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Bridging the gap between natural & artificial light-harvesting : a structure-function investigation with MAS NMR

Ganapathy, S.

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Chapter 1

A general introduction

1.1 Introduction

Nature over billions of years has achieved the extraordinary means of converting solar energy into fuel. Photosynthesis, wherein light-energy from the sun is captured and through a series of events converted into biochemical energy, is the process which sustains life [1]. The variety of organisms that carry out photosynthesis is large and is broadly classified into two categories. The first is the oxygenic group of organisms, which use water as the primary electron donor and produce molecular oxygen as a by-product of photosynthesis. This group includes plants, algae and the cyanobacteria [2]. The second group of organisms is the anoxygenic group, which uses compounds other than water as primary electron donors, like reduced sulfur compounds, molecular hydrogen, or simple organic compounds. Anoxygenic micro-organisms do not produce molecular oxygen as a byproduct and comprise the purple bacteria, the green sulfur bacteria, the green nonsulfur bacteria and the heliobacteria [3].

Photosynthesis is a multistage process and the first stage of this process involves the capture of light by the photosynthetic antenna systems, and the subsequent transfer of excitation energy to the photosynthetic RC. In most photosynthetic organisms from higher plants, algae, the heliobacteria, the purple bacteria, and the cyanobacteria, the antenna system is a chlorophyll-carotenoid-protein complex that is located in the photosynthetic membrane. In these organisms, energy-transfer is mediated by pigment-protein interactions.

From a biological perspective, photosynthesis is an expensive process in terms of resources required. Organisms evolve only the photosynthetic capacity that they need to survive, and functioning of the entire photosynthetic conversion chain is optimized against environmental

constraints. In a healthy, unstressed organism, the light energy conversion limits at the maximum rate of electron transport that the organism needs. This determines the biological design, starting from a fixed set of components. For most organisms the environmental constraints derive from a low concentration of nutrients, such as iron or nitrogen, or dilute CO₂ in the atmosphere, and rapid conversion of light is not a major factor in the biological design. Green sulfur bacteria and the green nonsulfur bacteria, however, are found in anoxygenic environments where the light intensities can be extremely low; with single chlorophyll photon absorption rates of less than 1 photon per hour. In order to survive under such low light intensities, green bacteria such as *C. tepidum* evolved chlorosomes, very efficient extra-membraneous antenna systems that consist of $\sim 2 \times 10^5$ self-organized chlorophyll molecules [4,5]. Chlorosomes accomplish their energy transfer via pigment-pigment interactions which flourish in the absence of a protein matrix.

There are many routes to a secure, sustainable and efficient energy supply, and in all scenarios solar energy plays an essential role. Recent advances in structural biology have provided profound insight into the structure and operational mechanisms of the molecular machinery of a variety of photosynthetic apparatus. This has set the stage for a technology push towards the construction of artificial biomimetic devices mimicking photosynthesis to collect, direct, and apply solar radiation to produce environmentally clean fuel.

The role of light-harvesting antennae as solar energy collectors makes their extensive characterization an obvious starting point towards construction of such a device. This thesis focuses on bridging the gap between natural and artificial systems by the structural and structure-function characterization of two kinds of natural photosynthetic antenna systems, a pigment-protein complex *i.e.* the LH2 complex, and the protein-free chlorosome supramolecular light harvesters. Chlorosomes contain the largest numbers of chromophores for any antenna system known in nature and are very efficient ultra-fast light harvesters. They provide an optimal starting point for a novel class of artificial antenna arrays for ultra-rapid feeding of energy into photocatalytic devices.

1.2 The LH2 complex

Purple bacteria have an antenna system that comprises two types of pigment-protein complexes called the LH1 and LH2 complexes [6]. The LH1 complex is the core (integral) membrane protein-pigment complex, while the LH2 complex is the accessory protein-pigment complex [1]. The structure of the LH2 complex has been resolved at high resolution by X-ray methods [6-8]. LH2 from the purple bacterium *Rps. acidophila* strain 10050 is built from nine identical monomeric repeat units forming a ring having nine-fold symmetry (Figure 1.2).

