A semisynthetic peptide-metalloporphyrin responsive matrix for artificial photosynthesis

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Chapter 1

A general introduction

In recent years, the transition to sustainable energy has become one of the most pressing issues in the world. The search for efficient, clean and renewable energy has become a common goal for scientists all over the world, and solar energy is favored for its renewability, it is environmentally friendly, and has low cost. In nature, light-harvesting complexes absorb sunlight to transfer energy and maintain the activity of life through chemical reactions. After billions of years of evolution, protein matrices have been optimized to cooperate with light-harvesting molecules to efficiently capture and transport solar energy until it can be stored in a chemical bond.[1-3] The natural light-harvesting antenna systems are thought to carry on lossless Non-adiabatic Conversion by Adiabatic Passage (NCAP) processes for high yield.[4-7] Scientists try to create artificial light-harvesting antenna systems to create solar fuel. However, the uptake rate of solar energy by artificial photosynthesis is still very low. At present, most chemists' approach for solar fuel production is focused on changing the properties of dyes and catalysts, thus ignoring the matrix. Engineering of a responsive matrix is likely of vital importance to improve the efficiency of artificial photosynthesis devices. This thesis makes a first step in this direction.

1.1 Moving from natural photosynthesis to artificial photosynthesis

In nature, plants, algae and some bacteria are able to perform photosynthetic processes, converting light energy into chemical energy. In most cases, photosynthesis includes two processes called the 'light' and 'dark' reactions. Both ATP and reduced NADP are produced in the light-reaction after absorption of a photon. And these two products are consumed by the Calvin–Benson–Bassham cycle to reduce carbon dioxide into carbohydrates in the dark-reaction (Figure 1.1).

Photosynthesis is a complicated process and a diversity of strategies is used by different organisms. Commonly, the photosynthetic reactions can be divided into four steps.[8] The light is absorbed by light-harvesting antenna systems in the first step. The harvested light is transferred to photosynthetic reaction centers where charge separation takes place. The positive charges extract electrons from water in the third step, and the negative charges reduce carbon dioxide to carbohydrates in the last step.

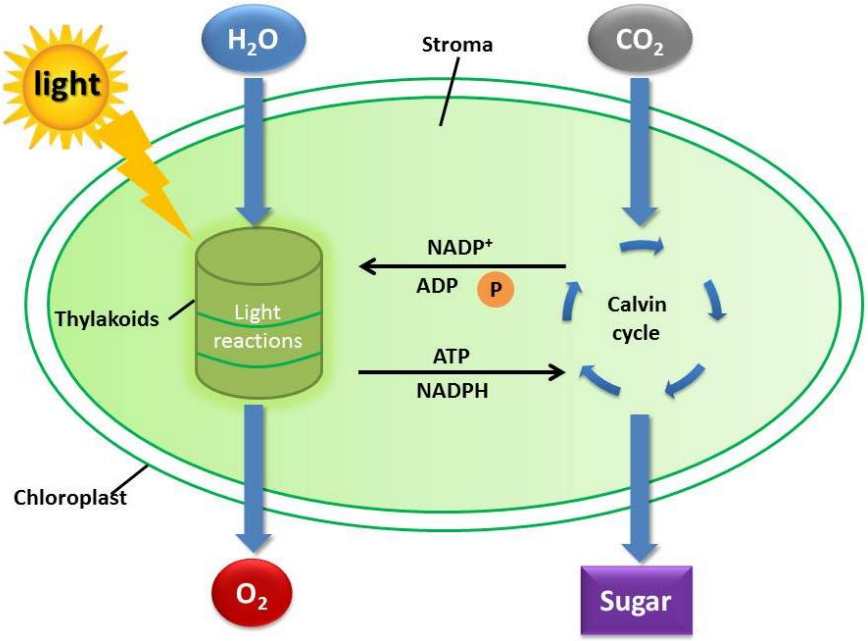

Figure 1.1 Diagram of the process of photosynthesis, showing the light reactions and the Calvin cycle.

During photosynthesis, light energy is converted into chemical energy. However, not all absorbed light energy finally converts into chemical energy, the overall photosynthetic efficiency normally reaches to 3% to 6% of the total incoming light energy.[9] The excess light energy dissipates as heat in a process called non-photochemical quenching, or is emitted as fluorescence to avoid damaging the photosynthetic apparatus. Although the final efficiency of photosynthesis is not high, the light energy can be transferred to reaction centers with nearly 100% efficiency.[10] Natural photosynthesis is highly conserved in evolution, and the biological success of natural photosynthesis inspires scientists to replicate nature and build an artificial photosynthesis system to convert sunlight energy into chemical energy.

To perform good artificial photosynthesis, two crucial factors, light harvesting and charge separation, should be optimized. The harvested light energy should be able to drive charge separation to an electron acceptor to promote chemical reactions. While many different artificial photosynthesis approaches are currently under development, genuinely efficient and robust components for capturing and storing solar energy in chemical bonds were not yet obtained. Research is mainly focused on designing and synthesizing electrocatalysts that can be linked to a light-driven charge separation system.[11-12] In this manner solar cells have been made that can achieve photo-electrochemical hydrogen production with photon to product yield, or external quantum efficiencies, as high as 60%.[13] A challenge is to build antenna complexes with highly ordered arrays of pigments which can transfer nearly 100% of the harvested energy to reaction center. This thesis focuses on necessary steps to be made towards the construction of an efficient and robust semisynthetic artificial light-harvesting antenna system.

