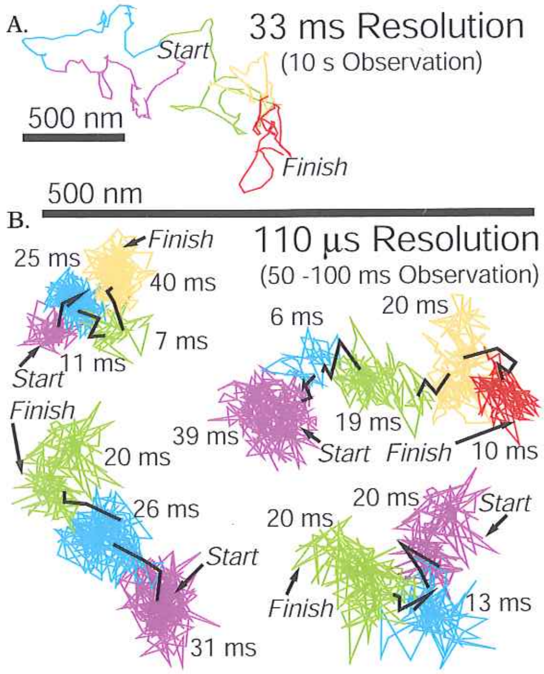
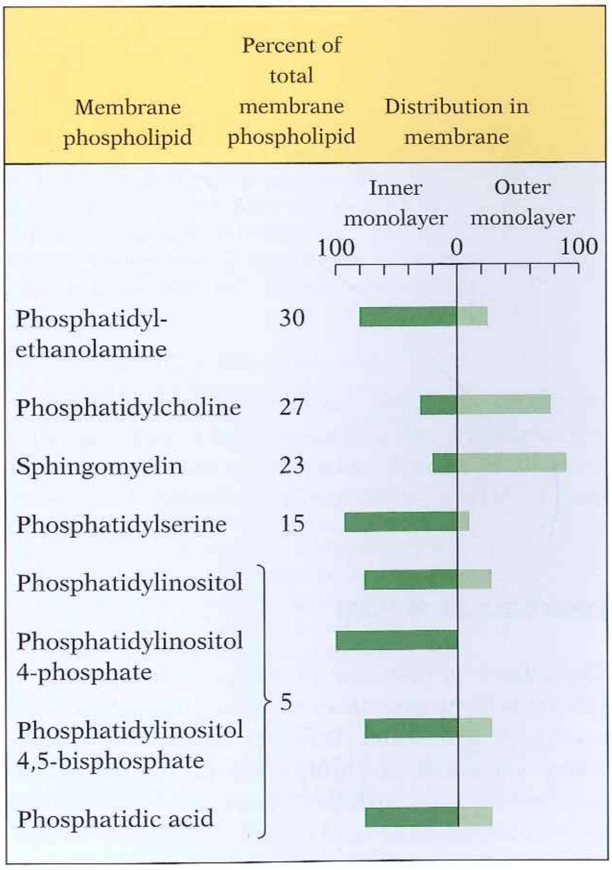


2.13. Hop diffusion of individual lipid molecules. Computerized time-resolved single-particle tracking was used to follow the diffusion path of a single gold-labeled DOPE molecule on the surface of the cell. **A.** At a resolution of 33 msec, the path appears to be simple brownian diffusion. Each color represents 60 step periods or 2 seconds. **B.** At a resolution of 110 microseconds (µs), the pattern of movement reveals the phenomenon of hop diffusion as the lipid hopped from one region to the next. Each color indicates confinement within a compartment, with black for intercompartmental hops. The residency time for each compartment is indicated. From Murase, K., et al., *Biophys J.* 2004, 86:4075–4093. © 2005 by W. H. Freeman and Company. Used with permission.

from the outer leaflet, as well as chemical labeling with nonpenetrating agents such as trinitrobenzenesulfonic acid (TNBS). A good example of lipid asymmetry (and the first observed) is the erythrocyte membrane, whose outer leaflet is enriched in sphingomyelin and PC, while the inner leaflet contains most of the PE and nearly all of the PS found in the membrane (Figure 2.14). Furthermore, the same phospholipid species may have acyl chains in the outer leaflet different from those in the inner leaflet. The concentration of sphingolipids is typically six-fold higher in the outer leaflet of membranes than in the inner leaflet; in contrast, cholesterol is commonly distributed in both leaflets of eukaryotic membranes. The consequences of lipid asymmetry are being explored *in vitro* with new techniques that produce supported bilayers with asymmetric lipid composition (see Chapter 3).

Numerous *in vitro* studies have shown that the two leaflets of a lipid bilayer can be coupled together by interdigitation produced when some of the acyl chains extend past the bilayer midplane, pushing their terminal methyl groups into the opposing leaflet. This may result from chain length asymmetry within individual lipid molecules (when a lipid bears one acyl chain that is much longer than the other), as frequently occurs in sphingolipids. Because the two monolayers become physically coupled, interdigitation may be observed as a distinct phase in calorimetric studies and as line broadening in the ^{31}P-NMR spectrum (see below). An important consequence of interdigitation is a decrease in the bilayer thickness, because it allows the two monolayers to approach each other more closely.

The thickness of the lipid bilayer is strongly influenced by the number of carbons and degree of saturation of acyl chains. In addition, much experimental evidence indicates that cholesterol increases the thickness of a lipid bilayer. Thickening by cholesterol is attributed to stabilizing the neighboring acyl chains in their most extended conformations (with all *anti* dihedral angles), thus increasing their effective length (see Figure 2.9A). However, a challenge to this view is presented by experiments that measure the thickness of various cell



2.14. The asymmetric distribution of lipids in erythrocyte membranes. The graph shows the content of each lipid type expressed as mol % in the inner and outer leaflets. Redrawn from Nelson, D. L., and M. M. Cox (eds.), *Lehninger Principles of Biochemistry*, 4th ed., W. H. Freeman, 2005, p. 373. © 2005 by W. H. Freeman and Company. Used with permission.