3.2.3   The Mn$_4$Ca cluster

The harsh reaction depriving electrons from water is run by a small cluster of 4 Mn and 1 Ca (Fig. 43) intra-connected with μ-oxo and di-μ-oxo oxygen bridges. Electrons are extracted from the cluster by oxidized P$_{D1}$ via redox-active Tyr161 of D1. By repeated excitation of the RC, the OEC (oxygen evolving complex) cycles through five successive oxidation states (Fig. 5 and section 1.2), called S$_i$-states ($i = 0 - 4$), leading to the oxidation of two water molecules to oxygen, four protons and four electrons in the *ms* time range [1, 33, 230, 253]. The exact structure of the Mn$_4$Ca cluster is the area of extensive research, employing different techniques like X-Ray diffraction, spectroscopy, EXAFS and many others. During last decade several models were suggested [41, 224, 254-257].

The Mn$_4$Ca cluster is located at the lumenal side of subunit D1. The shape of its electron density did not change compared to the previous 3.0 Å resolution structure. The Mn$_4$Ca cluster can be described by four Mn cations, arranged as "L" numbered 1-4, starting from the bottom of the "L" (Fig. 43).
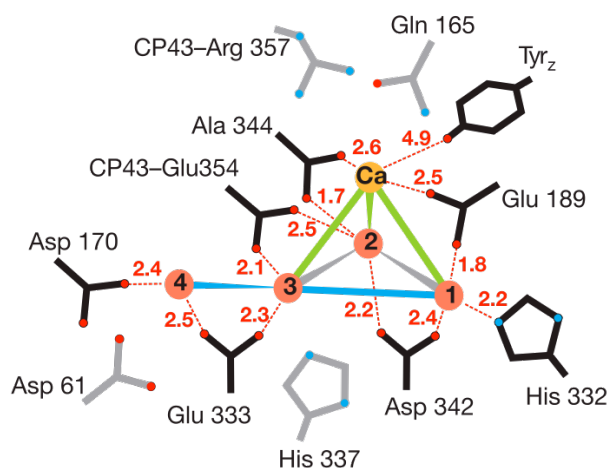

Figure 43. Schematic representation of the Mn$_4$Ca cluster. Distances between Mn (red spheres) and Ca (orange spheres) are indicated by the connecting lines (grey, 2.7 Å; blue, 3.3 Å, green, 3.4 Å). Amino acids of the first coordination sphere are in black; those of the second sphere are in grey; all are from subunit D1, except Glu354 from CP43. Adapted from [41].

The two pairs, Mn1-Mn2 and Mn2-Mn3, are spaced by 2.7 Å, indicating di-μ-oxo bridges, while the internal connection Mn1-Mn3 and the terminal Mn3-Mn4 are 3.3 Å long and likely represent mono-μ-oxo bridges. The Ca^{2+} is on the tip of the pyramid and equidistant (~3.4 Å) from three Mn cations, as shown by X-ray data [41]. The found

arrangement is different from the cubane-like arrangement proposed from the 3.5 Å resolution structure [255] as the Mn-Mn distances in the pyramid formed by three Mn and one Ca cations are not equal and the pyramid is connected asymmetrically to Mn4 [41].

The ligation of the Mn_4Ca cluster in the 2.9 Å resolution model is similar as observed previously (Figs. 43, 44 and Table 5) comprising several residues (D1-Glu189, Glu333, Asp342, Ala344, CP43-Glu354) which could act as bridging ligand connecting two different metals cations. For Mn1, ligation is possibly provided by D1-Glu189, D1-His332 and D1-Asp342. Mn2 is ligated by D1-Asp342, the C-terminus of D1-Ala344 and CP43-Glu354. Mn3 is probably ligated by D1-Glu333 and CP43-Glu354 and for Mn4 ligation by D1-Asp170 and D1-Glu333 is most probable. The Ca ion forms interactions with D1-Glu189 and the C-terminus of D1-Ala344.

