Resolving the dynamic structure of chlorosomes in green sulfur bacteria by MAS NMR

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Summary

This investigation into the structure and dynamics of chlorosomes is drawing to a close. My journey may have raised more questions than it answered, but I hope it sparks inspiration for future explorations.

Photosynthesis represents a complex, multistage process, with the initial phase involving light harvesting. In green bacteria, this pivotal stage is facilitated by chlorosomes. The bacteriochlorophyll molecules in chlorosomes possess a distinct characteristic in that they undergo self-assembly, independent of protein involvement in the scaffolding process. Chlorosomes are the largest photosynthetic light-harvesting antennae in nature and are capable of transferring excitation energy with near-unity quantum yield over distances of several hundreds of nm, much longer than generally considered possible for organic dye systems. This unique property of chlorosomes claims structure characterization and dynamics study to mimic them in artificial devices to harvest sunlight. This thesis aims to study the structure and dynamics using mainly magic angle spinning (MAS) nuclear magnetic resonance (NMR) and integrating it with other techniques such as cryo-EM, Optical spectroscopy, and modeling.

In **Chapter 1**, a general introduction to green sulfur bacteria and chlorosomes is presented. In addition, it provides a brief theoretical overview of the solid-state NMR techniques that have been extensively utilized for structure and dynamics studies. I adopted a dynamic spectral editing (DYSE) strategy to study the rigid and dynamic parts simultaneously. Along with it, various dipolar and scalar-based 2-D measurements, and relaxation measurements such as $T_{1\rho}$ and REDOR helped solve the structure and dynamics.

In **Chapter 2**, chlorosomes from the *bchQ* mutant are studied which are prepared biosynthetically from *Chlorobaculum tepidum (Cba. tepidum)*. This mutant is predominantly made of a single homolog, [8Et, 12Et]BChl *c*. The advantage of studying mutant chlorosomes is their reduced structural heterogeneity compared to their WT counterparts. This reduced structural inhomogeneity allowed us to deduce the *syn-anti* parallel stacking of the BChls in the chlorosomes of this mutant. In this chapter, the integration of two other techniques along with MAS NMR, cryo-EM and optical spectroscopy, is discussed. Cryo-EM revealed a layer line which gave the subunit axial repeat of 1.49 nm. This repeat distance was crucial in determining the distance between two chromophores in a stack. Optical spectroscopy, in

particular circular dichroism (CD), gave a chiral angle which helped in further revealing whether the stacks are running vertically, horizontally, or at an angle. Integration of these techniques gave us an understanding of the structure of *bchQ*. The obtained results were compared with the WT *Cba. tepidum* chlorosomes. The results suggested that both *bchQ* chlorosomes and WT chlorosomes have *syn-anti* parallel stacks, with the stacks in *bchQ* chlorosomes running vertically to the tube axis with a chiral angle of 90º and for WT at an angle to the tube axis of 112º. In this chapter, preliminary insights into the dynamics of *bchQ* chlorosomes and WT chlorosomes are also discussed, revealing that the farnesyl tail and side chains attached to the BChl macrocycle are dynamic.

To investigate the efficient energy transfer mechanism, it is important to study the dynamics of BChls in *bchQ* chlorosomes. In **Chapter 3**, we implement various temperatures along with 1-D and 2-D dipolar and scalar-based NMR measurements to see the effect of temperature on various functionalities within the BChls in *bchQ* chlorosomes. The results show that at reduced temperatures the tails and side chains that showed mobility get frozen. We also see lipid isomerization at elevated temperatures from all-trans to trans gauche. However, to study the libration of the BChls, a robust NMR technique called Rotational Echo DOuble Resonance (REDOR) was necessary to estimate the dipolar coupling strength. The obtained value was compared to the rigid limit value for CH dipolar coupling strength. The results showed a decrease in dipolar coupling strength from 22.7 kHz to 17.5 kHz indicating partially restricted motion. This was the first experimental evidence for underpinning the computationally obtained results for the chlorosomes of *Cba. tepidum* that converged upon librational motion of the macrocycle as the critical feature producing dynamic crossing of exciton levels and ultrafast delocalization of harvested light energy over the entire chlorosome for driving the photosynthetic conversion chain.

The libration/rotational mode seen in chlorosomes is directed towards the plastic crystallinity of chlorosomes. A plastic crystal is a material that possesses one conformational degree of freedom. In the case of chlorosomes, it is the librational motion. In **Chapter 4**, we discuss the dynamics of WT chlorosomes using a similar protocol from Chapter 3. Interestingly, the dynamic trends observed from *bchQ* chlorosomes were also seen in WT chlorosomes. Additionally, $T_{1\rho}$ relaxation measurements revealed similar trends in dynamics as seen from the DYSE strategy. REDOR datasets also revealed partial averaging of dipolar coupling strength. This reveals the plastic crystallinity of chlorosomes.

In **Chapter 5,** the first step in studying the structure of chlorosomes of *bchR* mutant are made. This mutant is prepared biosynthetically from *Chlorobaculum tepidum (Cba. tepidum)*.

This mutant has 3 homologs but is predominantly made of [8Et, 12Me]BChl *c*. Reductions in side chain variability rendered the mutant to be more homogeneous which in turn resulted well resolved NMR peaks in the 2-D data sets. Results showed alternating *syn-anti* parallel stacking of the BChls in the chlorosomes of this mutant.

In **Chapter 6**, the polarization transfer DYSE technique is extended to study the whole cells of *Cba. tepidum* using MAS NMR to study the chlorosomes in intact cells. The whole cell NMR procedure allows for fast detection of molecular structure and dynamics without needing the purification steps. Results showed almost all BChl signals of chlorosomes in whole cells. the study also revealed the conversion of bacteriochlorophyll to bacteriopheophytin due to oxidation of thiosulfate to sulfuric acid with decreasing pH.

Finally, in **Chapter 7,** a general discussion of the results is presented and addresses the importance of whole cell measurements. In addition to whole cells, I discuss the potential of studying *bchR* mutants for future study.

In our summary, we have evaluated the importance of studying the structure and dynamics of chlorosomes. This knowledge will help in replicating natural photosynthesis in artificial systems. Most of the thesis focuses on examining isolated chlorosomes, with a small section dedicated to studying whole cells. If successful, the study of whole cells can help answer questions about whether the energy transfer mechanism observed inside isolated chlorosomes is the same when they are in their natural form in whole cells and there is the possibility for a sink for excitons to be carried away into the photosynthetic conversion chain.