show specific binding of **1** only to CAI. Molecular modelling methods revealed different coordination types of the C=O group in compound **1** to the enzyme Zn^{2+} in CAI and CAII which explain different inhibitor properties.

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Cs221

Studies on Precatalytic Systems of Copper-Catalyzed 1,4-Addition Reactions of Trialkylaluminum Reagents

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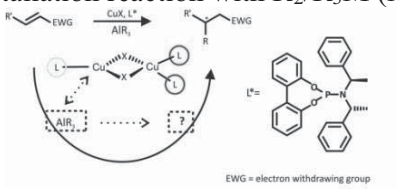
The progress in chemical industry and research was enhanced by the highly efficient synthesis of enantiopure compounds using non-chiral substances and cheap but selective reagents. For the C-C bond formation while creating a new chiral carbon atom the enantioselective catalytic 1,4-addition to α,β-unsaturated systems is one of the most attractive methods.[1-2] The usage of a catalytic system consisting of copper(I) salts and chiral phosphoramidite ligands offers a lot of advantages, like excellent enantioselectivity, high chemo- and regioselectivity and low costs compared to many other applied catalytic systems.

For the elucidation of the low temperature structures and the temperature dependency of those Cu(I) complexes, we performed NMR experiments with different copper(I) salts and phosphoramidite ligands. Recently we were able to identify a precatalytic structure with a mixed trigonal/tetrahedral coordination on the copper atom.[3] This complex structure provides also a free coordination site which is necessary for the assumed transmetallation reaction with R_2/R_3M (M=Mg, Zn, Al) species.



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Cs222

Catalytic oxidation of benzene on Cu-supported ZSM-5 and Y zeolites. Spin trapping of the transient radical species.

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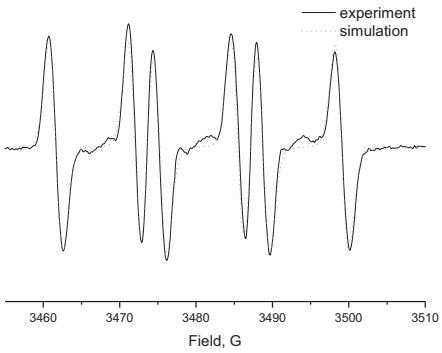
The formation of the transient radical species during the liquid phase adsorption of benzene on Cu-supported H,Na-ZSM-5 and H,Na-Y zeolites was investigated by EPR spectroscopy using the spin trap method. The liquid phase adsorption of benzene on Cu-containing ZSM-5 and Y zeolites leads to the formation of various transient radical species which play a crucial role in the oxidation of benzene to phenol with molecular oxygen. We have investigated the dependence of the various types and activation methods of the catalyst and the influence of oxygen on the formation of the transient radicals as well. $\cdot OOC_6H_5$ radicals were formed mainly when the acid and sodium form of Cu/ZSM-5 catalyst calcined in O_2 were used.

The initiation of bubbling oxygen to the benzene/catalyst suspension leads to the formation of other radical intermediates: $\cdot OC_6H_5$, $\cdot C_6H_5$ and di-tert-butyl-nitroxide derivatives that can form by oxidation of DMPO. No radical components could be detected after benzene adsorption on Cu/HY and Cu/NaY zeolites.



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Ct223

Similarity between NMR spectra: from binary trees to structure prediction

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Today NMR techniques are good candidates for high-throughput applications such as profiling of metabolic pathways, quality control, screening, etc. We present a method to estimate the similarity between NMR spectra based on binary trees and use this former to predict a vector of structural descriptors.

Nuclear spins are exquisitely sensitive to subtle modifications of their environments that reflect in the position of their NMR signals. Standard distance measurements fail to recognize those shifts and thus underestimate the real similarity. To address this issue, Pretsch and coworkers[1] introduced a comparison based on subsequent division or the spectrum into bins. Here we present a solution based on binary trees build from the position and intensity of the signals. Trees are more suitable for the analysis of multidimensional data, since they focus only on the regions of the spectra that contain signals, thus describing the data in a very compact form.

The accuracy of the described method allows its use to predict structural descriptors, such as aromatic rings, double bonds, etc. Therefore, the trees of 296 representative molecules were paired with their corresponding vector of structural descriptors and a joint-kernel[2] was calculated that correlates features of the spectra with features of the structure. This joint-kernel allows to predict the descriptors corresponding to any unknown tree. The predictions were found promising and represent a step toward structural elucidation.

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CT224

Using new computer approaches for biomedical researches.

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We suggested using as novel statistical insights a computer program for modeling different diseases. The important link in the biomedicine is an analytical processing of experimental results. By connecting fields such as medicine and biochemistry with mathematical simulation use MatLab computation program. Many attempts have been made to better characterize therapeutic properties of drug plant extracts for using in future by diseases. One of the stronger sites of suggesting computer program is repeated using of special program, getting up as M-files. Then by help MatLab-function`s plot we has obtained the graphical view of study biological processes at its development. The approximations of that kinetic curve allow us to make interpolation by polynomial-function with necessary power. Then we obtain a coefficient and roots of polynomials exactness and other statistical insights. At the same time testable and predictive models of pathways influense plant extracts on lipid peroxidation which are useful for efficient experimental design and bioengineering and the network-based design of drugs and therapies. The using of MatLab by analytical processing of experimental results to allow us with once program file to get the results, which is a basic at the same time evaluation a lot of kinetic parameters for studying processing in whole. That is methods for the modeling of living systems to applications in biotechnology and medicine.

CT225

Multiple quantum NMR dynamics of systems of equivalent spins: theory and computer simulations

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We consider the multiple quantum (MQ) NMR dynamics of a system of equivalent spins (s=1/2). A gas of spin-carrying molecules (atoms) in nanocavities can be considered as an example of such a system [1]. Since the averaged non-secular two-spin/two-quantum Hamiltonian describing MQ NMR dynamics of such systems depends on only one coupling constant, this Hamiltonian commutes with the square of the total spin angular momentum I^2 . We use the basis of common eigenstates of I^2 and the projection of I on the external magnetic field for an investigation of MQ NMR dynamics [1].

The developed approach allows us to find the dependencies of intensities of MQ coherences on their orders (the profiles of MQ NMR coherences) in systems consisting of 600 spins and even more. It is shown with computer simulations that the stationary MQ NMR profile in the considered system is an exponential one [1]. We investigate also the effect of the second order correction of the average Hamiltonian theory on the profile of MQ NMR coherences [2].

We study the decay of MQ NMR coherences due to dipole-dipole interactions on the evolution period of the MQ NMR experiment in systems consisting of 200-600 equivalent spins. It is shown that the relaxation time decreases with the increase in MQ coherence order and in the number of spins [3].

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CT226

Protein Structure Analysis via Chemical Shifts from a Side-Chain Perspective

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In contrast to the ever-growing use of protein backbone chemical shifts for biomolecular structure determination, there have been a few studies concerned with chemical shifts for side-chains. It would be highly desirable to extend such studies, since side-chains, naturally incorporated in proteins, are excellent probes of biomolecular structure, dynamics and recognition. In particular, proteins are rich of side-chain methyls and conjugated rings, many of which, owing to their hydrophobic nature, are more frequently placed at the inner core and protein-protein interfaces. This work presents the development and the use of the structure-based predictors for methyl and aromatic side-chain chemical shifts by incorporating terms convenient for the introduction of restraining forces in molecular dynamics simulations. The predictors are tested for their usefulness in validation of protein structures and dynamical ensembles and are of great importance for exploring protein-protein and protein-ligand assembly and interactions. Web server and stand-alone implementations are created to facilitate the usage of the developed tools. Their performance is demonstrated to already provide an opportunity for their immediate implementation in restrained simulations to refine protein structure and dynamics from the side-chain perspective.

CT227

A Tabu Search Approach for the NMR Protein Structure-Based Assignment Problem

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The key bottleneck of NMR studies is to map the NMR peaks to corresponding nuclei, also known as the assignment problem. Structure Based Assignment (SBA) is an approach to solve this computationally challenging problem by using prior information about the protein obtained from a homologous structure. Apaydın et al. (2010) formulated SBA as a linear assignment problem with additional Nuclear Overhauser Effect (NOE) constraints, which can be solved within Nuclear Vector Replacement's (NVR) (Langmead et al., 2003) framework. This approach (NVR-BIP) used NVR's scoring function and data types, and also gave the option of using CH and NH RDCs, instead of NH RDCs which NVR requires. In this paper, we prove that this problem is NP-hard and propose a tabu search algorithm (NVR-TS) equipped with a dynamic tabu list structure and guided perturbation mechanism to efficiently solve it. NVR-TS uses a quadratic penalty relaxation of NVR-BIP where the violations in the NOE constraints are penalized in the objective function. We also implemented a memory structure that reports k best solutions. Experimental results indicate that our algorithm finds the optimal solution on NVR-BIP's data set which consists of 7 proteins with 24 templates (31 to 126 residues). Furthermore, it achieves high assignment accuracies on two additional large proteins, MBP and EIN (348 and 243 residues, respectively), which NVR-BIP failed to solve.

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CT228

Robust Refocussing Pulses Which Facilitate Heteronuclear J-Evolution

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Coherence transfer elements like the INEPT-step are indispensible building blocks for multidimensional NMR spectroscopy as they efficiently transfer magnetization via heteronuclear J-couplings.

Two effects affect the performance of INEPT-transfer steps: on the one hand, pulse imperfections will lead to phase distortions and decreased signal-to-noise; on the other hand, relaxation causes magnetization losses during the transfer step that should be avoided by keeping the overall experiment time to a minimum.

A method is presented to numerically derive broadband 180° shaped pulses (e.g. for ^{1}H and ^{13}C), which compensate offset and B_1-inhomogeneity effects and at the same time allow heteronuclear coupling evolution during the pulse. Having the same effect as moving a part of the adjacent delay times into the shaped pulse, the overall length of the pulse sequence is decreased and corresponding losses due to relaxation are minimized.

CT229

NMR-Guided Protein-Ligand Docking

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State-of-the-art docking approaches used for the prediction of protein-ligand complexes in the drug development process are still only able to predict 60 to 80 % of the complexes correctly. This can be attributed to the severe approximations of the scoring functions due to efficiency reasons. As one possibility to overcome these deficiencies we will demonstrate that the combination of our docking tool PLANTS[1,2] with experimental data obtained from NMR experiments can highly increase the reliability of the predicted docking poses. For the complex of the antibody SM3 with its epitope, the ChemPLP scoring function[2] complemented with trNOE and STD distance constraints is able to predict the complex structure very close to the crystallographic one as the best-ranked docking pose[3]. The advantage of the new approach is that the used experimental data is relatively easy to obtain (compared to a full structure determination of the complexes by NMR or X-ray) since the experiments do not require isotope-labeled samples and have no size limitation for the receptor protein.

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CT230

Analytical Error Estimates for the Measurement of Scalar and Residual Dipolar Couplings in HCH-Type Spinsystems

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Scalar and residual dipolar couplings (RDC) of spin ½ nuclei are frequently used to determine conformation, configuration and constitution of organic molecules [1,2]. For ABX like spin systems like HCH and CH_2 groups, which are often seen in sugars, peptides and small molecules, the strong coupling derogates the possibility to easily evaluate the coupling by measuring the corresponding signal splitting. With the analytical solution of the ABX spin system [3-5], derived for a number of different operators active in various standard $^{1}H,^{13}C$-NMR experiments, it is possible to gain a closed expression for the experimental error. With this expression, an attempt is made to correct the error and gain the true underlying couplings.

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CT231

Chemical shift prediction for protein structure calculation and quality assessment using an optimally parameterized force field

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The exquisite sensitivity of chemical shifts as reporters of structural information, and the ability to measure them routinely and accurately, gives great import to formulations that elucidate the structure-chemical-shift relationship. Here we present a new and highly accurate, precise, and robust formulation for the prediction of NMR chemical shifts from protein structures. Our approach, shAIC (shift prediction guided by Akaikes Information Criterion), capitalizes on mathematical ideas and an information-theoretic principle, to represent the functional form of the relationship between structure and chemical shift as a parsimonious sum of smooth analytical potentials which optimally takes into account short-, medium-, and long-range parameters in a nuclei-specific manner to capture potential chemical shift perturbations caused by distant nuclei. shAIC outperforms the state-of-the-art methods that use analytical formulations. Moreover, for structures derived by NMR or structures with novel folds, shAIC delivers better overall results; even when it is compared to sophisticated machine learning approaches. shAIC provides for a computationally lightweight implementation that is unimpeded by molecular size, making it an ideal for use as a force field.

CT232

DFT Analysis of 3D Structure and NMR Parameters in Sulfated Oligosaccharides.

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Theoretical calculations were aimed at analysis of 3D structure of sulfated oligosaccharides and the effect of counter-ions upon their structures using density functional theory. Both B3LYP/6–311++G** and M05-2X/6–311++G** methods have been used for geometry optimization evaluating explicit solvent molecules. Optimized geometries showed considerable influences of counterions (Na^+ and Ca^{2+}) upon pyranose rings and the glycosidic linkage conformation. Solvent had only a limited influence upon magnitudes of proton-proton spin-spin coupling constants. Interatomic distances, bond and torsion angles indicated that the structure of the 2-*O*-sulfated iduronic acid residue influenced geometry of the N,6-sulfated glucosamine residue. Three-bond proton-proton spin-spin coupling constants agreed well with experimental data for both Na^+ and Ca^{2+} counterions. Analysis also showed that the Fermi contact term was not always dominant and that paramagnetic and diamagnetic contributions considerably influenced magnitudes of proton-proton spin-spin coupling constants.[1]

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CT233

EPR and DFT Study of Gamma Irradiated 2,6–di-tert-butyl-4-methylphenol Single Crystal

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Single crystal of gamma irradiated 2,6–di-tert-butyl-4-methylphenol was investigated using an electron paramagnetic resonance (EPR) technique at different orientations in the magnetic field at room temperatures. Taking into consideration the chemical structure and the experimental spectra of the irradiated single crystal of 2,6–di-tert-butyl-4-methylphenol, we assumed that one phenoxyl type paramagnetic species was produced having an unpaired electron localized at the methyl fragment side of the phenyl ring. Depending on this assumption, one possible radical was modeled using the B3LYP/6-311+G(d) level of density-functional theory (DFT). EPR parameters were calculated for these modeled radical using the B3LYP/TZVP and B3LYP/EPR-III level. The averaged value of isotropic hydrogen hyperfine coupling constants of a rapidly rotating methyl functional group of phenoxyl radical is calculated for the first time. Theoretically calculated values of the modeled radical are in reasonably good agreement with the experimental data determined from the spectra (differences in averaged coupling constant values smaller than 5 %, and differences in isotropic g values fall into 1 ppt).

CT234

Objective Identification of Residue Ranges for the Superposition of Protein Structures

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We present an automated and objective method for finding residue ranges for the superposition and analysis of structure bundles resulting from NMR structure calculations. The method is implemented in an algorithm, CYRANGE[1], that yields appropriate residue ranges in most commonly occurring situations, including low-precision structure bundles, multi-domain proteins, and protein complexes. Residue ranges are chosen to comprise as many residues of a protein domain that increasing their number would lead to a steep rise in the RMSD value. The ranges are determined by first clustering residues into domains, and then refining for each domain the initial choice of residues. A penalty for the opening of gaps favours contiguous residue ranges in order to obtain a result that is as simple as possible, but not simpler.

CYRANGE correctly identifies ordered regions, and global structure superpositions based on the CYRANGE residue ranges allow a clear presentation of the structure. In the majority of cases, the residue ranges from CYRANGE contain fewer gaps and cover considerably larger parts of the sequence than those from other methods, without significantly increasing the RMSD values. CYRANGE thus provides an objective and automatic method for standardizing the choice of residue ranges for the superposition of protein structures.

At **http://www.bpc.uni-frankfurt.de/cyrange.html** CYRANGE is freely available as a web service and as a stand-alone program.

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CT235

Optimal Control of Spins in the Presence of Relaxation and Radiation Damping: Applications in NMR Spectroscopy and Imaging

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In NMR, one important problem is how to steer a magnetization vector from a given initial state in minimum time or with minimum rf energy to a desired target state in the presence of relaxation and experimental bounds on the maximum rf amplitude. Here we present the solution of this problem based on recently developed analytical techniques of optimal control theory[1]. The analysis not only provides a thorough understanding of the design problems and of the structure of the optimal solutions, but also yields significantly improved pulse sequences for practical applications. The extensions of this work include radiation damping effects[2] and the simultaneous control of more than one spins[3]. The analysis of the optimal control of more than one spins can be applied to robust control problems and to maximize the contrast between spins with different transverse and longitudinal relaxation rates in magnetic resonance imaging. In addition, a comparison between the geometrical method and the numerical GRAPE algorithm[4] is presented.

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CT236

SIMPSON: Explore the limits of numerical simulations

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Over the years from the first release in 2000, the open-source SIMPSON simulation program for solid-state NMR spectroscopy[1,2] has attracted a significant attention in the community for its versatility and simplicity. Although other programs has been released (e.g. SPINEVOLUTION[3]), still a very high fraction of the solid-state NMR papers concerned with method developments or interpretation of data from simple to advanced experimental protocols is accompanied with simulations obtained using SIMPSON. Governed by development of increasingly powerful computer systems, simulation software packages, and efficient numerical algorithms, the area of applications of solid-state NMR simulations is steadily increasing. We wish to follow the recent trends and present here substantially speed-enhanced version of SIMPSON, really enabling to explore the limits of numerical simulations for rotating solids with time varying Hamiltonians. Through optimization of underlying matrix operations, internal data structures and exploitations of modern multiple cores computers as well as large-scale high-performance computing facilities (such as clusters and grids) it is now possible to simulate, as a selected example, proton driven spin diffusion experiment with 15 spins (2 carbons and 13 protons) in full detail of Hilbert space. SIMPSON remains an open-source program with all previous functionality (including optimal control tools[2]) and can be implemented on virtually every computer platform.

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CT237

Protein structure validation by multiple linear regression RMSD prediction

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Large-scale initiatives to obtain spatial protein structures by experimental or computational means have accentuated the need for the critical assessment of protein structure prediction methods. These include blind test projects like the Critical Assessment of protein Structure Prediction (CASP) and the Critical Assessment of protein Structure Determination by NMR (CASD-NMR). An important aim is to establish structure validation criteria that can reliably assess the accuracy of a new protein structure. A universal structural quality assessment method should combine multiple individual scores in a meaningful way, which is challenging because of their different measurement units. We present a method based on multiple linear regression (MLR) that combines diverse protein structure quality scores into a single quantity with intuitive meaning, namely the predicted coordinate RMSD value between the present structure and the (unavailable) "true" structure (MLR-RMSD). The correlation coefficients between actual (model vs. reference from PDB) and predicted (model vs. "true") RMSDs were 0.78 and 0.77, for two datasets from CASD-NMR and CASP, respectively, which is considerably higher than those for the individual scores (-0.24–0.64). The MLR-RMSD can thus predict the accuracy of protein structures more reliably than individual coordinate-based quality scores.

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CT238

Automated protein structure calculation from solid-state NMR data using the program CYANA

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It has been shown in several cases that protein structure calculation from solid-state NMR data is possible.[1–3] However, the majority of these calculations have been performed on microcrystalline model proteins of known structure and essential upper distance limits for the structure calculation were obtained by manual assignment of cross peaks. Due to broad lines and significant overlap, the manual assignment of cross peaks without using structural information is time consuming and prone to errors. First approaches to automation have been shown by Fossi *et al.*[4] and Van Melckebeke *et al*.[5]

We recorded a set of 2D solid-state NMR spectra of the microcrystalline model protein GB1 to investigate automated distance restraint assignment and structure calculation using the program CYANA, which is well established in solution NMR. As a consequence of broad lines, overlap and, so far, the limitation to two spectral dimensions, high assignment ambiguities make it challenging to obtain accurate structures. Several attempts to increase the reliability and accuracy of automated protein structure determination on the basis of solid state NMR data are presented.

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CT239

Automated Assignment of NMR Spectra

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Fully automated structure determination by NMR[1] requires accurate algorithms for the chemical shift assignment. These algorithms have to solve the problem of finding the correct chemical shift for the atoms of a protein using peak lists from a set of several multi-dimensional spectra.

Mapping a network of expected peaks, predicted on the basis of the protein sequence to appear in a spectrum according to its magnetisation transfer pathways, to the measured peaks in these spectra leads to an assignment of the measured frequencies to the atoms. This approach was first implemented in the software GARANT[2] (General Algorithm for Resonance Assignment) that gives reasonable solutions for good quality data and can in principle be used for every combination of spectra. The algorithm improves a randomly created starting generation by using a genetic and a local optimisation procedure iteratively.

To make the approach more applicable for common use we have reimplemented it as part of the CYANA[3] software and improved its reliability and efficiency. We enhanced the optimisation routine and introduced a new scoring scheme with the aim of improving the accuracy of the assignment. The algorithm is able to find the correct assignments of the atoms on the basis of manually or automatically prepared experimental peak lists for any combination of through-bond and through-space spectra. The reliability of the assignments depends on the extent and quality of the input data.

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CT240

MagRO-Assign: a tool for automated sequence specific assignment of chemical shifts for NMR signals of protein backbone

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The recent targets for biomolecular NMR spectroscopy tend to be complicated, such as protein oligomer, protein-ligand complex and multi-domain proteins. NMR signals from the polypeptide backbone are used to analyze basic properties of proteins such as folding/unfolding, protein-protein interaction and protein-ligand interaction. However, it would be very usual to encounter problems related to missing and duplication of NMR signals. These problems can be mainly caused by a chemical exchange phenomenon between a few conformational states such as *cis/trans* isomerization of proline, open/closed conformations and fold/unfold of proteins. In these cases, conventional automated assignment protocols require the number of conformational states *a priori*.

MagRO-Assign package includes programs for automated sequence specific assignments based on segment extension and simulated annealing using assignment matrix, which provides multiple sets of signal assignments corresponding to major and minor conformations without information of the number of conformational states. We have demonstrated the feasibility of our method for backbone assignments of two proteins: asymmetric dimer protein (124 amino acid residues) and 159 residue protein which are in slow exchange between two conformational states. We will show the detail of the program package and report present achievements in the poster presentation.

CT241

NMR structure calculation of protein symmetric aggregates

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Dealing with symmetric oligomeric structures is an important issue in the context of NMR for structural proteomics: it is estimated that about 60% of the proteins in every genome are homo-oligomers[1,2]. However, structure determination of symmetric aggregates by NMR is severely hampered by the difficulty to differentiate inter-monomeric and intra-monomeric correlations. Algorithmic support for assignment seems therefore particularly important for symmetric oligomers.

Several methods have been proposed but they generally rely on the knowledge of the monomer structure, which is unrealistic for *de novo* structure determination by NMR. A classical approach based on Ambiguous Distance Restraints[3] proved to be efficient for structure determination of symmetric homo-dimers from unassigned NOEs or solid-state NMR data. Still, this strategy has limited convergence properties and is somewhat difficult to apply for higher symmetry.

Here, we present a general method, based on strict symmetry relations, for structure calculation of high-order symmetric aggregates investigated by NMR[4]. The approach is not limited in terms of symmetry group and thus finds direct applications in both solution- and solid-state NMR for elucidation of oligomer, membrane protein or fibril structures.

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CT242

Comparative DEER- & FRET distance determination in simulation & experiment

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Since experimental inter label distances for structural elucidation of macromolecular complexes from DEER and or FRET cannot directly be translated to constraints in a structural model, different simulation techniques are used to provide orientation distributions of labeled side chains.

Here we present results on a model system, the subunits F and E from the archaeal RNA polymerase[1], which form a stable heterodimer (F/E).[2] We focus on a comparative evaluation of experimental and simulated distance distributions for DEER and FRET. Cysteine mutants within F/E were labeled for DEER- or FRET experiments. Accordingly, simulated orientation distributions of the labeled side chains are calculated using conformational sampling[3] by molecular dynamics- (MD) as well as by Monte Carlo (MC) simulations and, for the spin labels, by a rotamer library based approach[4]. This versatile approach allows not only for comparison of experimentally determined inter-label distances (or distributions) to the simulated orientation distributions for both EPR- & FRET labels, but additionally, it allows for cross validation of the different simulation techniques and protocols. For spin labels the comparison shows reasonable agreement, especially in terms of mean distances, while for FRET labels simulations prove to be possible yet more challenging.

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CT243

Promising new modules for data processing via the AUREMOL-SSA/ICA and protein quality control

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AUREMOL[1] is a software tool for protein structure determination from NMR data, which speeds up and automates the whole process. It is based on a new approach called "top down" where all given structural parameters (statistical databases, homology modeling, known torsions angles, known chemical shifts, etc.) provide a "starting structure" of the investigated protein. AUREMOL will then use experimental data to refine this structure and to calculate the assignment in an iterative process. The benefit of this approach is that the whole sequential assignment is not needed anymore.

The new tools AUREMOL-SSA/ALS [2, 3] and AUREMOL-ICA have been developed in order to automatically detect hidden resonances by the solvent. These fully automated procedures include the managing of digitally filtered, zero-filled data, the linear spline interpolation of the frequency domain base points and the phase correction consistent with respect to the group delay time shift due to the digital filtering. Once they have been applied the AUREMOL-QTA module allows comparing a set of spectra with the aim to quantify where and how much a molecule differs with respect to a target one, both in the spectra and in the three-dimensional structure.

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Hs244

A combined high-field ENDOR/DFT approach for proton-coupled electron transfer investigation in *E.coli* ribonucleotide reductase

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E.coli class I ribonucleotide reductase (RNR) is to date one of the most extensive studied enzymes in the field of EPR spectroscopy and quantum chemistry. Although many mechanistic aspects have been investigated, very less is known about the proposed proton-coupled electron transfer (PCET) process which occurs over > 35Å. The concomitant motions of electron and proton between the two homodimeric subunits α2 and β2 involve three conserved tyrosines: β2-Y_{356}, α2-Y_{730} and α2-Y_{731}. We incorporated the radical spin probe NH_2Y at position 730 and characterized it through multifrequency EPR spectroscopy, a precondition for the observation of a hydrogen-bonding network through ENDOR spectroscopy near α2-$NH_2Y_{730}^{\bullet}$ [1] [2]. Our high-field ENDOR experiments at 94 GHz and protein-like DFT cluster calculations support the model of a hydrogen atom transfer (HAT) mechanism to occur between the essential residues Y_{731}, NH_2Y_{730} (Y_{730}) and C_{439} with a nearby water molecule at the NH_2Y_{730} (Y_{730}) side which is potentially controlling the HAT transfer rates during nucleotide turnover.

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Hs245

The proton environment of the high-stability menasemiquinone intermediate in *E. coli* nitrate reductase A as resolved by pulsed EPR

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E. coli nitrate reductase A (NarGHI) is a suitable bacterial model enzyme for studying the reactivity of respiratory complexes towards membrane quinols. While no quinol is resolved in the available crystallographic structures of NarGHI, an EPR-detectable semiquinone intermediate (SQ_D) is very highly stabilized at the quinol oxidation site (Q_D) of the enzyme (1). HYSCORE spectroscopy at 3 and 9 GHz was used to show that the catalytic intermediate magnetically interacts with a ^{14}N nucleus assigned to the heme b_D axial ligand His66 of NarGHI (2,3). Recently, we showed that a cardiolipin molecule is tightly associated to the enzyme and is essential for quinol substrate fixation at the Q_D site (4). Herein, the radical proton environment is probed by ^1H-HYSCORE spectroscopy. Direct evidence for H-bonding to the radical is provided by combining H_2O/D_2O exchange experiments with 35 GHz Mims ENDOR experiments. Analysis of the spectroscopic data reveals an original binding mode of the radical at the Q_D site that might explain its peculiar properties.

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Hs246

Pulsed EPR investigations of model complexes of the [FeFe] hydrogenase active site: bulky bridges vs bulky ligands

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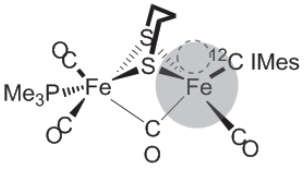

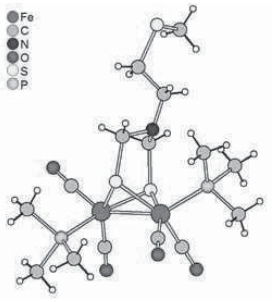

We present a detailed pulse EPR investigation on two compounds structurally resembling the H_{ox} state of the H-cluster in [FeFe] hydrogenases. These enzymes catalyze the reversible oxidation of molecular hydrogen. In addition, we studied a mimic of the CO inhibited state (H_{ox}-CO). This complex contains an *azadithiolate*-bridging ligand which is believed to be present also in the native system. EPR, ENDOR and HYSCORE experiments as well as DFT calculations show that both H_{ox}-like complexes have similar spin density distributions over the 2-iron core which differs drastically from that of the H_{ox}-CO like complex. The magnetic coupling parameters of the amino nitrogen in the azadithiolate bridge are quite similar to those observed for the native system in the H_{ox} state [1,2]. The confirmation of this amino function in the bridge is of great relevance to the proposed mechanism of [FeFe] hydrogenases since it may act as proton donor/acceptor.

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Hs247

Hydrogen-Bridged Nitroxide Spin Probe Model

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In EPR spectroscopy nitroxide molecules play an important role to probe structure and dynamics of nano scale systems in biology and material science. The basis for using the nitroxides as probes is that the spin density distribution in the molecule encoded in the g- and hyperfine-tensors reflect properties like proticity and polarity of the environment. Also the study of more specific interactions of the nitroxide molecule with other nearby molecules is of interest as they can affect the conformation and mobility of the probe. In this context, the possibilities of performing theoretical computations to find out the geometry and spin density distribution of nitroxides and the influence of the environment on them is very attractive, but it has to be carefully checked against experimental results.

In this contribution we present the theoretical and experimental result of geometry and spin distribution of di-tert-butyl-nitroxide (DTBN) in methanol. This simple system was chosen to focus on the most significant g- and hyperfine-tensors. The DTBN model was calculated with a hydrogen bridge in different geometries to the nitroxide by the ORCA software package, using an HF/MP2 approach and the COSMO solvation model. Multifrequency cw EPR measurements were done at high and low temperatures. ESEEM pulse techniques were done in X- and Q-band on DTBN in protonated and deuterated solvents to obtain the g-tensor, the intramolecular A-tensors of ^{13}C, ^{14}N and ^{1}H and the hydrogen bridged ^{1}H. The A- and Q-tensors of the ^{1}H and ^{2}H of the solvent reveal the geometry and length of the hydrogen bridge.

IM248

CPMG echo amplitudes with arbitrary refocusing angle: explicit expressions, asymptotic behaviour, approximations

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Carr-Purcell-Meiboom-Gill pulse sequence with arbitrary excitation and refocusing angles and resonance offset of RF pulses is considered. Exact explicit analytical formula for echo magnetization amplitudes was obtained. The echo-amplitudes are expressed in terms of Legendre polynomials. The dependence of asymptotic behavior of echoes on RF pulse refocusing angle was studied, analytical approximation for echoes was also obtained. It was shown that at refocusing angle $\alpha \neq \pi$ the echo amplitudes decay is defined not only by spin-spin relaxation time T_2 as usually but also spin-lattice relaxation time T_1 and the refocusing angle. Accuracy of assymptotics and approximations were tested by comparison with exactly calculated echo amplitudes.

IM249

Correlation of "Trumpet shaped" dilated cerebral aqueduct and cerebrospinal fluid (CSF) flow profiles in patients with highly elevated CSF flow

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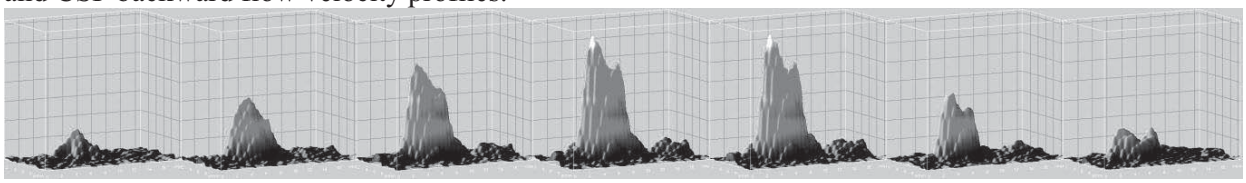
Hydrocephalus is an abnormal buildup of CSF in the ventricles of the brain. The fluid is often under increased pressure which can damage the brain. The aim of our study was to explore the relationship between aqueductal flow measurements of cerebrospinal fluid (CSF) and changes of the anatomical configuration of the cerebral aqueduct (AC) in patients with communicating hydrocehphalus.

We were interested in the cause of the dilatation of the AC so we analyzed 3D velocity plots of CSF measured at the AC over a heart cycle. We could demonstrate that there is a significant correlation between highly elevated absolute stroke volume of CSF and dilated, sometimes even "trumpet shaped" AC conformation. We could demonstrate that there is a significant difference in CSF velocity plots of normal and dilated AC. In cases of dilates AC we found differences in CSF forward and CSF backward flow velocity profiles.



IM250

Characterizing Hydrogel Microcapsules by NMR Microscopy

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Microcapsules consisting of natural polysaccharide hydrogels such as alginates or pectinates have a large potential as carriers for liquid drugs. If a controlled and site specific release of the encapsulated drugs is desired, they must be stored sufficiently long under alternating environmental conditions. We optimize the capsules with respect to the retention time of the encapsulated drugs and the chemical stability under gastrointestinal conditions. To achieve this, we modify the capsules by applying external coatings or additives to the hydrogel membrane.

We use NMR microscopy for the characterization of these microcapsules. With this technique, we can visualize differences in the structure of the capsules depending on their preparation and environment. The structure of the capsules is analyzed on a microscopic scale by obtaining two- or three-dimensional NMR images of the capsules, which allows measuring the thickness of the capsule membranes, the size of the capsules, the mechanical composition, etc. Parameter mapping of relaxation times as well as studies of diffusional processes also allows analyzing nanoscopic structure and dynamics. We can show that the hydrogels of the capsules have porous structures with poresizes of some nanometers, which vary with different preparations, modifications and environments.

A comparison of NMR imaging experiments with spectroscopic drug-release measurements shows how the structure influences the permeability and that pectinate capsules with a thin coating of shellac provide an excellent entrapment of cancer preventive anthocyanins, which were used as a model drug.

Leick et al., *Physical chemistry chemical physics,* 13, 2765-2773, (**2011**)

IM251

Biophysics of articular cartilage: What we can learn from spin relaxation?

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Articular cartilage (AC) is a connective tissue covering the articulating surfaces of long bones. Its structural basis is a biopolymeric extracellular matrix (ECM) based on collagen and proteoglycans. MRI provides a range of techniques for characterisation of the cartilage ECM and shows great promise for clinical diagnosis of osteoarthritis. Our research aims to develop MRI methodologies for studying the link between the ECM organisation and load carriage in cartilage.

Diffusion-Tensor Imaging enables direct measurement of the predominant collagen alignment in AC, as well as reorganisation of collagen fibres under mechanical load.[1,2] An alternative and potentially faster approach to mapping the collagen architecture is based on the anisotropy of the water ^{1}H transverse relaxation rate (R_2). R_2 depends on the predominant orientation of collagen fibres relative to $\mathbf{B}_0$ and usually exhibits the "magic-angle" dependence:[3] $R_2 = A + B(1 - 3\cos^2\theta)^2$. An added advantage of R_2 is its capacity to characterise compression-induced changes in the water content in different zones of cartilage. We discuss the use of R_2 mapping for studying load carriage in articular cartilage. Initial results show that the transitional and the radial collagen alignment zones participate in load processing in different ways: transitional zone absorbs much of the applied load at low loads (1-2 bar); while the radial zone significantly contributes to load processing at loads >3 bar.

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IM252

Magnetic resonance imaging study of the influence of incorporated drug on xanthan gel thickness

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Hydrophilic matrix tablets are widely used for controlled delivery of drugs. On contact with water or body fluids the outer surface of these tablets hydrate and swells, forming a hydrogel coat around the dry central core. The gel layer regulates the penetration of body fluids into the tablet and therefore limits the dissolution and diffusion of the incorporated drug. The drug release from such tablets is a very complex process and is to a great deal influenced by the gel layer structures [1,2].

Xanthan is an anionic poly-electrolyte that forms different complex structures depending on environmental conditions. A combination of different magnetic resonance methods [3] was used to study the influence of incorporated drug on xanthan swelling dynamics in media differing in pH and ionic strength. The drug has no effect on medium penetration into the tablet and on the position of swelling front (the interface between the glassy and rubbery states). The position of the erosion front (the interface between the swollen tablet and the bulk medium), on the other hand, is strongly dependent on the amount of the incorporated drug. The xanthan tablets containing higher amount of the incorporated drug forming the thinner gel layer.

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IM253

Specificity of Magnetization Transfer Parameters in Alzheimer Disease

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The protons in biological systems can be described as existing in two pools: the free (f) and the restricted (r) protons. It has been shown (1), that the restricted pool can be adequately described by modified Bloch equations. Patients with Alzheimer disease and Mild Cognitive Impairment (MCI) and healthy controls were compared with respect to model based magnetization transfer parameters (2) F (fractional part of the restricted pool), $T2_r$ (T2 restricted pool) and exchange parameters k_r and k_f and the relaxation parameters of the free pool (T1,T2). MT-Simulations and MT-spectra were used to investigate the specificity of the MT-parameters depending on the tissue compartmentalization. The comparisons of the simulations and experiments show that already small changes of the tissue compartmentalization resp. the presence of specific macromolecules have specifiable effects on the MT-parameters ($F,T2_r,k_f,k_r$)! This means that far from using the MT-technique for the presaturation of stationary tissue in angiography or in terms of the MT-ratio, the model based MT parameters are potentially able to classify healthy and pathological brain tissue. Further investigations will clarify to which degree model based MT can be used as a predictor for neurodegeneration.

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IM254

Assessment of Caries Lesions by ADC Mapping and T1-w MRI

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MRI provides excellent contrast between different soft tissues and is therefore an appropriate tool for assessment of various lesions including caries [1]. In this study feasibility of high-resolution MRI to assess caries lesions was tested.

The study was performed on 26 extracted human teeth of different dentine-pulp complex responses according to ICDAS scores. The teeth were imaged by 3D T1-weighted spin-echo and by diffusion weighted (DW) high-resolution MRI sequences. T1-weighted images were used to visualize the pulp chamber anatomy, to locate caries lesion and to determine the distance between the lesion and the pulp, while DW images were used to calculate the corresponding apparent diffusion coefficient (ADC) maps, which were then used to quantify responses of dental-pulp complexes. Measured ADC values of the responses were then compared with the corresponding ICDAS scores of the teeth [2].

Results of the study showed that average ADC values of the responses correlate with the corresponding ICDAS scores. ADC mapping, which is an instrumental method, may be an interesting and more precise alternative to ICDAS scoring, which lacks accuracy due to subjectivity of the observes. We found that ADC of $1.0 \cdot 10^{-9}$ m^2/s may represent a reasonable threshold value of the dentin-pulp complex below which stomatological treatment of caries is needed.

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IM255

Study of pulmonary emboli by diffusion spectroscopy MRI

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Diffusion spectroscopy MRI can be used to non-invasively obtain quantitative structural information of a biological tissue, which depends on its micro-structural properties. A promising method for studying diffusion properties of matter is diffusion-weighted imaging (DWI) based on the use of oscillating gradient spin-echo (OGSE) method [1-3]. Contrary to the well-established pulsed gradient spin-echo (PGSE) method, that is used primarily to determine the long time scale (zero frequency) apparent diffusion coefficient (ADC), the OGSE method enables studies of diffusion in much wider time scales of molecular motions, i.e., in a broader frequency range, and is therefore a convenient method for studying structural properties of heterogeneous samples.

In this study OGSE DWI was employed to study structural properties of pulmonary emboli. The emboli were imaged by OGSE DWI in a range of frequencies of oscillating gradients from 30-350 Hz. The corresponding high-resolution MR images had a frequency dependent signal attenuation that was varying quite substantially between different structural regions of the emboli. The differences were increasing with an increasing frequency of oscillating gradients.

Characterization of pulmonary emboli structural properties may be important in both clinical and research applications. Diffusion spectroscopy MRI can also help assessing a susceptibility of different blood clot regions to thrombolysis, which has an important clinical relevance.

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IM256

Redox Imaging of Lung Using Overhauser-MRI System and its Application to Lung Metastasis Model Mice

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Overhauser-enhanced MRI (OMRI) is a double resonance technique that uses the presence of paramagnetic agents such as nitroxyl radical to enhance the signal intensity from nuclear spins by means of a process known as dynamic nuclear polarization (DNP) or Overhauser effect. Recently many paper suggested that pulmonary disease such as COPD (chronic obstructive pulmonary disease), pneumonitis and lung cancer is related in breakdown of redox status in vitro study. In general, lung is one of the difficult organs to visualize by MRI due to low proton density by structure of alveoli. In contrast, our previous study suggested it has a possibility to obtain enhanced image by Overhauser enhancement using OMRI and nitorxyl radicals. In this study, we tested that possibility of lung imaging using OMRI and nitroxyl radicals and it application to lung metastasis model mice. Using newly developed surface coil for high sensitive detection of lung area, Overhauser-enhanced image was obtained. It was determined that image intensity of upper abdomen area by OMRI was derived from lung determined by imaging of isolated lung. In addition, redox status of the lung in mice 10 days after intravenously administration of B16F10 cell was clearly changed and lung metastasis was observed by histological observations.

IM257

Dynamics and Stability of Hydrogel Microcapsules

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Microcapsules consisting of natural polysaccharides like pectinates or alginates have a large potential for controlled delivery and release of liquid drugs in the intestine. The functionality of these capsules depends crucially on their stability in different environments. They have to be stable in the acidic stomach and must dissolve in the basic intestine for releasing the encapsulated drugs. To achieve this, we chemically modify the capsules by applying external coatings or additives to the hydrogel membrane.

NMR microscopy gives us the opportunity to characterize the geometry and the chemical stability of the microcapsules. By taking NMR images of capsules in media representing gastrointestinal conditions, we can show that shellac-coated pectinate capsules are stable under gastric conditions for many hours, but they disintegrate within a few minutes in the intestine. The stability of the capsules in different environments is correlated to their microscopic structure. Diffusion tensor images provide additional information about the structure of the polysaccharide hydrogels.

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IM258

Assessment of blood clot retraction by magnetization transfer MRI

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Magnetization transfer MRI (MT-MRI) can be used to obtain quantitative structural information of a biological tissue. As T_2 relaxation processes of the less mobile protons associated with macromolecules and membranes are too short to be directly seen by MRI (T_2 < 1 ms), the coupling between the macromolecular protons and the mobile protons associated with liquid water allows to measure the MR relaxation properties of the macromolecular pool through exchange processes. This can be done using offset RF irradiation prior to the imaging part of a MRI sequence [1]. MT-MRI provides a unique MRI contrast that can be therefore used to characterize tissue properties in a variety of clinical applications [2].

In this study MT-MRI was employed to study structural properties of model blood clots and of pulmonary emboli. The clots and emboli were irradiated by a sinc-like RF pulse with duration of 250 ms and with various offset frequencies in a range from 10 Hz to 100 kHz. This was followed by the fast single-shot RARE imaging sequence. Corresponding images, in which signal attenuation substantially increased with a decreasing offset frequency due to the exchange processes as well as due to the direct saturation of magnetization, were analyzed for contrast differences between the regions containing macromolecular and mobile protons as well as for their relaxation parameters.

As MT-MRI enhances discrimination of structural changes in biological tissues, this imaging technique may be clinically important for optimizing blood clot dissolution protocols.

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IM259

MRI of Hyperpolarized Protons Against a Large ^{1}H Background

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A major challenge in molecular imaging is the detection of tiny amounts of interesting molecules. In magnetic resonance imaging (MRI) their signals are usually concealed by the large background signal of the body. Hyperpolarization can overcome this issue by increasing the nuclear magnetic resonance (NMR) signals few orders of magnitude. For the most widely used NMR and MRI nucleus ^{1}H, however, this strategy is limited. The enormous number of thermally polarized protons in the body screens the small amount of hyperpolarized ones. In this work we present a simple approach dealing with parahydrogenated molecules (i.e., molecules generated in a reaction with Parahydrogen[1,2]), leading to a ^{1}H NMR/MRI contrast. It makes use of different temporal evolution of the antiphase signal of hyperpolarized ^{1}H generated with PHIP[3,4] compared to that of the thermal signal of the background. By choosing an optimal delay time for detection of the PHIP antiphase signals this new contrast can be simply implemented in any MRI pulse sequence.

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IM260

Redox mapping using Overhauser-enhanced MRI in colons of mice with DSS-induced colitis

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Ulcerative colitis is an inflammatory bowel disease characterized by acute inflammation, ulceration, and bleeding of the colonic mucosa, although its cause remains unknown. ROS are reportedly implicated as mediators of mucosal injury, and we reported direct evidences of *in vivo* ROS generation in living body using *in vivo* ESR/spin probe technique[1]. Overhauser-enhanced MRI (OMRI) that can get the images of nitroxyl spin probe with different isotopes, ^{14}N and ^{15}N, was newly established[2]. In this study, we applied an OMRI/spin probe technique to DSS–induced colitis model and investigated the relation of the ROS generation and mucosal injury.

Colitis was induced in male ICR mice by giving their drinking water with 5% DSS for 7 days. When ^{14}N-MC-PROXYL, a high membrane-permeable probe, was administered into the rectum of the DSS-treated mice, the intensity decay rate was increased in the distal colon of day 3, and the enhancement was increased in the overall colon of day 7. On the other hand, ^{15}N-carboxy-PROXYL, a membrane-impermeable probe, caused the enhancement of intensity decay on day 7. The simultaneous administration of DMSO suppressed the enhanced signal decay of ^{14}N-MC-PROXYL. These findings suggest that the ROS generation occurred in of epithelial cells of distal colon and the ROS producing area was expanding to both in and out of epithelium of overall colon as colonic damage was developed.

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IM261

High field OMRI scanner for imaging in vivo redox status

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Overhauser enhanced MRI (OMRI) is a technique for imaging free radicals in animals based on the Overhauser effect. Under physiological condition, the half-life of intrinsic free radical is too short to be directly measured and reporter molecule, “spin probe” is used to observe in vivo functional information. The spin probe itself has unpaired electron and can be measured with OMRI. We have developed molecular imaging and simultaneous assessment of redox processes by using OMRI with ^{14}N- and ^{15}N- labeled aminoxyl probes with different distribution properties. We developed a prototype of OMRI scanner with transport-system, in which the sample object was transported between ESR to MR magnets, to achieve field cycling. The OMRI scanner consisted of two kind of resistive magnets, which were operated at 1.5 T and 20 mT for MR detection and ESR excitation, respectively. The physical resolution of the OMRI image for the phantom object was less than 0.2 mm, indicating that the OMRI with transport-system would be useful for imaging redox reaction at high MR detection field. The developed OMRI system would have a significant advantage for imaging in vivo functional information.

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IM262

In vivo monitoring of absorption kinetics in human skin by single-sided NMR

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This work reports the continuous monitoring of the ingress of water and beauty cream into human skin. The measurements were performed with a single-sided NMR sensor generating a reduced but highly uniform static gradient, which allows recording in vivo skin profiles of up to two millimetres depth in experimental times of 30 s with up to 25 micrometers spatial resolution. With this performance absorption kinetics of water and cream in the skin tissue were measured. Characteristic times for the absorption of each skin layers are determined, and also changes in the thickness of the different layers during the exposition period can be quantified. As in the presence of a low gradient the relaxation time T_2 can be measured without strong diffusion contamination, it was also possible to spatially resolve both T_2 and diffusion coefficients along the depth direction. Then, by acquiring a CPMG echo train and combining a Fourier Transformation (FT) along the echo acquisition time with an Inverse Laplace Transform (ILT) along the echo decay time, the relaxation time and diffusion coefficients distribution at the different layers of skin were resolved.

IM263

CSI and Velocimetry of Enclosed Flames Using Hyperpolarized ^{129}Xe

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There is considerable interest in new methods to probe the chemistry and thermodynamics of enclosed combustion processes. Hyperpolarized (HP) ^{129}Xe gas MRI enables sensitive and noninvasive analysis of chemical composition and velocity[1] within opaque samples. By taking advantage of the both the temperature sensitivity of the chemical shift[2] as well as the inertness of ^{129}Xe, temperature and velocity distribution images of an enclosed flame can be acquired. Previous attempts to analyze high-temperature combustion reactions using NMR employed 2D-EXSY[2] of HP ^{129}Xe and SPRITE[3] proton imaging. In the present study, a homebuilt, water-cooled probe was fabricated, including electronics that are able to withstand high temperatures (> 2000 K). HP xenon is premixed with a combustible gas and meets with pure oxygen at an enclosed diffusion flame centered within a 15 (ID) × 25 (H) mm insulated saddle coil. A spin-echo pulse sequence with velocity and acceleration compensated phase encodes, is used to generate temperature-weighted, 3D chemical shift images (CSI) as well as 3D velocity maps of the flame region. This technique can be applied to studying confined combustion processes such as microturbine engines on MEMS devices.

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Cv264

Functional EPR and Proton-Electron Double-Resonance Imaging: *In Vivo* Application in Animal Model of Breast Cancer

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L-band electron paramagnetic resonance (EPR) approach for *in vivo* real-time assessment of tumor tissue extracellular pH (pH_e), redox and intracellular glutathione (GSH) based on the application of specially designed paramagnetic probes was developed. In addition to spectroscopic measurements, pH_e mapping was performed using recently proposed functional proton-electron double-resonance imaging (PEDRI)[1,2] and extracellular-targeted nitroxide pH probe allowing for MRI high quality spatial resolution and short acquisition time (≈10 s). The tissue parameters were monitored in PyMT mice bearing breast cancer tumors during treatment with Granulocyte Macrophage Colony-Stimulating Factor (GM-CSF). It was observed that tumor pH_e is about 0.4 pH units lower than in normal mammary gland tissue. Treatment with GM-CSF decreased the value of pH_e by 0.2 - 0.3 units compared with PBS control treatment. Tumor tissue reducing capacity and GSH were elevated compared with normal mammary gland tissue. GM-CSF treatment resulted in a decrease of the tumor tissue reducing capacity and GSH content. The PEDRI pH mapping supports probe localization in mammary gland/tumor tissues, shows high heterogeneity of tumor tissue pH_e and a difference of about 0.4 pH units between average pH_e values in tumor and normal mammary gland. *In summary*, the developed multifunctional magnetic resonance-based approaches allow for *in vivo*, noninvasive tissue pH_e, redox and GSH content monitoring during investigation of various therapeutic strategies for solid tumors. *This work was partly supported by NIH grants CA132068 and EB03519.*

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Cv265

NMR in Global Change Research

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Many burning questions in environmental sciences require input of molecular information. We show how NMR can make unique contributions, by bridging from lab studies to studies of natural ecosystems, or by linking short-term experiments to long-term responses.

The land biosphere currently absorbs a significant fraction of human CO_2 emissions, dampening Climate Change. However, this net CO_2 sink is a small difference of large fluxes of C uptake and release. We use solids and liquids NMR at natural abundance and with isotope labels to study C turnover in soils. We show that residual liquid water in frozen soils determines biological activity during the winter season, which so far could not be modeled[1]. Contrary to previous conclusions, microbial metabolism under frozen conditions is very similar to unfrozen conditions[2].

Besides defining current biogeochemical fluxes, forecasting them on time scales of centuries relevant for Climate Change is a key challenge. This challenge can be met by NMR in retrospective studies, exploiting NMR's unique ability to measure intramolecular isotope distributions. Lab experiments allow interpreting these distributions in terms of metabolic regulation[3]. Applied to historic or ancient plant samples, isotope distributions can track metabolic changes on otherwise inaccessible time scales. Examples include acclimation to increasing CO_2 or changing climate [4].

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Cv266

Evaluation of Spin Labels for In-Cell EPR

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Spin-label electron paramagnetic resonance spectroscopy has become a powerful and useful tool for studying structure and dynamics of biomacromolecules. However, utilizing these methods for in-cell studies is hampered by reduction of the nitroxide spin labels and thus short half-lives in the cellular environment. Consequently, reduction kinetics of two structurally different nitroxides was investigated in cell extracts of *Xenopus laevis* oocytes using rapid-scan cw-experiments at X-band. The data indicate an enzymatic origin of the reduction process and were analyzed according to the Michaelis-Menten model. The five member heterocyclic ring nitroxide PCA (3-carboxy-2,2,5,5-tetramethylpyrrolidinyl-1-oxy) under investigation features much higher stability against intracellular reduction than the six member ring analogue TOAC (2,2,6,6-tetramethylpiperidine-N-oxyl-4-amino-4-carboxilic acid) and is therefore a suitable spin-label type for *in-cell* EPR[1-3].

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Cv267

In situ NMR analysis of complex incubation media of *Bacillus* sp 3B6 on different saccharides.

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Recent research has shown that microorganisms are present in atmospheric waters and they participate on degradation process of organic compounds present there as biocatalysts. To understand better a role of bacteria in atmospheric chemistry the knowledge about their metabolism is indispensable. Metabolism of *Bacillus* sp. 3B6, isolated from cloud water collected in free troposphere of Puy de Dôme, France, was incubated on different sugars. Our previous study has shown that on sucrose *Bacillus* sp. 3B6 is producing fructans: levan polysaccharide and FOSs oligosaccharides of levan and inulin type[1]. Cellobionic acid was produced by *Bacillus* sp. 3B6 incubated on cellobiose[2]. Here we present results of „*in situ*“ NMR analyses of complex incubation media (IM) obtained during incubations of this bacterium on different saccharides with a special attention devoted to glucose substrate. Comparison of IM spectral patterns and obtained NMR data enabled an identification of more low and high molecular mass components in mixtures. Glycoprotein and polyhydroxybutyrate (PHB) were identified as high molecular mass exopolymeric substances produced by this bacterium.

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Cv268

Unusual variety of fructans produced by *Bacillus* sp. 3B6, a bacterium isolated from cloud water.

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Bacillus sp. 3B6, isolated from cloud water collected in free troposphere of Puy de Dôme, France, was incubated on sucrose for exopolysaccharide production. Dialysis of obtained mixture afforded dialyzate (DIM) and retentate (RIM). Both were separated by size exclusion chromatography and analysed by NMR spectroscopy. RIM afforded eight fractions: levan exopolysaccharide (EPS), fructooligosaccharides (FOSs) of levan and inulin types with different degree of polymerization (dp 3-6). Difructose anhydride DFA IV was present in a disaccharide fraction. In higher molecular mass DIM fractions 1–kestose, 6–kestose and neokestose, nystose and FOSs of levan type (2,6-βFruf) were identified. Identification of levan 2,6–βFruf and inulin 1,2–βFruf type oligosaccharides in the incubation medium suggests both levansucrase and inulosucrase enzymes activity in *Bacillus* sp. 3B6.

Identification of levan 2,6–βFru*f* and inulin 1,2–βFru*f* type oligosaccharides in the incubation medium suggests both levansucrase and inulosucrase enzymes activity in *Bacillus* sp. 3B6.

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Cv269

Endotoxin-induced alterations in renal oxygen consumption: an ESR oximetry study

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The kidney, one of the most injured organs in critically ill patients, is faced with unique challenges for molecular oxygen regulation**.** Recent research activities in the pathophysiological mechanism of acute renal injury (ARI) emphasize the central role of hemodynamic and inflammatory events in septic shock[1]. More particularly, two mechanisms have been postulated to explain the inability of the injured kidney to extract oxygen: tissue hypoxia and cellular energetic metabolism dysfunction[2].

The present investigation was carried out to characterize the effects of bacterial endotoxin on the oxygen consumption of human tubular proximal cell line (PTC) by using the very sensitive electron spin resonance oximetry method[3]. Oxygen consumption was shown to decrease quite markedly in cells treated with lipopolysaccharide (LPS) from 16.52 ± 2.51 (n=6) in the control group to: 12.94 ± 2.62 (n=3) in the short incubation time group (6h) and 10.86 ± 2.20 (n=3) in the long incubation time group (18h).

This decrease in oxygen consumption in renal cells after LPS challenge may be in relation with a metabolic down-regulation. Renal energetic are deranged in sepsis not just because O_2 delivery is impaired but perhaps also because the ability of cells to utilize available O_2 is compromised.

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Cv270

MOUSE meets HOG – Online-Investigation on a hog's small intestine with the *Profile* NMR-MOUSE®

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During a posed aorta surgery on a test animal (hog), we tested the use of the *Profile* NMR-MOUSE® in an operating room. We observed a changing diffusion coefficient during the surgery time, which is attended by the degeneration of the small intestine. This result is encouraging and represents a significant step towards qualifying mobile NMR systems for medical applications. During surgery on the abdominal or descending thoracic aorta the use of a heart-lung machine is unavoidable. For the cardiovascular system as well as for the various organs, this exposure is critical and needs to be as short as possible. The microcirculation in the small intestine is a parameter which can be observed with mobile NMR by measuring the diffusion coefficient in a defined layer of the tissue. Non-invasive online monitoring would allow giving a more precise prediction of the point-in-time for the switch back to the heart-lung machine. The three regions of intestinal villi (mucosa), the musculature, and the connective tissue can be discerned by a profile measurement taken with the *Profile* NMR MOUSE®. T_2 and diffusion measurements were performed as well as profiles on living and dead tissue of a hog's small intestine. We obtained significant results for the dead and the living hog, as well as for the online-monitoring of the diffusion coefficient.

Cv271

Towards High-Resolution in ^{1}H-NMR Spectroscopy of Glioma Cells

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High-resolution ^{1}H-NMR spectroscopy of whole cells constitutes the missing link between pure *in vitro* NMR spectroscopy of cell extracts and *in vivo* MR spectroscopy of animal models and, at clinical MRI scanners, of humans. Taking advantage of both methods, purified and cultured cells, e.g. glioblastoma cells or neural stem cells, can be investigated under *in vitro* conditions regarding magnetic field strength and sensitivity of cryo probes, and additionally, structural and dynamic subcellular information is maintained and ultimatively, can be transferred to *in vivo*.

However, NMR spectroscopy of whole cells faces the spectroscopist with substantial challenges. In this contribution, we present findings regarding cell immobilization, shimming artifacts, cell density-dependent line broadenings, intra- and extracellular compartments, NMR-visible intracellular macromolecules, temperature effects, and long-term stability. Regarding the latter, we describe a perfusion setup that enables a continuous supply of cells with oxygenated cell culture medium during the measurement within the 800 MHz Bruker Avance NMR spectrometer.

Based on our former investigations of stem cell-specific biomarkers in NMR spectra of neural stem cells [1] and of brain tumor-initiating cells [2], a metabolic and structural profiling (biomarker screening) *in vitro* will lead to both a better understanding of the fundamentals of stem/tumor cell metabolism and to a possible detection of neurogenesis and tumorigenesis non-invasively in humans.

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Cv272

In-cell NMR spectroscopy of nucleic acids inside *Xenopus laevis* oocytes

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In-cell NMR spectroscopy of proteins in different cellular environments is a well-established technique that, however, has not been applied to nucleic acids (NA) so far. Here, we show that isotopically labeled DNA and RNA can be observed inside the eukaryotic environment of *Xenopus laevis* oocytes by in-cell NMR spectroscopy (1). One limiting factor for the observation of nucleic acids in *Xenopus* oocytes is their reduced stability. We demonstrate that chemical modification of DNA and RNA can protect them from degradation and can significantly enhance their lifetime. Finally, we show that the imino region of the NMR spectrum is devoid of any oocyte background signals enabling the detection even of isotopically nonlabeled molecules. As a first application, we are investigating human telomeric repeat sequences to address the biologically relevant conformation(s) in vivo. In addition, a 22 kDa engineered 2'-deoxy-Guanine Riboswitch was characterized upon its binding behavior under cellular conditions.

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Cv273

In vivo high pressure ^{1}H NMR studies on oocytes of Xenopus laevis

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Oocytes of the African Clawed Frog Xenopus laevis are an excellent candidate for in vivo high pressure NMR studies. This is due to their relative good resistance against mechanical stress compared to other living cells and on the other hand their quite large cell size.

We studied the oocytes in the pressure range from ambient pressure to 180 MPa by ^{1}H NMR spectroscopy. The strongest signals come from the lipids contained in the oocytes. The signals of the lipids decrease with increasing pressure where the signals assigned to different groups behave differently. Signals due to protons in unsaturated fatty acids show a smaller pressure effect than signal arising from saturated fatty acids. The T_2-values measured by a Carr-Purcell sequence are only weakly dependent on pressure. The data can be explained by a pressure dependent phase transition in the lipid droplets. The pressure induced effects observed by NMR spectroscopy are completely reversible up to a pressure of 150 MPa, which agrees well with the vitality measurements on pressure treated cells by patch-clamp experiments on the membrane.

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Cv274

Cellular Oxidative Stress and alpha-Synuclein Aggregation

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Aggregation of human alpha-Synuclein (AS) into amyloid fibrils has been linked to Parkinson's disease (PD). Under isolated *in vitro* conditions, AS forms fibrils via a nucleation-dependent mechanism that includes an initial association of AS monomers into toxic oligomeric species ("on-pathway" aggregates). In cellular environments, with many other components present, a number of additional routes to aggregation are possible, several of which involve "off-pathway" species that do not form "classical" amyloid fibrils (1).

Here, we employ a set of biophysical tools, including high-resolution NMR spectroscopy, to characterize Cytochrome *c* (Cyt *c*)-mediated high-molecular weight (HMW) aggregates of AS that form under conditions of cellular oxidative stress (2). Our data indicate that these aggregates inhibit amyloid formation and possess neuroprotective properties. The possible physiological roles of these HMW species during AS-medited neurodegeneration will be discussed.

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Cv275

EPR study of cross-talk between superoxide anion and nitric oxide in vasculature.

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Endothelial dysfunction (ED) is developed under metabolic diseases such as atherosclerosis, diabetes and aging as a disbalance between vessel vasodilation and vasoconstriction. This is tightly regulated by nitric oxide bioavailability. Potential reaction between endothelial nitric oxide and superoxide anions, generated by NOS, NOX enzymes and mitochondria, with formation of peroxynitrite is often discussed as an important source of ED. Cyclic hydroxylamine, 1-Hydroxy-3-methoxycarbomyl-2,2,5,5-tertramethylpyrrolidine (CM-H), was reported to penetrate into tissue, and can be oxidized by superoxide or peroxynitrite anions to form paramagnetic nitroxide (A_N = 16.1 G) reflecting redox status of environment. CM-H as redox active spin probe was used in numerous biological studies, but selectivity was not deeply analyzed in tissues. We studied effects of modulation by SOD and NO in vitro and in situ, using aortic rings, isolated from C57Bl/6 mice (12-14 weeks old). 1. We observed that in oxygenated solution spontaneous CM-H oxidation was increased and partly SOD-sensitive. 2. Added NO-donor (up to 0.5 µmol/L/min) did not change auto-oxidation; 3. Additional radical formation in aortic rings in situ at 37°C was significantly decreased by preincubation with free superoxide dismutase (SOD, 100U/mL) or L-NAME, 2 mM, (to 16+/-16%, N=10, and 72+/-12%, N=6, respectively, $P<0.05$), indicating superoxide anion and discrete peroxynitrite formation. This method can add useful information about site and mechanism of radical formation, and a good basis for evaluation of ED model in situ, but needs careful analysis of the data.

Cv276

In-cell NMR spectroscopy: proof of principle towards general applicability

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Different in-cell NMR applications have successfully demonstrated the feasibility of the method, with proteins like NmerA, GB1 or intrinsically unfolded proteins that show fast tumbling even in the crowded environment of a cell (1,2). For these proteins detection of 15N labelled backbone resonances is the method of choice. However, the majority of cytosolic proteins act in larger molecular complexes, which leads to significant signal broadening or even disappearance caused by the increase of the rotational correlation time. An alternative labelling scheme with 13C on methyl groups in a prokaryotic and eukaryotic approach has been established and allows the investigation of molecular complexes and proteins that tumble slow. Specifically labelled methyl groups of methonines, alanin and delta-methyl groups of isoleucine turned out to be a successful strategy. The investigation of proteins in even larger macromolecular complexes by in-cell NMR is up to now impossible. We will present a mutational concept to reduce nonspecific binding events of intracellular proteins to the protein under study indicating an auspicious tool within liquid-state in-cell NMR experiments.

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Cv277

NMR Detection of Cellular PTMs at Physiological Substrate Concentrations

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In order to measure different post-translational protein modifications (PTMs) at low cellular substrate concentrations, we developed a series of NMR protocols that take advantage of the joint application of fast NMR pulse sequences and the use of paramagnetic T1 relaxation enhancement (PRE) agents in proton- and carbon-detected NMR experiments.

We demonstrate how this combination of advanced sampling conditions enables NMR recordings of cellular PTM events at ultra-low substrate concentrations. This opens the road to in-cell NMR measurements of biological processes at physiological protein concentrations.

Cv278

Direct ^{13}C Detection Methods for In-Cell NMR

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We use ^{13}C detection methods to study the structural properties of the Intrinsically Disordered Protein (IDP) Alpha-Synuclein (AS), whose aggregation into amyloid fibrils has been strongly linked to the etiology of Parkinson's Disease (PD). Direct ^{13}C detection is particularly useful for IDPs where the lack of well-defined structures results in narrow ^{1}H chemical shift dispersions and unfavorable chemical exchange properties under conditions of macromolecular crowding and at physiological temperatures and pH. Employing 2D ^{1}H(start) CON and CACO NMR experiments (1) we analyzed what kind of effects different solution environments (crowding and temperature) and various post-translational protein modifications (phosphorylation and oxidation) exerted on the structural features of AS. These factors are known to modulate the aggregation behavior of AS and are thought to be involved in the onset of PD (2, 3).

Here, we present benchmark data for direct ^{13}C-detected *in vitro* NMR experiments on AS under different solution conditions and provide an outlook for in-cell NMR applications in different eukaryotic cell types.

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Cv279

Characterizing Cancer Kinase Networks by NMR

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Eukaryotic signaling pathways rely on protein kinases and protein phosphatases to reversibly regulate site-specific phosphorylation events on target proteins. Thereby, these enzymes relay divers cellular messages and function in decision-making processes. Defects in cellular signaling pathways are associated with several diseases, most notably cancer. In cancer, aberrant kinase activities may be the result of mutations (e.g. tyrosine kinases EGFR, Src, Abl), or of oncogenic signaling cascades (e.g. serine kinase MAPK activities downstream of oncogenic Ras). As such, it is important to characterize both specific cancer-associated kinase mutations and the resulting changes in cellular kinase activity profiles.

Using peptide-based Kinase Activity Reporters (KARs) and NMR readouts we observe multiple site-specific phosphorylation events in real time. Being able to monitor multiple KARs within the same NMR experiment enables us to delineate several kinase activities simultaneously.

Here we demonstrate the application of KARs to characterize different kinase activities in human cancer cell lines and describe the recent expansion of our KAR library to also include Tyrosine Kinase Reporters. We discuss potential applications of this NMR technique to develop novel kinase inhibitors.

Cv280

In-cell NMR Spectroscopy in Mammalian Cells

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Recent advances in high-resolution in-cell NMR spectroscopy include methods to directly analyze proteins inside mammalian cells (1,2). To this end, two different protocols for the intracellular delivery of isotope-labeled proteins have been reported.

Here, we compare both delivery schemes with yet another protocol for intracellular protein deposition: Protein transduction via mammalian cell electroporation. Our data indicate that protein electroporation outperforms the above delivery schemes in terms of cell viability, transduction efficiency, linearity of intracellular sample deposition, reproducibility, general applicability and ease of use. Following a detailed comparison of the three delivery methods, we present a selection of in-cell NMR spectra of proteins inside live mammalian cells.

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Cv281

Cellular Kinase Activity Profiling by NMR

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Protein kinases orchestrate cellular processes that range from cell-cell communication to proliferation-, differentiation-, and programmed cell death. Aberrant kinase activities, in turn, are implicated in a number of human diseases including most prominently, cancer. It is hence not surprising that quantitative methods for annotating cellular kinase activities are heavily sought after.

Here, we introduce the concept of cellular kinase activity profiling by NMR spectroscopy. Using peptide-based Kinase Activity Reporters (KARs), multiplexed experimental setups and time-resolved NMR readouts, we directly monitor multiple cellular kinase activities in parallel. This approach is especially useful to qualitatively assign active kinases in complex environments such as cell extracts and whole live cells, to quantitatively compare cellular kinase activities in terms of specific enzymatic units (U) and to evaluate kinase inhibitor specificities under native cellular conditions.

Cv282

NMR Analysis of Phosphorylation Cross-Talk on Histone H3

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N-terminal histone tails are heavily post-translationally modified (PTM). Phosphorylation in particular controls a variety of cellular processes that range from general transcription to DNA repair and chromosome condensation. Many of these modifications influence one another and thereby establish well-defined PTM states of dynamic regulatory crosstalk.

Using time-resolved NMR detection of protein phosphorylation (1), we demonstrate that Ser10 modification of histone H3 abolishes the ability of PKC and Chk1 to phosphorylate their respective substrate sites at Thr6 and Thr11, respectively. We further delineate that both kinases exhibit cross-reactivity at Ser10 and thereby establish auto-inhibitory feedback states on individual H3 substrate molecules. In contrast, phosphorylation of Thr6 and Thr11 does not affect modification of Ser10 by either Msk1, or AurB, which defines a mechanistic hierarchy for H3 phosphorylation events and may suggest a revised paradigm for copy-specific histone modifications on single nucleosomes.

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Ls283

Some Recent Developments in COSY-type NMR Experiments applied to quadrupolar nuclei.

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Two steps in the phase cycle are necessary in the COSY NMR experiment to achieve phase modulation of the signal during t_1 and eight steps in the phase cycle are needed in the DQF-COSY NMR experiment to achieve double quantum filtration and phase modulation of the signal during t_1. This means that one needs to make the calculation ten times if one wants to simulate the COSY and DQF-COSY NMR spectra of any spin system by using either the density matrix or the product operator formalism. By using the density matrix, it will be shown in this presentation that one needs to calculate only one set of coefficients to simulate both the COSY and the DQF-COSY NMR spectra of an AX system of any spins. This contributes to a significant simplification of the calculations compare to a full density matrix treatment. The expressions found in the case of an AX system of any spins are also valid for an AMX system of three spins I=7/2. Some theoretical simulations for such a system in the case of slow, intermediate and fast relaxing quadrupolar nuclei will be presented to illustrate the theory. Finally, a practical case involving the determination of $^{1}J(^{59}Co\text{-}^{59}Co)$ scalar coupling constants in $HFeCo_3(CO)_{10}(PCyH_2)(PPh_2[CH_2C(O)Ph])$ from the simulation of the DQF-COSY NMR spectrum will be presented.

Ls284

NMR chromatography using microemulsion systems

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NMR spectroscopy is an excellent tool for structural analysis of pure compounds. However, for mixtures it performs poorly because of overlapping signals. Diffusion ordered NMR spectroscopy (DOSY) can be used to separate the spectra of compounds with widely differing molecular weights, but the separation is usually insufficient. NMR 'chromatographic' methods have been developed to increase the diffusion separation but these usually introduced solids into the NMR sample that reduce resolution.

Using nanostructured dispersed media, such as microemulsions, eliminates the need for suspensions of solids and brings NMR chromatography into the mainstream of NMR analytical techniques. DOSY was used in this study to resolve spectra of mixtures with no increase in line-width as compared to regular solutions. Components of a mixture are differentially dissolved into the separate phases of the microemulsions. Several examples of previously reported microemulsions and those specifically developed for this purpose were used here. These include a fully dilutable microemulsion, a fluorinated microemulsion and a fully deuterated microemulsion.

Log (diffusion) difference enhancements of up to 1.7 orders of magnitude were observed for compounds that have similar diffusion rates in conventional solvents. Examples of commercial pharmaceutical drugs were also analyzed by this new technique and the spectra of up to six components were resolved from one sample.

Ls285

Sharpening your NOEs: NOESY with pure shift in both dimensions.

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The complexity of NMR spectra has a variety of sources of which homonuclear multiplet structure is arguably the most important. Suppressing the multiplet structure, by refocusing ^{1}H-^{1}H scalar couplings, gives a decoupled (or pure shift) spectrum with an increase in resolution of almost an order of magnitude[1,2]. The principle can be extended to multidimensional NMR as demonstrated in the F_2 decoupled pure shift TOCSY experiment. Furthermore covariance processing can carry over the resolution gain in F_2 to F_1, giving a doubly pure shifted 2D spectrum[3]. Here we apply the same principle to 2D NOESY, demonstrating that pure shift NMR combined with covariance processing is an excellent method of gaining resolution for through space correlations.

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Ls286

Suppression of sparse sampling artefacts in multidimensional NMR spectra

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Multidimensional NMR experiments play a crucial role in research on structure and dynamics of complex biomolecules. According to the *sampling theorem*, indirectly detected dimensions have to be sampled with a fixed interval $\Delta t=sw^{-1}$. Consequently, max. evolution times are severely limited already for 3D experiments, resulting in broad spectral lines. Poor resolution of conventionally recorded 4D spectra makes them impractical to use unless extensive folding is applied.

Spectral resolution and dimensionality can be increased by use of *non-uniform sampling* of evolution time space, which in turn requires the appropriate processing methods.

Here we present the efficient algorithm for removal of artifacts in randomly sampled 3D and 4D spectra. The algorithm [1] follows the concepts behind CLEAN algorithm, which was previously adapted to NMR by other authors [2,3]. It was shown that it preserves relative peak intensities [1,4], and is therefore suitable even for spectra containing resonances of various amplitude.

The experimental examples include 4D HCCH-TOCSY or 4D C,N-edited NOESY for both well-folded and partially disordered proteins. We show how (i) high dimensionality, (ii) high-resolution and (iii) high redundancy of information in 4D HCCH-TOCSY can be utilized to overcome spectral crowding and unambiguously identify a spin system of particular a.a. residues.

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Ls287

Heteronuclear Detection of STD-NMR Spectra

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Saturation Transfer Difference (STD) NMR is a powerful tool to investigate protein-substrate interactions. STD-NMR has the same limitations standard proton NMR has: signal overlap or a strong solvent (e.g. water) signal can cause problems. With biological samples water suppression is required. To overcome those problems choosing heteronuclear detection is one possible method.

For the detection of STD NMR in the heteronuclear channel saturation has to be accomplished in the proton channel. After the saturation transfer magnetization is transferred to the non-hydrogen channel employing a transfer step (e.g. INEPT). So far we have been able to demonstrate the transfer of the STD-NMR signals to carbon[1]. First results indicate that it is also possible to use other nuclei (e.g. ^{31}P). Here the INEPT transfer has to be replaced by other methods.

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Ls288

NMR Structural Insights into the Plasmodium Falciparum P2 Protein

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Plasmodium falciparum P2 protein is a part of the eukaryotic ribosomal stalk. Eukaryotic ribosomal stalk function in translation mechanism is still not clearly understood. The Ribosomal P proteins from plasmodium falciparum have been thought to play an important role in protein synthesis. Thus it becomes important to understand its structural and dynamics features to get insights into mechanistic aspects of protein-protein interactions. From various biophysical methods (NMR, CD and DLS), we found that P2 is a mostly alpha helical protein and its C-terminal is a highly unstructured random coil. P2 protein tends to form aggregates. Removal of the last 40 residues from the C-terminal did not reduce aggregation unlike what was observed in human P2. Multidimensional NMR investigations have been carried out on full length P2 (FL). HSQC of FL shows only 43 peaks instead of expected 137. After assigning these 43 peaks we found that they belong to residues from the C-terminal. This clearly indicates that the C-terminal is exposed and flexible while the N-terminal is buried inside the core. CSI of this domain shows some helical propensities in solution. Various experimental conditions were tried without success to obtain monomeric P2 in solution. Hexafluoroisopropanol (HFIP) caused dissociation but exchange broadening resulted vanishing of many peaks in the HSQC spectrum. Urea denaturation dissociates the aggregate and all the expected peaks are observed. This paves the way for step-wise investigation of folding and self-association of this protein.

Ls289

Quality Control Mapping of Olive Oil by ^{1}H NMR and ^{1}H Diffusion Ordered-Spectroscopy (DOSY) NMR

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Olive oil, a natural fruit juice, has gained popularity due to potential health benefits [1]. The characterization of geographical origin of olive oil, which is to be marketed under a protected designation of origin (PDI) label according to European Union (EU) regulations, becomes more important [2]. In addition, classification of different grades of olive oil for instance as virgin, extra virgin, refined or pure is also essential [1].

In the current study, our goal is to characterize olive oils from countries such as Turkey, Jordan and Palestine whose olive oils are not well studied with respect to Spanish and Italian olive oils. We employ NMR spectroscopy, a useful technique to distinguish among different grades and geographical origins of olive oils [1]. ^{1}H NMR has been used in determining both major and minor components of olive oils such as triglycerides and squalene [2]. ^{1}H DOSY NMR is based on the fact that higher molecular weight compounds diffuse more slowly [3].

Quantitative ^{1}H NMR analysis distinguishes the contents of olive oils (fractions of saturated and non-saturated acids) from different cities in Turkey, and to differentiate Turkish olive oils from the samples of Jordan and Palestine. ^{1}H DOSY NMR results revealed the existence of minor components in some olive oils whose geographical origins are different. This is also very useful in determining geographical origins of olive oils.

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Ls290

^{1}H and ^{13}C NMR Characterization of Pyrazole Carboxamide Derivatives

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There is a recent interest in pyrazole derivatives, well-known nitrogen containing heterocyclic compounds, because antimicrobial and antidepressant type biological activities [1]. In the current study, our aim is to characterize 4-benzoyl-1-(3-nitrophenyl)-5-phenyl-*N*-(4-sulfamoylphenyl)-1*H*-pyrazole-3-carboxamide (**E1**) and its derivatives by solution ^{1}H and ^{13}C NMR spectroscopy.

NMR spectroscopy is utilized to elucidate chemical structures, to detect hydrogen bonding interactions of **E5-E7** that play important role in the properties of chemicals [2], and to differentiate between possible tautomeric structures of pyrazole carboxamide derivatives.

The corresponding structure elucidation of the derivatives from the NMR spectra is done referring to our previous work [3]. Hydrogen bonding established via N-H···O was observed as downfield shift of isotropical chemical shifts in the ^{1}H and ^{13}C NMR spectra. The difference between the possible tautomeric structures is clarified by ^{1}H NMR spectra. It was also possible to distinguish the ^{13}C resonances of nitrogen-containing heterocyclic structures from the rest of the resonances in the ^{13}C NMR spectra [4].

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Ls291

Interaction of procaryotic reggie-like Proteins with structurally refined Nfed-homologues

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Proteins containing the stomatin/prohibitin/flotillin/HflK/C (SPFH) domain are found in a diverse multitude of species from prokaryotes to eukaryotes. Reggie/Flotillin belongs to the SPFH protein family and like the other family members it is found to be enriched in so called 'lipid raft' membrane microdomains, in diverse subcellular localizations. Despite their widespread occurrence and their importance in several processes (ion channel regulation, vesicle and protein trafficking, membrane–cytoskeletal coupling) only little is known about the exact function at the molecular level. Prokaryotic SPFH proteins are often co-expressed with an NfeD-like protein and there are several hints for an interaction of the two proteins.

In this work we refined the NMR structures of NfeD domains of three homologueous proteins (YuaF, YuaF-Bst and YqiJ) to support the hypothesis that they all share a common OB-fold structure. Therefore, residual dipolar couplings (RDCs) in different alignment media were measured in order to arrive at high-quality cross-validated 3D structures. Furthermore, initial interaction studies between the NfeD protein YuaF and its Reggie-like partner protein YuaG will be presented.

Ls292

Alignment of small and medium sized molecules induced by strong magnetic fields.

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Molecules with anisotropy of magnetic susceptibility exhibit small partial orientation in strong magnetic fields. The resulting macroscopic anisotropy may become manifest anisotropic nuclear dipolar and quadruple coupling [1]. This very tiny effect can provide information on the spatial structure of molecules. New procedure ALIGN was suggested for quantitative simultaneous analysis of a series of strongly coupled high resolution NMR spectra recorded on spectrometers with different magnetic field strength [2]. This technique allows accurate separation of the field-independent spin-spin and field-dependent dipole-dipole coupling constants. It gives also estimates for anisotropy and rhombicity of magnetic susceptibility tensor, which independently can be calculated quntum-mechanically with CSGT technique [3]. We performed detailed experimental and theoretical (Gaussian-09 program suit) studies for a series of bicyclic carbo- and heterocycles. The results supported applicability of the method for structure applications.

Support: AvH and RFBR.

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Ls293

Broadband Platinum NMR Spectroscopy

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Platinum possesses an NMR-active nuclear spin ½ species, ^{195}Pt, which is well-suited for the characterization of Pt-complexes. Having a similar gyromagnetic ratio as ^{13}C but roughly 30 times the natural abundance, ^{195}Pt spectra can be obtained in reasonable time. However, the huge chemical shift range of nearly 14,000 ppm makes it impossible to acquire a full ^{195}Pt spectrum with conventional pulses. A new class of pulses, so-called xy-BEBOP pulses optimized using optimal control algorithms, allows now to acquire bandwidths of 3800 ppm and even higher in a single experiment.

Examples are shown for ^{195}Pt 1D and specifically designed ^{1}H,^{195}Pt-HMBC 2D experiments (*Fig. 1*).

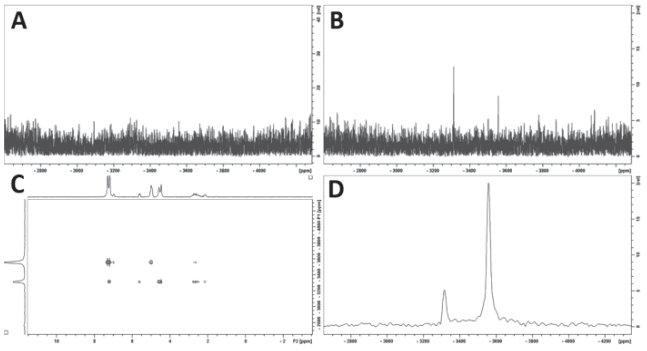

Figure 1: Example spectra for (COD)$PtCl_2$ with impurities; **A:** 1D-spectrum, 256 scans, 9 min 32 sec acquisition time, S/N = 1.86; **B:** 1D-spectrum, 1024 scans, 37 min 52 sec acquisition time, S/N = 7.13; **C:** ^{1}H,^{195}Pt-HMBC, 2 scans, 128 increments, 10 min 38 sec acquisition time, S/N = 102.05; **D:** slice of the 2D-spectrum at 7.20 ppm.

Ls294

Pulse Trains with Variable Nutation Angles for Homonuclear Decoupling

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The challenge of measuring transverse relaxation rates R_2 in homonuclear *J*-coupled systems lies in the presence of echo modulations. Recently, Carr-Purcell-Meiboom-Gill (CPMG) pulse sequences using moderate radiofrequency (*rf*) amplitudes $\omega_1 \geq \Omega_S$ relative to the off-resonance chemical shift Ω_S, were used to obtain modulation-free R_2 decays in homonuclear coupled systems.[1-3] The only pitfalls are 'recoupling conditions' at certain inter-pulse delays, where the *J*-modulations are reintroduced.

Experiments on protons in Cyclosporin A were performed with nutation angles of 360° instead of the classic 180°. The new sequence led not only to comparable R_2's but also did not show any recoupling conditions. In order to understand these observations, simple ^{1}H spin-systems in organic molecules were studied with a focus on the nutation angles. Experimental and simulated maps of signal intensities as a function of the angle and the inter-pulse delay showed many favorable conditions with arbitrary angles and were confirmed by consistent measurements of R_2's.

Where all these experiments are two-dimensional, a one-dimensional experiment could generate a homonuclear decoupled spectrum, as long as the chemical shift is not refocused. Window-acquired short tickling pulses with angles $\theta < 1°$ achieved this for selected spin systems.

