All of the Xe positions are at some distance away from cofactors of the ETC that are responsible for charge separation and electron transport in the RC. The distribution of Xe sites along the membrane normal is reminiscent of the distribution of O_2 in lipid bilayers [292] and suggests that there might be oxygen diffusion pathways through the interior of PSII provided by the high lipid content (see also section 3.2.1.1). The physiological significance of these pathways could be the following: even though O_2 is probably guided away from the OEC into the lumen via the putative channels B1 and B2 discussed above, it may accumulate in the vicinity of PSII. The pathways in the PSII interior will then guide O_2 into the membrane, where it can diffuse away faster and may exit elsewhere from the thylakoid membrane. Though the membrane surface is a barrier for oxygen diffusion, it is not insurmountable [220]. Thus, PSII itself could contribute to fast O_2 removal by facilitating O_2 diffusion into the membrane, while still minimizing the contact of O_2 with the RC. The latter effect is important as chlorophyll triplet states are likely formed in the RC and could react with O_2 to form harmful singlet oxygen.

No Xe was found in the calculated oxygen channels B1 and B2. Since the reason for this could be the size of the Xe atom, the experiment was repeated with Kr, which has a lower atomic weight (Kr 83.80, Xe 131.30) and smaller atomic radius of 2.02 Å. However, Kr has one significant disadvantage as the anomalous signal is three times less than that of Xe (11.830e for Xe versus 3.794e for Kr), complicating the assignment of Kr atoms at low resolution (the collected datasets are around 4 Å resolution). Despite this fact and taking into account the good (~70%) correlation between binding sites of Xe and Kr atoms, 12 binding sites of Kr were determined in each PSII monomer (23 for the dimer, one of them on the C_2 axis) (Appendix Table 7.9)). Indeed, two Kr binding sites were found in putative oxygen channels, Kr9 in channel B2 and Kr10 in channel B1 (Fig. 59).

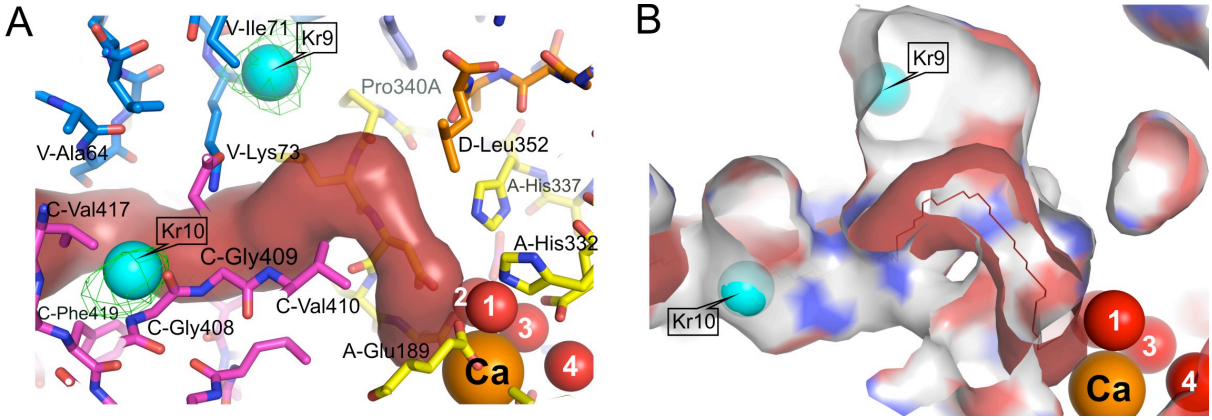

Figure 59. Kr positions in oxygen channel B1. (A) Difference anomalous electron density for Kr sites 9 and 10 contoured at 5σ level (green mesh). The calculated trajectory of channel B1 is indicated by the red surface. (B)

Surface of oxygen channel B1 (coloured according to polarity of amino acid side chains: grey - apolar, blue - positively, red – negatively charged) with CAVER trajectory (red line) and Kr sites (blue spheres).

It is puzzling that no Xe is located in any of the channels assigned to water or oxygen transport, as expected. Most probably this is associated with the properties of Xe atoms, which have a higher affinity for hydrophobic sites and are unable to penetrate into smaller cavities as compared to Kr atoms [72]. This difference of noble gas binding was shown for other proteins as well [68]. All the Xe binding sites found in PSII are purely non-polar (see Appendix Table 7.9) and formed either by non-polar amino acids or by non-polar amino acids and non-polar side chains of lipids or Chl *a* molecules, in some cases also by non-polar carotenoids.

By contrast, none of the channels for water and oxygen transport is entirely non-polar but contains also polar, sometimes even charged amino acid side chains that appear to be essential for efficient transport of these educt and product molecules, but are less well suited to accommodate the non-polar noble gases. This agrees with the finding that the additional Xe sites found at higher noble gas pressure are again exclusively non-polar.

