required flexibility to avoid too strong binding for efficient dissociation into monomers. Of the 14 lipids at the monomer-monomer interface, six form contacts with protein subunits from both monomers, for example the head group of SQDG13 forms hydrogen bonds with subunits PsbM, CP47 of the other monomer and PsbL of one and PsbL' of the other monomer (Fig. 31 and Appendix Table 7.6). Another six lipids mediate interactions between a subunit of one monomer and a lipid (or detergent) bound to a subunit of the other monomer. The remaining two lipids form only contacts to the same monomer.

There are some indications that PG may be involved in the dimerization of PSII because it was shown [208] for dimeric PSII from spinach that digestion of PG by added phospholipase resulted in the formation of PSII monomers. A similar effect was observed in *Synechocystis* when the biosynthesis of PG was genetically blocked, resulting in a higher percentage of monomeric PSII and a destabilization of dimeric PSII [209, 210]. In contrast, two recent studies on PSII from *T. vulcanus* [211] and from *T. elongatus* [212] showed that dimeric PSII did not dissociate into monomers upon treatment with phospholipase. In agreement with this finding, no PG molecule could be located at the monomer-monomer interface in the cyanobacterial PSII structure. In addition, the head groups of all detergent molecules localized at the monomer-monomer interface are at the lumenal side of the membrane, indicating that *in vivo* these positions are probably not occupied by PG that would rather prefer the positively charged cytoplasmic side. However, it cannot be excluded that additional lipids are present on the cytoplasmic side that are not yet resolved in the 2.9 Å electron density.

When PG was depleted in *Synechocystis* mutant strains, an increased amount of monomeric CP47 / RC complexes was found, indicating only loose attachment of CP43 to the D1/D2 core in these cells [210]. This agrees well with the location of the two PG molecules at the interface region between D1 and CP43 in the PSII structural model (Fig. 33).

A destabilization of the D1-CP43 interaction by depletion of PG could also be an indirect cause for the monomerization of the dimeric PSII complex. In addition the localization of both PG molecules at the outside of subunit D1 (Figs. 27, 33) is in good agreement with a study showing tight association of PG with D1 in cyanobacteria [213]. Furthermore, studies of *Chlamydomonas* mutants depleted in PG suggested that PG might be essential for the correct insertion of D1 into the thylakoid membrane and for the assembly of the complex during replacement of photodamaged D1 [214].

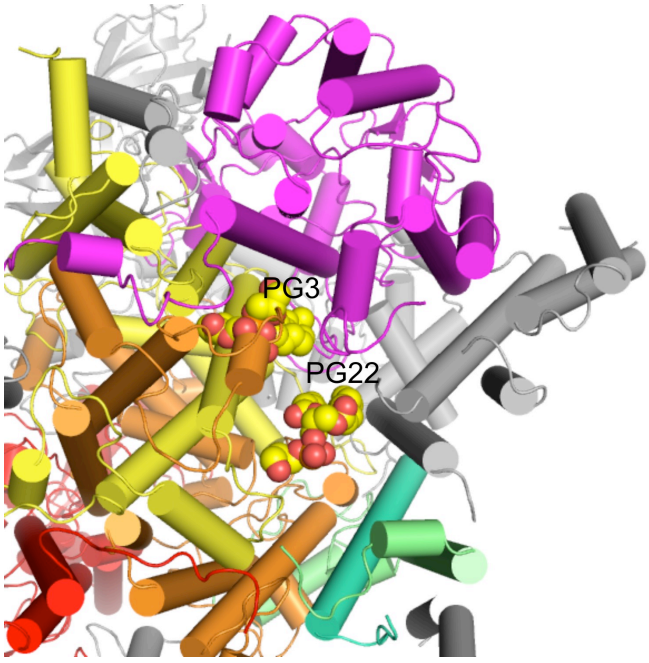

Figure 33. Location of two PG molecules in PSII. View from the cytoplasmic side, same as in Fig. 32. Colouring is as in Fig. 8. Only PG lipids are shown, the other lipids are omitted for clarity. See Appendix Table 7.6 for contacts with protein.

