

New approaches for study of substrate-supported phospholipid membranes

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Biology provides many examples of exquisite control of structure and dynamics in nanoscale assemblies. Examples of functions of these assemblies include energy harvesting and transduction, ultrasensitive chemical and biochemical recognition and sensing, and control of mineralization processes to produce nanostructured inorganic and composite materials with unusual combinations of strength and flexibility. All of these functions represent areas with important applications, and a major scientific challenge is to improve our understanding of how to control complex self-assembly processes at the molecular and supramolecular level in order to generate useful synthetic or biocomposite materials.

Some of our recent work in this area has focused on the development of new types of model phospholipid membrane assemblies. Phospholipids are amphiphilic molecules that contain regions that prefer to be exposed to water (hydrophilic) and regions that prefer to be exposed to oil or oil-like organic environments (hydrophobic). This combination of properties leads to a propensity for these molecules to assemble in structures with hydrophilic portions all exposed to water and hydrophobic portions all protected from water. In biology, phospholipids and related molecules assemble into cellular membranes, which provide the containers that define cells and sub-cellular components. Upon exposure to clean silica surfaces, phospholipids spontaneously assemble into planar bilayer structures (Figure 1)¹. These substrate-supported bilayer assemblies are amenable to surface characterization strategies such as atomic force microscopy, optical microscopy and ellipsometry, and hence are of great value for studies of the physical and biological properties of membranes. In addition, these structures are of interest for applications that require interfacing biological systems with synthetic materials, most notably for providing transducer interfaces in biosensors. A variant of substrate-supported bilayer membrane can also be prepared by exposing phospholipids to hydrophobic, solid surfaces prepared, for example, by functionalization of an oxide surface with a self-assembled monolayer. In this case, a single layer of phospholipids forms on the hydrophobic self-assembled monolayer (Figure 1), leading to a so-called hybrid bilayer.

Here, we present two recent advances in the study of substrate-supported model membrane architectures. The first is the development of new strategies for preparation of patterned mem-

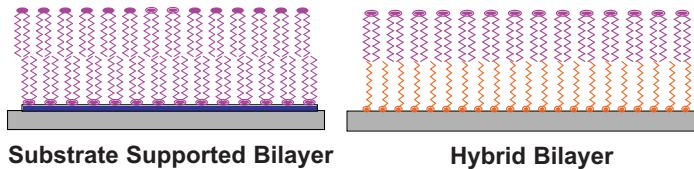


Figure 1. Schematic molecular architectures of substrate-supported bilayers (left) and hybrid bilayers (right). Phospholipid molecules (purple) contain two hydrophobic tails and a hydrophilic head group. Substrate-supported phospholipid bilayers are cushioned from silica substrates by a thin (approximately 1 nm) layer of water (blue). Self-assembled monolayers (orange) are typically formed from molecules with a single hydrophobic tail and a reactive group that interacts with the solid surface. Thicknesses of the architectures are approximately 5 nm.

