Supporting Information

Silica Colloidal Crystals as Three Dimensional Scaffolds for Supported Lipid Films

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Surfactant solubilized lipid solutions for CC modification

Surfactant solubilized lipid was incubated with CCs for 30 minutes before being placed in 500 mL of HEPES buffer (10 mM pH 7.4) overnight. The fluorescence image on the left (Figure S1) shows typical lipid films resulting from this approach. The region near the edge of the lipid/surfactant drop area is depicted. When films were subjected to a second incubation with lipid/surfactant and again rinsed overnight, less homogenous lipid coatings were observed (right), and the average fluorescence intensity increased by only 7%.

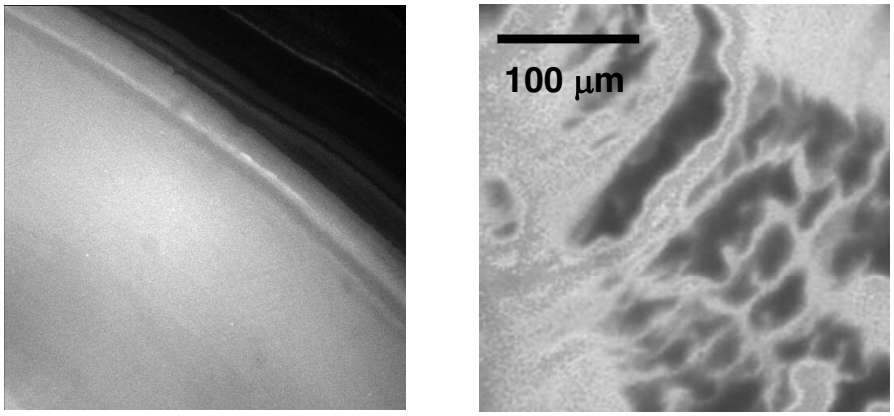

Figure S1

Fluorescence recovery after photobleaching data treatment

FRAP data were fit to the following single and double exponential models using Origin 70 software to derive three or five parameters respectively.

(1) $$F = A - B \cdot e^{(-t/\tau)}$$

(2) $$F = A - B(C \cdot e^{(-t/\tau 1)} + (1 - C) \cdot e^{(-t/\tau 2)})$$

F is fluorescence intensity at time t, A is the prebleach intensity, B is the postbleach intensity at time (t) = 0, C is the weighting factor for the first recovery time constant, and τ is the time constant.

It was observed that for some data, a double exponential provided a better fit, supported both by R^2 values and visual inspection. In some cases data noise caused R^2 to be less sensitive to overall fit, however the data clearly contained a recovery component with a slow time constant as indicated by the very slow approach to plateau. The fits to FRAP data for surfactant deposited lipid in CCs (Figure S2) illustrate this, the red line overlaid on the recovery data is a single exponential with $R^2 = 0.993$, and the blue line is the result of a double exponential with $R^2 = 0.996$. Time constants are used qualitatively, and no conclusions are drawn from the data regarding the structural and physical properties of the lipid modified CCs. Data in the manuscript text are the average of three separate CC films.

