1P-001 単粒子解析による TRP family 内での channel の構造比較

Structure of TRP channels revealed by single particle analysis using EM images

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Transient receptor potential (TRP) channels comprise a large family of cation channels. In mammals, nearly 30 TRP members have already been found, and are grouped into six subfamilies based on their amino acid sequences (TRPC, TRPV, TRPM, TRPA, TRPP and TRPML). TRP channels have common structural features with voltage-gated cation channels: six transmembrane (TM) segments, a pore-forming region between the fifth and sixth TM segments, cytoplasmic N- and C-terminal regions, and tetrameric subunit stoichiometry. Transient receptor potential melastatin type 2 (TRPM2) is a redox sensitive, calcium-permeable cation channel activated by various signals, such as adenosine diphosphate ribose (ADPR) acting on the ADPRpyrophosphatase (ADPRase) domain, and cyclic ADPR (cADPR). TRPC3 has been widely studied and is involved in various biological processes such as neuronal differentiation, blood vessel constriction, and immune cell maturation. We have recently determined the structure of two different TRP channels: the TRPC3 and TRPM2 channels, using single particle analysis from cryo-EM images. The basic structure of the molecules was shown to be similar: a bell-like shape composed of a relatively small extracellular (or luminal) domain, a protein-dense transmembrane domain, and an expanded cytoplasmic domain. However in detail, the cytoplasmic architectures are quite different from each other and are diversely evolved to their specific physiological functions.

1P-002 ラット肝臓由来 vault の X 線結晶構造

X-ray structure of the vault purified from rat liver

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Vaults are the largest cytoplasmic ribonucleoprotein particles found in numerous eukaryotic species. It has a barrel-shaped structure with two protruding caps and an invaginated waist and is composed of three proteins: the major vault protein (MVP), the vault poly(ADP-ribose) polymerase (VPARP), and the telomerase-associated protein 1 (TEP1) and small untranslated RNA (vRNA). Many different roles have been proposed for vaults since their first discovery in 1986. Several studies suggested that vaults may play an important role in the multidrug resistance (MDR) of cancer cells. Human vRNAs have the ability to bind mitoxantrone, a chemotherapeutic compound, and they may play an important role in the export of toxic compounds. The recent study showed that MVP is rapidly recruited to lipid rafts when human lung epithelial cells are infected with *Pseudomonas aeruginosa*, and vaults may be involved in innate immunity. However, their fundamental cellular function is still unknown. We have determined the X-ray crystal structure of rat liver vault. X-ray structure reveals that vault particle has 39-fold dihedral symmetry and shell is made up of 78 identical MVP chains, and a domain structure suggests a physiological role of the vault.

Reference: Kato K. *et al.* (2008) *Acta Crystallogr D Biol Crystallogr.*, **64**, 525-531.

1P-003 蛋白質のデコイ構造識別関数 DFMAC の原子分解能への拡張

An advanced version of the decoy-discriminating function of DFMAC with atomic resolution

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The structure-discriminating function for native or near-native structures from their decoy structures are useful for such as structure prediction. We have developed a knowledge-based pseudo-potential function of DFMAC, which can discriminate native or near-native structures from their decoys in reasonable accuracy. Since the DFMAC deal with the coarse-grained representation of the structure, the DFMAC was expanded into an advanced version with atomic resolution. In the new function, some components of DFMAC were included, which deal with the distance between two alpha carbons, the relative orientation between two vectors from alpha carbon to pseudo-beta carbon, and main-chain conformation. The additional components, which deal with the distances between two atoms, except hydrogen, the virtual hydration condition, and the electrostatic interaction, were also introduced. After simple tuning of the new function, improvement in discrimination efficiency from the DFMAC was successfully observed. The results of detailed analysis will be presented.

