intrinsic subunits. Interestingly, these lipids are always grouped together in clusters (red ellipses in Fig. 29): DGDG1 and DGDG2 (ellipse I) between D1 and CP43 and MGDG9, MGDG10, MGDG11 (ellipse II) plus SQDG16 and DGDG8 (ellipse III) between D2 and CP47. The remaining lipids PG3, SQDG4, PG22 and MGDG18 on the cytoplasmic side and MGDG19, DGDG5, DGDG6 and MGDG7 on the lumenal side are arranged as a bilayer island (blue rectangle in Figs. 29, 30), which is essential for plastoquinone exchange (*vide infra*).

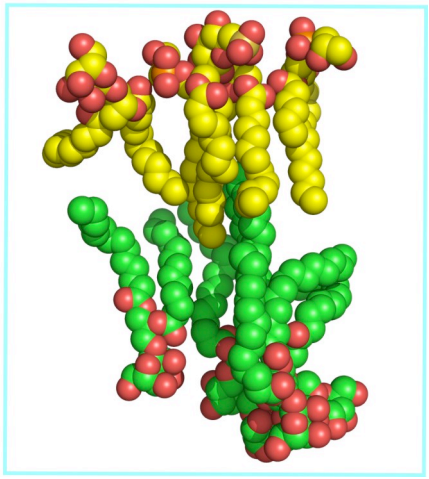

Figure 30. Lipid bilayer cluster. Enlarged blue rectangle (side view) from Fig. 29. The charged PG3, PG22 and SQDG4 and neutral MGDG are on the cytoplasmic (above; carbon in yellow) and the neutral MGDG19, DGDG5, DGDG6 and MGDG7 are on the lumenal (below) side (carbon in green).

The composition of lipids as derived from the crystal structure of PSII corresponds very well to the average lipid composition of the thylakoid membrane. In each PSII monomer eleven MGDG, seven DGDG, five SQDG and two PG molecules could be located corresponding to relative contents of 44%, 28%, 20%, and 8%, respectively, and reflecting the highly conserved lipid composition of cyanobacterial thylakoid membranes (≈45%, ≈25%, 15-25%, 5-15%, respectively) [200].

The distribution of lipids is asymmetric with the head groups of negatively charged PG and SQDG located exclusively at the cytoplasmic side, those of uncharged DGDG at the lumenal side and those of MGDG at both sides, similar to the situation in the other photosynthetic membrane protein complexes. There are several studies on the content of lipids in PSII preparations from plants and cyanobacteria. For *T. elongatus* and *T. vulcanus* between ten and 60 lipids have been reported per PSII monomer [61, 200, 201], and different compositions were found especially for the content of PG (varying from six to 30% [200] of

total lipid content). The large deviations from the number of 25 lipids (per monomer) described here for the PSII from *T. elongatus* might be due to differences in preparation, intrinsic errors in the quantification and that the used analytical methods cannot distinguish between free, peripheral and integral lipid molecules. The observed higher amount of PG in PSII preparations [200] is not reflected in the present structural data, because the presence of possible additional PG molecules in the lipid shell around the complex could not be resolved in the crystal structure.

Nonetheless, the total lipid content in PSII determined in this study yields 0.7 lipids per TMH. This value is high compared with other lipid-containing membrane complexes: PSI [37], *cyt* b_6f [40, 202], purple bacterial reaction center [203, 204] and cytochrome C oxidase [205] with 0.10, 0.23, 0.27, 0.46 lipids per TMH, respectively. This suggests a special role for these lipids in PSII function, as discussed below.

The head groups of all identified integral lipids are located near the membrane surface. As this coincides with the stromal or cytoplasmic end of the TMHs, Tyr and Trp that are found typically at the termini of TMHs, are present in the binding pockets of almost all lipid head groups (see Appendix Table 7.6).

3.2.1.2 Lipid binding sites

The detailed lipid-protein interactions are dependent on the nature of the head group of a lipid. Between two to six amino acids form polar interactions with each head group, yielding a total of four to eight (in some cases up to 16) hydrogen bond and salt bridge interactions between protein and the lipid head. The average number of hydrogen bond and salt bridge interactions is increasing from 4.5 for the smaller head group of MGDG to 8.5 for the larger DGDG. This situation is similar for the negatively charged PG and SQDG, where the smaller head group of PG shows on average ~4.3 and the larger SQDG shows ~6.5 polar interactions. The non-native detergent molecules show a lower number of polar lipid-protein interactions (~3.3 on average), indicating weaker binding to the complex compared to the natively bound lipids.

The amino acid compositions of the binding pockets are also different, depending on the lipid species. Positively charged residues Arg and Lys are nearly exclusively found in the binding pockets of the negatively charged SQDG and PG whereas MGDG and DGDG have relatively few Arg and nearly no Lys residues as their neighbours. On the other hand,

negatively charged Glu and Asp are only found in the binding pockets of MGDG and DGDG but not around PG and SQDG.