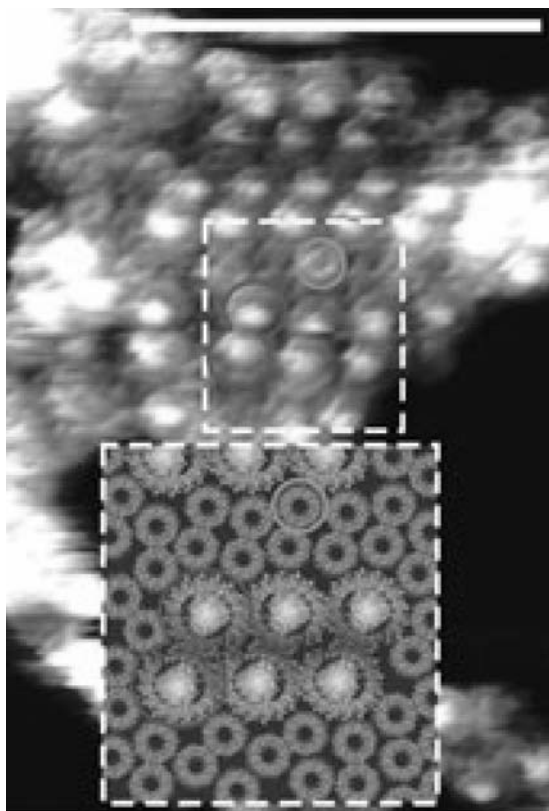


Figure 1.1 Native photosynthetic membranes from the wild-type purple bacterium *Rhodobacter sphaeroides* as imaged by AFM [9]. The inset at the bottom is a representation of the region denoted by the dashed box in the centre, using model structures derived from atomic resolution data (Reprinted by permission from Macmillan Publishers Ltd: Nature, ref. [9] © 2004).

