

University of Groningen

Toward understanding the S2-S3 transition in the Kok cycle of Photosystem II

Amin, Muhamed; Kaur, Divya; Gunner, M. R.; Brudvig, Gary W.

Published in:
 Inorganic Chemistry Communications

DOI:
[10.1016/j.inoche.2021.108890](https://doi.org/10.1016/j.inoche.2021.108890)

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
 Publisher's PDF, also known as Version of record

Publication date:
 2021

[Link to publication in University of Groningen/UMCG research database](#)

Citation for published version (APA):

Amin, M., Kaur, D., Gunner, M. R., & Brudvig, G. W. (2021). Toward understanding the S2-S3 transition in the Kok cycle of Photosystem II: Lessons from Sr-substituted structure. *Inorganic Chemistry Communications*, 133, [108890]. <https://doi.org/10.1016/j.inoche.2021.108890>

Copyright

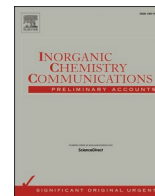
Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

The publication may also be distributed here under the terms of Article 25fa of the Dutch Copyright Act, indicated by the "Taverne" license. More information can be found on the University of Groningen website: <https://www.rug.nl/library/open-access/self-archiving-pure/taverne-amendment>.

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.

Downloaded from the University of Groningen/UMCG research database (Pure): <http://www.rug.nl/research/portal>. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.



Toward understanding the S₂-S₃ transition in the Kok cycle of Photosystem II: Lessons from Sr-substituted structure

Muhamed Amin^{a,b}, Divya Kaur^{c,d,e}, M.R. Gunner^{d,e}, Gary Brudvig^f

^a Rijksuniversiteit Groningen Biomolecular Sciences and Biotechnology Institute, University of Groningen, Groningen, Netherlands

^b Science Department, University College Groningen, University of Groningen, Groningen, Netherlands

^c Department of Chemistry, Brock University, 500 Glenridge Avenue, St. Catharines, ON L2S 3A1, Canada

^d Department of Chemistry, The Graduate Center of the City University of New York, New York, NY 10016, United States

^e Department of Physics, City College of New York, New York, NY 10031, United States

^f Department of Chemistry, Yale University, New Haven, CT 06520-8107, United States

ARTICLE INFO

Keywords:

Photosystem II
Density functional theory
Continuum electrostatics

ABSTRACT

Understanding the water oxidation mechanism in Photosystem II (PSII) stimulates the design of biomimetic artificial systems that can convert solar energy into hydrogen fuel efficiently. The Sr²⁺-substituted PSII is active but slower than with the native Ca²⁺ containing PSII as an oxygen evolving catalyst. Here, we use Density Functional Theory (DFT) to compare the energetics of the S₂ to S₃ transition in the Mn₄O₅Ca²⁺ and Mn₄O₅Sr²⁺ clusters. The calculations show that deprotonation of the water bound to Ca²⁺ (W3), required for the S₂ to S₃ transition, is energetically more favorable in Mn₄O₅Ca²⁺ than Mn₄O₅Sr²⁺. In addition, we have calculated the pK_a of the water that bridges Mn4 and the Ca²⁺/Sr²⁺ in the S₂ state using continuum electrostatics. The calculations show that the pK_a is higher by 4 pH units in the Mn₄O₅Sr²⁺ cluster.

The oxygen evolving complex (OEC) is a unique natural bioinorganic cluster that catalyzes the water oxidation reaction in the 5-step (S₀, S₁, S₂, S₃, S₄) Kok cycle [1,2]. The core of the OEC contains a metal cluster of four Mn and one Ca²⁺ connected through bridging oxygens [2–4]. Ca²⁺ depletion [5,6] blocks the S₂-S₃ transition, while replacing Ca²⁺ with Sr²⁺ reduces the catalytic activity [7–10]. In addition, in the absence of Ca²⁺, electron transfer from the OEC to Tyrosine (Y₂) becomes uphill [11].

Calcium and strontium belong to group 2 alkaline earth metals in the periodic table. Thus, they are chemically similar and have a stable oxidation state of +2. However, Ca²⁺ is a stronger Lewis acid, which indicates that aqua-Ca²⁺ compounds have a lower pK_a than aqua-Sr²⁺ (measured pK_a is 2 pH unit lower). This difference in proton affinity of the bound waters may be the reason for the difference in the catalytic activity in the Sr-substituted PSII [10,12,13]. Here, we use Density Functional Theory (DFT) to compare the energetics of the S₂-S₃ transition in native and Sr-substituted PSII.

