the chloride binding site. Mechanism of chloride binding will be discussed based on the difference ATR-FTIR spectra.

[1] Katayama et al. *Angew. Chem. Int. Ed.* 49, 891-894 (2010).

[2] Katayama et al. manuscript in preparation.

2P279 高速 AFM によるバクテリオロドプシン分子間相互作用の研究 1A1520 Role of aromatic residue for inter-molecular interaction between bacteriorhodopsin trimer studied by high-speed AFM

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Bacteriorhodopsin (bR), the light-driven proton pump, oligomerizes into a trimer, which naturally assembles into two-dimensional (2D) crystals, forming the purple membrane (PM) of *Halobacterium salinarum*. The mechanism of the 2D protein crystallization has been investigated by various techniques such as electron microscopy, X-ray crystallography and NMR. They have revealed that the membrane lipid composition, protein density and temperature are essential factors for crystallization. To obtain more critical insights into the 2D crystallization, we have used high speed AFM, which allows dynamic imaging of individual protein molecules in solutions with submolecular resolution. Thus far, we have succeeded to directly image the oligomeric structure and dynamic processes of photo-activated bR in PM. It has also showed association-dissociation dynamics of bR trimer occurring at the edges of a native PM crystal, suggesting the presence of a specific inter-trimer bond through aromatic residues on the extracellular side [1].

In this study, we observed bR mutants in which the aromatic residue is substituted. AFM movies of the mutant in which isoleucine is substituted for tryptophan 12 showed that the bR lattice was disassembled but bR diffused as keeping the trimer. On the other hand, the substitution of the tryptophan with phenylalanine did not change the lattice structure. These results provide direct evidence of a role of aromatic residue for inter-trimer interaction on the assembly of the bR lattice.

[1] H. Yamashita et al. (2009)

2P280 異常な pH 効果を用いたプロテオロドプシンの青吸収型から緑吸収型への転換 Conversion of Blue Proteorhodopsin into Green PR Using Abnormal pH Effect

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Proteorhodopsin (PR) is a light-driven proton pump found in marine bacteria, and thousands of PRs are classified into blue-absorbing (λ_{max} ~490 nm) and green-absorbing (λ_{max} ~525 nm) types. We recently found that replacement of Ala178 in the E-F loop of green PR exhibits spectral red-shift by ~20 nm and increase of the pKa of the counterion [1-3].

Here we report that an abnormal pH effect causes color change of blue PR into green-absorbing type. BPR in LC1-200, marine γ -proteobacteria, was expressed in *E. coli*, whose absorption properties were examined for the DM-solubilized sample. The λ_{max} was located at 497 and 514 nm at pH 7 and 4, respectively, and pH titration was reversible (pKa = 4.8). When pH was lowered from 4, the λ_{max} was further red-shifted (528 nm at pH 2). This is unusual, because blue shift occurs by chloride binding in the case of bacteriorhodopsin [4]. Surprisingly, when pH was then increased from 2 to 7, the λ_{max} of this BPR was further red-shifted to 540 nm. Thus, the >40-nm red-shift was achieved by abnormal pH effect, though the protonation of the Schiff base counterion yields only the <20 nm red-shift. PR₅₄₀ was stable, but eventually returned to the original BPR (half decay was one day and half). This unique pH dependence and possible molecular mechanism will be discussed.

[1] Yoshitsugu et al. *Angew. Chem. Int. Ed.* 47, 3923 (2008).

[2] Yoshitsugu et al. *Biochemistry* 48, 4324 (2009).

[3] Yamada et al. *Biochemistry* 49, 2416 (2010).

[4] D'ér et al. *Proc. Natl. Acad. Sci. USA* 88, 4751 (1991).

