

Molecular transport and organization in supported lipid membranes

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The mechanism by which vesicles spontaneously form supported lipid bilayer membranes on glass surfaces is becoming better understood and this knowledge is the basis of a technology of patterning membrane arrays and controlling composition. Controlled interactions between supported membranes and cells, particularly from the immune system, provide direct insight into cell–cell surface interactions.

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Abbreviation

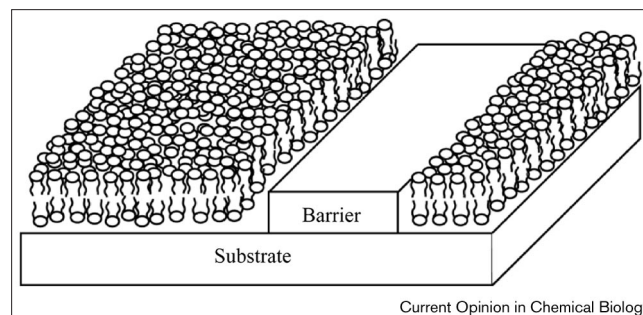
PDMS polydimethylsiloxane

Introduction

Cells and sub-cellular structures such as the nucleus and mitochondria are defined by their surrounding surfaces: these surfaces are biological membranes. Intercellular contacts, organization and communication are mediated by the cell membrane; transmembrane electric potentials are generated by energy transduction mechanisms to drive biosynthetic processes or generate impulses; and proteins are transported to specific locations by a fantastic network of internal membranes and membrane-mediated fusion processes.

The basic structure common to all biological membranes is the lipid bilayer. The membrane is essentially a two-dimensional fluid, with many components free to diffuse over the membrane surface within the plane of the membrane. This lateral diffusion is sometimes bounded into lipid domains, and understanding this organization and lipid-based structures such as lipid rafts within membrane domains [1] are active areas of study. Lateral diffusion is critical for many biological functions because function often depends upon the lateral association or clustering of several components. Most of the functions associated with biological membranes involve proteins: integral membrane proteins that span the membrane and membrane-anchored proteins. Examples of integral membrane proteins include ion channels and numerous proteins involved in energy transduction such as the photosynthetic apparatus and cytochrome oxidase. Membrane anchored proteins are attached to membranes either permanently or transiently by the addition of a lipid-like structure such as a fatty acid or glycosylphosphatidylinositol (GPI) tether. This includes many functionally important proteins such as the Ras protein, cell-surface proteins of the immune system, and prion proteins. Intermediate between these classes are membrane-anchored proteins that are held in the membrane by a

Figure 1



A lipid bilayer membrane supported on a glass substrate and patterned by a barrier. The bilayer is separated from the substrate by a thin (~ 10 Å) layer of water. The lipid molecules are laterally mobile. Barriers made from many types of material (see text) control the assembly and corral the lateral motion of the lipids.

transmembrane helix and that often have large and complex functional domains on both sides of the membrane. A common functional feature is the association of two or more membrane-anchored proteins by binding to a relatively small molecule (e.g. a hormone) on one side of the membrane, thereby activating function on the other side. Most signaling pathways are initiated in this way. Finally, there is a large class of molecules that function by interacting with or processing lipid head groups or clusters of head groups. Examples of the former are the Ca^{2+} -dependent C2 domains that interact with phospholipid head groups and cholera toxin binding to GM1; an example of the latter is head group processing by phospholipase D.

Lipid bilayers and membrane proteins are notoriously difficult to work with. Supported lipid bilayers are created by the self assembly of lipids into bilayers on solid supports, typically glass; this planar configuration is ideally suited for observation by fluorescence microscopy. A key finding is that the lipid molecules in supported membranes retain the lateral fluidity associated with lipid membranes in vesicles and in living cells. Furthermore, living cells recognize components displayed on the surface of supported membranes; thus, if the appropriate components are present, the supported membrane mimics a real cell membrane. It was shown both by neutron scattering [2] and by NMR on glass beads clad with supported membranes [3] that the bilayer is separated from the solid support by a thin layer of water approximately 10–15 Å thick (see Figure 1). This water provides a lubricating layer that maintains the lateral fluidity of both leaflets of the membrane. A poorly understood combination of hydration, van der Waals and electrostatic forces traps the bilayer at the surface, and it is indefinitely stable so long as the entire system is hydrated.

