## Chapter 1

## Introduction

In 1988, Johann Deisenhofer, Robert Huber and Hartmut Michel were awarded the Nobel price in chemistry for the determination of the three-dimensional structure of a photosynthetic reaction centre from the purple bacterium *Rhodopseudomonas viridis* [1]. In 1995 Richard Cogdell and coworkers were the first to resolve the x-ray structure of the peripheral light-harvesting 2 complex from another purple bacterium called *Rhodopseudomonas acidophila* [2]. The progress made in high-resolution structural studies of the photosynthetic unit of these bacteria has strongly stimulated experimental and theoretical investigations to understand the fast and efficient energy transfer and tunability of their spectral properties [3–10].

In purple bacteria the sunlight is absorbed by a network of antenna pigment proteins and subsequently the excitation energy is efficiently transfered to the photochemical reaction centre (RC) where a charge separation takes place providing the free energy for subsequent chemical reactions. It is known that most of these bacteria contain two types of antenna complexes, the central light-harvesting complex 1 (LH1) and the peripheral light-harvesting complex 2 (LH2) [11]. LH1 and the RC are closely associated and form the so called core complex, whereas LH2 is not in direct contact with the RC but transfers the energy to the RC via the LH1 complex [12]. LH2 is known to comprise two distinct bacteriochlorophyll (BChl) *a* pigment pools which are labelled B800 and B850, whereas the LH1 complex presumably comprises only one pigment pool labelled B870. The denotations of the pigment assemblies correspond to their room-temperature absorption maxima in the near infrared. Based on the homology between the LH1 and LH2 proteins together with theoretical modelling a scheme of the arrangement within a photosynthetic unit (PSU) has been proposed in which the core complex is surrounded by several LH2 complexes in a two dimensional structure [13, 14]. However, despite the tremendous progress in the field that has been achieved during the last decade important details, for example the structure of the LH1-RC complex or the precise supramolecular organisation of the PSU, are unknown and currently an issue of hot debate.

By now, it has been established that the spatial structure of photosynthetic complexes, especially the mutual orientation of the pigments, determine to a large extent their spectroscopic features and excited-state dynamics [15]. These assemblies of repeating non–covalently bound molecular units show intermediate features between an individual molecule and a crystal, which makes them suitable model systems to study different types of intermolecular interactions in great detail [16–23]. For the B870 system of LH1 and the B850 pool of LH2 the excited states can be described in terms of delocalised Frenkel excitons whereas for the B800 molecules the excitations can be treated in first approximation as being localised on an individual BChl *a* molecule [24–29].

Generally, information about the parameters that determine the description of the electronic structure of light-harvesting complexes can be obtained by optical spectroscopy. But even isolated protein-pigment complexes of photosynthetic systems are rather complex, and it has proven difficult to analyse the excited-state properties of these systems in all details. This is mainly caused by a pronounced disorder, which masks details in the steady-state optical spectra, even at low temperature. Therefore, in this thesis, the light-harvesting complexes from purple bacteria were investigated by applying single-molecule spectroscopic techniques. The intriguing feature of this technique is that it allows to elucidate information that is commonly washed out by ensemble averaging. Besides the possibility to circumvent spatial inhomogeneities it allows also the observation of dynamical processes which are usually obscured by the lack of synchronisation within an ensemble. A single molecule that undergoes a temporal development between different states is at any time in a distinct, well defined state and the whole sequence of steps can be studied. This allows in particular to identify short-lived intermediate states that might be essential for the understanding of the process under study but which would be completely masked otherwise.

Such dynamical processes can be observed in light-harvesting complexes when looking at the interaction of the chromophores with amino acid residues of proteins in their local environment. Conformational fluctuations of the backbone residues are equivalent to rearrangements of their atoms, and chromophores embedded in the protein experience those changes as fluctuations in the local interactions and react with changes of their electronic energies. This makes them suited to act as local probes for monitoring the dynamics of a protein and to test the validity of the model describing protein dynamics and folding put forward by Frauenfelder and coworkers, which proposes that the energy landscape of proteins is arranged in hierarchical tiers [30–33].

Since the beginnings of single-molecule spectroscopy in the late 1980s [34, 35] the field underwent a breathtaking progress away from its cryogenic roots and especially the application of single-molecule *detection* techniques under ambient conditions in biology and biochemistry has led to a revolution in these disciplines (see for some examples [36–42]). However, the low temperature approach allows to study single molecules over a very long observation period because photobleaching effects of the probe molecules, usually limiting the observation time to some tens of seconds under ambient conditions, are negligible. This offers the opportunity to determine the electronic eigenstates of an individual system, i.e., to perform single-molecule spectroscopy rather than merely detection and to apply many experimental techniques from the highly developed toolbox of spectroscopy also to single objects [43–48].

