3.2 Cofactors

In total, 92 cofactors (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) per monomer (Fig. 27) were indentified in the improved electron density and successfully refined. A detailed description and analysis of each class of cofactors is given below.

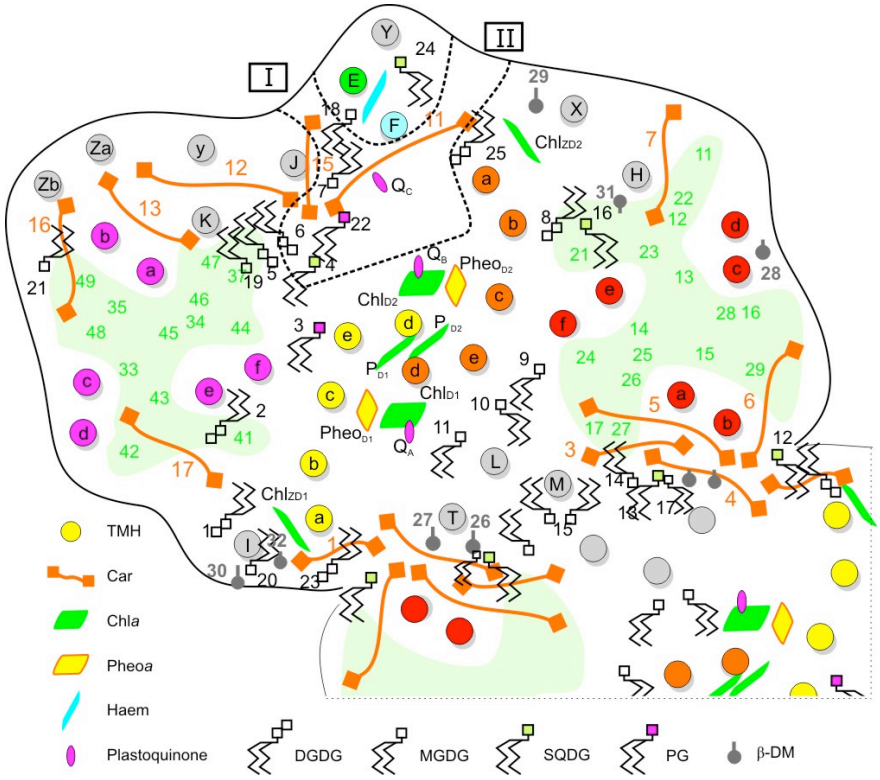

Figure 27. Schematic arrangement of cofactors in PSII. Protein subunits shown in the same way as in Fig. 15. Positions of Chls in CP43 and CP47 are indicated by green numbers. Lipid and detergent molecules located on the lumenal or cytoplasmic side are drawn with head groups pointing "downwards" or "upwards", respectively and numbered. The PQ/PQH_2 exchange cavity is indicated by dotted lines with entry/exit channels I and II labelled with boxed I, II.

3.2.1 Lipids

The electron density revealed the presence of 25 integral lipids and seven detergent molecules in each monomer (see Appendix Table 7.6 and Figs. 27, 28).

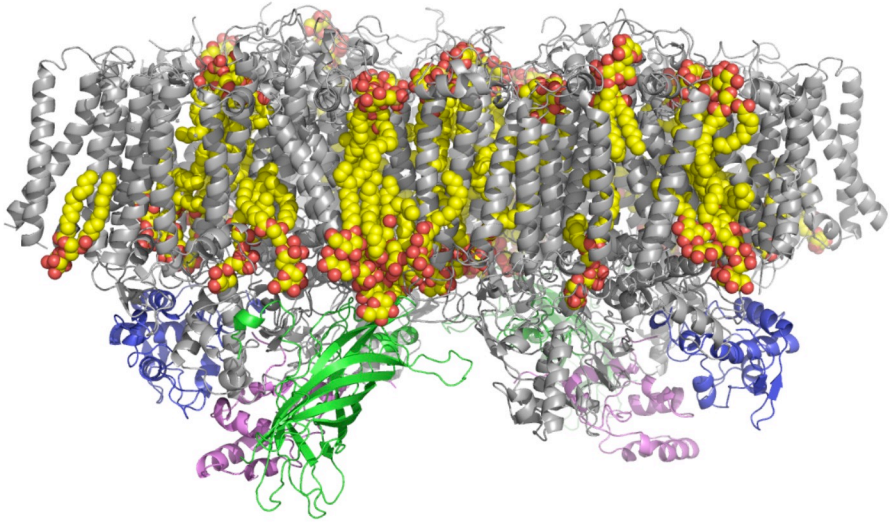

Figure 28. Lipids in PSII. View and colouring are the same as in Figure 23. Lipids are shown in space-filling mode (carbon in yellow, oxygen in red).

The previously found [41] 14 lipids (numbered 1 to 14) and two detergent molecules were confirmed, whereas eleven lipids (numbered 15 to 25) for each monomer are novel.

