

## Synthesis

Pad-Pad-C (**1**) was synthesized according to Holme et al. (Nature Nanotechnol., 2012, Supporting information)<sup>1</sup> and the crude product was purified by silica gel column chromatography (CH<sub>2</sub>Cl<sub>2</sub> / MeOH / H<sub>2</sub>O; 65:25:4; v/v/v). In order to further purify **1**, it was dissolved in a minimal amount of CHCl<sub>3</sub>/ MeOH (1:1) and precipitated with a 20-times volume excess of acetone. A final purification was done on a size exclusion column (Sephadex LH20; CH<sub>2</sub>Cl<sub>2</sub> / MeOH / H<sub>2</sub>O; 65:25:4; v/v/v). A white powder was obtained.

**<sup>1</sup>H NMR** (400 MHz; CDCl<sub>3</sub>)  $\delta$ = 7.86 (d,  $J$  = 6 Hz, 1H), 7.68 (t,  $J$  = 5.5 Hz, 1H), 4.39 (br s, 2H), 4.05-3.77 (m, 4H), 3.55-3.43 (m, 1H), 3.39 (s, 8H), 2.16 (quin,  $J$  = 8 Hz, 4H), 1.57 (q,  $J$  = 6.7 Hz, 4H), 1.25 (br s, 48H), 0.88 (t,  $J$  = 6.8 Hz, 3H) ppm.

**<sup>31</sup>P-NMR:** (121 MHz; CDCl<sub>3</sub>):  $\delta$ = -0.1 ppm.

**HRMS (ESI<sub>+</sub>):**  $m/z$  [M+H<sup>+</sup>]: calculated for [M+H<sup>+</sup>]: 732.6014 g/mol; found: 732.6008 g/mol.

**IR** (GoldenGate, cm<sup>-1</sup>) = 3281, 2915, 2849, 1639, 1546, 1467, 1231, 1058, 969, 833, 720, 504.

**$R_f$**  (silica gel; CH<sub>2</sub>Cl<sub>2</sub> / MeOH / H<sub>2</sub>O; 65:25:4; v/v/v): 0.45.

---

<sup>1</sup> M. N. Holme, I. A. Fedotenko, D. Abegg, J. Althaus, L. Babel, F. Favarger, R. Reiter, R. Tanasescu, P.-L. Zaffalon, A. Ziegler, B. Müller, T. Saxer, A. Zumbuehl, *Nat. Nanotechnol.* **2012**, 7, 536–543.

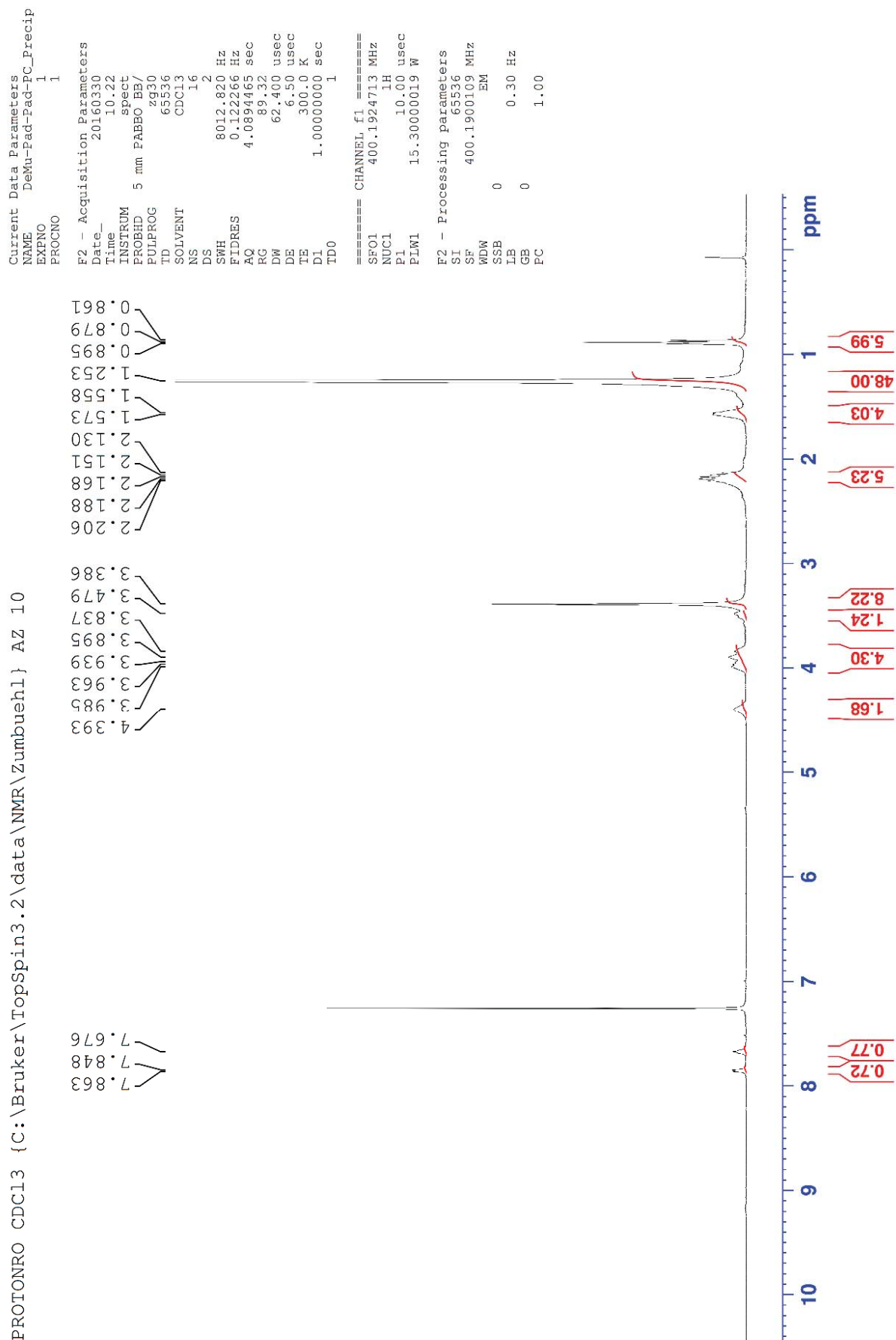


Figure SI 1: Proton NMR spectrum of Pad-Pad-PC (1).

