membrane and is anticipated to form a protein-transport channel. However, the molecular structures of the core complex and potential peripheral subcomplexes have not yet been revealed. We report here that two inner membrane spanning proteins, Dotl and Dot2, form a multimeric complex that associates with the inner-membrane components of the core complex. Dotl and Dot2 show a mutual dependency to maintain their protein levels in bacteria. From these results and other evidence, we hypothesize that Dotl and Dot2 form a ring like structure that associates with the core complex to function as a transport conduit on the inner membrane.


Alternative splicing (AS) is thought to be a major mechanism for diversifying transcriptome and proteome in metazoans. Especially, more than 90% of multi-exon genes in human genome are shown to undergo AS using a next-generation sequencing method. Although the accumulation of novel transcript data produced by AS are accelerated by new experimental technologies, a large portion of proteins encoded by the transcripts (termed "AS isoforms") is not known in their 3D structures and functions. Therefore, information whether AS events affect 3D structures and/or functional sites is expected to be useful for studies of novel AS isoforms. For this purpose, we have developed a pipeline that automatically annotates users’ uploaded transcript sequences. Given a transcript sequence, the pipeline identifies regions altered by AS events (termed "AS regions") in a putative protein sequence encoded by the transcript and assigns functional information such as domains and inter-molecular interaction sites obtained by the 3D structure information. Furthermore, the pipeline predicts the possible 3D structure of the AS isoform using structural constraints of hydrophobic core residues mainly. Based on the pipeline, we have been developing a web tool, named AS-EAST (Alternative Splicing Effects ASsessment Tools). Using our web tool, users can inquire whether their own transcripts encode a novel AS isoform and infer the impact of AS on its molecular function. AS-EAST is a freely available at http://as-alps.nagahama-ibio.jp/ASEAST/.

2P125 Dynamics and Structural Changes of ABCB1 transporter induced by binding of substrates and inhibitors
Wei-Lin Hsu, Yurika Watanabe, Tadaomi Furuta, Minoru Sakurai (Center for Biol. Res. & Inform., Tokyo Tech)

ATP-binding cassette (ABC) transporters form one of the largest protein families. ABC transporters translocate a number of substrates across cellular membrane by coupling with ATP hydrolysis. Several of these membrane proteins are clinically important in causing, e.g., multidrug resistance to cytotoxic pharmaceuticals.

The crystal structures of some ABC transporters have recently been determined. They reveal similar arrangements of the conserved ATP-hydrolyzing nucleotide-binding domains (NBD), but various architectures of the transmembrane domains (TMD), with the exception of common coupling helices that are necessary for transmitting conformational changes. Despite the fact that a wealth of research has been conducted on these structures to resolve the mechanistic problems about how ABC transporters couple with ATP hydrolysis in cytosolic domains to translocate substrates through the transmembrane pore, the specific process about how substrates bind to the proteins and the resultant protein structural change still remains elusive.

Our present study focuses on the mechanisms of protein structure changes induced by binding of ATP, substrates or inhibitors in the mouse ABCB1 transporter with an inward-facing conformation. The structure fluctuation simulated by the GROMACS package (version 4.0.7) shows the atomic-level description of the protein structural changes. The result sheds light on the mechanism about the drug translocation process of ABC transporter and the molecular design of inhibitors.

2P126 NMR study on DNA mismatch repair protein MutL
Ryota Mizushima1,2, Tomoyo Takai2, Young-Ho Lee2 (*Grad. Sch. Frontier Biosciences, Osaka Univ., 1PIR, Osaka Univ.)

