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DISCUSSIONS

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Biological approaches to artificial photosynthesis, fundamental processes and theoretical approaches: general discussion

Vincent Artero, Leif Hammarström, Fengtao Fan, Dong Ryeol Whang, Jose Martinez, Anthony Harriman, Takumi Noguchi, Joshua Karlsson, Peter Summers, Shigeru Itoh, Richard Cogdell, Alexander Kibler, Johannes Ehrmaier, Hitoshi Tamiaki, Etsuko Fujita, Seigo Shima, Shunya Yoshino, Haruo Inoue, Michael Wasielewski, Thomas Corry, Devens Gust, Flavia Cassiola, Hitoshi Ishida, Katsuhiko Takagi, Sang Ook Kang, Can Li, Licheng Sun, Hyunwoong Park, Hideki Hashimoto, Yutaka Amao, Eun Jin Son, Nobuo Kamiya, Jian-Ren Shen and Kizashi Yamaguchi

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Haruo Inoue opened the discussion of the paper by Seigo Shima: Thank you for your nice talk. The conversion process of CO_2 into methane takes 8 steps and the electrons of hydrogen are once converted in the form of organic hydride. It is a bit difficult for a chemist to imagine the molecular mechanism where and how the hydride interacts with CO_2 and how CO_2 is captured prior to their interaction. Could you explain about those points?

Seigo Shima answered: In the hydrogenotrophic methanogenic pathway, CO_2 is reduced with two electrons from ferredoxin. This reaction is catalyzed by formylmethanofuran dehydrogenase (Fwd). CO_2 reduction in this enzyme system is catalyzed by the subunits that are homologous to Mo- or W-containing formate dehydrogenase (Fdh). According to the proposed catalytic mechanisms of Fdhs, In Fwd, a hydride, or two electrons, appears to transfer from the tungsten complex to the activated form of CO_2, which was captured at the metal site or at the side chain of arginine located near the metal site.

Haruo Inoue asked: Again on the first step of the molecular mechanism of capturing CO_2, what is the role of tungsten in the enzyme?

Seigo Shima responded: Based on the proposed catalytic mechanisms of metal-dependent formate dehydrogenase, there are two possible roles of tungsten in formylmethanofuran dehydrogenase, which catalyzes the reduction of CO_2 in

the methanogenic pathway. (1) Binding of CO_2 for activation and electron transfer. (2) Supplying two electrons to reduce CO2.

Leif Hammarström asked: Have you been able to characterize how large is the fraction of enzyme that is reconstituted (functional)? Does your overall activity of 1% compared to wild-type reflect that only 1% was reconstituted, or is the intrinsic activity of the semi-artificial enzyme lower?

Seigo Shima answered: The infrared spectrum of the CO ligands on the Fe site of the reconstituted enzyme was analyzed. The data indicated that most of the reconstituted enzyme contains very similar infrared spectroscopic properties to the native enzyme. But the infrared spectrum of the reconstituted enzyme was distinct from that of the free mimic compound. This suggested that the specific activity of the reconstituted enzyme is 1% of the native enzyme.

Can Li then asked: If you do a comparison between the bio and chemical catalysts, you see that the active sites are very different between the two systems. Is there any correlation between the two catalysts in the mitigation reaction to methane. Could you show the reaction steps? Is it at least 8 steps? Most of the CH_2 and CH_3 species are very similar in the two systems; what can we learn from each other?

Seigo Shima replied: As I have shown in Fig. 2 in our paper, the biological reaction systems were evolved (designed) to conserve energy and give rise to a relatively strong reducing power using electrons from molecular hydrogen. The key enzymes of the hydrogenotrophic methanogenesis might be (1) the CO_2-reducing/fixing enzyme (Fwd), (2) energy-conserving sodium-ion-pumping methyltransferase (Mtr), (3) methane-forming enzyme (Mcr) and (4) electron-bifurcating enzyme to reduce ferredoxin (Hdr). Fwd has a tungsten-containing active site, where CO_2 is reduced to HCOOH and then, at the second Zn catalytic site of the same enzyme, HCOOH is fixed as a formyl group at the amino group of a C1 carrier. The formyl group is transferred to the second C1 carrier and there converted by two successive two-electron reductions to a methyl group. The methyl group is transferred to the third C1 carrier by the reaction catalyzed by Mtr; this exergonic reaction is coupled with sodium-ion translocation for conservation of energy to produce ATP. The methyl transfer reaction is catalyzed by the cobalt active site. Then, the methyl group is reduced to produce methane and a disulfide compound by the Mcr reaction. The active site of Mcr is nickel. Hdr catalyzes using the disulfide ($E^{\circ\prime} = -140$ mV) as an oxidant to reduce ferredoxin ($E' = \sim -500$ mV) with molecular hydrogen ($E^{\circ\prime} = \sim -414$ mV) by an electron bifurcation mechanism. Please refer to Fig. 1 in the manuscript for the other reactions. Chemical reactions, which aim purely for production of methane from CO_2, do not need to contain such reactions as those observed in the biological system. Therefore, the catalytic mechanisms are largely different to each other. However, even though the reactions and the catalytic mechanisms are different, we can learn the different ways of CO_2 reduction to methane from nature and how to develop new methane-forming chemical reactions.

Can Li also asked: Where was the temperature measurement taken?

Seigo Shima replied: The assay temperatures of the methanogenic enzymes tested in my lab are between 37 °C and 80 °C.

Etsuko Fujita asked: Can your Fe-guanylpyridinol model compound catalyze CO_2 hydrogenation to formic acid?

Seigo Shima responded: We have no experimental data of reactivity of the Fe-guanylylpyridinol model compounds to CO_2.

Richard Cogdell asked: Do you know how the reactions you measure depend on the concentration of carbon dioxide? Also, do they work at atmospheric concentrations of carbon dioxide?

Seigo Shima responded: The curves relating growth rate to the CO_2 concentration were hyperbolic. From reciprocal plots, the apparent K_s value of CO_2 is 11%,[1] which indicates the growth rate at atmospheric concentrations of carbon dioxide is very low.

1 P. Schönheit *et al.*, *Arch. Microbiol.*, 1980, **127**, 59–65.

Richard Cogdell asked: What is the concentration of bicarbonate in your growth media?

Seigo Shima replied: The culture medium for *Methanothermobacter marburgensis* contains 24 mM Na_2CO_3; the gas phase is 80%H_2/20%CO_2 and the growth temperature is 65 °C.

Richard Cogdell commented: Chemists have been making analogues of the metal co-factor sites seen in crystal structures of proteins for a long time. Uniformly these don't work as stand alone catalysts. In biology in enzymes the protein actively participates in the reaction. Look at how this is true for hydrogenase from the work of Wolfgang Lubitz. Chemists need to start trying to put these metal centres into a 'smart matrix' that begins to mimic the role of the protein.

Hitoshi Tamiaki asked: How efficiently does your reported system convert CO_2 to CH_4 based on consumed H_2? Please tell me the conversion yield.

Seigo Shima answered: Calculation of the conversion yield is not easy. Considering the free energy change and biosynthesis of the cells, the conversion yield could be approximately 70%. However, for the calculation of the real conversion yield, we need to consider leakage of gas from the cultivation system and the energy required for fermentation.

Haruo Inoue opened the discussion of the paper by Hideki Hashimoto: As regards the efficient energy transfer from the ICT state of Fucoxanthin to BCh, is it understandable for Fucoxanthin to borrow a larger transition moment of ICT than the smaller transition moment of the S_1 state?

