1P217 一分子 FRET 測定法を用いた、膜貫通ヘリックス間相互作用への GXXXG モチーフの寄与の解明**Contributions of GXXXG motif to transmembrane helical interactions as revealed by single molecule FRET****Kotaro Kondo**, Yoshiaki Yano, Katsumi Matsuzaki (*Grad. Sch. Pha., Univ. Kyoto*)

Transmembrane helix-helix interactions are important for folding and conformational changes of α -helical membrane proteins. These interactions are dependent on amino acid sequences, lipid compositions, and helical orientations, however, their mechanism is not fully understood. In this study, we focused on the transmembrane dimerization GXXXG motif to measure the association-dissociation dynamics of GXXXG-inserted transmembrane helices in lipid bilayers by single molecule FRET. We revealed that the insertion of the GXXXG motif significantly promoted the parallel association of helices compared with the control helices. We also found that not only amino acid sequences but also lipid compositions strongly regulated the transmembrane helix-helix interactions.

1P218 X 線 1 分子追跡法による 5 量体リガンド作動性イオンチャネル GLIC の pH 依存 3 D 分子内運動マップ**PH dependent 3D Motion Maps of GLIC from X-ray Single Molecule Observations****Yuji Sasaki**^{1,2}, Hiroshi Sekiguchi², Yufuku Matsushita¹, Keigo Ikezaki¹, Yuri Nishino³, Atsuo Miyazawa³, Christele Huon⁴, Jean-Pierre Changeux⁴, Pierre-Jean Corringer⁴ (¹*The University of Tokyo*, ²*Spring-8/JASRI*, ³*University of Hyogo*, ⁴*Pasteur Institute*)

GLIC is a proton-gated bacterial ion channel from *Gloeobacter violaceus* and is member of pentameric ligand-gated ion channels. Recently structural information of GLIC in activated state (acidic pH) and closed state (neutral pH) are available and the gating mechanism is discussed based on these information. However the dynamic information of each state in physical point of view was not available. Here we used Diffracted X-ray Tracking (DXT) method to detect rotational motion of extracellular or transmembrane domain of GLIC from neutral to acidic pH. We found that tilting motion of transmembrane domain of GLIC and the both tilting and twisting motions of extracellular domain of GLIC were enhanced in acidic pH. The detailed dynamic information will be discussed.

1P219 電位依存性 H⁺チャネルのゲート電流**Gating charge movement of the voltage-gated H⁺ channel****Yuichiro Fujiwara**, Yasushi Okamura (*Integrative Physiology, Grad Sch of Med., Osaka University*)

Voltage-gated channels are responsible for sensing membrane potential and generating electrical impulses in many organs. In the voltage sensing process, positively charged residues in the voltage sensor domain are known to sense membrane potential and move across the membrane electric field, generating a transient 'gating current' ahead of an ionic current. However, it is mysterious that the voltage-gated H⁺ channel (Hv) does not show the gating current. Here we report that we succeeded in recording the gating current, and it was affected by changing the pH_o/i gradient and Zn²⁺. This suggests that the voltage sensing and the H⁺ permeation can be considered separately in Hv, and the physiological modifiers affect the voltage sensing process.

**1P220 再構成した電位依存性プロトンチャネルの電気生理学的計測
The electrophysiological recording of the reconstituted voltage-gated proton channel in artificial membrane****Akira Kawanabe**, Yasushi Okamura (*Grad. Sch. Med., Osaka Univ.*)

The voltage-gated proton channel protein (VSOP/Hv1) is known to be controlled by membrane voltage and pH. A number of fundamental questions remain unanswered. These include mechanisms of voltage gating, sensing the pH difference across membrane and proton selective permeation. The reconstitution system is ideally suited for answering these questions. We therefore construct the reconstitution system of the expressed VSOP into the artificial membrane.

Mouse-VSOP/Hv1 protein heterologously expressed in *E. coli* was purified and reconstituted into the artificial membrane liposome. The activity of VSOP/Hv1 was confirmed upon liposomal pH change. We report here our trial of electrophysiological recording of VSOP/Hv1 in the reconstituted proteoliposome.

1P221 カリウムチャネル KcsA のゲート開閉と運動した膜中集合・離散**Gating-associated clustering-dispersion dynamics of the KcsA potassium channel in a lipid membrane environment****Ayumi Sumino**^{1,2}, Daisuke Yamamoto³, Masayuki Iwamoto², Takehisa Dewa⁴, Shigetoshi Oiki² (¹*JST/PRESTO*, ²*Facult. Med. Sci., Univ. Fukui*, ³*Facult. Sci., Univ. Fukuoka*, ⁴*Grad. Sch. Eng., Nagoya Inst. Tech.*)

The KcsA potassium channel is a pH-dependent channel, and the activation gate opens at acidic pH. Using atomic force microscopy (AFM), we revealed pH-dependent clustering-dispersion behavior of the KcsA channels on the membrane, which is associated with the gating. At neutral pH, the closed channels formed self-assembled nanoclusters. At acidic pH, the open-gated channels were dispersed as singly-isolated channels. High-speed AFM revealed that the clustering-dispersion dynamics were completed within several minutes. The interplay between the gating conformational change of individual channels and the collective behavior of the clustering-dispersion provides insight into understanding membrane-mediated protein-protein interactions and functional cooperativity.

**1P222 無細胞翻訳系による SecYEG トランスロコンの合成
In vitro synthesis of SecYEG translocon****Hideaki Matsubayashi**¹, Yutetsu Kuruma^{1,2}, Takuya Ueda¹ (¹*Graduate School of Frontier Sciences, The University of Tokyo*, ²*Earth-Life Science Institute, Tokyo Institute of Technology*)

SecYEG is the bacterial protein conducting channel which facilitates the transport of membrane proteins and secretory proteins. In contrast to the structural and functional analysis, its biogenesis is still unknown. In order to study how SecYEG subunits assemble into complex, we aimed to reconstruct SecYEG on lipid vesicles by synthesizing its subunit proteins in the PURE system: the reconstituted cell-free protein synthesis system. Synthesized subunit SecY, SecE, and SecG proteins spontaneously formed SecYEG complexes on lipid membrane and showed the protein translocation activity. The results suggested that SecYEG protein could form an active complex in self-assembly process.