Electronic Supporting Information (ESI):

Structures of malonic acid diamide/phospholipid composites and their lipoplexes

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This article is dedicated to Professor Andreas Langner on occasion of his 60th birthday. We are very grateful for the allocation of the innovative research topic.

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Detailed Information about the GAP fit.

The GAP fit was performed with the program GAP 1.3, written and provided by Georg Pabst. For more details we refer to the describing literature published by Georg Pabst:

1. Pabst, G., M. Rappolt, H. Amenitsch, and P. Laggner. 2000. Structural information from multilamellar liposomes at full hydration: full q-range fitting with high quality xray data. Phys. Rev. E 62:4000-4009.
2. Pabst, G., R. Koschuch, B. Pozo-Navas, M. Rappolt, K. Lohner, and P. Laggner. 2003. Structural analysis of weakly ordered membrane stacks. J. Appl. Crystallogr. 63:1378-1388.
3. Pabst, G. 2006. Global properties of biomimetic membranes: perspectives on molecular features. Biophys. Rev. Lett. 1:57-84.
4. Pabst, G., J. Katsaras, V. A. Raghunathan, and M. Rappolt. 2003. Structure and interactions in the anomalous swelling regime of phospholipid bilayers. Langmuir 19:1716-1722.

According to the manual, GAP fits the full q-range of SAXD patterns of lamellar phases, i.e., in particular for lipid/water systems the phases L_α, L_β, $L_{\beta'}$, and L_c using the equation:

$$I(q) = (1 - N_{diff})\frac{S(q)|F(q)|^2}{q^2} + N_{diff}\frac{|F(q)|^2}{q^2}$$

where $S(q)$ is the structure factor relating to the 1D lattice, $F(q)$ the form factor given by the Fourier transform of the electron density profile and N_{diff} a scaling constant that reports on the fraction of positionally non-correlated bilayers (e.g. unilamellar vesicles). $1/q^2$ is the Lorentz correction, where $q = 4\pi \sin(\theta)/\lambda$ is the wave vector as usual. For fitting the scattering intensity after background correction was used. We chose the fit settings with no structure factor used for systems that do not exhibit Bragg peaks but only diffuse scattering from positionally uncorrelated bilayers (e.g. ULVs). This set up is based on the experimentally observed scattering curves as well as on the structures found in cryo TEM (no multilamellar structures were observed).

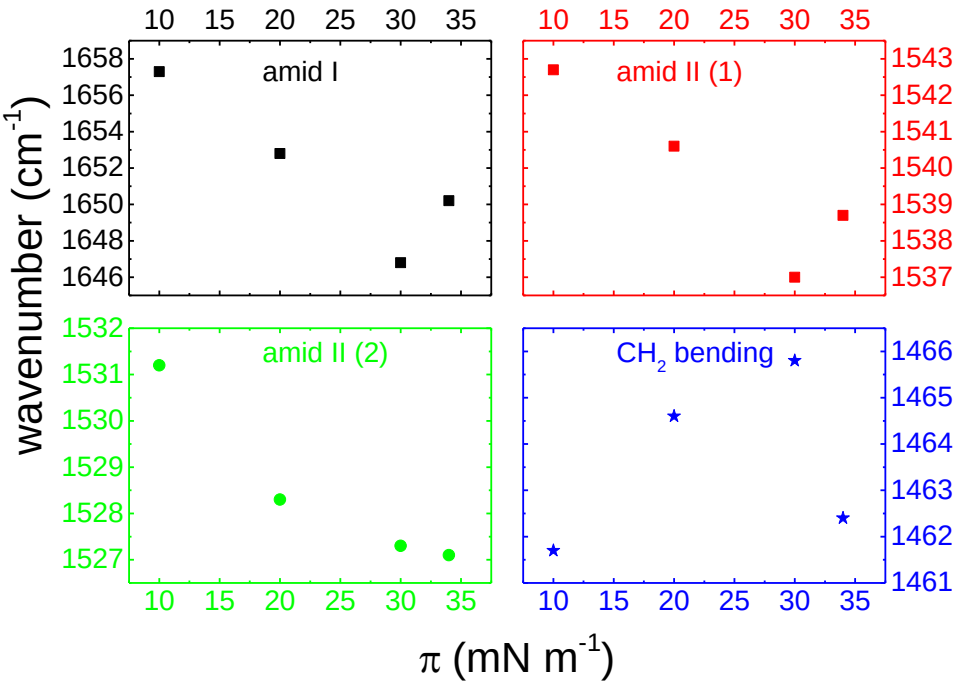

Fig. S1. Positions of selected peak maxima of IRRA spectra of **OH4** monolayers on MES buffer pH 6.5 as a function of the surface pressure.

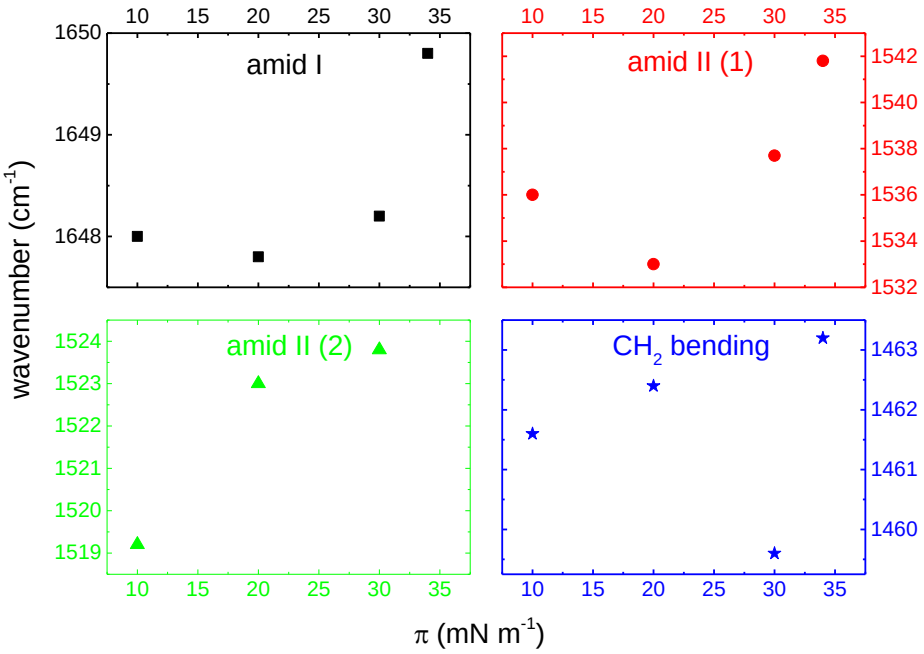

Fig. S2. Positions of selected peak maxima of IRRA spectra of **TH4** monolayers on MES buffer pH 6.5 as a function of the surface pressure.

