Nanoscale Patterning in Mixed Fluorocarbon-Hydrocarbon Phospholipid Bilayers

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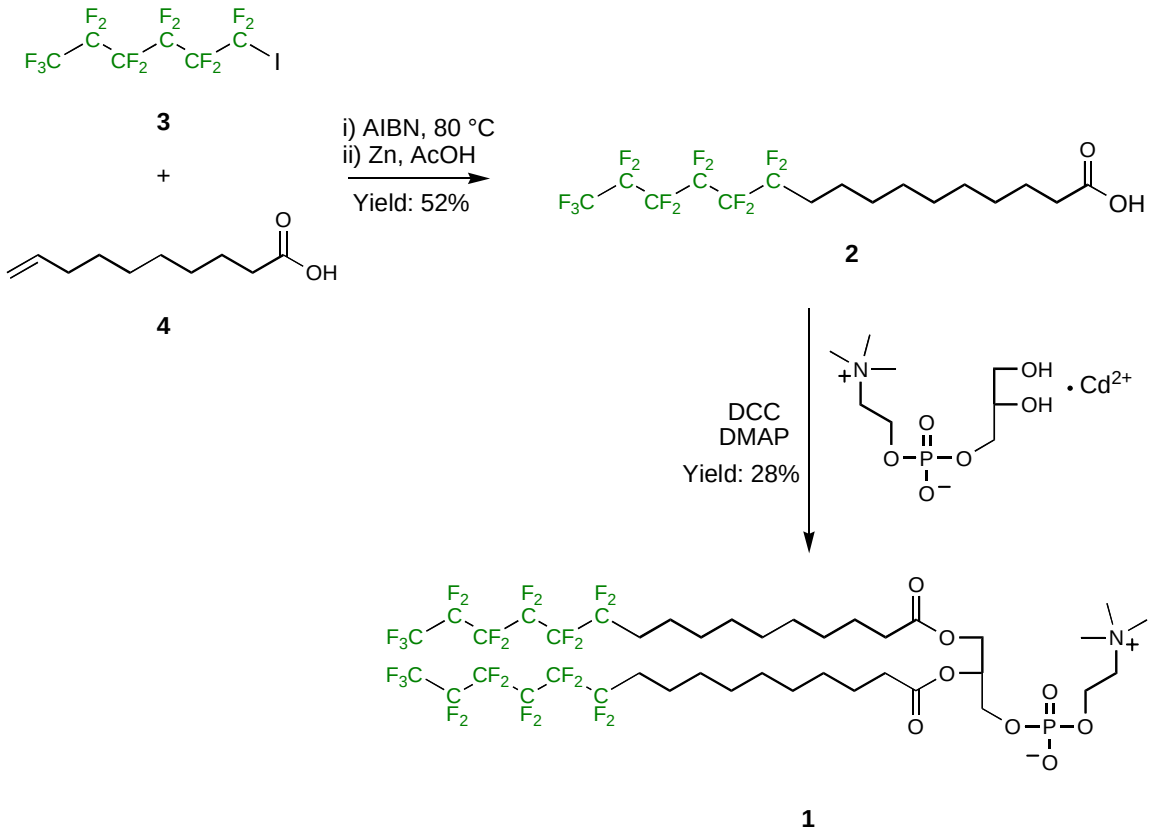
Supplementary Information

Experimental Methods

General Procedures. Flash column chromatography was performed on Kieselgel 60 silica gel (230-240 mesh, EM Science) using standard literature procedures.[1] Analytical thin layer chromatography was performed using E. Merck silica gel Kieselgel 60 F_{254} (0.25 mm) plates. Compounds were visualized by UV light, exposure to iodine vapor or by staining with a ninhydrin solution followed by heating. Reagents and solvents were of reagent grade or better and were obtained from Aldrich Chemical Co., Fluka Chemie AG, Fluorochem USA, Lancaster Synthesis or Novabiochem Corp. Deuterated solvents were obtained from Cambridge Isotope Laboratories.

Nuclear magnetic resonance spectra were recorded on a Bruker AM–300 or a Bruker DPX–300 instrument in standard deuterated solvents. ^{19}F NMR spectra were measured using $CFCl_3$ ($\delta = 0$) for organic solvents and CF_3CO_2H ($\delta = -76.50$)[2] for D_2O as the internal standards. Electrospray mass spectra (ESI-MS) were recorded using a ThermoQuest LCQ Deca.



Scheme S1. Synthesis of fluorinated phosphotidylcholine **1**.

