

Direct Patterning of Membrane-Derivatized Colloids Using In-Situ UV-Ozone Photolithography**

By Cheng-han Yu, Atul N. Parikh, and Jay T. Groves*

Supported membranes, consisting primarily of a bilayer assembly of phospholipids on a solid substrate, have been productively employed in a wide range of chemical, physical, and biological investigations of cell-membrane phenomena.^[1-4] Membranes can assemble onto solid materials such as silica by spontaneous adsorption and fusion of lipid vesicles. This process results in a single, continuous membrane coating and is equally applicable to monolithic substrates and micrometer-sized silica particles.^[5-7] In addition, these membrane-coated silica beads, namely membrane-derivatized colloids, are able to represent two-dimensional colloidal phase transitions.^[7]

A salient characteristic of the supported-membrane configuration is the preservation of natural membrane fluidity. Lipids and membrane-linked proteins diffuse freely over macroscopic distances with lateral diffusion coefficients generally in the range of $1-10 \mu\text{m}^2 \text{s}^{-1}$. The functional consequences of this fluidity can be dramatic. For example, supported membranes displaying cognate protein ligands for T cell surface receptors can effectively replace the antigen-presenting cell and form a synapse with a living T cell.^[8] During this interaction, proteins on the T cell surface become redistributed into distinctive patterns, dragging their cognate ligands in the supported membrane into complementary patterns. A large number of cellular processes involve spatial redistributions of proteins,^[9] and patterned supported membranes can be an effective way of studying and dissecting these events.^[4,10,11] The success of the hybrid live cell supported membrane synapse configuration underscores the growing need for methodologies to prefabricate spatial composition patterns in supported membranes.

A number of strategies for patterning membranes on monolithic flat surfaces have recently been introduced.^[12] The substrate can be pre-patterned by conventional lithographic techniques, and these patterns can subsequently guide the assembly of the supported membrane.^[13] Alternatively, a variety

of direct-membrane-patterning methodologies, including microcontact printing,^[14,15] microfluidic flow patterning,^[16,17] scanning probe lithography,^[11] and in-situ deep-UV photolithography,^[18,19] have also been employed. To date, however, membrane patterning has been restricted to monolithic and planar substrates. In the following, we demonstrate direct membrane patterning on the surface of micrometer-sized silica particles by in-situ three-dimensional (3D) UV photochemical lithography in an aqueous environment. This is accomplished by back-side contact lithography with self alignment. The photomask template consists of an array of micromasks, which are positioned in registry with surface topographical features that guide the gravitational settling of the beads into alignment with the mask pattern (Fig. 1). A variety of mask patterns can be implemented, and patterning resolutions down to micrometer dimensions are easily achieved.

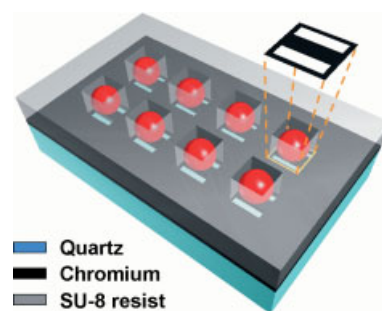


Figure 1. Schematic illustration of the in-situ UV-ozone lithography photomask template. Thin chromium serves as the masking layer, and a SU-8 resist layer creates an array of pits right on the top of chromium patterns. The size of the pits is matched to the diameter of the colloidal particles. (Inset: the chromium pattern underlying the pits.)

Deep-UV illumination (primarily 185 nm and 254 nm) generates ozone and other reactive radicals from oxygen. In particular, O_2 becomes reactive oxygen atoms under 185 nm wavelength UV exposure, and these oxygen atoms react with O_2 to form ozone (O_3). Moreover, O_3 itself absorbs 254 nm wavelength UV light and produces the reactive oxygen atom $\text{O}(^1\text{D})$ and singlet molecular oxygen $^1\text{O}_2^*$. In aqueous environments, a water molecule can then react with $\text{O}(^1\text{D})$ and $^1\text{O}_2^*$ and form another radical, H_2O_2 .^[20-23] These radicals chemically degrade and remove lipids with high spatial resolution, even in an entirely aqueous environment. In order to apply this photochemical process to the patterning of lipid membranes supported on the surface of colloidal particles, a photomask consisting of an array of micromasks overlaid with a corresponding array of topographically registered pits is employed to position the particles and to minimize free Brownian motion (Fig. 1). Using this technique with the mask described here, up to 810 000 membrane-coated beads can be patterned homogeneously and simultaneously. The resultant micrometer-resolution patterns of membranes on the surface of colloid particles can be observed by fluorescence microscopy. Figure 2 depicts post-exposure images of an array of mem-