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Ls295

$^{35}Cl/^{37}Cl$ isotope effects in ^{195}Pt and ^{103}Rh NMR: a fundamentally new tool for unambiguous speciation of the deceptively simple Pt^{IV} and Rh^{III} complex anions, in chloride-rich process solutions.

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High-resolution ^{195}Pt and ^{103}Rh NMR is a unique tool for the unambiguous spectroscopic speciation of the deceptively simple $[PtCl_n(H_2O)_{6-n}]^{4-n}$ (n = 1-5)[1], as well as on the basis of the $^{35}Cl/^{37}Cl$ isotope-induced shifts observed for each complex anion in solution, as relevant to the refining industry.[2] For the first time, the corresponding $[RhCl_n(H_2O)_{6-n}]^{3-n}$ complexes (n = 1 – 3) in acidic solution at 293 K have been have been identified by this means. A direct species distribution diagram for $[RhCl_n(H_2O)_{6-n}]^{3-n}$ anions has been constructed as a function of chloride-ion concentration in acidic solution.

Detailed analysis of the ^{195}Pt and ^{103}Rh resonances at 128.8 and 19.11 MHz respectively shows that well-resolved $^{35}Cl/^{37}Cl$ isotope-induced shifts for each complex anion, results in unique ^{195}Pt and ^{103}Rh NMR line-shapes under carefully controlled conditions, due to the individual *isotopologue* and *isotopomer* distributions observable for each of the complex species; the latter serve as a unambiguous 'fingerprints' for the assignment of all complexes in solution, notably also for the *stereoisomers* of the kinetically inert octahedral $[MCl_{6-n}(H_2O)_n]^{2/3-n}$ anions ($M=Pt^{IV}$, Rh^{III}) present at minor concentrations in these solutions at a given chloride concentration.

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Ls296

Quantitative metabolic profiling of body fluids by nonlinear sampling and forward maximum entropy reconstruction of 2D ^{1}H-^{13}C HSQC

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Two-dimensional (2D) NMR methods have shown to be an excellent tool for the identification and characterization of statistically relevant changes in low-abundance metabolites[1] in body fluid. Although 2D NMR data provides minimized ambiguities in peak assignment, aided in metabolite identifications and comprehensive metabolic profiling but it takes more time to collect the data, which make it inappropriate for metabolic profiling.

In this presentation, we report the application of two – dimensional ^{1}H-^{13}C HSQC NMR spectroscopy for the quntitative metabolic profiling[2] with reduction in the experimental time. Maximum Entropy Reconstruction and Non linear sampling techniques[3] have been investigated for the quntitataive metabolic profiling. Experimental results on the standard samples of mixture of metabolites and body fluid will be presented

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Ls297

A Plug 'N' Play Set of Optimal Control Pulses for Enhancing NMR performance

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The steady increase in the magnetic field strength of NMR spectrometers improves the sensitivity and resolution of the resulting NMR spectra. However, hard pulses, the commonly used workhorses of most NMR pulse sequences, cannot always excite spins effectively over this increased bandwidth. This is especially problematic for nuclei with large chemical shift ranges such as ^{13}C, ^{15}N ^{19}F, and ^{31}P. Additionally, hard pulses do not compensate for B_1-field inhomogeneity/miscalibration. In this poster, a first topic will be the design and experimental implementation of numerically optimized RF pulses for robust broadband excitation, inversion and universal rotation pulses based on optimal control theory [1-4] in multi-dimensional experiments such as 2D HSQC. These optimized pulses compensate for the B_1-field inhomogeneity/miscalibration and excite spins effectively over large chemical shift ranges hence enhance on average the S/N ratio compared to conventional 2D HSQC. Since these pulses designed for ^{1}H and ^{13}C nuclei are all of identical duration, they can be used directly as replacements for corresponding hard pulses in existing pulse sequences.

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Ls298

Intrinsic Disorder in Measles Virus Nucleocapsids

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The genome of measles virus is encapsidated by multiple copies of the nucleoprotein (N), forming helical nucleocapsids of molecular mass approaching 150 Megadalton. The intrinsically disordered C-terminal domain of N (N_{TAIL}) is essential for transcription and replication of the virus via interaction with the phosphoprotein P of the viral polymerase complex. The molecular recognition element (MoRE) of N_{TAIL} that binds P is situated 90 amino acids from the folded RNA-binding domain (N_{CORE}) of N, raising questions about the functional role of this disordered chain. Here, we report the first in situ structural characterization of N_{TAIL} in the context of the entire N-RNA capsid. Using solution NMR spectroscopy, small angle scattering, and electron microscopy, we demonstrate that N_{TAIL} is highly flexible in intact nucleocapsids and that the MoRE is in transient interaction with N_{CORE}. We present a model in which the first 50 disordered amino acids of N_{TAIL} are conformationally restricted as the chain escapes to the outside of the nucleocapsid via the interstitial space between successive N_{CORE} helical turns. The model provides a structural framework for understanding the role of N_{TAIL} in the initiation of viral transcription and replication, placing the flexible MoRE close to the viral RNA and, thus, positioning the polymerase complex in its functional environment.

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Ls299

Vibration effects in NMR spectroscopy for studies of ultra fast conformational dynamics

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Dynamic behavior of molecular systems is regularly associated with some sort of chemical reactions. There are still numerous examples of extremely rapid dynamics, which can't be sufficiently evaluated in terms of classical kinetic parameters. Accurate structure studies of open-chain compounds and saturated four- and five-membered cycles imply solving specific problem of quantitative description of dynamic processes with very low barriers. We developed a practical method for evaluation of the parameters of conformational dynamics in terms of vibrations with large amplitude. The method based on: (*i*) complete analysis of high resolution NMR spectra, (*ii*) ab'initio calculations of a reaction path and surfaces of spin-spin coupling constants, (*iii*) a numerical solution of vibration problem and (*iv*) refinement for the parameters of the potentials based on the best fit of experimental and calculated spin-spin couplings (see e.g. [1-2]). Advantages of the technique demonstrated on studies of pseudorotation in four- and five-membered cycles and internal rotation in acyclic systems: natural endogenic hormone adrenaline, styrene and substituted azobenzenes.

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Ls300

Rapid Backbone Assignment protocol based on 2D projections of HNN and HN(C)N suite of experiments

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Backbone assignment is the very first and key step in protein NMR research. Getting this information fast is crucial for structural and functional proteomics projects, especially dealing with unstable proteins. In this context, an efficient high-throughput protocol for assigning backbone resonances in $^{13}C/^{15}N$-labeled proteins has been designed. The protocol uses (i) protein primary sequence, (ii) sequential amide correlations from 2D-hNcocanH [1] and 2D-hNcanH spectra, (iii) self and sequential ^{13}C correlations from 2D-hnCOcanH [2] and 2D-hncoCAnH [2] spectra, and finally (iv) a few check points (glycines, alanines, serines/threonines, and the residues following them in the sequence) derived from variants of 2D-(HN)NH [3] and 2D-hncNH [1]. The protocol has successfully been used for assigning the backbone (^{1}HN, ^{15}N, $^{13}C^{\alpha}$ and $^{13}C'$) resonances in three small globular proteins: ubiquitin (76 aa), Calbindin-d9k (75 aa) and M-crystallin (85 aa). In each case, the whole exercise including experimentation and analysis was accomplished in less than a day. The protocol would be of immense value for high-throughput structural proteomics of small well-folded proteins. Additionally, the approach is most amenable for re-establishing sequential assignments lost by the complex formation with various ligands (proteins/metal ions/drug molecules) while investigating protein structure-activity relations. Further, the protocol will also be well suited for studying mutated proteins where the whole assignment can be re-established without recording the various 3D NMR experiments.

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Ls301

Spectral simplification by collapsing J-multiplet structures: an improved technique for dilute samples

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1D proton NMR spectra of small molecules in solution often suffer from complex multiplet structures due to a dense network of homonuclear scalar coupling interactions. The complexity and signal overlap resulting from such scalar couplings can seriously hamper spectral analysis. Several methods have been proposed previously to record simplified 1D-^{1}H NMR spectra in which the multiplets collapse into single lines[1,2]. However, due to their intrinsic low sensitivity, these methods can be applied mainly to samples with relatively high concentration (> mM). Here we present an optimized approach to record proton NMR spectra free from homonuclear J-couplings with much higher sensitivity compared to previous methods. By using our approach the acquisition of *pure-shift*[1] spectra can be extended to samples at more critical concentrations (sub-mM range).

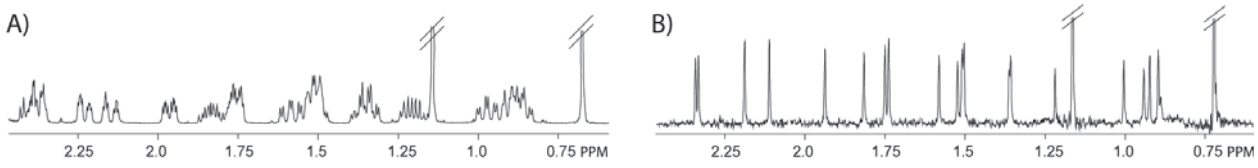

Figure 1; A) 1D proton NMR spectrum of testosterone B) Spectrum of 500µM testosterone free from homonuclear J-couplings

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Ls302

Three-Field NMR to Preserve Hyperpolarized Proton Magnetization as Long-lived States in Moderate Magnetic Fields

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Proton hyperpolarization by dissolution Dynamic Nuclear Polarization (DNP) can in principle provide enhancement factors up to $\varepsilon_{DNP} = 17000$ [1]. ^{1}H dissolution DNP is more attractive than ^{13}C because it has higher sensitivity ($\gamma H = 4\gamma C$) and does not require any isotopic labeling. However, the price to pay is the rapid return of the population differences to the Boltzmann equilibrium due to a faster longitudinal relaxation rates R_1. Long-lived States (LLS) offer a way to overcome this disadvantage by preserving magnetization for longer times $T_{LLS} >> T_1$ [2,3].

We developed a three-field experiment where inequivalent scalar-coupled pairs of spins are hyperpolarized at $B_0 = 3.35$ T and 1.25 K, rapidly transferred to high field ($B_0 = 7$ T) to prepare a suitable initial condition $I_z - S_z$ that is converted adiabatically into LLS by shuttling the sample to a moderate magnetic field ($2\ mT < B_0 < 7$ T). The polarization is preserved in the moderate field, where it slowly decays with $T_{LLS} >> T_1$. The sample is finally shuttled back to high field ($B_0 = 7$ T) for observation.

We were able to preserve the enhanced polarization of protons ($\varepsilon_{DNP} = 1700$, $T_{LLS} > 60$ s) at moderate fields ($B_0 < 0.1$ T).

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Ls303

Observing ipso-contacts at dimer interfaces with the novel diagonal-free 3D [H]C,CH-NOESY experiment

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NOESY spectra are the most important source of data for structure determination by NMR. Yet, critical NOE cross signals may be biased or concealed by the intense, redundant diagonal signals through direct spectral overlap, induced baseline distortion (from phase errors) and derived artefacts (e.g., decoupling sidebands, t_1 noise, truncation wiggles). Thus, diagonal signal suppression is of paramount importance for maximal data coverage and accuracy in NOESY spectra. In homoisotopic NOESY experiments, it may be achieved by applying *orthogonal spin state selection* (oS^3) across the NOE mixing time, exploiting the fact that ^{1}H spins can be magnetically distinguished by the spin state of a coupled spin-½ heteronucleus X (e.g., ^{13}C, ^{15}N). This principle has so far been applied only to ^{15}N-bound protons, ranging from 2D homonuclear NOESY [1] to maximum resolution 3D heteronuclear [H]N,NH-NOESY-TROSY [2]. Yet, the cited problems caused by diagonal signals are most pronounced in the crowded ^{13}C-edited NOESY, where further complications may arise from the heterogeneity of CH_n multiplicities and $^{1}J_{CH}$ couplings.

We here present the novel 3D [H]C,CH-NOESY experiment with diagonal signal suppression, and apply it to the homodimeric bacterial transcription repressor CopG (2 × 16 kDa). Thus, we were able to unambiguously detect various new NOE contacts close to, and even at the exact position of, the suppressed diagonal. Such degenerate NOE signals indicate *ipso*-contacts between identical residues in both monomers of a head-to-head dimer, providing a critical definition of the dimer interface.

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Ls304

Measurements of transverse relaxation rates in scalar coupled spin systems

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This work investigates the advantages provided by multiple quantum-filtered experiments for transverse relaxation rate measurements in homonuclear scalar coupled spin systems.

Recently, a solid-state NMR method based on a combination multiple quantum-filtered experiments, has been introduced for estimating small homonuclear dipole–dipole couplings.[1]

Here, we show that combination of multiple quantum-filtered experiments can also be used in solution-state NMR to suppress echo modulations coming from homonuclear scalar couplings. As a result, accurate relaxation rate measurements can be achieved in both weakly and strongly coupled spin systems.[2]

The scheme proposed here was explored theoretically and experimentally. The numerical simulations, assisted by the *SpinDynamica* code developed by M. H. Levitt,[3] investigated different motional and coupling regimes. Experimentally, we demonstrated that the methodology proposed here provides more accurate results than the conventional CPMG method without requiring any experimental optimization.

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Ls305

Efficient Indirect Quadrature Detection by Multiplex Phase Cycling

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Application of multiplex phase cycling(1) to multi-dimensional heteronuclear NMR significantly reduces the number of acquisitions required to achieve the desired resolution in the indirect dimensions.

In conventional *N*-dimensional pulse sequences the minimum number of one-dimensional FIDs required to obtain a phase-sensitive spectrum is determined by the basic phase cycle and the method for quadrature detection (e.g. States, TPPI, echo-/anti-echo). With multiplex quadrature detection (MQD) the tasks of coherence selection and quadrature separation are merged. The minimum total number of scans, which are combined per data point, is decreased by a factor $(3/4)^{N-1}$. This is especially interesting for three- and higher dimensional experiments since spectra with the same resolution in the indirect dimension can be obtained in less time (i.e: ca. 75%, 56%, 42%, 32% for 2D, 3D, 4D, 5D experiments, respectively) without introducing artefacts. Furthermore this approach can be straightforwardly combined with other time saving methods (e.g. sparse sampling techniques(2-4)).

As an example we could obtain MQD 3D triple resonance spectra of the photosynthetic protein PsbQ(5) with the same resolution and the same per-scan signal-to-noise ratio as in the standard experiment in only 56% of the usual time. The time saved could also be invested into additional resolution.

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Ls306

Design of Entry Inhibitors against Human Noroviruses

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Noroviruses from the family of *Caliciviridae* are the main cause of acute non-bacterial gastroenteritis worldwide. No direct treatment or vaccination strategy is yet available. Noroviruses bind to oligosaccharide structures found on the surface of human host cells, so called Histo-blood group antigens (HBGAs). Sequence alignment of a large number of norovirus strains of the currently dominating genogroup from the past 20 years[1] had shown a strict conservation of residues recognizing a fucose moiety. Utilizing STD and trNOESY NMR with VLPs and synthetic HBGAs we could elucidate the natural receptor binding pattern of a human norovirus at atomic detail and confirm a strong preference for fucosylated oligosaccharides. This structural information served as the basis for the synthesis of specific inhibitors. Further derivatization was done by covalent linkage with hits from small molecule and virtual library screening. Finally, synthesis of multivalent compounds yielded prototype inhibitors with affinities several orders of magnitudes higher than the monovalent interaction.[2] Binding affinities and HBGA competition were evaluated by direct and competitive titration with NMR and SPR as well as hemagglutination assays.

These are invaluable steps towards development of so-called entry inhibitors against this important group of human pathogenic viruses.

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Ls307

Dissecting the potential of solvent PREs for structural analysis of large proteins and their complexes using sparse data

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Determining structure and architecture of proteins and their complexes faces challenges when only sparse restraint sets are available. Here we present our efficient approaches for structural analysis of proteins and large protein complexes using paramagnetic relaxation enhancements (PREs) from a soluble paramagnetic agent[1,2]. We are showing highlights for the use of solvent PRE data in combination with sparse data in i) a scoring procedure and ii) direct refinement which significantly improves accuracy and convergence of structure calculation approaches (i.e. docking programs, CS-ROSETTA). The strength of solvent PRE methodology is that it is readily implemented (i.e. no covalent modifications needed), and that (transient) interactions and local structure can be efficiently detected and refined. We applied our protocol to the challenging ternary 150 kDa nuclear export complex[3,4] (docking) and a benchmark of ~50 medium- to large-size proteins (CS-ROSETTA). We show that solvent PREs are an excellent indicator of the quality of structural models and thus resolve ambiguities; direct refinement against solvent PRE data improves structural accuracy and precision. We show that solvent PREs provide a new class of restraints that are easily accessible and applicable to proteins and large protein complexes. In particular for challenging systems, our approach promises significant time-savings and significantly improved quality of structure calculations

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Ls308

Characterizing Aggregation of Murine PrP by Time-resolved NMR

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In this work, we address the aggregation behavior of the prion protein by NMR spectroscopy. We follow the fibril formation of wildtype mPrP(121-232) by time-resolved NMR. Site-resolved kinetic data for more than 50 residues can be characterized providing probes well distributed throughout the amino acid sequence. Aggregation of the monomeric protein as evidenced by losses in peak intensity is most pronounced for residues in the region of the disulfide bond encompassing many single-point mutations which are implicated in human prion diseases. Strikingly, peaks of the very C-terminal residues (residues 225-232) appear to increase in intensity. In addition, both Y225 and G228 show peak doubling indicating conformational heterogeneity which is in good agreement with recent fibrillization studies on hPrP(90-230) performed in our group (1).
In order to gain more insight into the peculiar behavior of the C-terminus, we synthesized a decapeptide corresponding to residues 223-232 of mPrP. As evident from ^{15}N HSQC, ^{13}C HSQC and TOCSY data, residues 223-228 show pronounced structural rearrangements whereas the remainder of the peptide is not affected. Based on these observations, we suggest that the decapeptide undergoes self-assembly via its N terminus building up the aggregate core while the C-terminal residues protrude into solution and remain flexible. This interpretation is supported by several algorithms allowing for the prediction of aggregation properties.

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Ls309

A robust, sensitive, and versatile HMBC experiment for rapid structure elucidation by NMR: IMPACT-HMBC

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In this communication, we describe a robust, sensitive, and versatile HMBC experiment for structure elucidation. The proposed IMPACT-HMBC experiment eliminates the weaknesses of the basic HMBC experiment and the overall performance of the pulse sequence is improved significantly. The IMPACT-HMBC is able to provide optimal $^{1}J_{CH}$ suppression, very good signal to noise ratio using minimum experimental time, and able to provide very high resolution in the ^{13}C dimension. Furthermore, the experiment allows the user to take advantage of the Ernst angle effect, which can significantly improve the sensitivity. Finally, the experiment can be recorded with very short recovery delays, without any detrimental effects. The technique may find application in routine analysis by NMR as well as in the structure elucidation of complex organic systems, and especially when the experimental time is limited. We also pay particular attention to the experimental set-up, with the intention that the proposed HMBC experiment can be used as it starts from standard parameters set by inexperienced users as well as under automated conditions.

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Ls310

Accordion-optimized DEPT experiments

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In this contribution, a pulse sequence is described for recording accordion-optimized DEPT experiments. The aim of the ACCORDEPT experiment is to afford data with improvement of the signal-to-noise ratio (SNR) for responses exhibiting significantly different couplings from the optimization of the static experiment. For this purpose, we first focus on the sampling technique of the accordion delay. This delay can be varied on the basis of equal steps in time or on the basis of equal steps in frequency (hertz). The nonlinear sampling of the desired coupling range is a result of equal *time* between decrementation steps, but not equal in frequency, while the decrementation technique on the basis of equal steps in hertz uniforms at best the polarization transfer efficiencies.

As a proof of concept, this strategy has been applied to a mesogen containing a large range of one-bond $^{1}J_{CH}$ coupling constants associated with the various structural elements. The ACCORDEPT experiment afforded significant enhancements for the resonances with the larger $^{1}J_{CH}$ couplings, similar SNR for aromatic resonances, but reduced SNR for aliphatic resonances as compared with the standard DEPT experiment. In addition, the ACCORDEPT is straightforward to implement, does not require any supplementary calibration procedures and can be used under automated conditions without difficulty by inexperienced users

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Ls311

Novel Dimerization Mode of a Sterile Alpha Motif

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SH3 containing lymphocyte protein 1 (SLY1) is a 380-residues protein exclusively expressed in T and B lymphocytes [1]. It plays an important role in adaptive immunity. SLY1 comprises of three domains, namely, SH3, nuclear localization signal, and SAM domain. The SAM domain is a common protein interaction module, found in highly diverse organisms scaling from bacteria to human. It consists of ~70 amino acids and is observed to participate in numerous biological processes including signal transduction and RNA binding. Homo-SAM- and hetero-SAM-domain oligomerization has been reported and may be crucial for the role of SAM domains in protein interactions. Here, we report the three-dimensional solution structure of the SAM domain of SLY1 using NMR spectroscopy. We found, that this SAM domain forms a symmetric and stable homodimer. Self association properties of SLY1 SAM were analyzed by dynamic light scattering, analytical ultracentrifugation, diffusion ordered spectroscopy (DOSY), NMR chemical shift perturbation analysis and thermophoresis. The fold of each SAM monomer consists of five well-defined helices packed into a compact globular structure. The relative orientation of the two monomers and the amino acid residues lining the SAM-SAM interface were determined based on filter-NOESY experiments. Formation and stabilization of the SAM dimer appears to arise from burial of hydrophobic residues in combination with electrostatic interactions.

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Ls312

Solving Problems in Natural Product Chemistry by NMR

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Natural products are highly evolved, specific, and effective gene products. Their diverse structural and stereochemical characteristics make them valuable templates. Nature has been the first source of medicines for diseases and discomforts which have led to the foundation of many empirical therapeutic interventions. Globally, there is a revival of interest in the use of natural products for the treatment of various ailments. This is mainly due to increased awareness of the limited horizon of synthetic pharmaceutical products to control major diseases, high cost of available synthetic medicines, adverse side-effects of modern medicines and perceived gentleness of natural medicines. Despite the tremendous potential of natural products, they have certain problems including availability in very low quantities, novel structures, complex stereochemistry, isolation from complex mixture, and unstable nature.

NMR spectroscopy has been extensively applied to solve above cited problems in the field of natural products and drug discovery. For example cryoprobe and inverse NMR spectroscopic techniques have been deployed to solve the problems associated with lower sensitivity due to limited quantities or lower abundance of nuclei. A number of NMR techniques have been developed to solve the structure of novel natural products. Overhauser effects and other relaxation techniques have been developed to determine the complex stereochemistry of natural products. DOSY has now been extensively used to analyze the mixture of natural products. Low temperature NMR can be used to handle unstable natural products. We have been using these technique for structure determination of small organic molecules, for monitoring ligand-receptor binding, to analyze mixtures using LC-NMR, in quality control, and rational drug designing. Results of these studies, along with an overview of new approaches and emerging technologies in natural product-based drug development, will be presented during this lecture.

Ls313

Sparse sampling and fast pulsing methods for unfolded proteins

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Resonance assignment of intrinsically disordered proteins is made difficult by the extensive spectral overlaps. High-resolution 3D and 4D spectra are thus essential for this purpose and made possible by relatively narrow line width of each resonance due to fast conformational averaging. We have adapted the series of 3D BEST experiments proposed by Lescop *et al* [1] to the case of unfolded proteins. As compared to standard triple-resonance experiments, a relaxation delay between transients as short as 0.2 sec can be employed. The BEST-approach is fully compatible with sparse random sampling techniques in the indirect dimensions [2]: improved digital resolution along these dimensions can be obtained by sampling longer time increments by means of semi-constant time evolution. Using 2D Maximum Entropy reconstruction [3] for the indirect dimensions, the artifact intensity due to sparse sampling can be reduced to a level similar to or below the standard noise (thermal and t_1 noise). If no cross-correlation effects would be present, these pulse sequences could even be more simplified by eliminating all refocusing pulses and removing the J-splittings by spectral deconvolution during the reconstruction. The reduction of the sampled increments and the shorter duration of individual transients make it possible to record a 4D experiment with reasonable resolution in less than 60 hours.

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Ls314

High performance NMR analysis of enantiomeric purity and absolute configuration of chiral alcohols and amines on a microgram scale

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Use of chiral derivatizing agents (CDAs), which transform enantiomers into magnetically nonequivalent diastereomers, has made NMR spectroscopy a powerful tool for analysis of chiral compounds. Though great number of various CDAs has been introduced over last years[1] the derivatization procedure remaines time- and reagent consuming operation which limits it's use as a routine tool for rapid analysis in asymmetric synthesis and catalysis.

Recently we have developed new derivatization procedure for analysis of chiral alcohols and amines which can be performed directly in NMR tube[2]. No preliminary purification of diastereomers formed is required to obtain various NMR spectra (both 1D and 2D) of high quality which allows determination of enantiomeric purity of various chiral alcohols and amines with high accuracy within several minutes including sample preparation time. 5-10 mg of chiral samples is enough for standard analysis in various deuterated solvents though even 0.01 mg of chiral sample can be successfully analyzed using standard NMR hardware. The "in tube" method developed is applicable to determination of absolute configuration using both double and single derivatization techniques[3].

Acknowledgments: The work was supported by the Research Grant MK-1434.2010.3.

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Ls315

High resolution characterization of intrinsic disorder in proteins: expanding the suite of ^{13}C direct detection NMR experiments

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Intrinsic disorder and flexibility have been recognized as important functional features in proteins and add a new dimension to the structure/function paradigm. Among the available methods to characterize protein intrinsic disorder at atomic resolution, NMR plays a key role thanks to the wide variety of observables that can be determined for providing structural and dynamic information. A set of experiments based on ^{13}C detection that only exploit heteronuclear chemical shifts in all detected dimensions (so-called *exclusively heteronuclear* NMR experiments) has recently been developed and provides a useful tool to study intrinsically disordered proteins (IDPs)[1;2] all the way to in-cell[3]. We present here a set of new experiments that enable the determination of key observables (solvent exchange and heteronuclear NOEs)[4] and/or provide additional tools to characterize intrinsically disordered proteins (experiments with improved chemical shift dispersion, aminoacid type selection, C^{α} direct detection).

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Ls316

Conformational Flexibility and metal binding studies of Anthrax Lethal Factor catalytic core

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Anthrax Lethal Factor (LF) is Zn-dependent highly specific metalloprotease that plays a vital role in the anthrax disease[1] and shows proteolytic specificity against vital cellular signal transducers, the family of mitogen-activated protein kinase kinases (MAPKKs)[2]. Its structure has already been analysed through X-Ray crystallography[1]. Here, we report the expression and structural analysis of a C-terminal part of LF ($LF_{672-776}$) that harbors the enzyme's core protease domain, using solution NMR spectroscopy. The biophysical characterization and backbone assignments (^{1}H, ^{13}C, ^{15}N) of the polypeptide in its apo-form revealed a stable structure over a wide range of temperature (through CD measurements), a flexible N- and a robust C-terminal segments. The NMR structure of the catalytic core polypeptide has been determined exhibiting a compact fold and great similarities with the crystal structures of the corresponding polypeptide, despite the absence of the metal cofactor [3]. Also, a series of single amino acid mutants of the $LF_{672-776}$ was produced and further characterized through NMR to gain additional insights of the conformational and functional properties of LF's catalytic core.

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Ls317

Spectral Analysis Using Nonnegative Matrix Factorization for Peak Decomposition

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Simple peak-picking algorithms, such as those based on lineshape fitting, perform well when peaks are completely resolved in multidimensional NMR spectra, but often produce wrong intensities and frequencies for multiple peak clusters. For example, NOESY-type spectra show considerable overlap leading to peak-picking intensity errors of twice or more, which can result in wrong structural restraints. Precise frequencies are critical for spectral assignment. To alleviate this problem, a more sophisticated peak decomposition algorithm, based on Nonnegative Matrix Factorization (NMF), was developed. Its performance is compared for several types of regularizations and divergences in the context of multidimensional decomposition (MDD). Apart from its main goal of deriving components from spectra and producing peak lists automatically, the NMF approach also allows to apply constraints if some information about the components is known a priori, e.g. the number of peaks or positions in some dimensions.

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Ls318

PHIPing styrene and biomolecules

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The para-hydrogen induced polarization (PHIP)[1] also known under the acronym PASADENA was discovered by Bowers and Weitekamp in the late 80s.[2] Shortly after this discovery, a related PHIP phenomenon called ALTADENA was investigated.[3]

With this method it is possible to observe enhanced NMR signals from samples with low amount and intermediates of reaction mechanisms as for example of the olefin isomerization.[4]

An important application of this effect is found in the sensitivity enhancement in Magnetic Resonance Imaging (MRI)[5]. Therefore research for special biomolecules which can be used as contrast agents is required. Here we present biomolecules which have a functional group like a triple or double bond. These molecules are hydrogenated with *para*–enriched hydrogen and show the PHIP effect. Such biomolecules can be attached to small peptides and therefore also to other molecules like enzymes which can pass through the blood-brain barrier.

Further we present the standard model styrene as substrate in several hydrogenation reactions employing *para*-enriched H_2 with homogeneous and heterogeneous catalysts like nanoparticles which can be separated easily from the product solution.

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Ls319

Mimicking the Ideal Frequency Sweep

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Using optimal control methods[1,2,3], robust broadband inversion pulses creating a defined second order phase shift can be designed. This phase shift corresponds to an offset-dependent effective evolution time with respect to chemical shift and couplings, thus recreating the effect of an ideal linear frequency sweep.

In combination with a magnetic field gradient, spatially different evolution times can be prepared and this novel class of pulses can replace non-ideal adiabatic frequency sweeps wherever this phase behavior is desired. Applications include single scan 2D-experiments[4] and z-filter-elements[5].

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Ls320

Fast High Resolution 2D-Spectra for RDC-Measurements by Spectral Folding

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To determine the residual dipolar couplings (RDCs) in the indirect dimension of F1 coupled HSQC spectra a high number of t_1 increments are necessary. This is very time consuming. Previous works of our group show, that one can reach highly resolved HSQC spectra in a short time employing spectral folding and post processing with a MATLAP-routine[1].

The acquisition of several folded HSQC spectra and the post processing reduces the measuring time by a factor of ten in contrast to a conventionally acquired spectrum with the same digital resolution. Compared to other methods for determination of RDCs, such as gated decoupled ^{13}C spectra, we have the advantage of edited spectra , well separated peaks and the sensitivity gain from the INEPT transfer.

We will demonstrate our method using a standard organic sample (50mg strychnine & swollen polystyrene stick in $CDCl_3$)[2].

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Ls321

Poly isocyanides as alignment media to measure RDCs for small organic molecules

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Residual Dipolar Coupling (RDC) is the magnetically induced dipolar coupling of spin active nuclei. RDC can be observed by NMR when molecules are anisotropically oriented in a solution. Liquid Crystals, bicells, and filamentous viruses have been developed as anisotropic media to enhance the magnetic orientation of bio molecules, but these methods are not well suited for small organic molecules due to the requirement of water as a solvent. Liquid crystals and stretched gels are being used as anisotropic medium to measure RDCs for organic molecules. But these are not well developed methods. Strong alignment by using liquid crystals and time consuming by using stretched gels are problems. So we are investigating new alignment medium like poly isocyanides.

Poly isocyanides are helical polymers; these can form liquid crystals in concentrated solutions of chloroform, tetra hydro furan (THF) and dichloromethane. We aligned these liquid crystals in magnetic fields and we measured deuterium quadruple NMR to find the alignment. Then we introduced a guest molecule (Strychnine) into this chiral solvent, then it transferred this alignment to the guest molecule and we measured RDCs for a guest molecule.

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Ls322

Automated platform for backbone assignment of globular and disordered proteins

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The platform targets the two major bottlenecks in the traditional procedure of protein signal assignment: the lengthy data collection and manual peak identification in series of 3D spectra. Our approach starts with rapid recording of high-resolution spectra using incremental non-uniform sampling (iNUS) in the process called targeted acquisition [1]. The spectra are co-processed [2] with multi-dimensional decomposition (MDD) [3]. Clean automated peak identification is performed in one-dimensional MDD shapes. The peak lists and automated assignment are validated directly versus gradually accumulating volume of experimental data. The method has been successfully used for de novo assignments of four 12-15 kDa globular proteins from BioNMR (EU FP7, www.bio-nmr.net) and AEROPATH (EU FP7, www.aeropath.eu) target lists, and for several intrinsically disordered cytoplasmic domains of T-cell and B-cell receptor complexes (fig. 1).

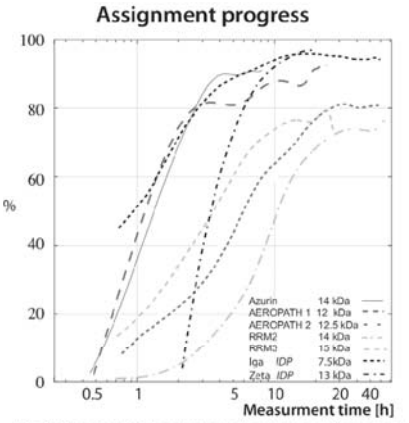

Fig. 1, Progress of the automated assignment for several protein systems. Slope of the curves is defined mainly by the S/N in the spectra. Level of the final plateau depends on the spectra intensity range and presence of weak or missing spin systems

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Ls323

NMR studies of the extracellular domain of a prokaryotic Ligand-Gated Ion Channel (LGIC)

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Pentameric ligand-gated ion channels of the Cys-loop family are of special importance for the rapid chemo-electrical signal transduction at synapses [1–3], but the mechanisms of ion permeation and gating of these membrane proteins remain elusive. Recently the X-ray structures of two prokaryotic homologues of the nicotinic acetylcholine receptor (nAChR), the best studied member of the LGIC family, have been determined: 1) the bacterial *Gloeobacter violaceus* pentameric ligand-gated ion channel homologue 4 (GLIC; 2.9 Å) in an open conformation [2] and 2) a homologue from the bacterium *Erwinia chrysanthemi* (ELIC; 3.3 Å) in a closed conformation [3].
The 200-residue extracellular domain of GLIC, which is found to be a monomer in solution, was cloned and expressed in high yields in *E. coli* and studied through multi nuclear and multidimensional NMR. Assignment efforts achieved the identification of the 80% of the backbone $^{13}C/^{15}N$ nuclei. Additionally, the dynamics of GLIC was studied through ^{15}N relaxation experiments and provide valuable insights about GLIC mobility (Chasapis C.T. *et al.* to be submitted).

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Ls324

Arkadia's RING Finger NMR structure and its interaction with E2 partner

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E3 ubiquitin ligases play a key role in the recognition of target proteins by catalyzing the covalent attachment of the ubiquitin and degradation by 26S proteasome [1]. Many known tumor suppressors or oncoproteins are RING type E3 ubiquitin ligases and among them the best studied are various RING finger domains. Arkadia is a relatively new E3 ubiquitin ligase and possibly the first example of an E3 ligase that positively regulates TGF–β family signaling, by inducing the ubiquitin-dependent degradation of TGF–β signaling negative regulators, through its C-terminal RING finger domain [2]. Arkadia RING finger, was cloned and expressed in its zinc-loaded form, and studied through NMR (BMRB acces.no. 15948) [3]. Additionally, the 3D NMR solution structure of Arkadia RING finger was determined (PDB 2KIZ) and its interaction with the E2 UbcH5B enzyme was studies through titration experiments monitored by NMR. The RING-E2 complex structure was also constructed through an NMR-driven docking protocol (using HADDOCK). Finally, various Arkadia forms are prepared bearing amino acid substitution inspired either by the atypical RING fingers sequences or driven by identified cancer-related mutations observed in human tumors.

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Ls325

High resolution 1D and 2D NMR spectroscopy in native ionic liquids systems

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Native state NMR spectroscopy in ionic liquids is a rapidly emerging field with several breakthrough findings reported recently[1,2]. High resolution NMR spectra became possible to acquire directly in ionic liquid after overcoming the difficulties associated with high viscosity, conductive ionic character of the medium, and adsorption of radiofrequency.

We have focused our attention on conversion of various carbohydrates into 5-hydroxymethylfurfural (5-HMF) – a single renewable biomass-derived building block, which can be obtained in high selectivity in ionic liquids (IL) media. The goal of our research was to develop reliable NMR approach for direct spectral investigation of 5-HMF production in IL in order to design efficient promoters and carry out a mechanistic study with characterization of intermediate species.

We have developed special NMR approach to record high resolution NMR spectra directly in IL/carbohydrate systems and to monitor formation of 5-HMF. Not only 1D, but also 2D (HMBC, HSQC, COSY) spectra were successfully acquired and utilized for line assignment of the NMR signals. The anomeric composition of the carbohydrates in IL was measured for the first time, with surprising difference compared to water solutions.

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Ls326

NMR-investigation of interactions and binding constants between antimicrobial actives and emulsifiers

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The efficacy of antimicrobial actives in emulsions depends strongly on the ingredients used in the formula. It is well known, that only the amount of active located in the water phase works against microbes. According to the literature, the amount of oil and emulsifier modifies the concentration of active in the water phase as well as the oil-water-partitioning coefficient and the binding constant of active and emulsifier.

In the study presented, the binding constants C of benzethonium chloride to glyceryl isostearate and polyethylene glycol isocetyl ether were determined, according to the relation between the binding constant and the chemical shifts δ or diffusion coefficients D and the concentration of emulsifier in micelles S_{mic}: $C = (\delta_{obs} - \delta_{free})/((\delta_{bound} - \delta_{obs})[S_{mic}])$ or $C = (D_{obs} - D_{free})/((D_{bound} - D_{obs})[S_{mic}])$.[1]

The chemical shifts were determined by ^{1}H-NMR spectroscopy, the diffusion coefficients by pulsed-field gradient NMR spectroscopy. δ_{free} and δ_{bound} as well as D_{free} and D_{bound} were determined in pure solutions of the active or the emulsifiers as a function of concentration below and above the CMC. δ_{obs} and D_{obs} were measured in solutions of active and emulsifier where the concentration of the active was constant and the concentration of the emulsifiers varied.

Benzethonium chloride interacts differently with the two emulsifiers. Differences were also observed between the hydrophilic and hydrophobic site of the molecule leading to the conclusion that benzethonium chloride is located at the interface.

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Ls327

Optimization of Sampling and Analysis of NMR Spectra for Automated Chemical Shift Assignment and Interaction Studies of Proteins

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Protein structure and interaction studies are complicated by spectral overlap. This frequently results in the incomplete assignment of backbone and especially side-chain atoms. Non-uniform sampling (NUS) and hyperdimensional (HD) methods can give high resolution spectra. We used these methods for a fused protein system that is designed to characterize a range of protein targets including partially folded peptides. We optimized the assignment for a set of 10-30% NUS experiments. Backbone and side-chain assignments were calculated fully automatically using the FLYA algorithm. In a first optimization we varied the total number of experiments from 2 to 12. A gradual increase in the number of assignments was observed. Second, an improvement of the assignment was observed as a result of simultaneous (HD) processing compared to treating the NUS spectra individually. A third optimization was performed by varying peak picking parameters. The optimum was found when the number of picked peaks was 80% of the peaks expected from the amino acid sequence which also gave the best S/N ratios for the peak lists. This occurred because of a flexible loop between fusion peptide and target protein was not observed. Overall, this approach is designed for the fast characterization of short-lived proteins, which requires fast NMR spectroscopy (1-2 days).

Ls328

Method for measuring of gas concentrations in solutions by qNMR

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Quantification using quantitative NMR (qNMR) is becoming more popular, as qNMR is a relative primary method, because the signal is in direct proportionality with the number of nuclei contributing to the resonance line. On the other hand, data sets of gas solubilities in solvents are sparsely[1], because the measurements generally need great effort. As you can find in the most publications, the currently methods base upon the changes of pressure or volume, that means these methods are indirect and a quantification of gases (e.g. hydrogen) in reaction solutions during a reaction seems to be impossible.

Therefore, the aim of this work was to develop a method by using the advantages of the NMR technology and to simplify the measuring of gas solubilites in solvents/solutions, with which it is possible to quantify gases in reaction solutions in-situ.

This method is based on the determination of absolute signal intensities (principle of reciprocity) as described by Gerhard Wider and Lars Dreier[2] as PULCON. But, reaction solutions contains a multitude of substances, the used solvents are normally undeuterated, and the signal you expect from your dissolved gas will be weak with the risk of distorting by effects like radiation damping, so it is hardly to get a reliable quantification. The solution is a combination of soft pulses, which are selective for the dissolved gas signal, with the PULCON method. Thus, you have a powerful tool to measure gas concentrations even in different undeuterated solutions, with accuracy about 3% (95% confidence level).

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Ls329

^{13}C Enrichment Degree of Organic Compounds Measured by NMR.

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Determination of isotopic enrichment of ^{13}C enriched organic compounds, important for medicine diagnostics, was performed by ^{1}H or ^{13}C NMR spectroscopy. Measurment of isotopic enrichment is to determine the ratio of isotopomers containing the isotopes ^{13}C (**A**) and ^{12}C (**B**).

^{1}H NMR spectroscopy may be used successfully when a compound studied contains protonated carbon atom. The signal of protons attached to enriched carbon in isotopomer **A** is split to doublet due to direct coupling $^{1}J(C\text{-}H)$ while the appropriate proton signal in **B** is singlet. The intensity ratio of these components characterizes ^{13}C enrichment degree. This way have been used in the case of (methyl-^{13}C)thymidine, (^{13}C-methoxy)metacetine and 1-^{13}C-D-glucose.

If the enriched carbon atoms had no attached protons, measurement of isotopic enrichment was performed by $^{13}C\{^{1}H\}$ NMR spectroscopy using the carbon signal of the atom adjacent to the enriched one and their direct $^{1}J(C\text{-}C)$. The signal of this carbon atom in isotopomer **A** is doublet, while in isotopomer **B** the appropriate carbon atom shows singlet signal. The intensity ratio of these signals gives the enrichment value. This procedure allowed us to determine the isotopic enrichment in 1-^{13}C-octanoic acid, 1,2,3-*tris*-(1'-^{13}C-octanoyloxy)propane, ($^{13}C_2$-carboxy)dimethylphthalate and 4-*tert*-butyl($^{13}C_2$-carboxy)dimethylphthalate.

The method requires a long term acquisition, careful selection of the solvent, a high degree of chemical purity of the substance studied.

Ls330

A development of a useful analytical methodology for the determination of the molecular recognition site of the phosphatases. The use of the NMR through the system ^{1}H - ^{31}P.

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Protein phosphorylation is a fundamental mechanism for numerous important aspects of eukaryote physiology such as cell division, metabolism, migration, transcription, etc. It becomes a target to favor or avoid those biological phenomena, as well as human health and disease[1,2] Almost all these phosphoproteins are modified on multiple serines, threonines and tyrosines. In order to achieve a contribution to the knowledge of the phosphatase reaction mechanism it is necessary to determine the recognition site of phosphatases through NMR. Saturation Transfer-Difference (STD) spectroscopy is one of the most useful methods to characterize binding in tightly bound ligand-receptor complex. This methodology has been employed to analyze the binding kinetics of protein-ligand complex formation.[3,4]

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Ls331

Protein-protein interaction between a homodimeric dynein light chain and a highly mobile myosin fragment

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The homodimeric, highly conservative dynein light chain (DYNLL2) is an essential hub protein which is responsible for various functions beyond being a tail-associated subunit of dynein and also of the myosin Va motor proteins. The binding grooves of the DYNLL surface interact with linear sequences, almost all of which are part of a disordered domain in the partner protein. Investigation of binding for a myosin M7 fragment showed that the 27 residue long region can be considered highly dynamic in free form, and becomes partly structured upon binding to DYNLL2. Binding positions on the M7 side were determined by chemical shift mapping. T_1, T_2 relaxation time and heteronuclear ^{1}H-^{15}N NOE measurements help us characterize the dynamics of the myosin fragment in free form and in the protein complex by reduced spectral density mapping analysis. Saturation transfer difference experiments probing ^{15}N group selective STD, ^{15}N-HSQC STD and ^{13}C-HSQC STD methods led to the conclusion that the hydrophobic interactions at the myosin terminal parts help in the positioning and steering of the binding partner.

Ls332

Chemical shift prediction for protein structure calculation and quality assessment using an optimally parameterized force field

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The exquisite sensitivity of chemical shifts as reporters of structural information, and the ability to measure them routinely and accurately, gives great import to formulations that elucidate the structure-chemical-shift relationship. Here we present a new and highly accurate, precise, and robust formulation for the prediction of NMR chemical shifts from protein structures. Our approach, shAIC (shift prediction guided by Akaikes Information Criterion), capitalizes on mathematical ideas and an information-theoretic principle, to represent the functional form of the relationship between structure and chemical shift as a parsimonious sum of smooth analytical potentials which optimally takes into account short-, medium-, and long-range parameters in a nuclei-specific manner to capture potential chemical shift perturbations caused by distant nuclei. shAIC outperforms the state-of-the-art methods that use analytical formulations. Moreover, for structures derived by NMR or structures with novel folds, shAIC delivers better overall results; even when it is compared to sophisticated machine learning approaches. shAIC provides for a computationally lightweight implementation that is unimpeded by molecular size, making it an ideal for use as a force field.

Ls333

Experiments to determine coupling constants in sugars of labeled RNA

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We present here a new J-modulated[1] experiment to measure H2'C2' coupling constants without the need of assigning the H2' and C2' resonances. The measurement of a large number of coupling constants in RNA molecules is usually difficult due to considerable signal overlap and the lack of protons within the bases. The sugar moieties contain a rather large number of protons but do still suffer from poor signal dispersion. However, the C1'H1' region of larger RNAs can be assigned when using selectively labeled samples. The presented pulse sequence measures H2'C2' coupling constants detecting on H1'C1' resonances.

Since the ^{1}J coupling constants themselves report on multiple aspects of the present sugar conformation in RNA molecules[2], this method can directly provide structural information even more accurate when combined with other measurements, e.g. the H1'C1' coupling constant.

We demonstrate the application to a larger RNA[3] (70 nucleotides). We could show that the described experiment is capable of providing coupling constants for such a large system with high precision and accuracy in a reasonable amount of experimental time.

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Ls334

^{31}P-NMR studies of $[P_9]^+$, an $A_2A_2'BC_2C_2'$ spin system.

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The search for homopolyatomic cations of phosphorus was a big challenge in inorganic chemistry in the past. The combination of a suitable oxidant ($[NO]^+$) and a very weakly coordinating anion ($[Al(OC(CF_3)_3)_4]^-$) was necessary to synthesize the homopolyatomic phosphorus cation $[P_9]^+$ – earlier only known from mass spectrometry [1]– for the first time in condensed phase. ^{31}P-NMR spectroscopy provided the unambiguous proof for the existence and structure of this cluster cation in CH_2Cl_2 solution [2]. The $A_2A_2'BC_2C_2'$ spin system expected for the predicted minimum structure (D_{2d} symmetry [3]) was identified and fully solved. Quantum chemical calculations of the coupling constants allowed a simulation of the NMR spectrum that was appropriate as a starting point for a successful iteration by the program PERCH© [4].

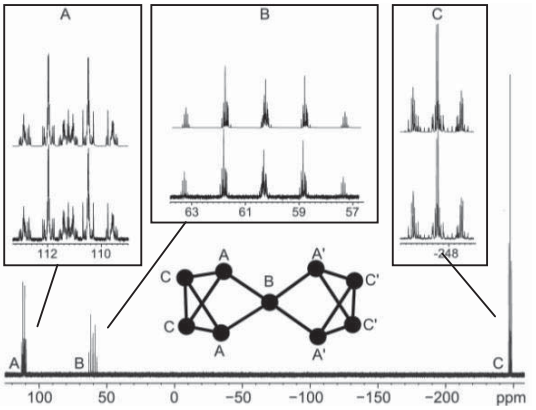

Figure 1 ^{31}P spectrum (161.99 MHz) of $P_9[Al(OC(CF_3)_3)_4]$ in CH_2Cl_2/CD_2Cl_2 at 298K (20000 scans, standard: aqua. H_3PO_4 (85%)). In the lower trace the experimental spectrum is shown. The boxes enlarge the individual signals of $[P_9]^+$ (lower trace: experimental spectrum, upper trace: calculated spectrum).

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Ls335

Using NMR for Direct Determination of K_M and V_{max} of Enzymes from Progress Curves Analysis utilizing the Lambert W Function

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For the determination of kinetic parameters of enzymes, K_M and V_{max}, many spectroscopic methods are applied frequently. NMR spectroscopy, by which substrates and products can directly be quantified, is however rarely used. NMR requires no labeling of the substrate(s)/product(s) and often allows a direct analysis of the stereochemistry of the initial reactions products.

^{1}H NMR spectroscopy was used to follow hydrolization of sucrose by invertase. The Michaelis Menten parameters were determined from progress curves at only one concentration. Furthermore, the reaction progress was monitored at various initial concentrations of 3.5 to 41.8 mM to apply an initial rate analysis. Using the Lambert W solution of the Michaelis Menten equation, K_M and V_{max} were fitted to obtain the experimental progress curves at each concentration.[1,2] The result was compared to the initial rate analysis and gave the same data, K_M= 28 mM and V_{max} = 13 µM/s, in much less time.

Progress curve analysis of enzyme reactions has the advantage that the reaction progress is monitored over the full reaction time, providing information about the dependence of reaction rates on substrate and product concentrations. Product inhibition is easily detected by recording progress curves at two initial concentrations. This approach can also be used for direct characterization of enzyme inhibition by NMR spectroscopy.

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Ls336

PCS NMR of DOTA-M8 tagged human Carbonic Anhydrase II

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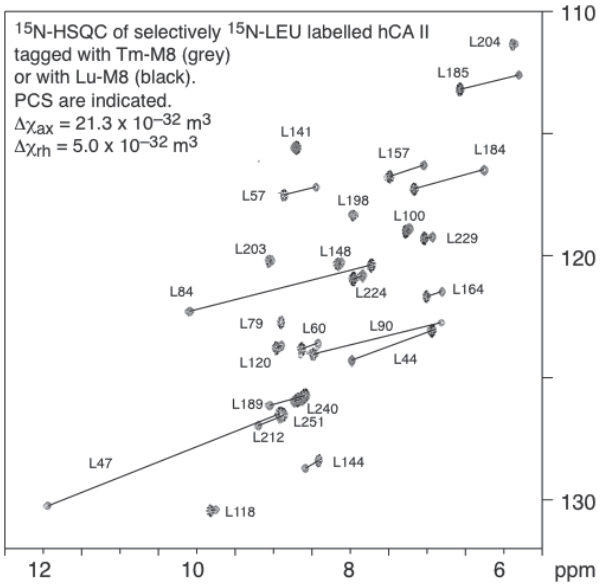
Synthetic lanthanide chelating tags (lct) that are site-specifically attached to a protein induce pseudo-contact shifts (pcs) in the nuclei of the protein. This long-range effect can be used to determine structure and dynamics of proteins and their complexes [1]. We have recently developed [2] an unusually rigid, high affinity chelating tag, DOTA-M8. Here we present applications of this tag to the monomeric 261 residue protein human carbonic anhydrase (hCA II). Host guest transition metal complexes bound to hCA II [3] have successfully been applied as synthetic metalloenzymes in homogeneous catalysis. The PCS NMR experiments aim at a structural characterization of these protein - ligand complexes in order to understand the outstanding catalytic properties of this complex system.



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Ls337

Photocaged puromycin for time-resolved experiments on nascent polypeptide chain release

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To study translation-arrested ribosome-nascent chain complexes (RNCs) and post-translational folding events, we have developed a trigger mechanism to facilitate a rapid, concerted release of nascent polypeptide chains from the ribosomal complex. By mimicking a tyrosyl-tRNA at the ribosomal A-site, the antibiotic agent puromycin acts as a covalent, codon-unspecific nascent chain acceptor and facilitates the release of nascent polypeptides from the ribosome. We have protected the nascent chain accepting amino functionality of puromycin by means of a widely used photocage to initiate nascent chain release by laser excitation at 350 nm inside an NMR sample tube.

We are currently applying the compound to investigate nascent chain release kinetics of two model systems: Venus YFP and firefly luciferase. Immediately after the laser-induced photorelease of puromycin, the folding degree of the released polypeptide chains is monitored directly by fluorescence spectroscopy and a chemiluminescence assay, respectively.

As proof of concept, we have prepared isotope-labeled RNCs of Venus YFP and are characterizing the folding state of the nascent chain by NMR spectroscopy using fast acquisition time-resolved NMR experiments. Furthermore, we are comparing the amount of native Venus backbone structure before and after the optically triggered uncaging of puromycin, which is embedded into the pulse sequence of the NMR-experiment.

Ls338

Enantiomeric discrimination using desktop NMR spectroscopy

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In this work we present NMR spectroscopy measurements of alanine in gelatine samples measured with a mobile 1 T magnet. The sensor, built from permanent magnets, is based on a recently proposed geometry[1] that achieves high homogeneity thanks to the implementation of a powerful mechanical shimming approach[2]. To improve its performance, the system was temperature stabilized. Samples of pure L-Ala and a mixture of L-Ala/D-Ala in 1:1 ratio were added to gelatine, which works as a chiral orienting medium[3]. Gelatine and solute were introduced into a flexible perfluorinated elastomer tube[4], which by a simple stretching action allows for a rapid and free scaling of the alignment to a desired degree. 1D and 2D spectra were measured for different extensions. The stability and homogeneity of the sensor made it possible to quantify the values of the homonuclear couplings ${}^3T_{ab}=D_{ab}+{}^3J_{ab}$ between alpha and beta protons. The induced anisotropy in stretched samples leads to different values of ${}^3T_{ab}$ for each enantiomer because of the presence of residual dipolar couplings. The possibility to obtain this information under simple experimental conditions and using a desktop NMR system is of great potential for the pharmaceutical industry.

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Ls339

High-resolution spectroscopy with a desktop NMR system

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During the last decade several magnet designs based on a Halbach arrays has been proposed for desktop NMR instruments. However, due to variations of the polarization of the magnet pieces used in the assembly, the magnetic field generated by real magnets is strongly inhomogeneous. The field variation is such that only a small fraction of the bore can be excited. To shim this type of magnets we have recently presented a robust method useful to correct strong field inhomogeneities of the order of several thousands of ppm. The first implementation of the method was to generate a spot of highly homogeneous field (fraction of ppm) outside the magnet for single-sided 1H spectroscopy. Later, the method was extended to correct the field inhomogeneities of a Halbach magnet for MRI. In that case, a second Halbach array made of movable pieces was placed in the bore of an existing Halbach to efficiently generate first and second order shim terms. Finally, the method was refined by incorporating the movable pieces into the main magnet design and extending the correcting shim terms to higher orders. Here we present the performance of our last magnet generation working at a field strength of 1 Tesla. To offer the highest performance the magnet is temperature stabilized and furnished with shim coils generating up to second terms. Moreover a lock system is used to correct magnetic field drifts.

Ls340

Microfluidic Remote Detection of a Xenon-Based Molecular Sensor

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Xenon-based molecular sensors (biosensors) have been developed to sensitively report on their chemical environment. In combination with lab-on-a-chip technology, these biosensors could be implemented in microfluidic-based chemical and biological assays. The sensitivity of hyperpolarized xenon (hp-Xe) to its local environment has been shown to distinguish high concentrations of ss- versus ds-DNA by a change in chemical shift[1]. Here detection of the biosensor on a microfluidic chip is demonstrated with chemical exchange saturation transfer of hp-Xe (Hyper-CEST)[2] and remote detection methods[3].

A microfluidic chip with a well was fabricated and affixed with ssDNA twenty basepairs long[4]. The complimentary strand of ssDNA was attached to a cryptophane cage. During detection, the MR signal was depleted by pulsing on the biosensor-associated ^{129}Xe resonance frequency. Immediately after the pulse, the xenon solution peak was stroboscopically detected with the resulting dip in the travelcurve showing the presence of the biosensor.

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Ls341

Modeling and Inhibitor Studies of Gamma-Butyrobetaine Hydroxylase (GBBH) and Trimethyllysine Dioxygenase (TMLD)

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GBBH and TMLD are enzymes that belong to the separate family. They participate in the biosynthetic pathway of L-carnitine and catalyze the formation of L-carnitine from gamma-butyrobetaine [1]. Gamma-butyrobetaine hydroxylase has the X-ray structure [2] which was used in calculations. TMLD does not have X-ray structure yet, so it was modeled using GBBH crystal structure as a template due to satisfying similarity. The possible steric strain in obtained TMLD structure was released by 2ns molecular dynamic calculations. The modeled structure was used for ligand docking by InducedFit docking program [3].

The GBBH enzyme interaction with potential inhibitors was also studied by NMR techniques. Several mixtures of corresponding ligands were prepared and NMR ST1D and $T_1\rho$ experiments were performed. The spectra obtained were analyzed and compared with computed data and known activity (IC_{50}) values.

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MP342

Structure - dynamics heterogeneity in amorphous polymers via the molecular vs. atomic probes: *cis-1,4-Poly(isoprene)*

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The continuing problem in physics of condensed matter is to understand the structure and dynamic evolution in disordered materials on going from their normal liquid state through the supercooled liquid one towards the glass. One way to characterize microscopically the structural and dynamic heterogeneity of glass-formers is to use special external probe techniques such as the atomic ortho-positronium (o-Ps) via positron annihilation lifetime spectroscopy (PALS) [1] and stable free radicals, the so-called spin probe by using electron spin resonance (ESR) [2]. In our contribution we report on a joint ESR and PALS investigation of the structural - dynamic state in a typical amorphous polymer of diene type: *cis-1,4-poly(isoprene) (cis-1,4-PIP)* via the above-mentioned molecular and atomic probes. By comparison of the ESR and PALS responses over wide temperatures ranges, a number of the mutual correlations between the annihilation parameters, being related to free volume, and the dynamic ones of both the external probes have been revealed and discussed in the structure - dynamic terms in detail.

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MP343

Nuclear Magnetic Resonance Study of Inorganic NanoMaterials

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Nuclear Magnetic Resonance (NMR) is an excellent tool in studying nanomaterials at the atomic level. I will report on a NMR study of the local crystal structure, electronic structure, nature of chemical bond and defects in boron nitride nanotubes, tungsten and molybdenum sulfide fullerene-like nanoparticles, dithallium selenide nanorods, and vanadium oxide nanotubes. The properties of the corresponding bulk samples vary from wide gap semiconductors to semimetals. The data obtained on the nanosized compounds will be compared with those of the bulk ones. Our research elucidates when the properties of nanomaterials differ significantly from those of bulk samples, and when this difference is small or nearly absent.

Our studies also show that some nanoparticles reveal core-shell structure, while the others do not. This problem will also be discussed in the presentation.

MP344

Diamond Nanoparticles with Functionalized Surface – a NMR Study

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On-purpose functionalization of the nanodiamond (ND) surface with targeted species allows preparation of NDs with specified chemical, physical and electronic properties. Here we review our recent NMR studies of structure, chemical bonding and defects in fluorinated (F-ND), hydroxylated (OH-ND) NDs and those with surface decorated by transition metal ions (Cu-ND and Co-ND).

In F-ND, we obtained formation of different fluorocarbon groups on the nanodiamond surface, which substitute for hydrocarbon and hydroxyl groups. Our data provide detailed information about the structure and bonding in both diamond core and surface of the F-ND particle. F-ND sample has a significant number of paramagnetic defects, resulting in fast ^{19}F and ^{13}C nuclear spin-lattice relaxation.

OH-ND reveals signals from the diamond core, surface CH_x, C-OH groups and adsorbed moisture. Its surface is greatly enriched with the OH-groups. To distinguish between contributions from ND particle and surface moisture, pumped samples were measured.

^{1}H, ^{13}C and ^{19}F spin-lattice relaxation is mainly dominated by the interaction of nuclear spins with the paramagnetic centers, represented by broken dangling bonds with unpaired electrons.

NMR studies of Cu-ND and Co-ND show the increase in the ^{1}H and ^{13}C spin-lattice relaxation rates with increasing transition metal amount, revealing appearance of paramagnetic Cu^{2+} and Co^{2+} complexes at the ND surface and their interaction with the hydrogen and carbon nuclear spins.

The magnetic resonance findings are well supported by EPR, FTIR, XPS and Raman studies.

MP345

Iron Phosphate Glass Modified Jute/PP Composite as Shielding Material

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Shielding design for spectrometers is relatively straightforward depending upon the type of radiation (gamma, neutron, alpha, beta). In this work, we have explored a pathway of fabricating composite material toward the design of magnetic resonance-compatible gamma shields. Jute fabrics (hessian cloth) reinforced polypropylene (PP)-based composites (30% by weight) were prepared by compression molding. Tensile strength (TS), tensile modulus (TM), bending strength (BS), bending modulus (BM) and impact strength (IS) of the composites were found to be 28 MPa, 280 MPa, 31 MPa, 440 MPa and 18 kJ/m^2, respectively. Iron phosphate glass (IPG) (compositions: Na_2O-CaO-MgO-Fe_2O_3-P_2O_5) powder was incorporated onto jute fabrics by hand lay-up technique for shielding purposes and then PP-based composites were fabricated. The mechanical properties of both composites (jute-PP and jute-IPG-PP) were compared. It was found that the values of TS, BS, TM, BM, and IS of jute-IPG-PP composite improved significantly than those of jute-PP composite. Jute-IPG-PP composite was found 85% increase in TS, 148% increase in BS over those of jute-PP composite. It was also found that TM, BM, and IS increased to 157, 172, and 94%, respectively. Water uptake and degradation tests of the composites were also performed. Mechanical and thermal properties of the composite shows that such composite materials can under some conditions reach very high shielding efficiencies while being easily moulded to any shape or dimension.

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MP346

Solid-State NMR Studies of Deuterated CMPs

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Conjugated microporous polymers (CMPs)[1] are a type of polyyne network that have advantageous properties, such as tuneable micropore size and surface area, and the ability to swell. Changes in porosity can be achieved by varying the aryleneethynylene strut lengths,[2] which may allow the networks to be used as gas storage materials.[3] Owing to limited solubility and the amorphous nature of these materials, little information is known about their structure and characterisation is dominated by NMR. In addition to previous structure elucidation by ^{1}H-^{13}C CP/MAS NMR,[2,3] we have used ^{2}H NMR to investigate these materials. This technique is sensitive to detailed molecular mobility and can reveal structural information and origins of porosity. We have observed immobile and mobile components of the network even at room temperature. For a partially deuterated CMP-1 network, the intensity of phenyl ring flipping has been shown to change with varying temperature (Figure). We will discuss swelling experiments for CMP-1 with benzene-d_6 to investigate changes in porosity for swollen and non-swollen networks. All of the information collected is complimented by molecular modelling. This data can be used give an overall picture of the origins of flexibility and physical properties for CMP networks, ultimately leading to the design of such materials.

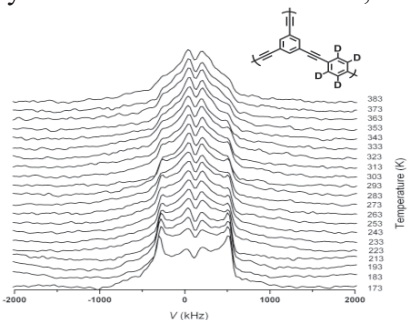

Figure: ^{2}H VT NMR and structure (inset) of deuterated CMP-1.

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MP347

EPR and luminescence of photoexcited states in NH_4BPh_4, $KBPh_4$

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Nowadays, the search for novel materials has a great importance, especially those of them whose properties could be easily changed by external influences. Such materials are potentially applied in the rapid developing areas of nanoelectronics and spintronics. Tetraphenylborate (TPhB) is a tetrahedral type of organoboron compounds, which are widely used as materials for optical and electronic applications. Meanwhile the luminescence effect of the complexes has not been properly studied. We revealed[1] for NH_4BPh_4, $KBPh_4$ at T=77 K a self-excitation luminescence with wavelength max at λ=460 nm and long relaxation times: t_1=0.33 s and t_2=0.053 s. Further investigation of the phenomenon has showed that photoexcitation at 77 K resulted in a set of levels appearing in the forbidden band, which are responsible for the appearance of intensive luminescence. Experiments showed that UV irradiation leads to the capture of the excited electrons on phenyl rings of neighboring molecules, thereby forming electron-hole pairs with different distance between an electron and a hole. For practical applications electron-hole pairs with large distance between an electron and a hole must be remove, therefore we used mesoporous frameworks to limit the sample size. Solutions studies of TPhBA, TPhBK have showed that UV irradiation at 77 lead to formation of phenyl radical, but for bulk samples it not observed in connection with the so-called "cage effect".

In this work we consider a question of O_2^- sorption on surface of mesoporous structures, and examine electron-hole recombination for all samples. Stable excited triplet states in TPhBA at low temperatures could provide a means of creating high-density recording optical memory elements.

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MP348

The Influence of impurity transition metal ions on the luminescent propirties of $Li_2Zn_2(MoO_4)_3$ crystals

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Extensive application of scintillation crystals in medicine, nuclear research and space programmes stimulates the search for new scintillation materials with higher light output, light emission in a definite wavelength range and shorter afterglow times. In recent years there have appeared publications on growing and studying properties of $ZnMoO_4$ crystals and $Li_2Zn_2(MoO_4)_3$ double molybdate crystals. This compounds began to attract research interest, in particular, due to the fact that they can solve the problem of registration of the neutrinoless double β-decay. In our work, the right conditions of $Li_2Zn_2(MoO_4)_3$ crystal growth were selected on the basis of corrected phase diagram and large optically homogeneous and virtually stoichiometric undoped and activated by transition metal ions (Cu, Cr, Fe, Ti) crystals were grown. Charge state and structural position of transition metal ions were established by EPR method. Investigation of luminescence shown that the luminescence with λ = 388 nm is observed for undoped crystals at room temperature. The luminescence lifetime is very short and described by two exponential components, with relaxation times τ_1 = 2 ns и τ_2 = 6 ns. The luminescence with λ= 560 nm and lifetime τ = 100 nm is observed as for undoped, so as for activated by transition metal ions crystals at 77K. Besides, the luminescence intensity with λ= 560 nm depends on nature and concentration of transition metal ions. It is supposed, that cation vacancies, which ensure for charge compensation of the impurity transition metal ions, are responsible for the low-temperature luminescence.

MP349

NMR Investigations of Coil-Globule Phase Transition in Aqueous Solutions of Thermoresponsive Polymers

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It is well known that thermoresponsive polymers show in aqueous solution a coil-globule transition followed by aggregation and formation of so-called mesoglobules. Their thermosensitivity makes these polymers interesting for miscellaneous biomedical and technological applications, e.g., as drug release polymers. NMR spectroscopy can play an important role in investigations of these systems [1].

Formation of globular structures results in a marked line broadening of a major part of polymer segments in NMR spectra. The fraction of units with significantly reduced mobility can be determined from integrated intensities in high-resolution NMR spectra. Application of this procedure on D_2O solutions of poly(*N*-isopropylmethacrylamide (IPMAm)-*co*-acrylamide (AAm)) random copolymers indicates that in respective mesoglobules there are domains where both hydrophilic AAm sequences (units) and surrounding IPMAm sequences are hydrated and therefore mobile.

The behaviour of water during the phase transition we mainly studied on poly(vinyl methyl ether) (PVME)/D_2O solutions. ^1H spin-spin relaxation measurements show that in semidilute solutions a portion of HDO is bound in mesoglobules with fast (ms) exchange between bound and free sites. In contrast, a 3 order of magnitude slower exchange in comparison with semidilute solutions was found in highly concentrated PVME/D_2O solutions (c = 20-60 wt%) using 1D NOE NMR experiment. These results can be explained by diffusion of water molecules through mesoglobules and by different size of mesoglobules in both cases as determined by optical microscopy.

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MP350

Phase Composition and Molecular Mobility in Polyamide Films in Relation to Oxygen Permeability

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The effect of phase composition and molecular mobility on oxygen permeability is studied for stretched films prepared from polyamide 6 (PA6) and a blend of PA6 with semi-aromatic amorphous polyamide (aPA) - PA6/aPa. Changes of these parameters upon strain of films are determined by ^{1}H NMR relaxometry. Molecular mobility in the amorphous phase of stretched films is largely restricted upon increasing strain. At temperatures well above T_g, the amorphous phase consists of two fractions: one behaves like glassy polyamides and the other one, a semi-rigid fraction, reveals largely hindered chain mobility. The amount of the glassy-like fraction increases proportionally to the strain. In addition, molecular mobility in the semi-rigid fraction decreases with increasing strain. It is shown that the immobilization of the amorphous phase has a large influence on the permeability of the films. It appears that oxygen permeability correlates well with a parameter describing strain-induced decrease in molecular mobility in the amorphous phase. This parameter is the reciprocal product of the amount of the semi-rigid fraction at temperatures well above T_g and molecular mobility in this fraction as determined by NMR T_2 relaxation time. It is suggested that the semi-rigid fraction of the amorphous phase could be considered as "channels" for diffusion of oxygen molecules. Despite lower oxygen solubility in PA6 films, as shown by low-temperature proton NMR T_1 relaxation data, the permeability of all PA6/aPA films under humid conditions is significantly lower than that of PA6 films. It is suggested that the lower permeability of PA6/aPA films is due to complex formation between oxygen molecules and aromatic rings of aPA, which slows down oxygen diffusion.

MP351

A Solid-state NMR Study of Boric Acid Doped in Poly(vinyl alcohol)

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A multinuclear solid-state NMR study of boric acid doped in poly(vinyl alcohol) (PVA) will be presented. Usually a small amount of a boric acid is added to modify the physical properties of PVA films, such as mechanical strength and water resistance. It is believed that the doped boric acid plays an important role in crosslinking. Thus far, various schematic representations for the crosslinked structures have been proposed, most of which are known as the di-diol model. In this model, a boron site involved in the formation of crosslinks has a coordination number of four.

Nevertheless, the detailed structure for the boric acid crosslink is not understood completely probably because it is difficult to obtain the molecular structure of a crosslinker in a polymer that contains both crystalline and non-crystalline regions. In the present study, the experiments used for PVA films include ^{1}H windowed Phase-Modulated Lee-Goldburg (wPMLG), and ^{11}B Magic-Angle Spinning (MAS), ^{11}B stationary NMR, ^{11}B Multiple-Quantum MAS, and ^{1}H-^{11}B CP and HETCOR at 14.1 and/or 21.6 T. The spectral simulations using density matrix calculations are carried out for obtaining ^{1}H and ^{11}B NMR parameters, and accurate quantitative information on boron sites in the PVA films. The present analysis strongly suggests the possibility that the boric acid serves as a crosslinker with a coordination number of three for the first time, contrary to expectations from the previously proposed di-diol model.

MP352

Structural, Dynamical and Morphological Studies of PE Pipes due to the Storage under Hydrostatic Pressure and at Elevated Temperatures

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Since many decades polyethylene (PE) plays an important role in our daily life. One of its main applications is for pipes to transport water, gas, and sewer. The standardized life time of this material is supposed to be 50~100 years under normal operation conditions. However, the actual service time of the pipe system depends on the local service conditions. In order to predict the remaining service time of the pipe systems and to search for ways of extending this time, the understanding of the material's aging mechanisms is necessarily.

In the present study, PE pipes aged for various times under hydrostatic pressure and at elevated temperature were investigated by a combination of ^{1}H and ^{13}C solid state NMR methods performed under static and MAS conditions. Based on the NMR data three different phases were observed based on differences in the chain dynamics: a rigid phase, an intermediate one, and a soft amorphous phase. It could be shown that not only the amounts of the three phases are changing with the aging time but also their chain dynamics. The most pronounced change was detected in the rigid phase whose amount and domain size are increasing with the aging time. This can be attributed to two aspects: (1) the intermediate and soft amorphous phases harden due to the creep of the chain segments caused by chain elongation under the pressure; (2) the elevated temperature gives rise to an annealing process which perfects the crystalline structure. As a consequence, the chain segments become more restricted with the aging time, as is also observed by relaxation measurements, which is the main reason of the embrittlement of the pipes eventually followed by complete failure.

MP353

EPR investigation of the evaporation-induced self-assembly of ordered mesoporous carbon

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A family of ordered mesoporous carbon prepared by a soft-templating approach similar to the synthesis of silica materials, has been reported by Zhao's group.[1,2] These materials were synthesised using poly(ethylene oxide)-poly(propylene oxide)- poly(ethylene oxide) (PEO-PPO-PEO) pluronic block copolymer as structure directing agent and resol as carbon precursor. They could be produced following a solvent evaporation induced self-assembly (EISA) process or in aqueous media. Using a polyurethane support to produce the material as a monolith,[3] we could investigate the EISA route at a molecular level, using in-situ X-band electron paramagnetic resonance (EPR), in combination with electron spin echo enveloppe modulation (ESEEM) and double electron-electron resonance (DEER) pulse experiments. Two spin probes derived from pluronics with PEO and PPO chains of different lenghts were used to probe different regions of the system. Experimental evidences were obtained, which demonstrate interactions between resol molecules and the PEO and the PPO blocks. The composite resol-pluronic self-organizes upon removal of the solvent in a different way as compare to pluronic alone. Upon thermopolymerization of the resol, the resol is driven out of the PPO part, and polymerizes around the PEO chains, similarly to what has been reported in the synthesis of pluronic templated ordered silica material.

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MP354

Evidence for the co-existence of distorted tetrahedral and trigonal bipyramidal aluminium sites in $SrAl_{12}O_{19}$ from ^{27}Al NMR studies

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$SrAl_{12}O_{19}$ is a ceramic material having the magnetoplumbite-type structure, similar to that of the well known hexagonal strontium ferrite used for permanent magnetic applications, with multiple Al coordination environments and finds applications in the field of photoluminescent materials. The aim of this study was to synthesize single phase $SrAl_{12}O_{19}$ and understand the coordination behavior of Al using ^{27}Al solid- state NMR techniques. An earlier ^{27}Al solid-state NMR study had reported that five different Al sites are present in this system: one AlO_4, one AlO_5, and three AlO_6 sites[1]. However, in a recent study, it has been argued that the AlO5 site is not really a five coordinated site but a distorted AlO4 with a very high quadrupolar coupling constant (~20MHz)[2]. This has been explained using the ''split atom model" for that particular Aluminium site. Our aim was to resolve the issue on the coordination environment of the AlO5 site and find out the exact number of Al sites in this system and their coordination behavior using MAS and 3QMAS NMR experiments. Single phase $SrAl_{12}O_{19}$, as evidenced from detailed powder XRD studies, was synthesized by a citric acid precursor method and by heating the calcined precursor at 1200°C. We have been able resolve five distinct Al sites clearly: one AlO4 and three AlO6 and the AlO5 site unambiguously from the 3QMAS NMR experiments at a field of 7.05T. In addition, we have found evidence for the presence of a distorted AlO4 site from the studies at high fields (16.4T and 17.6T), showing that both the distorted tetrahedral and trigonal bipyramidal aluminium sites are simultaneously present in this system[3].

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MP355

Probing the Time-Scale of Local Chain Dynamics in Poly(ethylene) Crystallites by Simple Low-Field NMR Investigations

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Helical jumps of poly(ethylene) (PE) chains in the crystallites based on 180° chain flip motions are a well-known phenomenom[1,2]. To directly measure a correlation time of this jump motion is difficult as most NMR interactions do not change under the 180° flip process. Early yet non-quantitative investigations revealed an effect of the flips on the ^1H line width[2], while recent advanced ^{13}C-based studies[1] were very time consuming. In our approach we detect changes of the dipolar coupling between protons of a flipping chain and protons of neighboring chains based on simple and fast low-field ^1H NMR methods. For this purpose we use FID component decomposition and magic-sandwich echo experiments to measure the relaxation behavior of the crystalline signal contribution of PE, possibly enabling quantitative results on flipping rates. Using this technique we compare the chain-flip motion in a variety of PE samples, differing with regard to morphology. According to our results the speed-up of long-range chain diffusion between crystalline and amorphous regions in adjacent-reentry-like morphologies is not correlated to faster dynamics of the elementary jump process as no essential difference is detectable in the time-scale of the chain flip process for adjacent-reentry and switchboard-model-like morphology. Results for a nanoparticle sample however hint at higher jump rates as compared to the other sample systems in combination with differences in lattice expansion.

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MP356

Self-Diffusion Coefficients of Small Penetrants in Semicrystalline Polymers using Single-Sided NMR

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The estimation of self-diffusion coefficients of small penetrants in semicrystalline polymer materials is of great importance in obtaining information about their interaction with the polymer matrix. However, few studies about such measurements by NMR are reported to date mainly due to the low values of these coefficients and therefore to the difficulty to estimate them using standard NMR setups. Here we show that such problems can be overcome by taking advantage of the strong and uniform gradient of a single-sided NMR sensor. The approach is demonstrated on polyethylene samples with different degrees of crystallinity which are fully saturated with n-hexane and toluene. The self-diffusion coefficients are estimated using a constant gradient stimulated echo (SGSTE) pulse sequence appended with a Carr–Purcell–Meiboom–Gill (CPMG) echo-train during the detection period. The obtained values are in good agreement with published results on similar samples.

Furthermore, it is also shown that one-dimensional profiles of the self-diffusion coefficients across the thickness of the polymer material can be obtained in a reasonable amount of time with demonstration on various polyethylene samples fully saturated with n-hexane. In such way, important information about the heterogeneity of the polymer can be gained.

MP357

Changes in microstructure of wood caused by thermal modification: PGSTE NMR, remote detection MRI and NMR cryoporometry studies

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Thermal modification is an environmentally friendly method for increasing the lifetime and properties of timber products. In this work, we investigate the changes in microstructure of *Pinus Sylvestris* pine wood caused by thermal modification using sophisticated NMR techniques.

The changes in the tracheid cell dimensions are studied by PGSTE NMR of a fluid absorbed in the cells [1]. The experiments show that thermal modification decreases the cell dimensions in all the three orthogonal dimensions. In addition, the results imply that the wood cell wall structure begins to be destroyed above a critical modification temperature [2].

Fluid flow paths in wood are investigated by remote detection MRI. Adjacent tracheid cells are connected by tiny pits, allowing water and nutrients to travel between the cells. Time-of-flight images of hyperpolarized xenon gas flowing through wood samples reveal that a large amount of the pits are closed in thermal modification [3].

Wood cell walls contain small pores in between microfibrils, whose size is on the order of nanometers. We investigate the size distribution and amount of the nanopores by NMR cryoporometry. Results imply that a major part of the nanopores are closed in thermal modification, preventing the swelling of wood when exposed to moisture.

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MP358

Identification of hydration residues in potato cell wall pectin by ^{2}H and ^{13}C solid-state MAS NMR spectroscopy

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Hydration of polysaccharides is an important aspect in a range of processes taking place in the biological processes in plants and also in relation to enzymatic processing as enzymes require water in order to function. Presently, rhamnogalacturonan-I (RG-I) (M_w ~1.5 MDa) from the main cell wall in potatoes was analyzed at various hydration levels (using D_2O) by solid-state ^{2}H and ^{13}C MAS NMR spectroscopy. In order to further elucidate the hydration mechanisms enzymatically modified versions of RG-I were explored by the same approach.

During the hydration process ^{2}H single-pulse (SP) MAS, ^{13}C SP/MAS and ^{13}C cross-polarization (CP) MAS spectra were recorded at each hydration level. This facilitated observation of the structural impact on the deuterium sites in HDO/D_2O as well as on carbon sites in the polysaccharides. Furthermore differences between the ^{13}C SP/MAS and ^{13}C CP/MAS spectra allowed for identification of more or less mobile carbon sites, since only the immobile sites were observed in the CP/MAS spectra, while all carbon sites were observed quantitatively by the SP/MAS experiments.

By this approach it was demonstrated that the arabinan side chains were easier to hydrate than the galactan side chains in native RG-I. Results from the modified RG-I's furthermore suggested that the hydration properties depended on the length and character of the side chains.[1]

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MP359

Polymers under mechanical stress

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The effect of mechanical stress on polymers has been investigated. Uniaxial stress on solid polymers has been studied in low field NMR using a Halbach magnet. Stretching of elastomers results in an instantaneous shortening of T_2 resulting from partial orientation of the polymer chains, which is seen in residual dipolar coupling measured in double quantum experiments. After releasing the stress both values return to their initial value on a time scale of minutes. For the time-dependent studies relaxation experiments are more suitable, because they maybe performed significantly faster. Stress on semi crystalline polymers results in a time-dependent behavior because the polymer chains will rearrange to release the stress and even creep through crystallites. Especially after the initial formation of a neck at constant extension the time dependences shows a return of both the shortened T_2 and the enhanced dipolar couplings to their initial values on a time scale of hours.

For the investigation of polymer melts a dedicated probehead for high-temperature rheo NMR has been developed. The polymer melt is sheared in the gap of a Couette cell. Temperature effects are excluded from the he strong temperature dependence of T_1. Residual dipolar couplings vanish upon melting of the polymer simultaneously with a prolongation of T_2. Shearing the polymer melt does not result in measurable residual dipolar couplings, while T_2 increases [2]. This implies, that no significant shear-induced orientation of the polymer chains is present, on the other hand shear results in a loss of entanglements and thus more flexible polymer chain segments.

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MP360

Assessing the Solution Shape and Size of Charged Dendronized Polymers Using Double Electron-Electron Resonance

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In this report, we present double electron-electron resonance (DEER) data that suggest that highly branched dendronized polymers (denpols) in solution are macromolecules with persistent shape, a well-defined envelope, and a size independent of their environment. Macromolecules exhibiting such properties can be considered as 'molecular objects'.(*1*) By determining the distance distribution of self-assembled dianionic spin probes (Fremy's salt dianion) on the surface of the cylindrically shaped and cationic denpols, we show that the measured solution radii are in excellent agreement with the solid state radii of the neutral denpol analogues (Figure). An analytic distance distribution of particles on the lateral surface of cylinders is developed for this purpose and fitted to DEER time traces. Such, DEER in combination with site-directed spin probing provides an indirect and simple method to determine the solution shape and size of macromolecules on the nanometer scale. It furthermore shows that at least generation 4 and 3 denpols in solution may already be described as molecular objects(*2*), while so far only the generation 5 denpol was explicitly mentioned in this context.(*3*)

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MP361

Assessment of the Specific Surface Area of fat crystal networks by Diffusion NMR

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The Specific Surface Area (SSA) is widely used to predict and control physical properties of structuring agents in product formulations. Different measurement methods are in use, but the application scope varies with matrix and underlying physical models used for interpretation of the data.

Two different model systems having a coarse and fine fat crystal network were made by dispersion of solid fat in water and oil respectively using different shear forces. D-NMR was used to assess the surface-to-volume ratio of the fat crystal network for short diffusion times from which the SSA was calculated. The latter was compared with indicative values obtained with XRD analysis for the fine fat crystal network model system dispersed in oil. The ratio solid fat versus liquid phase for the model systems were chosen such that both short and long diffusion time behaviour effects were observed within the diffusion range of the D-NMR experiments.

Results for the fine fat crystal network dispersed in oil showed that the calculated self diffusion coefficient of oil for short diffusion times did not match with the theoretical value. The obvious reason is non-elastic collision between the liquid oil and the solid fat surface. It is assumed that this does not influence the calculated SSA, which was comparable with XRD analysis results.

For the coarse fat crystal network dispersed in water results showed that the calculated self diffusion coefficient of water did compare with the theoretical value, meaning elastic collision between the water and the solid fat surface. The calculated SSA was lower than for the oil continuous fine fat crystal network due to the lower applied shear force and confirmed by SEM.

