1 Supplementary Information

1.1 Synthesis

1.1.1 Synthesis of cholesteryl-succinic acid propargylamide

2.5 g (5.1 mmol, 1.1 eq) of cholesteryl-hemisuccinate (Aldrich), 1.81 g (5.6 mmol, 1.3 eq) of *p*-toluenesulfonic anhydride (Aldrich) and 1.26 g (10 mmol, 2.2 eq) of DMAP are dissolved in 25 ml of chloroform and after 15 min 0.26 g (4.6 mmol, 1 eq) propargylamine (Aldrich) are added. After 1.5 h the mixture is quenched with 3 ml of a saturated solution of sodium bicarbonate ($NaHCO_3$). The solution is extracted with ethyl acetate and the combined organic layers are washed two times with 50 ml of sat. $NaHCO_3$ and two times with 50 ml of brine. The organic layer is dried over anhydrous Na_2SO_4. The crude product is purified by recrystallisation in ethyl acetate and freeze dried from cyclohexane.

- **Yield:** 1.25 g (55 %).
- ^{1}**H-NMR** (300MHz, $CDCl_3$): 6.2 ppm (1H, -NHCO-); 5.35 ppm (1H, H-6 Cholesterol); 4.6 ppm (1H, H-3 Cholesterol); 4.0 ppm (2H, -CH2 propargyl); 2.2 ppm (1H, H-alkyne); 0.65 ppm (9H, -CH3 cholesterol).

1.1.2 Synthesis of the SAPs

Prior to the experiment, solutions of $CuSO_4$ (c = 0.13 mol/l) and a THPTA (c = 63 mmol/l) are prepared with millipore water. The polyrotaxane (3 μmol) and the cholesteryl succinic acid propargylamide (6 μmol, 2 eq (per azide)) are dissolved in a mixture of 1.5 ml tBuOH/millipore water 8:2, sonicated for 5 min and heated for several minutes to ensure complete solubilisation of the compounds. Then the ligand solution (1 μmol, 0.3 eq) and the $CuSO_4$ solution (0.2 μmol, 0.06 eq) are added to the mixture to give a transparent solution. Sodium ascorbate (2.5 μmol, 0.8 eq) is added and the solution is left under stirring over night at room temperature. The transparent solution is diluted with 5 ml of Millipore water and dialysed (cut-off 2 kg/mol) twice with 2 l of millipore water for 24 h and freeze dried. The crude product is taken up in 5 ml of ether and centrifuged 3 times to eliminate the residual cholesteryl alkyne. The residue is dissolved in 10 ml of tBuOH/H2O 8:2 and freeze dried.

- **Yield:** 50-95 % (depending on MW of polyrotaxane and used stopper).
- ^{1}**H-NMR** (300MHz, $CDCl_3$) DMPE stopper: 8.3 ppm (s, 1H, -NHCO-); 7.8 ppm (s, 1H, H-triazol); 6.96 - 6.74 ppm (6H, aromatic HÕs DMPE); 5.86 ppm (4H, H-urea); 5.6 ppm - 5.4 ppm (12H, OH-2 and OH-3 CD); 5.3 ppm (1H, CH sp2 cholesterol); 5.0 ppm (1H, H-1 modified glucose unit CD); 4.8 ppm (5H, H-1 CD0); 4.5 ppm (6H, OH-6 CD); 3.1-3.9 (nH, -OCH2CH2- PEG and H-2, H-5, CH2-6 CD); 2 - 0.8 ppm (H cholesteryl moiety); 0.65 ppm (9H, -CH_3 cholesterol).

1.1.3 Synthesis of Cholesteryl α-CD

100 mg (50 μmol, 1 eq) ACDN3 and 29 mg (60 μmol, 1.2eq) cholesteryl succinic acid propargylamide are dissolved in 1.5 ml of ultrapure DMF and 160 μl of a CuI/PMDETA 1:1 solution (c = 57 mmol/l in DMF) are added under argon atmosphere. The solution is stirred for 24h. The solvent is evaporated and the compound is suspended in 4 ml of phosphate buffer solution (20 mM, $pH = 6.5$). Then the compound is centrifuged in 3 ml of millipore water, as well as 3 times in 2 ml of acetone. Finally the compound is taken up in 10 ml of millipore water and freeze dried.

- **Yield:** 35 mg (35 %).
- ^{1}**H-NMR** (300MHz, DMSO-D6): 8.30 (1H, -NHCO-); 7.8 ppm (1H, H-triazol); 5.6 ppm - 5.4 ppm (12H, OH-2 and OH-3 CD); 5.0 ppm (1H, H-1 modified glucose unit CD); 4.8 ppm (5H, H-1 CD); 4.4 ppm (6H, OH-6 CD); 3.2-3.7 (H-2 and H-5, CH2-6 CD); 2.5 ppm (residual H2O); 2 - 0.8 ppm (H cholesteryl moiety); 0.65 ppm (9H, -CH3 cholesterol).