3.2.1.4 Interactions with other cofactors

Interestingly, in PSII eleven of the 35 Chl per monomer have a lipid close to their chlorin ring, and two more are close to a β-DM which might be replaced by a lipid *in vivo*, yielding a total of 13 Chl (roughly 1/3 of all Chl) with a lipid group forming part of the binding pocket of the chlorin ring. In most cases the lipid contributions to the Chl binding pockets are from the fatty acid moieties and consequently apolar. However, in the case of six Chl (Chl7, 17, 24, 27, 37, 44) the glycerol moiety and the head group of lipids MGDG1, PG3, DGDG5, MGDG9, MGDG14, MGDG17 contribute to the chlorin ring binding pockets. Here, indeed, hydrogen bonds are formed between the head group of a lipid and the chlorin ring (including coordination of Mg^{2+}) or the carbonyl oxygen of the phytyl ester. An example for a typical Chl-lipid interaction is shown in Fig. 34.

The central Mg^{2+} of Chl17 and Chl37 are possibly ligated by a lipid. The carbonyl oxygen of one of the fatty acid esters of MGDG14 is found at 3.9 Å to the central Mg^{2+} in Chl17 of CP47. The only other possible axial ligand to this Mg^{2+} could be the sulphur of CP47-Met37, but here the distance is even larger (4.6 Å). These distances are rather long and not typical for the distances found for normal axial ligands to the Mg^{2+} (around 2.3-2.5 Å for

His-Mg^{2+}) but could indicate indirect ligand-Mg^{2+} interaction mediated by water molecules that could not be located at 2.9 Å resolution.

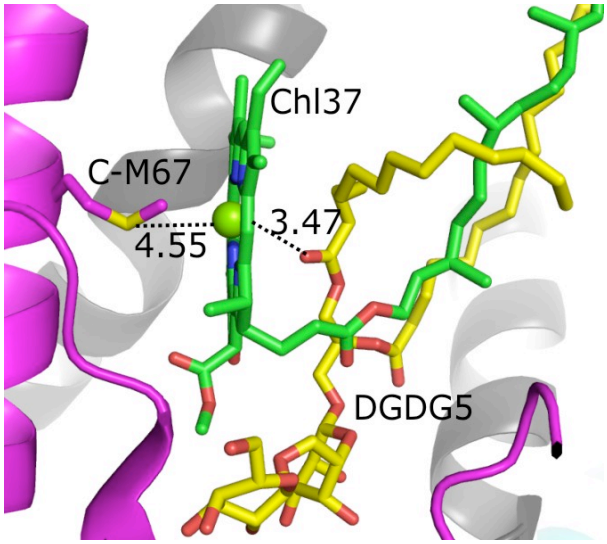

Figure 34. Coordination of the central Mg^{2+} of Chl37 (green) by the glycerol moiety of DGDG5 (yellow). Surrounding proteins shown in magenta (CP43) and grey (other subunits).

Similar as Chl17, Chl37 which is at the symmetry equivalent position in CP43 shows no direct ligand for the central Mg^{2+}. The glycerol moiety and one fatty acid of DGDG5 are at one face of the chlorin ring with the fatty acid carbonyl group in a position similar to the situation found for MGDG14, leading to an O-Mg distance of 3.5 Å (Fig. 34). At the other face of the chlorin ring the two nearest amino acids C-Trp63 (at 5.4 Å) and C-Met67 (at 4.5 Å) could also indirectly ligate the central Mg^{2+}.

The arrangement of lipids in the vicinity of Chl described above and Mg^{2+} ligation by lipid head groups found in different complexes of oxygenic photosynthesis suggest that the presence of a negative charge of SQDG or PG and hydrogen bond formation with lipids could influence the electronic distribution within the π-system of a nearby Chl or the energies of its excited electronic states, thereby tuning its spectral and redox properties. Using lipids as ligands for the Mg^{2+} in Chl allows to incorporate Chl into regions of a protein structure where no direct ligation by the protein is possible. In the light of these structural findings the ligation of Chl by lipid head groups could occur temporarily during the assembly of Chl-protein complexes, and lipids could play a chaperone-like role in this process.