Compound **1**:[3] L-α-glycerophosphocholine/$CdCl_2$ complex (50.0 mg, 0.11 mmol) was placed in a flame dried flask and dried overnight under high vacuum. Fluorinated fatty

acid **2** (222 mg, 0.45 mmol) and DMAP (24.2 mg, 0.22 mmol) in 2 mL of dry $CHCl_3$ were added to the flask. DCC (93.4 mg, 0.45 mmol) was added and the reaction mixture was sonicated for 6 h at room temperature and left stirring for 3 d at room temperature in the dark under N_2. The reaction was followed by TLC analysis; a single spot on the silica plate eluted with 65:25:4 $CHCl_3$/MeOH/H_2O (R_f 0.3) and stained orange with Dragendorff's reagent.[4] The reaction mixture was placed on a column and the product was eluted using $CHCl_3$ and MeOH mixture (80:20) to give fluorinated lipid **1**. Impurities were apparent in the aromatic region of the ^{1}H NMR spectrum. Therefore, the lipid was further purified by preparative TLC using a $CHCl_3$ and MeOH mixture (80:20) to give pure **1** in 28% yield. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.16 (m, 1H), 4.35 (dd, 1H, J = 12.0, 2.7 Hz), 4.28 (m, 2H), 4.08 (dd, 1H, J = 7.2, 12.0 Hz), 3.91 (m, 2H), 3.84 (m, 2H), 3.38 (s, 9H), 2.27-2.20 (m, 4H), 1.99 (tt, 4H, $^3J_{HH}$ = 9.6 Hz, $^3J_{HF}$ = 17.8 Hz), 1.53 (m, broad, 8H), 1.24 (s, broad, 20H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 173.50 (s, CO), 173.18 (s, CO), 70.57 (d, $^3J_{CP}$ = 7.6 Hz), 66.37 (d, $^2J_{CP}$ = 6.4 Hz), 63.54 (d, $^2J_{CP}$ = 4.7 Hz), 62.96, 59.45 (d, $^2J_{CP}$ = 4.7 Hz,), 54.45 (s, NCH_3), 34.32, 34.12, 30.85 (t, $^3J_{CF}$ = 22.3 Hz), 29.23, 29.12, 28.77, 24.96, 24.88, 20.11; ^{19}F NMR ($CDCl_3$, 282 MHz) δ –79.8 (m), –113.4 (m), –120.9 (m), –121.8 (m), –122.5 (m), –125.1 (m); ^{31}P{H1} NMR ($CDCl_3$, 121 MHz) δ –0. 84 (s); ESI-MS calcd for $[C_{40}H_{56}F_{26}NO_8P{\cdot}Na]^+$ 1224.8, found 1224.3.

Atomic Force Microscopy

For Figures **S1**–**S3**, supported lipid bilayers on mica were prepared as described in the main text, except for the changes in bilayer and buffer composition noted in the figure captions.

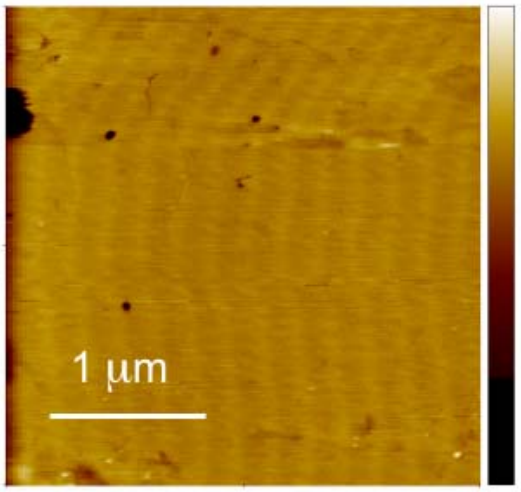


Figure S1. Tapping mode AFM (3 μm × 3 μm) AFM height image of a SLB composed of pure DPPC in standard buffer (10 mM Tris-HCl, pH 7.5, 100 mM NaCl). Height scale is 5 nm. The faint vertical lines are due to vibrational noise.

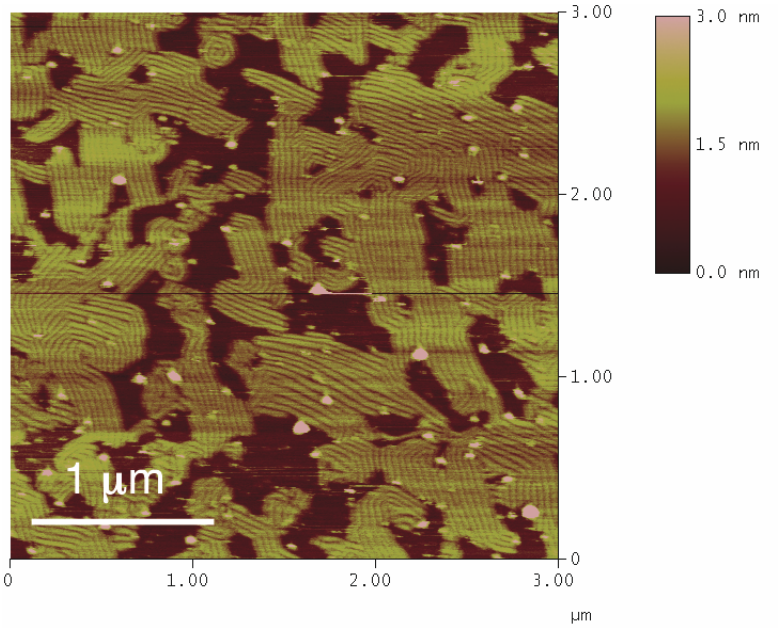


Figure S2. Tapping mode AFM (3 μm × 3 μm) height image of a SLB composed of 1:1 DPPC:**1**. Buffer is 10 mM Na-HEPES pH 7.5, 100 mM NaCl. Height scale is 3 nm, as indicated.

