

Imaging Domains in Membranes with Atomic Force and Fluorescence Microscopy

by A. R. Burns

Motivation—Lipid bilayer membranes in living systems have many critical functions in sensing and transport. Recently, it has been postulated that these functions occur within small domains of chemically or structurally distinct lipids that have undergone phase separation from the bulk membrane constituents. As schematically depicted in Fig. 1, these domains mediate the lateral organization required for complexes of transmembrane proteins and other membrane-associated proteins involved in membrane function. We are currently working on advanced imaging techniques to reveal the structure and properties of these domains in both intact biological and model membranes.

Accomplishment—Over the years, labeling of specific membrane lipids and proteins with fluorescent tags has enabled the use of optical imaging to visualize their location and to follow important dynamical processes such as diffusion. There are significant limitations, however, to fluorescence techniques. The small size (< 100 nm) of many membrane domains precludes lateral resolution with diffraction-limited optics (> 300 nm). Also, since it relies on specific fluorescent probes, optical imaging will not provide information with regard to the overall membrane landscape or topography. These limitations can be overcome by atomic force microscopy (AFM). We have shown that AFM has the ability to map topographic details of membrane organizational structure such as phase-separated lipid rafts and transmembrane proteins with 1 nm resolution. We are now able to perform *simultaneous* fluorescence and AFM imaging in order to provide full correlation of the location of fluorescently-tagged lipids or proteins with detailed membrane structure.

Simultaneous AFM and fluorescence images are shown in Fig. 2 for a model lipid bilayer supported on a glass substrate. The bilayer is a mixture of two lipids that have phase-separated into gel-like and liquid-like domains on the basis of structural differences in the long alkyl chains. As in Fig. 1, the gel-like lipids have straight chains (pink lipids), whereas the liquid-like lipids have bent chains (blue lipids). The straight chains result in densely-packed domains that are thicker or higher relative to the loosely-packed bent chains (AFM image in Fig. 2). A fluorescent probe lipid mixes with the liquid domains, but is excluded from the gel-like domain (fluorescence image in Fig. 2). Thus it verifies the identity of the lipids. These model bilayers are being used to determine the important chemical and physical factors that give rise to domain formation and probe partitioning. We are also working with the University of New Mexico Medical School on AFM/fluorescence imaging to map the location and follow the dynamic activity of specific membrane proteins involved in the immunological response of cells.

Significance—Mapping of membrane signaling pathways in biological systems is of great interest to the worldwide community. Knowledge of nanometer-scale lateral organization and its function in cellular membranes is required in the analysis of receptor-based signaling. Our unique imaging capabilities enable us to examine membrane structure and function with much greater detail than was previously possible since we are able to take advantage of both selective labeling and high topographic resolution.

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Contact: Alan R. Burns, Biomolecular Materials and Interfaces, Dept. 1141