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[**] This work was supported by the Director, Office of Science, Office of Basic Energy Sciences, Division of Materials Sciences and Engineering, of the U.S. Department of Energy under Contract No. DE-AC03-76SF00098. We also gratefully acknowledge funding from NSF Center for Biophotonics Science and Technology. All devices were fabricated at the UC Berkeley Microfabrication Laboratory.

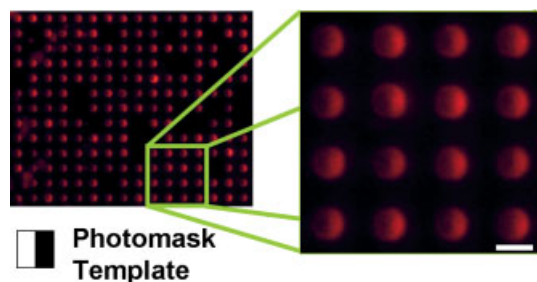


Figure 2. Fluorescence images of an array of membrane-coated silica particles in the registry pits on the colloid-patterning mask after in-situ UV-ozone photolithography. The particles are 6.8 μm diameter spheres, which have been patterned with a 50% left exposure pattern (as shown in the inset). The membranes contain the lipid probe Texas Red 1,2-dihexadecanoyl-*sn*-glycero-3-phosphoethanolamine (Texas Red DPPE), which can be seen to coat only the right half of each particle. The bar represents 7 μm. Membrane composition: 89% 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC), 10% 1,2-dioleoyl-3-trimethylammonium-propane (DOTAP), and 1% Texas Red DPPE.

brane-coated silica particles still in the registry pits on the colloid-patterning mask. The particles are 6.8 μm diameter spheres, which have been patterned with a 50% left exposure pattern (as shown in the inset). The membrane contains the lipid probe Texas Red DPPE, which can be seen to coat only the right half of each particle. After exposure, the particles can be released from the template by mild sonication and collected for use.

The patterns were generally stable for weeks and the membranes remained fluid when stored at 4 °C. The reason why the patterns were stable is not entirely clear.^[18,19] A likely possibility is the formation of stable hemimicellar edges at the pattern edges. Several recent theoretical studies confirm this prediction.^[24] Another stabilizing factor could be the increased frictional coupling between the membranes and solid substrates. This sort of coupling can impede large-scale movement, such as rearrangement of a boundary, while having only limited effects on individual molecular motions.^[11,25] Long-term stability can likely be achieved using membrane-stabilizing techniques, such as binding to a surface protein layer.^[26]

The fact that the membrane in the exposed region of the colloidal particle is removed is confirmed by the ability to refill these regions with another membrane type. Incubation of patterned colloids with new small unilamellar vesicles (SUVs) results in deposition of new membrane into the previously exposed regions. When both the newly deposited and the original supported membranes are fluid, they rapidly mix and all patterns are completely erased. Alternatively, the exposed regions on the originally patterned colloid surface can be refilled with non-fluid membrane or simply by protein deposition (Fig. 3), yielding stably patterned colloids. In Figure 3, fluorescence images of different post-exposure membrane patterns (Figs. 3A₂, B₂, C₂) on the surface of silica colloidal particles (5 μm in diameter) and the corresponding photomasks (Figs. 3A₁, B₁, C₁) are illustrated. After photopatterning of the membrane, fluorescein isothiocyanate (FITC) labeled

