Creating Spatially Addressed Arrays of Planar Supported Fluid Phospholipid Membranes

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Received May 3, 1999

The use of planar supports for presenting large arrays of spatially addressed molecules is one of the most powerful and versatile methods for creating combinatorial libraries.[1−3] These systems are starting to form the basis for a new generation of rapid screening assays and sensor devices in the biological and chemical sciences. Extending this approach to supported phospholipid bilayer membranes containing peptides, receptors, and integral membrane proteins is an especially valuable goal because of the ability of these systems to mimic many of the properties of native cell surfaces.[4] Addressing biomembrane mimics on planar supports, however, presents unique challenges, as the two-dimensional fluidity of the biomembrane must be preserved in many cases for it to function properly.[5−7] The bilayer deposition process must take place in an aqueous environment, and the entire system must continue to remain submerged under water to preserve the planar supported structure. Because of this physical constraint as well as the inherent complexities of biomembrane materials, traditional technologies such as light-directed synthesis for addressing peptide or DNA sequences onto solid supports are inherently difficult to apply.[1] We have, therefore, employed an alternate approach based upon depositing mesoscopic quantities of aqueous solution onto lithographically patterned hydrophilic surface well plates,[8] followed by the immersion of the entire substrate into buffer. This is a general and flexible method for directing chemically distinct phospholipid membranes into individually addressable surface sectors.

Previous studies have shown that patterned surfaces allow partitioning of one fluid lipid bilayer from the next.[9,10] Molecules within an individual membrane are free to move within the confines of a single partition but do not cross over to a neighboring region. In the experiments presented here, planar borosilicate substrates were partitioned into arrays of micrometer-sized hydrophilic boxes using standard photolithography. Patterning was achieved by exposing the surface to ultraviolet light through a lithographic mask consisting of an array of square boxes. Developing the pattern and cleaning the substrate formed well plates of hydrophilic glass onto which picoliter-sized droplets of liposome solution were placed (Figure 1). The liposomes, which were small unilamellar vesicles (SUVs) of phospholipids, were present at 1 mg/mL concentration in a pH 7.0, 100 mM sodium phosphate buffer solution.

Figure 2 shows the epifluorescence image of nine 50 μm × 50 μm well plates that have been addressed with three chemically distinct types of supported phospholipid bilayers. The boxes appearing red in color contain 1 mol % Texas Red DHPE fluorescent probes, while those appearing green contain 3 mol % NBD-DHPE probes. The center box, which appears dark yellow, contains both kinds of fluorophores. Fluorescence recovery after photobleaching (FRAP) demonstrated that the lipids were free to move throughout each two-dimensional box but were otherwise completely confined.[11,12] It should be emphasized that the bilayer in the center box was formed from a premixed solution of the NBD and Texas Red-labeled lipids. When the two solutions were

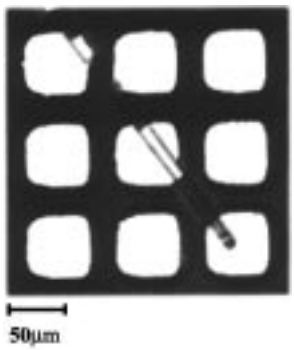

Figure 1. Bright field image of a microcapillary tube containing liposome solution situated over an array of patterned hydrophilic glass plates (50 μm × 50 μm). The tip of the capillary tube, which is directly over the center of the lower right-hand plate, is less than 5 μm above the surface. Microcapillary tubes of less than 10 μm in outer diameter were prepared by pulling in a standard micropipet puller. Prior to being filled with vesicle solution, the newly formed tubes were heated in an oven to 75 °C with 1,1,1,3,3,3-hexamethyldisilazane vapor. This process rendered the tubes hydrophobic, which facilitated liquid transfer to the surface. The glass substrate was cooled to the dew point (8−12 °C) during the transfer process to prevent the vesicle containing droplets from losing water through evaporation. The transfer process itself was achieved by positioning the capillary tip over the patterned oxide substrate using a linear travel stage and delivering an aliquot of liposome solution (typically 10−100 pL) onto the surface. Droplets of different composition could be placed in each hydrophilic well plate consecutively by changing tips or simultaneously by using multiple capillary tubes separated by 50 μm and brought into registry with the patterned substrate. After an array of vesicle droplets had been addressed on the surface, the entire system was immersed in buffer solution inside a flow cell and excess lipid vesicles were washed out.

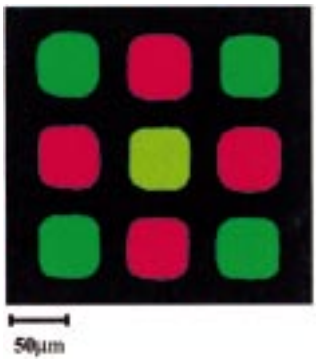

Figure 2. An epifluorescence image of a 3 × 3 array of glass well plates containing addressed egg phosphatidylcholine lipid membranes with various dyes. The image was obtained with an E800 fluorescence microscope from Nikon equipped with a black and white CCD camera. The image was processed by the technique of false color imaging.