In the cases where hydrogen bonding interactions between lipid head groups and substituents of the chlorin ring were found, lipids could be employed to fine tune the redox potential of the Chl if no hydrogen bond interaction by the protein is possible due to steric or

sequence reasons. The presence of the fatty acids of lipids in the binding pocket of a chlorin ring compared to a situation where only protein is forming the binding pocket, the overall hydrophobicity of the binding pocket would be increased, and the composite absorption spectrum of all Chl bound to a protein complex could be broadened, compared to a situation where only protein bound Chls are present.

Among the twelve Car found in PSII per monomer, nine (75%) have fatty acids of a lipid contributing to their binding pocket. The number of lipids contributing to each binding pocket is between one and five. For example, the binding pocket of Car15 is formed by only six amino acids, covering roughly less than 1/3 of this binding pocket. The other 2/3 of the binding pocket are contributed nearly exclusively by fatty acids from five different lipids. The two special carotenoids Car_{D1} and Car_{D2} (Fig. 14), bound to D1 and D2, respectively, are thought to be involved in possible secondary electron transfer reactions, and found in a lipid rich environment with four and five lipids, respectively, contributing about 50% to the Car binding pockets. Lipids are employed in some cases for the inclusion of carotenoids into protein complexes but the interactions between lipid and carotenoid are less specific compared to the situation for Chl.

There are several reports of changes in water oxidizing activity of PSII induced by changes in the lipid composition of the thylakoid membrane [215-217]. The catalytic site of water oxidation, the Mn_4Ca cluster, is located at the lumenal side of PSII close to the membrane surface but shielded from the lumen by large loop regions of subunits D1, D2, CP43 and CP47 as well as by the membrane extrinsic subunits PsbO, PsbU and PsbV. In the structural model no lipid was found in the direct vicinity of the Mn_4Ca cluster. The closest lipids are DGDG2, DGDG5 and DGDG6 (Fig. 35), all of them at distances of 15 Å and more from the Mn_4Ca cluster, indicating that depletion of DGDG might have a rather indirect effect on water oxidation and possibly is associated with conformational changes at the lumenal side of the PSII complex that are induced by depletion of a lipid exclusively located at the lumenal side. This might also be related to changes in binding of the membrane extrinsic subunits of PSII upon depletion of DGDG because DGDG head groups interact with PsbO and PsbV.

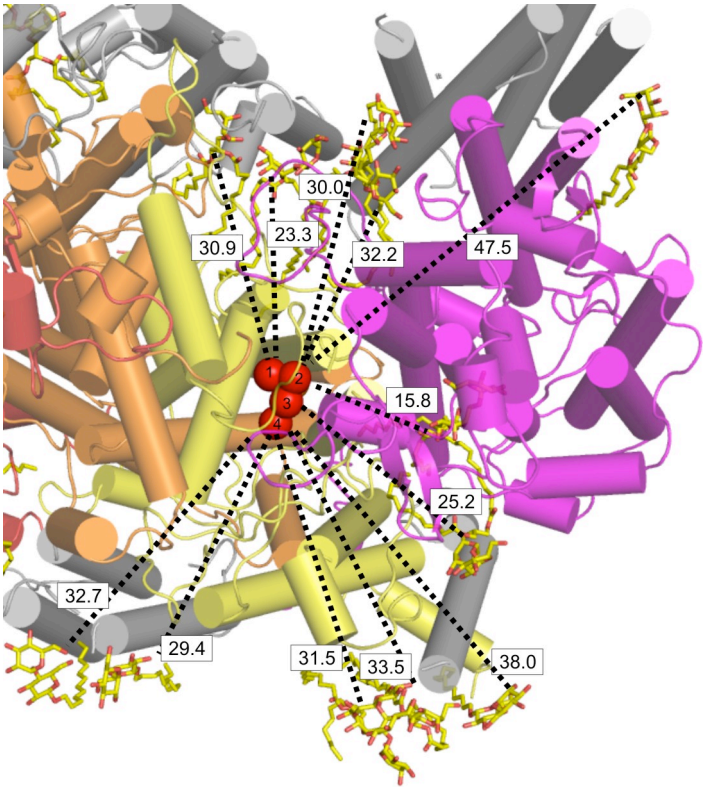

Figure 35. Lipids on the lumenal side in vicinity of the Mn_4Ca cluster (red spheres, numbered 1 to 4). Distances are given in Å.