1.2 Monolayer in-plane morphology

1.2.1 Brewster Angle Microscopy

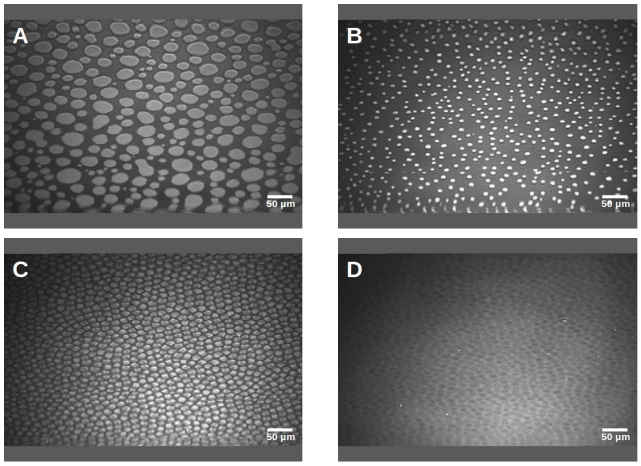

Figure 1: BAM images for the mixture 3 mol% SAP-6k/DPPC at: A) 5 mN/m; B) 9 mN/m; C) 13 mN/m and D) 30 mN/m.

The Brewster Angle Microscope (BAM), type PI, C-138K003, Optrel GBR, Berlin, coaligned with the Langmuir trough is based on the Hoenig and Moebius setup [1]. A green laser (LasNova series 50) with a wavelength of 532 nm is directed onto the water surface at the Brewster Angle (53.1°). The reflected light from the surface is imaged by means of a CCD camera (EHD®kamPro02) to give images of the monolayer morphology with a size of 480 µm× 599 µm and a resolution of 480 × 640 pixel.

1.2.2 Atomic force microscopy

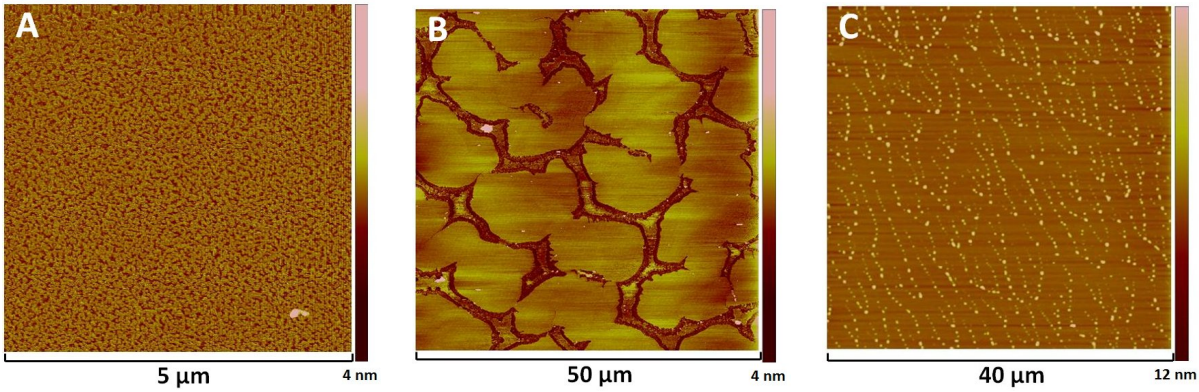

Figure 2: AFM images for monolayers of the mixture 3 mol% SAP-6k/DPPC deposited on mica at: A) 5 mN/m; B) 13 mN/m and C) 35 mN/m.

Mixed SAP/DPPC monolayers at several surface pressures were transfered from the air-water interface onto freshly cleaved, hydrophilic mica wafers (11x11x0.15mm, purchased from Agar Scientific), using the Langmuir-Blodgett (LB) technique (dipper speed: 1 mm/min). Transfer ratios close to 1 were obtained for good monolayer depositions.

The films are then examined in tapping mode with a Nanoscope V (Veeco) AFM. Standard cantilevers with a conical silicon etched probe tip (NSC15, μmasch) with typical spring constants in the order of 40 N/m, as determined by the thermal resonance method [2], and typical resonance frequencies in the order of 350 kHz are used. Images with scan sizes of 1μm×1μm and 10μm×10μm have been recorded with scan rates of 1 Hz and 0.5 Hz, respectively.

