Supplementary Material (ESI) for *Soft Matter*

Supplementary Information: Preparation of Fluid Tethered Lipid Bilayers on Poly(Ethylene Glycol) by Spin Coating

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Preparation of PEG Lipid Functionalized Surfaces (PLS)

Polished 6" silicon wafers with a thermally grown oxide layer of 100nm were cut into small pieces of size 20 × 15 mm^2 (Crystec GmbH, Berlin, Germany) and chemically cleaned before experiments. Substrates were sonicated in isopropanol for 10 minutes followed by rinsing with DI water. Then a three-stage chemical cleaning treatment was applied: first, the substrates were boiled in acetone for 10 min, then in 1/1/5 H_2O_2/HCl/H_2O by volume for 15 min, then in 1/1/5 H_2O_2/NH_4OH/H_2O for another 15 min. The substrates were finally rinsed thoroughly with DI water after each step and then used for silanization within 30 minutes of cleaning. PLS surfaces were prepared as described in a previous publication[1]. In summary, undecenyltrichlorosilane (UCT) silane films were prepared according to procedures described in the literature[2] and were then oxidized to the corresponding COOH-terminated films via a permanganate-periodate oxidation process[3]. The oxidized UCT wafers were then reacted with an aqueous solution of 75 mM 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimidehydrochloride (EDC) and 15 mM N-hydroxysuccinimide (NHS) for one hour. They were then thoroughly rinsed with water and reacted with a solution of amino-terminated DSPE-PEG_{2000} (0.5 mg/mL) in anhydrous DMF for 12 hours. After rinsing, the PEG-DSPE surfaces were again reacted with NHS as described above and then reacted with a solution of NH_2-PEG_{750} (10 mg/mL) in carbonate buffer at pH 8.5 for additional 12 hours. The wafers were finally sonicated in $CHCl_3$ for a few minutes and dried with nitrogen flow. Samples were dried in vacuum (1mbar) at room temperature for several hours and stored in vacuum until use.

X-Ray Reflectivity

X-ray reflectivity measurements were performed at the bending magnet beamline D4.1 at the Hamburg Synchrotron Radiation Laboratory (HASYLAB) in Hamburg, Germany. An X-ray energy of 19.9 keV (λ=0.623 Å) was chosen to allow for beam penetration through the water and to minimize the beam damage at the sample interface in the microfluidic chambers. Sample chambers were mounted in a horizontal scattering geometry with a beam cross section limited by a pre-sample aperture of 200 × 1000 μm^2 (vertical × horizontal). The large beam size ensures a wide illumination of the surface area even at high grazing angles of incidence. A small vertical post-sample aperture was used to completely suppress reflectivity from the thin top foil of the sample chamber. Evacuated beam guides with Kapton windows positioned close to the sample chamber minimized air scattering, and the reflected intensity was collected with a NaI detector.

Formation of a lipid bilayer on PEG lipid functionalized surfaces (PLS)

The initial steps of the equilibration process for the hydrated lipid films on PLS are monitored in detail by fluorescence microscopy. For this purpose, a circular spot on the inhomogeneous lipid film structure is photobleached directly after hydration using a 63× objective (Fig. S1,a). The partial fluorescence recovery (Fig. S1,b) indicates that the spin coated sample initially consists of roughly two types of lipid aggregates. Connected and highly fluid membrane areas co-exist with isolated, immobile lipid areas in this early stage of equilibration.

Fig. S2 shows a micrograph of the sample surface after a few hours equilibration at room temperature. A freshly photobleached spot on the surface is used to assess the membrane homogeneity (Fig. S2,a). A complete recovery of the photobleached spot is observed after 8h (Fig. S2,b), leaving no indication for the presence of membrane areas with different fluidity. This observation suggests that previously isolated lipid areas are now fully integrated into a closed and homogeneous lipid membrane surface.

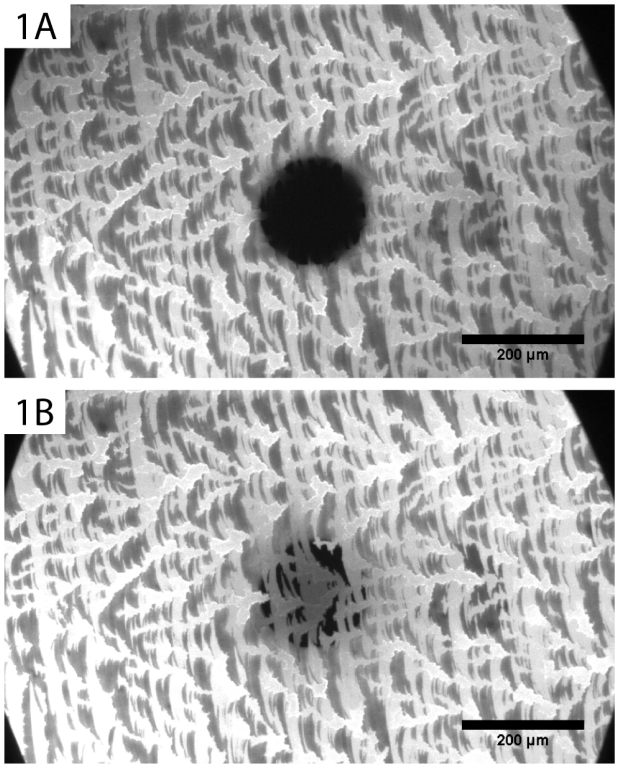

Fig. S1 Fluorescence microscopy images recorded with a 10× objective. (A) Photobleached spot. The circular area is bleached after 5min illumination using a 63× objective. (B) The same surface after 1 hour of equilibration without further illumination.

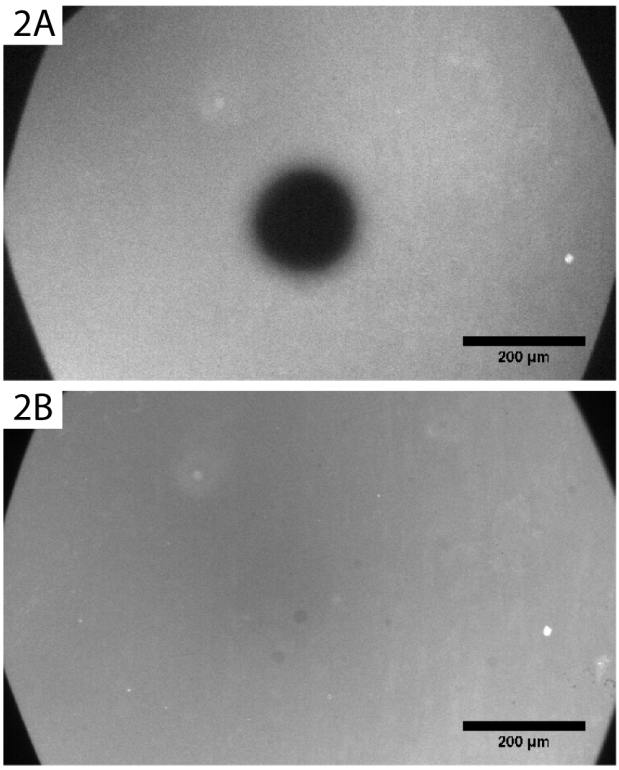

Fig. S2 (A) Sample surface after full equilibration. A freshly photobleached spot is shown on the surface. (B) After 8h, a complete fluorescence recovery is observed.

References

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