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10.1021/ja991453a CCC: $18.00
Published on Web 08/19/1999

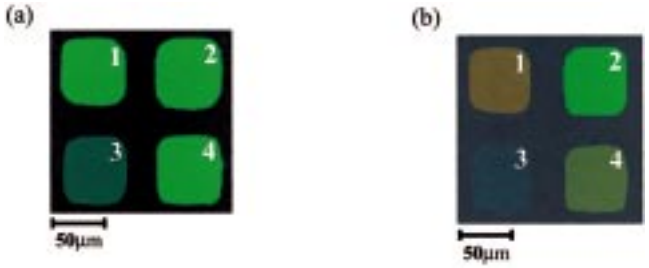

Figure 3. An epifluorescence image of membranes containing various concentrations of biotinylated lipids (a) before and (b) after exposure to streptavidin.

added to the same box sequentially, the bilayer consisted primarily of the lipids that were introduced first. This occurs because the self-assembly process is completed within a few seconds under the conditions used for these experiments. Lipids in the outer leaflet of the supported bilayer will slowly exchange with those from vesicles in solution, but this process usually takes tens of minutes to hours at 10 °C; moreover, the lipids in the inner bilayer leaflet are even less accessible.[13,14]

Selective incorporation of a protein receptor site into the patterned membranes demonstrates the viability of addressed supported bilayers as sensor devices. For this purpose we employed the biotin-streptavidin system.[15] Biotin can be covalently attached to the headgroup of phosphatidylethanolamine lipids and binds the aqueous protein, streptavidin, with a high affinity. Figure 3a shows the epifluorescence image of four 50 μm × 50 μm boxes. The membrane in box 1 was formed from egg PC vesicles containing 3 mol % NBD-PE and 2 mol % biotinylated PE. Box 2 contained an egg PC membrane with 3 mol % NBD-PE and no biotinylated PE. Box 3 was left empty and box 4 was similar to box 1, but the biotinylated PE concentration was only 1 mol %. Upon exposure of the substrate to a 10 μM concentration of Texas Red-labeled streptavidin solution, the membrane patches containing biotinylated lipids quantitatively absorbed the streptavidin, which remained mobile on the surface (Figure 3b). This contrasted with box 2 where no streptavidin was detected. As expected, a small quantity of protein absorbed to the bare glass surface of box 3 but was immobile as determined by FRAP. These results are depicted schematically in Figure 4. They not only demonstrate the high level of binding specificity of streptavidin to fluid supported membrane patches containing the biotin receptor site but also indicate a high level of resistance of fluid phospholipid membranes to nonspecific protein binding.

The methodology outlined above for the formation of spatially addressed phospholipid bilayers containing multifarious lipids, peptides, receptors, and membrane-associated proteins is straightforward and should prove to be widely applicable. Up to now we have experimented with square well plates from 25 μm × 25 μm to 250 μm × 250 μm with hydrophobic partitions ranging from 25 μm to 250 μm in thickness. All sizes appeared to work equally well as the data presented here. Incorporating new deposition technologies such as the chemical inkjet microdispenser should allow very large membrane libraries to be created on experimentally practical time scales.[16] In addition to being able to rapidly address a variety of membranes, the microdispenser could serve as a convenient method for depositing premixed concentration arrays of three or four component membranes analogous to the methods now being employed for materials discovery.[2,3] On the other hand, a method for parallel membrane deposition would be even more powerful. For this purpose an array of microreservoirs having the same dimensions as the hydrophilic well plates could be fabricated by soft lithographic techniques.[17] The reservoirs would be addressed with liposome solution and employed as a master from which to transfer solution aliquots to the well plates. Droplets of solution would be delivered in parallel by employing an array of glass probes that could be dipped into the aligned microreservoirs and then touched to the hydrophilic well plates following the procedure outlined in ref 17. Finally, because a planar support can accommodate a wide variety of surface-specific spectroscopies, biosensors can be developed from existing technologies such as plasmon resonance imaging without the need for fluorescence labeling of lipids and proteins.[18]

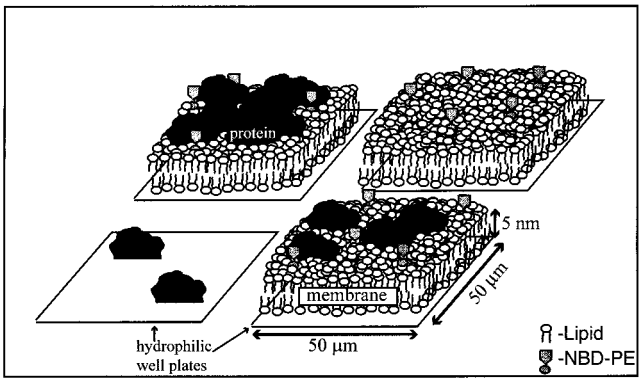

Figure 4. Schematic representation of the system presented in Figure 3b.

Acknowledgment. The authors thank Drs. Hagan Bayley, Orit Braha, Li Sun, and Richard Crooks for useful discussions. This work was supported by startup funds from Texas A&M University.

JA991453A

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