Our aim here is to suggest better model/theory to describe our assay results. First, we exhaustively searched better protocol to recognize hit compounds more efficiently by focusing solely the energy/score of the best docking mode. It was not successful. Instead one statically calculated value using many docking mode information showed a better result for predicting anti-prion activity. A statistical view of docking for prion protein may be more important than the case of enzyme.

[1] Kuwata *et al.* (2007) *PNAS* 104, 11921-26.

[2] Hosokawa-Muto, Kamatari, Nakamura, Kuwata (in preparation)

3S1-5 レア・イベントとしてのタンパク質ダイナミクスと複合体形成

Protein dynamics as rare event, in connection with complex formation

Akio Kitao (1,2). (1: *Inst Cell Biol Sci, Univ of Tokyo*; 2: *JST, CREST*)

If protein dynamics is examined energetically in close-up view, protein native state should be found to be highly frustrated as commonly seen in other condensed systems. This frustration also appears as multiple conformational substates on protein energy landscape. At room temperature, a protein molecule tends to be trapped around a single substate within Terahertz time range or so but conformational transitions from one substate to another are more likely to occur in slower time range.

In highly frustrated systems, it is expected that relatively weak perturbation can induce conformational change among substates. Also in the process of complex formation of proteins, inter-molecular interaction can play a role as a sort of perturbation and may induce conformational change.

Conformational change between substates separated by relatively high energy barrier should occur as "rare" events, which is expected to be essential to various protein functions including complex formation processes. In this talk, I am going to consider the connection between rare protein dynamics and complex formation.

3S1-6 量子化学計算に基づく創薬研究へのアプローチ - プログラム開発と生体分子への応用 -

Drug discovery based on quantum mechanical calculation: program development and application to a biomolecular system

Takeshi Ishikawa (1). (1: *Division of Prion Research, Center for Emerging Infectious Disease, Gifu University*)

In recent years, ab initio quantum mechanical calculations have been applied to large molecules including biomolecular systems. The fragment molecular orbital (FMO) method, which was developed by Kitaura *et al.*, is one of the most efficient approaches for the quantum mechanical investigation of such molecules. In the FMO method, a target molecule is divided into small fragments and only calculations of the fragments and pairs of fragments are required for evaluating the total energy or the total density. This feature reduces the computational effort significantly and enables us to carry out quantum mechanical calculations of large molecules. Additionally, the inter fragment interaction energy (IFIE) can be obtained in the FMO calculations. The IFIE is known to be very useful for analyzing the interaction in biomolecular systems such as ligand-protein interaction. In this speech, we will show the potential of the FMO method in the field of drug discovery. At the beginning of my speech, the theoretical aspects of the FMO method will be explained. Second, the results of calculations for the prion protein (PrP) complexed with a small molecule will be shown as an illustrative example of application to a real biomolecular system. Finally, we will introduce the drug discovery software developed in our laboratory.

3S2-1 Mg²⁺輸送体 MgtE による Mg²⁺ホメオスタシス機構の構造・機能解析 Structure and Mechanism of the MgtE Mg²⁺ transporter

Motoyuki Hattori (1). (1: *Graduate of School of Bioscience and Biotechnology, Tokyo Institute of Technology*)

The magnesium ion Mg²⁺ is a vital element involved in numerous physiological processes. It remains obscure how Mg²⁺ transporters selectively recognize and dehydrate the large, fully hydrated Mg²⁺ cation for transport. The MgtE family of Mg²⁺ transporters is ubiquitously distributed in all phylogenetic domains, and human homologues have been functionally characterized and suggested to be involved in magnesium homeostasis. Recently we reported the crystal structures of the full-length *Thermus thermophilus* MgtE at 3.5 Å resolution, and of the cytosolic domain in the presence and absence of Mg²⁺ at 2.3 Å and 3.9 Å resolutions, respectively (Hattori, *et al.*, 2007 *Nature*). Based on these structures, we proposed a Mg²⁺ homeostasis mechanism. However, the present resolution of the full-length structure does not allow a detailed discussion on its coordination chemistry and ion its ion selectivity. Moreover, functional analyses are necessary to provide evidence to support the proposed homeostasis mechanism. Here we solved the crystal structure of the full-length MgtE at 2.9 Å resolution. In this structure, we identified new putative Mg²⁺ binding sites. Moreover, a combination of genetic and biochemical techniques demonstrates that the proposed Mg²⁺ homeostasis mechanism, in which the cytosolic domain of MgtE function as a Mg²⁺ sensor regulating the gating of the channel by sensing the intracellular Mg²⁺ concentration.

3S2-2 神経伝達物質トランスポーターホモログに見る膜を介した物質輸送の構造基盤

Structural basis for substrate transport across a lipid bilayer by a neurotransmitter transporter homolog

Atsuko Yamashita (RIKEN Spring-8 Center)

Secondary transporters are integral membrane proteins which catalyze translocation of a wide array of chemical substances across a membrane bilayer utilizing pre-existing ionic electrochemical gradients. LeuT, a sodium-dependent leucine transporter from *Aquifex aeolicus*, is one amenable to high-resolution crystallographic structure analyses as well as detailed biochemical analyses.

