How to detect nonrandom motion of proteins in membranes

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Analysis of the lateral movements of proteins in cell membranes provides insight into the finer details of membrane structure and is also important for a quantitative analysis of many functional processes. It has been known for many years that the lateral mobility of most membrane proteins is highly restricted. Diffusion coefficients of proteins in cell membranes are often several orders of magnitude less than those measured in simple reconstituted systems and a significant immobile population is also frequently detected (1). These conclusions are based largely on the extensive application of the FRAP (Fluorescence Recovery After Photobleaching) technique. The precise molecular interactions responsible for restricted diffusion in cell membranes have in most cases proved elusive, although there are good grounds for supposing that cytoskeletal structures are involved.

FRAP provides information on the lateral movements of proteins over distances on the order of 1 μm. Recently, new experimental methods have been developed which offer much higher spatial resolution (10–20 nm) and thus are capable of providing a more detailed characterization of membrane protein dynamics (2). These methods employ digital imaging to visualize and accurately locate small particles bound to receptors on cell surfaces. A sequence of images then enables the trajectories of individual particles, and hence their receptors, to be mapped. The particles are either colloidal gold particles visualized by video enhanced-contrast techniques or fluorescent particles detected using highly sensitive imaging devices. In this issue, Saxton (3) makes an important contribution to the analysis of single particle tracking experiments.

Most FRAP measurements are interpreted on the basis of a random diffusion model (a notable exception is a study of the effect of the size of the photobleached spot on FRAP measurements (4)). The principal alternatives to random diffusion are diffusion limited to a domain and directed motion which could result from attachment to moving cytoskeletal elements or from bulk membrane flow. Simple considerations indicate that whereas plots of mean square displacement against time ($\langle r^2 \rangle$ v t) are linear for random diffusion, such plots exhibit upward curvature for directed motion and downward curvature for domain-limited diffusion. Observation of the trajectories of individual receptors may thus enable departures from random motion to be readily detected. There are, however, difficulties with this type of analysis. The problem is that because diffusion is a stochastic process, trajectories which resemble those expected for directed or domain-limited motion can be generated by purely random movements. This problem is directly addressed by Saxton (3), who proposes methods for reliably assigning observed particle trajectories to different types of motion.

Saxton simulates membrane protein diffusion by random walks on a triangular lattice. Obstacles are placed at random points, allowed to form clusters or arranged to produce bounded domains. Trajectories are obtained from Monte Carlo calculations and the probabilities of occurrence of different types of trajectories are determined. These simulations demonstrate that trajectories which would be mistaken for other types of motion on the basis of $\langle r^2 \rangle$ v t plots are generated by random unobstructed diffusion with "distressing frequency." Saxton goes on to develop a number of alternative analyses for discriminating different types of motion. One approach extends a method employed by Anderson et al. (5) of plotting histograms of particle displacements at different times. The results of Monte Carlo calculations are compared with expected probabilities for random and domain-limited diffusion. Another useful method is based on determining an asymmetry parameter for individual trajectories. Again, histograms of the asymmetry parameter calculated for different types of motion are compared with the expectation for random motion. Finally, Saxton considers how the results of these calculations can be used to determine whether an individual trajectory has a high probability of resulting from directed or domain-limited motion.

Application of Saxton's results will be of great help in the analysis of particle tracking experiments and will place their interpretation on a firmer footing than hitherto. Of course, problems of analysis still remain. Conceivably, proteins might switch from one type of motion to another during the time of observation, thus further complicating the analysis. The current model represents protein motion in membranes as either obstructed by randomly placed obstacles or confined to regular shaped domains. The real situation in membranes may well be a subtle mixture of order and disorder. Nevertheless, experimentalists will be encouraged to test to what extent the model accounts for observed protein movements and this in turn can only deepen our understanding of the complex dynamics of biological membranes.

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