Interestingly, many amino acids forming lipid-binding sites are conserved (see Appendix Table 7.6), indicating that the given lipid-binding sites might be present in other species.

The differences in binding pocket architecture and the observation that the head group of each bound lipid could be identified unambiguously in the electron density show that each binding pocket is highly specific for one type of lipid. There is no indication for chemical heterogeneity concerning the occupation of binding sites by the lipid head groups, even for the similar head groups of MGDG and DGDG. Since the detergent ß-DM features a maltoside head group instead of the similar digalactoside in DGDG, one could assume that ß-DM might have replaced DGDG.

The head groups of 18 of the intrinsic lipids found in PSII form hydrogen bonds or salt bridges with at least two different subunits, and three of them (DGDG1, DGDG6 and SQDG13) even connect four different subunits (Appendix Table 7.6, Fig. 31). This large number of intersubunit contacts confirms the importance of lipid head groups in promoting the assembly and providing stability for the PSII complex. In most cases these contacts are formed by residues at the ends of TMHs of membrane intrinsic subunits but for the two lumenally located DGDG1 and DGDG6 H-bonds with loop regions of the membrane extrinsic protein subunits PsbO and PsbV are found as well, indicating that lipids might contribute to the optimal binding of these subunits.

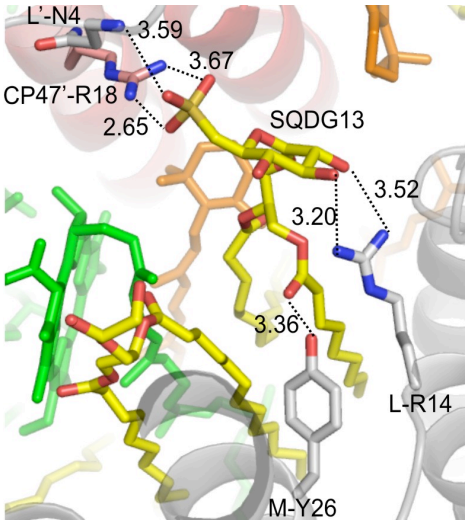

Figure 31. Example of lipid interactions. Interactions of the polar head group of SQDG13 (yellow) with amino acid side chains from PsbL, PsbM, PsbL' and CP47' (' from the second monomer). Possible hydrogen bond interactions are shown by dotted lines and distances are given in Å.

3.2.1.3 Lipids mediate monomer-monomer interactions and dimerization

The monomer-monomer interface in the homodimeric PSII complex is dominated by the presence of lipids and only very few direct protein-protein interactions are observed between the two monomers. These are mainly contributed by the TMH of the small membrane intrinsic subunit PsbM that is located close to the pseudo-twofold C_2 axis relating the monomers in the homodimer and shows a high content of leucine residues (see section on low molecular weight subunits and Fig. 21). The TMHs of PsbM and its symmetric counterpart PsbM' interact through a motif typical of membrane-spanning leucine zippers [160]. In addition there are polar interactions at the N-termini of the TMHs between symmetry-related PsbM-Gln5, PsbM-Gln5' and at the C-termini between related PsbM-Ser31, PsbM-Ser31' and PsbM-Gln33, PsbM-Gln33' (see Appendix Table 7.4).

The only other possible direct protein-protein contact between the monomers is found at the lumenal side between a protruding loop of subunit PsbO (from one monomer) and a membrane extrinsic part of subunit CP47 (from the other monomer) (see Appendix Table 7.5). Consequently only a total of six polar protein-protein contacts exist between the two monomers, which is surprisingly low considering the size of PSII. Since PsbM and PsbM' are located on the roughly planar surface forming the monomer-monomer contact there is a large gap between the two monomers that is filled by a total of 14 lipids, seven from each monomer (Figs. 27, 32). In addition, there are eight β–DM molecules that may have replaced galactolipids during preparation, suggesting the presence of even more lipids when PSII is embedded in the thylakoid membrane.

Such overall weak protein-protein interactions with a strong involvement of lipids in the interaction between the monomers suggest that lipids are an important constituent of this interface in PSII and are located for special reasons in this position.

Whereas PSI, LHCII and cyt b_6f do not normally dissociate into monomers, the PSII complex is experiencing a constant disassembly and reassembly process [43, 44, 135] that is necessary to provide replacement of photo-damaged parts of the photosystem caused by side reactions within the RC, namely the formation of long lived triplet states of Chl molecules and the subsequent generation of ROS [206] that is extremely reactive and leads to oxidative damage of cofactors and proteins of PSII [206]. In order to circumvent this damage problem a complicated repair system is implied in PSII, which enables the selective replacement of subunit D1, which is most prone to photo-damage because it harbours the

ETC. In the course of this exchange, termed D1 turnover [44], it is thought that the damaged dimeric PSII complex first dissociates into monomers, followed by detachment of subunit CP43 [135, 207]. Because the estimated half live time of subunit D1 in the thylakoid membranes can be shorter than 30 minutes under high light conditions, an easy way of separating the dimers into monomers has to be employed.

