interest is A-Glu65 located at the bottleneck of this channel. Mutation of this residue in PSII of *Synechocystis* sp. PCC6803 to Asp, which is also protonatable, has only a marginal influence on the O_2 evolution (93% of wild type). In contrast, mutation to the non-protonatable groups Gln or Ala reduces oxygen evolution to about 20% [289]. Taking into account the distance of A-Glu65 to the Mn_4Ca cluster of 11 Å, a direct involvement of this residue in the water oxidation chemistry can be excluded, whereas participation of A-Glu65 in proton transfer is very likely.

3.3.2 Experimental confirmation

To confirm possible hydrophobic pathways for oxygen transfer away from the Mn_4Ca cluster experimentally, we determined the structure of PSII crystals derivatized with Xe or Kr under pressure (see Appendix Table 7.8). The Xe atom has more electrons than Kr (thereby facilitating its detection with X-rays) and comparable van der Waals radius to O_2 (2.16 Å for Xe, and 2.13 Å for oxygen, along the O=O bond) so that it can mimic oxygen in X-ray crystallographic studies [64, 74, 290]. In total, ten Xe derivative datasets were collected, and three of them were used for the assignment (see Appendix Table 7.8 for statistics) and refinement of the Xe positions (see Appendix Table 7.9 for details on Xe and Kr positions in each dataset). Peaks lower than the 9σ level were excluded. The number of detectable Xe atoms in the structure increased with the pressure applied to the crystal. Pressurizing with ten bars allowed to resolve only 19 Xe in the PSII homodimer, while 35 and 53 Xe atoms were identified at 14 and 30 bar, respectively. These Xe sites have reasonably good correlation (~60%) with those obtained by Murray et al. [291] .

At all Xe pressures, one of the Xe atoms (Xe5) is riding on the non-crystallographic C_2 axis relating the monomers in the PSII homodimer (Fig. 55).

Among the 26 assigned Xe atoms located at 30 bar in each monomer, one (Xe2) is located in the hydrophobic interior of the β-barrel of PsbO (Fig. 56), but there is no direct connection between the internal cavity of PsbO and the Mn_4Ca cluster.

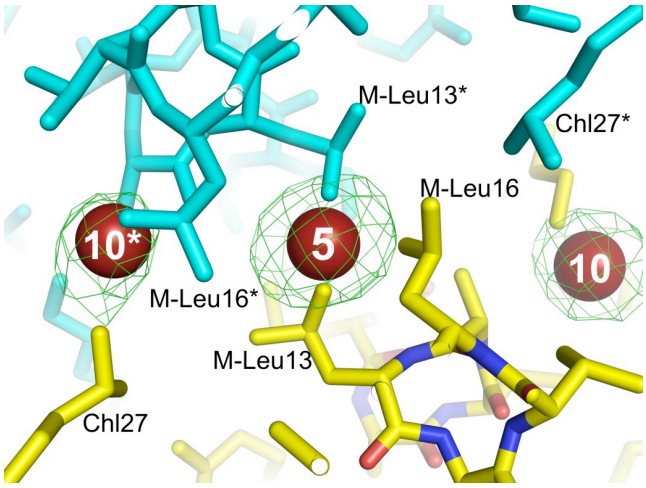

Figure 55. Example of difference anomalous electron density for Xe sites at ten bars pressure at the monomer-monomer interface (one monomer is in cyan, the second is in yellow) at the 7σ level (Xe5 is located on the local C_2 axis). View is from the cytoplasmic side.

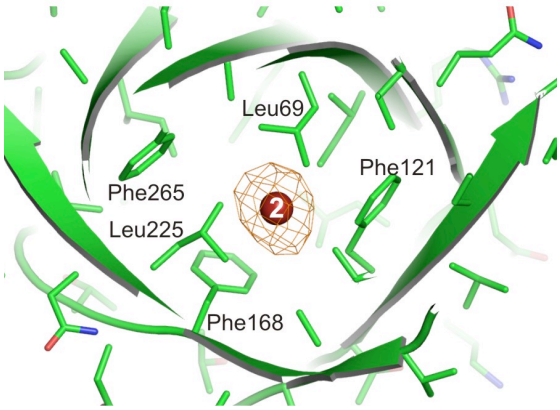

Figure 56. Position of Xe2 in the middle of the PsbO β-barrel (green). Anomalous map is countered at 7σ level.

