1B1400 “空隙”機能構造創出のための自然の戦略**Cavity—a Nature's strategy for functional sub states in proteins****Kazuyuki Akasaka** (*Kinki UNIV., Inst. Adv. Tech.*)

How Nature can provide proteins with the dynamics and sub states required for their specific needs for function is a fundamental question in protein science and evolution. To address the question, here we focus on cavities. Cavity is synonymous to void in folded proteins, often discernible as atom defect in crystal structures of proteins. Although cavity has long been known as a factor that affects thermal stability and compressibility of proteins (Gekko and Hasegawa, *Biochemistry* 1986), its specific role in protein dynamics and function remains unclear. In recent years, the question is pursued intently on pressure axis using NMR or X-ray crystallography in functional proteins including hen lysozyme, cMyb protein and OspA among others (1-3). The studies start revealing crucial roles of cavities in providing functional dynamics and sub states, and further suggest their roles in evolution.

References

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1B1412 HD-exchange motion of free heptameric GroES studied by the use of TROSY and DMSO quenching followed by 2D NMR

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The GroEL-GroES is a chaperonin complex which assists target proteins to fold into native state. To understand structural and functional relationship of GroES-GroEL complex, we studied the dynamic structural fluctuations of free heptameric GroES by the use of hydrogen/deuterium (H/D) exchange and 2DNMR techniques. H/D exchange analysis is used to trace the rate at which protein backbone amides undergo chemical exchange with deuterated solvent. NMR spectroscopy is used to study proteins having molecular weight up to 50KDa. However, as the molecular weight of the GroES complex is 70KDa, we cannot get well resolved NMR spectrum. So, we need a modified strategy to study this complex. In this study, we report the dynamic structural fluctuations of free heptameric GroES by the use of H/D exchange technique followed by: 1) Transverse relaxation optimized spectroscopy (TROSY) and 2) DMSO quenching with 2DNMR. In our study, we observed rapid exchange rate of backbone amide residues in a mobile loop region. During the GroES-GroEL complex formation, this mobile loop region forms contact with GroEL complex. Therefore, this result indicates that mobile loop is very flexible in free heptameric GroES.

1B1424 溶液 NMR を用いた Na⁺駆動型べん毛モーターの固定子タンパク質 FliG の C 末端領域構造解析**Solution NMR analysis of FliG C-terminal domain derived from Na⁺-driven motor of *Vibrio***

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In the bacterial flagellar motor, torque is generated by interactions between the rotor and the stator. In the rotor, a C ring component, FliG, mainly participates in torque generation, primarily through its C-terminal domain. In the H⁺-driven motor of *Escherichia coli*, mutational analyses revealed that the rotor-stator interaction is mediated by charged residues between the FliG C-terminal domain and the MotA cytoplasmic loop region. However, in the Na⁺-driven motor of *Vibrio*, the effects of mutations of the charged residues on the motility were very weak. Therefore, the mechanism of torque generation in the Na⁺-driven motor remains unknown. To investigate the interaction between FliG and the MotA ortholog PomA in the Na⁺-driven motor, we performed solution NMR analyses. We previously reported that drastically improved ¹H-¹⁵N TROSY-HSQC spectra were obtained with the N-terminally truncated FliG variant, G214-FliG (FliG_C). We'd now like to discuss the result of the 3D ¹H-¹³C-¹⁵N NMR

measurements and the on-going sequential signal assignments. Furthermore, we tried to create PomA fragments containing the cytoplasmic loop region, with which FliG is supposed to interact. We found that overexpression of the PomA fragment impedes the motility. We are currently trying to measure NMR spectra of FliG_C in the presence and absence of PomA fragments, and further results will be discussed at the meeting.

1B1436 酸化還元状態に依存してプロテインジスルフィドイソメラーゼの基質結合部位ドメインの空間配置が変化する仕組み**Molecular mechanism of redox-dependent domain rearrangement of the substrate-binding region of protein disulfide isomerase**

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Protein disulfide isomerase (PDI), a major protein in the endoplasmic reticulum, operates as an essential folding catalyst and molecular chaperone for disulfide-containing protein substrates. We have previously reported that b' and a' domains of PDI exhibit an intramolecular domain rearrangement depending on the redox state of the active site cysteine pair in a' domain, which is coupled with the shielding and exposure of substrate-binding hydrophobic surface spanning across these domains. To gain insights into the molecular mechanism of this domain rearrangement, we prepared mutated PDI-b' a' in which a cysteine pair was substituted for serine or alanine. By using fluorescent hydrophobic probe, we revealed that the serine- and alanine-substituted mutants mimicked the reduced and oxidized forms of wild-type protein, respectively, in terms of exposure of hydrophobic surface. By nuclear Overhauser effect experiments of NMR spectroscopy, we demonstrated that the side chain of the tryptophan residue located adjacent to the a' active site was in contact with the b' domain in the reduced form and serine-substituted mutant, whereas the tryptophan side chain was accommodated in the active site in the oxidized state and the corresponding site of the alanine-substituted mutant. Based on these results along with those obtained by X-ray crystallographic analyses, we proposed a mechanistic model of the domain rearrangement triggered by redox-dependent flipping of the tryptophan side chain.

1B1448 Transferred cross-saturation 法を用いた EB1 の CH ドメインの微小管との結合界面の特定**Microtubule-binding sites of EB1 CH domain revealed by transferred cross-saturation experiments**

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End-binding 1 (EB1) is a member of plus end tracking proteins which bind to microtubule (MT) plus end and regulate MT dynamics. EB1 binds to microtubule lattice with its N-terminal CH domain and stabilizes MT. EB1 is thought to be autoinhibited by the interaction between CH domain and C-terminal domain. Despite the importance of the EB1-MT interaction in regulation of MT dynamics, the molecular details of the EB1 autoinhibition remain unclear.

By comparison between CH domain K59E mutant (low affinity interactor) data and a competition data, we identified the binding interface of CH domain with polymerized tubulin by transferred cross-saturation (TCS) experiments. In addition, by chemical shift perturbation experiments, we identified the binding interface between EB1 CH domain and C-terminal domain. Interestingly, we found that the MT-binding sites and C-terminal domain binding sites of CH domain are closely located, indicating that the MT-binding sites are masked by the interaction between the C-terminal domain in an autoinhibited state. Our chemical shift perturbation experiments suggest that the CH domain binding region of C-terminal domain is almost identical to the SxIP motif of APC and CAP-Gly domain of p150^{glued} binding region. Autoinhibition may therefore be regulated through competition binding by such binding partners.

1B1510 再構築型生体外タンパク質合成系を用いた分子シャペロンによる凝集抑制効果の大規模解析**Global analysis of aggregation-inhibition effects of molecular chaperones using a reconstituted cell-free translation system**

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