ELECTRONIC SUPPORTING INFORMATION

Polymer-capped magnetite nanoparticles change the 2D structure of DPPC model membranes

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EXPERIMENTAL PART

Materials

10 The $Fe_3O_4@MEO_2MA_{90}$-co-$OEGMA_{10}$ NPs used in this study are Fe_3O_4 NPs (d = 6.4 nm) grafted with random copolymers of 2-(2-methoxyethoxy) ethyl methacrylate (MEO_2MA) and oligo(ethylene glycol) methacrylate (OEGMA), with molar fractions of 90% and 10%, respectively. The polymer (Mn = 15 39000 $g \cdot mol^{-1}$) shell thickness is 4.8 nm. These NPs were synthesized and characterized as previously reported.[1] The NPs are not charged, and they have a weight fraction of 29.5% of Fe_3O_4 (thermogravimetric analysis, TGA). The NP system does not contain free polymer.[1-2]

20 For the experiments, the NPs have been dispersed at different concentrations, either in Milli-Q Millipore water (specific resistance of 18.2 MΩcm) or in chloroform (HPLC grade).

Surface Pressure – Area isotherms

25 The pressure-area isotherms have been measured with a Langmuir trough system equipped with one (or two) moving barriers. The setup included a surface pressure microbalance with filter paper Wilhelmy plate. The results were plotted as surface pressure (π) versus the area per molecule (in respect to the DPPC 30 molecules). The bare water surface was proved to be clean by compression before each measurement. The temperature of the Milli-Q Millipore water subphase was maintained at 20 °C by using an external thermostat.

Pressure/area (π/A) isotherms were recorded during monolayer 35 compression on a computer-interfaced Langmuir trough (R&K, Potsdam, Germany). Each measurement was repeated at least 2 times to prove the reproducibility of the results. In order to avoid dust contamination of the interface and to ensure a constant humidity, the Langmuir trough was placed in a sealed box.

40 The mixed DPPC-NPs Langmuir layers have been prepared by co-spreading at the air/water interface chloroform solutions of DPPC (0.8 mM) and NPs (0.73 mg/ml) in a volume ratio of 2:1, respectively. Thus, the ratio of the two components is: 3.33×10^3 DPPC molecules per NP.

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Total reflection X-ray fluorescence (TRXF)

In situ total reflection X-ray fluorescence measurements at the air/water interface were performed at the beamline BW1, HASYLAB, DESY, Hamburg, Germany. The synchrotron X-ray 50 beam was monochromized at a photon energy of 14.2 keV by a Si (111) double monochromator. The beam was deflected from the horizontal plane by a gold-coated mirror. This beam touches the liquid surface at an incidence angle of 0.075° that is 80% of the critical angle of total reflection for the water surface. A 55 scintillation (NaI) detector for the reflected X-ray beam was used for the height adjustment of the liquid surface. The fluorescence

signal was measured by a Peltier-cooled VORTEX silicon drift detector (SDD) with an entrance window placed parallel to the liquid surface. The axis of the incident beam and the view 60 directions of the NaI and the SDD were lined up so that they crossed at the same point. The surface of the liquid was moved to this point before each measurement of the fluorescence signal.

Part of TRXF measurements was performed at the beamline ID10B at ESRF, Grenoble, France. The synchrotron X-ray beam 65 coming from the undulator was monochromized at a photon energy of 22.5 keV by a double crystal monochromator using symmetric Bragg reflection (220) from two diamond crystals. Higher harmonics were rejected by a platinum-coated double mirror system. Both mirrors reflect in the vertical plane and keep 70 the X-ray beam in the horizontal plane. The down stream mirror was bent to focus the X-ray beam at the sample (6 m from the mirror). The beam was deflected from the horizontal plane by rotation of the deflecting Ge crystal around the incident beam with keeping the Bragg reflection condition for Ge (111) 75 reflection. The deflected beam (0.017 mm height, 1 mm width) touches the liquid surface at a grazing angle of 0.022° that is 40% of the critical angle of total reflection for the water surface.

