Sub-100 nm Patterning of Supported Bilayers by Nanoshaving Lithography

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Supporting Information

Materials

Bovine serum albumin (BSA) and Texas Red-conjugated BSA were purchased from Molecular Probes, Inc. (Eugene, OR). 1-Palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC), 1,2-dihexanoyl-*sn*-glycero-3-phosphocholine (DHPC), and 1,2-dipalmitoyl-*sn*-glycero-3-phosphoethanolamine-*N*-(7-nitro-2-1,3-benzoxadiazol-4-yl) (Ammonium Salt) (NBD-PE) were purchased from Avanti Polar Lipids (Alabaster, AL). Phosphate buffered saline (PBS) solutions were used in all experiments. These solutions contained 20 mM sodium phosphate, 150 mM NaCl, and 0.2 mM sodium azide. The pH of the solution was set to 7.4 by the dropwise addition of 2.0 M NaOH. Purified water for these experiments came from a NANOpure Ultrapure Water System (≥18.2 MΩ·cm, Barnstead, Dubuque, IA). Borosilicate glass coverslips (VWR Scientific, No. 1, 22 × 22mm) were used as substrates for protein monolayer and lipid bilayer formation. It should be noted that the substrates were cleaned in a boiling 1:3 solution of ICN ×7 detergent and purified water. After cleaning, the substrates were rinsed with copious amounts of purified water, dried with nitrogen, and annealed in a kiln at 480 °C for 5 hours.

Vesicle Preparation

Small unilamellar vesicles (SUVs) were prepared by vesicle extrusion. Lipids dissolved in chloroform were dried under a stream of nitrogen followed by overnight vacuum desiccation. Next, the lipids were rehydrated in PBS solution. After five freeze-thaw cycles, the vesicles were extruded more than seven times through a polycarbonate filter (Whatman) containing 50 nm pores. These vesicles had a size distribution centered around 70 nm as determined by dynamic light scattering (Brookhaven Instruments 90Plus Particle Size Analyzer). These vesicles were employed to obtain all the data obtained in the main text. As a control experiment, POPC vesicles containing 2.0 mol% NBD-PE were also extruded more than seven times through a polycarbonate filter (Whatman) containing 30 nm pores. The center of the size distribution for these vesicles was approximately 34 nm. Again, it was found that lines with a ~ 55 nm width could be formed, but not lines with a ~ 36 nm width (Figure S1). We have also tried to use osmotic shock by adding 500 mM NaCl to the solution in an effort to form SLBs from POPC in the ~ 36 nm wide lines. Even under these conditions no evidence was found for SLB formation.

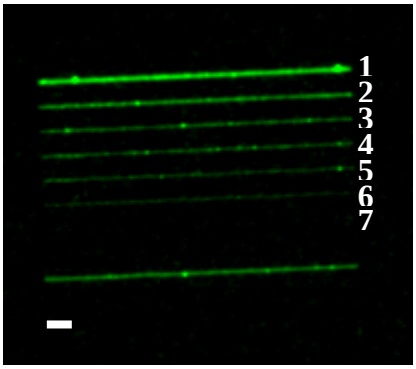

Figure S1. Epifluorescence image SLB lines. The bottom line, which is ~ 200 nm in width, was used as a reference marker. The widths of the shaved lines were (1) ~ 600 nm, (2) ~ 300 nm, (3) ~ 142 nm, (4) ~ 103 nm, (5) ~ 78 nm, (6) ~ 55 nm, and (7) ~ 36 nm, respectively. The scale bar is 3 μm.

Epifluorescence Microscopy and Lipid Mobility Measurements

Epifluorescence images of protein patterns and SLB lines were obtained using a Nikon E800 fluorescence microscope equipped with a MicroMAX 1024B CCD camera (Roper Scientific). Removal of BSA was confirmed by fluorescently tagging protein molecules at a low degree of labeling (1 molecule in 20) with Texas Red and visualizing the system under the epifluorescence microscope. To check the quality and fluidity of the SLB lines, one-dimensional fluorescence recovery after photobleaching (FRAP)[1] studies were conducted using an inverted epifluorescence Nikon Eclipse TE2000-U microscope equipped with a Nikon Plan Fluor 40× oil immersion objective (NA 1.30). Laser radiation (488 nm, 200 mW) from a mixed gas Ar^+/K^+ laser (Stabilite 2018, Spectra Physics) was used to bleach the lipid bilayer samples. Bleaching times were less than 1 second and FRAP images were captured with the MicroMAX CCD camera. Figure S2 shows time lapse FRAP images of SLB lines, which demonstrate the mobility of the lipid molecules.