This thesis is organised as follows: in chapter 2 the photo-physical and biological properties of the photosynthetic apparatus of purple bacteria are introduced. In chapter 3 sample preparation and the single-molecule setup is described. In chapter 4 the B800 band of LH2 is studied, and the pigment-pigment interaction, energetic disorder within and between complexes and the dynamics in the energy landscape of the proteins in the binding pockets are discussed. In chapter 5 a pattern recognition approach is employed to gain information on spectral diffusion processes in the B800 band as well as on the line shapes of the individual B800 absorption. Chapter 6 deals with the delocalised Frenkel excitons in the B850 band and touches on the types of energetic and structural disorder within the LH2 complex. The large structural heterogeneity of the core complex (LH1-RC) is looked at in chapter 7 and possible pathways of protons leaving the RC into the lipid phase of the membrane for further biochemical processing are discussed. Finally, in chapter 8 the observation of energy transfer from LH2 to the core complex within a single PSU is presented and the affinity of its building blocks is evaluated.

## Light harvesting complexes

### 2.1 Photosynthesis

Living organisms are commonly divided into two groups. The first one comprises the so called *autotrophic* organisms, which are capable of self-nourishment by using inorganic matter as the main source of carbon. They obtain the energy for life processes from the oxidation of inorganic elements (*chemotrophic*) or from radiant energy (*phototrophic*). The second group contains the so called *heterotrophic* organisms, which are capable of deriving energy for life processes only from the decomposition of organic compounds. They are unable to use inorganic compounds as sole sources of energy or for organic synthesis [49, 50].

The process by which phototrophic organisms can produce organic substances from pure inorganic compounds using electromagnetic radiation as energy source is called photosynthesis. Nearly all organic matter on earth is formed by these photosynthetic processes and life on earth would shortly come to a complete standstill if photosynthesis ceased to function.

In green plants carbon dioxide is reduced by water under the illumination with light finally leading to the synthesis of glucose:

$$6 \operatorname{H}_2 \operatorname{O} + 6 \operatorname{CO}_2 \xrightarrow{\operatorname{hv}} (\operatorname{CH}_2 \operatorname{O})_6 + 6 \operatorname{O}_2.$$

$$(2.1)$$

Here, the water molecule acts as electron donor and the carbon dioxide functions as electron acceptor. Other electron donors (e.g.,  $H_2S$ ) and acceptors (e.g.,  $NO_3^-$ ,  $N_2$ ,  $H^+$ ) also occur in nature. For more details on the photosynthetic process see references [49, 50].

The primary steps of photosynthesis comprise the absorption of a photon by a photosynthetic pigment such as a chlorophyll or carotenoid in a light-harvesting complex and the subsequent transport to a so called reaction centre in which the energy is stored by means of a long lived (>100 ms [51, 52]) charge separation which is necessary as the excited states of the pigments decay very rapidly in less than a few nanoseconds. This charge separation is ultimately used for the synthesis of even longer lived chemical storages such as the major energy source in biological systems, adenosine triphosphate (ATP) or the major electron donor in reductive biosynthesis, nicotinamide-adenine dinucleotide phosphate (NADPH) [53, 54].

Purple non-sulfur photosynthetic bacteria (i.e., *Rhodospirillaceae*) which were studied in this thesis depend on organic hydrogen donors and are therefore, strictly speaking, not autotrophic but photoorganotrophic. Following the primary steps of photosynthesis which take place in two different light-harvesting (LH) complexes and the reaction centre (RC), these purple bacteria feature a cyclic photoelectron transport leading to a proton gradient across the intracytoplasmic membrane (i.e., the membrane in which their photosynthetic membrane complexes are located) which is used for the synthesis of ATP [55].

In this section, the elements which constitute the photosynthetic membrane complexes as well as their supramolecular organisation will be discussed.

