Electronic Supplementary Material (ESI) for Chemical Communications
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Supplementary materials

Experimental:

Materials

Rhodamine B, Sodium hydroxide (99.99%), cysteamine (98%), tetrahydrofuran (THF 99%) and phosphate buffered saline (PBS 99% composed of 0.01M phosphate buffer, 0.0027M KCl and 0.137M NaCl) were purchased from Sigma. Polystyrene latex spheres (820 nm) were obtained from Duke scientific. L-α- Dimyristoyl phosphatidylcholine (DMPC) was purchased from Avanti Polar Lipids and the membrane probe 5-dodecanoylaminofluorescein (DAF) was obtained from Molecular probes.

Preparation of gold nanocavities

Gold nanocavities of 820 nm diameter were prepared using the nanosphere lithography technique similar to that described by Bartlett and co-workers.[i] Gold coated (400 nm) silicon wafers were first rinsed with acetone and then cleaned with piranha solution. Cysteamine was self-assembled onto the freshly cleaned smooth gold surface by immersing the substrate in a 10 mM ethanolic solution of cysteamine at room temperature for 24 hours. This chemical modification of the substrate with cysteamine increases surface hydrophilicity and increases the efficiency of the assembly of the sulphate functionalized polystyrene spheres on the gold substrate. Self-assembled monolayers of polystyrene spheres were prepared by placing the cysteamine modified gold substrates upright in an aqueous solution of polystyrene spheres (0.01%) and allowing the solvent to evaporate. Through these pre-assembled templates a gold film of controlled thickness (620 nm) was electrochemically deposited from a commercially obtained aqueous gold plating solution containing gold sulfite (Technic Inc., Cranston, RI, USA). In electrodeposition, the template-coated smooth gold substrates were incorporated as a working electrode in a three-electrode cell at room temperature. A platinum mesh was used as the counter electrode. A potential of -0.95 V versus an Ag/AgCl (sat. KCl) electrode was applied and potential was controlled by a CH Instruments Model 660 electrochemical workstation. After deposition the Au-covered electrodes were sonicated in THF for 1 hour to dissolve and remove the template spheres. Nanocavity arrays were filled with PBS buffer (pH 7.4) by sonication for 30 min before bilayer formation.

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Preparation of spanning lipid bilayers on pre-filled gold nanocavity arrays

Luminescent DMPC lipids were prepared incorporating 1 mole% DAF and were dissolved in chloroform in a beaker. The solvent was evaporated by blowing with N_2 gas onto the solution until a lipid film was formed. This lipid film was dissolved in PBS buffer solution (pH 7.4) to make a lipid suspension and this was sonicated for 30 min to form vesicles. The liposome solution was then extruded through a 100 nm polycarbonate filter. Liposomes without dye were prepared for both electrochemical and AFM measurements using the same procedure but without the addition of the membrane probe, 5-dodecanoylaminofluorescein (DAF).

The lipid bilayers were assembled at the gold nanocavity arrays using a liposome rupture by sonication of the arrays in liposome in PBS buffer for 30 min. Two approaches were taken and compared; in the first, dry nanocavity arrays were sonicated in PBS buffer for 30 minutes prior to treatment with the liposome solution, alternatively, the arrays were sonicated directly from dry in the liposome/buffer solution.

Instrumentation

Fluorescence microscopy was conducted using an LSM 50, Zeiss confocal fluorescence microscope using a 64x oil immersion objective (NA = 1.4), and 488 nm excitation light from an argon ion laser. The collected light was separated using a 505 nm long-pass filter, yielding reflectance (λ<505 nm) and fluorescence images (λ>505 nm).

