

Contents

1	Introduction	1
1.1	Electron Paramagnetic Resonance	1
1.2	Bioenergetics	2
1.3	Aim of This Work	3
1.4	Outline	3
1.5	Publications	5
2	Mitochondrial Respiration	7
2.1	The Respiratory Chain	7
2.2	Complex I	12
2.2.1	Structure and Function	12
2.2.2	Cofactors	14
2.3	Iron-Sulphur Clusters	18
2.3.1	Structure and Function	18
3	Theory	23
3.1	Static Spin Hamiltonian	23
3.1.1	Energy Level Diagramm	28
3.2	Spin Echoes – Classical Description	29
3.3	Density Matrix in EPR	33
3.3.1	Expectation Value	33
3.3.2	Statistical Ensemble	34
3.3.3	Liouville-von Neumann Equation	35
3.3.4	Application	36
3.4	2-Spin Model System $S = \frac{1}{2}, I = \frac{1}{2}$	40
3.4.1	Static Hamiltonian	40
3.4.2	Product Operators	42

3.5	Nuclear Modulation Effects	44
3.5.1	Two-Pulse ESEEM	48
3.5.2	Three-Pulse ESEEM	51
3.5.3	2D Three-Pulse ESEEM	58
3.5.4	HYSCORE	59
3.5.5	DONUT	65
3.6	Double Resonance Experiments	66
3.6.1	Davies-ENDOR	68
3.6.2	Mims-ENDOR	71
3.7	Relaxation	72
3.7.1	Longitudinal Relaxation	75
3.7.2	Transversal Relaxation	78
4	Materials and Methods	81
4.1	Instrumentation	81
4.2	Test Samples	82
4.2.1	Polystyrene Samples	82
4.2.2	Copper-doped <i>l</i> -Histidine Hydrochloride	83
4.2.3	DANO	83
4.2.4	Complex I	84
4.3	Spectral Analysis	84
4.3.1	Fourier Transformation	84
4.3.2	Apodisation	85
4.3.3	Mathematical Filtering	86
4.3.4	Data Processing of Time-Domain Data	87
4.4	Spectral Simulations	88
4.4.1	Orientation Selection	89
4.4.2	Simulation of ESEEM Spectra	91
4.4.3	Simulation of ENDOR Spectra	93
5	REFINE – Biological Application	95
5.1	Abstract	95
5.2	Introduction	96
5.3	Materials and Methods	99
5.4	Results	102
5.4.1	Model Compounds	102

5.4.2	Complex I	107
5.5	Discussion	109
5.5.1	Model Compounds	109
5.5.2	Complex I	111
5.6	Conclusions	114
6	REFINE – Methodology	115
6.1	Abstract	115
6.2	Introduction	116
6.3	Experimental	118
6.3.1	Sample Preparation	118
6.3.2	Instrumentation	118
6.3.3	Data processing	120
6.4	Results	120
6.5	Discussion	122
6.6	Conclusions	126
6.7	Acknowledgements	127
6.8	Appendix	127
6.8.1	Suppression of the fast-relaxing component	128
6.8.2	Suppression of the slowly relaxing component	129
7	Complex I studied by Pulsed EPR	131
7.1	Introduction	131
7.1.1	EPR Spectroscopy	131
7.1.2	Complex I	132
7.2	Materials and Methods	134
7.2.1	Preparation and Instrumentation	134
7.2.2	Spectral Simulations	134
7.3	Results	135
7.3.1	Temperature Dependency of the EPR Signal	135
7.3.2	One- and Two-dimensional ESEEM Spectroscopy	136
7.3.3	Comparison of the ^{14}N -ESEEM spectra at two different pH values	140
7.3.4	H ₂ O-to-D ₂ O Exchange	141
7.4	Discussion	142
7.4.1	Temperature Dependency of the EPR Signal	142

7.4.2	One- and Two-dimensional ESEEM Spectroscopy	143
7.4.3	Comparison of the ^{14}N -ESEEM spectra at two different pH values	149
7.4.4	H_2O -to- D_2O Exchange	150
7.5	Summary and Conclusions	151
8	Relaxation Filtered EPR Spectroscopy	153
8.1	Introduction	153
8.2	Data Inversion	154
8.2.1	Practical Aspects	157
8.3	Numerical Simulations	158
8.3.1	Two Species	158
8.3.2	More Species	161
8.4	Application	163
8.5	Conclusions and Outlook	164
9	Summary and Outlook	167
9.1	Summary	167
9.2	Outlook	170
10	Deutsche Zusammenfassung	173
A	Mathematics and Constants	179
A.1	Euler Angles	179
A.2	Spin Operators	181
A.2.1	Direct Product	181
A.2.2	Spin Matrices for a System $S = \frac{1}{2}$, $I = \frac{1}{2}$	181
A.3	Transformation of Propagators	182
A.4	Eigenfrequencies of the Model System	183
A.5	Constants	185
A.5.1	Magnetogyric Ratios for Some Selected Nuclei	185
B	Complex I	187
B.1	G-Tensor Values	187
C	Source Codes	189
C.1	OrientSelect	189

C.1.1	Input Parameter File	189
C.1.2	OrientSelect	190
C.2	AnalyseREFINE	195
C.2.1	AnalyseREFINE – Main Program	195
C.2.2	AnalyseREFINE – CalcAmp	198
C.2.3	Input File	198
D	REFINE-ESEEM – Phase Cycle Sequence	201
E	REFINE-PELDOR	203
E.1	PELDOR	204
E.2	REFINE-PELDOR	205
	List of Figures	227
	List of Tables	231
	Curriculum Vitae	233