The general area of supported membranes was reviewed in 1996 [4]; in the following we focus on key recent work on the properties of supported membranes themselves and highlight applications in diverse fields of biology and biotechnology.

Mechanisms of supported membrane formation and spreading

Supported membranes are most often prepared by vesicle fusion on glass or fused silica. There has been important progress during the past year in understanding the mechanism by which vesicles fuse to glass, rupture and then spread to form supported membranes. In a series of papers, Kasemo and co-workers [5,6,7] used the quartz-crystal microbalance (QCM) to monitor both the change in mass and the energy dissipation associated with structures on the surface (monitoring the energy dissipation is analogous to a blind person distinguishing cake from jello by shaking the plate). These measurements demonstrate that small unilamellar vesicles (typically 25–100 nm diameter) initially adsorb to the surface; at low surface coverage, these adsorbed vesicles are stable on the surface. At high coverage, the vesicles rupture and fuse to form the supported membrane. An intermediate in this process has been visualized by atomic force microscopy (AFM) on mica surfaces [8]. These measurements suggest that when small vesicles are close enough they fuse to form larger vesicles and that these rupture and ultimately fuse.

A key feature of supported membranes is that they are self-healing, that is the formation process minimizes defects on the surface. Bilayer spreading on the surface is important both from the perspective of self-assembly and for patterning, discussed below. This process has been studied by Rädler's group [9] and by our group [10]. Rädler and co-workers placed a blob of dry phospholipid on plain glass, dextran-coated glass or cellulose-coated glass, immersed the system in water, and then watched the moving front of supported membrane spreading out from this large source of lipid, visualized by the addition of a small amount of a fluorescently-labeled lipid. The spreading kinetics were analyzed using a theoretical model that accounts for the competition of favorable interactions between the membrane and solid support with hydrodynamic shear flow and inter-monolayer friction. When the leading edge encounters defects on the surface, the membrane flows around rather than over the defect. Our studies of membrane spreading [10] took a different approach: half of a glass slide coated with a supported membrane was removed from water, which leads to immediate removal of membrane from the exposed surface and the creation of a clean membrane boundary or edge on the surface. The slide was then returned under water and the spreading front was monitored by epifluorescence microscopy. Rapid spreading occurred with fingering at the edges, probably because of pinning defects, as observed by Rädler. Because the amount of lipid on the surface was fixed, however, the spreading slowed and then halted, presumably

because further spreading would result in the loss of intermolecular interactions within the membrane. Thus, Rädler's results show that spreading continues if a lipid reservoir is available, while our results show that spreading is self-limiting in the absence of a reservoir. The latter result is the basis for a key approach to membrane patterning discussed below [11].