4 Summary and Outlook

The here-described 2.9 Å resolution structure of Photosystem II is a milestone in the structural studies of oxygenic photosynthesis. It is the best-resolved structure of PSII up to date and allowed for a first time a complete assignment of all 20 protein subunits, and numerous newly found cofactors (92 cofactors per monomer in total; 35 chlorophyll molecules, two pheophytine and two haem molecules, twelve β-carotenoids, 25 lipids, seven detergent molecules, three plastoquinones, unique Mn_4Ca cluster, bicarbonate, two Ca^{2+}, one Fe^{2+} and one Cl^- ion) significantly enhanced the whole picture of this sophisticated natural machinery. The newly found third plastoquinone molecule Q_C in combination with the found channels connecting the Q_B-binding site with the thylakoid membrane allowed for the first time to describe possible mechanisms of plastoquinone / plastoquinol exchange in PSII. It also initiated crystallographic studies on herbicide binding to PSII, being performed now in collaboration with Prof. Dr. Athina Zouni (Technical University Berlin).

The detailed analysis of interactions between the protein matrix and newly assigned lipid molecules may serve as a basis for future mutagenesis studies to better understand the roles of lipids in PSII, although several proposals for lipids functions have been already made in this work. Lipids found at the monomer-monomer interface and around the reaction centre seem to be the ideal lubricant component in processes of dis- and reassembly of PSII needed for replacement of photodamaged subunit D1. The largest lipid cluster composed of eight lipids forms an intrinsic lipid-bilayer providing hydrophobic environment for plastoquinone / plastoquinol exchange and all lipid clusters within PSII may participate in auxiliary oxygen channelling as shown in the experiments with a gaseous xenon. Additionally, lipids close to the plastoquinone molecules could influence on the electron transfer between them, and some lipids could even ligate chlorophyll molecules if no ligating residue is available from the protein matrix.

Although the positions of cations of the Mn_4Ca have not changed significantly in comparison with the 3.0 Å resolution model, the localization of the chloride ion in the vicinity of the Mn_4Ca cluster is an important step in elucidating the exact structure of this unique catalyst.

The calculated and experimentally confirmed transport pathways provide a better understanding how water, oxygen and protons travel to and from Mn_4Ca cluster, and the

analysis of amino acids forming these channels may be used as the basis for point mutations to determine the exact involvement of every channel in the transport of given small molecules.

However, a model of PSII with a higher resolution is still very desirable. The most promising way to improve the resolution limit of given crystals is an attempt to get better crystal packing which can be achieved by varying of detergent, for example. The currently used β-DM detergent might substitute some lipid molecules, therefore application of chemically different detergent may give another benefit that the lipid content will remain unchanged. The search for a better detergent was recently initiated in the laboratory of Prof. Dr. Zouni.

Radiation damage is another serious issue leading to the deterioration of diffraction pattern. Although addition of radical scavengers and extremely low temperatures achievable with helium cryostat may slow down it, changes of oxidation states within the Mn_4Ca cluster occurs, not allowing to determine fine structure of the latter with X-ray diffraction. The available high resolution models of the Mn_4Ca cluster obtained with EXAFS are valuable indeed, but a detailed information about exact ligation and arrangement of cations of the Mn_4Ca cluster within PSII is still missing. To overcome this problem application of neutron diffraction is might be recommended.

5 Zusammenfassung

Photosystem II (PSII) - ein grosser homodimerer Protein-Kofaktor Komplex - ist in die photosynthetische Thylakoidmembran von Pflanzen, Algen und Cyanobakterien eingebettet und kann als lichtgetriebene Wasser-Plastochinon Oxidoreduktase beschrieben werden. Die einzigartige Reaktion der Wasserspaltung findet am Mn_4Ca-Zentrum statt und führt zur Bildung von atmosphärischem Sauerstoff, wohingegen bewegliches Plastochinon/Plastochinol zum Transfer von Elektronen von PSII über den Cytochrom *b6f* Komplex zu Photosystem I genutzt wird.

Das in dieser Arbeit beschriebene drei-dimensionale Strukturmodel des homodimeren PSII aus dem Cyanobakterium *Thermosynechococcus elongatus* bei einer Auflösung von 2.9 Å erlaubt die eindeutige Zuordnung aller 20 Proteinuntereinheiten und die vollständige Modellierung aller 35 Chlorophyll *a* Moleküle pro Monomer. Darüber hinaus konnten 12 Karotenoide, 25 integral gebundene Lipide und ein Chlorid-Anion in jedem Monomer modelliert werden. Das erstmalig gefundene dritte Plastochinon-Molekül Q_C zusammen mit einem neuen zweiten Kanal für den Transfer von Plastochinon bildet die Grundlage für Mechanismen des Plastochinon-Plastochinol-Austauschs in PSII. Die Struktur des Mn_4Ca-Zentrums konnte durch die Modellierung eines Chlorid-Anions in einem Abstand von 6.5 Å von Mn4, das wahrscheinlich über ein Wassermolekül mit dem Mn-Zentrum verbrückt ist, weiter verbessert werden.

Der ungewöhnlich hohe Anteil an integral gebundenen Lipiden in der Struktur hängt mit der dynamischen Natur von PSII zusammen: zum einen mit dem stetigen Ab- und Aufbau des PSII-Komplexes infolge von schädigenden Nebenreaktionen durch überschüssiges Licht; zum anderen, um eine hydrophobe Umgebung für den Austausch von Plastochinon-Plastochinol mit der Thylakoidmembran bereitzustellen und die Diffusion des gebildeten Sauerstoffs aus dem Lumen in das Stroma zu erleichtern. Die berechneten und teilweise experimentell bestätigten Kanäle erlauben es, den Transport von Wasser, Sauerstoff und Protonen zum und vom Mn_4Ca-Zentrum besser zu verstehen.