## Cryo-TEM

LUVET<sub>100</sub> of Pad-Pad-PC (**1**) were prepared by a standard extrusion protocol. First, 10 mg lipid were dissolved in CH<sub>2</sub>Cl<sub>2</sub> in a 25 ml round bottom flask. The organic solvent was evaporated and the resulting thin film of lipids was dried under high vacuum (40 mbar) overnight. Then the thin film was hydrated with 1 ml isotonic saline and subjected to at least 5 consecutive freeze (liquid N<sub>2</sub>) thaw (bath temperature 65 °C) cycles. Then the multilamellar vesicles were extruded 11 times with a Mini Extruder at 65 °C (Avanti Polar Lipids, USA) using a track-edged filter with a size of 100 nm (Whatman, USA). The liposome suspensions were let to reach room temperature and were diluted 1:1 with isotonic saline and were mounted on glow-discharged holey carbon grids, quickly frozen by a Cryoplunge 3 system (Gatan, USA) and transferred to a JEM2200FS transmission electron microscope (JEOL, Japan) using a Gatan626 cryo-holder. Cryo-electron micrographs were recorded at the acceleration voltage of 200 kV, x 20,000 magnification, 4-8 µm underfocus and a dose of 10 electrons/Å<sup>2</sup>, using a F416 CMOS detector (TVIPS, Germany). The tomography was acquired from -65° to +65° with a total dosage of 30 electrons/Å<sup>2</sup> using the SerialEM software.<sup>2</sup> The picture was reconstructed with the IMOD software<sup>3</sup> and the final picture was rendered using UCSF Chimera<sup>4</sup> and IMOD.

---

<sup>2</sup> SerialEM: D. N. Mastronarde. *J. Struct. Biol.* **2005**, *152*, 36–51.

<sup>3</sup> IMOD: J. R. Kremer, D. N. Mastronarde, J. R. McIntosh, *J. Struct. Biol.* **1996**, *116*, 71–76.

<sup>4</sup> Chimera: E. F. Pettersen, T. D. Goddard, C. C. Huang, G. S. Couch, D. M. Greenblatt, E. C. Meng, T. E. Ferrin, *J. Comput. Chem.* **2004**, *25*, 1605–1612.

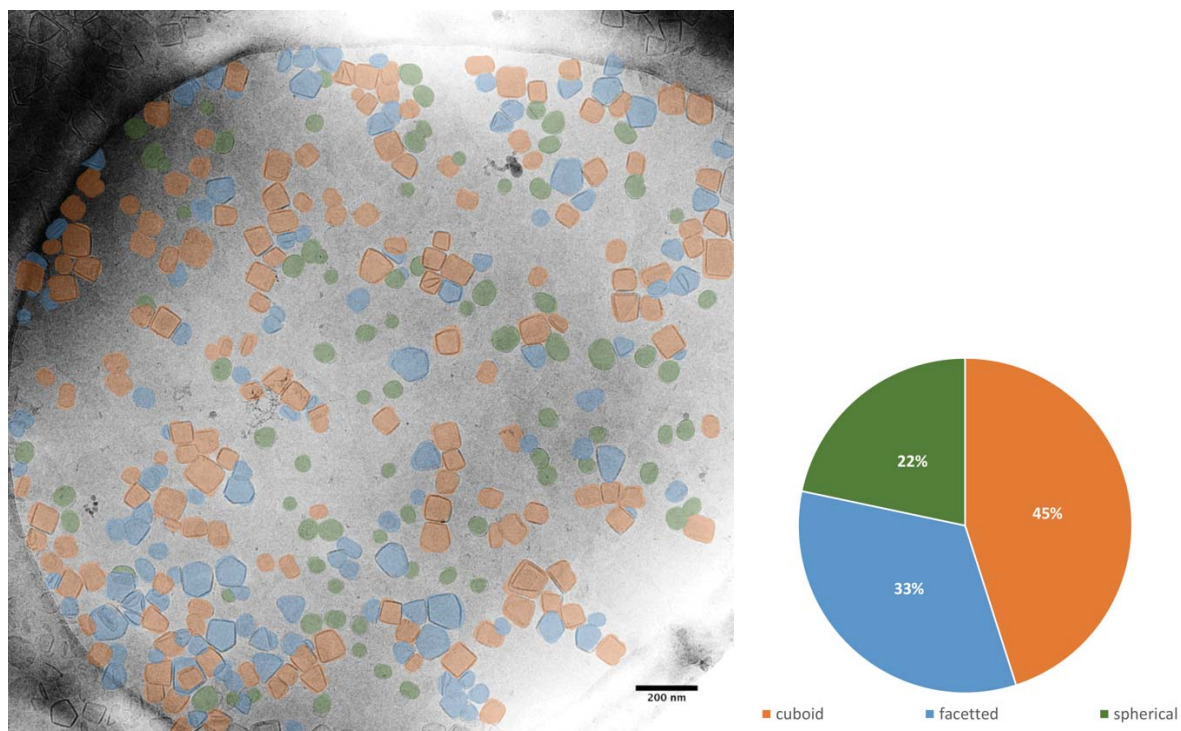
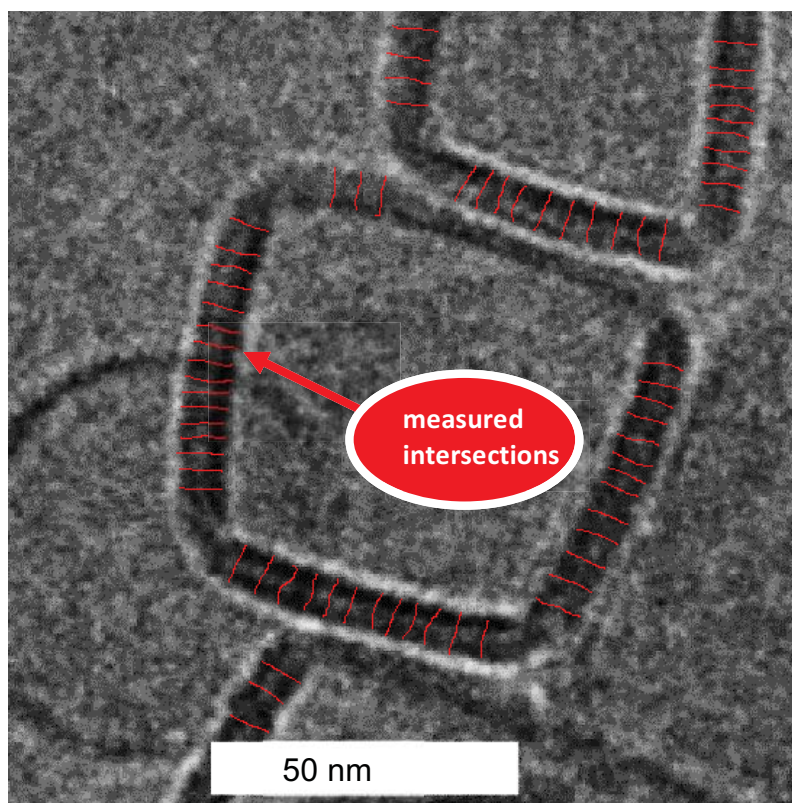