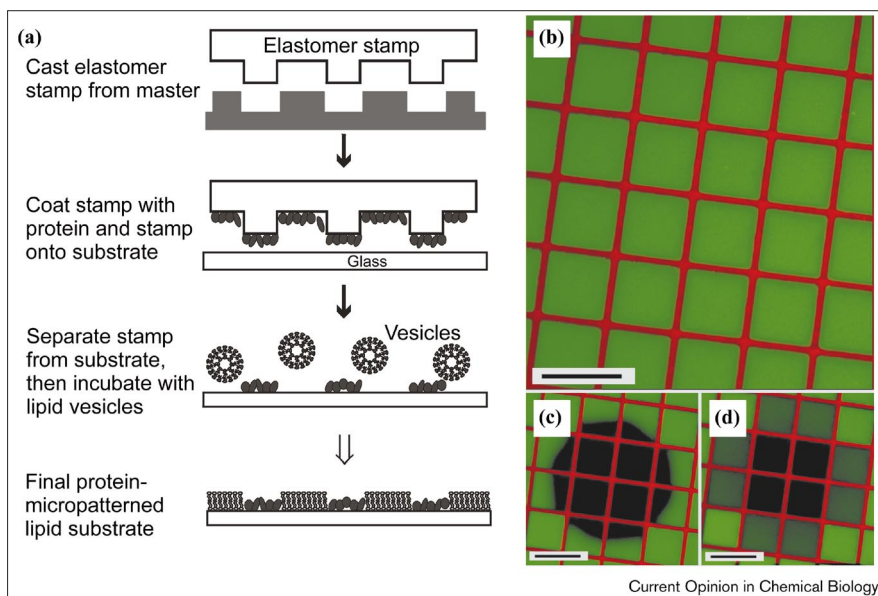
Softer surfaces and tethering

It has often been observed that proteins with large membrane anchors and integral membrane proteins are immobile or exhibit severely restricted motion in supported bilayers. It is presumed that this is a result of interactions between the membrane protein and the solid support; there have been relatively few careful studies of the functional consequences of this interaction, but it is generally thought that these interactions will reduce or eliminate protein function. This has led to widespread efforts to 'soften' the surface by coating it with a polymer and to more elaborate strategies that involve tethering the membrane to the surface, thereby creating a greater space between the solid support and fragile membrane components. Preassembled supported bilayers can be incubated with simple polymers that somehow find their way under the membrane, coating the solid support [12]. This simple strategy has not yet been tested with membrane proteins present. More complex polymers [13] and lipopolymers [14] have been attached to glass and provide a softer cushion for supported membrane assembly. Wagner and Tamm's work [13] demonstrates improved lateral mobility of cytochrome *b₅* and annexin on these polymer-supported membranes as compared with glass-supported membranes. Strategies have been developed for tethering membranes by using thiolipids on gold [15,16], which may provide additional space between the membrane and solid surface. A recent workshop dealt with these efforts in the context of building membrane-based devices of various types (Proceedings of the International Workshop on Biosensors using tethered Membranes, Mainz, April 22–25, 1999). Overall, progress has been slow and there has been little characterization of lateral mobility and function for integral membrane proteins or even for membrane-anchored proteins.

Motion within and on supported membranes

Lipid molecules in supported membranes diffuse in two dimensions with diffusion coefficients that are quite similar to those measured in vesicles or free-standing bilayers. Diffusion is typically measured by fluorescence recovery after photobleaching (FRAP) or by the rate of approach to steady-state for concentration gradients of charged molecules subjected to lateral electric fields and the relaxation time of these gradients after the field is turned off [17]. In a series of papers, Schmidt and co-workers [18–21] used single-molecule fluorescence methods to measure both the lateral and rotational motion of individual lipid molecules in supported membranes. These experiments demonstrated essentially free diffusion on a short timescale, with

Figure 2



Membrane patterning using proteins. **(a)** Method for patterning protein and assembly of supported membranes in open regions on glass. **(b)** Fibronectin grids (red fluorescence) used to corral supported membranes (green fluorescence from a small fraction of a fluorescently labeled lipid). **(c)** A circular region covering a number of grids is photobleached (dark circle). **(d)** After 20 minutes, the fluorescence is homogeneous because of diffusive mixing between bleached and unbleached lipids in each corral. The intensity of this homogeneous fluorescence is proportional to the area fraction originally illuminated. The scale bars in parts (b–d) represent 50 μm .

slower diffusion for longer trajectories, presumably because of defects or barriers on the surface. Related work has been done on cell and model membranes [22,23].

A novel application combining bilayer diffusion and single molecule measurements was reported by Maier and Rädler [24•,25]. Large double-stranded DNA was adsorbed electrostatically to fluid cationic lipid bilayers on solid supports, and the motion along the entire length of individual DNA molecules was monitored by fluorescence microscopy. The DNA was found to diffuse freely in the plane with a diffusion coefficient that depends inversely on the number of base pairs, as predicted by the Rouse model. Furthermore, the average radius of gyration follows a power law with an exponent near to that predicted for a self-avoiding random walk in two dimensions. These studies are interesting from the perspective of fundamental physics of two-dimensional systems and may have some relevance to interactions between DNA and cationic lipid systems used for transfection.

