2P025 計算と実験による黄色ブドウ球菌の Isd 蛋白質間へム輸送機構の解明

Structural insight into the heme-transfer mechanism between Isd proteins in *Staphylococcus aureus*

Yoshitaka Moriaki¹, Tohru Terada¹, Jose M. M. Caaveiro², Kouhei Tsumoto², Kentaro Shimizu¹ (¹*Dept. of Biotech., Grad Sch. of Agri. Life Sci., Univ. of Tokyo*, ²*Dept. of Bioeng., Sch. of Eng., Univ. of Tokyo*)

Although the tertiary structures of the IsdH-N3, IsdA-N, and IsdC-N domains of *Staphylococcus aureus* have been determined by X-ray crystallography, the mechanism of the rapid and unidirectional transfer of heme between these domains has remained unclear. We performed MD simulations on these domains and identified two distinct regions that may cause the difference in the affinity for heme. This prediction was validated by *in vitro* experiments. The rapid transfer of heme implies the existence of the heme-transfer intermediate complexes. Here, we propose novel structural models for them. We found that the complex structures were stable during 200-ns MD simulations. We will discuss how heme is transferred in each complex based on the results of QM/MM calculations.

2P026 滑走細菌 *Mycoplasma mobile* 由来新規シアル酸レセプターの結合活性

Binding activity of novel sialic acid receptor from gliding bacterium, *Mycoplasma mobile*

Tasuku Hamaguchi¹, Masaru Kawakami², Makoto Miyata¹ (¹*Grad. Sch. of Sci., Osaka City Univ.*, ²*Fac. of Eng., Yamagata Univ.*)

M. mobile binds to sialylated oligosaccharides and glides on the host cell. Gli349 composed of 3,183 amino acids acts as “leg” and has a sialic acid receptor domain at a C-terminal 463 amino acids region (foot). The binding activity of this receptor is critical for *Mycoplasma* gliding.

We prepared a soluble recombinant foot (rfoot) with high refolding efficiency and measured the binding force to sialylated glycoprotein by atomic force microscopy (AFM). The rfoot showed binding activity with the average force 64.5±34.0 pN, which was similar to that of Gli349 isolated from *M. mobile*. Crystallization of rfoot is undertaken for determination of three dimensional structure.

2P027 子囊菌由来不凍タンパク質の機能と構造 Function and Structure of Antifreeze Protein from Ascomycete

Daichi Fukami¹, Yuichi Hanada¹, Jing Cheng¹, Sakae Tsuda^{1,2}, Hidemasa Kondo^{1,2} (¹*Grad. Sch. Sci., Univ. Hokkaido*, ²*Bioproduction Research Inst., AIST*)

Antifreeze proteins (AFPs) bind specifically to ice. A novel AFP (AnpAFP) from ascomycete, *Antarctomyces psychrotrophicus*, has been found in Antarctica. AnpAFP shows a high homology to that from a fungus *Typhula ishikariensis* (TisAFP) at the amino acid level. The thermal hysteresis (TH) activity of AnpAFP was measured to be 0.8°C. In contrast, TisAFP is known to have a TH of 0.5°C. Fluorescence-based ice plane affinity analysis showed that AnpAFP bound to the prism plane of ice, whereas TisAFP binds to both the prism and basal planes. The ice-binding site of AnpAFP was deduced from the homology model obtained by using the crystal structure of TisAFP. These results would lead to understanding of the structural basis of AnpAFP to exhibit ice-binding function.

2P028 分裂酵母キネシン Cut7 の両方向運動性 Bidirectional motility of the fission yeast kinesin-5, Cut7

Masaki Edamatsu (*Department of Life Sciences, Graduate School of Arts and Sciences, The University of Tokyo*)

Kinesin-5 is a homotetrameric motor with its motor domain at the N-terminus. Kinesin-5 crosslinks microtubules and functions in separating spindle poles during mitosis. In this study, the motile properties of Cut7, fission yeast kinesin-5, were examined for the first time. In *in vitro* motility assays, full-length Cut7 moved toward minus-end of microtubules, but the N-terminal half of Cut7 moved toward the opposite direction. Furthermore, additional truncated constructs lacking the N-terminal or C-terminal regions, but still contained the motor domain, did not switch the motile direction. These indicated that Cut7 was a bidirectional motor, and microtubule binding regions at the N-terminus and C-terminus were not involved in its directionality.

2P029 第一原理フラグメント分子軌道法による ブチリルコリンエステラーゼと阻害剤間の特異的相互作用の解析

Ab initio fragment molecular orbital calculations on specific interactions between butyrylcholinesterase and its inhibitors

Takeru Murakawa¹, Tomoya Suzuki¹, Tareq Khan², Noriyuki Kurita¹ (¹*Toyohashi University of Technology*, ²*University of Tromsø*)

Butyrylcholinesterase (BChE) exists mainly at hippocampus and hydrolyses neurotransmitter acetylcholine. Many types of ligands have been produced to inhibit the activity of BChE. We here investigate the specific interactions between BChE and several types of ligands Kx, using *ab initio* fragment molecular orbital (FMO) calculations. The binding energies between BChE and Kx evaluated by FMO are well correlated with the IC50 obtained by the previous experiments. In addition, the FMO calculations highlight the amino acid residues of BChE important for the binding between BChE and Kx. Based on the results, we propose some novel ligands and elucidate that one of the ligands can bind more strongly to BChE than Kx.

2P030 *Ab initio* 分子シミュレーションによるがん細胞レセプターへのリガンド結合を阻害する新規ペプチド阻害剤の提案

Ab initio molecular simulation for proposing novel peptide inhibitors blocking the ligand-binding to the receptor of cancer cell

Tatsuro Mizushima¹, Ryushi Kadoya¹, Tomoyo Kasumi¹, Hiroshi Kobayashi², Noriyuki Kurita¹ (¹*Toyohashi University of Technology*, ²*Nara Medical University*)

Recent biochemical experiments have revealed that many proteases play important roles in cancer invasion and metastasis. Among these proteases, the binding of urokinase-type plasminogen activator (uPA) and uPA receptor (uPAR) existing on the surface of a cancer cell is a trigger for cancer invasion. Therefore, the blocking of the binding is expected to inhibit cancer invasion. In the present study, we created a various types of peptides for blocking the ligand-binding pocket of uPAR. The binding energies between uPAR and the peptides were evaluated by *ab initio* fragment molecular orbital calculations, and the peptide with the largest binding energy was proposed as a potent inhibitor to cancer invasion.