Chapter 1

Introduction

Energy conversion in the biosphere occurs mainly through photosynthesis and respiration, exceeding all anthropogenic energy usage by an order of magnitude. Mankinds' major sources of primary energy, however, are fossil fuels like coal, oil and natural gas. These materials are all derived from ancient plants and animals and the energy stored within them is chemical energy that originally came from sunlight processed by photosynthesis. Every year around 610 000 PWh [1] of solar energy reach the surface of the earth [1]. In 2006 more than 6 billion human beings consume food with the energy equivalent of about 13 PWh and are additionally responsible for a worldwide energy consumption of approximately 130 PWh of which more than 85% stem from burning fossil fuels [2, 3]. In comparison, photosynthesis stores approximately 880 PWh of energy in biomass every year [4]. Most of our current energy needs are met by photosynthesis, ancient or modern. These figures illustrate the ecological and economical importance of photosynthesis and explain the vital interest of scientists in understanding the basic principles of photosynthesis.

Living organisms are commonly divided, based on their metabolism, between heterotrophs and autotrophs [5]. Heterotrophic organisms on the one hand derive their energy for life processes solely from decomposition of organic components. Autotrophic organisms on the other hand are capable of nourishing themselves by using inorganic matter as the main source of carbon. They

[1] P (peta) is the abbreviation for $1 \cdot 10^{15}$

obtain the energy for life processes from the oxidation of inorganic elements (chemotrophic) or from radiant energy (phototrophic). The process by which phototrophic organisms can produce organic substances from pure inorganic components using electromagnetic radiation as energy source is called photosynthesis.

Photosynthesis can be divided into oxygenic - O_2 producing - photosynthesis carried out by cyanobacteria and plants and non-oxygenic photosynthesis conducted by e.g. purple bacteria or green-sulphur bacteria [5]. Among these various different classes of photosynthetic organisms a wealth of different light-harvesting and electron transport systems are used. However, they all use the same basic principle: whereby the light energy is initially absorbed, concentrated by an antenna system and then transferred to a specialized reaction center, where a charge separation takes place. The electron is then oxidized along a chain of redox enzymes to generate a proton translocation across the membrane. This gives rise to a transmembrane electrochemical proton gradient which drives further metabolic reactions such as the synthesis of ATP.

In this thesis I will concentrate on the light-harvesting complexes of purple non-sulfur bacteria, containing the most studied photosynthetic apparatus. In most purple bacteria the photosynthetic membranes contain two types of light-harvesting complexes: light harvesting complex 1 (LH1) and light harvesting complex 2 (LH2). In order to be able to capture sunlight, the light-harvesting complexes utilize bacteriochlorphyll *a* (BChl *a*) pigments and carotenoids. Generally, the pigments are non-covalently bound to proteins, forming the so-called pigment-protein complexes. Both of these pigment-protein complexes are membrane bound and form transmembrane proteins which are only stable in an amphiphilic environment such as a lipid bilayer. Within the photosynthetic membrane the complexes are structurally stabilized and spatially organized into supramolecular assemblies. Since the photosynthetic membrane comprises many different proteins, a systematic investigation of an individual type of LH complex is not possible within the native membrane. Hence, the transmembrane protein of interest has to be extracted out of the membrane and during the purification process stabilized by a suitable detergent.

By now it has been established that the spatial structure of photosynthetic complexes, especially the distance and the mutual orientation of the pigments, determine to a great extent their spectro-

scopic features and excited states dynamics. This assembly of non-covalently bound molecules offers the possibility to study different types of intermolecular interactions in great detail [6, 7].