	residue	Mn1	Mn2	Mn3	Mn4	Ca
Monomer 1 (pdb: 3BZ1)	Asp 170				2.31 (2.39) OD2	2.93 (3.47) OD1
	Tyr 161					4.75 (4.89) OH
	Gln 165					3.82 (4.11) NE2
	Glu 189	1.75 (1.81) OE2				2.35 (2.52) OE1
	His 332	2.17 (2.18) NE2				
	Glu 333			2.20 (2.31) OE1	2.81 (2.54) OE2	
	Asp 342	2.55 (2.42) OD2	2.28 (2.18) OD1			
	CP43-Glu 354		2.63 (2.48) OE1	2.26 (2.12) OE2		
	Ala 344		1.63 (1.77) OXT			2.50 (2.61) O
Monomer 2 (pdb: 3BZ2)	Asp 170				2.68 (2.72) OD2	2.78 (3.32) OD1
	Tyr 161					4.94 (5.08) OH
	Gln 165					3.91 (4.18) NE2
	Glu 189	1.81 (1.84) OE2				2.55 (2.74) OE1
	His 332	2.29 (2.28) NE2				
	Glu 333			2.06 (2.20) OE1	2.45 (2.17) OE2	

Asp 342	2.66 (2.49) OD2	2.24 (2.13) OD1			
CP43-Glu 354		2.49 (2.32) OE1	2.26 (2.12) OE2		
Ala 344		1.66 (1.69) OXT			2.41 (2.53) O

Table 5. Distances (in Å) between ligating amino acids (interacting atom is indicated) and cations of Mn_4Ca. In parentheses values for old (3.0 Å resolution) structure are given.
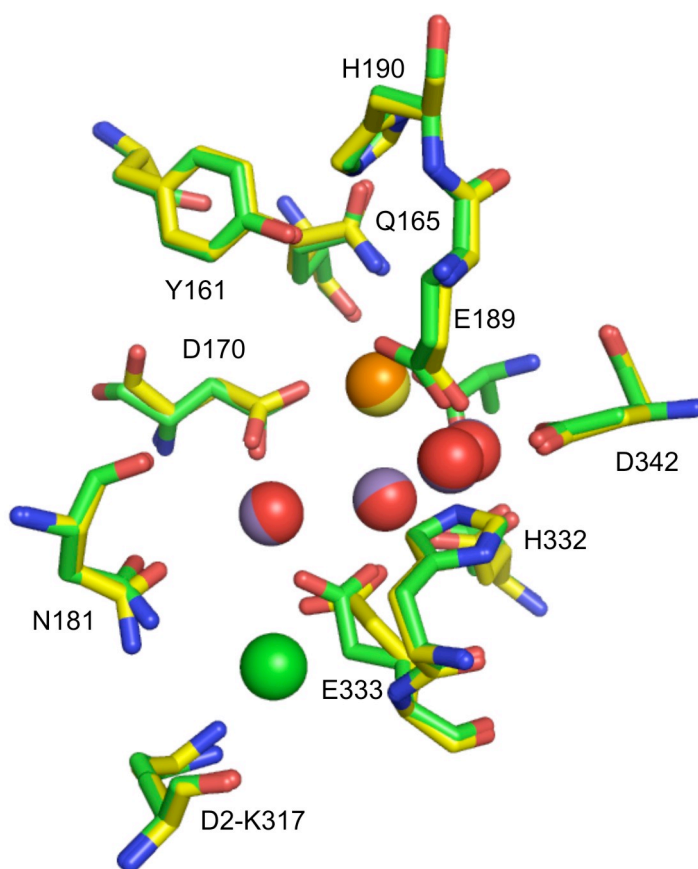

Figure 44. Superposition of the Mn_4Ca cluster and its ligating amino acids from 3.0 (green) and 2.9 (yellow) Å resolution models. Note the additional green sphere in the 2.9 Å resolution model, representing chloride ion.

The most intriguing part of the cluster is the widely debated chloride ion (Cl^-) which is believed to play a significant role during catalysis. The stoichiometry for Cl^- : OEC has been shown to be 1:1 [258] and the dependence of oxygen production on Cl^--depletion [259] as well as possible substituents of chloride ion [260-263] have been studied. Up to date there was no direct evidence about exact position of Cl^-, except from a X-ray absorption study

by Haumann [264], which revealed the putative position of Br⁻ (that had replaced Cl⁻) approximately 5 Å away from the Mn₄Ca cluster.

During modelling at 2.9 Å resolution a spherical patch of electron density in the vicinity of the Mn₄Ca cluster was revealed. As normally water molecules are not seen at this resolution it was assumed that this position is probably occupied by an ion, and to eliminate high-ordered water as a candidate, this site was refined as water against native data. A positive peak that remained in the difference f_o-f_c map confirmed that water has not enough electrons to describe the electron density completely. Therefore the Cl⁻ ion was assigned to this position and successfully refined with full occupancy.