The S₂-S₃ transition involves the insertion of a water molecule that binds to the OEC complex. Different mechanisms such as the pivot and carousel mechanisms have been proposed for water insertion [14,15]. The transition also depends on the specific S₂ spin state involved in the transition to the S₃ state. The S₂ state has two types of EPR signals:

multiline which corresponds to g = 2 and broad corresponding to g = 4.1 or higher depending upon the species and experimental conditions [16,17]. In the g = 4.1 EPR state, Mn1, Mn2, and Mn3 are in the IV oxidation state, while Mn4 is in the III state (Fig. 1) [18]. In the g = 2 redox isomer, Mn1 is Mn(III) while Mn4 is Mn(IV). However, the two S₂ spin states can interconvert [17–19].

Recent computational studies proposed different models for the S₂ spin states, which differ in either the protonation states of W1 and W2 of Mn4 or the protonation states of the oxygen bridges mainly O4 [20–22]. An open question is: Which spin and protonation states of the S₂ state are oxidized to the S₃ state? Different mechanisms have been proposed. For instance, experimental studies [16] including temperature dependence kinetics experiments and computational studies including those using DFT and continuum electrostatics based methods [23–25] have proposed that conversion of the S₂ g = 2 open cubane to the g = 4.1 closed cubane occurs before OEC oxidation to the S₃ state. The model used for both g = 2 and g = 4.1 spin isomers involves a deprotonated oxygen bridge O4. Another DFT study proposed the transition from a high spin open cubane S₂ state with O4 deprotonated to the S₃ state [26]. Recent studies using broken symmetry DFT and spin ladder calculations show that the g = 4.8/4.9 form observed at high pH corresponds to the high spin S = 7/2 species involved in the advancement to S₃. This model of

E-mail address: m.a.a.amin@rug.nl (M. Amin).

<https://doi.org/10.1016/j.inoche.2021.108890>

Received 21 May 2021; Received in revised form 27 August 2021; Accepted 29 August 2021

Available online 1 September 2021

1387-7003/© 2021 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

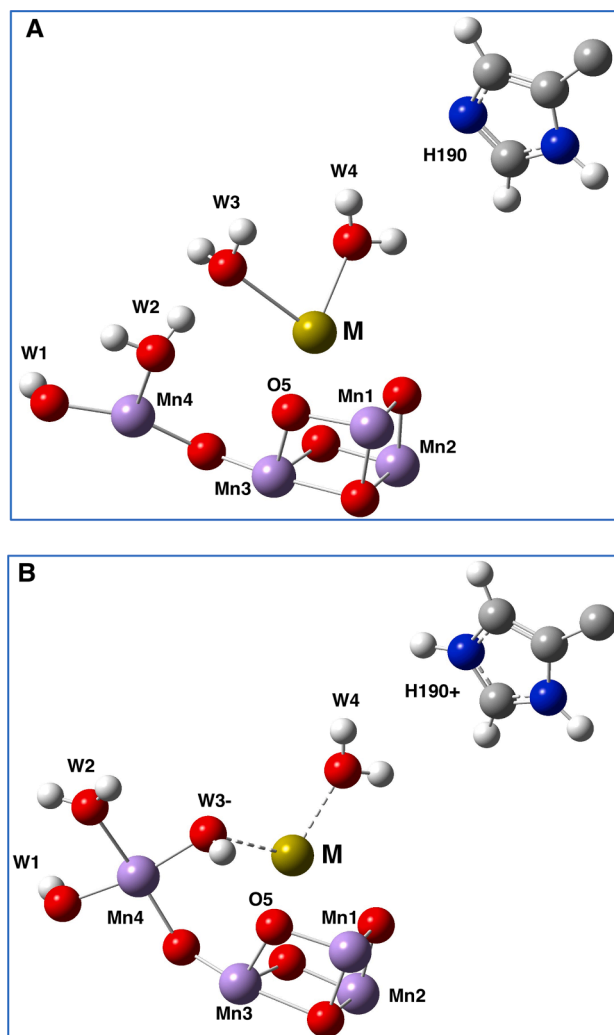


Fig. 1. **A** represents the S_2 state with HIS190 neutral. **B** represents the S_2 state with HIS190⁺ protonated. **M** is Ca²⁺ or Sr²⁺. Mn1, Mn2, Mn3 are in the IV oxidation state. Mn4 is III. The complete description of the model is included in the supplementary information.

the S_2 state proposes a proton shift where O4 is protonated and the water bound Mn4 W1 is a hydroxide [21,22]. Other studies involving time-resolved photothermal beam deflection measurements suggest that a proton is released from the OEC or surroundings when the nearby Tyr, Y_Z, is oxidized before Mn oxidation in the S_2 - S_3 transition [27,28].