Hideki Hashimoto responded: Yes, I think so, although it is still speculative at this moment. Please refer to our following work in the literature:

1 D. Kosumi, M. Kita, R. Fujii, M. Sugisaki, N. Oka, Y. Takaesu, T. Taira, M. Iha and H. Hashimoto, Excitation Energy-Transfer Dynamics of Brown Algal Photosynthetic Antennas, *J. Phys. Chem. Lett.*, 2012, **3**, 2659–2664, DOI: 10.1021/Jz300612c.

Haruo Inoue then asked: Is there any state-mixing among the strongly allowed S_2 state and the ICT state?

Hideki Hashimoto answered: Theoretically the state mixing between S_2 and ICT might be possible. However, we need to prove it.

Leif Hammarström asked: In Fig. 6 of your paper, the traces before and after incorporation look very similar. What are the relative amplitudes in the biexponential fit? How large a part of the carotenoid is incorporated?

Hideki Hashimoto replied: Fig. 7(b) of our paper shows the species associated difference spectra (SADS) of the fast and slow components of fucoxanthin that is reconstituted into the LH1 complex. The amplitude of the SADS shows the concentration of each component. Therefore, the ratio of the fast and slow components are estimated to be 1 : 2.

Devens Gust said: Do you see any evidence for energy transfer directly from the carotenoid S_2 state without relaxation to one of the other states?

Hideki Hashimoto responded: In the case of the natural FCP complex, yes, we already have observed it. The EET efficiency from the S_2 state of fucoxanthin to chlorophyll *a* is as low as 26%. However, the experiment is still ongoing for the reconstituted LH1 complex with fucoxanthin.

Hitoshi Tamiaki remarked: Excited energy transfer from carbonylated carotenoid to chlorophyll occurs in FCP, while energy transfer from non-carbonylated carotenoid to bacteriochlorophyll occurs in LH2. Please explain why the different caronotenoids are utilized as the energy donor.

Hideki Hashimoto replied: This is due to the biosynthetic pathways of carotenoids. The open end-ring type carotenoids are bound to the light-harvesting complexes in purple photosynthetic bacteria. When these bacteria are grown under anaerobic conditions, carbonyl containing carotenoids are not produced. However, when they are grown under semi-aerobic conditions, carbonyl-containing carotenoids are produced. In the case of cyanobacteria and higher plants that perform oxygenic photosynthesis closed end-ring type carotenoids are produced. These carotenoids usually do not contain carbonyl groups. On the other hand, diatoms and marine algae produce various species of carbonyl containing carotenoids.

Richard Cogdell commented: If you try to reconstitute carotenoids into antenna complexes *in vitro* it can be difficult. However, *in vivo* it can sometimes be

easier when all the natural assembly factors are present. In the LH2 complexes from purple bacteria we can exchange the B800 Bchl molecules to even incorporate Chl. This is not true for the B850 Bchls. However, in this case maybe again an *in vivo* approach may work?

Hideki Hashimoto answered: For example, please refer to the following works by Prof. C. Neil Hunter at University of Sheffield, UK. Molecular biology is indeed very useful.

1 P. L. Dilbeck, Q. Tang, D. J. Mothersole, E. C. Martin, C. N. Hunter, D. F. Bocian, D. Holten and D. M. Niedzwiedzki, Quenching Capabilities of Long-Chain Carotenoids in Light-Harvesting-2 Complexes from *Rhodobacter sphaeroides* with an Engineered Carotenoid Synthesis Pathway, *J. Phys. Chem. B*, 2016, **120**, 5429–5443, DOI: 10.1021/acs.jpcb.6b03305.
2 D. M. Niedzwiedzki, P. L. Dilbeck, Q. Tang, D. J. Mothersole, E. C. Martin, D. F. Bocian, D. Holten and C. N. Hunter, Functional characteristics of spirilloxanthin and keto-bearing Analogues in light-harvesting LH2 complexes from *Rhodobacter sphaeroides* with a genetically modified carotenoid synthesis pathway, *Biochim. Biophys. Acta, Bioenerg.*, 2015, **1847**, 640–655, DOI: 10.1016/j.bbabio.2015.04.001.
3 S. C. Chi, D. J. Mothersole, P. Dilbeck, D. M. Niedzwiedzki, H. Zhang, P. Qian, C. Vasilev, K. J. Grayson, P. J. Jackson, E. C. Martin, Y. Li, D. Holten and C. Neil Hunter, Assembly of functional photosystem complexes in *Rhodobacter sphaeroides* incorporating carotenoids from the spirilloxanthin pathway, *Biochim. Biophys. Acta, Bioenerg.*, 2015, **1847**, 189–201, DOI: 10.1016/j.bbabio.2014.10.004.
4 D. P. Canniffe and C. N. Hunter, Engineered biosynthesis of bacteriochlorophyll *b* in *Rhodobacter sphaeroides*, *Biochim. Biophys. Acta, Bioenerg.*, 2014, **1837**, 1611–1616, DOI: 10.1016/j.bbabio.2014.07.011.

Can Li asked: What is the difference between the charge transfer efficiency and energy transfer efficiency? Why it is different generally?

Hideki Hashimoto answered: The charge transfer efficiency is governed by Marcus theory, while the energy transfer efficiency is governed by Förster, Dexter, or Redfield theory. They are very different processes, although the mathematical expressions are somewhat similar.

Can Li then asked: Why does the longer chain make a difference in the efficiency?

Hideki Hashimoto replied: That is due to the difference in the energy of the singlet excited states (S_2, S_X, and S_1) of carotenoids. Any carotenoids whose number of conjugated C=C bonds is between 9 and 13 show a similar EET efficiency from the S_2 state to the Q_x level of bacteriochlorophyll (Bchl) *a*, but the EET efficiency from the S_1 state of the carotenoids to the Q_y level of Bchl shows a sudden drop off when *n* exceeds 11. This is regarded as the S_1 to Q_y EET process being closed, although the energy level of the S_1 state is slightly higher or almost equivalent to the Q_y state of Bchl. It is a great mystery how the EET efficiency of carotenoid to Bchl is so variable.

Haruo Inoue opened the discussion of the paper by Yutaka Amao: It is interesting to induce C–C bond formation by utilizing the two-electron reduced form of diphenyl viologen to be incorporated into the enzyme. In the wild species, they utilize organic hydride, NADPH as the two-electron-reduced mediator. How can we imagine the difference? What is the difference between the stepwise one

electron after another and simultaneous two-electron transfer? You mentioned that the one-electron-reduced methyl viologen cannot induce the reaction. Is it not incorporated into enzyme?

Yutaka Amao responded: The one-electron reduced form of methyl viologen acts as a co-enzyme for malate dehydrogenase (MDH) in the reaction of oxaloacetic acid to malic acid conversion. However, the one-electron reduced form of methyl viologen does not act as a co-enzyme for malic enzyme (ME) in the reaction of pyruvic acid and CO_2 to oxaloacetic acid or malic acid conversion. Thus, the one-electron reduced form of methyl viologen is usable for the reduction of the carbonyl group of oxaloacetic acid with MDH. By using the double electron reduced form of diphenyl viologen derivative (PV^{2+}), however, oxaloacetic acid production from pyruvic acid and CO_2 was observed. It is presumed that the oxaloacetic acid production from pyruvic acid and CO_2 was promoted by using the double electron reduced form of PV^{2+}.

Dong Ryeol Whang commented: In the electron transfer scheme in Fig. 1 of your paper, oxidative quenching from H_2TPPS to the electron shuttle was proposed. Is there any possibility that this system undergoes reductive quenching of H_2TPPS? The photoluminescence of H_2TPPS was not quenched either by MV^{2+} or PSV^{2+}.