1.2 The natural and artificial light-harvesting antenna

Light-harvesting antenna systems play a vital role in solar energy uptake by collecting photons and transferring excited state energy to the reaction center.[14] A wide variety of light harvesting complexes is found among the different photosynthetic species, *e.g.* green sulfur bacterial chlorosomes, cyanobacterial phycobilisomes, dinoflagellate peridinin-chlorophyll-protein, the Pcb protein, higher plant light-harvesting complex II (LHCII), and the purple bacterial light-harvesting complexes 1, 2 and 3 (LH1-LH3).[15-20] They all show similar properties for light harvesting. They all contain high-density arrays of pigments for exciton energy transfer via the Förster or Dexter exciton transfer mechanisms.[21-22] In these light-harvesting complexes, pigments are thought to be tuned by the matrix to perform non-adiabatic conversions that transiently violate detailed balance for high yield.[1, 3, 23] Among the high-density arrays inspired by nature that have been investigated for artificial photosynthesis, however, systems with high energy uptake efficiency have not yet been found.[24-26]

The smallest antenna motif among all natural light-harvesting complexes is the CsmA protein in the baseplate from *Chlorobaculum tepidum*. CsmA proteins (6.2 KDa) in complex with BChl *a* form a crystalline-like baseplate

for energy transfer, in between the surfaces of the chlorosome and the FMO protein complex (Figure 1.2). [27-29] Each CsmA only binds with one BChl *a* and self-assembles to form dimers and strands in the baseplate.[30]

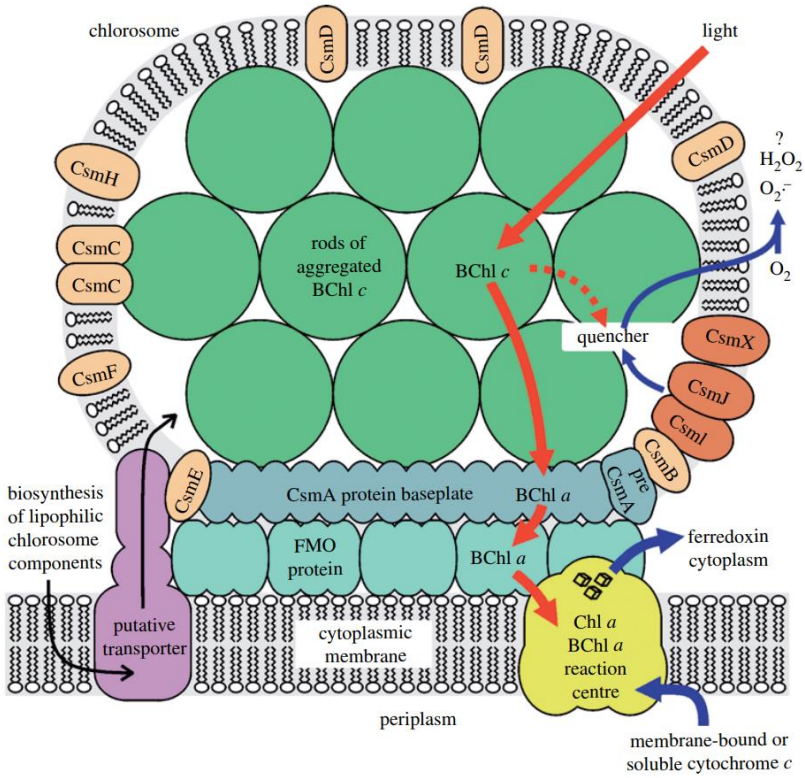

Figure 1.2 Schematic of the photosynthetic apparatus in *Chlorobium tepidum*.[31-32]

The liquid state NMR structure of the 59-residue CsmA protein shows a high α-helical content in the structure; the first and largest helical segment extends from residues 6–36, and contains a binding domain for BChl *a*. The other helical segment is shorter and extends over residues 41–49 towards the C-terminal part.[33] Amphipathic CsmA contains a hydrophobic and a hydrophilic side. With the exception of the only coordinating histidine, all potentially charged residues, e.g. lysine and arginine, are located at one side of the helix (Figure 1.3). The resulting strong hydrophobicity on the opposite side of the helix allows the protein to aggregate. The binding of the BChl *a* cofactor and the assistance of salt bridges formed by the charged residues on the hydrophilic side cause CsmA to self-assemble into long arrays resulting in the para-crystalline superstructure of the baseplate as observed by Cryogenic electron microscopy (cryo-EM).[30, 34-35]