DNA mismatch repair (MMR) works in the last process of gene repair system, enhancing the DNA replication fidelity up to 100 - 1000 fold. Familial non-polyposis colon cancers called Lynch-Syndrome (LS) caused by mutations of genes coding MMR proteins occupies 2 - 5 % of total colon cancers. About 80 % of those who hold such genetic mutations suffered from colon cancers in their life. I am currently engaged in studying one of the MMR proteins called MutL, in terms of structural biology by the method of NMR and other biophysical techniques. MutL homologs can be divided into two groups: E.coli. and Eukaryotic MutL. The former does not possess endonuclease activity (MutL does not exist) and the latter does. In this research, I deal with the latter. MutL is comprised of three parts: dimerized and endonuclease C-terminal domain (CTD), ATPase N-terminal domain transiently dimerized thorough ATP-binding and hydrolytic cleavage and the linker region connecting CTD and NTD. Inter-domain configuration between CTD and NTD is thought to be regulated thorough ATP hydrolysis. Moreover, this configuration dynamics regulates endonuclease activity of MutL and interaction with other MMR proteins. No NMR study has been officially published on both E.coli. and eukaryotic MutL, so far. The purpose of this research is to elucidate the regulatory mechanisms of MutL endonuclease activity though analyzing the interaction between CTD and NTD at the residue level by means of NMR.

2P128 EGFR 分子 C-末端の天然変異ドメインの分子拡散 FRET 時間変域 {Single-molecule FRET} measurement of a intrinsically disordered C-tail domain of an epidermal growth factor receptor
Kenji Okamoto, Yasushi Sako (RIKEN)

Structural dynamics of biomolecules, especially proteins, has been known to have indispensable roles in living systems. While conventional X-ray crystallography or NMR is powerful tools to investigate a molecular structure, single-molecule FRET (smFRET) measurement has advantages in observing its dynamics. smFRET can investigate structures of, for example, intrinsically disordered proteins (IDP) and its time resolution enables real-time observation of structural changes. Epidermal growth factor receptor (EGFR), which is a membrane protein and triggers cellular signal transduction pathways, has an intrinsically disordered (ID) C-tail domain in its intracellular part. The C-tail domain has several phosphorylation sites, one of which binds a protein Grb2 after phosphorylation. Years ago, Grb2-binding kinetics was found to be nonlinear system and it has been suggested that structural dynamics of this ID domain plays an important role. We introduce smFRET measurement of EGFR C-tail domain using recently developed apparatus and statistical data analysis, which is based on the hidden Markov model and the variational Bayes. Time series of 2ch fluorescence signals are obtained from single EGFR C-tail molecules immobilized in vitro. Obtained data are analyzed to reconstruct the state transition trajectory (STT) while the number of states is also estimated from data. Further analysis on those STTs gives us details of dynamics, such as possible paths or rates of state transitions.

2P129 タンパク質の変性及びクロステラト転移に対する浸透圧効果
Effect of osmotic pressure on protein unfolding and cross-beta transition
Kazuki Takeuchi, Mitsuhito Hirai (Grad. Sch. Eng., Gunma Univ.)

[Introduction]
Proteins are designed to function in crowded environments. However, how a protein folds into its native structure under crowded macromolecular condition is still ambiguous. To clarify the crowding effect on the thermal stability of proteins, we carried out synchrotron radiation wide-angle and small-angle X-ray scattering (WAXS, SAXS) experiments. We employed so-called "osmotic stress" method by using a high molecular weight neutral polymer (osmolyte) in solutions.

[Experimental]
Polyvinylpyrrolidone (PVP) with Mw 40,000 was used as an osmolyte. The protein was myoglobin (MY) from horse skeletal muscle and hemoglobin (HM) form bovine blood. The proteins were dissolved in 10 mHEPES buffers at pH 7.4 with different PVP concentration (0 % to 25 % w/v). WAXS and SAXS measurements were performed both at Spring-8 and PF. The temperature of the samples were controlled from 25℃ to 85℃.

[Results]
The radius of gyration of the protein at 25℃ showed a minimum at a low PVP

2P127 選択的スライディングによる新規タンパク質アイフォームの機能構造予測を目的としたツールの開発と公開
Development of a functional and structural annotation tool of novel protein isoforms produced by alternative splicing

NII-Electronic Library Service