MP362

Solid-State NMR studies of structure, dynamics, and host-guest interactions of Metal-Organic-Framework compounds (MOFs)

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Solid-State NMR spectroscopy is an excellent tool for the characterization of structure, dynamics, and host-guest interactions of Metal-Organic Frameworks such as DUT-8(Ni) or UMCM-1. The novel flexible MOF $Ni_2(2,6\text{-ndc})_2(dabco)$ (DUT-8(Ni)) exhibits a pronounced gate pressure effect during adsorption/desorption of different molecules, i.e., a reversible structural transition from a narrow pore system ("closed") to a wide pore state ("open") [1, 2]. This behavior is accompanied by significant changes of the electronic structure and magnetic properties as detected by ^{1}H and ^{13}C MAS NMR spectroscopy. The ^{13}C CP MAS spectrum of the "closed" compound is well resolved and shows narrow signals in contrast to broadened lines in the "closed" state (Fig. 1). This well-resolved spectrum allows the application of the full arsenal of 1D and 2D NMR techniques in order to study structure and dynamics.

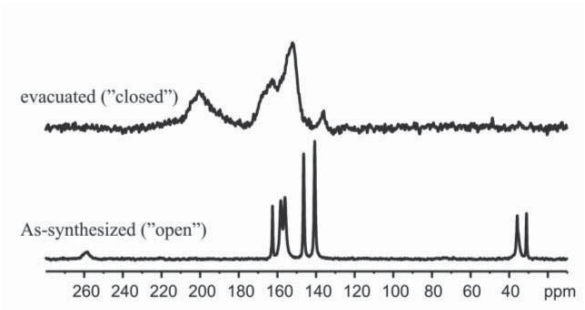

Fig. 1 ^{13}C CP MAS NMR spectra of DUT-8 (Ni)

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MP363

CW and Pulsed 180GHz HF-EPR Study of Carbon Nanomaterials

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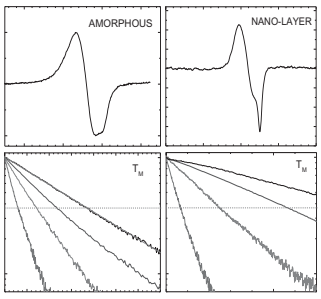
Carbon nanomatrices have been reported to show magnetic-correlation behavior, localized paramagnetic moments or spin exchange phenomena. The magnetic properties of Carbon nanomaterials are determined by the configuration of the solid matrix. Local symmetry [sp^2, sp^3], localised *pi*-networks, and metal-like energy band formation are the underlying physical mechanisms determining electrical and magnetic properties. We have used 180GHz HF-EPR spectroscopy to study the electronic configuration and lattice dynamics of three types of carbon-nanomaterials: [i] Amorphous and Nanofoam carbon [ii] Layered carbonaceous nanosheets [iii] Crystralline nanodiamonds. Using HF-EPR the *g*-values were resolved and T_1 and T_M values were recorded in the temperature range 4-300K. The HF-EPR data reveal that the principal *g*-values of the stable radicals have significant *g*-anisotropy. In these Carbon centered radicals *g*-anisotropy originated from sp^3 configurations of the unpaired electron in the solid matrix. In layered materials a perfectly flat geometry would correspond to 100% sp^2 configurations. Thus an important conclusion by the present data is that at the radical centers in the layered nanosheets are localized on sites which deviate significantly from planarity. The temperature dependence of T_1 reveals significant differences between the three types of carbon matrices.



Acknowledgments: *This work has been supported by the Greece-German IKYDA-2010 program for the promotion of the exchange and scientific cooperation [Project Contract #150].*

MP364

Study of molecular packing and dynamics of luminescent phenylene-vinylene/aliphatic multiblock copolymers by Solid State NMR

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Optoelectronic devices with polymer films as active layers have become an important topic in science and technology of today´s electronic engineering. Chemical modifications to confine the degree of the polymer conjugation to well defined values opens the possibility of engineering materials with tunable gaps to control the color of the devices emission. One way of achieving that is preparing multiblock conjugated/non-conjugated copolymers where the non-conjugated units are aliphatic spacers. However, the insertion of aliphatic chains may change the chain aggregation, which may influence the polymer emission. Thus, we present a spectroscopy study of copolymers built by conjugated p-phenylene type units (PV) of variable length and an aliphatic spacers, namely, poly[1,8-octanedioxy-2,6-dimethoxy-1,4-phenylene-1,2-ethenylene]. Solid-state NMR, Fluorescence Spectroscopy, Wide angle X-ray and Raman scattering were used to characterize the composition, molecular dynamics and packing of copolymers with increasing PPV units. The results show that the distribution of PPV chain lengths together with the formation of molecular aggregates produces pathways for exciton migration, which induces strong red shift in the photoluminescence. Besides, the molecular dynamics of the aliphatic spacers in the neighborhood of the PV units favors the appearance of non-radiative paths and phonon modes, which decrease and broaden the polymer luminescence.

MP365

Radical Photoinitiators with β-phenylogous cleavage

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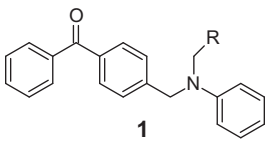
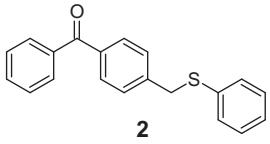
Photoinitiators are key compounds in light curable polymerization systems as they generate the reactive (radical) species and thereby have a great impact on the resulting polymer properties. Typical photoinitiator systems are bimolecular systems consisting of benzophenone and tertiary amines. However those systems have back draws because of reaction kinetics and other limitations stemming from the bimolecularity. To overcome this problem the two units have been covalently bound together[1] to from form initiators like **1** which show high reactivity and a similar reaction mechanism[2] as previously investigated compound **2**.[3]



1



2

We present results to substantiate the proposed cleavage mechanism and to discern the different products and intermediates, which lead to the high efficiency of the initiator. To that effect, the photoinduced reactivity of **1** and **2** was investigated using time resolved EPR (TR-EPR) and Chemically Induced Dynamic Nuclear Polarization (photo-CIDNP) spectroscopy.

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MP366

Structure Study on an Aluminum Phosphonated Framework Using Solid-State NMR

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Metal organic frameworks [1] (MOF) have gained increasing interest due to their potential applications as sorbents, ion exchangers, ionic conductors and catalysts. The various ordered MOF structures can meet different target demands. Therefore, study on MOF structures plays a key role in its application development.

In this work, an aluminum phosphate hybrid, prepared from aluminum nitrate and 1,3,5-tris(p-phosphonatophenyl)benzene (TPB)[2], was investigated by using solid-state NMR techniques. From 1D ^{1}H, ^{13}C and ^{31}P spectra as well as ^{13}C CP dynamics studies, it was revealed that a new, well ordered crystalline material (named Al-4) was formed after the complexation of TPB with Al^{3+}-ions. The molecular dynamics at different sites of TPB and Al-4 was quantitatively analyzed using $^{13}C\{^{1}H\}$REPT-HDOR sideband patterns, which confirmed the crystalline structure of Al-4. To further elucidate the structure of aluminum sites in Al-4, ^{27}Al MQMAS spectrum has been acquired .Combined with the previous X-Ray work[2], a structural model of Al-4 was proposed, where each oxygen is coordinated to two aluminum ions, forming an octahedral aluminum columns connecting organic TBP layers. To support this model and to refine the x-ray distance information, the ^{31}P DQ build up curve has been recorded using an improved Back-to-Back sequence [3].

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MP367

Electron Magnetic Resonance Studies of Magnetic Nanoparticles encapsulated in Novel Multifunctional Microcontainers

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Recently, significant progress has been made in the field of microcontainers responsive to a variety of stimuli. Due to their unique features, such as size, shape, morphology, and reliable response to various external or internal stimuli, they are utilized in a wide range of biomedical applications.[1] Temperature and pH responsive hollow microspheres (microcontainers) were prepared using the distillation precipitation polymerization method with magnetic nanoparticles (Fe_3O_4) encapsulated either in the shell or in the core.[2] Here we study the superparamagnetic properties of the encapsulated nanoparticles by electron magnetic resonance (EMR) methods at X-band (9.5 GHz) in the temperature range 100-400 K. The temperature dependence of some characteristic EMR parameters like peak-to-peak linewidth (ΔH_{PP}) and resonance field are in line with recently developed theoretical models for superparamagnetic nanoparticles.[3]

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MP368

Naturally Occurring Alignment media for Biomacromolecules

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In high-resolution NMR spectroscopy, anisotropic parameters like RDCs or RCSA play an important role for structure determination. For macromolecules a weak alignment is necessary to avoid extensive line-broadening. Naturally occurring polymers like gelatin and gellan gum form gels at relatively low polymer concentration. Gelatin is a well studied alignment medium for small organic molecules.[1] Another polymer gellan gum that polymerizes at much lower concentration than other common gelating agents. The alignment can be tuned depending on gellan gum concentration and composition.[2] We will introduce the alignment media and show first examples for their application to proteins.

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MP369

PEO as an Alignment Medium for Measuring Residual Dipolar Couplings in Small Molecules

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For measuring complex molecular structures residual dipolar couplings are a useful tool to define and/or verify conformation and configuration. For obtaining RDCs it is necessary to partially orient the molecules via a so-called alignment medium. A number of gel-based alignment media like polydimethylsiloxane[1] (PDMS), polyacrylnitrile[2] (PAN) or poly(methmethylacrylate)[3] (PMMA) have been developed during recent years. All these alignment media are suitable for a relatively small range of NMR solvents.

In contrast to these alignment media, PEO forms gels with a large variety of solvents ranging from apolar over polar organic solvents to water. Furthermore, PEO is applicable for a wide range of molecules like sugars, peptides, and other small molecules.

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MP370

Molecular rulers for the spectroscopic rulers DEER and FRET

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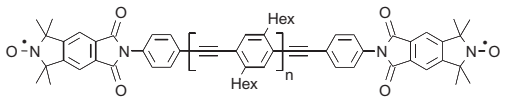
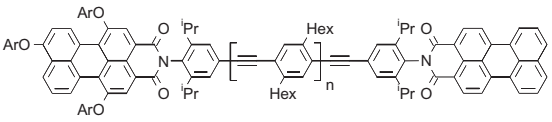
Double electron-electron resonance (DEER) and Förster resonance energy transfer (FRET) have proven to be powerful tools to obtain structural data of (supra)molecules in the amorphous state. With DEER we determined the end to end distance distributions of rod-like molecules and thus the flexibility of these compounds.[1,2] The technique was also applied to gain insight into the co-conformation of catenanes. [3] With our present work we intend to compare the two spectroscopic rulers DEER and FRET using the well-characterised rod-like molecules, either spin labeled or chromophore labeled, as molecular rulers.





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MP371

Electron Paramagnetic Resonance studies of copper (II) sorption by methafilcon A from single and multi-ions solution

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Methafilcon A is a copolymer which is used to production of the hydrogel contact lenses. By the presence of methacrylic acid in its structure, it is ionic material. In an aqueous environment, due to the negatively charged surface, is a good sorbent for metal cations.

The aim of this study was to investigate the sorption of copper ions from aqueous solution by methafilcon A using EPR spectroscopy. This method allows to identify the local structure of paramagnetic ions. EPR spectra exhibits axial pattern with $g_{II}>g_{\perp}$ (g_{II}=2,357 $g_{\perp}$=2,073) and A_{II}=130G. In this study investigated effect of concentration of the solution single-ion (Cu^{2+}) and the competitiveness of other metals (Cr^{3+}, Mn^{2+}) in solution multi-ions on the intensity of the EPR signal of Cu(II)-methafilcon A complexes. There examined also the stability of Cu(II) in the matrix of copolymer in aqueous solution.

MP372

Solid State NMR for Structural Analysis and Understanding of Adsorbate-Adsorbent Interaction of Metal Organic Frameworks

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Metal-organic frameworks (MOFs) are porous crystalline materials designed from organic linkers and inorganic connectors having 3-D network structure and high surface area.[1] $Cu_3(btc)_2$, [2] is one of the well investigated materials of this family in terms of crystal structure and surface area, and has already shown its enormous applicability in gas storage, separation, catalysis etc.

Here we present the application of solid-state NMR (SSNMR) to study $Cu_3(btc)_2$ as well as mixed metal $Cu_{3-x}Zn_x(btc)_2$. ^1H and ^{13}C SSNMR are performed, even though the presence of paramagnetic Cu^{2+} makes the NMR characterization complicated. Likewise, adsorption of small molecules, e. g., CO, CO_2 and H_2O, their interaction with the MOF framework, and the dynamics of the adsorbed molecules and the adsorption sites are investigated. It is observed that the presence of water leads the structural decomposition and the stability depends on different water contents.[3] The adsorption process of CO and CO_2, is followed over a large temperature range and multiple processes happen in this process such as increased adsorption, phase transition, and changes to the magnetic state of the MOF.

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MP373

NMR-Investigations on Local Ion Coordination Motifs in Polymer Electrolytes

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The ever increasing demand for battery systems for mobile electronics and the automotive industry (electric vehicles) has triggered a wealth of studies aiming at the development of a solid electrolyte meeting the complete property profile for an application as electrolyte in Li battery systems, which includes a high ionic conductivity, a large electrochemical window, chemical inertness as well as environmental compatibility and safety [1,2]. Especially polymer based solid electrolytes have evolved as rather promising materials, especially due to their low weight, mechanical flexibility and enormous compositional flexibility. Despite intensive research efforts during the last two decades, however, the optimization of the key property, the ionic conductivity, has not succeeded to date. Consisting of a polymer, e.g. polyethylene oxide or polyacrylonitrile, a lithium salt and variable additives, in polymer electrolytes the ionic conductivities are governed by a delicate interplay of the various electrostatic interactions between the constituents, i.e. polymer – Li-salt, polymer – additive, additive – Li salt. A specific fine tuning of these interaction may pave the way to a new generation of polymer electrolytes with optimized property profile. In our approach, these interactions are analyzed for a variety of different polymer electrolyte systems employing a range of advanced dipolar based solid state NMR methods including ^{13}C-{^1H}-CPMAS-{^7Li}-REDOR, ^7Li-{$^1H/^2H$}-CPMAS and ^7Li-{^1H}-CPMAS-REDOR.

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MP374

Continuous wave (cw) and pulsed ESR spectroscopy of metal organic framework (MOF) compounds and their adsorbate interactions

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Metal organic framework (MOF) compounds are 3D porous coordination networks consisting of metal ions or clusters connected by multifunctional organic molecules as linkers.[1]

HKUST-1 is one of the first MOF built from Cu^{2+} ions, which are linked by 1,3,5-benzene-tricarboxylate (*btc*) molecules forming so called binuclear paddle wheel units.[2] With the substitution of 1 % of the Cu^{2+} ions by Zn^{2+} ions we have successfully synthesized $Cu_{2.97}Zn_{0.03}(btc)_2$. Besides the antiferromagnetically coupled Cu···Cu pairs the structure also consists of mixed Cu···Zn pairs with paramagnetic Cu(II) centers (d^9, S = ½).[3] The Cu(II) centers are an excellent sensor for studying the interactions between adsorbed molecules like $^2H_2$, ^{13}CO and CH_3OH.

We used *cw* and pulsed ESR techniques like FS ESE, pulsed ENDOR and HYSCORE experiments to determine the isotropic and dipolar ^1H, ^2H and ^{13}C hyperfine coupling parameters of the framework nuclei as well as of the adsorbed molecules. We found $^2H_2$, ^{13}CO and CH_3OH weakly coordinated to the Cu^{2+} ions On the basis of these data we propose structural models of the adsorption complexes.

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MP375

Microstructure determination of Brominated Butyl Rubbers

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Brominated butyl rubbers (BIIR) are the most commercially important derivatives of butyl rubber. They possess higher adhesion characteristics and vulcanization rate, compared to original butyl rubber.

Variations in the type and content of unsaturated functionalities can have profound effects on vulcanization process and properties of the resultant crosslinked polymer networks. Therefore, accurate knowledge of the amount and types of unsaturation in BIIR is important. Several methods have been developed to quantitate these functionalities, but most of these procedures are too time-consuming and complex. Besides, these techniques measure total unsaturation, and are not capable of distinguishing among several types of unsaturation present in bromobutyl rubbers.

We propose a rapid procedure for simultaneous determination of total unsaturation, mole fraction of exomethylene allylic bromide structure and residual unsaturation (mole fraction of non-halogenated isoprenyl units) in one ^1H NMR experiment. Solidphase brominated butyl rubbers were chosen as targets of research, by addition to standard bromobutyl rubbers, previously investigated by the other researchers in detail[1].

NMR spectra of BIIR in $CDCl_3$ were obtained with Bruker Avance III spectrometer operating at 400 MHz. The abovementioned parameters can be determined by utilizing the olefinic (3.6-6.0 ppm) and aliphatic (0.5-2 ppm) regions of the NMR spectrum. We proposed formulas which allow calculation of unsaturation in of BIIR samples. Besides, this method makes it possible to estimate the important characteristics of BIIR even in the presence of significant amount of carbon black as a filler.

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MP376

Lysozyme sorption in hydrogel contact lenses: a study using electron paramagnetic resonance spectroscopy

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The interactions of proteins with hydrophilic materials is of importance in the contact lens industry due to their implication in allergic and inflammatory reactions. Lysozyme is one of the major protein components of the human tear fluid and the most prevalent protein adsorbed onto hydrogel contact lenses. Lysozyme is constituted of 129 amino acids, which results in a molecular weight of 14.5 kDa. Electrostatic interaction between the negatively charged hydrogel and the overall positive charge of lysozyme is thought to be the cause of lysozyme uptake by hydrogels.

The aim of this study was to investigate the practical role of electron paramagnetic resonance spectroscopy (EPR) in the lysozyme sorption in hydrogel contact lenses. We investigated hydrogel etafilcon A at soaked in lysozyme solution and UV exposure. The solution lysozyme was prepared at a concentration of 0,1mg/ml, 0,5mg/ml, 1mg/ml, 2mg/ml, 3mg/ml and doped the lenses for 24 hours. Dried samples were irradiated during the time of 1 hour. Were observed the changes in the characteristics of the EPR spectrum with increasing concentration lysozyme solution.

MP377

Investigation of the structural phase transition of MIL-53($Al_{0.98}Cr_{0.02}$) during adsorption of CO_2 with Electron Paramagnetic Resonance

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MIL-53(Al/Cr), a Metal Organic Framework (MOF) build up from chains of $Al_{1-x}Cr_x O_6$ – octahedra (x = 0.02) , which are connected with BDC (*benzendicarboylat*)-ions, was investigated by Electron Paramagnetic Resonance (EPR) at X-band (f = 9.6 GHz) after adsorption of CO_2 at pressures ranging from 0 to 2.5 bar. It is known from this material, that it changes its structure from an empty large pore phase (LP) to a filled narrow pore phase (NP) during the adsorption process at low pressure.[1] Indeed, in this work it was possible to identify the LP- and NP-phase using their characteristic EPR-Signals of the paramagnetic Cr^{3+}- centres having electron spin S = 3/2. The spin Hamiltonian parameters of these centres were determined. At low CO_2 pressures (between 200 mbar and 400 mbar) the expected change from the LP-signal to the NP-signal was observed. Furthermore it was possible to distinguish NP-phases by EPR due to different loadings of CO_2 (i. e. different pressures of CO_2). Hence in this work it was shown, that EPR can be a highly sensitive approach for the investigation of the structural transition of MIL-53 during the adsorption of guest-molecules such as CO_2. Therefore this method may be extended to investigate the behaviour of MIL-53 during the adsorption of other guest-molecules. Such projects are in progress in our group.

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MP378

UV radiation effect on contact lenses by electron spin resonance spectroscopy

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The materials which contact lenses are made can be sensitive and degradated by UV irradiation. In this paper hydrogel contact lenses were investigated using electron spin resonance (ESR) method to find the types and concentration of free radicals generated by UV irradiation and to check the degradation process due to UV radiation.

To increace the resolution of ESR spectra the computer resolution enhancement method (CREM) was used and Gaussian program for quantum-chemical calculations to confirm the obtained types of free radicals. It was stated that in some materials of contact lenses based on HEMA UV-radiation generates different types of free radicals. Two types of free radicals have been identified, one resulting from a scission of main chain polymer C-C; the second is due to attachment the hydrogen atom to the carbonyl group >C=O of the polymer.

The dynamics of free radicals in contact lenses was also investigated. It was found that total recombination of free radicals in contact lenses occurred during 24 hours after radiation. Such dynamics of free radicals suggests their potentially high biological activity and requires further study.

MP379

^{1}H and ^{13}C Solid State NMR Investigations of Zn/Co Heteronuclear MOFs

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Metal Organic Frameworks represent a new class of porous materials with potential applications[1] in, e. g., hydrogen storage, catalysis, and gas separation. Apart from the commonly studied polyfunctional carboxylate ligands, we are interested on Cd, Zn and Co MOFs based on various substituted triazole linkers[2,3]. ^{1}H and ^{13}C chemical shifts for a series of Zn MOFs are reported and compared to their corresponding free ligands. Unlike the ligand spectrum, the ^{13}C NMR spectrum of the MOF is more complicated showing a doubling of certain resonances which indicates different relative orientation of ligand units around the metal ion. Additionally, we investigated a series of Zn MOFs partially substituted with paramagnetic Co^{2+}. ^{1}H spin-lattice relaxation studies on such heteronuclear systems indicate the uniform distribution of Co^{2+} in the Zn lattice.

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MP380

Spin-Probing Room Temperature Ionic Liquids: Supramolecular Structures in Aqueous Solution

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Room temperature ionic liquids (RTIL) have emerged as intriguing reaction media in chemical engineering, in particular in the context of green chemistry.[1] Here, we report on the structure of aqueous solutions of 1-buthyl-3-methyl imidazolium tetrafluoroborate. On dilution with water, this particular RTIL forms meso-structures assembled from micelle-like aggregates of solvent cations. The spin probe methodology is used to study this process by means of EPR spectroscopy. Neutral, anionic, and cationic nitroxides, some of which resemble the imidazolium cation, are used as probes. cw-EPR spectroscopy makes assessable the micro-viscosity and polarity.[2] A marked thermal hysteresis and memory effects are found using temperature dependent studies. High-field pulsed EPR spectroscopy at W-band frequencies is employed to determine hyperfine interaction tensors and g-matrices. Polarity-proticity plots (g_{xx} vs. A_{zz}) reveal the interaction of the nitroxide moiety with apolar and ionic nanodomains, depending on the probe structure.[3] A more detailed picture of the structure of the RTIL in the immediate surroundings of the probe is obtained by means of high-field, pulse (Mims- and Davies-type) ENDOR spectroscopy and three-pulse ESEEM/HYSCORE spectroscopy. Accessibility studies are conducted on the basis of the modulation depth observed in ESSEM spectra of RTIL/D_2O mixtures. Using ^{15}N-Fremy's salt as probe, all major isotopes (^{1,2}H, ^{14}N, ^{10,11}B, ^{19}F) associated with the solvent system can be studied as a function of dilution with D_2O.

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MP381

Structural Characterization of Lithium Silicides using Solid State NMR

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Local environments and lithium ion dynamics in binary lithium silicides [1] have been studied using variable temperature static and MAS NMR experiments, showing Li site averaging on the kHz timescale at temperatures higher than 200 K, as well as phase transitions in Li_7Si_3 and $Li_{13}Si_4$. The observed shift ranges from -20 to 50 ppm indicate significant amounts of charge stored at Li as well as diamagnetic shifts induced by the Si building blocks. In the case of $Li_{12}Si_7$ the presence of the five-membered ring suggests the possibility of aromatic ring currents in this structural element, whereas for $Li_{15}Si_4$ the theoretically calculated difference in charge at the two Li sites [2] is found to have a severe influence on the shift as well. The ^{29}Si MAS spectra and 2D experiments such as JRESolved, INADEQUATE, RFDR and CP-HETCOR can be utilized to differentiate the Si sites within the framework. The static temperature dependent spectra reveal the onset of strong motional narrowing effects, illustrating high ionic mobilities. However, $Li_{15}Si_4$ seems to be geometrically restricted, which may result in a significant impediment for the use of Si as anode material.

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MP382

Elucidation of the Chemical and Morphological Structure of Double Network (DN) Hydrogels by HRMAS NMR

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^{1}H HRMAS NMR spectroscopy is applied to gain insight into the structure of double-network (DN) hydrogels, prepared from poly(2-acrylamido-2-methyl-1-propanesulfonic acid) (PAMPS) and poly(acrylamide) (PAAm), using N,N'-methylene *bis*(acrylamide) (MBAA) as a cross-linker. Diffusion filtered HRMAS spectra, HSQC and ROESY were used to address both structure and dynamic properties of the DNs. The results confirm the formation of covalent bonds between the two polymer networks through the non-reacted double bonds of the cross-linker MBAA. Evidence to the existence of strong hydrogen bond networks based on the N-H group of the PAMPS as a hydrogen bond donor and the C=O group of the PAAm as a hydrogen acceptor is provided. The findings contribute to clarify the origin of the toughening mechanism of DN gels and their unusual mechanical properties.

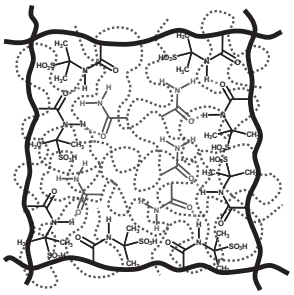

Figure: schematic illustration of the proposed hydrogen bonds formation between side chain fragments in the PAMPS/PAAm DN hydrogel. The polymer chain of PAMPS is presented with a black solid curved line, the PAAm polymer chain is given with a dash curved line.[1]

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MP383

Host-guest Interactions in Poly(N-isopropylacrylamide) Hydrogel Seen by One- and Two-dimensional ^{1}H CRAMPS Solid State NMR Spectroscopy

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The most attractive property of poly(N-isopropylacrylamide) (PNIPAAm) is its lower critical solution temperature (LCST) at 32-33 ^{o}C in pure water. Under different physical and chemical effects, small molecules can modify this temperature. In general, phenols decrease the LCST of the swollen hydrogel, but dopamine, a catecholamine, increases the LCST. In this work we compare the influence of chatechol and dopamine. The results from one- and two-dimensional ^{1}H CRAMPS spectroscopy studies suggest that chathecol acts very similarly to phenol[1]. It is attached to the acrylamide side-chains by second-order bonds. Distances were calculated from the two-dimensional correlation spectra by rate matrix analysis approach. Although dopamine forms strong H-bonds, as revealed by a highly shifted (8.5 ppm) signal, two-dimensional correlation ^{1}H spectra show that this signal does not arise from dopamine – polymer interactions, i.e., no direct relationship was found between dopamine molecules and the polymer network. It was found that the structure of dopamine in the PNIPAAm matrix differs from that either in the dry state or in aqueous solution.

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MP384

Elucidation of Host-Guest Interactions in Functionalized MIL-53 MOFs by High-Resolution Solid-State NMR Spectroscopy

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Metal-organic frameworks (MOFs) offer a huge potential for different applications like drug delivery, gas separation or gas storage and sensing. All these applications rely crucially on the interaction between the framework and the incorporated guest molecules. To obtain a better understanding of such interactions, we performed a case study on a series of functionalized MIL53 topologies. Beside MIL53, also $MIL53NH_2$ and MIL53NHCHO[1], with anchor groups providing different hydrogen bond donor acceptor pattern, were investigated. Acetone was chosen as guest molecule because of its ability to act solely as hydrogen bonding acceptor.

Both the structural aspects of the host-guest systems as well as the dynamical properties of the guests within the pores were studied based on solid-state NMR spectroscopy. For the structural studies, we make use of high-resolution ^{1}H solid-state NMR techniques at high magnetic fields, taking advantage of CRAMPS (combining rotation and multiple pulse sequences) decoupling schemes for improved ^{1}H resolution. High-resolution 2D ^{1}H-^{13}C HETCOR spectra recorded with a selective PRESTO transfer was employed to, unambiguously, assign the observed ^{13}C resonances. The protons which are not bound to Carbon atoms where assigned with the help of ^{1}H-^{27}Al und ^{1}H-^{14}N D-HMQC. To probe nuclear proximities between the protons of the different host-guest system, 2D ^{1}H-^{1}H DQ CRAMPS[2] and 2D ^{1}H-^{1}H proton-driven spin-diffusion spectra were recorded.

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MP385

NMR and HPLC as complementary methods to characterize the structure and composition of surfactants

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Ethoxylated and propoxylated fatty alcohols are known to be used as surfactants in emulsions. The impact of the surfactant structure on the stability and the properties of emulsions makes it necessary to characterize this class of chemicals consisting of hydrophilic and hydrophobic parts. The surfactant structure can be complex, due to the fact that different hydrophilic structures can be included (ethylene oxide ("EO") and propylene oxide ("PO") as two of the most applied examples), its chain length varies, the hydrophobic aliphatic part can be linear or branched or a mixture of both, and overall, the surfactant can be a blend of several single surfactants. For these reasons, separation (by HPLC) and structure analysis (by NMR) are very valuable tools for surfactant characterization.

By HPLC, the molecules can be separated on the basis of either its hydrophobic part (alkyl chain lengths) or hydrophilic part (the number of ethoxylated/propoxylated groups). Since ethoxylated/propoxylated alcohols do not contain UV-absorbing groups, they can only be detected by bulk property detectors, like evaporative light scattering detector (ELSD).

Using ^{13}C NMR the average chain length of the hydrophilic EO/PO part can be determined as well as the chain length and the branching level of the aliphatic part. The ratio of end groups of the EO/PO part versus the aliphatic part can be used to calculate the content of free EO/PO and to compare that with the complementary information from HPLC.

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MP386

NMR investigation of the state of aggregation of water in 1-butyl-3-methyl-imidazolium acetate

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Ionic liquids (IL) are generally hygroscopic and the presence of traces of water which possibly influences their thermodynamical and physical chemical properties is a matter of concern. The characterization of the state of water is therefore of relevance not only from the viewpoint of fundamental researches but has also has incidence in engineering applications.

However, only a few systems have been studied so far and as a matter of fact investigations in this domain are scarce. These studies have been mostly performed using Raman scattering and ATR infrared and mainly addressed this subject on Imidazolium based IL in which the anion was of inorganic type such as BF_4^- and PF_6^-. We have therefore undertaken an investigation of the state of water using ^1H and ^{13}C NMR spectroscopy on 1-butyl-3-methyl-imidazolium acetate, ionic liquid in which the anion is a moderate base. We have studied the evolution of the chemical shift of the resonances lines upon adding water to this IL at increasing content ranging from the dried IL up to 99% molar fraction. We found that the evolution of the chemical shifts is governed by the competition between two mechanisms namely hydrogen bonding and stacking of the rings.

MP387

Rheo-NMR investigations on polymer melts

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Polymer processing occurs mostly in the molten state. Mechanical shear influences polymer dynamic and chain order in the melt and thus materials properties. The flow behaviour of polymer melt is essential for polymer processing and end-use properties.

We apply high-temperature rheo- NMR for in-situ investigations of polymer melts under shear. For the high-temperature rheo NMR experiments a dedicated probe head operating at 220°C with an integrated Couette cell has been developed. The influence of temperature and mechanical shear on chain mobility and ordering is investigated by relaxation measurements and double quantum experiments. Imaging and velocity profiles, derived from a combination of NMR imaging with PFG NMR, have been used to control the shear.

Both T_1 and T_2 relaxation show a strong temperature dependence. Increased mobility in the melt is reflected in reduced dipolar couplings and longer relaxation times. The double quantum intensity diminishes upon melting, no significant double quantum signal has been observed under shear. Shearing the polymer results in longer transverse relaxation time, indicating enhanced polymer mobility. The heating effect from shearing is excluded because there is no change of T_1 under shear.

MP388

Ionic liquids for the analysis of sparingly soluble cellulose acetates by solution-state NMR spectroscopy

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Cellulose acetate has widespread application, but to date the characterization of their microstructures is not possible. Until now there is a lack of analytical techniques to characterize the substitution pattern of cellulose acetates which in turn limits to establish proper structure↔property relationships. Solution-state NMR spectroscopy is an important method in polymer analysis to determine the average chemical composition, the microstructure and the topology of polymers. However, the sparse solubility of cellulose acetates with a low degree of substitution (DS < 2.0) in common NMR solvents prevented so far the access with the NMR spectroscopy.

On the other hand ionic liquids (IL) are well known solvents with excellent properties (low melting point, non volatile, stable towards air and hydrolysis). They aroused an enormous interest in a broad variety of industrial applications in the last two decades. Due to their solvation properties IL are often used in cases where commonly familiar organic liquids fail to dissolve polymers.

The first results that have been achieved by using 1-ethyl-3-methylimidazolium acetate (EMIM acetate) as NMR solvent for the characterization of cellulose acetates will be presented. The dissolving power of EMIM acetate was compared with that of common solvents and the resolution of the ^{13}C-NMR spectra will be discussed. Furthermore the characterization regarding both the DS and the distribution of acetate substituents will be shown.

MP389

NMR Crystallography on Semi-Crystalline Poly(triazine imide)

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Carbon nitride materials have spurred marked interest within the material science community due to their wide range of properties and applications, e.g as catalysts or electronical and optical sensors. As the inherent low crystallinity of carbon nitrides limits the use of standard X-ray diffraction structure analysis, we apply a combined analytical approach called NMR crystallography. Combining information gained from X-ray diffraction, solid-state NMR, TEM and quantum chemical calculations allows for the buildup of refined structure models. Here, we discuss the structure elucidation of semi-crystalline Poly(triazine imide) as investigated via the NMR crystallographic method. A first structure model is com-posed of electron and X-ray diffraction data[1]. Standard 1H, 7Li, 13C, 15N spectra are recorded and signal assignment confirmed by common NMR techniques (e.g. CPPI or HETCOR). Connectivity in-formation, specific crystallographic site occupancy and distance constraints between selected nuclei are probed for further structure refinement. 1H-DQ experiments are used to measure 1H-1H proximities, while REAPDOR experiments help approximate 1H-7Li, and 15N-7Li distances. Furthermore, LGCP build-up curves allow for the extraction of 1H-15N distances. Lastly, refined structure models are then evaluated by quantum chemical calculations via CASTEP. The obtained calculated parameters (e.g. isotropic shifts, CSA and CQ values) are then compared to experimental data.

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MP390

Multifrequency EPR and Raman: Unique tools to address the role of conjugated bridges in biradicals

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By changing the length of a phenylene-vinylene bridge (n=1,3,5) a series of polychlorotriphenylmethyl (PTM) biradicals (Fig. 1) has been prepared in order to study the role of diamagnetic conjugated bridges on the intramolecular spin delocalization and hence on the final magnetic properties of these stable and persistent biradicals. Herein we present a multifrequency continuous wave EPR study carried out to determine the extent of the electron conjugation of the two PTM radical sites as well as the preliminary results of the performed simulations. Interestingly, a strong dependence on solvent, attributable to conformational changes, has been observed making us suggest that both the length of the bridge as well as its conformation play a fundamental role on the spin delocalization. Raman studies on the same system are discussed for comparison.[1]

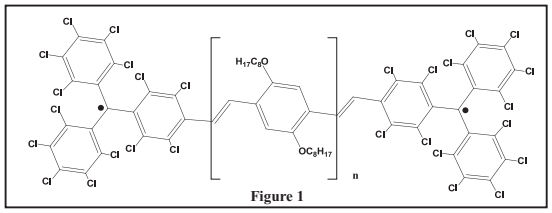

Figure 1

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ME391

Structural characterization of HAMP domain-membrane interaction

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The environment is constantly changing and hence prokaryotes need to adapt their gene expression profiles and their regulation of gene product activity to match current circumstances. The main system used by bacteria for this task is the two-component signal transduction system. Within more than 5500 different proteins in these systems a domain called the HAMP domain is found. The HAMP domain consists of two amphipathic segments (AS1 and AS2), and a control cable, connecting the two segments. HAMP domains are responsible for converting extracellular sensory input into an intracellular signaling response in predominately membrane spanning signal transduction proteins. Since the membrane has been suggested to take an active part in HAMP domain signaling, we wanted to study the influence of membrane model systems on peptides containing either AS1 or AS2 from different well characterized HAMP domains. Far-UV CD and NMR spectroscopy were used to monitor the induction of secondary structure upon association with neutral or acidic LUVs and bicelles. Upon association, we observed significant increases in α-helicity within AS1 from $NarX_{Ec}$ and Tar_{Ec}. The association with a neutral membrane mimetic was of a hydrophobic character while an electrostatic interaction enabled stronger interaction with acidic membrane mimetics. The solution structure of AS1 from Tar_{Ec} associated with acidic bicelles was determined. AS1 formed an amphipathic α-helix while the control cable remained unstructured. Two positively charged amino acids are flanking the hydrophobic part of the amphipathic helix and are thought to account for the electrostatic interactions. Our results conclude that the membrane is very likely to take an active part in the signal transduction of a subgroup of HAMP domains.

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Spin labeled WALP - A ruler for peptide immersion depth in membranes for ESEEM spectroscopy

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Obtaining information regarding peptides orientation and immersion depth in membranes is important for understanding the mode of action of membrane active peptides. Pulse EPR techniques, particularly ESEEM (electron spin-echo envelope modulation) methods, can be used to probe the position and orientation of peptides within membranes. These are based on the measurement of weak dipolar interactions between nitroxide spin labels attached to the peptide and deuterium atoms on isotopically enriched lipids that constitute the membrane and in water molecules (D_2O). Here we explore whether the well-known, α-helix transmembrane peptide, WALP, already used as a ruler for EPR measurements based on relaxation times and exposure to paramagnetic quenchers, can be used as a ruler for ESEEM as well. WALP was labeled using standard site directed spin labeling technique at positions 9, 12, 14, 17 and 20. The measurements were performed on a model membrane of DPPC/PG (7:3 w/w) in H_2O solutions with deuterated methyl groups of the choline segments of DPPC ($(CD_3)_3NCH_2CH_2$). We developed a quantitative method that enables a direct fitting of ESEEM time domain trace yielding the depth of the spin label with respect to the membrane surface and the density of the deuterons around it. In addition, the trans-membrane water profile was obtained from samples with natural abundance lipids and D_2O. We found that it is more reliable to determine the penetration depth for from deuterated lipids than for D_2O samples.

ME393

Towards a structural and dynamic characterization of the HIV viral coat protein gp41

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A homotrimeric construct encompassing residues 1-194 of the HIV viral coat protein gp41 is being investigated with TROSY-based NMR methods. Backbone assignment could be achieved for the fusion peptide, first N-terminal residues of the HR1 ectodomain region, the immunodominant loop region, parts of the adjacent ectodomain regions and the C-terminal part of the HR2 ectodomain region close to the MPER region. NMR-relaxation methods have been adapted and optimized for the dynamic characterization of these regions. Different detergents and small bicelle systems are tested to optimize the quality of NMR spectra under as close as possible membrane-like conditions. Methods for the measurement of RDCs in membrane proteins are being optimized and extended. RDCs will provide essential orientational information as well as dynamic insight, with the aim of generating a detailed structural model of gp41 including the fusion peptide and the ectodomain region. The current state of research will be presented.

ME394

Characterization of Membrane Anchored GABARAP in Nanodiscs

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Autophagy is a degradation mechanism, which sequesters retired organelles and bulky proteins by a double membrane vesicle, termed autophagosome. Formation of the autophagosome requires a number of membrane-anchored proteins. A comprehensive understanding of autophagy requires structural information of these proteins in their membrane-attached state. While conventional membrane protein solubilization methods like detergent micelles or bicelles can be employed in solution NMR, they may destabilize the native structure and thus affect protein activity. Here we applied a new membrane system, termed nanodiscs, to solubilize one of the key lipidated-proteins, γ-aminobutyric acid type A receptor-associated protein (GABARAP), which is recruited to the autophagosome membrane. In this case, ubiquitin-like conjugation of the GABARAP C-terminus to a membrane lipid was replaced by conventional thiol-maleimid chemistry. An essential component of the nanodisc system is an apolipoprotein A-I derivative, which scaffolds the lipid bilayer and the lipidated-protein to form a flat discoidal structure. Despite the molecular weight of about 160 kDa for the whole nanodisc complex, the ^{1}H line widths of the rigid GABARAP residues are below 50 Hz in our study, making them amenable to solution NMR. ^{1}H^{15}N-HSQC based structural comparison indicates that lipidation only affects the residues close to the C-terminus but not the hydrophobic pockets implicated in protein-protein interactions. Accordingly, functional studies revealed that the hydrophobic pockets are still able to selectively bind to established ligands. Our data demonstrate the superior properties of nanodiscs for investigations of membrane-anchored proteins by solution NMR.

ME395

Heptahelical membrane proteins and non-conventional membrane mimeticks

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The choice of an adequate membrane-mimicking environment is of central importance for structural and functional characterization of specific membrane proteins. However, it has been shown that the most commonly used detergent micelles are often not optimal for stability and activity of the system of interest (1).

Here we report on recent progress in studying heptahelical membrane proteins in different soluble environments using NMR. Our approach is based on the combination of cell-free protein expression (2) and the use of non-micellar environments such as lipid bilayer nanodiscs and Amphipols (3,4). Although the overall molecular weight increases by about 70% as compared to a detergent micelle, we show that high-resolution NMR studies of heptahelical membrane proteins are still well feasible in these environments. Our data also indicate that for the tested proteins the non-micellar systems are able to provide a more stable environment than the micelle. Besides to their stabilizing effect, the biggest advantage of the amphipols is their superior refolding property, whereas our experimental data suggest that the protein functional state is most stable in the lipid bilayer environment.

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ME396

Structure and topology of a membrane-bound Shaker B inactivating peptide obtained by combining solid state NMR and MD simulations

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Solid state NMR provides a powerful tool to study membrane peptides and proteins in their native environment[1-2]. Still, these systems remain notoriously challenging to study by experiments alone. In particular, information about the system topology, i.e., the orientation to the membrane or the insertion depth, not to mention lipid-protein or lipid-peptide interaction, is often sparse if not elusive. Here, we demonstrate on the membrane-bound inactivating peptide of the potassium channel Shaker B how a joint approach of solid state NMR (ssNMR) experiments and atomistic Molecular Dynamics (MD) simulations allows obtaining high resolution structure and topology of the membrane-bound peptide and how to cross validate ssNMR and MD results. In particular, by back-calculating the peptide's water accessibility[3] over the simulated trajectory, we present a new method for establishing the topology of membrane-bound peptides and proteins.

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ME397

A 3D structural view of ion channel inactivation in lipid bilayers from solid-state NMR

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X-ray crystallography has made rapid progress in the determination of membrane protein structures. However, the role of the surrounding lipid bilayer for protein structure and function has largely remained elusive. We present a solid-state NMR-based hybrid strategy that allowed us to determine 3D structures of a membrane-embedded potassium channel in two different functional states. For this purpose, we produced uniformly labeled [^{13}C,^{15}N] and fractional deuterated [^{2}H,^{13}C,^{15}N] variants of the tetrameric KcsA-Kv1.3 channel for which ssNMR resonance assignments have been reported[1,2]. Structurally relevant constraints of intra and intermolecular nature were obtained using high-resolution CHHC[3] and CC correlation experiments. Unlike to crystallographic work on the parent KcsA channel, we find distinct structural changes in the pore loop region that connects transmembrane helices 1 and 2 via the selectivity filter[4]. Further ssNMR work and electrophysiological studies identifies this channel segment as a critical protein unit that controls channel architecture and mediates functional coupling.

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ME398

Investigation of Structural Changes upon Ligand Binding of the Methylated Neuropeptide Y Receptor Type 2

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G protein-coupled receptors (GPCRs) represent very important drug targets. For any pharmacological interference, detailed knowledge about the structure and dynamics of the molecules are essential.

We are able to produce large amounts of the receptor in a prokaryotic expression system as inclusion bodies. Subsequently, the receptor was refolded into its functional state tested by phosphorylation. Our aim is to investigate ligand-specific conformational changes of the receptor by NMR spectroscopy. Therefore, we used the reductive methylation of lysine residues to introduce ^{13}C-methyl groups. Due to their favorable relaxation properties, these methyl groups allow for sensitive NMR-measurements. We detected ^{1}H-^{13}C HSQC NMR spectra, which provide some resolved NMR signals of the respective methyl groups. Chemical shift changes of some signals are observable, which are induced by ligand binding. These changes could be related to alterations of salt bridges or ring current effects. A very astonishing side effect is that the functional methylated receptor shows a dramatic increase in the stability.

ME399

Interphase Kinetics of an Integral Membrane Kinase by Time-Resolved Solid-State NMR

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The integral membrane protein Diacylglycerol Kinase (DGK) from *E.coli* is a lipid regulator and as such in focus of structure-function studies in its native environment, the lipid bilayer, by solid-state NMR. DGK catalyses the transfer of the γ-phosphate of Mg*ATP to diacylglycerol (DAG) to generate phosphatidic acid (PA) at the interface membrane-cytoplasm.

The label-free direct and simultaneous observation of interdependent reactions both within the aqueous and the lipid phase catalyzed by membrane bound enzymes is still a challenging task. A possible solution is offered by a time-resolved MAS-NMR approach which allows probing enzymatic reactions at interfaces. This technique is of particular relevance for and applicable to all types of proteins whose reactions take place in the lipid bilayer itself or at the membrane interface [1]. Based on these novel results, a model of DGK's mechanism is presented in context with the available 3D structure [2].

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ME400

Extensive conformational and dynamic changes throughout the ATP hydrolysis cycle of the ABC multidrug transporter LmrA

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Members of the integral membrane protein ATP binding cassette (ABC) transporter superfamily translocate their respective substrates across the membrane at the expense of ATP hydrolysis. To this end, the transporter passes through a number of conformational states. These states have not yet been characterized structurally and a dynamic picture throughout the catalytic cycle is still amiss. LmrA is a homodimeric multidrug ABC transporter from L. lactis and shares the typical ABC architecture with six transmembrane α-helices forming the transmembrane domain and a nucleotide binding domain (NBD). It has been strongly debated whether the power stroke for substrate translocation stems from the event of ATP binding or hydrolysis. Using solid-state NMR as well as site directed spin labeling for pulsed EPR, we were able to obtain detailed structural data on full-length LmrA throughout the ATP hydrolysis cycle: The apo state protein is highly flexible with large amplitude fluctuations of both NBD and TMD. The nucleotide-dependent transformation to the nucleotide-bound state requires large domain movements. Upon nucleotide binding the transporter progresses to a structurally more defined state where the large fluctuations are strongly restricted in both domains. Studies on the isolated soluble nucleotide binding domain with solution NMR reveal an intricate inter- and intra-domain interaction network dependent on the nucleotide bound status of the nucleotide binding domain.

ME401

α-Synuclein on vesicles: horseshoe or extended? It depends …

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Electron paramagnetic resonance (EPR) is rapidly gaining ground for structure determination in biological systems [1]. For intrinsically disordered proteins, structure determination is a particular challenge, because they adapt to their environment, can interact with many different proteins, and require flexibility for their function. Pulsed double electron-electron spin resonance (DEER or PELDOR) on spin labelled variants of such proteins presents an outcome. We demonstrate this for α-Synuclein (αS)[2-5], a protein implicated in Parkinson's disease and suggested to interact with the membranes of synaptic vesicles. On vesicles, the protein can bend into a horseshoe shape [2]. On small unilamellar vesicles, its interaction with the membrane seems to be so strong that it can partially break up the membrane, and, under these conditions, the protein forms well defined aggregates, in which two horseshoes come together in an entangled form [4]. On larger vesicles, the horseshoe and the extended forms occur side by side [5]. We speculate that the coexsistence of the two forms derives from the energetic proximity of these conformations and that it is a subtle function of the environment.

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ME402

Analysis of orientation and dynamics of transmembrane peptides using solid state ^{2}H- and ^{15}N-NMR

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Membrane-bound peptides can have important biological functions, like fusion peptides or host defence peptides, or therapeutic potential like cell-penetrating peptides. To understand the function, the orientation and dynamics of such peptides in membranes can give important clues, and this can be studied in detail using solid state NMR. We have studied transmembrane (TM) model peptides similar to the WALP family using both ^{2}H-NMR in the GALA (geometric analysis of labeled alanines) and ^{15}N-NMR in the PISA (PISA wheel analysis of PISEMA-type 2D spectra) methods and compared the influence of dynamics on the analysis (1, 2).

From a re-analysis of ^{2}H-NMR data of several peptides in up to four different lipid systems, a total of 31 peptide-lipid systems were used to get a good picture of hydrophobic mismatch-dependent tilt of TM peptides. We show that by including dynamics in the analysis in the form of distributions of orientational angles, a clear mismatch dependence is seen for WALP peptides, as expected from simple theory. However, when dynamics is ignored this mismatch response is missing. PISA analysis can give additional understanding, especially by better distinguishing between different dynamics models. We present a comparison of GALA and PISA analysis for two systems, WLP23/DMPC and GWALP23/DLPC. When dynamics is included, both methods gives almost identical results.

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ME403

Detection of water binding to Photosystem II, a multifrequency ^{1}H/^{2}H/^{15}N/^{17}O-ENDOR study; experimental determination of the protonation of the S_2 state

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The first definitive assignment of an exchangeable substrate water of the S_2 of the OEC in Photosystem II (PS II) is reported. PS II samples were suspended in $H_2{}^{17}O$ and D_2O. Hyperfine couplings of more than one coordinating ^{17}O (I = 5/2) nucleus were detected using Mims and Davies-ENDOR at 94 GHz. Three classes of nuclei are tentatively assigned: i) μ-oxo bridge(s), ii) terminal Mn-$^-$OH/H_2O ligand(s); and iii) second shell OH/H_2O ligand(s). The assignments are based on comparison to model complex data performed in tandem with measurements on Photosystem II and the comparison to the recent high resolution crystal structure reported by Umena et al.[1] The model complex data used includes the bent, bis-μ-oxo, μ-carboxylato bridged $Mn^{III}Mn^{IV}$ (DTNE) complex. Universal ^{14}N/^{15}N (I = 1, 1/2) labeling of the PS II excludes the possibility that these signals are attributable to a nitrogen nucleus. ^{1}H/^{2}H (I = 1/2, 1) ESEEM/ENDOR data of the S_2 state performed at X-, Q- and W-band complement the above findings. These measurements are fully consistent with the current electronic model of the S_2 state; the tetramer model, derived from ^{55}Mn-ENDOR studies, where all Mn ion contribute approximately equally to the ground electronic state.

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ME404

Explaining colour tuning in Green Proteorhodoposin

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Green Proteorhodopsin (PR) is a bacterial retinal protein which upon light activation is able to transport protons. Naturally, the amino acids close to the chromophor retinal influence the colour of the protein. However, recently it has been discovered that a single mutation (A178R) in the EF loop causes a redshift of the absorption maximum by 20 nm[1]. This study aims to elucidate how a change in a loop far away from the chromophor can cause a colour change of the protein.

$^{13}C^{15}N$ PR A187R and $^{13}C^{15}N$ PR wild type were expressed in E.coli and reconstituted into lipid membranes. PDSD spectra recorded on these samples show that some parts of the protein differ in those two samples while others overlay very well. Using the published assignment[2] several amino acids could be identified which are sensitive to the mutation. Indeed, the mutation affects amino acids near the chromophor. Thus the conformational change caused in the loop by the mutation is transmitted into the centre of the protein by rigid body movements. This is in line with recent results which show that the EF loop is not flexible[3]. There have been hints that PR not only serves as a proton pump but also as a sensor[4]. The EF loop could play a role in activating a potential signalling pathway as in related G-protein Coupled Receptors. This leads to the question whether a change in the chromophore influences also the EF loop structure. Retinal cis-trans isomerisation is caused by illumination of PR. Thus wild type PR was studied in the dark and with illumination, respectively.

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ME405

Structural Basis for Tail-Anchored Membrane Protein Biogenesis by the Get3-Receptor Complex

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Tail-anchored (TA) proteins are involved in cellular processes including trafficking, degradation and apoptosis. They contain a C-terminal membrane anchor and are post-translationally delivered to the endoplasmic reticulum (ER) membrane by the Get3 ATPase interacting with the hetero-oligomeric Get1/2 receptor. We have determined crystal structures of Get3 in complex with the cytosolic domains of Get1 and Get2 in different functional states. NMR titration experiments together with biochemical data show that Get1 and Get2 use adjacent, partially overlapping binding sites and that both can bind simultaneously to Get3. Docking to the Get1/2 complex allows for conformational changes in Get3 creating a force, which is directly transferred to the TMDs of the receptor and thereby could contribute to TA protein insertion. These data suggest a molecular mechanism for nucleotide-regulated targeting and receptor-assisted insertion of TA proteins (1). In future experiments it will be important to dissect the precise proceedings within the receptor´s transmembrane parts.

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ME406

Polymerisation mechanism at the outer membrane usher of Type 1 pili from *E.coli* investigated by SDSL-EPR

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Bacterial infection by uropathogenic *Escherichia coli* (UPEC) is the primary cause of urinary tract infections in Europe and North America. There is an increase in resistance to antibiotics by these bacteria and studies of the onset of bacterial infection are gaining importance. The surface fiber type 1 pili are important attachment devices that target UPEC to the bladder epithelium. These pili are assembled by the chaperone-usher pathway, involving a periplasmic chaperone (FimC) and an outer-membrane assembly platform, the usher (FimD). We are using site-directed spin labelling (SDSL) in combination with continuous wave (cw) and pulsed electron paramagnetic resonance (EPR) spectroscopy to investigate the polymerisation mechanism at the usher. Starting with the usher-chaperone-subunit complex FimD:FimC:FimH, the next chaperone-subunit FimC:FimG is added. Spin labels are positioned on the C-terminal domain (CTD) of the usher FimD and the chaperone FimC of FimC:FimG. By measuring distances between spin labels, we could show by EPR that after polymerisation, the subunit FimG binds to the CTD.[1] This result is comparable to the recently solved crystal structure of FimD:FimC:FimH where the previous subunit FimH is bound to the CTD of the usher. The involvement of the N-terminal domain (NTD) in the recruitment of the subunits is still not understood. Results on the intermediate state of the FimD:FimC:FimH:FimG complex, right after the addition of the subunit FimG and before it binds to the previous subunit FimH, will be presented.

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ME407

Solution structure of proteorhodopsin

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Green-absorbing proteorhodopsin (PR), a light-driven proton pump, shows a strong dependence of its function on the pH. The primary proton acceptor D97 has an unusually high pKa value around 7.5 and its protonation state affects the absorption characteristics of the retinal cofactor. Furthermore, the direction of proton pumping switches in response to pH between an outward directed transport at alkaline pH and an inward directed transport at acidic pH. The potential function of this pH-dependency including the changing vectoriality is, however, still debated and a possible regulatory activity cannot be excluded. For a further insight into the structure-function relationship, we have solved the solution NMR structure of detergent-solubilized PR at acidic pH. NOE data was obtained with the help of stereo-array isotope labeling (SAIL) as well as selective labeling and complemented with a large number of distance restraints derived from paramagnetic relaxation enhancement (PRE). Additionally, restraints from residual dipolar couplings (RDCs) served to improve the structural accuracy of this seven-helix-bundle. The three-dimensional structure of PR reveals differences from its homologues such as the absence of the extended β-sheet in the B-C loop and enables insight into the mechanisms of color-tuning and proton transport.

ME408

Combinatorial triple-selective labeling as a tool to assist backbone resonance assignment in membrane proteins

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Backbone resonance assignment of detergent-solubilized membrane proteins usually employs deuterated samples to overcome difficulties due to slow tumbling. Disadvantages of deuteration are high costs, decreased protein stability and partial back-exchange of α/β-deuterons in H_2O-based cell-free expression media which deteriorates relaxation properties and spectral resolution. For proteins of moderate size 3D HNCA/HN(CO)CA spectra of reasonable quality can often be obtained without deuteration. Due to $^{13}C^{\alpha}$ chemical shift degeneracy, however, the information obtained is by no means sufficient for complete backbone assignment. Combinatorial dual-selective 1-^{13}C/^{15}N labeling in conjunction with cell-free expression has been proposed to provide anchor points for sequential assignment via the C^{α} pathway. It features both combinatorial selective ^{15}N labeling for efficient amino acid type identification and dual amino acid-selective 1-^{13}C and ^{15}N labeling to obtain sequence-specific information and involves recording of [^{15}N,^{1}H]-HSQC and 2D HN(CO) spectra.

We present an extended protocol, combinatorial triple-selective labeling, which employs fully ^{13}C/^{15}N labeled amino acids together with 1-^{13}C and ^{15}N labeled ones. Additional 2D HN(CA), HN(COCA), and DQ-HN(CA) experiments allow differentiation of the fully labeled amino acid type from both 1-^{13}C and ^{15}N amino acids due to the presence of a label at the C^{α} position. As a consequence, for a given number of samples more residue types and sequential pairs can be identified. The method is demonstrated with two helical membrane proteins, KvAP voltage sensor domain and proteorhodopsin.

ME409

Dynamics of VPU Protein in Absence and Presence of Human CD4 Peptide by solid state NMR Spectroscopy

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It's well known that the viral protein VPU of HIV-1 directly interacts with the human T-cell co-receptor CD4 and subsequently induces the degradation of this protein in the endoplasmic reticulum[1,2,3]. Towards the study of this interaction on a residue-specific level, shorter constructs of these proteins comprising the cytoplasmic domains with and without the transmembrane part have been expressed and studied [4,5] . To get closer to the picture of the interaction between VPU and CD4 in the membrane of the endoplasmic reticulum, a full-length VPU protein was reconstituted into POPC lipid bilayers in absence or presence of the CD4 peptide[4]. In addition, VPU dynamics were probed using solid state MAS NMR spectroscopy. Our results suggest that in absence of the CD4 peptide, the VPU transmembrane domain is rather rigid whereas the cytoplasmic domain is flexible. However, in presence of the CD4 peptide, the dynamic behavior of the VPU is altered, particularly in its cytoplasmic domain.

In this contribution, we report on changes of NMR parameters of VPU amino acid residues observed after addition of the CD4 peptide and discuss effects of the CD4 on the dynamic behavior of the VPU in both its transmembrane and cytoplasmic domains.

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ME410

Site-directed EPR Studies Reveal Cholesterol Dependent Conformational Equilibrium of M2 Protein from Influenza A

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ABSTRACT. M2 is a homotetrameric membrane protein from influenza A that plays multiple roles in viral replication (1-2). Structural studies have shown that the conformation of the M2 protein is dependent on the hydrophobic environment (3). Using side directed spin-labeling EPR, we have studied the conformation of a 38-residue M2 peptide spanning the transmembrane region and its C-terminal extension in a range of different lipid bilayers. We have previously shown using EPR that the C-terminal region of the M2 peptide forms a membrane surface helix. (4) More recently we have collected both CW and pulsed EPR spectra which demonstrate evidence that M2 adopts multiple conformational states in bilayers, and that the cholesterol content of the membrane lipid bilayers dictates the relative populations of the states.

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ME411

Solution structure of MscS determined by PELDOR spectroscopy

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Cells are protected from the life threatening risks of hypo-osmotic shock by a family of membrane spanning mechanosensitive ion channels, including MscS, MscM and MscL. These proteins act like pressure-release valves, but the mechanism by which they "sense" the change in pressure is still unclear. MscS, a homoheptameric inner membrane protein, has been crystallised in two different conformational states [1,2], but their relevance has been questioned by chemical cross-linking, CW EPR and molecular modelling studies [3]. Our aim is to use site-directed spin labelling and PELDOR spectroscopy to elucidate the solution state(s) of MscS and clarify the mechanism by which it gates, i.e. opens its valve.

We have prepared single-cysteine mutants at different positions within each of the transmembrane helices and at some of these positions PELDOR time traces with clear dipolar oscillations were observed, from which reliable distance distribution data could be extracted. These distance distributions have been used to evaluate which of the structural models (X-ray or molecular dynamic simulations) of MscS most resembles the detergent-solubilised solution state of MscS.

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ME412

Optimization of amino acid type-specific ^{13}C and ^{15}N labeling for the backbone assignment of membrane proteins by solution- and solid-state NMR with the UPLABEL algorithm

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We present a computational method for finding optimal labeling patterns for the backbone assignment of membrane proteins and other large proteins that cannot be assigned by conventional strategies. Following the approach of Kainosho and Tsuji (Biochemistry 21:6273–6279 (1982)), types of amino acids are labeled with ^{13}C or/and ^{15}N such that cross peaks between $^{13}CO(i-1)$ and $^{15}NH(i)$ result only for pairs of sequentially adjacent amino acids. Unambiguous sequence-specific assignments can be obtained for unique pairs of amino acids that occur exactly once in the sequence of the protein. Our algorithm UPLABEL maximizes the number of unique pair assignments with a minimal number of differently labeled protein samples for a given sequence. Various auxiliary conditions, including labeled amino acid availability and price, previously known partial assignments, and sequence regions of particular interest can be taken into account when determining optimal amino acid type-specific labeling patterns. The software is available as a part of the CYANA package (Güntert, Methods Mol Biol. 2004; 278:353-78), as a standalone program and through a webportal: http://www.bpc.uni-frankfurt.de/guentert/wiki/index.php/UPLABEL .

ME413

Membrane protein investigations in native lipid environment by DNP Solid-State NMR.

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Membrane proteins are a large and diverse class of proteins that are vital for numerous cell functions. Prokaryotic and eukaryotic genome searches show that roughly 30% of all encoded proteins are membrane proteins and more than 50% of all administrated drugs are targeted to these proteins. Though their importance is great, it has proven to be difficult to obtain high-resolution structural data, only 1.7% of all deposited protein structures in the RSCB databank are membrane proteins. The preparation of samples with suitable qualities for structural biology studies is a tedious task and is a bottleneck that has not yet been circumvented. Secondly, *in-vitro* prepared systems does not contain all endogenously needed components for protein stability and function. Here, we present an alternative sample preparation with a low initial threshold for high-resolution categorization of integral membrane proteins in native environment. For this study, native membranes from *E. coli* containing an over-expressed membrane protein could be assessed by biochemical assays and by DNP Solid-State NMR within a week of the inception.

ME414

Protein-membrane interactions of Alpha-Synuclein monitored by spin-label-EPR and FCS

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A key feature in Parkinson's disease is the deposition of Lewy bodies. The major protein component of these intracellular deposits is the 140 amino acid protein α-Synuclein (αS) that is widely distributed throughout the brain. Although not yet fully understood, the physiological function of αS is likely to involve a role in modulating synaptic plasticity, presynaptic vesicle pool size, and neurotransmitter release, as well as vesicle recycling.

Many of the proposed physiological functions of αS are related to its ability to interact with phospholipids. To better understand the membrane binding and the conformational changes of monomeric αS, we performed EPR (Electron Paramagnetic Resonance) and FCS (Fluorescence Correlation Spectroscopy) of spin and fluorescently labelled αS derivatives with different lipid membranes.

ME415

Solid-state NMR studies on substrate-EmrE interactions watched from the ligand side

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EmrE is a secondary small multidrug efflux pump from *E. coli*. It consists of four transmembrane helices and functions as a dimer. A highly conserved Glu14 residue is essential for substrate binding and transport. During the transport cycle the protein adopts an occluded state [1]. We used solid state NMR to directly monitor protein-substrate interaction in membrane embedded protein samples. In the past we have studied EmrE complexed with ethidium by ^{13}C CP MAS NMR [2].

Here we report novel data on the high affinity ligand tetraphenylphosphonium (TPP) and its structural analogue methyl-triphenylphosphonium (MTP). We have chosen these substrates since both contain a phosphorus atom and can be easily studied by ^{31}P NMR. Two signals of the substrate could be detected in the presence of the protein for both TPP and MTP; which were assigned on the basis of their chemical shifts and CP build-up data as free and protein-bound populations. The K_d determined from the NMR data for TPP is in the nanomolar range (34 nM) indicating TPP bound to dimeric EmrE. Competitive titrations indicate that these substrates share the same binding pocket. Relaxation data measured on the free and bound substrate will be presented as well. Measurements on ^{13}C labeled TPP suggest a fast rotation of the structurally symmetric substrate in the binding pocket of the protein.

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ME416

Importance of Conformational Restraints from Paramagnetic Relaxation Enhancement for Membrane Protein Structure Determination by NMR

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Paramagnetic relaxation enhancement (PRE) has long been recognized as an approach for obtaining conformational restraints that can complement NOE restraints, which are limited to distances of up to 5 Å.[1] Paramagnetic nitroxide spin labels that can be attached to a target protein produce distance-dependent line-broadening in the resulting NMR spectra that can be translated into distance restraints for the structure calculation. The paramagnetic influence ranges up to 25-35 Å.[2] PRE restraints are of particular interest for the structure determination of membrane proteins, for which the large effective size of the protein-micelle system often precludes the collection of long-range NOE distance restraints. We present a study to evaluate the impact of PRE-derived distance restraints on structure calculations with limited or no available long-range NOEs. The evaluation was done using simulated structural restraints derived from the crystal structures of three membrane proteins: the protein GlpG, halorhodopsin, a member of the rhodopsin family, and DsbB, a 4-transmembrane-helix protein.

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ME417

Throughput strategies for the optimization of membrane protein samples by cell-free expression

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The open nature of cell-free expression systems enables throughput screening approaches as a rational strategy for protein expression and protocol development. Quality and production of membrane proteins could thus be optimized by extensive modification of their direct expression environment.

We established a throughput platform for the linear as well as for the correlated screening of compounds supplied into cell-free expression reactions. In linear screens, the concentrations of compounds are evaluated in one dimension. However, the concentration optima of different compounds can often correlate with each other. Such interfering compounds can be optimized in correlated screens using both dimensions of the microplates. Reaction condition could later be scaled up to at least 1 ml volumes without losses in yields.

This throughput sample production will provide a fast and convenient method for NMR study, especially, for special labeling strategy, i.e., transmembrane segment enhanced labeling or combinatorial selective labeling.

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ME418

Membrane microcompartmentalization can enhance a "weak" dimerization of RTK transmembrane domains: structural and kinetic insight into self-association process of the ErbB4 transmembrane domain

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Specific helix-helix interactions of single-span transmembrane domains of receptor tyrosine kinases are believed to be important for their lateral dimerization and signal transduction. Establishing structure-function relationship as well as rational drug design requires precise structural-dynamic information about this class of biologically significant bitopic membrane proteins. ErbB4 is a ubiquitously expressed member of the HER/ErbB family of growth factor receptor tyrosine kinases that is essential for normal development of different adult and fetal human tissues and plays a role in pathobiology of organism. Spatial structure of dimeric ErbB4 transmembrane domain embedded into lipid bicelle was obtained by solution NMR and revealed a right-handed parallel packing of the membrane-spanning α-helices $(651\text{-}678)_2$ through the N-terminal double GG4-like motif $A^{655}GxxGG^{660}$ in a fashion believed to allow proper kinase domain activation. The helix association undergoes through a structural "tuning" of the dimer subunits with the formation of the net of inter-monomeric polar contacts. The quantitative analysis of observed monomer-dimer equilibrium gives an insight into kinetics and thermodynamics of folding processes of helical transmembrane domain in model environment and perhaps in cellular membranes. It suggests that the lipid bicelles singly occupied by ErbB4 transmembrane domain behave as an ideal solvent while multiply occupied ones resemble microdomains of cellular membrane providing seemingly substantial kinetic enhancement of the weak helix-helix interactions that can be critical for membrane protein functioning.

ME419

Relaxation time dependent separation of EPR signals of the membrane-bound [NiFe] hydrogenase from *Ralstonia eutropha*

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Understanding the unusual robustness of certain [NiFe] hydrogenases towards oxygen is of high importance given their potential future application as catalysts for H_2 production. [NiFe] hydrogenases mainly consist of a large subunit with the [NiFe] active site and its ligands and a small one containing [FeS] clusters as part of the electron-transfer chain. While standard hydrogenases are inactivated by traces of O_2 there are exemplars sustaining high activity even under ambient oxygen levels.

Since many redox states of the named cofactors are paramagnetic, EPR spectroscopy is well suited to characterise them and their surroundings. Here we focus on the oxygen-tolerant membrane-bound hydrogenase from *Ralstonia eutropha*. The enzyme exhibits in its (resting) Ni_r-B state a magnetic coupling between the [NiFe] centre and two paramagnetic species, a distinct feature not found in standard hydrogenases. Previous research revealed high similarity of the [NiFe] site in both standard and O_2-tolerant hydrogenases [1], leaving the [FeS] region as possible origin of the O_2 tolerance.

Overlapping of characteristic signals of diverse paramagnetic species often complicates EPR spectra. However, in several cases these species show different relaxation behaviour. Therefore, by using 2D-REFINE spectroscopy [2], we aim to distinguish between crossing features of the [NiFe] site, the [FeS] region and the yet unknown paramagnetic center.

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ME420

Membranes or micelles cause structural changes on GCAP-2

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GCAPs are neuronal calcium sensor proteins (NCS) located at the membrane of the outer segments of the photoreceptor cells, where they regulate, in a Ca^{2+}-dependend manner, the transmembrane retinal guanylate cyclase (RetGC), and therefore play a central role in light adaptation of the visual process. GCAPs are posttranslationally modified by N-terminal myristoylation, and the specific role of this myristoyl moiety is still unclear. For some NCS, the Ca^{2+} binding leads to an extrusion of the myristoyl chain and this direct the protein to the membrane (Ca^{2+}-myristoyl-switch) (1). Typically, lipid modifications serve as membrane anchors, but the published crystal structure of GCAP-1 shows the myristoyl moiety buried inside the hydrophobic core; therefore a protein structure stabilizing function was suggested (2). Furthermore, non-myristoylated GCAP-2 also binds to membranes. In our studies we investigate the Ca^{2+}-dependent localization and the dynamics of GCAP-2's myristoyl moiety in the presence of membranes (POPC liposomes) and micelles (DPC, CHAPS) by solid-state and solution NMR; additionally we address structural changes in the N-terminal region of GCAP-2 caused by an increasing amount of DPC or CHAPS. Our results indicate that membrane binding causes the release of the myristoyl moiety from the protein and its insertion into the lipid bilayer (3).

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ME421

Structural and Dynamics Studies of Amyloid Precursor Protein's Transmembrane Domain

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For appropriate therapy of Alzheimer's disease which affects people all over the world regardless of nation and social status it is essential to determine the molecular basis of the pathogenesis. Amyloid β-peptide which forms amyloid plaques in brain during Alzheimer's disease is the product of sequential cleavage of a single-span membrane amyloid precursor protein (APP). More than half of mutations of APP found to be associated with familial forms of Alzheimer's disease are located in its transmembrane domain. The pathogenic mutations presumably affect structural-dynamic properties of the APP transmembrane domain, changing its conformational stability and/or lateral dimerization. In the present work was studied the structure and dynamics of recombinant peptide, corresponding to APP fragment Gln686-Lys726 including the APP transmembrane domain with adjacent N-terminal juxtamembrane sequence. The peptide's investigations were performed for its dimeric form in membrane mimetic environment composed of detergent micelles using NMR spectroscopy methods. It was found that the structure slightly alters from monomeric state. Major changes could be found in the juxtamembrane region with its melting to disordered form which maybe pH induced. The transmembrane domain lacks its bending as reported for monomer.

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ME422

Production of transmembrane segments of receptor tyrosine kinases for NMR structural studies

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Malfunctions of epidermal growth factor receptors (ErbB1-ErbB4), Eph receptors (EphA1, EphA2) and fibroblast growth factor receptor 3 (FGFR3 in norma and with G380R or A391E point mutations), including dysfunction of their transmembrane (TM) region, lead to variety of human diseases. Relatively small size of complexes of TM peptides (TMPs) of these receptors with detergents/lipids allows one to study their detailed spatial structure using heteronuclear 3D NMR spectroscopy. An effective protocol for preparative-scale production of TMPs (including ^{15}N- and ^{15}N-/^{13}C- labeled) for structural and functional studies were developed. The recombinant TMPs were produced in *Escherichia coli* BL21(DE3)pLysS as C-terminal extensions of thioredoxin A. The fusion proteins cleavage was accomplished with the light subunit of human enterokinase. Up to 10-30 milligrams of purified TMPs were isolated with the use of immobilized metal affinity and ion-exchange chromatographies, reconstituted in lipid/detergent micelles and characterized using dynamic light scattering, CD and NMR spectroscopy. Data obtained indicate the suitability of the purified TMPs for NMR studies. Acknowledgments: supported by FTPs “Scientific and scientific-pedagogical personnel of the innovative Russia in 2009-2013” (P1276 and 16.740.11.0195).

ME423

Expression, purification and refolding of bacteriorhodopsin for NMR application

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Unique biochemical and biophysical properties and GPCR-like structure of bacteriorhodopsin (BR) make it an attractive sample protein for NMR structural study of GPCRs. An effective system for preparative-scale production of functionally active BR for NMR structural study was developed. The BR was expressed in *Escherichia coli* as C-terminal extension of Mistic and purified by immobilized metal-affinity chromatography. Thrombin was used to cleave BR from the fusion partner moiety. Pure target protein was refolded in DMPC/CHAPS mixed micelles. The yield of functionally active BR (including isotope-labeled derivatives) was not less than 15 mg/L of M9 minimal salt medium. After incorporation into different lipid/detergent micelles, the BR samples were studied by NMR spectroscopy. We found that the DMPC/DHPC appeared to be optimal for solubilization of BR for further investigations. Number of cross peaks in glycine region, good signal dispersion and width, reasonable line shape, and number of signals observed in the [1H-15N]-HSQC NMR spectrum of DMPC/DHPC/BR complexes are consistent with that expected from the theoretical data, indicated that the BR is stable, homogeneously folded in the DMPC/DHPC micelles and suitable for NMR structure-dynamic studies. Acknowledgments: supported by FTPs “Scientific and scientific-pedagogical personnel of the innovative Russia in 2009-2013” (16.740.11.0195 and P1276).

ME424

Cell-Free Expression of GPCRs: an approach for NMR structural investigation

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G-protein-coupled receptors (GPCRs) are cell-surface membrane proteins that mediate various signal transduction processes through G-protein activation. The human endothelin receptor type A (ETA) and B (ETB) are prototypic GPCRs distributed among multiple endothelial cell types as well as in smooth muscle cells. The ubiquitous distribution of these receptors implicates their involvement in a wide variety of physiological and pathological processes In our laboratory we have established protocols for high-level production of ETA, ETB and other GPCRs in an individual Continuous Exchange Cell-Free System (CECF). This expression system, based on Escherichia coli cell extracts, has been demonstrated to provide a highly promising tool for structural investigation and in particular for NMR studies. The possibility to obtain selective labeling of certain amino acid types without metabolic scrambling can enable an efficient backbone assignment. Direct solubilization of membrane proteins in mild detergents, avoiding cell-disruption or time-consuming unfolding and refolding procedures, is an additional advantage over cellular based expression systems.

In this work we demonstrate that different cell-free expression modes (P-CF, D-CF and L-CF) can be successfully used for the production of ligand-binding competent ETA and ETB receptors in quantities sufficient for structural approaches. In order to obtain functional receptor an extensive detergents screening has been performed and sample quality has been evaluated by protein homogeneity, stability and ligand binding competence.

ME425

Study of interaction between rhodopsin and arrestin by solution NMR

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Arrestin upon binding to photoactivated phosphorylated rhodopsin inactivates the photo-transduction cascade in photoreceptor cells. We used solution NMR to study the receptor-bound arrestin peptide Arr (170-182) in order to characterize the molecular interaction between rhodopsin and arrestin. Two main aim of this study is to find out the effect of binding of peptides to meta II state of rhodopsin and also to find out the transient structure of peptides upon binding.

We investigate the changes in structure of free form peptides upon binding to both dark state and photoactivated rhodopsin by comparing their 2D NOESY spectra. We will also investigate the formation of extra meta II induced by tritration of rhodopsin with Arr (170-182) peptide in order to study the kinetics of meta II state of Rhodopsin. In future we will study the interaction of full length arrestin with rhodopsin by studying the changes in dynamics of the C-terminus of rhodopsin.

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ME426

Investigation of the POTRA Domains from Cyanobacterial Omp85 by PELDOR Spectroscopy

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Omp85 proteins contain a C-terminal transmembrane β-barrel and a soluble N terminus with a varying number of polypeptide-transport-associated (POTRA) domains.[1] N-terminal POTRA domains (P1 and P2) of Omp85 from the cyanobacterium *Anabaena sp.* PCC 7120 might have functions in substrate recognition and heterooligomerization.[2] P3 is implied in regulation of protein transport by its L1-loop. Molecular dynamics (MD) simulations predicted that P2 and P3 are fixed in orientation, consistent with a short connection and a large interface between both domains, and that there is a hinge between P1 and P2.[2] The flexibility between P1 and P2 is matching the observation of the smaller interface combined with a longer linker, compared with P2-P3. In this study we used site-directed spin labeling (SDSL) to investigate the conformational flexibility between POTRA domains by PELDOR (pulsed electron-electron double resonance) spectroscopy.[3-5] The experimental results will be compared with the MD calculations and X-ray structures.[2] Further studies of the interaction of POTRA domains with chaperones and substrates are underway.

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ME427

Lipid interactions of the malaria antigen MSP2 revealed by NMR

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With more than half the world's population living at risk of malaria infection, there is a strong demand for the development of an effective malaria vaccine, however this has proven elusive [1]. One promising vaccine candidate is merozoite surface protein 2 (MSP2), which is among the most abundant antigens of the blood stage of the parasite [2]. To ensure an efficient immunogenic response when applying MSP2 in a vaccine formulation, knowledge of the native state of the protein is extremely important. In solution, MSP2 appears to be intrinsically unstructured, whereas cross-linking studies suggest it forms homo-oligomers on the surface of the parasite [3]. Furthermore, MSP2 is prone to form amyloid fibrils, and this fibril-propensity is linked to the presence of a highly conserved N-terminal domain in an otherwise mainly variable and highly polymorphic protein [4].

Initial NMR studies from our group have shown that MSP2 interacts with lipid micelles through the conserved N-terminal domain [4]. We have continued these studies by investigating how different lipid environments (micelles, bicelles and bilayers) affect the protein structure. In summary, all tested lipid preparations perturbed only the N-terminal part of MSP2, which in DPC micelles adopts an α-helical structure. Moreover, some lipids were able to induce oligomer formation, consistent with the N-terminal domain having an important function in maintaining the native organization of MSP2 on the parasite surface.

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ME428

Easy method for signal enhancement and progress in the structure determination of the helical human membrane protein Hv1

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Sensitivity enhancement in liquid state nuclear magnetic resonance (NMR) triple resonance experiments for the sequential assignment of proteins is important for the investigation of large proteins, protein complexes or membrane proteins. We present here the 3D TROSY-MQ/CRINEPT-HN(CO)CA[1] which makes use of a ^{15}N-^{1}H-TROSY element and a ^{13}C'-^{13}CA CRINEPT step combined with a multiple quantum coherence during the ^{13}CA evolution period. Because of the introduction of these relaxation-optimized elements and ten less pulses required, when compared with the conventional TROSY-HN(CO)CA experiment an average signal enhancement of a factor of 1.8 was observed for the membrane protein-detergent complex KcsA with a rotational correlation time τ_c of around 60 ns. In addition to that this sequence was used to investigate a helical human membrane protein, the human voltage gated proton channel (Hv1[2] or VSDO[3]). Hv1 plays an important role in the human innate immune system. Its predicted structure differs considerably from other cation channels. The goal is to understand the voltage-sensing and the proton permeation pathway of this unique channel.

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ME429

Substrate-dependent binding of MalE to the ABC transporter MalFGK$_2$

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The ubiquitous ABC-transporter family is involved in the ATP-dependent uptake or export of a large variety of substrates. In the case of importers, a specific substrate-binding protein (SBP) exists, which captures the substrate in the periplasm and delivers it to the importer.

The ABC type I maltose import system of *E.coli* is structurally and biochemically well characterized and recently several new crystal structures of the transporter were published [1]. The aim of this study is to characterize the formation of the complex between the SBP MalE and the transporter MalFGK$_2$ during the nucleotide cycle in the presence and absence of maltose, to complement the available structural data and to obtain new insights into the role of the substrate in the mechanism of import.

Our method of choice is site directed spin labeling (SDSL) in combination with double electron electron resonance (DEER) to obtain precise distance information between selectively labeled sites. Our results clearly show that MalE interacts with the transporter independently of the presence of maltose. However, the interaction in the apo state in the absence of maltose seems to mostly take place via the N-lobe of MalE and the P2-loop of the transporter, whereas in the presence of maltose we have evidences that the interaction involves both N- and C-lobe of MalE. In contrast, the conformation of the MalFGK$_2$-E complex in the ATP-state is only driven by binding of ATP, regardless of the presence or absence of maltose. The EPR results are confirmed by cross linking data and by comparison to the available crystal structures. A more complete model of the substrate import cycle of the type I ABC importer is presented.

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ME430

Structural and dynamical model of transmembrane domain of fibroblast growth factor receptor 3

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Homodimerization and dynamic properties of the transmembrane segment of fibroblast growth factor receptor 3, participating in signal transduction through the cell membrane, were characterized by heteronuclear NMR spectroscopy in the membrane-mimicking environment. The helix-helix dimerization heptad motif ($YA^{374}X_2L^{377}X_2G^{380}X_2FF^{384}X_2IL^{388}X_2A^{391}X_2TL^{395}$) is employed for a left-handed parallel packing with crossing angle of ~20^o and helix-helix distance of ~9 Å. The central region of the dimer is characterized by relatively tight packing stabilized by intra- and intermolecular stacking interactions of aromatic rings (Y^{379}–FF^{384}–F^{386}), whereas N-terminal part of transmembrane helix is stabilized upon dimer formation that can be related with the receptor activation. The pathogenic mutations Y373C, G380R and A391E are located precisely in the in the helix-helix interface assuming that the obtained dimer conformation is important for receptor functioning. The NMR results combined with molecular modeling data providing detailed analysis of conformational space as well as influence of mutations on structure, stability and dynamic properties of the dimer.

ME431

LT-MAS NMR measurements on multi drug transporter EmrE

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With multidrug resistance (MDR) being an increasing challenge for modern medicine it is vital to understand its mechanisms. One important aspect is the efflux of drugs via membrane proteins. We are studying the “Small multi drug resistance” protein EmrE (UniProt P23895) by low temperature solid-state NMR. Low temperature measurements become more important in the context of dynamic nuclear polarization and freeze-quenching experiments. The temperature range down to 100 K is reliably accessible in long-term experiments with commercial equipment.

EmrE is a secondary transporter that uses the proton gradient across the cell membrane to export drugs from the cell. Typical substrates are aromatic, cationic compounds like TPP^+ or ethidium. It consists of 110 amino acids with a highly conserved glutamate as the only charged residue residing inside the membrane with a proposed pk_a of 7.5. Understanding its protonation behavior is critical for understanding the transport mechanism of EmrE. In order to minimize scrambling we have used cell free expression to label a single glutamate mutant of EmrE with ^{13}C. Solid state NMR analysis of the cell free produced protein-precipitate shows high alpha helical content. The protonation state of EmrE has been assessed by ^{13}C-DQF experiments conducted at 100K and at different pH values indicating a protonation dependent shift of the C_δ signal below pH 6.

ME432

A Solid-state NMR Study of the isotope labelled Multidrug Efflux Pump EmrE in Complex with Substrates

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Multidrug transporters (MDTs) are responsible for the removal of a wide range of drugs from the cytoplasm of cells hence contributing to the rise of multidrug resistant bacteria. A prototype of such MDTs is EmrE (*Escherichia coli* multidrug resistance transport), which belongs to the family of Small Multidrug Resistance (SMR) transporters. Here, we investigate the interaction of this membrane protein with its substrates in its lipid environment using MAS solid state NMR(ssNMR). Two-dimensional ^{13}C-^{13}C PDSD correlation experiments of selective-extensively labelled EmrE in its apo state and in complex with its substrates tetraphenylphosphonium (TPP), methyltriphenylphosphonium (MTP) and ethidium are compared. Although in all cases, significant line broadening is observed, probably arising from intermediate timescale domain movements, differences in cross-peak volumes at different mixing times for different substrates are observed. This indicates that substrate binding triggers changes in dynamics and structure of EmrE. For example, the intraresidual cross peak pattern involving Cd1 of isoleucines (Ile) showed significant reduction in signal intensities in either TPP- or MTP-bound ^{13}C-EmrE . This observation is likely to be due to a change in dynamics (and probably orientation) of the side chains of Ile within the protein helices upon substrate addition. Direct protein ligand interactions using 13C-TPP in complex with SE-13C-EmrE have been probed at low temperature using cwDNP enhanced MAS-NMR. These preliminary data indicate that DNP may be the method of choice for specific interaction studies within membrane protein-substrate complexes.

