Here we studied similar mutation effect for blue-absorbing PR (BPR). Thousands of PR are classified into GPR ($\lambda_{\text{max}} \sim 520$ nm) and BPR ($\lambda_{\text{max}} \sim 490$ nm). GPR and BPR possess Leu and Gln at position 105, and L/Q GPR and Q/L BPR absorb blue and green lights, respectively (L/Q switch)³. In case of BPR, distant mutation effect was only observed for the C-D loop, not E-F loop, suggesting that large-scale structural rearrangement is absent for the E-F loop of BPR. In case of GPR, distant mutation effects of the C-D and E-F loops were not additive, suggesting that common structure around the retinal chromophore is formed. Molecular mechanism of mutation effect in the cytoplasmic loops will be discussed on the basis of the present results.

1. Yoshitsugu et al. *Angew. Chem. Int. Ed.* **47**, 3923 (2008).
2. Yoshitsugu et al. *Biochemistry*. **48**, 4324 (2009).
3. Man et al. *EMBO J.* **22**, 1725 (2007).

2F1424 キメラタンパク質を用いた *Rhodobacter capsulatus* 由来 Photoactive Yellow Protein の相互作用部位の解明

Analysis of interaction sites on the Photoactive Yellow Protein of *Rhodobacter capsulatus* with chimeric proteins

Mayu Shimad, Yoichi Yamazaki, Hironari Kamikubo, Mariko Yamaguchi, Mikio Kataoka (*Grad. Sch. Mat. Sci., NAIST*)

Photoactive Yellow Protein (PYP) is a photoreceptor protein that absorbs blue light with p-coumaric acid as a chromophore. We identified the target protein of PYP named PBP from *Rhodobacter capsulatus* (Rc-PYP). PBP interacts with Rc-PYP in light dependent manner. This is the only target protein of PYP homologues revealed so far. Rc-PYP shows no substantial conformation changes upon absorption of light. The observation raised a serious question how PBP recognizes the photolyzed state of Rc-PYP. In order to clarify the mechanism of light dependent interaction of PBP with Rc-PYP, we aimed to identify the interaction sites on Rc-PYP by using chimeric mutants between Rc-PYP and *Halorhodospira halophila* PYP (Hh-PYP). Rc-PYP and Hh-PYP are separated into four segments considering secondary structure and amino acid conservation. The first block contains N-terminal 30 residues, the second block contains 31st -57th residues, the third block contains 58th - 86th and the fourth block contains remaining residues. Chimeric proteins were designed to replace some of four segments into a parent protein. Binding ability of PBP was verified spectroscopically, since the complex formation brings distinguishable spectral shift. The complex formation was also examined by photocycle kinetics the size exclusion chromatography. As a result, chimeric proteins having the second block of Hh-PYP depressed binding ability. While chimeric proteins having the other block(s) of Hh-PYP did not bind PBP. The further analyses are required to identify interaction sites.

2F1436 FTIR study of Aureochrome that possesses LOV domain at the C-terminus

Shota Ito¹, You Zhang¹, Tatsuya Iwata², Osamu Hisatomi³, Fumio Takahashi⁴, Hironao Kataoka⁵, Hideki Kandori¹ (¹*Nagoya Inst. Tech.*, ²*Ctr. Fost. Yng. Innov., Nagoya Inst. Tech.*, ³*Grad. Sch. Sci., Osaka Univ.*, ⁴*PRESTO, JST*, ⁵*Botanical Gardens, Tohoku Univ.*)

LOV domain is a blue-light sensor that binds an FMN. In many blue-light sensors, LOV domain is located at the N-terminal side. For example, phototropin possesses two LOV domains at the N-terminal side and regulates serine-threonine kinase domain at the C-terminal side¹. By means of light-induced difference FTIR spectroscopy, we have studied photoactivation mechanism of LOV domains in phototropin². Aureochrome, a novel photosensor protein from *Vaucheria frigida*, possesses a LOV domain at the C-terminus, while bZIP domain is located at the N-terminal side³. It is reported that the bZIP domain of aureochrome can recognize a DNA sequence (TGACGT), when its LOV domain is photoactivated. An interesting question is how signal transductions in LOV domains differ between the N- and C-termini? In this study, we measured light-induced structural changes of aureochrome (bZip-LOV) by low-temperature FTIR spectroscopy, where temperature-dependent structural changes were observed at 150, 200 and 295 K. The obtained spectra were compared with those of an isolated LOV domain, and those with DNA. We also measured the mutants of Phe298 and Gln317, which are located at the β -sheet near the FMN and identified as the important residues for the structural changes of LOV domain in phototropins². Molecular mechanism of the light-switch in aureochrome will be discussed.

