

REPLY TO WANG ET AL.:

Clear evidence of binding of Ox to the oxygen-evolving complex of photosystem II is best observed in the omit map

Mohamed Ibrahim^a, Nigel W. Moriarty^b, Jan Kern^b, James M. Holton^{b,c,d}, Aaron S. Brewster^b, Asmit Bhowmick^b, Uwe Bergmann^{e,f}, Athina Zouni^g, Johannes Messinger^{g,h}, Vittal K. Yachandra^b, Junko Yano^{b,1}, Holger Dobbek^g, Nicholas K. Sauter^b, and Paul D. Adams^{b,i}

Wang et al. (1) performed their own analysis of our crystallographic data (2) and questioned our, and similar previous (3–5), results that a newly inserted water (Ox) in the catalytic center of photosystem II (PSII) can be uniquely identified in the room temperature crystal structures taken at various time points during the S₂-to-S₃ transition of the water oxidation reaction in PSII. They suggest that the results can be explained by the movement of the existing ligand, O5, without incorporating a new water (Ox). The following four points describe why their claim is not valid.

- 1) Wang et al. claim that they can fit our data using a single population, but their approach ignores the experimental validation. We reported independent experimental evidence to support the population of each flashed state (MIMS, EPR, Mn K β XES) (2) showing that two conformer refinement is the valid approach.
- 2) Wang et al. claim that the isomorphous difference peak position (Fig. 1 in ref. 1) does not coincide with where we modeled Ox. However, the center of the positive peak position of the isomorphous difference map cannot be simply used to determine atomic positions (due to the convolution of positional changes). The computed F_{model}-F_{model} difference map using our published structural models for the 0F and 2F state shows good agreement with the experimental F_{obs}-F_{obs} map, whereas a computed F_{model}-F_{model} map using a model similar to the one suggested by Wang et al. is missing important features (Fig. 1).
- 3) Chemical restraints are required for refinements at this resolution (~2.1 Å). Ignoring chemical knowledge will produce models with poor stereochemistry. To replicate the results by Wang et al. we had to apply implausible chemical restraints relating to O5 (Fig. 2 A and B). Indeed, we observe that in the refined model by Wang et al., all of the O5-Mn distances are longer than expected (2.63, 2.25, and 2.54 Å), implying that O5 is not ligated by any of the Mn. This is not chemically feasible given the proximity to the Mn atoms and knowledge from related inorganic model compounds (6, 7). Furthermore, relaxing all O5 restraints resulted in the O5 closely approximating our model (Fig. 2A). It should be noted that both refinement approaches resulted in higher R_{free} values compared to our original model [24.8% and 24.5% vs. 24.1% (2)].
- 4) Wang et al. do not discuss the electron density omit maps we used in our analysis. At crystallographic resolutions of ~2.1 Å, it is still challenging to accurately determine light atom positions that are close to heavy metal atoms. Therefore, we used omit maps (2, 3) to locate O5 and Ox positions (figure 4C of ref. 2). These clearly show that one oxygen alone cannot explain the observed electron density, and modeling of two oxygens (O5 and Ox) is required (Fig. 2 C and D).

Based on these observations, we conclude that the suggested model of a modified Mn₄O₅Ca cluster (1) cannot explain the PSII 2F state data (2–5) and that a model containing an additional oxygen (Ox) is necessary to fit the data.

^aInstitut für Biologie, Humboldt-Universität zu Berlin, D-10115 Berlin, Germany; ^bMolecular Biophysics and Integrated Bioimaging Division, Lawrence Berkeley National Laboratory, Berkeley, CA 94720; ^cStanford Synchrotron Radiation Lightsource, SLAC National Accelerator Laboratory, Menlo Park, CA 94025; ^dDepartment of Biochemistry and Biophysics, University of California, San Francisco, CA 94158; ^eStanford PULSE Institute, SLAC National Accelerator Laboratory, Menlo Park, CA 94025; ^fDepartment of Physics, University of Wisconsin, Madison, WI 53706; ^gDepartment of Chemistry - Ångström, Molecular Biomimetics, Uppsala University, SE 75120 Uppsala, Sweden; ^hInstitutionen för Kemi, Kemiskt Biologiskt Centrum, Umeå Universitet, SE 90187 Umeå, Sweden; and ⁱDepartment of Bioengineering, University of California, Berkeley, CA 94720

Author contributions: M.I., J.K., U.B., A.Z., J.M., V.K.Y., J.Y., and N.K.S. designed research; M.I., J.K., A.S.B., A.B., U.B., A.Z., J.M., V.K.Y., J.Y., N.K.S., and P.D.A. performed research; M.I., N.W.M., J.K., J.M.H., A.S.B., A.B., J.Y., H.D., and N.K.S. analyzed data; and M.I., N.W.M., J.K., J.M.H., U.B., A.Z., J.M., V.K.Y., J.Y., H.D., N.K.S., and P.D.A. wrote the paper.

