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Scanning Probe Lithography on Fluid Lipid Membranes

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The membranes of living cells, based exclusively on the lipid bilayer motif, are essentially two-dimensional fluid emulsions. Spatial organization and dynamic rearrangement of proteins and other molecules within the membrane environment is a significant aspect of their biological functionality in vivo. Correspondingly, there is growing interest in the role of spatial organization as a mechanism of regulating differential outcomes from chemically equivalent systems.[1−4] The coupling of spatial patterns to reaction outcome is a widespread physical phenomenon with important implications outside biochemical systems, such as in heterogeneous catalysis.[5] However, the most elaborate examples of spatially regulated reaction systems are certain to occur within living cells. Experimental techniques that permit controlled spatial organization of relevant biological molecules on subcellular length scales (≤ 1 μm) can be of great utility in the study of these phenomena in live cell systems.

Applications of scanning probe lithography (SPL) to pattern organic and biological molecules on surfaces have experienced rapid growth in recent years. In one common implementation of SPL, the tip is used to remove or modify portions of a first molecular layer, such as alkane thiol or siloxane self-assembled monolayers, from the surface. Exposed regions of the substrate can then be refilled with other molecules.[6,7] Dip-pen nanolithography (DPN) is a variation of SPL in which molecules on the tip are transported to the substrate via a solvent meniscus.[8] Proteins[9] and DNA[10] have been successfully patterned by DPN. Such SPL techniques can produce molecular patterns with nanometer lateral dimensions. Immobilization of the molecules by direct coupling to the solid substrate preserves their spatial configuration. Although some proteins naturally function from within a solid matrix, such as the extracellular matrix proteins,[11−13] the vast majority of cell surface interactions involve proteins associated with the fluid cell membrane.

In the following, we describe an application of SPL to create spatially patterned fluid membrane surfaces. Patterning is achieved through a two-step process in which a supporting substrate is prepatterned with grids of chromium lines by electron-beam lithography. A supported membrane can then be deposited uniformly over exposed regions of the substrate by vesicle fusion. In this supported membrane configuration, a combination of attractive van der Waals forces, repulsive hydration forces, and electrostatic forces trap the membrane in a plane, ~1 nm away from the solid substrate. A layer of water fills the intervening space and allows the membrane to retain its fluid state. Whereas diffusive mixing occurs freely within each corral, the chromium boundaries prevent mixing between adjacent corrals; the membrane is pixilated by the grid.[14] A critical feature of this strategy is the ability to juxtapose fluid membranes with differing composition within nanometers of each other; 100 nm separation is demonstrated here.

In the second patterning step, SPL is used to selectively remove membrane from specified corrals in the array. Subsequently, new membrane is deposited into the exposed corrals by a second round of vesicle fusion. Multiple distinct membrane types can be deposited with spatial resolution determined by the grid size on the supporting substrate. This SPL membrane-patterning process is illustrated schematically in Figure 1. Supported membranes can also be patterned by other lithographic techniques including microcontact printing,[15] polymer lift-off stenciling,[16] microfluidic flow patterning,[17] and in situ UV degradation.[18] However, a combination of fundamental and practical limitations has generally restricted these alternative methods to the patterning of distinct fluid membrane compositions on multimicrometer-length scales.

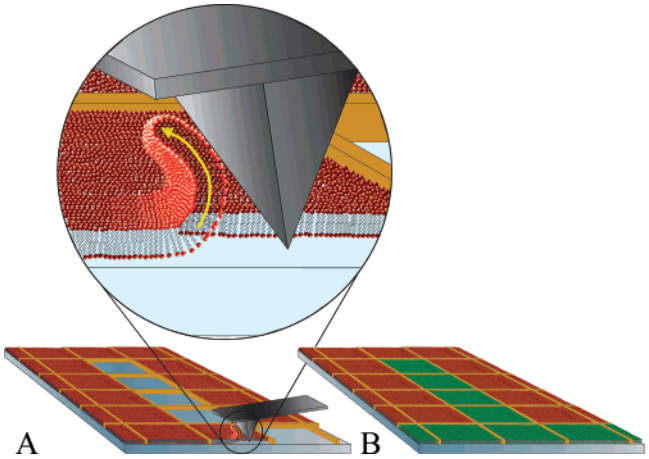

Figure 1. (A) Schematic of scanning probe lithography on a supported bilayer. (B) Bilayer of different composition fuses over exposed substrate. Chromium grids prevent diffusive mixing between separate corrals.