Figure SI 2: LEFT: This is the same figure as figure 1 with an overlay depicting the different vesicle geometries: cuboid in orange, strongly faceted in blue, and spherical in green. RIGHT: Counting 45 % cuboid structures, 33 % highly faceted geometries, and 22 % spherical.

Table SI 1: Measured membrane thickness of Pad-Pad-PC (1) large-unilamellar-vesicles in a cryo-TEM image.

Pixels	distance/nm	Pixels	distance/nm
212	50	212	50
28,6	6,745283019	29,7	7,004716981
26,9	6,344339623	27,2	6,41509434
26	6,132075472	27,9	6,580188679
27,9	6,580188679	30,1	7,099056604
28,6	6,745283019	28,6	6,745283019
29,2	6,886792453	28,3	6,674528302
29,7	7,004716981	29,1	6,863207547
28,2	6,650943396	28,4	6,698113208
30,7	7,240566038	28,6	6,745283019
27,7	6,533018868	29,4	6,933962264
30,1	7,099056604	29	6,839622642
31	7,311320755	28,2	6,650943396
29	6,839622642	28,6	6,745283019
30	7,075471698	29,4	6,933962264
29	6,839622642	26,5	6,25
29	6,839622642	28,6	6,745283019
28,2	6,650943396	28,4	6,698113208
29,6	6,981132075	28,6	6,745283019
28,9	6,816037736	28,1	6,627358491
28,5	6,721698113	29,1	6,863207547
29,3	6,910377358	30,4	7,169811321
28,2	6,650943396	28,4	6,698113208
28,5	6,721698113	28,2	6,650943396
28	6,603773585	29,3	6,910377358
26,3	6,202830189	28,1	6,627358491
28,4	6,698113208	28,3	6,674528302
28,8	6,79245283	29,1	6,863207547
29,2	6,886792453	28,4	6,698113208
29,2	6,886792453	29,2	6,886792453
29,3	6,910377358	29,1	6,863207547
26,9	6,344339623	30	7,075471698
27,5	6,485849057	29,4	6,933962264
29,5	6,95754717	27,5	6,485849057
29,5	6,95754717	27,7	6,533018868
29,7	7,004716981	27,9	6,580188679
28,2	6,650943396	28,6	6,745283019

<b>6,76329927</b>
<b>0,1747849</b> <b>2,58%</b>





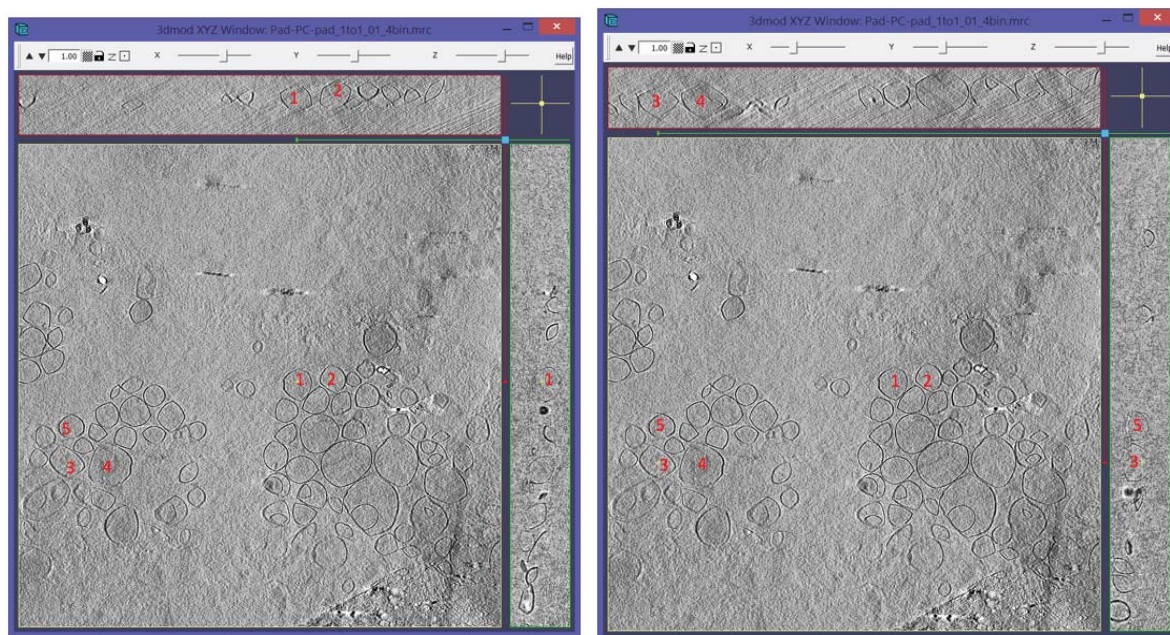


Figure SI 3: Depiction of vesicle orientation in 3D using IMOD. The presentation of the data is as follows: XY (widest panel), XZ (top) and YZ (right) sections. Yellow crosses show where we sectioned. Five vesicles were randomly chosen. #1 has tilted walls in the XZ plane. #2 has an edged tip at the top in the XZ plane. #3 and #4 stand on their edges in the XZ plane. #5 lies flat in the YZ plane.

