F1-ATPase is a rotary motor in which the central γ subunit rotates inside a stator ring made of α subunits. ATP hydrolysis in the β subunit generates torque for γ rotation by a mechanism not fully understood. The γ rotor consists of a globular domain that protrudes from the stator and a slender shaft that penetrates the αβ3 ring. The shaft is an anti-parallel coiled coil of the N- and C-terminal helices of γ. Previously we have deleted either or both of these terminal helices genetically. Surprisingly, all mutants rotated in the correct direction, showing that the shaft portion is dispensable. Here we inquire if the rest of the γ rotor, the globular protrusion that accounts for ~70% of the γ residues, is also dispensable. Keeping the N- and C-terminal helices that constitute the shaft, we have replaced the middle ~200 residues with a short helix-turn-helix motif borrowed from a different protein. Furthermore, 14 residues of the remaining N- and C-terminal helices have been replaced with unrelated residues such that the resulting construct and the previous shaft-truncated mutants (ΔC36 and ΔN29) that generated ~half the wild-type torque do not share common residues. This construct retained a high ATPase activity and rotated fast in the correct direction, generating a sizable torque. We conclude that none of the residues of the rotor are needed for the generation of finite torque.

3A1058 イソサイクリプティコン・スターチースのイクソディア活動 \[ \begin{align*} &\text{Detection of steps of Mycoplasma mobile gliding ghost} \\
&\text{Yoshiaki Kinoshita, Daisuke Nakane, Kana Mizutani, Makoto Miyata, Takayuki Nishizaka} \\
&\text{Mycoplasma mobile is a pathogenic flask-shaped bacterium about 0.8 μm long and has prominent gliding activity, on the glass surface coated with siacids at velocities of 2.5 μm/sec. Previous studies showed that the gliding motion is driven by many legs which repeatedly catch and release siacids on the glass surface, accompanied by ATP hydrolysis. To comprehend a unique mechanism of the gliding machinery, the step size and the kinetics per one ATPase cycle should be clarified. Nakane et al. have shown that the living M. mobile takes 85-nm step during gliding. Next challenge is to elucidate the kinetics of ATP hydrolysis in the stepwise motion. Here we performed the improved motility assay by using "gliding ghosts" of which the cell membrane was permeabilized by triton X-100. The ghosts allow us to control the gliding speed by modifying ATP concentrations in solution. The cell membrane was stained with Cy3 so that we could observe the gliding motility at the time resolution of 2 ms without a huge probe. Furthermore, to reduce the number of the legs contributing the gliding motion, free siacic acids was added to the assay buffer. Through these improvements we successfully observed the stepwise motion of the ghosts. A parwise distance analysis showed that the M. mobile gliding ghosts took 60-100 nm steps, which are consistent with that of the living cell. Michaelis-Menten constant and Vmax were 59 μM and 2.5 μm/sec. Now we are analyzing the dwell time.} \end{align*} \]