For FRAP experiments, the same experimental set up was used with a bleach spot size of 2 μm. The total bleach time was 4 seconds, the resolution of the FRAP plot was 1.5 s and the total collection time was 40 s. The FRAP experiment was repeated 5 times. The equation below was used to fit the FRAP curve and is applicable to two dimensional diffusion of molecules to a circular bleached spot from a plane or a line source[ii] ;

$$I(t,r) = I_0 - \frac{M}{(4\pi Dt)^{d/2}} \cdot e^{\frac{-r2}{4Dt}} = I_0 - A(t).e^{\frac{-r2}{4Dt}}$$

where I and I0 are the fluorescence intensity after and before bleaching respectively. M is the fluorescence intensity corresponding to the bleached fluorophore; D is the diffusion

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The mobile fraction was determined by comparing the fluorescence in the bleached region after full recovery (I_∞) with the fluorescence before bleaching (I_i) and just after bleaching (I_0). The mobile fraction R defined as

$R = (I_\infty - I_0)/((I_i - I_0)$

Was determined to be 1 for the bilayer on the fluid filled array indicating the bilayer is 100% mobile across the array, but was only 0.38 for the lipid assembled on the dried array.

For FRET measurements, the gold nanocavities were pre-filled with rhodamine B (1 μM) in PBS buffer by sonication in this solution for 30 min prior to assembly of the lipid bilayer.

Capacitance measurements were carried out using AC voltammetry on a CH Instruments Model 660 electrochemical workstation at a frequency of 527 Hz and using an excitation signal of 5 mV. The contact angle for the buffer prefilled cavity array was approximately 89±9°, while for the dry cavity array a value of approximately 105±11° was obtained.

AFM images and force curves were recorded with a Veeco Bioscope II in contact mode using commercial Si_3N_4 cantilever tips. These tips are pyramidal in shape with spring constants between 0.04 and 0.08 N/m, and tip sizes were nominally less than 20 nm radius. All images were recorded with the AFM cantilever and substrate immersed in phosphate buffered saline.

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Supplementary Data

Figure S1 Capacitance measurements of (----) buffer prefilled gold nanocavity array, (----), bilayer formed on top of buffer prefilled gold nanocavity array and (----) bilayer formed inside the dry nanocavity array.

