Supporting Information

Nonlamellar Lipid Liquid Crystalline Model Surfaces for Biofunctional Studies

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Preparation of lipid formulation
Soy phosphatidylcholine (SPC), glycerol dioleate (GDO), ethanol and propylene glycol were purchased from Lipoid, Danisco, Solveco and Fisher, respectively, and used without further purification. All components were weighed and mixed on a roller-table until complete mixing.

Substrate preparation
Lipid liquid crystalline films were formed on both hydrophilic and hydrophobic substrates. Polished and thermally oxidized silicon wafers (p-typed, boron-doped, <100> oriented) with a ~300 Å thick SiO_2 layer were purchased from Semiconductor Wafer Inc., Taiwan. The substrates were cut in ~1cm^2 pieces and cleaned, first in a base mixture of (1/1/5, by volume) 25% NH_4OH (Merck), 30% H_2O_2 (Burdick and Jackson, Honeywell) and H_2O (MilliQ, 18 MΩcm at 25 °C) at 80 °C for 5 minutes, rinsed in MilliQ, followed by an acidic mixture of (1/1/5, by volume) 37% HCL (Merck), H_2O_2 and MilliQ H_2O at 80 °C for 5 minutes. The hydrophilic silica substrates were thoroughly rinsed in MilliQ water and stored in ethanol. Hydrophobic substrates were prepared by gas phase silanization of plasma cleaned (5 minutes in Harrick Scientific Corp., model PDC-3XG plasma cleaner) hydrophilic silica surfaces in a desiccator, containing approximately 2 ml of dimethyloctylchlorosilane (DMOCS) from Fluka. The desiccator was evacuated and left over night at room temperature. The substrates were sonicated in tetrahydrofurane (Burdick and Jackson, Honeywell) and 99.7 % ethanol (Solveco) to remove any unreacted DMOCS and then stored in ethanol. In case of small angle X-ray diffraction studies, freshly cleaved mica was used as a substrate because it is permeable for the X-rays.

Formation of lipid film by spin-coating
The lipid films were prepared in ambient air by adding 50 µl of lipid formulation square substrate (~1 cm^2) to cover almost the whole surface, followed by spin-coating in a LabSpin system (SUSS Micro Tec Lithography GmbH). All substrates were first rotated for 5 seconds at 1000 rpm followed by 5 s at 4000 rpm to spread the material uniformly over the whole surface. A rotation speed of 8000 rpm for different periods of time was then used to obtain lipid films of various thicknesses. An acceleration of 4000 rpm/s was used when spin velocity was altered. The films were immersed in excess (> 5 ml) of MilliQ water immediately after (within 3s) completing the spinning program. The film-covered substrate was released 1-2 cm above the water level in a perpendicular orientation relative to the water surface. This hydration method resulted lipid LC films that appeared visually smooth.

Spectroscopic Ellipsometry, SE
SE is an optical technique, in which changes in polarization of a light beam upon reflection to a film-covered substrate are used to extract information about the optical constants and thickness of the film. The electrical field of the incident light beam can be decomposed into a

parallel (*p*-) and a perpendicular (*s*-) component relative to the plane of incidence.
The complex Fresnel reflection coefficients, R_p and R_s, gives the amplitude of the reflected *p*- and *s*-polarized light, respectively, relative to corresponding amplitude of incident light.[1] The measured parameters in ellipsometry, Δ and Ψ, are defined by the ratio (*p*) of the reflection coefficients:

$$p = \frac{R_p}{R_s} \cdot \tan \Upsilon \cdot e^{i\Delta}$$

The values of Δ and Ψ are dependent on the optical properties (complex refractive index, $\tilde{N} = n+ik$)) and thickness of the film. The optical constants (*n* and *k*) and the thickness of the film can thus be extracted by model-based analysis of the acquired data using optical physics.
All spectroscopic ellipsometry measurements were accomplished in the UVISEL instrument from SA Jobin Yvon-Horiba using an angle of incidence (AOI) of 70° and modulator and analyser angle of 0 and 45°, respectively. The windows of the liquid cell (~10 ml) were adapted for measurements at AOI= 70° and transparent for light in the actual spectral range. All data was analysed in the DeltaPsi2 software. The silicon oxide layer and dimetyloctylchlorosilane layer of the clean substrates were characterized prior deposition of the lipid film. A silicon oxide layer was modelled on top of an infinitely thick silicon crystal (Figure SI 1A). Software default values of the optical constants were used for both materials (Figure SI 1B) and the thickness of the SiO_2 was fitted. Thicknesses (300 ± 20 Å) consistent with the thickness specified by the supplier were obtained. Due to the very thin thickness of the DMOCS layer and its optical similarities with silicon oxide, it was not modelled as an individual layer but instead accounted for as an additional thickness (~ +20 Å) of the SiO_2 layer. The fitted thickness of the SiO_2 layer (alone or including the DMOCS layer) was then kept fixed in the analysis of the liquid crystalline lipid film.

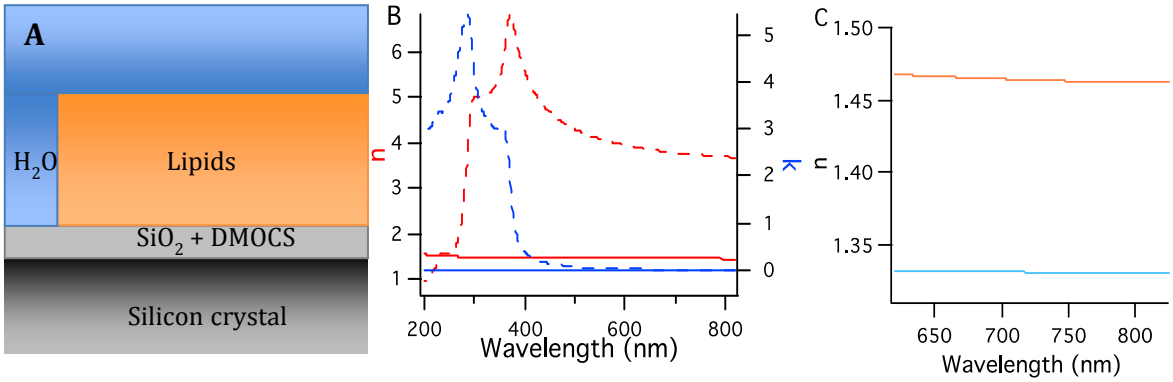

Figure SI 1 A) The layered optical model of the lipid LC film on a silica substrate. B) The n (red) and k (blue) used for silicon crystal (dashed lines) and SiO_2 and DMOCS layer (solid lines) to model the substrate properties. C) The refractive index, n, used to represent lipids (red) and H_2O (blue) in the optical model of the lipid LC film. All material constants are default in the DeltaPsi2 software.

The liquid crystalline lipid film was modelled as a single optical layer on top of the substrate (Figure SI 1A). The thicknesses of the lipid LC film and the volume fraction of water in the film were fitted using a Marquardt minimization algorithm. A layer with optical constants (described by a Lorentz oscillator dispersion formula) typical for lipids[2] was used to represent the lipid film. Software default optical constants based on a dispersion formula were use also for H_2O. Although dispersion formulas were used, *n* is almost constant in the spectral range recorded for the lipid film (620-825 nm) both for lipids and H_2O, see Figure SI 1C. No light adsorption by the lipids or H_2O was assumed (k=0) in the spectral range. The error bars of the

fitted thickness in Figure 1B includes both variations within a single film (typically < 0.1 μm) and separately prepared films.

Small Angle X-ray Diffraction (SAXD)

Synchrotron SAXD was performed at beamline I911-4 at MAX-lab, Sweden. Thin mica sheets with lipid LC films were mounted in a steel sample holder, filled with H_2O and sealed with kapton windows. The sample was mounted 1952 mm from the 1M PILATUS 2D detector. The x-ray wavelength was 0.91 Å and the size of the beam at the sample was approximately 0.25 x 0.25 mm. Diffractograms were recorded during 5 minutes at 25 °C. The intensities recorded by the 2D detector were integrated using Fit2D provided by A. Hammersley (http://www.esrf.eu/computing/scientific/FIT2D/). The peak positions were noted and indexed as a cubic phase of space group *Fd3m*. The lattice parameter was calculated according to:

$$a = \frac{2\rho}{q}(h^2 + k^2 + l^2)^{\frac{1}{2}}$$

The temperature response (at 25, 37 and 42 °C) for the bulk LC phase of 35/65 wt%/wt% SPC/GDO in excess of water was also studied. Kapton windows were used to enclose the sample in the steel sample holder. The I_2-phase present at 25 °C was preserved also at 37 and 42 °C. However, the I_2–reflections were superimposed on an, with temperature increasingly intense, broad shoulder (~1.2 nm^{-1}) arising from diffuse scattering of the disordered L_2-phase.

