Oral Presentations

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Amazing molecular motors in *Mollicutes* !!!

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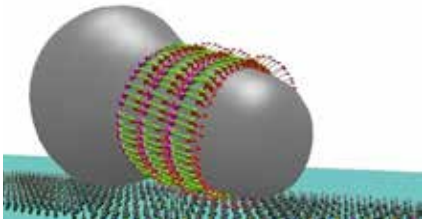
There are numerous species of lifes on the earth. However, if we focus on the structure of force generating molecules, the motility discovered so far can be classified into just 18 types. Interestingly, three of them are found in a small group of bacteria called the class *Mollicutes*. The class *Mollicutes* is thought to have been established after unique evolution through parasitic on higher animals and plants in a short time from Gram positive bacteria, represented by *Bacillus subtilis var. natto*. Recently, we succeeded in clarifiying the core part of the motilities, through a process including (i) analyzing the movement of cells and molecules in detail, (ii) identifying protein molecules involved, (iii) clarifying the structure of motility machinery. Then, we saw unimaginable secrets!

Spiroplasma eriocheiris (right figure) has a regular helicity in the cell and pushes water backward by reversing the handedness from front to back, and swims forward. We isolated the ribbon structure responsible for helix formation and its component "Fibril protein", and clarified their structures at nanometer level. We found that the reversal of cell helicity is caused by the structural switch in Fibril protein, and its structure depends on MreB, which is an actin homolog dictating the shape of cells in other bacteria, and the intracellular energy state.



Mycoplasma mobile (right figure) forms an organelle on a cell pole, binds to the host surface and shows gliding motility. Previously, we revealed that a complex including legs composed of four huge proteins works on the surface of cell membrane, and motors hydrolyze ATP on the inside of cell membrane. We isolated the motor and clarified its structure to the atomic resolution by using electron cryomicroscopy. A “Monster" appeared as the combination of phosphoglycerate kinase and ATP synthase which are responsible for ATP synthesis in many organisms, associated with several novel proteins.

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Animal opsins: non-visual functions and optogenetic applications

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Most animal opsins bind to a retinal as a chromophore to form photosensitive pigments and serve as light-sensitive G protein-coupled receptors (GPCRs), which constitute a large protein family of receptors that sense molecules outside the cell and activate inside signal transduction pathways and, ultimately, generate cellular responses.

Many animals capture light through opsin-based pigments and utilize the light information for visual and non-visual functions including regulation of circadian rhythms. Thousands of opsins have been identified from a wide variety of animas thus far. We have characterized diverse opsins, including novel ones, spectroscopically, biochemically, molecular physiologically and evolutionally [1-4], and also investigated their contribution to biological function [5, 6]. Interestingly, we found that some non-visual opsins have unique molecular properties and such unique peroperties might be suitable for optogenetics applications, which are the combination of genetics and optics to control well-defined events within specific cells of living tissues.

O-2

In lower vertebrates, pineal and its related organs in the brain discriminate UV and visible light. A pineal UV-sensitive opsin, parapinopsin has been considered to be involved in this color discrimination [1, 3]. We recently found that a signle photoreceptor cell containing parapinopsin alone generates color opponency, which is essential cellular photoresponse to detect different wavelenght of lights [6]. Because it has been discussed that maltiple opsins are requiered for color opponency, this finding could propse a new concept for the mechanism of color detection and its evolution. We also found that UV and green light illuminations activate and deactivate parapinopsin, respectively to regulate G protein-mediated signal transduction cascade in the mammalian cultured cells [7]. Therefore we suggest that parapinopsin has optogenetic potentials to control cellular responses and animal behaviours in a light wavelength-dependent manner.

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New insights on the Photosystem II O_2 evolution mechanism: The S_2 to S_3 transition.