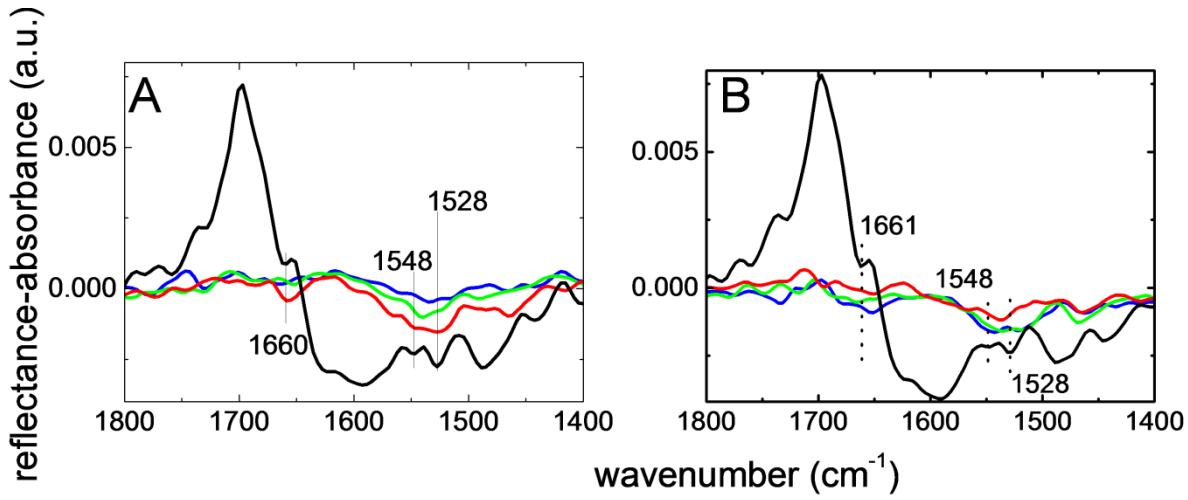

Fig S3. Sections of IRRA spectra of **OH4** (A) and **TH4** (B) at 25 °C (p-polarized light, incidence angle 40 °) at different surface pressures (10 mN m^{-1}, blue; 20 mN m^{-1}, green; 30 mN m^{-1}, red; 34 mN m^{-1}, black) of monolayers prepared on MES buffer pH 6.5. Sections of the amide I and amide II region including the CH_2 deformation band are presented.

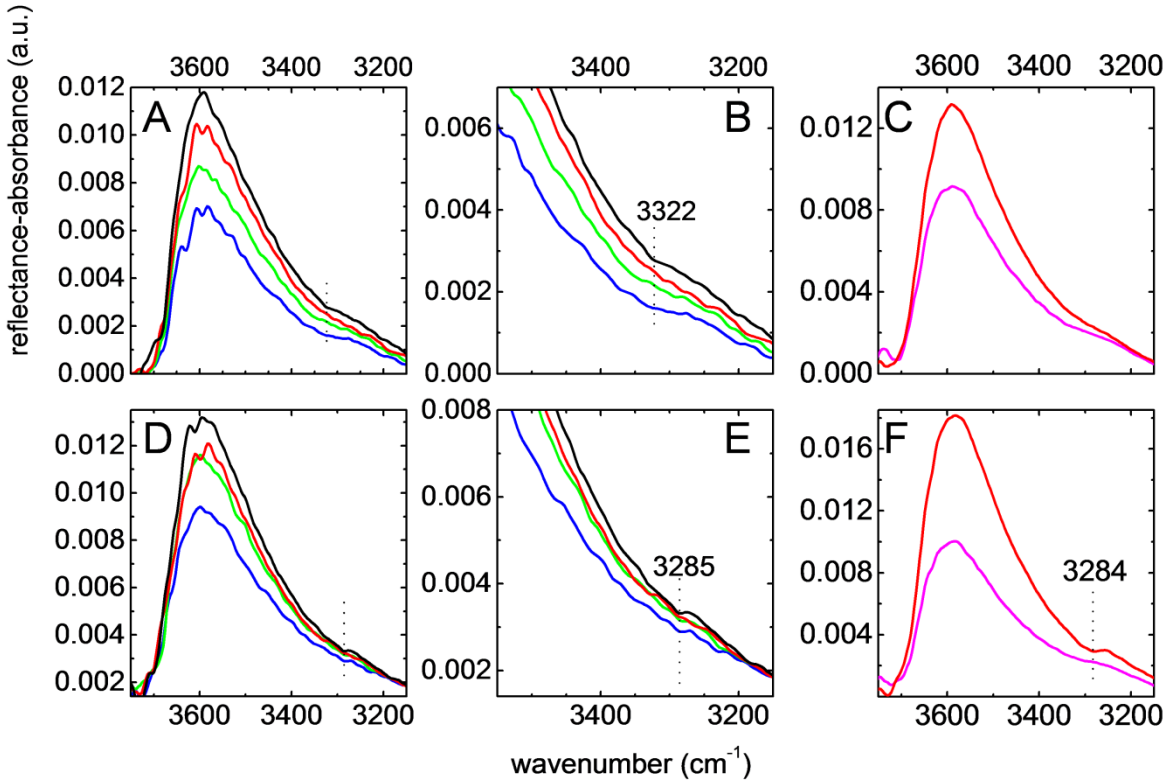

Fig S4. Sections of IRRA spectra of **OH4** (A, B, C) and **TH4** (D, E, F) at 25 °C (s-polarized light, incidence angle 40 °) at different surface pressures (5 mN m^{-1}, magenta; 10 mN m^{-1}, blue; 20 mN m^{-1}, green; 30 mN m^{-1}, red; 34 mN m^{-1}, black) of monolayers prepared on MES buffer pH 6.5 (A, B, D, E) and water (C, F). The peaks marked with a dotted line result from the NH stretching of amides (amide A). The position of the band indicates NH groups involved in hydrogen bonds.