Yutaka Amao replied: In the system consisting of triethanolamine (TEOA), H_2TPPS and bipyridinium salt, oxidative quenching from H_2TPPS to the electron shuttle was proposed. If this quenching process undergoes reductive quenching of H_2TPPS by an electron donor, the rate of reduction of the bipyridinium salt depends on the electron donor. However, the rate of reduction of the bipyridinium salt was independent of the electron donor reagents. Thus, we proposed the oxidative quenching from H_2TPPS to the electron shuttle. Moreover, the photoluminescence of H_2TPPS was quenched by MV^{2+} or PSV^{2+} due to stacking between porphyrin and bipyridium salt.

Hitoshi Ishida asked: Production of malic acid requires electrons, but formation of oxaloacetic acid does not require any electrons. How can we understand the reaction? The reaction needs the reduced species of the viologen derivative, doesn't it?

Yutaka Amao replied: In the carbon–carbon bond formation with malic enzyme, the double electron reduced form of the viologen derivative will be involved in the tautomerism of pyruvic acid for production of the enol type. The enol type of pyruvic acid is needed during formation of the carbon–carbon bond with malic enzyme in the presence of NADPH.

Hitoshi Ishida said: Does the reduced species of the viologen derivative interact with the enzyme for the reaction to occur?

Yutaka Amao responded: By using natural co-enzyme NADPH for CO_2 and pyruvic acid to malic acid conversion with malic enzyme, the role of NADPH for carbon–carbon bond formation with malic enzyme has not been elucidated. In

the carbon–carbon bond formation with malic enzyme, the double electron reduced form of the viologen derivative will be involved in the tautomerism of pyruvic acid; the enol type of pyruvic acid is needed for formation of the carbon–carbon bond.

Hitoshi Tamiaki asked: Photoinduced CO_2 fixation on pyruvic acid to oxaloacetic acid was reported in your talk. Please tell me the efficiency for the transformation including quantum yield.

Yutaka Amao answered: In the reaction system of photoinduced CO_2 fixation on pyruvic acid to oxaloacetic acid, the efficiency for the transformation of CO_2 and pyruvic acid to oxaloacetic acid was estimated to be less than 1.0%. Moreover, the efficiency for the transformation with visible-light was estimated to be less than 0.1%.

Devens Gust opened a general discussion of the papers by Seigo Shima, Hideki Hashimoto and Yutaka Amao: In an artificial photosynthetic system, why would one choose to use carotenoids as antennas? Of course, they are very useful for photoprotection from singlet oxygen damage (triplet state processes), but their photophysics is not at all well suited for singlet–singlet energy transfer. They have extremely short excited singlet state lifetimes and are virtually non-fluorescent. Why not choose another chromophore for which it is much easier to design efficient singlet–singlet energy transfer?

Hideki Hashimoto answered: Nature does not use the fluorescent chromophore as a light-harvester. One exception is phycobilins.

Richard Cogdell commented: I agree that they are not promising when you first look at them. But biology has learnt how to use them by packaging them correctly with proteins so that they are positioned to allow them to function at even 100% efficiency as light harvesters.

Michael Wasielewski remarked: Chemists frequently face the problem of whether to develop biomimetic or bio-inspired systems. An important concern with biomimetic systems is that we have not yet developed the appropriate scaffolds and secondary structures that proteins provide to control active site function and stability. Thus, the development of robust bio-inspired systems seems more promising, but both directions are important and useful to pursue.

Hideki Hashimoto replied: I completely agree with your opinion.

Seigo Shima remarked: Modification of proteins to change the catalytic properties theoretically and experimentally became easier and cheaper. There are many examples of such protein-modification experiments, for example, changing the substrate into another substrate. Improving the protein active site appears to be very promising and we should try.

Vincent Artero commented: This is related to the smart matrixes, we previously discussed, that could augment the efficiency of catalytic/photocatalytic systems.

This is a true issue for chemists to design such matrixes and we cannot just mimic natural systems in that regard because we also want to integrate our systems into technologically relevant devices. To do that, we have to cope with specifications in terms of operating conditions (pH, pressure, temperature) that are quite distinct from those experienced by enzymes or other natural systems.

Richard Cogdell remarked: The difference between biomimicry and bio-inspiration needs to be examined carefully. Biology has learnt how to make proteins very tough and robust, *e.g.* in the case of thermophilic proteins. However, this can now be extended by using modern molecular techniques to incorporate non-natural amino acids into proteins. This can produce proteins that appear to be like rocks! If you need something like this ask a friendly molecular biologist.

Devens Gust responded: Your comment that proteins can be "tough as old boots" is very apt, since old boots are indeed made of protein (although totally denatured, of course).

Flavia Cassiola addressed Hitoshi Tamiaki: Is the use of GMOs (as a whole organism or its products, such as enzymes or biomolecules) the future of artificial photosynthesis in hybrid systems? How can the scientists assist on the development of regulations that will make GMO acceptable by the consumers?

Hitoshi Tamiaki replied: A whole organism has been already used for one of the artificial photosynthetic systems.[1] Such hybrid systems including the utilization of any useful enzymes would be spread in the future and genetically mutated organisms should be used in a strictly closed space.

1 C. Liu *et al.*, *Science*, 2016, **352**, 1210.

Flavia Cassiola posed some questions for the future: Are there any other features in biology that we are not considering? For example: CO_2 capture from the atmosphere. Is there any hope that we are going to mimic nature and be able to capture CO_2 from the air, in the rates needed for the target efficiency for AP systems? Ideally, the use of an air concentrator of any sort would be avoided, reducing costs and saving energy.

How can biology help us to improve CO_2 solubility and address the mass transfer issues in the AP system? Here the target is to avoid any type of extra homogenization (stirring, agitation) of the system.

Haruo Inoue opened a general discussion of the paper by Jian-Ren Shen and Kizashi Yamaguchi: Congratulations on your findings, Professor Shen! It is really impressive to find the appearance of an O–O bond in the S_3 state of PSII. May I ask a question to Professor Kizashi Yamaguchi from the theoretical viewpoint? What is the difference between $O_{(4)}$ and $O_{(5)}$, especially on their electronic structures in the S_2, S_3 states? Both oxygen atoms are sandwiched by manganese atoms and could be activated by the stepwise oxidation of the Mn-cluster. If anything is revealed or even prospected, could you provide your opinion?

Jian-Ren Shen replied: Thank you! Regarding the difference between $O_{(4)}$ and $O_{(5)}$ from the geometric point of view, $O_{(4)}$ is ligated to two Mn ions ($Mn_{(3)}$ and $Mn_{(4)}$), whereas $O_{(5)}$ is ligated to 3 Mn ions ($Mn_{(1)}$, $Mn_{(3)}$ and $Mn_{(4)}$) and also the Ca ion. Corresponding to this, the bond distances between $O_{(4)}$ and the two Mn ions are short and fall in the typical distances found for Mn oxides, whereas the bond distances between $O_{(5)}$ and its nearby Mn ions are much longer, indicating the uniqueness of this oxygen atom relative to the other oxo-bridged oxygen atoms. This may also result in a difference in the electronic structures between these two oxygen atoms, as our previous theoretical calculations have suggested that $O_{(5)}$ may exist as a hydroxide in the dark-stable S_1 state, whereas $O_{(4)}$ should be a typical oxygen dianion.