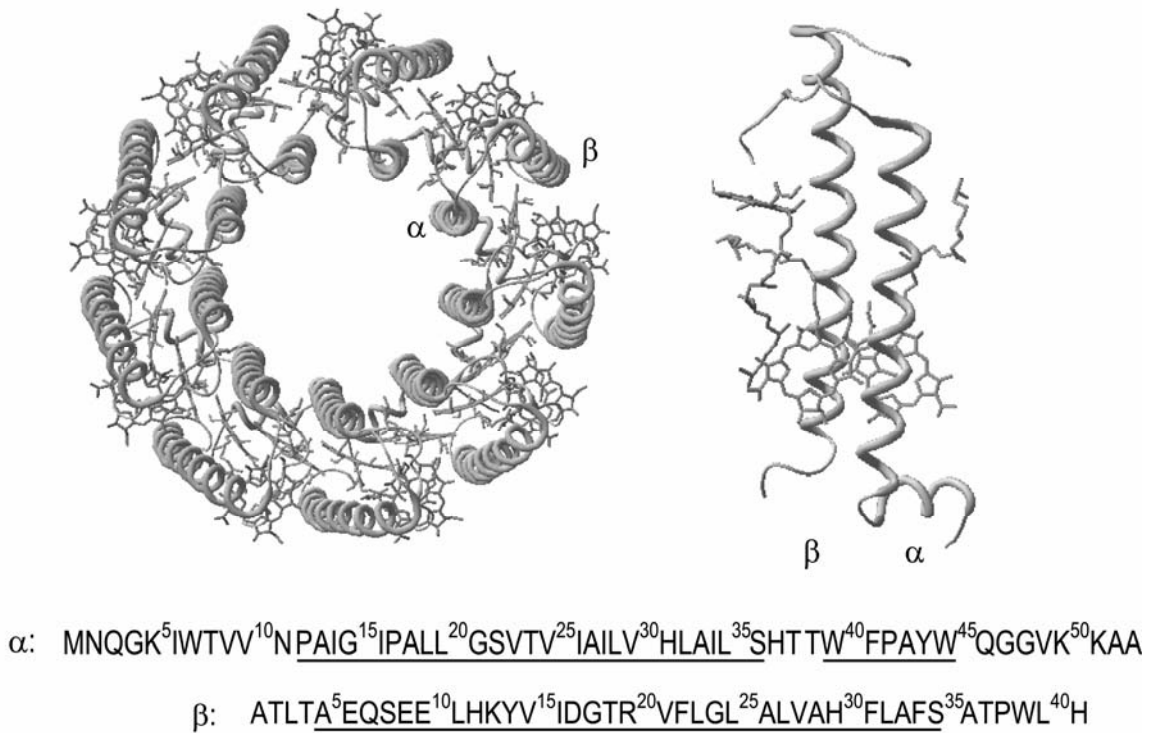


Figure 1.2 Left: The X-ray structure of the nonameric LH2 complex from *Rps. acidophila* strain 10050. Right: the structure of the monomeric LH2 complex derived from the 1NKZ PDB file [8]. Bottom: the primary sequences of the α - and β -subunit of the monomeric LH2. Reprinted from ref. [10].

Each monomer consists of two helical polypeptide subunits, three molecules of BChl a , and one carotenoid [11]. The polypeptide segments are called the α -subunit and β -subunit and consist of 53 and 41 amino acid residues, respectively. The BChl a cofactors are denoted by their prominent absorption maxima as B800, α B850, and β B850. The B800 pigments are axially coordinated at their central Mg ion by the carboxyl- α M1 at the N-terminus of the α -subunit, forming a weakly coupled nine-membered ring where the separation between each B800 molecule is approximately 21 Å. Their spectral properties are consistent with their being individual molecules. The pigments which absorb at 850 nm are arranged quite differently. α B850 and β B850 are arranged as a closely coupled dimer, are sandwiched between each α - and β -subunit pair, and

are axially coordinated at their central Mg ion by β H30 and α H31 respectively. In LH2 antennae these dimers form a continuous overlapping ring of 18 pigments that is subject to moderate structural heterogeneity according to optical spectroscopy, while appearing nearly crystalline in the NMR [12-14]. The B850 pigments in the ring are strongly exciton-coupled, and excitations are effectively delocalized over much or the entire B850 ring, rather than being effectively localized to a single pigment for a short while before jumping to the next pigment as is the case with the B800 pigments, which are not exciton-coupled [12,13].

The LH2 complex has proven to be an excellent model for studying membrane proteins by using MAS NMR spectroscopy [14-16]. A sequence-specific assignment of the NMR response was recently obtained for 76 of the 94 residues of the monomeric unit of the LH2 complex using MAS NMR in combination with pattern labeled samples developed with site-specifically labeled precursors in the expression medium [14,15]. It was not possible, however, to get a structure from chemical shifts alone. In Chapter 3 it is shown how structure defining distance constraints can be obtained for the LH2 complex with MAS NMR.

1.3 The chlorosomes

Chlorosomes are the largest light-harvesting antennae, found in green sulfur bacteria (*Chlorobi*), some green filamentous anoxygenic phototrophs of the phylum *Chloroflexi*, and the recently discovered aerobic anoxygenic phototroph *Candidatus Chloracidobacterium thermophilum* [3,17-19]. They are characterized by the self-organization of thousands of BChl *c*, *d*, or *e* molecules forming co-axial cylinder like vesicles via pigment-pigment interactions with little or no protein involvement [18-20]. Chlorosomes are found attached to the cytoplasmic side of the cell membrane via the baseplate to a BChl *a* containing protein complex called the FMO protein complex [1,21]. Via the FMO protein complex, photonic excitations are funneled into membrane bound RCs. A schematic representation of the chlorosome containing photosynthetic apparatus is given in Figure 1.3.