Phone: (505) 844-9642, Fax: (505) 844-5470, E-mail: aburns@sandia.gov

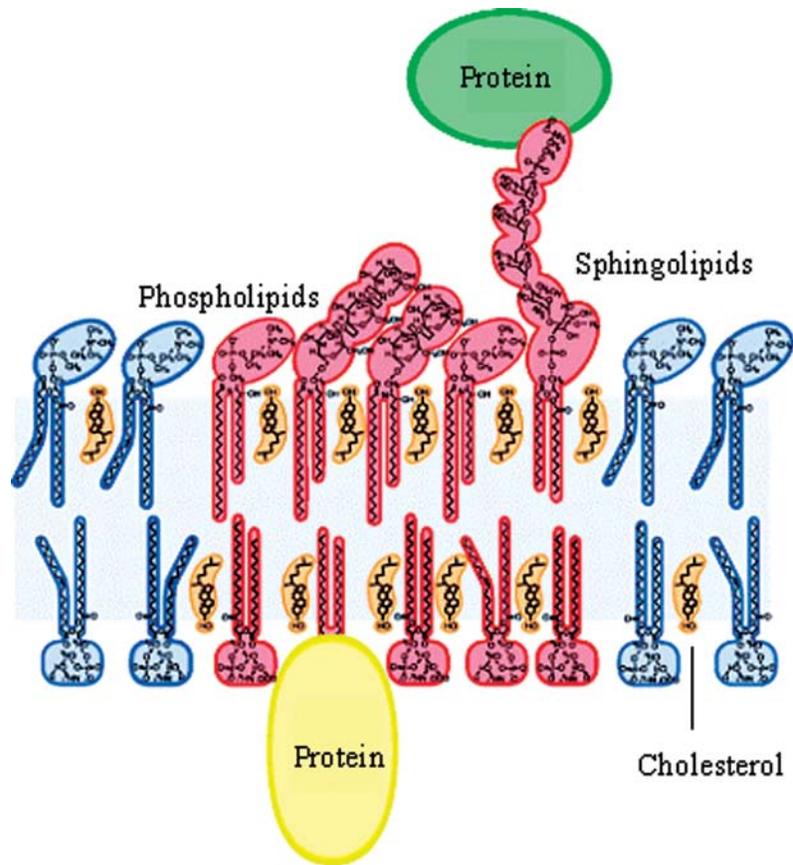


Figure 1. Membranes are organized by phase separations of phospholipids into liquid-like (blue) and gel-like (pink) domains. Proteins involved in cellular processes tend to associate with the gel-like domains or "rafts." Cholesterol molecules stabilize the rafts.

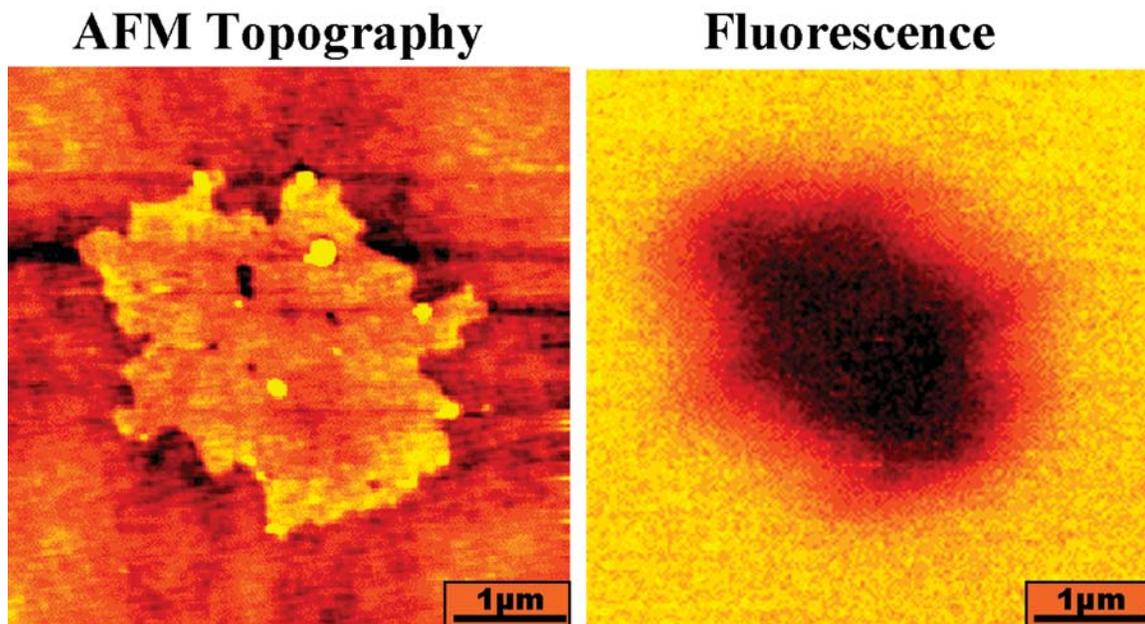


Figure 2. Simultaneous AFM topography (left) and fluorescence (right) image of a gel-like lipid raft in a model membrane. The raft is 10 Å higher than the surrounding liquid phase. A fluorescent probe is excluded from the raft and thus the raft appears darker than the liquid phase. Scale bar = 1 μm.