Kizashi Yamaguchi responded: Your question is very important for elucidation of the mechanism of water oxidation in the oxygen-evolving complex (OEC) of PSII. Fig. 2 in our paper illustrates possible protonation states (O^{2-}, OH^{-}) of the $O_{(5)}$ and $O_{(4)}$ sites of the $CaMn_4O_5$ cluster in the S_0, S_1 and S_2 states by several theoretical groups involving us. Frontier MO investigation[1] based on the high-resolution XRD structure[2] revealed possibilities of the participation of the $O_{(5)}$ and $O_{(4)}$ sites for the O–O bond formation as illustrated in Scheme 1. At the moment, available S_3 structures by SFX (XFEL) are not completely converged yet (see ESI SIV of the paper), and therefore the experimental results are not conclusive to decide which theoretical S_3 structures in Fig. 3 of our paper are the most probable. Therefore we have considered possible reaction pathways under the assumption of (i) no water insertion and (ii) water insertion at the $S_2 \rightarrow S_3$ transition as illustrated in Fig. 6 and 7 of our paper. Further details of our opinions concerned with possible roles of the $O_{(5)}$ and $O_{(4)}$ sites including the internal oxo bonds, $Mn{=}O_{(5)}$ and $Mn{=}O_{(4)}$, are given in the figures of the ESI of our paper.

1 S. Yamanaka, H. Isobe, K. Kanda, T. Saito, Y. Umena, K. Kawakami, J.-R. Shen, N. Kamiya, M. Okumura, H. Nakamura and K. Yamaguchi, *Chem. Phys. Lett.*, 2011, **511**, 138.
2 Y. Umena, K. Kawakami, J.-R. Shen and N. Kamiya, *Nature*, 2011, **473**, 55.

Anthony Harriman remarked: It is not clear if the calculated structures are static or allow for dynamic exchange. I wonder if there is any information about the transient stability of the many intermediates associated with a complete cycle of catalytic activity. If an intermediate state is left in the dark, and is not oxidized to the next level, would the geometry remain unchanged or might you expect structural changes? My interest in this point relates to those occasions when the flow of oxidizing equivalents is intermittent.

Kizashi Yamaguchi answered: Our QM and QM/MM computations[1–12] have elucidated that the nature of chemical bonds in the $CaMn_4O_5$ cluster is labile, and therefore confinements of the $CaMn_4O_5$ cluster by the hydrogen bonding networks and amino acid residues of protein (see Fig. S1 in the ESI of our paper) are crucial for catalytic functions (activity) in water oxidation in OEC of PSII. Therefore our pictures of OEC at room temperature are consistent with the dynamic exchange in your terminology although stable static structures have been detected by the EPR methods at low temperatures (see Fig. 5 in our paper).

Very recently Kamiya *et al.*[13] have elucidated two different S_1 structures having different hydrogen bonding patterns for the A- and B-monomers in the dimer structure of OEC.[1,2] Their results demonstrated subtle structural controls of the $CaMn_4O_5$ cluster by protein (see Fig. S23 in the ESI of our paper) and important roles of the system structures of OEC of PSII (see Fig. S1 in the ESI of our paper).[1] Furthermore their results may indicate that possible intermediates in Fig. 2 of our paper are dynamically interconvertible, depending on environmental conditions. Further theoretical calculations using the large-scale QM/MM models[1] are crucial for obtaining reliable answers for your questions.

1 M. Shoji, H. Isobe, S. Yamanaka, Y. Umena, K. Kawakami, N. Kamiya, J.-R. Shen, T. Nakajima and K. Yamaguchi, *Adv. Quantum Chem.*, 2015, **70**, 325.
2 M. Shoji, H. Isobe, T. Nakajima and K. Yamaguchi, *Chem. Phys. Lett.*, 2016, **658**, 354.
3 M. Shoji, H. Isobe, S. Yamanaka, M. Suga, F. Akita, J.-R. Shen and K. Yamaguchi, *Chem. Phys. Lett.*, 2015, **623**, 1.
4 M. Shoji, H. Isobe, S. Yamanaka, M. Suga, F. Akita, J.-R. Shen and K. Yamaguchi, *Chem. Phys. Lett.*, 2015, **627**, 44.
5 H. Isobe, M. Shoji, J.-R. Shen and K. Yamaguchi, *J. Phys. Chem.*, 2015, **B119**, 13922.
6 H. Isobe, M. Shoji, J.-R. Shen and K. Yamaguchi, *Inorg. Chem.*, 2016, **55**, 371.
7 M. Shoji, H. Isobe and K. Yamaguchi, *Chem. Phys. Lett.*, 2015, **636**, 172.
8 S. Yamanaka, H. Isobe, K. Kanda, T. Saito, Y. Umena, K. Kawakami, J.-R. Shen, N. Kamiya, M. Okumura, H. Nakamura and K. Yamaguchi, *Chem. Phys. Lett.*, 2011, **511**, 138.
9 H. Isobe, M. Shoji, S. Yamanaka, Y. Umena, K. Kawakami, N. Kamiya, J.-R. Shen and K. Yamaguchi, *Dalton Trans.*, 2012, **41**, 13727.
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11 K. Yamaguchi, S. Yamanaka, M. Shoji, H. Isobe, Y. Kitagawa, T. Kawakami, S. Yamada and M. Okumura, *Mol. Phys.*, 2014, **112**, 485.
12 S. Yamanaka, K. Kanda, T. Saito, Y. Umena, K. Kawakami, J.-R. Shen, N. Kamiya, M. Okumura, H. Nakamura and K. Yamaguchi, *Adv. Quantum Chem.*, 2012, **64**, 121.
13 A. Tanaka, Y. Fukushima and N. Kamiya, *J. Am. Chem. Soc.*, 2017, **139**, 1718.

Anthony Harriman commented: My earlier question arises because it is clear that biology, and most certainly in the case of photosynthesis, employs an elaborate light-harvesting machine to collect and focus photons. A primary purpose of this machinery is to ensure that photons (excitons if you prefer) arrive at the catalytic sites at frequent time intervals. A crude calculation suggests that photons reach the reaction centre, under average light exposure, with a frequency of about 1 ms. This situation will be very challenging to achieve with an artificial device. It could be argued that the natural systems are trying to avoid having highly energetic intermediate states hanging around for too long. Is there any way, from your calculations, that you can explore what might happen to these intermediate species if left alone for any significant time? I think this is an important point to clarify.

Kizashi Yamaguchi responded: Our static theoretical calculations at the QM and QM/MM models[1–12] (see Fig. S23 in the ESI of our paper) cannot provide reliable answers for your questions relating to time-dependent processes in water oxidation in OEC of PSII. We have not examined larger QM models involving the P680 site other than QM model V (380 atoms) in Fig. 4 of our paper: P680-Tyr-161-$CaMn_4O_5$ cluster is desirable for elucidation of the photo-induced electron transfer process. Moreover the QM/MM/MD simulations are crucial for elucidation of the dynamical mechanisms of water oxidation as illustrated in Fig. S23 in the ESI of our paper. Unfortunately the MD simulations of OEC of PSII at a msec

time scale are hardly possible even in our KEI computer system. After the construction of a post KEI computer at Kobe Japan, long-time simulations of complex systems such as OEC of PSII will become feasible for elucidation of dynamical mechanism of the O–O bond formation in water oxidation in OEC of PSII in combination with experimental big data by SFX (XFEL) at SACLA.