Seven atoms (Xe5, Xe9, Xe10, Xe14, Xe21, Xe24 and Xe25) and their symmetry related mates are found at the monomer-monomer interface (Fig. 58). The remaining Xe sites are located in the membrane spanning part of PSII at approximately half height of the membrane (Fig. 57) (see Appendix Table 7.9 for a detailed description of the Xe positions).

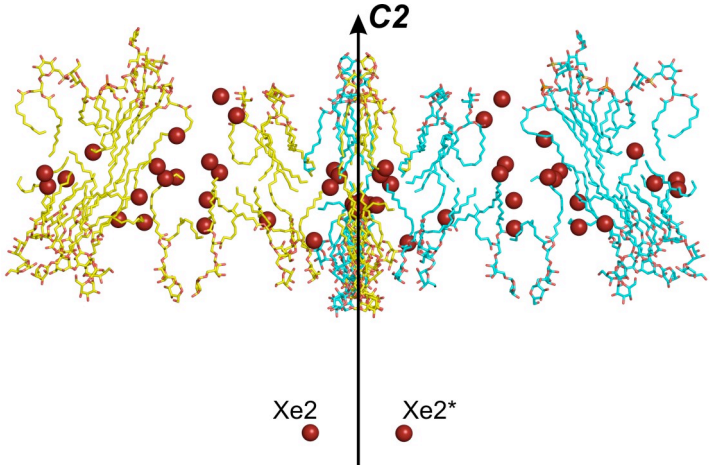

Figure 57. View of the Xe sites in dimeric PSII in the membrane plane showing the distribution along the membrane height. The C_2 axis relating the two monomers is indicated by the black arrow. Note the predominant location of Xe sites in the middle of the membrane between fatty acid chains of lipids.

To understand how Xe (and O_2) could escape from the lumen into the membrane (as thylakoid membrane is the bag-like and is a closed compartment), possible pathways with CAVER were calculated starting from Xe5 and using the same restrictions as employed for the calculations of oxygen channel (*vide supra*). The analysis yielded three main trajectories that are wide enough for oxygen diffusion (cyan arrows), two of them connecting various Xe sites and ending in the membrane interior (yellow in Fig. 58), while one entrance channel for oxygen initiates in the lumen (red in Fig. 58) and is connected with the exit channels (yellow).

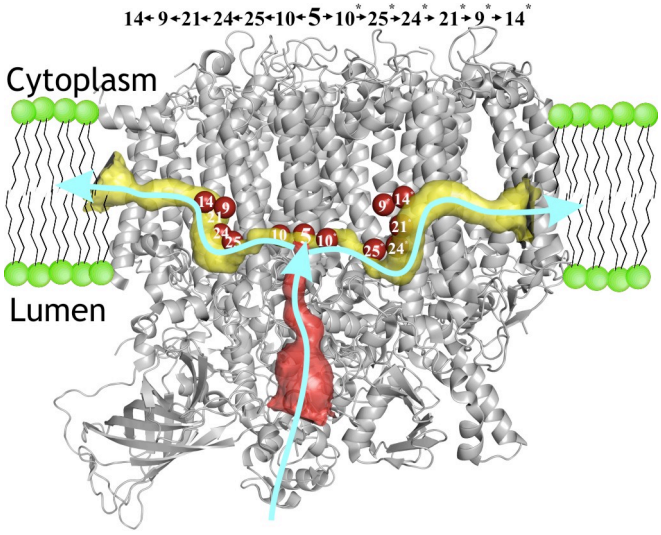

Figure 58. Xe positions at the monomer-monomer interface and possible oxygen escape routes from the lumen via PSII, shown by cyan arrows. Possible trajectories of diffusion channels for Xe (or oxygen) connect Xe sites 5→10→25→24→21→9→14→membrane interior (yellow) and Xe5 with the lumen (red).