Interactions with cells

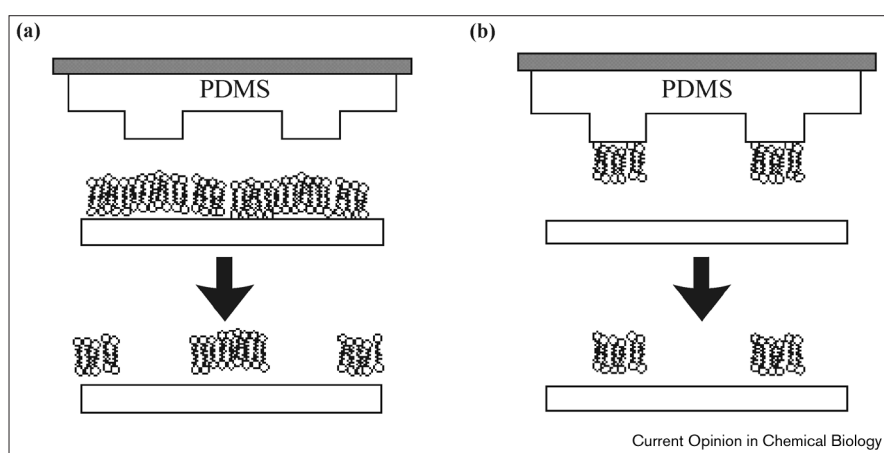
When cell recognition components are incorporated in the supported membrane, cell-surface interactions and their functional consequences can be studied. Such studies complement surface patterning with ligands that direct cell growth and/or stimulate function [26–29]. These bioactive ligands are attached covalently to the surface and consequently are laterally immobile. Ligands of the type used in some of these experiments have recently been attached to lipid-like molecules and incorporated into supported membranes [30]. These surfaces were then used to study factors that control cell adherence and spreading. We have recently created hybrid surfaces in which some regions are fixed and others are mobile [31].

Supported lipid bilayer membranes inhibit interactions between cells and the underlying glass surface. Cell-surface interactions may occur with defects in the bilayer or with proteins from the medium or excreted by the cells that bind to defects and create adhesion sites; these defects can be reduced by adding bovine serum albumin (BSA) prior to seeding the surface with cells [31]. A model for cell–cell and cell–supported-membrane interactions was recently described that uses giant vesicles and supported membranes each decorated with receptor proteins [32]. By using interference microscopy, it was possible to observe the structure and dynamics of the interface between these two cell-surface mimics with high precision and insight. Segregation of receptors into domains was observed in response to the adhesion event and the strength and sources of the adhesion were evaluated quantitatively.

Supported membranes were originally developed for studies of immune recognition [33], and they have recently been applied to study the specialized junction between T lymphocytes and antigen-presenting cells, known as the immunological synapse [34•]. Uncovering the nature of the recognition of peptide-carrying MHC complexes on antigen-presenting cells by T-cell receptors and the subsequent cellular response are central problems in immunology. The interactions between proteins on both cell surfaces involve multiple copies of each protein, each capable of moving laterally, along with additional mobile factors in each membrane and the actin cytoskeleton in the T cell. By direct visualization of each component using fluorescent video microscopy, the relative motion of T-cell receptor ligands and critical clustering with receptors were monitored. This study probably represents the most sophisticated use of supported bilayer membranes to probe a real biological

Figure 3

Supported membrane patterning by blotting and printing. **(a)** Schematic of supported membrane patterning by blotting away material using a PDMS. **(b)** Schematic of supported membrane printing to create arbitrary shaped membrane patches on a clean glass surface.



system and should serve as a model for many related types of cell–cell recognition studies.

Micropatterned supported membranes and devices

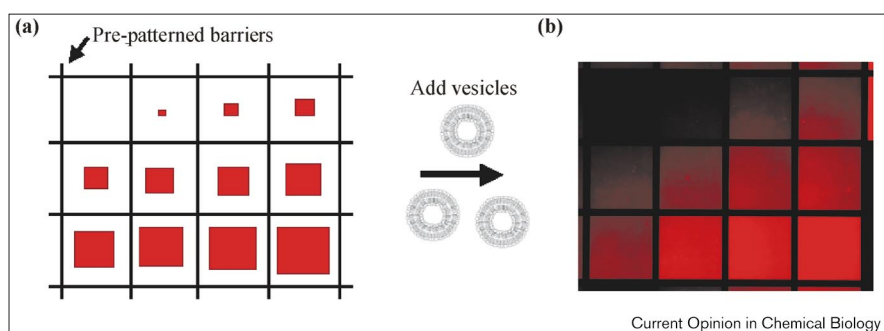
Most of the work on supported membrane patterning comes from our laboratory and this work is reviewed briefly. The chance observation that mechanical scratches prevent lateral diffusion across the scratch boundaries [17], led us to pattern a variety of materials on the surface and spatially control the assembly, organization and lateral mobility of supported membranes [35]. Many methods now exist for patterning bilayer surfaces including photolithography, electron-beam lithography, and microcontact printing. Photolithography can be used to pattern hard materials such as photoresist (plastic) and a variety of metals and metal oxides. Barriers to lateral diffusion can also be created by printing proteins on the surface using microcontact printing [36]. We found that effective barriers can be formed from a single monolayer of protein, suggesting that the vertical dimensions in Figure 1 are not unrealistic in this case (the barrier edges are not well understood). An example of the use of a patterned protein to control the assembly of supported membrane patches is shown in Figure 2, which also