Based on our earlier classical electrostatic calculations and DFT study [23], we proposed that the S_2 - S_3 transition starts with the transition from the $g = 2$ to $g = 4.1$ state followed by deprotonation of the W3 Ca²⁺ ligand [29]. This is coupled to the protonation of HIS190 upon the oxidation of the secondary donor Yz*. The deprotonated W3 moves toward Mn4 adding the sixth ligand to its coordination shell to facilitate its oxidation to the IV state. Similar mechanisms have been proposed by previous theoretical [30–33] and experimental [10] studies.

Here, we compare the energies of a proton shift between OEC cluster in the S_2 $g = 4.1$ state and His190 in both Mn₄O₅Ca²⁺ and Mn₄O₅Sr²⁺ clusters. In structure **A** HIS190 and W3 are neutral (Fig. 1A) and in **B** HIS190⁺ is protonated and W3 is a OH⁻ bridge between Mn4 and Ca²⁺ (Fig. 1B). The structures were optimized at the DFT level using the B3LYP functional and 6-31G(d) basis sets for N, O, C and H atoms, while SDD basis sets are used for Mn, Ca and Sr. All the Mn ions are in the high spin state. Furthermore, the energies are compared using different levels of theory; B3LYP/6-31G+(d) and B97D/6-31G+(d) [34,35].

The energy differences between the **A** and **B** states ($\Delta G_{(B-A)}$) at

different levels of theory are shown in Table 1. In general, the **B** state (protonated HIS190 and hydroxyl on W3) is always more favorable for the Mn₄O₅Ca²⁺ than the Mn₄O₅Sr²⁺ cluster. The large energy difference obtained for the Mn₄O₅Sr²⁺ cluster using the B3LYP/6-31G(d) level of theory indicates the importance of including diffuse functions in the basis sets when modeling large ions. These diffuse functions provide a flexible representation to the tail part of the atomic orbitals further from the nucleus [34,35].

Sr²⁺ is larger than Ca²⁺ by 0.1 Å, which elongates the interatomic distances between the Sr²⁺ and the rest of the atoms in the Mn cluster. This is seen in the optimized structures of the **A** and **B** states with Ca²⁺ and Sr²⁺ clusters (Table 2). In addition, the dispersion interaction between the metal and the water ligand is expected to push the water away in case of Sr²⁺, which will result in smaller electrostatic interactions and a higher pK_a. This is found for aqua-Ca²⁺ and aqua-Sr²⁺ compounds, where the water bound to Sr²⁺ has a higher pK_a than those bound to Ca²⁺. Thus, the Sr²⁺ structure is more stable with neutral W3 (Fig. 1A). However, with Ca²⁺, W3 deprotonates forming a hydroxide that moves to bridge Mn4 and Ca²⁺ (Fig. 1B).

The optimized DFT structures show that Mn-Sr²⁺ distances are in general longer than Mn-Ca²⁺. In the **A** state the Sr²⁺-W3(HOH) distance is 0.1 Å longer than Ca²⁺-W3(HOH). In the **B** state the Sr²⁺-W3(OH)⁻ distance is 0.2 Å longer because Ca²⁺ moves significantly toward Mn4 after the deprotonation of W3. To further compare the Mn₄O₅Ca²⁺ and the Mn₄O₅Sr²⁺ clusters, we calculated the pK_a of W3 in the **A** state with HIS190 protonated for both clusters using Monte Carlo sampling with continuum electrostatics and molecular mechanics energies [36,37]. MCCE (Multi-Conformer Continuum Electrostatics) [37] is used to calculate the pK_a starting with the DFT optimized structure by calculating the difference in the free energies $\Delta\Delta G$ of the protonated and deprotonated conformers. The $\Delta\Delta G$ includes the electrostatics and the desolvation energies calculated using DELPHI [38] (see supporting information). The surroundings of the isolated DFT structure are given a dielectric constant of 80. Thus, the model removes long-range interactions from the protein. The high dielectric solvent around the isolated cluster decreases the pairwise electrostatic interactions within the cluster, which can be balanced by the solvent stabilizing the cluster charges.