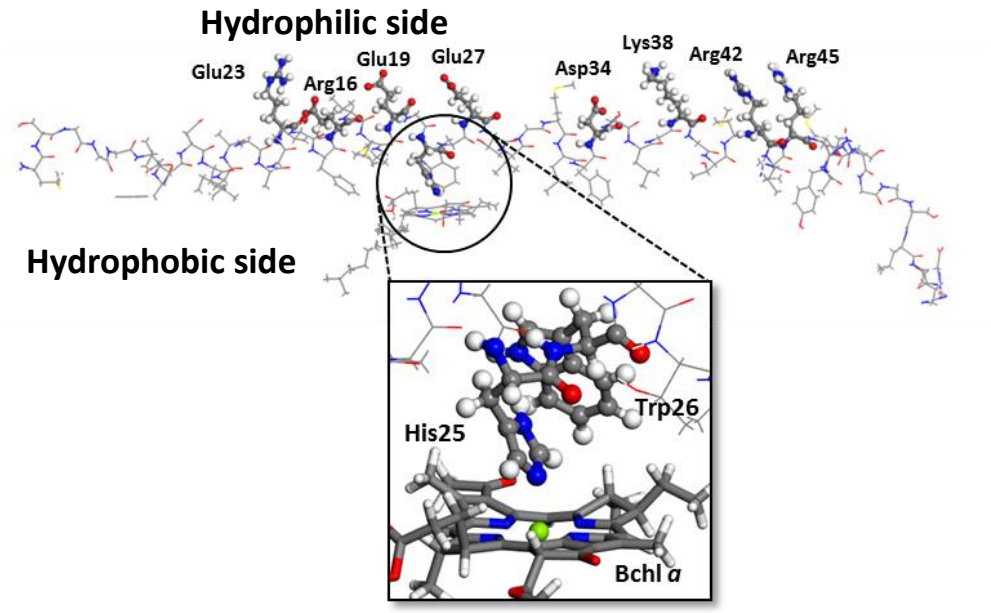

Figure 1.3 The CsmA–BChl *a* baseplate complex

Here we investigate a semisynthetic CsmA (tCsmA) construct, containing 43 amino acids, that was obtained by removing the flexible segments from the N and C termini and leaving the central α-helical part with the two functional helical segments. tCsmA can be synthesized using standard solid-phase Fmoc based chemistry and purified by Reverse phase High-performance liquid chromatography (RP-HPLC). For pigment, we use the chemically robust zinc-protoporphyrin IX (ZnPP) , a compound found in red blood cells that is often used in artificial light harvesting studies, to replace BChl *a*.[35-37]

Most research aiming for artificial antennae focuses on building entirely synthetic peptide scaffolds, such as four helix bundles, instead of modifying a natural complex.[37-41] Although bottom up design is not always straightforward, it allows for flexibility in the incorporation of chromophores. Often the stability is worse than for natural systems and it is difficult to match the ability of light harvesting compared to the performance of natural systems. In contrast, the semisynthetic tCsmA-ZnPP complex builds a bridge between the natural system and fully artificial systems. Using the outcome of evolution in biology as a starting point, with subsequent modification afterwards, we can possibly build a simple, stable and efficient semi-artificial light-harvesting antenna system.

1.3 Non-adiabatic Conversion by Adiabatic Passage (NCAP) processes

Traditionally, transmission of exciton energy is thought to proceed via the Förster or Dexter exciton transfer mechanisms.[21-22, 42] Most photosynthetic light harvesting complexes are chiral "responsive" matrix assemblies. It is believed that they can undergo a light-driven conformational change and self-select vibrations to develop vibronic states for driving semi-classical coherent transfer with nearly 100% yield in lossless Non-adiabatic Conversion by Adiabatic Passage (NCAP) processes.[4-7, 43]

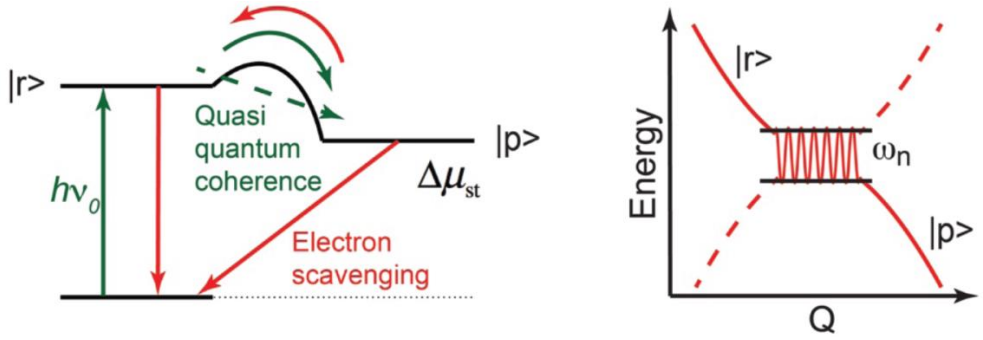

Figure 1.4 Conversion from a reactant state |r> to a product state |p>. Forward processes are shown in green and reverse processes in red. [43]

The Pauli Exclusion Principle states that two or more identical fermions cannot occupy the same quantum state within a given quantum system simultaneously. Thereby crossing of the electronic states is forbidden. The adiabatic conversion from a pure reactant state |r> into a product state |p> normally has a barrier to overcome, which is unfavorable with respect to energy efficiency. However, in a chiral "responsive matrix" the transfer from |r> to |p> proceeds non-adiabatically. A transient semi-classical channel through the barrier opens up when anticorrelated |r> and |p> states approach each other and resonant coupling to a vibrational mode producess off-diagonal elements in the Hamiltonian. This can occur most effectively in a chiral natural or semisynthetic matrix that is subject to a conformational change upon excitation. (Figure 1.4). The NCAP process differs from the adiabatic (Born-Oppenheimer type) process which is shown as a trajectory over the barrier (Figure 1.4, green curved arrow, right). The adiabatic process may be subject to back transfer (Figure 1.4, red curved arrow) and

subsequent relaxation (Figure 1.4, red downwards arrow) or direct recombination as a result of electron scavenging on the nanoscale (Figure 1.4, red diagonal arrow). However, due to dynamic symmetry breaking in chiral light-harvesting complexes and resonant coupling to a vibration, the NCAP process may proceed in a unidirectional lossless manner, driven by a ω_n twisting mode strengthened by the specific ordering of the system matrix (Figure 1.4, right).[43] The resonant coupling to a vibrational mode is a Goldilocks-type self-selection process, which is implicitly verified in this thesis that focuses on a chiral matrix to produce rapid energy transfer between cofactors.[3]

1.4 Constitution of an artificial light-harvesting antenna complex

1.4.1 The synthesis of tCsmA

There are two major paths to obtain protein products, these are chemical synthesis and protein biosynthesis, and both have their own advantages and disadvantages.