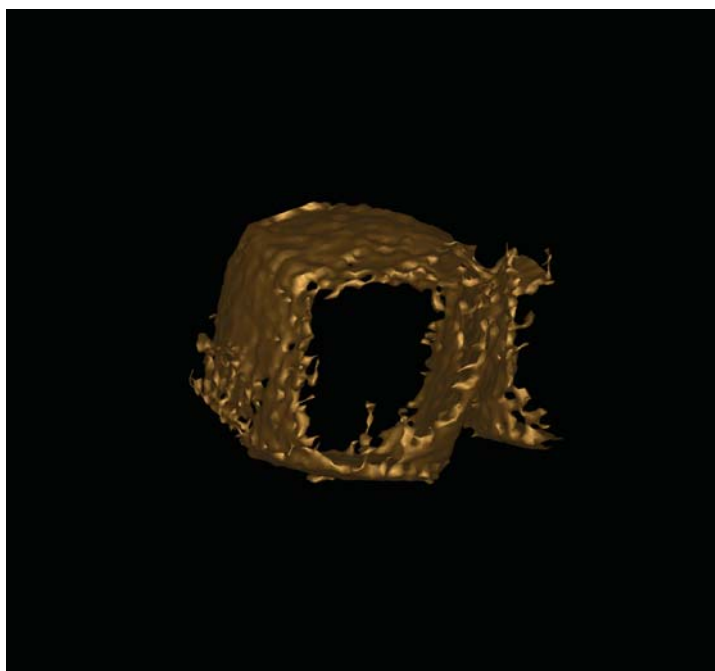


Figure SI 4: Cryo-Tomogram of a cubic unilamellar vesicle formulated of Pad-Pad-PC (1).

## Surface-pressure/area isotherm measurements

Molecular area / surface pressure isotherms were recorded on a custom made computer interfaced Langmuir-Pockels trough (Riegler&Kierstein, Germany) with a surface area of  $194\text{ cm}^2$ . The Wilhelmy method was used to measure the surface pressure with a filter paper as Wilhelmy plate with an accuracy of  $\pm 0.3\text{ mNm}^{-1}$ . Each measurement was repeated at least two times. The Langmuir-Pockels trough was filled with ultrapure water (specific resistance of  $18.2\text{ M}\Omega\cdot\text{cm}$ ). Stock solutions ( $1\text{ mgmL}^{-1}$ ) of the lipids prepared in chloroform were used for the spreading process. In order to let the system equilibrate and for complete solvent evaporation, the compression ( $3.12\text{ cm}^2\text{min}^{-1}$ ) was started 5–15 min (depending on the temperature) after spreading the lipid solution.

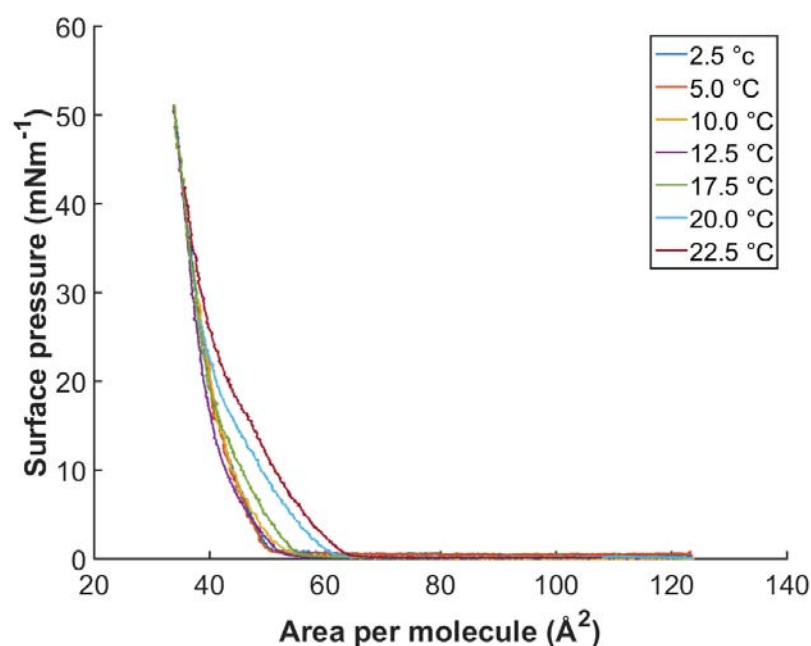


Figure SI 5: Langmuir Isotherms of a Pad-Pad-PC (**1**) containing monolayer at the air/water interface. Measured at different subphase temperatures. Compressed with  $3.12\text{ cm}^2\text{min}^{-1}$  on a  $200\text{ cm}^2$  PTFE-trough.

## Grating-incidence X-ray diffraction (GIXD)

The GIXD measurements were performed at the PETRA III beamline P08 at the German Synchrotron DESY-Hamburg, Germany. A photon beam of 15 keV was applied for the measurements. The monolayers were prepared on a Langmuir-Pockels trough with a fully extended area of 440 cm<sup>2</sup> and 500 mL subphase volume at 295 K air temperature and different subphase temperatures between 10-25 °C on an antivibrational table. The trough chamber was flushed with prewetted Helium throughout the whole measurement. A linear position-sensitive MYTHEN detector system (PSI, Villigen, Switzerland) measured the diffracted signal and was 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 measured the out-of-plane  $Q_z$  component of the scattering vector between 0.0 and 0.75 Å<sup>-1</sup>. The intensities of the scattered radiation were corrected for polarization, footprint area, and powder averaging. Model peaks taken to be Lorentzians in the in-plane direction (Bragg peaks) and Gaussians in the out-of-plane direction (Bragg rods) were fitted to the corrected intensities. The in-plane lattice repeat distances  $d$  of the ordered structures in the monolayer were calculated from the Bragg peak positions,  $d = 2\pi/Q_{xy}$ .