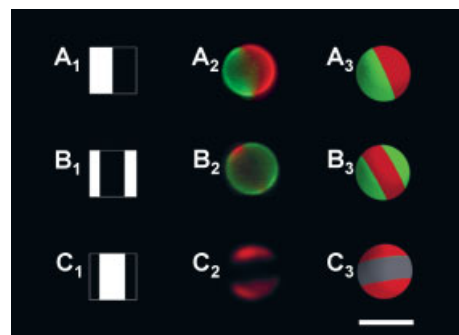


Figure 3. Pattered membranes on colloidal particles: A₁, B₁, C₁) Photomask template patterns. A₂, B₂, C₂) Fluorescence images of membrane patterns on 5 μm colloidal-silica particles. A₃, B₃, C₃) Simulated images of membrane patterns corresponding to the each photomask. We found that fluorescein isothiocyanate (FITC) labeled bovine serum albumin (BSA) only binds to UV-patterned areas (e.g., A₂, B₂). This suggests that the lipid bilayer membranes in the exposed regions were largely removed. The image C₂ represents a bare membrane pattern without adding BSA. 5 μm membrane-coated beads are demonstrated here. Even under aqueous environments, the resolution of this system can be up to 1–2 μm. The bar represents 5 μm. Membrane composition: 89% DMPC, 10% DOTAP, and 1% Texas Red DPPE.

bovine serum albumin (BSA) is added and can be seen to selectively bind to the exposed regions of the colloid. Refilling the patterned membrane with BSA provides a simple example of the types of photopatterns that can be produced.

By tracking a single membrane-coated colloidal particle lying across the substrate, the membrane patterns on its surface can be observed at different angles of view by digital video microscopy. This allows full resolution of the 3D surface patterns. For example, Figure 4 illustrates a 3D fluorescence image analysis of patterned membranes on the surface of a single colloidal-silica particle (Fig. 4A), along with simulated views of the same 3D pattern from various angles (Fig. 4B). The supported membrane is seen to be uniformly patterned three-dimensionally throughout the colloidal surface, and the membrane patterns are in good agreement with the simulations. The homogeneity of membrane patterns over the surface of the colloidal particle suggests the achievement of 3D photolithography.



Figure 4. 3D analysis of patterned membranes in different view angles with digital video microscopy. A) Fluorescence time-lapse images of membrane patterns on a single 7 μm colloidal-silica particle lying on the substrate. B) Simulated images in each corresponding angle of view. Membranes are seen to be nicely patterned three-dimensionally throughout the colloidal surface, and membrane patterns are in good agreement with simulations. The bar represents 7 μm. Membrane composition: 99% DPPC and 1% Texas Red DPPE.

We have demonstrated a simple method for direct patterning of lipid bilayer membranes on the surface of colloidal-silica particles by in-situ UV photochemical lithography and conventional microfabrication technology. The key features of this photopatterning technique are that it occurs entirely in an aqueous environment, does not require development steps, and the generality of the UV-ozone chemistry renders it applicable to a wide range of lipid compositions.^[16,17] Highly parallel generation of micrometer-resolution membrane patterns is achieved with biocompatible processes. A unique aspect of this technique is the ability to define patterns on small 3D substrates. Particularly, it enables presentation of biomolecules in a controllably asymmetric manner. By protecting the exposed area with BSA or other stable silica-binding proteins, the original patterned lipid bilayer membrane can be removed by weak detergent, and the inverted protein patterns on silica particles can be preserved for future use. Geometrically asymmetric membranes on colloid particles are of utility for presentation of biological signals (e.g., cell-surface signaling molecules) and may find applications in a variety of bead-based assays for ligand–receptor interactions (e.g., flow cytometry). They also create new possibilities for studying symmetry-dependent behavior in two-dimensional colloidal systems in general.