1.2.3 Discussion

Using BAM and AFM the in-plane film morphology can be investigated from µm to nm scale. Mixtures with SAPs of different MW give comparable results for similar surface densities. At

low surface pressures large bright domains of $\sim$ 50 µm in diameter are visible (Figure 1 A). Complementary AFM images show that there is also a heterogeneity at much smaller scale in the order of tens of nanometers (Figure 2 A). With further compression those domains vanish and starting at $\Pi > 8$ mN/m small (10 µm) brighter domains appear, which grow in number (Figure 1 B). These brighter domains, visible for all molar ratios and regardless the MW of the SAPs, correspond to the LE-LC phase transition, typical for DPPC [3]. These domains are also displayed in corresponding AFM images (Figure 2 B).

The domains become denser with further rise of Π (Figure 1 C and Figure 2 C), and an inversion of contrast occurs for $\Pi \sim 20$ mN/m, resulting in a honeycomb-like pattern, which prevails up to very high surface pressures (Figure 1 D). This is in contrast to pure DPPC, where a uniform surface is obtained. For higher SAP molar ratios the bright domains visible at 13 mN/m are less dense and the inversion of contrast occurs at lower surface pressure to give the same honeycomb-like pattern only with higher contrast. Interestingly for molar ratios $>$ 30 mol% BAM images do not display a contrast for high surface pressures. The AFM images for high surface pressures do not display the patterns observed in BAM, but many aggregates are visible (Figure 2 D). The aggregates (height 5 - 10 nm) increase in number with SAP molar ratios and they are oriented in the direction of deposition.

1.3 Neutron reflectivity

Table 1: Selected SLDs, taken from references [4–7], for the different components. The SLD for the α-CD part of the SAP is calculated, using the molecular volume $V_m = 1000$ Å^3 and the scattering length $b = 189$ fm. To account for the complexation of α-CD and PEG in the SAP the SLD of the inserted PEG is added to the α-CD.

material	$SLD[10^{-6}\text{Å}^{-2}]$
Si	2.07
SiO_2	3.47
D_2O	6.34
4MW	4
ZMW	0
H_2O	-0.56
DPPC-alkyl tail	-0.4
DPPC-d_{62} alkyl tail	6.82
DPPC-PC head	1.74
DSPE-alkyl tail	-0.4
DSPE-PE head	2.66
PEG	0.6
α-CD head + inserted PEG	2.4
β-CD head	1.9
cholesteryl anchor	0.5

		bilayer 10 mol% SAP-6k		bilayer 20 mol% SAP-6k	
layer		25°C	50°C	25°C	50°C
water	thickness [Å]	4.6 ± 1	5.3 ± 1	4.0 ± 1	6.3 ± 1
	SLD [Å^{-2}]	-	-	-	-
	water [v/v%]	100	100	100	100
	roughness [Å]	7.1 ± 2	7.1 ± 2	6.3 ± 2	6.5 ± 2
heads DPPC	thickness [Å]	9.1 ± 1	9.12 ± 1	9.0 ± 1	9.2 ± 1
	SLD [Å^{-2}]	1.7	1.7	1.7	1.7
	water [v/v%]	30 ± 5	35 ± 5	28 ± 5	28 ± 5
	roughness [Å]	7.8 ± 2	6.6 ± 2	7.0 ± 2	7.5 ± 2
tails	thickness [Å]	30 ± 1	25 ± 1	29.7 ± 1	25.5 ± 1
	SLD [Å^{-2}]	6.81 ± 0.2	6.6 ± 0.2	6.55 ± 0.2	6.37 ± 0.2
	water [v/v%]	10 ± 5	10 ± 5	10 ± 5	10 ± 5
	roughness [Å]	8.5 ± 2	8.3 ± 2	9.0 ± 2	8.0 ± 2
heads mixed	thickness [Å]	10.2 ± 1	10.0 ± 1	12.2 ± 1	12.0 ± 1
	SLD [Å^{-2}]	1.8	1.8	1.8	1.8
	water [v/v%]	25 ± 5	25 ± 5	22 ± 5	21 ± 5
	roughness [Å]	8.5 ± 2	8.5 ± 2	8.0 ± 2	8.5 ± 2
polymer	H [Å]	84 ± 10	75 ± 10	110 ± 10	95 ± 10
	SLD [Å^{-2}]	0.6	0.6	0.6	0.6
	Φ_0 [v/v]	0.05 ± 0.02	0.041 ± 0.02	0.11 ± 0.02	0.11 ± 0.02
	roughness [Å]	9 ± 2	9.2 ± 2	9.5 ± 2	10 ± 2

Table 2: Neutron reflectivity results for supported bilayers with a first monolayer DSPE as well as a second mixed monolayer DPPC/cholesteryl β-CD and DPPC/SAP-10k, respectively.

References

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