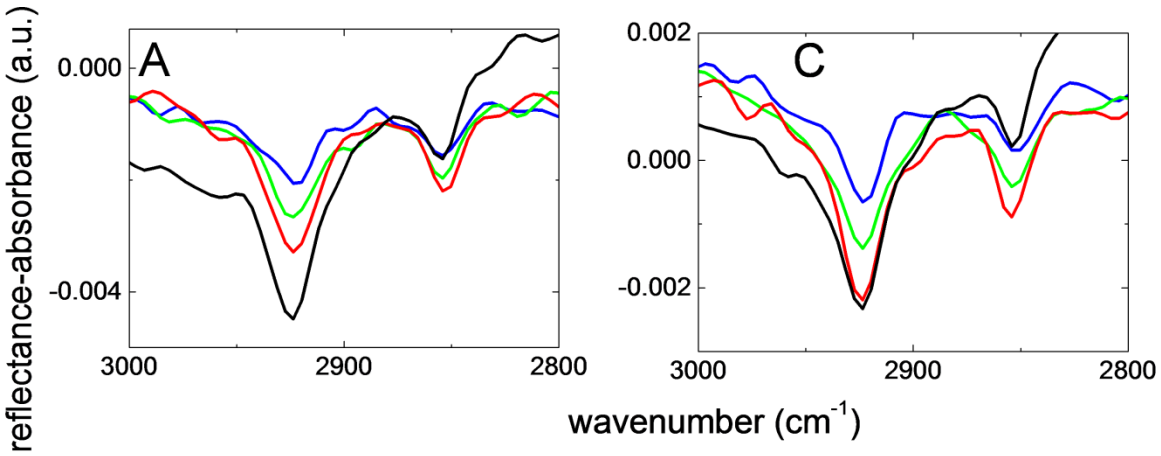

Fig S5. Sections of IRRA spectra of **OH4** (A) and **TH4** (B) at 25 °C (p-polarized light, incidence angle 40 °) at different surface pressures (10 mN m^{-1}, blue; 20 mN m^{-1}, green; 30 mN m^{-1}, red; 34 mN m^{-1}, black) of monolayers prepared on MES buffer pH 6.5. Sections of the CH stretching region are presented.

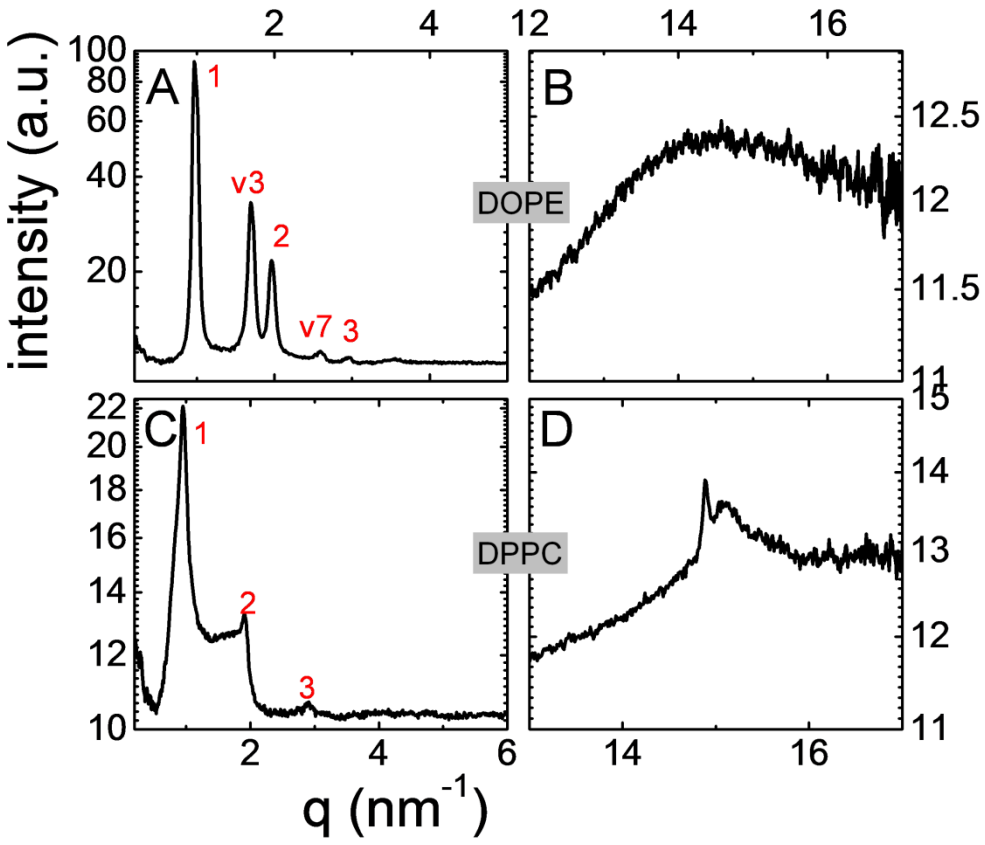

Fig. S6. SAXS (A, C) and WAXS (B, D) patterns of aqueous dispersions (10 wt%) of DOPE (A, B) and DPPC (C, D) in MES buffer pH 6.5 at 25 °C measured at the BESSY II facility.