To calculate the value of the diffusion constant, a one dimensional diffusion equation[1] was employed:

$$C_{0,t} = C_{0,0} \times R_o(R_o^2 + 8Dt)^{-1/2} \qquad \text{(eqn. 1)}$$

where $C_{0,0}$ is the concentration of the bleached dye at the center point of the bleaching profile (Figure S2) at 0 sec, $C_{0,t}$ is the concentration of the bleached dye at the center point of the bleaching profile as a function of time, R_o is the half-width ($1/e^2$) of the bleach spot, and D is the diffusion constant. In the case of Figure S2, the half-width of the bleach spot is ~ 7.2 μm as calculated by fitting the initial bleaching profile (t = 0 sec) to a Gaussian profile. From eqn. 1, a plot of $(C_{0,0}/C_{0,t})^2$ *vs.* time t should yield a straight line with a slope of $8D/R_o^2$. Figure S3 shows the linear relationship with a slope of 0.35 sec^{-1}. Hence, $D = 2.3 \times 10^{-8}$ cm^2/s. The average of 3

trials yielded a diffusion constant of 2.5 ± 1.4 × 10^{-8} cm^2/s. The mobile fraction was calculated by comparing the fluorescence intensity of the bilayer region before photobleaching and after recovery. The bottom line in Figure S2 was used a reference marker for the calibration of excitation light intensity and dye bleaching by continued imaging. Note that the bleached area must also be considered for the mobile fraction calculation, because the bleach spot occupied ~ 16% of the top line in Figure S2. Taking this into account yields a mobile fraction of ~ 0.97.

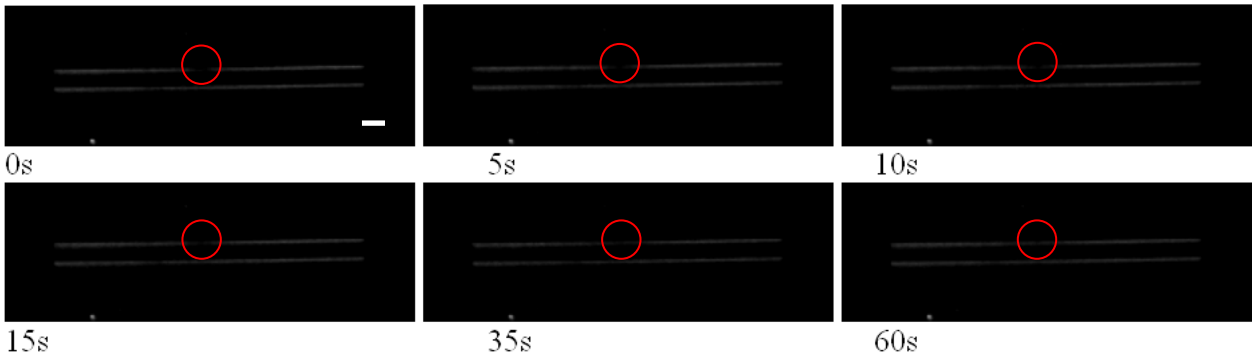

Figure S2. FRAP images of 2.0 mol% NBD-PE/POPC bilayer lines as a function of time. The top line was bleached at the center, and the bottom one was used as a reference marker. The scale bar is 6 μm.

