LUND UNIVERSITY

Simulation of the isotropic EXAFS spectra for the S2 and S3 structures of the oxygen evolving complex in photosystem II.

Li, Xichen; Siegbahn, Per E M; Ryde, Ulf

Published in:
Proceedings of the National Academy of Sciences

DOI:
10.1073/pnas.1422058112

2015

Link to publication

Citation for published version (APA):
Li, X., Siegbahn, P. E. M., & Ryde, U. (2015). Simulation of the isotropic EXAFS spectra for the S2 and S3 structures of the oxygen evolving complex in photosystem II. *Proceedings of the National Academy of Sciences*, *112*(13), 3979-3984. https://doi.org/10.1073/pnas.1422058112

Total number of authors:
3

General rights
Unless other specific re-use rights are stated the following general rights apply:
Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.
• Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
• You may not further distribute the material or use it for any profit-making activity or commercial gain
• You may freely distribute the URL identifying the publication in the public portal

Read more about Creative commons licenses: https://creativecommons.org/licenses/

Take down policy
If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

LUND UNIVERSITY

PO Box 117
221 00 Lund
+46 46-222 00 00

A Simulation of the Isotropic EXAFS Spectra for the S_2 and S_3 Structures of the Oxygen Evolving Complex in Photosystem II

Xichen Li*

College of Chemistry, Beijing Normal University, 100875, Beijing, China

Per E. M. Siegbahn*

Department of Organic Chemistry, Arrhenius Laboratory, Stockholm University, SE-106 91, Stockholm, Sweden.

Ulf Ryde

Department of Theoretical Chemistry, Lund University, Chemical Center, P.O. Box 124, S-22100 Lund, Sweden.

Abstract.

Most of the main features of water oxidation in photosystem II are now quite well understood, including the mechanism for O-O bond formation. For the intermediate S_2 and S_3 structures there is also nearly complete agreement between theory and experiments. Given the present high degree of consensus between theory and spectroscopic experiments for these structures, it is of high interest to go back to previous suggestions concerning what happens in the S_2 to S_3 transition. Analysis of EXAFS experiments have indicated relatively large structural changes in this transition, with changes of distances sometimes larger than 0.3 Å and a change of topology. In contrast, our previous DFT(B3LYP) calculations on a cluster model showed very small changes, less than 0.1 Å Siegbahn, P.E.M. *Biochim. Biophys. Acta* **2013**, *1827*, 1003-1019. The main purpose of the present study was therefore to investigate whether the DFT structures are consistent with the EXAFS spectra for both the S_2 and S_3 states, or if there are differences that suggest significant structural discrepancies. The present EXAFS refinement of the DFT structures now indicates that the DFT structures are topologically correct but that one Mn-Mn distance should increase by +0.13 Å while another one should decrease by -0.09 Å during the S_2 to S_3 transition, in better agreement with the EXAFS interpretations, and fully consistent with normal errors of DFT(B3LYP). Our results highlight the fact that it is often possible to fit several different structures to the same EXAFS spectrum, a general problem noted before.

Keywords: Water oxidation, Density functional theory, S-state structures, EXAFS

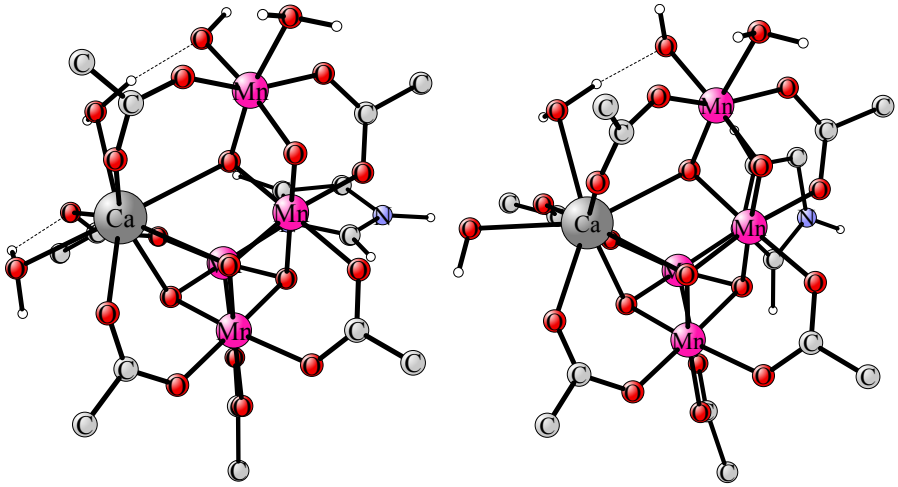

Figure 1: The previously DFT(B3LYP)-optimized structure for the S_2-state [11, 12] is shown to the left and the structure suggested after a spectroscopic analysis is shown to the right [10]

refinement.

I. Introduction

The knowledge of the different steps of water oxidation in photosystem II has increased rapidly the last years. After the first low-resolution X-ray structures that appeared about 10 years ago [1, 2, 3] quantum chemical studies using Density Functional Theory (DFT) have played a major role for obtaining a mechanistic understanding. First, an O-O bond formation mechanism was suggested in 2006 [4], in which a terminally bound oxyl radical in the center of the oxygen evolving complex (OEC) was attacked by a manganese-bridging oxo-group. Second, an improved structure was suggested in which, most importantly, the outer manganese was placed quite differently from where it was placed in the previous X-ray structures [5]. This position led to an open space in the center of the OEC, which is critical for allowing the low-barrier O-O bond-formation suggested earlier [4].

In 2011, a major experimental breakthrough occurred when the first high-resolution X-ray structure at 1.9 Å was presented by Umena et al, which essentially confirmed the quantum chemical structure of the OEC [6]. The main difference was that Asp170 was found to bind in a bridging mode between the terminal manganese and calcium instead of only terminally to the manganese as in the quantum chemical structure. The rest of the structure is very similar, including the critical positions of the outer manganese and the