A scintillation (NaI) detector for the reflected X-ray beam was used for the height adjustment of the liquid surface. The 80 fluorescence signal was measured by the Peltier cooled ROENTEC silicon drift detector (SDD) with an entrance window placed parallel to the liquid surface at a distance of 22 mm.

The axis of the incident beam and the view directions of the NaI and ROENTEC detectors were lined up so that they crossed at the 85 same point. The surface of the liquid was moved to this point before each measurement of the fluorescence signal.[3]

X-ray fluorescence is an element-specific technique which permits the identification of elements due to their characteristic fluorescence spectra.[3-4]

90 The grazing incidence angle of the X-ray limits the penetration depth to approximately 8 nm. The fluorescence signal arises therefore from elements enriched at the surface and is much less sensitive to contributions from the diluted bulk solution.

95 Grazing incidence X-ray diffraction (GIXD)

The grazing incidence X-ray diffraction measurements were carried out at the undulator beamline BW1 using the liquid surface diffractometer at HASYLAB, DESY (Hamburg, Germany). The experimental setup and evaluation procedures are 100 described in detail elsewhere.[5-8]

The setup is equipped with a temperature controlled Langmuir trough (R&K, Potsdam, Germany), which is enclosed in a sealed, helium-filled container. The synchrotron X-ray beam is monochromized to a wavelength of 1.304 Å and is adjusted to 105 strike the helium/water interface at a grazing incidence angle α_i = 0.85α_c (α_c = 0.13° is the critical angle for total reflection) illuminating approximately 2×50 mm² of the monolayer surface.

A MYTHEN detector system (PSI, Villigen, Switzerland) measures the diffracted signal and is rotated to scan the in-plane Q_{xy} component values of the scattering vector. A Soller collimator in front of the MYTHEN restricted the in-plane divergence of the diffracted beam to 0.09°. The vertical strips of the MYTHEN measure the out-of-plane Q_z component of the scattering vector between 0.0 and 0.75 Å^{-1}. The diffraction data consist of Bragg peaks at diagnostic Q_{xy} values. These peaks are calculated by summing the diffracted intensity at each in-plane Q_{xy} value over a defined vertical angle or Q_z-window. The in-plane lattice repeat distances d of the ordered structures in the monolayer are calculated from the Bragg peak positions: $d = 2\pi/Q_{xy}$. The diffracted intensity normal to the interface is integrated over the Q_{xy} window containing the diffraction peak to calculate the corresponding Bragg rod. The thickness of the monolayer is estimated from the FWHM of the Bragg rod using $0.9(2\pi)/\text{fwhm}(Q_z)$.

AFM measurements

Atomic force microscopy (AFM) was used for the characterization of the transferred Langmuir layers of mixed DPPC/NPs. The samples were prepared by Langmuir-Blodgett transfer of the mixed interfacial layer (at different surface pressures) on freshly piranha cleaned silica plates and dried under a flux of nitrogen. The measurements have been conducted in tapping mode using a Veeco (MultiMode) AFM device. The silicon AFM probes are from NanoWorld (Germany) and have the following characteristics: Length - 160 μm, width - 45 μm, thickness - 4.6 μm, reflex Al coating on the backside, resonance frequency - 285 kHz, force constant - 42 N/m, tip radius - 10 nm.

Brewster angle microscopy (BAM)

The morphology of the monolayer was imaged with a Brewster angle microscope, model BAM2plus from NanoFilm Technologie (Göttingen, Germany), equipped with a miniature film balance from NIMA Technology (Coventry, UK), both mounted on an antivibration table. The microscope was equipped with a frequency-doubled Nd:YAG laser (532 nm, ~50 mW), a polarizer, an analyzer, and a CCD camera. When p-polarized light is directed onto the pure air/water interface at the Brewster angle (~53.1°), zero reflectivity is observed. The presence of a monolayer causes light to be reflected because of the changed refractive index of the surface layer, which is then registered by the CCD camera after passing the analyzer. BAM images of 355 × 470 μm^2 were digitally recorded during compression of the monolayer. The lateral resolution was ~2 μm.