ME433

NMR studies of porin A

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We have investigated the ß-barrel membrane protein porin A that is involved in the pathogenesis of the meningitis causing bacteria *Nesseria meningitidis*. The protein has been over-expressed as inclusion bodies in *E. coli* and successfully refolded in several detergents. To overcome problems with broad and overlapping signals in the 2D 15N HSQC spectrum, cell-free protein synthesis (CFPS) was evaluated as an alternative expression system. In order to generate a sample using CFPS several different lipids and detergents, as well as nanodiscs, were screened to find an expression condition that produced levels of protein suitable for NMR purposes.

NMR studies of the interaction between porin A and domains 1-2 from Complement Protein 4B and the binding between porin A and chitobiose were initiated

ME434

Mechanosensation at a molecular level studied by EPR spectroscopy

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Mechanosensitive (MS) channels have a major role in cellular homeostasis and in maintaining the physical integrity of bacterial cells. One of the best characterized MS is MechanoSensitive Channel of Large conductance. The channel functions as a safety valve to protect bacterial cell against osmotic shock. If cell faces sudden hypoosmotic stress, the bacterial membrane stretches resulting in opening of nonselective pore in the protein. It is still unknown how this channel works. To understand the mechanism of channel opening a strategy is needed that will allow us to understand the helical movements of the protein, since it converts the mechanical force directly into helical movement.

Our strategy combines the power of EPR spectroscopy with a biochemical approach to activate the channel in a controlled way without applying a membrane tension.

The method, based on Site Directed Spin Labeling (SDSL) technique, allows us to introduce artificially a nitroxide radical to desired position of the protein. The EPR of the attached nitroxide provides information about the mobility and polarity of the local environment and allows distance measurements between two or three paramagnetic centers. It is known from the crystal structure that the channel is a homopentamer with two transmembrane helices per subunit: inner and outer (TM1, TM2) and the narrowest part (where the gate starts formation) is about 3Å in a close state. By labeling all five Cys residues in this region the strong dipolar interaction between unpaired electrons cannot be avoided. To overcome this problem we develop method in which we can control the number of Cys residues per pentamer. We also optimized the SDSL procedure and perform EPR studies.

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ME435

Helix-bundle structure of sodium/proline symporter PutP revealed by Double Electron-Electron Resonance (DEER) in conjunction with a modelling approach

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Relative arrangement of 13 transmembrane helices – a helix bundle – of Na^+/proline symporter PutP, whose structure is currently unknown, was determined by means of coarse-grained modelling constrained by experimentally derived distance information. For that nitroxide spin labelling (with MTS spin labels) along with distance measurements by pulse EPR was utilised. Double Electron-Electron Resonance (DEER) experiments were performed at X- and Q-band and mean interspin distances obtained from the experiment were used as constraints during modelling. Spin labels were placed such that the distances between helix ends within the periplasmic as well as the cytoplasmic side of the protein, complemented by additional transmembrane distances, were available. Spin label conformations were predicted by the rotamer library approach[1]. The number of available experimental distances (about 70) was found to be not sufficient to fully constrain the problem, therefore additional internal protein restraints as well as those generated from the template structure (crystal structures of vSGLT[2,3]) were used together with the experimental constraints. A fitting algorithm based on matrix geometry approach[4] provides an ensemble of possible helix bundle arrangements.

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MB436

Selective Excitation of Metabolite Signals for *in vivo* ^{1}H MRS

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Selective excitation of metabolite signals in *in vivo* MRS is important for the correct and robust quantification of the content of some key metabolites. This is of special importance under conditions of low field strength in common clinical MRI scanners, where considerable signal overlap combined with low signal intensities impedes quantification of in vivo MRS signals.

Here we develop pulse sequences which improve sensitivity and selectivity in *in vivo* MRS. With the use of optimal control theory we calculate pulse shapes which allow for a robust selective excitation in the presence of experimental imperfections such as RF- and static field inhomogeniety. For the optimization we use the Krotov approach and perform simulations and experimental tests of the resulting pulses. The aim is to develop spectral editing techniques for the selective excitation of individual metabolites in the human brain, that perform better compared to standard selective excitation methods.

As a model system we examine the metabolites lactate and alanine combined with lipid which have overlapping signals in human tissue.

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MB437

NMR Analyses of Milk Metabolites Allow Disease Prediction for Dairy Cows

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Milk is an easy-to-collect target for metabolic analyses and NMR has been previously successfully applied on cow's milk[1]. Metabolic diseases in dairy cows have become more and more wide-spread during the last decades, leading to animal suffering and economic loss, with ketosis being one of the most common metabolic diseases. Although biomarkers for ketosis are known, for example acetone and beta-hydroxybutyric acid (BHBA) levels in blood or milk, disease prediction is still a hard task. In this contribution we show that using a combination of different milk metabolites measured by NMR allows the reliable prediction of disease risk. The advantages of this method include that it is not dependent on acute disease status, such as acetone or BHBA based methods, and that it allows an early prognosis for the disease risk of an animal. The method may also be used for the selection of metabolically stable cows for breeding purposes.

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MB438

Of Mice and Men – NMR-based metabonomics from Animal Systems to Clinical Science

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We use NMR-based metabonomics to study how disease, genetics and environment influence metabolic profiles in animals and humans. **Mice:** Growth hormone (GH) is the key factor regulating postnatal growth and an important regulator of metabolism. As a model system for obesity we have analysed the metabolic profiles of transgenic mice with truncations in the intracellular GH receptor domain. These mice develop late-onset obesity. NMR profiling identified metabolites, including taurine, trimethylamine, creatine/creatinine and branched-chain amino acids. These metabolites were correlated with genetic data from microarray profiling and indicate significant changes in choline and lipid metabolism leading to the observed phenotype. They support a potentially important role for taurine in developing obesity. **Men:** We have analysed the stability of human urine samples at room temperature. We show that the standard method of sample preservation with 0.06% NaN_3 does not prevent sample degradation over 24h. This can be a source of error in human clinical studies if subjects fail to store samples at 4°C before delivery to the clinic. We solve this problem by preserving urine samples in >100 mM sodium borate upon collection. Following this newly established standard procedure, samples will remain stable and an accurate metabolic fingerprint can be recorded.

These results highlight the versatility of metabonomics in studying complex biological problems.

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MB439

Altered Fatty Acid Metabolism in Long Duration Road Transport: An NMR-based Metabolomics Study in Sheep

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Ruminants experience metabolic/endocrine changes during and after road transport due to a combination of food/water deprivation with stress. The recovery time and how to improve recovery are of interest to livestock industries. However, traditional clinical indicators are relatively insensitive to subtle metabolic changes. We investigated the metabolic responses of merino ewes allocated to 12 and 48 h road transport under standard industry conditions with NMR-based metabolomics. Analysis of NMR spectra from urine and serum collected at pre-transport, on arrival, and up to 72 h post-transport revealed changes in several metabolites between treatment groups and time points. The metabolic responses involved metabolites associated with several metabolic pathways, especially carbohydrate, and lipid metabolism. The animals also experienced alterations in gut metabolism, protein catabolism and possibly a renal response. The longer transport duration caused an amplified and different perturbation of both serum and urinary profiles. During the recovery period, the metabolism of the animals returned to a new stable state. Intriguingly, excretion of acyl glycines and a dicarboxylic acid was observed after transport and during recovery, implicating peroxisomal fatty acid oxidation as metabolic response to transport-induced stress.

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MB440

State-of-the art data normalization methods improve NMR-based metabolomic analysis[1]

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Extracting biological and medical information from NMR derived metabolomic datasets by multivariate data analysis is of substantial complexity. Common challenges include for example screening for differential metabolites, estimation of fold changes, and sample classification. Prior to these analyses, it is important to minimize contributions from unwanted biases and experimental variance. This is the intention of data preprocessing. Here, two different types of data normalization methods were compared systematically. The first type of algorithms aims to remove unwanted sample-to-sample variation, while the second type is aimed at adjusting the variance of the different metabolites. The latter approaches include variable scaling methods and variance stabilizing transformations. The effect of the various methods on sample classification was evaluated on urinary NMR data obtained from healthy volunteers and patients suffering from autosomal polycystic kidney disease (ADPKD). Performance in terms of screening for differentially produced metabolites was analyzed on a dataset following a Latin-square design, where varying amounts of 8 different metabolites were spiked into a human urine matrix. In conclusion, preprocessing methods originally developed for DNA microarray analysis performed best in reducing bias, accurately detecting fold changes, and classifying samples.

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MB441

Metabolic profiling and antiradical activity of honeys and herbal honeys studied by NMR and EPR spectroscopy

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Since a special emphasis is put on finding suitable methods for the detection of geographical and botanical origin of honeys, twenty-seven samples of honeys and herbal honeys from Poland have been studied by means of ^{1}H and ^{13}C NMR. Multivariate statistical techniques used to investigate metabolic profiles of food and plant extract have been shown to be useful tools for the investigation of their botanical source[1], as well as of aging processes that can alter the composition of the studied sample. Therefore principal component analysis (PCA) was used to analyze the NMR spectra of the honeys of different botanical origin. In order to detect both sugars, which represent the main components of honey, and constituents from plant extracts, which are also of importance in case of herbal honeys, analysis in two different solvents: D_2O and DMSO was performed. In addition, samples of two chosen honeys after storing in accelerated aging conditions were studied in order to establish whether the NMR/PCA method can be applied for tracking aging changes.

Antiradical activity of honeys was studied by EPR spectroscopy to establish if the substances from plant extracts enhance the radical scavenging properties of the samples. It was also of interest to check whether the accelerated aging conditions influence in any way the antiradical activity of honeys.

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MB442

Insight into neural cell metabolism by NMR – employing UDP-GlcNAc as a metabolic marker

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UDP-N-acetylglucosamine (UDP-GlcNAc) is an activated sugar nucleotide produced during hexosamine biosynthetic pathway (HBP) [1]. This activated sugar is the key substrate for posttranslational glycosylation and O-GlcNAcylation of proteins [2,3]. Other activated sugars (e.g. UDP-GalNAc, UDP-Glc, UDP-Gal and CMP-NeuNAc) are being produced in salvage pathways of the HBP [4]. Changes in flux through the HBP either increase or decrease UDP-GlcNAc levels, affecting glycosylation and O-GlcNAcylation of many proteins. These modifications not only play important roles in many fundamental cellular processes, but also their dysregulation can lead to human diseases such as diabetes, Alzheimer's disease and cancer [5,6,7,8]. Alterations of UDP-GlcNAc levels may provide an indication of the development of metabolic disorders, making UDP-GlcNAc an ideal metabolic marker.

Perchloric acid (PCA) is widely used for extraction of water-soluble metabolites. We found that PCA extraction is not suitable for detection of activated sugar nucleotides in cell extracts, since most of these sugars are being decomposed. However, methanol/chloroform extraction is well suitable for quantitative detection of these metabolites, as shown by nuclear magnetic resonance (NMR).

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MB443

Painless T_2 filtration.

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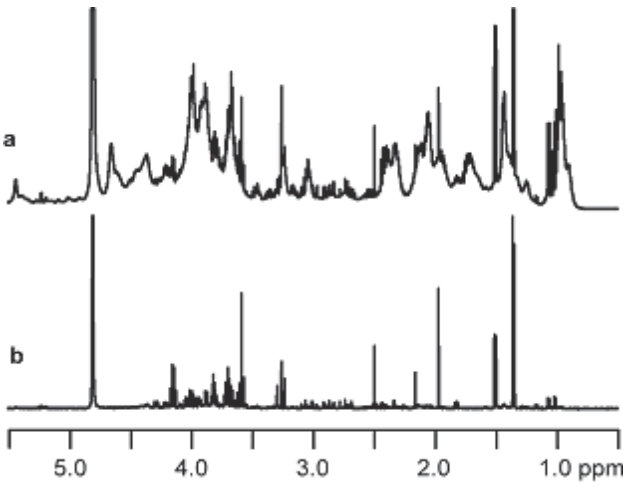

T_2 filtration is used in fields such as metabolomics where broad signals need to be suppressed in order to see underlying sharp resonances.[1] It typically employs a high duty cycle Carr-Purcell-Meiboom-Gill (CPMG) pulse sequence to quench J-evolution. The high average RF power needed creates problems with hardware and with sample heating, causing lines to shift and broaden. Here a novel variant of the CPMG pulse sequence is presented that efficiently refocuses J-evolution, allowing clean T_2 filtration with minimal heating. The method uses a quadrature 90° pulse every two echoes to refocus J modulation, as in the "perfect echo" sequence of Takegoshi et al.[2]. The perfect echo was originally reported to be restricted to AX spin systems, but has been used to reduce modulation in larger sytems.[3] Here it is shown that the effect is in fact general for refocusing intervals short compared to *J*.

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MB444

Metabolomic studies on *Isatis tinctoria* – Comparison of different origins, harvesting dates, and the effect of repeated harvesting

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Isatis tinctoria (Brassicaceae) is an ancient dye and medicinal plant with potent anti-inflammatory and anti-allergic properties.[1, 2] We investigated metabolic differences of plants grown on experimental plots at the Agricultural Field Station of Thuringia under identical conditions. Comparisons were carried out for plants of different geographic origins, different harvesting dates, and between once and repeatedly harvested plants. Leaf samples were shock-frozen with liquid nitrogen immediately after harvest, freeze-dried, and cryomilled prior to extraction. Extracts were prepared by Accelerated Solvent Extraction (ASE) with EtOAc and CH_3OH.

EtOAc extracts were dissolved in $CDCl_3/CD_3OD$ (7:3) with TMS as internal standard for NMR measurements, and spectra analyzed by multivariate analysis. The score plots produced by Principal Component Analysis (PCA) revealed differences in the metabolic profile between the origins and harvesting dates. Partial Least Square Discriminant Analysis (PLS-DA) plots exhibited differences between the once and repeatedly harvested plants, if the harvesting dates were plotted separately. Its loading plots showed mainly the aliphatic region to be responsible for these differences. Additionally, a part of the samples showed higher quantity of indoles. Assignement of signals in the interesting regions is ongoing.

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MB445

^{1}H NMR-based Metabonomics for Drug Testing

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During the last decade, metabonomics became a very attractive field of research and has been studied by means of various analytical techniques. More recently, the advent of metabonomics by NMR led to a growing number of applications in medicine.[1] ^{1}H NMR spectra of biological materials are advantageous to quickly detect a large range of low MW metabolites in a single experiment as opposed to other biophysical techniques and changes in the biochemical composition of body liquids caused by exposition to drugs can be evaluated.[2] This may serve to supplement conventional targeted detection techniques e.g. for steroids in doping control[3] in a non-targeted design.[4] Metabonomics studies of biofluids, such as urine, also represent a complex problem for NMR spectroscopy. Detection is hampered e.g. by the presence of water (main component of any biological fluid), whose dominating signal needs to be eliminated to detect the several orders of magnitude weaker signals of dissolved metabolites. Different pulse sequences were tested for the solvent suppression. ^{1}H NMR spectra of a range of metabolites were obtained using signal suppression under the same conditions as test spectra from urine samples to create the basis for urinary NMR profiling.

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MD446

A Dual-mode Microwave Resonator for Double Electron-Electron Resonance in W-band

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High-frequency PELDOR is an important technique to deliver information about the relative orientation of paramagnetic species, which is essential to study conformational changes of labelled biomolecules [1]. Furthermore, dual frequency experiments can be performed to determine the effective saturation factors of polarizers utilized for dynamic nuclear polarization (DNP) [2]. However, the execution of such experiments at high fields is usually aggravated by a narrow bandwidth of available single mode resonators. One way to overcome this limit is to employ a dual mode resonator.

We present a resonator that operates at W-band microwave frequencies and supports two microwave modes with the same field polarization at the sample position [3]. The resonator was designed for dual-frequency experiments with a variable separation of probe and excitation frequencies up to several hundreds MHz. It has been applied for orientation-selective PELDOR on the labelled RNA and peptide molecules. Furthermore, it was used in W-band dual frequency experiments to study the saturation behavior of polarizers for DNP in liquids.

The resonator design, its numerical analysis as well as some experimental aspects of its applications will be discussed.

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MD447

Orientation analysis of rigid nitroxide spin-labels in RNA duplex by high-field pulsed electron-electron double resonance

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Recently pulsed electron-electron double resonance (PELDOR) spectroscopy at high magnetic fields has been successfully employed not only for distance measurements but also for the mutual orientation between two tyrosyl radicals [1, 2]. For the applications of distance measurement in biological macromolecules, however, the nitroxide spin-label is widely used as a paramagnetic center. The method to acquire the orientation between the two nitroxide labels is more challenging due to the flexibility of the nitroxide side-chain. Thus, we present in this work the analysis of PELDOR data to determine the orientation of double nitroxide spin-labels in an RNA duplex. Two rigid nitroxides were incorporated in the RNA duplex with approximately 3.1 nm apart. Two sets of PELDOR experiments were performed at 94 GHz with a commercial high-Q resonator and a home-built dual mode resonator [3]. The analysis was implemented to fit with both data sets. The orientation of the radicals is reported in Euler angles of the nitroxide coordinates with respect to the dipolar vector. The details of the data analysis with the model will be discussed as well.

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MD448

μ-Freeze quench technique combined with EPR spectroscopy: a powerful tool to investigate enzymatic reactions

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Electron Paramagnetic Resonance (EPR) spectroscopy is ideally suited to the study of many enzymatic processes since the substrate conversion often involves electron transfer reactions, via radical intermediates formation and/or via changes on the oxidation states of transition metal cofactors.

During catalysis the half-life ($t_{1/2}$) of these transient species lies typically in the millisecond time scale, making their EPR characterization in the steady state not feasible.

Mechanistic and kinetic studies can be achieved by coupling EPR with Rapid Freeze Quench (RFQ) techniques,[1] a suitable method to trap these meta-stable reaction intermediates.

Here we report a description of our setup on the μ-RFQ apparatus optimized to be coupled with (X-, Q- and W-band) EPR and its application on the study of PpoA enzyme, in which the transient behavior of three paramagnetic centers (two hemes and a tyrosyl radical) is observed.[2]

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MD449

Non-Canonical DNA Structures Studied by Spin-Label EPR

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In contrast to simple and predictable duplex structures, non-canonical DNA shows a high degree of polymorphism with respect to topological features, such as the orientation of individual strands and the connectivity of the loops. Therefore, the high-resolution methods are not always able to decipher the exact nature of these structures since they require the presence of single species. We have introduced EPR distance measurements for the investigation of highly polymorphic DNA structures.

For example, the human telomeric repeat adopts drastically different conformations depending on parameters such as the type of monovalent ions coordinated by the quadruplex and the slight changes in the nucleotide sequence. Double electron-electron resonance (DEER or PELDOR) spectroscopy is ideally suited to distinguish between the different conformations.

DNA three-way junctions can be used for the design of nanoscale assemblies because of their conformational versatility. In our case, an external stimulus, the addition of a small molecule, triggers a conformational change which can be monitored by spin-label EPR.

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MD451

Distances between paramagnetic metal centers and spin labels in proteins by pulsed EPR: The RIDME method as a new tool

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Structure determination in biological systems by electron paramagnetic resonance (EPR) is becoming increasingly popular. Distances in the nm range between spin labels in proteins yield structure restraints[1]. Transition metal-ion centers abound in proteins, but their potential as markers for distance determination is limited by their large g-anisotropies and fast relaxation times. For many of these centers, the known pulse sequences for e.g. DEER or PELDOR cannot be applied because of excitation bandwidth limitations. The RIDME method[2] circumvents this problem by making use of the spin-lattice relaxation (T_1)-induced spin-flip of the transition-metal ion. Designed to measure distance between such a fast relaxing metal center and a radical, it suffers from a dead time problem. This disadvantage can be avoided by the five-pulse RIDME (5p-RIDME) sequence. An Fe(III)-spin label distance in ths protein cytochrome *f* is determined.[3]

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MD452

Structure and Dynamics of Nanotubular Lipid Bilayers by Spin-labeling EPR

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Lipid nanotube arrays represent a new type of substrate-supported lipid bilayers (SSB) that are formed by self-assembling phospholipids into tubular structures inside ordered nanochannels of anodic aluminum oxide (AAO). These structures exhibit several important advantages over conventional SSB formed on planar substrates. Among those are very high surface area, long-term stability of aligned lipid assemblies under exceptionally wide range of temperatures, pH, and salt concentration, high hydration level of lipid bilayers, and protection from surface contaminations. Here we employed an arsenal of spin-labeling and high field EPR as well as other magnetic resonance methods to study structure, dynamics, and local interfacial polarity of nanotubular lipid assemblies formed in nanochannels with well-defined diameters of 21.0±3.2, 37.0.0±3.0, and 58.3.0±3.5 nm fabricated at NCSU. Specifically, synthetic phospholipids with pH-reporting nitroxides covalently tethered to the lipid polar head were used for evaluating surface potentials of lipid nanotubes when confined to AAO nanopores. Alternatively, alumina surface has been modified with pH-sensitive nitroxides to report on interfacial electrostatics. Spin-labeling continuous wave EPR was used to access membrane insertion and assembly of transmembrane peptides. Finally, DEER measurements have been performed to determine the distance constraints of peptide assemblies formed in lipid nanotubes.

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MD453

High-Field EPR Methods to Probe Electrostatics and Hydrogen Bonding in Protein Systems.

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Nitroxide spin-labeling in combination with EPR spectroscopy has found many applications in studying structure and dynamics of proteins and biological membranes. Recently, there has been a substantial interest in utilizing High Field and pulse EPR to characterize local effects of polarity and hydrogen bonding in proteins and biological membrane systems. Here we report on employing an arsenal of advanced spin-labeling EPR methods to address two questions: 1) to characterize binding of lipids by Sec14 protein and 2) to profile heterogeneous dielectric and hydrogen bonding environment along the α - helical chain of an alanine-rich WALP peptide that is anchored in a lipid bilayer in a transmembrane orientation. The measurements of local polarity and hydrogen bonding from characteristic changes in EPR spectra were enhanced by use of perdeuterated and ^{15}N-substituted nitroxides and high field EPR at 130 GHz (D-band). Formation of hydrogen bonds between the nitroxides and membrane-penetrating water molecules was observed directly in HYSCORE X-band experiments. Such measurements allowed us to derive experimental profiles of hydrogen bonding environment along a typical transmembrane α - helix.

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MD454

Spin-dependent transport in *μc*-Si:H silicon thin-film solar cells

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Microcrystalline silicon (μc-Si:H) is a promising material for thin-film solar cells[1]. It is characterized by a mainly disordered structure with embedded silicon crystallites. Defects in the bulk and at interfaces as well as localized states in the near of the energy bands give rise to charge carrier loss and hopping processes influencing the device efficiency. In order to elucidate the correlation between morphological structure and the electrical properties, charge carrier transport channels and the EPR fingerprints of contributing defect centres were analyzed by electrically detected magnetic resonance (EDMR). This technique detects changes in sample conductivity induced by spin manipulation rather than absorption of microwave resulting in significantly enhanced sensitivity. EDMR was applied to μc-Si:H fully processed thin-film solar cells to analyse the charge transport and recombination processes and the nature of the contributing states in the device. Furthermore an a-Si:H/c-Si interface was studied serving as a model system of crystallites in the amorphous silicon (a-Si:H) matrix revealing partly similar EDMR structures.

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MD455

Distance, Orientation and Structure Determination using a divalent spin label on fully deuterated histone Chaperone Vps75

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Histone chaperones physically interact with histones to direct proper assembly and disassembly of nucleosomes, regulating diverse nuclear processes such as DNA replication, promoter remodeling, transcription elongation and DNA repair. Nucleosome assembly proteins (Nap proteins) represent a distinct class of histone chaperone[1]. We recently turned our attention to the examination of the Vps75 chaperone and demonstrate that the protein undergoes a transition from dimer to tetramer on going from high to low salt conditions. We have used a divalent MTSSL like spin label to label and crosslink at the dimeric dyad axis of fully deuterated[2] Vps75. PELDOR spectroscopy demonstrated a distance of almost 80Å between 2 spin labels defining the tetrameric ring form at physiological salt concentration. Due to the restricted motion of the spin labels used we have been able to demonstrate and measure significant label orientation using W-band measurements[3].

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MD456

PELDOR Data Base

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Pulsed Electron-electron Double Resonance (PELDOR) is a method frequently used to determine distances between paramagnetic centers in bio-macromolecule on nanometer scale [1, 2]. A standard algorithm for determination of distances from the experimental data assumes that all possible mutual orientations of spin labels are equally probable. However, in many applications mobility of spin labels attached to large molecules can be significantly restricted [3]. In order to determine the total PELDOR signal in this case, individual contributions of each rigid biradical should be explicitly calculated for given frequencies of probe and pump pulses. Solution of the inverse problem or determination of ensemble of molecular structures that fit experimental PELDOR data acquired at multiple mw-frequencies and magnetic fields has proven to be an non trivial task, especially, when no information about molecular structure under study is available.

In this work we present a fitting algorithm that reconstruct experimental data by searching for an optimal combination of presimulated PELDOR time traces for nitroxide biradicals with all relative orientations and with inter-spin distances in the experimentally accessible range. The generated library of PELDOR time traces has been employed to excellently fit experimental data containing orientation selection effects gathered on model biradical systems and rigidly labeled DNA molecules.

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MD457

Design and Implementation of Optimal Control Based Broadband Excitation Pulses for EPR Spectroscopy

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Shaped pulses can significantly improve the performance of classical rectangular pulses to steer a spin from a given initial state to a desired target state, as demonstrated in NMR. In EPR spectroscopy, the creation of arbitrary shaped pulses is more difficult since the EPR timescale is about three orders of magnitudes faster than the NMR timescale. Our setup consists of a Bruker Elexsys pulsed X-band EPR spectrometer where we added an arbitrary waveform generator to be able to shape microwave pulses. Our waveform generator provides two independent programmable channels with 1GHz DAC's, which makes it possible to generate waveforms with arbitrary amplitude and phase on a 1ns timescale. For excitation bandwidths on the order of the bandwidth of the electronics and the resonator, transient effects play an important role, resulting in systematic distortions of the pulse shape experienced by the electron spins, compared to the ideal pulse shape. These effects can be characterized using a protocol to measure the experimental impulse response of the probe with a pickup coil. Based on the measured impulse response, the transient effects can be taken into account in the optimal control based GRAPE optimization algorithm, resulting in significantly improved experimental performance of broadband pulses. We present the application of broadband (200 MHz) excitation pulses to a quasi isolated spin ½ and an isotropically coupled spin ½ system.

MD458

Numerical Analysis of DEER in Gd^{3+}-Nitroxide Spin Pairs

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In last few years Gd^{3+} centers have attracted attention as possible probes for DEER-based distance determination, especially suitable for high field experiments[1,2]. In particular, DEER in Gd^{3+}-nitroxide spin pairs has been recently tested in our group and it shows surprisingly good performance down to X-band frequencies. No deviation of detected distances from the expectations has been observed so far even for Gd^{3+} complexes with moderately strong zero field splittings.

In this work we analyse the transitions excited and observed in the Gd^{3+}-nitroxide DEER experiment with the particular pulse settings used in our work[2]. We aim to see how much the distortion of the observed dipolar frequencies is reduced for this particular setup compared to the general case.

In addition to the dipolar frequency analysis, we perform spin-dynamics simulations for this type of pulse sequence in order to understand the experimentally observed echo reduction phenomena. The detected signal is usually dominated by the coherences on $|-1/2\rangle \leftrightarrow |1/2\rangle$ transitions of Gd^{3+} centers. The DEER echo reduction seems to occur when the pump pulse excites transitions that have a level in common with the $|-1/2\rangle \leftrightarrow |1/2\rangle$ transition of Gd^{3+} centers. This effect strongly influences sensitivity of the distance measurements with Gd^{3+}-based spin labels.

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MI459

Spin Relaxation in Trinuclear Clusters Comprising Half Integer Spin Ions

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The spin relaxation properties of Polynuclear Transition Metal Clusters (PTMCs) constitute a major field in Molecular Magnetism, especially in relation to Single Molecule Magnets (SMMs). The spin relaxation behavior of such systems is often assessed by Alternating Current (AC) magnetic susceptibility measurements while critical parameters affecting the relaxation mechanisms are determined by Electron Paramagnetic Resonance spectroscopy (EPR). Since the early nineties the combination of these two techniques has been established as a standard methodology for the study of SMMs. However, such combined studies on PTMCs that do not exhibit SMM behavior are rather scarce in the literature. In the present work we apply this methodology to the two trinuclear clusters $[Bu_4N]_2[Cu_3(\mu_3\text{-}Cl)_2(\mu\text{-}pz)_3Cl_3]$ (pz = pyrazolato anion) (**1**) and $[Fe_3(O_2CPh)_6(H_2O)_3]ClO_4\cdot py$ (**2**). We demonstrate that the relaxation properties of both clusters may be monitored by AC susceptibility measurements in the presence of moderate external magnetic fields. It is found that the relaxation follows a thermally activated process for both clusters.[1,2] In **1**, ferromagnetic interactions lead to an S=3/2 ground state. EPR reveals an almost axial zero field splitting tensor for this state with D ~ +0.1 cm^{-1}. In **2** the interactions are antiferromagnetic resulting in an S = 1/2 ground state. EPR indicates the presence of antisymmetric exchange [$\mathbf{d_{ij}}\bullet$ ($\mathbf{S_i} \times \mathbf{S_j}$)]. In SMMs, generally, the thermally activated relaxation is related to transitions within the same (ground state) spin manifold. For both **1** and **2** the analysis of the relaxation data indicates the involvement of different spin manifolds sets.

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MI460

Correlation of the EPR properties of polychlorotriphenylmethyl radicals and their efficiencies as DNP polarizer

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Recently, water soluble polychlorotriphenylmethy (PTM) radicals have been introduced as polarizers for ^{13}C nuclei in dynamic nuclear polarization (DNP) measurements[1, 2]. A mechanism has been proposed where the polarization transfer between the electron spins and the bulk ^{13}C nuclei takes place via the chlorine atoms of the PTM radicals. In this work we have investigated the EPR properties at W-band (~95 GHz, ~3.5 T) of two PTM radical derivatives, substituted with three and six carboxylate groups. Analysis of the EPR lineshape showed that although the solid effect mechanism in DNP is operational, contributions from EPR forbidden transitions involving Cl nuclear flips are likely. This point is further substantiated by ELDOR (electron-electron double resonance) detected NMR, time dependent ELDOR measurements and theoretical analysis of the Cl nuclear polarization based on the spin Hamiltonian parameters. All of the above studies provided support for the unique Cl assisted polarization transfer mechanism in these radicals.

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MI461

A paramagnetic DPPH crystal deposited on the crystal of single molecular magnet; EPR evidence on the proximty effect

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Single molecule magnets (SMM) are candidates for many applications, such as quantum computation, high-density magnetic data storage and magnetoelectronics[1]. In order to develop these applications, it is important to investigate when they are in contact with other dissimilar materials because it is expected that their wave functions and/or magnetic fields extend considerably outside the physical structure[2]. This also implies to improve detection of magnetic field on their surface *i. e.* magnetic field generated by the crystal in the vicinity of its surface. Thus, at the surface of the SMM crystal one expects time dependent as well as magnetic field depended distribution of magnetic fields. EPR spectroscopy accompanied with a local paramagnetic probe will be employed as an alternative simple detection method. Detection of such local fields on surface at very short time interval (~0.1 ns), as well as linewidth anisotropy of the probe, as function of orientation of SMM crystal in the external magnetic field are in the focus of this presentation. The broadening and splitting properties of the DPPH linewidth are considerably changed because of proximity with a molecular magnet. It is suggested that proximity effect on DPPH linewidth is produced by modulation of the zero field splitting of excited triplet state in DPPH crystal due to presence of local fields at the surface of molecular magnet.

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MI462

Light-Induced Spin State Switching and Relaxation in Copper-Nitroxide based Molecular Magnets Studied by EPR

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Exchange-coupled spin triads nitroxide-copper(II)-nitroxide are the key building blocks of polymer-chain molecular magnets $Cu(hfac)_2L^R$ exhibiting unusual magnetic switching similar to a spin crossover. EPR allows one to study this spin state switching induced by temperature or light [1]. The observed photoswitching is analogous to a well-known light-induced excited spin state trapping (LIESST) in spin-crossover complexes of iron(II), the promising effect for potential applications in light-operated magnetic nanodevices. In this report we discuss general trends, characteristics and mechanism of light-induced spin state switching in molecular magnets $Cu(hfac)_2L^R$ and following relaxation to the ground state using continuous wave X/Q-band and time-resolved (TR) W-band EPR. The formation of metastable light-induced state occurs on a nanosecond timescale and can be studied by TR EPR. Then this state slowly relaxes back to the ground spin state on a timescale of hours. This long relaxation occurs mainly in the tunneling regime with the thermally-activated region at elevated temperatures. Remarkably, the observed relaxation shows pronounced self-decelerating character for all studied compounds. This trend can be described assuming the distribution of activation energies due to unusual structural and magnetic characteristics of these 1D materials. LIESST-like phenomena in $Cu(hfac)_2L^R$ is an interesting topic for future research in field of molecular magnetism. This work was supported by RFBR (№ 11-03-00158), RF president grant (MK-4268.2010.3) and FAE (P 1144).

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MI463

HF-EPR study of Tetrairon(III) Single-Molecule Magnets

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Molecules showing slow relaxation of the magnetization at low temperature, known as Single-Molecule Magnets (SMM), are attracting continuing interest in molecular magnetism. Slow relaxation results from a large spin ground state S associated to an Ising type magnetic anisotropy, leading to the presence of a barrier to the reversal of the magnetization. Axial anisotropy terms govern the height of the barrier whereas transverse magnetic anisotropy terms influence the quantum tunneling of the magnetization. HF-EPR spectroscopy has demonstrated to be a key tool to provide precise information on the magnetic anisotropy of SMM and thus to understand the dynamics of their magnetization.

Tetrairon(iii) complexes with a propeller-like structure, of formula $[Fe_4(L)_2(dpm)_6]$, are providing an important class of SMM displaying synthetic flexibility and ease of functionalization (H*dpm* = 2,2,6,6-tetramethyl-heptane-3,5-dione), where L stands for the tripodal bridging ligand. HF-EPR spectra at low temperature have been collected on polycrystalline samples of several complexes in order to determine the zero-field splitting (zfs) parameters in the ground $S = 5$ spin state. In all these compounds, a remarkable correlation is found between the axial zfs parameter D and the pitch γ of the propeller-like structure.We report on our latest results obtained on new derivatives.

NA465

Structural and dynamic characteristics of an A→I edited RNA duplex as revealed by site-specific labeling and NMR spectroscopy

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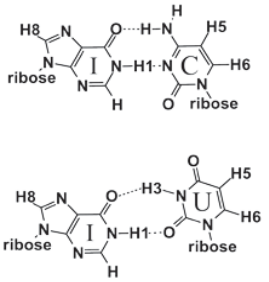

Adenosine (A) deaminases acting on RNA (ADARs) are responsible for the hydrolytic deamination of A to Inosine (I). As shown in the figure, a stable I:C base pair can form in a Watson-Crick geometry, while the I:U base pair can only occur as a wobble base pair. As a result it is interpreted by cellular machineries as G and Thus A → I editing can alter codon identity which may result in the alteration of various downstream regulatory events such as regulation of gene expression and RNA interference.

Surprisingly, this wobble pair has a highly destabilizing effect in the interior of the duplex as compared to other wobble base pairs like G:U. However, structural data explaining this phenomenon is lacking yet.

We employ NMR spectroscopy to analyze the structure and dynamics of an RNA duplex containing multiple I:U base pairs. For the NMR studies, a site-specifically isotope-labeled inosine phosphoramidite building block was synthesized and incorporated into the sequence. Thereby the inosines can be unambiguously assigned. Surprisingly, the central IIUI of the sequence is found to be highly dynamic and partly unstructured. This is corroborated by base pair lifetime measurements of the edited sequence in comparison with the native Watson-Crick duplex. A severe destabilization of the central part of the sequence is observed and the destabilization quantified base-pair-specifically.

NA466

Telomeric DNA coexists in two distinct G-quadruplex conformations

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In vertebrates, telomeric DNA consist of tandem repeats of the hexanucleotide $d(TTAGGG)_n$. Structural investigations have shown that these sequences form G-quadruplexes with various folding topologies under *in vitro* conditions. So far, experiments on telomeric sequences capable to form intramolecular monomeric G-quadruplexes have demonstrated four different structures in dilute solution including two hybrid parallel-antiparallel (3+1), one 2- and one 3-tetrad antiparallel folds (1). In addition, studies simulating molecular crowding and crystallographic data (2) suggest that the parallel G-quadruplex structure is the physiological relevant conformation *in vivo*.

We have investigated various telomeric sequences capable of forming monomeric, dimeric and trimeric intramolecular G-quadruplexes by means of CD, NMR and EPR spectroscopy and native PAGE under physiological relevant conditions. Data will be presented showing that telomeric DNA coexists in two distinct conformations.

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NA467

Characterization of hydrogen bond networks in nucleic acid and their ligand complexes by direct determination of XH - O=P and XH - - N type hydrogen bonds

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Hydrogen bonds are of fundamental importance for the formation of functional nucleic acid structures. Functional RNA molecules such as tRNAs, self-splicing introns, ribozymes, riboswitches and ribosomal RNAs adopt intricate three dimensional folds held together by non-canonical hydrogen bonds involving the Hoogsteen- and sugar-edges of the nucleobases, the ribose 2'-OH and the oxygen of the phosphate backbone. In their ligand complexes, hydrogen bonds are often formed between the ligand functional groups and free acceptor and donor groups of the RNA. Although the presence of these hydrogen bonding interactions is often indirectly inferred from the distance and geometry of the involved atoms, both intra-RNA and RNA-ligand hydrogen bonds have so far eluded direct detection. Here, we present the first direct characterization of such hydrogen bonding interactions by virtue of the through bond scalar coupling correlating the donor hydrogen to the acceptor group ($^{2h}J_{HX}$). Thus, we could verify the NH - - O=P imino-backbone and a 2'-OH - - N interaction in the U-turn motif, NH_2 - - O=P amino-backbone hydrogen bond in the YNMG tetraloop family and a 2'-OH - - O=P interaction in a looped-out base motif by application of long-range ^1H, $^{31}P/^{15}N$ HSQC. In addition, interactions between OH – and NH_3^+- functionalities of an aminoglycoside ligand and the RNA phosphodiester backbone or nitrogen nuclei in the base moieties could also be detected. The size of the through-bond hydrogen bonds was quantified by 1D ^1H $\{^{31}P\}$- and 1D ^{31}P $\{^1H\}$ spin-echo difference spectra.

NA468

Long-lived water molecules found within a G-quadruplex structure

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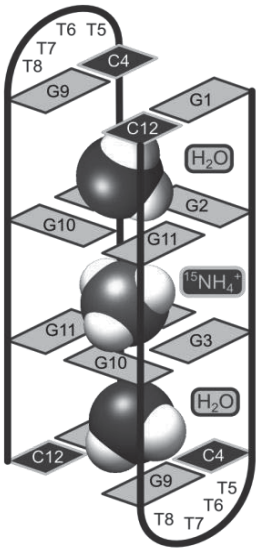
G-rich oligonucleotides containing cytosine residues can form G-quadruplexes where G- quartets are flanked by G·C Watson-Crick base pairs. Solution state NMR was used to study the folding of d($G_3CT_4G_3C$) oligonucleotide into a G-quadruplex upon addition of $^{15}NH_4^+$ ions. The topology is equivalent to the solution state structure of the same oligonucleotide in the presence of Na^+ and K^+ ions[1,2]. A single ammonium ion binding site was identified between adjacent G-quartets although three sites were expected. The remaining potential cation binding sites between G-quartets and G·C base pairs are occupied by water molecules. To the best of our knowledge this is the first observation of long-lived water molecules within a G-quadruplex structure[3]. The flanking G·C base pairs adopt a coplanar arrangement and apparently do not require cations to neutralize unfavorable electrostatic interactions amongst proximal carbonyl groups. A relatively fast movement of ammonium ions from the inner binding site to bulk with the rate constants of 21 s^{-1} was attributed to the lack of hydrogen bonds between adjacent G·C base pairs and the flexibility of the T_4 loops.



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NA469

The Hunt for the demon: Is there a general mechanism of RNA chaperones ?

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The obtainment of a defined functional structure at a distinct temporal and spatial position is essential for proper function of RNA molecules. Unfortunately, the conversion between off-pathway structures and the native correctly folded ones is a critical and slow step in RNA folding. But proteins have evolved that facilitate the folding of RNA molecules: RNA chaperones. These proteins have by any mechanism to elevate the free-energy of the RNA in such a way that the RNA is able to enter successfully the folding pathway that leads to its functional conformation. The C-terminal domain of the *E. coli* RNA chaperone StpA (CTD-StpA) displays both RNA annealing and strand displacement activities. Using NMR approaches we defined the sources of energy needed for the structural destabilization of the RNA. Contrary to computational predictions, CTD-StpA is a well folded but dynamic protein. Its structural fold is similar to that of its homolog protein H-NS presenting a positively charged surface of high plasticity, which interacts with the RNA. Although CTD-StpA interacts only transiently with the RNA, cross-links showed that the RNA is completely coated by peptides. Complex formation occurs via electrostatic interactions with the RNA backbone and can be modulated by ions. In presence of RNA the protein becomes structurally less flexible. In contrast, the RNA gains conformational entropy enhancing the ability to refold properly. We intend to discuss the results obtained for StpA with results for other RNA chaperone proteins and try to derive a general mechanism of RNA chaperoning by proteins.

NA470

The structure of SRSF1 pseudo-RRM in complex with RNA revealed an unexpected mode of recognition

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Among RNA recognition motifs, three different classes have been reported, namely the canonical RRMs[1], the quasi-RRMs[2] and the pseudo-RRMs. Using NMR, we solved the first structure of a pseudo-RRM in complex with RNA. This domain is found in SR (Ser-Arg rich) proteins, one of the most important families of factors that control gene expression in metazoans by regulating splicing events. In this study, we focus on the pseudo-RRM of SRSF1, which is the prototypical SR protein in humans.

Using 40 intermolecular NOEs, we solved with a high precision the solution structure of this domain in complex with a purine-rich RNA sequence. Instead of interacting with the β-sheet surface or loops as previously shown for RRMs[1] and quasi-RRMs[2], respectively, SRSF1 pseudo-RRM uses a completely different interaction surface centered on the negatively charged α1-helix. Three conserved residues (one serine and two aspartates) are directly involved in the specific recognition of a 5'-GGA-3' motif. The importance of these residues for binding RNA is further validated by affinity measurements and *in vivo* splicing assays. Importantly, we found a NMR signature characteristic of this interaction and could test RNA binding with seven pseudo-RRMs (SR protein homologs in fly and yeast and the remaining human SR proteins). Remarkably, all the tested pseudo-RRMs bind GGA-containing RNAs using the same binding surface.

Altogether, these structural data reveal a very unexpected mode of RNA recognition for one pseudo-RRM that can be extended to the whole family.

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NA471

NMR analysis of HAR1F RNA models

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Human accelerated regions (HARs) are parts of human genome which display a significantly accelerated nucleotide substitution rate (1). The majority of these regions are transcribed into non-coding RNAs. HAR1 encodes for 118-nt RNA and has 18 substitutions instead of the expected 0.27 substitutions since our last common ancestor with chimpanzees. HAR1F RNA is involved in brain development. Something caused our brains to evolve to be much larger and have more function than the brains of other mammals. The function of HAR1F RNA in development of human consciousness is unknown. Two different cloverleaf-like secondary structure models have been offered for the human HAR1F RNA. We used NMR spectroscopy techniques to determine secondary and 3D solution structures.

Different RNA models were prepared for human and chimpanzee HAR1F RNA sequences. Resonance assignment was performed using ^{15}N-HSQC, HNN-COSY and NOESY spectra. The NOE imino proton resonance patterns of the 37-nt RNAs were comparable to the patterns of the whole length human and chimpanzee HAR1F RNAs, respectively. We studied the dynamic properties of the 37-nt RNA constructs with the help of ^{13}C- and ^{15}N-relaxation NMR measurements and estimated the fast internal motions in the 37-nt RNA constructs by the measurement of longitudinal and transverse relaxation rates, along with heteronuclear NOEs. We were able the assign almost all aromatic and sugar proton resonances which allowed us to perform a complete NOE sequential walk. Calculated 3D structures show interesting structural features..

NA472

The bacterial second messenger c-di-GMP: slow kinetics of oligomer dissociation and monomeric state at physiological conditions

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Cyclic di-guanosine-monophosphate (c-di-GMP) is a bacterial intracellular signaling molecule that triggers the switch from motile to sessile lifestyles. C-di-GMP signaling is of considerable pharmaceutical interest, since it is related to bacterial virulence, biofilm formation and persistence of infection. Previously, c-di-GMP has been reported to display a rich polymorphism at millimolar concentrations, involving various oligomeric forms, due to base stacking and G-quartet formation. Here, we have analyzed the equilibrium exchange kinetics between the various forms of c-di-GMP by NMR spectroscopy. At low micromolar concentration and in a buffer that mimics cytosolic conditions c-di-GMP is predominantly in its monomeric state. This finding is important for the understanding of c-di-GMP recognition by protein receptors. On the chemical shift timescale, the c-di-GMP monomer is in fast equilibrium with a dimeric form, with a relatively large dissociation constant of about 1 mM. Above a concentration of 100 µM tetramers and octamers are present and octamers dominate above about 0.5 mM. In contrast to the monomer/dimer equilibrium, formation and dissociation of both tetramers and octamers occurs on a timescale of several hours to days, as revealed by 1D NMR spectroscopy after equilibrium perturbation. Kinetic parameters have been determined by fitting a kinetic model to the time course of the NMR peak intensities. After dilution from millimolar concentrations, the UV absorbance of c-di-GMP increases slowly at 253 and 277 nm and decreases at 300 nm over the time course of several hours, presumably caused by the destacking of bases during dissociation of the c-di-GMP oligomers. The extremely slow kinetics of oligomer formation/dissociation can generate severe artifacts for enzymatic characterizations, depending on the cation type and concentration of the reaction buffer. A protocol is proposed to minimize the problem of artifacts caused by oligomers.

NA473

Strategy to improve therapeutic siRNA by fragment-based screening and structural biology

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A therapeutic siRNA is a duplex of a 21-nucleotide RNA oligonucleotide that mediates gene silencing of its target mRNA by association of one RNA strand with the Argonaute-2 protein (Ago2) in the siRNA silencing complex (RISC). The ends of the guide strand are recognized by distinct domains of Ago2: the 5'-end binds in the MID domain, whereas the 3'-terminal two nucleotides are associated in a pocket of the PAZ domain. In order to improve the siRNA stability as well as their immune stimulatory properties, we addressed the possibility to substitute the 3'-dinucleotide overhang by a mimetic that still interacts with the PAZ domain. To this end, the crystal structure of human Ago2 PAZ domain was obtained, a fragment library was designed by in-silico screening using this structure, and these fragments were screened for binding to the PAZ domain by NMR spectroscopy. Novel ligands for the PAZ domain were identified in this way. For these hits, building blocks for oligonucleotide synthesis were derived and the corresponding conjugates were synthesized. The best conjugates derived using this approach, have enhanced chemical stability, without losing the binding properties of the modified siRNA to the PAZ domain. This results in novel fully active siRNA molecules as demonstrated by in vitro and in vivo model systems.

NA474

Pre-organization and ligand-induced folding in the *mfl*-2'-deoxyguanosine aptamer and a GC-mutant aptamer by solution NMR spectroscopy

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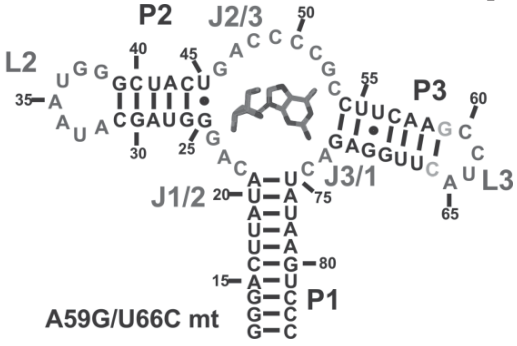
ABSTRACT The 2'-deoxyguanosine riboswitch from *Mesoplasma florum* (*mfl*) belongs to the class of purine-sensing riboswitches[1)]. We characterized the influence of Mg^{2+} on structural aspects of folding and complex formation for the *mfl*-aptamer and a stabilizing mutant (*mfl*GC) by NMR spectroscopy. Mg^{2+} titrations of the free native *mfl*-aptamer reveal a supporting role of Mg^{2+} in terms of structural motif stabilities. Stabilization of the peripheral long-range interaction by a conservative double mutation (A59G/U66C) results in a significantly weaker Mg^{2+}-dependence of tertiary structure pre-formation for the free *mfl*GC aptamer. The majority of the free aptamer conformational ensemble consists of pre-organized species even without any Mg^{2+}, whereas the native mfl-aptamer requires moderate Mg^{2+} concentrations to establish the loop-loop interaction. Interestingly, neither Mg^{2+} nor the stabilization of the loop-loop interaction affect the binding pocket which remains unstructured in the absence of 2'-dG.



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NA475

Sequence-specific local stability and dynamics of DNA double-helix studied by hydrogen NMR

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We have found an analytical solution of Bloch-McConnell equations describing general two-site chemical exchange for free induction decay, which has never been fully done before. The result gives us spectral lineshape with no presumptions made to T_2's or the ratio of magnetizations of the two sites. The explicit formula allows experimental spectra to be fitted by this function with no further numerical calculations. In this way, reliable values of relative populations and exchange rates can be obtained. [1]

This approach was applied to study the flexibility of various DNA duplexes. Base-pair breaking and corruption of regular geometry at particular location of DNA chain is accompanied by chemical exchange influencing the NMR lineshapes. Analysis of aromatic regions of ^1H spectra acquired in a broad temperature range covering the duplex melting yielded thermodynamic description of the base-pair breaking independently for each spectral line. Differences from the common course present a sensitive indicator of a local deviation from the overall duplex geometry and flexibility, which plays a major role in immune response in vertebrates caused by CpG containing oligodeoxynucleotides [2].

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NA476

Questioning the molecular crowding effect on structure and stability of human telomeric G-repeat sequences

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We have investigated G-quadruplex topologies of telomeric repeat sequences from vertebrates in the presence of molecular crowding mimetics, namely PEG 200, Ficoll 70 as well as *Xenopus laevis* egg extract by CD and NMR spectroscopy and native PAGE. Data will be presented showing that the conformational behaviour of the telomeric repeats in *X. laevis* egg extract or in Ficoll is notably different from that observed in the presence of PEG. While the behaviour of the telomeric repeat in *X. laevis* egg extract or in Ficoll resembles results obtained under dilute conditions, PEG promotes the formation of high-order parallel topologies. Our data suggest that PEG should not be used as a molecular crowding mimetic and that the parallel G-quadruplex structure of vertebrate telomeric repeat sequences is not the preferred folding topology under physiological conditions (1).

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NA477

Pushing the size-limitation of NMR of RNA.

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RNA structure analysis by NMR suffers from the drawback of small ^{1}H-chemical shift dispersion. This problem is enhanced by the fact that RNA consists of only four different nucleotides. An alternative to proton detection is the use of heteronuclei with larger chemical shift dispersion than ^{1}H. We synthesized ^{2}H, ^{13}C, ^{15}N labeled RNA by incorporating isotopically labeled nucleotides using *in vitro* transcription.

Taking advantage of higher sensitivity by using cryogenically cooled probeheads we performed predominantly ^{13}C-direct detected NMR experiments and developed new assignment strategies for RNA. It turned out, that the use of deuterium improved the relaxation properties and simplified the spectra remarkably. In future we want to benefit from these advantages in solution NMR as well as solid state NMR studies of RNA.

NA478

A combined X-ray and NMR study of the loop-loop mutant of the guanine riboswitch

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One method of gene regulation is the specific binding of small metabolite molecules to riboswitch RNA elements. Riboswitches are located in the 5' untranslated regions of messenger RNA and consist of an aptamer domain responsible for ligand binding and an expression platform. The guanine riboswitch binds guanine and related compounds with high specificity. The aptamer domain consists of a three-helix junction which is stabilized by long-range base pair interactions between two loops. Previously, using NMR-spectroscopy, we have found that the long range interactions are already present in the free RNA and that magnesium-ions are not required for ligand binding and structure formation but stabilize the tertiary structure (1,2). Mutating one of the essential long range base pair in the loop-loop region we found, that the long range interactions are disrupted in the free form and that ligand binding and structure formation depend on the presence of Mg^{2+}. Here, we present the X-ray structure of this loop-loop mutant and discuss the influence of the loop-loop interactions on the three-dimensional structure.

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NA479

NMR-based structural investigations of a minimalistic neomycin sensing riboswitch in complex with different aminoglycosids

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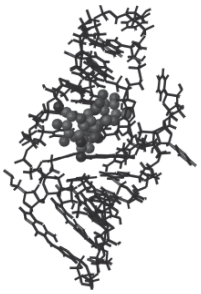

The 27nt engineered neomycin riboswitch (N1) is the smallest known riboswitch.[1] N1 represses gene expression upon binding of the aminoglycosides neomycin B and ribostamycin. In contrast, the closely related paromomycin, which differs from ribostamycin in only one OH-group, also binds N1 but does not inhibit gene expression.
NMR-structures have been solved for the N1-ribostamycin-and the N1-paromomycin complex[2]. To identify the structural and dynamical basis for their different regulatory activities, we meticulously examined their structural and dynamic differences. Our detailed NMR-investigations allow an understanding of the relation between ligand binding and regulatory activity in vivo.

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NA480

Time-resolved NMR-studies of competitive DNA i-motif formation

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Beside the well known double helix conformation DNA is able to build structures stabilized by non-Watson-Crick base pairs like G-quadruplexes[1] and i-motifs.[2] Those structures can be formed by guanosine- and cytidine-rich strands, respectively. In nature these sequences are found e.g. in telomeres, the ends of eukaryotic chromosomes.[3] They present a good target to address them e.g. concerning cancer therapy. Quarduplex structures are also found in the field of nanodevice applications.[4] The cytidine-rich strand forms its intercalated structure in slightly acid environment.[2] In the so-called i-motif two parallel-stranded duplexes with hemiprotonated $C{\cdot}C^{+}$ base pairs are fully intercalated.

We investigate structural changes and the kinetics of pH-induced folding of an i-motif at atomic resolution. For this, we used a rapid-mixing device which allows us to mix two solutions at a defined time directly inside of the NMR-spectrometer.[5] We could show that after induction of the folding with acid, first two competitive i-motif structures with different intercalation topology are formed. In a second slower step refolding of the less stable conformation to the thermodynamically more stable conformation occurs.

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NA481

Divide and Conquor: Structural studies of HAR1F RNA by NMR Spectroscopy

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The human accelerated regions (HARs) are a group of 49 segments in the human genome, which are ranked by their underlying fast mutation rate compared to homologous chimpanzee segments.[1] The 118bp HAR1 region has with 18 substitutions the highest mutation rate in the *homo sapiens* genome. HAR1F is coexpressed with Reelin in Cajal-Retzius cells which is a regulator in human cortical development. HAR1F RNA is involved in Huntington's disease.[2] A clear structure-function relation has not been identified yet. Secondary structure models of human and chimpanzee sequence of HAR1F are developed by DMS treatment[1], chemical and enzymatical probing[3], which differ to each other.

Here, we investigated a secondary structure elucidation of human and chimpanzee HAR1F RNA using CD and NMR spectroscopy. With this sizable 118-nt RNA, NMR spectroscopic assignment is challenging due to considerable spectral overlap. To overcome incomplete sequential connectivities we pursued a "divide-and-conquer" strategy by utilizing model hairpins that mimic structural elements of the full length RNA. We investigate the structures of model hairpins **c37**, **c47**, **c54**, **h37** and **h47** by NMR spectroscopy. We derived from our NMR data that model hairpins **c37**, **c47** and **h37** RNA are folded like the corresponding structural elements in the full length secondary structures by Beniaminov *et al.* and are able to disprove the chimpanzee structure model which was published by Pollard *et al.*

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NA482

Direct observation of the temperature-induced melting process of the *Salmonella* fourU RNA thermometer at base-pair resolution

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Many microorganisms change their gene expression pattern upon changes of the environmental conditions like metabolite concentration or temperature. FourU RNA thermometers are capable of sensing temperature. They repress translation initiation by blocking the Shine Dalgarno (SD) sequence at low temperatures.

In the present study the thermodynamic stability of the temperature labile hairpin 2 of the *Salmonella* fourU RNA thermometer was investigated at base-pair resolution [1]. Free energy, enthalpy and entropy values for the base-pair opening of individual nucleobases were determined from the temperature dependence of imino proton exchange rates measured by NMR spectroscopy. Nucleobase stabilities were mapped for the wildtype sequence and the temperature stable A8C mutant. Enthalpy and entropy values for base-pair opening are correlated linearly in both RNAs. However, the slopes of these correlations are different and coincide with the respective melting point determined by CD spectroscopy for both RNAs. Hence, RNA unfolding in both RNAs occurs at temperatures where all nucleobases have equal thermodynamic stabilities. Temperature-dependent *in vivo* gene expression analysis performed on three different fourU RNA thermometer constructs significantly differing in thermal stability (wt, A8C mutant, G14A-C25U mutant) confirmed the correlation of base pair stabilities, global thermal stability and *in vivo* gene expression levels.

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NA483

NMR structural investigations on the functional primase domain of an archaeal replication machinery

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Primases are single-stranded DNA dependant RNA polymerases that synthesize RNA during DNA replication. A primase, a DNA polymerase and an helicase compose the replication machinery of the archaeal plasmid pRN1[1]. The structure of the archaeal functional primase domain has been solved recently by X-ray crystallography[2,3] and it revealed an heterodimeric structure with a catalytic prim/pol domain and a novel helix bundle domain.

We investigated the NMR structure of the functional pRN1 primase domain in complex with a single-stranded DNA template containing the GTG motif[4]. On this 38 kDa enzyme, we localized the interaction site and we showed that 2 α-helices of the helix bundle domain are involved in DNA binding. Intermolecular contacts detected exclusively between the helix bundle domain and the DNA template led us to isolate specifically this structurally independent protein. We solved the solution structure of the helix-bundle-domain in complex with the single-stranded DNA template and we performed affinity measurements to confirm the importance of residues located in the helices 10 and 12 for the interaction with the GTG motif.

In association with functional assays, this novel transient structure will allow us to decipher the series of reactions required for replication initiation.

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NA484

Co-transcriptional folding studies of the guanine-sensing riboswitch by use of time-resolved NMR spectroscopy

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Co-transcriptional binding of guanine to the guanine-sensing riboswitch causes premature transcription termination of the mRNA under control. The ligand binding state of the 5' located aptamer domain of the riboswitch RNA decides whether the adjoining expression platform folds co-transcriptionally into a terminator or an antiterminator conformation. The latter conformation allows the polymerase to transcribe beyond the riboswitch element, thereby leading to the expression of downstream located enzymes. Static and dynamic NMR studies suggest that regulation by the guanine-sensing riboswitch RNA is under kinetic control, meaning that the riboswitch aptamer does not reach its equilibrium before the genetic decision has been made. However, if *in vitro*-transcribed guanine-sensing riboswitch RNA is purified and refolded into a single conformation in the absence of ligand, we always obtain the thermodynamically more stable terminator hairpin conformation. In the present study, NMR spectroscopy is employed to analyze the guanine-sensing riboswitch RNA molecules for their conformation(s) *in situ*, without subjecting the *in vitro* transcribed riboswitch to any refolding step or buffer exchange.

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NA485

Mechanism of ligand recognition in the tetracycline-riboswitch

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Riboswitches are highly structured non-coding RNAs that are involved in gene regulation. Here gene regulation is mediated by a structural rearrangement in the riboswitch architecture, which is induced on ligand binding. The tetracycline-riboswitch is an *in vitro* selected aptamer with the highest known affinity of an artificial RNA for a small molecule. In addition, it is one of only few *in vitro* selected RNAs capable of acting as engineered riboswitches[1]. The X-ray-structure of the aptamer in complex with tetracycline reveals an intricate three-helix junction architecture that is more complex than those of most aptamers and is reminiscent of natural riboswitches[2].

We investigated the role of Mg^{2+}-ions dependency for the structuring and stability in the ligand-induced folding of the tetracycline Riboswitch using high-resolution NMR and fluorescence spectroscopy in solution. Our data reveal that Mg^{2+}-ions induce long-range base pairing interactions and a variety of non-canonical structural elements in the core region of the ligand free RNA and pre-organize its global fold. Furthermore, we found that upon ligand binding only certain regions of the aptamer were folded and stabilized by tetracycline which resulted in the thermodynamically stable, regulatory active conformation.

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NA486

DNA Condensation With Spermine Dendrimers: Interactions in Solution, Charge Inversion, and Morphology Control

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Positively charged condensation agents are able to neutralize > 90% of the DNA phosphate charge and cause DNA condensation into tightly packed structures [1]. In this study we characterize the formation of compact complexes of 884 base pair, double stranded DNA and spin-labeled, second generation dendrons (SL-G2, 27 positive charges per molecule) using continuous wave electron paramagnetic resonance spectroscopy (CW EPR) and transmission electron microscopy (TEM) [2].
TEM shows that at a charge ratio of 2.3 of positive charges of the dendrimers to negative charges of the DNA strands the condensates form rod-like structures. Adding small concentrations of monovalent salt resulted in aggregation of the rod-like condensates and partial transformation to toroids. By adding manganese(II) salts charge inversion of DNA could be observed by CW EPR at the specific charge ratio of 2.3. At a charge ratio of 2.3 the Mn(II) ions are expelled from the formed DNA dendriplexes, which indicates an inverted charge of the DNA. Also, the observation of the Mn(ll) signal is indicative for the rod-like shape in the condensate. At varying charge ratios the Mn(II) ions are bound to the DNA. With increasing temperature the Mn(ll) signal becomes stronger but vanishes completely at monovalent salt concentrations above 35 mM. CW EPR observation of the rotational dynamics of SL-G2, shows that the condensation agent sticks tightly to DNA regardless of ionic strength concentration or temperature. Thus all obtained effects under varying conditions can be correlated with the interaction between dendriplexes of DNA and dendrons.

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NA487

New Insight Into The Structure of the Free State Of The Aptamer Domain Of The Adenin Binding Riboswitch

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Riboswitches are part of mRNAs that regulate gene expression upon specific binding of a small metabolite (vitamin cofactors, nucleobases, amino acids or even metal ions).[1] Conformational changes upon ligand binding transmit the ON or OFF signal for further transcription or translation. Binding region, so called *aptamer domain*, is adopting a sophisticated 3D structure able to bind the ligand with high specificity and affinity (K_D= nM - mM). After analysis by NMR-spectroscopy, mutation studies and successful crystallization of numerous complexes ligand binding mode is well understood.[2] Latest biophysical studies aim to solve the structure of the free aptamer domain and to elucidate the complex folding paths by identifying intermediate states. [3] In this work free state of the aptamer domain of the adenin binding riboswitch (*pbuE*) is investigated. By NMR-spectroscopy structures of two constructs with different lengths of the P1-helix are characterized. Results show that the elongation of the P1-helix has an unexpected effect on the structure of the free aptamer domain. By analyzing the structural differences and further comparing kinetic and thermodynamic data new insight into the relationship between structure and function is revealed. [4]

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NA488

Spectroscopic and molecular modeling studies on the interaction modes between natural alkaloids of potential pharmaceutical interest and telomeric G-quadruplex DNA

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During the past decade, interest toward the telomeric DNA and its quadruplex structures has significantly grown and many efforts have been recently devoted in the investigation of G-quadruplex stabilizing molecules as potential anticancer drugs.[1,2] However, the structural information reported till now is very little, thus impairing a comprehensive description of the ligand-quadruplex binding modes.

Among the potential G-quadruplex binders, we have studied the binding ability of sanguinarine and berberine, belonging to the alkaloids' family, an important class of natural products used by humankind as medicine since millennia.[3,4,5]

Spectroscopic (NMR, CD and fluorescence), calorimetric and computational approaches have been used to understand quadruplex–ligand interaction.

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NA489

Bifunctional Ligands Targetting RNA Identified and Optimized by NMR Spectroscopy and X-Ray Crystallography

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The essential role of ribonucleic acid-ligand interactions in the regulation of the retroviral life cycle, as in HIV, makes RNA an attractive drug target. Therefore, we use a FRET-assay to identify small molecules that interact specifically with the HIV TAR RNA and characterize these interactions by NMR spectroscopy and x-ray crystallography. Due to the unusual binding properties of RNA rational drug design was only modestly successful in identifying new RNA-specific ligands. The negatively-charged phosphate backbone indeed provides strong but unselective coulomb interactions with positively charged groups, such as amine or guanidinium groups. To increase selectivity and maintain binding strength, small molecules targetting different regions in one RNA will be connected and investigated. NMR spectroscopy helps to map the binding sites on the RNA of the individual ligands, to determine the binding stoichiometry and to clarify possible sites of modification to connect and optimize ligands. These bifunctional ligands should provide high selectivity and strong binding characteristics. The RNA-ligand complexes will be further investigated by X-Ray crystallography.

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NA490

Structural studies on tertiary folded DNA molecules

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Pulsed Electron-Electron Double Resonance (PELDOR) is a well-established method to gain structural and dynamic information of biomolecules on a nanometer scale. The dipolar coupling between two spins used in PELDOR gives rise to distance determinations in the range of 1.5 to 8 nm. Moreover, the magnetic tensor orientation can be unraveled in the case of rigid spinlabels.[1] The aim of this work is to understand the structure and conformational dynamics of double-stranded bulge DNAs. Such bent DNAs can serve as a model of complex nucleic acids, containing bulges, loops, junctions and kinks, and their interaction with proteins. Here we analyze three bulge DNA motives, in which the rigid Cytidin-analogue (**Ç**) was incorporated into chosen positions of DNA. This allows studying the dynamics of the biopolymer directly due to the rigid nature of **Ç**.[2] In order to gain information regarding the distances and the relative orientations of the paramagnetic centers we perform orientation selective measurements at X-, Q- and G-Band. The experimental PELDOR data are analyzed quantitatively by a home-written program using a simple geometric model, as well as molecular dynamic trajectories. Comparison of our results with NMR structures[3] and a combination of both constraints are under development and will be discussed.

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NA491

Structural and Kinetic Investigation of the full - length adenine - sensing riboswitch RNA by NMR - spectroscopy

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Riboswitches are recently describes RNA-elements which are located in the 5'-UTR of bacteria, fungi and plants. Due to specific ligand binding to the aptamer domain structural changes occur in the expression platform, which results in gene regulation [1, 2]. The gene expression is reported to be affected either at the level of transcription or translation[3].

As there is a lack of knowledge concerning full length riboswitches and the characterization of the folding pathways, this work focuses on the full length adenine-sensing riboswitch of *V. Vulnificus.* First we assigned the imino protons of the 112nt construct using the divide-and-conquer strategy.

With NMR spectroscopy we characterized the binding mode of the ligand to the RNA. Our data support the assumption of an induced-fit binding mode[4].

We now investigate the ligand-induced folding kinetics in atomic resolution with real-time NMR using a mixing device [5], in order to understand, whether the adenine full-length constructs follow a similar kinetic and structural mechanism of folding as the aptamer domain.

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NA492

Individual Base Pair Stability of DNA and RNA studied by NMR-detected Solvent Exchange

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Base-pair opening of nucleic acids is of fundamental importance during transcription or translation. Previously, we have characterized base-pair opening from NMR analysis of the temperature dependence of imino proton exchange rates (1).

Here, we study the thermodynamics of base-pair opening in DNA and RNA duplexes, deduced from the temperature-dependence of imino proton exchange rates monitored by NMR spectroscopy with individual base-pair resolution (2). We find that base pairs in RNA are more stable than in DNA, in general, and that enthalpy and entropy values of base-pair dissociation are correlated linearly. In case of RNA, the slope of the correlation coincides with the melting temperature as determined by CD spectroscopy, duplex unfolding occurs at the temperature at which all base pairs are equally stable. For the DNA duplex, these temperatures differ significantly. Increase of the deuteration level of the solvent stabilizes base pairs in DNA and RNA. By contrast, solute-solvent interactions are not influenced by the deuteration level as deduced from enthalpy-entropy correlation. Solute intrinsic interactions are enhanced in DNA with increasing protium/deuterium ratio. The impact of protium-deuterium exchange of the imino hydrogen on free energy of RNA base-pair opening is investigated. In the RNA duplex, two types of A·U base pairs can be distinguished based on fractionation factor analysis.

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NA493

Tetramolecular G-quadruplexes in solution: new insights

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G-quadruplexes are higher order secondary structures formed by guanine-rich nucleic acid streches in the presence of cations. G-rich segments are found in biologically significant regions of the genome such as telomeres, immunoglobulin switch regions and promoter regions of eukaryotic cells. NMR is superior tool to ascertain the nature of Hoogsten hydrogen bonds within G-quartets and for 3D structure determination of the G-quadruplex structures. Sequence details and the nature of metal ions play a major role in formation and stabilization as well as structural diversity of G-quadruplexes. Oligonucleotides containing only a single run of guanines form G-quadruplex structures where four strands come together. Early NMR spectroscopic studies, which were performed almost twenty years ago revealed that d(TG_4T) in the presence of Na^+ ions forms a tetramolecular G-quadruplex consisting of four G-quartets with all guanine residues in anti conformation.[1,2] We herein report on undescribed minor form, which is in slow exchange with the known topology.[3] An additional aspect of the study involves the evaluation of dynamics of cation movement in tetramolecular quadruplexes.

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NA494

Elucidating RNA dynamics with residual dipolar coupling restrained molecular dynamics simulations

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NMR spectroscopy can provide information about the structure and dynamics of nucleic acids over a wide range of biologically relevant timescales. Experimental data like Residual dipolar couplings (RDC) calculated by NMR are being widely used in determination and refinement of structure of proteins and nucleic acids. We discuss an approach to describe conformational fluctuations in RNA by incorporating RDCs as structural restraints in molecular dynamics simulations. This strategy has been demonstrated for proteins, but so far applications to nucleic acids have been limited. Here we perform molecular dynamics simulations with restraints derived from RDCs on different RNA molecules to characterise their dynamical properties by generating conformational ensembles representing the range of structures that they populate. The RNA molecules that we consider cover various aspects of RNA behaviour, including RNA-protein interactions, RNA-DNA interactions and RNA catalysis, and help understand the role of dynamics in these processes.

Keywords: RNA dynamics; Residual dipolar couplings; Molecular dynamics simulations

PS495

An EPR and ENDOR Study of the Frozen Ammoniated Electron at Low Alkali-Metal Concentrations

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Ammoniated electrons in dilute frozen solutions are examined using EPR spectroscopy under conditions where the formation of metallic nanoparticles is avoided. Two signals from two different species have been observed. One signal is metastable and decays irreversibly upon annealing. The metastable species saturates at a spin concentration of 10 nM. The annealing temperature for this species amounts to 60 K for frozen solutions of sodium in neat ammonia and is raised upon addition of metal iodide. The observed g value is smaller than the free electron g value and is compatible with a cluster-anion radical rather than with a cavity electron. The wave function of the unpaired electron contains about 6 % - 10 % of 2p character at nitrogen. The observed g shift is fully compatible with previously reported theoretical calculations.[1] The second signal cannot be annealed in the frozen state. The lineshape is homogeneous and its width depends on the identity of the metal and at large metal concentrations on the metal concentration itself. Upon increasing alkali metal concentration above 0.15 MPM, the lineshape changes from Lorentzian to Dysonian, indicating the presence of metal nanoparticles. A new ENDOR pulse sequence is introduced to investigate the presence of weakly coupled nuclear spins for homogeneous EPR lines.

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Ps496

Multifrequency Electron Paramagnetic Resonance Characterization of PpoA, a CYP450 Fusion Protein that Catalyses Fatty Acid Dioxygenation

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PpoA is a fungal dioxygenase that produces hydroxylated fatty acids involved in the regulation of the life cycle and secondary metabolism of *Aspergillus nidulans*. It was recently proposed that this novel enzyme employs two different heme domains to catalyze two separate reactions: within a heme peroxidase domain, linoleic acid is oxidized to (8*R*)-hyderoperoxyoctadecadienoic acid [(8*R*)-HPODE]; in the second reaction step (8*R*)-HPODE is isomerized within a P450 heme thiolate domain to 5,8-dihydroxyoctadecadienoic acid.[1] We observe EPR resonances of two distinct heme centres with *g*-values typical for Fe(III) $S = 5/2$ high-spin and Fe(III) $S = ½$ low-spin hemes. ^{14}N ENDOR spectroscopy on the $S = 5/2$ signal reveals resonances consistent with an axial histidine ligation. Reaction of PpoA with the substrate leads to the formation of an amino acid radical on the early ms time scale concomitant to a substantial reduction of the $S = 5/2$ heme signal. High-frequency EPR (95- and 180-GHz) unambiguously identifies the new radical as a tyrosyl, based on *g*-values and hyperfine couplings from spectral simulations. Further, EPR distance measurements revealed that the radical is distributed among the monomeric subunits of the tetrameric enzyme at a distance of approx. 5 nm.

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Ps497

DISCRIMINATION BETWEEN IRRADIATED and UN-IRRADIATED COFFEE MATE POWDER BY ESR SPECTROSCOPY

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Un-irradiated coffee mate (CM) powder (Nestlé Company) do not exhibit any ESR signal. A large ESR signal with unresolved shoulders was observed for the samples exposed to γ-irradiation by a ^{60}Co γ-cell having a dose rate of 1.0 kGy/h. The signal intensity variations versus to the exposure time (0-1.0 h) and radiation doses (0.1-5.0 kGy) were constructed for the UV and γ-irradiated samples, respectively. The dose-response curves of the CM samples exposed to UV and γ-radiations were found to be described well by an exponential and a linear function, respectively. The results of the present work show that, the discrimination between un-irradiated and irradiated CM samples can be done just comparing their ESR spectra. However, determination of the radiation dose received by the sample cannot be possible because of the fast decay of signal intensity at room temperature.

Keywords: ESR; coffee mate; irradiation

Ps498

Why H_2O is a Good Hydrogen Atom Donor in Radical-Based Epoxide Opening Catalyzed by Titanocene

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Abstract: The binding of H_2O, D_2O, CH_3OH, CD_3OD, CH_3OD and CD_3OH to Cp_2TiCl is studied on the electronic level by EPR spectroscopy and quantum chemical calculations in order to obtain structural insight pertinent to radical based epoxide opening. The wave function of the unpaired electron is found to be insensitive to the binding of small molecules and is characterized by a d^1_{z2} ground state at Ti^{III}. In addition, the hyperfine resolving ENDOR and ESEEM spectra indicate that a hydrogen bond to chloride is formed by H_2O, D_2O, and methanol at low concentration. At larger concentrations, complexation occurs concomitant with dissociation of the chloride. The visible absorption responsible for the color of the respective complexes is ascribed to a changed ligand-to-metal charge transfer transition of the dimeric catalyst. The observations provide new insight into the mechanism of the recently described hydrogen atom transfer from these reagent mixtures to alkyl radicals and opens perspectives for the design of more efficient reagents.

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Electron Paramagnetic Resonance and Magnetism of Small Platinum Clusters in Zeolites

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In its bulk metallic form platinum is not magnetic. However, in the pores of zeolites 13-atom clusters can be prepared quantitatively, and they show unusual and interesting magnetic behaviour that includes a fraction of diamagnetic Pt_{13}, a second fraction of high-spin paramagnetic and a small fraction of EPR active spin-½ clusters, all with essentially the same near-icosahedral structure with 12 atoms coordinating one that sits in the center [1-3]. Structural characterization was performed with EXAFS [2], magnetic measurements using XMCD [3] and SQUID magnetometry [2]. At room temperature, super-diamagnetism dominates over paramagnetism. Hydrogen desorption, readsorption and exchange are monitored using EPR spectroscopy [1]. More than 30 H atoms per 13-atom Pt cluster are found, and paramagnetism oscillates with hydrogen coverage. After desorption and readsorption one observes two types of surface-adsorbed H.

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Ps500

Huge Isotopically Induced Nuclear-Nuclear Interactions in Solids

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Dipolar nuclear-nuclear interactions in solids are generally too weak (<10 kHz) to be easily observed in EPR or ENDOR spectroscopy. Nevertheless this kind of interaction can be enhanced by one order of magnitude when it is mediated by an electron spin. In the special case of two nuclei linked by an inversion center, this so-called pseudo-dipolar interaction can be further enhanced by another order of magnitude. In this work we extensively investigated the effect of this interaction in a linear cluster of Ga-Ti^{3+}-Ga present in titanium doped gallium oxide β-Ga_2O_3 in which the electron spin is equally delocalized over the neighboring nuclei[1]. The ENDOR spectra of the three isotopic configurations ^{69}Ga-Ti-^{71}Ga, ^{69}Ga-Ti-^{69}Ga and ^{71}Ga-Ti-^{71}Ga were analyzed in details focusing on the isotopic effect. The effect of this interaction on the ENDOR spectra is drastically different between symmetrical (^{69}Ga-Ti-^{69}Ga and ^{71}Ga-Ti-^{71}Ga) and asymmetrical clusters (^{69}Ga-Ti-^{71}Ga). The interaction is one order of magnitude larger (1 MHz) for the symmetrical clusters than for the asymmetrical one (<0.1 MHz)[2]. These symmetrical clusters thus combine a resolved nuclear-nuclear spin interaction, a nuclear spin monitoring by an unpaired electron, and a large nuclear spin quantum register, which make them attractive for quantum information processing whereby nuclear qubits can be monitored by electronic spin and controlled by short selective radio-frequency pulses. In this prospect, we performed electron and nuclear Rabi oscillations both on symmetrical and asymmetrical systems.

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Ps501

The ID card of a nitronylnitroxide monoradical

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The synthesis of nitronylnitroxide radicals was first described in 1968 by J. H. Osiecki and E. F. Ullman[1]; a thorough characterization of these molecules was subsequently reported in 1972 in a paper whose title is "Studies of Stable Free Radicals. X. Nitronylnitroxide Monoradicals and Biradicals as Possible Small Molecule Spin Labels"[2]. Since then nitronylnitroxides have been mainly used as building blocks for molecular magnets[3] or radical scavengers against nitric oxide[4], yet to our knowledge their use as spin labels – despite being suggested in the title of reference 2 – is rather uncommon, albeit reported in the literature[5].

Within this context we try to outline a sort of «ID card» of nitronylnitroxide monoradicals with the aim to show that these molecules are well-suited to be applied as spin labels along with the commonly used nitroxide radicals; in this respect we will report a CW-EPR, pulsed EPR and CW-ENDOR characterization of Nit-Ph-CH_2-SAc. It will be shown that even though the more complicated spin system of nitronylnitroxides with respect to nitroxides could in principle discourage from the use of the former ones, still nitronylnitroxides are appealing spin probes especially when techniques requiring long spin relaxation times – such as pulsed EPR and CW-ENDOR – are considered.

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Ps502

Fitting the puzzle pieces together: the cage complexes with encapsulated cobalt(II) ion as new paramagnetic labels

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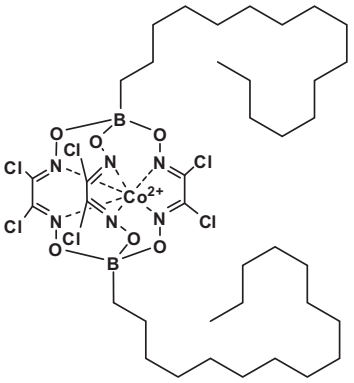
Macrobicyclic tris-dioximate complexes with an encapsulated cobalt(II) ion are perspective paramagnetic labels owing to the complete isolation of the metal ion [1]; hence the stability of the complex and its magnetic characteristics do not depend on the environment. The functionalization of such complexes by six ribbed and two apical substituents gives a room for fine tuning the characteristics of an encapsulated ion to achieve the desired features. In particular, the molecular design allowed obtaining macrobicyclic complexes with a high affinity to proteins (for example, T7 RNA polymerase). At the same time, the choice of dioximate ribbed groups affects tremendously the magnetic properties of an encapsulated ion, enabling the spin transition in these complexes. The pseudocontact interactions with the cobalt(II) ion leads to the paramagnetic shift of distant (>2.5 nm) nuclei signals in NMR spectra, paving the way for the use of the cobalt(II) cage complexes as non-covalent paramagnetic tags.



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Ps503

CO_2 Fixation: Why Nature Chose Oxazoline and Thiazole Rings.

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Lissoclinum patella is a colonial ascidian found on the Great Barrier Reef and lives in a symbiotic relationship with prochlorin, a cyanobacterium which is known to produce the cyclic octa-peptides patellamide D and ascidiacyclamide which incorporate oxazoline and thiazole rings. These ascidians also accumulate high concentrations of copper(II) from the seawater. Utilizing multifrequency EPR spectroscopy, circular dichroism (CD), UV-vis, mass spectrometry (ESI-MS) and X-ray crystallography we have shown that these cyclic peptides can form mono- and di-nuclear Cu(II) complexes, with the latter being able to fix CO_2 from the atmosphere.[1] Subsequently, we have undertaken systematic studies[2,3] of a series of synthetic analogues to examine the importance of stereochemistry, ring choice, and steric bulk in influencing the Cu(II) coordination chemistry and the ability of the dinuclear Cu(II) complexes to fix CO_2 from the atmosphere. The spectroscopic and computational chemistry results of the Cu(II) coordination chemistry indicate that the oxazoline and thiazole rings and backbone stereochemistry introduce greater conformational flexibility enabling CO_2 fixation to occur in the native systems. The biological implications of these observations will also be discussed.

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Ps504

Identification of the Active Eu^{2+} Centres in CsBr Storage Phosphors for Medical X-ray Radiography

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Classical film-screen X-ray radiography is gradually being replaced by technologies that provide the X-ray image in digital form. Computer radiography presents by far the cheapest solution : traditional photographic films, which require a wet development step, are replaced by image plates (IPs) and a scanner, while the same X-ray table can still be used. At the heart of the IP is a storage phosphor layer. X-ray irradiation creates stable defects in this phosphor, which recombine in the scan-out process after photo-stimulation in the (infra)red, resulting in green-blue photo-stimulated luminescence (PSL). In order to solve resolution problems of the first-generation IPs, Agfa Healthcare developed CsBr:Eu needle IPs with sensitivities comparable to those of BaFBr:Eu. This in itself is quite remarkable, as large Eu^{2+} doped CsBr single crystals grown from the melt exhibit only poor PSL. Our EPR and ENDOR study of CsBr:Eu IPs and crystals has been directed towards explaining the differences between these systems and concentrated on the structure of the active Eu^{2+} PSL centres.

In contrast with large single crystals, CsBr:Eu needle IPs do exhibit an intense room temperature stable Eu^{2+} related EPR spectrum. This undergoes dramatic changes when cooling the sample from 300 to 4 K. The ENDOR spectra not only reveal interactions with the central $^{151/153}$Eu and matrix ^{133}Cs and $^{79/81}$Br nuclei, but also with protons. A model in which Eu^{2+} replaces a Cs^+ ion, with a H_2O molecule and a Cs^+ vacancy in nearest neighbour cation positions explains all these findings. The EPR and ENDOR study explicitly makes use of the partially ordered needle structure of the specimens, rendering single crystal like spectra for specific plate orientations.

