small but significant amounts of sliding displacement between the anti-parallel coiled-coil helices as a means of communication between the AAA core and the microtubule binding head (Gibbons *et al., J. Biol. Chem.*, **280**, 23960-23965, 2005). However, the structural basis of how to slide the two long strands of coiled-coil helices in the opposite directions and couple the microtubule binding is still unknown. In order to address these questions, we expressed several recombinant proteins of the microtubule binding domain of mouse cytoplasmic dynein with the different lengths of stalk anti-parallel coiled-coil, and crystallized them for the X-ray structure analysis. Here we will discuss the coiled-coil structure of the stalk domain connecting the microtubule binding domain and the AAA core of cytoplasmic dynein.

2S8-1 MEMSによるベシクル形成

MEMS-based vesicle formation

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Membrane proteins play very important roles in cells. They are also useful in various industrial fields, including next-generation diagnosis techniques, drug discovery, and highly-sensitive biosensors. Here, I will introduce our microfluidic technologies to form lipid bilayer membrane and vesicles. These technologies are useful for producing a membrane protein chip: an array of single-species-specific membrane proteins reconstituted into (1) planar lipid bilayers formed in microfabricated holes and channels and (2) giant vesicles. In our first approach, a highly reproducible method was developed for planar lipid bilayer reconstitution. Planar lipid bilayers are formed at micro apertures, by flowing lipid organic solution and buffer alternately into an integrated microfluidic channel. Using this technique, multiple lipid bilayers are formed simultaneously in a single chip, and channel currents through peptide ion channels was recorded in parallel. In the second approach, we try to prepare monodisperse giant vesicles by "blowing" a planar lipid bilayer and deforming it into vesicles. We have also developed a simple method to make an array of the vesicles on a microfluidic chip. We believe that these devices are useful for an efficient and rapid analysis of single-species-specific membrane proteins.

2S8-2 巨大リポソームを用いて観察された様々な条件下で起きる膜穿孔

Membrane pore formations that were observed by using giant liposomes

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In our studies, dynamic behaviors of unlabelled giant liposomes caused by interactions between liposomal membranes and surfactants, amphiphilic peptides or membrane-interacting proteins were directly visualized by optical high-intensity dark-field microscopy. They are thought to induce many events into lipid bilayer membranes, transformation, solubilization, pore formation or fusion and fission; however, the actual process has not been clarified.

We found that liposomes exposed to melittin, an amphiphilic peptide from bee venom toxin, or proteins possessing the membrane-binding FERM domain in their N-terminal, exhibited opening large membrane hole. It has been well known that large pores can be formed in liposomal membranes using an electro-permeabilization technique; the formed pores are quasi-stationary and they closed spontaneously. In contrast, our found protein- or peptide-induced holes in giant liposomes are extremely stable. When the FERM-domain proteins or melittin were added to a liposome solution, liposomes opened stable holes and transformed into cup-shaped liposomes. The size of holes was depending on the concentration of proteins or peptide. The proteins localized mainly along the membrane verges, and presumably prevented exposure of the hydrophobic portion of lipid molecules at the edge of the lipid bilayer. This was the first demonstration that a lipid bilayer can stably maintain a free verge in aqueous solution, and refutes the established dogma that all lipid bilayer membranes inevitably form closed vesicles.

2S8-3 単一GUV法を用いたペプチド/蛋白質と脂質膜の相互作用の研究

The Single GUV Method Reveals Interaction of Peptides/Proteins with Lipid Membranes

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Interaction of substances such as peptides/proteins has been investigated using a suspension of many small vesicles such as LUVs. In these studies, the average values of the physical parameters of vesicles have been obtained from many vesicles, and thereby much information has been lost. Recently we have proposed a novel method, the single GUV method [1-6]; we observe interactions of substances with single giant unilamellar vesicles (GUVs) with a diameter of >10 μm, and measure their physical properties' changes, and analyze these results over many "single GUVs" statistically.

The measurement of leakage of internal contents from the inside of LUVs has been extensively used to investigate the interaction of various kinds of substances (e.g., antimicrobial substances, peptides/proteins with hemolytic activity and pore-forming activity) with lipid membranes. For this leakage experiment, the single GUV method can provide new information such as direct evidence for the cause of the leakage, detailed elementary processes, and their rate constants [5]. I show several examples (e.g., effects of antimicrobial peptides, and other peptides/proteins, and also antibacterial substance on lipid membranes), and discuss the advantage of the single GUV method.

