New and Notable

Round-up at the Bilayer Corral

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Pattern formation in biological systems is an area of long term study. However, the systems under study are usually forming patterns themselves, rather than being forced to form patterns that might be more consistent with practical applications. For example, spontaneous pattern formation occurs in a variety of phase-separated lipid and lipid-protein monolayers at the air-water interface (Möhwald, 1991); such patterns can be transferred to substrates via the Langmuir-Blodgett or other techniques (Chi et al., 1993) to provide a template for the growth of materials or to serve as a site for specific attachment as the basis for a biosensor (Fang and Knobler, 1995). Theoretical considerations suggest that the shape of a lipid domain depends on a competition between the dipole repulsion of the lipids, which causes the domains to elongate, and the line tension (the two dimensional analog of surface tension), which minimizes the domain boundary (McConnell, 1991). Domain sizes and shapes can be manipulated to some extent by the application of an external electric field, and transitions between domain shapes can be induced by applied surface pressure (Lee et al., 1994). Line tension active agents, such as lung surfactant protein SP-B, can also induce a transition from circular domains to stripe domains, or a systematic decrease in the size of domains (Lipp et al., 1996). To some extent, these patterns can be manipulated by changes in subphase ionic strength, monolayer composition, and pressure, which suggests that these stripe patterns can be used to template a substrate. However, the shapes and length scales are governed by material parameters that are hard to control, and arbitrary or complex patterns would be difficult to create.

In this issue, Groves et al. (1996) show a much more general method of creating patterns and concentration differences in supported bilayer membranes using two-dimensional electrophoresis and remarkably simple bilayer "corrals." Glass-supported bilayers are prepared by adsorption of vesicles from solution, but any equivalent method of preparing the bilayer film would probably be as useful. Instead of using the lipid and protein interactions to create patterns spontaneously, an applied electric field in the plane of the membrane was used to induce a concentration gradient in an initially homogeneous lipid-protein bilayer by causing an electrophoretic flow of charged molecules in the membrane. As the authors point out, not all membrane-embedded proteins are sufficiently mobile for this scheme to work—many transmembrane proteins are slowed down or immobilized by contact with the glass substrate. To overcome this problem, the authors have genetically manipulated several proteins so that they were tethered to the membrane by glycan-phosphatidylinositol (GPI) linkages. The GPI-tethered proteins were sufficiently mobile at fields of 10–60 V/cm to be observed by following the motion of fluorescent antibodies to the proteins (or fluorescent lipids in the bilayer) with conventional optical microscopy. The proteins could travel anywhere on the glass support where there was a bilayer, with the positively charged proteins drifting toward the cathode and the negatively charged fluorescent lipid drifting toward the anode.

The authors have taken advantage of this by creating barriers or "bilayer corrals" to trap the concentration gradient by scratching away the bilayer from the glass support. Although it is somewhat surprising given the mobility of the lipids and proteins within a bilayer domain, once a scratch is made through the bilayer, it apparently never heals. The bilayer does not seem to be able to spread over the scratch, at least over the time scale of the experiments. The proteins are confined more and more by the action of the electric field against these barriers, which creates a concentration gradient in the charged components of the membranes. The magnitude and shape of the concentration gradient is determined by a competition between the protein diffusivity and the electric drift velocity, hence by a combination of the protein charge and size, the applied field, and the bilayer fluidity. The authors report that the proteins were concentrated up to 50 times within the corrals. At these concentrations, the proteins would be close packed and perhaps starting to be forced into ordered arrays. The field-induced concentration gradients were reversible after the field was turned off, indicating that there were no electric-field-induced changes in the proteins or lipids.

Several potential applications of this phenomena suggest themselves, especially should future advances show how to shut the door on the corrals to preserve the concentration gradients for subsequent analysis. The authors suggest integrating patterns of electrodes in the substrate to open the corral door and then shut it after sufficient protein or lipid has been captured. In this way, a pattern of protein concentration reflecting the underlying substrate could be created and maintained. Other potential applications would be the forced two-dimensional crystallization of membrane proteins for x-ray or electron diffraction, or even atomic force microscopy while under electrical confinement. Many membrane associated proteins do not crystallize on their own; but at the appropriate density within the appropriately shaped corral, crystallization might be forced

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on the proteins, leading to much better structural determination via microscopy techniques. In combination with spontaneous patterns of lipid or protein distribution in the membrane, the microelectrophoresis might show how lipid domains alter the diffusion and transport of proteins in bilayers. It may also be possible to manipulate the spontaneous patterns mentioned earlier into more useful structures and shapes with an electric field. Membrane proteins, perhaps the most difficult to organize and corral in the past, may have met their match and are heading for "the last round-up."

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