1 M. Shoji, H. Isobe, S. Yamanaka, Y. Umena, K. Kawakami, N. Kamiya, J.-R. Shen, T. Nakajima and K. Yamaguchi, *Adv. Quantum Chem.*, 2015, **70**, 325.
2 M. Shoji, H. Isobe, T. Nakajima and K. Yamaguchi, *Chem. Phys. Lett.*, 2016, **658**, 354.
3 M. Shoji, H. Isobe, S. Yamanaka, M. Suga, F. Akita, J.-R. Shen and K. Yamaguchi, *Chem. Phys. Lett.*, 2015, **623**, 1.
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7 M. Shoji, H. Isobe and K. Yamaguchi, *Chem. Phys. Lett.*, 2015, **636**, 172.
8 S. Yamanaka, H. Isobe, K. Kanda, T. Saito, Y. Umena, K. Kawakami, J.-R. Shen, N. Kamiya, M. Okumura, H. Nakamura and K. Yamaguchi, *Chem. Phys. Lett.*, 2011, **511**, 138.
9 H. Isobe, M. Shoji, S. Yamanaka, Y. Umena, K. Kawakami, N. Kamiya, J.-R. Shen and K. Yamaguchi, *Dalton Trans.*, 2012, **41**, 13727.
10 K. Kanda, S. Yamanaka, T. Saito, Y. Umena, K. Kawakami, J.-R. Shen, N. Kamiya, M. Okumura, H. Nakamura and K. Yamaguchi, Chem. Phys. Lett., 2011, **506**, 98.
11 K. Yamaguchi, S. Yamanaka, M. Shoji, H. Isobe, Y. Kitagawa, T. Kawakami, S. Yamada and M. Okumura, *Mol. Phys.*, 2014, **112**, 485.
12 S. Yamanaka, K. Kanda, T. Saito, Y. Umena, K. Kawakami, J.-R. Shen, N. Kamiya, M. Okumura, H. Nakamura and K. Yamaguchi, *Adv. Quantum Chem.*, 2012, **64**, 121.

Licheng Sun asked: In general, there are 2 pathways for O–O bond formation – nucleophilic attack, and radical coupling. 1st question: in your work, which reaction mechanism do you think is taking place?

Jian-Ren Shen responded: Our results indicate a mechanism more similar to a radical coupling one.

Licheng Sun also asked: Do you know from which side the proton leaves, and which in which step does water come in?

Jian-Ren Shen responded: In total 4 protons must leave during the whole reaction cycle, and our results suggested that at least one proton leaves *via* the site of $O_{(4)}$. There must be additional sites/pathways for the proton release, which we do not know at present.

Takumi Noguchi opened a general discussion of the paper by Nobuo Kamiya: Does the presence of monodentate bicarbonate indicate that the non-heme iron was somehow oxidized in the crystal?

Nobuo Kamiya replied: Our description in the manuscript is only one possibility based on the other reports. We have at present no evidence for the oxidation of the non-heme iron.

Takumi Noguchi remarked: You found monodentate ligation of the bicarbonate in the crystal structure. Please tell me the individual distances between Fe and bicarbonate oxygens.

Nobuo Kamiya responded: In the A-monomer, two bicarbonate ions are assigned as disordered. Please note that the distances in the disordered structure are not so reliable. The two distances of the bicarbonate ion ligated in the monodentate mode are 2.46 and 2.29 Å. In the B-monomer, the bicarbonate is assigned as a unique ion but having a larger temperature factor. The two distances of the bicarbonate ion ligated in the monodentate mode are 2.35 and 2.59 Å.

Takumi Noguchi asked: Was the tyrosine removed in the case of monodentate bicarbonate? What is actually happening to the tyrosine in this situation?

Nobuo Kamiya answered: In the A-monomer, Y244/D was assigned with temperature factors around 30 $Å^2$, and Y246A with those around 80 $Å^2$. In the B-monomer, Y244/D was assigned with slightly larger temperature factors around 40 $Å^2$, and Y246A could not be assigned because of the absence of the electron density distribution corresponding to the tyrosine side chain. Especially in the B-monomer, Y246A may have a highly flexible conformation.

Shigeru Itoh asked: What kind of mechanism do you expect to change the electron transfer rate from Q_A to Q_B? Although your structure does not show the large geometry changes around Q_A and Q_B, you detected that the rate decreased to 1/2. However, with the fixed geometry and distance of Q_A and Q_B, Marcus theory predicts no big change of the rate in general. So do you mean the small structural changes around the His–Fe–His portion that connects Q_A and Q_B affected the super-exchange type electronic coupling between Q_A and Q_B to affect the rate, even with almost unaltered local geometry of Q_A and Q_B?

Nobuo Kamiya responded: We do not know about "the super-exchange type electronic coupling", but we expect that the changes of the bicarbonate protonation states or of the non-heme iron balance numbers will affect the electron transfer rate from Q_A to Q_B.

Alexander Kibler asked: At what pH did you recrystallise your protein? Did you try varying the pH to obtain crystal structures that reflect the change in proton concentration in the lumen and stroma occuring during photosynthesis? If the crystal structure obtained has the same pH environment at both the lumenal and stromal side of the transmembrane protein, does this truly reflect the structure of the protein during photosynthesis?

Nobuo Kamiya replied: Our condition for PSII crystallization was around pH 6.1. Yes, the structure determinations of PSII crystals at different pH conditions are the next research target. No, at present, we have not detected clearly the pH-dependent structure changes yet.

Katsuhiko Takagi remarked: By removing the subunit, PsbM, from the wild PSII dimer, the crystal structure should be destabilized, I think. But, the resolution of the PsbM deleted crystal (ΔPsbM-PSII) was shown to improve from 4.2 Å to 2.2 Å. In spite of destabilization of the structure, why does the resolution of the

PsbM-deleted crystal improve considerably? Please explain the reason why the structure resolution was improved.

Nobuo Kamiya responded: The destabilization of the PSII dimer was really induced by the deletion of the PsbM subunit. But the destabilization was not so large as to damage the PSII dimer structure completely, as indicated by the fact that the PsbM-deleted PSII structure was very similar to the wild-type one. The same crystal packing was retained and the same space group, $P2_12_12_1$, was maintained. In these conditions, the crystal quality and resolution was determined by the purity of the PSII sample, crystallization protocol, post-crystallization treatment, *etc.* These points were very much improved from the previous 4.2 Å resolution case. We consider that this is the reason for the resolution improvement.

Richard Cogdell asked: Do you know the redox state of the quinones in your crystal structures? Maybe you would see changes in the structures with the quinones in different redox states. This could be tested by soaking your crystals in reductants.

Nobuo Kamiya replied: No. The candidates of the species are quinone, semiquinone, and quinole. Because our structure has no information about the protons, we cannot say the real redox state of quinone. Thank you very much for your comment, and we would like to test the reductant soaking as the next research target.

Joshua Karlsson opened a general discussion of the papers by Takumi Noguchi: Your gold nanoparticles (GNP) are 20 nm in diameter. Is there a particular reason why you chose this nanoparticle size? Can you comment on what change you would expect to see by altering the nanoparticle size (larger or smaller) and number of Photosystem II units attached to it?

Takumi Noguchi answered: We selected 20 nm GNP, because it is a similar size to a PSII dimer and handling is relatively easy (*e.g.*, for separation of PSII–GNP from free PSII by centrifugation). The change in the overlap of the PSII absorption with the plasmonic absorption of GNP by changing the GNP size could affect the rate of fluorescence quenching.

Shigeru Itoh asked: You have shown very fast decay kinetics of fluorescence for chlorophyll of PSII attached to the surface of gold nanoparticles. It means that the excitation energy on PSII chlorophylls is quenched very rapidly. The fast quenching process should have also decreased the forward electron transfer rate competitively, and decreased the charge separation efficiency. Did you detect it? Did quantum efficiency decrease? Did you measure the reaction rate at room temperature?

Takumi Noguchi responded: Charge separation takes place at an early picosecond region, whereas the decay rates of the main fluorescence components are tens or hundreds of picoseconds, even in PSII–GNP. Thus, as long as the excitation light is saturated, the effect of faster quenching may be limited. Although we

did not detect charge separation, we already showed that O_2 evolution activity of PSII–GNP measured at 25 °C was similar to that of free PSII under the same salt condition.[1]

1 T. Noji, H. Suzuki, T. Gotoh, M. Iwai, M. Ikeuchi, T. Tomo and T. Noguchi, *J. Phys. Chem. Lett.*, 2011, **2**, 2448–2452.

Shigeru Itoh asked: Did the rate vary depending on the size of gold nanoparticle?