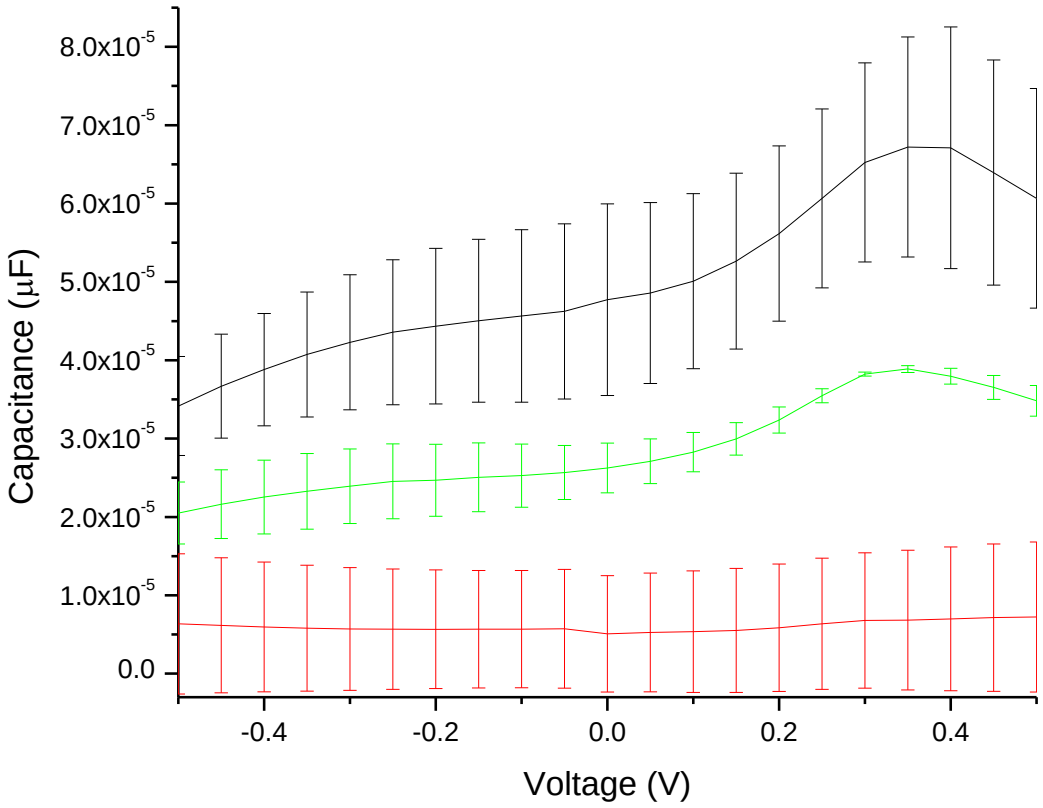

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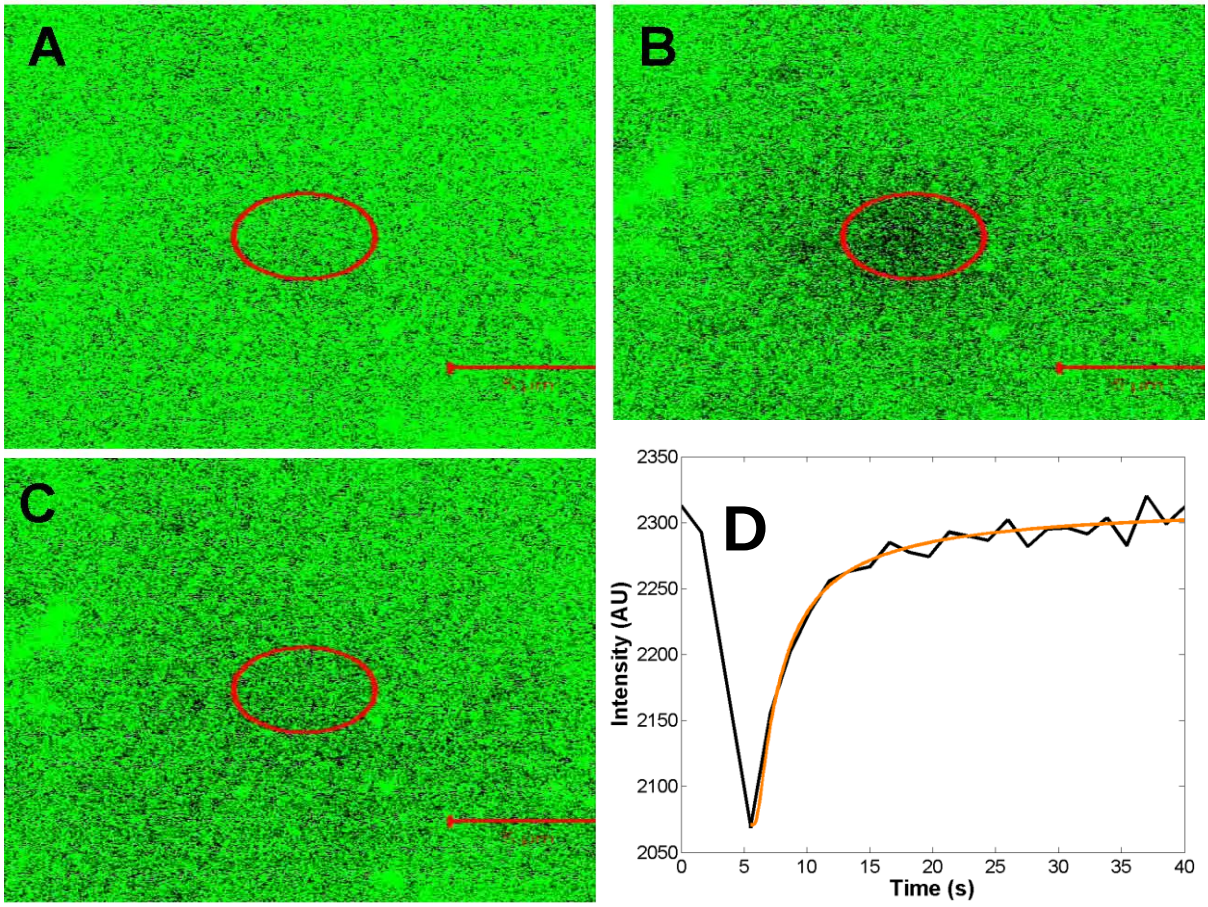
Figure S2 Fluorescence images taken during FRAP experiment: (A) before bleaching the fluorophore, (B) after bleaching and (C) after the fluorescence recovery. The red ellipse indicates the bleached area. (D) depicts the fluorescence recovery curve and the the red line is the best fit of Equation 1 (above) to this data (R^2 = 0.99).