Ps505

Gd(III) Chelates as NMR Probes of Protein-Protein Interactions

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Two cyclen-derived Gd probes, $[Gd\text{-}DOTAM]^{3+}$ and $[Gd\text{-}DOTP]^{5-}$, were assessed as paramagnetic relaxation enhancement (PRE)-inducing probes for characterization of protein-protein interactions. Two proteins isolated from *Desulfovibrio gigas* rubredoxin and cytochrome c_3, were used as model partners[1]. $[Gd\text{-}DOTAM]^{3+}$ caused PREs on a well-defined patch near the metal center of rubredoxin (especially the patch constituted by residues D19 to G23 and W37 to S45, which broaden beyond detection). This effect was partially reversed for some resonances (C6 to Y111, in particular) when cytochrome c_3 was added to this system. In a ^{1}H NMR titration it was shown that $[Gd\text{-}DOTP]^{5-}$ binds to cytochrome c_3 near heme IV, also causing pronounced PREs, characterized by line width broadenings of its heme methyl resonances at ratios as low as 0.50. A K_d of 23 ± 1 μM was calculated based on chemical shift perturbation of selected heme methyl resonances belonging to three different heme groups, caused by allosteric effects upon $[Gd\text{-}DOTP]^{5-}$ binding at a stoichiometry of 2:1. Both probes were successful in causing reversible PREs at the partner binding site, thus showing to be good probes to identify partners' binding sites, and since the interaction was reversible, to structurally characterize protein complexes[2].

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Ps506

Synthesis of new Lanthanide Tags for RDC measurement in small organic molecules

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Lanthanide Ions are attractive tools in NMR analysis of large biomolecules. Most of these ions are paramagnetic, thereby generating different effects in the spectra. Residual dipolar couplings (RDCs) result from partial alignment of the molecule with respect to the magnetic field. The incorporation of a lanthanide ion can be realized either by natural ion binding sites or by attaching an ion binding tag to the sample molecule.[1]

While RDCs are arising to expanded application for large molecules, especially proteins, they are fairly less utilized for small organic molecules due to the fact that many existing alignment media are not suitable to organic solvents.

The aim of our project is to design lanthanide binding tags that are soluble in organic media and to check the possibilities of measuring RDCs of small organic molecules.

There are several molecules with ion binding properties that could serve as promising templates for new lanthanide tags (Figure 1) Already several decades ago DPM was used as lanthanide shift reagent to separate overlying signals but RDCs were never observed. Closer investigation and derivatisation at one side chain have to be performed to evaluate the opportunities to design a tag based on DPM. Dipicolinic acid is already well established as tag for the investigation of RDCs in biomolecules.[2] By esterification it can be transformed in derivatives which are soluble in organic media and functionalization in 4-position gives room for introduction of linkers or sample molecules.

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Ps507

EPR and DFT Study of Gamma Irradiated 2,6–di-tert-butyl-4-methylphenol Single Crystal

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Single crystal of gamma irradiated 2,6–di-tert-butyl-4-methylphenol was investigated using an electron paramagnetic resonance (EPR) technique at different orientations in the magnetic field at room temperatures. Taking into consideration the chemical structure and the experimental spectra of the irradiated single crystal of 2,6–di-tert-butyl-4-methylphenol, we assumed that one phenoxyl type paramagnetic species was produced having an unpaired electron localized at the methyl fragment side of the phenyl ring. Depending on this assumption, one possible radical was modeled using the B3LYP/6-311+G(d) level of density-functional theory (DFT). EPR parameters were calculated for these modeled radical using the B3LYP/TZVP and B3LYP/EPR-III level. The averaged value of isotropic hydrogen hyperfine coupling constants of a rapidly rotating methyl functional group of phenoxyl radical is calculated for the first time. Theoretically calculated values of the modeled radical are in reasonably good agreement with the experimental data determined from the spectra (differences in averaged coupling constant values smaller than 5 %, and differences in isotropic g values fall into 1 ppt).

Ps508

EPR study of unstable Eu^{2+} centres in CsBr

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In contrast with physical vapour deposited CsBr:Eu image plate (IP) X-ray storage phosphors, melt-grown Eu^{2+}-doped CsBr single crystals do not exhibit a room temperature stable Eu^{2+}-related electron paramagnetic resonance (EPR) spectrum (Eu^{2+}: $^8S_{7/2}$ ground state). However, after heating CsBr:Eu crystals in vacuum above 800K and rapid quenching to 77K or 273K, a spectrum exhibiting hyperfine structure typical for a natural abundant mixture of ^{151}Eu/^{153}Eu is produced. In a previous investigation, the intensity decay of this spectrum was interpreted as aggregation of Eu^{2+}-V_{Cs} (V_{Cs} - cesium vacancy) dipoles with trimer formation as an initial step [1]. However, the spin Hamiltonian analysis and consequences for the centre's model (Eu^{2+}-V_{Cs} orientation) remained unrevealed in this study. From the angular dependence of the EPR spectra, we determine that the dominant Eu^{2+} centre produced after quenching has cubic symmetry, which indicates a nonlocal charge compensation of the Eu^{2+} ion substituting for Cs^+. The broadening of certain EPR transitions is indicative of a random distribution of lattice imperfections near the Eu^{2+} ion. Both the thermal stability and angular dependence of this EPR spectrum are completely different from those of the Eu^{2+} centres stable in as grown IPs. Nonetheless an EPR spectrum with the same characteristics as in CsBr crystals can be produced in IPs by subjecting them to the same annealing and quenching procedure in vacuum.

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Ps509

Transferred RDCs, Induced by designed Lanthanide Binding Peptides

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A lanthanide-binding peptide tag with nanomolar affinity to trivalent lanthanide ions is attached to a short peptide that binds to ubiquitin. The alignment induced by the anisotropic magnetic susceptibility of the bound lanthanide ion when exposed to a strong external magnetic field arises residual dipolar couplings not only in the peptide but also in the coordinated protein and may therefore be used to investigate protein-ligand complexes. Utilizing the UIM1 sequence from hepatocyte growth facor-regulated tyrosine kinase substrate and loaded with one equivalent of Terbium (III) we were able to measure residual dipolar couplings as well as paramagnetic relaxation enhancement and paramagnetic pseudocontact shifts on ubiquitin.

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Ps510

Influence of the axial ligand on agostic interactions in paramagnetic Nickel complexes

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The impact of agostic interactions (3-center–2-electron M-H-C bonds) on the geometry of Ni-center catalysts is under investigation[1]. In case of an agostic interaction, the C-H bond tilts to close proximity with the metal center. Through this unusual interaction the metal center activates inert C-H bond, which is in potential interest for organometallic catalytic reactions. The synthesized Ni-complexes in our study carry three different axial ligands (Pyridine, DMF and Chloride) which can influence the agostic interaction.

In this work, we use low temperature CW and pulsed EPR experiments to study the electronic and geometric structure of the Ni-centers with different axial ligands. To this end, the carbon and hydrogen atoms participating in the agostic interaction were isotope-labeled with ^{2}H and ^{13}C. 4-pulse standard and matched and 6-pulse HYSCORE measurements have been performed[2]. The EPR parameters were compared to DFT calculations (ORCA) for modeled Ni-complexes[3]. The distances between the Ni-center and the atoms in C-H bond were predicted using equation for the anisotropic hyperfine interaction as described in Koh and Miller, 1985[4].

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Ps511

Anionic effects observed in complexes of trivalent lanthanides with partitioning relevant N-donor ligands

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World-wide efforts are being made to optimize separation technologies for removing trivalent actinides (An) from their chemically similar lanthanides (Ln) from nuclear waste streams. This is a key step in the partitioning and transmutation (P&T) strategy for reducing the long term radiotoxicity of spent nuclear fuel. Separation of An from Ln in nitric acid solution can be performed by liquid-liquid extraction using selective N-donor agents, e.g., alkylated bis-triazinyl-pyridines (BTP) in organic solvents. In efforts to optimize such separations, comparative investigations of An and Ln interaction with different extracting agents, solvents and solution components are performed.

Previous studies show that An- and Ln-BTP complex interaction with different charge compensating anions varies. To elucidate the nature of the interaction of (application relevant) nitrate anions, a number of BTP complexes with different anions are studied using NMR techniques. From the beginning of the '90s, anions are known to interact with electron poor aromatic systems. Associated changes in electron density on the aromatic ring are expected to affect changes in the chemical shift of nuclei in direct proximity. By separating the anionic effect from other parameters influencing chemical shifts of selected ligand nuclei using comparative NMR data on "reference complexes" with a non-coordinating anions, we are able to differentiate modes of interaction; chloride anions coordinate directly and nitrate anions insert between ligands in the complexes.

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A comparative NMR study of complexes of trivalent actinides and lanthanides with partitioning relevant N-donor ligands

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Partitioning and transmutation (P&T) is a strategy of reducing the long term radiotoxicity of spent nuclear fuel, thereby minimizing the required storage time. P&T involves separating actinides and fissioning them into shorter-lived fission products. In this context the separation of trivalent actinides from the chemically similar lanthanides is a key step. This separation can be performed by liquid-liquid extraction using selective N-donor extracting agents, e.g., alkylated bis-triazinyl-pyridines (BTP). These have high separation factors (>100) for trivalent americium over europium. However, little is known of the origin of their selectivity.

Previous studies have shown that actinide and lanthanide complexes of BTP are isostructural. Therefore a comparative study of the different binding modes of actinides and lanthanides is possible, as every observable effect on the electron density of the ligand may be attributed to a change in the central metal ion of the complex. NMR spectroscopy, as a tool to study such effects on the electron density around the observed nuclei, is ideally suited to address this question. By separating different contributions to the overall chemical shift, especially separating and monitoring the dipolar and Fermi-contact part of the paramagnetic chemical shift on selected ligand nuclei, it is possible to elucidate the ratio and nature of interactions between the ligands and the chelated trivalent actinide or lanthanide. A comparison of complexes with different *f*-element metal ions is revealing different degrees of covalency in the interaction between metal ions and BTP.

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Structural characterization of the NS2B-NS3 dengue virus protease in the presence of an inhibitor using pseudocontact shifts

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The two-component dengue virus NS2B-NS3 protease (DENp) is an established drug target but inhibitor design is hampered by the fact that no three-dimensional structure of the protease has been determined in the presence of an inhibitor. In contrast to the highly homologous West Nile virus protease (WNVp), the functionally important C-terminal segment of the dengue virus NS2B is dissociated from the NS3 in the absence of inhibitor (open conformation)[1]. A large structural change to a closed conformation is necessary for enzymatic activity.

In this work, paramagnetic tags were introduced at different positions in DENp to assess the fold in solution with a bound inhibitor. Pseudocontact shifts (PCSs) observed for the NS2B co-factor are in apparent agreement with a closed conformation modeled on the crystal structures of WNVp with different inhibitors. Therefore, the homology model based on the closed conformation of WNVp may be a better template for rational drug design than the open conformation of the available crystal structures.

On a technical note, PCSs from paramagnetic tags at different sites allowed the assignment of well-resolved NMR cross-peaks that could not be unambiguously identified through conventional 3D experiments. This presents a novel way of NM resonance assignments for difficult proteins.

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Ps514

Dynamics of loop-Lanthanide-Binding-Tags in Interleukin-1β

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Lanthanide-binding-tags (LBTs) are valuable tools for investigation of protein structure, function and dynamics. Their versatile use in NMR spectroscopy, X-ray crystallography and luminescence studies could be demonstrated for several proteins attached as fusion tags to the termini of proteins or chemically attached to cysteine residues.[1-3] Our new strategy involves the incorporation of encodable LBTs into loop regions of proteins. We could previously demonstrate the feasibility of this approach using a small library of Interleukin-1β constructs (loop-IL1β), which were designed to bear LBTs of varying length at different loop positions. Incorporation of the LBT neither impairs the binding affinity of Ln^{3+} to the LBT nor the overall fold of the protein. Moreover, we were able to measure paramagnetic effects such as residual dipolar couplings (RDCs) or pseudo-contact shifts (PCSs).[4] However, the magnitude of these effects differs with respect to the choice of the loop for LBT-insertion. Intrinsic motions of the protein backbone reduce the size of the measurable parameters. Here, we investigate the dynamic properties of three different loop positions (denoted L2, R2 and S2) of the loop-IL1β and compare it to the wildtype IL1β. The calculated generalized order parameter S^2 from $\{^1H\}$-^{15}N HetNOE, ^{15}N longitudinal relaxation rates (R_1) and ^{15}N transversal relaxation rates (R_2) will provide insights into the loop-LBT dynamics and the best loop architecture.

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Ps515

Trityl: A New Spin Label for Nanometer Distance Measurements

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We have used pulsed electron double resonance (PELDOR) and double quantum coherence (DQC) for nanometer distance measurements on novel trityl-trityl and trityl-nitroxide biradicals. The advantage of the trityl radical is its narrow linewidth of 2G, its large intensity and its slow relaxation at elevated temperatures, making it a promising candidate as a new spin-label for such measurements.

We performed PELDOR measurements on the trityl-nitroxide biradical with the inversion pulse set on the trityl spectrum and detection on the nitroxide which results in orientation selective time traces with a modulation depth of up to 100%. DQC measurements on the same biradical, using a commercial X-band spectrometer, gave a time trace with a very strong, well-defined modulation and yields the same distance distribution as the PELDOR experiment. On the trityl-trityl biradical the PELDOR experiment is not the prefered option but the DQC experiment can be easily performed on a commercial X-band spectrometer. These measurements show that trityl radicals have some advantages over the commonly used nitroxides, at least with respect to distance measurements.

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Potentialities of EPR in the characterization of the petroleum residues

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The petroleum residues are a complex mixture of high molecular weight compounds which contain pollutants as sulfur or metallic ions which must be removed by hydrodesulfuration and hydrodemetallation. The most abundant paramagnetic ion is vanadium (IV) which is mainly present in the asphaltenes. This corresponds to the petroleum residue fraction which precipitates in paraffinic solvent[1]. The chemical structures of the metallic compounds are not well known and understood yet[2] because of the complexity of the matrix and the investigation of the structure of the metallic complexes in asphaltenes is still an active domain of research. Indeed, it will permit to improve process and catalysts of petroleum residue conversion into valuable cuts which becomes of major importance considering the world increasing energy needs.

We will show that the continuous wave and pulsed EPR characterizations of the asphaltenes of several feeds and hydrotreated effluents reveal that the electron is a very sensitive probe of the vanadium and even the radicals which are naturally stable in the asphaltenes. Indeed, regarding the radicals, it permits to correlate the hydrodesulfuration depth with the g factor and enhance the different structures according to their geographic origin. Furthermore, regarding the vanadium, this technique turns out to be a powerful tool to characterize the environnement of the first coordination sphere (porphyrinic or non porphyrinic) which considerably evolves during hydrodemetallation. Thus, EPR allows giving crucial information on the structures which are the more resistant to the hydrotreatment.

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Rc517

Investigation of free radical activity of Artemisia absinthium for clinical using

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It is known that free radicals play an important role in a number of biological processes, some of which are necessary for life. In our experimental works we try to understand why not thermal treatment extract (NTTE) Artemisia Absinthium has is pro-oxidant in free radical processes, but after heat treatment of extracts (TTE) that activity became as antioxidant.In the first experiment we investigated the influence plant extract depended of kind of preparation on lipid peroxidation (LPO) using lipid contained biological target. The obtained results show that NTTE decreasing the concentration of both product of LPO- diene conjugate and malone dialdehyde. Then we investigated the level of luminescence of extracts. The NTTE had spontan chemiluminescence level at 5000 impulse/10sec, and TTE had only 75 impulse/10sec (the background of chemiluminescence was 30 impulse/10sec). For understanding the mechanism of influence plant extracts of free radical processes we used electron spin resonance (ESR) technique that gave some quality changes of a spectrum consistent, which indicate parameter of microviscousity of biological target after adding of plant extracts. We also used ESR to detect and identify free radical metabolites of drug extract to unravel the molecular mechanisms that lead to oxidative stress. ESR spectrum of drug plant extract pointed on displacement of maximum meaning of spectrum when we change concentration of plant. Our result can be help by preparation of drug for clinical using. Supported by ANSEF Grant1440-NS-biochem.

Rc518

Evidence of the formation of free-radical species in color degradation of hydrocracked streams

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Under some conditions, the hydrocracking gasoil streams has been observed to change color from a clear transparent hue to yellow until the formation of brown sediment at longer times.

The change of color occurs usually in the presence of air and light [1]. Different oxidation mechanisms have been proposed to explain the fuel instability involving the formation of free-radical hydroperoxide, the electron-transfer and the formation of soluble macromolecules.

In this study the free-radical oxidation mechanism was observed experimentally by *in-situ* irradiation of hydrocracked stream with a Xenon lamp (1000 Watt) in an EPR resonant cavity. Two main radical species were observed: peroxides and aromatic/alkoxides. The two radical species show different time evolution. The peroxide radicals appear immediately after light irradiation and tend to decrease at longer time; the concentration of the aromatic/alkoxide radicals increases more slowly until a plateau is reached under illumination and decreases only when the light is turned off. On the basis of this study a mechanism for the formation of color and deposit is proposed consisting in an early formation of peroxide radicals, followed by a propagation step in which aromatic/alkoxide radicals are produced. Subsequently, the products of oxidation can combine with aromatic compounds in the hydrocracking streams to form sediment. The increase in aromatic size and the formation of oxygen groups are confirmed by synchronous fluorescence and IR spectroscopies. The amounts of radical species and deposit seem to be related to the polyaromatic content.

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Rc519

Origin of Light-induced Spin-Correlated Radical Pairs in Cryptochromes

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Blue-light excitation of cryptochromes (Crys) and homologs uniformly triggers electron transfer (ET) from the protein surface to a flavin-adenine dinucleotide (FAD) cofactor [1]. A cascade of three con–served tryptophan (Trp) residues has been considered to be critically involved in this photoreaction. Starting from fully oxidized FAD, light-induced ET via the Trp triad generates a series of short-lived spin-correlated radical pairs (RPs) comprising of an $FAD^{\bullet}$ and a $Trp^{\bullet}$. Coupled doublet-pair species of this type have been proposed as the basis, e.g., of a biological magnetic compass in migratory birds, and were found critical for some Cry functions *in vivo* (see, e.g., [2]). In this contribution, we examined a Cry from *Xenopus laevis* as paradigm system. Light-generated $FAD^{\bullet}\cdots Trp^{\bullet}$ RP states have been characterized in detail by time-resolved EPR (TREPR) at various microwave frequencies. Different RP precursor states – singlet versus triplet – have been considered in spectral simulations of the experimental spin-polarized TREPR signals. Conclusively, we present evidence for a singlet-state precursor of $FAD^{\bullet}\cdots Trp^{\bullet}$ RP generation because at the different magnetic fields, where the RPs have been studied, net-zero spin polarization was observed. Neither a spin-polarized triplet precursor nor a triplet at thermal equilibrium can explain such a polarization pattern. It turns out that a multi-frequency TREPR approach is essential to draw conclusions on the nature of the precursor electronic states in light-induced spin-correlated RP formations [3].

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Rc520

EPR Study of Gamma Irradiated Single Crystal Cholesteryl Heptanoate

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Cholesterol take part significantly in many biological mechanisms and an important component for manufacture of bile acids, steroid hormones, and several fat-soluble vitamins. To determine magnetic properties of cholesteryl heptanoate ($C_{34}H_{58}O_2$) which is an important cholesteryl ester in human life and new technology; the single crystals of cholesteryl heptanoate were grown by slow evaporation of concentrated ethyl asetat solution and the grown single crystals were irradiated at room temperature with ^{60}Co–γ ray. The radical produced by gamma irradiation has been investigated in the range of temperatures 123–330 K for different orientations of the crystal in a magnetic field by EPR. Radiation damage center was attributed to radical $\dot{C}H_\alpha CH_{2\beta}$. The g factor and hyperfine coupling constants have slight dependency on temperature and evident dependency on the orientation of the magnetic field. Determined g factor and hyperfine coupling constants for the radical $\dot{C}H_\alpha CH_{2\beta}$ were found to be anisotropic with the average values g_{av}=2.00361, $a_{CH\alpha}$ = 14.52 G, $a_{CH2\beta}$ = 25.78 G. The EPR parameters which were compared and found to agree with the values given in the literature are given, the angular variations of the hyperfine interaction tensor, *A(θ)* and spectroscopic splitting factor tensor, *g(θ)* for the radical $\dot{C}H_\alpha CH_{2\beta}$ produced in cholesteryl heptanoate are shown in the presentation.

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Rc521

ESR Investigation of Stable Organic Radicals in Soils

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ABSTRACT. The role of stable radicals on the sorption and irreversible binding of organic chemicals (OC) to soil [1] were investigated by ESR spectroscopy. Analyzing ESR spectra of two soils, a luvisol (ME) and a cambisol (KA), at X- and W- bands, we observed a complex signal [2] of organic radicals (OR) (g_{Ph}=2.0039; g_{Ar}=2.0025 ÷ 2.0035) as well as of those of paramagnetic defects in the mineral phase, such as E'$_1$ and E'$_2$ paramagnetic centers (g_z=2.002) that can influence the binding of OC to soils. Soil moisture between 5 and 30% and treatment with oxygen and argon had no significant influence on the OR concentration whereas the shape of the signal changed in dependence on the previous drying of sample. Samples dried in a furnace at 105° C after moistening showed a significant sharpening of the complex signal whereas the drying of soil samples at 200° C resulted in an insensibility to moistening and the identity of the signals from ME and KA. To investigate the molecular mechanism and kinetics of sorption and irreversible binding of OC to soils, we applied a method of paramagnetic probes, i.e. nitroxide spin labels (SL) with different substitutes, to the soils, and found a substantial decrease in the radical concentration of Amino Tempo, Tempo and Hydroxy Tempo SL during the first two days of incubation with both soils, an enhanced appearance and quenching of OR of soil ME (during 1 – 2 days) and KA (during some first minutes) by interacting with Amino Tempo, and Hydroxy Tempo partitioned in sites of different polarity.

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Rc522

Electron Paramagnetic Resonance of free radicals in atherosclerosis and abdominal aortic aneurysm in patients treated surgically

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The project aims to examine the type and concentration of free radicals and iron ion complexes using electron paramagnetic resonance in blood in patients with atherosclerotic arterial obstruction in aorto-iliac segment, and femoro-popliteal segment treated surgically.

Increasing levels of free radicals is the main cause of complications occurring in the ischemia and reperfusion. So far, studies suggest that some deaths due to multi-organ failure may be related to damage arising from reperfusion after temporary clamping of the aorta during vascular reconstructive procedures.

The measurements of blood samples were performed using EPR spectrometer from Bruker working at 9.4 GHz frequency. All samples were investigated at 170 K temperature.

The samples from 25 patients treated at the Department of General and Vascular Surgery, Medical University of Poznan, both with femoro-popliteal occlusion and occlusion of the arteries in the aorto-iliac segment were examined. Blood test for free radicals were taken before surgery, 30 minutes after restoration of blood flow (reperfusion) and 24 hours after surgery. The results show increasing of free radicals concentration after surgery depending on time and type of medical treatment.

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Rc523

Combination of spin trapping ESR and ESR oximetry to study the effect of plant extracts on Fenton reaction

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The anti-oxidant properties of various compounds could be shown by their influence on the formation of free radicals in Fenton reaction. The formation of free radicals during the Fenton reaction is usually studied by the ESR spin trapping technique. Spin trapping utilizes the reaction of unstable free radicals with nitrone or nitroso spin traps, resulting in the production of spin adducts that can be detected by ESR spectroscopy. However, several possible pathways proposed for the Fenton reaction complicate the direct interpretation of the anti-oxidant properties of the studied compounds. Therefore, in addition to spin trapping the information about variation of oxygen concentration during the Fenton reaction is used. One of the methods, which enable these measurements is ESR oximetry.

In this work the anti-oxidant properties of glucose and extracts from typha pollen and onion seeds are shown by their influence on the kinetics of 4-hydroxy-5,5-dimethyl-2-trifluoromethylpyrroline-1-oxide (FDMPO) adducts formation. Additionally, the ESR linewidth of perdeuterated 2,2,6,6-tetramethylpiperidine-N-oxyl(PD-TEMPONE) spin probe is analyzed in order to monitor changes in oxygen concentration. Our results indicate that the addition of various plant extracts and glucose dissolved in water influence the Fenton reaction in different ways. This experiment reveals the possible application of this method to study antioxidant activity of plant extracts.

Rc524

Radical cations and diradical dications of trimethylene and dimethylated-trimethylene mono-bridged bis-*p*-phenylenediamines

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Hole transfer between *p*-phenylenediamine units plays a decisive role for the conductivity in polyanilines. Our systematic investigation of specifically connected *p*-phenylenediamines provides the intrinsic factors of charge delocalization connected with the relative orientation between these moieties.

The radical cations and dications of singly bridged bis-*p*-phenylenediamines *N,N'*-Di-(n-dimethylaminophenyl)-1,3-propanediamine (**HMPD**) and *N,N'*-Di-(p,p'-dimethylaminophenyl)-3,3'-dimethyl-2,4-propanediamine(**OMPD**) (the PD dimers bound by a flexible linker) were investigated by EPR and UV-Vis spectroscopy.

The delocalization of the charge and the interaction between the electron spins in the dications were found to be exceptionally temperature sensitive, depending on the change of an extended conformation (doublet-doublet state) predominant at room temperature and a π-stacked conformation (singlet state) being populated when the temperature is lowered. This process is also dependent on the flexibility and the shape of the interconnecting alkyl chain. Moreover ion-pairing phenomena influence the amount of charge delocalization.

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Rd525

Nuclear spin-lattice relaxation in carbon nanostructures

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Interpretation of nuclear spin-lattice relaxation data in the carbon nanostructures is usually based on analysis of fluctuations of dipole-dipole interactions of nuclear spins and anisotropic electron-nuclear interactions responsible for chemical shielding, which are caused by molecular dynamics. However, many nanocarbon systems such as fullerene and nanotube derivatives, nanodiamonds and carbon onions reveal noticeable amount of paramagnetic defects. The interaction between nuclear and electron spins strongly influences the nuclear spin-lattice relaxation. However, it is usually not taken into account, thus the relaxation data are not correctly interpreted. Here we report on the temperature dependent NMR spectra and spin-lattice relaxation measurements of intercalated fullerenes $C_{60}(MF_6)_2$ (M = As and Sb), where nuclear relaxation is caused by both molecular rotation and interaction between nuclei and unpaired electron spins. We present a detailed theoretical analysis of the spin-lattice relaxation data taking into account both these contributions, as well as our NMR relaxation measurements of $C_{60}(MF_6)_2$. The developed approach would be useful in interpreting the NMR relaxation data in different nanostructures and their intercalation compounds.

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RD526

A theoretical and experimental study of NMR contrasting properties of nanocomposites based on ferric oxides stabilized by arabinogalactan matrix

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NMR relaxational properties of aqueous solution containing nanocomposites based on magnetite and maghemite nanoparticles stabilized by arabinogalactan obtained from Baikal larch (Larix sibirica) wood matrix were investigated. The relaxational properties of the solutions, namely viscosity dependences of T_1 and T_2 and magnetic field dependence of T_1, were studied experimentally. Two models of the nanocomposite granular structure corresponding to two limiting cases of ferromagnetic material distribution over the arabinogalactan matrix were considered. The first one assumes a homogeneous distribution of magnetite and maghemite nanoparticles over the spherical arabinogalactan matrix, while the second one considers a single hard ferromagnetic core at the centre of the spherical arabinogalactan matrix. Theoretical fitting of the experimental results within these models was performed.

RD527

Correlated motions in biomolecules by HDR methods

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In numerous biological processes that constitute the base of living organisms, protein function is fundamentally related to internal dynamics occurring on μs-ms timescales, that can give rise to chemical exchange contributions to relaxation. In a heteronuclear two-spin system (e.g., $^1H^N$-^{15}N), correlated motions of the two nuclei induce cross-relaxation between multiple-quantum coherences that can be quantified using new Heteronuclear Double Resonance (HDR) techniques [1,2]. An analytical model describes the effect of an applied rf field on the relaxation rate of interest in the presence of fast exchange [3], providing accurate information on the kinetics of correlated processes. We show that motions on similar timescales are present in the two different bonding surfaces in human ubiquitin and in the linker between them (Phe45), suggesting the presence of a possible global motion. Additional applications of HDR give insights into the dynamics undergoing in the KID-binding domain (KIX) of the CREB-binding protein. We identified the presence of exchange processes, faster than the one reported in [4], outside the main helices of KIX. These fast motions are most likely the sign of conformational disorder within the native state, which may promote the transition to the unfolded ensemble.

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RD528

Novel "order-disorder-order" mechanism for protein conformational change

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Conformational changes are intimately coupled to the biological activity of many proteins. It is a challenge to unravel molecular mechanisms of conformational change since intermediate states are short-lived and usually not observable by spectroscopy. Adenylate kinase (adk) is an enzyme that undergoes a large conformational rearrangement in response to ATP binding. Thus, it is an excellent model to study the interplay between structure, dynamics and activity.

To study the mechanism of adk conformational change we have combined protein engineering with solution state NMR and other biophysical techniques. We have identified a novel mechanism involving local unfolding/refolding of a segment in the ATP binding subdomain in an otherwise folded enzyme. Thus, the mechanism can be denoted as an "order-disorder-order" transition. Only "order-disorder" transitions have been observed experimentally for other proteins previously.

Apparently, in the case of adk, the functional and folding energy landscapes clearly overlap. An appealing hypothesis based on our results is that Nature has made use of cooperative unfolding/refolding reactions to add functional properties (such as conformational plasticity) to proteins.

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RD529

The effect of matrix disorder on electron spin relaxation: EPR study of solid ethanol

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Amorphous solids in comparison with crystals exhibit anomalous dynamics at low temperatures. However, the microscopic nature of processes governing molecular dynamics at temperatures well below the glass transition is still in the focus of the research interest [1]. In this context the application of pulsed electron paramagnetic resonance (EPR) spectroscopy offers the advantage of studying local properties in the vicinity of paramagnetic center. Since solid ethanol can be prepared in phases characterized by different types of disorder [2], it has been used as a very convenient model system for studying low-molecular weight solids by EPR upon incorporation of paramagnetic nitroxyl radicals [3]. In this study ethanol host was doped with TEMPO while electron spin-lattice and phase-memory relaxation times, measured in the temperature interval between 5 and 80 K, were compared for crystalline and glassy states of solid ethanol. In order to delineate the impact of matrix protons on electron spin relaxation the comparison was made between the data acquired with protonated and deuterated host. The largest difference between spin-lattice relaxation rates was detected at lowest temperatures studied and discussed in terms of two level system/boson peak excitations [4].

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RD530

Modeling of Microstructure of Ion Hydration Shells on the Basis of NMR Data and Quantum-Chemical Calculations

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Investigations of the magnetic relaxation processes of nuclei belonging to solvent molecules and ions give information about the microstructure in different substructures in solutions. On the basis of the developed NMR-relaxation method it was possible to determine the coordination numbers of about 30 ions and to estimate the reorientation times of water molecules near ions [1].

Spin-lattice relaxation rates of ^{1}H, ^{2}H, ^{7}Li, ^{23}Na, ^{35}Cl, ^{81}Br and ^{127}I nuclei in salt and acid solutions were measured in a wide temperature range using the home-built relaxometer, Bruker SXP 4-100 and AVANCE 500 spectrometers.

In order to conciliate the data obtained from proton and deuteron resonances the electric field gradients and the QCC of deuterons from different molecular complexes were estimated on the basis of quantum-chemical calculations. Quantum-chemical calculations of different ionic-water clusters were carried out to study peculiarities of hydration shell structure in aqueous solutions using Gaussian 98 and Gaussian 03 program suites. The DFT method with hybrid functional B3LYP was chosen to take into consideration a non-local electronic correlation. The basis set 6-31++G** augmented by diffuse functions was chosen to outline hydrogen bonds structure after the additional investigation of the influence of a basis-set choice on calculation results. In particular, the coordination number of COO^- group was obtained and compared with experimental NMR results.

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RD531

NMR Relaxation: a Key to Domain Mechanics in Proteins

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Knowledge of protein dynamics in the multi-domain enzymes is essential for understanding biocatalysis. Domain/loop motions might be responsible for substrate recognition, positioning, binding, and product release. Here we demonstrate the capabilities of the ^{15}N spin relaxation and ^{15}N relaxation dispersion to study catalysis-related domain motions and conformational exchange with the aid of adenylate kinase from *E. coli* (AKeco) and its complex with the two-substrate inhibitor AP_5A, which was shown to be a transition state mimic. In the course of the phosphorylation reaction domains AMPbd and LID of AKeco execute collective *ns* motions with the activation energy E_a in the range of 53–64 kJ/mol. The helix α_3 (AMPbd) and strands β_5, β_6 and β_8 (LID) are associated with E_a = 80–100 kJ/mol, typical of the AKeco chemical reaction, which was estimated by biochemical methods to be on the order of 90 kJ/mol. At the same time, domains AMPbd and LID experience site-specific *ms* motions with the average activation energy of 90 kJ/mol to configure an active site. Hence, domain motions may provide the respective limiting stages of the enzymatic reaction. These domain motions affect the activation energy of global tumbling. For AKeco and the two-domain Ca^{2+}-calmodulin, this activation energy is 3.3 and 2.8 kJ/mol less than for their substrate complexes. This indicates that domain motions facilitate protein tumbling that may affect the transmission factor.

The chemical exchange associated with the active site, consisting of collectively exchanging residues H-bonded to the inhibitor, was detected within AKeco*AP_5A. This process may coordinate the two spatially separated reaction centers. Specific residues are implicated tentatively in substrate capturing and positioning, cleavage of the P_2–P_3 ATP bond and phosphorylation of AMP, yielding the ADP product.

RD532

High Speed Sample Transportation Apparatus Applying on Field Dependent Relaxation Study – Design and Experimental Results

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In order to investigate molecular motions and biomolecular structural information, a high speed sample transportation apparatus in a high field superconducting magnet (NMR 600MHz, 14.1T) has been built. The magnetic field gradient along the vertical axis of the magnet allows NMR parameters to be determined at 0-14 T magnetic field strength in high resolution from heteronuclear multidimensional NMR spectra. In order to achieve this field dependent measurement, our designed apparatus, named "field cycler", is driven by a high power servo motor and shuttles the sample stably center of the magnet at 14.1T to the top of fringe field at 0.01T in 100 ms which permit us to determine NMR dynamics parameters up to the order of 10^{-1}s in a routine basis. This opens up many experiments which could not be done previously, thus extends the capability of NMR on determining macromolecular dynamics. Some promising preliminary experimental data will be discussed.

RD533

Magnetic Cellulose Microbeads as effective relaxation agent for MRI contrast Enhancement

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Porous cellulose micronized particles are the convenient matrix for incorporation of superparamagnetic ferumoxide for MRI. Magnetic Cellulose Microbeads were synthesized by alkaline coprecipitation of magnetite in cellulose pores from Fe(2) and Fe(3) salt solutions after preliminary diffusion saturation of microporous beads. The deposited particles contained the magnetic material at concentration 64 mM/l. Magnetic relaxation of water protons was strongly disturbed by magnetic cores due to intensive diffusion in nonhomogeneous magnetic field. T_2 was measured by spin-echo and T_1 by inversion-recovery method at 7,1 T. The cellulose magnetic particles displayed the high rates of magnetic relaxation per mmol of Fe which was more higher than in suspensions of ferumoxides stabilized by low-molecular dextran at comparable concentrations. The line of proton resonance of cellulose preparation had the form of wide nonlorenzian curve. The analysis of relaxation behavior and line form points to the static dephasing mechanism in averaged magnetic field. MR images of fantom 2% agar-agar gel and mouse stomach with magnetic micronized cellulose were made by «Avance II 500» (Bruker)

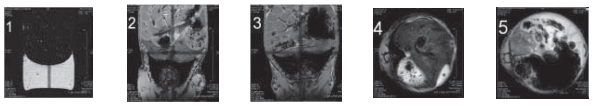

Fig. MR images: sample vial with micronized cellulose(100-200 nm, 3mg/ml) with ferrumoxide (64 mM/l) dispersed in agar-agar 2% - (1), mouse stomach before and after peroral administration of 75 mg micronized cellulose (D=30-10 μg) (gefi orto, sagittal) – (2, 3), (MGE TE=3 ms, axial) – (4,5).

RD534

Investigation of Correlated Protein Motions by the Measurement of Cross-Correlated Relaxation Rates

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The internal motion of biomolecules is essential for biological functionality like enzyme catalysis or molecular recognition. Recently it has become possible on the basis of residual dipolar couplings (RDCs) to create conformational ensembles of the protein ubiquitin. These ensembles reflect ubiquitins's protein dynamics up to the millisecond time scale. In addition to residue specific motion, these conformational ensembles suggest a high degree of correlated motions. Correlated motions between distinct sites in biomolecules have been proposed to play a major role in important processes like allostery and signal transduction. They are particularly important for a protein like ubiquitin which has to be able to assume multiple conformations in order to interact with many different proteins. However, the experimental validation of these predicted correlations is difficult. Since, cross-correlated relaxation (CCR) rates are highly sensitive to the angle between two involved dipoles; the measurement of CCR rates can be a useful probe for correlated motions.

CCR rates of scalar coupled nuclei pairs can be obtained in a straight forward manner. Of special interest are CCR rate measurements of nuclei pairs in parts of the protein like β-strands, which are proximal in space to each other, but are not scalar coupled. Here a newly developed NMR experiment is presented based on two relaxation-allowed coherence transfer (RACT) steps for the measurement of CCR rates between the CαHα groups of residues in separated β-strands.

RD535

Towards a Comprehensive Elucidation of Motion in Proteins by Ensemble-based Structure Calculation Using Exact NOEs

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Standard NMR structure determination of biomolecules is mainly based on the collection of many nuclear Overhauser enhancements (NOEs) that are translated semi-quantitatively into upper limit distances. This practice dates back to the 1980s when it proved difficult to determine NOE rates quantitatively and convert them into exact distances. We recently demonstrated that $^1H^N$ – $^1H^N$ NOE rates distances up to 5 Å obtained from both ubiquitin and GB3 have an experimental random error of < 0.1 Å which is smaller than deviations between high-resolution NMR or X-ray structures[12]. Extension of this approach to aliphatic protons yielded the collection of 850 eNOEs for GB3. Upon translation of eNOEs into exact distances standard structure calculation by CYANA yields a well defined NMR structure with an rms deviation of 0.54 Å (bb 0.08 Å) to a RDC-refined X-ray structure. However, many experimental restraints are violated due to the nature of the NOE being an ensemble-averaged probe. Thus, we developed a novel CYANA structure calculation protocol which enables an ensemble calculation that takes into account several simultaneously present structural states of the protein. The structure calculation of GB3 shows that at least 3 structural states are required to fulfill the experimental restraints. Analysis of the 3-states ensemble shows the presence of correlated dynamics of the beta sheet and of multiple rotamers in the side chains.

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RD536

Cold-Hot Unfolding and Dynamics of a Higly Stable Antifungal Protein

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Many water soluble proteins exist at thermal equilibrium as mixture of folded and unfolded conformers. While the highly populated folded conformation can be observed and characterized by NMR, the less populated conformers often coexist as hidden exchange partners. In order to disclose which form is important for the biological effect, we studied the dynamic behavior of the antifungal protein PAF, that is stabilized by three disulfide bonds. Single cysteine mutations of PAF resulted in unfolded structures, though they preserved some activity. Mutation of critical lysines caused loss of function, though the fold was kept. Conventional and relaxation interference data were interpreted using the model-free and reduced spectral density methods. Possibility of putative disulfide bond rearrangement was checked. Temperature induced reversible unfolding/folding of PAF was evaluated in the –10 … +80^{o} C range using ^{15}N-^{1}H HSQCs. Enthalpy, heat capacity, low and high temperature melting points of unfolding were determined residue by residue, using either two or three-state models (one intermediate state). It turned out that cold denaturation happens in a concerted way in contrast to heat-shock, and „hot-spots" were found in the non-conserved loop regions of the sequence. The oligomeric state of heat-denatured (inactive) PAF was also investigated.

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RD537

Capturing multiple scale dynamics in proteins through NMR relaxation analyzed by a fractional Brownian dynamics approach

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Internal dynamics represents a crucial ingredient of molecular function at the biological level. This has been the impulse to many methodological developments of spin relaxation measurements. Interpretation of experimental data in terms of dynamics is based on assumptions about the underlying physics. One of the difficulties is to provide a motional model that is both physically sound and in statistical agreement with experimental data. Our approach uses a Fractional Brownian dynamics (FBD) model of internal motions in proteins, which is a simple way to introduce *multiple scale dynamics*[1,2]. Therefore, based on general physical considerations about stochastic motions we propose to model internal correlation functions of bond vectors by Mittag-Leffler functions, which arise in a natural way in the theory of fractional diffusion. We used MD simulations to demonstrate the capacity of FBD to correctly model various correlation functions and the superiority of Mittag-Leffler functions over the usual multi-exponential models[1]. We then used relaxation rates predicted by MD simulations to develop a strategy to analyze NMR relaxation data[2]. The methods will be illustrated by applications to experimental measurements on the protein 6PGL at 14.1 and 21.1 T[3].

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RD538

Binding of a viral protein to a PDZ Domain studied by NMR and ITC underlines the role of domain flanking sequences

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Carcinogenesis mediated by the human papillomavirus oncoprotein E6 involves its binding to a subset of PDZ domains. In order to understand the molecular basis of the recognition between these PDZ domains and the E6 binding motif, a biophysical study was undertaken using NMR and ITC. While only limited structural changes of the PDZ domain were observed upon viral motif binding, major changes of backbone dynamics in the C-terminal flanking region of the PDZ domain was revealed by ^{15}N relaxation measurements[1]. This dynamical transition correlates with a significant change of the heat capacity between the free and bound PDZ domain as revealed by ITC experiments. Mutations of the C-terminal region of the domain, which is disordered in the apo state, were shown to affect the binding process, highlighting its role in the molecular recognition process. Our study provides original insights on the complexity of protein-protein interactions mediated by PDZ domains and illustrate the valuable complementarity between thermodynamics and dynamics information provided by ITC and NMR studies.

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RD539

Microstructure of complex solutions with organic molecules

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Approach developed in the Department of Quantum Magnetic Phenomena (SPbSU) is based on two independent and complementary research methods: NMR-relaxation and quantum chemical calculation. This approach allows us to get the solvation and hydration properties of the organic molecules in salt solutions.

It was found, that the ions can be used as probes in the study of complex solutions. Researches of the NMR ion nuclei are useful for studying the hydration environment of organic molecules. The investigation of the aqueous solutions of salts, containing the Na+, Cl-, surfactants (SDS) and different organic compounds, has been carried out in a wide range of concentration and temperature.

Hydrophobic properties of surface of some amino-acids were studied. Comparison of spin-lattice and spin-spin relaxation gives information on the mechanisms of exchange in solution and the protein surface. The results of NMR investigation suggest that there are two classes of chlorine-binding sites on BSA molecules: a small number of strong binding sites and weak binding sites.

The experimental results have been compared with the data obtained by quantum-chemical calculations. The combination of two methods, one of which is experimental and another is theoretical, offers obvious advantages.

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RD540

Natural porous media investigated with low-field NMR

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Mobile NMR has its origin in well-logging. By now there are numerous applications of mobile NMR in materials analysis and chemical engineering where, for example, unique information about the structure, morphology and dynamics of matter is obtained, and new opportunities are provided for geo-physical investigations.[1] In particular, dynamic information can be retrieved by two-dimensional Laplace exchange NMR, where the initial NMR relaxation environment is correlated with the final relaxation environment of molecules migrating from one environment to the other within a so-called NMR mixing time t_m.[2]

Relaxation-relaxation exchange experiments were performed with saturated [3] and un-saturated soil samples at low field with a simple, homemade, portable Halbach-Magnet. By executing such exchange experiments for several mixing times and inverting the results to 2D T_2 distributions (reminiscent of joint probability densities of transverse relaxation times T_2) with the help of the inverse 2D Laplace Transformation (ILT), we observed characteristic exchange processes: The results from fully saturated samples are compared to exchange at different saturation levels.

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RD541

Recent Improvements in Production and Transport HP 129-Xe

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Hyperpolarized (HP) ^{129}Xe is applied successfully as a contrast agent in MRI with clear advantages over ^{3}He, like low price, high availability and solubility in some liquids (e.g. blood) [1]. Also, HP ^{129}Xe is used in fundamental physics research [2]. Typically, these experiments require polarization levels higher than 20% and long relaxation times (T_1) for storage and transport. To improve the first, a freezing/thaw system was developed based in the ideas of Ruset *et al* [3]. This system minimizes polarization losses due to diffusion in inhomogeneous fields and lingering in liquid phase for too long. A signal improvement of about six was achieved with this system over previous procedures with reproducibility around 80%. To increase the T_1 times, different storing vessels, environments and gas mixtures will be tested. Therefore, a low field NMR spectrometer was build which allows T_1 measurements at the transport conditions (~25G) and to avoid magnetizing ferromagnetic impurities inside the glass walls. The main disadvantage of working at such low fields is the low signal to noise ratio. An improved aluminum shield and some strategies for noise reduction are described. Finally, HP ^{129}Xe T_1 measurements in different storage vessels are presented together with some considerations for achieving longer T_1 times.

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RD542

Frequency Dependent NMR Relaxation of Oil Molecules Confined Inside Polymeric Micro-Capsules for Drug Delivery

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Polymeric micro- and nanocapsules are often used as drug delivery systems [1]. Their main benefit is that various active substances such as drugs or even proteins can be transported directly to the diseased tissue, completely bypassing the healthy ones. Understanding the surface effects on the dynamics of molecules confined inside polymeric capsules is important both for obtaining of theoretical scientific knowledge and for designing of new capsules to be used in controlled drug delivery. In order to extract information on molecular dynamics in a wide range of characteristic times the Fast Field Cycling (FFC) nuclear magnetic resonance relaxometry [2] has proven to be a valuable tool. In our work frequency dependent nuclear magnetic resonance (NMR) relaxation studies have been carried out on Miglyol molecules confined inside core shell polymeric capsules to obtain a correlation between capsules dimension, relaxation time and diffusion coefficient. The polymeric capsules were prepared using an interfacial polymerization technique for three different concentrations of Miglyol. It was shown that the variation of Miglyol concentration influences the capsules dimension. The relaxation dispersion curves were obtained at room temperature by a combined use of a fast field cycling instrument and a high field instrument. The frequency dependence of relaxation rate shows a transition from a diffusion-limited to a surface-limited relaxation regime [3].

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RD543

A Mean field Brownian Dynamics simulation to explore intermolecular nuclear spin relaxation mechanisms in solution

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The study of solvent effects on nuclear spin relaxation have a long history and have been explored, mainly, with analytical models and numerical solution of Smoluchowski equation to compute spectral densities (1). For instance, the effects of translational motions have been studied using either force free diffusion models or radial mean field potential approaches. The effects of spins that are eccentric in molecules have been accounted for in these calculations but only in an approximate way (2).

We introduce a mean field Brownian dynamics simulation method significantly cheaper, in computational cost, than atomistic molecular dynamics simulations. This simulation technique allows us to explore spin eccentricity and rotational diffusion in an exact form and under different dynamical regimes. The method is flexible in allowing for higher dimensions and therefore a wider range of relaxation effects become accessible. So far, we have exploited these potentialities exploring nuclear spin relaxation, out of the spherical molecule approximation and using mean field results from atomistic molecular dynamics.

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RD544

NMR Study on Thermal Phase Behaviors of Ionic Liquid 1-Butyl-3-methylimidazolium Hexafluorophosphate

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^{1}H-T_1, T_2 of 1-butyl-3-methylimidazolium hexafluorophosphate [C_4mim]PF_6 were measured as a function of temperature in the range from 173 to 413 K. There was no discontinuous change in ^{1}H-T_1, T_2 traces in the cooling process. In the heating process, the^{1}H-T_1, T_2 traces changed discontinuously at 233 and 253 K. The first discontinuous change of ^{1}H-T_1, T_2 values at 233 K is due to the crystallization and the second change of ^{1}H-T_1, T_2 is a phase transition between crystalline phases. Furthermore, the third phase is obtained after the sample in the crystalline state is cooled in a freezer for 24 hours. These three crystal phases correspond to gauche-trans (GT), trans-trans (TT) and gauche'-trans (G'T) conformations of the butyl group in the cation, respectively, which was shown in the previous report[1)]. These three phases have different ^{1}H-T_1 traces. Second moment calculations indicated that there were different segmental motions in every phase.

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RD545

NMR Study on Thermal Phase Behaviors of 1-Alky-3-methylimidazoliums Bromides Depending on Alkyl Chain Length

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In previous work, we investigated the thermal phase behaviors of ionic liquids 1-alkyl-3-methylimidazolium bromides [$C_n$mim]Br where n is 2, 3 or 4 by ^{1}H-T_1,T_2 measurements. [C_2mim]Br crystallized at 290 K in the cooling process. [C_3mim]Br does not crystallize in the both cooling and heating processes. On the other hand, [C_4mim]Br does not crystallize in the cooling process but crystallized at 273 K in the heating process[1)]. Its crystallization speed was extremely slow that is it took about 1 hour for 0.5 ml sample. In this study, ^{1}H-T_1,T_2 of [C_5mim]Br and [C_6mim]Br in the wide temperature range were also investigated. [C_5mim]Br showed the cold crystallization in the same monner as [C_4mim]Br. The crystallization speed was very slow and almost similar to that of [C_4mim]Br. The phase behaviors of [C_6mim]Br was the same as that of [C_3mim]Br, namely, it did not crystallize in the both cooling and heating processes.

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RD546

Influences of Ceramides on POPC Membrane Dynamics from ps to ms Timescales: A Solid-State NMR and MD Study

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Ceramides are one of the important membrane component that play a crucial role in many physiological processes like cell growth, apoptosis and signalling. However, the mechanism of these processes is not known. Thus, It is of interest to understand the effects of ceramide on membranes.
The aim of this study was to monitor the complete dynamic profile of membranes in the presence of ceramide from ps to ms by solid-state NMR techniques and molecular dynamics simulations. NMR experiments were performed on a pure POPC bilayer, and on POPC-cer-4 and POPC-cer-12 systems to obtain order parameters, rotating and laboratory spin-lattice relaxation times for 13C and 1H atoms. MD simulations were performed of a pure POPC bilayer and a set of POPC bilayers that contain different amounts of cer-4 and cer-12, ranging from 5 % to 20 % in number. The collected data from SS-NMR and MD simulations methods reveals how ceramide effects the dynamics of membranes.

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RD547

^{23}Na NMR study of intelligent hydrogels

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Hydrogels are three-dimensional polymer networks which have the ability to absorb high amounts of water. Due to this property they are used as diapers, contact lenses, sealing materials, ameliorates, drug delivery systems, sensor and actuators. In response to an external stimulus such as pH, ion concentration, or temperature, hydrogels can reversibly change their shape. Depending on their chemistry, they may swell or shrink upon external stimulation.

Different kinds of hydrogels are presented based on acrylic acid and maleic acid for a high charge density and on vinylphosphonic acid for a high ionic strength. The particular chemical composition also influences the mechanical properties and the swelling behaviour. The new types of hydrogels could be employed as sensors, actuators, and switchable porous media. The mobility of sodium ions is investigated by NMR interns of the ^{23}Na self-diffusion coefficient and the relaxation times T_1 and T_2 for a better understanding of the swelling and the switching processes.

Furthermore the ion dynamics of thermo sensitive copolymers on N-isopropylacrylamide and sodium acrylate were investigated by ^{23}Na relaxometry as a function of temperature.

RD548

Characterisation of the dynamics in the bound state of the I-domain of LFA-1 with lovastatin using computational docking and RDC-restrained molecular dynamics

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We have recently developed an approach to determine ensembles of structures representing the conformational equilibrium dynamics of proteins by exploiting the information provided by residual dipolar couplings (RDCs). In this approach, RDCs are calculated from the shape and charge of individual structures, and employed as ensemble-averaged restraints in molecular dynamics simulations. Since the RDCs of the individual conformations making up a heterogeneous state of a protein can differ very significantly, this approach enables to achieve an accurate description of the statistical weights of the different conformers that are populated during the dynamics. Here we use this approach to explore the idea that conformations populated during the equilibrium dynamics of a protein could be used in the search for novel pockets for ligand docking. The inclusion of the protein flexibility during protein-ligand docking is a crucial problem in rational drug design, and it represents one of the great challenges for the structural biology community. The combination of in silico techniques with experimental NMR data is a promising alternative to X-ray crystallography screening of ligand fragments. To illustrate our approach we present our preliminary results of a study of the dynamics of Lymphocyte Function Associated Antigen-1 (LFA-1) Inserted domain (I-domain) and its interaction with lovastatin.

RD549

Characterization of Nonnative States by NMR: Hen and Human Lysozyme

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Unfolded and partially folded states of proteins play a crucial role in human neurodegenerative diseases. Single point mutations are linked to these diseases but the structural code for the onset is still unclear. One protein known to form amyloids are mutants of the extremely stable human lysozyme, whereas this is not known to date for the highly homologous hen lysozyme. Here, nonnative states of hen[2, 3] and human lysozyme[1] and single-point mutants of both proteins have been investigated using NMR spectroscopy. Secondary structure propensities, relaxation data, RDCs and R_h were compared. All proteins exhibit α-helical propensities, higher in hLys and its mutants than in hen lysozyme and its mutants. Most significantly dynamics and aggregation propensities vary between the proteins: High aggregation propensities and restricted dynamics are found in amyloidogenic mutants of human lysozyme, while W to G mutants in hen lysozyme disrupt dynamic restrictions.

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RD550

Dynamics in the Catalytic Cycle of a β-Phosphoglucomutase

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Of all the reactions that occur in biology, phosphate monoester hydrolysis is among the slowest to proceed without a catalyst. The enzymes which catalyse these reactions are able to achieve rate enhancements on the order of 10^{21}, the highest acceleration factor currently identified[1]. Trifluoromagnesate (MgF_3^-) ions are both isosteric and isoelectric with a transferring phosphate ion and therefore make excellent transition state analogues for these reactions[2].

β-Phosphoglucomutase (βPGM) catalyses the interchange between β-Glucose-1-Phosphate and β-Glucose-6-Phosphate (βG6P). In the presence of βG6P and both Mg^{2+} & F^- ions, a βPGM-MgF_3^--βG6P transition state analogue complex spontaneously forms, allowing probing of an active conformation. Study by either ^{15}N Relaxation Dispersion or ^{19}F Selective Exchange SpectroscopY (SEXSY) reveals the presence of 2 conformations which interchange at the same rate as catalysis, suggesting the minor conformer is of catalytic importance

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RD551

ENDOR study of Jahn-Teller effect in irradiated L-alanine crystal probed by Second stable radical

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L-alanine has been widely studied as basic biological molecular system as well as due to the application in dosimetry. Until now extensive investigations of L-alanine crystal have been undertaken, pointing out very interesting physical properties[1,2]. Despite the fact that x-ray and neutron single-crystal diffraction measurements support the same crystal structure at low (20 K) and at room temperature, there are several other experimental evidences[3] for lattice instability at around 220 K such as birefringence, light depolarization measurements and ^1H NMR relaxation measurements around the same temperature.

In this study dynamical properties of the second stable L-alanine radical ($NH_3^+C^\bullet(CH_3)COO^-$), R2, induced by γ-irradiation have been investigated by the electron nuclear double resonance technique (ENDOR). The study focuses on the properties of three amino protons of R2 radical in the temperature range from 120-300 K. The motional dynamics of the amino group is impotrant due to its involments in intermolecular interactions through the hydrogen bond network in the crystal lattice. The results of the temperature dependence of the ENDOR linewiths show phase transition around 220 K for all three amino protons and additionally confirm the strong dynamic Jahn-Teller effect in the L-alanine lattice and support evidence for properties related to lattice instability at around 220 K.

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RD552

Relaxation Dispersion of Multiple Quantum Coherences

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Measuring the dispersion of the R_2-rate represents a powerful method to examine exchange processes in proteins, which lie in the time domain range of µs to ms. The underlying experiments are based on Carr-Purcell-Meiboom-Gill (CPMG)-sequences of constant duration, and can be performed using single- or multiple-quantum coherences.

Here we present pulse sequences to determine R_2-dispersions with the help of zero- and double quantum transitions (based on previously published experiments by Lewis Kay [1]). With the experiments, the folding and unfolding rates of the Cold Shock Protein of *Bacillus subtilis* could be determined. To counteract the high transverse relaxation rates of multiple quantum coherences, a partially deuterated protein was used for the experiments.

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RD553

Solvent Dynamic Effects Studied by High Pressure ESR Spectroscopy

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Solvent dynamic effects are of interest since they provide information about the role of the solvent during an electron transfer reaction [1]. Such effects have been observed in many self-exchange systems, whose kinetics is often studied by ESR line broadening experiments [2]. The obtained results are treated within the framework of the Marcus Theory, yielding valuable information on the reaction, such as the activation parameters, and on the influence of the solvent [3].

When using self-exchange reactions involving an uncharged species, the volume of activation is determined by two separate contributions. One is owing to the reorganization of solvent molecules during the reaction, in analogy to the reorganization energy of Marcus (λ_o), while the other involves the solvent dynamic effects:

$$\Delta^{\ddagger}V = -RT\left(\frac{\partial \ln k_{ex}}{\partial P}\right)_T = \Delta^{\ddagger}V_o + \Delta^{\ddagger}V_{SD}$$

Experimental volumes of activation have been obtained for a number of combinations of solvents and exchange systems and subsequently been compared with the predicted ones. Additionally, we have been able to reinvestigate the solvent dynamic effect and make a comparison with results obtained using more traditional methods.

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RD554

Investigation of Ionic Liquids with Spin Probes Bearing Neutral or Ionic Substituents

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Spin probes have received increased attention as tools to probe the mobility in materials on a molecular level. Among various materials, ionic liquids have gained increasing interest because of their nonvolatility and temperature stability in many cases. Ionic liquids are composed of cations and anions interacting via Coulomb forces, hydrogen bonding, and van der Waals forces. Therefore, the spin probes used for investigation of ionic liquids should bear substituents that can undergo similar interactions with the individual ions of the ionic liquids. The spin probes give information about microviscosity of ionic liquids and about their micropolarity if the mobility of the spin probes is sufficient in the ionic liquids.

2,2,6,6-Tetramethylpiperidine-1-yloxyl and substituted derivatives of this spin probe bearing a hydroxy group or an ionic substituent in the 4-position were selected to investigate imidazolium and pyrrolidinium based ionic liquids. Significant differences were found in the mobility of these spin probes in the ionic liquids that are caused by the additional interactions of the substituent in the 4-position of the spin probe with the individual ions of the ionic liquids. Furthermore, viscosity of the ionic liquid also influences the mobility of the spin probes. The viscosity differs as result of modifying the structure of the ionic liquid cation on the one hand and by temperature variation on the other hand.

RD555

Recoupling non-secular part of the magnetic dipole interaction

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NMR spin-spin relaxation in solids in strong static fields is normally described only with the help of the secular part of the full spin-spin interaction Hamiltonian. This truncation procedure is associated with the averaging of the spin-spin interaction over the fast Larmor precession. Here we report a set of conditions when the above averaging procedure continuously and selectively preserves some of the non-secular terms entering the full Hamiltonian. These conditions relate the value of the static magnetic field with the frequency and the amplitude of the radio-frequency field. When the above conditions are satisfied, the effective recoupled spin-spin interaction Hamiltonians have an unconventional form with tunable parameters. These tunable Hamiltonians offer interesting possibilities to manipulate nuclear spins in solids and can help the progress of the fundamental studies of the nuclear spin-spin relaxation phenomenon.

RD556

Comparison of X- and Q-band stimulated electron spin echo data on molecular motions of spin labels

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A few years ago new method of investigation of molecular motion was proposed [1], which is based on analyzing transverse relaxation in stimulated electron spin echo experiment (π/2-τ-π/2-T-π/2-τ-echo), when T is constant and τ is varying. The extraction of the anisotropic part of transverse relaxation, which is done by comparing relaxation in hyperfine components of different anisotropy, allows investigating independently nanosecond librations and microsecond small-angle reorientations of nitroxide spin probes and labels in organic glasses [2] and lipid bilayers [1, 3, 4]. So far, these experiments have been done at X-band only [1–3].

Q-band (35 GHz) seems to be more convenient for these studies, because here EPR spectrum possesses a low-field narrow hyperfine component that is almost isotropic, which facilitates extraction of the relaxation anisotropy. In this work, comparative X- and Q-band stimulated echo investigations of molecular motions of spin-labeled lipids in model membranes are performed. It is shown that at Q-band the motions manifest themselves more pronouncedly. The models of motions used previously [1–4] may fit fairly well experimental results at both bands. Additionally, the distinct advantages of Q-band studies are much higher sensitivity and absence of the proton ESEEM.

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RD557

Influence of paramagnetic impurities in porous media on the NMR relaxation signal

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In porous media, different processes contribute to the transverse relaxation rate. Besides pore size these are diffusional motion in internal gradients caused by magnetic susceptibility differences between solid and fluid [1], and presence of paramagnetic impurities [2].

Aim of the present study is the investigation of the relative contributions of internal gradients due to susceptibility differences and the contributions of paramagnetic impurities. We performed CPMG-measurements of quartz sand and goethite (FeOOH) coated quartz sand samples with known paramagnetic content at high and low magnetic field strength with different echo-spacings (T_E). The analysis showed multiple relaxation processes which are accelerated by the presence of iron oxides at the surface of the quartz particles while iron ions in the core of the particles have negligible influence on the relaxation behavior.

Summarizing, we observed that, besides the pore size, the iron content has the major influence on the transverse relaxation of the investigated porous media whereas the effect of the magnetic field strength is smaller. The dependence of all relaxation rates of the pure and the coated sands on T_E^2 is approximately linear as expected. However, internal gradient strengths obtained from these slopes are in the same order of magnitude for both types of porous media.

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RD558

Crystal Structure and Proton Dynamics of $(ImH_2)_2SeO_4 \cdot 2H_2O$

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Fuel cells are promising electrochemical devices for electrical power generation because their high efficiencies and low pollutions. Interest in *hydrogen fuel cells* as energy conversion systems stimulates the search for appropriate *proton exchange membranes* which perform the function of *solid electrolytes* for proton transport as well as separators for hydrogen and/or oxygen. Among the different kinds of *proton conductors*, the materials based on the *nitrogen-containing heterocycle molecules* (e.g., *imidazole rings*) have a promising future as the proton exchange membranes for fuel cell applications.

In the present work, we have studied (i) the crystal structure, (ii) proton conductivity and (iii) molecular dynamics in *Imidazolium Selenate Dihydrate*: $(ImH_2)_2SeO_4 \cdot 2H_2O$. (i) The crystal synthesized contains eight imidazole molecules in the unit cell ($Z = 4$). Imidazole nitrogen atoms form strong and linear N-H···O hydrogen bonds to the selenate ions and water molecules. The selenate ions have tetrahedral symmetry and the crystalline water is present in the form of H_3O^+. (ii) The bulk *dc* conductivity of $(ImH_2)_2SeO_4 \cdot 2H_2O$ was measured as a function of temperature. The process observed is thermally activated and fulfils the Arrhenius law (E_a = 1.16 eV). At 60 °C the value of the electric conductivity equals ca. 10^{-1} S/m. (iii) ^{13}C MAS NMR spectra proved that all imidazole rings are dynamically equivalent and they undergo a reorientation around C_2 axis at room tempetarure. ^1H NMR spin-lattice relaxation measurements evidenced that two dynamically different types of protons: mobile and rigid, exist in $(ImH_2)_2SeO_4 \cdot 2H_2O$. Mobile protons play a crucial role in the proton transport.

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RD559

Unfolded State Dynamics of Single Disulfide Mutants of Hen Egg Whithe Lysozyme

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Hen egg white lysozyme (HEWL) contains eight cysteine residues forming four disulfide bonds in its native structure at positions [6-127], [30-115], [64-80] and [76-94]. During oxidative folding of HEWL, the four disulfide bonds form consecutively in a cooperative manner from 0SS-HEWL – where no disulfide bonds is present - over 1SS-, 2SS- and 3SS- to 4SS-HEWL - where all disulfide bonds are formed correctly. Up to now, there is only scarce data on the conformational dynamics of these intermediates and how the formation of disulfide bonds governs the folding process towards the correct folded form. Recent studies on 0SS-HEWL and several tryptophan mutants show that hydrophobic residues in the unfolded state of 0SS-HEWL modulate long-range interactions and that these hydrophobic patches influence disulfide bond formation during HEWL folding. The modulation of these dynamics, however, is influenced by the formation of disulfide bonds. In order to unravel the changes in dynamics upon disulfide bond formation, the characteristics of the unfolded polypeptides with only one disulfide bond are investigated. Therefore, the four 1SS mutants with native disulfides (1SS[6-127], 1SS[30-115], 1SS[64-80] and 1SS[76-94]) have been studied by NMR in detail by the use of R_2, R_1, $R_{1\rho}$ and hetNOE as well as RDC and DOSY data.

RD560

The Influence of Polymer-Solid Contacts on Chain Order and Local Dynamics Studied by Using Static ^{13}C-NMR Spectroscopy

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Many macroscopic properties of polymeric materials are determined by structure and dynamics of the polymer chains on the nano scale. Surfaces and interfaces are supposed to have a strong influence on both, local structure and local molecular dynamics.

In this work the impact of spherical silica surfaces on the dynamics of grafted poly(ethyl methacrylate) (PEMA) was studied by using ^{13}C NMR spectroscopy. Simulation of static CSA tensor orientations of the ^{13}C carbonyl site in PEMA is well suited for the analysis of motions and local ordering on the nano scale. By carrying out static variable temperature ^{13}C NMR experiments the dynamics in various regions were monitored and could be compared to the bulk polymer. Four systems were investigated, all isotopically enriched at different regions of the PEMA chains, i.e. directly at the surface (system 1), in the middle (system 2), and at the chain ends (system 3). System 4 was a mixture of unlabelled particles with labelled bulk polymer. While system 1 showed no difference in dynamics compared to the bulk polymer, the molecular dynamics of the other systems were shifted to higher temperatures indicating an impact of the surface on the local ordering. The fact that system 4 showed similar behavior a chemical bonding effect can be excluded.

RD561

NMR Investigations on Fluoride conducting Electrolytes

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Recently, there is an increasing interest towards solid state batteries based on fluoride ion transfer. These electrochemical cells offer high energy density and have a wide range of operating temperature: up to 500 ºC [1]. Conventional solid state fluoride batteries consist of a metal anode, a fluoride conducting electrolyte and a metal fluoride cathode [2]. The aim of the present study is the investigation of the intrinsic fluoride mobility of solid state electrolytes such as $La_{1-x}Ba_xF_{3-x}$ ($x \leq 0.15$) by temperature dependent relaxation NMR spectroscopy. The fitted NMR ionic conductivity is compared to values compiled by electrochemical impedance spectroscopy.

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SE562

Investigations on Polarization Enhancement

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The inherently low sensitivity of NMR experiments is still a limiting factor in solving complex problems by NMR spectroscopy. Due to this fact there exist several methods to achieve an increase in signal intensity by hyperpolarization, i.e. to enlarge the difference in population far away from Boltzmann distribution at thermal equilibrium. One possible approach to realize a so called dynamic polarization was discovered by *Haupt* [1] in 1972. He applied a sudden change in temperature from 8 K to 30 K and observed a 100-fold signal enhancement for the ^{1}H-resonances of gamma-picoline. A fundamental condition for substances showing the so called Haupt effect is the presence of molecular groups with a low rotational barrier, e.g. methyl groups referred to as quantum rotors. [2] At low temperatures the system can tunnel through the rotational barrier which enhances the number rotational states. A fast change in temperatur leads to relaxation effects where a coupling between rotational and nuclear spin states of the free rotating group can proceed leading to hyperpolarized spins. Till now there are only a few compounds known that show the effect. [3,4] Our aim is to identify more such substances and to transfer magnetisation from hyperpolarized compounds to other analytes dissolved in that matrix.

Furthermore some experiments using para-hydrogen-induced polarization (PHIP) are performed investigating possibilities of magnetisation transfer.

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SE563

Double Electron-Nuclear Magnetization Transfer Application in Low-Field MRI

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A new method for low-field MRI sensitivity enhancement and contrast improvement is developed. This technique combines 3 methods: magnetization transfer (MT), dynamic nuclear polarization (DNP) and spin probes method. A pulsed DNP has been chosen for the electron magnetization transfer. For nuclear MT we used an off-resonance pulse technique, where multiple, short duration, high intensity RF pulses irradiate a sample at a frequency offset on several kHz from the free water resonance. This causes saturation of the bound pool biomacromolecules protons while leaving the free pool protons unaffected. This saturation effect is transferred subsequently (by dipolar or chemical exchange interactions) to protons in free water, and is proportional to the relative sizes of the pool, individual proton relaxation rate, and cross-relaxation rate. MT imaging has been quantitatively investigated and computer-simulated as a function of the number, amplitude, offset and duration of the off-resonance pulses using a special computer simulated program. Off-resonance saturation method has been investigated to improve contrast sensitivity by using of superparamagnetic iron oxide (SPIO) nanoparticles in low field MRI too.

In our DNP-MT studies we used simple phantoms as a model biopolymer-water systems, containing some spin probes. All experiments have been performed on home-built low-field MRI scanner in a magnetic field of 7 mT.

SE564

High sensitivity and flexibility of DEER experiment on a nitroxide radical pair at Q-band frequency

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Quality of distance information obtained by pulse EPR methods plays a crucial role in studying conformational dynamics of proteins. One of the most commonly techniques used to this end – the Double Electron Electron Resonance (DEER) experiment – under common (standard) conditions (X-band frequencies of ~9.5 GHz, commercial hardware) provides distance information in a range of 2..5-6 nm, whereby at the longer distance edge often only *mean* interspin distances can be extracted with confidence. When precise knowledge on the *shape* of the distance distribution is required, an effective distance limit may drop down to even 4-4.5 nm, in particular for membrane proteins.

Here we report on significant improvements of the DEER experiment on nitroxide spin pair when performed at Q-band frequencies (~34.5 GHz). Using a home-built spectrometer together with a custom probe head for oversized samples we were able to achieve a 3-7 fold sensitivity increase with respect to standard operation at X-band thus providing access to longer distances, increased quality of data as well as significantly reduced measurement time. In addition, by varying excitation bandwidth of the microwave pulses we were able to switch on the same very sample between orientationally selective and non-selective operation, allowing for either probing geometry of the spin pair or getting precise and undistorted distance information from the pair even at a high degree of geometrical correlation.

SE565

NMR Microcoils for reaction conditions screening in single flow experiments.

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Herewith we present the use of an NMR-chip (rf-planar microcoil integrated on top of a glass substrate) for rapid reaction optimization of continuous-flow microwave-assisted chemical processes. Monitoring reactions under microwave irradiation with the aim of optimizing the reaction conditions is a complex task. Traditional methods are not adequate because they require stopping the reaction and the time required for analysis is longer than the reaction time. The methodology developed within our group is focused on the optimization of microwave-assisted reactions in short times and with low cost. This methodology implies the use of NMR-chips (detection volume 6 nL) hyphenated to a continuous-flow microwave reactor (reaction volume in the microliter range) for on-line monitoring and rapid optimization of the reaction conditions. Firstly with the monitoring of Diels-Alder reactions to prove the concept, and secondly, extending the scope of the system with the preparation of a small library of heterocycles[1] compounds with significant biological activity. This is the first time that a microwave reactor has been coupled to an NMR-probe.[2] The NMR-chip, as a consequence of having a smaller active volume than the reaction volume, provides several data points just from a single constant-flow experiment what accelerates the optimization process. The applicability of the NMR-chip has been further illustrated with the study of supramolecular interactions by ^{1}H- and ^{19}F-NMR spectroscopy.[3]

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SE566

Microfluidic gas-flow reactor imaging utilizing parahydrogen-induced polarization and remote-detection NMR

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Microfluidic devices provide control over a process with capabilities that exceed those for conventional macroscopic systems. NMR imaging is one of the rare candidates for *in situ* monitoring of physico-chemical processes, since it has versatile and rich toolkit for mass transport visualization. However, conventional NMR imaging techniques suffer from low sensitivity, which makes studies of microfluidic reactors practically impossible. Substantial sensitivity boost can be achieved by combining remote-detection (RD) NMR and parahydrogen-induced polarization (PHIP) as has been demonstrated recently for microfluidic gas flow [1]. In the current study, we used this technique for imaging of mass transport and progress of gas-phase hydrogenation reaction inside cylindrical micro-scale packed-bed reactors of 150-800 μm in diameter. At the same time, we introduce the concept of microfluidic PHIP polarizer as a methodology for continuous production of hyperpolarized substances. We show that the combined PHIP-RD technique can provide information about reaction product distribution, mass transport and adsorption effects in the model micro-scale reactors during their *in situ* operation.

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SE567

Liquid State Dynamic Nuclear Polarization for MRI applications: An In-bore Approach

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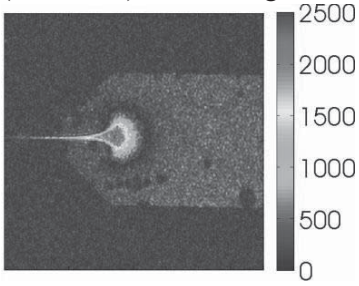
Contrast and scan time being key issues in Magnetic Resonance Imaging (MRI), several approaches can be taken to improve the Contrast-to-Noise Ratio (CNR) of MRI images. Among those, hyperpolarization techniques are very promising, one of which is Dynamic Nuclear Polarization (DNP).

In DNP, hyperpolarization of nuclei is achieved by microwave irradiation of electron spins ("radicals") transferring their larger Boltzmann polarization to the nuclei – in this study via the Overhauser Effect.



We present a liquid state DNP polarizer operating in flow through mode at a magnetic field strength of 1.5 T (42 GHz microwave frequency), compatible with a standard 1.5 T medical imaging magnet. Compared to other approaches, where the polarization of the sample takes place in a separate magnet placed outside the imager bore, there are major technical differences. Liquid state polarization buildup time is in the range of seconds, allowing for a flow through design providing a constant flow of polarized sample. Additionally, the shuttling distance is minimized and shuttling through a magnetic field profile is avoided. The work presented outlines the design of the DNP system, comprising of the flow-through resonator and an appropriate microwave source, and demonstrates its performance in enhancing MRI signal.

SE568

Solid-state NMR and Dynamic Nuclear Polarisation on Membrane Proteins

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We describe cwDNP enhanced MAS-NMR experiments on two different membrane proteins reconstituted into lipid bilayers. The experiments were carried out using a non-commercial DNP setup consisting of a high power 258 GHz gyrotron (Gycom, Nizhny Novgorod, Russia) and a specially modified Bruker 3.2mm cryo-MAS probehead operating at 100 K at a 393 MHz Bruker Avance II spectrometer.

Proteorhodopsin: we show cwDNP enhanced double-quantum filtered experiments on ^{13}C labeled retinal in proteorhodopsin and TEDOR experiments on His75 and Asp97 selectively labeled samples.

SecYEG: The bacterial protein translocation complex SecYEG contains three subunits and has a total size of 72 kDa. This complex forms the core of a conserved machinery, which transports proteins across or into membranes. We have performed first cwDNP MAS NMR experiments on SecYEG reconstituted into lipid bilayers with the ^{13}C-labeled signal peptide LamB . The natural abundance background from the SEC-YEG necessitates the use of double quantum filtered experiments to observe signals from the peptide. Conventional NMR experiments are not feasible, as they would require over 3 weeks for a simple 2D spectrum, but only 20 hours when using DNP.

Our first data on two very different membrane proteins show the great potential of cwDNP-MAS. NMR to obtain very specific data from large membrane protein complexes.

SE569

Mobile DNP polarizer for continuous flow applications

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Despite its wide applicability in natural science, NMR still suffers from its inherently low sensitivity. This could be overcome by hyperpolarization of molecules via dynamic nuclear polarization (DNP).[1] Here, we introduce a mobile DNP polarizer, based on an inexpensive Halbach magnet operating at 0.35 T. It shows an almost vanishing magnetic flux at its outer side and is not disturbing other instruments. We will show that it can be placed directly next to a superconducting magnet, thus limiting the transport time of the hyperpolarized sample. Moreover, it will be demonstrated, that the Halbach magnet has the same DNP performance like an electromagnet. However, two problems for DNP applications remain: Firstly radicals are needed, which are mostly toxic. This problem becomes crucial with regard to medical applications. Secondly, the sample must be transported from the polarization magnet to the place of detection like a MRI scanner and polarization losses due to T1 occur. We implemented a flow system into the mobile DNP polarizer, which overcomes both obstacles. The radicals are immobilized in a gel matrix and the hyperpolarized radical free fluid is pumped subsequently directly in the MRI scanner.[2,3] It will be shown, that even at flow conditions the NMR signal is enhanced in the Halbach magnet as well as in the MRI scanner (4.7 T) in 1.4 m distance. Acquired images will demonstrate the use of enhanced and, due to dipolar coupling, inverted NMR signals which provides even for small enhancements an excellent MRI contrast.

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SE570

Overhauser DNP of ^{19}F and ^{13}C using a mobile X-Band polarizer

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Direct NMR signal enhancements of ^{19}F and ^{13}C obtained by Overhauser DNP in a mobile X-Band DNP polarizer [1] are presented. The setup is based on a Halbach arrangement of permanent magnets which is creating an adjustable magnetic field [2] with a field strength corresponding to 14.7MHz ^{1}H Larmor frequency and leading to a spectral line width (FWHM) of approx. 400ppm in a cylindrical sample of 4mm in height and 1mm in diameter.

Samples were made of solutions of nitroxide radicals in hexafluorobenzene (for ^{19}F measurements) and in an aqueous solution of ^{13}C-enriched urea (for ^{13}C measurements), respectively. A systematic study of the DNP enhancement with different radical concentrations and varying microwave power was performed. The highest enhancements achieved were -68 for ^{19}F (190mmol/l TEMPO concentration, 11 Watt microwave power) and -987 for ^{13}C (100mmol/l 4-Hydroxy-TEMPO concentration, 3.8 Watt microwave power)

For further evaluation of the results, experimental influences like heating of the sample and the microwave cavity as well as the effects of varying sample height and position were investigated. EPR spectra are compared to DNP signal intensities at varying magnetic field strength to demonstrate power broadening effects.

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SE571

Observation of signal enhancement via PHIP in a symmetric molecule. Experiments and a preliminary model.

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Two parahydrogen protons inserted in a molecule with symmetric structure occupy magnetically equivalent positions and, consequently, should remain in a singlet state, an NMR silent state. Surprisingly, this is not always the case and hyperpolarized signals from symmetric molecules can be observed[1]. Particularly, we have observed significant NMR signal enhancement during the parahydrogenation of acetylene dicarboxylic acid dimethylester, even though it is a symmetrical molecule and the same remains true for its product after a hydrogenation reaction. In this work we present new experiments where the behavior of this symmetric molecule in presence of different magnetic fields and for different evolution periods is studied. In this way, we were able to monitor the spin system evolving as a weak or as a strongly coupled one. By means of these experiments we obtained some important hints to model in a simple way the dominant relaxation mechanisms in the molecule[2,3] in order to explain the unexpected observations.

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SE572

Para Hydrogen Induced Polarization in fluorous/organic phases

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Since the discovery of the Para Hydrogen Induced Polarization (PHIP) method [1-3] a lot of efforts have been put to develop this technique for practical and commercial applications in the medicine and the industry [4,5]. However, there are still some problems which hamper the PHIP utilization. For example, the problem of fast product/catalyst separation is one of special concern [6,7].

In this presentation this problem is addressed. We demonstrate that the PHIP effect can be observed in different fluorous solvents. Further, we present that the PHIP signal can be observed in fluorous/organic systems. These systems are homogenous at high temperature and biphasic at low temperature. This temperature behavior of fluorous/organic phases can be of crucial importance for product/catalyst separation in PHIP.

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SE573

Quantum Rotor Induced Hyperpolarization

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Polarization techniques attract significant interest in NMR spectroscopy spurred by the promise of enormously enhanced sensitivities. Hyperpolarization is commonly generated by polarization transfer from electrons or by utilizing parahydrogen in chemical interactions. A less well-known method of generating hyperpolarization is the Haupt effect[1,2], previously known for γ-piccoline, which generates polarization from quantum tunneling systems. Mechanistically the mechanism can be seen as the C_3 symmetry equivalent to triplet-singlet transitions.

We have recently shown that quantum rotor polarization (QRP) is not limited to γ-picccoline but can be observed for a range of molecules at low temperatures. The pure QR effect was observed for many molecules with methyl-groups and arises in absence of any radical or MW irradiation[3]. Time courses of the signal recorded after leaving substances for varied periods of time in the polarizer allowed us to calculate the rates of the build-up relative to the relaxation rates[3]. Using substances like pentanol with a methyl group on only one end of the molecule it could be shown that polarization is transferred entirely via protons by a fast spin diffusion mechanism[3]. Polarization transfer is also possible between protons of different molecules in glassy samples[3], the essential component of any polarization technique. These findings show that QRP can serve as an alternative method to obtain NMR hyperpolarization.

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SE574

Fabry-Perot Resonators for Liquid-State DNP at 9.2 Tesla

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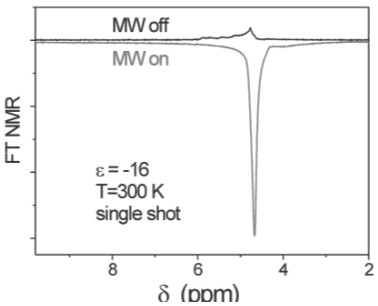
In-situ DNP experiments on physiological aqueous solutions of biomolecules at ambient temperatures are a challenging task due to high microwave losses in water causing excessive sample heating. To avoid heating a typical sample volume can be reduced to keep it in a position away from electrical component of the microwave field.

We showed with a helical double resonance structure [1] that indeed, substantial DNP enhancements can be achieved at high magnetic fields in liquid water solutions. On the other hand the sample size in the helical resonance structure is restricted to very small volume below 3 nl. Together with poor spectral resolution it results in small signal amplitudes causing observation of NMR on biomolecules problematic.

Recently we developed a new type of the double resonance structure employing Fabry-Perot resonators, that allowed to improve spectral resolution and increase aqueous sample volumes up to 30-fold reaching 100 nl. Design, MW and RF performance, and first DNP applications at 9.2 Tesla will be presented.



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SE575

400 MHz High Field Liquid State Dynamic Nuclear Polarization

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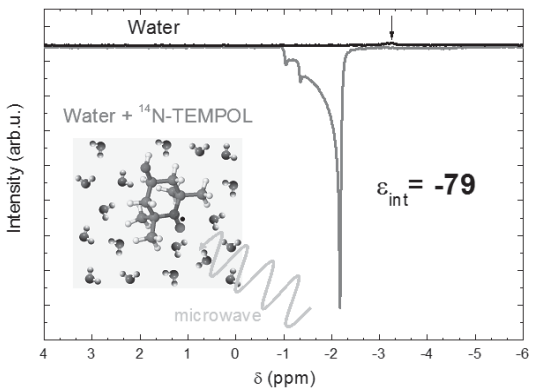
Liquid state NMR is a powerful and versatile spectroscopic technique to study structure and dynamics of liquid systems. However, it suffers from its intrinsically low sensitivity, due to the small energy gap between nuclear spin states (Boltzmann population). Therefore, improving its sensitivity is a key issue in NMR spectroscopy. One of the ways of tackling this issue is hyperpolarization by Dynamic Nuclear Polarization (DNP). In DNP, hyperpolarization of nuclei is achieved by microwave irradiation of electron spins ("radicals") transferring their larger Boltzmann polarization to the nuclei – in the liquid state this is mediated by the Overhauser Effect. Based on theoretical models the Overhauser Effect was predicted to become very inefficient at high magnetic fields. However, recently ample signal enhancements up to -80 in aqueous solution with ^{14}N-TEMPOL radical have been observed in our homebuilt 392 MHz ^{1}H-DNP spectrometer operating at 9.4 T which uses a highly powered 260 GHz gyrotron microwave source for continuous wave sample irradiation.