1. Briggs, R. W., et al. *Trends Plant Sci.* **7**, 204-210 (2002).
2. Iwata, T., et al. *BIOPHYSICS* **7**, 89-98 (2011).
3. Takahashi, F., et al. *Proc. Natl. Acad. Sci. USA* **104**, 19625-19630 (2007).

2F1448 LOV ドメインを鋳型としたレドックス感受性蛍光タンパク質の開発 Construction of LOV-based redox-sensitive fluorescent proteins

Yukiko Ono¹, Tatsuya Iwata², Hideki Kandori¹ (¹*Nagoya Inst. Of Technol.*, ²*Ctr. Fost. Yng. Innov. Res., Nagoya Inst. Tech.*)

LOV domain is a blue-light sensor domain that was first found in phototropin. LOV domain binds a flavin mononucleotide (FMN) as a chromophore, and its sensor function is initiated by the adduct formation with an S-H group of Cys. It was proposed that the C/A-based mutant LOV domains (iLOV) could be better fluorescent probe than GFP because of smaller size [1]. In such case, highly fluorescent iLOV proteins are desired, where the oxidized FMN emits green light. It should be however noted that FMN is a redox active cofactor, and photoreduction of the oxidized form causes fluorescence quenching. In other words, this fact suggests that redox-sensitive fluorescent proteins may be designed from the C/A mutants of LOV domains. The purpose of this research is to construct redox-sensitive probes using random mutagenesis into Arabidopsis phot2-LOV2 C426A mutant. The candidate plasmids were transformed into JM109 E. coli cells. The bacteria were grown in liquid media in the dark, and the fluorescence quenching by light illumination was used as a screening method to find desired proteins. Consequently, we found a mutant showing faster photoreduction and lower fluorescence level compared to C426A. Another mutant showed slower photoreduction than C426A. Interestingly, the mutated amino acid residues were distant from FMN. Highly redox-sensitive fluorescent proteins are searched by this method, and the molecular properties of the obtained mutant proteins will be discussed.

- [1] Chapman S et al. *PNAS USA* **105**, 20038 (2008)

2F1510 クラミドモナス由来の全長フォトトロピンが示す光反応ダイナミクスの時間分解検出

Time-resolved study on photoreaction dynamics of full-length phototropin from *Chlamydomonas reinhardtii*

Yusuke Nakasone¹, Koji Okajima², Yusuke Aihara³, Akira Nagatani³, Satoru Tokutomi², Masahide Terazima¹ (¹*Grad. Chem. Sci., Kyoto Univ.*, ²*Grad. Sci., Osaka Prefecture Univ.*, ³*Grad. Biol. Sci., Kyoto Univ.*)

Phototropin (phot) is a blue-light dependent kinase which regulates phototropism of higher plants. It contains two LOV domains (LOV1 and LOV2) as light sensor domains and adduct formations between their chromophores and Cys residues upon light illumination are essential for the signal transduction. So far, biophysical studies of phot have been restricted to shorter fragments such as the LOV domains, LOV2-linker, and the LOV1-LOV2 tandem protein, because of the difficulty of purification of the full-length protein. Kinase activation of phot has been studied in vitro by autophosphorylation assays, which, however, did not provide any information about the signal transfer to the kinase after the activation of LOV domains. In this study, we used full-length *Chlamydomonas* phototropin (Cr-phot), which regulates sexual differentiation. In order to understand the mechanism of the kinase activation, we have investigated the reaction dynamics of full-length Cr-phot by using the transient grating (TG) method. The TG signal showed that the adduct formation was completed within few microseconds, which is similar to the case of shorter constructs. Additionally, we have detected global conformational changes of protein moiety in milliseconds time scale as a decrease of diffusion coefficient. CD measurements also showed that the secondary structure was partially changed upon light illumination. We will discuss the photochemistry of intact protein on the basis of these findings.

