- 3P028 時間領域テラヘルツ分光によるバクテリオロドプシンの低振動スペクトル
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Low-Frequency Spectrum of Bacteriorhodopsin Studied by Terahertz Time-Domain Spectroscopy
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When proteins express their functions, large conformational changes often occur. These conformational changes result from collective motions of a large number of atoms. Such motions of proteins have characteristic frequencies in the low-frequency region below a few tens of wavenumbers. In this work we have measured the low-frequency spectra of bacteriorhodopsin (BR) in the ground state using terahertz time-domain spectroscopy (THz-TDS) to investigate relation between the low-frequency motions and expression of their function. BR is a light-driven proton pump protein, and photoisomerization of retinal activates a photocycle. Therefore, in the ground state BR does not express its function. In a future plan we aim to measure the THz spectra of the M intermediate state in the photocycle, where it is reported that numerous conformational changes occur. One of the key issues in this experiment is to prepare a uniform sample. We prepared several samples in different methods and compared their THz spectra. One method was to add a small amount of glycerol to obtain a uniform film sample. Another was to prepare a pellet sample after drying BR. From the obtained THz spectra we calculated a physical quantity which is proportional to vibrational density of states (VDOS) in the low-frequency region. It was found that the VDOS of all the BR samples show power-law behavior, and the exponents are close to two. From the observation we discuss the low-frequency dynamics and couplings among the low-frequency motions.

- 3P030 細胞性粘菌アクチンの部位特異的変異を用いたアクチンSSダイマーの研究
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A study on actin SS dimer by site-directed mutagenesis of *Dictyostelium* actin.
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As reported previously (Ishiwata, S., 1976), the freeze-thawing of actin solution induced the oxidation of sulfhydryl groups, which results in the formation of an intermolecular disulfide bond to yield an actin dimer (SS dimer); we showed the evidence that at least the C374 is involved in this SS bond. The SS dimer has anomalous physico-chemical properties and a different conformation from normal F-actin when it is polymerized. To identify which sulfhydryl group of actin monomers is involved in the intermolecular SS binding, we here focused on cysteine residues of *Dictyostelium* actin, and genetically engineered one of them (C258) by site-directed mutagenesis. Two mutant *Dictyostelium* actins (E360H & C258A/E360H) were prepared because the E360H mutation was needed as a tag to separate the mutant actin from endogenous wild-type actin by using an anion-exchange column chromatography (Saeki, K. et al., 1996), and performed freeze-thawing. As a result, while the E360H actin formed SS dimer as well as rabbit-skeletal native actin or *Dictyostelium* wild-type actin, the C258A/E360H actin did not form SS dimer. These results strongly suggest that the C258 of *Dictyostelium* actin is oxidized and involved in an intermolecular SS binding, so that the SS bond is formed between C374 and C258. We plan to prepare the actins of genetically engineered with other cysteine residues, C18, C286, and C374, and make sure this conclusion.

- 3P029 トランスサイレチン変異体の細胞毒性
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Cytotoxicity of transthyretin variants
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Transthyretin (TTR) is the major component of amyloid deposits in human tissue that putatively cause a wide range of amyloid diseases, including senile systemic amyloidosis (SSA), familial amyloid polyneuropathy (FAP), familial amyloid cardiomyopathy (FAC), and central nervous system-selective amyloidosis (CNSA). SSA is a late-onset sporadic disease associated with amyloid deposition of wild-type TTR. On the other hand, the familial TTR amyloidoses (FAP, FAC and CNSA) result from tissue-selective deposition of one of over 100 TTR variants. Although it is not known whether the protein aggregates are involved in the disease directly, recent studies have suggested that pre-fibrillar aggregates or spherical aggregates rather than mature fibrils are toxic to neuronal cells. In FAP caused by the L55P mutation, non-fibrillar aggregates accumulate in nerve tissues before development of amyloid depositions and nerve degeneration. We have examined the cytotoxic effects of seventeen different TTR variants including V30M and L55P. Some of the variants exerted the cytotoxic effect on neuroblastoma cells, but others did not. Cytotoxic effect of TTR variants did not correlate with tendency of aggregation. The cytotoxic species of TTR variants will be discussed.

- 3P031 哺乳類のキサンチン脱水素酵素とバクテリア由来のキサンチン脱水素酵素との間における動的構造の差異
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Difference in the dynamic structure between mammalian and bacterial xanthine oxidoreductases
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Xanthine oxidoreductase (XOR) is an enzyme, found in a wide range of organisms from bacteria to man, which catalyzes the hydroxylation of xanthine to uric acid. The XOR is a target of drugs against gout and hyperuricemia. The recently introduced inhibitors of the mammalian enzymes, TEI-6720 [1] and FYX-051 [2], have been reported to display potent structure-based inhibitors that fill the substrate channel almost fully to obstruct the approach of the substrate to the catalytic site of the molybdenum cofactor. However, these compounds do not inhibit at all even at 10⁻⁶ M concentration the XOR from *Rhodobacter capsulatus*, although the three-dimensional structure of bacterial XOR is very similar to that of the bovine milk XOR, and all the important amino acid residues are conserved in the active site molybdenum channels of these XORs.

To clarify the cause of the difference in behavior between the bacteria and mammalian XORs, we investigated their fluctuations and correlated motions using dihedral angle normal mode analysis [3]. We found, only for the mammalian XOR, significantly correlated motions among several amino acid residues around the substrate channel, which would favor an inhibitor to interact with the mammalian XOR. In this meeting, we discuss a detailed mechanism of inhibition by TEI-6720 & FYX-051 for the two XORs.

[1] Okamoto et al., J. Biol. Chem. 278 (2003) 1848.

[2] Okamoto et al., Proc. Natl. Acad. Sci., USA, 101(2004) 7931.

[3] Kikuchi et al., Biophys. J. 79 (2000) 1587.