Takumi Noguchi responded: We did not try different sizes of GNP. However, the change in the size of GNP alters the wavelength of the plasmon absorption, and hence the quenching rate should also be changed.

Haruo Inoue said: You have tried to collect electrons from PSII by gold nanoparticles, but actually the emission of PSII was quenched by energy transfer. To avoid the energy transfer and enhance the electron transfer, is it possible in future to shift the plasmonic absorption of the gold nanoparticle to the blue by downsizing the diameter of the gold nanoparticle?

Takumi Noguchi replied: Indeed, changing the size of GNP to eliminate the overlap of the plasmon absorption with Chl fluorescence may be effective to avoid quenching of the excited states of Chls. This is a good strategy for the future study of the development of a functional PSII–GNP nano-device.

Haruo Inoue enquired: May I ask about the adsorption of PSII on the gold nanoparticle? What is the anchoring site of PSII? Is it the thiol group?

Takumi Noguchi responded: PSII is attached to GNP through a His-tag (on the C-terminus of the CP47 subunit) bound to a Ni–NTA, which is self-assembled on the GNP surface by a thiol group.

Fengtao Fan asked: I am curious about the correlation between the plasmonic effect and the charge separation in PSII. What happens if you shift the excitation light out of the plasmonic absorption region and further into the UV region, *e.g.* 400 nm? Will this excitation also affect the charge separation process in PSII?

Takumi Noguchi replied: We did not observe the effects of binding to GNP on charge separation in PSII; we studied the effects on excitation energy transfer. Because the energy transfer and quenching by GNP take place in the Q_y transitions of Chls at 670–680 nm, the excitation wavelength may not affect the observations as long as it is shorter than the Q_y region.

Fengtao Fan remarked: It might be possible that the driving force between PSII and Au NP determines the charge transfer directions rather than plasmonic hot carriers. So, the impact of the plasmonic effect should be carefully studied through well designed experiments, such as changing the excitation wavelength to a shorter wavelength.

Takumi Noguchi answered: We did not detect charge transfer between PSII and GNP. The plasmonic effect was rather small in our system judging from the relatively small change in the Q_y absorption band upon GNP binding (Fig. 1B of the paper, inset).

Seigo Shima commented: Lipids (amounts and structure) binding to PSII differ between the enzyme in the cells and purified enzyme as well as that bound to nano-particle. Do you have any information about the potential effects of lipids bound to PSII on the spectroscopic data?

Takumi Noguchi replied: Lipids strongly bound to the PSII proteins may have some specific roles in the reaction, but this is not well understood yet. We currently have spectroscopic data that replacement of sulfoquinovosyl diacylglycerol (SQDG) with phosphatidyl glycerol (PG) near Q_B retards its reaction.[1]

1 Y. Nakajima *et al.*, abstract of the 58th annual meeting of The Japanese Society of Plant Physiologists, PF-115, 2017.

Shigeru Itoh asked: Are particles strong under some specific condition?

Takumi Noguchi answered: Although free GNPs readily aggregate in a salt solution, PSII–GNPs are relatively stable, probably because of the protective effect of PSII proteins assembled on a GNP.

Johannes Ehrmaier opened a general discussion of the paper by Eun Jin Son: The question concerns Fig. 7A of the paper. For the different experiments, the same amount of a sacrificial electron acceptor is used, but different electron mediators. Why is the final amount of evolved oxygen so different for the different mediators? I would have expected them to be similar, as the final amount of evolved oxygen should only depend on the amount of the sacrificial electron acceptor.

Eun Jin Son answered: The difference in charge separation of $[Ru(bpy)_3]^{2+}$ by each mediator may change the amount of sacrificial electron acceptor consumed, which can affect the efficiency of photocatalytic water oxidation. And the difference in ability of extracting photoexcited electrons from $[Ru(bpy)_3]^{2+}$ by each mediator may determine the final amount of evolved oxygen. Even though RGO showed much higher conductivity than PNE/RGO through cyclic voltammetry, the oxygen yield with RGO-only was lower than that with PNE/RGO. And we consider it is because RGO may also be conducting in the back electron transfer direction. Also, we attribute the enhanced oxygen evolution with PNE/RGO over that of PNE to the formation of a multistep electron transfer pathway by RGO and a greater amount of redox-active quinone groups on the RGO scaffold than free PNE, which increases forward charge transfer while suppressing charge recombination.

Can Li asked: What happens if the RGO is reduced and what is the role of RGO?

Eun Jin Son answered: The reduction of GO to RGO occurred simultaneously during the functionalization with PNE, by accepting electrons released from the

process of polymerization of NE to PNE. And we considered the role of RGO from two perspectives. First, the formation of a multistep electron transfer pathway made by conductive RGO can increase forward charge transfer while suppressing charge recombination. Second, when we calculated the ratio of electroactive quinone groups that can act as electron acceptors, the amount was greater in PNE on the RGO scaffold (*i.e.*, PNE/RGO) than that in free PNE. This can possibly be another factor that leads to the enhanced oxygen evolution efficiency compared with free PNE.

Peter Summers remarked: In Fig. 7(c) of your paper, looking at the decay profiles from the Ru photosensitiser excited state. What do the decay profiles for other control experiments look like? For example, in the presence of just PNE or RGO, are they both mono-exponential?

Eun Jin Son responded: We haven't measured the decay profiles for other electron mediators, but I guess they may be both mono-exponential because in the previous work using polydopamine as an electron mediator, which is very similar to PNE, the decay profile is mono-exponential.[1] In the case of RGO, it is because I would not expect that $[Ru(bpy)_3]^{2+}$ could bind to the RGO due to its 3-D molecular structure, which can cause fitting with double exponential decay.[2]

1 J. H. Kim, M. Lee and C. B. Park, *Angew. Chem.*, 2014, **126**, 6482.
2 A. Wojcik and P. V. Kamat, *ACS Nano*, 2010, **4**, 6697.

Peter Summers asked: In Fig. 7(c) of your paper, looking at the decay profiles from the Ru photosensitiser excited state, do you know what is responsible for the fast component of the decay profile in the presence of PNE/RGO (red trace)? Has this fast component been included in the fit for the calculation of the 368 ns lifetime, when compared to 374 ns for just the photosensitiser?

Eun Jin Son responded: Yes, the fast component was included when calculating with mono-exponential fitting. When we recalculated the lifetime excluding the initial fast component, the tendency of decreased lifetime of the photosensitizer with PNE/RGO was still maintained, which indicates the fast charge separation of $[Ru(bpy)_3]^{2+}$ in the presence of the electron mediator. The interaction between graphene part of the mediator and the photosensitizer may be one of the possible reasons for the phenomenon to occur.

Alexander Kibler commented: During your oxygen evolution measurements you analysed the oxygen evolved over 1400 s, during which the last 200 s saw no further oxygen evolution for each species. Is this due to catalyst deactivation, sacrificial electron acceptor consumption or water consumption? As water is oxidised, the pH of your system will decrease, which may cause removal of the PNE from the surface.[1] Have you performed any stability measurements over longer time frames to investigate this?