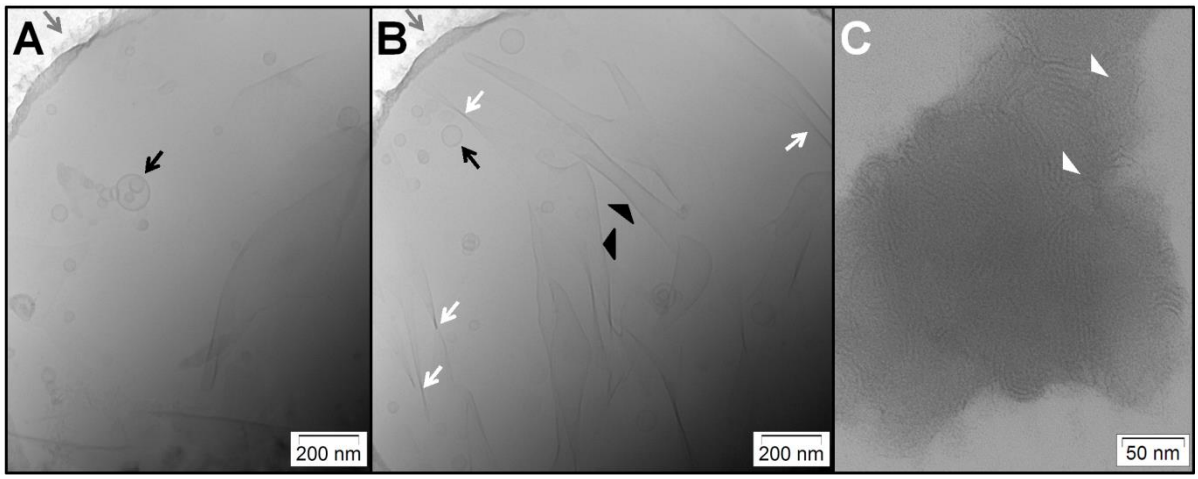

Fig. S7. CryoTEM images of 2 mg mL^{-1} (0.2 wt%) lipid dispersions (A,B) of **OH4**/DOPE 1/1 (n/n) in 10 mM MES buffer. The black arrows indicate vesicles. The white arrows show the pitch if twisted ribbons. Black triangles point to sheets. Grey arrows indicate the rim of the supporting film. TEM image of negatively stained **OH4**/DOPE 1/1 (n/n) lipoplexes N/P 4 (D).

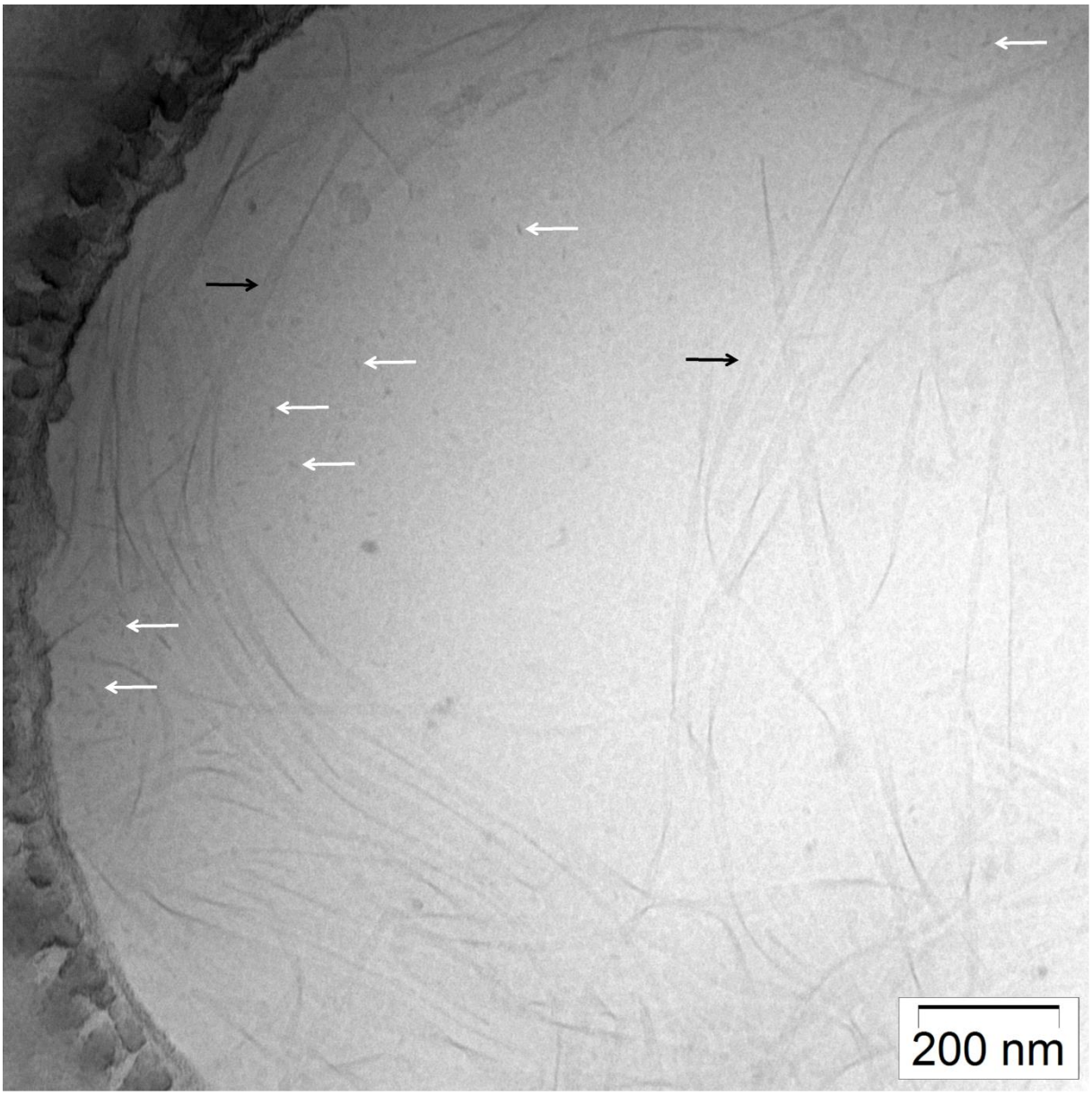

Fig. S8. Cryo TEM image of **OH4**/DPPC 1/1 (n/n). Black arrows indicate discoid aggregates and white arrows indicate twisted ribbons.