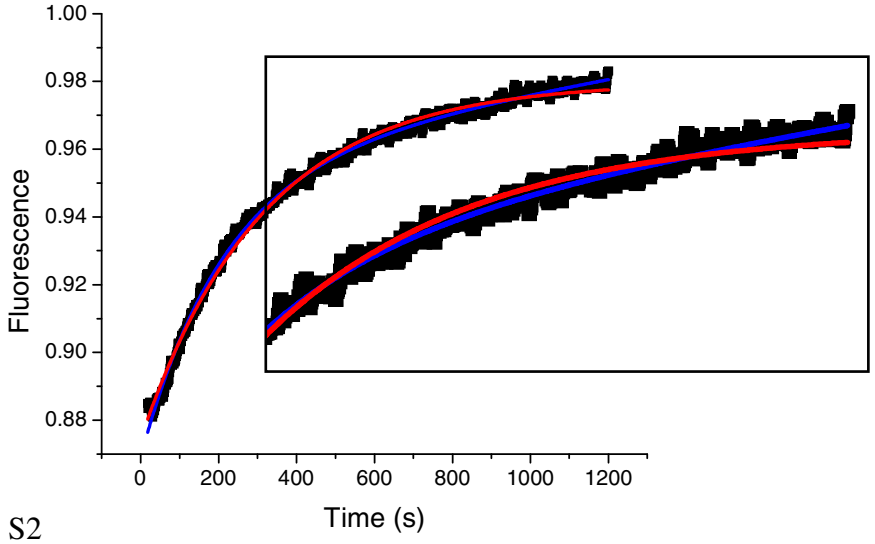

Figure S2

Example FRAP data for a colloid supported bilayer (vesicle fusion to CC with void filled with buffer) is shown below (Figure S3). For these films, R^2 is limited by noise, and single and double exponential fits return equivalent values. Visually, the double exponential (left plot) appears to better account for a fraction of rapid recovery (small time constant) which is apparent at t = 0.

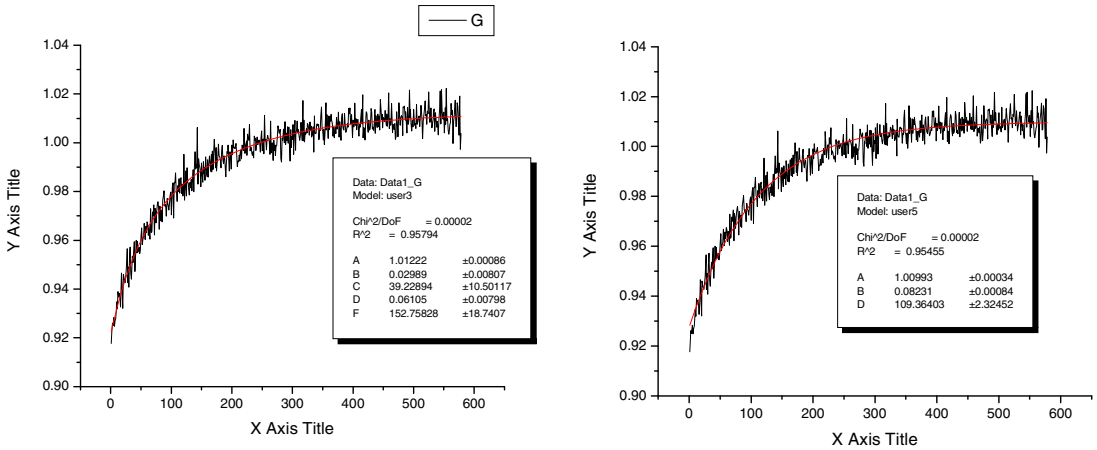

Figure S3

Fluorescence intensity after drying and rehydration

Fluorescence images were collected before and after drying the lipid modified CCs. The sequence below (Figure S4) shows images collected before (far left) and after 2 dry/rinse cycles described in the text (center). The circled regions demonstrate contrast features of that were used to locate the same areas for quantitative measurements. A directed stream of nitrogen at high pressure was directed at the crystal after removal from buffer (far right). The lipid films were found to be affected by such a procedure and a decrease in fluorescence homogeneity is observed. It is proposed that this procedure removes void buffer, and consequently lipid, by drainage rather than by evaporation. Fluorescence intensities before and after dry/rinse cycles are shown in Figure S5.