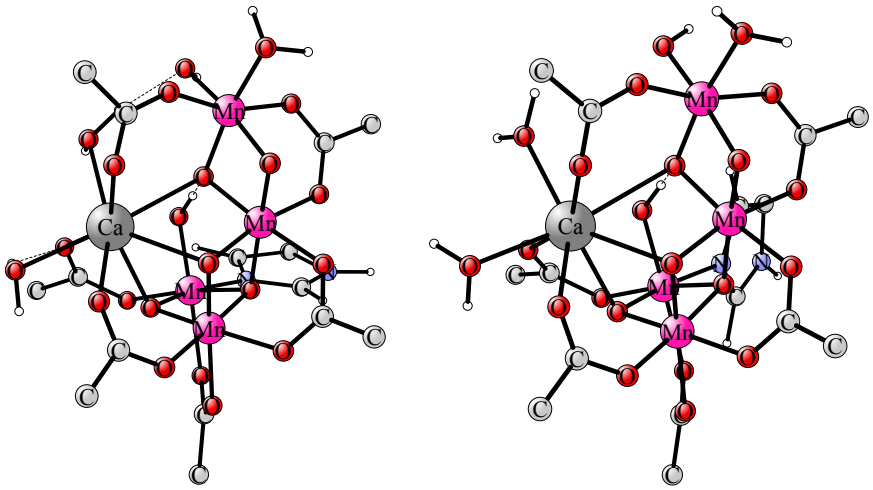

Figure 2: The previously DFT(B3LYP)-optimized structure for the S_3-state [16, 12] is shown to the left and the structure suggested after a spectroscopic analysis is shown to the right [15]

oxo groups, and the ligand connections. A minor problem with the X-ray structure is that it is most probably reduced by X-ray radiation [7, 8, 9], indicating that it is unlikely to be in the S_1-state as claimed. More recently, spectroscopic studies have played a major role by confirming the most important aspects of the quantum chemical suggestions. On the basis of the new X-ray structure and old DFT(B3LYP) structure [5], using EPR, ENDOR, and DFT, a detailed structure of the OEC in the S_2 state was reached [10] that agrees almost perfectly with a structure obtained independently by a DFT(B3LYP) energy minimization [11, 12], see Fig.1. The positions of the oxo groups and the protonation states, even including which ligands are water and which are hydroxides, agree, also which manganese is Mn(III) and which are Mn(IV) at that stage. Also, the DFT(B3LYP) structure from 2009, before the high-resolution structure, is very similar [13]. Two years ago, also the substrate oxygen positions have been suggested for S_2 using a W-band ^{17}O-ELDOR detected NMR spectroscopy [14]. The position for the slowly exchanging substrate agrees with the one suggested by the DFT studies [4, 11, 12, 13], but there is still a minor possible disagreement for the fast exchanging substrate. Very recently, a combined experimental and theoretical study appeared in Science [15], where EPR and ^{55}Mn-EDNMR spectra were used to suggest an S_3 structure which is almost identical to the structure suggested by DFT(B3LYP) two years ago [16], see Fig.2, again very similar to the one from 2009 [13]. It was claimed that only this structural model fits the measured spectra.

Even though the major features of water oxidation can now be claimed to be reasonably

well understood, additional studies are required to sort out details of the mechanism. A puzzling observation stems from previous EXAFS studies of the S_2 to S_3 transition. In one EXAFS study by Yachandra et al [17, 18, 19], three short distances of 2.7-2.8 Å were found in S_2. In another EXAFS study by Dau et al [20], only two short Mn-Mn distances of 2.7 Å were suggested. Instead, two of the Mn-Mn distances were proposed to be longer than 3 Å. For the S_2 to S_3 transition, the discrepancies were even more marked. In the studies by Yachandra et al, it was concluded that there is a *lengthening* of one of the 2.7-2.8 Å distances to 3.00 Å. In the study by Dau et al it was instead suggested that there is a *shortening* of one of the distances which was >3 Å down to 2.7 Å, indicating a formation of an additional Mn-Mn bis-μ-oxo bridge in S_3. Perhaps the most noteworthy of the differences of the suggested S_3 distances is the one which is 2.80 Å in the Dau et al study, and as long as 3.0 Å in the Yachandra et al study. The suggestions from both these studies give larger deviations to the DFT/spectroscopy structure than are expected from DFT, greater than 0.1 Å on some distances and a topology change. The two different EXAFS interpretations led to quite different proposals for the water oxidation mechanism. It should in this context be mentioned that for S_2 the raw data from the two groups are the same but not for S_3. In the present work the EXAFS information from Dau et al has been used. Quite recently, after the theoretical and spectroscopic consensus structure of S_2 had appeared, a reanalysis of the EXAFS spectra was made by the group of Yachandra et al [21]. For the S_2 structure, they now find full agreement between the EXAFS analysis and the DFT/spectroscopic structure in Fig.1. For the S_3 structure two alternatives were given, one with essentially four equivalent distances and one where one distance is longer.

It is not straight-forward to compare DFT and EXAFS data, because the latter are very sensitive to the metal-ligand distances (with an accuracy of 0.01-0.02 Å), whereas DFT calculations often give about 0.05 Å too long metal-O bonds and even larger deviations in the metal-metal distances. Therefore, an EXAFS spectrum calculated directly on a DFT structure will be poor, and a direct comparison of DFT and EXAFS distances will also show quite extensive deviations. Instead, it is better to perform a combined EXAFS/DFT refinement of the EXAFS spectrum, in which the EXAFS raw data (not only the EXAFS distances) are used as a restraint in the DFT geometry optimization [22, 23, 24]. Thereby, DFT will determine the general structure of the complex, whereas the EXAFS data will determine the detailed distances involving the metals. Such calculations are presented in this study for the S_2 and S_3 states, for a large DFT model used previously [11, 12], and

also for a much smaller model. The results are compared to the two different experimental EXAFS analyses. The main purpose of the present study is to investigate whether the discrepancies to experiments for the computational model, concerning the structural changes in S_2 to S_3, really indicate significant differences in the structures, or if they are mainly due to minor differences in bond lengths and technical differences in how the spectra are analyzed. The present study agrees with earlier ones [25, 26] in that a major problem of interpreting EXAFS spectra of complicated molecules is that it is possible to fit several quite different structures to the same spectrum.

It should finally be emphasized that a comparison to other theoretical and experimental work is not part of the purpose of the present paper, which is instead focused on the EXAFS results, see above. However, other theoretical work on water oxidation in PSII has been discussed in detail in recent reviews [12, 27, 28].

II. Methods and models

In the previous studies where the present starting structures were obtained, the DFT method used was the hybrid functional B3LYP [29] with the lacvp* basis set. For the present EXAFS refinement analysis the non-hybrid functional BP86 [30] has been used instead for technical reasons. The structures were therefore first re-optimized with this functional using a def2-SV(P) basis set. BP86 is known to give structures of similar quality as B3LYP, and the structural changes were indeed small. It has previously been emphasized that for the present system, B3LYP gives much better energetics than BP86 [31]. However, the comparison made concerned relative energies for different structures, while in the present study the energy differences entering the fitting procedure concern points in the same local minima. Since the equilibrium geometries are very similar using B3LYP and BP86, these energy differences should also be very similar. The cluster type modeling of the active site was used [28], with a model shown in Fig.3. The details of the model, which is the same as used in previous studies [12], are described in the supplementary material.

The EXAFS/QM refinements were performed with the ComQum-EXAFS software [23, 24]. This method is a combination of a quantum mechanical (QM) geometry optimization and an EXAFS structure refinement. This method is the same as has been used in previous studies [22, 23, 24]. The technical details are given in the supplementary material.