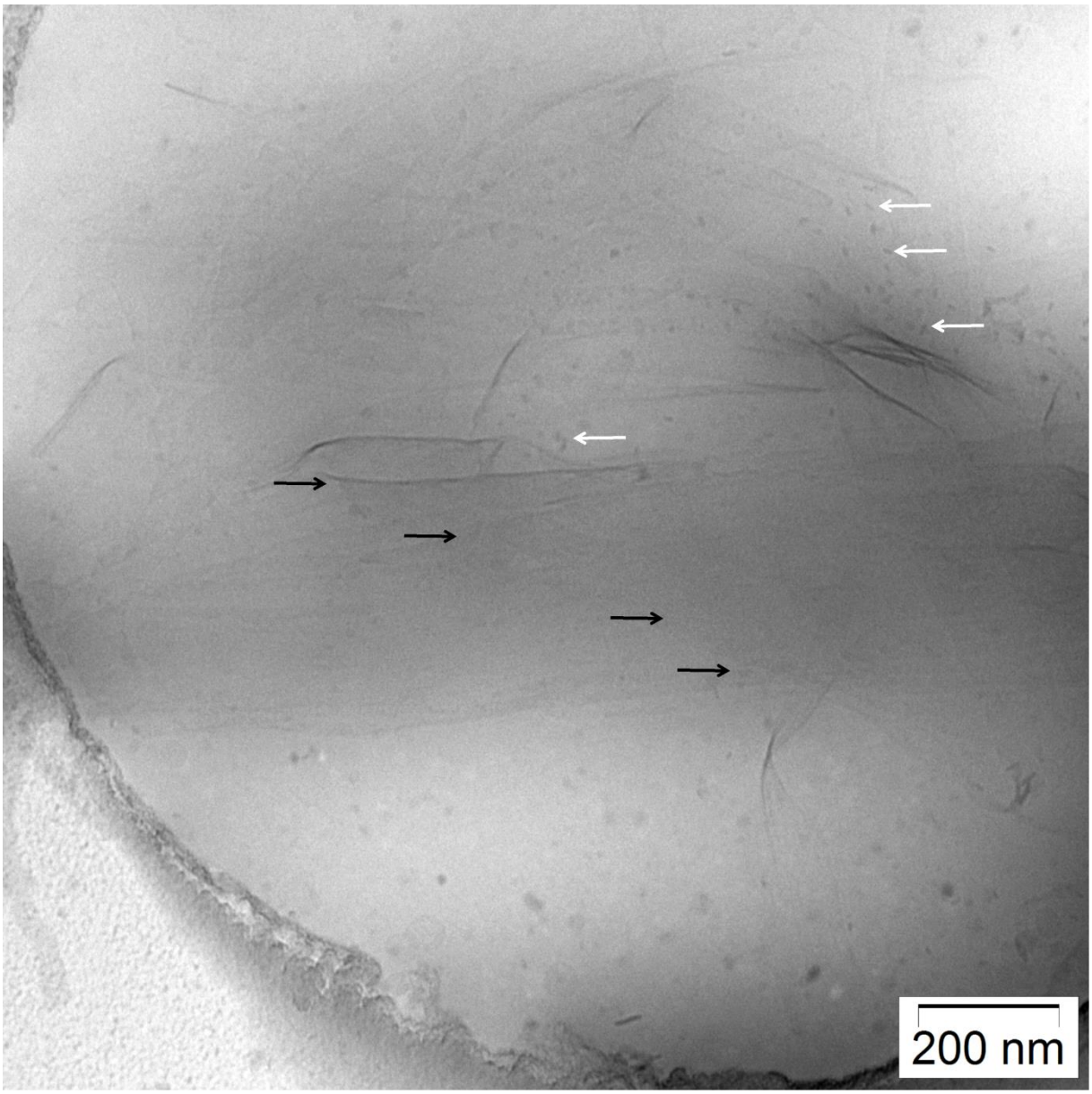

Fig. S9. Cryo TEM image of **TH4**/DPPC 1/1 (n/n). White arrows indicate discoidal aggregates and black arrows indicate sheet-like layers.

