chain and a ubiquitin-like (Ubl) domain. Thus, arrangement and location of the proteasome initiation region affect a protein's fate and play a central role in selecting proteins for proteasome-mediated degradation.

1P035 **3α-hydroxysteroid dehydrogenase の二量体界面にある Arg251 と Phe255 の役割**
Roles of Arg251 and Phe255 at the dimer interface of 3α-hydroxysteroid dehydrogenase

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3α-hydroxysteroid dehydrogenase from *Pseudomonas* sp. B-0831 (Ps3αHSD) forms a dimer composed of two 26-kDa subunits, and catalyzes the oxidoreduction of hydroxysteroids in a nucleotide cofactor-dependent manner. The crystal structure of its complex with NADH indicates that the structural change of one subunit induced by the cofactor binding affects the conformation of another subunit through the C-terminal dimer interface in a negative cooperative manner. At the dimer interface, the side chain of Arg251 of one subunit forms a unique salt bridge with the carboxyl group of Phe255 of another subunit. In this study, to analyze the roles of Arg251 and Phe255 for dimer formation, cofactor binding, and catalytic activity, we overexpressed the truncated or Ala substituted mutants of Ps3αHSD (R251A, F255A, F255del, R251A/F255A, R251A/F255del) in *E. coli* with N-terminal His-tag. The analytical ultracentrifugation experiments showed that 5 μM F255del was a mixture of monomer (8%) and dimer (89%) while most of wild-type formed dimer. The monomer content of F255del was dependent on its concentration which was also observed in other mutants. The fluorescence measurements showed that the NADH binding affinity to mutant was much lower than that to wild-type and that the negative cooperativity was disappeared. Together with the decreased catalytic activities of mutants, the present results indicate that the interaction of Arg251 and Phe255 is critical for dimer formation, which is correlated with the cofactor binding and the catalytic activity.

1P036 **基質タンパク質のフォールディングにおけるシャペロニン GroES の役割**
Role of the chaperonin GroES in the folding of substrate proteins

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The *Escherichia coli* chaperonin proteins, GroEL and GroES, assists in folding non-native polypeptides. GroEL is a large cylindrical complex with two heptameric ring, stacking back to back. GroES is a single heptameric ring of identical 10-kDa subunits, which caps a GroEL central cavity induced by ATP binding to GroEL. It is known that non-native substrate proteins are recognized and captured by the apical domain of GroEL. Efficient folding of substrate proteins is shown to occur inside the *cis* cavity of GroEL encapsulated by GroES in the presence of ATP. Previous study demonstrated that a high-speed AFM (HS-AFM) imaging revealed an entirely occupied protein inside the GroEL cavity or a nano-particle cluster like a large aggregated protein bound on the GroEL surface. In this study, to monitor the interaction between substrate proteins and the chaperonin proteins, we prepared a substratum with urea denatured substrate proteins on the mica surface. Interestingly, we found that GroES bound the substratum with the unfolded substrate protein imaged by HS-AFM. Besides, GroEL also bound the substratum with the unfolded substrate protein. On the GroES attached substratum, the addition of the GroEL and ATP, GroES was released from the surface with the unfolded substrate proteins by the chaperonin reaction cycles. Our results suggest that GroES binds the unfolded substrate protein and is positively involved in the folding of substrate proteins.

1P037 **Effect of cavity on the overall and local stability of c-Myb R2 sub-domain**

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c-Myb is a transcription factor involved in proliferation and differentiation of hematopoietic cells. The DNA-binding domain of c-Myb consists of three structural repeats, R1, R2 and R3, of 51-52 amino acids, each forming a helix-turn-helix related motif containing three helices. Only R2 sub-domain has an internal cavity and the presence of this cavity was reported to be very important for the DNA-binding activity of c-Myb. In order to understand the effect of cavity on the overall and local stability of R2 sub-domain, we have designed

two mutants, one with C130I mutation (to avoid complications due to side chain oxidation) and the second one with C130I and V103L (to fill the cavity) mutations. The local and overall stability of the two proteins were determined by variable temperature 1D-¹H NMR and CD analysis. The analysis revealed that the cavity filled mutant has higher T_m value than that of the wild-type, which indicated the higher stability of cavity filled mutant. Determination of line broadening by peak half-width analysis of variable temperature ¹H NMR spectra showed that helix1, which is very close to the internal cavity, is more mobile than the other two helices, while this mobility is less in cavity filled mutant. Proximity of the internal cavity may be responsible for the more dynamic nature of helix1. It has been reported that Trp95 side chain is sliding into the R2 cavity upon binding to DNA. The higher mobility of helix1 might augment the sliding process of Trp95 residue into the cavity.

1P038 **リガンド結合によるタンパク質立体構造変化のデータベース解析**
Database analysis of protein structural changes upon ligand bindings

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Proteins are flexible molecules that undergo significant structural changes upon ligand bindings to control their functions. Those structural changes can be explained by the linear response theory based on the view of the causal relationships. Protein Data Bank (PDB) contains a number of redundant entries of identical proteins whose structures were determined separately in different conditions, for example, with/without ligands. Accumulation of such information about structural variations has constructed valuable resources for understanding of the protein structural change in general. The purpose of this study is to assess and clarify the causal relationship between ligand binding and structural change. We prepared a dataset of monomeric or homodimeric proteins having both ligand-free and ligand-bound structures in PDB. The 839 protein pairs in the dataset were first classified into 5 motion classes, i.e., domain motion (115 protein pairs), domain+loop motions (89), loop motion (333), other type motion (51), and no motion (251). Then, we examined whether protein pairs are directly relevant to ligand binding, and whether these motions can be explained by the linear response theory. We further examined the relevance between protein motions and their functions. Proteins undergoing disorder-order transition were also included in the targets of the analyses. The results will be explained in the poster session.

1P039 **抗生物質代謝酵素の基質結合シミュレーション**
Molecular Simulation of Substrate Binding by Antibiotics Acylases

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Antibiotics acylases are key enzymes for industrial production of antibiotic drugs and have been targeted to be engineered for improvement of their stabilities and catalytic activities. Cephalosporin acylase (CA) is one of the most intensively studied enzymes that catalyze deacylation of β-lactam antibiotics. These enzymes are known to be synthesized in vivo as the inactive precursor form and to be transformed into the active form by cleavage of a short intervening amino-acid sequence. In this study, substrate binding by CA and CA mutants were simulated based on their X-ray crystallographic structures. In activated forms of the enzymes, docking poses were obtained with substrate conformations suitable for the catalytic reaction, whereas no such docking poses were obtained for the precursor enzymes. Binding affinities of the activated enzymes for their substrates were estimated as the docking energies for the poses, and were compared with experimental values of Michaelis-Menten constant (K_m). The calculated energies were found to be well correlated with the experimental K_m values. The docking simulation was further performed for 3D homology models of aculeacin A acylase (AAC), which is in an alternative acylase class catalyzing the deacylation of cyclic lipopeptide antibiotic molecules as the substrate. The results obtained for the AAC models were also consistent with the experimental data. The present study demonstrates the usefulness of our computational approach for engineering antibiotic acylases.

1P040 **サツマハオリムシ V2 ヘモグロビンの oxy-deoxy 状態での構造変化**
Structure transition between oxy and deoxy states of V2 hemoglobin from *Lamellibrachia* *Satsuma*

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