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Abstract:

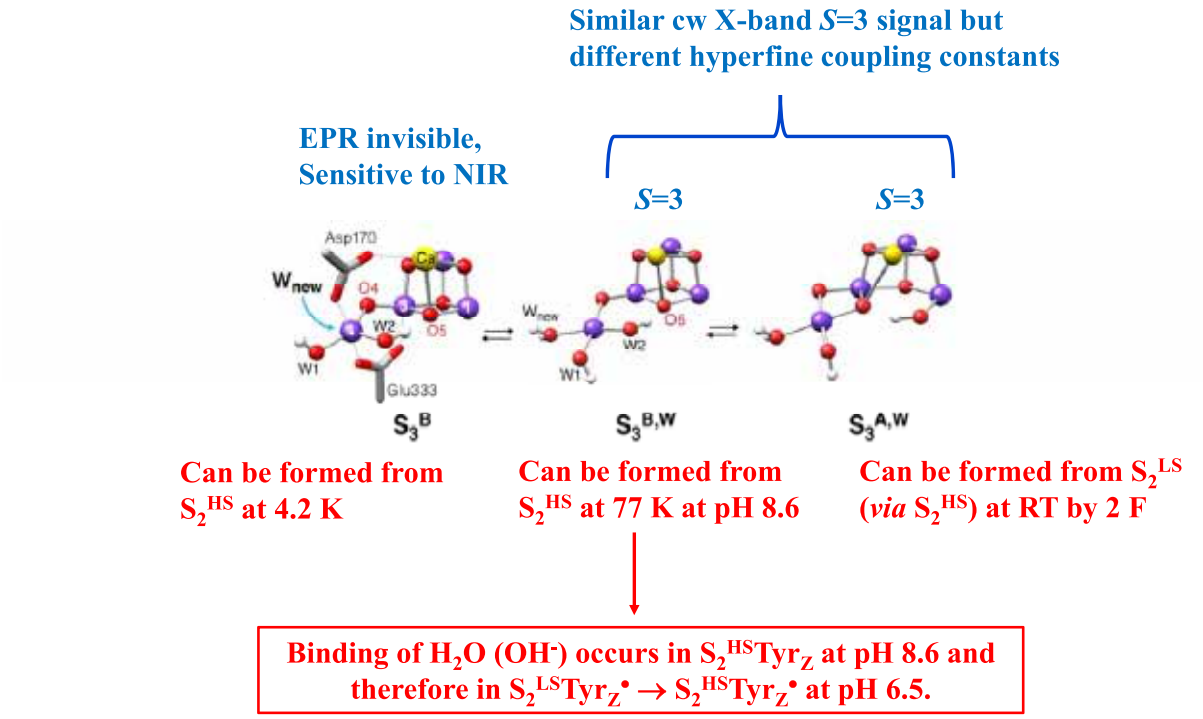
Oxygenic photosynthesis provides the main input of energy into biology. This process produces food, fibers and fossil fuels, and energizes the atmosphere with O_2. Photosystem II (PSII) is the unique water-oxidizing enzyme of the cyanobacteria, algae and higher plants that is at the heart of oxygenic photosynthesis. The active site of PSII, a Mn_4CaO_5-cluster, advances through five sequential oxidation states (S_0 to S_4) before water is oxidized and O_2 is generated. Both the S_2-state and S_3-state exhibit different EPR properties showing there are several structural conformations for both of them. The S_2-state can be present in either a low spin (S=1/2) or a high spin (S=5/2) configuration. The S_3-state has at least two configurations. one exhibiting an EPR signal from a spin S=3 state and one that is EPR silent but detectable upon near-infrared illumination at 4.2 K by inducing a so-called "split signal". The S_2 to S_3 transition is a complex state because several events occurs as the preloading of water, the binding of water to the cluster, deprotonation steps and possible structural changes in the cluster. In the present work, the transition between the S_2 and S_3 state has been studied by using EPR spectroscopy, quantum chemical calculations using Density Functional Theory (DFT), and time-resolved UV-visible absorption spectroscopy.