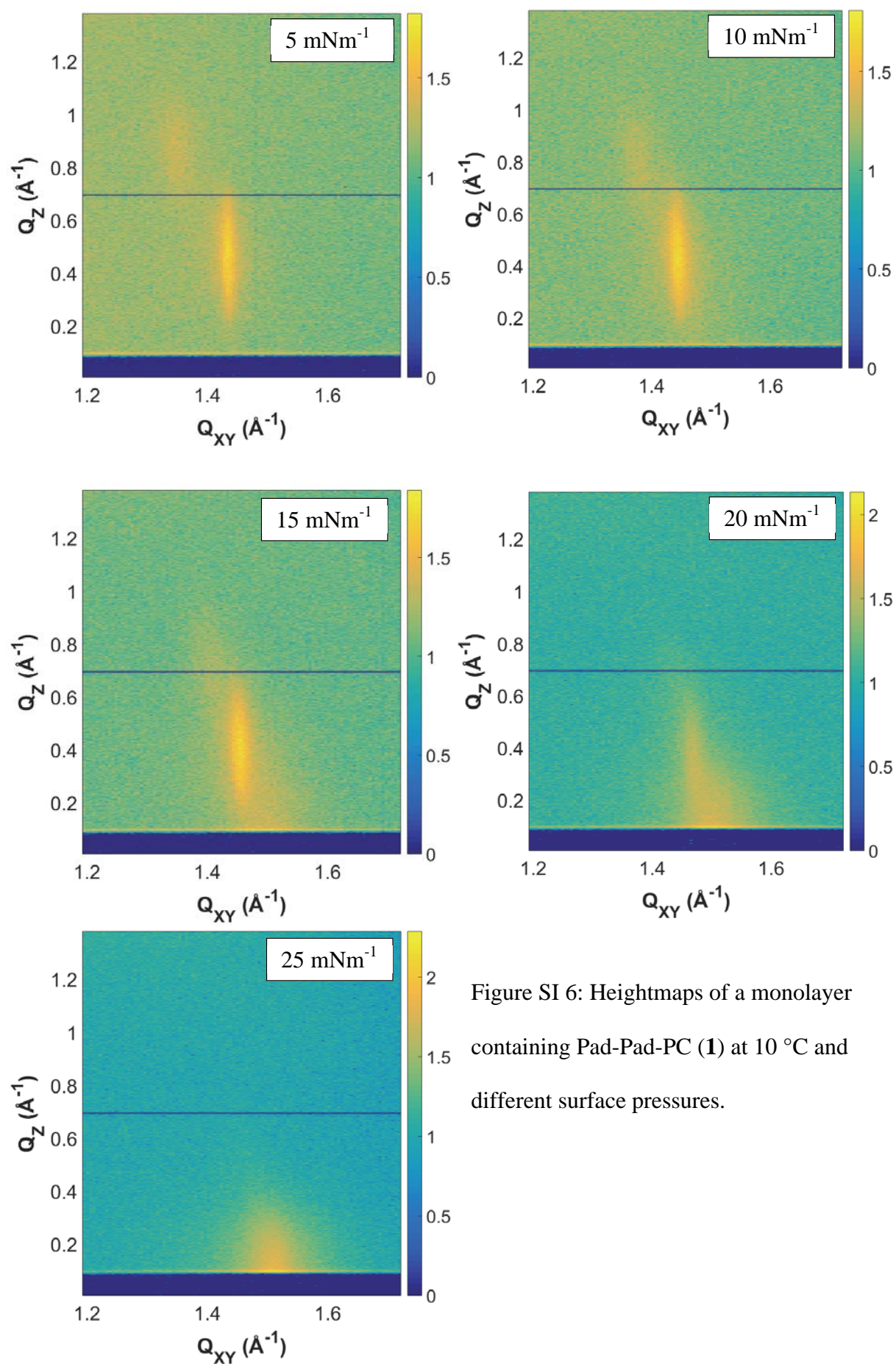


Figure SI 6: Heightmaps of a monolayer containing Pad-Pad-PC (**1**) at 10 °C and different surface pressures.