A challenging aspect of achieving controlled removal of supported membrane by SPL is the self-healing characteristic of membranes in the fluid state.[19] Although it is well-known that a scanning probe tip can remove membrane from a supporting substrate, the tip can be also be dragged through the membrane without removing material or inducing defects. We find that these differential results from tip interactions with the supported membrane can be controlled by adjusting the pH and ionic strength of the bulk aqueous phase. In the sequence of images depicted in Figure 2, A−C and G, the probe tip is dragged through the supported membrane under 15 mM NaCl at pH ~7. Under these neutral pH conditions, mechanically induced gaps in the supported membrane produced by the tip rapidly heal, and the membrane remains continuous. At elevated pH (~10), the equilibrated surface charge on the silica and its associated hydration layer inhibits membrane spreading and also prevents supported membrane deposition from negatively charged vesicles.[20] Whereas preexisting membrane is metastable at elevated pH, perturbation with a probe tip triggers a collective dissociation of the membrane from the substrate (Figure 2, D−F and H). The resulting gaps in the membrane do not heal. Consequently, membrane can be cleanly

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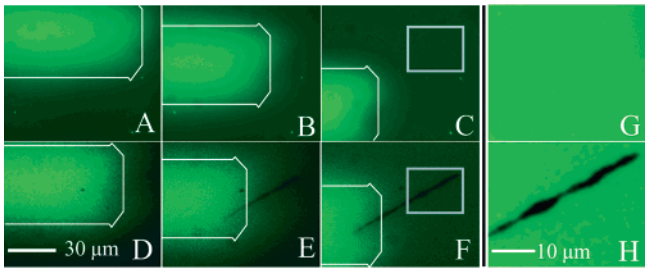

Figure 2. Epifluorescence images of an SPL tip moving through an NBD-PE labeled bilayer on an unpatterned substrate in 15 mM NaCl at pH ~7 (A−C) and pH ~10 (D−F). A diffuse reflection off the cantilever identifies its position, also outlined with white line. The inset gray box in C and F outlines the enlarged and recontrasted region shown in G and H, respectively.

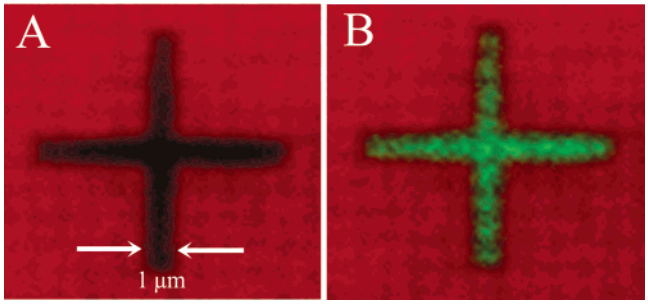

Figure 3. Fluorescence images of distinct fluid membrane compositions on a silica substrate within a grid of 100 nm wide chromium lines, which form an array of 1 × 1 μm membrane pixels. (A) Pixilated membrane after SPL has been used to remove membrane from selected pixels (dark). (B) After exposure to a vesicle suspension of a different membrane composition (green), new membrane is deposited into the exposed pixels.

removed from selected corrals of a gridded substrate at elevated pH while membrane in undisturbed corrals remains intact.

It can also be seen in Figure 2H that the gap in the membrane produced by the probe tip is substantially wider than the tip itself. This is a general phenomenon, and is pH dependent. At even higher pH of ~11, membrane disruption from the probe tip can propagate many micrometers from the point of contact. Such membrane disruptions do not propagate over the chromium grids. Thus, in addition to preventing diffusive mixing between membrane corrals, the grids confine tip-induced membrane disruption. The membrane remains fluid under all of the above-mentioned solution conditions, as can be assessed by fluorescence recovery after photobleaching (see Supporting Information).

After SPL patterning, the pH and ionic strength are again adjusted to favor supported membrane formation (pH ≈ 7, 100 mM NaCl), and a second membrane composition is refilled into the exposed corrals. Figure 3 illustrates an example membrane pattern produced by this process. First a membrane containing the fluorescent probe Texas Red is removed by SPL (3A). A second membrane, containing NBD-labeled lipids (green) is subsequently refilled, producing stable patterns of distinct bilayer compositions (3B). The substrate is borosilicate glass, which has been patterned with chromium lines (100 nm wide and 10 nm high), forming grids of various sizes. The SPL patterning and refill sequence can be repeated to add additional membrane types to the pattern. Grid sizes down to 1 × 1 μm were used in the present study, but this does not represent a fundamental limit of pattern size. Both SPL and electron-beam lithography can be scaled down by an order of magnitude, suggesting that this technique may be extendable to 100 nm pixel sizes with 10 nm separation distances.

Supported membrane patterning on submicrometer-length scales is, perhaps, most applicable to cellular studies in the context of live cell-supported membrane junctions. Specifically, functional interactions between living cells and supported membranes have now been observed in a variety of cell−cell contact systems.[21] The use of a fluid membrane display environment has proven instrumental in reconstituting protein functionality. The ability to produce defined mosaic patterns of membrane composition in the fluid state offers significant advantages for studies of cell membrane organization and corresponding function.

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Supporting Information Available: Complete epifluorescence image series from Figure 2 (movies) and fluidity measurements for bilayers at elevated pH, and materials and methods. This material is available free of charge via the Internet at http://pubs.acs.org.

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