The EPR experiments show that the equilibrium between S_2^{LS} and S_2^{HS} is pH dependent [1], with a pK_a ≈ 8.3 (n ≈ 4) for the native Mn_4CaO_5 and pK_a ≈ 7.5 (n ≈ 1) for Mn_4SrO_5. The DFT results suggest that exchanging Ca with Sr modifies the electronic structure of several titratable groups within the active site, including groups that are *not* direct ligands to Ca/Sr, *e.g.*, W1/W2, Asp61, His332 and His337. This is consistent with the complex modification of the pK_a upon the Ca/Sr exchange. By studying a Val185Thr mutant [2] it is shown that Val185 contributes to the stabilization of the S_2 into the low spin configuration. Indeed, in the V185T mutant a high proportion of S_2 exhibits a high spin, S = 5/2, configuration. By using bromocresol purple as a dye, a proton release was detected in the $S_1Tyr_Z^{\bullet} \rightarrow S_2^{HS}Tyr_Z$ transition in the V185T mutant in contrast to the WT*3-PSII in which there is no proton release in this transition. Instead, in WT*3-PSII, a proton release kinetically much faster than the $S_2^{LS}Tyr_Z^{\bullet} \rightarrow S_3Tyr_Z$ transition was observed, and we propose that it occurs in the $S_2^{LS}Tyr_Z^{\bullet} \rightarrow S_2^{HS}Tyr_Z^{\bullet}$ intermediate step before the $S_2^{HS}Tyr_Z^{\bullet} \rightarrow S_3Tyr_Z$ transition occurs.

S-1

By studying the temperature dependence of the S_2^{HS} to S_3 transition [3] it is found that *i*) upon illumination at 77 K, the S_2^{HS} state is able to progress to the S_3 state ($S_3^{S=3}$) state in a proportion of centers and *ii*) in another proportion of centers, illumination at low temperature advances the S_2^{HS} state to form the S_3 state that lacks the $S_3^{S=3}$ EPR signal (S_3^{inv}), and this still occurs down to 4.2 K.

On the basis of the model proposed (in blue) and drawn by Pantazis [4] and from the present data, the following suggestions are made (in red) for the S_2 to S_3 transition.



In conclusion we suggest from the present study that both the proton release and the water binding occur in the $S_2^{LS}Tyr_Z^{\bullet} \rightarrow S_2^{HS}Tyr_Z^{\bullet}$ step. It would remain to establish by Pulsed ENDOR measurements if the S_3 state induced at low temperature exhibits or not the same hyperfine coupling constants than the S_3 state induced at room temperature (see [4] for a discussion on this point).

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New insights on Chl_{D1} function in Photosystem II from site-directed mutants

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The monomeric chlorophyll, Chl_{D1}, which is located between the $P_{D1}P_{D2}$ chlorophyll pair and the pheophytin, $Pheo_{D1}$, is the longest wavelength chlorophyll in the heart of Photosystem II and is thought to be the primary electron donor. Its central Mg^{2+} is liganded to a water molecule that is H-bonded to D1/T179 [1]. Here, site-directed mutants on D1/T179H and D1/T179V, were made in the thermophilic cyanobacterium, *Thermosynechococcus elongatus*, and characterized by a range of biophysical techniques [2]. The Mn_4CaO_5 cluster in the water-splitting site is fully active in both mutants. Changes in thermoluminescence indicate that *i*) radiative recombination occurs *via* the repopulation of $*Chl_{D1}$ itself; *ii*) non-radiative charge recombination reactions appeared to be faster in the T179H-PSII; and *iii*) the properties of $P_{D1}P_{D2}$ were unaffected by this mutation, and consequently iv) the immediate precursor state of the radiative excited state is the $Chl_{D1}^{+}Pheo_{D1}^{-}$ radical pair. Chlorophyll bleaching due to high intensity illumination correlated with the amount of $^1O_2$ generated. Comparison of the bleaching spectra with the electrochromic shifts attributed to Chl_{D1} upon Q_A^- formation, indicates that in the T179H-PSII and in the WT*3-PSII, the Chl_{D1} itself is the chlorophyll that is first damaged by $^1O_2$, whereas in the T179V-PSII a more red chlorophyll is damaged, the identity of which is discussed. Thus, Chl_{D1} appears to be one of the primary damage site in recombination-mediated photoinhibition. Finally, changes in the absorption of Chl_{D1} very likely contribute to the well-known electrochromic shifts observed at ~430 nm during the S-state cycle.