Experimental

Lipids: 1,2-Dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC), 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC), 1,2-dioleoyl-3-trimethylammonium-propane (DOTAP), and 1-palmitoyl-2-[12-[(7-nitro-2-1,3-benzoxadiazol-4-1)amino]dodecanoyl]-*sn*-glycero-3-phosphocholine (NBDPC, 16:0-12:0, tail-labeled) were purchased from Avanti Polar Lipids (Alabaster, AL). Texas Red 1,2-dihexadecanoyl-*sn*-glycero-3-phosphoethanolamine (Texas Red DPPE) was purchased from Molecular Probes (Eugene, OR). The lipid composition of this study is just for demonstration, and is not case specific.

Lipid Vesicles and Silica Colloids: For preparation of small unilamellar vesicles (SUVs), lipids in chloroform were first evaporated onto the wall of a round-bottomed flask, and dried in nitrogen for 5 min. The lipids were then resuspended in distilled, deionized water by vortexing moderately for several minutes. SUVs were generated by extruding the lipid suspension 10 times through a 0.1 μm polycarbonate filter using a pressure-regulated Lipex extruder (Northern Lipids, Canada). During extrusion, the temperature was maintained at 50 °C by warm-water circulation. SUVs were stored at 4 °C and could typically be used for several weeks. Before usage, the SUV suspensions were diluted into the buffered solution, typically phosphate buffered saline (PBS), to a final lipid concentration. Single lipid bilayer membranes were deposited on 5 μm or 6.8 μm silica microspheres (Bangs, Fishers, IN) by spontaneous fusion of SUVs [7]. Membrane-coated colloid particles were then allowed to settle gravitationally onto microfabricated photomask templates. Ice water could be used to decrease the fluidity of membranes during the 6 min backside UV exposure.

Deep-UV Illumination: A mercury-discharge grid lamp with emission peaks at 185 nm and 254 nm (UVP, Upland, CA) served as the UV source. A closed homemade chamber in a chemical hood was designed for this study, preventing exposure to the UV radiation and ozone. Direct exposure to short-wavelength UV or a high concentration of ozone must be avoided. 0.1 ppm of ozone can cause serious irritation.

Fabrication Process: High-UV-transmission fused-quartz wafers (4 inch [10.16 cm] diameter; HOYA, San Jose, CA) were used as substrates for mask fabrication. A conventional process with two lithography steps was utilized for fabricating the photomask templates, as

sketched in Figure 5. The substrates were prepared by a 10 min etch in piranha solution (5:1 H₂SO₄/H₂O₂), followed by dehydration in an oven (120 °C for 30 min) and then hexamethyldisilazane (HMDS) vapor immersion (10 min). S1818 photoresist (Shipley, Marlborough, MA) was spun onto wafers to a thickness of 1.8 μm. After a soft bake at 90 °C for 5 min, the wafers were exposed to UV light through the first photolithographic mask and developed. This mask establishes the pattern that forms the array of photomasks in the finished device. Next, a 150 nm thick chromium layer was deposited by electron-beam thermal vapor deposition and patterned by a lift-off with acetone in a

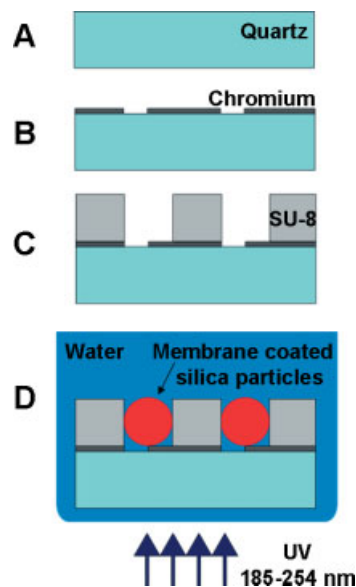


Figure 5. Process flow of photomask templates fabrication. A) A quartz wafer is used as the substrate. B) A chromium layer is deposited by an electron-beam thermal evaporator and defined by the lift-off method. C) SU-8 resist is precisely aligned to the underlying chromium layer and cured after development. D) Membrane-coated colloidal-silica particles settle onto the photomask templates by gravity, and are aligned into the SU-8 pits. Deep-UV (185–254 nm) illumination from the back-side patterns the membranes via a contact-printing mechanism.