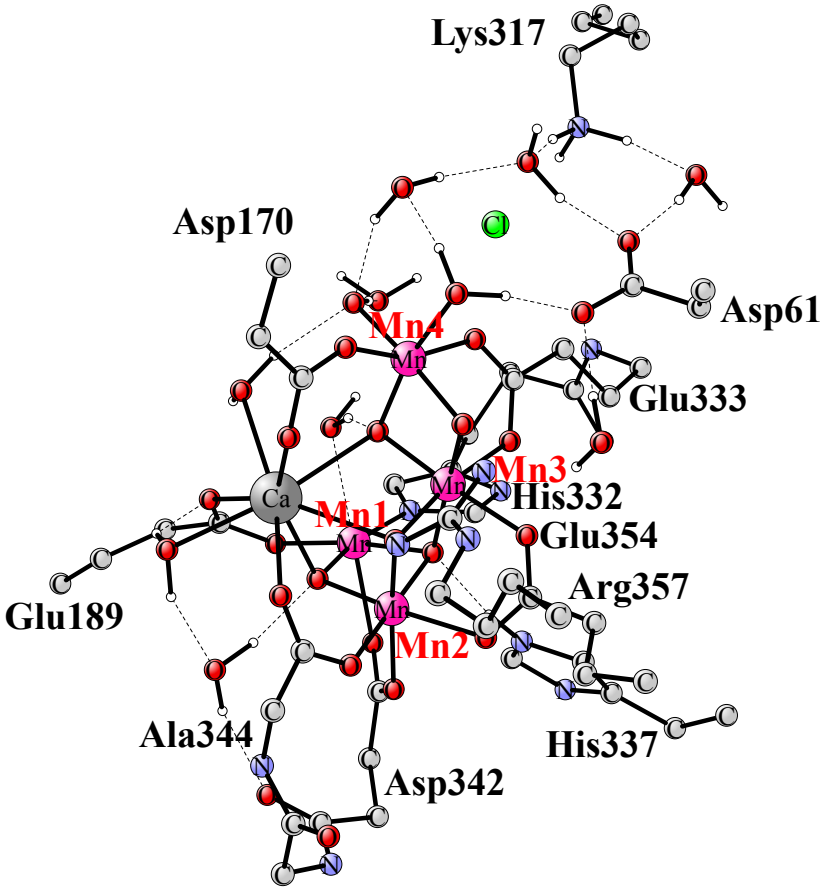

Figure 3: The previously optimized structure for the S_2-state [12]. The numbering of the Mn-atoms is the one used throughout the present paper, and is the same as in the high-resolution X-ray structure. Amino acid hydrogens are removed.

III. Results

The results from the present optimizations and refinements for the S_2 and S_3 states are given in Table 1, where also the results from the experimental EXAFS analyses are shown. For the S_3-states, results for two topologically different structures are given, see Fig.4, the first ones for the energetically optimal structure with the central oxo-group closer to the outer manganese Mn4. The second set of results given are for a local minimum where this oxo group is closer to Mn1. These two types of structures have been known to be nearly degenerate (outer oxo preferred) for the S_2 state [13], and recently they have been shown to correspond to the two states observed by EPR [32]. The refined S_3 spectrum for the "outer" oxo position is shown in Fig.5 together with the one for the S_2 state. The refined spectra are superimposed in Fig.6. The spectrum for the "inner" oxo position is shown in Fig.7. The optimized distances for the S_2-state from the previous study [12] using the large

Table 1: Mn-Mn distances obtained for S_2 and S_3 using different methods and models.

Method	Reference	Mn1-Mn2	Mn2-Mn3	Mn3-Mn4	Mn1-Mn3
S_2					
EXAFS	[17]	2.82	2.73	2.73	3.30
EXAFS	[21]	2.7	2.7	2.7	3.2
EXAFS	[20]	2.69	2.74	> 3	> 3
B3LYP	[12]	2.83	2.80	2.74	3.46
BP86		2.87	2.81	2.77	3.44
Refined[a]		2.83	2.74	2.68	3.62
Truncated, refined[b]		2.74	2.83	2.69	3.60
S_3					
EXAFS	[17]	2.73	2.80	3.00	3.30
EXAFS	[21]	2.7	2.7	3.2 (2.8)	2.8
EXAFS	[20]	2.73	2.73	2.77	> 3
Outer oxo					
B3LYP	[12]	2.84	2.81	2.76	3.55
BP86		2.88	2.82	2.79	3.53
Refined[c]		2.74	2.73	2.81	3.78
Truncated, refined[d]		2.72	2.79	2.79	3.80
Inner oxo					
B3LYP		2.75	2.78	3.18	2.89
BP86		2.78	2.80	3.19	2.92
Refined[e]		2.72	2.75	3.15	2.81

