1P235 センシロードプシントランスデューサーの一分子 FRET 観察
Single-molecule FRET study of the sensory rhodopsin-transducer
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Sensory rhodopsin I, SRI, is a dual photo-receptor membrane protein regulating both positive- and negative phototaxis in collaboration with its cognate transducer protein, HtrI. Here we used single-molecule fluorescence-resonance-energy-transfer (FRET) method to observe conformational changes and structural fluctuations in the complex. We designed the fusion complex of SRI and HtrI. The protein was labeled with dyes, which are expected to work as FRET-donor and the acceptor. We obtained the fluorescence signals from the labeled SRI-HtrI by illuminating with evanescent wave produced by the total internal reflection of the laser beam. As the result, single-molecular FRET between SRI and HtrI was successfully observed by this system. Its implications will be discussed.

1P236 海洋緑藻 Ostreococcus tauri 由来の光修復酵素 (CPF1, CPF2) における FAD を介した光反応中心の分光解析
Spectroscopic analysis of FAD photoreaction center in two photolyase (CPF1, CPF2) from a marine green alga
Ostreococcus tauri

Cryptochrome/Photolyase-Family (CPF) is widely distributed from bacteria to plant and animal kingdoms. Generally, photolyase binds two chromophores, a flavin adenine dinucleotide (FAD) and a N5,N10-methylenyl-5,6,7,8-tetrahydrofolate (MTHF) non-covalently. Recently, five CPF genes were identified in marine green alga Ostreococcus tauri (Ot). It was reported that Ot_CPF1 and Ot_CPF2 have (6-4)-photolyase activity cyclobutane pyrimidine dimer (CPD)-photolyase activity, respectively. We measured photochemistry and structural change of the chromophores using UV-Vis absorption and circular dichroism (CD) spectrocopies, respectively. In comparison of these results, the difference in MTHF binding and FAD conjugation between Ot_CPF1 and Ot_CPF2 will be discussed.

1P237 光修復酵素のDNA修復能と光反応中心FADのコンフォメーションとの関相
Correlation of DNA repair type with FAD conformation in the photoreaction center of photolyases

Far-UV light produces two major DNA photoproducts, cyclobutane pyrimidine dimer (CPD) and pyrimidine-pyrimidone (6-4) photoproduct. Most organisms have two photolyases to repair these photoproducts using near UV and blue light. They cannot repair different types of photoproduct each other. Both binds a flavin adenine dinucleotide (FAD) non-covalently in the photoreaction center. We studied the FAD conformations of a variety of photolyases with circular dichroism (CD) spectroscopy and found that CPD- and (6-4)-photolyases show a negative and a positive CD signal of FAD in its fully reduced form, respectively.

1P238 チャネルロドプシンの機能理解への理論的アプローチ
Theoretical approach toward an understanding of molecular functions of channelrhodopsin
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Channelrhodopsins (ChRs), light-gated cation channels, were originally found in green algae where they serve as sensory photoreceptors for photophobic response. Recent establishment of ChR expression in mammalian neurons enables optical control of nerve impulse on intact brain. We succeeded in structural modeling for unknown structures of ChRs with theoretical computations. Then, we attempted to describe their functional mechanism to extend optogenetic toolbox in combination of the computational model and a later published x-ray structure at the atomic level. On Basis of the computational analysis, we present several structural characteristics that are highly involved in the channel function including ion selectivity, photocycle kinetics and color-tuning mechanism.

1P239 Photoactive Yellow Protein におけるアルギニン 52 のプロトン化状態
Protonation State of Arginine 52 in Photoactive Yellow Protein
Kenji Yonezawa, Hironari Kamikubo, Keito Yoshida, Yoichi Yamazaki, Mikio Kataoka (Grad. Sch. Mat. Sci., NAIST)

We have revealed the deprotonated R52 and low barrier hydrogen bond (LBHB) near the chromophore in photoactive yellow protein. We also revealed that the R52 of the LBHB lacked E46Q is protonated. Based on the results, we consider that the formation of LBHB is coupled to the deprotonation of R52. In this study, the effect of the mutation on the IR bands is observed to examine the protonation state of R52, in order to further confirm our hypothesis. The difference spectra between 15N labeled R52 and unlabeled PYP’s showed substantial differences between WT and E46Q. The difference comes from their dark states, suggesting that the protonation state of R52 at the dark state is different between WT and E46Q. The assignment of the bands will be discussed.

1P240 PYP-Phytochrome Related Protein の 2 つのセンサードメインで生じる光反応の調節性
Relationship of the photoreactions between two sensor domains in PYP-Phytochrome Related Protein
Keito Yoshida, Hironori Kamikubo, Kento Yonezawa, Yoichi Yamazaki, Mikio Kataoka (Grad. Sch. Mat. Sci., NAIST)

PYP-Phytochrome Related Protein (Ppr) is comprised of two light sensor domains, PYP and Bph, and a His-kinase domain. The PYP and Bph domains show similar photoreactions to Hh PYP and other bacterial phytochromes, respectively. In order to reveal the physiological roles of two photosensor domains, it is essential to understand the relationship of their photoreactions. We examined the blue-light induced photoreaction of holo-holo Ppr with and without red-light pre-irradiation. While an M-like intermediate was accumulated in PYP without pre-irradiation, an L-like intermediate was observed with pre-irradiation. Solution structural changes are also different. We conclude that the photoreaction of Bph controls the photo-induced domain rearrangement of Ppr.