of the export process. However, the efficiency of finding the basal body with the FliH/FliI complex attached is still low. The search for optimum mixing ratio and solution conditions for structural analysis by electron tomography of the basal body complex at different stages of the export process is in progress.

3P-071 重水素標識を利用した中性子小角散乱法によるプロテアソーム構成タンパク PA28 および α 7 サブユニットの溶液構造解析

Solution structural analyses of PA28 and α 7 subunit of the 20S proteasome by SANS in conjunction with deuterium labeling techniques

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Small-angle neutron scattering (SANS) is a very powerful tool to obtain the structural information of large protein complexes, since it allows discrimination between protonated and deuterated subunits. The objective of this study is to understand the assembly mechanisms of proteasome complexes by SANS analyses of PA28 and α 7 subunit of the 20S proteasome. Although the crystal structure of the homo oligomer of α subunits of PA28 (PA28 α) has been resolved, they actually form a hetero-oligomer together with homologous β subunits in vivo and its packing structure remains to be elucidated. On the other hand, the α 7 subunit of the 20S proteasome forms a self-assembling ring-like structure, which, however, remains to be characterized in detail. These proteins were produced in *E. coli* and subjected to SANS measurements in solution. Our simulation of the SANS profiles based on the previously reported crystallographic data revealed that PA28 assumes a 14mer in which the heptameric rings are dimerized through their interfaces, which are also used for the interaction with the 20S proteasome. Furthermore, the subunit arrangement in the hetero-oligomer was characterized by a contrast variation method of SANS using deuterated α subunits assembled with protonated β subunits. The SANS analysis also revealed that the α 7 subunits of proteasome form a homo-heptameric rings that are stacked back-to-back. Subunit exchange process of this α 7 oligomer in solution will be discussed on the basis of SANS data obtained by the deuteration technique.

3P-072 バルク脂質膜表面積を再現する膜タンパク質系の分子動力学シミュレーション法

Development of the method reproducing the bulk lipid surface-area in the molecular dynamics simulation of the protein-membrane complex

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In the all-atom molecular dynamics (MD) simulations of lipid bilayers or membrane proteins in the bilayers, a surface area per lipid is one of the most important parameters. In the simulations of lipid bilayers, the surface area is taken from the experimental value of the bulk membrane system and fixed to the value during the simulation in most cases. However, this approach, which is called as NPAT ensemble, cannot be directly applicable to the simulations of membrane proteins, because no experimental values for the surface area around the proteins are measured.

In this study, we propose a new method to reproduce a structural feature of bulk membrane, especially a surface area of lipid, in the MD simulation of membrane proteins. Our method is based on the combination of NPT and NPAT ensembles, where the lipid surface area was calculated by the Voronoi tessellation. We performed all-atom model MD simulations of the SecY protein-conducting channel and Ca²⁺-ATPase in phospholipids and analyzed the lipid surface area. From the simulation, it was found that when we used the usual modeling and equilibration method, the lipid surface area was close to that of the bulk lipid but slightly greater than the experimental value. In contrast, our proposed method could reproduce the exact surface area of bulk lipids. These results suggest that by using our method we can perform a more reliable simulation of the membrane protein system.

3P-073 逐次モンテカルロ法による FRET 時系列解析

Analysis of single-molecule FRET trajectories by sequential Monte Carlo method

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Förster resonance energy transfer (FRET) is a powerful method for determining various mechanistic properties of biomolecules. However, except for the simple cases of well-separated, low-noise, and limited to only a few states, it is usually

difficult to determine the configurational states and the transition rates between them from FRET trajectories.

The general state space hidden Markov models have opened a possibility to analyze such a complex time series. With the help of the recent advances in computer power and sampling techniques, it has become possible to determine the hidden states of the high-dimensional models even from low-dimensional information. In this study, we propose the use of the sequential Monte Carlo method to identify the conformational changes of protein based on FRET trajectories. We applied the method to the high-resolution single-molecule FRET trajectories (J. A. Hanson et al., *PNAS* 2007) of the domain movements in adenylate kinase.

3P-074 *Paracoccus denitrificans* チトクロム c 酸化酵素の大腸菌由来無細胞系での合成

Synthesis of *Paracoccus denitrificans* cytochrome c oxidase in the *E. coli* cell-free system

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The proton pumping mechanisms of bovine heart and *P. denitrificans* cytochrome c oxidase (CcO) have been advocated from the fine X-ray structural analyses and structure-based mutagenesis studies. However, to clarify the reaction mechanism, changes in protonation state and polarity of the amino acid functional groups at the proton pumping pathway should be examined during the proton pumping process by time-resolved infrared spectral measurements. The spectral data could be assignable by site-specific stable isotope labeling of the functional amino acid residues, which is currently achievable only by the cell-free protein synthesis. We have already succeeded in the synthesis of *P. denitrificans* CcO by an *E. coli* cell-free system. Here, we report kinetic constant (kcat) of the synthesized protein, which were 140 sec⁻¹ comparable to 200 sec⁻¹ of the authentic enzyme. This result is consistent with normal Soret/vis spectrum of the synthesized protein. In our system, efficiency of functionalization for the synthesized protein is still less than 1 %. In an attempt to increase the efficiency, *E. coli* cell membrane fraction, where synthesized proteins are assembled in our cell-free system, were enriched with an assembly factor Surf1 to facilitate the CcO assembly. However, noticeable increase in the efficiency was not obtained similarly to the previous result of enrichment of YidC that accelerates membrane insertion, suggesting that the efficiency is limited by neither the assembly- nor membrane insertion-step.

3P-075 基質カンファー結合によるチトクロム P450cam の構造変化

Substrate *d*-camphor binding induces structural change of cytochrome P450cam

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Cytochrome P450cam (P450cam) is a thiolate-heme containing monooxygenase that catalyzes the regino- and stereo-selective hydroxylation of *d*-camphor to produce 5-*exo*-hydroxycamphor. The reaction is initiated by the binding of camphor to the resting state of the enzyme. *d*-Camphor binding excludes a cluster of waters at the active site, raising the redox potential of heme by an extent that allows efficient reduction by the electron transfer system. This binding was reported to involve no significant structural changes in the protein. Here, two ferric P450cam structures partially complexed with *d*-camphor (occupancies were 65 and 80 %, respectively) were determined by X-ray crystallography at 1.30–1.35 Å resolutions, revealing the structures of the substrate-free and -bound forms. In the substrate-free state, the active site hydroxyl group of Tyr96 forms a hydrogen bond (H-bond) with Thr101 side chain. Upon substrate binding, the hydroxyl group of Tyr96 forms a H-bond with the carbonyl group of *d*-camphor, while the Thr101 side chain rotates around its C ^{α} -C ^{β} bond by breaking the H-bond with Tyr96 and forms a H-bond by donating the hydrogen with the peripheral heme-6-propionate. This bonding contributes to the redox potential change together with spin state change of heme iron from low to high occurring associated with water exclusion from the active site.

3P-076 チトクロム P450cam の活性部位からの水クラスター排出機構

Mechanism of the water exclusion from the active site of cytochrome P450cam

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A cluster of waters (6 molecules) located at the active site of P450cam is not accessible from the protein surface. However, this water cluster is expelled from the site upon substrate camphor binding, which changes the spin state of heme iron from low to high. Thus, the water exclusion from the active site is essential to the catalytic reaction. We have proposed that Asp297-Glu322-heme-7-propionate hydrogen bond networks constitute a gate controlling water passage to the bulk water. Mutation of Asp297 to bulkier Leu lowered the camphor binding affinity