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Scheme S1 Schematic representation of FRET pair in first approach to assembly lipid bilayers on top of gold nanocavity arrays. The orange star indicates the location of rhodamine inside the array and the green star indicates the presence of 5-dodecanoylaminofluorescein (DAF) in the spanned lipid (DMPC) bilayers.

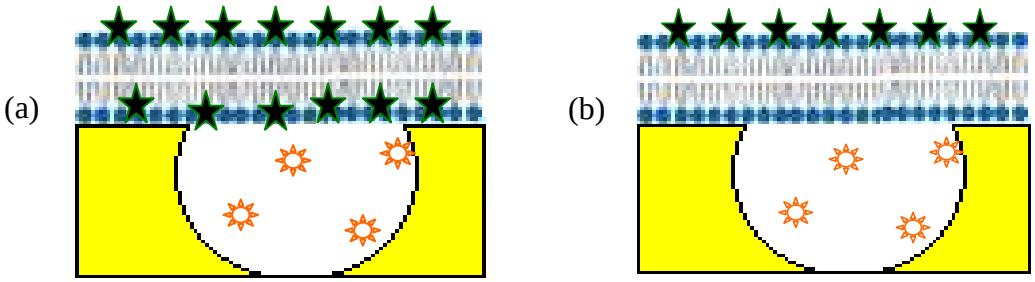

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Figure S3 AFM images of height and friction. (A) Height and (B) friction images of prefilled gold nanocavities before liposome rupture. (C) Height and (D) friction images of spanning lipid bilayer formed by liposome rupture on prefilled cavities.