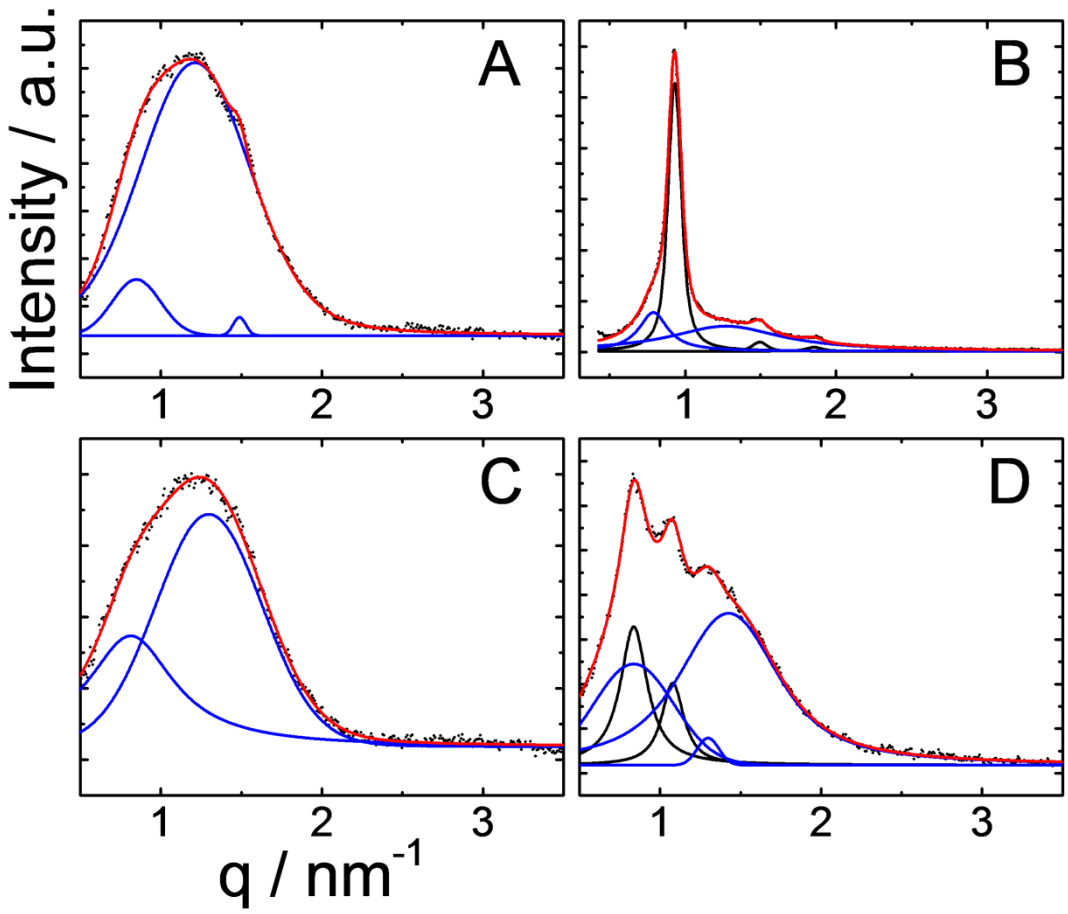

Fig. S10. SAXS curves (dots) of **OH4**/DPPC (A), **OH4**/DPPC with DNA N/P 4 (B), **TH4**/DPPC (C), **TH4**/DPPC with DNA N/P 4 (D) and the results of peak deconvolution using Voigt fits. The red line is the sum of the deconvoluted Voigt peaks. Peaks ascribed to the lipid assemblies without DNA are blue and peaks ascribed to lipid assemblies with DNA are black.

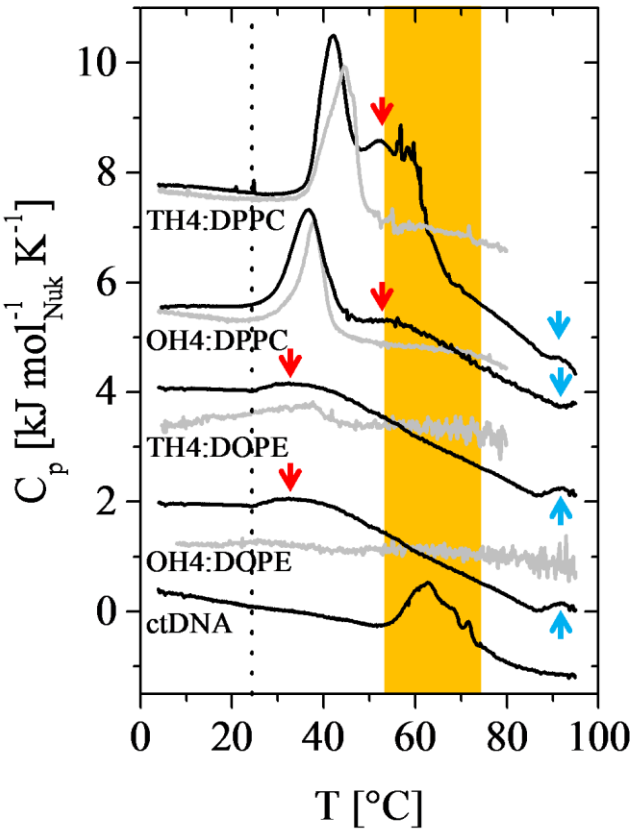

Fig. S11. DSC heating scans of lipid mixtures as well as of calf thymus (ct) DNA pure and in complex with the lipid mixtures (N/P 4) in MES buffer pH 6.5. The DNA concentration in every experiment with DNA was 3.03 $mM_{nucleotides}$. The amount of lipid is given with the N/P ratio. The curves are shifted along the y-axis for clarity. The scanning rate was 60 K h^{-1}, and only the first scan is shown. The equilibration time before each scan was 10 min. C_p is the molar heat capacity under constant pressure. The grey curves are the lipid formulation without DNA while the black curves are from DNA containing samples. The line marks the 25 °C and the orange area the range of the melting temperature of pure ctDNA. The red arrows indicate transitions assigned to DNA melting. There is a certain uncertainty that these transitions might be connected with structural changes in the lipids, but the fact that these transitions do not occur in the second scan (because DNA did not anneal to double strands in these time scale) and that the **OH4**:DOPE mixture alone shows no transition, but the broad transition occurs only in the presence of DNA supports the assumption that the red labelled transitions are connected with the DNA melting.

Experimental: The DSC measurements were performed on a MicroCal VP-DSC (MicroCal Inc., Northampton, MA, USA). The heating rate was 60 K h^{-1}, each heating and cooling scan was repeated confirming reproducibility. The first scan was abolished except for DSC experiments with DNA and lipoplexes. The scanned temperature range was between 2 and 95 °C. The reference cell was filled with pure solvent. The buffer−buffer baseline was subtracted from the thermograms of the samples, and the DSC scans were evaluated using MicroCal Origin 8.0 software.