LeuT is a stable homolog protein of Na⁺/Cl⁻-dependent neurotransmitter transporters (neurotransmitter sodium symporters; NSS). NSS plays significant roles for termination of synaptic transmission in the central nervous system of vertebrates by uptake of neurotransmitters, including the biogenic amines (serotonin, dopamine, etc.) and amino acids (γ-aminobutyric acid, glycine, etc.), from the synapse to the cytoplasm of neurons and glia. Dysfunction of these transporters causes various psychiatric disorders, such as depression. NSS members are the targets of therapeutic compounds for these diseases, such as antidepressants.

The first crystal structure of LeuT was solved at 1.65 Å under a state with the substrates, L-leucine and sodium ions, binding to the molecule. We recently found that tricyclic antidepressants (TCA), which target NSS, non-competitively inhibit the LeuT transport, and solved the structure of LeuT under an "inhibited" state in complex with TCA. Structural bases for the LeuT transport will be discussed based on these crystal structures under different functional states, and results from accompanying functional analyses.

3S2-3 細菌型 Sec トランスロコン複合体の結晶構造解析

Crystal structure of bacterial Sec translocon

Tomoya Tsukazaki (1), Hiroyuki Mori (2), Shuya Fukai (3), Ryuichiro Ishitani (4), Anna Perederina (5), Dmitry G. Vassilyev(5), Koreaki Ito (2), Osamu Nureki (1,4). (1 : *Graduate School of Bioscience & Biotechnology, Tokyo Institute of Technology*; 2 : *Institute for Virus Research, Kyoto Univ.*; 3 : *SRRO, Univ. of Tokyo*; 4 : *Institute of Medical Science, Univ. of Tokyo*; 5 : *Dep. of Biochemistry & Molecular Genetics, Univ. of Alabama*)

The Sec translocation machinery facilitates the movement of newly-synthesized secretory and membrane proteins across and into membranes. The prokaryotic SecYE complex, embedded in the cytoplasmic membrane, functions as an evolutionarily conserved core component of the protein-conducting channel, the Sec translocon. Here, we present the first crystal structure, at 3.2 angstrom resolution, of the SecYE complex from a SecA (bacterial translocation motor)-containing organism, *Thermus thermophilus*, in complex with a specific Fab fragment. Comparisons with the previously reported structures of the Sec translocon revealed a significant conformational change in the transmembrane helices, which creates a hydrophobic crevasse that is open to the cytoplasm and is probably used for signal peptide entry. Interestingly, the Fab fragment interactions with SecY involve the SecA-binding interface at the tip of the cytoplasmic protrusion, possibly mimicking the SecY-SecA contacts and stabilizing this "pre-open" state of SecY. Intramolecular disulfide cross-linking experiments revealed that the pre-open state also exists in physiological membranes, and it probably represents the first conformational transition of the Sec translocon during protein translocation.

3S2-4 Bacterial multidrug efflux transporter, AcrB - The pumping mechanism

Bacterial multidrug efflux transporter, AcrB - The pumping mechanism

Satoshi MURAKAMI (1) (1: *Dept. Life Science, Tokyo Institute of Technology*)

Resistance nodulation cell-division (RND) transporters are one of the main cause of the bacterial multidrug resistance. They pump a wide range of antibiotics out of the cell by proton motive force. AcrB and its homologues are the major RND transporter in *Escherichia coli* and other Gram-negative bacteria. Recently, the crystal structures of AcrB have been determined by different space groups. All these structures are consistent with asymmetric trimer. Each monomer has different conformation corresponding to one of the three functional states of the transport cycle. Transporting hydrophobic drug was bound in the periplasmic domain on one of the three monomers. The transport pathway with alternating access mechanism is located at the hydrophilic domain protruded into the periplasmic space while this mechanism of other transporter families like ABC and MFS transporter is located in the membrane embedded region. For the RND, protonation might take place also asymmetrically at the functionally important charged residues in the transmembrane region. The structures indicate that drugs are transported by a three-step functional rotation in which substrates undergo ordered binding change.

[1] Murakami, S. *et al.*, *Nature*, 1997, **419**, 587.[2] Murakami, S. *et al.*, *Nature*, 2006, **443**, 173.

3S2-5 古細菌型ロドプシンと無脊椎動物のロドプシンとの構造比較

Structural comparison of archaeal and invertebrate rhodopsins

Tsutomu Kouyama (1) (1: *Dept Physics, Graduate School of Science, Nagoya University*)

For structural investigation of oligomeric complexes of archaeal rhodopsins, we have previously developed the membrane fusion method, by which the trimeric bacteriorhodopsin-lipid complex was crystallized into the hexagonal P622 crystal. Recently, we applied this method to prepare the P321 and P63 crystals of archaeorhodopsin-2 without destroying the trimeric structure [1]. These crystals provided information as to the physiological roles of native lipids including bacterioruberine. Structural comparison of archaeal rhodopsins showed that the structure of the retinal-binding pocket was well conserved, while the structure of the protein-lipid interface was more variable during the evolution. For structural investigations of invertebrate rhodopsins, we also tried to develop a