Infrared Reflection Absorption Spectroscopy (IRRAS)

Infrared reflection absorption spectra[9-12] have been recorded using the IFS 66 FT-IR spectrometer (Bruker, Germany), equipped with a liquid-nitrogen cooled MCT detector and coupled to a Langmuir film balance, which was placed in a sealed container to guarantee a constant vapor atmosphere. Using a KRS-5 (thallium bromide and iodide mixed crystal) wire grid polarizer, the IR-beam was polarized perpendicularly (p) or vertically (s) and focused on the fluid subphase at an angle of incidence of 40°.

A computer controlled "trough shuttle system"[13] enables us to choose between the sample (subphase with spread layer) and a reference (pure subphase). The single-beam reflectance spectrum from the reference trough was taken as background for the single-beam reflectance spectrum of the monolayer in the sample trough to calculate the reflection absorption spectrum as $-\lg(R/R_0)$ in order to eliminate the water vapor signal. FTIR spectra were collected at a resolution of 8 cm^{-1} using 200 scans for s-polarized light and 400 scans for p-polarized light.

All the experiments were performed at a temperature of the subphase of 20 °C.

GIXD tables

Table 1. GIXD data of mixed DPPC/ Fe_3O_4@MEO_2MA_{90}-co-$OEGMA_{10}$ NP layers measured on water at 20°C
Peak positions and full-widths at half-maximum

π (mN/m)	Q_{xy_1} (Å^{-1})	Q_{z_1} (Å^{-1})	Q_{xy_2} (Å^{-1})	Q_{z_2} (Å^{-1})	Q_{xy_3} (Å^{-1})	Q_{z_3} (Å^{-1})
10	1.458 0.013	0.093 0.29	1.322 0.069	0.671 0.29	1.297 0.045	0.764 0.29
15	1.460 0.012	0.078 0.29	1.368 0.032	0.648 0.29	1.342 0.037	0.726 0.29
20	1.465 0.012	0.082 0.29	1.355 0.037	0.641 0.29	1.333 0.06	0.723 0.29
25	1.468 0.011	0.084 0.29	1.382 0.047	0.635 0.29	1.352 0.054	0.719 0.29
30	1.463 0.030	0.152 0.29	1.385 0.106	0.562 0.29	1.350 0.057	0.714 0.29
40	1.467 0.033	0.130 0.29	1.432 0.097	0.546 0.29	1.376 0.059	0.676 0.29
45	1.473 0.022	0.02 0.27	1.401 0.071	0.57 0.27	1.371 0.046	0.59 0.27

Table 2. Lattice parameters a, b, c and α, β, γ of the unit cell, lattice distortion, chain tilt t in respect to the normal to the interface, in-plane area A_{xy} per chain, cross sectional area A_0.

π (mN/m)	a/b/c (Å)	α/β/γ (°)	distortion	tilt (°)	A_{xy} (Å^2)	A_0 (Å^2)
10	5.138 5.237 5.776	124.6 123.0 112.3	0.1492936	33.6	24.9	20.7
15	5.060 5.158 5.505	123.4 121.7 114.8	0.1039432	31.2	23.7	20.3
20	5.074 5.157 5.576	123.7 122.3 113.9	0.1193255	31.3	23.9	20.4
25	5.018 5.130 5.449	123.4 121.5 115.0	0.1000520	30.6	23.3	20.1
30	5.019 5.149 5.439	123.5 121.2 115.3	0.09605236	29.5	23.36	20.3
40	4.926 5.126 5.252	123.3 119.6 117.0	0.07407418	27.6	22.5	19.9
45	4.977 5.086 5.336	123.0 121.0 115.9	0.08319804	26.4	22.7	20.3

Table 3. GIXD data of mixed DPPC/ MEO_2MA_{90}-co-$OEGMA_{10}$ layers measured on water at 20°C
Peak positions and full-widths at half-maximum