Information about the parameters that determine the electronic structure of light harvesting complexes can be obtained by optical spectroscopy. However, even isolated pigment-protein complexes are rather sophisticated and it has been proven to be difficult to analyze the excited state properties in detail on the ensemble level. In this thesis the LH complexes are investigated by single-molecule spectroscopy elucidating information that is commonly washed out by ensemble averaging. The technique of single-molecule fluorescence-excitation spectroscopy exhibits a superior signal-to-noise ratio as compared to absorption spectroscopy if the fluorescence is collected efficiently and scattering sources are minimized [8]. For this purpose a confocal microscope is suited best. This microscope uses point illumination and a pinhole in an optically conjugate plane in front of the detector to eliminate out-of-focus information. Only the light within the focal plane can be detected, so the image quality is much better than that of wide-field images of a conventional microscope. In the fluorescence-excitation spectrum the number of absorption lines, their spectral position and their homogeneous linewidth provide information about the electronic structure of the different pigment pools. However, at room temperature details in the optical spectra of single molecules are masked due to pronounced disorder. Studying them at low temperatures the homogeneous line broadening effects are dramatically reduced and more information can be extracted from the spectra.

This thesis is structured as follows: In chapter 2 the photo-physical and biological properties of the photosynthetic apparatus of purple bacteria are introduced. In chapter 3 the sample preparation and the single-molecule setup is explained. The LH2 complexes are reconstituted into a lipid bilayer in chapter 4 in order to compare the influence of the detergent and the lipid environment on the spectroscopic properties of the pigment-protein complex. Chapter 5 reveals the influence of a symmetry breaking in the BChl *a* pigment arrangement on the spectroscopic features of the LH1 core complexes. Finally, by combining single molecule spectroscopy with numerical simulations, differently refined structural models for the BChl *a* configuration of the core complex from *Rps. palustris* are evaluated.

Chapter 2

Bacterial Photosynthesis

In photosynthesis solar radiation is absorbed by the light-harvesting (LH) apparatus and the excitation energy is then transferred efficiently to a reaction center (RC), where it is used to create a charge separated state that ultimately drives all the subsequent metabolic reactions. Most purple photosynthetic bacteria contain two types of antenna complexes, the light-harvesting 1 (LH1) complex and the light-harvesting 2 (LH2) complex [5, 9]. The LH1 complex surrounds the RC, whereas the LH2 complexes are arranged around the perimeter of the RC-LH1 complex in a two-dimensional array, the structure of which depends on how the bacteria are grown [10–12].

2.1 Pigment molecules

Chlorophylls are the most ubiquitous pigments in photosynthesis as they serve multiple functions such as light harvesting, excitation energy transfer in the antenna system, and charge separation in the reaction center. In purple bacteria the chlorophyll pigments are non-covalently bound to the protein scaffold, forming the so-called pigment-protein or light-harvesting complexes. Chlorophyll pigments are planar molecules, where the conjugated double bounds in the ring like porphyrin derivate bacteriochlorin are responsible for the characteristic light absorption. Chlorophylls generally contain a magnesium atom in the center of the bacteriochlorin and a long hydrophobic phytol chain in the peripheral region that serves as the anchor for the pigment in the

protein environment. Metal free chlorophyll molecules are generally called pheophytins.

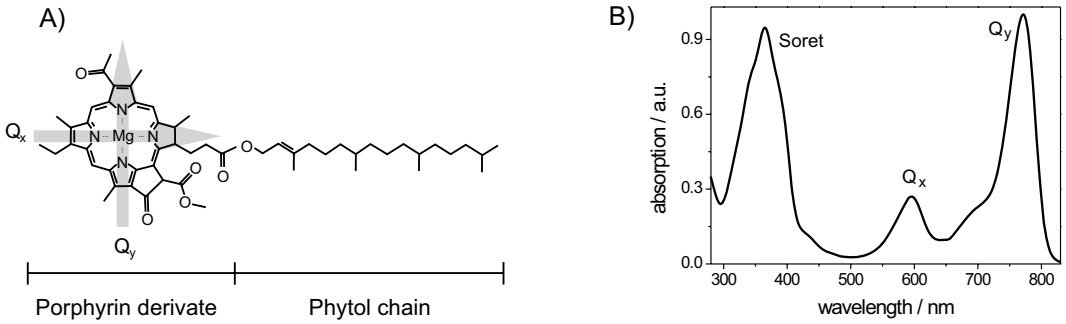

Figure 2.1: A) Molecular structure of a bacteriochlorophyll *a* (BChl *a*) pigment together with the orientation of the Q_x and Q_y transition-dipole moments are indicated with arrows. The BChl *a* pigment can be divided into the photophysical active part of the porphyrin derivate and the hydrophobic phytol chain. B) Room temperature absorption spectrum of BChl *a* in ethanol.