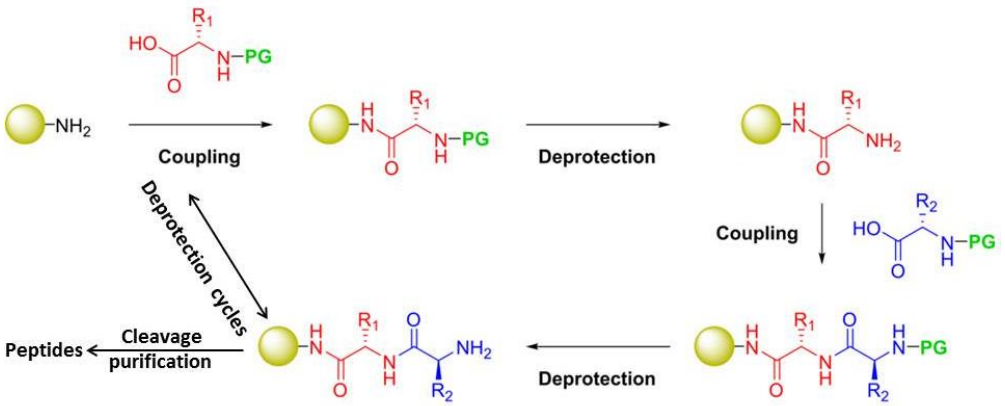

Figure 1.5 Solid-phase synthesis of peptide.

The most common and efficient chemical synthesis is known as solid-phase peptide synthesis (SPPS).[44-45] The SPPS procedure consists of repeated cycles of alternate N-terminal deprotection and coupling reactions (Figure 1.5). Amino acids are coupled to a resin which is washed before each step.[46] Subsequently, the deprotected amine can couple with the free acid of the

second amino acid. After a number of cycle repeats the desired peptide is stripped of all its protecting groups using a strong acid reagent, *e.g.* trifluoroacetic acid or a nucleophile and the resulting deprotected peptide strand is purified by RP-HPLC. However, the production of long protein sequences or isotopically labelled protein via SPPS is challenging due to the high costs of isotope labelled chemicals as well as due to technical limitations.

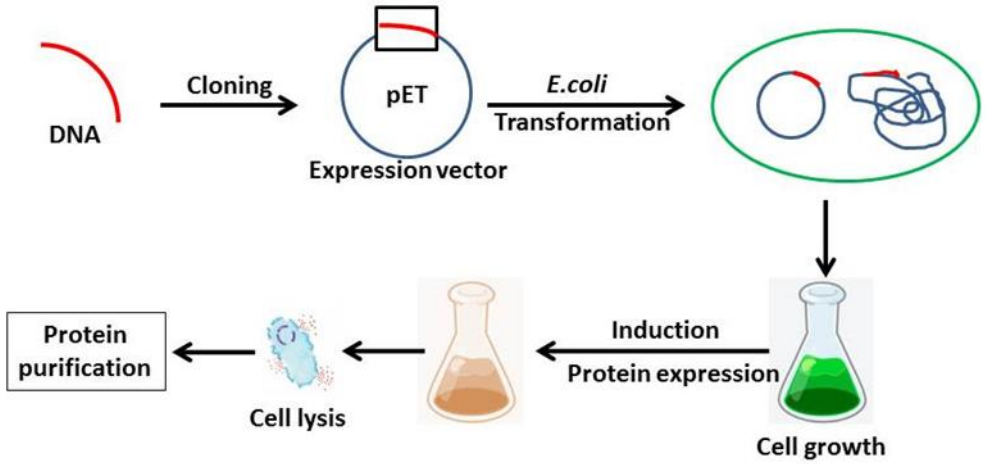

Figure 1.6 Steps for synthesis of the recombinant proteins in *E. coli*.

On the other hand, via protein biosynthesis large or isotope labelled proteins are more easily obtained. Generally, the gene of interest is cloned into the expression vector followed by transformation into the desired *Escherichia coli* (*E.coli*) strain. Upon induction, the cell will begin to overexpress protein. Other cell components are to be removed by purification after cell lysis (Figure 1.6). Protein expression in *E.coli* has been the most popular means of producing recombinant proteins for over the past two decades. *E.coli* is a well-established host that offers short culturing time, easy genetic manipulation and low cost media. Membrane proteins have been synthesized in the past using *E.coli* strains such as BL21.[47] Using these strains has the advantages of rapid, inexpensive and simple protein expression with a possibility of achieving high yields, even with proteins which are not native to *E.coli*. However, not all protein can be produced or easily purified. In addition, the extra residues can stay in the sequence if using fusion protein.

