tural motif between each subunit. In particular, the D subunit, which is main component of the central stalk in V_1 -ATPase, shows apparently more straight conformation than the γ subunit of F_1 -ATPase. This difference can influence on the generating and transmitting torque during the rotation. Moreover, little conformational differences are observed among the three catalytic A subunits of V_1 -ATPase. In contrast, quaternary changes around nucleotide binding sites are quite similar to both the enzymes. Therefore, the common property between V_1 - and F_1 -ATPases is only in the subunit interfaces around the active sites, strongly suggesting that the rotation of V_1 -ATPase is primarily driven by the quaternary changes around the interface of nucleotide binding sites.

2P007 大腸菌由来スperlミジンアセチルトランスフェラーゼの X 線結晶構造解析
Crystal structure of the *Escherichia coli* spermidine acetyltransferase in complex with spermidine and coenzyme A

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Polyamines such as spermidine, putrescine, and spermine are essential for cell growth. Intracellular polyamine levels are regulated by a variety of metabolic pathway such as biosynthesis, degradation, uptake, and excretion. Spermidine acetyltransferase (SAT) is a key enzyme in the control of polyamine levels in procaryotic cells. SAT transfers the acetyl group from acetyl-CoA to either the N-1 or N-8 position of the spermidine to produce acetylspermidine and CoA, thereby reducing the intracellular polyamine level. Although the physiological role of SAT has been extensively studied, it remains to be investigated how the enzyme catalyze and how the activity of the enzyme is regulated at the molecular level.

To determine the crystal structure of SAT, the recombinant SAT was over-expressed and purified from *Escherichia coli*. The purified enzyme was crystallized with the sitting-drop vapor-diffusion method in the presence of spermidine and CoA. X-ray diffraction data were collected by using beamline BL41XU of the SPring-8 synchrotron source. The crystal structure of SAT in ternary complex was determined at 2.5 Å resolution. The SAT complex is a dodecamer made up of six dimers. Interestingly, a spermidine molecule is bound approximately 13 Å apart from the CoA binding site in our crystal structure. The spermidine is located dimer-dimer interface, whereas CoA binds to the active site of each monomer. We employed structure-based mutagenesis and biochemical analysis to elucidate the molecular mechanism of SAT.

2P008 蛋白質の ω -ヘリックス
Omega-helices in Proteins

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A modification of the α -helix, termed the omega-helix, has four residues in one turn of a helix. We searched the ω -helix in proteins by the HELFIT program which determines the helical parameters - pitch, residues per turn, radius, and handedness - and $p = \text{rmsd} / (N - 1)^{1/2}$ estimating helical regularity, where "rmsd" is the root mean square deviation from the best fit helix and "N" is helix length. A total of 1,496 regular ω -helices 6 to 9 residues long with $p \leq 0.10$ Å were identified from 866 protein chains. The statistical analysis provides a strong evidence that the frequency distribution of helices versus n indicates the bimodality of typical α -helix and ω -helix. Sixty-two right handed ω -helices identified (7.2% of proteins) show non-planarity of the peptide groups. There is amino acid preference of Asp and Cys. These observations and analyses insist that the ω -helices occur really in proteins.

2P009 *Halomonas* sp.593 由来ヌクレオシドニリン酸キナーゼ (HaNDK) の多量体構造
Oligomeric structure of nucleoside diphosphate kinase from *Halomonas* sp.593 (HaNDK)

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The nucleoside diphosphate kinases (NDKs) are known to have a tetrameric or hexameric oligomer structure formed by association of common dimeric components. In order to understand the oligomeric structure of NDK, the

wild-type NDK from *Halomonas* sp. 593 (HaNDK) was crystallized into two different space groups, R3 and C2, and the crystal structures were determined by X-ray crystallography to 2.4 Å and 2.9 Å resolution, respectively. In both crystal forms, the wild-type HaNDK only exhibited a dimeric form that is the same as a common dimer unit seen in other NDKs, but neither tetrameric nor hexameric assemblies were observed. This is the first evidence of a dimeric structure for HaNDK. To explore the effect of mutation on the assembly form of HaNDK, E134, which is a key interface residue for other tetrameric NDKs, was replaced with alanine, and the crystal structure was determined by X-ray crystallography. Two kinds of tetrameric assemblies, Type I found in the *Myxococcus* NDK tetramer and Type II found in the *E.coli* NDK tetramer, appeared in the asymmetric unit of the E134A mutant HaNDK. The change from dimeric to tetrameric assembly is attributed to the removal of negative charge repulsion caused by the E134 in the wild-type HaNDK, suggesting that Type I is a tetramer which forms in a solution. Change in the assembly form may be an effective way for NDK to acquire molecular characteristics suited to various circumstances.

2P010 X-ray Crystallography of PDZ domains from LNX1, an E3 ubiquitin ligase regulating intercellular adhesion machinery

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A tight junction (TJ) is an intercellular adhesion machinery located in the most apical part of epithelial cells, such as an intestine, as well as endothelial cells in CNS vessels. TJ functions as the impermeable barrier of fluid. Recently, Furuse et al. discovered E3 enzyme, ligand-of-Numb X (LNX) 1, that acts against TJ component protein, claudins (CLDs). We proposed a dynamic equilibrium mechanisms of TJ homeostasis, in which TJ was maintained in an equilibrium between biogenesis and degradation. TJ biogenesis is promoted by ZO-1/2/3, a family of PDZ domains proteins. LNX1 is an E3 enzyme harboring four PDZ domains. Thus, both biogenesis and degradation are likely to depend on the interaction between the PDZ domain(s) to C-termini of CLDs.

Nevertheless, it remains unclear that which one out of the four PDZs of LNX1 is responsible for CLD-recognition. To answer this question, we started structural study of PDZ domains of LNX1.

We succeeded in constructing several protein expression vectors of the PDZs of LNX1. Among them, mouse PDZ2 (mLNX1-PDZ2) showed the highest expression level and solubility. After extensive searching for crystallizing conditions, we obtained a single crystal diffracting at 2.8 Å resolution. Further improvement of the crystal quality is under way. Simultaneously, we are preparing more than ten recombinant peptides of LNX1-binding proteins, which include CLDs, JAMs and ESAM. A mechanism for the molecular recognition of mLNX1-PDZ2 to those junctional related proteins will be discussed.

2P011 X線自由電子レーザーを用いた生体単粒子イメージングにおいて期待される分解能の理論的見積もり
Estimation of attainable structural resolution by computer simulation for single biomolecule imaging with X-ray Free Electron Laser

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New bright light sources by X-ray Free Electron Laser (XFEL) are under construction in Europe, USA and Japan. XFEL can potentially offer a new mean for single biomolecule 3D-imaging. Measured intensity, which is a 2D diffraction pattern per one observation without phase information is extremely low even using XFEL because sample is a single molecule in a single shot and incident intensity cannot make stronger than a certain intensity due to suppress molecule decay. Under this conditions, we consequently have to use extremely weak signals with quantum-noise effectively to reconstruct a 3D molecule structure using multiply measured many 2D patterns.

We have developed a two-step algorithm for reconstructing a 3D diffraction data. Once a 3D data obtained, the 3D molecule structure can be obtained by applying oversampling method. The first step is a classification of 2D patterns into groups according to similarity and an averaging within each group to reduce the noise. The second step is a calculation of mutual location between 2D patterns in the 3D space. The developed algorithm enables us to detect