In order to properly understand and model these findings, we investigate the DNP effect in aqueous solutions at this field, probing its dependences on temperature, radical concentration and saturation of the electron's transition.

SE576

Shuttle DNP Spectrometer with a Two-Center Magnet

Philip Lottmann[3], Alexander Krahn[1], Thorsten Marquardsen[1], Andreas Tavernier[1], Maria-Teresa Türke[2], Marcel Reese[3], Andrei Leonov[3], Marina Bennati[2], Peter Hoefer[1], Frank Engelke[1], Christian Griesinger[3]

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A DNP setup is described where a liquid sample is hyperpolarized by the electron-nucleus Overhauser effect[1] in a field of 0.34 T and transferred to a field of 14.09 T for NMR detection. A ferroshim system was inserted into the bore of a 14.09 T shielded cryomagnet to provide a homogeneous low-field region in the stray field 47 cm above the magnetic center. A dedicated EPR cavity was used for low-field polarization. The ferroshim system and the EPR cavity were cooled to 290 K. In this way sample heating caused by microwave irradiation could be reduced to 5 to 10 K and a higher reproducibility was achieved. Afterwards the sample is transferred to the high-field magnetic center within 40 ms by a pneumatic shuttle system. In our setup a standard high-resolution inverse $^{1}H/^{13}C$ selective probe was used for NMR detection and experimental data are presented on various samples[2]. We observed a maximum proton Overhauser enhancement of up to ε_{HF} = -4.2 in the high field position for a 10 mM 4-Oxo-TEMPO-D,^{15}N (TEMPONE)/D_2O sample. In addition we can demonstrate for the first time Overhauser enhanced high resolution proton spectra of glucose and 2,2-dimethyl-2-silapentane-5-sulfonic acid sodium salt (DSS) in D_2O, where the high resolution spectrum was acquired in the high-field position after polarizing the sample in the low-field. Further results on even larger biomolecules will be presented.

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SM577

Quantitative "long ultrafast" NMR as a valuable alternative to conventional 2D NMR

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Ultrafast (UF) 2D NMR[1] is a very promising methodology enabling the acquisition of 2D spectra in a single scan. The analytical performances of UF 2D NMR have been highly increased in the last few years, and the potentialities of ultrafast 2D NMR for precise and accurate quantitative analysis have been demonstrated.[2,3] However little is known about the sensitivity of ultrafast experiments versus conventional 2D NMR. A fair and relevant comparison has to consider the Signal-to-Noise Ratio (SNR) per unit of time, in order to answer the following question: for a given experiment time, should we run a conventional 2D experiment or is it preferable to accumulate ultrafast acquisitions? To answer this question, we perform a systematic comparison between accumulated ultrafast experiments and conventional ones, for different experiment durations. Sensitivity issues and other analytical aspects are discussed in the context of quantitative analysis. The comparison is perfomed on biologically relevant model metabolic mixtures. The results highlight the high analytical performance of the "long-ultrafast" approach versus conventional 2D NMR acquisitions. This result is mainly attributed to the absence of t_1 noise in spatially-encoded experiments.

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SM578

Simultaneous enhancement of chemical shift dispersion and diffusion resolution in mixture analysis by diffusion-ordered NMR spectroscopy

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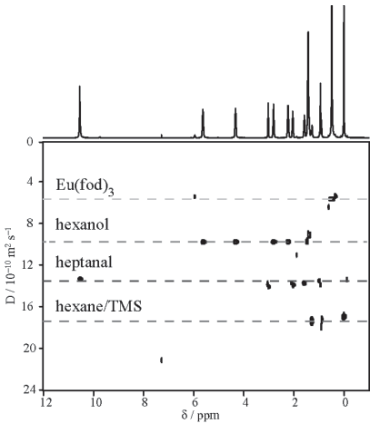
NMR spectroscopy is a powerful tool for the elucidation of molecular structure, but is relatively little used for mixture analysis. High resolution diffusion-ordered spectroscopy (HR-DOSY)[1] allows the separation of signals from different species, but for the best results requires both that the mixture components have different diffusion coefficients, and that their signals do not overlap. The two requirements can be addressed simultaneously using lanthanide shift reagents[2] (LSRs): the chemically-selective binding of solutes to an LSR both increases chemical shift dispersion, reducing signal overlap, and changes the diffusion coefficients seen for the different species. The latter effect is an example of matrix-assisted DOSY[3,4], in which the relative diffusion coefficients of different mixture components are manipulated by changing the matrix in which they diffuse.



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SM579

Analysis of small molecule mixtures by Chromatographic- and MaxQ-NMR

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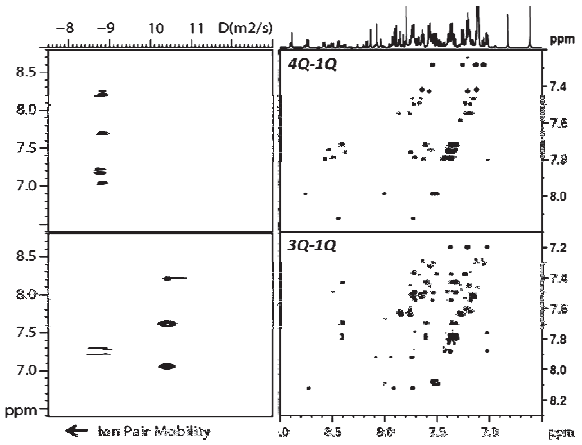
Chromatographic and MaxQ NMR are recent additions for the analysis of mixtures of small molecules, producing a simple 2D NMR layout in which the second dimension serves to separate the 1D spectrum of the mixture components. We illustrate examples of these techniques, their benefits and limitations. **Chromatographic NMR** merges chromatography inspired separation and NMR structural analysis.[1] We apply it to diastereomers of Fe^{2+} ion pairs in slow exchange, eluding chromatographic separation.[2] **MaxQ NMR** simplifies ^{1}H-spectra by a correlation with the highest MQ order, as demonstrated on PAHs and phenolics in a standard set and in extra virgin olive oil.[3] Improvement of the experiment through optimal control pulse sequence design[4] and non-uniform sampling will be demonstrated.



Chromatographic NMR on F^{2+} pairs (left), MaxQ on 16 PAHs (right).

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SM580

The smaller the better in an optimization process

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From a few years to now the mass and volume limited samples analysis by NMR spectroscopy has been possible and perfectly demonstrated by the miniaturization of coils, knowing different microcoil geometries (microsolenoids, planar spiral microcoils, striplines, and microslot waveguides).[1] The reduced size of these microcoils allows to achieve detection volumes in the range of nanoliter and the detection of picomole amount of materials.[2]

In line with, the best approximation to analyze mass and volume limited samples are the flow techniques. Microcoils can be integrated on lab-on-a-chip devices and hyphenated to others techniques when the whole system works on continuous-flow. In this sense, we previously reported the hyphenation of a radiofrequency planar microcoil integrated on a glass substrate (NMR-chip) with a microwave microreactor for on-line monitoring and rapid optimization of a cycloaddition reaction.[3]

In this presentation we will report a new different methodologies of monitoring reactions by the called NMR-chip, taking advantage of the fact that the detection volume has been chosen lower than the reaction volume providing different data points, corresponding to different reaction times, within a single on-flow NMR experiment. The setup has been optimized with the lower reaction volume and lower interface volume as possible for an optimization of the reaction conditions with low cost and with the lower interface volume as possible for detection of reaction intermediates respectively.

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SM581

"Through space" *J*-coupling between hydrogen nuclei

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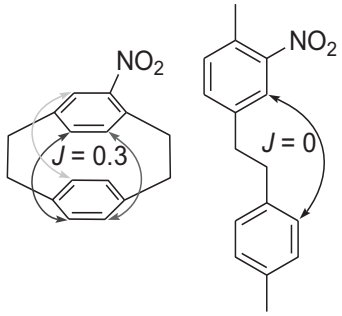
The indirect spin-spin NMR band splitting (*J*-coupling), known to be mostly mediated by covalent bonds, is an indispensable probe of fine molecular structure. However, some of the magnetic interaction may not be dependent on the covalent pattern, which should be taken into account in accurate conformational studies. This coupling contribution was quantitatively investigated on newly synthesized model molecules consisting of rigidly and loosely connected aromatic rings. The measured coupling constants clearly indicate that the rigid and flexible systems differ in the magnitude of the through-space coupling components. The trend was in agreement with the values calculated by quantum-chemical methods.



The calculated carbon and hydrogen coupling maps and perturbed electronic densities suggest that the aromatic system is responsible for a part of the non-covalent contribution.

ACKNOWLEDGEMENT:
Financial support from the Grant Agency of the Academy of Sciences of the Czech Republic through Project KJB400550903 is acknowledged.

SM582

Computer-assisted complete assignment of ^{1}H and ^{13}C NMR data for a poorly functionalized kaurane diterpene.

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Diterpenes (DT) are an important class of biologically active natural products.[1] Regarding this, DT are also the target in several phytochemical, pharmaceutical and biological studies. The unequivocal assignment of the diterpene structures is an important step for studies on structure-activity relationships of such class of compounds. One can find in the literature some ^{1}H and ^{13}C NMR data complete assignments for polifunctionalized DT, but for poorly functionalized structures, the ^{13}C NMR structural determination is fairly common. In this work, we have performed a detailed NMR data study of methyl kauranoate (**1**) by ^{1}H NMR, $^{13}C\{^{1}H\}$ NMR, gCOSY, gHMQC, gHMBC experiments. NMR spectra were recorded on a Bruker Avance DRX400 spectrometer, 5 mm probehead (BBI 1H-BB). Samples were 20-25 mg mL^{-1} in $CDCl_3$ with TMS. Signals were simulated in SimEsp_NMR program[2] to verify their distortions and to turn possible *J* values determination. All hydrogen signals were assigned separated from their geminal hydrogen in each case, and some new signal multiplicities were also determined. In comparison to previous works,[3] here is presented eighteen new assignments and seven new signal multiplicity were determined. This is an essential contribution to the literature, especially for structure-activity studies.

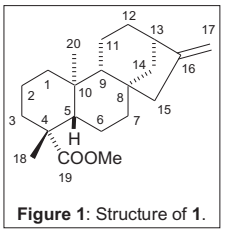

Figure 1: Structure of **1**.

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SM583

Complete structural assignment of ^{1}H and ^{13}C NMR data for two prepared furanoheliangolides and their products obtained with Stryker's reagent.

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Sesquiterpene lactones (SL) are a class of Natural Products with structural variety and several known biological activities.[1] During our synthetic research with SL,[2] we have obtained substances **3** to **6** by reduction of **1** and **2** with Stryker's reagent, as shown on figure 1. We now present firstly, the ^{1}H and ^{13}C detailed NMR data assignment for all the substances on figure 1. Compounds **1** to **4**, as well as the previously unpublished substances **5** and **6** were all submitted to the experiments: ^{1}H NMR, $^{13}C\{^{1}H\}$ NMR, gCOSY, gHMQC, gHMBC, *J-res* and NOESY. NMR spectra were recorded on a Bruker Avance DRX500 spectrometer, 5 mm probehead (BBI 1H-BB). Samples were 20-25 mg mL^{-1} in $CDCl_3$ with TMS. ^{1}H and ^{13}C NMR signals were fully and unequivocally assigned; all hydrogen homonuclear coupling constants were measured, with all ^{1}H signals multiplicities clarified. NOE was also verified, and all signal intensities are in agreement with the presented structures. These results are an important contribution as they can be considered an useful data base to be available in the literature and help future assignments.

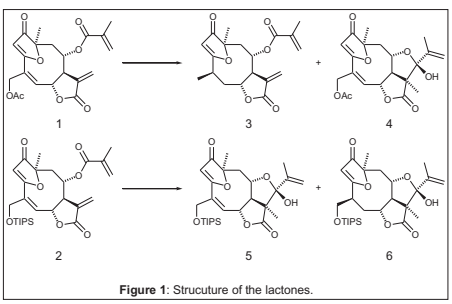

Figure 1: Strucuture of the lactones.

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SM584

Combined use of filtered and edited ^{1}H NMR spectroscopy to detect ^{13}C enriched compounds in complex mixtures

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ABSTRACT: In conventional metabolism and pharmacokinetic studies, radioactive isotopes are used to identify and quantify the breakdown products of xenobiotics. However, synthesising the radiolabelled xenobiotics needed for such studies is extremely expensive, and careful safety precautions are required when using them. The stable isotope ^{13}C provides an alternative method of tracing xenobiotic metabolism. Because it is non-radioactive, ^{13}C enriched xenobiotics are cheaper to synthesise and using them requires no special precautions. Metabolites of the enriched xenobiotic can then be traced, quantified and identified by ^{13}C filtered NMR spectroscopy.

One obstacle in using ^{13}C is its high natural abundance (1.1%). This produces a background signal in ^{13}C filtered NMR spectra of crude biological extracts. This background makes it difficult to distinguish resonances from ^{13}C enriched xenobiotics from endogenous metabolites unrelated to the xenobiotic.

This poster demonstrates that ^{13}C filtered and edited NMR spectra can be combined to separate the 13C background from the resonances of ^{13}C enriched xenobiotics. The theory underlying the approach is described, and the method is demonstrated in a study of the metabolism of ^{13}C -enriched Phenacetin in hepatocyte microsomes.

SM585

Towards a better understanding of the selectivity of an organocatalyst

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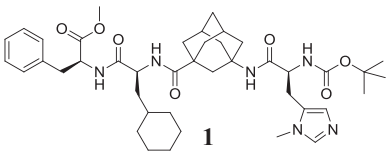
The tetrapeptide **1** is used as organocatalyst for the enantioselective monoacylation of *trans*-cyclohexane-1,2-diol, yielding exceptionally high selectivities.[1] To understand the observed selectivity, quantum chemical calculations have been used,[1,2] but so far no experimental evidence towards the existence of the proposed intermediate nor towards the solution structure of **1** itself is available. Thus we started to investigate the solution structure of **1** by NMR spectroscopy. Since calculations suggest conformational flexibility for the organocatalyst, it is difficult to determine the structure with routine procedures.



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We started using a combination of NOE and RDC[3] data to describe the structure in solution. As orienting media to measure RDC, the homopolypeptide poly-γ-benzyl-D-glutamate[4] is used.

Our investigations so far show that the structure of the tetrapeptide in solution does not match one of the proposed quantum chemical structures and show strong signs of conformational flexibility. It is thus very unlikely that the structure in solution can be described by one single conformer. We are currently trying to generate a conformer or an ensemble of conformers that is in agreement with the experimental data.

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SM586

Analysis of Fatty Acids in Chiral Oriented Systems by NAD 2D-NMR: Optimisation of Enantiodiscrimining conditions and First Experimental Application of the "Non-Uniform Sampling/Covariance" Approach

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Natural abundance deuterium 2D-NMR (NAD 2D-NMR) in chiral liquid crystals (CLC), made of organic solutions of polypeptide (PBLG, PCBLL) is an original and powerful approach for analyzing (pro)chiral molecules or investigating the (D/H) isotopic fractionation in biointerest molecules. In this work, we describe the recent experimental and methodological advances of NAD 2D-NMR dedicated to the investigation of (un)saturated C-18 fatty acids such as stearic, linoleic or vernoleic acid. The first achievement concerns the optimisation of enantiodiscrimination conditions of all deuterium inequivalent sites in fatty acids in order to extract all possible information relative to the (D/H) ratios. The second one reports the possibility to reduce significantly the experimental time of acquisition of NAD NMR data in CLC by processing non-uniform sampling (NUS) of data sets with covariance transform (Cov). The combination of NUS and Cov methods allow a decrease in measurement time by a factor of 2 compared to Cov applied to unifor mly-sampled (US) data and a factor of 4 compared to FT applied to US data.

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SM587

RDC-based Determination of the Relative Configuration of the Fungicidal Cyclopentenone "Hygrophorone A"

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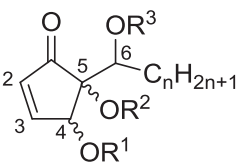
After their isolation from fungi of the *Hygrophorus* family, the Hygrophorones **1** have been subject of intense study due to their structural similarity with the antibiotic pentenomycin and antifungal activity.[1] The relative configuration of C4 and C5 was established by comparison of the $^{3}J_{H3\text{-}H4}$ and $^{4}J_{H2\text{-}H4}$ coupling constants with the known (epi-)pentenomycin structure and NOESY measurements. However the relative configuration of C6 remained unknown and also the proposed 4,5-*trans* configuration has not yet been determined unambiguously.



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We used RDCs[2] to determine the relative configuration of all three stereogenic centers at once. By aligning about 2 mg of hygrophorone A in a liquid crystalline phase of high-molecular-weight PBLG in CD_2Cl_2[3] we were able to measure eight one-bond and long-range C-H RDCs. We studied possible conformational flexibility in the five membered ring and along the C5-C6 bond by conventional force-field and DFT methods. Fitting these calculated structure models with our RDC module in the software hotFCHT,[4] we found only a single relative configuration reproducing the experimental data.

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SM588

THE ROLE OF SOLVENT IN SUGAR-BASED ORGANOGELS

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The organogels composed by the sugar-based organogelators: methyl-4,6-O-(*p*-nitrobenzylidene)-α-D-glucopyranoside and 1,2-O-(1-ethylpropylidene)-α-D-glucofuranose with a variety of solvents were the subject of our studies. We focused our attention on the role of solvent on the gel formation, the thermal stabilities of the gels, the microstructure of 3D fibrillar network formed by the gelator molecules in the gel and in particular, on the interaction of the solvent molecules with gelator aggregates.[2-4] Different solvent parameters, such as dielectric constant, the one-component solubility parameter, the polarity parameter and the Kamlet-Taft parameters were considered to quantify solvent effects on the gelation. Thanks to the ^{1}H FFC relaxometry we were able to evidence the interaction between the solvent and gelator aggregates.[4]

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ACKNOWLEDGMENTS
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SM589

Separation of natural product epimer signals by chiral matrix-assisted diffusion-ordered NMR spectroscopy

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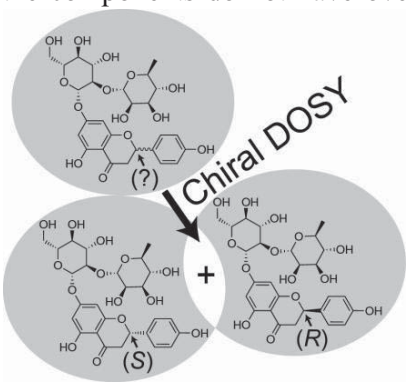
High resolution diffusion-ordered NMR spectroscopy[1] allows the separation of signals from different species based on their diffusion coefficients. In general this requires that the NMR spectra of the components do not have overlapping signals and that the diffusion coefficients are significantly different. Modifying the solvent matrix in which a sample is dissolved can change the diffusion coefficients observed, allowing resolution ("matrix-assisted DOSY").[2] Some matrices can change both diffusion coefficients and chemical shifts. We show that dissolving the two naturally-occurring epimers of naringin,[3] the component of grapefruit juice responsible for its bitterness, in an aqueous solution of β-cyclodextrin causes both shift and diffusion changes, allowing the signals of epimers to be distinguished. Chiral matrix-assisted DOSY has the potential to allow simple resolution and assignment of the spectra of epimers and enantiomers, without the need for derivatisation or for titration with a shift reagent.



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SM590

ENANTIO DISCRIMINATION AND CONFIGURATIONAL ASSIGNMENT OF CARBOXYLIC ACIDS BY ^{77}Se NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY

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In this work, we show results that point to the efficiency of chiral β-arylselanyl alcohols, when employed as chiral derivatizing agents (CDA), in the enantiodiscrimination of α–, β–, γ–substituted chiral carboxylic acids through ^{77}Se NMR. We also propose an empirical model for the assignment of the absolute configuration of α-substituted carboxylic acids by ^{77}Se NMR. The experiment described here was performed in the NMR tube, by mixing and shaking the commercially obtained carboxylic acids in the presence of the chiral selenium auxiliary β-arylselanylalcohols, synthesized in our laboratory. As a consequence, diastereomeric esters were obtained as products. Finally, ^{77}Se NMR spectra were acquired. Large anisocronies of the two selenium signals were observed, revealing a capability of this procedure to usefully distinguish between alkyl substituents, up to seven bonds of distance to the selenium nucleus. In order to assign the absolute configuration to the carboxylic acids, we used a methodology similar to the one described above. However, this time, the ^{77}Se NMR spectra acquisitions were registered for both enantiomers, (*R*)- and (*S*)-, one at a time, for several α-substituted carboxylic acids. Based on the ^{77}Se NMR spectra of the resulting diastereomeric esters, we were able to successfully propose an empirical model for the determination of the absolute configuration of these compounds, by taking into consideration the shielding/unshielding effect over the selenium nucleus and the association based on the conformational equilibria led by steric interactions and/or electronic effects.

Financial Support : FACEPE, CNPQ, PRONEX/FACEPE (Brazilian Agencies)

SM591

Discovery and Characterization of Small-Molecule Inhibitors of PDZ-mediated Protein-protein Interactions

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Protein-ligand interactions are investigated by a variety of NMR techniques. ^{1}H- ^{15}N Heteronuclear Single Quantum Correlation (^{1}H-^{15}N-HSQC) is widely used due to its importance in the mapping of the binding site of the ligand by observation of CSP (Chemical Shift Perturbations). This approach is applied to study interactions between PDZ (Post-synaptic density-95, Drosophila discs large, Zonula occludens -1) domains and the small molecules that bind to it.
PDZ domains are protein-protein recognition modules that play a central role in organizing diverse cell signaling assemblies. PDZ binding specificity involves the recognition of the C-terminus of protein, belonging to receptor and ion channel families. Antagonizing PDZ-mediated interactions may allow for the treatment of several human disorders such as neuropathic pain, congenital diseases, psychiatric disorders, and cancers. In our study, the PDZ domain´s of interest are DVLPDZ and AF6PDZ.

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SM592

Combining ^{1}H high-resolution solid-state NMR methods and *in silico* approaches to study Pharmaceuticals

Luís Mafra[1,2], Sérgio M. Santos[1] and Renée Siegel[1] and Dmytro Dudenko[2]

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Understanding the effect of water on the transformation of drug hydrates to anhydrates, and exploring the correlations between the water environment in the crystal lattice and the hydration/dehydration behavior, are non-trivial matters of particular concern in the study of such multiple-component crystals and a field of great importance where some research efforts have been employed [1].

Here, we present an experimental and computational study of distinct fluorinated pharmaceutical crystalline forms with Z'=1 and =3. Multinuclear (^{1}H, ^{19}F, ^{13}C) 2D high-resolution NMR and various computational approaches were used to investigate these pharmaceutical systems. Combining a toolbox of advanced 2D ^{1}H CRAMPS-based [2] NMR experiments at high fields and fast MAS rates, aided by GIPAW and NICS calculations, we have achieved in some cases, the full assignment of 51 and 54, ^{13}C and ^{1}H resonances respectively, and the quantification of the packing interactions by means of a stepwise *in silico* dismantlement of the 3D crystal packing [3]. we also discuss how the presence/absence of water in two hydrates affects the packing interactions by computing NMR chemical shifts in hydrated and *in silico*-dehydrated crystal structures.

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SM593

Study of Stannanes by NMR Spectroscopy and Quantum Calculations

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Discussions concerning the synthesis of functionalized homoallylic alcohols promoted by tin have to deal with the stereo and regioseletivity of the products. For instance, the allylic halide γ-substituted system, such as crotyl bromide, reacts with aldehyde producing α- and γ-adducts [1]. However, the diastereoseletivity control depends upon the reaction conditions. Most likely, these reactions involve organometallic species and the investigation of organostannanes preformed is crucial to understand which species actually takes part in the addition reaction.

In this communication we described an example where NMR techniques, namely J-Resolved, ^{1}H-^{13}C HMQC and HMBC, and ^{1}H-^{119}Sn HMQC were employed to elucidate all isomers produced in the reaction by crotyl bromide with tin in basic media. By ^{1}H NMR we were unable to assign the species formed. In contrast, by ^{119}Sn NMR we have observed five crotyl stannanes species. In addition, 2D experiments were necessary to assign these five different isomers. The experimental data were supported by quantum chemical calculations that include relativistic effects (scalar and spin-orbit). The calculated NMR parameters with the calculated isomer structures correlated quite well with the observed ones, and such a combination provides a useful tool for structural determination of organotin.

Acknowledge: Brazilian agencies CNPq, PRONEX-FACEPE-CNPq (APQ-0859-1.06/08; APQ 0788-1.06/08), CAPES, FINEP, inctINAMI.

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SM594

How reliable is the configurational assignment of complex natural products by NMR spectroscopy?

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The configurational assignment of natural products (relative and absolute configuration) is essential to understand their biological activity on a molecular level and to allow their procurement through total synthesis. The structural elucidation of amorphous molecules with several unknown stereogenic centers would benefit greatly from a method that could simultaneously analyze all configurations. The so-called fc-rDG/DDD method[1] was successfully applied in 2007 to the palau'amine congener tetrabromostyloguanidine[2] leading to a revision of the relative configuration of palau'amine and its congeners[3]. The reassignment of the relative configuration was finally proven by the total synthesis of palau'amine.[4] The application of the fc-rDG/DDD method to four further examples are discussed. Three of the compounds (axinellamine A[5], 3,7-*epi* massadine[5], and donnazole C[6]) are pyrrole-imidazole alkaloids with 8 stereogenic centers as the palau'amine congeners. The fourth compound is an intermediate in the total synthesis of palau'amine, 20-deoxymacropalau'amine azide.[7] Application of the fc-rDG/DDD method to the configurational of natural products could prevent future misassignments.

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SM595

NMR reveals the origin of environmental contaminants

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Pollutants have severe toxic effects on the environment and pose risks to all living organisms. Understanding their fate in the environment and tracing them back to their sources is important, but difficult. NMR can address these challenges by analysis of heavy stable isotopes. The abundances of these isotopes are not constant, but are influenced by synthesis, transport and transformation processes. These processes leave a fingerprint, in the form of the isotope abundance for each intramolecular position of a compound. NMR is the most powerful technique to measure intramolecular isotope abundances; here we show how NMR can explain sources and transformation processes of pollutants.

First, we analyzed whether bromophenoles and –anisoles used in brominated flame retardants have a common source. These compounds showed distinct isotope patterns in the structurally common parts, ruling out a common source[1]. Second, we studied the infamous chloropesticide DDT and its congener DDD. The deuterium abundance of these compounds differed only in a structurally different C-H group. This difference was traced to the technical DDT synthesis, as opposed to biodegradation in the environment[2]. Thus, NMR yields unique mechanistic information to determine transformations of persistent pollutants and to distinguish natural and anthropogenic sources.

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SM596

Speciation of Organic Phosphorus in P-immobilizing soils: A ^{31}P NMR study

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Phosphorus (P) limits plant growth in many parts of the world, and it has been predicted that agriculture will run out of P fertilizer with dire consequences for food production[1]. Most soil P occurs as organic P species, but there is a severe lack of knowledge about the molecular processes controlling the reactivity of organic P species and their resulting bioavailability. Our aim is to develop solution and solid state ^{31}P NMR techniques to identify P species in soils; information ideal for correlating different organic P species to plant and soil processes[2]. Unfortunately, NMR studies on soil P are compromised by the line broadening caused by paramagnetic ions in soils. For liquid-state NMR, soil P is commonly extracted with NaOH-EDTA, leading to co-extraction of heavy metals. We reasoned that to improve resolution in ^{31}P NMR and allow the application of 2D ^{1}H,^{31}P NMR, paramagnetic impurities must be completely removed from these extracts. We find that sulfide precipitation removes Fe and Mn ions without affecting the P-composition, and dramatically improves the line widths from over 100 Hz down to 2 Hz (to be submitted). This resolves the crowded monoester region for quantification. In the first practical use of 2D ^{1}H,^{31}P correlations on soil science, we show how organic P species can be identified by the combination of P and H chemical shifts and coupling constants.

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SM597

Crosslinked helically chiral poly-(γ-benzyl-L-glutamate) as alignment medium

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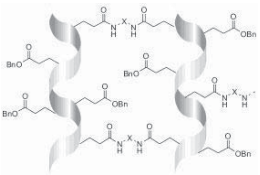
The development of new alignment media – for the structure determination of small molecules by RDCs[1] – is of great interest. For the alignment of organic compounds mainly liquid crystals like homopolypetides[2] or polymer gels like crosslinked polystyrene[3] are used. Polymer gels have several advantages.[4] Unfortunately most organic solvent based gels are achiral.[5] However homopolypeptide based media do have the benefit of a helically chiral structure, which allows enantiodiscrimination.[2, 6]

Therefore we studied the syntheses of polymer gels based on crosslinked helically chiral poly-(γ-benzyl-L-glutamate) (PBLG)[7] which swell in many common organic solvents ($CHCl_3$, DCM, THF, dioxane, toluene, benzene). We analyzed the alignment properties with the help of (+)- and (-)-isopinocampheol and see first indications of enantiodifferentiation for the two enantiomers.



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SM598

Matrix-assisted diffusion-ordered NMR spectroscopy of flavonoid mixtures

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Flavonoids are an important group of natural products, but often their characterization in mixtures is complicated by their structural similarity.[1] Diffusion–ordered spectroscopy (DOSY) is a family of NMR spectroscopic experiments that facilitates mixture analysis by separating signals of species with different diffusion coefficients.[2] However, DOSY struggles to resolve mixtures when there is signal overlap and/or the diffusion coefficients of the species involved are very similar. To overcome these limitations, we use a matrix-assisted DOSY (MAD) approach that exploits differential interactions with sodium dodecyl sulphate (SDS) micelles to resolve flavonoid mixtures.[3]

The analysis of mixtures of fisetin, flavone, catechin and quercetin using MAD experiments is presented, and we demonstrate for the first time that MAD experiments are compatible with the use of mixed solvents to enhance the solubility of flavonoids.

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SM599

Pure shift 2D NMR: simplifying structural analysis and improving resolution

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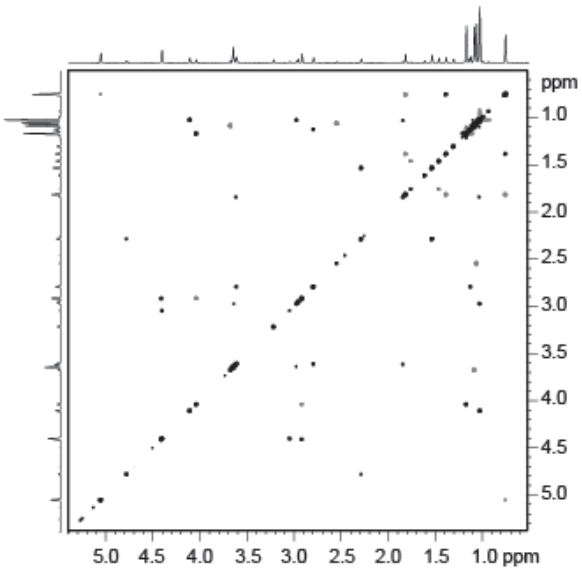

Homonuclear couplings are fundamental to 2D correlation methods such as COSY and TOCSY, but the multiplet structure they cause increases signal overlap and degrades resolution. Multiplet structure can be informative but is usually just a nuisance, especially in through-space experiments such as NOESY or ROESY. 2D correlation spectra can be greatly simplified, and resolution improved, by collapsing all multiplets to singlets. A simple and general way to do this is to use a constant time (CT) evolution period t_1, and then perform covariance processing[2]. This produces a correlation map in which both diagonal and cross-peaks are fully homodecoupled. Results from CT COSY-nQF,TOCSY, NOESY and ROESY experiments will be presented.

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SM600

A DOSY experiment without exchange effects, with improved sensitivity, and with convection compensation

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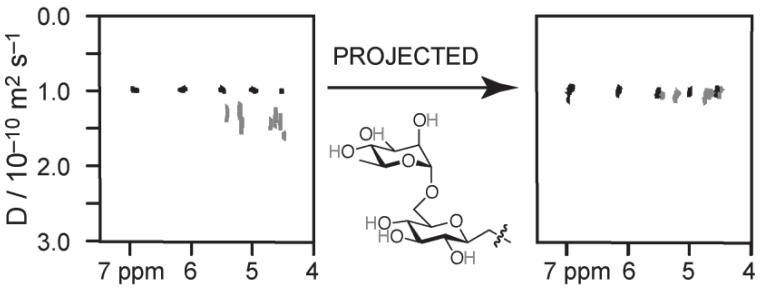

Diffusion-Ordered SpectroscopY (DOSY)[1] is a family of experiments used in mixture analysis that allows signals belonging to the same species to be correlated through their diffusion coefficients. Most pulse sequences use the stimulated echo (STE) or double stimulated echo (DSTE) rather than the spin echo (SE). The STE sacrifices at least half of the available signal compared to the SE, the DSTE three quarters. Moreover, in STE and DSTE experiments, chemical exchange commonly causes confusing results, as exchange during the diffusion delay can mix the signals of species with different diffusion coefficients (as with the OH signals highlighted above). Such problems could be avoided by using SE-based sequences, but J modulation normally prevents this. Here a new DOSY pulse sequence PROJECTED, based on a train of J-refocused double spin echoes, is presented. It suppresses the confusing effects of chemical exchange, suppresses J-evolution, is convection compensated, and (for small molecules with long T_2s) is more sensitive than STE sequences.

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SM601

Sialic acid fingerprints of viral cell attachment proteins

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Sialic acid (Neu5Ac) is an essential component of many glycoproteins and glycolipids, and different viruses have evolved to use sialylated carbohydrates as host cells receptors. The exact chemical environment of Neu5Ac rules these interactions on a molecular level, leading to an astonishing fine-tuning in receptor preference. In extension, this specificity determines which organism and cell type can be infected by individual viral strains. We characterized the carbohydrate receptor binding sites of several Polyoma- and Reovirus capsid proteins by X-Ray crystallography and saturation transfer difference (STD-)NMR. We also demonstrate how a single amino acid mutation can re-target a Polyomavirus to a different Neu5Ac receptor. A large variety of orientations and interactions of Neu5Ac and neighboring carbohydrate rings is observed in our crystallographic models. This diversity is reflected well in STD-NMR spectra, leading to a scenario in which viral proteins can be identified through characteristic magnetization "fingerprints" on their carbohydrate ligands. While hydrogen bonds and salt bridges are readily identified by crystallography, STD-NMR can be used to map non-polar interactions, screen potential carbohydrate receptors, rank affinities and discard models biased by crystal contacts. Our study underlines how both techniques complement each other in the field of carbohydrate-protein interactions.

SM602

Characterization of low molecular – weight gelator methyl-4,6-O-(p-nitrobenzelidene)-α-D-glucopyranoside in toluene and diphenyl ether gels

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The presented work focuses on the thermal properties, the microstructure, and the molecular dynamics of toluene and diphenyl ether in the gels (1.5, 2, and 2.5% [g mL^{-1}]) formed by sugar-based low molecular-weight gelator methyl-4,6-O-(p-nitrobenzylidene)-α-D-glucopyranoside. The diffusion coefficients of solutions molecules inside the gel network were detected by PGSE ^{1}H NMR method for different diffusion time Δ (30–250 ms) and temperatures (230 – 300 K). For larger Δ values, so-called restricted diffusions are observed
and manifested in the linear decreases of the diffusion coefficient with diffusing time Δ.

In a room temperature for a small value of Δ the toluene diffusion (in 2% gel) is almost free and characterized by the diffusion coefficient in the range from 1.86×10^{-9} to 1.96×10^{-9} m^2 s^{-1} for studied gels. The situation is much different in the case of diphenyl ether. Thus the diffusion coefficient of solution molecules in the gel is almost 20% smaller in the gel than in a bulk state.

Optical Polarization Microscopy investigation (OPM) was performed with a JENAPOL microscope operating in different contrast and polarization mode. The microstructure of the 1.5% [g mL^{-1}] gel shows a characteristic fibril structure of the gel network with individual gel fibers, the junction points of thicker fibers, and pores occupied by solution.

SM603

NMR-Study of uptake–release processes in the {Mo_{132}} cluster

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The present study considers porous inorganic molecular capsules of the $\{(Mo^{VI})Mo^{VI}_5O_{21}(H_2O)_6\}_{12}\{Mo^{V}_2O_4(ligand)\}_{30}$ type, which contain organic anions (acetates, propionates, butyrates and valerates) as ligands. An investigation of these $\{Mo_{132}\}$ clusters by NMR spectroscopy provides important information about structure, stability and host-guest interactions.

EXSY/ROESY NMR spectra demonstrate that, in the abovementioned system, exchange equilibrium occurs between the "free" anions in the solutions and the internal ligands. This occurrence is dependent on temperature, pH value and anion size[1-3]. As a carboxyl group of organic ligands is coordinated to an $\{Mo_2\}$-linker, the alkyl tails of the ligands form a hydrophobic cavity, which was expected to show a strong tendency for uptake of hydrophobic species (e.g. long-chain alcohols) from an aqueous solution via the flexible pores[3]. Guest molecules demonstrate positive ROE peaks both along the carbon chain and with the alkyl tails of ligands and are in good accordance with results from ^{13}C-chemical-shift-mapping; these peaks indicate the close packing of the molecule in the capsule interior. Moreover, the DOSY spectra permit the identification of the captured molecule and ligands with the same diffusion coefficient; these spectra correlate with the X-ray data.

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SM604

Conformational analysis of menthol by elucidation of high resolution ^{1}H NMR multiplet structure and FPT DFT calculations

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Natural (-)-menthol is widely-used in NMR spectroscopy as a standard reference compound for testing new experiments due to relatively simple almost first-order ^{1}H NMR spectrum. Most of those studies were devoted to assignment of ^{1}H and ^{13}C NMR signals (see e.g. [1-2]). However, exact values of ^{1}H-^{1}H spin-spin couplings have not been published in the literature yet. For this reason, here we report results of lineshape analysis of high resolution ^{1}H NMR multiplets in menthol, which provide accurate values of these couplings. Relative signs of couplings were experimentally determined by COSY-45 and multiplet-selective 2D-COSY with soft-pulse excitation [3]. Simultaneously we performed FPT DFT (B3LYP/6-311++G(d,p)) calculations of spin-spin coupling constants for all reasonable conformers of menthol. Comparison of experimental and calculated couplings allowed us to establish the preferable orientation of isopropyl and hydroxyl substituents relatively to a cyclohexane moiety.

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SM605

Dynamics of internal rotation in styrene through analysis of high resolution ^{1}H NMR multiplet structure and dynamic FPT DFT calculations

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Styrene is a classical system with hindered internal rotation of vinyl group around C_1–C*i* bond. Previously attempted studies of this dynamics problem gave inconsistent results (see discussion in [1]). Here we report results based on a detailed analysis of ^{1}H NMR spectra and a series of quantum mechanics calculations for dynamic systems vibrating with a large amplitude. ^{1}H NMR spectral analysis for styrene was performed with total lineshape iteration program VALISA [2]. For determitation of relative signs of ^{1}H-^{1}H couplings between aromatic and olefinic protons we calculated spin systems with all possible combinations of these couplings. Simultaneously we performed dynamic FPT DFT (B3LYP/aug-cc-pVTZ) calculations of the couplings. Dynamics of internal rotation was evaluated by a numerical solution of the vibrational problem. Calculated spin-spin coupling constants were obtained by averaging of corresponding conformational dependencies with vibrational distribution function. Experimental and calculated spin-spin coupling constants are in a reasonable agreement with each other. The best-fit potential has a minimum for skewed conformation with dihedral angle 30^o. The "plane" and "orthogonal" forms have energies of 0.66 and 2.64 kcal/mol.

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SM606

Stereochemistry of 2-fluoro-ribose-derivatives - NMR, Molecular Modeling and Outcome

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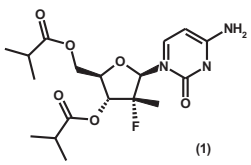
In ongoing studies on the synthesis of the Hepatitis C Virus (HCV) NS5B RNA polymerase inhibitor RG7128 (**1**) the stereochemistry of the intermediate 3,5-dibenzoyl-1-bromo-2-deoxy-2-fluoro-2-C-methyl-D-ribose (**2**) was to be assigned by NMR.[1] ^{1}H, ^{1}H-NOESY-NMR as well as ^{1}H, ^{19}F- HOESY-NMR spectra did not provide unambiguous results. Therefore molecular modeling techniques [PERCH MMS and Quantum Mechanic calculations using Jaguar][2,3] were applied to several related molecules and expected NMR coupling constants and NOE constraints were calculated. Based on the X-ray diffraction data of a deprotected intermediate related to its ^{3}J-^{1}H-^{19}F-coupling constants in NMR, the stereochemistry assignment of the particular representatives of this structural class could be done. Moreover the quality of both molecular modelling approaches was assessed by means of these examples.



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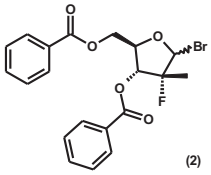

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SM607

1,4-DHP CATIONIC LIPIDS STUDIED BY NMR

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Cationic lipids synthesized on the base of 1,4-dihydropyridine (1,4-DHP) are capable to self-association and formation of nanoparticles in solutions. These compounds are promising gene delivery agents *in vivo* [1]. The structural organization and stability of sonicated aqueous dispersions of cationic lipids (1,4-DHP-derivatives) were characterized by combined use of NMR, DLS, AFM and molecular dynamics techniques. It was found that NMR signal intensities reflect the size and degree of aggregation of the obtained vesicles. Sharp signals were observed for the small vesicles with high surface curvatures that undergo fast rotational motion and the effects of lateral diffusion became important averaging out chemical shift anisotropies and dipolar interactions.

1,4-DHP nanoaggregates behave differently as compared with phospholipids and their mixtures subjected to the same sonication. Starting from certain sonication cycles aggregates formed by cationic lipids reach a relatively small size and remain unchangeable.

DNA is absorbed on the surface of 1,4-DHP cationic lipid aggregates by electrostatic binding. Broad signals of DNA:DHP lipoplexes can be registered and controlled in ^{1}H NMR spectra along with the signals of 1,4-DHP vesicles. Relatively slight structural modifications of 1,4-DHP aggregates significantly affect the DNA complexation ability of the compounds

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SM608

Molecular Fragments inhibiting Human Blood Group B Galactosyltransferase

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Glycosyltransferases (GTs) are an important family of enzymes. They are involved in the biosynthesis of oligosaccharides, polysaccharides and glycoconjugates. They catalyze the transfer of a monosaccharide from a donor substrate to other carbohydrates, lipids, proteins or DNA, that serve as acceptors. Since GTs are related to different pathological pathways and could serve for the directed design of glycosylation patterns of recombinant glycoproteins, developing specific inhibitors is of great interest. A previous ligand-based NMR screening identified acceptor site ligands for the human blood group B galactosyltransferase (GTB) from a fragment library[1]. A combination of STD NMR, spin-lock filtered ^{1}H spectra, surface plasmon resonance (SPR) and activity assays was used.

Here, for further investigations of the top hits, we used STD NMR for epitope mapping and SPR for the determination of binding constants. These studies and enzymological inhibitor studies for the best hit with GTB, human blood group A N-acetyl galactosaminyltransferase (GTA) and a GTA/GTB chimeric enzyme show highly specific binding to GTB. The crystal structure of GTB and the chimeric enzyme show a new inhibition mode for the top compound and therefore represents a first prototype for the development of a specific GT inhibitor.

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SM609

Stereochemistry of tropane *N*-oxide derivatives studied by experimental and theoretical NMR

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The calculation of the NMR parameters (shielding and coupling constants) has nowadays found application in various areas of chemistry including stereochemical problems e.g. conformation[1] or configuration.[2] This poster presents the usefulness of the computation/experiment comparison approach[3] for determination of N-O center configuration in selected tropane derivatives.

Tropane derivatives (tropane, tropinone, pseudopelletierine and cocaine) were oxidized *in situ* in an NMR tube by MCPBA to protonized isomeric *N*-oxides. For comparison, the authentic *N*-oxide samples were prepared by oxidation of the corresponding amines by hydrogen peroxide in ethanol. All ^{1}H and ^{13}C resonances were assigned using standard 1D and 2D (COSY, HSQC, HMBC, ROESY) experiments. The NMR parameters were then calculated on the optimized geometries using DFT GIAO (OPBE/6-31G** and 6-311++G**) methods and the calculated chemical shifts were compared with observed values. It was found that the calculated data correlate well with the experiment and the approach can be successfully used for the prediction of *N*-oxide configuration.

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SM610

Determination of sulfoxide configuration in five-membered rings using NMR spectroscopy and DFT calculations

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Recently, we have shown that computation/experiment comparison of the chemical shifts can be used for determination of configuration at S or N atom in sulfoxides and N-oxides.[1] This poster presents our study of sulfoxides and sulfones with five-membered ring (thia-norbornane derivatives, 1,6-anhydro-1-thio-β-D-hexopyranoses and cyclodipeptides containing thia-proline). The sulfoxides and sulfones were prepared by a stepwise *in situ* oxidation of corresponding sulfides with *meta*-chloroperbenzoic acid in an NMR tube. The oxidation was followed by NMR spectra. All ^{1}H and ^{13}C resonances were assigned using standard 1D and 2D (COSY, HSQC, HMBC, ROESY) experiments. The geometries of all compounds were optimized using the DFT B3LYP/6-31G** method and chemical shifts were calculated for geometry-optimized structures with the DFT B3LYP/6-31++G** method. The calculated ^{13}C chemical shifts induced by oxidation ($\Delta\delta$ values) show good agreement with the experimental data and can be used to determine the oxidation state of the sulfur atom (S, SO, SO_2). The characteristic differences of the oxidation induced chemical shifts of carbon atoms in the α– and β–position to sulfur were successfully used for distinguishing the diastereoisomeric sulfoxides.

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SM611

NMR studies of organometallic chalcogenides and similar compounds

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So-called N,C,N chelating ligands (NCN = -(2,6-di(Me_2NCH_2)$_2C_6H_3$)) stabilize low valence metals (Sb, Bi, Se, Te, Sn) bound in position 1. These compounds can react with many reactants to give rather unusual structures [1-4]. X-ray data proved the new structures undoubtedly. Multinuclear NMR was used to study the constitutions in solution. The ^{1}H, ^{13}C, ^{31}P, ^{77}Se, ^{125}Te and ^{119}Sn NMR spectra were used for such a purpose. In some cases, the different constitution or dynamic behaviour was observed comparing the situation in the solid state and in solution. The examples will be shown.

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SM612

Synthesis and Spectroscopic Investigations of Acylguanidine Derivatives

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Acylguanidines, which can be considered as less basic isosters of guanidines, have numerous applications in the field of organic and medicinal chemistry. Due to their ability to form strong fork-like hydrogen bonds, they are able to take part in various molecular recognition processes. Despite the crucial role of H-bonding networks in ligand recognition, enzymatic reactions and organocatalysis, the detailed understanding of H-bonding in solution is rather limited. Recently we have been able to detect the first scalar couplings across hydrogen bonds in artificial, high affinity arginine receptor systems. [1, 2] These investigations allowed for the first time insights into the H-bonding networks and the binding geometry of these pharmacologically important arginine derivatives. Moreover the conformational preferences of acylguanidines in solution were studied by NMR in order to validate the driving forces for the formation of the distinct interaction pattern and the binding geometry between the acylguanidines and their receptors. [3] However, in contrast to the detailed NMR-investigations of these complexes, so far nothing is known about the binding constants of the investigated acylguanidine complexes. Due to the fact that the binding constants of the complexes are far too high to be determined via NMR titration, fluorescence labelled acylguanidines were synthesized, in order to determine the binding constants via fluorescence titrations experiments.

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SM613

Complete Determination of Absolute Configuration and Conformation of a Homodimeric Alkaloid using Residual Dipolar Couplings and Circular Dichroism

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NMR-based stereochemical analysis of dimeric structures[1] is a difficult problem in NMR due to ambiguities in NOE correlations. To assess the utility of RDC-enhanced NMR in this context, the relative configuration and conformation of a homodimeric bispyrrolinoindoline alkaloid has been determined by the only use of $^1D_{CH}$ residual dipolar couplings. Conformational ensembles were obtained by molecular mechanics molecular modelling for the four possible diastereoisomers. RDCs were obtained by alignment in a $CDCl_3$ swollen polydimethylsiloxane gel and recording of CLIP-HSQC experiments. Single-shot RDC analysis of these ensembles proved the configuration of the homodimer and indicated the dominance of an *anti* disposition around the C–C bond connecting monomer units. Further RDC multitensor analysis allowed quantification of the conformational distribution. Based on the so obtained structural information, circular dichroism spectrum was computed using TD-DFT and, by comparison with the experimentally obtained one, the absolute configuration of the homodimer was unequivocally determined.

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SM614

Investigation of the Interaction between two Intrinsically Disordered Proteins. The Cytoplasmic Tail of Stabilin-2

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Thymosin-β4 is a short intracellular intrinsically disordered protein that is crucial in the organization of the cytoskeleton and provides a link between the cytoplasmic tail of stabilin-2 and actin. A physiological relevance of this interaction is to regulate endocytosis of certain ligands (e.g. LDL). Stabilin-2, a multifunctional scavenger receptor, has a major role in the process at the membrane bilayer, whereas thymosin-β4 provides not only a physical link but also a signal toward downstream events. The interaction of these two proteins was invoked in an earlier study and mapped to the residues 2504-2514 at the cytoplasmic tail of stabilin-2[1].

Based on these observations, we have used biochemical and spectroscopic approaches in order to further characterize the interaction between these two IDPs. The study is an early stage effort to examine this interaction.

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SM615

Exotic helical foldamers structures driven by a novel turn inducer

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Foldamers are oligomers which adopt well-defined secondary structures (1). Numerous pseudopeptides have been designed such as β-peptides, m-phenyleneethylenes, oligoureas, etc (2-3). Small conformational constraints motives able to induce secondary structures are particularly interesting since they are essential building blocks to provide unique structures. In this context, we present helical foldamers structures containing an original bicyclic β-amino acid: the (*S*)-ABOC ((*S*)-aminobicyclo[2.2.2]octane-2-carboxylic acid) (4). Various sequences have been synthesized and the pseudopeptides conformations have been characterized by CD, X-ray crystallography, infrared spectroscopy and NMR. ^{1}H, ^{13}C and ^{15}N complete resonances have been assigned in methanol using ^{13}C and ^{15}N natural abundance and ROESY spectra have numerous (i, i+2) and (i, i+3) characteristic NOE correlations which allow us to solve good quality solution structures. A typical strong H-bond pattern is described for each foldamer and CD spectroscopy at variable temperature points out very stable conformations.

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SM616

NMR elucidation of molecular structure of the appetite receptor from Hoodia Gordonii.

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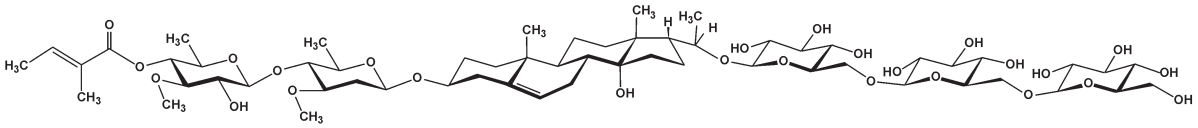
A plant Hoodia Gordonii is nowadays regarded as a source of biologically active compounds of natural origin with appetite-suppressant effect [1]. Interdependency of the biological activity of the extracts with the presence of a steroid-based compound of the pregnene series was postulated, and structure of few compounds was elucidated by a variety of techniques [2, 3]. Here we present results of detailed NMR study of major component of the alcohol extracts from Hoodia Gordonii. The structure was elucidated using the whole set of appropriate 1D and 2D NMR techniques: (3β,20S)-20-{[6-O-(6-O-D-glycero-hexopyranosyl-β-D-glycero-hexopyranosyl)-β-D-gly-cero-hexopyranosyl]oxy}-14-hydroxypregn-5-en-3-yl 2,6-dideoxy-4-O-{6-deoxy-2-O-methyl-4-O-[(2E)-2-methyl-2-butenoyl]-β-L-erythro-hexopyranosyl}-3-O-methyl-β-L-erythro-hexopyranoside.



It differs from previously reported in [2].

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SM617

Dimerization of N-methylindole

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It has been shown that structures of many biological systems are stabilized not only by strong hydrogen bonds with nitrogen and/or oxygen atoms playing the role of the donor (N-H or O-H groups) but also by much weaker hydrogen bonds in which carbon atoms act as donors (C-H groups). Among hydrogen bonds of that type those with π-electron acceptors (C-H···π)are the weakest but strong enough to assemble the secondary structures. C-H···π interactions stabilizing structures of very short peptides seem to belong to the most interesting and important. It has been found that side chain - side chain interactions of aromatic amino acids (Phe, Tyr, Trp) are of great importance in the formation of β-hairpins. Therefore, a deep insight into aromatic ring contribution in the energetic balance and geometry of such interactions observed in biological systems is of great importance. It may become possible by studying simple models. For this purpose we have chosen N-methylindole as a model compound mimicking a tryptophan ring. NMR studies of N-methylindole have shown that some of its proton and carbon chemical shifts are significantly concentration dependent. Also the diminution of dipole moment has been observed for this compound with the increase of its concentration. These findings have been accompanied with the blue shift of the C-H stretching vibrations observed in the IR spectra. These observations suggest the presence of a reversible self-association process in which unsymmetrical dimers are formed. Ab initio calculations have confirmed the possibility of the existence of the stable unsymmetrical dimer of N-methylindole.

SM618

HydroNMR Applied to Ethanol Cluster Size Investigation

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Molecules of simple alcohols as well as water form hydrogen bonded clusters that are subject to fast reorganization in liquid state. The properties such as the cluster size, structure, lifetime, the free energy of its formation are necessary for explanation of often non-ideal macroscopic thermodynamic characteristics of the respective liquids.

Approach combining NMR translational diffusion measurements, DFT quantum chemical calculations and hydrodynamic simulations was used to investigate the properties of hydrogen bonding of ethanol in non-polar solvent (hexane). Hydrodynamic calculations were conducted by HydroNMR [1], a computer program intended for the calculation of hydrodynamic quantities of small, quasirigid macromolecules. NMR diffusion measurements of tetramethylsilane (TMS) and hexamethylenetetramine (HMTA) dissolved in hexane and D20/DMSO, respectively, were carried out at wide temperature range in order to find optimal settings for small molecule calculation.

The mean size of ethanol hydrogen bonded clusters present under different conditions was determined. The clusters consisting of several units are present at decreased temperatures. The pure monomer was found at temperatures above 315 K in 0.16 mM ethanol solution. Its hydrodynamic radius was correctly predicted by the calculations.

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SS619

NMR investigation of novel hydroxides $MO(OH)_2$ (M = Ti, Zr, Hf)

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The most extensively studied crystalline oxyhydrates of IV-group elements are hydrated dioxides $MO_2 \cdot H_2O$ (M=Ti, Zr, Sn), widely used as selective sorbents possessing high chemical resistance and showing high sorption activity in relation to rare earths elements However, the lack of sorbents based on hydrated dioxide is that synthesis conditions and spontaneous aging process affect composition, morphology and particle size [1]. Previously, we reported synthesis and crystalline features of a new group of hydroxides $MO(OH)_2$ (M = Ti, Zr) exhibiting a high sorption activity to f-elements [2, 3].

This purpose of this study is to estimate the acid-base characteristics of oxyhydroxides Ti, Zr, Hf by spectroscopic methods.

IR, Raman and NMR ^1H (MAS) spectra allow establishing differences in the states of structural protons in hydroxides. The chemical shifts values correlate with the positions of absorption bands of O- H stretching vibration in the oxyhydroxides $MO(OH)_2$ (M = Ti, Zr, Hf) and reflect the changing nature of hydrogen bonds and acid-basic properties of the compounds. In $TiO(OH)_2$ the structural protons form strong hydrogen bonds resulting in total chemical shift value is 11.2 ppm., and are able to ion exchange for Li^+ in salt water solutions. Lower chemical shift value and the absorption bands position of O-H stretching vibrations for oxyhydroxides Zr and Hf indicate a more basic nature of the OH-groups.

The work has been supported by the UB RAS grants (nr. M-10-Б, nr. 09-C-3-1006).

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Ss620

Chemical shift powder spectra obtained by using ROtor-Directed Exchange of Orientations Cross-Polarization (RODEO-CP)[1]

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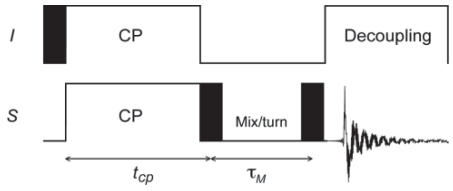
We present a new simple and robust cross-polarization (CP) scheme, called ROtor-Directed Exchange of Orientations CP (RODEO-CP), which permits to obtain undistorted chemical shift powder spectra with short contact times, i.e., when a (quasi-)equilibrium state polarization is not reached. RODEO-CP uses slow magic-angle spinning to suppress lineshape distortions caused by the orientation dependence of CP. The reliability of the method is demonstrated on a powder sample of ferrocene and RODEO-CP is shown to remove the « magic-angle hole » that distorts CP spectra of non-oriented membrane-associated peptides undergoing fast rotational diffusion around the bilayer normal.



The RODEO-CP pulse sequence

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Ss621

Membrane protein structure information by solid state NMR

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We have been working on the preparation and analysis of membrane proteins reconstituted into lipid vesicles by magic angle spinning (MAS) solid state NMR. Although a powerful technique; solid state NMR when applied to studying small transmembrane proteins is disadvantaged when compared to traditional techniques by the lack of established sample preparation protocols and quite often the requirement to use elaborate labelling schemes due to spectral crowding caused by inherently broad line widths. Using the well characterised protein Glycophorin A (GpA) as a model transmembrane protein, we set out to identify simple reliable and reproducible methods for reconstitution of small transmembrane proteins into lipid vesicles for study by solid state NMR. Having shown that we can use 2D ^{13}C-^{13}C DARR experiments to observe interactions between two uniformly labelled amino acids at the GpA dimer interface, we can now show that the same correlations can be also observed when combining differing peptides with amino acids labelled at single positions within the homo dimer interface. In addition we have also been measuring the effect of membrane lipid composition on the resolution of NMR spectra obtained for samples prepared using this method. We now hope to apply these sample preparation and experimental methods to other similar small transmembrane proteins in order to gain new structural information.

Ss622

Numerically exact REDOR calculations of infinite spin systems

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In solid-state NMR under MAS conditions REDOR is one of the most prominent pulse sequences to recouple heteronuclear dipolar interactions. Comparison of experimental and simulated data provide distance information. Density-matrix based programs impose a limit on the number of accounted spins, due to their exponential behavior. Therefore, often only information about the effective dipolar interaction can be gained. In this work, a program that can efficiently calculate numerically exact REDOR curves of large spin systems for the entire time-period was developed.[1,2] It shows a linear scaling behavior with respect to the number of spins and fast convergence behavior. We show for the first time a perfect agreement between experimental and theoretical data for spin systems in crystalline materials with "infinite" spin-systems. Our approach is based on an improved C-REDOR sequence.[3,4] We present data for ^{31}P-^{1}H, ^{19}F-^{31}P and ^{13}C-^{1}H spin systems.[4] Such calculations of REDOR curves can be used to localize NMR-active nuclei in nanoparticles.[5,6] Since the heteronuclear dipolar interactions relate to the crystal structure, our approach might be an interesting add-on to NMR crystallography.

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Ss623

Measurement of ^{15}N-^{13}C heteronuclear dipolar couplings for high resolution solid state NMR spectroscopy of aligned proteins

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High resolution NMR spectrum of membrane proteins can be obtained by orienting them in suitable environment. Structural dependent orientation constraints can be measured by Seperated Local Field (SLF) experiments. Most of these experiments have been developed to measure ^{1}H – ^{15}N dipolar couplings and ^{15}N chemical shifts in uniformly ^{15}N labeled oriented proteins[1-3]. The technique suffers from poor resolution for protein of larger size. We present here a new class of solid state NMR (ssNMR) experiments to measure ^{15}N – ^{13}CO and ^{15}N – ^{13}C$_\alpha$ dipolar couplings in oriented proteins. The experiments are based on separating the hetronuclear dipolar couplings in second dimensions with ^{13}C chemical shifts. The experiments are developed for the uniformly ^{15}N labeled oriented proteins. Experiments are demonstrated on ^{15}N labeled N-Acetyl Valine crystals and ^{15}N labeled bactereophage Pf1. These measured dipolar couplings will provide extra constraints for high resolution structure determination of oriented proteins. Experimental results and numerical simulations carried out by SIMPSON and SIMMOL will be presented.

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Ss624

Molecular Dynamics of F8BT Polymer Film: Correlation with Opto-Electronic Properties

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Poly(9,9 '-dioctylfluorene-*co*-benzothiadiazole) (F8BT) is currently one of the most promising material for use as active layers in polymeric electronic devices, such as polymer light-emitting diodes (PLEDs) and polymer field effect transistor (P-FET). It is well known that both polymer structure and dynamics affects either the luminescence or transport properties in thin films, making worthwhile to investigate them. Dynamical aspects of F8BT were first investigated by Dynamical Mechanical Thermal Analysis (DMTA), Differential Scanning Calorimetry (DSC) and ^{1}H Nuclear Magnetic Resonance (NMR). The results revealed the presence of two main relaxation process, which occurs at about 225 K (β-relaxation) and 370 K (α -relaxation). The molecular processes responsible by such relaxations were investigated by specific NMR experiments, such as Dipolar Chemical Shift correlation (DIPSHIT) and Centerband Only Detection of Exchange (CODEX). The results showed that, in the temperature range of 220 to 373 K, the lateral chain execute molecular rotations with average correlation times ranging from 10^{-4} – 10^{-7} seconds. On the other hand, from 300 to 350K the backbone carbons execute slow libration motions with reorientation angles that increase as a function of temperature. Those results could be correlated with the Current-Voltage characteristics of thick ITO/F8BT/Al devices carried out a several temperature (70 until 490 K).The drift charge mobility in the device was studied as a function o temperature by Time of Flight techniques (TOF), showing relatively abrupt change on the activation energy near both relaxations (β and α). Based on these results, a model that explains the behavior of the charge transport as a function of temperature based on the occurrence of the molecular relaxation was proposed.

Ss625

Amplifying the effects of molecular motion in DIPSHIFT-like experiments

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Dipolar Chemical Shift Correlation (DIPSHIFT)[1] is frequently used to study molecular motions by probing the reorientations through the modulation introduced in the dipolar CH coupling and T_2.[2] In systems where the coupling is weak or the reorientation angle is small, a variant of the DIPSHIFT experiment, where the effective dipolar coupling is amplified by a REDOR π - pulses train, is applied. However, the previously described experiment is not sensitive to the T_2 modulation introduced by the motion,[3] which avoid the observation of motions with rates ranging from hundreds of Hz to few kHz. We present a DIPSHIFT implementation which amplifies the dipolar couplings and is sensitive to T_2 effects. Spin dynamics simulations, analytical calculations and experiments demonstrate the sensitivity of the technique to molecular motions and the best experimental conditions to avoid imperfections. Furthermore, an in-depth analysis of the DIPSHIFT experiments was done, which allowed explaining the physical origin of many artifacts found in the literature data. We also show that in DIPSHIFT-like experiments, simple Lee-Goldburg homonuclear decoupling may perform as good as, or even better than, its more intricate variants, such as Frequency Switched and Phase Modulated Lee-Goldburg. To demonstrate the use of the technique, we show recent applications to membrane lipids

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Ss626

Structural investigations of Frustrated Lewis Pairs by modern Solid State NMR techniques and DFT calculations

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In this contribution we present detailed Solid State NMR investigations of intramolecular phosphine borane adducts[1] that are of high interest in the field of Frustrated Lewis Pair (FLP) chemistry due to their capability of activating small molecules such as dihydrogen[2,3]. Solid State NMR is well-suited for the structural characterization because of the large number of NMR active nuclei present in these compounds (as ^{1}H, ^{13}C, ^{19}F, ^{11}B and ^{31}P). By exploiting double resonance techniques such as **R**otational **E**cho **DO**uble **R**esonance (REDOR)[4], the interaction between Lewis acid and Lewis base is analyzed with the general aim of obtaining a deeper understanding of the mechanism of the dihydrogen activation.
A main focus of this work lies especially on the detection of $^{11}B\ldots^{31}P$ internuclear distances and weak B-P covalent interactions through modification of NMR parameters such as quadrupolar coupling constants, isotropic chemical shifts and indirect spin-spin-couplings. DFT calculations of NMR parameters allow a more profound understanding of their correlations to structural characteristics and therefore help to establish Solid State NMR as an extremely sensitive spectroscopic method for providing useful information on a local level.

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Ss627

High-Resolution Solid State NMR Analysis of Surfaces and Interfaces in Aluminum Doped ZnO Nanoparticles

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Aluminum doped ZnO nanoparticles (ZnO:Al) are precursors for Transparent Conducting Oxide (TCO) materials used in many electronic devices. Here, we study ZnO:Al nanoparticles, which are prepared via a microwave-assisted polyol process. Solid-state NMR offers tools to study the local structure of the dopant in the host material and the surface coverage of particles e.g. by solvent molecules using ^{13}C, ^{1}H, ^{27}Al NMR, qNMR[1] and quantitative REDOR experiments. These techniques have proven to be useful in earlier investigations of nanoparticles.[2,3] The doping concentration and the defect distribution of ZnO:Al as well as its effect on conductivity is studied by ^{27}Al MQMAS and ^{27}Al semi-quantitative NMR analyses. Information about the neighborhood of different Al-dopant sites is obtained by HETCOR, REDOR and PRESTO experiments. Based on experimental results and quantum chemical calculations[4], we discuss different models which explain the increase in conductivity of different defect models of ZnO:Al.

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Ss628

Composite pulses for efficient excitation of the central transition of half-integer quadrupolar nuclei

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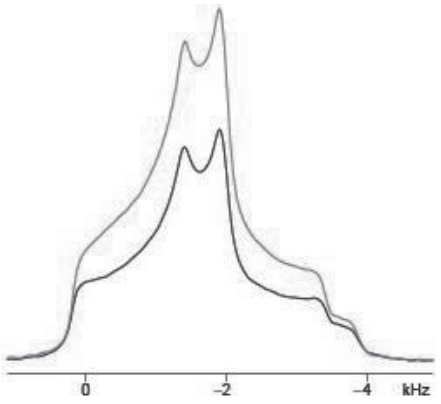

We present a new scheme using composite pulses for the excitation of the central transition of half-integer quadrupolar nuclei. Previous methods achieved signal enhancement via inversion[1,2] or saturation[3] of the satellite transitions in order to enhance the population difference across the central transition. In contrast, our method is based on a more efficient conversion of the population difference across the central transition into single-quantum coherences that avoids spurious excitation of higher coherence orders. All crystallite orientations are uniformly excited and no distortion of the lineshape is observed. Numerical simulations agree remarkably well with experimental results. Enhancement factors of 1.35 can be obtained. The implementation of the experiment is extremely simple and depends only on the optimization of a single parameter. The method has been tested on samples containing ^{23}Na (I = 3/2), ^{27}Al (I = 5/2) and ^{45}Sc (I = 7/2).

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Ss629

Solid state NMR at cryogenic temperatures

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Solid state NMR at cryogenic temperatures offers an increase in signal, a reduction in noise, and the opportunity to study a wide range of interesting and technologically important physical phenomena. Examples include superconductivity, quantum tunnelling and quantum molecular rotation. On the biological side, cryogenic NMR has a major potential in the study of protein assembly or membrane proteins. Furthermore, cryogenic NMR coupled to DNP is expected to give rise to an enormous sensitivity gain with an even stronger impact in those applications.

We have constructed cryogenic NMR systems working at a magnetic field of 14.1 T and able to perform both static and magic-angle-spinning solid state NMR experiments at very low temperature. Our static cryoprobe is able to perform solid state NMR (without sample rotation) at temperatures down to 1.8 K. Our cryogenic MAS probe can spin a 2mm zirconia rotor at 15 kHz while keeping the real sample temperature at 13K. The sample temperature and spinning frequency may be adjusted independently, since the bearing, turbine and sample cooling lines are well separated. The cooling is done using He gas produced in a custom-made boiler by evaporating liquid He in the supercritical regime. In this contribution we will show the first results obtained with this setup run at the most extreme working conditions. We use the longitudinal relaxation constant of ^{127}I in cesium iodide as a convenient NMR thermometer for temperatures down to about 15K.

Ss630

Direct Estimation of Spin-Diffusion Coefficients and Homonuclear Coupling by a Cross-Polarization Based Method

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Spin diffusion appears as an omnipresent effect in solid-state NMR whose efficiency depends on structural and dynamic properties of the sample. Therefore, this phenomenon can be used to characterize heterogeneities[1]. However, knowledge of the spin-diffusion coefficient of each investigated domain is always a prerequisite for data evaluation. In this context, we present a cross-polarization (CP) based method by which spatially well-defined proton "magnetization holes" can be created. (An alternative procedure is based on REDOR[2].) By following the re-equilibration of such locally inverted magnetization, direct observation of spin diffusion is enabled.

As a second effect, for very narrow initial holes produced by Lee-Goldburg CP it is possible to obtain magnetization exchange pattern between close-by nuclei. In case of no powder averaging as given in oriented liquid crystals, this can results in long-lasting oscillations giving a quantitative spectrum of the local homonuclear dipolar couplings.

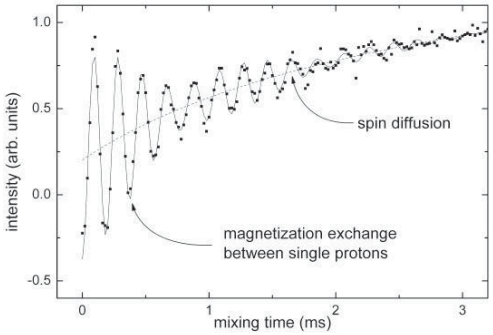

Fig.: Local proton magnetization exchange pattern

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Ss631

Switched angle spinning solid-state NMR as a tool for internuclear distance determination

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Dipolar couplings are a valuable source of structural information in solid-state NMR. However, most applications require the use of MAS which averages anisotropic interactions, such as dipolar couplings, and thereby loses the distance information contained therein. Commonly, dipolar couplings are selectively reintroduced by a sequence of rf-pulses that suspend the averaging effects. A much simpler approach to prevent complete averaging is off-magic angle spinning (off-MAS) whose potential for distance estimations in multiple-spin systems has been demonstrated, recently.

Off-MAS is typically accompanied by a loss in spectral resolution especially at large magic-angle deviations as they are necessary to determine structurally interesting long internuclear distances >4 Å. Here we present an experiment that combines a frequency-selective spin-echo — applied off-MAS —with data acquisition on the magic-angle. The frequency-selective pulses therein can be calibrated to isolate any desired internuclear distance. Subsequently, the experiment is carried out at several spin-echo evolution times, which yields characteristic build-up curves for each selected component of the NMR signal. This build-up is sensitive to the dipole-dipole coupling strength; a phenomenon that is demonstrated to determine reliable internuclear distances in multiple-spin systems and a protein sample of rhodopsin.

Ss632

Analytical fitting functions for correlated 2D spectra of disordered materials

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Homonuclear 2D correlation NMR spectroscopy is a routine tool for the analysis of solid disordered materials. Examples can be found for double-quantum and triple-quantum [1] filtered ^{1}H, ^{13}C, ^{29}Si, ^{31}P NMR on various materials: inorganic polymers [1], glasses or disordered nano-scale [2,3] materials.

Here we present the theory [4] to extract lineshape parameters and correlation factors by fitting experimental spectra with analytical functions. These functions have been derived from normalized 2D and 3D probability density functions. Projections of these functions for 1D spectra, for 2D double-quantum single-quantum and triple-quantum single-quantum correlation spectra have be calculated. A fitting tool has been implemented in a program which can read in 2D spectra generated from standard spectrometer software. We demonstrate our approach on 2D ^{31}P NMR spectra of a series of phosphate glasses including ultraphosphate compositions, which have been obtained by pulse-transient optimized multi-quantum symmetry-based pulse-sequences [5,6]. The advantage of this approach is that partially overlapping peaks can be deconvoluted and we hope to generate parameters which can finally be correlated with structure.

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Ss633

Dipolar Recoupling Involving Quadrupolar Nulcei in Magic-Angle-Spinning NMR

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Firstly, we demonstrate the estimation of homonuclear dipolar couplings, and thereby internuclear diastances, between half-integer spin quadrupolar nuclei by central-transition (CT) double-quantum (2Q) sideband magic-angle-spinning (MAS) NMR spectroscopy [1]. The rotor-encoded sideband amplitudes from CT 2Q coherences are sensitive probes of the magnitude of the homonulcear dipolar coupling, but significantly less affected by other NMR parameters such as the magnitudes and orientations of the electric field gradient tensors.

Secondly, we demonstrate high-resolution proton-detected solid-state NMR multi-quantum (MQ) correlation spectroscopy, in which the indirect dimension corresponds to the high-resolution dimension of a split-t_1 3QMAS experiment. The figure shows an example for ^{17}O-L-tyrosine obtained at 21.1 T external field and 62.5 kHz MAS frequency. Other examples will be shown for ^{87}Rb and ^{27}Al. Inversely (proton) detected MQMAS spectroscopy possesses great potential, especially for insensitive nuclei.

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Ss634

Exploiting Paramagnetic Effects in Solid State NMR Using a Membrane Binding Heme Protein Nitrophorin 7

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Nitrophorins represent a group of ferriheme *b* proteins designated NP1-4, which were found in the blood-sucking insect *Rhodnius prolixus*[1]. Recently, a novel form NP7, which is able to attach to negatively charged phospholipid membranes, was established by recombinant protein expression[2, 3].

In contrast to its homologues, liquid state NMR studies of NP7 did not yield well-resolved resonances probably due to the low molecular tumbling of the transient aggregates formed in solution[3]. A viable alternative was therefore the study of NP7 by means of solid-state NMR spectroscopy.

Here, we report an initial solid state NMR studies of NP7 in the Fe^{III} low-spin state (S = ½) and the diamagnetic (S = 0) NO bound form. Our results show that SSNMR has sufficient sensitivity and resolution and can be successfully applied to gain structural insights of relatively large heme proteins (21 kDa), including spin states that typically create difficulties for NMR spectroscopy.

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Ss635

Internuclear Distance Measurements Between Half-Integer Quadrupolar Nuclei

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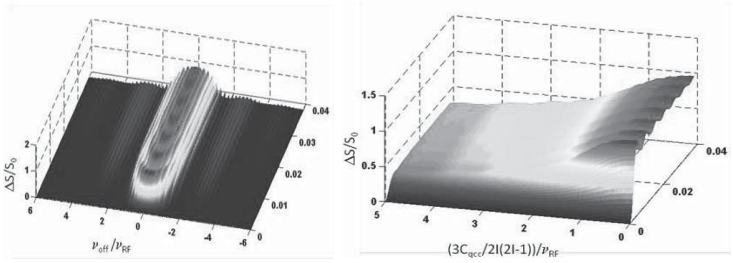
A new methodology to extract internuclear distances between half integer quadrupolar nuclei from REDOR (REAPDOR or TRAPDOR) dephasing curves is introduced. From the universal REDOR dephasing curve for two I=1/2 spins, the effect of offset irradiation on one of the I=1/2 spin is investigated numerically and approximated with a sinc-type function weighting the universal REDOR curve.



The dephasing is drawn in a three-dimensional map function of dephasing time and function of the offset irradiation. When one of the spins is quadrupolar the effect of the quadrupolar interaction is numerically compared with the effect of an offset irradiation for the case when both spins have I=1/2. Different orientations in a powder feel different quadrupolar interactions and therefore an empirical formula that sums over the different orientations/offsets is introduced and compared with experimental results and numerical simulations. The advantage of our empirical formula is that it contains factors that take into account the size of the quadrupolar interaction and/or the adiabaticiy parameter. Results obtained on ^{15}N-^{17}O enriched Glycine; ^{27}Al-^{17}O $LaAlO_3$ and ^{71}Ga-^{17}O $LaGaO_3$ are presented and analyzed.

Ss636

Quantitative aspects of solid state NMR: Quantitation of polymorphic and solvate impurities using MAS ^{19}F & ^{2}H NMR

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Understanding and control of solid state form is vital to the development of drug products in the pharmaceutical industry. In this context, it is imperative that the nature of solid state impurities such as undesired polymorphs and solvates are characterised and ultimately controlled. Solid state NMR (ssNMR) is not only sensitive to changes in crystal packing (*i.e*. can differentiate polymorphs), but is also inherently quantitative. The ssNMR spectra of these solid state impurities need to be understood to allow quantitative methods to be developed and potentially validated.

This poster describes the use of solid state ^{19}F NMR to characterise and quantify a polymorphic impurity in the active pharmaceutical ingredient (API); a discussion of method validation is also presented.

In a second example, solid state ^{2}H NMR was used to characterise a labelled solvate and discriminate free *vs*. bound solvent. In tandem with solution state ^{1}H NMR, it was possible to quantify the solvate as a solid state impurity in API.

Ss637

Solid-state ^{31}P NMR strategies for self-assembled polymers based on [(η^5-$(PhCH_2)_5C_5$)Fe(η^5-P_5)] and [{(η^5-Me_5C_5)Mo}$_2$(μ-η^6:η^6-P_6)] building blocks

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The self-assembly of smaller components into extended structures is a fascinating and rewarding field of contemporary chemistry. Metal atoms and ions have proven to be particularly interesting building blocks [1]. In this contribution, we report our investigations of the coordination polymers formed by reacting the pentaphosphaferrocene [(η^5-$(PhCH_2)_5C_5$)Fe(η^5-P_5)] ("*cyclo*-P_5 compounds") [2] or the triple-decker hexaphosphabenzene complex [{(η^5-Me_5C_5)Mo}$_2$(μ-η^6:η^6-P_6)] ("*cyclo*-P_6 compounds") [3] with various transition metals. To distinguish the different crystallographic phosphorus sites, solid-state ^{31}P MAS NMR spectra were examined; in addition, the suitability of various two-dimensional techniques for providing connectivity information and site assignments was explored, in an effort to develop a comprehensive spectroscopic strategy for similar materials without availability of crystallographic information. The title compounds are particularly challenging candidates for 2-D NMR studies, owing to very short spin-spin relaxation times and long spin-lattice relaxation times. For the rigid *cyclo*-P_5 materials, preliminary results were obtained using radiofrequency-driven dipolar recoupling (RFDR). In contrast, the spectra of *cyclo*-P_6 compounds are generally affected by fast molecular motion on the NMR timescale, which was studied further by variable-temperature MAS NMR and spin-lattice relaxation time measurements.

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Ss638

Phosphorus-31 and Vanadium-51 Solid-State NMR Spectroscopy of β-Vanadyl Phosphate: Homo- and Heteronuclear Spin-Spin, Electrostatic and Paramagnetic Interactions

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Vanadium phosphorus oxide catalysts have received widespread industrial attention due to their highly active and selective catalytic behaviour in the oxidation of paraffins. Depending on precursors and synthetic route chosen, a variety of different materials have been obtained. The characterization of these materials and their precursors by means of NMR spectroscopy presents a challenge to the NMR spectroscopist. The present study investigates the various nuclear spin interactions that contribute to the linewidths of ^{31}P and ^{51}V solid-state NMR spectra of the title compound, a prototypal diamagnetic vanadyl phosphate.[1]

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Ss639

Speeding up ssNMR on Membrane Proteins – Paramagnetic Doping of Proteorhodopsin

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Solid-state NMR is an established method for hypothesis-driven biophysical studies on membrane proteins and an emerging technique for structural biology. Its major strength is the possibility to investigate membrane proteins directly within the lipid bilayer. However, many protein systems of interest, like GPCRs, pose a great experimental challenge because of the low sensitivity of ssNMR. Therefore, large amount of the protein sample and/or long data collection time is needed to overcome this bottleneck. One possible route for improvements is the enhancement of the signal-to-noise ratio per unit time. Most of the experimental time is required for the ^{1}H spin system to return to equilibrium (^{1}H T_1) in between each scan, i.e. 2-5s or 95% of the experimental time. Mechanisms for reducing this time would result in a better S/N ratio per unit time and are searched for other studies, e.g. faster data acquisition schemes in time-resolved ssNMR, as well. In a comprehensive study the 7TM light-driven proton pump proteorhodopsin, reconstituted into lipid vesicles, is doped with Cu-EDTA and a Cu-Tag aiming at the reduction of ^{1}H T_1. Furthermore, a promising approach with a Gadolinium complex as a paramagnetic relaxation agent is presented. Our data reveal that in principle paramagnetic relaxation enhancement is applicable to membrane proteins, but large differences exist between the paramagnetic agents used in this study.

Ss640

Solid state NMR investigation on phase separation, crystallization and structural changes in disilicate glasses and ceramics

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Disordered materials having a wide range of application are often phase separated on the micro- or nanoscale. One representative for this are lithium disilicate glass-ceramics which are of interest in restorative dentistry. As the degree and type of crystallization influences the chemical and physical properties of glass-ceramics it is essential to have a controlled process of crystallization.[1] To get a deeper insight into the structural and chemical aspects of the process of crystallization on different length scales solid state NMR spectroscopy is a useful tool.[2]

The local structure of several binary silicate glasses and ceramics has been investigated by solid state NMR spectroscopy. The investigated systems have a composition near or of lithium disilicate. During the process of quenching and additional heat treatment the formation of lithium metasilicate and lithium disilicate can occur.[3] Characterization of the initial stages of crystallization by standard X-ray diffraction and solid state NMR techniques is often hindered by the low concentration of the crystalline species formed. The present contribution introduces $^{29}Si\{^{7}Li\}$ cross polarization magic angle spinning NMR (CP-MAS) as a new approach for detection incipient crystallization processes in these ceramics.

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Ss641

Recoupling of Native Homonuclear Dipolar Couplings in Magic-Angle-Spinning Solid-State NMR by the Double-Oscillation Field Technique

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A double-oscillating field technique[1] (DUO) that produces an effective Hamiltonian proportional to the native high-field homonuclear dipole-dipole coupling under magic-angle spinning (MAS) conditions is presented. Basically, DUO uses one part of the radio frequency (rf) field to recouple the dipole-dipole coupling interaction. This part of the rf field does also modulate the chemical shift interactions, thereby eliminating offsets effects over the aliphatic region on a 700 MHz (Larmor frequency of ^{1}H) spectrometer. The other part of the rf field averages all non-secular terms and in addition ensures stability towards rf inhomogeneity and rf miscalibration. Experiments on a two spin system confirm the theoretical results.

We have compared the capability to transfer polarization through longitudinal mixing of the DUO sequence to that of finite pulse rf driven recoupling (fpRFDR) with π pulses lasting one third of a rotor period and to that of dipolar-assisted rotational resonance (DARR) with a short mixing time (10 ms). For most of the one-bond transfers, DUO gives more intense cross-peaks. 2D recoupling experiments were conducted on antiparallel amyloid fibrils of the decapeptide SNNFGAILSS with the FGAIL part uniformly labeled with ^{13}C and ^{15}N. With its high stability towards rf inhomogeneity and good one-bond transfers, we anticipate that DUO will find applications within the field of biological ssNMR spectroscopy, e.g. as an assignment experiment.

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Ss642

Resolution Enhancement of Solid-State NMR Correlation Spectra by Optimal Control Based Dipolar-Driven Spin-State Selective Filtering

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In solid-state magic-angle spinning (MAS) NMR spectra, an increase in the linewidths in the direct dimension due to unresolved J-couplings is observed. This results in poorer resolution, overlapping peaks and ambiguous resonance assignments. Here a new method to out-compensate the linebroadening effect of the J_{CC}-couplings on the carbonyl resonances is presented. Pre-acquisition preparation of single quantum coherence spin-states is achieved using dipolar-driven magnetization transfers.

