Commentary

A New Technique for Probing Inter-Membrane Interactions

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It remains an important and challenging problem to characterize the interactions between biomembranes. In vitro, nonspecific interactions can lead to membrane-bound states, fusion, and budding (cf. Lipowsky, 1995; Chernomordik and Zimmerberg, 1995; and references therein). In addition, living cells possess a variety of protein-based mechanisms for performing and regulating such tasks as endocytosis, exocytosis, fusion, and adhesion. Whether or not cells utilize nonprotein-based mechanisms, the underlying (nonspecific) interactions will be present and must be elucidated in the course of unraveling the complexities of the cell membrane.

The general features of membrane interactions are well understood. The free energy governing the conformations of single bilayers contains curvature, stretching, and entropic contributions. Intermembrane interactions are mediated by the ambient solvent and include Van der Waals (fluctuating dipoles), electrostatics (charge), hydration (water ordering), and Helfrich entropic repulsion (excluded volume interaction between fluctuating membranes). Dissolved ions affect the electrostatic interaction through screening, condensation, and (as we see below) cross-bridges between bilayers. Scattering and force measurement techniques have gone far in characterizing these interactions (cf. Israelachvili and Wennerström, 1996; and Safinya, 1989), but it has proved difficult to explore the detailed structure in the region of contact between membranes. New approaches are needed.

An impressive step in this direction is described in the article by Niles et al. (1996) in this issue of the *Journal of General Physiology*. These investigators use resonance energy transfer (RET) between donor and acceptor fluorophores to gain information on the distribution of membrane separation. The donor is incorporated into the membrane of a vesicle that contains the negatively charged phosphatidyl serine (in addition to other lipid components); the acceptor is incorporated into a planar bilayer that also contains phosphatidyl serine. To optimize delivery, vesicles are ejected from a pipette that is placed near the planar membrane. At solvent calcium concentrations of a few millimolar or greater, vesicles are bound to the planar membrane, whereas in the absence of divalent cations, vesicles are unbound.

The acceptor fluorophores are excited by the donors with a probability that falls off with the sixth power of their separation. The characteristic length at which the probability of resonance transfer is 0.5 is ~3 nm. Nevertheless, for separations below ~35 nm, RET fluorescence provides a sensitive measure of distance. RET thus produces a two-dimensional map of intermembrane separation in a relatively noninvasive manner; no solid probes or substrates are needed. The local distance sensitivity is limited by the intensity calibration (>0.5 nm for distances ≥1.0 nm); the lateral distance resolution is limited by diffraction and imaging (~0.5 μm).

Niles et al. find that increasing $[Ca^{2+}]$ up to ~15 mM increases the area of contact and decreases the distance between membranes monotonically, presumably as a result of Debye screening. Beyond ~15 mM $[Ca^{2+}]$, however, the contact area decreases; the decrease is ascribed to charge reversal. Throughout, punctate regions, which are areas of close membrane apposition (<2 nm), are observed, with the density of punctate sites increasing with increasing $[Ca^{2+}]$. They suggest that these sites correspond to calcium cross-bridges between phosphatidyl serine headgroups in the apposed membranes. From the intensity autocorrelation function of the fluorescence images, Niles et al. also find an apparent order in the arrangement of punctate sites. Surprisingly, the autocorrelation function is not rotationally symmetric.

These punctate sites are reminiscent of other mobile junctional contacts between membranes such as gap-junction channels and ligand receptor–mediated membrane attachments (Chiruvolu et al. 1994). A theoretical analysis shows that there is a strong tendency for such junctional contacts to aggregate (R. Bruinsma et al., 1994), and it is possible that ordered structures will arise. An alternative interpretation is that the pipette ejection of vesicles might produce strong lubrication forces and a hydrodynamic instability, which could result in ordering of the punctate sites. It is more difficult

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to account for the lack of rotational symmetry in the intensity autocorrelation function. This could be a result of the video imaging or the relative arrangement between micropipette and planar membrane.

The tantalizing results of this paper stimulate many new questions. What is the precise nature and origin of the higher order organization of punctate sites? Is there structure within the sites? Do the different lipid components in both the vesicles and planar membrane play a role? To address these questions, one needs improvements in resolution, especially within the plane of the membranes—a formidable challenge. With advancements in near-field microscopy, it may be possible to measure the lateral distribution of intensity with greater sensitivity.

This experiment provides a remarkable view of the region of inter-membrane contact at a resolution that hitherto was unavailable and is an important step toward a detailed understanding of local membrane interactions. Applications to (protein-mediated) cell adhesion and fusion, along with the inevitable refinements in the technique, promise to open a new experimental window on cell-membrane structure and function.

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