[a] χ^2=337, [b] χ^2=169, [c] χ^2=174,[d] χ^2=73, [e] χ^2=169

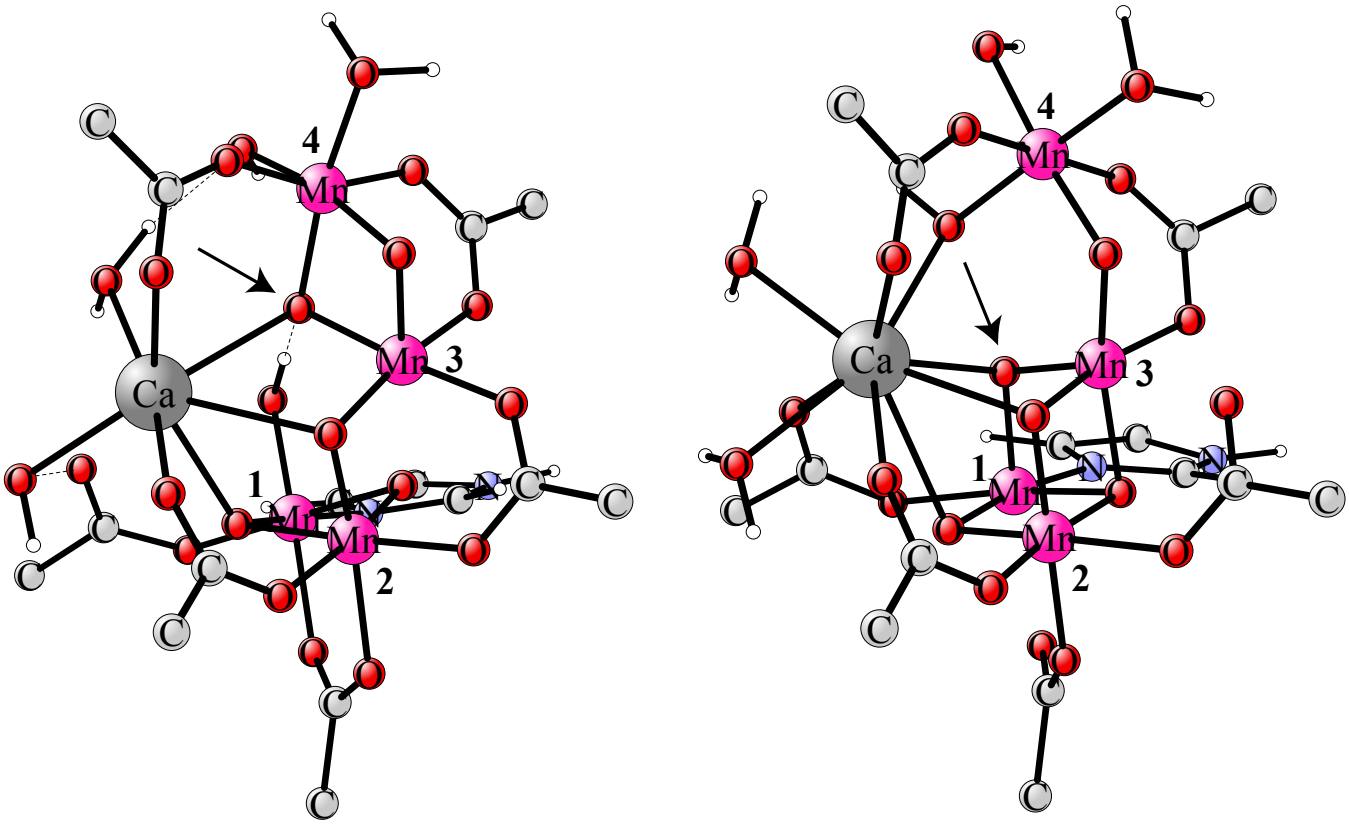

Figure 4: The two topolgically different S_3-states discussed in the text, the "outer" oxo position to the left and the "inner" one to the right.

model were Mn1-Mn2 = 2.83 Å, Mn2-Mn3 = 2.80 Å, Mn3-Mn4 = 2.74 Å, and Mn1-Mn3 = 3.46 Å. These results were obtained using B3LYP with the lacvp* basis set. For the present study, a re-optimization using BP86 was done. The differences to the B3LYP results are very small (0.01-0.04 Å) with the BP86 results being Mn1-Mn2 = 2.87 Å, Mn2-Mn3 = 2.81 Å, Mn3-Mn4 = 2.77 Å, and Mn1-Mn3 = 3.44 Å. The calculated results compare very well with the EXAFS analysis by Yachandra et al [17], where the distances were suggested to be two of 2.73 Å, one of 2.82 Å and one of 3.30 Å. It should be noted that the EXAFS analysis could not distinguish between the different Mn-Mn distances. In the more recent study, the results of the analysis was quite similar with three distances of 2.7 Å and one with 3.2 Å [21]. As usual, the DFT(B3LYP) distances are somewhat long. In contrast, the EXAFS analysis by Dau et al suggests one distance of 2.69 Å, one of 2.74 Å and two distances larger than 3 Å. From the nearly perfect agreement between the general DFT structure and recent results of EPR and ENDOR spectroscopy for the S_2-state [10], it must be concluded that the assignment by Dau et al of only two short Mn-Mn distances is unlikely to be correct.

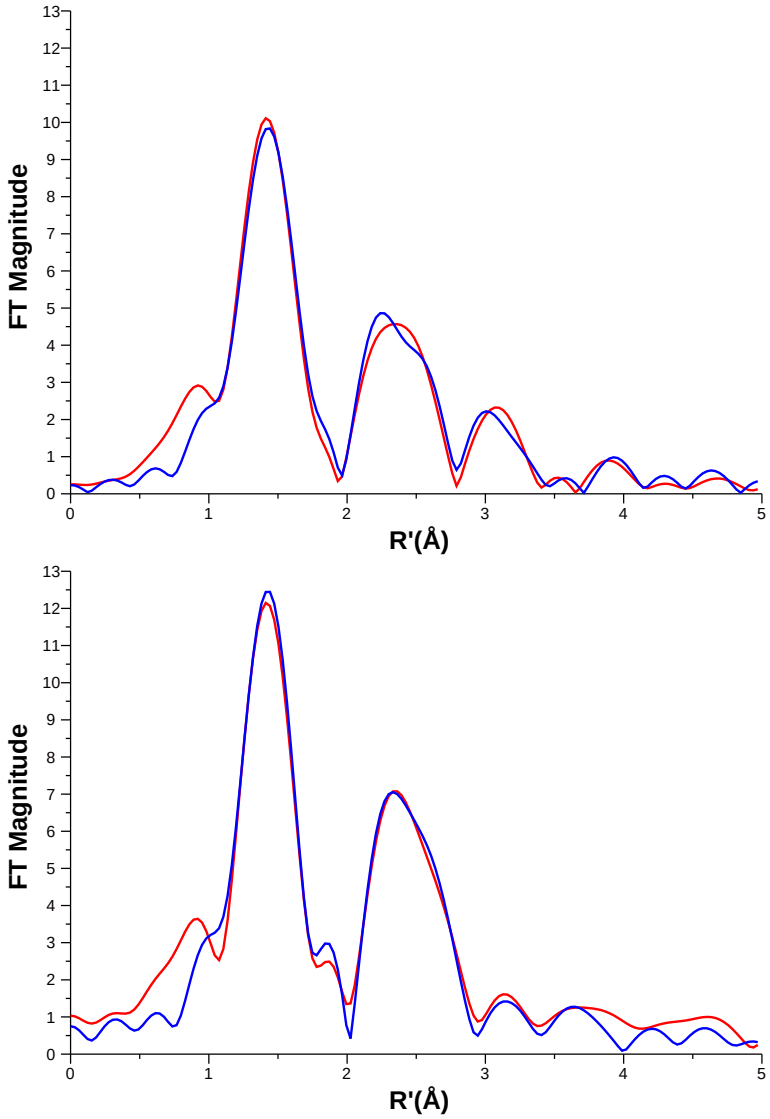

Figure 5: The refined EXAFS spectrum for the S_2-state is shown on top. The one for the S_3-state is shown at the bottom, for the case with the central oxo close to the outer manganese Mn4, the "outer" oxo position. The experimental spectra are in red.

The DFT/EXAFS refinement leads to the expected shortening of the three short Mn-Mn-distances. The refined results are Mn1-Mn2 = 2.83 Å, Mn2-Mn3 = 2.74 Å, Mn3-Mn4 = 2.68 Å, and Mn1-Mn3 = 3.62 Å. The three short distances are now in even better agreement with the analysis by Yachandra et al. For the long Mn-Mn distance, the refined distance is slightly longer than the experimental one, but this can not be regarded as a serious difference since this distance is extremely sensitive to details in the models used in the calculations.

