2P109 サイズの異なるナノポアの脂質二分子膜への再構成
Reconstitution of various-sized nanopores in lipid bilayer

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It is well known that nanopore sensing is strong tool in the field of high sensitive biosensing. To date, α -hemolysin (1.4 nm in diameter) pore has been used in the most of the nanopore sensing. This size of pore can detect a single-stranded DNA (1 nm in diameter) molecule because of the great size-matching. The size of the nanopores often used is nearly 1 nm scale in diameter. However, much larger nanopore is required for detecting proteins, secondly-structured DNA/RNA, and antigen, a few examples have been reported previously. In this study, we show two different proteins, magainin (2 nm in diameter) and human perforin (~10 nm in diameter). We believe that these nanopores can detect the molecules of which size is too large to be detected by α -hemolysin pore.

2P110 多剤輸送担体 EmrE の pH 依存性基質結合駆動力に対する酸性残基の役割

Role of acidic residues of multidrug resistance transporter, EmrE for the pH dependent driving force of substrate binding

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EmrE is H⁺/lipophilic cations antiporter, which has three acidic residues (Glu14, Glu25 and Asp84). We reported previously the pH dependent driving force of substrate binding to EmrE. Here, we had demonstrated with isothermal titration calorimetry (ITC) how substrate binds to the EmrE mutants (E14D, E25Q and D84N). Asp84 is a mainly key residue to drive the substrate binding, because in D84N, the driving forces of substrate binding were enthalpy at pH 6.5 and 7.4, not shifted. These results indicate that Asp84 might become the protonated form at the intermediate state between inward-facing (entropy-driven binding) and outward-facing (enthalpy-driven binding) conformation. The thermodynamics-based mechanism of H⁺/substrate transport in EmrE will be discussed.

2P111 Fast measurements of membrane transporter activity with attoliter-sized arrayed lipid bilayer chamber system

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Transporters play pivotal roles by transporting molecules across bio-membranes. We recently developed the femto-liter sized arrayed chambers sealed by lipid bilayers (ALBiC), which enable the fluorescent detection of single transporter activity. However, it still had some drawbacks, i.e. slow detection (>1 h). To address this issue, we in this study developed the attoliter-sized ALBiC (aL-ALBiC). aL-ALBiC demonstrated the fast detection of passive transport activity by α -hemolysin within hundreds seconds, which was 10-fold faster than that of conventional ALBiC. This fast detection of single transporter molecules would reduce possible artifacts such as photobleach. Thus, aL-ALBiC would be the platform for single-molecule analysis of the various membrane proteins.

2P112 新世界ザルの苦味受容体 TAS2R1 および TAS2R4 のリガンド感受性の種間差と進化

Interspecific variation of ligand sensitivity and evolution of bitter taste receptors TAS2R1 and TAS2R4 in New World monkeys

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Bitter taste is mediated by bitter taste receptors (TAS2Rs). In recent years, sensitivity of human TAS2Rs to various bitter compounds has been elucidated. However, it remains largely unknown how far this knowledge is applicable to other primates and how variable the sensitivity is among species. In this study we focused on TAS2R1 and TAS2R4 of New World monkeys with dietary and sensory (visional) diversity. By calcium imaging in HEK293T cells, we demonstrated interspecific variation of sensitivity to some bitter tastants in both receptors. Furthermore, we applied the functional assay to ancestral receptors reconstructed based on the maximum likelihood phylogenetic inference and identified amino acid substitutions relevant to the evolutionary shift of ligand sensitivity.

2P113 新規 Halorubrum 属菌のもつバクテリオロドプシン類タンパク質の研究

Study on the microbial rhodopsins from the cell membrane of Halorubrum sp.ejinoor

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We have identified the new bR-like and pR-like genes from a halorubrum species (*H.sp.ejinoor* ; (*H.e.*) isolated from Ejinoor salt lake in Inner Mongolia and reported last year. The brown membrane was isolated from *H.e.* by a step sucrose gradient centrifugation like purple membrane. The photoreaction and ion-pump activities were measured on the brown membrane. The photoreaction cycle with M, O-intermediate absorbance was observed, and kinetics were close to that of Xanthorhodopsin. Measurement of ion transportation suggests the existence of proton pump and chloride pump activities in the membrane vesicles of *H.e.*. The results suggest that the brown membrane contains not only bR and pR-like pigments, but also chloride pump, hR-like pigment.

2P114 NpHR の三量体安定化に寄与するアミノ酸残基の特定
Specification of amino acid residues which stabilize trimer formation of halorhodopsin

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Halorhodopsin (NpHR) from *N. pharaonis* is a retinal protein and forms trimer as a unit. At room temperature, the trimer of NpHR is retained both in the presence and absence of anion although the former is more stable. In this study, we aimed to specify the amino acid residue which stabilizes the NpHR trimer. Mutation of N145 or T147 located in the hydrophilic region of the molecular interface to A led to dramatic destabilization of the NpHR trimer in the absence of anion, compared to the case of the wild type. On the other hand, the trimer structures of those mutants were stable similarly to that of the wild type in the presence of anion. These results suggest that N145 and T147 have important role to keep the packing between NpHRs especially in the absence of anion.