2F1522 N 末アミノ酸残基の有無は AppA BLUF ドメインの保存されたトリプトファン残基の位置に影響しない

N-Terminal Truncation Does Not Affect the Location of a Conserved Tryptophan in the BLUF Domain of AppA from *Rhodobacter sphaeroide*

Masashi Unno¹, Yuuki Tsukiji¹, Kenshuke Kubota¹, Shinji Masuda^{2,3} (¹*Depart. Chem. Appl. Chem., Saga University*, ²*Center for BioRes. & Inform., Tokyo Inst. Tech.*, ³*PRSTO, JST*)

The flavin-binding BLUF domains are a class of blue-light receptors, and AppA is a representative of this family. Although the crystal and solution structures of several BLUF domains have already been obtained, there is a key uncertainty

regarding the position of a functionally important tryptophan (Trp104 in AppA). In the first crystal structure of an N-terminally truncated BLUF domain of AppA133 (residues 17-133), Trp104 was found in close proximity to flavin (Trp_{in}), whereas in a subsequent structure with an intact N-terminus AppA126 (residues 1-126), Trp104 was exposed to the solvent (Trp_{out}). A recent study compared spectroscopic properties of AppA126 and AppA133 and claimed that the Trp_{in} conformation is an artifact of N-terminal truncation in AppA133. In this study, we compared the flavin vibrational spectra of AppA126 and AppA133 by using near-infrared excited Raman spectroscopy. In addition, the conformations as well as the environments of Trp104 were directly monitored by ultraviolet resonance Raman spectroscopy. These studies demonstrate that the N-terminal truncation does not induce the conformational switch between Trp_{in} and Trp_{out}.

2F1534 Substrate-Dependent Measurement Revealed Different Protein Conformations on Photoactivation and DNA Repair of *E. coli* CPD Photolyase

I Made Mahaputra Wijaya¹, Yu Zhang¹, Junpei Yamamoto², Kenichi Hitomi³, Shigenori Iwai³, Elizabeth D. Getzoff³, Hideki Kandori¹ (¹Nagoya Institute of Technology, ²Osaka University, ³The Scripps Research Institute, La Jolla, USA)

Photolyases (PHRs) are flavoproteins that repair UV-induced damaged DNA by the use of near UV/blue light. PHR that repair cyclobutane pyrimidine dimers (CPDs) and (6-4) photoproducts are called CPD-PHR and (6-4) PHR, respectively. In order to study protein-mediated chemical reactions on PHRs, light-induced difference FTIR spectroscopy was applied. Previously, we have successfully measured FTIR difference spectra of the light-induced activation and DNA repair of *X. laevis* (6-4) PHR [1] and *E. coli* CPD-PHR [2]. CPD-PHR can repair not only cyclobutane thymine dimer, but also cyclobutane cytidine dimer and dimers from combination thymine-cytidine and vice versa. In the present study, we introduced those different dimer compositions as DNA substrates and observed FTIR light-induced activation and DNA repair spectra of CPD-PHR. From these substrate-dependent measurements, we successfully observed different signals of peptide backbone, α -helices, and β -sheets of the enzyme, suggesting different protein conformations occurred during photoactivation and photorepair, which depend on the substrates. In addition, we also successfully observed difference spectra on binding of each different substrate to CPD-PHR enzyme. Enzyme conformations difference of photoactivation, photorepair, and substrate-enzyme binding will be discussed on the basis of the spectral comparison among each substrate.

[1] Zhang et al. *Biochemistry* 50, 3591 (2011)

[2] Wijaya et al. to be submitted

2F1546 アニオンラジカル型の(6-4)光回復酵素による DNA 修復 Repair of damaged DNA by the anion radical form of (6-4) photolyase

Daichi Yamada¹, Yu Zhang¹, Tatsuya Iwata², Junpei Yamamoto³, Kenichi Hitomi⁴, Shigenori Iwai³, Elizabeth Getzoff⁴, Hideki Kandori¹ (¹Nagoya Inst. Tech., ²Ctr. Fost. Yng. Innov. Res., Nagoya Inst. Tech., ³Grad. Sch. Eng. Sci., Osaka Univ., ⁴The Scripps Res. Inst. USA)

Photolyases (PHRs) are repair enzymes of UV-damaged DNA by utilizing near-UV/blue lights, which maintain genetic integrity by reverting UV-induced photoproducts into normal bases. The FAD chromophore of PHRs has four different redox states: oxidized (FAD^{ox}), anion radical (FAD⁻), neutral radical (FADH[•]) and fully reduced (FADH⁻) forms, and the enzymatically active state of PHR is FADH⁻. Mechanism of photoactivation from FAD^{ox} to FADH⁻ by proton-transfer coupled electron transfer through FAD⁻ and FADH[•] is intriguing, and we recently obtained difference FTIR spectra among four redox states of *Xenopus* (6-4) PHR by selecting temperature and illumination conditions [1]. According to the FTIR analysis, the protein structure of the FAD⁻ form trapped at 200 K is very similar to that of the FADH⁻ form, suggesting that FAD⁻ might have the DNA repair activity. To test this hypothesis, we attempted to stabilize the FAD⁻ state at room temperature, by introducing the N392C mutant that mimics insect cryptochromes and cyanobacterial cryptochrome mutant [2, 3]. The FAD⁻ form is observed even at room temperature, and FTIR spectroscopy detects possible photorepair signals of (6-4) photoproduct by the FAD⁻ form. Activation and repair mechanism of the N392C mutation of (6-4) PHR will be discussed on the basis of the present spectroscopic observations.