W3 has a pK_a of 6.5 in the Mn₄O₅Ca²⁺ and 10.3 in the Mn₄O₅Sr²⁺ clusters. Because the Sr²⁺ has a larger ionic radius than Ca²⁺, the optimized structures show that the W3 water ligand is closer to Ca²⁺, Mn4 (III) and Mn1(IV) (Table 2), which explains the difference in proton affinity with Ca²⁺ or Sr²⁺ in the cluster. This is supported by the DFT calculations, which show that with Ca²⁺ **B** has a lower energy than **A** indicating an easier deprotonation of W3. The calculated pK_a of W3 is significantly lower than 14, the value obtained by Saito et al. [39,40]. However, the pK_a of W3 is expected to be lower than the aqua Ca²⁺ pK_a (~12) due to the positively charged Mn cluster. In addition, the nearby positively charged HIS190⁺, favors the hydroxide conformer and reduces the pK_a of W3.

An open question is: what is the source of the proton which is released after Y_Z is oxidized but before the OEC advances to the S_3 state [3,41]. As there are no protons bound to the bridging oxygens in the S_2 state, the donors are likely to be terminal water ligands bound to Mn4 [42,43] or to Ca²⁺ [44]. Previous studies have shown the Mn4-bound water W1 is deprotonated upon formation of the tyrosyl radical, however the proton is trapped by the nearby acceptor D61 in the S_2 state

Table 1
The $\Delta G_{(B-A)}$ DFT energies.

	B3LYP/6-31G(d)	B3LYP/6-31G+(d)	B97D/6-31G+(d)
Mn ₄ O ₅ Ca ²⁺	-2.3	-8.7	-6.8
Mn ₄ O ₅ Sr ²⁺	13.0	-8.0	-6.4

Energy differences are expressed in Kcal/mol. The transition from **A** to **B** state is more favorable in the Mn₄O₅Ca²⁺ cluster than the Mn₄O₅Sr²⁺

Table 2
Interatomic distances in A and B states.

	A		B	
	Ca ²⁺	Sr ²⁺	Ca ²⁺	Sr ²⁺
Mn1	2.50	3.58	3.49	3.67
Mn4	4.35	4.51	3.58	2.64
W3	2.55	2.66	2.39	2.58
W4	2.33	2.50	2.35	2.53
O5	2.66	2.78	2.66	2.77

All distances are reported in Å. In general, the interatomic distances are longer for Sr²⁺.

[14,25,45–47].

The present study utilizes the S₂ g = 4.1 models for Ca²⁺ and Sr²⁺ containing PSII to understand the nature of deprotonation event. Our DFT calculations support the deprotonation of W3 in the S₂ to S₃ transition, which is also supported by the XFEL structures comparing the S₁, S₂ and S₃ states [48]. In conclusion, the above calculation shows that the S₂-S₃ transition occurs upon the loss of a proton from Ca-ligated W3 in the presence of HIS190⁺ maintaining the hydrogen bonding network necessary for the proton transfer. In the Sr²⁺-substituted structure, the energy barrier for deprotonating W3 is higher due to the weaker electrostatic interactions that enhance proton affinity.

CRedit authorship contribution statement

Muhammed Amin: Conceptualization, Methodology, Writing – original draft. **Divya Kaur:** Methodology, Writing – original draft. **M.R. Gunner:** Writing – review & editing. **Gary Brudvig:** Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors acknowledge computational resources from the support by the U.S. Department of Energy, Office of Science, Office of Basic Energy Sciences, Division of Chemical Sciences, Geosciences, and Biosciences via Grants DESC0001423 (M.R.G. and V.S.B.), and DE-FG02-05ER15646 (G.W.B.).

Appendix A. Supplementary material

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.inoche.2021.108890>.

