1F1510 ASR の Pro206 はレチナル構造と光反応を制御する**Pro206 of Anabaena sensory rhodopsin is responsible for the isomeric composition of retinal and photochromism**

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Anabaena Sensory Rhodopsin (ASR) is a microbial rhodopsin found in *Anabaena* (*Nostoc*) sp. PCC7120, a freshwater cyanobacterium. ASR is believed to function as a photochromism sensor. Microbial rhodopsins possess all-trans and 13-cis retinal as the chromophore, but only the former is important for function. In bacteriorhodopsin (BR), the stable photoproduct at the end of the functional cycle of the all-trans form is 100% all-trans. Nevertheless, we found that the photoreaction of ASR is completely photochromic; the stable photoproduct of the all-trans form is 100% 13-cis, and that of the 13-cis form is 100% all-trans [1]. It is intriguing that BR and ASR exhibit similar protein architecture, but photoreactions are entirely different.

One of key amino acids may be Pro206 of ASR, because the corresponding amino acid of other microbial rhodopsins is Asp (Asp212 in BR). In the present study, we replaced Pro206 of ASR into 19 amino acids and examined their molecular properties. Interestingly, all mutants were properly expressed and folded, including P206R and P206K. HPLC analysis shows that the isomeric composition between all-trans and 13-cis forms is highly amino-acid dependent, and some mutants contain 9-cis and 11-cis forms under light conditions. P206D contains 13-cis retinal dominantly under both dark and light conditions. We thus conclude that Pro206 is a responsible residue for the photochromic reaction in ASR, and the mechanism will be discussed.

[1] Kawanabe et al. *J. Am. Chem. Soc.* 129, 8644 (2007).

1F1522 Large deformation of helix F upon formation of the M intermediate of the azide-bound purple form of pharaonis halorhodopsin

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Halorhodopsin(HR) utilizes light energy to transport chloride ions from the extracellular side to the cytoplasmic side. When the chloride ion bound to the active site is replaced with an azide ion, it acquires the ability to transport protons. To clarify this proton pumping mechanism, we investigated light-induced structural changes in pharaonis halorhodopsin (pHR) using the C2 crystal that was prepared in the presence of chloride ion. When the C2 crystal was soaked in a solution containing azide ion, the chloride ion and one water molecule in a cavity between the Schiff base and Thr126 were replaced with an azide ion. When the azide-bound purple form of pHR was exposed to orange light, an M-like yellow species containing 13-cis retinal was generated efficiently in one of the three subunits in the asymmetric unit. This subunit has the EF loop facing a free space, so that a profound outward movement of the cytoplasmic half of helix F was induced upon formation of the M state. This structural change was accompanied by a large swing of the phenyl ring of Phe259 in helix G, creating a large cavity in the cytoplasmic side of the retinal Schiff base, where a linear cluster of water molecules were incorporated. Simultaneously, the middle moiety of helix C distorted largely, leading to a significant shrinkage of the primary anion binding site (site I). As a result, the azide ion that was initially located in site I was forced to move to a large cavity created by a flip-flop motion of the side chain of Glu234 in the anion uptake channel.

1F1534 センサリーロドプシン II-トランスデューサー複合体の比較シミュレーションにより明らかにされた基底状態と M 中間体の差異**Differences between the ground state and the M-intermediate of Sensory Rhodopsin II-Transducer complex revealed by comparative simulations**

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The complex of sensory rhodopsin II (SRII) and its cognate transducer HtrII (SRII:HtrII/2:2) is a photosensor associated with negative phototaxis in extreme halophiles. To examine how photoexcitation of SRII influences the overall

structure of the complex, we performed comparative molecular dynamics simulations of the complex of SRII and truncated HtrII (residues: 1-136) from *N. pharaonis* of two photochemical states, the ground state and the M-intermediate, in explicit solvent and lipid bilayer. The simulation systems consist of each crystal structure appended with the models of the missing residues and the N-terminal HAMP domain. The simulation results showed significant increases in the structural differences compared to the two crystal structures: the outward bending motions of F-helix of SRII and the rotation of the C-terminal region of TM2 helix. The most remarkable change was found in the overall orientation of the two SRII molecules: the cytoplasmic side was closed in the ground state and open in the M-intermediate. This change was attributed to the differences in the side-chain packing of the 4-helix bundle of the HtrII dimmer. We will discuss the results in detail with additional simulations of the complex without the HAMP domain. Finally, we will propose a hypothesis for the signal transduction mechanism of SRII-HtrII system.

1F1546 Effect of potential on the photo reaction of Sensory rhodopsin II monolayer studied by Surface Enhanced IR absorption Spectroscopy

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Membrane potential is one of important factors that set out the function of many membrane proteins. Although an importance of this factor is widely accepted, experimental method that can access directly to the effect is limited. Here, we introduce a method tackling this problem, in which exploit a membrane protein monolayer tethered on a metal solid-support and surface enhanced infrared absorption spectroscopy (SEIRAS). To reproduce highly orientated protein layer as mimic of the cellular membrane, we employed a tactic that the membrane protein bind through recombinant histidine tags with Ni-NTA self-assembled monolayer modified solid support. After orientated binding of the protein to the surface, full functional integrity is regained by embedding into a lipid layer. SEIRAS provides specific enhancement of the signal from the tethered membrane protein, in which is difficult to detect by conventional IR technique due to a scarce amount of monolayer as few as pico-mol/cm². Advantage of SEIRAS becomes most beneficial when the functional studies of the tethered membrane proteins are performed by the application of external stimuli (light, voltage, chemicals etc.) to initiate the reaction. Functional difference spectroscopy between initial non-active and active state can extract the contributions from functional groups that are involved in the reaction. Application on the study of a photo-cycle reaction of sensory rhodopsin II under potential control will be discussed.

**1F1558 In-situ 光照射固体 NMR による光受容膜蛋白質の光活性状態の解明
Light activated states of photoreceptor membrane proteins as revealed by in-situ photo-irradiated solid-state NMR**

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Pharaonis phoborhodopsin (ppR or sensory rhodopsin II) is a negative phototaxis receptor of *Natronomonas pharaonis* and forms a 2:2 complex with the cognate transducer (pHtrII), which transmits the photosignal into cytoplasm. Light absorption of ppR initiates *trans-cis* photo-isomerization of the retinal chromophore followed by cyclic chemical reaction consisting of several intermediates (K, L, M and O). The M intermediate is thought to be an active state for signal transduction. We have successfully observed the M intermediate by using photo-irradiated solid-state NMR, because the life time of the M-intermediate is much longer than the other intermediates. ¹³C NMR signal from [20-¹³C] retinal-ppR and ppR/pHtrII revealed that multiple M-intermediates (M1, M2 and M3) with 13-*cis*, 15-*anti* retinal configurations coexisted under the photo-irradiated condition. Further, since the life time of M3 state was much longer than those of the other M states, the M3 state could be distinguished from the other M states. Carbonyl carbon signals of [1-¹³C] Tyr174 in ppR also showed that multiple structures coexisted in the protein side. Light induced structural changes were further observed in [3-¹³C]Ala-pHtrII/ppR complexes. Particularly, helix structure of the interfacial region in pHtrII changed significantly. Detailed structure of photo-intermediate in photoreceptor membrane proteins is now possible to gain insight into the signal transduction mechanism by using in-situ photo-irradiated solid-state NMR.