2P288 ドロップレット法を用いたアクトヘビーメロミオシン封入巨大リボソームの構築
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ActoHMM-containing giant liposomes constructed from water-in-oil droplets
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Liposome is a micro-compartment made of lipid bilayer membranes with characteristics quite similar to those of biological ones. To form an artificial cell model being able to reproduce morphology and motility of the living cells, giant liposomes containing cytoskeletal proteins have been tried to construct. Using dark-field optical microscopy, Honda et. al. have visualized dynamic behaviors and shape changes of the liposomes caused by mechanical forces generated by the assembly of encapsulated proteins (Honda et al., J. Mol. Biol., 1999; Nomura et al., J. Biol. Phys., 2002).

However, no one has succeed to construct efficiently giant liposomes that contained molecular motor, such as myosin, and their fuel ATP, owe to the difficulty in preparing giant liposomes at physiological salt condition, especially in the presence of divalent cation, and under the high viscosity of actomyosin solutions.

Recently, it has been found that water-in-oil phospholipid-coated micro-droplets at an oil/water interface are spontaneously transformed into giant liposomes ever in the presence of Mg²⁺ (Hase & Yoshikawa, J. Chem. Phys., 2006; Yamada et al., Langmuir, 2006). By adopting this methodology, we have tried to construct giant liposomes containing both F-actin and HMM in the presence of 5mM MgCl₂ and 50mM KCl. Successful generation of giant liposomes with the high concentrations of actoHMM is reported, showing the formation of self-organized actin network-like structures. This is the first step for developing the motile artificial cell model.

2P290 原核生物由来膜電位依存性ナトリウムチャネルのチャネル特性の多様性
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2aK13 **The bacterial voltage-gated sodium channel family has various channel properties.**
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Voltage-gated sodium channels (Navs) generate the rapid upstroke of action potentials in axons. Complexity and instability render structural studies of Navs difficult. NavBacH, the bacterial voltage-gated sodium channel from *Bacillus halodurans*, is highly simpler than eukaryotic Navs. NavBacH is a six-transmembrane protein and forms a homo-tetramer. Despite both being Navs, NavBacH and eukaryotic Navs differ greatly in their kinetics.

Here we report, newly cloned voltage-gated sodium channels from three different bacteria (NavBacL from *Bacillus licheniformis*, NavSheP from *Shewanella putrefaciens* and NavRosD from *Roseobacter denitrificans*). Their sequence homologies with NavBacH were 49.4, 36.7 and 32.8% respectively. When expressed in HEK293 cells, all three bacterial Navs were Na⁺-selective and voltage-dependent. However their kinetics and voltage dependence differed significantly from each other. Activation and inactivation of NavBacL were as slow as NavBacH's, while those of NavRosD and NavSheP were faster than those of NavBacH and close to eukaryotic Navs. At the point of voltage dependence, inward currents of NavBacL and NavSheP peaked at -70mV and NavRosD's peaked at 0mV. Their various properties are thought to be optimized for each bacterium's habitat.

We show that bacterial Navs, notwithstanding their sequence homologies, exhibit great electrophysiological diversity. Further studies of structure and function considering similarity among them will be helpful to understand the basic mechanism of all Navs.

2P289 システインスキャン実験に見られる電圧感受性イオンチャネルの S4 ヘリックスの大きな動き
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2aK12 **Large mobility of S4 helix in voltage-gated ion channel revealed by cysteine-scanning**
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Voltage-gated ion channel consists of two modules: voltage-sensor (S1-S4 transmembrane helices) and gating-pore (S5-S6 helices). The sensor is located around the pore that sits in the center of the functional tetramer. A previous study of *shaker* channel with cysteine-mutations showed that the first arginine residue in S4-helix, the heart of voltage-sensor, becomes close to a residue at S5-helix of the adjacent subunit in the inactivated state. Assuming that the sensor shows dramatic conformational change depending on membrane potential however, some other pairs of residues that are close to each other should be found. We introduced two cysteine mutations into bacterial voltage-gated Na channel (NavBacH) and prepared its *E. coli* expression vector; the closer the two mutated points, the more channel tetramerized by intersubunit disulfide bond upon expression. 62 pairs were surveyed by checking tetramerization on SDS-PAGE. For the residues at S5-helix, exclusively M164 was found close to residues in the sensor part. In contrast, a wide range of residues at the extracellular of S4-helix were close to M164. Three of them, Q107, T110 and R113, are noticeable because they are closer to M164 than the others and appear every 3 resiudes. T110 is the closest to M164, and the second is R113 which is the first arginine in NavBacH. This cysteine-scanning study clearly shows how largely S4-helix can move as membrane-crossing, and also may give structural information about the extracellular of S4-helix in this bacterial channel.

2P291 細胞接着部位へ停留する膜伸展依存性 BK チャネルの 1 分子可視化解析
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2aK14 **Immobilization of Stretch-Activated BKca channels to focal contacts of cell membrane as studied by single fluorophore video imaging**
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Stretch activated and Ca²⁺-activated big K channel (SAKCaC) identified by our group, is one form of splice variants of the BKca channel encoded by *Slo-1* gene, and has the STREX-sequence, a 59 amino-acid splice insert located in the cytoplasmic side of the channel, which may responsible for the membrane stretch sensitivity of SAKCaCs. Recently, we found that most SAKCaCs exhibited simple diffusion on the plasma membrane but that approximatly 25% of the channels was immobilized on or near focal contacts (FCs), suggesting that SAKCaCs are associated with the FC components such as adhesion molecules and cytoskeletons to form a mechanosensing device. However, the mechanisms of such channel immobilization on FC remains unclear. To address this issue, we examined the role of STREX on the channel immobilization. We expressed GFP-tagged STREX and observed using the single fluorophore video imaging technique. Simultaneous observation with RFP-tagged paxillin showed that STREX molecules were recruited and diffused rapidly on the plasma membrane but significant fraction of them was immobilized on FCs, indicating that STREX itself could associate with certain FC components. However, STREX-deleted SAKCaCs were also immobilized on or near FCs, indicating that SAKCaCs could be associated with FCs via STREX and/or other sites of the channel.