π (mN/m)	Qxy_1 (Å^{-1})	Qz_1 (Å^{-1})	Qxy_2 (Å^{-1})	Qz_2 (Å^{-1})	Qxy_3 (Å^{-1})	Qz_3 (Å^{-1})
10	1.464 0.012	0.089 0.29	1.372 0.05	0.618 0.29	1.348 0.024	0.707 0.29
15	1.467 0.012	0.085 0.29	1.369 0.058	0.637 0.29	1.344 0.036	0.722 0.29
20	1.463 0.012	0.087 0.29	1.350 0.049	0.668 0.29	1.328 0.049	0.755 0.29
25	1.471 0.013	0 0.27	1.379 0.06	0.707 0.27		
30	1.465 0.018	0.111 0.29	1.369 0.08	0.582 0.29	1.346 0.048	0.693 0.29
40	1.472 0.025	0.126 0.29	1.424 0.061	0.439 0.29	1.393 0.051	0.565 0.29

Table 4. Lattice parameters a, b, c and α, β, γ of the unit cell, lattice distortion, chain tilt t in respect to the normal to the interface, in-plane area A_{xy} per chain, cross sectional area A_0.

π (mN/m)	a/b/c (Å)	$\alpha/\beta/\gamma$ (°)	distortion	tilt (°)	A_{xy} (Å^2)	A_0 (Å^2)
10	5.048 5.137 5.482	123.3 121.7 114.8	0.1023300	30.2	23.5	20.3
15	5.045 5.139 5.507	123.5 121.9 114.5	0.1088108	30.9	23.5	20.2
20	5.085 5.170 5.602	123.8 122.4 113.7	0.1226081	32.5	24.0	20.2
25	5.386 5.049	115.5 122.2	0.08788156	31.2 (NN)	23.0	19.8
30	5.051 5.137 5.498	123.4 121.9 114.7	0.1056464	29.4	23.6	20.5
40	4.952 5.062 5.233	122.5 120.5 117.0	0.06445230	23.2	22.3	20.5

REFERENCES

1. M. Chanana, S. Jahn, R. Georgieva, J.-F. Lutz, H. Baumler and D. Wang, *Chem. Mater.*, 2009, **21**, 1906-1914.
2. C. Stefaniu, M. Chanana, D. Wang, D. V. Novikov, G. Brezesinski and H. Möhwald, *Langmuir*, 2011, **27**, 1192-1199.
3. V. L. Shapovalov, M. E. Ryskin, O. V. Konovalov, A. Hermelink and G.Brezesinski, *J. Phys. Chem. B*, 2007, **111**, 3927.
4. N. N. Novikova, S. I. Zheludeva, O. V. Konovalov, M. V. Kovalchuk, N. D. Stepina, I. V. Myagkov, Y. K. Godovsky, N. N. Makarova, E. Y.Tereschenko and L. G. Yanusova, *J. Appl. Crystallogr.*, 2003, **36**, 727.
5. J. Als-Nielsen, D. Jacquemain, K. Kjaer, F. Leveiller, M. Lahav and L. Leiserowitz, *Phys. Rep.*, 1994, **246** 251.
6. K. Kjaer, *Physica B* 1994, **198**, 100.
7. V. M. Kaganer, H. Möhwald and P. Dutta, *Rev. Mod. Phys.*, 1999, **71**, 779.
8. M. Lepere, C. Chevallard, G. Brezesinski, M. Goldmann and P. Guenoun, *Angew. Chem., Int. Ed.*, 2009, **48**, 5005.
9. R. Mendelsohn, J. W. Brauner and A. Gericke, *Annu. Rev. Phys.Chem.*, 1995, **46**, 305.
10. C. R. Flach, A. Gericke and R. Mendelsohn, *J. Phys. Chem. B* 1997, **101**, 58.
11. T. D. Andreeva, J. G. Petrov, G. Brezesinski and H. Möhwald, *Langmuir* 2008, **24**, 8001–8007.
12. M. Lepere, A. H. Muenter, C. Chevallard, P. Guenoun and G. Brezesinski, *Colloids Surf., A* 2007, **303**, 73.
13. C. R. Flach, J. W. Brauner and R. Mendelsohn, *Biophys. J.*, 1994, **66**, A373.