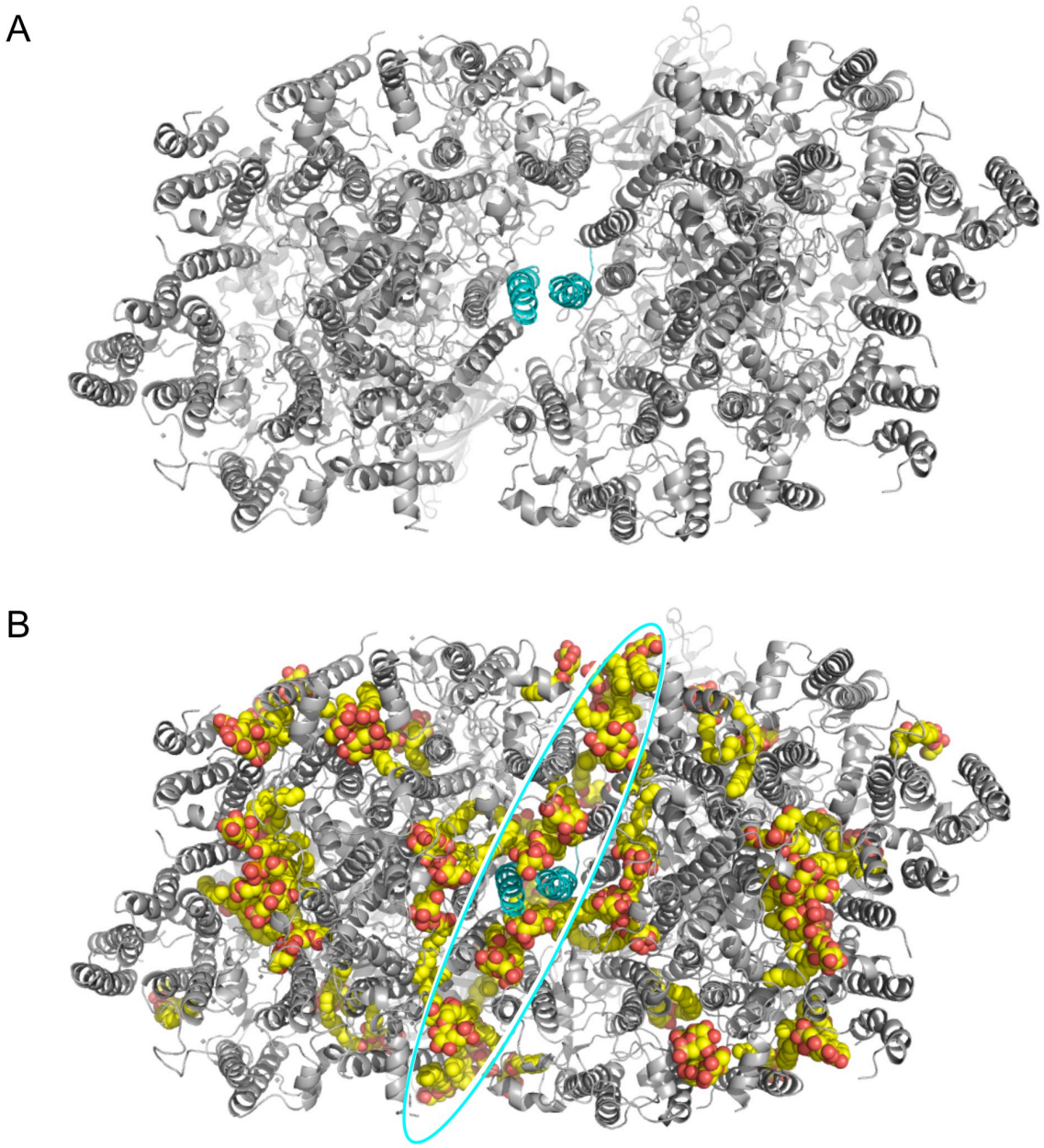

Figure 32. Lipids at the monomer-monomer interface. A) "Bare" (without cofactors) PSII is shown, all protein subunits in grey, except PsbM in both monomers in cyan. View from the cytoplasmic side. B) Lipids are shown (carbon in yellow, oxygen in red). Notice the lipid layer between two monomers, encircled with an ellipse.

Lipids appear to be the ideal components for providing both, the specific interaction necessary for association of two monomers into a dimeric complex as well as the