3.2.1.1 Lipid positions and content

The electron density of detergent β-DM15 [41] at the monomer-monomer interface was reassigned to MGDG15. The head group of SQDG16 lies in a positively charged pocket between subunits D2 and CP47. SQDG16 is accompanied by detergent β-DM31, which indicates either the presence of two lipids in this pocket under *in vivo* conditions or that SQDG16 adopts two alternative positions. At the cytoplasmic side, MGDG17 is close to SQDG13 at the monomer-monomer interface, whereas MGDG18 is located near the PQ / PQH_2 exchange cavity (see section 3.2.2.4). The head group of MGDG19 on the lumenal side of PSII is near the putative Ca^{2+}-PsbK ion at a possibly water-mediated distance of 6.7 Å. MGDG19 is close to DGDG5 in the pocket formed by CP43 and the small subunits PsbJ, PsbK and ycf12. MGDG20 is close to PsbI and $ChlZ_{D1}$, and lipid MGDG21 is near the periphery of PSII between CP43 and TMHs Z_A and Z_B of PsbZ (Fig. 27). PG22, the second PG molecule newly identified in PSII, is on the cytoplasmic side close to Q_B, SQDG4, MGDG18 and PG3. DGDG23 is on the lumenal side close to PsbI and TMH **a** of D1, with its head group forming polar contacts with sugars from MGDG20 and β-DM32.

SQDG24 is embedded between PsbY, PsbF and PsbX. The DGDG25 head group is in contact with the C-terminal loop of PsbE and loop **ab** of D2, with its fatty acid interacting with Car_{D2}. The five newly assigned detergent molecules β-DM28 to β-DM32, except β-DM31 (*vide supra*), are at the periphery, close to the membrane.

Of all found 25 lipid and seven detergent molecules the head groups face towards the membrane surface (lumenal or stromal) (Fig. 27) and their hydrophobic tails are within the membrane spanning part of PSII, as expected (Fig. 28). Twelve of the lipid and one detergent molecules have their head group oriented towards the cytoplasm whereas 13 lipid and six β-DM molecules are oriented with their head group pointing towards the lumen (Fig. 27). A further classification is possible based on the lipid positions within the complex. Only three of the 25 lipids and one β-DM molecule are found at the membrane exposed periphery of the complex (peripheral lipids) whereas seven lipids and five β-DM from each monomer are located at the interface between the two PSII monomers, and 15 lipids and one β-DM are embedded between different subunits within each monomer.

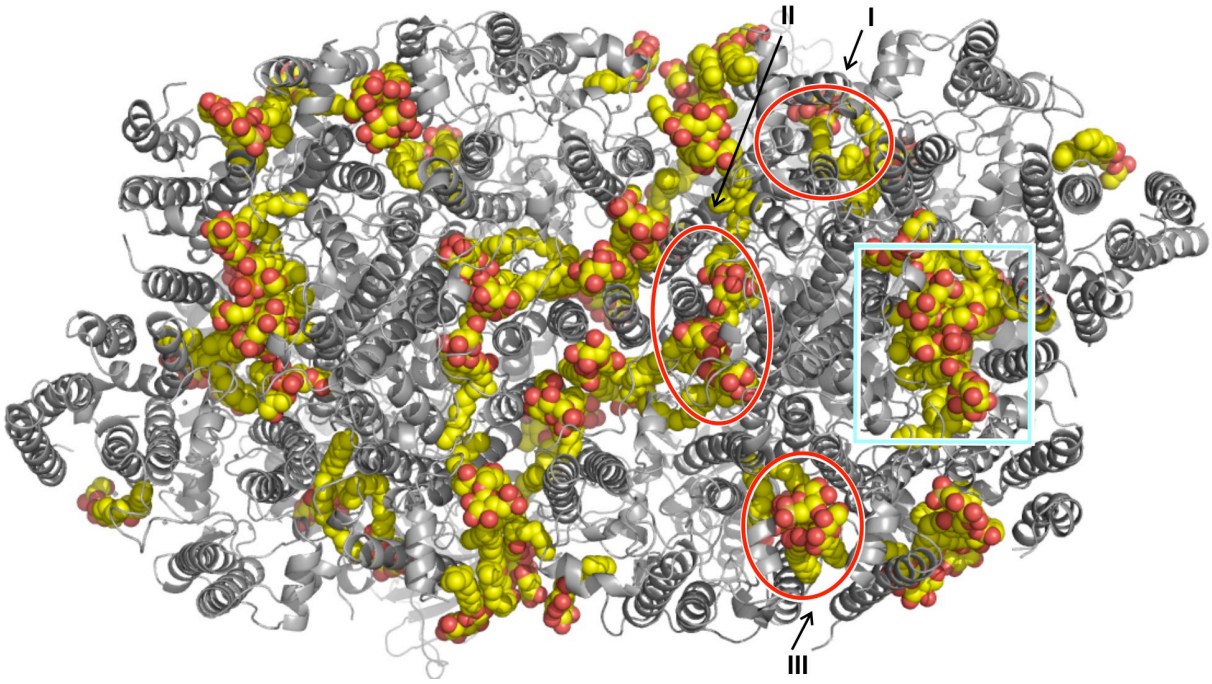

Figure 29. Lipid clusters in PSII. The red ellipses I, II, III and the blue rectangle encircle the lipid clusters mentioned in the text.

These intrinsic or non-peripheral lipids are arranged roughly in a belt surrounding the RC formed by the D1/D2 heterodimer and separating it partly from the other membrane