Turning to the results for the S_3-state, the B3LYP distances for the energetically optimal

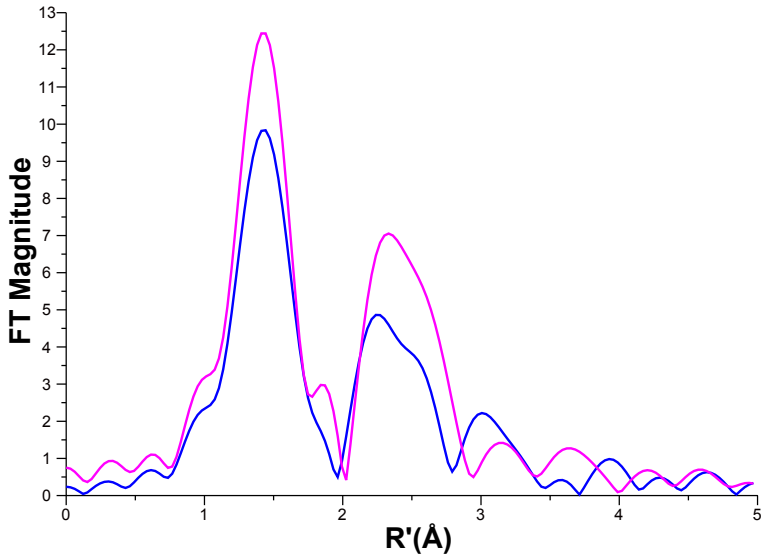

Figure 6: The refined EXAFS spectra for the S_2- and S_3- states superimposed. The one for the S_3-state is shown in purple.

"outer" position of the oxo group, are Mn1-Mn2 = 2.84 Å, Mn2-Mn3 = 2.81 Å, Mn3-Mn4 = 2.76 Å, and Mn1-Mn3 = 3.55 Å. The differences to the short S_2 distances are +0.01 Å, +0.01 Å and +0.02 Å, respectively. The similarity of these Mn-Mn distances to the ones for the S_2-state are surprising from two aspects. First, both EXAFS studies suggest much larger changes of these Mn-Mn distances. Second, since Mn1 is oxidized from Mn(III) to Mn(IV) in this transition, there is a loss of a Jahn-Teller axis which leads to a significant shortening of an Mn-O bond along this axis by as much as 0.6 Å, from 2.4 to 1.8 Å, but this does not seem to affect the Mn-Mn distances. The re-optimized BP86 distances show the same tendency with Mn1-Mn2 = 2.88 Å, Mn2-Mn3 = 2.82 Å, Mn3-Mn4 = 2.79 Å, and Mn1-Mn3 = 3.53 Å. The differences to the short S_2 distances are +0.01 Å, +0.02 Å and +0.02 Å, respectively, almost exactly the same differences as for B3LYP. The DFT/EXAFS refinement changes the picture somewhat, with distances of Mn1-Mn2 = 2.74 Å, Mn2-Mn3 = 2.73 Å, Mn3-Mn4 = 2.81 Å, and Mn1-Mn3 = 3.78 Å, leading to differences for the short distances of -0.09 Å, -0.01 Å and +0.13 Å, respectively, compared to the S_2 distances, instead indicating two notable changes of the short Mn-Mn distances. An even larger difference is found for the long distance after the refinement, from 3.62 to 3.78 Å. However, it should be noted in this context that the long distance is much more sensitive to details of the structures.

The EXAFS analysis by Yachandra et al [17] led to suggested distances for the S_3-state of

2.73 Å, 2.80 Å, 3.00 Å and 3.30 Å. There is one rather large difference to the DFT(B3LYP) results and this is the 3.00 Å distance, which is only 2.81 Å using DFT/EXAFS. As mentioned above, DFT(B3LYP) normally overestimates distances, so this would be a surprising difference. In the EXAFS analysis by Dau et al, the suggested distances are two of 2.73 Å, one of 2.77 Å, and one larger than 3 Å. Notably, the longest of the short distances is only 2.77 Å in comparison to 3.00 Å in the analysis by Yachandra et al. On the other hand, the results of the Dau et al analysis are well in line with the DFT results. Again, it should be pointed out that the EXAFS information from the two experimental studies is different for the S_3 state, and that the information from Dau et al was used here. In the recent reanalysis by the Yachandra group, two alternatives were given, one where all four distances are short, and one where one distance is longer [21]. For the first of these suggestions there is thus a discrepancy to the present analysis. In the second one there is a discrepancy concerning which distance is the long one, see further below.

From the above, it can be concluded that the agreement between the DFT(B3LYP) and EXAFS results is quite good compared to the Yachandra et al analysis [17] for the S_2-state. For the S_3-state the agreement is quite good compared to the Dau et al analysis. For the earlier analysis of the Yachandra group [17], the agreement is not as good. In the more recent reanalysis there are still disagreements, see further below [21]. Since the two EXAFS analyses differ substantially to each other for each state, the discrepancies between the results of DFT(B3LYP) and EXAFS, become more pronounced when the *changes* of the distances between S_2 and S_3 are compared. The DFT changes of the short Mn-Mn distances are very small as mentioned above. On the other hand, it is interesting to note that the DFT/EXAFS refinement led to somewhat larger changes of +0.13 and -0.09 Å for two distances. The refined results therefore agree better qualitatively with the interpretations of the EXAFS studies, indicating some structural changes in this transition. A conclusion that appears clear is that DFT (B3LYP or BP86) by itself is not accurate enough for predicting the detailed changes in the distances, see further below. It can be noted that two of the distances (Mn1-Mn2 and Mn2-Mn3 in the cube) show the same type of shortening of the (short) distances after refinement as in the case of the S_2-state. This is in line with previous experience. However, the third distance (Mn3-Mn4) to the outside manganese shows an unexpected lengthening. This may be a sign of a slightly worse description by DFT(B3LYP) for the S_3-state. It can be added that errors of 0.1 Å are not unusual for DFT optimized geometries. The typical accuracy of DFT calculations

for metal-ligand distances is about 0.06 Å, while the accuracy of DFT(B3LYP) is lower for non-bonded distances such as Mn-Mn and Mn-Ca (probably 0.1-0.2 Å).