In purple bacteria the main chlorophyll is the bacteriochlorophyll *a* (BChl *a*) pigment. The photophysical active part is highlighted in Fig. 2.1A. The main electronic transitions are termed Q_x and Q_y. The absorption spectrum of BChl *a* in ethanol (Fig. 2.1B) shows three main absorption maxima. Generally, a combination of two higher singlet states (S_3 and S_4) give rise to the Soret band at 365 nm, while the Q_x (S_2) and Q_y (S_1) bands have mutually perpendicular transition dipole moments centered at around 595 nm and 772 nm, respectively [13]. In vivo, with the pigments embedded in a protein environment, the spectral properties of BChl *a* can be altered by interactions with neighboring pigments as well as with the protein. Pigment-pigment interactions, hydrogen bonding or electric field interaction with the protein matrix can cause shifts of their absorption bands up to 80 nm [14, 15]. Of major importance in bacterial photosynthesis is the efficient energy transfer between the Q_y transitions of the BChl *a* pigments. This energy transfer is fast enough, in the order of picoseconds or even faster, in order to outpace wasteful processes such as fluorescence or relaxation to the triplet state [16].

Carotenoids (Car) are another class of key pigments in photosynthesis, which are in general linear molecules. The basic building block of the carotenoid molecules is the isoprene unit. Typically, carotenoids involved in photosynthesis have 9 to 13 conjugated C-double bounds formed by these isoprene units which determine their optical properties [17]. They often act as efficient light harvester that fill the spectral gap left open by the BChl *a* pigments. Rhodopin glucoside

e.g. (Fig 2.2) is the carotenoid identified in the LH2 complex from *Rps. acidophila* [18].

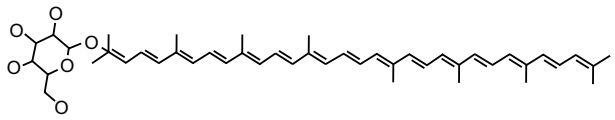

Figure 2.2: Molecular structure of the carotenoid rhodopin glucoside in the all-trans configuration found in the LH2 complex from *Rps. acidophila*.

The main absorption band of carotenoids is generally in the visible range around 350-570 nm, which originates from dipole allowed electronic transitions from the S_0 ground state to the second electronically excited state S_2. These states are classified according to the idealized C_{2h} point group of linear polyenes. The lowest singlet state (S_1) has the same inversion symmetry as S_0, and, therefore, it is inaccessible by a one-photon transition from the ground state. Carotenoids increase the spectral cross section of the overall system for light absorption and by transferring excitation energy to the neighboring bacteriochlorophylls a complementing spectral region can be accessed for light-harvesting processes [19–21]. Carotenoids are also necessary to assure a correct assembly of the photosynthetic pigment-protein complexes, as some pigments will not be properly incorporated in their absence [22]. Furthermore, they are indispensable in their photoprotective function in both the antenna system and the RC complexes [23]. Excited BChl *a* pigments have a small probability of inter-system crossing to the triplet state. The triplet excited BChl *a* molecule lasts long enough to react with molecular oxygen to generate singlet oxygen (^{3}BChl* *a* + $^3O_2 \rightarrow {}^1$BChl *a* $+ {}^1O_2^*$). Singlet oxygen is a very powerful oxidizing agent and rapidly kills cells that are exposed to it. Carotenoids can prevent these harmful effects of singlet oxygen in two ways. The carotenoid either quenches the BChl *a* triplet state (^{3}BChl* *a* + ^{1}Car $\rightarrow {}^1$BChl *a* $+ {}^3$Car*) by generating an additional decay channel or by directly quenching the singlet oxygen state ($^1O_2^* + {}^1$Car $\rightarrow {}^3O_2 + {}^1$Car*). In vivo, the major protective effect is the rapid quenching of BChl *a* triplet state so that no singlet oxygen is produced.