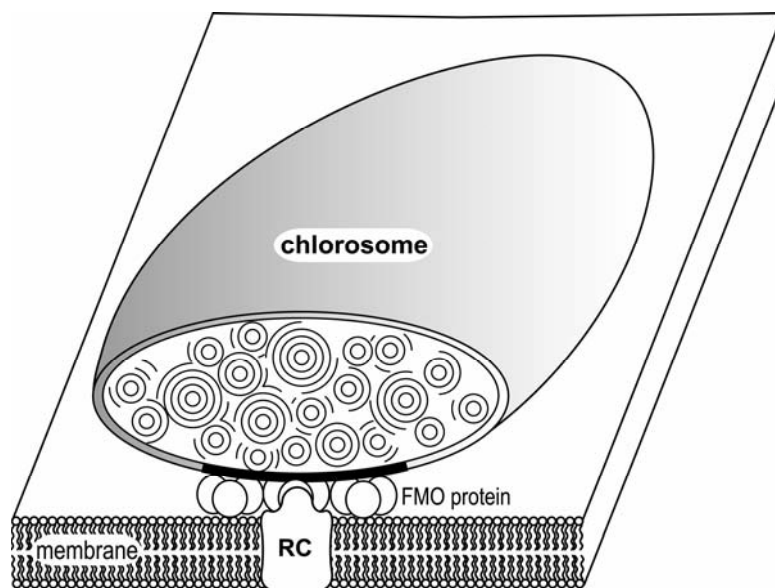


Figure 1.3 Schematic representation of chlorosomes from the green sulfur bacteria *C. tepidum*.

The self-organization of the BChl is thought to be driven by three noncovalent interactions: coordination of the 3¹-oxygen unit to the magnesium ion, hydrogen bonding between the 3¹-alcohol to the 13¹-keto group and π - π stacking [17,22]. It is already known that *in vitro* aggregation of BChl *c* molecules leads to a structure that is spectroscopically very similar to the BChl *c* in chlorosomes [21,23,24]. Over the last decade several solid-state NMR and EM studies of chlorosomes have resulted in different structural models, addressing both the microscopic and macroscopic structure of chlorosomes but none providing conclusive evidence on their structure, which is subject to considerable heterogeneity and variability [22,24-28].

Recent advances in understanding the biosynthesis of BChl *c* in the green sulfur bacteria *C. tepidum* has led to a well characterized set of mutants [29,30]. A recent breakthrough through a cryo-EM study of chlorosomes from the WT and from the *bchQRU* triple mutant of *C. tepidum* led to the first end-on images of chlorosomes (Figure 1.4), revealing that chlorosomes contain several multilayered tubules with a 2.13 nm layer separation, rather than single-layer tubules or exclusively undulating lamellae as previously proposed [19,28].

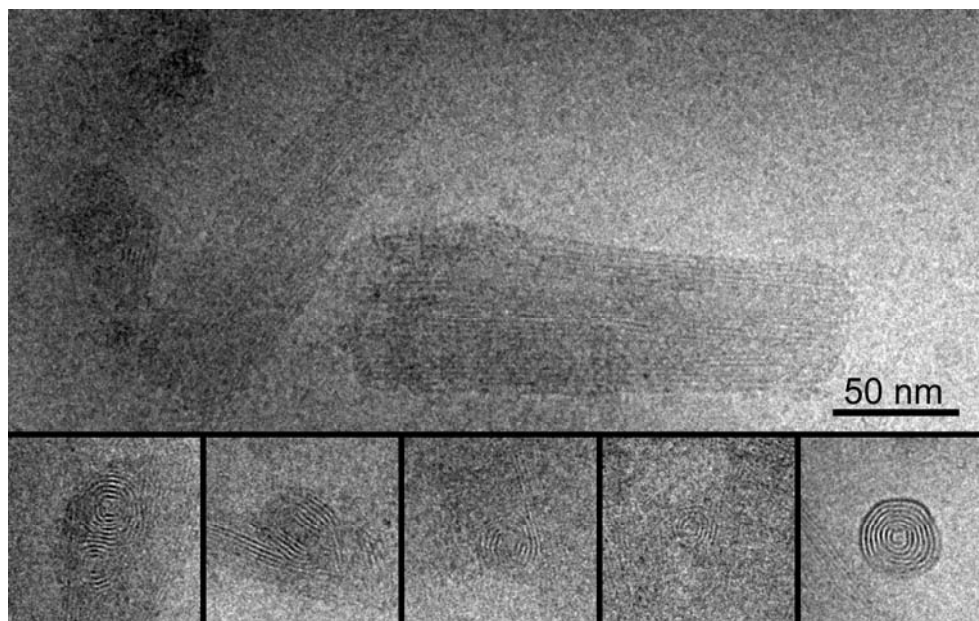


Figure 1.4 Cryo-EM images of isolated chlorosomes from the *bchQRU* triple mutant of *C. tepidum* embedded in amorphous ice. In the upper frame chlorosomes are seen in side-on position; the lower row presents end-on views. Reprinted from ref. [19].