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Infrared analyses of photoreactions in photosystem II

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Abstract:
Photosystem II (PSII) is an enzyme that has a function of water oxidation and quinone reduction utilizing light energy. It provides electrons necessary for CO_2 fixation, and molecular oxygen that is the source of oxygen in the atmosphere. The structure and the reactions of PSII have long been studied using various spectroscopic methods such as fluorescence, electron spin resonance, X-ray absorption, and Fourier transform infrared (FTIR) absorption measurements. In 2011, Kamiya, Shen, and coworkers resolved the X-ray crystallographic structure of the PSII core complexes from a thermophilic cyanobacterium at a high resolution of 1.9 Å [1]. In this structure, oxygen atoms in the water-oxidizing Mn cluster and water molecules were first resolved and the overall structure of the catalytic center of water oxidation was revealed. After the report of this structure, PSII researches, especially on the water oxidation mechanism, entered a new era. Quantum chemical calculations using the density fictional theory (DFT) and quantum mechanics/molecular mechanics (QM/MM) methods based on the atomic coordinates of the X-ray structure became available, and detailed analyses of spectroscopic data have been performed to provide accurate information on the structure and reactions in PSII. Here, I will introduce our results of FTIR studies on PSII in combination of QM/MM calculations based on high-resolution X-ray crystallographic structure. FTIR analysis of vibrations of water molecules around the Mn cluster provided crucial information of the proton transfer mechanism during water oxidation [2]. In addition, vibrations of carboxylate groups and histidine residues interacting with the Mn cluster revealed the role of amino acid residues in the water oxidation mechanism [3, 4]. Furthermore, our FTIR study in combination with genetic introduction of a hydrogen bond to P_{D1} and P_{D2} and QM/MM analysis clarified the distribution of a positive charge on a chlorophyll dimer P680 (P_{D1}/P_{D2}) [5].

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Light Conversion in Bacterial Photosynthesis

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Abstract: In bacterial photosynthesis, light harvesting antennae and reaction centers are responsible for harnessing sunlight. The photosynthetic bacterium *Thermochromatium* (*Tch*.) *tepidum* is a thermophile growing at optimal temperature of 48–50 ºC. Its light harvesting antennae exhibit remarkable thermal stability and nearinfrared absorption. It is important to study the primary excitation dynamics of both isolated and membrane-embedded light harvesting complexes of *Tch. tepidum*, so as to understand the mechanisms of light absorption, thermal stability, photoprotection and resistance to environmental stresses.

We have investigated, by the use of triplet excitation profiles (TEPs),[1] the roles of multi-compositional carotenoids (Cars) in the core light harvesting complexes (*m*-LH1-RCs) from a mutant strain of *Rhodobacter* (*Rba*.) *sphaeroides*.[2] Transient absorption kinetics revealed the triplet excitation transfer from spheroidene (Spe, major composition~85%) to spirilloxanthin (Spx, minor composition ~8%), implying that the two different kinds of Cars coexist in individual *m*-LH1-RC complexes. TEP results showed that Spx is involved in photoprotection by quenching ^{3}BChl*, whereas Spe does so merely for BChls of relatively low site energy. The Spe-to-Spx triplet excitation transfer and their inequivalence in quenching ^{3}BChl* constitute a mechanism of cooperative photoprotection.

We have also investigated, by the using femtosecond time-resolved absorption spectroscopy, the uphill excitation energy transfer (EET) from the core antennae (LH1s) to the reaction centers (RCs) by comparing the *m*-LH1-RC to the native LH1-RC of *Tch. tepidum*. The former exhibits a substantially large RC-LH1 energy difference (ΔE = 630 cm^{-1}, ~3k_BT). The semilogarithmic plot of the EET rate is found to be invresely proportional to ΔE, which consolidates a thermal activation mechanism for the uphill EET. The results are discussed on the basis of the newly reported LH1-RC structure of *Tch*. *tepidum*,[3] which allows us to propose the presence of specific doorway BChls in LH1 in promoting the uphill EET.

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Mechanism of water-splitting catalyzed by the Mn_4CaO_5-cluster of photosystem II