By use of optimal control, spin-state selective sequences for biological solid-state NMR have been designed. The optimal control based development has facilitated the use of the strong dipolar couplings among neighboring carbon nuclei to achieve spin-state selective coherences, which previously only have been accessible through J-based transfers. The use of the dipolar couplings for spin-state manipulation shortens the pulse sequence and decreases demands for very intense proton decoupling as compared to previous J_{CC}-based methods.

For spin-state selective coherence transfer[1], a doubling of the resolution in the detection dimension is achieved for the carbonyl region of homonuclear correlation spectra.

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Ss643

Random deuteration of proteins studied by ssNMR spectroscopy

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Biological solid-state nuclear magnetic resonance (ssNMR) spectroscopy emerged as an important tool for structural biology. However, experiments, which rely on carbon or nitrogen detection, suffer from low sensitivity. To achieve high spectral quality and sensitivity, we introduced the RAP (Reduced Adjoining Protonation) labeling scheme [1], which yields randomly protonated samples in a deuterated matrix. Deuteration reduces the dipolar $^{1}H,^{1}H$ network and facilitates ^{1}H-detected high-resolution ssNMR spectroscopy of aliphatic resonances. Further characterization of RAP samples displays their utilizability for various issues, as retrieving of dynamical parameters, determination of tertiary structure information and resonance assignments, as presented here.

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Ss644

NMR Analysis of Trace Elements in Speleothems

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Speleothems can grow for thousands of years. During that time they encode palaeoclimatic information in their geometry and geochemistry. A great work has been done over the past decade with a variety of techniques that have been used to investigate trace elements included in the structure of speleothems ($CaCO_3$) or isolated within the crystal lattice[1]. Solid state NMR can bring new information for high resolution palaeoclimate and palaeoenvironmental reconstruction. Until now, MAS NMR was used to investigate ^{31}P in speleothems[2], with conclusion that calcite speleothems can contain phosphate in several forms, including phosphate defects in calcite and several types of coexisting crystalline phosphate inclusions. The aim of our work is to determine the possibility of investigating other trace elements in the structure of speleothems such as ^{23}Na and ^{27}Al using solid state NMR in order to contribute to the understanding of the conditions of their formation. Samples in the study are taken from various locations in Croatian karst that is characterized by complex speleogenesis influenced by different climatic, geological, hydrological and geomorphological environmets.[3]

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Ss645

27Al NMR investigation of V phase Al-Cu-Mg intermetallic

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The V phase $Al_5Cu_6Mg_2$ single crystalline sample is a cubic phase (*Pm3*) and has 39 atoms per unit cell with the cell edge of 8.3 Å. The long dimension of our sample was oriented along [100] axis and the perpendicular edge was along [110] direction. The unit cell contains three nonequivalent Al (Al-1, Al-2, Al-3) crystallographic positions, where the number of atoms at a given crystallographic site is 1, 6, 8 respectively.

The sample was placed in the magnetic field with the [100] axis perpendicular to the field. We measured the temperature dependence of the T_1 relaxation time by using the inversion-recovery pulse sequence with the 90-degree pulse length D1 = 2.4 μs and the irradiation frequency was set to υ_0 = 104.295 MHz. The measured signal exhibited two peaks at any temperature. We integrated each peak separately at a given temperature to produce the magnetization-recovery curves. We fitted the curves by using the formula for spin I = 5/2 central line irradiation.

For the measurement of angular dependence of the spectra, we have constructed an epoxy and fiberglass coil holder that enabled us to fix the sample in the exact orientation – rotation around the [100] axis. We used frequency sweep from 102.4 MHz to 106.2 MHz at the temperature of T = 80 K. The spectrum was recorded every 3 degrees of orientation ranging from 0 to 180 degrees. A two pulse sequence (solid echo) with two 90-degree pulses of 2.4 μs was used.

We analyzed the spectra by simulating the angular dependence of the central lines and the sattelites. Due to three crystallographicall nonequivalent Al positions in the unit cell, there are three EFG and three anisotropic Knight shift tensors, which were extracted from the spectral analysis.

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Multiple-acquisition method exploiting polarization transfer from ^{1}H and ^{2}H in solid state NMR spectroscopy

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In solid state NMR spectroscopy an increasing interest for extensive ^{2}H labeled samples, as a means to dilute proton spins, is observed. This labeling scheme places a huge amount of information on the deuteriums and optimal ways of achieving this knowledge, while still obtaining the proton based information, is therefore of interest.

We present a novel interleaved sampling strategy to simultaneously acquire pairs of one- or multiple dimensional spectra utilizing polarization from both ^{1}H and ^{2}H spins, which results in improved spectral information content or sensitivity. This so-called RAPID (Relaxation-optimized Acquisition of Proton Interleaved with Deuterium) ^{1}H-^{13}C/^{2}H-^{13}C CP/MAS multiple-acquisition method is exploiting, that the relaxation time for deuterium is orders of magnitudes faster than the relaxation time for protons and during the repetition delay for a standard proton CP experiment a second experiment utilizing polarization-transfer from deuteriums[1] can be acquired. The experiments can be chosen to fulfill different needs, resulting in spectra that provides additional information about the chemical shifts of ^{2}H, the molecular dynamics provided from the ^{2}H quadrupolar coupling size, as well as which ^{13}C spin is in proximity of ^{1}H or ^{2}H. If the spectra are simply added together, this will provide a significant sensitivity gain.

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Ss647

Sensitivity enhancement using STMAS and its application to characterization of trace amounts of boron in coal

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MQMAS and STMAS spectra for calcium aluminosilicate glass and kaolin have been compared in sensitivity and resolution.[1] STMAS implemented with double quantum filtered soft-pulse-added mixing (DQF-SPAM) pulse sequence[2] provides the most enhanced sensitivity without impairing spectral resolution.

Based on these results, ^{11}B DQF-SPAM STMAS experiment has been applied to characterization of trace amounts of boron (0.001-0.01*mass*%) in coal.[3] As seen in Fig. 1, on the ^{11}B-STMAS NMR spectrum of coal (carbon content of 70-90 *mass*%), nonequivalent four-coordinated boron ($^{[4]}$B) sites with small quadrupolar coupling constants (≤ 0.9 MHz) are clearly distinguished. Two of the $^{[4]}$B sites in down field are considered organoborons with aromatic ligands, and the other in the most upper field is assigned to inorganic tetragonal boron (BO_4).[2] The assignment of organoborons has been confirmed by ^{11}B{^{1}H} CPMAS experiments. Furthermore the ^{11}B STMAS spectrum of coal ash demonstrates that a part of organoborons is converted to trigonal species (BO_3) during the combustion process.

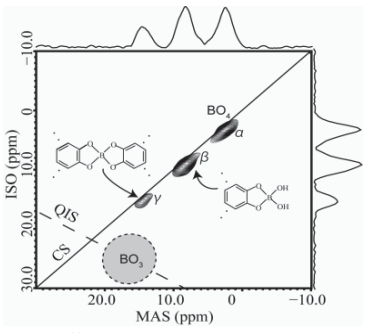

Fig. 1. ^{11}B STMAS NMR spectrum of coal acquired with 1.6 ms acquisition time for F_1 dimension, spinning rate of 20kHz, and

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Ss648

Supramolecular packing and hydrogen bonding in organic functionalized materials from first principles

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In the emerging field of organic-based electronics the application of functional materials such as low band gap polymers and molecular stacks has become one of the central goals of current research activities. The perspective of using self-assembling, self-healing supramolecular assemblies appears to be extremely promising for photovoltaic nano-scale devices. Combining ab initio Car-Parrinello Molecular Dynamics (CPMD) [1,2] with subsequent sampling of trajectories by linear response magnetic property calculations gives an excellent tool to learn about NMR spectroscopic signatures of molecular packing, especially in cases where long-range crystalline order is absent. To get knowledge about the local packing motifs and interactions we combine solid-state NMR measurements with in silico packing models. We also demonstrate the potential of this combination by using Nuclear Independent Chemical Shifts (NICS) [3] calculations together with liquid-state ^{1}H NMR measurements for obtaining a molecular picture of local packing that is consistent with solid-state NMR experiments [4]. Furthermore, via dispersion corrected DFT method (DC-DFT) we show that is possible to investigate the competition between van der Waals forces and hydrogen bonding forces in molecular packing.

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Ss649

Constant Time REDOR NMR Spectroscopy

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Among the various pulse sequences available for the investigation of heteronuclear couplings the **R**otational **E**cho **Do**uble **R**esonance (REDOR) experiment has evolved as today's standard method. In the archetypical REDOR approach, introduced by Schaefer and Gullion [1], the dipolar coupling is reintroduced during an increasing evolution time. However in case of strong dipolar interactions, especially in multiple spin systems, severe problems in the data analysis are encountered. Here the Constant Time (CT) approach can be applied as an alternative [2,3]. In the CT-REDOR experiment the evolution time is kept at a constant value and the dipolar interaction is only partially reintroduced by varying either the position of the dephasing π-pulses over the entire rotor period or their pulse length. Furthermore a combination of both approaches may be used. The applicability of the CT-REDOR method is validated with different reference substances, including isolated two spin systems as well as multiple spin systems.

As demonstrated with these model compounds accurate values for the second moments can be obtained without the need to consider the detailed spin geometry, what is of special interest in case of unknown multiple spin systems.

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SP650

Investigation of the ESR in Pnictide and Boride of Europium

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The ESR spectrum of conducting magnets contains a wealth of information not only about the resonance ions, but also the magnetoresistance, magnetic phase transitions, skin layer. The present study is an attempt to understanding all this information as a whole. The ESR measurements of the $EuZn_2As_2$ (powder) and $EuB_{6-x}C_x$ (single crystal) were performed on frequency 9.3 GHz in TE_{102} rectangular cavity in the temperature range from 4.2 to 300 K. $EuZn_2As_2$ (space group **P-3m1**) is anti-ferromagnetic (AFM) with $\boldsymbol{\theta_p}$ ~ - $\mathbf{T_N}$ = -16.5 K and its resistance is semiconductor-like [1]. $EuB_{6-x}C_x$ (space gr. **Pm-3m**) is semi-metal, FM or AFM ($\boldsymbol{\theta_p}$ ~ ±16 K) depending on content x of carbon. The observed resonance lines of the Eu^{2+} ions best fit by a Lorentzian lineshape with linewidths ~700 Oe at temperatures above 150 K. Surprising, but it is quite explainable that the paramagnetic temperatures $\boldsymbol{\theta_p}$, obtained for $EuZn_2As_2$ from ESR data, have positive sign. For $EuB_{5.98}C_{0.02}$ $\boldsymbol{\theta_p}$ is ~ +8 K in case of a magnetic field along [111] axis, and $\boldsymbol{\theta_p}$ ~ -7 K for the field along [100] axis. Large deviation ($\Delta g \sim 0.03$) of the g-factor relative free Eu^{2+} ion indicates on the strong hybridization of the f-states Eu with the p- s-states of the band electrons and possible formation of Kondo-like bound states. The obtained data are interpreted in terms of indirect exchange interaction between localized magnetic moments of Eu^{2+} by means electrons of a valence band (Bloembergen-Rowland's modified RKKY interaction [2]).

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SP651

Electronic structure of the nitrogen donors in quasi-cubic positions in 6H SiC studied by General TRIPLE ENDOR method

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Despite of considerable efforts undertaken in the study of the nitrogen (N) superhyperfine (shf) structure some of the fundamental questions related to the spatial distribution of the electronic wave function (EWF) of the isolated N donor residing two quasi-cubic ('k1', 'k2') sites in 6H SiC lattice are not well understood yet. This is related to the fact that the shf lines with large coupling constants observed in pulsed EPR and ENDOR spectra [1] were not assigned to the specific ^{13}C and ^{29}Si atoms located around N_{k1}, N_{k2} in 6H SiC as well as the relative sign of their shf coupling constants were not determined. In this work the relative sign of the shf coupling constants and spatial distribution of EWF for N_{k1}, N_{k2} in 6H SiC was obtained by General TRIPLE ENDOR. It was found that the N_{k1} and N_{k2} in 6H SiC with very close values of the hyperfine constants (a_{k1} = 33.564 MHz, a_{k2} = 33.221 MHz, [1]) reside at two different atom sites in 6H SiC lattice. Si atoms are located in the nearest neighborhood (nn) of N_{k1} while in case of N_{k2} C atoms are found in the nn of N. This result is consistent with the fact that there are two positions for N within the 6H SiC unit cell with the same distance to the nearest three C (Si) planes which become nonequivalent if N resides at C and Si sites in the lattice.

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SP652

Localization effects induced by decoherence in superpositions of many-spin quantum states

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The spurious interaction of quantum systems with their environment known as decoherence leads, as a function of time, to a decay of coherence of superposition states. Since the interactions between system and environment are local, they also cause a loss of spatial coherence: correlations between spatially distant parts of the system are lost and the equilibrium states are localized. This effect, which has not been studied in detail so far, limits the distance over which quantum information can be transmitted, e.g., along a spin chain. We investigate this issue in a nuclear magnetic resonance quantum simulator, where it is possible to monitor the spreading of quantum information in a three dimensional network: states that are initially localized on individual spins (qubits) spread under the influence of a suitable Hamiltonian apparently without limits. If we add a perturbation to this Hamiltonian, the spreading stops and the system reaches a limiting size, which becomes smaller as the strength of the perturbation increases. This limiting size appears to represent a dynamical equilibrium.

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SP653

Ferroelectric nanosized materials charachterization by EPR

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Inhomogeneous charged defects distribution within particles leads to a strong difference between local and average properties, which are strongly size dependent. In composites based on nanomaterials interfaces are known to concentrate charged defects what results in dramatic changes in the properties as compared to the ones of the parent materials. A detailed knowledge of the nature of such defects as well as their correlation with the materials composition, preparation conditions and properties is thus of key importance for prospecting the macroscopic properties expected by industry. We are reporting on nanosized ferroelectrics and composites characterization using Electron Paramagnetic Resonance method. With examples of barium titanate based materials the questions of charged defects location, stabilization and mobility/dynamics linked to their influence on properties are discussed. In particular, the nature of the conduction mechanism within BT grains [1] and of the charge accumulation at the grains boundaries [2], nanoparticles' surface [3] or composites interfaces is proposed.

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SP654

^{27}Al NMR Investigation of γ- $Mg_{17}Al_{12}$ Intermetallic

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γ–$Mg_{17}Al_{12}$ phase plays an important role in Al–Mg alloys and it has been studied in numerous investigations. According to structure analysis **Ref. 1**, γ–$Mg_{17}Al_{12}$ has space group I-43m with lattice constant a = 1.05438 nm and contains 58 atoms in the unit cell. Atoms are distributed over three Mg and one Al crystallographically inequivalent sites that are fully occupied. In order to obtain NMR parameters (EFG tensor and isotropic magnetic shift) for Al we measured rotation patterns of the ^{27}Al (I = 5/2) NMR spectra of monocrystalline sample around [100] and [011] crystallographic directions. The NMR spectra are inhomogeneously broadened by the electric quadrupole interaction and the line shapes are featureless and powderlike but still exhibit significant orientation-dependent variation of the intensity in the magnetic field. We were able to theoretically reproduce rotation patterns and determine EFG tensor (η = 0.40; ν_Q = 0.100 MHz) and the isotropic magnetic shift (S_{iso} = 1072 ppm). The EFG tensor elements are Gaussian distributed with standard deviations as large as the values themselves. This indicates that either the sample contains many defects or there are many different Al sites in the unit cell, suggesting larger unit cell of the investigated compound.

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SP655

Static ssNMR and NQR investigation of hexanuclear metallic clusters

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Hexanuclear metallic clusters $[(M_6L^i_{12})L^a_6]^{n+}$ (n = 2, 3, 4) are worth studying due to their interesting physical properties which depend on oxidation states n as well as on dimensionality of structures they form. They consist of six metallic atoms M (Nb or Ta), twelve inner ligands L^i (usually Cl or Br) and six apical ligands L^a (H_2O, OH, CH_3OH, Cl...). Especially interesting are paramagnetic clusters with n=3 which have one unpaired electron delocalised over whole cluster unit [1].

Here we present both theoretical NMR/NQR calculations and experimental results on two diamagnetic clusters $[Nb_6Br_{12}(H_2O)_6][HgBr_4]\cdot 12H_2O$, $Nb_6Cl_{12}(H_2O)_4(OH)_2\cdot 4H_2O$ and paramagnetic one $[Et_4N][Ta_6Br_{12}(H_2O)_6]Br_4\cdot 4H_2O$ whose structures were previously determined [2]. Static ^{93}Nb and ^{35}Cl NMR spectra were obtained in 12 T field. Zero field NQR measurements on ^{93}Nb, ^{79}Br and ^{81}Br were performed in order to fix EFG parameters for simulation of NMR spectra [3]. Independent GIPAW-DFT calculations of NMR/NQR parameters using CASTEP [4] are in good agreement with experimental results.

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P_b like paramagnetic center at a-Si:H/c-Si interface detected by EDMR

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Hydrogenized amorphous silicon (a-Si:H)/crystalline silicon (c-Si) hetero-junction solar-cells reach the highest efficiency of silicon solar-cells in mass production. Due to the high quality of the silicon wafer material and the low thickness of a-Si:H used, the passivation quality of interface defects is the process that ultimately determines device efficiency. Furthermore state-of-the-art hydrogenized microcrystalline silicon (μcSi:H) solar-cells intrinsically possess a high number of a-Si:H/c-Si interfaces at the grain boundaries, and an a-Si:H/c-Si heterojunction can be regarded as an appropriate model system for μcSi:H. In this study the spectroscopic properties of paramagnetic defects at the a-Si:H/c-Si interface are investigated by low temperature electrically detected magnetic resonance (EDMR). This technique is best suited due to its high sensitivity and the ability to study fully processed solar-cells [1]. Spin-dependent recombination between conduction band tail states in the a-Si:H bulk and a-Si:H/c-Si interface dangling bonds is detected. By taking a rotation pattern it could be shown that the signal originates from the a-Si:H/c-Si interface. The g-tensor of this site on the [111] oriented silicon wafer resembles the values of the well-known P_b center [2] at the SiO_2/Si interface. By way of sample preparation it can be excluded that natural SiO_2 is present at the interface.

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EPR on TM ions-doped hydrothermally-grown TiO_2 Nanoparticles

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Transition-metal (TM) ions-doped semiconducting oxides, such as TiO_2, ZnO, and SnO_2, have received considerable attention in the last years [1], due to many reports of room-temperature ferromagnetism (RTFM). However, their magnetic properties still remain a controversial issue, since the observed magnetic behaviour appears to be strongly dependent on the preparation methods.

In this work we report structural investigations on TiO_2 nanoparticles prepared by a hydrothermal route, with different doping content of transition-metal Fe, Mn, and Co at low concentration (0.1 – 2% at.). X-ray diffraction and transmission electron microscopy revealed the formation of pure anatase TiO_2 phase devoid of elemental TM clusters in all doped samples. Mean crystallite sizes of almost all TiO_2 grains were in the range of 13 - 23 nm. Electron paramagnetic resonance (EPR) was used to investigate the impurity sites, their local environments and interactions between TM ions. The spectra were recorded at different temperatures (100 - 550 K) in X- and Q-band, on as-prepared and annealed doped samples. Each EPR spectrum of Fe^{3+}, Mn^{2+}, and Co^{2+} is complex and consists of an overlap of spectra, either due to some intrinsic paramagnetic defects (initially existing in the undoped TiO_2 samples) and the incorporated TM ions (interstitially and substitutionally), and/or to the ferromagnetic resonance lines (well seen in annealed samples). A careful EPR characterization of local magnetic properties in TiO_2 samples, as revealed by the temperature dependence of EPR spectra [2], is consistent with the bound magnetic polaron (BMP) model of RTFM. It is strongly related to the paramagnetic defects (oxygen vacancies) formed during the preparation of doped TiO_2 nanoparticles.

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SP658

Phase transition and conductivity mechanism in the ionic conductor Cu_2HgI_4

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The first multinuclear, frequency-dependent NMR investigation of the ionic conductor Cu_2HgI_4 is presented, giving new insights into the ion dynamics in this compound. At 344 K it undergoes a transition from insulator to ionic conductor, which we were able to follow in detail. Pretransitional fluctuations are observed in copper NMR, with a mean-field theory providing an adequate description. Spin-lattice relaxation measurements provide information about defect motion below the transition, with discernible effects from both copper and mercury. At the transition temperature the copper sublattice is disordered, line broadening is observed and the relaxation time drops abruptly for a factor of 50. In contradistinction, the mercury line shows a substantial narrowing in the conductive phase, indicating that Hg^{2+} ions are the main contributors to conduction. This is in disagreement with the consensus view that conduction is dominated by copper ions [1], so we performed constant field gradient diffusion measurements to confirm mercury motion. In the extreme narrowing regime (above ~380 K) the signal decay exactly follows the prediction for diffusing spins [2], providing unambiguous evidence of mercury diffusion. We discuss the role of dynamic correlations in enabling the motion of the large and heavy mercury ions.

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SO659

Backbone assignments and structural analysis of the second catalytic cysteine half-domain (SCCH) of mouse ubiquitin-activating enzyme E1

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Proteins degradation by ubiquitin-proteosome pathway requires jointed action of three enzymes responsible for ubiquitin-activation (E1), conjugation (E2) and ligation (E3). Second catalytic cysteine half-domain (SCCH) is a 276 amino acid residues long fragment of the mouse ubiquitin-activating E1 enzyme contained catalytic cysteine. As demonstrated previously, SCCH is folded autonomously and formed stable 3D structure which was solved by an X-ray and available in pdb databank (1Z7L) [1].

In spite of existence structural data the initial stages of ubiquitin activation process are still not understood in details. In presented work we perform structural analysis of SCCH sub-domain in solution in regard of possibility to obtain complex with ubiquitin in future. The sequence-specific assignments were achieved on a sample of ^{2}H,^{13}C,^{15}N-triple labeled recombinant SCCH using standard heteronuclear HNCA, HN(CO)CA, HNCACB and CB(CO)NH NMR experiments. All data sets were acquired at 298 K on Varian VNMRS 800 NMR spectrometer equipped with cryogenic probe head. At the moment, backbone resonances for 213 residues are successfully assigned. Further structural analysis is performed by TALOS+ and CS23D programs and will be presented on a poster.

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So660

Structural basis of cyclic nucleotide-activated ion channel gating

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Cyclic nucleotide-activated ion channels, known as HCN and CNG channels, play an important role in signal amplification pathways. To elucidate the structural basis of the underlying gating mechanism, high resolution structures of the cyclic nucleotide-binding domain (CNBD) in the apo and holo state are required. Until recently, however, only crystal structures of CNBDs from HCN2 and the prokaryotic CNG channel MloK1 have been known. In the case of HCN2, the apo and holo state did not reveal substantial differences[1,2]. For MloK1, structural information for the apo state has only been gained from a non-functional mutant CNBD[3]. In the present study, we describe the solution structures of the apo and holo wild-type CNBD of MloK1[4,5]. A comparison of these structures reveals large conformational rearrangements upon ligand binding. These structures provide important insights into conformational events that accompany channel gating within the ligand-binding site.

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So661

Structural and membrane interaction studies of the antimicrobial peptide alyteserin-1c and its bio-active analogue

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Cationic antimicrobial peptides (AMP's) are of particular interest as potential antibiotics due to their broad spectrum of activity [1]. The selectivity of AMP's is a combination of charge, hydrophobicity, secondary structure and target membrane composition, and as a result it is difficult for microbes to acquire resistant to the peptides. The biggest limitation of these peptides is their cytotoxic activity [2]. The aim of this study is to investigate the structure and conformation of the AMP alyteserin-1c and its analogue [E4K]alyteserin-1c in anionic and zwitterionic membrane mimetic media, to represent the bacterial and mammalian cell membranes respectively, in an attempt to gain insight into the activity of the peptides.

The structures of alyteserin-1c and its analogue [E4K]alyteserin-1c were investigated by proton NMR spectroscopy and molecular modelling. The solution structures are characterized by an extended alpha helix in membrane mimicking environments for both peptides. Positional studies indicate that the C-terminal residues do not interact with the micelles while the N-terminal and central residues reside within the micelle and parallel with the polar head groups.

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So662

Mechanism of Non-specific Inhibitors of Amyloid Assembly: Interactions of Lacmoid with the Amyloid β peptide

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Increasing evidence shows a strong link between the self-assembly of the amyloid β peptide (Aβ) and the pathogenesis of Alzheimer's disease. Soluble oligomeric Aβ assemblies are thought to be the toxic species causing synaptic and neuronal injury in the patient's brain. Many inhibitors for the oligomerization and/or fibrillation process of neurodegenerative diseases have been reported, yet only little is known about the mechanistic details of these compounds. The present studies concern the interaction of one such inhibitor, Lacmoid (Lac). We investigated this interaction by a broad biophysical approach revealing similar binding characteristics to Aβ as has been reported for detergents. Furthermore, we show that Lac has the ability to inhibit both oligomeric assembly and fibrillation of Aβ. Nuclear magnetic resonance experiments show an overall signal decrease upon addition of Lac while the chemical shifts only display small changes. High Lac concentration causes a loss of the major part of NMR signals including ^{1}H-^{15}N-HSQC, ^{1}H-^{15}N-TROSY and ^{1}H-^{13}C-HSQC cross-peaks. Circular Dichroism spectroscopy was applied to monitor the kinetic aggregation process of Aβ in presence of Lac. Low Lac concentrations slow down the conversion towards a β-sheet state while at high concentration Lac completely prohibits secondary structure changes.

Taken together, these findings provide the basis for a simple model which could explain how non-specific interactions with small molecules may interfere with amyloid formation. Understanding the mechanistic details is potentially helpful for future drug design of small molecule therapeutics targeting amyloid disorders, such as Alzheimer's disease.

So663

Insights in domain interactions within type VII collagen studied by NMR

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Type VII collagen (Col7) is the major component of the anchoring fibrils of the skin. These fibrils link different skin layers together and contribute essential to skin stability. Mutations within Col7 lead to a heritable skin blistering disease. In addition, an autoimmune disease exists that is characterized by autoantibodies against Col7. Despite the biological importance of Col7 no detailed structural information has been available so far. Col7 has a central collagenous domain that is flanked by two non-collagenous domains with subdomains homolog to fibronectin III (FNIII) and the A3 domain of the von-Willebrand-factor.

Here, we report the cloning, expression and purification of the von-Willebrand-factor-A-like domain 2 of murine Col7 (mvWFA2). Resonance assignment of the domain was performed. Secondary structure prediction by TALOS+ is in agreement with a homology model of mvWFA2 domain. We further analyzed the interaction of mvWFA2 with the neighboring FNIII-like domain 9 (FNIII-9). Chemical shift mapping identified the binding site for FNIII-9 at the N- and C-terminal part of mvWFA2 enabling a first structural insight into the domain architecture of Col7. The interaction site is opposite to potential collagen I binding sites. It might be of more importance in the native molecule due to the direct linkage of the two domains. In addition, this interaction might be influenced by binding of extracellular matrix components.

Our results provide the basis for further structural and functional studies of Col7 and will lead to a better understanding of the pathogenesis of related skin blistering diseases.

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So664

Characterisation of the Interactions between Integrin α1β1 and Collagens

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Integrins comprise of a large family of heterodimeric cell surface receptors consisting of an α and a β subunit. They are responsible for mediating cell-extracellular matrix (ECM) and cell-cell interactions, as well as transmitting bi-directional signals across the plasma membrane. Four members of the family are categorized as collagen-binding integrins and are characterized by their ligand specificity for different collagens. These receptors contain an inserted domain (I-domain) in their α-subunit, which is the primary binding site of the receptor for extracellular ligands. Studying the I-domain and its interaction with collagen allows understanding of the nature of the essential structural changes that govern the collagen-binding and signal transmission of the receptor. (1)
The aim of this project is to investigate the interaction between the α1β1 and collagen by studying the specific collagen recognition by the α1 I-domain. The study employed a range of biophysical techniques including surface plasmon resonance (SPR) and isothermal titration calorimetry (ITC) that provided kinetic and thermodynamic information of the binding, gel filtration chromatography and SAXS that revealed unexpected stoichiometry of the binding and NMR experiments that provided detailed information on the collagen binding-induced structural changes of the protein. Combining the information generated from all these techniques in conjunction with some mutagenesis studies provided a detailed insight into the interaction. These results are valuable for understanding the biological roles and the regulation of the receptor.

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So665

Solution NMR and biophysical analysis of the cataract-associated R76S mutant of human ©D-crystallin

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Although a number of ©D-crystallin mutations have been associated with cataract formation, there is no clear understanding of the molecular mechanism(s) that lead to cataracts. As part of our ongoing studies, we investigated the recently discovered Arg76 to Ser (R76S) mutation that is correlated with childhood cataract in an Indian family. We expressed R76S γD-crystallin protein in *E. coli* and characterized it by CD and fluorescence spectroscopy, as well as determined its thermodynamic stability with respect to thermal and chemical denaturation. No significant biochemical/biophysical differences were observed between the wild type protein and the R76S variant. As expected, replacement of the positively charged arginine side chain by the neutral serine lowered the pI of the protein, and the experimentally determined value was 6.8 compared to the wild-type value of 7.3. We also characterized the solution structure of R76S ©D-crystallin by NMR. Using residual dipolar couplings (RDCs) we showed that overall structure of the mutant is very similar to the wild type structure. Likewise, the protein's dynamics are also unaffected by the mutation: 15N relaxation data, in particular R_2 values are essentially the same as in wild-type ©D-crystallin. Overall, our results suggest that neither structural nor stability changes in the protein are responsible for the R76S ©D-crystallin mutant's association with cataract. Further studies will be necessary to evaluate the functional relevance of the R76S γD-crystallin mutation.

So666

Structure and dynamics of the UNR-Sxl-msl-2-mRNA complex in Drosophila dosage compensation

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The protein Upstream to N-Ras (UNR) is the key to understand regulation of gene expression on the translational level. UNR has been implicated in the regulation of mRNA translation in humans, and as such has a direct link to viral diseases and cancer. In *Drosophila*, its role in dosage compensation is well characterized. Sex-lethal (Sxl), only present in females, binds to msl-2 mRNA and UNR cooperatively, represses translation of msl-2 mRNA and thus expression of X linked genes. In order to understand the molecular mechanism of this process, it is important to understand the structural basis for the cooperative assembly of the Sxl-UNR-msl-2-mRNA complex. UNR consists of five cold-shock domains, and it has been shown that only the first of these domains (CSD1) is needed for the interaction with msl-2 mRNA and Sxl. The region of the mRNA interacting with the proteins has been mapped to involve 18 nucleotides. Importantly, chemical shift titrations revealed that both protein subunits interact in the complex with each other and contact to msl-2 mRNA. Based on chemical shift perturbations, a HADDOCK model has been calculated in order to rationally design cysteine mutation sites for spin labeling. PREs derived from these spin labels together with intermolecular NOEs, RDCs and solvent PREs will be used to determine the high resolution structure of this complex. Static light scattering revealed a 1:1:1 binding stochiometry for the protein-RNA complex, with RNA binding affinity in the nanomolar range. The results of our NMR and structural studies will be presented.

So667

Finding active ligands to kRAS using solution state NMR

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RAS has been the classical oncology target for many years and yet to date; no small molecule drug has been described that targets this protein directly. RAS mutations are drivers for a number of cancers (1) and kRAS (2) is mutated in the majority of pancreatic cancers. We have used a fragment based lead discovery (FBLD) workflow (3) to identify and biophysically characterize binders to kRAS from a library of diverse fragments. Solution state ligand-detected NMR methods were used to identify fragments that bind to the protein. The binders were validated using isotope-labeled protein and amide proton and nitrogen chemical shift perturbations. Ligands were identified that bind to a pocket on a functionally relevant face of kRAS. This region is involved in the interaction of the GTPase activating protein (GAP), the GTP exchange factor (SOS) and the effector (PI3K). The ligand-based screen of 3300 compounds produced 786 primary hits; a cascade of follow-up measurements validated 83. Of those, 25 compounds showed similar chemical shift perturbations indicating one preferred binding site. High-resolution RAS/ligand complex structures were determined by crystallography. Guided by the complex structures, we have identified a small molecule ligand that shows activity in a biochemical assay, underlining the functional relevance of the binding site.

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So668

Structure and Molecular Interactions of the RRMs of the splicing factor TIA-1

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Recognition of the short RNA regions for alternative splicing by cognate splicing regulators is important, but many of the versatile interactions remain poorly understood. T-cell intracellular antigen 1 (TIA-1), also known as an apoptosis-promoting factor, can regulate alternative pre-mRNA splicing by binding uridine-rich RNAs and thereby facilitate 5' splice site recognition by U1 small nuclear ribonucleoprotein (snRNP). TIA-1 promotes the inclusion of exon 6 during splicing of human FAS, which encodes a transmembrane protein that triggers programmed cell death. Alternative splicing with skipping of Fas exon 6 results in a soluble anti-apoptotic form that is linked to autoimmune responses. TIA-1 is composed of three RNA recognition motifs (RRM1, RRM2 and RRM3) with a C-terminal glutamine-rich domain. Here, we employ solution state NMR and segmental isotope labelling methods towards understanding the molecular basis of TIA-1 mediated alternative splicing. NMR relaxation data and chemical shift comparisons indicate that the three RRMs of TIA-1 are largely independent structural modules in the absence of RNA. However, a tandem RRM23 construct of TIA-1 shows different tumbling rates for RRM2 and RRM3 in the absence of RNA while a uniform tumbling correlation time is found when bound to a pre-mRNA ligand. We report our NMR structural analysis towards describing the multi-domain arrangement of free and RNA bound TIA-1 and its role in TIA-1-mediated regulation of Fas alternative splicing.

So669

Architecture of the chromatin factor HMGN2-nucleosome complex as revealed by methyl-TROSY NMR

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Chromatin structure and function are regulated by numerous proteins through specific binding to nucleosomes. The structural basis of many of these interactions is unknown, as in the case of the high mobility group nucleosomal protein family (HMGNs) that regulates various chromatin functions, including transcription. We report the architecture of the HMGN2-nucleosome complex determined by a combination of methyl-TROSY nuclear magnetic resonance spectroscopy and mutational analysis.[1]

NMR methyl-TROSY spectra of nucleosome (200 kDa) are of excellent quality and allowed us to assign nearly all histone isoleucine, leucine and valine methyl groups. Using these methyl groups as 'reporters' we studied the interaction with HMGN2. We found that HMGN2 binds to both the acidic patch in the H2A-H2B dimer and to nucleosomal DNA near the entry/exit point, thereby "stapling" the histone core and the DNA.

Our results provide insight into how HMGNs regulate chromatin structure through interfering with the binding of linker histone H1 to the nucleosome as well as a structural basis of how phosphorylation induces dissociation of HMGNs from chromatin during mitosis. Importantly, our approach is generally applicable to the study of nucleosome-binding interactions in chromatin and holds great promise for study of nucleosome dynamics.

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So670

Molecular basis of viral pathogenicity: interaction of the Rabies virus glycoprotein with host neuronal proteins.

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Rabies virus hijacks survival regulation of infected neurons to favor its propagation. Virulent strains have optimized the C-terminal sequence of their G protein, encompassing a binding site for PDZ domain (PDZ-BS), to interact with the PDZ domain of the human MAST2 kinase (MAST2-PDZ) triggering neuronal survival. Upon viral infection, we showed that the G protein could disrupt the complex between MAST2 and PTEN, a partner of the kinase and an inhibitor of neuronal survival. We characterized here the unusual binding mode of the MAST2-PDZ/PTEN-C_{TER} and MAST2-PDZ/G-C_{TER} for which structures were solved by NMR. Our structural analyses revealed that viral PDZ-BS binding mimics the mode of interaction of the endogenous ligand PTEN to MAST2-PDZ with the conservation of a large surface of interaction and an original mode of interaction involving two anchorage points. Combining *in vitro* and *in cellulo* results, we concluded that survival phenotype in infected cells is a consequence of the dissociation of MAST2-PDZ/PTEN-C_{TER} complex by the viral G protein that excludes nuclear PTEN.

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So671

Interaction of Na^+-translocating NADH:quinone oxidoreductase with quinones characterized by NMR spectroscopy

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The sodium ion-translocating NADH:quinone oxidoreductase (Na^+-NQR) from *Vibrio cholerae* is a respiratory membrane protein complex that couples the oxidation of NADH and the reduction of membrane-bound quinone to the transport of Na^+ across the bacterial membrane. The Na^+-NQR is composed of the six subunits NqrA-F and contains at least five cofactors. The entire mechanism of sodium translocating is still enigmatic and constitutes the major objectives of this project[1].

Here, we present results obtained with the soluble NqrA subdomain of Na^+-NQR. Protocols for expression and purification of unlabeled, as well as ^{15}N-labeled NqrA were developed and improved. By saturation transfer difference NMR spectroscopy we could show that ubiquinone Q1 and the inhibitor HQNO bind to the NqrA with the quinone head group and the terminal methyl groups having closest contact to the protein. Surface plasmon resonance and fluoroscence titration experiments indicate that NqrA harbours two sites for quinone binding. This will be investigated further by NMR interaction studies with isotope-labeled NqrA. An initial, promising ^{1}H-^{15}N TROSY NMR spectrum has been obtained from fully protonated ^{15}N-NqrA. Using a perdeuterated NqrA and optimized NMR methods it should be possible to identify the quinone binding pockets of NqrA and to characterize changes of protein structure and dynamics upon ligand binding.

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So672

Structure Determination of Neurolin Ig Domains

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The neuronal receptor neurolin is a member of the superfamily of immunoglobulin like receptors. Like it's human homologues ALCAM/RAGE, it plays a central role during neuronal development in the model organism goldfish. Intraocular application of antigen binding fragments and domain-specific antibodies against the first three immunoglobulin domains cause severe pathfinding and fasciculation errors of growing axons in the retina. Especially, the second immunoglobulin domain of neurolin seems to be of great importance for axon pathfinding. However, the molecular mechanisms of these processes are still unclear. Our working hypothesis is that the protein interacts with molecular guiding cues, which lead the growing axons through specific routes to their targets in the tectum opticum. Up to now, neither a structure of the protein is known, nor have interaction partners been identified.[1]

The goal of the present study is to approach both problems using NMR spectroscopy. Protocols for high level expression and purification of different neurolin domains have been developed including a cleavable N-terminal SUMO-tag, serving as an expression and solubility enhancement. High quality NMR spectra could be obtained for the second Ig domain. The resonance and NOE assignment is currently in progress. Further work will aim at the structure determination using NMR based constraints including RDC based refinement. In addition, ligand capture experiments will be performed, in order to identify a potential molecular axon guiding cue. Using methods like *STD NMR*, *Transfered Cross Saturation* or *chemical shift perturbation mapping,* it should be possible to determine the binding mode between the two interaction partners.

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So673

Direct NMR Evidence for Non-native Interactions in the Denatured State of an Ultrafast-folding Mini-protein

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Deciphering the process of how nascent polypeptide chains attain a native fold only within a few tenths of a second in the crowded environment of a living cell has recently been acknowledged as a key element for better understanding how the formation of pathogenous protein aggregates occurs *in vivo*. Residue-specific spectroscopic information on unfolded protein states, however, remains sparse due to their intrinsic conformational heterogeneity and dynamic nature.

Here, we present NMR data on the 6 M urea-denatured state of the ultrafast folding TC5b molecule, a small peptide exhibiting natively a globular fold and long-range interactions. By combining 2D NMR spectroscopy together with ^{15}N relaxation experiments on the unfolded state of TC5b and a structurally optimized point mutant, we are able to highlight the importance of both native *and* non-native interactions for ultrafast and productive refolding. Among other things, NOE contacts between Trp and aliphatic amino acids exhibiting both native *and* non-native character were identified. Moreover, a mutationally induced enhancement of the nucleation site's hydrophobicity led to the detection of not only additional non-random interactions but also a concomitant acceleration of refolding rates. The results are complemented with ^{15}N $R_{1,2}$ and het. NOE measurements thus providing distance-independent sources of structural information.

Hence, our data productively contribute to the ongoing discussion of how only a few sequence determinants can direct the entire folding pathway of globular proteins starting from the very early stages of structure formation.

So674

Structural basis of galectin-1 dependant pre-BCR activation

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During B cell differentiation in the bone marrow, pre-B cells express a pre-B cell receptor (pre-BCR) composed of an immunoglobulin heavy chain (Igμ) and a surrogate light chain made of the λ5 and VpreB proteins. Expression and activation of this receptor constitutes a crucial checkpoint for B cell development. Pre-BCR interacts, via the unique region of λ5 (UR-λ5), with the β-galactoside binding protein, galectin-1 (GAL1) (1). This interaction is associated to the formation of a synapse between pre-B cell and stromal cell leading to pre-BCR relocalization and activation. First, the structure we solved by NMR revealed that UR-λ5 interacts with GAL1 via a stable previously undescribed α-helix which lies on a mostly hydrophobic patch at the surface of GAL1. Second, we have investigated Gal-1/oligosaccharide specificities using an NMR/molecular dynamics combined approach (2-3). Finally, we are planning lectin microarrays experiments to investigate the glycosylation state of pre-B cell and stromal cell, with the aim to determine the galectin-1 specificity involved in cell/cell recognition necessary for synapse formation.

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So675

Protein dynamics modulate function in case of cellular retinol-binding proteins

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Vitamin A trafficking and metabolism are regulated inside the cell primarily by specific high-affinity carriers called cellular retinol-binding proteins types I and II (CRBP-I, CRBP-II). They belong to a large family of ubiquitous intracellular lipid-binding proteins (i-LBPs) that feature a β-barrel structure with an internal water-filled cavity where the hydrophobic ligand is bound. Expression patterns, affinities for retinoids, as well as interactions with enzymes and membranes are unique to each CRBP. Although both homologs exhibit the same structural topology and identical retinol-binding motifs, the K_d values for example differ by two orders of magnitude. The goal of our research is to elucidate the molecular basis of the dramatic discrepancies between CRBP-I and CRBP-II.

Based on ^{15}N relaxation dispersion, line-shape analysis as well as H/D exchange, we recently have demonstrated major differences in the backbone dynamics of these two CRBP types, which indicated diverging mechanisms of ligand entry into the binding cavity[1,2]. Here we continue to investigate retinol uptake and release in the presence of membrane mimetics by examining the effects of lipid bilayers on CRBP structure and dynamics.

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So676

Structural insights into a class of fibril-forming fungal proteins

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Hydrophobins are small fungal proteins that self-assemble into amphipathic monolayers at hydrophobic:hydrophilic interfaces. Hydrophobins are secreted by all filamentous fungi and play diverse roles in their development and reproduction. There are two classes of hydrophobins that are characterised by different monolayer morphologies and stabilities. Class I hydrophobin monolayers are formed by the lateral assembly of amyloid-like fibrils called rodlets. These hydrophobins are stable and monomeric in solution, but at interfaces they undergo a conformational change which allows formation of the rodlet monolayer, however, structural information about this process is lacking. We have solved the monomeric solution structure of a class I hydrophobin, DewA from *Aspergillus nidulans*, using solution NMR spectroscopy. There is only one other class I hydrophobin, EAS from *Neurospora crassa*, for which the structure has been elucidated. These two proteins have been found to form monolayers with the same morphology, and are known to fulfill similar roles *in vivo*; however, their sequences and structures are very different. We are now studying the assembled forms of these proteins using magic-angle spinning solid-state NMR in order to understand the structural conversions that take place in this family of unique proteins.

So677

'Prepared orientation' of tandem domains in HMGB2 revealed by DIORITE

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HMGB2 protein consists of tandem HMG-boxes that function as DNA binding domains. These two domains are linked by a short linker. We found the inter-domain linker has specific contact to the N-terminal HMG-box *via* CH-π interactions. Although the inter-domain linker is essentially flexible as characterized by low ^{15}N {^{1}H}NOEs, we found through the NMR analyses on weakly-aligned samples that the CH-π interactions define a preferential relative domain orientation of the tandem boxes as a dynamic average. The tandem domains linked by the short linker, therefore, should have '*prepared orientation*'; in the *prepared* domain orientation, the DNA binding surfaces of the HMG-boxes face to the same side to prompt their cooperative DNA binding.

The mutant losing the CH-π interactions has shown the different domain orientation relative to the wild-type, in which the DNA binding surfaces of the boxes face differently to each other. The DNA bending activity of the mutant, which was assayed by the DNA circularization, significantly reduced from that of the wild-type. Overall, present work has revealed that the inter-domain linker in HMGB2 has a role to make '*functionally prepared domain orientation*', even though the orientation is not defined in static but in a dynamic averaging.

As a technical aspect of this work, because of the highly anisotropic shape of HMGB2, our devised DIORITE[1] (Determination of the Induced ORIentation by Trosy Experiments) was essential in determining the relative domain orientation. This will be also described in the presentation.

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So678

Ras Homolog Enriched in Brain (Rheb) Enhances Apoptotic Signaling

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Rheb is a homolog of Ras GTPase that regulates cell growth, proliferation, and regeneration via mammalian target of rapamycin (mTOR). We found that overexpression of lipid-anchored Rheb enhanced the apoptotic effects induced by UV light, TNFα, or tunicamycin in an mTOR complex 1 (mTORC1)-dependent manner. Knocking down endogenous Rheb or applying rapamycin led to partial protection, identifying Rheb as a mediator of cell death. Ras and c-Raf kinase opposed the apoptotic effects induced byUV light orTNFα but did not prevent Rheb-mediated apoptosis. We have determined the structure of Rheb-GDP by NMR spectroscopy. The complex adopts the typical canonical fold of Ras GTPases and displays the characteristic GDP-dependent picosecond to nanosecond backbone dynamics of the switch I and switch II regions. NMR spectroscopy revealed Ras effector-like binding of activated Rheb to the c-Raf-Ras-binding domain (RBD), but the affinity was 1000-fold lower than the Ras/RBD interaction, suggesting a lack of functional interaction. shRNA-mediated knockdown of apoptosis signal-regulating kinase 1 (ASK-1) strongly reduced UV orTNFα-induced apoptosis and suppressed enhancement by Rheb overexpression. Pharmacological regulation of the Rheb/mTORC1 pathway using rapamycin should take the presence of cellular stress into consideration, as this may have clinical implications.

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So679

Different DNA Polymerase Conformations When Encountering Matched, Mismatched, and Abasic Template Sites

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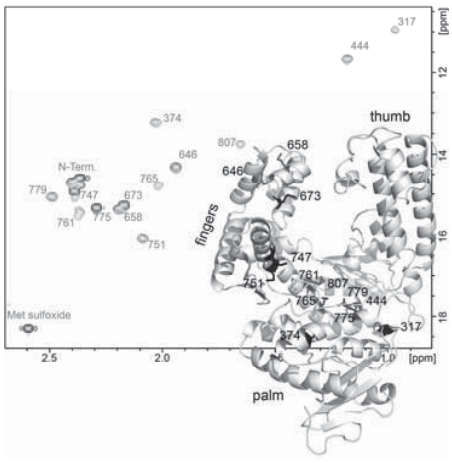

The intrinsic accuracy of DNA polymerases is key to maintaining correct genome replication. Discrimination between correct and incorrect nucleotides is believed to be based on a series of conformational alterations leading from a conformation with an open, solvent exposed active site to a closed conformation triggered upon nucleotide binding. To investigate conformational changes of the KlenTaq DNA polymerase we introduced 13 spin probes using [methyl-^{13}C]methionine. Following the chemical shift changes of these labels allowed us to monitor primer/template complex and nucleoside triphosphate binding as clearly distinguishable events. Mismatched complexes were found to adopt unique conformations for incorrect base pair formation that are clearly distinct from the match case and are rather related to the binary, open complex. Similar results were obtained when we investigated incorporation opposite an abasic site template. Our observations suggest that in non-canonical cases the transition from an open complex into a closed, productive complex is hampered explaining the significantly reduced catalytic efficiencies for catalyzing non-canonical nucleobase pairs.

So680

Mechanism of Multivalent Carbohydrate-Protein Interactions Studied by EPR Spectroscopy

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The inhibition of carbohydrate-protein interactions by tailored divalent ligands is a powerful strategy for the treatment of many human diseases.

A series of spin labeled divalent N-Acetylglucosamine (GlcNAc) derivatives were synthesized and the interaction with plant lectin wheat germ agglutinin (WGA) [1] was studied with cw- and pulsed two-frequency EPR-spectroscopy.

Chelating binding of the divalent ligand bridging adjacent binding sites can be directly distinguished from monovalent binding of other ligands with a linker too short to bridge binding sites. In addition, analysis of intermolecular spin interactions between different ligands bound to the same multivalent protein provides hints which of the eight proposed carbohydrate binding sites are preferentially occupied.

In summary, experimental data show a detailed picture of the molecular binding mechanism of these mono- and divalent ligands to WGA and for the first time structural evidence of multivalent protein-ligand interaction in solution can be observed.[2]

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So681

NMR characterization of hPEBP bindin with locostatin and analogs

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The Phosphatidyl Ethanolamine Binding Protein (PEBP) family of proteins is widely distributed, from bacteria to mammals. In mammals, PEBPs have been described to modulate important cell mechanisms, such as the control of heterotrimeric G-proteins, the inhibition of Mitogen-actived protein-kinase . Human PEBP affects various cellular processes and is implicated in metastasis formation and Alzheimer's disease.

Locostatin is the only known ligand of PEBP with an effect on its activity. Locostatin was found in a chemical genetics screen in a cell migration assay, and PEBP identified as the relevant cellular target.

Locostatin is a michael acceptor for which the nuclophilic reactant on the protein is unknown.

We have used 13C labelled locostatin and the combination of HCCH tocsy and 13C HSQC to identify the different locostatin addition products.15N labelled protein has been used to identify the locostatin and locostatin analogs binding sites.

So682

Conformational consequences of binding of aggregation inhibitors to monomeric tau

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In many neurodegenerative disorders wrongly folded species form abnormal deposits in the brain. In this study we focus on the intrinsically disordered protein Tau (1), that is one of the main agents associated with Alzheimer disease. The physiological role of Tau is the stabilization and regulation of microtubules and the support of the outgrowth of axons (2,3). In Alzheimer disease an extensively phosphorylated Tau no longer binds to microtubules and aggregates into intracellular neurofibrially tangles. There is great interest to find small molecules that can inhibit tau aggregation and to understand their mechanism of action. Here we probed the impact of a selected Tau aggregation inhibitor on the conformational sampling of the Tau backbone using residual dipolar couplings. Quantitative analysis of $N\text{-}H^N$, Cα-Hα, CO-Cα, $CO\text{-}H^N$ RDCs provided first insights into its mechanism of action.

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So683

Searching for low molecular weight ligands stabilizing membrane proteins fold – aid in structural biology studies by means of NMR

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Structure determination of membrane proteins using X-ray crystallography and NMR is often complicated by the intrinsic dynamics of membrane proteins. At the same time, high-affinity ligands might stabilize the membrane protein fold upon binding and thus allow structure determination of the resulting complex. A biologically highly important membrane protein, for which the high-resolution structure is not yet known due to its dynamic nature, is the mitochondrial 18 kDa translocator protein (1,2). Here we demonstrate that the tertiary fold of the translocator protein might be stabilized by selected natural and synthetic ligands such as cholesterol, etifoxin and PK11195 (1) as monitored by solution-state NMR. On the basis of 2D [^{1}H,^{15}N] HSQC spectra of ^{15}N-labelled translocator protein in the presence of ligands we estimate theirs suitability for structural studies by means of solution NMR techniques.

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So684

NMR-compartible expression tag provides new perspectives in the study of proteins and their interactions.

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Expression, isolation and purification of the peptides and proteins are the crucial initial steps in the studies of their structural and functional organization. In the last years many reports showed the progress in efficient expression and isolation of peptides and proteins using fusion technology with various expression and solubility tags – GST, NusA, SUMO, ubiquitin, GB, etc. However, most biophysical applications require to remove the tag and to operate with the highly purified target peptides and proteins. Therefore, benefits of the well expressed authentic polypeptides within fusion construct could be lost after the rigorous procedures of its purification. Especially it is a main concern in the case of short peptides. In order to overcome this unfavorable situation, we have engineered an expression vector, possessing an N-terminal modified Ub tag for effective expression of fused peptides and proteins, an internal His(6) tag, and the TEV cleavage site. Using the new vector, we have expressed in *E.coli* more than 10 different targets (peptides and proteins) with an increased yield and used them later in our NMR, CD and ITC studies with and without TEV protease cleavage and following isolation of the target polypeptides. We have found that the fusion constructs are in all cases identical in biophysical properties to the isolated and purified original peptides and proteins. This observation opens an avenue for the complete, fast and efficient characterization of peptides/proteins structures, intermolecular interactions and functional features.

So685

Investigation of HSA binding to skin sensitisers by STD-NMR

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Human Serum Albumin (HSA) is used as a tool to investigate protein haptenation mechanisms, particularly by skin allergens[1], since approximately 40% of extravascular HSA is located in the skin. Aldehydes react reversibly with nitrogen bionucleophiles (e.g. lysines) yielding a Schiff base covalent adduct[2] which can be at the genesis of adverse allergenic reactions promoted by this class of compounds. In this work we report the determination of dissociation constants of two very weak skin sensitisers: benzaldehyde (I) and vanillin (II), and of two moderate skin sensitisers: cinnamaldehyde (III) and phenylacetaldehyde (IV) with HSA protein using the STD method[3]. STD titration with the two food flavouring aldehydes (I) and (II) yielded K_D 21870μM and K_D 9512μM. Similarly, for aldehydes (III) and (IV) we got K_D 7826μM and K_D 76256μM, respectively. Our results indicated that STD spectroscopy can be applied to the measurement of Schiff base and Michael addition aldehyde dissociation constants with HSA and that a clear relationship between the protein reactivity pkD, the aldehyde hydrophilicity logP(octanol/water) and the allergenic potency of each aldehyde log1/EC3 can be drawn.

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So686

NMR Structure of Protein NP_888769.1, a Phage-Related Protein in the *Bordetella bronchiseptica* Genome

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The NP_888769.1 is hypothetical phage-related protein, isolated from Gram-negative bacterium *Bordetella bronchoseptica*. As a result of our work, the complete three-dimensional dynamic structure of the first member of this novel protein family PF13554 of unknown structures was determined by using automated NMR protocol, which include APSY-NMR experiments and the software UNIO for the backbone and side chain assignments. CYANA was used for the structure calculation of NP_888769.1. The protein showed a well-structured core comprising a five-stranded, antiparallel β-sheet packed on one side against two α-helices and a short β-hairpin with three flexibly disordered loops extending from the central β-sheet. The protein was functionally annotated as Mg^{++} binding protein using NMR experiments. The homolog of Np_888769.1, identified by software DALI with Z-scores > 8, was found to be oligomerized with the 40 mM Mg++, while No_888769.1 did not show oligomerization even at 200 mM Mg^{++}. A sequence-homology analysis confirmed that NP_888769.1 represents the first three-dimensional structural representative of a new protein family, which includes so far about 20 prophage proteins encoded in bacterial genomes.

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So687

The oxidative protein folding pathway in mitochondria by NMR

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The oxidative protein folding in the mitochondrial intermembrane space requires the transfer of a disulfide from MIA40 to the substrate. During this process MIA40[1] is reduced and regenerated to a functional state following interaction with the flavin-dependent sulfhydryl oxidase ALR. We describe, for the first time at the molecular level, the folding process of a MIA40-substrate, COX17, starting from its unfolded state through all of the intermediate steps[2]. We found that COX17 is largely unfolded in the cytoplasm and that MIA40 in the IMS induces a conformational transition in a specific targeting region of the protein, from an unstructured to an α-helical state, upon the formation of an intermolecular disulphide bonded COX17-MIA40 complex.

The molecular basis of ALR-MIA40 interaction that regenerates MIA40 in its functional state has been characterized at atomic resolution by biochemical and structural analyses of the mitochondrial ALR isoform and its covalent mixed disulfide intermediate with MIA40[3].

We found that the hydrophobicity-driven binding in both cases ensures precise protein–protein recognition needed for an efficient electron transfer process.

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So688

The DNA binding RecQL4 N-terminal domain

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RecQ helicases (BLM, WRN, RecQL4) are essential for the maintenance of genomic integrity and their dysfunction in DNA replication results in defined syndromes such as cancer predisposition and premature ageing. Mutations in the human RecQL4 gene cause the Rothmund-Thomson, the RAPADILINO and the Baller-Gerold syndromes[1]. Moreover, it was reported that RecQL4 is involved in prostate carcinogenesis[2]. Recently, we have determined and refined the solution structure of a functional part of the N-terminal domain of RecQL4 in *E. coli* (PDB code: 2KMU, r.m.s.d. 0.68 Å), which is essential for viability in mouse models. Despite low sequence homology it exhibits a surprising structural similarity to homeodomain proteins but lacks their archetypical minor groove binding N-terminal extension. Our NMR and binding data indicate a non–sequence specific DNA–binding similar to homeodomains with a major contribution of helix α3. We also observe a higher affinity and involvement of a subset of Arg residues in binding of Y-shaped DNA as compared to regular dsDNA.

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So689

NMR Analysis on the Interactions between a Mitochondrial Oxidative Translocator Tim40/Mia40 and a FAD-Linked Sulfhydryl Oxidase Erv1

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An oxidative folding system has been recently discovered in the mitochondrial inter membrane space (IMS). Most mitochondrial IMS proteins are synthesized in the cytosol as a precursor protein and imported into mitochondria. Tim40/Mia40 introduces a disulfide bond into these precursor proteins as a substrate by forming a mixed disulfide-bond intermediate, which allows their oxidative folding. Erv1 is a FAD-linked sulfhydryl oxidase and specifically oxidizes reduced Tim40. We have succeed in determination of the crystal structure of the "Tim40-substrate complex" as a Tim40 fusion protein with a substrate peptide at N-terminus (MSP1-Tim40). Although high-resolution structures of Tim40[1-3] and Erv1[4] are available, little is known about the recognition and interactions between Tim40 and Erv1. Here we report the interaction analysis for Tim40 and Erv1 using PRE-NMR.

We prepared spin-labeled Erv1 (Erv1-MTSL ((1-oxyl-2,2,5,5-tetramethyl-3-pyrroline-3-methyl)-methanesulfonate)) and analyzed its interactions with Tim40 and MSP1-Tim40. When we added Erv1-MTSL to ^{15}N-labeled Tim40, its HSQC spectra with or without ascorbic acid were only slightly affected. In contrast, when we added Erv1-MTSL to ^{15}N MSP1-Tim40, we observed significant changes in its HSQC spectra with or without ascorbic acid. In particular, signals from MSP1 and its nearby region of Tim40 were affected by Erv1-MTSL. These results suggest that Erv1 binds to the substrate and/or substrate-binding region of Tim40 and the substrate bound to Tim40 may enhance the interaction between Erv1 and Tim40.

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So690

Metal chaperons and their metal binding sites

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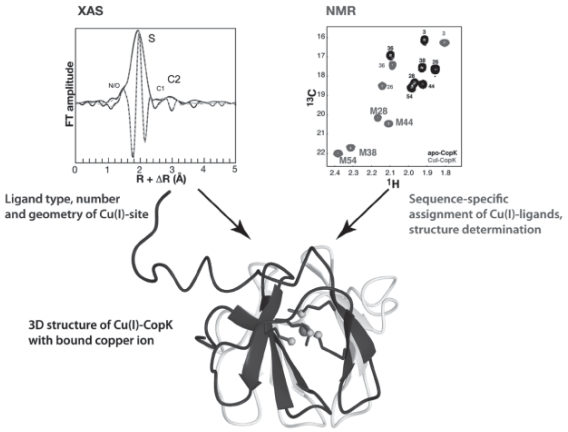
ABSTRACT NMR is a powerful technique for the study of molecular complexes. We are interested in metal proteins and the chosen examples will illustrate identification of Cu^{+}, Ag^{+}, and Zn^{2+} binding sites in metal chaperons using mainly NMR data. In addition, other spectroscopic techniques as for example X-ray absorption or Electron Paramagnetic Resonance spectroscopy can provide geometric information on the ligand sphere that complete distance information from NMR experiments. We will also report on the conformational changes proteins undergo upon metal binding.



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So691

From Proteins To Cancer: Structure and Interaction of the HPV Oncoprotein E6

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High-Risk Human Papilloma Virus (HR HPV) infections of basal epithelial cells of the cervix cause cervical cancer. The oncoproteins E6 and E7 are responsible for inducing and maintaining the malignant phenotype by reprogramming host cells through interaction with a variety of key regulatory cellular proteins.

Here, we present the solution structure of the C-terminal domain of the HR HPV51 E6 (RMSD 0.55 Å). Also, we have addressed the interaction of E6 with hDlg/SAP-97. This interaction is thought to induce E6 mediated degradation of hDlg, which in turn contributes to progression from G0/G1 to S phase and to subversion of cell polarity, thereby possibly promoting metastasis.

A method for the production of ^{13}C- and /or ^{15}N-labelled peptides without undesired residues was developed and exploited for the generation of an E6-derived peptide. A full structural analysis of a complex consisting of that peptide bound to hDlg PDZ domain 2 is ongoing. It already became clear that more E6 residues than anticipated contribute to complex formation and significantly enhance the affinity of the E6-hDlg PDZ2 interaction.

So692

Structural organisation of the box C/D s(no)RNP complex in solution

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Post-transcriptional RNA modifications in eukaryots are carried by RNA-protein complexes, where the specificity is ensured by small guide RNAs.

Our research is focused on the 2'-OH methylation of RNA which is mediated by the box C/D sRNP complex. The archaeal sRNP complex is constituted by three different proteins – L7Ae, which recognizes the box C/D RNA motif, Nop5 and Fibrillarin, which is a SAM-dependent methyltransferase. Proteins assemble around the methylation guide RNA containing two similar conserved motifs: box C/D and box C'/D'. The guide RNA in sRNP forms base pairs with complementary target RNAs and selects the methylation site.

In the past year two groups presented two different structures-models of the box C/D sRNP complex. In the classical mono-RNP model, the catalytically active complex is constituted by two copies of each protein assembled around one molecule of guide RNA (~200 kDa) [1], whereas in the di-RNP model the complex is constituted by four copies of each protein and two copies of guide RNA (~ 400 kDa) [2].

We aim at solving the structure and the mechanism of the box C/D sRNP complex **in solution** by using a combination of solution state NMR, SANS and molecular modeling.

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So693

Influence of endogenic post-translational *S*-nitrosylation on human S100A1 protein structure under physiological conditions

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S100A1 is a small EF-hand containing Ca^{2+}-binding protein belonging to S100 protein family. It exists as a homodimer. The molecular basis of S100A1 protein regulation is not fully understood. *In vitro* experiments on Ca^{2+} binding show a significant decrease in binding affinity in buffers with close to physiological ionic strength in comparison to low ionic strength buffers. Previous studies demonstrate that post-translational modifications of unique Cys85 result in increase of Ca^{2+} affinity and notable structural changes of S100A1 protein[1-3]. Estimation of changes induced by *S*-nitrosylation, predominant modification of conserved cysteine, is thus important for understanding the mode of cellular signal transduction mediated by nitric oxide. Therefore, we determined the 3D structure of human *apo*-S100A1 protein with C-terminal *S*-nitroso Cys85 (*apo*-S100A1-*NO*) and compared this structure to that of *apo*-S100A1 protein at the same conditions. *S*-nitrosylation provided structural differences which could explain higher Ca^{2+} affinity. Also, we have noticed some features which imply that *S*-nitrosylation can stimulate Ca^{2+}-independent interactions with target proteins as well. The obtained results suggest that *S*-nitrosylation can regulate the activity of an important subclass of S100 proteins including S100A1.

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So694

Structure of an ACP domain and insights into the molecular basis for interaction specificity with the cognate halogenase

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Polyketide (PK)- and non-ribosomal peptide (NRP)- synthetases are large multidomain proteins present in microorganisms that produce bioactive compounds. Curacin A is such a bioactive compound with anti- proliverative activity. In this work we investigate a triplett ACP $T_{I,II,III}$ present at the C-terminus of CurA. We show that the ACPs are independent of each other even upon dimerization and report the high resolution nuclear magnetic resonance (NMR) structure of the isolated TI ACP loaded with the unlabelled substrate 3-hydroxy-3-methylglutaryl (HMG). Recently, the role, timing and structure of the curacin halogenase (Cur Hal) could be established in the biosynthesis of curacin A[1,2]. We identified the interaction surface important for ACP recognition by the halogenase Hal using mutational analysis. These investigations give a new insight into the molecular basis modulating and regulating the specificity of the interaction between two domains of this important mega- synthase.

So695

NMR-spectroscopic Investigations of Protein Phosphorylation

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Phosphorylation of biomolecules is one of the most important reactions within living cells. Nearly all biochemical pathways are more or less affected by alterations of protein structure and activity due to site-specific phosphorylation of proteins. Therefore, ^{31}P-NMR is an interesting tool to investigate reaction rate constants of kinases and phosphatases, since there is no need for isotopic labeling. Unfortunately, one-dimensional ^{31}P-NMR is not able to identify phosphorylation sites and suffers from signal overlap if several phosphorylation sites appear.

To overcome these disadvantages, a new ^{1}H, ^{13}C-detected 2D triple resonance NMR pulse program was developed, which is able to discriminate phosphorylated and non-phosphorylated amino acids.[1] The aim of our work is to show the applicability of this pulse program to ^{13}C-labeled proteins in order to reduce singal overlap and allow kinetic investigations of protein phosphorylation. Several promising model system candidates, like Calmodulin or the peptidylprolyl *cis*/*trans* isomerase Pin1, are under investigation.

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So696

Characterization of the interaction of GABARAPL-1 with the LIR motif of NBR1

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Selective autophagy requires the specific segregation of targeted proteins into autophagosomes. The selectivity is mediated by autophagy receptors, such as p62 and NBR1, which can bind to autophagic effector proteins (Atg8 in yeast, MAP1LC3 protein family in mammals) anchored in the membrane of autophagosomes. Recognition of autophagy receptors by autophagy effectors takes place through an LC3 interaction region (LIR). The canonical LIR motif consists of a WXXL sequence, N-terminally preceded by negatively charged residues. The LIR motif of NBR1 presents differences to this classical LIR motif with a tyrosine residue and an isoleucine residue substituting the tryptophan residue and the leucine residue, respectively. We have determined the structure of the GABARAPL-1/NBR1-LIR complex and studied the influence of the different residues belonging to the LIR motif for the interaction with several mammalian autophagy modifiers (LC3B and GABARAPL-1). Our results indicate that the presence of a tryptophan residue in the LIR motif increases the binding affinity. Substitution by other aromatic amino acids or increasing the number of negatively charged residues at the N-terminus of the LIR motif, however, has little effect on the binding affinity due to enthalpy-entropy compensation. This indicates that different LIRs can interact with autophagy modifiers with unique binding properties.

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So697

Structural Analysis of phosphorylated Complex of Cardiac Troponin C-Troponin I by Double Quantum Coherence-EPR

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The contraction and relaxation of cardiac muscle is regulated by a response of intracellular Ca^{2+} ion concentration and phosphorylation. The switch between muscle contraction and relaxation occurs with a structural change in cardiac troponin (cTn). The details changes in a molecular level have not been made clear to understand the regulatory mechanism. cTn consists of three subunits; cTnC, cTnI and cTnT. We chose the cardiac TnC-TnI (cTnC-TnI) complex and used double quantum coherence (DQC)-EPR technique to measure the difference of distances between N and C-terminal domains of cTnC in non-phosphorylated and phosphorylated cTnC-TnI complexes.

In this paper, the distance between two nitroxide labels in the cTnC-TnI complex was measured by using a Ku-band (17.5 GHz) pulsed EPR spectrometer. We evaluate the mean distance between spin labels and its distribution by using the Tikhonov Regularization method. We found that the structural change of cTnC with Ca^{2+} binding in the phosphorylated complex is slightly smaller than that in non-phosphorylated. It is known that phosphorylation reduces the Ca^{2+} binding affinity in the N-terminal domain of cTnC with disassociation of the N-terminal region of cTnI from the N-terminal domain of cTnC[1]. Our results show that the structural change of cTnC is regulated by binding between the N-terminal region of cTnI and the N-terminal domain of cTnC. We will discuss the details of these structural changes of the cTnC-TnI complexes.

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So698

Aprataxin: a recovery enzyme for single strand break repair

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Genetic defects in the repair of DNA damage are implicated in a number of diseases, which include neurological dysfunction and cancer. Single strand breaks (SSBs) are the most frequent type of damage [1] (>10.000 per cell and day). Inefficient repair of SSBs in postmitotic cells such as neurons may lead to neurodegenerative diseases, e.g. ataxia oculomotor apraxia (AOA1). AOA1 is linked to mutations in aprataxin (APTX), which functions in concert with PARP, XRCC1 and PNKP during SSB repair by removing obstructive adenylate 5'-ends [2]. APTX comprises two structural domains: an N-terminal XRCC1–binding FHA domain and a C-terminal catalytically active HIT domain including a Zn–finger. We established recombinant expression, purification for both domains and the *in vitro* production of the natural substrate (rAppDNA). The HIT domain alone is sufficient for multi-turnover deadenylation catalysis, indicating that the structure of this domain will be of functional significance. We will present the current status of the ongoing solution state NMR-based structure determination using reverse labelling as well as perdeuteration.

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So699

The HPV E4 protein: characterisation of an aggregating protein

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Human papilomavirus (HPV) infect stratified epithelia and cause lesions that range in severity from benign warts to invasive carcinomas. HPV E4 is an abundant, predominantly cytoplasmic protein, that accumulates to very high levels in upper epithelial layers [1]. It is suggested that E4 accumulation plays an important role in the HPV life cycle by supporting virus release *via* inducing cytoskeletal changes and degradation. A common hallmark of E4 from different HPV types is that it forms supramolecular aggregates in infected cells. Aggregation appears to be intimately linked to the cytoskeleton compromising activity of E4. To form higher order complexes the C-terminal part of E4 is required. However, there seem to be significant differences in the morphology of such aggregates depending upon the HPV type. *E.g.*, E4 from HPV-16 is reported to form amyloid fibre-like aggregates [2] whereas E4 derived from HPV-1a appears to form more globular assemblies [3]. To assess the structural properties of E4, several mutants were generated, purified and characterised by CD spectroscopy, electronmicroscopy and NMR. The oligomeric high molecular weight nature of HPV-1a E4 protein in solution allowed only for the assignment of some N-terminal flexible residues by liquid-state NMR. Hence, solid-state NMR analysis appears necessary and initial spectra indicate a good signal/noise for E4 oligomeric preparations, which, however, require further efforts to optimise conditions.

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So700

Effect of Mg^{2+} Binding on the Receiver Domain of the CKI1 Sensory Histidine Kinase from *Arabidopsis thaliana*

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In *Arabidopsis thaliana*, cytokinin signal transduction is mediated by a two-component phosphorelay system, which consists of a phosphate transfer from a sensor histidine-kinase, to a histidine-phosphotransfer protein, and then to a response regulator.

The sensory histidine kinase consists of a sensory extracellular domain which binds the ligand and leads to autophosphorylation of the intracellular histidine kinase (HK) domain. The phosphate is then transfered to the active site aspartate in the receiver domain.

We studied the effect of magnesium, a cofactor for HK-mediated phosphorelay, on the function of the receiver domain by NMR. Assignment of the receiver domain was obtained for 91 % of the residues [1]. The missing assignment corresponds to the loop L3, following the aspartic acid in the active site. Upon addition of magnesium, new peaks appear that could be assigned to loop L3, stabilized upon addition of Mg^{2+}, using amino-acid selective isotope labeling.

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So701

Modulation of the activity of the hybrid antibiotic peptide Cecropin A - Melittin [CA(1-7)M(2-9)] by residue modification

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The manifest increase of bacterial resistance against traditional antibiotics has stimulated the design of novel antimicrobials acting on non-conventional targets. Thus, antimicrobial peptides (AMPs) of eukaryotic origin represent a promising alternative.[1] Moreover, hybrid peptides consisting of fragments of natural AMPs as cecropin A (CA) and melittin (M) exhibit substantial improvement in their antibacterial, antiparasitic and antifungal activities compared with their parent structures.[2]

Herein, a NMR based structural study of several linear pentadecapeptides derived from the cecropin A- melittin antimicrobial peptide CA(1-7)M(2-9) [KWKLFKKIGAVLKVL-NH_2] is presented. These analogues are modified by either ε-NH_2 trimethylation of lysines or acylation with saturated linear fatty acids at the N-terminus and showed variation in both antimicrobial and cytotoxic activities. We employed membrane mimetic media for the NMR experiments and paramagnetic probes to explore the mode of interaction of the peptides with prokaryotic membranes.

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So702

The alpha-defensin HNP2 specifically binds N-acetyl-neuraminic acid containing oligosaccharides - an NMR, SPR and molecular docking study

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In this study we have analyzed the carbohydrate specificity of the alpha-defensin HNP2 and related peptides with Nuclear Magnetic Resonance (NMR), Surface Plasmon Resonance (SPR) and molecular modeling methods in order to correlate the defensin structure with its ability to bind certain carbohydrate moieties. In addition to the HNP2 wild-type, a synthesized HNP2 mutant in which residues Tyr2 and Tyr20 are replaced by alanine was tested. Furthermore, the carbohydrate binding behavior of a mixture of HNP2 and the structurally similar defensins HNP1 and HNP3 from a clinical source was analyzed with SPR methods. The results clearly identify which functional groups of N-acetylmuramic and N-acetylneuraminic acid are necessary to establish specific interactions with essential amino acids in the carbohydrate binding pocket of the HNP-defensins under study (Ref. 1 - 3).

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So703

Molecular organization of different collagen hydrolysates and collagen fragments as revealed by a combination of Atomic Force Microscopy (AFM) and Diffusion Ordered NMR Spectroscopy (DOSY)

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ABSTRACT: Collagen hydrolysates are heterogeneous mixtures of collagen-like proteins or peptides with a high potential as nutrition supplement and agents for ointments with therapeutic relevance in wound healing. Since the precise composition of collagen hydrolysates is generally unknown it is of interest to analyze such samples with appropriate biophysical methods. Any product optimization without this knowledge is nearly impossible. It turned out that a combination of AFM methods with NMR techniques is extremely suited to scrutinize the size-range and the aggregation behavior of collagen hydrolysates from fish, jellyfish, chicken, pig and bovine origin. Such information is an essential prerequisite for a detailed study concerning the molecular basis of the bio-medical effectiveness of collagen hydrolysates as well as certain collagen fragments of defined mass. References please find in: http://geb.uni-giessen.de/geb/volltexte/2011/8119/

So704

Structural Basics of Interleukin-10 – Glycosaminoglycan Interactions

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The protein Interleukin (IL)-10 is a key regulator of the innate and adaptive immune system, which prevents an overwhelming immune reaction and tissue damage. IL-10 inhibits the synthesis of pro-inflammatory cytokines and of cell surface molecules. Thereby, cellular immune responses mediated by macrophages and T cells are inactivated[1].

There exists strong evidence that IL-10 acts over short distances *in vivo*[2]. Glycosaminoglycans (GAGs) of the extracellular matrix have been suggested as possible binding partners that restrict IL-10 to the vicinity of the secreting cell. GAGs are a class of highly sulfated carbohydrate molecules that are known to bind and regulate a number of distinct proteins[3]. Sequence similarity to other GAG interacting proteins and molecular docking calculations strongly predict a specific GAG binding site within IL-10, in particular a cluster of basic amino acid residues in helix D and the DE loop.

A His-tagged version of mouse IL-10 was recombinantly expressed in *E.coli* and was purified and refolded from inclusion bodies. One dimensional ^{1}H NMR and CD spectroscopy demonstrated that the protein is well-folded and has a significant α-helix content of 66%. Future studies will include the full isotopic labelling of IL-10 for resonance assignment and GAG titration experiments, which will help to unravel the GAG binding site in IL-10 and the structural basics of that interaction.

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So705

Structure-Function relationship of the Sterol Carrier Protein from the yeast *Yarrowia lipolytica* (YLSCP2)

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The sterol carrier proteins (SCP2) are small soluble intracellular proteins highly conserved in all biological kingdoms. The SCP2 spectrum of ligands is broad, including fatty acids, acyl-CoAs, sterols and phospholipids. The L-shaped ligand binding site shows a high conformational plasticity, which could be related to the binding mechanism [1]. Our aim is to study the structure-function relationship of YLSCP2, a yeast member of the sterol carrier protein family [2].

The effect of ligand binding on the structure and stability of the protein was evaluated by several techniques (CD, fluorescence, SAXS, and thermal unfolding). The stability of YLSCP2 decreases after *cis*-parinaric acid and ANS binding. The secondary structure content of YSCP2 decreases with palmitic acid and cholesterol binding. The intrinsic fluorescence decreases with palmitoyl-CoA.

The differential effects of ligand binding on YLSCP2 structure and stability could be correlated with the conformational plasticity of the binding site. To elucidate the ligand binding mechanism we are planning to perform NMR experiments, in order to identify the residues that define the binding site for each ligand.

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So706

Molecular Mimicry-Based Repositioning of Nutlin-3 to Anti-Apoptotic Bcl-2 Family Proteins

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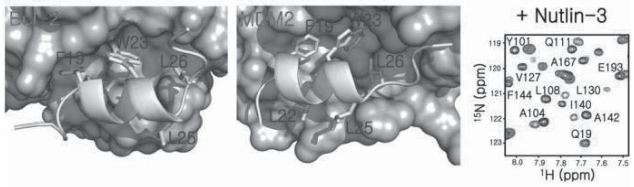
Drug repositioning has been an emerging strategy in drug discovery as understanding of novel usages for existing drugs could provide insightful information into molecular mechanism of drugs and safety of drugs[1]. The identification of off-target binding of drugs is a key to repositioning drugs to new therapeutic categories. Here we show the universal interactions of the p53 transactivation domain (p53TAD) with various anti-apoptotic Bcl-2 family proteins via a mouse double minute 2 (MDM2) binding motif, which play an important role in transcription-independent apoptotic pathways of p53. Interestingly, our structural studies reveal that the anti-apoptotic Bcl-2 family proteins and MDM2 share a similar mode of interaction with the p53TAD. On the basis of this close molecular mimicry, our NMR results demonstrate that the potent MDM2 antagonist Nutlin-3[2] bind to the anti-apoptotic Bcl-2 family proteins in a manner analogous to that with the p53TAD.



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So707

Characterization of the assocation of the FATC domain of TOR with membrane-mimetic systems

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Today, many studies focus on the kinase target of rapamycin (TOR) as a therapeutic target for diseases such as cancer, autoimmunity, and metabolic disorders. TOR is a structurally and functionally conserved serine/threonine kinase that plays a key role in a signaling network for the control of cell growth and proliferation in yeast and higher eukaryotes. The highly conserved C-terminal FATC domain contains 33 amino acids and was shown to be essential for the regulation of TOR function. The solution NMR structure of the FATC domain of yeast TOR1 (y1fatc) features an α-helix and a disulfide-bonded loop. Furthermore, a mutagenesis study in yeast cells illustrated that the cellular stability of TOR is depending on the redox state of the disulfide bond of the FATC domain (1). A solution NMR spectroscopy study that probed the association of y1fatc with membrane-mimetics delineated a lipid binding motif consisting of a hydrophobic bulb that has a rim of charged residues. Whereas the aromatic and aliphatic residues of the membrane anchor can interact with the fatty acid moieties of DPC, the polar residues can interact with the charged head groups (2).

Using oriented circular dichroism spectroscopy the orientation of y1fatc in small unilamellar vesicles could be characterized more precisely. In order to elucidate the association of y1fatc with membrane-mimetic systems a set of mutations was generated and investigated by solution NMR and circular dichroism.

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So708

Regulation of the bacterial phosphotransferase system by Mlc and glucokinase

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The global repressor Mlc is involved in the regulation of several metabolism processes like the regulation of maltose, mannose and glucose metabolism. In contrast to other repressors the activity of Mlc is controlled by the activity of the phosphotransferase system (PtsG).

While the PtsG is actively transporting glucose Mlc remains bound to the dephosphorylated IIB^{Glc}-domain and thus becomes inactive. The mechanism of inactivation and whether a conformational change occurs upon binding is still largely unkown. The comparison of the crystal structure of the repressor Mlc and the glucokinase Glk reveals great similarities. Overexpressed Glk binds to dephosphorylated IIB^{Glc}-domain and thus releases Mlc into the cytoplasm.

The goal of this project is to characterize the binding of Mlc and Glk to the IIB^{Glc}-domain of PtsG by different biophysical methods. Glk is a homodimer forming 35 kDa protein from E. coli. Protocols for the expression and purification of isotope labelled Glk and IIB^{Glc} in minimal medium were established and samples with sufficient concentration for NMR-experiments were obtained. First HSQC and TROSY-HSQC experiments revealed that both proteins are folded and well suited for NMR spectroscopy. Chemical shift pertubation experiments and NMR titrations with spin labelled Glk indicated a transient interaction between the IIB^{Glc}-domain and the glucokinase.

In order to disclose the nature of the binding of Mlc and Glk to IIB^{Glc} surface plasmon resonance (SPR) and isothermal calorimetry (ITC) experiments were performed. These experiments revealed that tetrameric Mlc binds with high affinity to IIB^{Glc} in a multivalent fashion whereas Glk showed only weak binding to immobilized IIB^{Glc}.

So709

Structure of SpaI: The protein conferring autoimmunity against the lantibiotic subtilin in *Bacillus subtilis*

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The careless use of many antibiotics in the past lead to an emerging resistance even against 'last resort' drugs such as vancomycin. Thus, there is an urgent need for structurally novel antimicrobial agents. Lantibiotics are small ribosomally synthesized peptide antibiotics with posttranslational modified amino acids resulting in the characteristic lanthionine and methyllanthionine bridges.

Bacillus subtilis ATCC 6633 produces the lantibiotic subtilin which damages the cell wall of gram-positive bacteria. SpaI is a 16.8 kDa lipoprotein which is part of the self-protection system of *B. subtilis* against subtilin. It is attached to the outside of the cytoplasmic membrane via a covalent diacylglycerol anchor. Together with the ABC-transporter SpaFEG SpaI protects the membrane from subtilin insertion and presumably it interacts directly with subtilin.

Here we present the solution NMR structure of a 15 kDa biologically active fragment of SpaI. Our data show that SpaI has a mainly β-strand structure with seven β-strands and two α-helices[1]. $^{15}N\{^1H\}$ heteronuclear NOE and H/D exchange data suggest a flexible loop between strands β3 β4 which might insert into the membrane. So far no structural information for any LanI (lanthionine immunity) protein of lantibiotic producing strains is available and a search in the DALI database showed no homologues structures to our structure indicating a new fold for SpaI.

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So710

Expression and characterisation of the fibronectin-III-like-domain-9 of type VII collagen

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Dermis and basal lamina of the skin are connected through anchoring fibrils with type VII collagen (Col7) as major component. In the autoimmune disease epidermolysis bullosa acquisita IgG antibodies bind to Col7 that is no longer able to retain its function. Contact and friction lead to blistering of the skin. FNIII-9 is one of nine fibronectin-like domains in the 145 kDa non-collagenous part of collagen VII. Previous experiments showed an interaction of FNIII-9 and its neighbouring domain, the von-Willebrand-factor-A-like domain 2.[1]

To obtain insight into the quaternary structure of Col7 FNIII-9 was expressed and purified following the IMPACT-TWIN protocol. 3D NMR spectra were obtained and the backbone resonance assignment is to 97 % complete. Additional heteronuclear NOE experiments were performed. Secondary structure prediction was done with TALOS+. A structural model was built with Rosetta showing a β-sheet containing structure.[2]

With the assigned FNIII-9 domain further information about the interaction with the von-Willebrand-factor-A-like domain 2 can be attained thus providing the basis for detailed insight into the organisation of the skin.