2P281 固体NMRを用いた圧力誘起によるバクテリオロドプシン中レチナールの異性化機構の解析 Pressure effect on retinal isomerization in bacteriorhodopsin as studied by solid state NMR

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Bacteriorhodopsin (bR) in purple membrane from *H. salinarum* shows a light-driven proton pump activity by photoisomerization of retinal. The all-*trans* and 13-*cis*, 15-*syn* retinal configurations in the dark-adapted bR coexist with the isomeric ratio close to 1:1. It is reported that the population of 13-*cis*, 15-*syn* retinal can be increased under the pressurized condition. In this study, we applied pressure to bR using fast magic angle spinning (MAS) in the solid state NMR (35 bar at 9 kHz). We first investigated dynamics of retinals with two configurations showing different ¹³C chemical shifts. NMR signal intensities and various nuclear spin relaxation parameters of ¹³C retinal in bR were determined by solid state MAS NMR. The ¹³C spin-lattice relaxation time (T₁) of 13-*cis*, 15-*syn* state was shorter than that of all-*trans* state. It is noticed that dynamics of retinal in 13-*cis*, 15-*syn* state is more flexible than that of all-*trans* state. Second, it was found that the 20-C methyl carbon signal intensity of 13-*cis*, 15-*syn* retinal at 22.0 ppm was increased under the fast MAS condition. It is indicated that the isomerization from all-*trans* to 13-*cis*, 15-*syn* state was generated by pressure application. This result suggests that a large negative molar volume change of protein is caused by moderately high pressure. It is also indicated that significant local structural changes of protein in the vicinity of retinal occur and consequently isomerization of retinal is generated by pressure application.

2P282 ウシロドプシンの細胞質側第3ループを含む光駆動プロトンポンプの性質 Chimeric proton-pumping rhodopsins containing the third cytoplasmic loop of bovine rhodopsin.

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G-protein-coupled receptors (GPCRs) transmit various stimuli to the intracellular signaling systems, and rhodopsin is the best studied GPCR. Archaeal-type rhodopsins, similar heptahelical membrane proteins, do not activate G-proteins, but it was reported that bacteriorhodopsin (BR) chimeras containing the third cytoplasmic loop of bovine rhodopsin are able to activate G-protein [1], where the activation level of the chimera was about 30 % of that of bovine rhodopsin.

We recently designed chimeric proteins from *pharaonis* phoborhodopsin (ppR, also called pharaonis sensory rhodopsin II; pSRII). The ppR chimeras containing the same region of the BR chimeras activated G-protein, but their levels were 10⁴-times lower than that of bovine rhodopsin. To gain further insights, here we prepared similar chimeras from proton-pumping rhodopsins such as proteorhodopsin (PR) and *Gloeobacter* rhodopsin (GR) as well as BR. Compared to the wild type, photocycle became 15-times longer for the PR chimeras, but no change for the GR chimeras. Interestingly, G protein activation was observed for the GR chimeras, but not for the PR chimeras, which might be correlated with the helix opening motion at the cytoplasmic side. We confirmed G-protein activation by the BR chimera, but its level was 10⁴-times lower than that of bovine rhodopsin. Spectroscopic measurements of the chimeras from proton-pumping rhodopsins are now in progress, and activation mechanism of visual and archaeal rhodopsins will be discussed.

[1] Geiser et al. *Protein Sci.* 15, 1679 (2006).

2P283 ハロロドプシン変異体 S130D, S130E の研究 Properties of S130D and S130E mutants of *pharaonis* halorhodopsin

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Nature created two kinds of light-driven ion pumps, outward proton pump and inward chloride pump such as bacteriorhodopsin (BR) and halorhodopsin (HR), respectively. It is known that BR can be converted to a chloride pump by a D-to-T mutation at position 85 [1], but HR cannot pump protons by the reverse T-to-D mutation. Our FTIR analysis of the Schiff base N-D and water O-D stretches in D₂O showed that both hydrogen bonds are strong in BR, while both are weak in HR [2,3]. We recently introduced BR-like 6 mutations into *pharaonis* HR, but it did not pump protons, where water hydrogen bonds were weak. Therefore, trial to convert HR into a proton pump is still great challenge, and we presumably need to control water-containing hydrogen-bonding network in the Schiff base region.

Here we study the role of Ser130 in pHR. Thr89 in BR, forming a hydrogen bond with Asp85, is important for the proton pump function [4,5]. Ser is conserved at this position in HR, which is the nearest amino acid from the Schiff base. We replaced Ser130 into Asp or Glu, which could work as the proton acceptor during photocycle. Photocycle and light-induced structural changes