11. DiI was incorporated into the plasma membrane by incubating the cells with 1 ml of dye (3.0 to 6.6 μg/ml) in Hanks balanced salt solution (BSS) containing 1 percent ethanol for 15 minutes at 37°C. In the L-6 cells used for our diffusion experiments, only peripheral diI fluorescence was visible by fluorescence microscopy. We believe that only this peripheral fluorescence contributed significantly to the observed diffusion. Round mitotic L-6 cells showed the characteristic "fluorescent ring." The laser beam focus could be scanned from the top to the bottom of the cells to see that virtually all of the fluorescence was confined to the cell membrane. In cells with membranes that contain different proportions of various phospholipids, a noticeable fraction of diI was internalized, and the nucleus and cytoplasmic granules were visible by fluorescence microscopy. Fluorescence recovery in FPR experiments on these cells was incomplete, indicating that some of the probe was trapped presumably within organelles smaller than the laser beam diameter. In macrophages, internalization of plasma membrane substantially reduces the fluorescence recovery of diI. Prevention of the internalization by inhibiting endocytosis permitted complete recovery (J. Reidler, unpublished data). It should be noted that diI exists in water only as micelles with low fluorescence quantum efficiency; only from our dilute alcohol solution could the diI be induced to label the cell membrane.