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2S8-4 モデル生体膜における相分離と浸透圧差による変形ダイナミクス

Shape deformation of ternary vesicles coupled with phase separation

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We report shape deformation branches of the ternary vesicle induced by the competition between the phase separation and the osmotic pressure difference. If we add salts outside of the vesicle, the vesicle deforms to various shape with repeating bifurcations. These various shapes are described by the area difference elasticity (ADE) model using two parameters, i.e. the excess area, defined by the total area to total volume ratio, and the intrinsic area difference between upper and lower leaflets. On the other hand, the ternary vesicles composed of saturated phospholipid, unsaturated phospholipid and cholesterol form domains below the immiscible transition temperature. The domains show coarsening and shape deformation called budding to reduce the boundary energy. In this study, we prepared the spherical ternary vesicles in homogeneous one phase region and added salts outside of the vesicles. When the vesicles deformed to appropriate shapes, we decreased the temperature to the coexisting two phase region where the shape deformation induced by the osmotic pressure difference couples with the phase separation. In the domain coarsening stage, every polygonal vesicle showed a shape convergence to discocytes with two large domains, whereas a tube vesicle deformed to a modulated structure, like a pearling instability. After the coarsening, domains started to bud toward inside or outside of the vesicle depending on excess area. These unique shape-deformation branches can be explained by the free energy analyses based on the ADE model.

2S8-5 新しい人工細胞モデル：分子コンピュータ内蔵リポソーム

Calculating liposome as a model system of artificial cellular life

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Recently, bio-molecular reactions (transcription, translation, PCR, etc.) in liposome have been reported, studies for bottom-up type artificial cellular life are just on the move. One of the characteristic which artificial cell models should aim to realize is a response to the environment. This response consists of the sensing of environmental information and the production of corresponding materials. We considered this reaction process to be a calculation, and had already developed RTRACS (Reverse transcription-and-TRanscription-based Autonomous Computing System) which was a molecular computer containing RTase and T7 RNA polymerase.

A liposome with the built-in RTRACS is our new artificial cell model which responds to the environmental information. Since RTRACS does not need additional materials and manipulations, it is suitable molecular computer in liposome which represses the permeability of materials across the membrane. In order to incorporate an input molecule into the liposome efficiently, the liposome has to be a giant unilamellar vesicle (GUV). Therefore a W/O emulsion centrifugation method is applied to construct GUV encapsulating RTRACS. In this method, a W/O emulsion containing lipid molecules is placed on a buffer solution, the water droplet in oil transfers to the buffer solution under the centrifugal force. In this study, we challenge to run RTRACS in GUV with an input oligonucleotide as a model material of environmental information from exterior of the liposome.

2S8-6 蛍光セルソータを用いたリポソーム構造および内部反応のダイナミクス計測

Dynamics of structure and internal reactions in liposomes explored by fluorescence-activated cell sorter

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Liposomes are widely used as a compartment of model cell system to mimic the physiological phenomena in living cells. Utilization of such a model system should allow us to extract the essential characteristics in a complex system in nature. Previously, we showed that the functional proteins can be synthesized in liposome containing gene and cell-free translation system. Our next step is to quantitatively investigate the dynamics of the internal biochemical reaction encapsulated, which may behave differently as a result of compartmentalization in the lipid membrane. Here, we addressed two aspects in liposome containing the cell-free translation mixture using the fluorescence-activated cell sorter (FACS). First, we examined the property of liposome as a microreactor. We show that the transcription and translation reaction, which involves numbers of reaction steps, does not occur uniformly in the entire volume of multilamellar liposome. However, quantity of the partial volume where the reaction took place was proportional to the entire volume irrespective of the liposome size. It indicates that the reaction volume can be estimated by conversion. Second, we studied how the presence of the lipid membrane affects the internal reaction. It was explored by statistically analyzing the dynamics of reaction in liposome composed of various phospholipids. Our measurement and analysis method enables us to understand the high-dimensional information present in the model cell system.