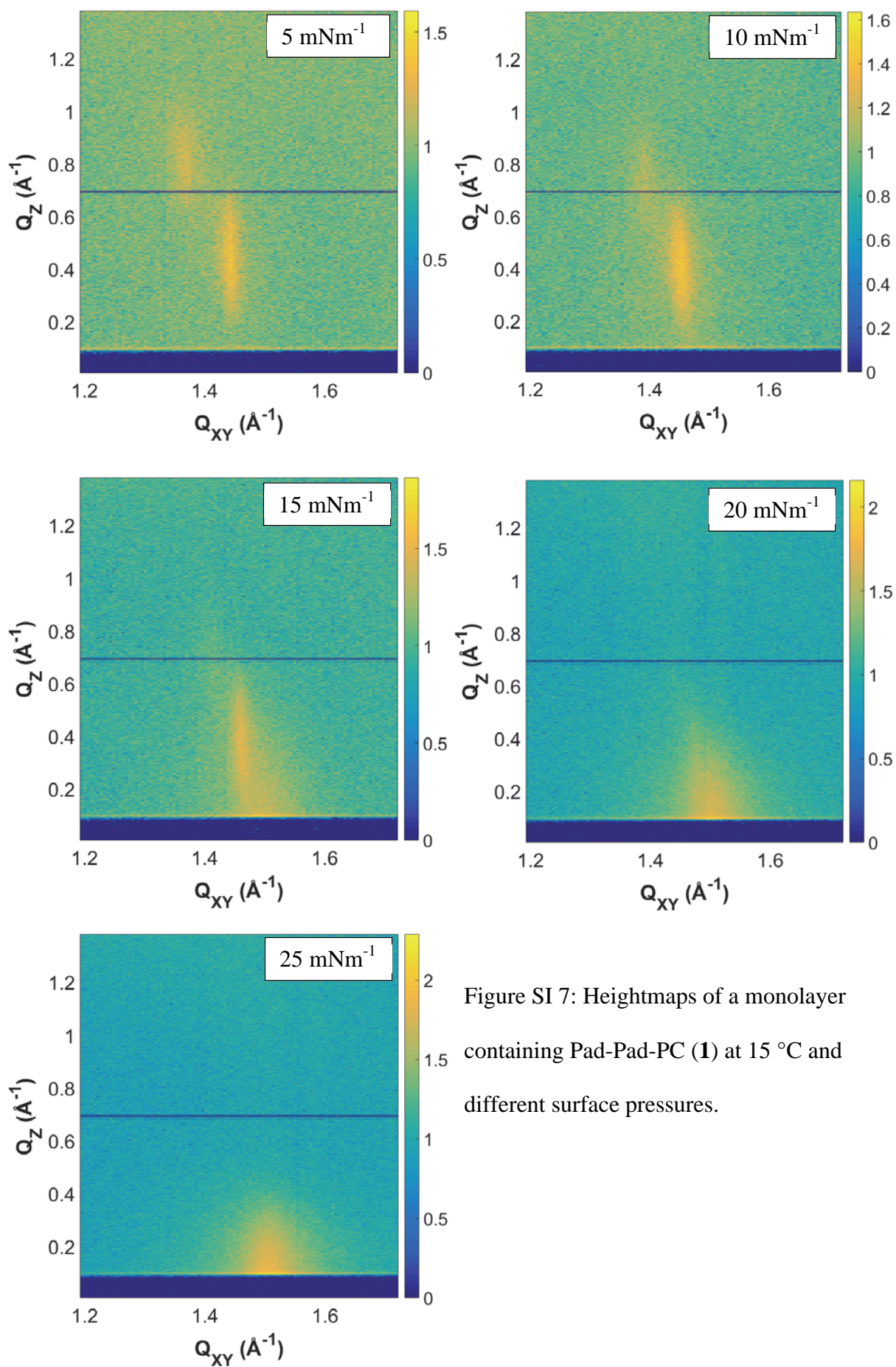


Figure SI 7: Heightmaps of a monolayer containing Pad-Pad-PC (**1**) at 15 °C and different surface pressures.



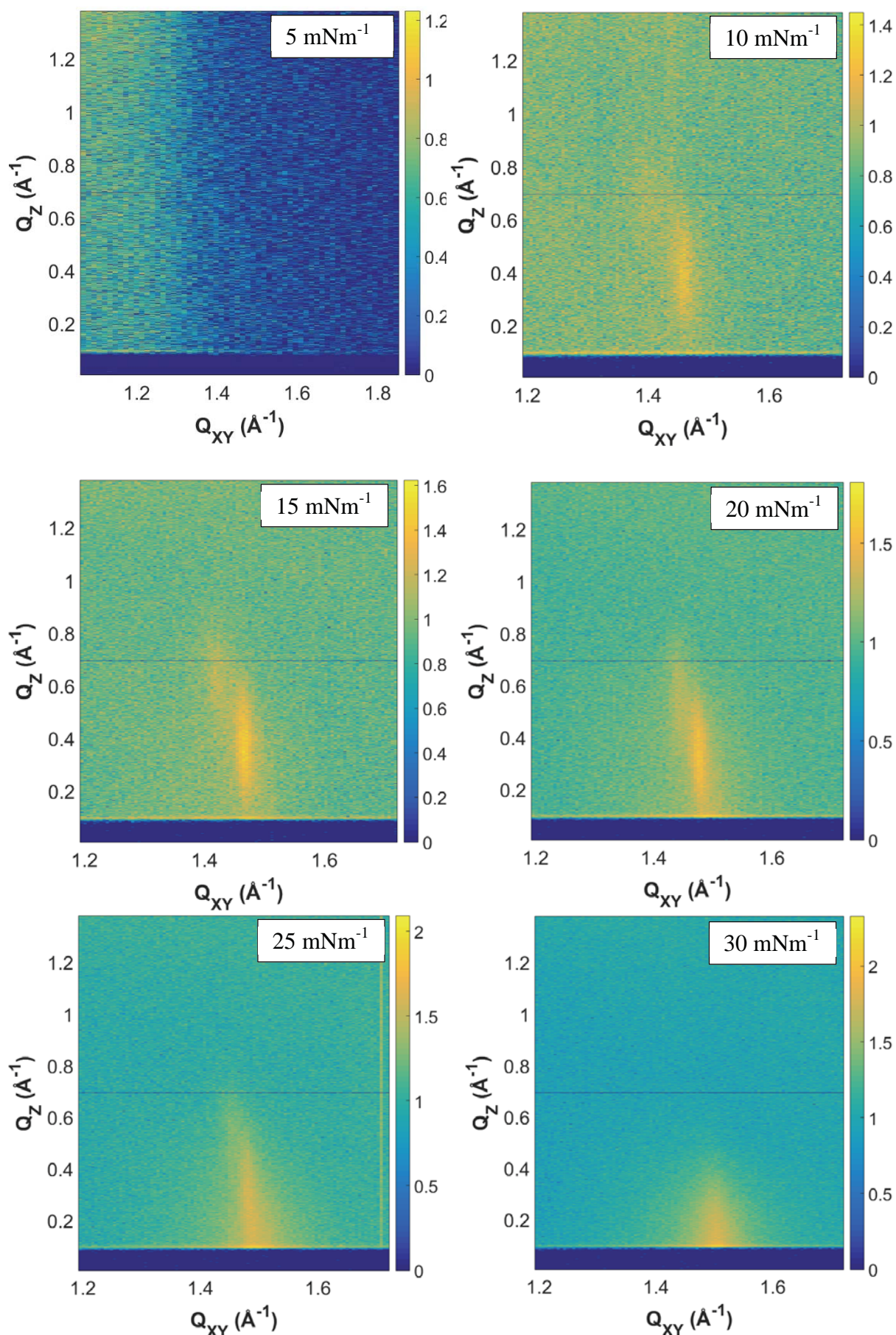


Figure SI 8: Heightmaps of a monolayer containing Pad-Pad-PC (**1**) at 20 °C and different surface pressures.