1 S. M. Kang , J. Rho , I. S. Choi , P. B. Messersmith and H. Lee, *J. Am. Chem. Soc.*, 2009, **131**, 13224–13225.

Eun Jin Son responded: For your first question, it is a well-known fact that $[Ru(bpy)_3]^{2+}$ photosensitizer is not photostable in highly concentrated phosphate

buffer containing $Na_2S_2O_8$.[1,2] The rapid decomposition of $[Ru(bpy)_3]^{2+}$ during the test is caused by nucleophilic attack of water on the bpy ring of the oxidized photosensitizer (*i.e.*, $[Ru(bpy)_3]^{3+}$), and its further oxidation by a sulfate radical. In addition, it has been reported that the rate-limiting step is the production of oxygen at the catalyst in the model system we employed (*i.e.*, Co–Pi as a water oxidation cocatalyst, $[Ru(bpy)_3]^{2+}$ as a photosensitizer, and $Na_2S_2O_8$ as a sacrificial electron acceptor).[2] Thus, the accumulation of oxidized photosensitizer (*i.e.*, $[Ru(bpy)_3]^{3+}$) can occur due to the mismatch of simultaneous reduction of $[Ru(bpy)_3]^{3+}$) by the cocatalyst, which may lead to fast decomposition of the photosensitizer, and thus limit the oxygen evolution yield. And, although we haven't performed stability tests over longer times, we consider that the pH change may be able to affect the activity of PNE as an electron mediator, because the driving force of reduction of quinone moieties to catechol is decreased as the pH value is decreased.[3]

1 M. Hara, C. C. Waraksa, J. T. Lean, B. A. Lewis and T. E. Mallouk, *J. Phys. Chem. A*, 2000, **104**, 5275.
2 B. Limburg, E. Bouwman and S. Bonnet, *ACS Catal.*, 2016, **6**, 5273.
3 I. L. Medintz, M. H. Stewart, S. A. Trammell, K. Susumu, J. B. Delehanty, B. C. Mei, J. S. Melinger, J. B. Blanco-Canosa, P. E. Dawson and H. Mattoussi, *Nat. Mater.*, 2010, **9**, 676.

Dong Ryeol Whang asked: Can you compare the turnover frequencies (TOFs) of your systems (ie, PNE/RGO, RGO reduced by hydrazine, PNE, and without any mediator)? And can you correlate them to the charge transfer rate of the systems? From Fig. 7a of your paper, the TOFs of PNE/RGO and RGO seem more or less the same.

Eun Jin Son responded: The calculated TOFs for PNE/RGO, RGO reduced by hydrazine, PNE, and without any mediator during the first 50 s are 0.349, 0.234, 0.168, and 0.141 s^{-1}, respectively. Although we did not calculate the exact electron transfer rate, we attribute the higher TOFs with each mediator than without any mediator to the ability of each mediator to take electrons from the $[Ru(bpy)_3]^{2+}$ photosensitizer. On the other hand, the final oxygen yield with PNE was higher than that with RGO, while the TOF value with RGO was higher than PNE. It may be because RGO is more conducting in the back electron transfer direction than PNE.

Leif Hammarström remarked: Your reported excited state lifetimes of 374 and 368 ns are not significantly different. Instead, there appears to be a rapid (*ca*. 10 ns) part representing 10–20% of the trace that might represent a fraction of excited states undergoing ET with graphene oxide much more rapidly than you think, while the main part does not react at all. Do you agree?

Eun Jin Son replied: As you pointed out, the initial rapid part may reflect the rapid ET with graphene. In my opinion, if the fast electron transfer can occur between the photosensitizer and graphene, the $[Ru(bpy)_3]^{2+}$ may be in a bound state to the graphene. But considering the 3-D molecular structure of $[Ru(bpy)_3]^{2+}$, I guess it may be hard to have happened and we need to check. On the other hand, when we recalculated the lifetimes excluding the initial rapid part, the difference of the photosensitizer's lifetime without and with PNE/RGO was 18 ns. The

tendency was not changed and it indicates that fast electron transfer from $[Ru(bpy)_3]^{2+}$ can occur in the presence of the PNE/RGO electron mediator.

Hyunwoong Park asked: In slide 10, the system is only three minutes, the reduced species will take the hole. That's why it does not work anymore. Electron shuttling. Is it only 3 min for that system? Is there an interaction with phosphate?

Eun Jin Son answered: The oxygen evolution reaction was continued for ~3 min with or without electron mediators, where we consider the use of $[Ru(bpy)_3]^{2+}$ significantly affected the stability of the system. Thus, we will need to employ another system using a different photosensitizer (*e.g.*, metalloporphyrin) to evaluate the exact effect of the quinone-based electron mediator on the stability of the system. And, to find out about the interaction between PNE/RGO with phosphate, further control experiments using buffer with different sources (*e.g.*, borate buffer) should be conducted.

Shunya Yoshino asked: Why was the C=O peak not observed in the XPS spectrum of graphene oxide (GO) before the surface treatment of GO with PNE?

Eun Jin Son responded: When we fitted the C=O peak of GO and PNE/RGO, the peak existed but was very minor. Thus, we focused on representing the clear difference between the samples, because we intended to show the newly detected C–N bond and the decrease of oxygenated species after the surface treatment of GO by PNE, through the XPS analysis.

Haruo Inoue returned to the discussion of the paper by Jian-Ren Shen: Amazing findings! Congratulations again! The beautiful structure of S_3 indicating definite O–O bond formation would imply that the S_2 state is prepared for the attack of water or OH^{-1} against $O_{(5)}$. If possible, could you comment on the prospect that everything will be revealed in the near future?

Jian-Ren Shen replied: Thank you! We think that we have found the site for O=O bond formation in the oxygen-evolving center of PSII. The remaining issues include how the O=O species are cut out and leave the Mn-cluster, where are the protons going out, and how the next water molecule will come in to replace the $O_{(5)}$ atom released, *etc.* Since we have established the experimental procedures for solving the intermediate structures of water oxidation by using the X-ray free electron lasers, we expect that these remaining issues will be revealed with the same approach in the future. These results will also promote theoretical studies by quantum mechanical approaches to elucidate the detailed mechanism of water oxidation by the oxygen-evolving complex of PSII.

Licheng Sun asked: In the bond formation between $O_{(5)}$ and $O_{(6)}$, where does the $O_{(6)}$ come from? Is it *via* the water bound to Ca?

Jian-Ren Shen responded: The origin of $O_{(6)}$ is not clear at present; however, it is not the water bound to Ca nor those bound to $Mn_{(4)}$. All of the 4 waters coordinated to Ca and $Mn_{(4)}$ are not changed, so $O_{(6)}$ must come from a site beyond the first coordination sphere.

Thomas Corry said: The bond distance between $O_{(5)}$ and $O_{(6)}$ is given as 1.5 Å, which implies that the bond is a peroxide. Other studies have suggested that $O_{(6)}$ should be a hydroxide in the S_3 state. If so, this would be expected to have a longer O–O bond length. How would you explain this observation?

Jian-Ren Shen answered: Since the $O_{(5)}$ and $O_{(6)}$ are clamped by two Mn ions in the two sides, I expect that a hydroxide would also fit with this distance. Alternatively, there may be some uncertainties in the bond lengths determined at this level of resolution, and this may hamper the identification between peroxide and hydroxide.

Thomas Corry remarked: It has been observed that the Sr-substituted OEC has reduced oxygen evolving activity. Is it possible to look at the crystal structure of a Sr substituted complex in the S_3 state?

Jian-Ren Shen responded: In principle it is possible to look at the crystal structure of a Sr-substituted complex in the S_3 state. However, since the Sr-substituted cells grow slower than the Ca-containing cells, and the Sr-substituted PSII is more difficult to purify, determination of the Sr-substituted structure in the S_3 state will be much more difficult, and we focused on the S_3 state structure of the native PSII right now.

Haruo Inoue returned to the discussion of the paper by Takumi Noguchi: I remember that recently Professor Noguchi has found an interesting deuterium isotope effect on the transient IR observation during the transition from S_2 into S_3 state. May I ask Professor Noguchi's opinion on my previous question?

Takumi Noguchi responded: Using time-resolved infrared spectroscopy, we have recently detectes a ~100 μs phase before the electron transfer phase with a ~350 μs time constant[1] and assigned this ~100 μs phase to water movement from the Ca site to the Mn site. This spectroscopic observation could be confirmed by time-resolved XFEL crystallographic analysis in the future.