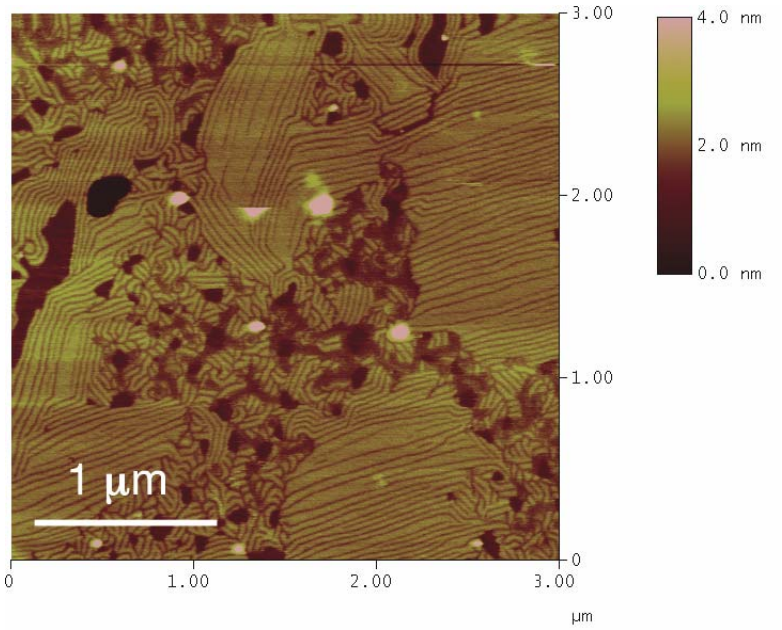


Figure S3. Tapping mode AFM (3 µm × 3 µm) height image of a SLB composed of 1:1 DPPC:**1**. SLB prepared in unbuffered water with 100 mM NaCl (pH ~6.3). Height scale is 4 nm, as indicated.

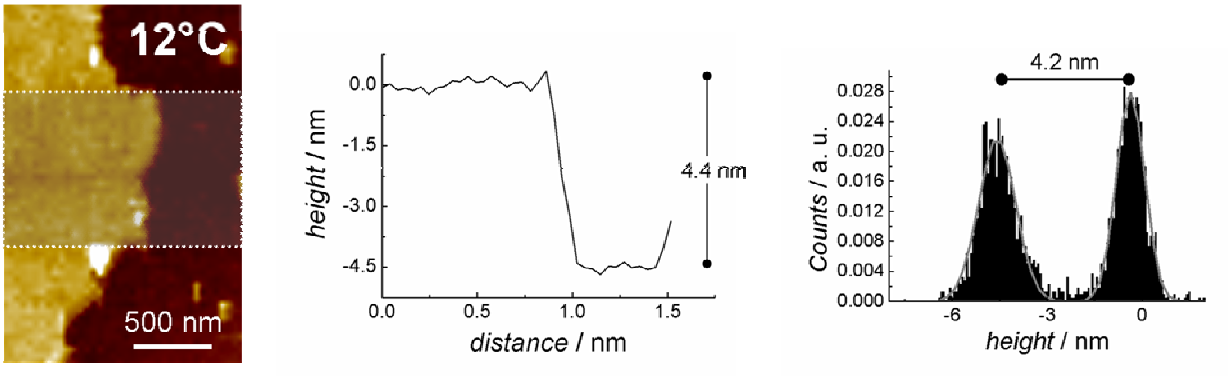


Figure S4. AFM image and height analysis of a SLB composed of pure **1** at 12 °C formed on glass via capillary micromolding.[4] The highlighted area in the center of the image (left) marks the area from with the averaged line scan was calculated (center). The histogram (right) was calculated from the entire image. A vesicular suspension of **1** was formed from a dried lipid film produced as described in the main text. The film was hydrated in standard buffer at 65 °C for 45 min, followed by vortexing for 20 seconds. This step was repeated once with a reduced hydrating time of 15 min, resulting in a homogeneous, turbid suspension. SUVs were prepared by ultrasonification for 20 min in a beaker resonator.

References

(1) Still, W. C.; Kahn, M.; Mitra, A. *J. Org. Chem.* **1978**, *43*, 2923.
(2) Prosser, R. S.; Luchette, P. A.; Westerman, P. W. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 9967-9971.
(3) Liu, X.; Stocker, B. L.; Seeberger, P. H. *J. Am. Chem. Soc*. **2006**, *128*, 3638-3648.
Menger, F. M.; Wood, M. G.; Richardson, S.; Zhou, Q.; Elrington, A. R.; Sherrod, M. J. *J. Am. Chem. Soc.* **1988**, *110*, 6797.
(4) Chakrabarti, P.; Khorana, H. G. *Biochemistry* **1975**, *14*, 5021.
(4) Schuy, S.; Janshoff, A. J. *J. Colloid Interface Sci.* **2006**, *295*, 93.