Even though the two experimental EXAFS analyses agree that there should be a significant structural change in the S_2 to S_3 transition, the details of this change, and the consequences of it, are quite different in the two studies. The earlier analysis by Yachandra et al [17], gave one change of 0.3 Å (or one of 0.2 and one of 0.1 Å). This change, supported by results of earlier and later XANES spectra [33], led to the conclusion that an oxygen rather than a manganese is oxidized in this transition, which strongly affected the suggested O-O bond formation mechanism [34, 35]. The conclusion from the XANES spectra has been questioned experimentally [36], and also recently on the basis of DFT model calculations [37]. The Dau et al results, on the other hand, gave one change from a distance larger than 3 Å down to 2.77 Å. This led to a suggested structural change in this transition with a formation of an additional Mn-Mn bis-μ-oxo bridge in S_3 [38]. With the present knowledge, both from model calculations and spectroscopy experiments, none of these suggestions are likely to be correct. One conclusion that can be drawn is that it appears to be very difficult to obtain detailed structural information from EXAFS spectra, even if these are accurately measured, of such a complicated multi-metal complex like the OEC, without detailed information from quite similar model complexes where the structures are known. Such information is presently missing.

There are two different alternatives to explain the changes of the short Mn-Mn distances that are implicated by the DFT/EXAFS-refinement, assuming that it is reliable. These changes could either stem from a minor error in DFT, or from something missing in the chemical model used. Even before the recent spectroscopic verification of also the S_3-state [15] (when the present study was performed), the first possibility appeared to be by far the most likely explanation. One interesting aspect is that in this transition a strong hydrogen bond is introduced from the substrate hydroxyl group bound to Mn1 and an oxo group bridging Mn3-Mn4. The hydrogen bond is very short, only 1.49 Å, and a lengthening effect on the Mn3-Mn4 bond could have been expected, but the DFT(B3LYP) optimized structure shows only a small lengthening of +0.02 Å. The EXAFS refinement indicates that it should be larger with +0.13 Å. The actual error for the DFT bond distances implied by the refinement is +0.06 Å for one distance and -0.05 Å for the other one. DFT errors like these can not be excluded. At the same time Mn1 is oxidized which could implicate a shortening of the Mn1-Mn2 bond distance. Instead, DFT surprisingly shows a small

increase by 0.01 Å. The EXAFS-refinement shows the expected trend with a shortening of -0.09 Å with individual errors of 0.00 Å and +0.10 Å. Again, a DFT error of this size cannot be excluded.

The second alternative to explain the effects on the distances from the DFT/EXAFS-refinement would be some sort of error in the model used for the DFT calculations. Since there is a very large degree of consensus between theory and spectroscopic experiments concerning both the S_2 [10] and the S_3 structure [15], an error of this type appears very unlikely. Another question in that case is what type of defect this could be. An effect from the surrounding, not included in the DFT model, can most likely be ruled out on the basis of previous experience. Inside the model, a protonation of one of the μ-oxo bonds between Mn3 and Mn4 could explain the lengthening of this bond distance, but could hardly explain the shortening of the Mn1-Mn2 bond. A protonation of an oxo bond in the S_2-state is very unlikely on the basis of the present knowledge of this state. In the S_2 to S_3 transition a water binds to the OEC and a proton from that water could in principle protonate one of the oxo-bonds, but this would require a lack of proton release in this transition. However, there is convincing experimental evidence that a proton release does occur [38]. A protonation of a μ-oxo bond in the S_2 to S_3 transition would furthermore complicate O-O bond formation substantially. In all known mechanisms for O-O bond formation, natural or biomimetic, an oxygen radical is critically needed. At the stage the oxygen radical is formed, the presence of another protonated group than a substrate should therefore preferably be avoided, since that group could be deprotonated instead.

Another alternative to the present optimal S_3-structure could have been the one where the central oxo group is close to Mn1, see Fig.4. This has, for example, been suggested in one of the alternatives in the recent reanalysis of the EXAFS spectra [21], but this analysis used a definition of the "outer" structure with a 5-coordinated Mn4 (present numbering) in contrast to the structure here. Also, it can now be added that the "inner" structure was ruled out by the recent spectroscopic analysis [15]. The B3LYP distances, see Table 1, with the energetically less optimal "inner" position for the oxo group, are Mn1-Mn2 = 2.75 Å, Mn2-Mn3 = 2.78 Å, Mn3-Mn4 = 3.18 Å, and Mn1-Mn3 = 2.89 Å. Since EXAFS can not identify which Mn-Mn distance is which, a comparison to the S_2 distances should be made with a rearrangement of the assignments for the bonds. The differences to the short S_2 distances are then +0.01 Å, -0.02 Å and +0.06 Å, respectively. These changes are again, like for the

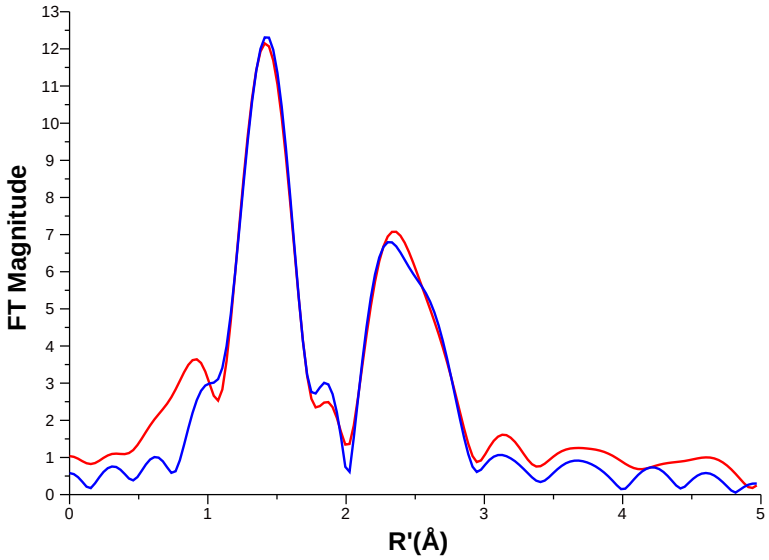

Figure 7: Refined EXAFS spectrum for the S_3-state with the central oxo close to manganese Mn1, the "inner" oxo position. The experimental spectrum is in red.

"outer" model smaller than the suggestions made by EXAFS. For the refined distances the corresponding differences are +0.03 Å, +0.01 Å and -0.02 Å, which thus show even smaller differences to the ones in the S_2 state. This is different to the case of the "outer" oxo where the refined distance changes showed a better correspondence with EXAFS. However, the results for the "outer" and "inner" structures show too small differences between each other to allow any conclusion of which structure should be best, just on the basis of the EXAFS analysis. Energetically, the "outer" minimum is preferred with a margin of 4.9 kcal/mol at the B3LYP level. In line with the excellent agreement obtained so far between the DFT results and spectroscopic experiments, the suggestion based solely on the calculations would therefore be that the "outer" minimum is the actually preferred one. For example, it has in this context been shown that the correct order, with a reasonable energy separation, compared to experiments is obtained by DFT for the corresponding states in S_2 [13, 32]. At the BP86 level, the preference for the "outer" minimum is even larger by another 5 kcal/mol. As discussed in detail before, the choice between these minima does not have any major effects on the actual O-O bond formation mechanism [13].