1.4.2 The constitution of the tCsmA-ZnPP complex

Amphipathic tCsmA and ZnPP have very poor solubility in water. To constitute the tCsmA and ZnPP, they should be co-dissolved with assistance of organic additives. However, if too much of these organic additives is present, the binding cannot easily take place. For instance, a detergent can be used for mediating constitution.[48] The amphiphilic nature of a detergent allows it to interact with hydrophobic membrane proteins and aqueous solvent to keep the protein in the dissolved state. Detergents are widely used for extraction, purification, and manipulation of membrane proteins.

Generally, detergents contain segregated polar and apolar domains (Figure 1.7 left).[49] In aqueous solution, a detergent is able to form micelles once a concentration above the critical micelle concentration (CMC) is reached. The shape and size of a micelle are a function of the molecular geometry of the detergent and solution conditions such as concentration, temperature, pH, and ionic strength. In addition, the concentration is important to the formation of the micelle. At below a certain temperature, the so called Krafft temperature, a saturated detergent solution will be in equilibrium with solid surfactant. In this case the temperature is too low for micelles to form. Above this temperature it is possible to form micelles and the detergent becomes much more soluble (Figure 1.7 right).[49]

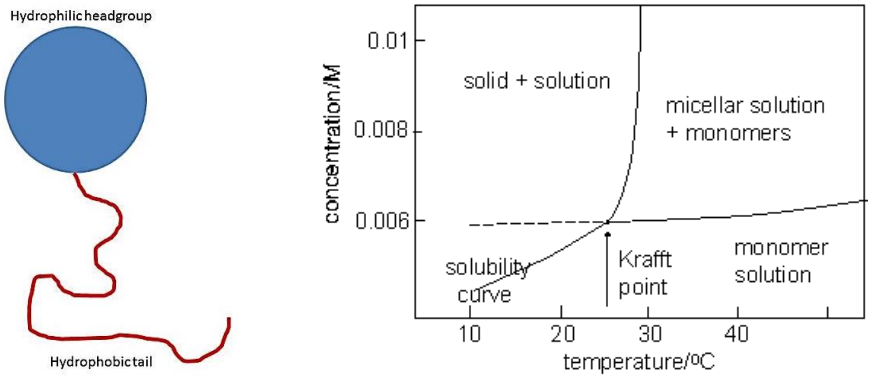

Figure 1.7 left : A typical illustration of a surfactant molecule. right : Temperature dependence of detergent solubility.[50]

Using these known properties we can control the solution conditions to solubilize the tCsmA and ZnPP above the CMC and while binding is allowed to take place below the CMC by dilution at low temperature. Here, detergents with high CMC such as Octyl β-D-glucopyranoside (OG) and Hexyl

β-D-glucopyranoside (HG) are a better choice for the constitution than detergents with low CMC, since a high enough final concentration of complex can be maintained in this manner. The solubility of tCsmA is proportional to the concentration of detergent (Figure 1.8). However, the detergent is difficult to remove while concentrating the protein due to the micelle formation.

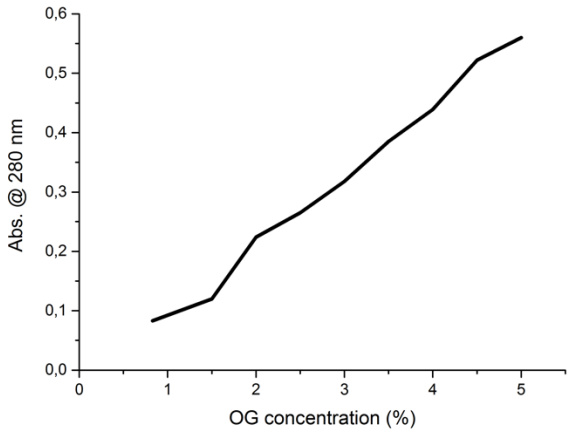

Figure 1.8 The OG-concentration dependent solubility of tCsmA.

In addition to dissolving the components with detergents, dialysis can also be used to control the solution conditions via buffer exchange. Compared to detergents, the complex can be obtained in pure buffer without dilution. Moreover, detergents are not necessary during dialysis for solubilization of protein, other compounds such as 1-propanol can also solubilize tCsmA well. In addition, compounds such as 1-propanol cannot form micelles which results in a faster dialysis speed compared to the dialysis of detergents.

1.5 Structure determination

X-ray crystallography, NMR spectroscopy, and electron microscopy are mainly used to determine the structures of proteins. X-ray crystallography can show atomic details in crystallized molecules. For NMR spectroscopy, the signal from the elements with nuclear spin ½ are able to offer information on the local conformation and distance between atoms that are close to one another to solve the structure. With 3D Electron Microscopy (3DEM) the 3D structure can be obtained from 2D projection images produced by transmission electron microscopy (TEM).