The authors declare no competing interest.

Published under the [PNAS license](#).

¹To whom correspondence may be addressed. Email: jyano@lbl.gov.

Published June 11, 2021.

Acknowledgments

This work was supported by the Director, Office of Science, Office of Basic Energy Sciences, Division of Chemical Sciences, Geosciences, and Biosciences of the US Department of Energy (J.Y., J.K., and V.K.Y.), by NIH Grants

GM055302 (V.K.Y.), GM110501 (J.Y.), GM126289 (J.K.), GM117126 (N.K.S.), GM124149 and GM124169 (J.M.H.), Vetenskapsrådet 2016-05183 (J.M.), and Sfb1078 (Humboldt Universität Berlin) and TP A5 (A.Z., H.D., and M.I.).

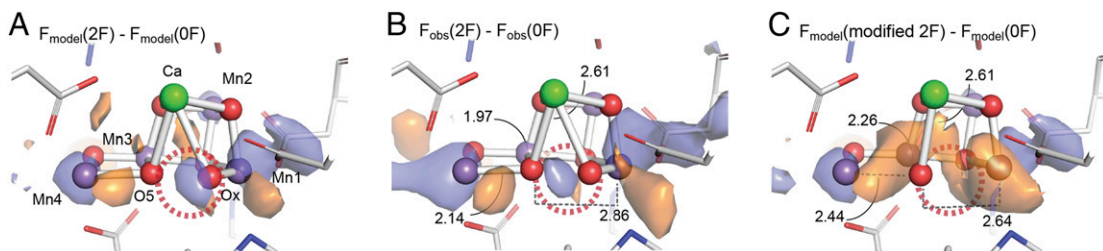


Fig. 1. Comparison of isomorphous difference electron density maps. (A) Theoretical isomorphous difference map using published models for the 2F and 0F state contoured at $\pm 4\sigma$ (blue/orange) [$F_{\text{model}}(6w1v) - F_{\text{model}}(6w1o)$, 6w1o.pdb for phases, 6w1v.pdb model, 1% error, 2.09-Å resolution]. (B) Observed isomorphous difference map at $\pm 3.5\sigma$ [$F_{\text{obs}}(6w1v) - F_{\text{obs}}(6w1o)$, 6w1o.pdb for phases, 6w1v model]. (C) Theoretical isomorphous difference map using a changed O5 position and no Ox, as suggested by Wang et al. (1) at $\pm 4\sigma$ [$F_{\text{model}}(6w1v \text{ modified}) - F_{\text{model}}(6w1o)$, 6w1o.pdb for phases, modified 6w1v model]. Metal-O5 distances are indicated in Å in B and C. Note the clear absence of a positive peak feature (red dashed circle) near the Ox position in the theoretical isomorphous difference map based on the model suggested by Wang et al. (1) (C), while this feature is strongly visible in both the observed $F_{\text{obs}} - F_{\text{obs}}$ map (B) or the theoretical $F_{\text{model}} - F_{\text{model}}$ map (A) based on the published structural models for the 2F and 0F state (2). F_{model} were generated from structural models using *phenix.fmodel* (8). Isomorphous difference maps were generated with *phenix.fobs_minus_fobs_maps* either from the F_{model} obtained in this way or using the structure factors deposited in the PDB for 6w1o and 6w1v. A structural model approaching the suggested model by Wang et al. was generated by modifying the 6w1v model in the following way. Atom O6 (Ox) of residue OEY, chain A, was deleted from the PDB file and the position of atom O5 of the OEY residue was adjusted manually to obtain distances to Mn1, Mn3, Mn4, and Ca as close as possible to the values reported by Wang et al. This resulted in distances of 2.64, 2.26, 2.44, and 2.61 Å between O5 and Mn1, Mn3, Mn4, and Ca, respectively. It was not possible to achieve the exact distances reported by Wang et al. (2.63, 2.25, 2.54, and 2.60 Å) without distorting the entire Mn cluster, and hence this was used as the closest possible approach. From this structural model, theoretical structure factors F_{model} were generated with *phenix.fmodel* and used for subsequent generation of the $F_{\text{model}} - F_{\text{model}}$ map in C. The two $F_{\text{model}} - F_{\text{model}}$ maps in A and C were contoured to a sigma level to approximately match the volume of the main positive and negative peaks near Mn1 and Mn4 in the experimental $F_{\text{obs}} - F_{\text{obs}}$ map at 3.5 σ contour level (B).