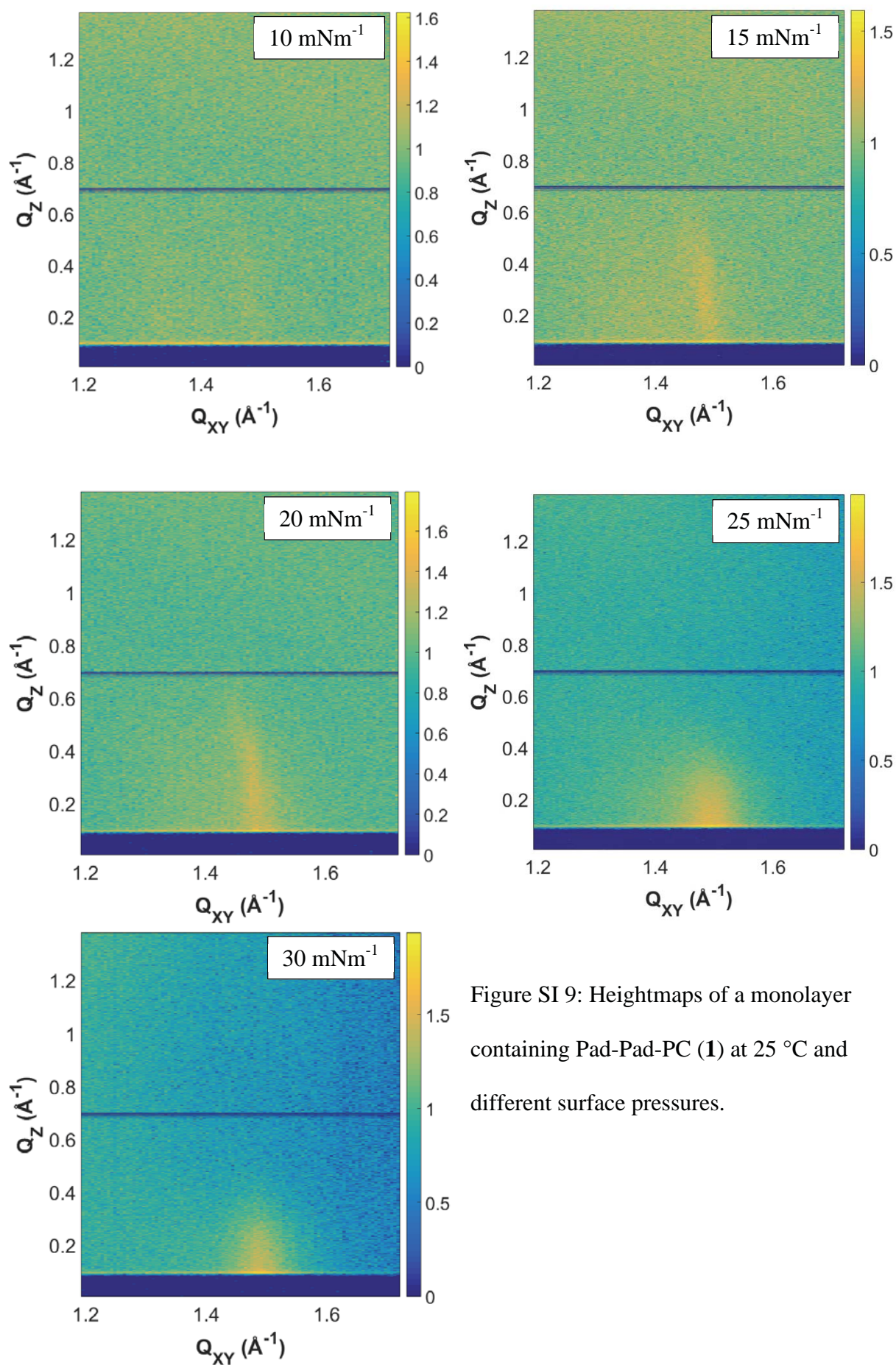


Figure SI 9: Heightmaps of a monolayer containing Pad-Pad-PC (**1**) at 25 °C and different surface pressures.



Table SI 2: GIXD data summary.

T/°C	Π/mNm-1	Non-degenerate		degenerate				gamma	tilt angle	tilt direction	distortion	dist direction	Axy	A0	2D crystal lattice			
		Qz	Qxy	Qz	Qxy	a	b									c	alpha	beta
10	5	0,94	1,352	0,47	1,431	4,982	5,273	5,273	123,6	118,2	118,2	34,8	NNN	7,42E-02	NNN	23,2	19,0	2 - rect NNN
10	10	0,84	1,378	0,42	1,442	4,96	5,19	5,19	122,9	118,5	118,5	31,4	NNN	5,96E-02	NNN	22,6	19,3	2 - rect NNN
10	15	0,6	1,397	0,3	1,452	4,936	5,13	5,13	122,5	118,8	118,8	23	NNN	5,08E-02	NNN	22,2	20,4	2 - rect NNN
10	15	0	1,501	0,4	1,452	5,055	4,89	4,89	117,8	121,1	121,1	17,8	NN	4,47E-02	NN	21,2	20,1	4 - rect NN
10	20	0	1,513	0,32	1,468	4,994	4,846	4,846	118	121	121	14,3	NN	4,07E-02	NN	20,7	20,1	4 - rect NN
10	25	0	1,51	0,19	1,479	4,94	4,839	4,839	118,6	120,7	120,7	8,5	NN	2,78E-02	NN	20,6	20,3	4 - rect NN
15	5	0,88	1,366	0,44	1,44	4,956	5,225	5,225	123,4	118,3	118,3	32,8	NNN	6,91E-02	NNN	22,8	19,2	2 - rect NNN
15	10	0,82	1,389	0,41	1,45	4,936	5,153	5,153	122,8	118,6	118,6	30,6	NNN	5,65E-02	NNN	22,3	19,2	2 - rect NNN
15	15	0,56	1,409	0,28	1,458	4,922	5,093	5,093	122,2	118,9	118,9	22,4	NNN	4,50E-02	NNN	21,9	20,3	2 - rect NNN
15	15	0	1,499	0,37	1,458	5,024	4,887	4,887	118,1	120,9	120,9	16,5	NN	3,73E-02	NN	21,1	20,2	4 - rect NN
15	20	0	1,508	0,23	1,475	4,956	4,848	4,848	118,5	120,7	120,7	10,3	NN	2,97E-02	NN	20,65	20,3	4 - rect NN
15	25	0	1,521	0,14	1,495	4,882	4,798	4,798	118,8	120,6	120,6	5,8	NN	2,31E-02	NN	20,2	20,1	4 - rect NN
15	25	0	1,503			4,827	4,827	4,827	120	120	120	0				20,2	20,2	5 - hex
20	5	0	0	0	0							-	-					1 - gaseous
20	10	0	0	0	0							-	-					1 - gaseous
20	15	0,285	1,464	0,57	1,42	4,908	5,06	5,06	122	119	119	21,9	NNN	4,03E-02	NNN	21,7	20,2	2 - rect NNN
20	20	0,23	1,475	0,46	1,436	4,877	5,009	5,009	121,7	119,1	119,1	17,8	NNN	3,54E-02	NNN	21,3	20,3	2 - rect NNN
20	25	0,21	1,479	0,42	1,454	4,878	4,962	4,962	121,1	119,4	119,4	16,1	NNN	2,26E-02	NNN	21,1	20,3	2 - rect NNN
20	25	0	1,504	0,31	1,479	4,934	4,852	4,852	118,9	120,6	120,6	13,7	NN	2,47E-02	NN	20,6	20	4 - rect NN
20	30	0	1,502	0,23	1,49	4,882	4,843	4,843	119,5	120,3	120,3	10,1	NN	1,07E-02	NN	20,4	20,1	4 - rect NN
25	10	0	0	0	0							-	-					1 - gaseous
25	15	0,21	1,477	0,42	1,411	4,842	5,069	5,069	122,9	118,5	118,5	16,6	NNN	6,00E-02	NNN	21,6	20,7	2 - rect NNN
25	20	0,18	1,479	0,36	1,428	4,851	5,024	5,024	122,3	118,9	118,9	14,1	NNN	4,62E-02	NNN	21,3	20,7	2 - rect NNN
25	25	0	1,498	0,19	1,465	4,99	4,88	4,88	118,5	120,7	120,7	8,6	NN	2,99E-02	NN	20,9	20,7	4 - rect NN
25	30	0	1,509	0,13	1,481	4,93	4,839	4,839	118,7	120,6	120,6	5,8	NN	2,51E-02	NN	20,5	20,4	4 - rect NN