Table 1.1 Comparison of different techniques[51-54]

Methods	Advantages	Disadvantages
X-ray	1. High resolution can be obtained. 2. It costs less than other techniques. 3. The method for analysis is fully completed.	1. Crystallization is necessary. 2. We cannot examine the behavior of the molecules in solution. 3. Studying of motions is not possible. 4. Not all proteins crystallize well, the quality of the crystals influences the accuracy. 5. Cannot observe the hydrogen atoms. 6. The native state may be not observed.
NMR	1. The motion of protein can be examined. 2. The activation-thermodynamics and certain kinetic data can be resolved. 3. The influence of the dielectric constant, the polarity and any other property of the solvent or added material can be tested. 4. The sample can be in the solution phase or in the solid state so that the native state can be observed for both membrane and soluble proteins. 5. NMR can be used to obtain abundant information, *e.g.* angles, distances, coupling constants, chemical shifts, rate constants.	1. NMR is limited to relatively small proteins or parts of proteins 2. Costly. 3. Normally isotope labelling is usually necessary for the structure determination of protein. 4. High purity of sample is necessary. 5. Large amounts of sample are necessary.
3DEM	1. It is possible to observe large molecules or self-assemblies. 2. The native state of molecules can be observed. 3. It becomes possible to view cells, cell organelles as well as macromolecular complexes. 4. Only small amounts of sample are needed for 3DEM.	1. A large number of images is needed for 3D structure analysis. 2. Not easy to obtain images from a tilted specimen. 3. The electron-beam is easy to destroy the specimen. 4. Costly.

Each method has advantages and disadvantages (Table 1.1). Dependent on the type of sample and the experimental conditions, one or more methods can be chosen for structure determination. In this thesis, 3DEM is chosen for structure determination.

1.6 Single particle analysis for helical structure

TEM uses an electron beam to examine the structures of molecules and materials at the atomic scale. However, traditional TEM is not good enough for biomolecules due to the evaporation of surrounding water, and the risk of damage from the high energy electrons burning the sample. Hence, cryo-EM is used for structure determination of biomolecules to overcome these problems by using frozen samples, weaker electron beams with a low dose of electrons, and advanced image processing. Single-particle analysis (SPA) and electron tomography (ET) are two common techniques for cryo-EM to solve the structure puzzle of biomolecules. The technique used in this thesis is single particle analysis.

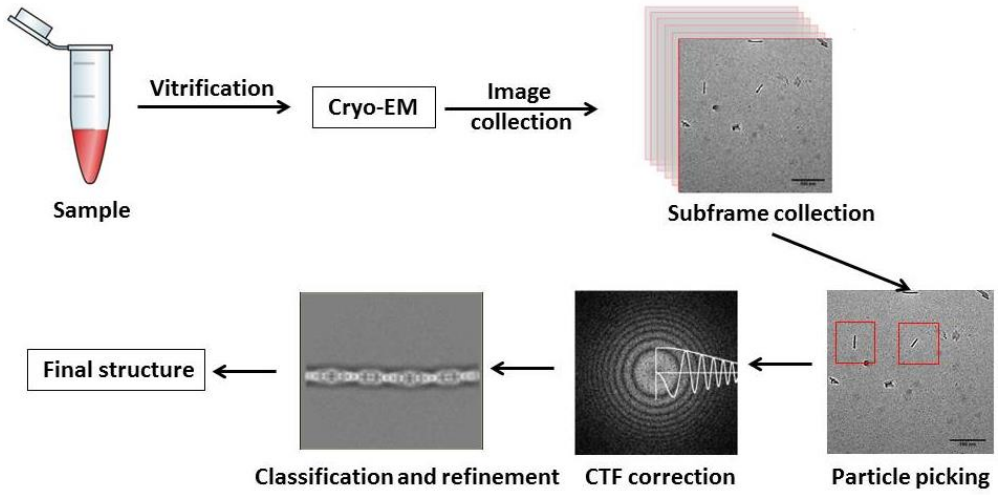

Figure 1.9 The process of single-particle cryo-EM.

The principle of SPA is based on averaging multiple views of a single molecule. A large number of images of the molecule is necessary for 3D reconstruction. The collected images from cryo-EM reflect the electron density of sample. To get less noise and higher resolution, the blotting and

vitrification process should be rapid to ensure a thin ice layer. Cryo-EM images are usually collected in defocused condition to increase phase contrast. The collected data is a series of images that need to be aligned to compensate for sample shift and beam-induced movement to reduce image blurring. These images are modulated in frequency space by the phase Contrast Transfer Function (CTF). By aligning several similar images to each other, an image with a higher signal to noise ratio can be obtained. There are several software packages such as SPIDER, EMAN2, RELION, SPARX and SPRING to process the collected images.[55-59] Generally, the particles should be picked and extracted for further classification and refinement (Figure 1.9).

Except for the single molecule or complex, SPA can be used for helical filaments if the accurate helical symmetry is applied to the 3D construction process by using RELION.[60] SPRING is a single-particle based helical reconstruction package for electron cryo-micrographs and has been used to determine the precise helical symmetry by using a class-based helical reconstruction scheme that explores the helical symmetry by reconstructing the 3D structures from a large number of symmetry combinations using a single class average. Afterwards, according to the cross-correlation of the power spectra of the class average and projections of the resulting 3D models, the helical symmetry can be extracted from a large number of possible symmetry combinations.

1.7 Scope of this thesis

This thesis is concerned with the constitution of chiral responsive matrix assemblies that may undergo light-driven conformational changes and self-select vibrations to develop vibronic states for driving semi-classical coherent transfer with nearly 100% yield in a lossless NCAP process. In this work, a truncated CsmA derived from the CsmA protein from the baseplate of the *Chlorobaculum tepidum* has been used to engineer a chiral matrix around a zinc protoporphyrin IX (ZnPP) chromophore (Chapter 2). After the study of the specific structure of tCsmA-ZnPP complex, the mechanism of self-assembly was resolved (Chapter 3 and Chapter 4). Expression and purification of tCsmA in *E.coli* was explored to pave the way for other methods, such as NMR (Chapter 5).