The chloride ion is located 6.5 Å from the OEC and has a positively charged environment provided by amino acids D2-Lys317, D1-Asn181 and the backbone N of D1-Glu333 (Fig. 45).
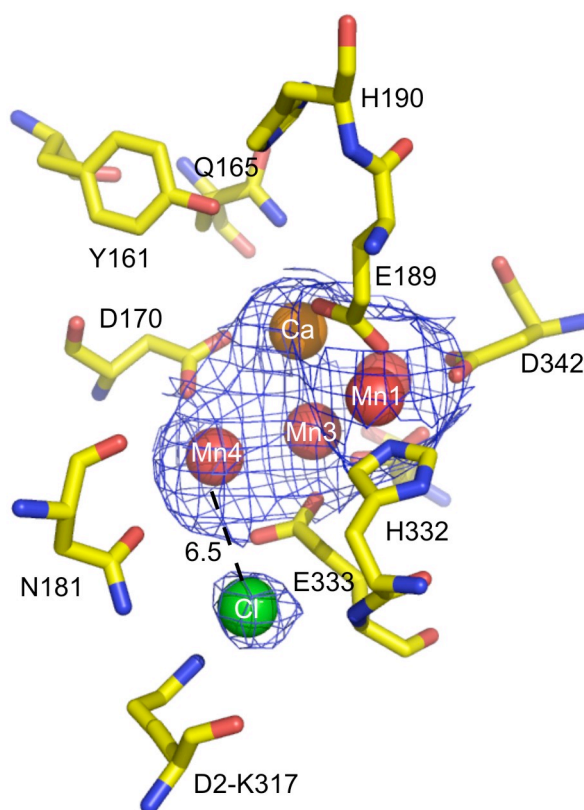

Figure 45. The Mn₄Ca cluster of PSII and the Cl⁻-binding site. Position of Cl⁻ (green sphere) located in the native electron density (blue mesh, contoured at 1.2 σ level) close to the Mn₄Ca cluster (red and orange spheres; Mn2 is partially hidden behind Mn1). All coordinating amino acids are from D1 except for D2-Lys317.

A random analysis of more than 40 crystal structures containing Cl⁻ deposited in the pdb repository [106] showed that an arrangement involving backbone nitrogens and

positively charged residues (His, Lys, Arg and sometimes Asn and Gln) is common in Cl⁻ coordination. Moreover residues D1-Asn181 and D2-Lys317 are strictly conserved. An additional non-described patch of electron density in between the Mn₄Ca cluster and the given Cl⁻ could possibly arise from water molecule(s). If water is placed in this electron density, distances Cl⁻···water and water···Mn4 are 3.3 and 3.5 Å (Fig. 46), respectively, and additional ligation of the water by conserved D1-Glu333OE2 and D1-Asp181ND2 is possible.
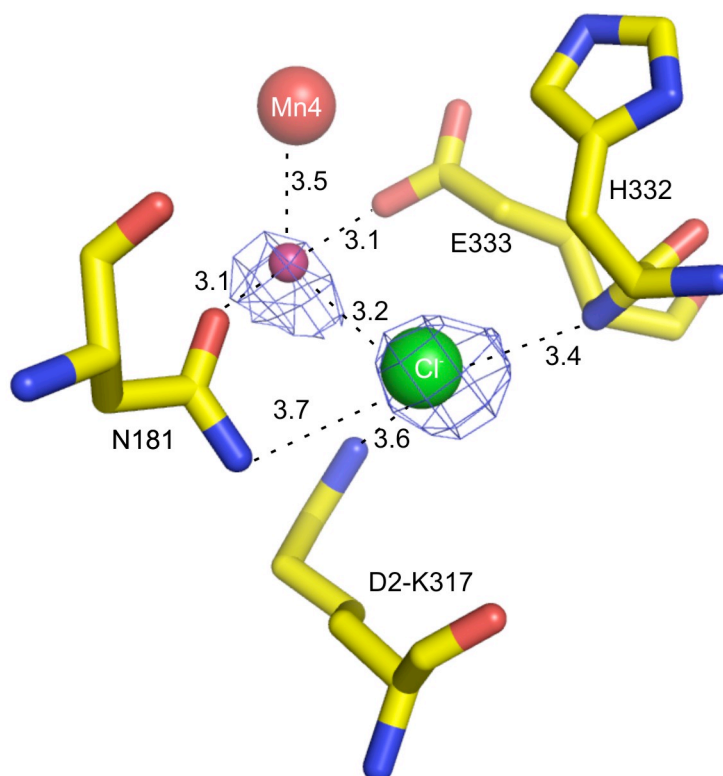

