2P268 生体模倣非対称モデル膜におけるドメイン構造形成
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The formation of raft domains in asymmetric vesicles.
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Since giant liposomes exhibit essentially the same bilayer structure as living cell membranes, they have been actively studied as a model of cell membranes. Although various methods for preparing liposomes were developed, difficulties in capturing the asymmetric nature of the lipid bilayers have not been fully resolved. Some of the current efforts in our laboratory are focused on formation of the giant liposomes with asymmetric lipid bilayers. The asymmetry between bilayer leaflets is largely related to physico-chemical properties of biomembranes, such as microdomain structure called rafts due to the interaction between lipid molecules. Here, we examine the effect of the asymmetric nature on the stability of the microdomains. The observation chamber consisted of a cylindrical hole in a Silicon rubber sheet on a glass slide. A thin layer of water phase was introduced at the bottom of the cylinder and then topped by a thin layer of the oil phase containing phospholipids (to be outer leaflet). A third layer of W/O emulsion (to be inner layer) was added above the first two layers. It is found that W/O micro-droplets spontaneously transferred across the oil/water interface from the oil phase to the water phase. During this process, W/O micro-droplets were transformed to be liposomes with asymmetric bilayer. The membrane fluctuation of thus obtained liposomes indicates that the bilayer membrane has little oil phase. Then, we adopted ternary lipid mixture system (DOPC, DPPC, cholesterol) to produce raft-exhibiting giant liposomes.

2P270 細胞サイズリボソームにおける内部区画を考慮した体積分布
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Inner aqueous volume distributions of cell-size liposomes in consideration of the inner-compartmentalization
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We investigated the inner aqueous volume distribution of cell-size liposomes. Cell-size liposomes have been used as a micro-reactor, as a capsule for drug delivery, or as a cell model: however, their heterogeneity in shape, especially in inner-compartmentalization, always lie in the way for those applications. Uncovering the basis underlying the inner aqueous volume distribution of inner-compartmentalization (i.e., a physically connected single liposome with multiple partial compartments) allows the physical understanding of the liposome formation process. So far, little work has been done to understand the basis of inner-compartmentalization. We evaluated liposomes with two parameters, which can be obtained experimentally by analyzing liposomes with flow cytometry (FCM): "whole volume" and "partial volume". We then found that the resulting distribution of these two volumes can be explained by a simple model for liposome formation, which gave us a clue to the physical basis of liposome formation. Based on these results, we developed a strategy to estimate the partial volume distribution from the data of the whole volume distribution. These estimations allowed us to detect and analyze a single molecule biochemical reaction in liposome.

2P269 環状骨格の剛直性は環状脂質の側方拡散に如何に影響を及ぼすか
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2aK04 Effect of rigid cyclic framework on lateral diffusion of cyclic lipid membranes
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Considerable resistance of archaea to harsh environments is derived from unique cyclic lipids observed in their biomembranes. We have synthesized artificial cyclic lipids that mimic the natural lipids for the purpose of constructing mechanically and thermally stable membranes. The structural features of these lipids include (i) a cyclic framework, (ii) two diacetylenic units, (iii) ether bonds and (iv) two hydrophilic groups. These are the minimum structural elements that would be necessary in order to create functional equivalents of the archaeal lipids. We have observed that the artificial lipids construct various types of self-assemblies. Specifically, a cyclic lipid containing 20-carbon long alkyl chains (C20) forms planar membranes on solid supports as well as vesicles and tubules. Since this planar membrane has membrane fluidity at ambient temperature, we consider that such a supported membrane system is a novel biomembrane model. Recently, a fundamental question occurred to us. How do the diacetylenic units affect on the membrane fluidity? To answer this question, we have synthesized a C20 derivative that contains saturated long alkyl chains (i.e., saturated C20), and measured membrane fluidity of the membranes composed of this lipid by using FRAP. In this paper, we wish to report the diacetylenic effect on the fluidity of cyclic lipid membranes by comparing a lateral diffusion constant between C20 and saturated C20.

2P271 INTERMOLECULAR RELATIONS AND HYDROGEN BOND DYNAMICS AT PHOSPHOLIPID MEMBRANE INTERFACE
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INTERMOLECULAR RELATIONS AND HYDROGEN BOND DYNAMICS AT PHOSPHOLIPID MEMBRANE INTERFACE
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Carbonyl, phosphate, and choline are the structural moieties at cellular interface participating in molecular recognition and signal transduction. Our knowledge on their relative arrangement and dynamics is essentially limited due to disorder in the polar crust of membrane. To learn about structural relations at interface of phospholipid membrane fragments we employ methods of nonlinear two colour two-dimensional infrared (2D-IR) spectroscopy. As a femtosecond analogue of 2D-NMR, 2D-IR disentangles inhomogeneities contributing along spectral excitation and probe axes and reveals distinct cross peak pattern due to anharmonic coupling. Using 2D line-shape of carbonyls, we estimate IR delocalization beyond 4 neighbouring C=O moieties via transition dipole moment coupling. Diagonal resonance of D₂O stretching modes reveals structural heterogeneity of aqueous species in the polar compartment of bilayer. Properties of water in membrane environment differ remarkably from those observed in bulk water, and in water segregated in reverse micelles. Exploring the perturbation of C=O absorption upon excitation of D₂O stretching modes, we demonstrate that sn-1 carbonyl has a higher propensity to form hydrogen bonds with water comparing to sn-2. The ultrafast water response, when energy is invested into the C=O stretching, characterizes the structural dynamics in C=O...D-O pairs. Two-colour 2D-IR spectroscopy provides a unique opportunity to review intermolecular structural properties and local hydrogen bond dynamics in disordered environment.