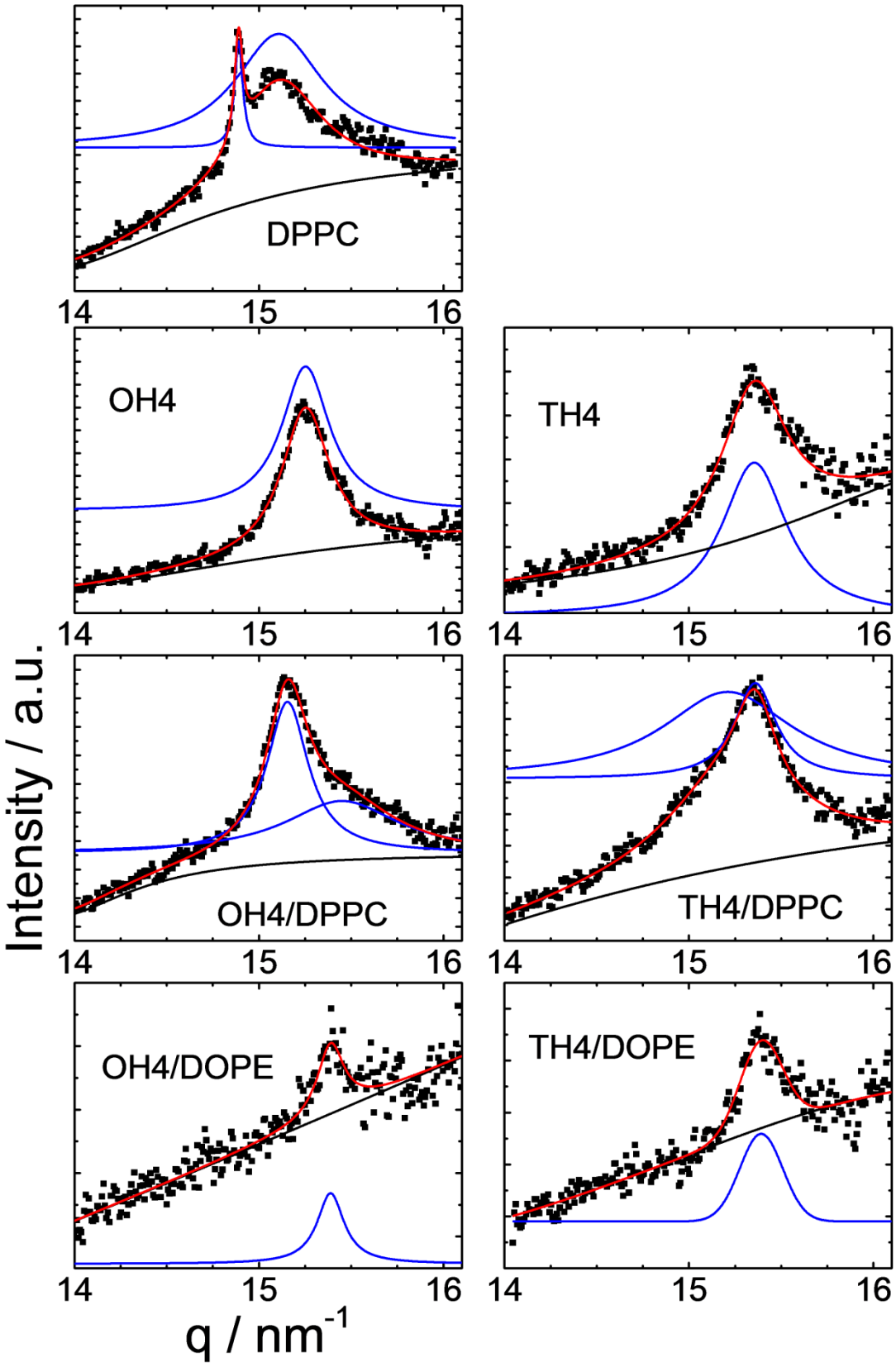

Fig. S12. Section of WAXS curves (squares) and the results of peak deconvolution using Voigt fits. The red line is the sum of the deconvoluted Voigt peaks. Peaks ascribed to the lipid assemblies are blue and the baseline peak is black.