illustrates the concept of lateral confinement or corralling of fluid membranes by patterning. The use of proteins also introduces a greater degree of lateral complexity into the patterned membrane system because the protein may provide interesting function; this has been used to pattern cell growth over fluid membrane surfaces [31].

As discussed above, in the section on membrane spreading, if lipid material is removed from the surface, the bilayer expands, but only to a limited extent unless a lipid reservoir is present. We recently discovered that bilayer can be removed from the surface by ‘blotting’ with a polydimethylsiloxane (PDMS) stamp [11••] (see Figure 3). If sufficient bilayer is removed from the surface, then the self-limiting lateral expansion on the surface prevents the re-joining of the blotted sections leaving a patterned membrane where the pattern is maintained by the gaps between adjacent membrane sections. Remarkably, it also proves possible to ‘print’ membranes that have been blotted from a supported bilayer surface onto a clean glass surface or to preassemble a supported bilayer on an oxidized PDMS stamp and print it in any desired pattern on a clean glass surface (Figure 3b). Fixed barriers made from hard materials or proteins can be combined with patterned membrane

Figure 4

Schematic diagram of a method to create composition arrays by printing membrane patches onto a surface that is **(a)** pre-patterned, followed by filling the remaining surface with vesicles. **(b)** Image of a dye-labeled lipid concentration array created using this method.



stamping, followed by filling in open, unstamped regions with fresh vesicles to create complex concentration arrays of essentially arbitrary composition as illustrated in Figure 4. All of these methods can be combined with secondary photolithography in which caged functionality is released by spatially directed photolysis [37]. These patterned arrays can be used for parallel studies of variations in membrane composition, for displaying membrane-associated functionality in an array format for high-throughput screening, and for organizing or probing cells interacting with membrane surfaces. Arrays of supported membranes in a microtitre well format have also been described [38].

Finally, we comment briefly on the combined use of micropatterned surfaces and electrophoresis in the plane of the membrane to create a 'geometric Brownian ratchet', a device for separating molecules associated with membrane surfaces [39**]. In the presence of a lateral electric field, charged molecules associated with the membrane experience a force and move with a drift velocity that depends on their charge and the magnitude of the field [17]. At the same time, the molecules are freely diffusing, and the competition between diffusion and forced motion leads to gradients of the charged components in confined regions. In the geometric Brownian ratchet, charged, fluorescently-labeled phospholipids are driven in one direction by a tangential electric field through a two-dimensional periodic array of asymmetric barriers to lateral diffusion fabricated from Ti-oxide on glass. By diffusion, the phospholipid molecules spread out in the orthogonal direction exploiting thermal fluctuations to drive directional transport. The effects of the asymmetric geometry of the barriers and the magnitude of the driving force were investigated quantitatively and were shown to agree well with a simple model for the properties of the ratchet. We showed a model application of the geometrical ratchet as a continuous molecular sieve to separate mixtures of phospholipid molecules that differ in electrophoretic mobility and diffusion coefficient [39**]. This proof of concept suggests that micropatterned membranes and electrophoresis might be used to separate membrane-associated molecules in their native environment.

Trends

The challenge of creating soft surfaces for supported membranes is an ideal area for combining sophisticated synthetic methods, physical characterization of surfaces and interfaces, and biology with potentially great impact on functional membrane protein biophysics, biology and biotechnology. If functional membrane proteins such as ion channels and hormone receptors could be displayed in arrays, this would be of widespread interest in the pharmaceutical industry for high-throughput screening of membrane-associated drug targets, a huge area of interest. It may also prove possible to integrate patterning, electrophoresis and electronic detection methods (see for example [40]) on a single surface. As fabrication methods for controlling membrane assembly and composition become

more sophisticated, one can imagine using supported membranes as a template for the assembly of more complex structures combining synthetic and living components.

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