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So711

NMR Investigation of Isoform specific differences in the binding of Tau to Microtubules

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The microtubule associated protein Tau has six different isoforms, which are expressed in neurons of the central nervous system and are responsible for the promotion of microtubule (MT) formation and stabilization[1]. Binding of Tau to microtubules is mediated by the C-terminal microtubule binding domain[1, 2]. Here we present an NMR-based characterization of the interaction between two different isoforms of the Tau protein, htau40 and htau23 which have either 3 or 4 pseudo-repeats, and MTs stabilized by taxol. As variation in the signal intensities and change in chemical shifts of the particular amino acid residues in the heteronuclear ^{1}H-^{15}N correlation spectra of Tau proteins in complex with MTs is due to the direct interaction of the specific residues with the MT binding site, we could accurately define the MT-interacting regions of Tau. The nature of interaction was studied by varying the stoichiometric ratios between Tau and MTs.

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So712

Perturbation of the Ras effector interaction by small ligands stabilizing a low-populated conformational state in Ras protein

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Ras protein is an essential component of signal transduction pathways within the cell, controlling proliferation, differentiation and apoptosis. It acts as molecular switch by cycling between a GDP-bound "off" and a GTP-bound "on" state. Only in the latter one Ras is able to bind effector molecules like Raf-kinase via its switch I region with high affinity. ^{31}P NMR spectroscopy reveals the existence of a dynamic equilibrium between at least two distinct conformational states of active Ras with different physiological properties. One of these states, namely state 1(T) shows a drastically reduced affinity to effector molecules [1,2]. Previously we identified Zn^{2+}-cyclen as a compound, which distinguishes between these two states [3]. It binds selectively to state 1(T) leading to a complete shift of the dynamic equilibrium towards this weak effector-binding state at higher concentrations. Thus it represents an attractive lead compound for the inhibition of aberrant Ras signalling, which is found to be intensively involved in human malignancies. The binding site of the ligand could be identified [4]. Here we report on perturbation of the association between Ras and its major downstream target Raf-kinase modulating intrinsic conformational equilibria by suitable small compounds.

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So713

Structural analysis of N-terminal PIN domain from Rrp44 protein based on multidimensional NMR data sets

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The exosome complex is multi-protein major eukaryotic exonuclease complex capable of degrading various types of RNA molecules. The Rrp44 protein is the associated subunit of the exosome which contain two major domain: catalytic RNB domain responsible for the activity of hydrolytic RNase II/R family members and highly conserved N-terminal PIN domain. Our recent results shows that PIN domain is responsible also for endoribonucleolytic activity of the yeast exosome [1]. Therefore Rrp44 is a unique example of a RNase in which endoribonucleolytic and exoribonucleolytic catalytic sites exists in a single protein.

204 amino acid residues long fragment of PIN domain was expressed in *E.coli* and purified in both ^{2}H, ^{13}C, ^{15}N-triple and ^{13}C, ^{15}N-double labeled forms. Acquired heteronuclear NMR data sets provide to assignments of ^{1}H, ^{13}C, and ^{15}N backbone resonances for 151 residues which were confirmed later by analysis of 4D HNCACO spectrum. Now we perform assignment procedure of ^{1}H, ^{13}C resonances in side chains on base 4D HCCH-TOCSY and ^{13}C, ^{15}N NOESY-HSQC spectra. Obtained data were used as input for TALOS+ and CS23D programs to extract information about 3D structure of PIN domain.

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So714

Activation of the p38α MAP kinase investigated by NMR spectroscopy

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Protein kinases function as molecular switches that due to their conformational plasticity are capable of switching between catalytically inactive and active structures depending on their phosphorylation status. Upon activation they are able to bind ATP and catalyze the transfer of the γ-phosphate of ATP to a down-stream target. Proper regulation of these biological switches is of crucial importance in eukaryotic organisms and misregulation is known to cause a wide variety of diseases such as cancer, rheumatoid arthritis, asthma and psoriasis as well as cardiovascular and neurological disorders. p38α is a mitogen activated protein kinase (MAPK), a serine/threonine kinase that is expressed in most cell types and appears in several signal pathways. It has been intimately linked with inflammation and p38α inhibitors are known to block the production of inflammatory cytokines e.g. tumour necrosis factor α (TNF-α) and inter-leukin-1(IL-1). p38α is activated by double phosphorylation of T180 and Y182 in the activation loop by upstream members MKK3 or MKK6 of the MAPK cascade. In this work activation is achieved by dual expression of p38α and MKK6 in *E.coli*. We present in-vitro studies of the activated p38α kinase analysed by high-field, liquid-state NMR spectroscopy and the differences derived from the assignments of both activated and inactivated p38α, revealing changes throughout the kinase in both structure and dynamics upon activation.

So715

Characterisation of Conopeptides by NMR

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Venom peptides present enormous opportunities in the discovery of novel therapeutics. Their efficient bioactivity on a wide range of ion channels has already led to the clinical development of new drugs in treatment of severe pain. The CONCO project focuses on therapeutically relevant peptides from venomous cone snails, particularly of the species *Conus consors*. These conopeptides are constrained by well-conserved cysteine frameworks (1–5 disulfide bridges). Sequence variations in the inter-cysteine loops and post-translational modifications confer structural and functional diversity. Structure elucidation using NMR combined with bioactivity assays will lead to a better understanding of their structure-function relationship. CONCO, the cone snail genome project for health, is funded by the European Commission: LIFESCIHEALTH-6 Integrated Project LSHB-CT-2007-037592 www.conco.eu

So716

Azurin in reversed micelles - Micelle size and protein-solvent interactions

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The impact of different solvents (hexane, octane and decane), water content and micelle size on azurin-containing reversed micelles composed of AOT was studied by NMR. Pulsed field gradient spin-echo (PFG-SE) NMR was used for determining the micelle diffusion constant and 2D ^{15}N HSQC for characterizing the protein with respect to water content, size of the micelles and the solvent used.

The fastest diffusion was obtained for hexane after disposing almost 75% of initial water. In contrast, decane resulted in two times lower diffusion constant. Independent on solvent, the protein structure was not affected by freezing or the amount of water in the micelle. However, the results indicated different protein-AOT-solvent interactions depending on the solvent used.

So717

A new approach to study dynamics of structural rearrangements upon ligand binding to a protein

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The understanding of protein function is still dominated by the static pictures that are provided by NMR and X-ray spectroscopy. However, it is clear that the function of a protein is ultimately governed by its dynamic character. Addressing this aspect experimentally is challenging because available techniques are either limited to steady-state observations or rather suited for very particular systems[1]. Here, I show how to study the dynamics of ligand-gated proteins.

As a model system I have chosen a cyclic nucleotide binding domain (CNBD). Cyclic nucleotides control a multitude of cellular responses via several distinct targets, i. e. protein kinases or ion channels. A common feature shared by these proteins is a CNBD that hosts the ligand and relays the binding event to an activated state of the protein[2].

To study the kinetics of the conformational rearrangements that lead from the open, ligand-free state to the closed, ligand-bound state of a CNBD protein we want to use the double electron-electron resonance (DEER) technique. If combined with rapid mixing and hyper-freeze quenching techniques[3], we will be able to derive a time-resolved picture of the structural transition upon ligand binding.

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So718

STAT6 - NCoA1 PAS-B domain interaction: understanding the structural and dynamic behavior of the complex by NMR

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Signal transducer and activator of transcription 6 (STAT6) regulates transcriptional activation in response to interleukin-4 (IL-4) by direct interaction with coactivators, an event which plays a crucial role in several biologically important processes[1]. The CREB-binding protein and the nuclear coactivator 1 (NCoA1) bind independently to specific regions of STAT6 and act as coactivators. STAT6-NCoA1 interaction is mediated by a short region of the STAT6 transactivation domain that includes the motif LXXLL[2]. The crystal structure of $STAT6^{794-814}$ in complex with NCoA1 PAS-B $domain^{257-385}$ revealed that the leucine side-chains of the motif are deeply embedded in the hydrophobic groove of the NCoA1 surface[3]. Recently, it has been demonstrated by a fluorescence polarization binding assay that additional residues, flanking the LXXLL motif in STAT6, may play an important role in stabilizing the protein binding to NCoA1[4]. In the current study, we have undertaken the structural and dynamic characterization by NMR of $STAT6^{783-814}$-NCoA1 PAS-B domain complex to address the mechanism of coactivator recognition in a more detailed level. Upon completion of structural calculation, analysis of the three dimensional structure of the complex will shed further light on the structural rearrangements that characterize this protein-protein interaction.

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So719

Effect of zinc binding on beta amyloid structure and dynamics: Implications for beta amyloid aggregation

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Assembly of beta amyloid (Aβ) peptide into toxic oligomers is widely believed to initiate Alzheimer's disease (AD) pathogenesis. Under in vitro physiological conditions, zinc (Zn(II)) can bind to Aβ and redirect its assembly from amyloid fibrillar toward less toxic amorphous aggregation. Propensity of Aβ to go toward a specific form of aggregate state is determined by structural and dynamical properties of the initial monomeric as well as the aggregate state. Here we probe the structural and dynamical impact of binding of Zn(II) to monomeric Aβ40 using NMR spectroscopy. To obtain further support for the importance of intrinsic dynamics in the aggregation precursor, 15N relaxation measurements were also performed for Aβ42, the more fibrillar aggregation-prone variant of Aβ. The combined data suggest that, upon Zn(II)-binding to the N-terminus of Aβ40, a relatively rigid turn-like structure is induced at residues Val24-Lys28 while the residues flanking this region become more mobile on the ps-ns time scale. This is in contrast to the increased rigidity of Aβ42 at the C-terminus, and proposed to be linked to the higher propensity of Zn(II)-bound peptide to form "amorphous" aggregates with less entropic penalties than their fibrillar counterparts.

So720

DC-SIGNR: the protein traitor which aids viral invaders.

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The C-type lectin DC-SIGNR (dendritic cell specific ICAM3 grabbing non-integrin related) has been shown to interact with a range of deadly diseases via specific glycosylation patterns on the surface of pathogenic glycoproteins.[1,2] The ability of DC-SIGNR to increase the rate of infection of viruses such as human immunodeficiency virus (HIV)[3] and hepatitis C virus (HCV)[4] make the study of DC-SIGNR/oligosaccharide interactions very attractive. This research aims to utilise solution state heteronuclear nuclear magnetic resonance (NMR) spectroscopy in order to obtain a detailed understanding of the DC-SIGNR binding domain structure along with information on binding affinities, the residues involved in binding, dynamics, and kinetics. It is hoped that an extensive knowledge of DC-SIGNR/oligosaccharide interactions will drive the development of novel antiviral drugs. This poster summarises progress in the assignment of the NMR spectra.

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So721

Structure and backbone dynamics of human *apo*-S100A1 protein and its two mutants, C85M and E32Q

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S100A1 is a homodimeric calcium binding protein of 93 residues per subunit. It is stabilized by noncovalent interactions at its dimer interface with each subunit containing two EF-hand motifs linked by a flexible linker. Chemical modification of Cys 85 residue in the C-terminal part of S100A1 protein by disulfide bond formation with small thiols leads to the conformational changes of the protein. It is caused, most probably, by an interaction of a thiol molecule attached to Cys 85 with aromatic rings of Phe 88 and Phe 89 promoting α-helical conformation in the 85-89 segment of the protein.
Two mutants of human S100A1 protein in the *apo* state, S100A1(C85M) and S100A1(E32Q), have been studied and their NMR-derived structures compared with that obtained recently for the wild type protein. The rationale for choosing C85M mutant is introducing more bulky side chain at residue 85, whereas in the E32Q mutant only one of two EF-had motifs can bind calcium ions. Both mutants display small but distinct structural changes localized in the vicinity of the mutations. Structural studies were complemented by ^{15}N nuclear magnetic relaxation data at multiple magnetic fields.

This work was supported by a grant N301 031234 from the Polish Ministry of Science and Higher Education.

So722

Changes in structure and dynamics of human *apo*-S100 protein induced by calcium binding and Cys 85 modification

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S100 is a multigenic family of calcium-modulated mostly homodimeric proteins of the EF-hand superfamily implicated in intracellular and extracellular regulatory activities.

Goal of our present study is to discuss structural differences between human S100A1 protein in the *apo*- and *holo* states with structure of human S100A1 modified with homocysteine in calcium bound form, and to study how protein backbone dynamics is affected by calcium binding.

For *holo*-S100A1 protein we observe typical for S100 proteins reorientation of helix III by almost 90°, exposing large hydrophobic cleft. Modification of the protein unique cysteine with homocysteine introduces only minor but possibly important structural change.

For the *apo*- and *holo*- forms of human S100A1 protein ^{15}N nuclear magnetic relaxation were obtained and their backbone dynamics elucidated. The *apo* form displays significantly increased mobility of both binding loops and linker joining two binding EF-hand domains in comparison with helices mobility. In the *holo* form mobility of the loops is partially restricted but the linker, on the other hand, displays greater mobility than in the *apo* form.

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So723

Domain organization of YtvA studied by EPR

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The arrangement of the blue-light sensitive LOV and the STAS effector domain of the blue-light activated YtvA protein from B. subtilis was studied by EPR techniques using site-directed spin labeling with MTSL. MMM simulations were employed for data analysis [1].

Pulsed ELDOR on singly labeled protein showed a specific dimerization of YtvA in solution. Differences in the spin label mobility at various postions of the proein deduced from *cw*-EPR yield a binding model for LOV-STAS involving the LOV □□sheets in contact with STAS T179. The distance observed by pulsed ELDOR between the LOV domains of the YtvA dimer results with this LOV-STAS arrangement in a LOV-LOV dimerization of YtvA, markedly different from previously published results [2][3].

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So724

Design and characterization of a dimeric inhibitor of PSD-95 protecting against ischemic brain damage.

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Inhibition of the ternary protein complex of nNOS, PSD-95 and the NMDA receptor is a potential strategy for treating ischemic brain damage but high affinity inhibitors have so far not been described. Here we report that a novel dimeric inhibitor, Tat-NPEG4(IETDV)$_2$ (Tat-N-dimer) binds the synaptic scaffolding protein PSD-95 with an affinity that is three order of magnitude higher than monomeric peptides (K_d = 4.6 nM). Tat-N-dimer reduces infarct volume in mice by 40% and restores motor function following ischemic brain damage. Hence, Tat-N-dimer is a highly efficient neuroprotective molecule with therapeutic potential in stroke.

The structures of free and ligand bound PSD-95 have been characterized by x-ray crystallography, small angle x-ray scattering and NMR spectroscopy. By NMR experiments we show that the Tat-N-dimer binds the first two domains of PSD-95 and that the stoichiometric ratio is 1:1. Furthermore we show that Tat-N-dimer binds the consensus binding pocket of both domains and adopts an extended conformation upon binding. Relaxation experiments interestingly demonstrate that interdomain motions of PSD-95 increase upon binding of dimeric peptide.

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So725

NMR solution structure of the Protein Tyrosin Phosphatase A of *M. tuberculosis* (MptpA)

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Tuberculosis (TB) remains one of the most infectious diseases today. The World Health Organization estimates that about one third of the world's population is currently infected with *Mycobacterium. tuberculosis* (*Mt*) and that about 2 million people die of TB every year [1]. Knockout strains of *Mt* show that MptpA is essential for long-term infection [2]. MptpA clearly qualifies as a potential target for inhibitors, but essential knowledge which could lead to the development of anti-TB drugs targeting MptpA is missing. Here we present the NMR solution structure of MptpA based on the nearly complete resonance assignment (93%). Automated NOESY assignment was achieved using the program CYANA 3.0 [3]. Additionally relaxation data (R_1, R_2, HetNOE) and residual dipolar couplings (RDC) were used for structure validation and refinement. Recently the MptpA complementary protein tyrosine kinase A (PtkA) was determined. We revealed the interaction site between MptpA and the complementary kinase PtkA by means of ^{1}H,^{15}N-HSQC NMR titration experiments. In further studies we will proceed our investigations of this complex using NMR techniques, in order to gain substantially more insight into the regulatory mechanism of MptpA.

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So726

Structural studies of the fibronectin III - immunoglobulin A67-A68 A-band domain tandem from the giant muscle protein Titin

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Titin is the largest known polypeptide (3.5 Mda) and the third most abundant protein present in vertebrate striated muscle after myosin and actin. The titin molecule spans between the M- and Z-lines in the sarcomere and is mostly folded as a string of ~300 immunoglobulin and fibronectin domains. In the A-band titin is integral with thick filaments and interacts with myosin, C-protein and other filament components. Through much of the A-band the domains are arranged in patterns of eleven, Ig-Fn-Fn-Fn-Ig-Fn-Fn-Fn-Ig-Fn-Fn, giving rise to a super-repeat that is itself repeated 11 times, making up nearly half the titin molecule. In order to determine the supra-molecular structure of this region we are looking at the structures and inter domain flexibility in domain tandems from this super-repeat. For the 215 residue A67-A68 domain tandem, initially structural models of the domains were obtained using CS-rosetta and the inter-domain orientation is established using residual dipolar couplings and paramagnetic relaxation enhancement. Side chain assignments of the methyl groups have been obtained by producing the protein in D2O using protonated glucose. Full structure calculations are underway combining the inter domain restraints with amide-amide, methyl-amide and methyl-methyl NOE restraints. Indications are that the linker in this domain tandem is rigidly oriented and there is very little inter-domain flexibility. How general this feature is in this repeat awaits the elucidation of more tandem domain structures.

So727

PELDOR Spectroscopy on a Protein/RNA-Complex: the RNA-Methyltransferase Nep1 Bound to its Target RNA

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PELDOR[1] spectroscopy is frequently used to measure distances and distance distributions in proteines[2] and nucleic acid molecules[3]. Here we show first PELDOR results on short RNA molecules bound to the dimeric Nep1 protein. Nep1 (Nucleolar Essential Protein 1) is a highly conserved eukaryotic protein essential in ribosome biogenesis. Its X-ray structure was recently published[4] and it was shown to act as a sequence specific pseudouridine N1-methyltransferase involved in the biosynthesis of a hypermodified nucleotide in helix 35 of 18S rRNA[5]. Furthermore, short single-stranded high affinity substrates of Nep1 have been identified[5]. Several spin labeled mutants of Nep1 as well as spin labeled RNA molecules have been used to determine the structure of dimeric Nep1 bound to two copies of its target RNAs. We will show our first PELDOR results on measured protein-protein, protein-RNA and RNA-RNA distances within this complex

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So728

GABARAP, Nix and Bcl-2: Structural basis of molecular interactions at the interface of autophagy and apoptosis

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Autophagy, a pathway primarily relevant for cell survival, and apoptosis, a process leading to cell death, are the two main mechanisms of self-destruction at cellular and subcellular levels. Currently, a potential crosstalk between apoptosis and autophagy is intensively discussed, the respective protein-protein interaction network, however, remains to be elucidated in detail.

The γ-aminobutyric acid type A ($GABA_A$) receptor-associated protein GABARAP belongs to a family of proteins implicated in intracellular transport events and autophagy[1].

We identified two new interaction partners of GABARAP, namely the pro-apoptotic nip-like protein x (Nix)[2] and Bcl-2 (B-cell lymphoma 2)[3], which is one of the most prominent anti-apoptotic regulators. The interactions of GABARAP with Nix and Bcl-2 form new molecular links between autophagy and apoptosis. Using solution NMR, we further characterized the structural basis underlying the interactions of GABARAP with Nix, an intrinsically disordered protein, as well as with Bcl-2. The binding modes of these proteins allow competitive as well as non-competitive interactions between those proteins, thus providing new possibilities for the regulation of autophagy and apoptosis.

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So729

Structural study of sulfiredoxin Srx from *Saccharomyces cerevisiae*

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H_2O_2-dependent post-translational overoxidation of the catalytic Cys of eukaryotic typical two cysteine peroxiredoxins (Prxs) to the sulfinic state ($PrxSO_2$) has been hypothesized as a switch between the peroxidase/redox relay/chaperone functions of Prx, allowing cellular adaptation to the dual toxic and beneficial effects of H_2O_2 through the control of H_2O_2 fluxes. The reversibility of this process depends on Sulfiredoxin (Srx), which catalyzes the ATP-dependent reduction of overoxidized $PrxSO_2$. Human sulfiredoxin hSrx structures alone or in complex with Prx have been previously solved, shedding light on the structural basis of the peroxidase activity restoration (1). Recently, the catalytic mechanism of the scSrx from *S. cerevisiae* has been deciphered, showing distinct features from the human one, particularly the formation of an oxidized scSrx intermediate with a disulfide bridge between the catalytic Cys84 and the resolving Cys48 (2-3). In comparison to hSrx, scSrx possesses a large flexible loop (17 residues) containing Cys48, which destabilizes the overall structure and speeds up the transversal relaxation. To gain insight into the molecular basis of the Prx reduction by the scSrx, we have determined the NMR solution structure of the reduced and oxidized scSrx. We present here the preliminary NMR solution structure of the reduced scSrx.

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So730

NMR studies of the ribosomal translation initiation factor IF2

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The translation initiation factor IF2 plays an essential role in the protein biosynthesis of bacteria. IF2 in complex with cofactors like GTP, fMet-tRNA, and with other initiation factors (IF1, IF3) and 30S ribosome stimulates the 70S complex formation. This event is followed by GTP hydrolysis and release of all other initiation factors.

The bacterial IF2 have been biochemically well characterized. IF2 has a fMet-tRNA binding C-domain and a nucleotide binding G-domain, which undergoes conformational changes upon ligand binding. Major conformational changes in response to cellular GTP, GDP and ppGpp levels could regulate the cell functioning. Although the NMR structures for individual IF2 domains are available, the effect of fMet-tRNA, GTP, GDP and 30S binding on the domain rearrangements and it's mechanism are still unclear.

Using NMR spectroscopy and other complementary techniques we are studying the regulatory role of the large G-domain of IF2. For this we have purified and partially assigned the G3 and G2G3 subdomains of IF2. In addition to the assignments we have performed titration experiments of IF2G23 domain against 30S ribosome and have been able to identify the 30S binding interface for IF2. These findings represent our initial attempts in a complete assignment and structure determination of the protein with its different ligand bound states and interaction partners.

Td731

A Powerful New Tool for Mixture Analysis: Local Covariance Order Diffusion-Ordered Spectroscopy (LOCODOSY)

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Diffusion-ordered spectroscopy (DOSY) is a useful, widespread tool for analyzing mixtures. In general, one aims to separate out the NMR spectra of individual mixture components, gaining information on the physical and chemical properties of the analytes: namely hydrodynamic radii, diffusion coefficients and intermolecular interactions. The two most common data processing approaches are univariate (e.g. HR-DOSY[1]) and multivariate methods (e.g. SCORE[2] and DECRA[3]). The former of these breaks down where peaks from different components overlap and the latter struggles with increasing number of components (2-4 is a practical limit). A hybrid approach has been developed[4] combining the strengths of both, the principle of which is to break the spectrum into regions for independent multivariate analysis before rebuilding the dataset from the processing results. The total number of components resolvable rises dramatically, as fewer components will be present in each spectral window. LOCODOSY allows the clean resolution of significantly more chemical components than previously possible.

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TD732

NMR on Emulsions: Examination of complex structures

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Emulsions are of high interest in industry and research. With progress in emulsion technology, more complex structures are realized and require adequate measuring techniques. Double emulsions are an example for complex emulsion system. Determining the droplet size distribution (DSD) of the encapsulated droplets is a challenge for common optical measuring techniques. PFG-NMR, which is well known in quality control of single emulsions, fulfills the requirements for gaining information about the encapsulated droplets.

Commonly known PFG-NMR techniques can be applied to the study of diffusion processes in double emulsions [1]. Methodological and analytical developments are presented with special emphasis on double emulsions. For example, the modality of the distribution is investigated. PFG-NMR results on DSD determination of encapsulated droplets [1] are compared with statistical image processing of Confocal Laser Scanning Microscopy (CLSM) images. It is shown that already with a bench-top NMR double emulsions can be analyzed with respect to the inner emulsion's DSD. Apart from the proof of concept and reproducibility, a new approach on basis of a combined CPMG PFG-NMR sequence for S/N improvement has been investigated on the 20 MHz device, limiting factors are discussed.

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TD733

Effective Calculation of the Echo decay in the SGP-limit

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We have recently developed a novel perturbation method to calculate quick and effective estimates of the echo decay in diffusion NMR [1,2]. More precisely the method calculates an estimate of the diffusion propagator, using a mixed basis formulation, from where the echo decay in the short gradient pulse limit is formed using few computational steps. The mixed basis method has been validated using Neumann boundary conditions defining the material and periodic boundary conditions are used on the computational cell. The mixed basis method is easily implemented and proposes a general tool to investigate diffusion in large structures and geometry dependence on the echo decay, due to the fact that several eigenvalues and eigenvectors of the diffusion propagator are retrieved. Since the method is entirely formulated on the boundaries it may complement other efficient techniques for solving diffusion problems such as boundary element methods, Trefftz methods and fast multi pole techniques.

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TD734

Molecular weight determination and component quantification of block copolymer samples using diffusion NMR

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Block copolymers constitute a fascinating class of polymeric materials used in a large range of applications, from advanced nanomaterials to biocompatible drug delivery systems.[1] The performance of these materials is highly coupled to the chemical composition and molecular weight distribution of the constituting block copolymers. Traditionally, the molecular weight of block copolymers is obtained by SEC. However, wrong estimates may be achieved when analyzing amphiphilic copolymers, for instance due to undesirable interactions with the stationary phase. In this context, diffusion NMR[2] is shown to be a faster technique for estimating the weight average molecular weight of a series of amphiphilic poly(ethylene oxide)-*b*-polystyrene (PEO-*b*-PS) block copolymer samples, obtained by controlled free-radical nitroxide mediated polymerization.[3] Moreover, alternatively to the quantitative DECRA method introduced by Antalek,[4] an analytical strategy is proposed to quantitatively analyze block copolymer mixtures by diffusion NMR. While being generalizable, this strategy is here illustrated by quantifying the amount of the functionalized PEO macroinitiator in a mixture with the PEO-*b*-PS copolymer sample.

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TD735

Predicting Diffusion Coefficients for Small Molecules

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Diffusion-ordered NMR spectroscopy (DOSY) has found increasing use as an analytical tool, capable of identifying components in a mixture[1]. However, DOSY has limited use in quantitative measurement because there is a poor understanding of the relationships between the diffusion coefficient of a molecule in solution and its size and shape. Diffusion coefficients are often predicted using the most basic relationship, the Stokes-Einstein equation[2], which balances the kinetic energy of the system against the friction acting on the molecules. Unfortunately this is a very poor approximation for small molecules. Various refinements of the original equation have been suggested[3, 4, 5, 6, 7] but none have been fully satisfactory.

Here a new model is proposed for predicting the diffusion coefficients of small molecules. It has a simple physical basis, uses only one adjustable parameter, and is shown to allow diffusion coefficients to be predicted to an accuracy of around 10%.

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TD736

Dendrimers as globular charged molcules and the formation of complexes

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PAMAM dendrimers as well-defined globular molecules contain only a few building blocks. Primary and tertiary amino groups are the only charged groups. Their large fractal dimension shows, that they are rather compact molecules [1]. Therefore they are used as a model system for charged globular molecules. Depending on pH the effective charge increases from the natural pH of the PAMAM down to pH 3. The nominal charge is determined from the degree of protonation of the amino groups inferred from the proton chemical shift of the adjacent CH_2 groups [2]. The effective charge is determined from a combination of diffusion and electrophoresis NMR [3, 4]. It is limited by the condensation of counterions on the macromolecule.

In contact with a strong polyanion like poly(styrenesulfonate) PAMAM dendriemrs form complexes depdending on their charge, which is understood as a model for the binding of ligands to globular molecules like proteins. Short polyelectrolytes bin to the dendrimer compensating the charge eventually leading to precipitation. Longer polyelectrolyte chains become more compact upon formation of the complex with the dendrimers.

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TD737

Dendrimers as globular charged molcules and the formation of complexes

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In contact with a strong polyanion like poly(styrenesulfonate) PAMAM dendriemrs form complexes depdending on their charge, which is understood as a model for the binding of ligands to globular molecules like proteins. Short polyelectrolytes bin to the dendrimer compensating the charge eventually leading to precipitation. Longer polyelectrolyte chains become more compact upon formation of the complex with the dendrimers.

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TD738

NMR Studies on the C39-like domain of HlyB reveal distinct interaction with the haemotoxin HlyA

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ABC transporter accomplish the translocation of substrates over biological membranes under consumption of ATP. Their substrates reach from ions to proteins of several hundred kDa. Our research is focused on the type 1 secretion system of the toxin HlyA in *E. coli*. As its central machine it contains the ABC transporter HlyB, which assembles the secretion complex together with the membrane fusion protein HlyD and the outer membrane protein TolC. By this a continuous connection between the cytosol and the exterior is formed, promoting the one-step translocation of HlyA without any periplasmic intermediate. The general setup of an ABC transporter is composed of four modules, two nucleotide binding domains and two transmembrane domains. HlyB, as a bacteriocin transport, exhibits two additional domains, which are classified as C39 peptidase. These thiol-proteases are thought to cleave the transport substrate after a conserved GG-motif. However, in HlyB the catalytic key cysteine is exchanged by a tyrosine residue rendering the domain proteolytic inactive, nevertheless the domain was proven to be essential for the transport.

We will present the solution structure of the isolated C39-like domain of HlyB and the details of the rearrangement in the triad. Furthermore, we will reveal novel insights in the interaction of HlyA with C39-like and its importance during the transport process.

TD739

Flow of Non-Newtonian Fluids in Porous Media with Transverse Permeability Discontinuity.

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Flow of non-Newtonian fluids in inhomogeneous porous media is of particular interest not only from the applied point of view but also from the fundamental perspective. In the presence of a permeability discontinuity in a porous medium volume averaged flow equations and corresponding boundary conditions require redefinition. Analytical flow description in these systems has been attempted before but the lack of experimental data makes it difficult to choose a proper theoretical model. We report for the first time experimental flow fields of non-Newtonian fluids in an open channel bound to a saturated porous medium measured by MRI velocimetry methods. Axial velocity profiles exhibit non-zero velocities at the interface with the porous medium for all flow regimes studied. Rheology of the fluids confirming their shear thinning behavior is also reported. Fundamentally, the obtained data can be used in refining present theoretical models whilst practical aspect can be related to oil recovery and biological flow in various porous media for example blood flow in vessels. Moreover MRI velocimetry methods are shown to be unique in tackling this fundamentally and practically important problem while other conventional velocimetry methods (optical, ultrasound and labeling) seem to fail.

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TD740

Permeability of polyelectrolyte multilayer capsules: A PFG-NMR diffusion- exchange study

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For colloidal carrier systems to be applied in drug delivery or other controlled delivery applications, the distribution of active molecules and their exchange dynamics is of major interest.

We employ here poly(ethyleneoxide) chains as guest molecules in dilute dispersions of hollow polymeric capsules consisting of polyelectrolyte multilayers as the wall material and an aqueous interior allowing molecular encapsulation. ^{1}H PFG- NMR can differentiate between encapsulated and free chains by their respective diffusion coefficients. Upon variation of the observation time, a transition from fast exchange to slow exchange is observed. Employing a two-site model to echo decay data sets obtained at different observation times, exchange rates of the chains through the capsule walls can be extracted.

Exchange rates determined in this way are not consistent with a chain permeation through a dense material, but rather through larger nanopores. Electron microscopy proves the existence of nanopores with sizes of about 16 nm.[1] It is shown that the nanopores develop during the process of capsule formation by core removal. The combination of PFG-NMR diffusion-exchange analysis and electron microscopy provides a powerful tool to correlate permeation rates and pore sizes, such that the effect of different post-preparative treatments of the capsules can be understood.

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TD741

Rehydration behaviour of freeze-dried vegetables assessed by NMR and MRI

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Consumers have a high appreciation of healthy meals rich in fruits and vegetables. Lack of convenience in preparing meals rich in fruits and vegetables is impeding consumers to reach their recommended daily intake of fibers and nutrients. This is addressed by developing food products consisting of dried fruits and vegetables that are rehydrated shortly before consumption. A main challenge is to develop products which deliver health benefits and authentic sensory properties without compromising the convenience of fast cooking. We adopted a microstructure-driven research strategy to investigate the structural impact upon freeze-drying of vegetables and to model their rehydration behaviour. Dried and rehydrated microstructures were assessed in a multi-scale manner (nm-mm) by means of complementary imaging techniques like SEM, μCT and MRI. Quantitative image analysis revealed the pore space topology in the dry state and NMR diffusometry and imaging allowed us to determine cell wall behaviour upon wetting. These features were used to construct a microstructure-based model for rehydration. Water kinetics during rehydration was assessed in real-time and in situ by means of RARE imaging[1] thus enabling model verification[2].

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TD742

^{1}H-DOSY NMR Aggregation Studies on Flavins

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Lately flavins have attracted much attention as catalysts for chemical reactions. After irradiation with visible light flavin becomes strongly oxidising and can convert benzyl alcohols into the corresponding aldehydes. The reduced flavin is instantly reoxidised by aerial oxygen. Although it was investigated and optimized for homogeneous reaction in acetonitrile the reaction turned out to be much more efficient in aqueous solution.[1,2] This could be explained by self-aggregation studies on riboflavin tetraacetate and association studies on riboflavin tetraacetate and p-methoxybenzyl alcohol. These studies were conducted by ^{1}H-DOSY NMR experiments by measuring diffusion coefficients of the analytes at different conditions and comparing them. Riboflavin tetraacetate turned out to form dimers in acetonitrile, whereas in water it appears as a monomer.

Motivated by these observations new sterically demanding flavins were synthesized with the aim to make them aggregate less and thus increase their photocatalytic activity. The aggregation behaviour of these new flavins was studied again with ^{1}H-DOSY NMR experiments by measuring diffusion coefficients, calculating the hydrodynamic volumes of the new flavins from the diffusion coefficients and comparing them to theoretical expected values. The new flavins showed less aggregation than riboflavin tetraacetate and were thus tested as catalysts for the photooxidation of benzyl alcohols.

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TD743

Water permeability through lipid membrane of salmonid intestine studied by ^{17}O NMR

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The enhancement of ^{17}O relaxation rates by paramagnetic ions e.g. Mn^{2+}, has been employed to distinguish between intestinal water exchange of Salmon smolt in fresh and sea water. Lipids of anterior and posterior intestine were obtained by mucosal scrapings and combined with cholesterol in sodium chloride buffer to form vesicles by ultrasonication. After extrusion, vesicle size and unilamellarity have been determined by dynamic light scattering (DLS) and cryogenic transmission electron microscopy (cryo-TEM).

The mean life time of water molecules within the vesicles were measured by taking advantage of the impermeability of the lipid membrane to paramagnetic ions. External vesicle water relaxes rapidly while internal water is unaffected. The mean life times were obtained from the ^{17}O line widths and when combined with the vesicle size from DLS the permeability through the membrane could be calculated.

We observed a clear difference in permeability for posterior intestine compared to the anterior intestine.

TD744

Anomalous diffusion in gelatin gelation

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We present a study of the gelation of water solutions of collagen-derived gelatin using pulsed field gradient NMR. For the first time, the complete dynamic structure factor is obtained in dependence on temperature for several gelatin concentrations. This enables us to follow the gelation process with great precision, on length and time scales relevant for self-organization.

We found that the material is homogeneous above ~10 μm, with well-defined effective diffusion coefficients mostly following activation temperature dependence. A decrease of those coefficients at the overlap concentration of ~35 g/L [1] is clearly observed, and an anomaly appears close to the sol-gel transition. At smaller scales anomalous diffusion sets in [2], and power-law tails are observed in the dynamic structure factor. Their nature changes abruptly at the transition. While fractal diffusion seems to provide a good description in the sol state, it is not applicable in gel; to explain this we suggest that correlated motion plays an important role, in analogy with systems such as glass-forming materials [3] and colloidal gels [4]. It is known that during gelation helical segments form on the polypeptide chains, thus forming interchain bonds [1]; we discuss the significance of hydrophobic interactions between these segments in mediating correlations at micron scales.

This work is enabled by the European Commission through FP7 project #229390 SOLeNeMaR.

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TD745

Dynamics and Hydration of PAMAM_G5 dendrimers as PGSE NMR sees

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NMR spectroscopy has a high potential to study self-diffusion. Self-diffusion plays important role in characterization of colloid (nano) systems. The dendrimer concentration dependences of apparent self-diffusion coefficients for either the PAMAM (polyamido(amine))_G5(5^{th} generation)_X (amino or carboxylate termini) in dilute aqueous solution or for the solvent have been determined. By application the basic theory of dynamics of colloid systems[1] we have shown that the hydrodynamic radius corresponds to the "effective" hard sphere radius, that is these branched organic polymers (30-40 kDa) in hydrated form behave as hard sphere colloids.

The concentration dependence of the self diffusion of water molecules in the presence of dendrimer decreased with the increase of the dendrimer concentration. Application of the cell model[2] of liquids we determined the number of water molecules moving with dendrimers but exchanging fast with bulk. ^{31}P NMR diffusiometry and relaxometry allowed us to characterize the interaction between PAMAM_G5_NH_2 dendrimers and analytically and biologically important phosphate ions.

These studies may give important information for drug delivery behavior of dendrimers and may serve a good model for the hydration of biological macromolecules, taking into account the large number of peptide bonds in them.

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TD746

Permeation of polymer chains through nanopores – timescales and mechanismen

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We investigate the permeation of polymers through the nanoporous wall of hollow capsules, in particular the correlation between polymer molecular weight and exchange time τ, by using Pulsed Field Gradient (PFG) NMR diffusion. With this method it is possible to distinguish polymers in different environments by their respective diffusion coefficients, here the free and the encapsulated chains. This exchange system can be described by the two-site model. In a previously analysed system[1] consisting of polyelectrolyte multilayer capsules and poly(ethylene glycol) as probe molecules, the chain permeation was described by a scaling law $\tau \propto N^b$.

In the present study, we combine experiments of chain permeation by diffusion time dependent PFG-NMR diffusion studies with Monte Carlo Simulation of a polymer chain threaded through a nanopore. On the one hand we analyse the chain permeation by diffusion experiments for poly(ethylene glycol) and poly(N-isopropylacrylamide) in aqueous solution. On the other hand we investigate the correlation between the exchange time and several parameters, in particularly the chain length and the wall thickness by simulations. This is a first step to understand the whole mechanism of polymer permeation through a nanopore. The final goal of this study is to identify general laws governing chain permeation.

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OT747

Globally Phase-encoded, Echo-based FT- EPR imaging for High Resolution and Quantitative in vivo Oximetry

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Low frequency *in vivo* EPR imaging is an emerging field of non-invasive functional imaging in probing oxygen distribution in tumor tissue, pharmacokinetics of free-radical probes, peripheral vasculature, etc. Oxygen mapping is based on Heisenberg exchange broadening of free radical resonances by oxygen *in vivo*. Oximetry is carried out by T_2 or T_2* dependent contrast. The three methods in vogue are (a) FID based filtered back-projection where one can impart T_2*-dependent contrast by varying the acquisition delay. Resonator size and gradient magnitude dependent calibration is necessary for oximetry and resolution is affected by susceptibility artifacts. Large dead time and magnitude mode processing limit resolution. (b) One can perform 90-τ-180 echo sequence and construct T_2-weighted images as a function of τ. Oximetry here is reliable and reproducible, but the resolution of image resolution is still governed by T_2* and susceptibility artifacts. (c) One can perform Single Point or Constant Time imaging (SPI) with pure phase encoding in all dimensions leading to very high resolution images, but the oximetry is based on T_2* is a problem. Here we combine the high resolution of the SPI mode with the T_2-weighted oximetry capability of the echo strategy to obtain high resolution in vivo EPR images with quantitative oximetry. We believe that such an approach holds promise to reliable anatomical and functional physiological imaging that can be co-registered with MRI / CT and will aid in cancer treatment by radiation and chemotherapy.

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OT748

Intermolecular self-assembly of $[Pt^{II}(phen)(L\text{-}S,O)]^+$ through cation-π interactions, studied by high resolution ^1H NMR and DOSY Spectroscopy

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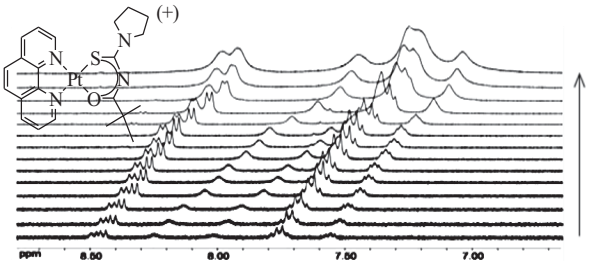
Non-covalent cation-π interaction have been the subject of extensive interest in the last decade in view of their well established role in biological systems, supra-molecular chemistry and molecular recognition phenomena.[1] In contrast the non-covalent self-assembly of planar complex cations such as $[Pt^{II}(diimine)(L\text{-}S,O)]^+$ (where diimine is 2,2'-bipyridine or 1,10-phenanthroline and HL-*S,O* represents various chelating *N*-acyl-*N,N'*-dialkylthioureas) have received comparatively little attention in the literature.[2] On varying the concentration of the $[Pt^{II}(phen)(L\text{-}S,O)]Cl$ complex significant changes in the ^1H NMR chemical shift were observed (see figure) which suggests that a self-association/aggregation processes occur as we have demonstrated for related complexes.[2]



We here show that in water/acetonitrile solvent systems $[Pt^{II}(phen)(L\text{-}S,O)]^+$ self-assemble to form super-'structures' from presumably higher order aggregates. Above a certain critical concentration of $[Pt^{II}(phen)(L\text{-}S,O)]^+$ (critical aggregation concentration) a type of micelle formation takes place in solution, leading to the formation of nano-sized aggregates as demonstrated by ^1H DOSY NMR and TEM images.

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OT749

CHELATE PHOSPHINE LINKERS WITH LONG ALKYL CHAINS FOR IMMOBILIZING CATALYSTS ON OXIDE SUPPORTS

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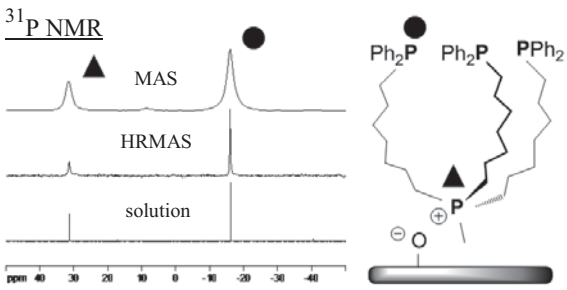
A new class of tridentate phosphine ligands with the general formula $[MeP\{(CH_2)_xPPh_2\}_3]^+I^-$ (x = 4, 7, 11), and $[MeP(CH_2PPh_2)_3]^+OTf^-$, have been synthesized and fully characterized.[1] The linkers have been immobilized on silica with their phosphonium moieties via electrostatic interactions, and their mobility and leaching was studied by solid-state HRMAS (high-resolution MAS) NMR. Immobilized Wilkinson-type Rh catalysts have been obtained by ligand exchange with the surface-bound linkers. Their activities and lifetimes have been tested for the hydrogenation of 1-dodecene. Their selectivity was investigated by ^1H NMR. The Rh catalyst surface-bound by the linker $[MeP\{(CH_2)_7PPh_2\}_3]^+I^-$ led to the highest activity and lifetime, as it could be recycled for a record 30 times. For all catalysts the formation of Rh nanoparticles with narrow size distributions around 4 nm has been proven.



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OT750

CARBON-13 NMR STUDIES OF PROTEASES INHIBITED BY SPECIFIC GLYOXAL INHIBITORS

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The rate limiting step in catalysis by proteases involves formation or breakdown of a tetrahedral intermediate [1]. In the serine [1,3,5,6] and cysteine proteases [2] an enzyme nucleophile reacts with the peptide carbonyl carbon to form a tetrahedral intermediate while with the aspartyl and metalloproteases water reacts with the peptide carbonyl to form the tetrahedral intermediate [4]. Therefore transition state analogues that mimic these tetrahedral intermediates are expected to be potent protease inhibitors.

We will show how we have used ^{13}C NMR to show that the glyoxal group (RCOCHO) is a versatile inhibitor warhead, which by adjusting its hydration state can mimic tetrahedral intermediates formed by different classes of proteases [1,2,3,4,5,6].

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Acknowledgements

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OT751

A high-frequency (210) GHz EPR study of Mn3+ in a MnMo6Se8 single crystal at low temperature (10 K): Jahn-Teller effect

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A high-frequency (208-GHz) EPR-study on Mn^{3+} ($3d^4$, S = 2) ions imbedded in a $MnMo_6Se_8$ single crystal at 10 K has been performed. The experimental spectra indicate an overlap of EPR lines from three magnetically inequivalent Mn^{3+} ions, whose magnetic axes are oriented along the three edges of the crystal in the form of a rectangular parallelepiped. The spin-coupling parameters were determined by the method of least-squares, fitting all the observed line positions simultaneously to the spin-Hamiltonian model. The symmetry of the spin Hamiltonian at the sites of the Mn^{3+} ions has been deduced from the parameters as determined from the EPR spectra. The differences in the values and signs of the zero-field splitting parameters for the three sites have been explained in terms of the Jahn-Teller distortions experienced by the Mn^{3+} ions in the $MnMo_6Se_8$ crystal.

OT752

Using NMR to study how aqueous solutions of Carbon-13 enriched bicarbonate react and/or interact with an azacryptand scaffold

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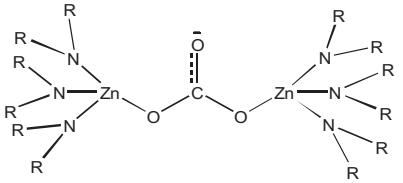
Earlier crystallographic studies have shown that in crystals the azacryptand scaffold binds a carbonate molecule between two zinc atoms. Each of the two zinc atoms is coordinated to 3 secondary nitrogen atoms of the azacryptand scaffold [1].



We dissolved the azacryptand scaffold in an aqueous solution at pH 10 containing 50% (v/v) DMSO and Carbon-13 enriched bicarbonate.Carbon-13 NMR has been used to observe the species formed in the presence and absence of zinc. The effect of the concentrations of bicarbonate and zinc on the stoichiometry of the species formed has been determined. HOESY has been used to determine how the Carbon-13 enriched carbon atom interacts with the protons of the scaffold and NOESY has been used to determine how the structure of the azacryptand scaffold is affected by added bicarbonate.

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OT753

Investigations of Flow in Quantitative NMR

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NMR as a non-invasive method provides qualitative and quantitative information from complex reacting multi-component mixtures for equilibrium or reaction kinetic studies [1–2]. Quantitative on-line NMR spectroscopy equipped with a flow probe allows the investigation of reaction progress almost in real time and under process conditions, in a wide range of temperatures and pressures. The delay time caused by the bypass realization in on-line NMR is an important issue. The non-ideal flow (transfer and spreading) of the sample from the reactor to the active region of the NMR probe is described by the residence time distribution (RTD) function [3]. It can be obtained from pulse tracer or step tracer experiments, where either a concentration pulse or a concentration step is produced in the reactor and the NMR-signal is monitored. The flow within the active region of the NMR cell should be characterized to assure that there are no stationary fractions of the sample remaining in the NMR sensitive region. This can be examined *in-situ* by MRI either in time-of-flight or in velocity imaging.

The contribution describes the residence time distribution and its consequences for a typical on-line NMR set-up. Pulse tracer experiments as well as tomographic flow studies are presented. The results provide detailed insight into flow properties and allow an optimization of the sample cell, which may be of general interest for other analytical on-line techniques using a sample cell within a bypass.

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OT754

Progress in SEC-medium resolution NMR

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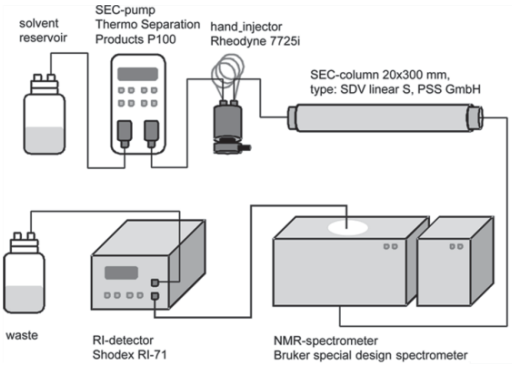
3: Bruker BioSpin GmbH, Silberstreifen, 76287 Rheinstetten, Germany
4: PSS Polymer Standards Service GmbH, In der Dalheimer Wiese 5, 55120 Mainz, Germany

A bench-top medium resolution prototype MR-NMR spectrometer has been adapted for online coupling with Size Exclusion Chromatography (SEC). Its sensitivity and selectivity are appropriate for polymer characterization. A great challenge of this online coupling is solvent suppression as polymers are investigated in solution using protonated solvents. T_1 relaxation differences can be exploited additionally to mathematical data treatment.



A dedicated steady state pulse sequence in combination with pre-experimental solvent degassing allows the measurement of the polymer with sufficient S/N under SEC-conditions (flow rate and concentrations), thus avoiding highly expensive deuterated solvents.

Chromatograms and the corresponding NMR spectra are measured online. On the one hand polymers could be separated from each other by a single preparative column and characterized chemically. On the other hand current limits are exploited: Sensitivity in NMR spectra and resolution in chromatograms.

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OT755

Long coherence lifetimes for Zeeman and hyperfine transitions in $Nd^{3+}:Y_2SiO_5$ measured by pulsed EPR

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Rare earth ions in inorganic crystals are promising candidates in the areas of quantum computing and quantum state storage. For example, storage of entangled photons has been recently demonstrated in $Nd^{3+}:Y_2SiO_5$ [1]. In these experiments, the upper Zeeman level was used as a shelving state for spectral tailoring, although it could be interesting to take advantage of the coherence properties of the Zeeman or hyperfine transitions. Indeed, the former could be efficiently used as a quantum bit [2].

We performed pulsed EPR experiments to measure the coherence lifetimes of a 30 ppm - doped $Nd^{3+}:Y_2SiO_5$. For Zeeman transitions, results show strong angular dependence and T_2 up to 30 µs at 5 K. Pulsed ENDOR experiments allowed transferring the coherence from the electronic spin to the nuclear spin [3] with a hyperfine T_2 of 330 µs, which is promising for quantum storage. Last, a study on the sources of spectral diffusion in the material has been carried out.

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От756

Investigation of kinetik and dosimetric potential of gamma-irradiated calcium ascorbate

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In the present work, radiosensitivity and dosimetric potential of solid calcium ascorbate dihydrate (CaAs) were explored through a detailed electron spin resonance (ESR) study performed at various temperatures. Irradiated CaAs was observed to exhibit an ESR spectrum consisting of two main strong lines spread over a magnetic field range of 5 mT and centered at g = 2.0052. An evaluation technique based on the variations of the characteristic resonance line intensities and the spectrum area under different experimental conditions was adopted, to determine the spectroscopic, kinetic and dosimetric features of radical species responsible for the observed experimental ESR spectrum. Radicals exhibiting similar ESR characteristics to those reported in the literature for irradiated ascorbic acid and its sodium salt (1, 2) were shown to be also produced in gamma-irradiated CaAs.

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ACKNOWLEDGEMENTS:
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От757

Environmental response on seed germination potential by low frequency of electromagnetic field (ELF) and ELF modulated millimeter waves

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Our research work focuses on a comparative study of extremely low frequencies of electromagnetic field (EMF) and ELF-modulated millimeter waves (MMW) effects on winter-wheat seed germination potential. Different frequencies were used in the experiment, particularly 14, 15, 16 Hz, with the exposition lasting 15 minutes. The seeds are incubated in aqua medium having composition NaCl-4,680g; KCl-0,298g; CaCl2-0.777g; MgCl2-5,66g; C6H12O6-1,860g; pH -8,8-8,9 in 1000 ml distilled water for 72 hours. The mechanism of observable and changing effects modulations caused by structural changes of the processed water by LF EMF and ELF-modulated MMW are discussing.

The data obtained helped observe ELF EMF and modulated MMW has a frequency-dependent character of producing effect. On the other hand, it was observed that modulated MMW has significantly higher effect than ELF EMF, compared to the control group. The results obtained during of the experiments may give some of clarifications of the activation effect mechanisms of 15 Hz EMF on seeds germination, plants growth, which will show the efficiency of this method, in the field of food safety and environmental conservation.

OT758

EPR in the Structure Analysis of Human Serum Albumin

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Human serum albumin (HSA) is the most abundant protein in human blood plasma which serves as a carrier of fatty acids (FAs) and a diverse range of metabolites from the blood stream to target cells. Using spin-labeled FAs, 5- and 16-doxylstearic acid (DSA), the uptake of the FAs by the protein and their spatial distribution in the protein can be monitored by CW EPR, and nanoscale distance measurements can be obtained with DEER spectroscopy. The FA distribution provides an indirect yet effective way to characterize the structure of the protein in solution. While the distribution of 5-DSA is mainly consistent with the crystallographic data, 16-DSA is distributed much more homogeneously on the protein surface than suggested by the crystal structure [1]. Furthermore, the effect of several ionic liquids (ILs) on the solution structure of HSA is revealed by EPR. Addition of imidazolium-based ILs to an aqueous solution of HSA/FA conjugates is accompanied by significant destabilization and unfolding of the protein's tertiary structure. In contrast, HSA maintains its tertiary structure when choline dhp is added. The comparison of FA distance distributions in HSA with and without choline dhp surprisingly revealed that with this IL, the FA anchoring units are in better agreement with the crystallographic data. These results indicate that choline dhp as a cosolvent may selectively stabilize HSA conformations closer to the crystal structure out of the overall conformational ensemble [2].

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OT759

NMR characterization of the interaction of TOR regulatory domains with membrane-mimetic systems

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The target of rapamycin (TOR) is a highly conserved multidomain ser/thr kinase that regulates eukaryotic cell growth and survival in response to nutrient availability and environmental factors. TOR forms two functionally distinct complexes and has been localized at different cellular membranes and in the nucleus. However, the atomic details of TOR membrane-association and its dependence on the composition of each TOR-complex as well as on stimuli known to regulate TOR function are not known. The N-terminal HEAT repeats and presumably also the following FAT domain were suggested to mediate interactions with membrane and other cellular proteins. The FRB domain between the FAT and the catalytic domain provides the binding site for the TOR-specific inhibitor complex rapamycin-FKBP12 and was suggested to interact with the negatively charged phospholipid phosphatidic acid (PA). The C-terminal FATC domain plays an important role for the regulation of TOR function. Based on the NMR structure of the oxidized form and *in vivo* mutagenesis data, the cellular stability of TOR depends on the redox state of two conserved cysteines in the FATC domain (1). In order to better understand how TOR can associate with different cellular membranes and whether this is influenced by the presence of PA in cellular membranes or by redox-sensitive stimuli, the membrane-binding properties of the yeast TOR1 FATC domain (y1fatc) (2) and of the human TOR FRB domain (hFRB) have been characterized by solution NMR spectroscopy and additional biophysical methods.

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1.

OT760

Structure, lipid-binding, and cellular loczalization of the putative GTPase-binding domain of the formin family protein ForC

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Formins are widely expressed proteins that govern cell shape, adhesion, cytokinesis, and morphogenesis by remodeling the actin and microtuble cytoskeleton. *Dictyostelium discoideum* ForC lacks a canonical formin homology 1 (FH1) domain and has been shown to mediate cell movement during the multicellar stages of this social amoeba. Here, we present the structural and functional characterization of the N-terminal putative GTPase-binding domain (GBD) of ForC. Similar to the GBD of the human Formin FHOD, this domain shows an ubiqitin-like fold with an unusually long loop between the first two beta-strands. The positively charged surface area is considerably larger than that of other GBDs. Based on NMR- and CD-detected lipid-binding studies, the ForC GBD undergoes large structural changes if immersed in dodecylphosphocholine micelles, which occur at lower micelle concentrations if negatively charged phosphoinositides are present. However, differently composed bicelles and micelles composed of short-shain Diacylphosphocholines have not such an effect. This suggests that the ForC GBD may be sensitive to membrane patches with specific curvatures and compositions. Additional binding studies evaluated the role of the long loop for DPC micelle-induced structural changes. Based on live cell imaging, both the GBD and the subsequent Formin homology 3 (FH3) domain are required for subcellular targeting to cell-cell contact sites and actin-rich crown-like structures representing the precursors of phagocytic cups and macropinosomes.

OT761

Local Structure and Dynamics of silica/polyol hydrogels: A solid-state NMR study

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Organic-inorganic materials obtained by sol-gel processing are widely used in chemical, physical and mechanical applications. Such materials include siloxane polymers, periodic mesoporous organosilicas, organically functionalised porous silicas, silicon polyoxolates and silica hydrogels.[1-5] These materials are structurally and dynamically heterogeneous and show a lack of long range ordering, which precludes the use of standard diffraction techniques. Solid-state NMR is an ideal technique to probe amorphous hybrids on the molecular level

We have used solid-state NMR to investigate the organisation of silica/polyol hydrogels and correlate molecular structural information with temperature induced structural changes. Variable temperature ^{1}H and ^{13}C MAS NMR were used to monitor changes in local structure whilst T_1 and $T_{1\rho}{}^{H}$ relaxation times provided an insight into dynamics and the presence of domains. ^{1}H-^{13}C HECTORs gave a unique insight into the organic-inorganic spatial proximity. ^{23}Na MQMAS experiments tracked the change in sodium coordination upon formation of crystalline layered silicates, precluding application of such hydrogels.

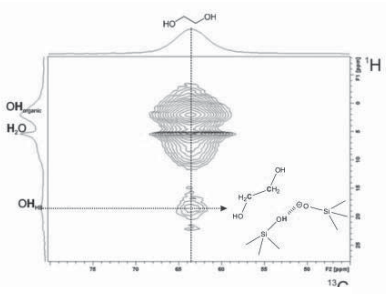

Figure. ^{1}H-^{13}C HETCOR NMR spectra of a silica/ethylene glycol hydrogel.

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OT762

The complex of Nitrite Reductase and Cytochrome c_{551}: a W-band EPR study of a single crystal.

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Copper Nitrite Reductases (CuNiR) are enzymes active in the catalysis of nitrite to nitrogen monoxide. In general, CuNiR folds in a structure that consists of three monomers, and each unit contains two distinct copper sites: a type1 site (T1Cu), where the oxidation of a redox-partner protein takes place, and a type 2 site (T2Cu), where the reduction of the nitrite takes place. The CuNiRs are further classified in two subgroups (blue and green), based on the spectroscopic properties of the T1Cu site, an indication that structurally similar sites may show profound differences in their electronic structure. In order to deepen our understanding of the biological activity of CuNiR, we have studied the complex of the blue CuNiR from *Achromobacter xylosoxidans* with its redox-partner Cytochrome c_{551}, which carries a heme as the active site[1]. Here we report on the first EPR study of a single-crystal of such a complex. At W band the T1Cu and T2Cu spectra overlap, so the copper in the T2Cu site of the CuNiR was substituted by zinc. The g matrix of the T1Cu was determined, both the principal values and the direction of the principal axes, which is most informative as regards the electronic structure. The heme-g_z transition is found to be shifted towards higher fields in the complex, which might well be related to the fact that a methionine residue of the CuNiR provides the sixth ligand for the heme-iron. The direction of the heme-g_z principal axis was determined. The mutual orientation of the g axes of the redox-partners provides insight into the change of their electronic structure upon complex formation.

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OT763

Light-induced EPR study of charge transfer in polymer/oligomer blends for organic photovoltaics

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Organic conjugated compounds are promising for the realization of low-cost, large-area electronic products such as organic solar cells. The 3D bulk heterojunction (BHJ) concept for solar cells starts from an intimate blend of donor ('p-type') and acceptor ('n-type') compounds. While a large number of p-type compounds is now available, reports about n-type organic oligomers are still scarce.

We present a light-induced electron paramagnetic resonance (EPR) investigation of a series of hexyl-substituted bisthiophene compounds (D1-D4) containing a thiazolothiazole(5,4-*d*) unit [1] . Compounds D1-D4 were blended in different ratios with the standard p-type polymer MDMO-PPV (poly[2-methoxy-5-(3,7-dimethyloctyloxy)]-1,4-phenylenevinylene) to investigate electron transfer under illumination, an essential step in the photovoltaic process. X-band (9.44 GHz) EPR of these polymer/oligomer blends were measured before and after illumination (argon ion laser, λ = 488 nm). This is compared with the results in pure compounds D1-D4 and in MDMO-PPV and related to photoluminescence quenching experiments. The acceptor capacity as observed in the light-induced experiments in the blends is anti-correlated with the efficiency of iodine doping of the pure oligomers.

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OT764

Quantitative Online NMR Spectroscopy for Reaction Monitoring in Chemical Engineering

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Online NMR spectroscopy facilitates monitoring processes under industrially relevant conditions in measurements that are non-invasive (no change of temperature, pressure or sample composition). A typical setup is an external reactor directly coupled to an NMR spectrometer equipped with a flow cell. Without any sample preparation the reaction mixture is pumped through the NMR flow cell, spectra are recorded and the mixture flows back to the reactor. This set up is ideal for studies of reactions with half-times down to several minutes [1].

For monitoring faster reactions a static micro-mixer is directly mounted below the NMR magnet; syringe pumps provide a pulse free continuous flow of the reactants through the NMR flow cell. This new development allows monitoring reaction kinetics with half-times of less than one minute.

A new design of a fully liquid-thermostated NMR flow probe head is presented that contains a micro-mixer coupled with a capillary NMR probe. The combination of a solenoidal micro-coil flow probe with a micro-mixer within the probe head allows monitoring very fast reactions. The design and the manufacturing of the new probe head were carried out in a co-operation with the Institute of Micro Mechanics (IMM), Mainz. It is shown that this new probe head is suited for obtaining reliable quantitative data on complex reacting mixtures with reaction times of the order of seconds.

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OT765

α-Lactalbumin, engineered to be non-native and inactive, kills tumor cells when in complex with oleic acid: A new biological function resulting from partial unfolding

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HAMLET (human alpha-lactalbumin made lethal to tumor cells) is a biomolecular assembly consisting of partially unfolded protein and fatty acid and has been demonstrated to be tumoricidal in > 40 cancer cell lines while leaving healthy, differentiated cells intact. Because native α-lactalbumin itself cannot trigger cell death, HAMLET's remarkable tumor-selective cytotoxicity has been correlated with the partially unfolded nature of the protein, but whether a recovery to the native state is required upon entering the tumor cell is yet unclear. Here we utilize pulsed-field diffusion NMR techniques to estimate the hydrodynamic properties of a recombinant variant of human α-lactalbumin ($rHLA^{all\text{-}Ala}$) that renders the protein non-native and biologically inactive under all conditions. While this analogue protein-fatty acid complex ($rHLA^{all\text{-}Ala}$–OA) exhibited tumoricidal activity equivalent to HAMLET, the fatty acid-free $rHLA^{all\text{-}Ala}$ protein lacked any cytotoxic activity. Structurally, NMR experiments show that $rHLA^{all\text{-}Ala}$-OA showed significant differences to HAMLET, devoid of any tertiary packing. The results identify α-lactalbumin as a protein with strikingly different functions in the native and partially unfolded states. We posit that partial unfolding offers another significant route of functional diversification for proteins within the cell.

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OT766

Exploring Quadrupolar Surface Sensitivity with Hyperpolarized ^{131}Xe

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Noble gas isotopes have received increased attention for their use in exploring materials, surfaces, and as a biomarker for lung magnetic resonance imaging (MRI). Of the noble gas isotopes that are available, three isotopes (^{21}Ne, ^{83}Kr, ^{131}Xe) are quadrupolar (spin I > ½) and therefore are sensitive to changes of the surrounding environment, particularly the surfaces the gases briefly adsorb onto. During these brief adsorption periods, changes in surface chemistry, temperature, surface-to-volume ratio, and the presence of co-adsorbing species influence the quadrupolar behavior of these isotopes. In the case of ^{131}Xe, these changes can be observed in the nuclear magnetic resonance (NMR) spectrum directly as a quadrupolar splitting ($2\nu_Q$). This splitting is study here, utilizing hyperpolarized ^{131}Xe, to understand the effects of the surface on the quadrupolar splitting and to study the use of surface sensitive probes for material and biomedical diagnosis.

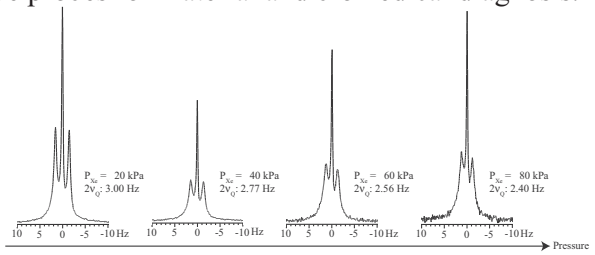

Fig. 1. NMR spectra of hp ^{131}Xe at different pressures on a glass surface. The quadrupolar splitting of the ^{131}Xe decreases as the pressure of xenon gas increase. This decrease is attributed to changes in the gas phase contribution of the $2\nu_Q$ given that the surface is unchanging between experiments.

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OT767

EPR CHARACTERIZATION OF [HEME-IMID-PYR] MODEL COMPOUNDS

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Hemeproteins are ubiquitous in living organisms and act in many biological processes as charge carriers, catalysts and other. Heme model compounds are useful for understanding the relationship between heme center structure and biological functions of hemeproteins. Heme model compounds can be characterized by different EPR techniques, including CW-EPR and advanced EPR methods as ESEEM and ENDOR.

Many different heme centers with bis-imidazole derivatives and bis-pyridine derivatives have been characterized[1]. In this work, we present the EPR characterization of a heme model compound with one imidazole and one pyridine as axial ligands. This is a model for the structure of natural existing hemeproteins, as the nicotinate-ligated leghemeglobin[2]. The synthesis of this model compound depends on the affinities of imidazole and pyridine, and on the solvents. The different chemical affinities of the ligands, both as first and second ligand, were evaluated by means of quantitative EPR measurements, and a CW-EPR spectrum corresponding to the heteroligated compound was obtained and analyzed. From this study, parameters characterizing the ligand enviroment and the electronic structure of iron within the heme center were obtained. Also pulsed EPR measurements were performed. The analysis of the results gave insights on the hyperfine couplings of the unpaired electron with nearest nuclei.

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OT768

Electronic structure of Mn bound to modified bacterial reaction centers

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The properties of Mn bound to highly oxidizing reaction centers of *Rhodobacter sphaeroides* were studied in a mutant modified to possess a metal binding site at a location comparable to the Mn cluster of Photosystem II. The modified highly oxidizing reaction center, identified as the M8 mutant, has a binding site with two carboxylates (Glu M168 and Glu M192) and two histidine residues (M173 and M193). In addition, the binding site contains Tyr M164, which is located in a similar position to Y_z in photosystem II,[1,2] Gly M288 and Asp M292. Steady state light-minus-dark optical spectra demonstrate the binding of Mn^{2+} ions to the M8 mutant. The dissociation constant, K_D, was found to be pH independent above pH 8.2 (at ~1.5 μM) and below pH 8.2 increases with decreasing pH, to a value of 85 μM at pH 7.6. This Mn-binding mutant shows a Mn^{2+} electron paramagnetic resonance (EPR) signal in the dark with no EPR signal from the manganese after illumination while mutants with only the binding site or only a highly oxidizing donor showed no difference upon illumination. The EPR spectrum of the bound metal has a distinctive spin 5/2 signal that can be fitted as arising from multiple "allowed" and "forbidden" electronic transitions. Electron spin echo envelope modulation (ESEEM) spectra showed the involvement of two different histidine nitrogen ligands (N_ε and N_δ) in the binding. Together, the data demonstrate the tight binding of $MnCl_2$ to the reaction center with the metal being redox active and serving as a secondary electron donor.

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OT769

Rapid measurement of methyl pseudo-contact shifts in paramagnetic proteins

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Pseudocontact shifts (PCSs) are important structural constraints in the calculation, refinement and validation of high-resolution 3D structures of metalloproteins. In an attempt to assign the PCSs of ^{1}H and ^{13}C spins belonging to various methyl groups present in the core of paramagnetic proteins, we propose a reduced-dimensionality based methodology denoted as the (3, 2)D CT-HCCH-COSY[1] experiment. This enables rapid data collection and assignment of the PCSs of CH_3 groups of Ala, Ile, Leu, Met, Thr and Val residues in paramagnetic proteins. This methodology is based on the spectral editing the individual ^{13}C-^{1}H signatures of all the methyl resonances of Ala, Ile, Leu, Met, Thr and Val residues in both uniformly and fractionally (10%) ^{13}C-labelled protein. By using this methodology the inherent problem of resonance overlap, a major problem in this spectral region, which is further aggravated due to the presence of peaks both from diamagnetic and paramagnetic species, could be overcome. We will demonstrate the utility of this experiment on the protein calbindin ($[Ca^{2+}][Yb^{3+}]$Cb).

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OT770

Compact rheo-TD-NMR System for Online Monitoring of Polymerization

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Significant macroscopic and microscopic information regarding the progress of a polymerization process is gained form the flow curve and transverse relaxation, respectively. The presented work aims at the development of a reliable system suited for process analytics in an industrial environment.

Whereas measurements at several flow rates and iterative corrections are required for conventional capillary rheometry, NMR-based capillary rheometry yields the flow curve at a single flow rate. This requires the measurement of the velocity profile, of its Abel transform, or of the velocity probability density function (VPDF) [1].

Decay of transverse magnetization as measured by a CPMG sequence on a liquid flowing through an inhomogeneous rf field is enhanced compared to the result in the case of a static fluid and homogeneous field. A correction is presented based on the rf profile and the VPDF.

In order to achieve a velocity-independent polarization, a pre-polarization pipe consisting of Halbach arrays was realized. A flow cell with an inductively coupled rf coil for pressures up to 2 MPa at 423 K was developed. The permanent-magnet system provided by Bruker, Rheinstetten is equipped with a three-axis gradient system.

A characterization of the system and first results are presented.

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OT771

Oil Demulsification Investigation: D-T_2 Low Field NMR Mapping Analysis

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The aim of this work was to use low field NMR[1,2] in demulsification studies through D-T_2 mapping. Emulsions were prepared using a crude oil (°API 28.9) and a 50 ppm $MnCl_2$ aqueous solution, using 20 wt.% of the solution. A 3 minutes of stirring, in 6.500 rpm, with a Ultra-Turrax homogeneizer methodology was applied. The D-T_2 mapping was made for three differrent situations: a) the pure emulsion; b) the emulsion with the demulsifier A and c) the emulsion with the demulsifier B. In each case, the sample was monitored for 14.5 hours, resulting in 25 maps spaced by 35 minutes. Commercially available demulsifiers were used at ~400 ppm. Reference experiment shows an undefined shape distinguishing oil and water signals since the beginning (Fig. 1a). The slightly difference comes from the T_2 distribution axis, but the addition of demulsifier (Fig. 1b and 1c) avoided this behaviour by reducing T_2 from water hydrogens. Demulsifier A breaks the emulsion, giving similar maps for subsequent experiments. Smoother demulsification happens when using demulsifier B (Figure 1c). Clearly, demulsifier B performance was overcomed by the demulsifier A, considering their demulsification rates. This effect could be easily noticed by D-T_2 maps, and these results highlighted its potential in such studies.

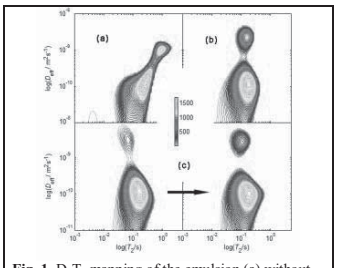

Fig. 1. D-T_2 mapping of the emulsion (a) without demulsifier, 67 min, (b) demulsifier A, 33 min and (c) demulsifier B, at 102 min and 664 min

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OT772

Characterization of the Ribosomal Binding Site for Thiostrepton

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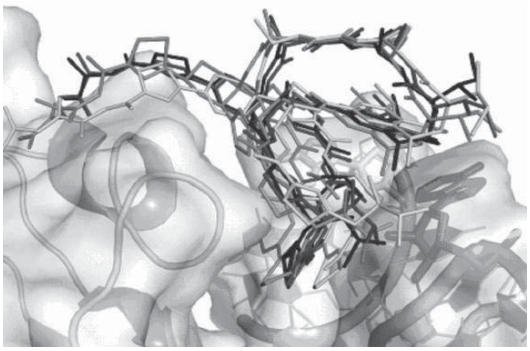
The thiazole-containing antibiotic thiostrepton prominently inhibits protein biosynthesis by tightly binding to the ribosomal GTPase-associated region (GAR). The conformation and dynamics of the ternary GAR RNA - L11 protein - thiostrepton complex was studied before by NMR, explaining how the elongation factor function gets disrupted [1]. To investigate the molecular scaffold in more detail, the bioactivity of thiostrepton and several derivatives with selected molecular changes were determined at the ribosomal binding site [2]. The compounds were obtained by semisynthesis and characterized using NMR structure determination, docking and biological evaluation studies. The combination of these techniques revealed important conformational and structural features for molecular recognition within the embedded pharmocophore of the target and facilitates future compound optimization.



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OT773

A tunable general purpose Q-band Resonator for CW and pulsed EPR/ENDOR experiments for large samples and optical excitation.

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We describe a frequency tunable Q-band cavity (34 GHz) designed for CW and pulsed Electron Paramagnetic Resonance (EPR) as well as Electron Nuclear Double Resonance (ENDOR) experiments [1]. The (self supporting) ENDOR coil consists of four 0.8 mm silver posts at 2.67 mm distance from the cavity center axis, penetrating through the plunger heads. The coil is very robust and electrically well shielded in order to enable CW ENDOR experiments with high RF power (500W). The top plunger of the cavity is movable to allow a frequency tunability of ± 2 GHz. Microwave matching to the resonator is accomplished through an iris in the cylinder wall and a sliding short in the coupling waveguide. Optical excitation is enabled through slits in the cavity wall (transmission ~60%). The resonator accepts 3 mm o.d. sample tubes. This leads to a favorable sensitivity especially for pulse EPR experiments of low concentration biological samples. It is demonstrated that, due to the relatively large active sample volume (20-30 µl), the described resonator has superior concentration sensitivity over commercial pulse Q-band resonators. The quality factor (Q_L) of the resonator can be varied between 2600 (critical coupling) and 1300 (over coupling). Shortest achieved π-pulse durations are 40 ns for microwave (3W) and 20 µs for radio frequency (ENDOR) pulses (^{1}H, 300W), respectively, allowing for excellent performance both for pulsed EPR and ENDOR spectroscopy.

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OT774

Kinetics and activation parameters of the reaction of pentavalent organic arsenic compounds with glutathione

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The investigation of the interaction of inorganic and organic arsenic compounds with biomolecules such as glutathione (GSH) is still of high interest. Structures of the conjugates formed in the reaction of GSH with several phenylarsonic acids were elucidated by routine 2D-NMR methods, revealing remarkable spectra due to diastereotopicity. After the NMR assignment we have investigated the systems' reaction kinetics.

The reaction consists of two steps: the reduction of pentavalent arsenic compounds to their trivalent analogues and the subsequent formation of the conjugate with GSH. Interestingly, in all cases the redox reaction is the rate determining step whereas the conjugation step is remarkably fast.

Former investigations dealt with the reaction order and the half life values, showing that phenylated arsenicals react faster with GSH than methylated compounds. These results are maybe due to steric or electronic reasons. The ranking of the reaction rates, which is at least within the aromatic compounds correlating with their toxicities, is now investigated concerning the activation parameters of the reactions. Therefore the temperature dependence of the reactions was monitored online by ^{1}H-NMR. The obtained data were evaluated according to Arrhenius and Eyring theory, with focus on the activation energy, activation enthalpy and entropy. With the help of these results we tried to gain new insight into the mechanism of the reaction of arsenic compounds with GSH.

OT775

NMR “Crystallography” of Enzyme Models

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Structural studies of enzymes and enzyme models are often restricted to the use of crystalline samples. Unfortunately, sometimes it may not be possible to crystallize a certain compound. Or, the crystalline structure of a compound differs from its native form in meanings of conformation and dynamics, respectively. In contrast, the REDOR (Rotational Echo Double Resonance) NMR experiment is a robust method to study samples in solid state that do not need to be crystalline at all.(1)

The aim of our work presented here is to investigate the structure of nickel superoxide dismutase (NiSOD). With respect to the active site of this enzyme, appropriate and functional peptide-based model compounds are synthesized and studied.

Because of the unique role of Proline in biochemistry, e.g. in peptide folding and function of biochemical processes, its influence on the active site is of particular interest.(2) Proline’s most distinct property is the unusual distribution of *cis-/trans*-isomers in amide bonds, that is, next to other reasons, dependent on crystallization conditions.(3) To get informations about the actual role of Proline in the active site of NiSOD, triazole-based *cis*-Prolyl mimetics are inserted into the peptide sequence and investigated for their effects on conformation and activity.(4)

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OT776

Parahydrogen signal enhancement for zero-field NMR: NMR without magnets.

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Now that the gigahertz (23.5T) barrier for protons has been broken in the quest for higher-field NMR, several groups have presented alternative approaches, where NMR information is obtained in fields as low as the earth field, or even in the absence of any magnetic field at all [1-5]. These approaches show advantages such as high resolution and potential portability, however, when thermal polarization is used, they all suffer from low sensitivity. We address this shortcoming by adapting the hyperpolarization techniques based on parahydrogen [6] to zero-field NMR with detection based on an atomic magnetometer[7]. We show that it is possible to get high sensitivity either with hydrogenative (similar to ALTADENA) or non-hydrogenative (SABRE [8]) processes. Chemical fingerprinting is achieved by measuring zero-field J-coupling parameters. The result is NMR without any magnets.

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OT777

From chemical shift data through prediction to assignment and NMR LIMS - multiple functionalities of nmrshiftdb2

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Nmrshiftdb2 has been available as a community-based NMR database since 2002. During that time a continuously growing set of currently more than 40000 structures with 48600 spectra could be established. These data are freely available (*nmrshiftdb.org*) and cannot only be searched but can also be downloaded and used for scientific investigations. Supplementary to the database, supplementary software was developed including an NMR lab administration system.

Recently, there were some changes in the team and we had a rebranding to *nmrshiftdb2*. Now, we would be very interested to enter discussion about the project and its future perspectives by receiving feedback from former, current and potential users of different areas. We will discuss the state of this project, e.g. software functions, data collection, and use for research.

An important new feature is the laboratory information management system (LIMS) which has been developed with the intention to better integrate the database functionality into an academic NMR laboratory environment. *Nmrshiftdb2* now allows NMR laboratories at the same time to administer and account for their users, instruments and measurements and use the known database functions from the same surface.

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OT778

Hyperpolarization of noble gases via spin-exchange optical pumping using line-narrowed laser sources to achieve high levels of spin polarization

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The use of hyperpolarized gases such as ^{129}Xe (spin I = 1/2) and ^{83}Kr (spin I = 9/2) allow for enhanced signal in a number of NMR and MRI applications. There has been significant interest in recent years for using these gases to investigate pulmonary diseases such as chronic obstructive pulmonary disease (COPD) and asthma[1].

Spin-exchange optical pumping (SEOP) can be used to generate hyperpolarization in these gases. It is desirable to optimize this process so that the greatest hyperpolarization, and therefore signal strength, can be achieved. This work investigated the SEOP process using a line-narrowed laser source under various conditions. Previous works have reported ^{83}Kr polarizations of up to 4.4%[2]. Optimized SEOP parameters based on physical effects investigated during this study have allowed polarizations exceeding 10% to be achieved.

T_1 weighted quadrupolar relaxation had previous been reported for ^{83}Kr making it a sensitive surface probe[3]. This work has observed that T_2 measurements in model systems for ^{83}Kr are surprisingly long, making it favourable for MRI studies.

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OT779

Slim-line logging NMR tool to measure soil humidity in situ

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Soils are an important factor for the food production and for the balance in many ecosystems. In areas where water is scarce, the need to improve its usage has sparked the research of flow in soils. Nowadays, as the demand for reliable on-field characterization of soils grows, the realization of heterogeneity in the soil features has fuelled the development of experimental techniques and theoretical models, which can help elucidating the inherent character of the soil under research.

The measurement of soil moisture via NMR was among the first applications of ex-situ instruments [1]. In the last years the first prototype of a slim-line NMR logging tool has been reported to measure the soil water content in the vadose zone [2]. Following a procedure similar to the one developed for well-logging NMR, a cylindrical sensor is lowered into the ground through permanently installed plastic pipes. The sensor reported here differs from commercial oil-well logging sensors in its much smaller diameter and the fact that the measurement is conducted in an environment naturally unshielded from electro-magnetic noise.

In this work we report the optimization of a large rf coil designed to provide large penetration depth at high sensitivity. Considering that the outer diameter (50 mm) of the sensor is fixed the diameter of the magnet was optimized taking into account the trade off between the field strength (increases with the diameter of the magnet) and the effect of the coupling between the rf coil and the shielding covering the magnet. As the size of the magnet increases the coupling also does, lowering the efficiency of the rf coil. The sensitivity improvement achieved by increasing the size of the rf coil to excite a larger volume allowed us to set the measurement depth at 20 mm into the soil. At this position the effect of the perforation and pipe instalation on the soil structure is expected to be negligible.

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OT780

Study of the structure activity relationship of an enzyme model with YASARA

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The enzyme model studied is based on 7 residues of the nickel-superoxide-dismutase (NiSOD). This SOD-class enzyme (EC 1.15.1.1) was discovered in 1996.[1] The active site is formed by the first 6-12 N-terminal amino acids.[2] This fact enabled the development of biologically active enzyme models, based on these residues.[3] The interaction between one of these models and the substrate was studied in our group by inserting cyanide as inhibitor.[4] The resulting complex was studied by several spectroscopic methods, in particular REDOR-NMR.[5]

This solid state NMR technique allows calculating distances between adjacent spins via determining the strength of dipolar coupling between these spins.[6] The resulting distances are used to determine the exact position of the substrate analogue in the active site. The enzyme-substrate-adduct was studied with YASARA[7] to shed new light on the mode of action of NiSOD. YASARA (Yet Another Scientific Artificial Reality Application) is a program designed for MD-simulations, energy minimizations and visualization of several properties of proteins and small molecules.

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OT781

Domain-selective and amino acid-specific labeling of multi-domain proteins for NMR studies

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Besides enhanced spin relaxation rates, structural studies of large proteins by NMR are often complicated by significant overlap of signals in the NMR spectra. Segmental labeling offers an avenue to reduce spectral overlap by providing domain-specific isotope labeling[1]. However, overlap can still prevail in proteins possessing extended segments of α-helical or non-regular secondary structure. Here we present a strategy which combines amino acid-specific labeling achieved using cell-free expression with domain-specific labeling obtained by expressed protein ligation, which yields multi-domain proteins with selective amino acid-type labeling of a single one of these domain. We have optimized our cell-free expression strategy to obtain milligram amounts of ligation-competent proteins containing isotope labels on one or several amino acid types. These are then ligated with unlabelled domains produced either in cells or by cell-free expression. To test our protocol, we have applied the strategy to the RRM domains 3 and 4 of the human polypyrimidine tract binding protein and to two helix-turn-helix domains from human glutamyl-prolyl tRNA synthetase. The results are documented with NMR spectra.

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OT782

Study of provenance of river sediments: Applications of ESR and TLCIs

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Quartz is one of the most abundant minerals on the surface of the earth. ESR dating of quartz, therefore, has been applied to a wide variety of samples, such as fault gouge, volcanic tephra, sediments, and flint artifacts.[1] Another direction of studies is to utilize the ESR signals as a marker of the samples like isotope analysis. The intensity of the E_1' center in quartz is shown to be a useful parameter to investigate the provenance of Aeolian dust as well as of sediments.[2] As the intensity correlates with the age of the host granite in the range 10 Ma to 1 Ga, it is possible to distinguish quartz grains of Tertiary origin from those of Cretaceous. The Al, Ti-Li and E_1' center signal intensity of natural quartz grains were irradiated with 2.5kGy gamma doses, as a means of estimating sediment provenance.[3] Various samples were prepared from the host rocks and the sediments of Tertiary and Quaternary around Kizu River and Saho River in Japan. In this study, it will be discussed to estimate those sediments provenance. If this technique is established, it will be useful to elucidate the provenance of river basin and the encroachment of mountain.

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