Previously, we studied correlation between the melting of 2D-crystalline array of bR and the photobleaching. The results suggested that the weakening of intermolecular interaction is responsible for irreversible photobleaching. In this work, we carried out the measurement of visible circular dichroism (CD) spectra and the photobleaching experiment of bR reconstituted into three kinds of phosphatidylcholine (PC) vesicles with different acyl-chain length. Visible CD spectra showed an exciton-to-positive transition of bR reconstituted into dimyristoyl-, dipalmitoyl- and distearoyl- PC vesicles around 17, 35 and 50 °C, respectively. These transition temperatures were close to the main transition of the reconstituted vesicles measured by calorimetric measurement. Irreversible photobleaching was started to occur at the same temperature regions as the change in the visible CD transition, clarifying the correlation between the melting of crystalline assembly and the photobleaching of bR. On the other hand, the thermal bleaching in the dark was not observed below 60 °C for each sample. The plausible model for irreversible photobleaching is discussed, based on these experimental results.

3P120 NADH-ユビキノ酸化還元酵素の FMN と鉄-硫黄クラスターの酸化還元状態による共鳴ラマンスペクトルの変化
Resonance Raman spectrum changes of FMN and iron-sulfur clusters of NADH-ubiquinone oxidoreductase upon redox states

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NADH-ubiquinone oxidoreductase (complex I) located at the entrance of mitochondrial electron transfer chain is the largest component of the respiratory chain with the proposed mass of 1000 kDa. The enzyme transfers electrons from NADH to ubiquinone with 10 isoprene unit (Q10) coupled with proton-pumping. The redox-active sites include one FMN, eight iron-sulfur clusters and one tightly bound ubiquinone. In order to elucidate the reaction mechanism of complex I, spectral characterizations of these redox sites are prerequisite as well as structural analyses at high resolution. Resonance Raman spectroscopy has provided a practical method for observing selectively the vibrational spectra of chromophoric groups of biomolecules. The resonance Raman spectra of the FMN and iron-sulfur clusters of complex I upon excitation at 441.6 nm and 488 nm have been determined by using our highly improved enzyme preparation and resonance Raman apparatus. In order to detect the changes of the spectra upon the change of redox states, the NADH-reduced complex I was prepared. For preventing the reoxidation of reduced enzyme, a spinning cell was filled with Ar gas and sealed with silicon cover. NADH concentration have to be maintained constant during the spectrum accumulation for 180 min. The addition of malic acid, malic dehydrogenase, citrate synthase and Acetyl CoA to NADH-reduced complex I was effective for maintaining the NADH concentration constant to keep complex I reduced.

3P121 Conformational change of mouse ABC transporter ABCB1 from an inward-facing conformation as studied by molecular dynamics simulation

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ATP-binding cassette (ABC) transporters are a large family of membrane proteins which transport various substrates by using MgATP hydrolysis. ABCB1 plays an important role in acquiring multidrug resistance. The x-ray structure of ABCB1 (PDBID 3G5U) was reported in 2009 for the first time, which enables us to study how it works based on the atomic-level structure. In this study, we use molecular dynamics simulation to follow conformational changes of ABCB1 starting from an inward-facing conformation and to reveal what intermediates it goes through in the transition from the inward-facing conformation to an outward-facing conformation. In addition, we also discuss the effect of an inhibitor on such structural changes. After 20 ns MD simulation, it was found that nucleotide-binding domains (NBD) underwent large conformational changes. Consequently, two NBDs were dimerized in a unique fashion that only one of two ATP binding pockets was close. In order to analyze what mode of molecular motion induces such conformational changes, we carried out principal component analysis for the trajectory data. As a result, it was found in the first principal component that one of the two NBDs rotate in clock-wise fashion and the other in anticlock-wise one with respect to the membrane normal, leading to the asymmetric contact between the two NBDs as described above. These results were obtained for the ATP-unbound state of ABCB1 transporter. We are under investigation now for the ATP-bound state.

3P122 全原子分子動力学シミュレーションによる多剤排出トランスポーター AcrB の解析
All-atom molecular dynamics simulation of multidrug efflux transporter AcrB

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In E. coli, it is known that the tripartite multidrug efflux system (AcrB/ AcrA/ TolC) exists, and AcrB resides in the inner membrane region and take part in substrate recognition and energy transduction for drug export through proton transfer. Recently, x-ray structures provided that AcrB forms trimeric protein where each subunit is different conformation, "binding state", "extrusion state" and "access state". Especially, only extrusion state subunit has different side chain conformation of residues (Asp407, Asp408 and Lys940), which are essential for proton translocation (protonation site). These results suggest that drugs are exported by a three-step structural change involved in proton motive force which is inferred to be caused by change of protonation states of the protonation site residues. However, the structural change process which involved in proton motive force is still unclear. In the present study, we performed 100 ns all-atom molecular dynamics (MD) simulations of three types of AcrB-membrane-water systems which are different from protonation state of Asp407 and/or Asp408 in extrusion state protomer. During all of the 100 ns MD simulations, the global structures of each subunit were conserved. However, in extrusion state protomer, the conformation of the protonation site residues were changed to those of other state protomers only when Asp 407 and 408 residues were deprotonated. In this presentation, we will discuss the structural change and proton transfer mechanism.

3P123 膜貫通タンパク質ドメインアーキテクチャー解析のための新規ドメインファミリー定義
Defining Novel Domain Families for Comprehensive Analysis of Domain Architectures in Transmembrane Proteins

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Transmembrane (TM) proteins have the characteristic domain architectures, consisting of a TM domain and a few soluble domains carried on long loops with more than 60 residues in most cases. Having the domain architectures determined for a large number of TM protein sequences, we would be able to understand functional makeup of TM proteins and evolutionary pathways to extant TM proteins. We first, in this study, assigned the Pfam and SCOP domains to loop regions of 62,348 TM protein sequences from 25 genomes, and then we defined novel domain families on the remaining long loop regions without assigned Pfam nor SCOP domains.

A total of 29,807 sequences (47.8%) have at least one Pfam or SCOP domain assigned, and the remaining 32,541 sequences have no assigned domains. After removing the domain-assigned segmental loop regions, we had still 66,258 loop segments remaining, which are longer than 60 residues with being able to carry soluble domains. Applying the MKDOM algorithm to these remaining loop segments, we defined 17,015 novel domain families (with ≥2 family size), with the result that multi-domain TM proteins to be the targets of the domain architecture analysis increased 10,487 (16.8%, Pfam/SCOP) to 14,249 (22.9%, considering the novel domain families).

We are now trying to analyze comprehensively the domain architectures in TM proteins, and also to investigate the evolutionary pathways of TM topologies through analyzing domain family sharing among TM proteins with the different number of TM segments.

3P124 全原子および粗視化MD計算による F_o のプロトン経路と c-ring 回転運動の解析
Proton pathway and rotary motion of c-ring in F_o analyzed by all-atom and coarse-grained molecular dynamics simulation.

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F_o is a membrane-embedded rotary motor, functioning as a part of ATP synthase, and is composed of *a*, *b* and *c* subunits. Transmembrane electrochemical potential of proton (or sodium ion) drives the rotational motion of *c*-ring relative to the *a*-subunit. The torque-generation mechanism, however, remains speculative and even the proton translocation pathway is not clear. To shed light on these issues by a computational approach, we conducted molecular dynamics (MD) simulations employing all-atom and coarse-grained models. In the all-atom model, *a*-subunit (PDB ID:1C17) and five *c*-subunits (PDB ID:2WIE) were embedded into DOPC bilayer sandwiched by water layers