Chapters 4 and 5 are devoted to the structural assessment of chlorosomes from two BChl *c* biosynthesis mutants and the WT of *C. tepidum* with a combination of MAS solid-state NMR, cryo-EM, and DFT calculations.

1.4 Pigment aggregates forming π -stacks: Artificial antenna

Chlorosomal light harvesting systems are highly aggregated, facilitating ultrafast inter-pigment electron transfer [31]. In addition they have the longest exciton diffusion lengths of any known pigment assembly [32-34]. This property is paramount in the light-harvesting efficiency of these systems and is an important factor to be considered in the design of an antenna assembly for an organic/biomimetic solar cell. It is known that the self-assembly of pigments observed in chlorosomes is replicated by BChl *c* aggregates in non-polar solvents like hexane [24]. This phenomenon implies that the noncovalent interactions of the metal-3¹-OH

coordination and π - π overlap, which keep a single stack intact, and the 3¹-OH-13¹=O hydrogen bond which binds the stacks together are reproduced in the artificially aggregated systems.

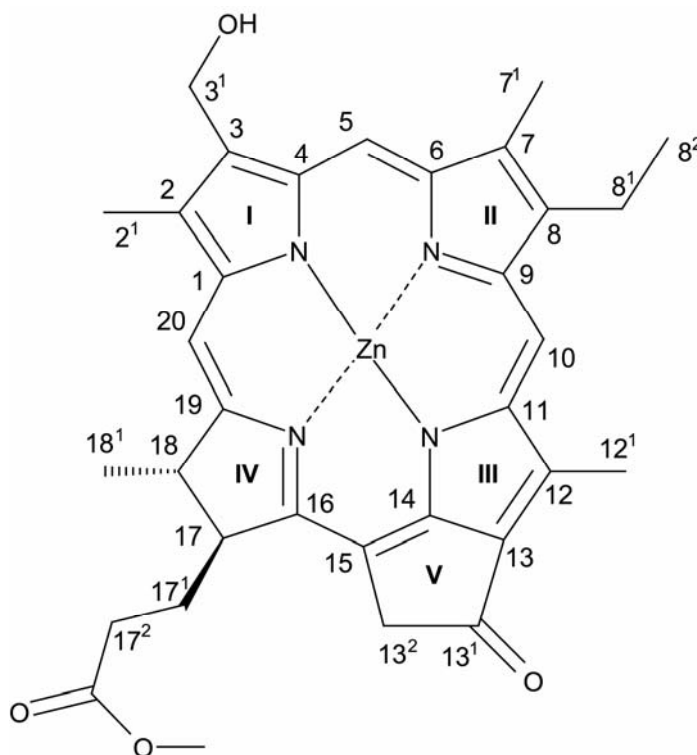


Figure 1.5 Chemical structure of a model chlorin which is an analogue of BChl *d* [35].

Tamiaki and co-workers demonstrated that the zinc chlorin compound shown in Figure 1.5, a BChl *d* analogue, replicates the aggregation behavior of BChl *c* in non-polar solvents [35]. Also in this compound the three primary non-covalent interactions known to hold the aggregates in the cylindrical form remain intact. The artificial antennae that can be prepared this way have been taken to the next level by Würthner and co-workers, who synthesized modified forms of the zinc chlorin in Figure 1.5. Modifications in the ester tails provide compounds with enhanced solubility that form rod shaped aggregates in non-polar solvents, which could be verified through AFM images [36,37]. The aggregation in these compounds is completely reversible and triggered by a change in solvent polarity. An additional modification of the hydroxy functionality at 3¹, to a

methoxy functionality, eliminates the H-bonding interaction of $3^1\text{-O—H}\cdots\text{O=13}^1$ that holds the stacks together. As a result, this compound forms independent stacks when solubilized in a non-polar solvent, which could be verified by AFM and STM analyses [37]. The STM data have also indicated that the nature of the stacking interaction is dependent on the concentration of the compound in the solvent. In Chapter 6, we have assessed the micro structure of a 3^1 -hydroxy and 3^1 -methoxy zinc chlorins using 2D solid-state NMR.