[1] Yamada D. et al, submitted.

[2] Kao Y.-T. et al, *J. Am. Chem. Soc.* 130, 7695-7701 (2008).

[3] Iwata T. et al, *Biochemistry* 49, 8882-8891 (2010).

2F1558 光センサータンパク質 UVR8 の解離・回復反応カINETICSS の測定

The study of the dissociation and recovery reaction kinetics for photo-sensor protein UVR8

Takaaki Miyamori¹, Yusuke Nakasone¹, Kenichi Hitomi², John M. Christie³, Elizabeth D. Getzoff², Masahide Terazima¹ (¹Grad. Sci. Chem., Univ. Kyoto, ²Scripps Research Inst., ³Univ. Glasgow)

Plants can perceive and respond to UV-B in order to protect them from UV-light. UVR8 from *Arabidopsis* has been found as a UV-light sensor protein, which mediates gene expression responses to establish the UV protection. Dimers of UVR8 are dominant under dark condition in the living cells. Doubly hydrogen-bonded salt bridges, which link R286 with D107 and R146 with E182 is thought to make the dimer stable. Upon UV-B light illumination, the dimer dissociates into the monomer, and this dissociation reaction is believed to be important for the biological function. Generally, light sensor proteins possess specific chromophore molecules to absorb light and initiate photochemical reactions for signal transduction. Interestingly, however, the UVR8 protein employs Trp residues, particularly W285 to sense the environmental light condition instead of having such specific chromophores. We investigate the kinetics of its dissociation reaction by the time-resolved transient grating method (TG method). In order to examine the importance of above key residues (R286, R146, W285), we performed the same kinetic measurements for the following mutants; R146A/R286A and W285F. Furthermore, the dark recovery reaction (dimerization) was studied by the circular dichroism method (CD method).

2G1400 膜融合におけるストーク構造からの変形：分子動力学シミュレーション Morphology change of fusion stalk studied by molecular dynamics simulation

Shuhei Kawamoto, Wataru Shinoda (*Nano. AIST*)

The life cycle of living organisms involves membrane fusion. In eukaryotes, membrane fusion is ubiquitous. As a plausible fusion pathway, the stalk-pore pathway has been identified and explored theoretically. However, stability of transiently appeared morphologies and driving force of the morphology change are not clear yet. We have investigated the fusion pore formation process from the stalk structure by coarse-grained molecular dynamics simulations. We found that the stalk was stabilized when the lipid membranes contain phosphatidylethanolamine (PE), which are more likely found around the stalk. By applying tension selectively on the proximal leaflet, we observed the progress of pore formation. The energy required for the fusion pore formation is estimated as about 50 $k_B T$, which is close to that obtained from the previous theoretical study.

2G1412 蛍光検出と電気計測による膜タンパク質計測に向けた脂質膜チャンバレイ

Lipid bilayer chamber array toward fluorescent and electrophysiological measurement of membrane proteins

Taishi Tonooka¹, Ryuji Kawano², Koji Sato¹, Toshihisa Osaki², Shoji Takeuchi¹ (¹Institute of Industrial Science, The University of Tokyo, ²Kanagawa Academy of Science and Technology)

Artificial lipid bilayer chamber, which is a small chamber sealed with lipid bilayer, is an attractive tool for membrane protein analysis because of its purity. Previous studies of artificial lipid bilayer chambers for membrane protein analysis had been focused on two main streams: one was for fluorescent measurement, the other for electrophysiological measurement. Recently, the devices for two-way measurement of fluorescent imaging and electrophysiological recording have been developed. These devices allow us to combine the information of channel conformational change resulting in electrical signals and molecular interaction between channels and ligands. Therefore, they have potential to demonstrate novel membrane protein properties. Here, we propose an array system leading to multi-channel two-way measurement as a next challenge for high-throughput membrane protein analysis. In this study, we successfully demonstrated the function of the pore-forming membrane protein, α -hemolysin, by electrophysiological measurement, and succeeded in Ca^{2+} ion concentration measurement in the chambers by using Ca^{2+} ion fluorescent indicators. We believe that this technique will be a strong tool for basic research or drug screening of membrane proteins.