1.8 References

1. Chin, A. W.; Datta, A.; Caruso, F.; Huelga, S. F.; Plenio, M. B., *New Journal of Physics* **2010,** *12* (6), 065002.
2. Chin, A.; Prior, J.; Rosenbach, R.; Caycedo-Soler, F.; Huelga, S.; Plenio, M., *Nature Physics* **2013,** *9* (2), 113.
3. Huelga, S. F.; Plenio, M. B., *Contemporary Physics* **2013,** *54* (4), 181-207.
4. Mathies, R. A., *Nature chemistry* **2015,** *7* (12), 945.
5. Purchase, R.; De Groot, H., *Interface Focus* **2015,** *5* (3), 20150014.
6. Yin, X.; Struik, P. C., *Journal of experimental botany* **2015,** *66* (21), 6535-6549.
7. Hoffman, D. P.; Mathies, R. A., *Accounts of chemical research* **2016,** *49* (4), 616-625.
8. Cogdell, R. J.; Brotosudarmo, T. H.; Gardiner, A. T.; Sanchez, P. M.; Cronin, L., *Biofuels* **2010,** *1* (6), 861-876.
9. Hall, D.; House, J., *Biomass and Bioenergy* **1994,** *6* (1-2), 11-30.
10. Millan-Almaraz, J. R.; Guevara-Gonzalez, R. G.; Romero-Troncoso, R.; Osornio-Rios, R. A.; Torres-Pacheco, I., *African Journal of Biotechnology* **2009,** *8* (25).
11. Tran, P. D.; Wong, L. H.; Barber, J.; Loo, J. S., *Energ Environ Sci* **2012,** *5* (3), 5902-5918.
12. Barber, J.; Tran, P. D., *Journal of The Royal Society Interface* **2013,** *10* (81), 20120984.
13. Nann, T.; Ibrahim, S. K.; Woi, P. M.; Xu, S.; Ziegler, J.; Pickett, C. J., *Angewandte Chemie International Edition* **2010,** *49* (9), 1574-1577.
14. Blankenship, R. E., *Molecular mechanisms of photosynthesis*. John Wiley & Sons: 2014.
15. Adir, N., *Photosynthesis Research* **2005,** *85* (1), 15-32.
16. Larkum, T., *Trends in Plant Science* **1996,** *1* (8), 252.
17. Liu, Z.; Yan, H.; Wang, K.; Kuang, T.; Zhang, J.; Gui, L.; An, X.; Chang, W., *Nature* **2004,** *428* (6980), 287.
18. Pšenčík, J.; Ikonen, T.; Laurinmäki, P.; Merckel, M.; Butcher, S.; Serimaa, R.; Tuma, R., *Biophysical journal* **2004,** *87* (2), 1165-1172.
19. Ting, C. S.; Rocap, G.; King, J.; Chisholm, S. W., *Trends in microbiology* **2002,** *10* (3), 134-142.
20. van Grondelle, R.; Novoderezhkin, V., *Biochemistry* **2001,** *40* (50), 15057-15068.
21. Van Grondelle, R., *Biochimica et Biophysica Acta (BBA)-Reviews on Bioenergetics* **1985,** *811* (2), 147-195.
22. van Grondelle, R.; Dekker, J. P.; Gillbro, T.; Sundstrom, V., *Biochimica et Biophysica Acta (BBA)-Bioenergetics* **1994,** *1187* (1), 1-65.
23. Chin, A.; Prior, J.; Rosenbach, R.; Caycedo-Soler, F.; Huelga, S.; Plenio, M. B., *Nature Physics* **2013,** *9* (2), 113.

