2P103 Direct monitoring of membrane protein folding process during in-vitro expression by Surface Enhanced IR spectroscopy

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Surface Enhance Infrared Absorption (SEIRAS) have unique properties that enhances signals at vicinity (in an order of several nm's) of a substrate metal. This property is useful to determine solely the surface chemical process distinguished from that occurred in bulk phase. Therefore when a biological sample of interest is confined to such surface, one can selectively monitor a chemical reaction of the target regardless of the complex ensemble biological process in the bulk phase. We present an application of SEIRAS to in-situ investigation for a folding process of membrane protein (bacteriorhodopsin) during cell free expression on the nano-disc artificial lipid bilayer.

2P104 等温滴定型熱量計による多剤輸送担体 EmrE の基質結合様式の解析

Thermodynamics analysis of substrate binding mode of multidrug resistance transporter, EmrE by Isothermal Titration Calorimetry (ITC)

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EmrE is a substrate/proton antiporter, which extrudes a wide range of lipophilic cations. Here, we had demonstrated with ITC how tetraphenyl phosphonium ion (TPP⁺) binds to the wild-type and mutants (E14Q, D, E25Q). As pH increase, the driving force of EmrE/TPP⁺ binding shifted from enthalpy-driven to entropy-driven and the binding affinity was more potent, inferring that EmrE has a more open conformation at acidic pH, and a closed conformation at neutral and alkaline pH. Especially, Glu25 might be one of determinant residues to drive the substrate binding, because in E25Q, at pH 7.4, the contribution of enthalpy to interaction energy remained a little, but significantly. The mechanism of proton coupled substrate transport will be discussed here.

2P105 多剤排出トランスポーター AcrB の Motion Tree 法による解析

Motion Tree analysis of the multidrug transporter AcrB

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The multidrug transporter AcrB actively exports a wide variety of noxious compounds using the proton-motive force as an energy source in Gram-negative bacteria. AcrB adopts an asymmetric structure comprising three protomers with different conformations that are sequentially converted during drug export. These cyclic conformational changes for drug export are called the functional rotation. The Motion Tree is a new method to describe the structural change between two states of proteins in a hierarchical manner. This method can detect the rigid body motions and clarify the relations among them. In the present study, we applied the Motion Tree analysis to the cyclic conformational changes of AcrB. As a result, the key motions for the functional rotation were observed.

2P106 ABC トランスポーターにおける薬剤結合の影響：分子シミュレーションによる研究

The effects of substrate binding in ABC transporter: A simulation study

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ATP-binding cassette (ABC) transporters constitute a large family of membrane proteins that transport diverse substrate across the cell membrane with the help of ATP hydrolysis. It is known in some ABC transporters that substrate binding promotes the ATPase activity. The effect of substrate binding is thus an important clue to understanding the functional mechanism of the ABC transporter. In present study, we performed all-atom molecular dynamics simulations of the system comprising ABC transporter of the inward-facing form, lipid bilayer, and water, both in the substrate bound state and in the apo state. The substrate binding effect was studied by comparing the simulation results in the substrate bound state with those in the apo state.

2P107 紫膜表面において観測される隆起構造体の曲率に対する溶媒 pH やイオン強度の影響

Curvature of Bump Structures on Purple Membrane Depending on pH and Ionic Strength Analyzed by Atomic Force Microscopy

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Purple membrane (PM) is a membrane patch which is formed by self-assembly of membrane protein bacteriorhodopsin with archaeal lipids. Atomic force microscopy (AFM) studies for PM supported onto mica substrate shows dome-like “bump” structures on the surface. Since PM must have a curvature to form the bump structure, bump formations will be related to a mechanism for structural formations via self-assembly. To elucidate effects of asymmetric distribution of charged residues between two aqueous domains on bump curvature, AFM observations were carried out toward identical PM sheets with varying solvent pH and ionic strength. The bump curvature studies indicated large influence of electrostatic interaction. The model for the bump structure will be discussed.

2P108 ナノディスクを用いたセンサリーロドプシン I Photoreaction dynamics of sensory rhodopsin I in nanodiscs

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Sensory rhodopsin I (SRI) is a member of microbial rhodopsins, functioning as a phototaxis sensor by interacting with a cognate transducer protein. In this study, we have tried to study photoactivation dynamics of SRI by spectroscopic methods, but the measurements in lipid bilayer, the native environment, was difficult, particularly for transient grating method. Here we reconstitute SRI from *Haloarcula vallismortis* (HvSRI) into nanodiscs, and photoreaction dynamics are studied by both transient grating and transient absorption methods. Low scattering of the sample into nanodiscs, allowed us to obtain signals, from which we reveal that the dynamics of HvSRI significantly differ between membrane and detergent.