S1e1-4
Active membranes: compositional segregation, budding and shape fluctuations
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The surface of living cells are subject to a variety of active forces which modulate its composition and shape. I will briefly discuss the results of some recent experiments on the dynamics of clustering of lipid anchored proteins at the cell surface and their relation to local morphology of the cell membrane. In order to understand these experiments we need a framework of active membranes. I will discuss such a framework in the context of compositional segregation, budding and shape fluctuations.

S1f1-1
X-ray structural determination of hydrogen atoms in bovine heart cytochrome c oxidase
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X-ray structure at the hydrogen atom level is indispensable for elucidation of the reaction mechanism of cytochrome c oxidase. In general hydrogen atoms become visible at above 1.4Å resolution. In the case of crystals of bovine heart cytochrome c oxidase, the ratio of number of data points (reflections) at 1.4Å resolution to atomic parameters to be determined is 5.5. However it is not easy to solve X-ray structure of large membrane protein at 1.4Å resolution. Because number of reflections depends on the resolution of X-ray diffraction experiments, the parameter ratio could be increased by reducing number of atomic parameters to be determined. TLS refinement is a method for reducing the number of temperature factor, considering the restraints in atoms of the ring structures like aromatic amino acids and imidazole groups. 7,844 parameters could be reduced by this method. Refinement of local isotropic temperature factor is also effective for reducing atomic parameters. The temperature factors of main chain atoms are approximately with isotropic temperature factors, since these atoms are tightly fixed. 37,000 parameters could be reduced by this method. Number of atomic parameters could be reduced from 138,429 to 93,585 with these two approximations. By reducing atomic parameters, the ratio of number of reflections at 1.6Å resolution to atomic parameters to be determined become 5.4. This result shows hydrogen atoms could be determined at 1.6Å resolution. The example of these approximations will be presented.

S1e1-5
Raft and non-raft molecules undergo very similar diffusion in the time scales between 25 microseconds and 2.5 seconds
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We investigated the rates of diffusion of putative raft-associated GPI-anchored protein molecules, including CD59 in unstimulated cells, and compared them with those of typical non-raft molecules, an unsaturated phospholipid, dioleoylphosphatidylethanolamine (DOPE) and a transmembrane protein transferrin receptor (TIR). Both single fluorescent molecule tracking at video rate (33-ms resolution) and single gold particle tracking at a 25-μs resolution were employed. In human T24 epithelial cells, all GPI-anchored molecules and DOPE undergo short-term confined diffusion within 110 nm compartments and long-term hop diffusion over those compartments once every 25 ms on average. TIR exhibited similar compartment size but much longer residency time (62 ms on average). In other cell types studied here (NRK, etc.), all of these test molecules exhibited similar compartment sizes for the same cells, either before or after the treatment for partial cholesterol depletion, and GPI-anchored proteins and DOPE exhibited similar hop rates under the same conditions. Based on our previous research, it is concluded that the movements of both putative raft-associated and non-associating molecules are largely determined by partitioning of the plasma membrane due to membrane-skeleton "fences" and transmembrane protein "pickets" anchored to and lined up along the membrane-skeleton, and that the effects of putative raft association on diffusion will be small until the rafts are enlarged and stabilized, as triggered by intracellular or extracellular queues.

S1f1-2
Crystallization of membrane protein complexes
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Membrane protein complexes perform a wide range of biological important functions. Integral membrane proteins possess hydrophobic surfaces that are in contact with lipid bilayer and hydrophilic surfaces exposed to the aqueous environment. Upon disruption of the membrane and removal of the lipid, these proteins tend to aggregate unspecifically and to precipitate. When membrane lipids are replaced by detergents the proteins can be solubilized in aqueous solution. For solving the functions of these membrane protein complexes, the most important information is the three-dimensional structure of these proteins at atomic resolution. However, purification and crystallization of the membrane proteins are difficult, because of their amphipathic natures. The most critical point in handling the membrane proteins is to find a detergent which replaces the lipids of the membrane without decrease in stability and change in its structure and function. Once the protein has successfully solubilized and purified with suitable detergent, crystallization trials may proceed. We have studied about cytochrome c oxidase, the terminal enzyme of mitochondrial respiratory chain. Since the crystal structures of beef heart enzyme at 2.8 Å resolution were obtained from the crystals stabilizing with decyl maltoside, we have improved the crystallization conditions to provide crystals that diffract x-rays up to 1.59 Å resolution. Some aspects of detergent structure and our experiences in improving the quality of crystals will be discussed.