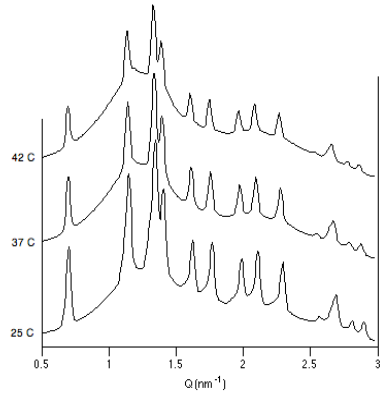

Figure SI 2. Small angle X-ray diffractograms of 35/65 wt%/wt% SPC/GDO bulk LC phase in excess of water at 25, 37 and 42 °C.

Non-contact mode AFM

The topography of the liquid crystalline lipid film in water was mapped by non-contact (tapping) mode AFM (A XE-100 Park system). To avoid structurally devastating tip-induced effects during imaging, soft Multi75AI probes (force constant: 3 N/m, resonance frequency in air: 75 kHz) with tip radius <10 nm were used. The substrate with the hydrated lipid film was mounted to the AFM liquid cell (Park system) and equilibrated for at least 15 minutes at the actual temperature, set by a high precision temperature controller (LDT-5948).

Figure SI 3 show the topography of the lipid LC film prepared at 25 °C, heated to 37 °C and re-cooled to 25 °C. The lipid film was allowed to equilibrate for 1 hour before imaging.

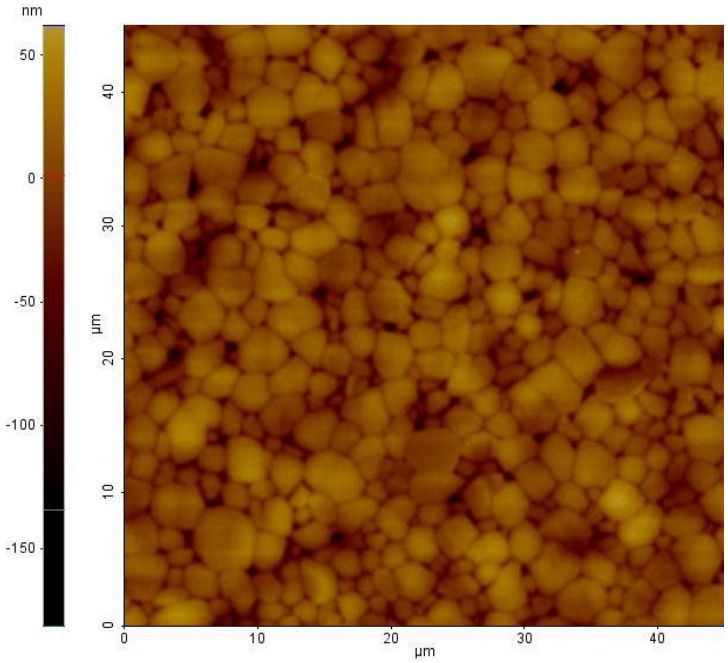

Figure SI 3. Non-contact mode AFM (45x45 µm) images of the lipid LC film in H2O on hydrophobic substrates after heating to 37 ℃ and re-cooling to 25 ℃.

References

1. Tompkins, H. G.; McGahan, W. A., 1999, Spectroscopic Ellipsometry and Refletctometry, John Wiley & Sons, Inc., New York.

2. O'Keefe, S. F.; Pike, O. A., Food Analysis, Food Science Texts Series, ed. Nielsen, S.S., Springer, USA, 2010, ch. 14 Fat characterization, 239-260