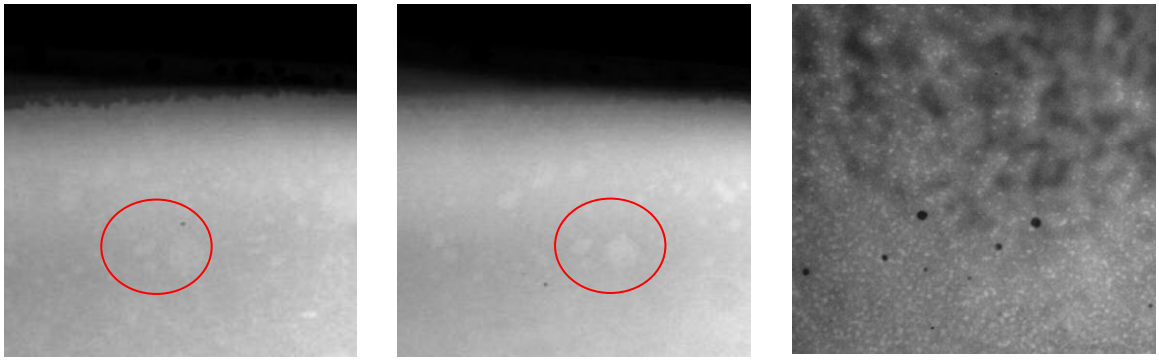

Figure S4

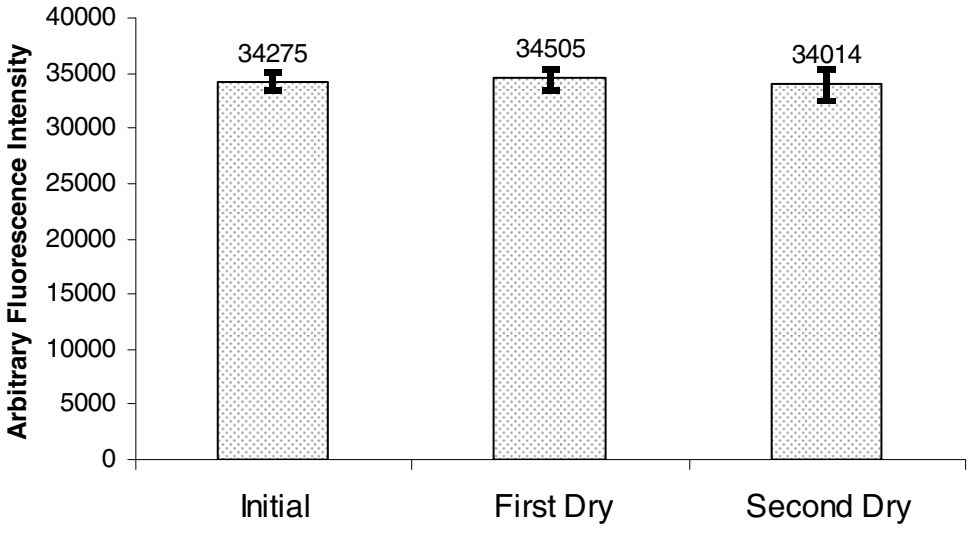

Figure S5. Fluorescence intensity observed from a chloroform deposited lipid film on a CC after desiccation and rehydration cycles.

Crystal thickness from microscopy images

Crystal thickness was measured by optical microscopy. CC on quartz slides were scored and snapped in half to allow profile images to be collected. Using a white light source (flashlight) directed at the crystal (perpendicular to imaging axis) to illuminate the crystal and cause scattering, we found images to be quite resolvable with adequate resolution to estimate film thickness. In a similar manner, a fluorescent probe such as rhodamine labeled BSA adsorbed onto a clean CC allowed the use of fluorescence microscopy to measure film thickness. Images are shown below, white light of a 14 micron thick CC on left, and fluorescence image of adsorbed BSA-TMR on a 11 micron (center) and 6.6 micron (right) CC.

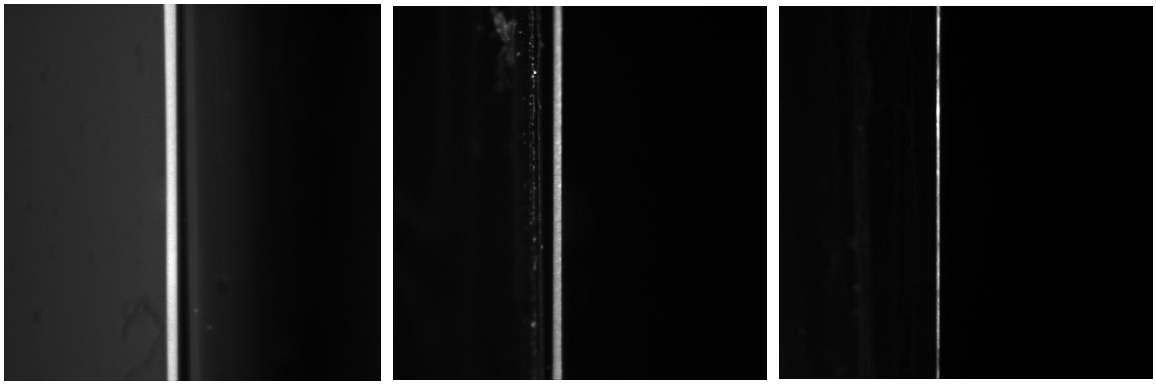

Figure S6

Estimation of surfactant solubilized lipid required for bilayer formation.