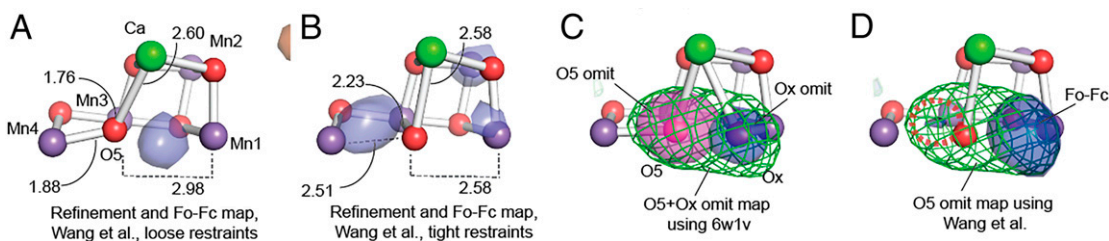


Fig. 2. Results of different refinements for the OEC in PSII and omit maps. (A and B) To explore the feasibility of a model for the OEC in the 2F data that contains no additional bridging oxygen ligand Ox, we performed refinements against our 2F data (6w1v; ref. 2) starting with a $\text{Mn}_4\text{O}_5\text{Ca}$ cluster with O5 placed in a position approximately similar to the position suggested by Wang et al. (1) and explored different refinement strategies. Upon refinement with *phenix.refine* (8), employing loose restraint settings (sigma values of 0.5 Å), the O5 atom moved back into a position closer to Mn4 and Mn3 and more distant from Mn1, closer to the model we obtained in our original work (2). The resulting refined model (final R_{free} of 0.2483) with O5 distances in Å and the Fo-Fc density contoured at $\pm 3\sigma$ (blue/orange) is shown in A. To force O5 to stay close to the starting position, we had to tightly restrain it to this position (sigma values of 0.005 Å). The resulting structural model (final R_{free} of 0.2454) together with the Fo-Fc density is shown in B. Upon tightly restraining O5, a new positive Fo-Fc peak is visible between O5 and Mn4, indicating that this model does not properly explain the experimental data. We further evaluated the proposed model by generating omit maps (C and D). We first used our published model 6w1v (C) and omitted O5 and Ox separately. The resulting omit map is shown in magenta (O5) and blue (Ox) at 5 σ contour level. In addition, both O5 and Ox were omitted together, and the resulting omit map is shown as green mesh at 3 σ contour level. The shape of this density clearly indicates that the experimental data cannot be described by a single atom. In comparison, we computed Fo-Fc maps assuming a position of O5 as suggested by Wang et al. (D). The resulting Fo-Fc map with O5 placed in the model is shown in blue/orange at $\pm 3\sigma$. In addition, the omit map obtained by removing O5 from the model is shown as green mesh contoured at 3 σ . The Fo-Fc map with O5 present in a position close to what Wang et al. suggested shows a strong positive peak to the right (toward Mn1), indicating that an additional atom needs to be included in the model. In addition, a weaker positive peak is visible to the left of O5 (toward Mn4, red dashed circle), indicating that O5 should move closer to Mn4.

- 1 J. Wang, W. H. Armstrong, V. S. Batista, Do the crystallographic XFEL data support binding of a water molecule to the oxygen-evolving complex of photosystem II exposed to two flashes of light? *Proc. Natl. Acad. Sci. U.S.A.*, 10.1073/pnas.2023982118 (2021).
- 2 M. Ibrahim et al., Untangling the sequence of events during the $S_2 \rightarrow S_3$ transition in photosystem II and implications for the water oxidation mechanism. *Proc. Natl. Acad. Sci. U.S.A.* **117**, 12624–12635 (2020).
- 3 J. Kern et al., Structures of the intermediates of Kok's photosynthetic water oxidation clock. *Nature* **563**, 421–425 (2018).
- 4 M. Suga et al., Light-induced structural changes and the site of O=O bond formation in PSII caught by XFEL. *Nature* **543**, 131–135 (2017).
- 5 M. Suga et al., An oxyl/oxo mechanism for oxygen-oxygen coupling in PSII revealed by an x-ray free-electron laser. *Science* **366**, 334–338 (2019).

- 6 S. Mukhopadhyay, S. K. Mandal, S. Bhaduri, W. H. Armstrong, Manganese clusters with relevance to photosystem II. *Chem. Rev.* **104**, 3981–4026 (2004).
- 7 C. Zhang *et al.*, A synthetic Mn_4Ca -cluster mimicking the oxygen-evolving center of photosynthesis. *Science* **348**, 690–693 (2015).
- 8 D. Liebschner *et al.*, Macromolecular structure determination using X-rays, neutrons and electrons: Recent developments in Phenix. *Acta Crystallogr. D Struct. Biol.* **75**, 861–877 (2019).