Figure 46. Enlarged view of the neighbourhood of Cl⁻ showing coordinating amino acids and electron density (blue mesh, contoured at 1.2 σ level) for a putative water molecule (purple sphere) located between Mn4 and Cl⁻ . Distances are given in Å.

To confirm the position of the Cl⁻ ion two approaches were used: 1) Br⁻-substituted crystals were grown and datasets from them were successfully collected and characterized; and 2) datasets from Xe-derivative crystals were thoroughly inspected for the presence of Cl⁻ contribution.

As the anomalous signal from chloride ion is rather low (Fig. 47A) and not easily detectable, several datasets from crystals of PSII substituted with Br⁻ (which has a detectable signal, see Fig. 47B) were collected to confirm the chloride-binding site, as it is known that Br⁻ behaves similarly as Cl⁻ and does support oxygen evolving activity of PSII [57].
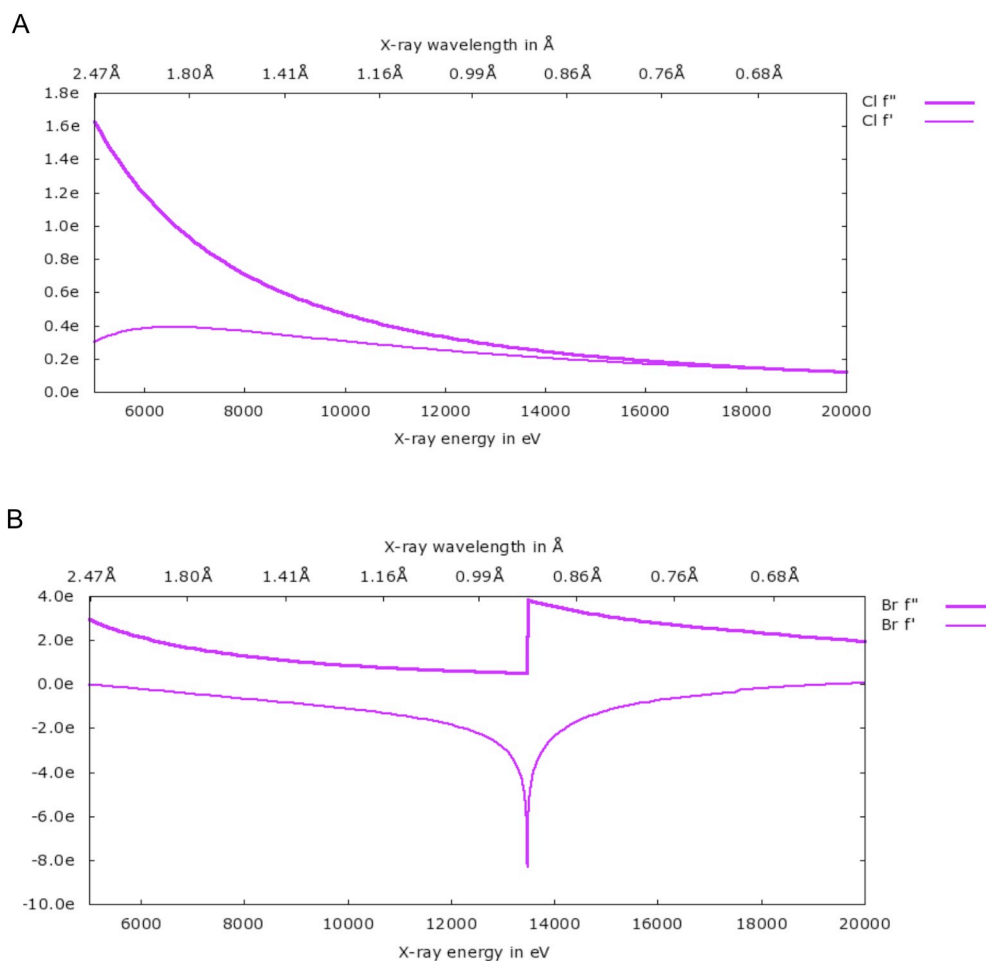
Figure 47. Theoretical plots of f ′ and f ″ for chloride (A) and bromide (B) respectively.