References

- D.J. Vinyard, G.W. Brudvig, Progress toward a Molecular Mechanism of Water Oxidation in Photosystem II, *Annu. Rev. Phys. Chem.* 68 (1) (2017) 101–116, <https://doi.org/10.1146/annurev-physchem-052516-044820>.
- J. Kern, R. Chatterjee, I.D. Young, F.D. Fuller, L. Lassalle, M. Ibrahim, S. Gul, T. Fransson, A.S. Brewster, R. Alonso-Mori, R. Hussein, M. Zhang, L. Douthit, C. de Lichtenberg, M.H. Cheah, D. Shevela, J. Wersig, I. Seuffert, D. Sokaras, E. Pastor, C. Weninger, T. Kroll, R.G. Sierra, P. Aller, A. Butryn, A.M. Orville, M. Liang, A. Batyuk, J.E. Koglin, S. Carbajo, S. Boutet, N.W. Moriarty, J.M. Holton, H. Dobbek, P.D. Adams, U. Bergmann, N.K. Sauter, A. Zouni, J. Messinger, J. Yano, V.K. Yachandra, Structures of the Intermediates of Kok's Photosynthetic Water Oxidation Clock, *Nature* 563 (7731) (2018) 421–425, <https://doi.org/10.1038/s41586-018-0681-2>.
- M. Suga, F. Akita, K. Yamashita, Y. Nakajima, G. Ueno, H. Li, T. Yamane, K. Hirata, Y. Umena, S. Yonekura, L.-J. Yu, H. Murakami, T. Nomura, T. Kimura, M. Kubo, S. Baba, T. Kumasaka, K. Tono, M. Yabashi, H. Isobe, K. Yamaguchi, M. Yamamoto, H. Ago, J.-R. Shen, An OxyL/Oxo Mechanism for Oxygen-Oxygen Coupling in PSII Revealed by an x-Ray Free-Electron Laser, *Science* 366 (6463) (2019) 334–338, <https://doi.org/10.1126/science.aax6998>.
- Y. Umena, K. Kawakami, J.-R. Shen, N. Kamiya, Crystal Structure of Oxygen-Evolving Photosystem II at a Resolution of 1.9 Å, *Nature* 473 (7345) (2011) 55–60, <https://doi.org/10.1038/nature09913>.
- A. Boussac, J.L. Zimmermann, A.W. Rutherford, EPR Signals from Modified Charge Accumulation States of the Oxygen Evolving Enzyme in Ca²⁺-Deficient Photosystem II, *Biochemistry* 28 (23) (1989) 8984–8989, <https://doi.org/10.1021/bi00449a005>.
- M.J. Latimer, V.J. DeRose, V.K. Yachandra, K. Sauer, M.P. Klein, Structural Effects of Calcium Depletion on the Manganese Cluster of Photosystem II: Determination by X-Ray Absorption Spectroscopy, *J. Phys. Chem. B* 102 (1998) 8257–8265.
- D.F. Ghanotakis, G.T. Babcock, C.F. Yocum, Calcium Reconstitutes High Rates of Oxygen Evolution in Polypeptide Depleted Photosystem II Preparations, *FEBS Lett.* 167 (1) (1984) 127–130, [https://doi.org/10.1016/0014-5793\(84\)80846-7](https://doi.org/10.1016/0014-5793(84)80846-7).
- A. Boussac, A.W. Rutherford, Nature of the Inhibition of the Oxygen-Evolving Enzyme of Photosystem II Induced by Sodium Chloride Washing and Reversed by the Addition of Calcium²⁺ or Strontium²⁺, *Biochemistry* 27 (9) (1988) 3476–3483, <https://doi.org/10.1021/bi00409a052>.
- A. Boussac, F. Rappaport, P. Carrier, J.-M. Verbavatz, R. Gobin, D. Kirilovsky, A. W. Rutherford, M. Sugiura, Biosynthetic Ca²⁺/Sr²⁺ Exchange in the Photosystem II Oxygen-Evolving Enzyme of *Thermosynechococcus elongatus*, *J. Biol. Chem.* 279 (22) (2004) 22809–22819, <https://doi.org/10.1074/jbc.M401677200>.
- C.J. Kim, R.J. Debus, Evidence from FTIR Difference Spectroscopy That a Substrate H₂O Molecule for O₂ Formation in Photosystem II Is Provided by the Ca Ion of the Catalytic Mn₄CaO₅ Cluster, *Biochemistry* 56 (20) (2017) 2558–2570, <https://doi.org/10.1021/acs.biochem.6b0127810.1021/acs.biochem.6b01278.s001>.
- K. Saito, M. Mandal, H. Ishikita, Energetics of Ionized Water Molecules in the H-Bond Network near the Ca²⁺ and Cl⁻ Binding Sites in Photosystem II, *Biochemistry* 59 (35) (2020) 3216–3224, <https://doi.org/10.1021/acs.biochem.0c0017710.1021/acs.biochem.0c00177.s00110.1021/acs.biochem.0c00177.s002>.