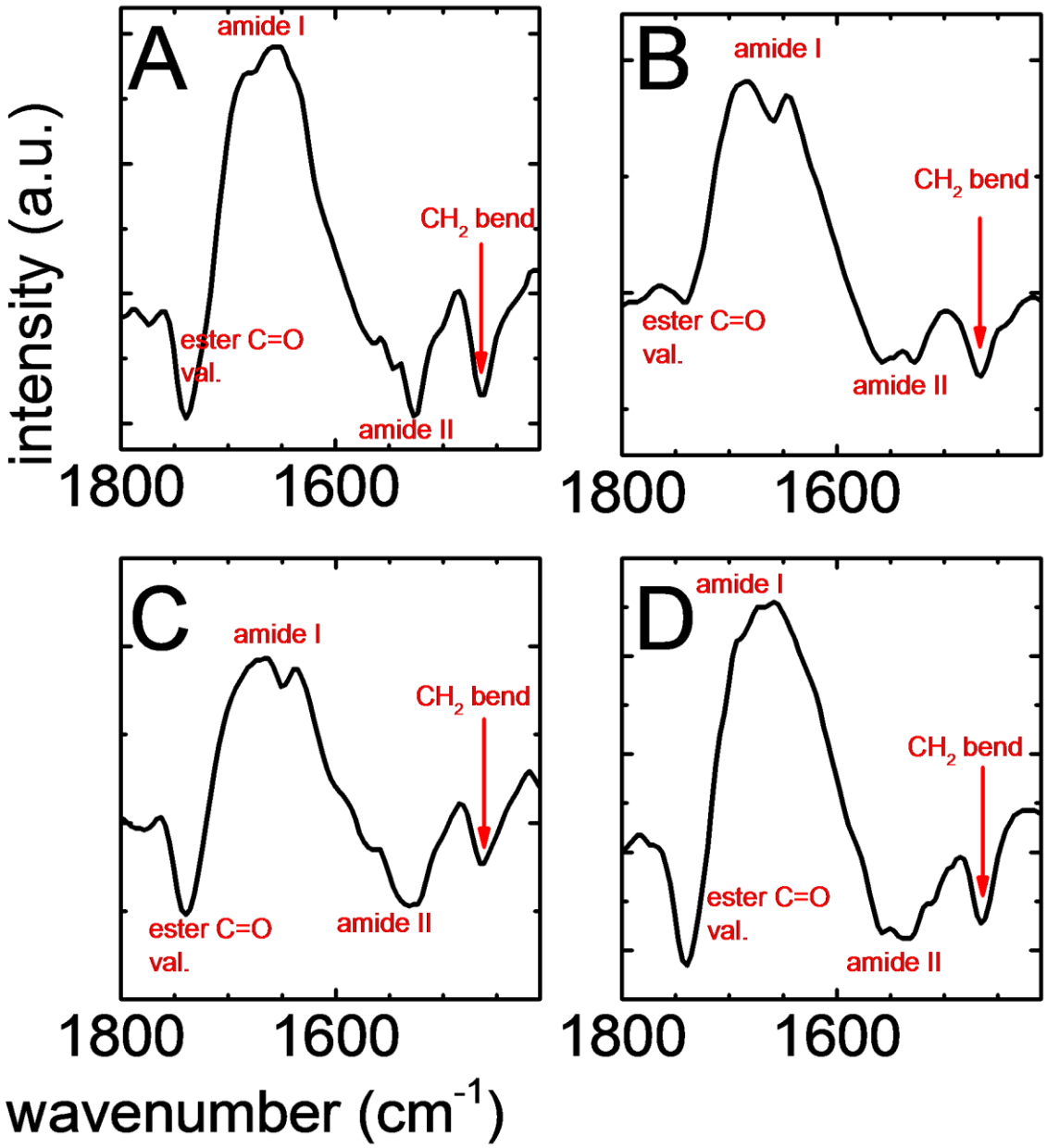

Fig. S13. Sections of IRRA spectra of **OH4**/DOPE (A), **OH4**/DPPC (B), **TH4**/DOPE (C) and **TH4**/DPPC (D) at 25 °C (s-polarized light, incidence angle 40 °) of monolayers prepared on MES buffer pH 6.5 at 30 mN m^{-1}. Sections of the amide I and amide II region including the CH_2 deformation band are presented.

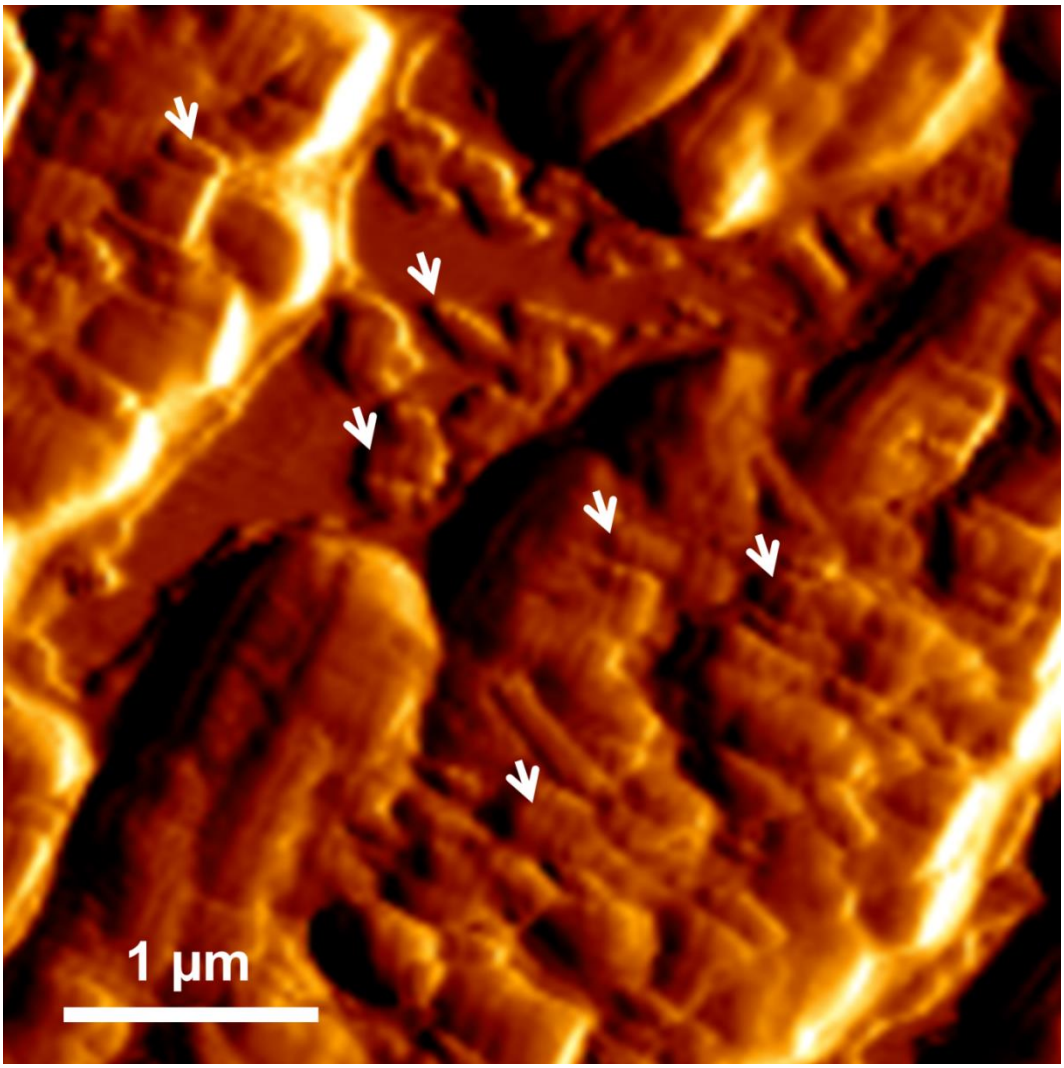

Fig. S14. Atomic-Force Microscopy (AFM) image of an **OH4**/DPPC 1/1 (n/n) mixture. Stacks of discs are indicated by arrows.

AFM Measurements: Atomic force microscopy was performed on a NanoWizard® III (JPK instruments, Berlin, Germany) equipped with an inverse microscope (AxioObserver® Z1, Zeiss, Germany). The microscope was vibration-damped in an acoustic enclosure. Commercial pyramidal n-type silicon tips (HQ:NSC16/Al BS, µmasch, Estonia) mounted to a cantilever with a length of 125 nm, a resonance frequency of about 160 kHz and a nominal force constant of about 5 N/m were used. To avoid damage of the sample, measurements were performed in intermittent contact mode. The scan speed was proportional to the scan size and the scan frequency was between 0.5 and 1.5 Hz. Images were obtained by displaying the height signal in the trace direction (512×512 pixel).