An estimate of the surface area of the CC is made using arguments presented in reference 6. The amount of lipid required to cover this area requires an estimate for the area occupied by each lipid. 60 $Å^2$ was used for POPC (Mukhopadhyay, P.; Monticelli, L.; Tieleman, D. P. *Biophys. J.* **2004**, 83, 1601.). Each lipid occupies 60 x 10^{-16} cm^2, which requires 1.1 x 10^{16} lipids, multiplied by 2 to account for both leaflets of a bilayer, which equates to 3.6 x10^{-8} moles of lipid. For a 1 cm^2 area of a 6.6 micron thick crystal composed of 330 nm collods, 88 cm^2 is the surface area estimate. The void volume is estimated to be 0.26 of the crystal volume (6.6 μm x 10000 μm x 10000 μm), or 0.17 μL. To deliver enough lipid to cover this area with a bilayer would require 0.14 M lipid, which is approximately 100 g/L.

Estimation of inaccessible surface area to lipid bilayers.

A bilayer film (5 nm thick) would theoretically be in steric conflict with a bilayer on an adjacent colloid at a distance of 42.9 nm from the colloidal contact points based on geometric considerations. A comparison of the surface area exluded to adjacent bilayers to the area available is found by ratio of the representative angles in the Figure S7. 46% of the surface area of the crystal composed of 330 nm colloids would theoretically be sterically excluded to bilayers on adjacent colloids. The red line represents the radius of the colloid plus a lipid bilayer. Based on right angle trigonometry, the surface area represented by a cone of 13.9^o would be excluded. There are 12 such contact points for each colloid in a *fcc*-packed CC. Within the excluded area for adjacent bilayers, a fraction of this area would support a single bilayer in the absence of a bilayer on an adjacent colloid. Using as the

hypotenuse a length of 167.5 nm to represent the colloid radius plus one-half of a bilayer, an area excluded to a single bilayer between two adjacent colloids is represented by a cone with angle 9.91°. This is an area fraction of 13.3%, that can only be realized for one of the colloids of the contacting pair, so an effective total area of 6.65% of the crystal. The total area that can be estimated available for bilayer coverage is therefore 62.65%.

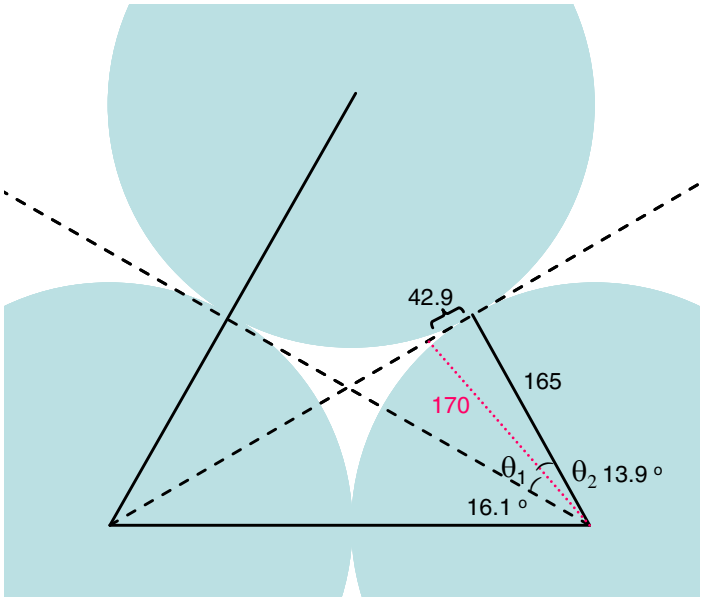

Figure S7.