- F.H.M. Koua, Y. Umena, K. Kawakami, J.-R. Shen, Structure of Sr-Substituted Photosystem II at 2.1 Å Resolution and Its Implications in the Mechanism of Water Oxidation, *Proc. Natl. Acad. Sci. U.S.A.* 110 (10) (2013) 3889–3894, <https://doi.org/10.1073/pnas.1219922110>.
- L. Vogt, M.Z. Ertem, R. Pal, G.W. Brudvig, V.S. Batista, Computational Insights on Crystal Structures of the Oxygen-Evolving Complex of Photosystem II with Either Ca²⁺ or Sr²⁺ Substituted by Sr²⁺, *Biochemistry* 54 (3) (2015) 820–825, <https://doi.org/10.1021/bi5011706>.
- M. Retegan, V. Krewald, F. Mamedov, F. Neese, W. Lubitz, N. Cox, D.A. Pantazis, A Five-Coordinate Mn(IV) Intermediate in Biological Water Oxidation: Spectroscopic Signature and a Pivot Mechanism for Water Binding, *Chem. Sci.* 7 (1) (2015) 72–84, <https://doi.org/10.1039/C5SC03124A>.
- M. Askerka, G.W. Brudvig, V.S. Batista, The O₂-Evolving Complex of Photosystem II: Recent Insights from Quantum Mechanics/Molecular Mechanics (QM/MM), Extended X-Ray Absorption Fine Structure (EXAFS), and Femtosecond X-Ray Crystallography Data, *Acc. Chem. Res.* 50 (1) (2017) 41–48, <https://doi.org/10.1021/acs.accounts.6b00405>.
- D.J. Vinyard, S. Khan, M. Askerka, V.S. Batista, G.W. Brudvig, Energetics of the S₂ State Spin Isomers of the Oxygen-Evolving Complex of Photosystem II, *J. Phys. Chem. B* 121 (5) (2017) 1020–1025, <https://doi.org/10.1021/acs.jpcc.7b0011010.1021/acs.jpcc.7b00110.s001>.
- A. Boussac, I. Ugur, A. Marion, M. Sugiura, V.R.I. Kaila, A.W. Rutherford, The Low Spin - High Spin Equilibrium in the S₂-State of the Water Oxidizing Enzyme, *Biochim. Biophys. Acta, Bioenerg.* 1859 (5) (2018) 342–356, <https://doi.org/10.1016/j.bbabi.2018.02.010>.
- J.L. Zimmermann, A.W. Rutherford, Electron Paramagnetic Resonance Properties of the S₂ State of the Oxygen-Evolving Complex of Photosystem II, *Biochemistry* 25 (16) (1986) 4609–4615, <https://doi.org/10.1021/bi00364a023>.
- D.A. Pantazis, W. Ames, N. Cox, W. Lubitz, F. Neese, Two Interconvertible Structures That Explain the Spectroscopic Properties of the Oxygen-evolving Complex of Photosystem II in the S₂ State, *Angew. Chem.* 51 (39) (2012) 9935–9940, <https://doi.org/10.1002/anie.201204705>.
- W. Ames, D.A. Pantazis, V. Krewald, N. Cox, J. Messinger, W. Lubitz, F. Neese, 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 (49) (2011) 19743–19757, <https://doi.org/10.1021/ja2041805>.
- T.A. Corry, P.J. O'Malley, Proton Isomers Rationalize the High- and Low-Spin Forms of the S₂ State Intermediate in the Water-Oxidizing Reaction of Photosystem II, *J. Phys. Chem. Lett.* 10 (17) (2019) 5226–5230, <https://doi.org/10.1021/acs.jpcclett.9b0137210.1021/acs.jpcclett.9b01372.s001>.
- T.A. Corry, P.J. O'Malley, Molecular Identification of a High-Spin Deprotonated Intermediate during the S₂ to S₃ Transition of Nature's Water-Oxidizing Complex, *J. Am. Chem. Soc.* 142 (23) (2020) 10240–10243, <https://doi.org/10.1021/jacs.0c0135110.1021/jacs.0c01351.s001>.
- M. Amin, D. Kaur, K.R. Yang, J. Wang, Z. Mohamed, G.W. Brudvig, M.R. Gunner, V. Batista, Thermodynamics of the S₂ to S₃ State Transition of the Oxygen-Evolving Complex of Photosystem II, *Phys. Chem. Chem. Phys.* 21 (37) (2019) 20840–20848, <https://doi.org/10.1039/c9cp02308a>.
- D. Kaur, W. Szejgis, J. Mao, M. Amin, K.M. Reiss, M. Askerka, X. Cai, U. Khaniya, Y. Zhang, G.W. Brudvig, V.S. Batista, M.R. Gunner, Relative Stability of the S₂ Isomers of the Oxygen Evolving Complex of Photosystem II, *Photosyn. Res.* 141 (3) (2019) 331–341, <https://doi.org/10.1007/s11120-019-00637-6>.