3B9090 原核生物由来ナトリウムチャネルにおけるC末端4-リリースパドル ティンダル素を用いたゲーティング制御 \[ \begin{align*} &\text{The cytosolic C-terminal four-helix bundle regulates the gating of prokaryotic sodium channel} \\
&\text{Katsumasa Irie, Takushi Shimomura, Yoshinori Fujiyoshi (CeSPL, Univ. Nagoya)} \\
&\text{Voltage-gated sodium channels play essential roles in many important physiological processes, including electric signalling and muscle contraction. Therefore their activation and inactivation are needed to be strictly regulated. Here, we show that the cytosolic C-terminal region of Nav1.5, a prokaryotic voltage-gated sodium channel cloned from Sulfitobacter pontiacus, accelerates the inactivation rate and keeps the voltage dependent activation positively. The crystal structure of the C-terminal region of Nav1.5 grafted into the C-terminus of Nax channel at 3.2 Å resolution revealed that the C-terminal region forms a four-helix bundle. Point mutations of the residues of the four-helix bundle destabilised channel tetramer and reduced the inactivation rate. The four-helix bundle was directly connected to the inner helix of the pore domain. The increase of the rigidity of the inner helix with the glycine-to-alanine mutation also reduced the inactivation rate, but did not destabilize the tetrameric channel. Moreover, all of these mutants shifted the voltage dependent activation negatively, which indicates that the four-helix bundle suppress the opening of the pore domain. These findings suggest that the Nav1.5 four-helix bundle plays important roles not only in stabilising the tetramer, but also in regulating the channel gating by the restriction of the conformational change of the inner helix. The four-helix bundle could reduce excess ion flux after activation and also suppress accidental ion flux even in the resting state.} \end{align*} \]
HypF are involved in the biosynthesis of the nitrile group as a precursor of the cyano groups. HypF catalyzes S-carbamoylation of the C-terminal cysteine of HypE via three steps using carboxyphosphate and ATP, producing two unstable intermediates: carbamoylcarbamoyladenylate. It remains unclear how the consecutive reactions occur without the loss of unstable intermediates during the proposed reaction scheme. We have recently determined the crystal structures of full-length HypF both alone and in complex with HypE at resolutions of 2.0 and 2.6 Å, respectively. Three catalytic sites of the structures of the HypF nucleotide- and phosphate-bound forms have been identified, with each site connected via channels inside the protein. This finding suggests that the first two consecutive reactions occur without the release of carbonate or carbamoyladenylate from the enzyme. The structure of HypF in complex with HypE revealed that HypF can associate with HypE without disturbing its homodimeric interaction and that the binding manner allows the C-terminal Cys351 of HypE to access the S-carbamoylation active site in HypF, suggesting that the third step can also proceed without the release of carbamoyladenylate.

3B0924 Dynamic structural and antigen binding analyses of antibody single-chain Fvs

We generated single-chain Fvs (scFvs) of anti-(4-hydroxy-3-nitrophenyl)acetetyl (NP) antibodies, N1G9 and C6, which are germline and affinity matured IgG1 antibodies. to analyze the change of structural fluctuation upon the antigen binding and through the affinity maturation. We overexpressed the scFvs in E. coli, and refolded by stepwise-dialysis method, followed by purification using NP-conjugated column. The scFv-antigen interactions were analyzed using surface plasmon resonance biosensor, Biacore, in which NP conjugated proteins were immobilized on sensor chip. The equilibrium association constant of C6 scFv for NP was about 10 times higher than that of N1G9 scFv, mainly due to the decreased dissociation rate constant. The kinetic parameters were similar to those of corresponding Fab and intact antibody, indicating that the scFvs are refolded correctly. The structural fluctuations of scFvs were measured using diffracted X-ray scattering (DXT) system at single-molecule level. Upon the binding of NP-C-epsilon caproic acid, the fluctuations were decreased in either N1G9 or C6 scFv, indicating that the antigen binding stabilizes the scFv structure. In comparison of N1G9 and C6 scFvs in the absence of antigen, the fluctuation of C6 was lower than that of N1G9, which could be correlated with the different antigen binding mechanism such as “induced-fit” and “lock-and-key” types. On the advantage of scFv, whose amino acid could be mutated, we try to obtain more quantitative and site-specific information.

3B0936 PYPの光制御機能の観点からの制御
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Proteins are formed from polypeptides by folding into the specific conformations, by which the physiological roles of the proteins are determined. The structure of the protein closely correlates with the physiological activity, and the activity of the protein is regulated by conformational change. For example, the receptor proteins alter their conformations in response to the stimuli to achieve the cellular transaction system. Thus, if the protein conformation is artifically regulated, one would control the protein activity. Here we tried to establish the technique to regulate the protein activity by changing the conformation. Photactive yellow protein (PYP), a bacterial blue-light sensor protein, was used as the model protein. Two amino acid residues present in the surface of PYP molecule were replaced by cysteines and crosslinked by an azobenzene linker, whose length is reversibly changed by trans/cis photoisomerization. We found that the velocity of photocycle was varied by 10-100 times by the isomerization of azobenzene when Met100 loop and Pca loop were crosslinked. Since the global protein conformational change involving Met100 loop takes place in the photocycle, it is likely that the protein conformational changes were perturbed by the isomerization of azobenzene linker. This technique is possibly applied to switch the enzymatic activity of the protein by light.