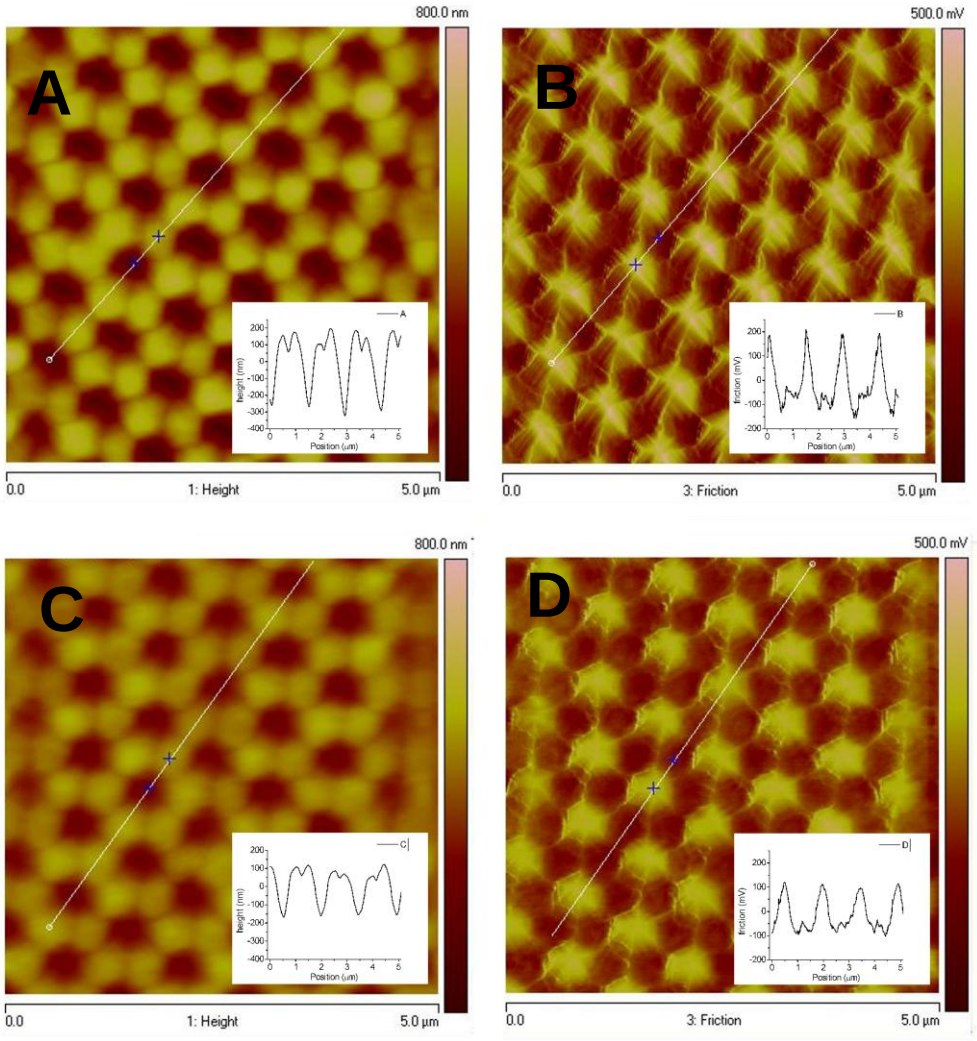

Fig. S3 shows height and friction images of prefilled cavities before and after liposome rupture, taken with the same AFM tip under identical loading force and feedback parameters. Cross-sections of the height images (insets of Fig. S3A and S3C) show that

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the depth probed by the AFM tip after liposome rupture is reduced from ~400 nm to ~250 nm. Correspondingly, the overall standard deviation of height (Rq) of 119 nm before the deposition of lipids is reduced to 70.8 nm afterwards. The compliance of a suspended lipid bilayer over the gold cavities appears to be great enough to allow some penetration of the AFM probe into the cavities, and the difference in heights is far too great to be attributed to a single collapsed bilayer. As shown in both the height and friction images, the suspended lipid bilayer also broadens features and decreases their range. The range of torsional flexure of the AFM tip (Table S1) that provides contrast in the friction images is reduced from an Rq of 0.0744 V to 0.0571 V. The height and friction AFM images support an interpretation of suspended lipid bilayers that resist z-directional forces from the AFM tip, and result in a lowered Rq of the height channel, and decrease shear forces, essentially lubricating the x-y motion of the tip, lowering Rq of the friction channel.

It is important to note that the aspect ratio of the AFM tip prevents its penetration to the full depth of the cavity, which is 620 nm deep. The SEM of these arrays, including side-on imaging, which confirms depth, can be seen in supplemental materials of B. Jose, U. Neugebauer, R Forster, T E. Keyes. Phys. Chem. Chem. Phys., 2009, 11, 10923

Table S1 The standard deviation of height and friction before and after lipid deposition on top of buffer filled cavity from AFM studies.

	Rq (height)	Rq (friction)
Bare surface	119 nm	0.0744 V
Lipid treated surface	70.8 nm	0.0571 V

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[i] Cintra, S.; Abdelsalam, M. E.; Bartlett, P. N.; Baumberg, J. J.; Kelf, T. A.; Sugawara, Y.; Russell, A. E., Faraday Discussions 2006, 132, 191-199.

[ii] Seiffert, S., Oppermann, W. Journal of Microscopy-Oxford 2005, 220, 20.