- [25] D. Narzi, D. Bovi, L. Guidoni, Pathway for Mn-Cluster Oxidation by Tyrosine-Z in the S₂ State of Photosystem II, *Proc. Natl. Acad. Sci. U.S.A* 111 (24) (2014) 8723–8728, <https://doi.org/10.1073/pnas.1401719111>.
- [26] P.E.M. Siegbahn, The S₂ to S₃ Transition for Water Oxidation in PSII (Photosystem II), Revisited. *Phys Chem Chem Phys* 20 (35) (2018) 22926–22931, <https://doi.org/10.1039/c8cp03720e>.
- [27] I. Zaharieva, H. Dau, Energetics and Kinetics of S-State Transitions Monitored by Delayed Chlorophyll Fluorescence, *Front. Plant Sci.* 10 (2019) 386, <https://doi.org/10.3389/fpls.2019.00386>.
- [28] I. Zaharieva, H. Dau, M. Haumann, Sequential and Coupled Proton and Electron Transfer Events in the S₂ → S₃ Transition of Photosynthetic Water Oxidation Revealed by Time-Resolved X-Ray Absorption Spectroscopy, *Biochemistry* 55 (50) (2016) 6996–7004, <https://doi.org/10.1021/acs.biochem.6b01078>.
- [29] M. Pérez-Navarro, F. Neese, W. Lubitz, D.A. Pantazis, N. Cox, Recent Developments in Biological Water Oxidation, *Curr. Opin. Chem. Biol.* 31 (2016) 113–119, <https://doi.org/10.1016/j.cbpa.2016.02.007>.
- [30] D. Bovi, D. Narzi, L. Guidoni, The S₂ State of the Oxygen-Evolving Complex of Photosystem II Explored by QM/MM Dynamics: Spin Surfaces and Metastable States Suggest a Reaction Path towards the S₃ State, *Angew. Chem.* 52 (45) (2013) 11744–11749, <https://doi.org/10.1002/anie.201306667>.
- [31] M. Shoji, H. Isobe, K. Yamaguchi, QM/MM Study of the S₂ to S₃ Transition Reaction in the Oxygen-Evolving Complex of Photosystem II, *Chem. Phys. Lett.* 636 (2015) 172–179, <https://doi.org/10.1016/j.cpl.2015.07.039>.
- [32] H. Isobe, M. Shoji, J.-R. Shen, K. Yamaguchi, Strong Coupling between the Hydrogen Bonding Environment and Redox Chemistry during the S₂ to S₃ Transition in the Oxygen-Evolving Complex of Photosystem II, *J. Phys. Chem. B* 119 (43) (2015) 13922–13933, <https://doi.org/10.1021/acs.jpbc.5b05740>.
- [33] I. Ugur, A.W. Rutherford, V.R.I. Kaila, Redox-Coupled Substrate Water Reorganization in the Active Site of Photosystem II—The Role of Calcium in Substrate Water Delivery, *Biochim. Biophys. Acta, Bioenerg.* 1857 (6) (2016) 740–748, <https://doi.org/10.1016/j.bbabi.2016.01.015>.
- [34] S. Grimme, Semiempirical GGA-Type Density Functional Constructed with a Long-Range Dispersion Correction, *J. Comput. Chem.* 27 (15) (2006) 1787–1799, [https://doi.org/10.1002/\(ISSN\)1096-987X10.1002/jcc.v27:1510.1002/jcc.20495](https://doi.org/10.1002/(ISSN)1096-987X10.1002/jcc.v27:1510.1002/jcc.20495).
- [35] A.D. Becke, Density-functional Thermochemistry. III. The Role of Exact Exchange, *J. Chem. Phys.* 98 (7) (1993) 5648–5652, <https://doi.org/10.1063/1.464913>.
- [36] M. Amin, L. Vogt, W. Szejgis, S. Vassiliev, G.W. Brudvig, D. Bruce, M.R. Gunner, Proton-Coupled Electron Transfer during the S-State Transitions of the Oxygen-Evolving Complex of Photosystem II, *J. Phys. Chem. B* 119 (24) (2015) 7366–7377, <https://doi.org/10.1021/jp510948e>.
- [37] Y. Song, J. Mao, M.R. Gunner, MCCE2: Improving Protein pK_a Calculations with Extensive Side Chain Rotamer Sampling, *J. Comput. Chem.* 30 (14) (2009) 2231–2247, <https://doi.org/10.1002/jcc.21222>.
- [38] C. Li, Z. Jia, A. Chakravorty, S. Pahari, Y. Peng, S. Basu, M. Koirala, S.K. Panday, M. Petukh, L. Li, E. Alexov, DelPhi Suite: New Developments and Review of Functionalities, *J. Comput. Chem.* 40 (28) (2019) 2502–2508, <https://doi.org/10.1002/jcc.v40.2810.1002/jcc.26006>.
