9 Summary and outlook

The here described X-ray crystal structure at 3.2 Å resolution of cyanobacterial PSIIcc is a big step forward (compared to the earlier 3.8 Å resolution structure) to better understand oxygenic photosynthesis, as the ~133,000 data allow for the first time crystallographic refinement of the structural model and provide a new quality of reliability of the electron density that shows many novel details on organic and inorganic cofactors and their coordinating amino acids and surroundings.

PSIIcc with a molecular weight of 750 kDa occurs as homodimer and is composed of 17 different subunits forming a field of 36 transmembrane α-helices per monomer. A total of 44,000 atoms (~75% of all atoms of PSIIcc) could be modelled in the electron density that is, at 3.2 Å resolution, still not good enough to see all atoms of the structure. In contrast to the previous structural model at 3.8 Å resolution, most of the amino acids within the membrane spanning region of the reaction centre proteins (D1 and D2), the antenna proteins (CP47 and CP43) as well as the α- and β-chain of cyt *b*-449 could be identified in the monomer.

The structure provides the protein scaffold for 46 embedded and identified cofactors. The cofactors of the electron transfer chain could be identified except for the primary and secondary plastoquinones Q_A and Q_B, the former being modelled only with the benzene ring and the latter was lost during purification. This opened the possibility to study the interplay of the cofactors and modulatation of the properties of the cofactors by the surrounding protein matrix. Anomalous X-ray diffraction data collected beyond the Mn-edge confirmed the presence of a Ca^{2+} close to the Mn_4-cluster, but the limited resolution did not allow to clearly indicate the metal centres that are about 3 Å apart. A total of 39 chlorophyll *a* (Chl*a*) molecules were assigned. The core antenna subunits CP47 and CP43 harbour 16 Chl*a* in CP47 and 13 Chl*a*, respectively, in CP43 that are arranged in two layers near the cytoplasmic and lumenal sides of the membrane. Additionally we could locate a carotenoid close to protein D2 and cytochrome *b*-449 that might be involved in secondary electron transfer during light-induced stress conditions. For cytochromes *b*-449 and *c*-550 the haem porphyrins were positioned. Due to the still limited resolution several elongated patches of electron density could not yet be assigned. They could represent carotenoids, lipids or the phytol chains of Chl*a*.

Based on the result of this thesis, H. Ishikita, a member of the group of Prof. W. Knapp, initiated model calculation on the redox potentials of cofactors along the ETC. I. Samish, a member of the group of Prof. A. Scherz at the Weizmann Institute of Science in Jerusalem

(Israel) is using the coordinates to develop a new algorithm to compute multiple structural alignments of photosynthetic reaction centres. Furthermore they plan to study the regulation of protein-gated electron transfer by H-bonding and packing between protein subunits.

The current model of PSII must be considered as an intermediate step, the aim being to obtain a structure of PSII at atomic detail that requires X-ray diffraction data to a resolution of 2.8 Å or better to answer a number of questions are still open. The detailed structure of the Mn_4-Ca-cluster and its coordination by amino acids has to be elucidated to understand the catalytic mechanism of water oxidation. Many still unconnected, elongated patches of electron density have to be assigned to organic cofactors which could be involved in the transfer of excitation energy from the antenna system to the electron transfer chain. The definite assignment of the amino acid sequence to the membrane-extrinsic subunits PsbO and PsbU as well as to the membrane-intrinsic unassigned low molecular weight subunits which thus far could only be interpreted with their polypeptide main chain atoms could help in understanding their function and the fifth direct ligand to the non-haem Fe^{2+} has to be determined. The plastoquinones Q_A and Q_B have to be located and their coordination as well as the H-bonding to the protein has to be verified.

The 3.2 Å resolution structure of PSIIcc is the best determined to date and will help to better understand the molecular mechanism of water oxidation and to provide insights in related fields like artificial photosynthesis, function of amino acid radical enzymes, and light adaptation processes in plants.