3B0948 αカテーテンによるアクトミオン反復の阻害
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α-catenin is known as a linker protein between cell adhesion and the actin cytoskeleton. It has been believed that monomer of α-catenin forms a complex with β-catenin and E-cadherin in cell-cell adhesions and plays an essential role as an anchor, directly or indirectly, of E-cadherin-mediated adhesions and cytoskeletal F-actin networks. Dimer of α-catenin, which is formed by the dissociation from the complex, binds to F-actin with μ affinity and bundles it, locally inhibiting the Arp2/3 complex. This reorganization of actin dynamics decreases membrane activity at sites of cell-cell contact and stabilizes cadherin-mediated cell-cell adhesion. In addition, inhibition studies of myosin ATPase activity suggested that the intracellular actomyosin contractile force also contributes to the assembly and extension of adhesions. In present study, we report that α-catenin inhibits actomyosin contraction in the dose-dependent manner, which was characterized by an in vitro F-actin gliding assay with myosin II HMM. Furthermore, no direct significant association between α-catenin and myosin II was observed in immunoprecipitation assay, indicating that α-catenin may compete with myosin II for binding to actin, resulting in a decrease in the number of myosin heads able to propel actin filaments, or may indirectly, through an actin filament, inhibit myosin activity. Herein, we will discuss how α-catenin inhibits actomyosin contraction and the implications of that in maintaining adhesions.

3B1010 ダイナモーダモデルの溶液中の分子動力学シミュレーション
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Dyneins are large microtubule motor proteins that play important roles in various biological movements. Cytoplasmic dynein is responsible for cell division, cell migration and other basic cellular functions. The motor domain of dynein consists of a ring-shaped six ATPases called AAA+ modules. Recently, an ATP-bound high-resolution structures of cytoplasmic dynein have revealed the organization of the motor domain that comprises the AAA+ ring, the linker, stalk/strut and C sequence (PDB IDs = 3vk8, 3vk9). However, the high-resolution structure of an ATP-bound dynein remains unclear. Here, we carried out molecular dynamics (MD) simulations of both ATP and ADP-bound forms to examine their structures and dynamics.

We built initial structures for MD as following. A higher resolution structure (3vk9), which is a truncation mutant, was chosen. Then, we modeled missing residues and added a truncated domain from the wild type structure (3vk8). Four ATP molecules were placed to their original positions in the ADP bound form. One of the ATP molecules, bound to the main ATPase site (AAAx), was replaced to ATP in the ATP bound form. A rectangular water box was placed around dynein. Finally, the systems consisted of approximately one million atoms. Electrostatic interactions were treated with zero-dipole summation method, and their computation was accelerated using GPGPU. We will discuss the effect of ATP on the structure and dynamics of dynein by comparing the trajectories between the ADP- and ATP-bound forms.

3B1022 A computational investigation into the MHC-I Recognition Mechanism of MIR2 from Kaposis Sarcoma-Associated Herpesvirus
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Kaposi's sarcoma-associated herpesvirus, a human tumor virus, encodes two membrane-bound E3 ubiquitin ligases, modulator of immune recognition 1 (MIR1) and MIR2, to evade host immune system through the ubiquitination-mediated endocytosis and lysosomal degradation of the proteins involved in immune recognition. MIR2 downregulates the surface expression of MHC I (major histocompatibility complex class I), co-stimulatory proteins (B7-2, ICAM-1), and platelet endothelial cell adhesion molecule 1 (PECAM-1). Recent