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Light-driven water-splitting catalyzed by photosystem II (PSII) produces molecular oxygen indispensible for aerobic life on the earth. The structure of dimeric PSII from a thermophilic cyanobacterium *Thermosynechococcus vulcanus* has been solved at a resolution of 1.9 Å [1] using synchrotron radiation (SR) X-rays, which revealed a clear picture of a Mn_4CaO_5-cluster, the catalytic center for water-splitting assocaited with the protein matrix of PSII. The Mn_4CaO_5-cluster is organized in a distorted chair form in which, a Mn_3CaO_4 cluster constitutes the main body of the chair with a cubic shape, and the forth Mn ion is attached to the outside of the cubane by two μ-oxo-bridges O4 and O5. Due to high sensitivity of the Mn ions to radiation damage, some of the inter-atomic distances within this cluster were suggested to be slightly longer than those deduced from EXAS spectroscopic measurements as well as QM/MM calculations. In order to avoid possible radiation damages and eliminate the uncertainties in the inter-atomic distances, we used femtosecond X-ray pulses from an X-ray free electron laser (XFEL) facility SACLA, Japan, to solve the structure of PSII. To obtain a high resolution structure, we used large PSII crystals, and adopted a "fixed-target rotational method" to collect the X-ray diffraction data. This approach required a huge number of large, isomorphous PSII crystals, but allowed us to solve the PSII structure at 1.95 Å resolution [2]. This structure confirmed the unique position of O5, and therefore suggested its possible involvement in the O=O bond formation. We further used a pump-probe approach with a combination of small PSII crystals and serial femtosecond crystallography (SFX) by XFEL to solve the structure of the S_3 intermediate-state induced by 2-flash illumination [3]. The results showed an insertion of a new oxygen in the S_3-state, giving rise to a Mn_4CaO_6 cluster. The newly inserted O6 is located close to O5, suggesting a mechanism of O=O bond formation between O5 and O6.

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Structure of the oxygen-evolving complex and valences of the manganese atoms in Photosystem II functioning in photosynthesis

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Oxygen-evolving complex (OEC) of photosystem II (PSII) extracts electrons from water molecules using solar light energy in photosynthesis. Crystal structureS of PSII and the OEC have been resolved at a high resolution of 1.9 Å [1]. Based on the structural information (PDB-ID: 3WU2), researchers make efforts to elucidate the mechanism of oxygen-evolving reactions in the OEC according to the Kok cycle model. Because the OEC is highly sensitive to X-ray irradiation, X-ray reduction and structure changes induced on the OEC have been discussed on the results mainly from DFT computational calculations. In order to overcome the X-ray reduction problem, we improved the quality of PSII crystals and the procedure of post-crystallization treatments, and succeeded in collecting datasets from highly isomorphous crystals at extremely low X-ray doses. By using 10 and 3 of the isomorphous crystals at pH 6.6, we collected two datasets of diffraction intensities at 0.03 and 0.12 MGy (0.43 MGy for the 3WU2 dataset) and at resolutions of 1.87 and 1.85 Å, respectively [2]. Obtained structures were compared with each other, and a threshold of X-ray dose was found below 0.12 MGy, under which large structural changes were not observed. Furthermore, two alternative OEC structures were found in the two PSII monomers (A, B) in an asymmetric unit of crystal.

According to the same strategies, we collected six datasets from several isomorphous PSII crystals, pH values of which were adjusted at 5.0, 5.7, 6.0, 6.3, 7.0 and 8.0. X-ray doses were at 0.21-0.11 MGy and resolutions, 2.19-1.85 Å. In comparisons of the six structures against the 0.03 MGy structure mentioned above (pH 6.6 and resolution, 1.87 Å), atomic distances in the OEC showed very interesting behaviors depending on the pH values. The five oxo-bridging oxygen atoms (O1-O5) bind four Mn and one Ca atoms in the OEC. The hydrogen-bond distances from O1 to one water molecule (W5) elongated at pH 6.0 from 2.5 Å to 2.8 Å in both of OECs in the A- and B-monomers. The similar behavior was found at the distance from O4 to another water molecule (W6) in the A-monomer, but no change was observed from 2.4 Å in the B-monomer. In the pH range of 5.0-8.0, the distances from O3 to Nε atom of D1-His337 were constant as 2.4 Å and 2.8 Å for the A- and B-monomers, respectively. These are indicating the pH-dependent structural flexibility of the OEC in PSII.

The anomalous dispersion studies have been conducted recently in order to determine valences of the Mn atoms directly in the OEC. These studies showed that the S-states of the Kok cycle were S_1 and S_0 for the A- and B-monomers, respectively. Based on the pH-dependent flexibility and the S-states of the OEC, the oxygen-evolving mechanism of PSII will be discussed in my talk.

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