Another comment can be made concerning the present EXAFS analysis. It is a fact that both the experimental EXAFS spectra show large differences between the S_2 and S_3 states, which was the reason for suggesting a much larger structural change than found both here and in the recent EPR spectroscopic analysis. However, the present analysis shows that

it is indeed possible to obtain quite different EXAFS spectra for two structures with quite similar Mn-Mn distances, see Fig.5 and 7.

A remaining question is whether the DFT/EXAFS refined distances would have significant effects on the energetics, and thereby the mechanism. To test this, the S_2 and S_3 structures were re-optimized keeping the short Mn-Mn distances fixed to the values obtained after the refinement. By definition, this is less optimal energetically for DFT, but the difference for S_2 is quite small with an energy increase of only 1.3 kcal/mol. The same small difference of 1.3 kcal/mol is found for the "inner" minimum of S_3. For the "outer" minimum of S_3, the effect is slightly larger with 2.2 kcal/mol. In line with previous experience, the other detailed differences in the geometries do not matter much. Previously, it has for example been shown, that the choice of basis set for the geometry optimization does not have significant effects on the energetics, even though this could change the distances by even more than the difference between the optimal and refined structures discussed here [39]. Finally, the energies were calculated with fixed distances from the different EXAFS studies [17, 20, 21]. Again the energy differences to the optimized structures are quite small, in the range 1-2 kcal/mol. The exceptions are when EXAFS has suggested only two, rather than three, short distances, where the energy difference goes up to 10 kcal/mol. For the most recent EXAFS study the energy differences are also somewhat larger with 4-5 kcal/mol for the outer oxo structures. It is not possible to draw any conclusion from these values, except that the energies are quite insensitive to the details of the Mn-Mn distances, and these details certainly do not affect the mechanism.

Results for a quite truncated model (see section II) are also shown in Table 1. In general, the full and the truncated structures are quite similar. However, concerning the changes of the distances from S_2 to S_3, the truncated model is too small to give reliable results. For example, the truncated model gives a shortening (after refinement) of only -0.01 Å for Mn2-Mn3, while the full model gives -0.08 Å. Still, the lengthening of the distance to the outer manganese (Mn3-Mn4) is similar in the two models with +0.13 and +0.10 Å, respectively.

IV. Conclusions

In the present study, a DFT/EXAFS refinement procedure has been applied to DFT structures for the S_2 and S_3 states, previously presented [12]. Previous experimental spec-

troscopic studies have confirmed the details of the DFT(B3LYP) structure for the S_2-state [10], see Fig.1. Very recently (after the present study was made), a similar confirmation also exists for the S_3 state [15], see Fig.2. The oxidation states of the four manganese atoms, the ligation of the amino acids, the identification of oxo-, hydroxide- and water ligands have for both states been confirmed by the experiments. Also the mechanism for O-O bond formation has been essentially confirmed by experiments [14]. With this background it was now of high interest to go back to the analysis of the EXAFS spectra. Over many years, EXAFS studies have been made on the OEC, mainly by two groups reaching rather different conclusions. However, both groups have agreed that there should be a significant structural change in the S_2 to S_3 transition, since the EXAFS spectra for the two states are quite different. However, the details of this change have been suggested to be quite different. In one of the studies, a major *lengthening* of one Mn-Mn distance by more than +0.2 Å was suggested [17, 18, 19], while in the other one a major *shortening* of one distance by at least -0.2 Å was instead suggested [20]. In contrast, the DFT(B3LYP) optimizations showed only quite limited changes of the Mn-Mn distances in this transition [12]. The main purpose of the present study was therefore to investigate whether the DFT structures could be considered consistent with the EXAFS spectra. The conclusion from the study is that the DFT structures could be very well fitted to match the spectra keeping the same topological structures and with only minor distortions, within the normal errors of DFT. This also means that it is indeed possible to obtain EXAFS spectra that agree very well with those obtained experimentally, even from structures of the S_2 and S_3 states that are quite similar.

The DFT/EXAFS refinement modified the previous DFT(B3LYP) results somewhat. Instead of the small changes in the S_2 to S_3 transition obtained previously, one Mn-Mn distance was suggested to increase by +0.13 Å, while another one decreased by -0.09 Å. The by far most likely explanation for this correction are minor errors in DFT. Errors in metal-metal distances of this magnitude have been noticed several times before and the energetic consequence of these errors is minor. Defects in the chemical model used are considered much less likely, in particular after the recent spectroscopic verification of also the S_3 state. This gives another support for the previously suggested O-O bond formation mechanism, with an attack by an oxyl radical, bound to Mn1, on a μ-oxo-ligand bound between Mn3 and Mn4 [4, 13, 12].

Acknowledgements.

This work was generously supported by the Knut and Alice Wallenberg Foundation and by grants from the Swedish research council. Computer time was provided by the Swedish National Infrastructure for Computing.

Supplementary material.

Coordinates for the structures in the figures and diagrams are given as supplementary material.

References

[1] Ferreira KN, Iverson TM, Maghlaoui K, Barber J, Iwata S (2004) Architecture of the photosynthetic oxygen-evolving center. *Science* 303: 1831-1838.

[2] Loll B, Kern J, Saenger W, Zouni A, Biesiadka J (2005) Towards complete cofactor arrangement in the 3.0Å resolution structure of photosystem II. *Nature* 438: 1040-1044.

[3] Guskov A, et al (2009) Cyanobacterial photosystem II at 2.9Å resolution and the role of quinones, lipids, channels and chloride. *Nat Struct Mol Biol* 16: 334-342.

[4] Siegbahn PEM (2006) O-O bond formation in the S4 state of the oxygen-evolving complex in photosystem II. *Chem Eur J* 12: 9217-9227.

[5] Siegbahn PEM (2008) A structure-consistent mechanism for dioxygen formation in photosystem II. *Chem Eur J* 14: 8290-8302.

[6] Umena Y, Kawakami K, Shen J-R, Kamiya N (2011) Crystal structure of oxygen-evolving photosystem II at a resolution of 1.9Å. *Nature* 473: 55-60.

[7] Grundmeier A, Dau H (2012) Structural models of the manganese complex of photosystem II and mechanistic implications. *Biochim Biophys Acta, Bioenergetics* 1817: 88-105.

[8] Luber S, et al (2011) S1-state model of the O_2-evolving complex of photosystem II. *Biochemistry* 50: 6308-6311.

[9] Galstyan A, Robertazzi A, Knapp EW (2012) Oxygen-evolving Mn cluster in photosystem II: The protonation pattern and oxidation state in the high-resolution crystal structure. *J Am Chem Soc* 134: 7442-7449.