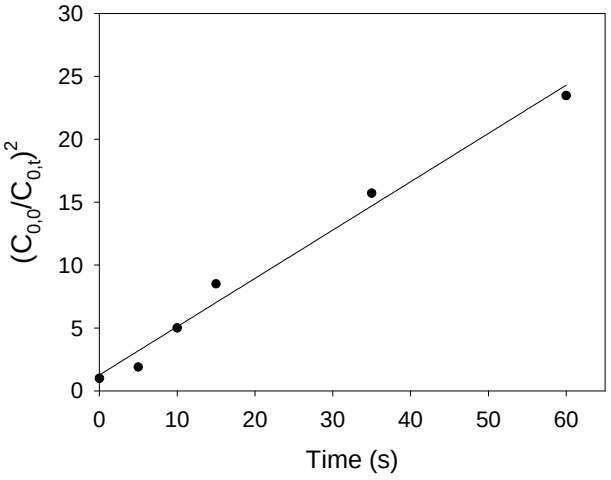

Figure S3. $(C_{0,0}/C_{0,t})^2$ *vs. time for the calculation of the diffusion constant.*

Lipid Composition Effects

To demonstrate that the inherent size limitation of bilayer lines is specific to the lipid composition of the membrane, we modulate the membrane chemistry which should lead to changes in the edge energy (γ). To do this, SUVs comprised of POPC with 6.0 mol% 1,2-dihexanoyl-*sn*-glycero-3-phosphocholine (DHPC) and 2.0 mol% NBD-PE were prepared by vesicle extrusion using a polycarbonate filter containing ~ 50 nm pores. Supported POPC/DHPC bilayers were expected to have a lower edge energy due to a high concentration of short-chain DHPC along the edge.[2] Figure S4 shows that SLBs made from POPC/DHPC/NBD-PE can be created within ~ 36 nm wide lines by vesicle fusion. We have directly verified by FRAP that the lipids in this narrowest line are mobile. The result demonstrates that the inherent size limitation of SLBs can be modulated by changing the lipid composition of the membranes.

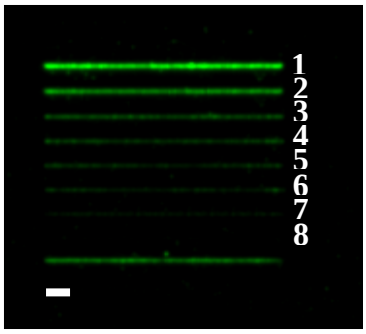

Figure S4. Epifluorescence image SLB lines. The bottom line, which is ~ 200 nm in width, was used as a reference marker. The widths of the shaved lines were (1) ~ 600 nm, (2) ~ 300 nm, (3) ~ 142 nm, (4) ~ 103 nm, (5) ~ 78 nm, (6) ~ 55 nm, (7) ~ 36 nm, and (8) ~ 15 nm, respectively. The scale bar is 3 μm.

Atomic Force Microscopy

A Nanoscope IIIa Multimode SPM (Digital Instruments, Santa Barbara, CA) equipped with a J-type scanner was used for nanoshaving lithography and AFM imaging of BSA monolayers. An etched silicon tip (NSC15/NoAl; spring constant: ~40 N/m; MikroMasch,

Wilsonville, OR) was used as the AFM probe. All images were obtained in tapping mode at a scan rate of 0.5 Hz. The only treatment applied to the images was flattening. Figure S5 shows AFM images and line scans for a series of nanoshaved BSA monolayers as a function of line width.

