2P277**Promiscuous ligand binding of β -lactoglobulin allowed by hydrophobic interactions and structural variability**○Tsuyoshi Konuma¹, Kazumasa Sakurai¹, Yuji Goto^{1,2}¹Institute for Protein Research, Osaka University, ²CREST/JST

Although bovine β -lactoglobulin (β LG) binds a variety of hydrophobic ligands, the underlying mechanism is unknown. To understand the structural basis of the ligand binding of β LG, we studied the binding of palmitic acid (PA) using heteronuclear NMR spectroscopy. Upon the binding of PA, signal disappearances and large changes in chemical shifts were observed for the residues located at the entrance of the cavity and those at the bottom, respectively. This observation indicates that the bottom region makes rigid interaction with PA whereas the entrance becomes more fluctuating. The result is in contrast to the ligand binding of intestinal fatty acid binding protein, another lipocalin family protein, in which structural consolidation occurs upon ligand binding. On the other hand, the ability of β LG to accommodate various hydrophobic ligands resembles that of GroEL, in which a huge hydrophobic cavity and flexible binding site confers the ability to bind various hydrophobic substrates. Considering these observations, it is suggested that, in addition to the presence of the large hydrophobic cavity, the ability of entrance region to assume various conformations makes possible the bindings of various shapes of hydrophobic ligands. Thus, in contrast to the specific ligand binding seen in many enzymes, β LG provides a model of another type of ligand binding with low specificity or promiscuity, which may play an important role in protein-ligand and protein-protein networks.

2P279**Formation of Ultra Giant Vesicles from a Planar Lipid Membrane**

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We report a simple method to create lipid vesicles. Lipid vesicles are the most fundamental structures in biology as a cell membrane models. Here, we utilize the planar bilayer to create lipid vesicles, similar to blowing soap bubbles from a soap film. When micro jets are applied from the one side of the lipid membrane, vesicles are ejected toward the opposite side and desired reagents can be encapsulated into vesicles directly. A planar lipid bilayer was formed at the interface of two droplets immersed in the organic solvent containing phospholipids. A chip device with the gourd-shaped well was fabricated. At first, the well was filled with lipid solution, and two water droplets (15 μ l) were then introduced with micro pipette. The bilayer was formed when the surfaces of the droplets contacted each other. Then, a nozzle made of a glass capillary was brought near the lipid membrane from the lateral side. It was connected to the air dispenser. As pulsed jet flows containing fluorescent molecules were ejected, bright and ultra-sized lipid vesicles were formed repeatedly (mean diameter; 480 μ m). We captured the sequence of lipid vesicle formation with a high speed camera. With an applied jet flow, the center of the lipid membrane dramatically deformed and ejected, resulting in formation of vesicles. Small satellites were sometimes created depending on ejecting conditions. By incorporating membrane proteins into the planar lipid membrane, the formed vesicles will be used as a tool for membrane protein analysis.

2P278**Giant Liposomes in Micro-Fluidic Device - Towards an efficient preparation, manipulation and immobilization method -**○Kaori Kuribayashi¹, Bruno Le Pioufle², Shoji Takeuchi¹¹CIRMM-IIS, The University of Tokyo, ²LIMMS/CNRS-IIS, The University of Tokyo

This abstract describes a new approach of micro-fluidic device (μ FD) for producing, handling and arraying giant liposomes. Benefits of using μ FD for such liposome application include (i) only minute amount of reactants is needed for liposome preparation, and (ii) parallel micro channels and chambers allow several liposome operations to be performed simultaneously. The usage of giant liposomes has increased in various fields. However, presently, there exist no simple all-in-one microdevice for preparation, manipulation and immobilization of giant liposomes, and such a device could greatly advance research in these fields. In this research, we prove this new concept using two prototypes. Using the first device of PDMS channels and chambers sandwiched between two ITO substrates, we succeeded in electroforming simultaneously two types of fluorescently-stained giant liposomes at bottom of each micro chamber. These liposomes were transported to top of the chamber by applying DC voltage (2 V). Finally, giant liposomes were collected through the microfluidic channels using a microfluidic flow. The second device has micro-hole array etched onto Parylene film formed over microchannels fabricated using DRIE process. In this way giant liposomes dyed red and green were successfully immobilized at different regions of the array by the negative pressure. This work demonstrates electroforming and handling (i.e. transporting, collecting and arraying) giant liposomes in microdevices.

2P280**Asymmetric fusion between a giant unilamellar vesicle and large unilamellar vesicles applied to controlling a cell-sized microreactor**

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Giant unilamellar vesicles (GUV; $\sim 10 \mu$ m) have membrane structures and cell-sized closed spaces, which are supposed to be microreactors suitable for modeling cells. To understand properties of biochemical processes entrapped in a microcompartment, it is necessary to make GUV inner systems more controllable. Here we studied the fusion of a GUV and large unilamellar vesicles (LUVs, ~ 100 nm), which can be used as carriers transferring reagents into a GUV inner space. GUVs and LUVs with various DOPC/DOPG contents were mixed in the presence of fusogenic amphipathic peptides composed of alternating lysines/leucines (KL-22), and then a favorable condition for the fusion was determined by lipid and content mixing assays through a fluorescence microscopic observation on individual GUVs. Interestingly, the fusion with a content mixing occurred often when the DOPG composition (90%) of GUVs was higher than that (50%) of LUVs, suggesting that a great deal of acid phospholipid in GUVs and a certain amount in LUVs are appropriate to chiefly induce the asymmetric fusion between a GUV and LUVs. For the attempt to construct a cell-sized microreactor, we prepared GUVs encapsulating resorufin- β -glucoside, and then mixed them with LUVs containing β -glucosidase in the presence of KL-22. Under the above condition leading to the asymmetric fusion, ongoing hydrolysis of resorufin- β -glucoside was observed. Here, we will also discuss a model of the fusion between a GUV and LUVs.