1.5 Solid-state NMR for the structural and structure-function investigation of biological and biomimetic systems

Over the last decade MAS solid-state NMR spectroscopy has rapidly developed as a technique for the investigation of the structure and function of biological systems, particularly those inaccessible to solution-state NMR or X-ray crystallography [38-42]. The scope of this thesis is to refine and improve these methods to a stage where they can be used for structure determination and structure-function studies of biological and biomimetic systems, bridging the gap between the life sciences and the nanosciences.

Due to dipolar truncation in homonuclear dipolar recoupling sequences like the RFDR sequence or the DARR sequence, it is notoriously difficult to obtain through space distance constraints, which is the cornerstone of any structural assessment [43,44]. The PDSD experiment, with long mixing times, is effective in obtaining through space constraints, but when performed on a uniformly enriched samples of larger systems, the resulting spectra comprises of many cross peaks [45]. The PDSD experiment works better on smaller systems or non-uniformly labeled or pattern labeled samples in which case the resultant spectra are less crowded and it is easier to assign the resultant cross-peaks [14,15,38,46].

The ^1H transfer is much faster than for ^{13}C and protons form a densely coupled network in space which makes them optimal for intermolecular transfer. The obvious disadvantage of the strong ^1H - ^1H dipolar couplings

has been overcome by encoding the ^1H - ^1H mixing period in a ^{13}C evolution and detection step. In this way it is possible to get a well resolved two-dimensional ^{13}C - ^{13}C spectrum with indirect proton spin-diffusion. This is the CP³ experiment that is more commonly referred to nowadays as the CHHC experiment [47-50]. de Boer and co-workers successfully utilized this experiment to get through-space distance constraints which they used to determine the microcrystalline structure of aggregated cadmium chlorin [51,52]. In addition, the CHHC/CP³ (hereafter CHHC) experiment has been successfully applied for obtaining distance constraints for protein structure determination [40-42,53].

In this thesis it is demonstrated how progress in solid-state NMR techniques for structure determination of biological solids, and the guidelines established for solving the structure of pigment assemblies by the use of spin diffusion MAS NMR, in combination with ring current shift calculations, can be adapted and exploited for the structural characterization of supramolecular assemblies of pigments having biological origin or those that are chemically synthesized [52].

Chapter 2 provides a brief theoretical overview of MAS NMR and the methods that were used to get chemical shift assignments. The CHHC experiment that was used to obtain structure-defining through-space distance constraints is also discussed in this chapter.

In **Chapter 3** it is described how the CHHC experiment has been successfully tested for the detection of through-space intermolecular distance constraints on a uniformly ^{13}C enriched preparation of the LH2 complex. This was done to gauge the effectiveness of the experiment as well as to get an estimate of the polarization transfer range.

Access to distance constraints and DFT validated ring current shifts in combination with cryo-EM data has led to the best possible suprastructural model that has been seen till date for the chlorosomal assembly, albeit for the chlorosomes from the [E,M] BChl *d* producing *bchQRU* mutant of *C. tepidum* (**Chapter 4**). In **Chapter 5** this model has been adapted to describe the suprastructural assembly of another mutant of *C. tepidum*, which is the [E,M] BChl *c* producing *bchQRU* mutant.

In **Chapter 6** the transition from natural to artificial antenna systems is made and using the ^1H chemical shifts that are assigned from natural abundance samples of ^3H -hydroxy and methoxy zinc chlorins, the supramolecular structure of these pigment aggregates is assessed.

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