The best obtained resolution for derivatized crystals was 3.9 Å (see Table 6). A search of peaks around the Mn_4Ca cluster in the difference f_o-f_c electron density map was performed and one prominent peak with 7.2σ level identified (Fig. 48), corresponding to the Cl^- position in native data. Refinement of the Br^- position yielded 0.7 occupancy of this site.

Inspection of the corresponding location in Xe datasets revealed a positive difference peak. Though the used wavelength of 2.1 Å is far away from the Cl-edge (4.39 Å), weak anomalous contribution from chloride could be expected and was found (Fig. 47). This position is unlikely to be occupied by a Xe atom as it has unfavourable environment (positively charged side chains, while Xe tends to stay at hydrophobic areas [64]), and the σ level of the respective peak is lower ($5.4\ \sigma$) than that observed for the Xe atoms (10σ or above), confirming the presence of Cl^- at this position.

	Bromide (1)	Bromide (2)
Space Group		P 21 21 21
Unit cell dimensions		
a, b, c (Å)	126.1, 223.7, 304.3	132.9, 229.4, 309.0
α, β, γ (°)	90.0, 90.0, 90.0	90.0, 90.0, 90.0
Resolution (Å)	30–3.9 (4.0–3.9)[*]	30–4.0 (4.1–4.0)[*]
R_{sym}	0.106 (0.362)	0.073 (0.532)
I / sI	7.52 (2.73)	9.83 (2.19)
Completeness (%)	82.5 (67.8)	89.2 (82.8)
Redundancy	2.40	3.10

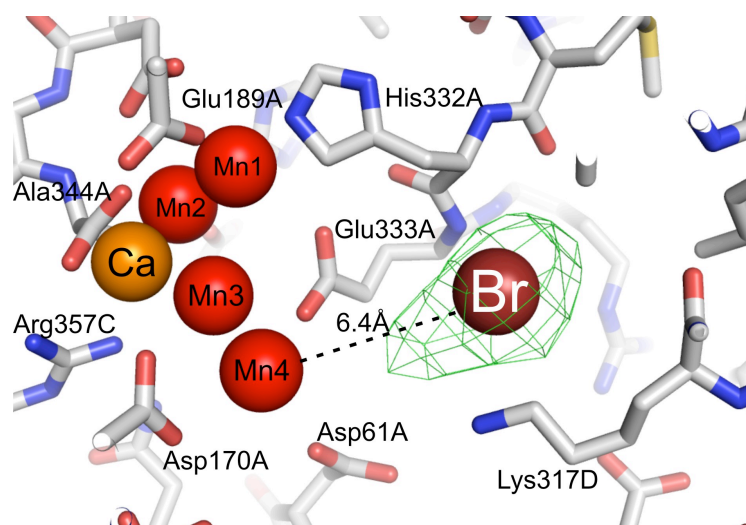Table 6. Data collection statistics for Br⁻-derivatized crystals. * values for highest resolution shells.

Figure 48. Bromide position near the Mn₄Ca cluster. The anomalous difference peak is shown as a green mesh with 7σ level.

The found position of Cl⁻ near the OEC and close to the entrance of the possible proton channels (see section 3.3) and the presence of a lysine residue as direct ligand which possesses unusual protonation properties [265], might support the idea of Cl⁻ involvement in the hydrogen bond network for fast proton removal from the Mn₄Ca cluster, as proposed earlier [261]. However, it is unlikely that the Cl⁻ interacts directly with substrate water bound to the Mn₄Ca cluster because the Cl⁻ is located opposite to the proposed water channels (*vide infra*) and the closest Mn-cation is Mn4, which was suggested to be redox-inactive during the S-state turnover [266, 267].

Although for spinach PSII it was demonstrated that only one functional Cl⁻ can be exchanged [261] and the determination of the PSII:Cl⁻ stoichiometry on ³⁶Cl⁻ grown spinach yielded about one Cl⁻ per OEC [259], the presence of additional Cl⁻ ions at the Mn₄Ca cluster can not be completely excluded from our data. If Cl⁻ would be a direct ligand to Mn, as