Moreover, we constructed and classically simulated the complex structure combined with CoA and an Mg ion in explicit water model, indicating that a water molecule specifically associated to the Mg ion consistent with the previous reports and predictions. In the presentations, we will show our results from the classical and the combined simulations with respect to the proton transfer processes and discuss the free energy landscapes.

2P085 種々の分子指標による化合物分類に基づく結合自由エネルギー予測における自由エネルギー変分原理の有効性の検討 Application of the free energy variational principle to prediction of relation binding free energies based on several molecular indices.

Ashida Takeshi, Kikuchi Takeshi, (Dept. Bioinformatics, Col. Life Sci., Ritsumeikan Univ.)

In drug discovery process, we must predict precise binding free energies between a target protein and a ligand. Therefore, it is desirable to use a technique with low computational costs and no empirical parameter, for a computation of binding free energies. We have proposed an efficient method to calculate accurate binding free energies based on a free energy variational principle. In this study, we try to classify ligands with three molecular indices and examine validity of the free energy variational principle.

The free energy variational principle can be expressed as the following formula; $\langle H_1 - H_0 \rangle \leq G_1 - G_0 \leq \langle H_1 - H_0 \rangle$ where G_0 and H_0 denote free energy and Hamiltonian of a unperturbed system. G_1 and H_1 denote free energy and Hamiltonian of a perturbed system respectively. When the perturbation is small, i.e., the difference in physico-chemical property between ligands is small, the above formula can be approximated by $\langle H_1 - H_0 \rangle \approx G_1 - G_0 \approx \langle H_1 - H_0 \rangle$. In this study, we use the immunosuppressant binding protein, FKBP12, and inhibitors and calculate relative values of binding free energy using a molecular dynamics. We make a linier combination of three molecular indices, shape similarity, charge similarity and logP, and classify 40 inhibitors with a cluster analysis. As a result, it is demonstrated that the free energy variational principle is applicable to the wide region in the classification rather than a method before. Therefore, it is expected that a more precise binding free energy value is predicted with the present criterion.

2P086 The analysis of the interaction between Sup35 protein aggregate and molecular chaperon Hsp104 by FCCS

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Budding yeast have some non-Mendelian inheritance phenotypes. These phenotypes are controlled by proteins called "yeast prion" that has two forms, normal and aggregation conformations. The difference of infectious ability between normal and aggregation form has been reported in view point of biochemistry. On the other hand, the prion protein dynamic state in live yeast is less known yet. The aim of our study is to reveal the protein dynamic state in living system. In budding yeast, the [PSI⁺] phenotype is controlled by Sup35 protein which is a famous yeast prion protein. Sup35 protein is regulated by molecular chaperon Hsp104. This chaperon has disaggregation ability for Sup35 aggregate. In our study, the interaction of Sup35 protein and Hsp104 was analyzed by Fluorescence Cross Correlation Spectroscopy (FCCS). Because evaluation the dynamic state of Sup35 protein and Hsp104 quantitatively, the size of aggregate, interaction intensity and the change of interaction intensity with inhibitor were analyzed assessed by FCCS.

The results analyzed by FCCS in yeast lysate was obtained as follows ;

1. In [PSI⁺], the interaction between Sup35 protein aggregate and Hsp104 is detected by FCCS.
2. In [psi⁻](no prion phenotype), that interaction is not obtained by FCCS.
3. In the case of guanidine hydrochloride(GdnHCl) addition to the Hsp104 disaggregation, that interaction is lower than 1.(Sup35aggregate and Hsp104 in [PSI⁺]).

In order to clarify the interaction, FCCS measurement in live yeast should be carried out near future.