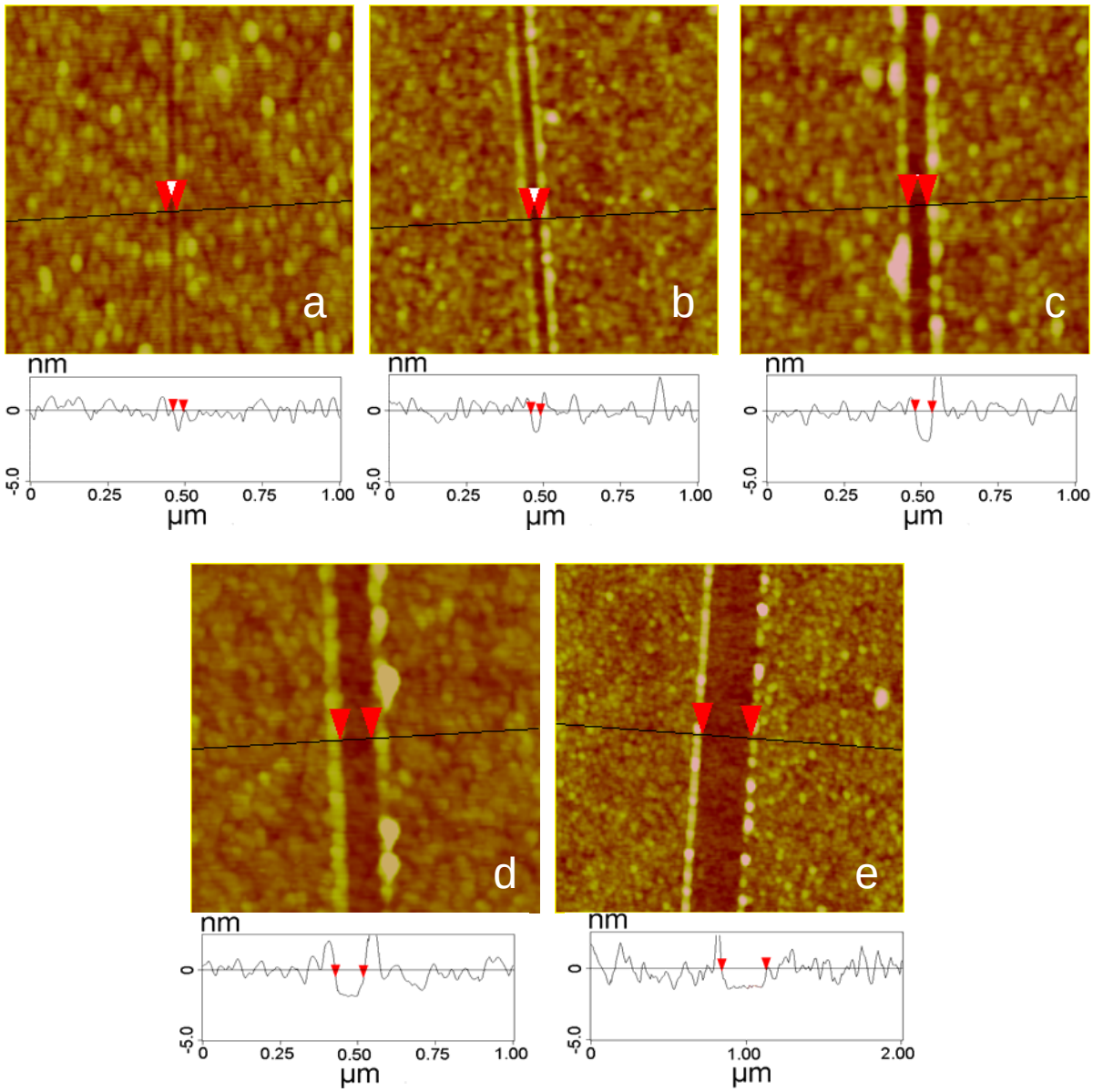

Figure S5. AFM images of a series of nanoshaved lines in a BSA monolayer. The width of the lines are: (a) ~ 15 nm, (b) ~ 36 nm; (c) ~ 55 nm; (d) ~ 103 nm; and (e) ~ 300 nm. The image size of (a), (b), (c), and (d) is 1 µm × 1 µm. The image size for (e) is 2 µm × 2 µm.

Choice of BSA for Nanoshaving

It is well known that a number of different materials are suitable for use as resist layers for patterning lipid bilayers on surfaces. For example, metals, oxides, polymers, as well as proteins have been employed.[3-5] For the nanoshaving experiments, we chose to use BSA because it forms single monolayer protein coatings when deposited from solution onto glass surfaces.[6] These very thin layers are conducive to shaving very narrow lines. Other proteins such as fibrinogen may work equally well. However, proteins that consistently lead to multilayer formation might be somewhat more difficult to use. Moreover, polymer layers such as spun-on films of photoresist may also be more difficult to employ. Indeed, it is difficult to shave 15 nm and even 36 nm lines in photoresist films that are 20 nm thick or more.

References:

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