Several lipids are close to plastoquinones Q_A and Q_B (Fig. 36), though direct interactions are not present. The head group of PG22 is only ~ 8 Å apart from the head group of Q_B and forms a hydrogen bond with conserved Asn266 (PG22-O1 ⋯ ND2-Asn266-D1, 3.3 Å).

It seems likely that in PSII the following scheme is used: by employing a lipid as cofactor to influence the redox properties of a quinone, changes in the lipid environment without changing the binding pockets of Q_A or Q_B could be used. Therefore, changes in the lipid environment could be important for modulating the properties of the two quinones in different ways depending on their location in PSII. This could affect the redox potentials of the quinones and allow fine-tuning of electron transfer from Q_A to Q_B in PSII by the lipid environment.

Depletion of PG in *Synechocystis* PSII leads to changes in the redox potential of Q_B and slows down the $Q_A \rightarrow Q_B$ electron transfer rate [209]. In mutants of *C. reinhardtii* depleted in SQDG, the interaction of the Q_B site with artificial electron acceptors was changed, indicating a conformation or electrostatic change due to the absence of SQDG [218].

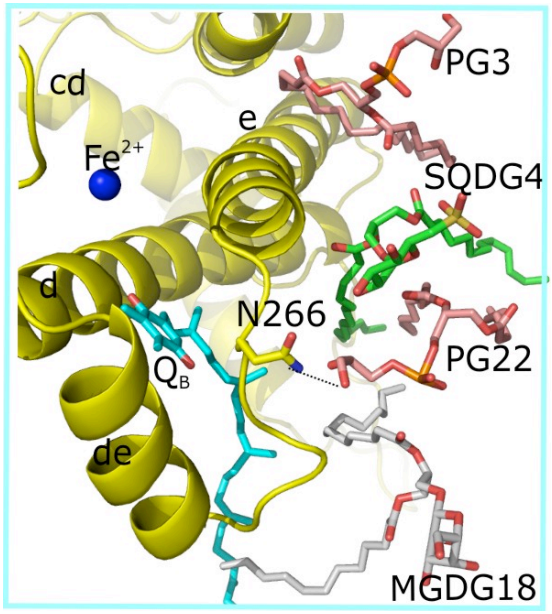

Figure 36. Lipids positioned around the Q_B binding site formed by subunit D1 (yellow). The non haem Fe^{2+} is shown as blue sphere, the PQ bound to the Q_B-site in light blue, PG3 and 22 in salmon, SQDG4 in green and MGDG18 in grey.

3.2.1.5 Oxygen diffusion via lipids

It is well known that oxygen can dissolve in the lipid bilayers that are permeable for oxygen [219, 220]. Measuring the oxygen concentration profile within a bilayer using spin labels at different positions revealed an accumulation of oxygen in the middle of the bilayer within the hydrophobic environment of the fatty acids [221]. As oxygen is constantly produced near the lumen of PSII during oxygenic photosynthesis [222] and the bag-like thylakoid membrane is a closed compartment, oxygen has to leave the thylakoids via the lipid bilayer towards the cytosol.

Xe might be used as an oxygen analogue in crystallographic studies [64, 72, 74]. X-ray diffraction data collected from Xe-derivatized PSII crystals yielded information on the location of Xe sites within the complex (see section 3.3.2 for detailed analysis and ref [223] for an independent study) and shed light on the oxygen escape routes. All the Xe were located in hydrophobic environments formed by fatty acids from lipids, phytol chains from Chl molecules, carotenoids or hydrophobic amino acids (Val, Gly, Leu, Ala, Ile, Phe, Trp). With the exception of one Xe located in the hydrophobic interior of the β-barrel of PsbO, all Xe sites are located in the membrane spanning part of PSII at approximately half height of the membrane. The sites are predominantly at the monomer-monomer-interface, at the interface