1 H. Sakamoto, T. Shimizu, R. Nagao and T. Noguchi, *J. Am. Chem. Soc.*, 2017, **139**, 2022–2029.

Anthony Harriman opened a general discussion of the papers by Jian-Ren Shen, Nobuo Kamiya, Takumi Noguchi and Eun Jin Son: Following on from your work, we now have a wonderful understanding of the structure and functions of the natural water oxidation catalyst. The level of detail is really exciting and we can now wonder about the mechanism and perhaps even think about the evolution patterns that resulted in this structure. On the other hand, there are few, if any, reports at this conference describing a manganese-based artificial catalyst. In many respects, manganese would be an excellent choice for a catalyst for artificial photosynthesis. So why is no one working with manganese and how might research in this area be promoted? I believe there are large deposits of manganese-rich nodules that could be exploited. It's not necessary to duplicate the natural system but I would think manganese is a more appropriate material

than ruthenium, iridium or even cobalt. So how might you use your findings to popularise research into manganese-based catalysts?

Eun Jin Son responded: Firstly, the reason why we used cobalt ions rather than manganese ions in our experiment was just because of the efficiency of oxygen evolution. But, I believe that our strategy of using a quinone-based redox mediator, to promote the electron transfer rate, can also be applied to other photocatalytic water oxidation systems with a manganese-based catalyst. In that case, manganese oxide or a manganese cluster should be utilized, rather than manganese ions, to achieve proper efficiency.

Nobuo Kamiya responded: Manganese is characterized by the ability of multinuclear complex formation as the oxygen-evolving complex (OEC) in PSII. We imagine that the OEC structure might be constructed by the hydrophilic protein environment in PSII including water molecules. Our suggestion for the R&D of manganese-based catalysts is to design a relatively large ligand system. The system should be stable and flexible, and have the ability to give different functions on the manganese atoms in the multi-nuclear complex.

Jian-Ren Shen answered: Although there were no reports on Mn-based catalysts in this conference, there are several groups working on this project, and indeed some compounds mimicking the structure and properties of the natural Mn-cluster have been reported. These include the group of Agapie,[1] Christou,[2] Zhang[3] *etc.* I am sure that there will be more Mn-based compounds that may possess catalytic activity for water oxidation.

1 J. S. Kanady, E. Y. Tsui, M. W. Day and T. Agapie, *Science*, 2011, **333**, 733–736.
2 S.Mukherjee, J. A. Stull, J. Yano, T. C. Stamatatos, K. Pringouri, T. A. Stich, K. A. Abboud, R. D. Britt, V. K. Yachandra and G. Christou, *Proc. Natl. Acad. Sci. U. S. A.*, 2012, **109**, 2257–2262.
3 C. Zhang, C. Chen, H. Dong, J.-R. Shen, H. Dau and J. Zhao, *Science*, 2015, **348**, 690–693.

Licheng Sun remarked: Harriman's question about why we are investigating Mn is highly relevant. Today's discussion is highly relevant because nature still keeps some secrets which we don't know. More information about Mn catalysts can provide a route to prepare more efficient water oxidation catalysts, and manganese can become a more efficient photocatalyst.

Sang Ook Kang returned to the discussion of the paper by Eun Jin Son: The data appears to show that your photosensitisers are not working properly. Have you looked at a Pourbaix diagram to increase reactivity/stability? From the stability point of view, the Ru species seems to be stable at pH 8.

Eun Jin Son responded: Yes, according to the Pourbaix diagram of $[Ru(bpy)_3]^{2+}$ in aqueous solution,[1] the photosensitizer itself seems to be stable at pH 8. The main reasons for the inactivation of the photosensitizer may be its decomposition during the reaction due to nucleophilic attack of water molecules on the bpy ring in its oxidized state and interaction with sulfate radical.[2,3]

1 A. R. Parent, R. H. Crabtree and G. W. Brudvig, *Chem. Soc. Rev.*, 2013, **42**, 2247.

2 M. Hara, C. C. Waraksa, J. T. Lean, B. A. Lewis and T. E. Mallouk, *J. Phys. Chem. A*, 2000, **104**, 5275.
3 B. Limburg, E. Bouwman and S. Bonnet, *ACS Catal.*, 2016, **6**, 5273.

Hitoshi Tamiaki continued the general discussion of the papers by Jian-Ren Shen, Nobuo Kamiya, Takumi Noguchi and Eun Jin Son: PSII uses Mn clusters for an oxygen-evolving complex. Why does nature utilize Mn? Are any other components useful for the alternative? Please tell me the candidates if possible.

Nobuo Kamiya responded: We cannot answer your questions exactly. Manganese is abundant on the Earth and has the ability of multi-nuclear complex formation as the oxygen-evolving complex (OEC) in PSII. We imagine that the multi-nuclear complex of manganese might have a "soft" nature although manganese itself is categorized as having "hard" atoms. At present, there is no evidence that indicates the "soft" nature of the OEC, and we have no idea for the useful alternatives.

Jian-Ren Shen replied: This is an important and often-asked question. I can say that the properties of Mn well match the requirements for a water-splitting catalyst; among which, the presence of its multiple oxidation states, its redox-potential, its abundance and non-toxic nature, and probably its ionic size and ligand requirement, can be immediately listed. However, we do not know if there are any alternatives that may be used in place of Mn. Anyway, it is the selection of nature through a long time of evolution, and thanks to this, we know that light energy can be converted to chemical energy indispensable for sustaining life on the earth.

Jose Martinez continued the discussion of the paper by Eun Jin Son: At the end of your photocatalytic experiments have you tried adding more $[Ru(bpy)_3]^{2+}$ and seeing if oxygen continues to be evolved? Is there potentially another photosensitizer you could try that doesn't degrade as fast or isn't as pH sensitive?

Eun Jin Son replied: Considering our closed experimental set-up for oxygen detection, it is impossible to add more $[Ru(bpy)_3]^{2+}$ during the oxygen evolution test. In this paper, because we focused on observing the effect of the quinone-based electron mediator to the enhancement of oxygen evolution efficiency, we employed a well-known photocatalytic water oxidation model system (*i.e.*, Co–Pi as a water oxidation cocatalyst, $[Ru(bpy)_3]^{2+}$ as a photosensitizer, and $Na_2S_2O_8$ as a sacrificial electron acceptor). But this system has an issue of pH-dependent photostability of the $[Ru(bpy)_3]^{2+}$ photosensitizer, as you pointed out. According to the literature,[1] Pt(II)-porphyrin photosensitizer exhibits higher stability than $[Ru(bpy)_3]^{2+}$ in neutral phosphate buffer. Thus, it can be a good strategy to use the metalloporphyrin as the alternative to improve the stability of the system.

1 H.-C. Chen, D. G. H. Hetterscheid, R. M. Williams, J. I. van der Vlugt, J. N. H. Reek and A. M. Brouwer, *Energy Environ. Sci.*, 2015, **8**, 975.

Licheng Sun said: After light driven water oxidation, did you measure the pH value of the solution? Depending on how long you shined the light, pH 3 or even lower could be obtained. By adding base into the solution, recovering the initial activity of the system can be anticipated.

Eun Jin Son answered: No, we didn't measure the pH value of the solution after the water oxidation test. As the driving force of reduction of quinone groups to catechol is decreased as the pH value decreased, increasing the pH value of the solution after the reaction may possibly affect the activity of PNE. But, the reaction mixture cannot be further reused, even with the addition of base, due to the decomposition of $[Ru(bpy)_3]^{2+}$ photosensitizer in phosphate buffer containing $Na_2S_2O_8$ in the first run.