- [39] K. Saito, M. Nakagawa, M. Mandal, H. Ishikita, Role of Redox-Inactive Metals in Controlling the Redox Potential of Heterometallic Manganese-Oxido Clusters, *Photosynth. Res.* 148 (3) (2021) 153–159, <https://doi.org/10.1007/s11120-021-00846-y>.
- [40] K. Saito, M. Nakagawa, H. Ishikita, pK_a of the Ligand Water Molecules in the Oxygen-Evolving Mn₄CaO₅ Cluster in Photosystem II, *Commun Chem* 3 (1) (2020) 1–7, <https://doi.org/10.1038/s42004-020-00336-7>.
- [41] M. Askerka, J. Wang, D.J. Vinyard, G.W. Brudvig, V.S. Batista, S₃ State of the O₂-Evolving Complex of Photosystem II: Insights from QM/MM, EXAFS, and Femtosecond X-Ray Diffraction, *Biochemistry* 55 (7) (2016) 981–984, <https://doi.org/10.1021/acs.biochem.6b00041.1021/acs.biochem.6b00041.s00210.1021/acs.biochem.6b00041.s003>.
- [42] M. Askerka, D.J. Vinyard, G.W. Brudvig, V.S. Batista, NH₃ Binding to the S₂ State of the O₂-Evolving Complex of Photosystem II: Analogue to H₂O Binding during the S₂ → S₃ Transition, *Biochemistry* 54 (38) (2015) 5783–5786, <https://doi.org/10.1021/acs.biochem.5b00974>.
- [43] J. Wang, M. Askerka, G.W. Brudvig, V.S. Batista, Crystallographic Data Support the Carousel Mechanism of Water Supply to the Oxygen-Evolving Complex of Photosystem II, *ACS Energy Lett.* 2 (10) (2017) 2299–2306, <https://doi.org/10.1021/acscenergylett.7b00750.1021/acscenergylett.7b00750.s001>.
- [44] C.J. Kim, R.J. Debus, One of the Substrate Waters for O₂ Formation in Photosystem II Is Provided by the Water-Splitting Mn₄CaO₅ Cluster's Ca²⁺ Ion, *Biochemistry* 58 (29) (2019) 3185–3192, <https://doi.org/10.1021/acs.biochem.9b00418>.
- [45] K. Kawashima, T. Takaoka, H. Kimura, K. Saito, H. Ishikita, O₂ Evolution and Recovery of the Water-Oxidizing Enzyme, *Nat. Commun.* 9 (1) (2018) 1247, <https://doi.org/10.1038/s41467-018-03545-w>.
- [46] K.R. Yang, K.V. Lakshmi, G.W. Brudvig, V.S. Batista, Is Deprotonation of the Oxygen-Evolving Complex of Photosystem II during the S₁ → S₂ Transition Suppressed by Proton Quantum Delocalization? *J. Am. Chem. Soc.* 143 (22) (2021) 8324–8332, <https://doi.org/10.1021/jacs.1c00633.1021/jacs.1c00633.s00110.1021/jacs.1c00633.s00210.1021/jacs.1c00633.s00310.1021/jacs.1c00633.s004>.
- [47] H. Kuroda, K. Kawashima, K. Ueda, T. Ikeda, K. Saito, R. Ninomiya, C. Hida, Y. Takahashi, H. Ishikita, Proton Transfer Pathway from the Oxygen-Evolving Complex in Photosystem II Substantiated by Extensive Mutagenesis, *Biochim. Biophys. Acta, Bioenerg.* 1862 (1) (2021) 148329, <https://doi.org/10.1016/j.bbabi.2020.148329>.
- [48] M. Ibrahim, T. Fransson, R. Chatterjee, M.H. Cheah, R. Hussein, L. Lassalle, K. D. Sutherlin, I.D. Young, F.D. Fuller, S. Gul, I.-S. Kim, P.S. Simon, C. de Lichtenberg, P. Chernev, I. Bogacz, C.C. Pham, A.M. Orville, N. Saichek, T. Northen, A. Batyuk, S. Carbajo, R. Alonso-Mori, K. Tono, S. Owada, A. Bhowmick, R. Bolotovskiy, D. Mendez, N.W. Moriarty, J.M. Holton, H. Dobbek, A.S. Brewster, P.D. Adams, N.K. Sauter, U. Bergmann, A. Zouini, J. Messinger, J. Kern, V.K. Yachandra, J. Yano, Untangling the Sequence of Events during the S₂ → S₃ Transition in Photosystem II and Implications for the Water Oxidation Mechanism, *Proc. Natl. Acad. Sci. USA* 117 (23) (2020) 12624–12635, <https://doi.org/10.1073/pnas.2000529117>.