[10] Ames W, et al (2011) Theoretical evaluation of structural models of the S2 state in the oxygen evolving complex of photosystem II: Protonation states and magnetic interactions. *J Am Chem Soc* 133: 19743-19757.

[11] Siegbahn PEM (2011) The effect of backbone constraints: The case of water oxidation by the oxygen-evolving complex in PSII. *ChemPhysChem* 12: 3274-3280.

[12] Siegbahn PEM (2013) Water oxidation mechanism in photosystem II, including oxidations, proton release pathways, O-O bond formation and O_2 release. *Biochim Biophys Acta, Bioenergetics* 1827: 1003-1019.

[13] Siegbahn PEM (2009) Structures and energetics for O_2 formation in photosystem II. *Acc Chem Res* 42: 1871-1880.

[14] Rapatskiy L, et al (2012) Detection of the water-binding sites of the oxygen-evolving complex of photosystem II using W-band ^{17}O electron-electron double resonance-detected NMR spectroscopy. *J Am Chem Soc* 134: 16619-16634.

[15] Cox N, et al (2014) Electronic structure of the oxygen-evolving complex in photosystem II prior to O-O bond formation. *Science* 345: 804-808.

[16] Siegbahn PEM (2012) Mechanisms for proton release during water oxidation in the S2 to S3 and S3 to S4 transitions in photosystem II. *Phys Chem Chem Phys* 14: 4849-4856.

[17] Liang W, et al (2000) Structural change of the Mn cluster during the S2→S3 state transition of the oxygen-evolving complex of photosystem II. Does it reflect the onset of water/substrate oxidation? Determination by Mn X-ray absorption spectroscopy. *J Am Chem Soc* 122: 3399-3412.

[18] Robblee JH, et al (2002) The Mn cluster in the S0 state of the oxygen-evolving complex of photosystem II studied by EXAFS spectroscopy: Are there three di-μ-oxo-bridged Mn2 moieties in the tetranuclear Mn complex? *J Am Chem Soc* 124: 7459-7471.

[19] Pushkar Y, et al (2007) Structure and orientation of the Mn_4Ca cluster in plant photosystem II membranes studied by polarized range-extended X-ray absorption spectroscopy. *J Biol Chem* 282: 7198-7208.

[20] Haumann M, et al (2005) Structural and oxidation state changes of the photosystem II manganese complex in four transitions of the water oxidation cycle (S0 $\rightarrow$ S1, S1 $\rightarrow$ S2, S2 $\rightarrow$ S3, and S3,4 $\rightarrow$ S0) characterized by X-ray absorption spectroscopy at 20 K and room temperature. *Biochemistry* 44: 1894-1908.

[21] Glöckner C, et al (2013) Structural changes of the oxygen-evolving complex in photosystem II during the catalytic cycle. *J Biol Chem* 288: 22607-22620.

[22] Li X, Sproviero EM, Ryde U, Batista VS, Chen G (2013) Theoretical EXAFS studies of a model of the oxygen-evolving complex of photosystem II obtained with the quantum cluster approach. *Int J Quantum Chem* 113: 474-478.

[23] Hsiao Y-W, Tao Y, Shokes JE, Scott RA, Ryde U (2006) EXAFS structure refinement supplemented by computational chemistry. *Phys Rev B* 74: 214101.

[24] Ryde U, Hsiao Y-W, Rulíšek L, Solomon EI (2007) Identification of the peroxy adduct in multicopper oxidases by a combination of computational chemistry and extended X-ray absorption fine-structure measurements. *J Am Chem Soc* 129: 726-727.

[25] Fonda E, Michalowicz A, Randaccio L, Tauzher G, Vlaic G (2001) EXAFS data analysis of vitamin B12 model compounds - a methodological study. *Eur J Inorg Chem* 2001: 1269-1278.

[26] Hsiao Y-W, Ryde U (2006) Interpretation of EXAFS spectra for sitting-atop complexes with the help of computational methods. *Inorg Chim Acta* 359: 1081-1092.

[27] Gatt P, Stranger R, Pace RJ (2011) Application of computational chemistry to understanding the structure and mechanism of the Mn catalytic site in photosystem II - A review. *J Photochem Photobiol B: Biol* 104: 80-93.

[28] Blomberg MRA, Borowski T, Himo F, Liao R-Z, Siegbahn PEM (2014) Quantum chemical studies of mechanisms for metalloenzymes. *Chem Rev* 114: 3601-3658.

[29] Becke AD (1993) Density-functional thermochemistry. III. The role of exact exchange. *The Journal of Chemical Physics* 98: 5648-5652.

[30] Perdew JP (1986) Density-functional approximation for the correlation energy of the inhomogeneous electron gas. *Phys Rev B* 33: 8822–8824.

[31] Siegbahn PEM, Blomberg MRA (2013) Energy diagrams for water oxidation in photosystem II using different density functionals. *J Chem Theory Comput* 10: 268-272.

[32] Pantazis DA, Ames W, Cox N, Lubitz W, Neese F (2012) Two interconvertible structures that explain the spectroscopic properties of the oxygen-evolving complex of photosystem II in the S2 state. *Angew Chem Int Ed* 51: 9935-9940.

[33] Messinger J, et al (2001) Absence of Mn-centered oxidation in the S2 $\rightarrow$ S3 transition: Implications for the mechanism of photosynthetic water oxidation. *J Am Chem Soc* 123: 7804-7820.

[34] Yachandra VK, Sauer K, Klein MP (1996) Manganese cluster in photosynthesis: Where plants oxidize water to dioxygen. *Chem Rev* 96: 2927-2950.

[35] Messinger J (2004) Evaluation of different mechanistic proposals for water oxidation in photosynthesis on the basis of Mn_4O_xCa structures for the catalytic site and spectroscopic data. *Phys Chem Chem Phys* 6: 4764-4771.

[36] Iuzzolino L, Dittmer J, Dörner W, Meyer-Klaucke W, Dau H (1998) X-ray absorption spectroscopy on layered photosystem II membrane particles suggests manganese-centered oxidation of the oxygen-evolving complex for the S0-S1, S1-S2, and S2-S3 transitions of the water oxidation cycle. *Biochemistry* 37: 17112-17119.

[37] Brena B, Siegbahn PEM, Ågren H (2012) Modeling near-edge fine structure X-ray spectra of the manganese catalytic site for water oxidation in photosystem II. *J Am Chem Soc* 134: 17157-17167.

[38] Klauss A, Haumann M, Dau H (2012) Alternating electron and proton transfer steps in photosynthetic water oxidation. *Proc Natl Acad Sci* 109: 16035-16040.

[39] Siegbahn PEM (2001) Modeling aspects of mechanisms for reactions catalyzed by metalloenzymes. *J Comput Chem* 22: 1634-1645.