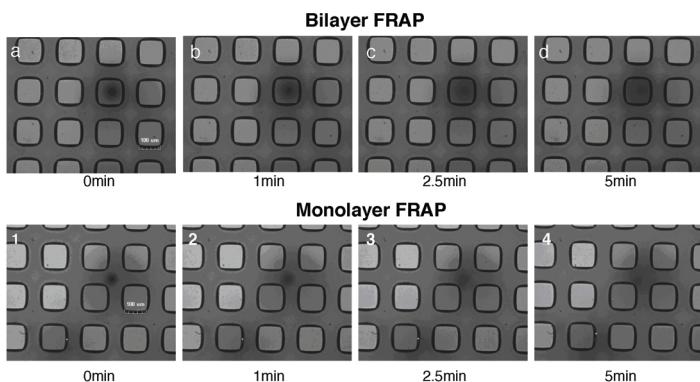


Figure 2. A fluorescence recovery after photobleaching (FRAP) experiment on patterned supported membranes that contain a small percentage of fluorescent labels. The bright regions are supported bilayers, the dimmer areas are hybrid bilayers, and the dark regions are boundary areas where no phospholipids are found. Focused illumination photobleaches a small spot at time zero (panels a and 1). Lateral diffusion in the membranes leads to evolution of the spatial profile of the initially photobleached spot (panels b-d, 2-4). In panels a-d, the photobleached spot is in an isolated supported bilayer region, and the photobleaching is contained within that region. In panels 1-4, the photobleached spot is in the hybrid bilayer region, and diffusion leads to a general, widespread, loss of intensity. Diffusion between the isolated supported bilayer regions and the surrounding hybrid bilayer regions is not observed.

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branes. Self-assembled monolayers, phospholipid bilayers, and other thin-film organic materials can be spatially patterned by exposure to deep-ultraviolet light through an appropriately patterned contact mask². One application is the generation of vacancies in otherwise uniform substrate-supported bilayers. These vacant regions can then be refilled with different phospholipids to create patterned composite membranes. Study of their structure and dynamics provides insight into domain formation in multi-component membrane systems, which is an important consideration for biological function in processes such as cell-signalling. Of equal interest is the formation of isolated regions of supported bilayers that could be used for applications such as high throughput screening platforms for interactions with membrane-bound or membrane-associated proteins. We have discovered a new route to such materials³. We start with a uniform self-assembled monolayer and pattern the monolayer using masked UV exposure. Under appropriate conditions, near the edges of the exposed regions a surface is formed that is incompatible with phospholipid bilayers. Exposing the patterned surface to phospholipids then leads to an architecture in which isolated regions of a supported phospholipid bilayer are present within a background of a hybrid bilayer. Fluorescence experiments (Figure 2) indicate that there is no lateral diffusion between the supported bilayer and surrounding areas. This overall architecture provides a reliable means of forming isolated and addressable regions of supported

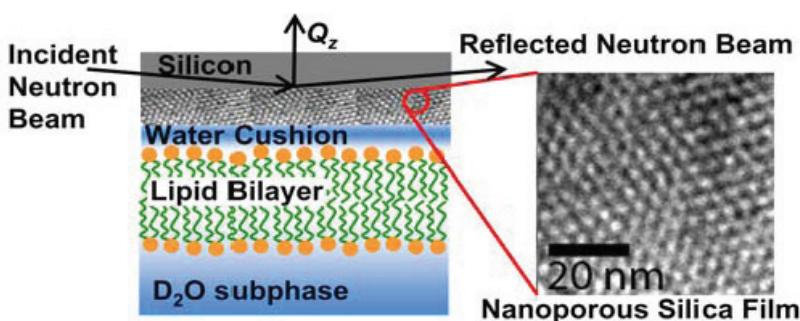


Figure 3. Schematic geometry of neutron reflectivity experiment for lipid bilayers assembled on nanoporous and nanocomposite silica thin films. Expanded view shows a transmission electron microscope image of a nanoporous silica film.

phospholipid bilayers on surfaces.

A second advance in the study of substrate-supported membrane architectures has been to develop nanocomposite silica materials as supporting substrates. A limitation of standard substrate-supported membranes is that the solid substrate can perturb both the membrane and proteins that are incorporated into the membrane. In addition, lack of a substantial fluid reservoir between the membrane and the underlying substrate leads to difficulty in electrochemical measurements. The use of porous or composite substrates could address both of these limitations. Nanocomposite and nanoporous silica thin films can be formed via an evaporation induced self-assembly process. Working with investigators at the Los Alamos Neutron Science Center, we have used neutron reflectivity to characterize phospholipid bilayers formed on these porous and composite substrates (Figure 3)⁴. Neutron reflectivity is a powerful tool for characterizing model membrane architectures such as these due to its sensitivity to

organic materials. By analysis of reflectivity data, the density profile of layers added onto surfaces has been determined to verify the formation of true supported-bilayers on these substrates.

References

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