sonication bath. The substrate was then prepared for the second lithographic step, creation of the alignment topography, by spinning a 7 μm thick SU-82007 photoresist layer (Microchem, Newton, MA) onto the wafer and soft-baking at 65 °C for 1 min and then at 95 °C for 2 min. The second photolithographic mask was carefully aligned with the underlying array of 810 000 micromasks. After a post-exposure baking (65 °C for 1 min and then 95 °C for 1 min), developing, and a final hard baking, the patterned SU-8 films with nearly vertical sidewalls provided the registry pits for alignment of membrane-derivatized colloids. The size of the pits was matched to the diameter of the colloidal particles to be patterned. These quartz photomask templates served as photomasks for the in-situ colloid UV lithography process.

Received: September 27, 2004

Final version: March 2, 2005

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Bottom-Up Fabrication of Carbon-Rich Silicon Carbide Nanowires by Manipulation of Nanometer-Sized Ethanol Menisci**

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The development of nanometer-scale lithographies is the focus of intense research activity because progress on nanotechnology depends on the capability to fabricate, position,

and interconnect nanometer-scale structures.^[1] The unique imaging and manipulation properties of atomic force microscopy (AFM) has prompted the emergence of several scanning-probe-based nanolithographies.^[2–5] Here we describe an atomic force nanolithography based on the spatial confinement of an electrochemical reaction within a nanometer-sized ethanol meniscus. The meniscus is induced by the application of an electrical field. The end result is the formation of a nanometer-sized electrochemical cell (nanocell) that contains about 5×10^4 molecules. The reduced number of molecules and the reaction kinetics allows a bottom-up fabrication of several types of nanostructures. We show, using spatially resolved photoemission spectroscopy, that the nanostructures are made of carbon-rich SiC. The nanolithographic possibilities are illustrated by the fabrication of nanowires with widths and positioning below 45 nm, both singly or forming arbitrarily shaped networks.

AFM nano-oxidation^[4] (local oxidation nanolithography) has allowed the fabrication of a variety of nanometer-scale devices such as 0.2 terabit cm^{-2} memories,^[6,7] superconducting quantum interference devices,^[8,9] or templates for driving the growth of organic molecules.^[10] Parallel patterning based on local oxidation has also been demonstrated by replacing the AFM tip with a stamp with multiple lines or protrusions.^[11,12] AFM local oxidation is based on the spatial confinement of the electrochemical oxidation between the tip and the sample surface.^[6,13] This requires the formation and manipulation of tiny water bridges.^[14,15] The directly fabricated structures are oxides of the sample surface that may serve as a tunnel barriers in nanoelectronic devices, templates, or masks for further processing.^[16–20] The local oxidation process is robust, low-cost, and compatible with ambient operation. However, it is severely limited because it only generates oxides of materials that are amenable for anodic oxidation.

We have developed a method to obtain a variety of nanometer-sized carbide structures. The method is based on the ability to form and manipulate the properties of ethyl alcohol nanometer-sized bridges. The ethanol bridge is formed by the application of an electrical field between an n-doped silicon tip and a silicon surface. Once an ethanol nanocell has been formed, a voltage pulse drives the ethanol molecules to a Si(100) interface where the electrochemical reaction occurs. The experimental set-up consists of a conductive dynamic AFM which is enclosed in a chamber filled with N_2 and $\text{CH}_3\text{CH}_2\text{OH}$ vapor. A voltage pulse applied between tip and sample condenses the vapor underneath the AFM tip, giving rise to the formation of a nanometer-sized liquid bridge. Tip-surface separation, voltage strength, and pulse duration control the meniscus size and hence the electrochemical cell's size, which in turns will control the nanostructure size.

Some elements of the experimental set-up and the steps involved in the formation of nanometer-sized liquid bridges are shown schematically in Figure 1. The instantaneous motion of the AFM tip is recorded in an oscilloscope screen. Before the application of a voltage pulse, the tip oscillates above the sample surface. The electrostatic interaction deflects the tip's

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[**] This work was supported by Ministerio de Educacion y Ciencia (Spain) contract number MAT2003-02655 and the European Commission, contract number NAIMO Integrated Project No NMP4-CT-2004-500355.