# 2.1.1 Building blocks of the photosynthetic apparatus in purple bacteria

### Pyrroles and chlorophylls

Although n-heterocyclic pyrrole (Fig. 2.1A) is a very stable chemical molecule it does not exist naturally as mono-, di- or trimer. Linear tetrapyrroles do occur but only cyclic tetrapyrroles, e.g., porphyrines or chlorophylls whose structures are based on the macrocyclic porphine system (Fig. 2.1B) play a major role in living organisms [56]. The carbon atoms in these rings can be labelled according to two different conventions of which the Fischer labelling system [57]



**Figure 2.1**: Pyrroles in Photosynthesis. (A) n-heterocyclic pyrrole. (B) Porphine molecule consisting of four pyrroles connected by methine bridges. (C) Bacteriochlorophyll *a* molecule based on a phorbine skeleton with side groups and a central Mg-ion. The atoms are labelled according to the Fischer system [57]. The arrows indicate the orientations of the  $Q_x$  and  $Q_y$  transition-dipole moments. (D) Absorption spectrum of BChl a in diethyl ether.

will be used throughout this thesis. The structural skeleton of chlorophyll (Chl) molecules (from Greek  $\chi\lambda\omega\rho\delta\varsigma$ , green and  $\varphi\delta\lambda\lambda\sigma\nu$ , leaf) is phorbine, which has an extra isocyclic ring (V) compared to porphine. Common features of the many existing different chlorophylls are a central Mg-ion that can be used as a ligand binding site and a phytyl group at position 7 of the ring system that serves as an anchor for the pigment in the protein environment. Chlorophylls differ by the types of further side groups that are attached to the macrocycle [49, 53, 58]. Metal-free chlorophylls are known as pheophytins. The purple bacteria which are looked at in this thesis all express the bacteriochlorophyll (BChl) *a* derivate whose structure is depicted in Fig. 2.1C. The absorption spectrum in Fig. 2.1D displays three broad bands belonging to electronically excited singlet states (S<sub>x</sub>) which are the Soret bands (S<sub>4</sub> / S<sub>3</sub>) at around 400 nm and the Q<sub>x</sub> (S<sub>2</sub>) and Q<sub>y</sub> (S<sub>1</sub>) bands in the visible and near infrared, respectively [15, 59]. The transition-dipole moments related to the latter two absorption bands are mutually orthogonal and their orientation within the plane of the phorbine molecule is given in Fig. 2.1C.

Once the pigments are embedded in a protein environment as in the light-harvesting complexes, their absorption maxima can be altered by more than 100 nm due to interactions with the proteins as well as with neighbouring pigment molecules [15, 60]. In this way nature has the possibility to fine-tune the absorption characteristics of the different organisms to match the environment in which they live. Chlorophylls in green plants, for instance, will not absorb at wavelengths longer than 680 nm, which corresponds to the amount of energy needed for water oxidation. Purple bacteria do not need these high energies and their large red-shifted absorption

allows them to live at the bottom of ponds, providing them with a niche that is not taken up by plants or algae

### Carotenoids

Another important class of photosynthetic pigments is formed by the carotenoids (Car) which are basically linear molecules. They consist of a polyene chain with alternating single and double bonds, the number of which can vary typically from eight to eleven between different species of carotenoids [61]. In Fig. 2.2 lycopene is shown which is the major carotenoid in LH2 from *Rhodospirillum molischianum*. Carotenoids generally absorb in the visible around 350-570 nm where chlorophylls do not absorb and transfer their excitation energy to neighbouring chlorophylls thus increasing the spectral absorption cross section of the overall system. They are responsible for the bright variety of colours in flowers and all other plants.



**Figure 2.2**: Structure of lycopene which is the major carotenoid molecule in LH2 from Rhodospirillum molischianum.

Apart from light-harvesting they fulfil the more important role of protection against photodamage [62]. Excited chlorophyll has a small probability of inter-system crossing to triplet states. Then, the triplet state can transfer its energy to oxygen, producing singlet excited oxygen  $({}^{3}Ch|^{*} + {}^{3}O_{2} \rightarrow {}^{1}Ch| + {}^{1}O_{2}^{*})$  which is a highly reactive free radical that can damage the organism. The carotenoids provide a solution for this problem as they quench the chlorophyll triplet  $({}^{3}Ch|^{*} + {}^{1}Car \rightarrow {}^{1}Ch| + {}^{3}Car^{*})$ . The generated triplet excited state of the carotenoids is lower in energy than that of singlet oxygen so that the danger is banned [63].

Carotenoids are also necessary to assure a correct assembly of the photosynthetic pigmentprotein complexes, as some pigments will not be properly incorporated in their absence [64].

#### The reaction centre and the antenna complexes

The photosynthetic apparatus in purple bacteria comprises several transmembrane protein and pigment-protein complexes and is located in the intracytoplasmic membrane.