24. Hu, S.; Chi, C.-Y.; Fountaine, K. T.; Yao, M.; Atwater, H. A.; Dapkus, P. D.; Lewis, N. S.; Zhou, C., *Energ Environ Sci* **2013,** *6* (6), 1879-1890.
25. Yalamanchili, S.; Emmer, H. S.; Fountaine, K. T.; Chen, C. T.; Lewis, N. S.; Atwater, H. A., *Acs Photonics* **2016,** *3* (10), 1854-1861.
26. Gao, L.; Cui, Y.; Wang, J.; Cavalli, A.; Standing, A.; Vu, T. T.; Verheijen, M. A.; Haverkort, J. E.; Bakkers, E. P.; Notten, P. H., *Nano Lett* **2014,** *14* (7), 3715-3719.
27. Montano, G. A.; Wu, H.-M.; Lin, S.; Brune, D. C.; Blankenship, R. E., *Biochemistry* **2003,** *42* (34), 10246-10251.
28. Staehelin, L. A.; Golecki, J. R.; Drews, G., *Biochimica et Biophysica Acta (BBA)-Bioenergetics* **1980,** *589* (1), 30-45.
29. Sprague, S.; Staehelin, L.; DiBartolomeis, M.; Fuller, R., *Journal of bacteriology* **1981,** *147* (3), 1021-1031.
30. Nielsen, J. T.; Kulminskaya, N. V.; Bjerring, M.; Linnanto, J. M.; Rätsep, M.; Pedersen, M. Ø.; Lambrev, P. H.; Dorogi, M.; Garab, G.; Thomsen, K., *Nature communications* **2016,** *7*, 12454.
31. Cogdell, R. J.; Gardiner, A. T.; Cronin, L., *Phil. Trans. R. Soc. A* **2012,** *370* (1972), 3819-3826.
32. Blankenship, R. E.; Olson, J. M.; Miller, M., Antenna complexes from green photosynthetic bacteria. In *Anoxygenic photosynthetic bacteria*, Springer: 1995; pp 399-435.
33. Pedersen, M. Ø.; Pham, L.; Steensgaard, D. B.; Miller, M., *Biochemistry* **2008,** *47* (5), 1435-1441.
34. Pedersen, M. Ø.; Linnanto, J.; Frigaard, N.-U.; Nielsen, N. C.; Miller, M., *Photosynthesis research* **2010,** *104* (2-3), 233-243.
35. Frigaard, N.-U.; Li, H.; Martinsson, P.; Das, S. K.; Frank, H. A.; Aartsma, T. J.; Bryant, D. A., *Photosynthesis research* **2005,** *86* (1-2), 101-111.
36. Luo, L.; Chang, C. H.; Chen, Y. C.; Wu, T. K.; Diau, E. W., *J Phys Chem B* **2007,** *111* (26), 7656-64.
37. Fry, H. C.; Garcia, J. M.; Medina, M. J.; Ricoy, U. M.; Gosztola, D. J.; Nikiforov, M. P.; Palmer, L. C.; Stupp, S. I., *J Am Chem Soc* **2012,** *134* (36), 14646-14649.
38. Gibney, B. R.; Rabanal, F.; Reddy, K. S.; Dutton, P. L., *Biochemistry* **1998,** *37* (13), 4635-4643.
39. Sharp, R. E.; Moser, C. C.; Rabanal, F.; Dutton, P. L., *Proceedings of the National Academy of Sciences* **1998,** *95* (18), 10465-10470.
40. Grosset, A. M.; Gibney, B. R.; Rabanal, F.; Moser, C. C.; Dutton, P. L., *Biochemistry* **2001,** *40* (18), 5474-5487.
41. Koder, R. L.; Valentine, K. G.; Cerda, J.; Noy, D.; Smith, K. M.; Wand, A. J.; Dutton, P. L., *J Am Chem Soc* **2006,** *128* (45), 14450-14451.
42. McConnell, I.; Li, G.; Brudvig, G. W., *Chemistry & biology* **2010,** *17* (5), 434-447.
43. R. Purchase, R. C., F. Breitling,V. Stadler,; N. van Hulst, G.-J. K., A. Ramirez,; R. Zwijnenberg, A. K., J. B. de Baan,; Groot, I. R. a. H. J. M. d., Semi-Synthetic Responsive Matrices for Artificial Photosynthesis. In *In Bioinspired Chemistry: From Enzymes to Synthetic Applications*, Reglier, M., Ed.

44. Synthesis—A, F. S. P. P., Practical Approach. Chan, WC: 2000.
45. Fields, G. B., *Peptide characterization and application protocols*. Humana Press: 2007; Vol. 386.
46. Chan, W. C.; White, P. D., *Fmoc solid phase peptide synthesis*. Oxford University Press: 2000.
47. Miroux, B.; Walker, J. E., *Journal of molecular biology* **1996,** *260* (3), 289-298.
48. Pedersen, M. O.; Pham, L.; Steensgaard, D. B.; Miller, M., *Biochemistry* **2008,** *47* (5), 1435-41.
49. Kronberg, B.; Lindman, B., *Surfactants and polymers in aqueous solution*. John Wiley & Sons Ltd., Chichester: 2003.
50. https://nova.disfarm.unimi.it/probuilder.htm.
51. Milne, J. L.; Borgnia, M. J.; Bartesaghi, A.; Tran, E. E.; Earl, L. A.; Schauder, D. M.; Lengyel, J.; Pierson, J.; Patwardhan, A.; Subramaniam, S., *The FEBS journal* **2013,** *280* (1), 28-45.
52. Cheng, Y.; Grigorieff, N.; Penczek, P. A.; Walz, T., *Cell* **2015,** *161* (3), 438-449.
53. Warren, B. E., *X-ray Diffraction*. Courier Corporation: 1990.
54. Coates, G. R.; Xiao, L.; Prammer, M. G., *NMR logging: principles and applications*. Haliburton Energy Services Houston: 1999; Vol. 344.
55. Frank, J.; Shimkin, B.; Dowse, H., *Ultramicroscopy* **1981,** *6* (4), 343-357.
56. Hohn, M.; Tang, G.; Goodyear, G.; Baldwin, P. R.; Huang, Z.; Penczek, P. A.; Yang, C.; Glaeser, R. M.; Adams, P. D.; Ludtke, S. J., *Journal of structural biology* **2007,** *157* (1), 47-55.
57. Tang, G.; Peng, L.; Baldwin, P. R.; Mann, D. S.; Jiang, W.; Rees, I.; Ludtke, S. J., *Journal of structural biology* **2007,** *157* (1), 38-46.
58. Scheres, S. H., *Journal of structural biology* **2012,** *180* (3), 519-530.
59. Desfosses, A.; Ciuffa, R.; Gutsche, I.; Sachse, C., *Journal of structural biology* **2014,** *185* (1), 15-26.
60. He, S.; Scheres, S. H., *Journal of structural biology* **2017,** *198* (3), 163-176.