2P087 Growth defect caused by the in-frame deletion mutant of PomB, a Na⁺-driven stator component, in *Vibrio alginolyticus*

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Stator protein PomA and PomB form a complex that functions as a Na⁺ channel and couples Na⁺ influx to torque generation in the polar flagellar motor of *Vibrio alginolyticus*. This stator complex must be anchored to an appropriate place around the rotor through a putative peptidoglycan-binding domain in the periplasmic region of PomB (PomB_C). Crystal structure of the corre-

sponding domain of H⁺-driven *Salmonella* MotB (MotB_C) indicated that this domain must dimerize to form the H⁺-conducting channel and conformational changes in its N-terminal portion are required both for PG binding and the H⁺-channel activation. To understand the role of PomB_C in the Na⁺-driven PomA/PomB stator complex, we carried out its biochemical and mutational characterizations based on the MotB_C structure. We constructed PomB_C fragments in various sizes, and found that one of them consisting of residues 135 through 315 inhibited the motility of the wild-type cells when overexpressed in the periplasm. We also found that an in-frame deletion (Δ 41-120) variant of PomB retained its function. Overproduction of PomB (Δ 41-120) severely impaired cell growth and reduced motile fraction of the cells. A point mutation in the N-terminal region of PomB_C (L168P) gave only a minor effect on this growth defects but restored motile fraction significantly. These results suggest that in-frame deletion may unplug the stator channel, and conformation of the N-terminal PomB_C affects stator assembly. Functionally critical Asp24 of PomB will be examined and discussed in the poster.

2P088 無脊椎動物由来抗菌ペプチドと脂質膜の相互作用解析 Analysis of interactions between antimicrobial peptides from invertebrates and lipid membranes

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Invertebrates lack an acquired immune system seen in higher vertebrates, but they can protect themselves from microbial infection by potent innate immune system. Antimicrobial peptides are an important component of the innate immune systems. They exhibit a relatively broad range of antimicrobial activity against bacteria, fungi and viruses. It has been believed that antimicrobial action of antimicrobial peptides depends on perturbation of the target membrane via electrostatic interactions promoting initial binding to the surface and hydrophobic interactions for pore formation into the membrane. However, more detailed mechanism of action is still not well understood. To gain more insight into mechanism of antimicrobial action, we examined interactions between several antimicrobial peptides from invertebrates and lipid membranes by using surface plasmon resonance (SPR) biosensor. SPR biosensor has the ability to readily compare the binding affinity of antimicrobial peptides for lipid membrane. Some antimicrobial peptides have a strong affinity to zwitterionic lipid membrane, while others have not. It suggest that these strong affinity to zwitterionic lipid membrane may be correlated with hemolytic activity. It was reported recently that lysozymes, which are believed to act as cell wall lytic enzyme, have antimicrobial activity independent of its catalytic activity. So, we also measured interaction between lysozymes and lipid membrane.

2P089 Single particle analysis of the 26S proteasome by high-resolution electron cryo-microscopy and advanced image analysis

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The 26S proteasome mainly functions to degrade proteins in cytoplasm. The core of the 26S proteasome is composed of one 20S CP(core particle) and two 19S RPs(regulatory particles). The 19S RPs cannot bind to the 20S CP tightly and tend to disassociate from it. Moreover, the 19S RPs dynamically change in structure with respect to the stationary 20S CP. These structural flexibilities make structural analysis more difficult. Thus, high-resolution structure of the 26S proteasome has not revealed yet. Here, we also examined the 26S proteasome structure by single particle analysis. The images of 26S proteasomes were acquired by electron microscopy under cryo conditions at the liquid helium temperature. Although the EM images contain higher resolution signals, they have lower contrast than those at the liquid nitrogen temperature. Thus, it was too difficult to pick the complexes of 20S and 19S which bind each other. Thus, we processed pairs of EM images with different defocusing values to emphasize their contrast. Both signals of low frequency regions and high frequency regions were improved by Wiener filter with estimated SN information. As a result, particles were visible enough to be picked. We are currently, picking the particles of the 26S to obtain higher resolved structure.

2P090 ランダムコンニカルティルト法によるタンパク質構造解析の半自動化 Semiautomated structure analysis of protein by random conical tilting method

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