1P-004 GroES アミロイド線維形成初期段階の NMR 測定

NMR measurements of an initial stage of GroES amyloid fibril formation

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Heat shock protein 10 (hsp10) is a member of the molecular chaperones and works with hsp60 in mediating various protein folding reactions. GroES is a representative protein of hsp10 from *Escherichia coli*. Previously, we found that GroES formed a typical amyloid fibril from a guanidine hydrochloride (Gdn-HCl)

unfolded state at neutral pH, and determined the core sequence of this fibril. In this study, we monitored the early stages of amyloid fibril formation using solution NMR spectroscopy. First, we have assigned individual peaks in the proton-nitrogen (HSQC) spectrum of Gdn-HCl denatured GroES uniformly labeled with stable carbon and nitrogen isotope by three-dimensional NMR methods. Then, real-time HSQC measurements were performed under conditions of amyloid fibril formation. Interestingly, while the overall peak intensities gradually decreased during fibril formation, several noticeable instances where the intensities decreased more rapidly, as well as some newly detected peaks denoting increase in intensity were observed and were mapped to specific amino acid residues. This suggested the formation of a soluble intermediate species during mature fibril formation. We are currently studying the relationship between the significantly highlighted amino acid residues detected in HSQC spectra and the fibril core region of hsp10 fibrils determined previously.

1P-005 タンパク質の立体構造予測に向けた新規スコア関数

Novel Scoring Function for Discriminating the Native Fold of a Protein from Misfolded Decoys

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The predicting the native structure of a protein from its amino-acid sequence is a challenging task for modern science. It appears that a breakthrough is not likely to be obtained unless a unique concept that is totally different from the conventional approaches is employed. We developed a new energy function F that is crucially important in arguing the structural stability of a protein:

$$F = (-TS + \xi) / (k_B T_0), \quad T_0 = 298 \text{ K}, \quad k_B: \text{ Boltzmann constant.}$$

The hydration entropy S is calculated using the multipolar model for water and the angle-dependent integral equation theory combined with the morphometric approach. The calculation of S , which is usually much more demanding, is also performed quite rapidly despite of a realistic molecular model for water by using morphometric approach. The ξ corresponds to the sum of the hydration energy and the intramolecular energy when the all the atoms exposed to water is chosen as the standard one. Then, calculation of both terms is quite rapid. Great advantage is that we can avoid uncertainty for the results depending on the force parameter sets. We have tested F as the scoring function for decoy sets of seven proteins, which are provided by B. Park and M. Levitt. It has been found that our scoring function takes the lowest value for the native structure and that the native fold can be discriminated from the misfolded decoy structures with 100% accuracy. We will report the recent results of attempting other decoy sets and how we can discriminate the native fold from a huge number of misfolded structures.

1P-006 結晶構造が結晶化によって誤って得られた構造か否か、簡単に判定する方法

The simple method to estimate crystal structures are caused by crystal packing effects or not

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Although most proteins are naturally found in an aqueous environment, X-ray crystallography, in which a protein is studied within a crystalline environment, has emerged as the principle means of protein structure determination. In general, it has been shown that the overall fold of a protein is the same whether it is in a crystal or in solution. However, several studies showed that local regions, such as side chains and loops at the surface of the protein could have notable differences due to crystal packing effects. For example, Simmerling C. and coworkers studied some crystal conformations of apo HIV protease, one was semi-open form and the other was wide-open form, and showed wide-open structure (PDB:1TW7) was caused by crystal packing effect.

They reported this result by using the molecular dynamics simulation in the crystal and non-crystal environment. Because the system of the crystal environment contains some proteins located in central and neighboring position, it is too huge to simulate.

To evaluate artifacts in crystal structures easily and shortly, we propose the simple method based Gaussian network model. This method can reproduce that the wide-open form of apo-HIV protease is stabilized by crystal packing. Moreover, it takes only ~1 minute to calculate. It should be useful to estimate that the crystal structure is caused by crystal packing effects or not.

1P-007 マルチカノニカル分子力学シミュレーションによる 57 残基蛋白質の構造探索

Multicanonical molecular dynamics simulation of a protein with 57 residues in explicit water

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Multicanonical molecular dynamics (McMD) simulation is a powerful technique to sample conformational space of a protein and can predict the native structure or the structural ensemble of the protein only from the amino acid sequence. We have so far applied the McMD method to various peptides and proved that this technique can reproduce the native structure of the peptides. However, we have not used this technique for proteins longer than 50-residue length. In the current study, we performed conformational sampling of a 57-residue protein by