**S1D-3-1** ATP hydrolysis-driven gating of an ABC transporter CFTR channel: From stills to movies.

**Yoshiro Sohma**


Cystic Fibrosis Transmembrane conductance Regulator (CFTR), a member of the ABC transporter superfamily, is a small conductance, PKA-regulated, ATP-dependent anion channel, which is very valuable as a model protein for investigating the mechanism of ABC transporters beside a product of the Cystic Fibrosis gene. Although many biochemical and electrophysiological functional studies had been performed on CFTR proteins, the accumulated functional data were too puzzling to give us a unified view for the ATP-dependent gating mechanism. However, recent solutions of the atomic structures in several ABC transporters suggested that two Nucleotide Binding Domains might form a dimer with sandwiching two ATP molecules in the dimer interface. Now the NBD dimerization hypothesis allows us to make a comprehensive understanding of CFTR ATP-dependent gating. Thus only a few snapshots of the molecular structures have provided a great advance in the research field.

Recent advances in the single molecule biophysics such as the high-speed AFM, are going to enable us to shoot movies of single molecules in action. In this symposium, we will introduce and discuss about the forefront of science and technology for "viewing" the molecular mechanism.

**S1D-3-2** Structure basis of a regulatory module of TRP channel homolog

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Transient receptor potential (TRP) channels play various physiological roles by responding to wide arrays of extracellular stimuli, such as temperature, membrane stretch, and chemical substances, as well as intracellular signaling, mediated by Ca" and phosphatidylinositol. They are attracting interests as potential drug targets for analgesia, and neuronal, bladder, dermatologic, or pulmonary disorders. We identified a fungus membrane protein homologous to a yeast-vacular osmosensitive TRP channel. The fungus protein turned out to share common functional features of TRP channel families, and thus is expected to serve as a prototypical protein for understanding TRP channel regulations. The integrated approaches by biochemical, biophysical, and crystallographic structure analyses elucidated a modular architecture of the C-terminal cytosolic region of the TRP channel homolog, a high-resolution structural snapshot of the region crucial for osmosensitve channel activity in a presumable active state, and dynamic characteristics of regulation on the channel activities by the C-terminal region upon various stimuli.

**S1D-3-3** Single-molecule analyses of the reactions and movements of ErbB receptors

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The ErbB family consists of four members of integral membrane proteins in the plasma membrane of various types of mammalian cells and is responsible to signal processing of cell proliferation and differentiation. ErbBs belong to the receptor protein tyrosine kinase superfamily, which form homo- and hetero-dimers upon association of the extracellular ligands and the dimerization induces mutual phosphorylations on the cytoplasmic tail of ErbB molecules. The phosphorylations of ErbB will be recognized by various species of cytoplasmic proteins. In this presentation, we will show the results of single-molecule analyses of the reactions and movements of ErbB1, B3, and B4 molecules observed in living cells. Using fluorescent labeling of ligands, receptors, and the cytoplasmic proteins, we can detect interactions between proteins on the membrane for kinetic analyses and movements of proteins along the membrane to see protein dynamics. Single-molecule analyses are revealing mechanism of signal processing by the ErbB system.

**S1D-3-4** Immuno-EM of cells in solution using the Atmospheric Scanning Electron Microscope (ASEM)

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Cell activity involves many proteins dynamically associating and dissociating, creating various orders of protein complexes. Understanding these mechanisms requires observation of their localization and their structural changes. Using single particle reconstruction EM, we observed the dynamic rearrangement of membrane protein complexes. Immuno-EM is an essential tool for high-resolution imaging of protein distribution. However, observation of samples in vacuum requires exacting and time-consuming pretreatment. The ASEM addresses this with an inverted electron microscope having an open sample dish [1]. The ASEM dish has a pressure-resistant 100nm SIN film window, allowing electron beam scanning of the aqueous sample from below. Surface coatings such as poly-L-lysine and collagen can be used, allowing culture of various types of cells in a CO2 incubator, including neural cells. Immuno-EM with the ASEM dish allows direct observation of cells labeled with gold-tagged antibodies in buffer, so epitopes are well preserved. Using the ASEM, we determined the localization of various proteins and their associations [2, 3, 4].[1] J. Struct. Biol. 169 (2010) 438-449. [2] J. Biol. Chem. 286 (2011) 1999-2007. [3] Ultramicroscopy, 111 (2011) 1650-1658.