## Matlab® code to process the GIXD measurements

```
% It is important to set the following variables in forehand: energy
% (beamenergy), offset (the mythen-channel offset), dfpm (distance
% footprint to mythen), angle (the incidence angle), planck (planck
% constant), cspeed (speed of light), chheight (channel height) and preset
% the following arrays depending on your measured range (Qxy, mline (number
% of mythen channels), Qz)
% for efficiency purposes. Also path (is the path to your datafile), path2
% (is the path to your datafolder in which all raw data are stored, so that
% you don't have to open every file on it's own)
[data]=load_FIO(filename);

for i=1:length(data.cols)
if(~isempty(strfind(data.cols{i},'Attenuation
Factor'))))atten_col=i;break;elseif(~isempty(strfind(data.cols{i},'atten_pos
ition'))))atten_col=i;break;end
end

x=data.numbers{dataCol};
attenfac=data.numbers{atten_col};

matrix=zeros(1280,length(x));
cd(path2);

for i=1:length(x)
idx_s=strfind(filename,'/');
idx_bs=strfind(filename,'\');
idx=max([idx_s idx_bs]);
mythen_name=[filename(1:end-4) filename(idx:end-4) '_' num2str(i) '.raw'];
tmp=load(mythen_name,'-ASCII');
matrix(:,i)=tmp(:,2)'.*attenfac(i);
end
matrix=flipud(matrix);

%% Data Processing
for i=1:length(x)
Qxy(i)=4*pi*energy/(planck*cspeed)*sind(0.5*x(i))/1e10;
end
for i=1:length(mline)
Qz(i)=4*pi*energy/(planck*cspeed)*sind(0.5*atand((mline(i)-
offset)*chheight/dfpm)+angle)/1e10;
end

%% Output
op=figure('Visible','on','Name',filename,'Toolbar','none');
pa=axes('Parent',op);

if(calcbutton.Value)p=pcolor(pa,Qxy,Qz,log10(matrix));else
p=pcolor(pa,x,mline,log10(matrix));end

set(p,'linestyle','none');
colorbar

%% Integration
% To integrate over a certain area on the plot you have to set Qxys to
% the lower Qxy value and Qxye to the upper Qxy value of the rectangle
% you are going to integrate and you have to do the same for Qzs and Qze.
% TO integrate over Qxy you have to set Qsel to 1 (or 'true'). The results
```

% will be automatically saved in a .txt-file.

```
function integrate (~,~)
intnum = intnum+1;
if(Qsel) result=zeros(2,length(mline));for(i=Qzs:Qze)
result(1,i)=Qz(i);result(2,i)=trapz(matrix(i,Qxys:Qxye));end;tmp='Qz/A^-'
1';else result=zeros(2,length(x)); for(i=Qxys:Qxye)
result(1,i)=Qxy(i);result(2,i)=trapz(matrix(Qzs:Qze,i));end;tmp='Qxy/A^-'
1';end
result(:,~any(result,1))=[];
assignin('base','result',result);
savename = '';
savename = ['integration_' filename '_' int2str(intnum) '.txt'];
cd(savedir);
fileID = fopen(savename,'w');
fprintf(fileID,'      %s\t Integration\r\n',tmp);
fprintf(fileID,'%0.8e\t%0.8e\r\n',result);
fclose(fileID);
cd(path);
end
```

## Small Angle X-ray Scattering (SAXS) and Wide Angle X-ray Scattering (WAXS)

SAXS spectra were recorded with a NanoMax-IQ camera (Rigaku Innovative Technologies, USA). The samples were kept in vacuum at a constant temperature of approximately 18 or 59 °C, respectively, during the measurement. The raw data were processed according to standard procedures, and the scattering spectra (1D) are presented as a function of the momentum transfer  $q = 4\pi \cdot \lambda^{-1} \cdot \sin(\theta/2)$ , where  $\theta$  is the scattering angle and  $\lambda = 0.1524$  nm is the photon wavelength.

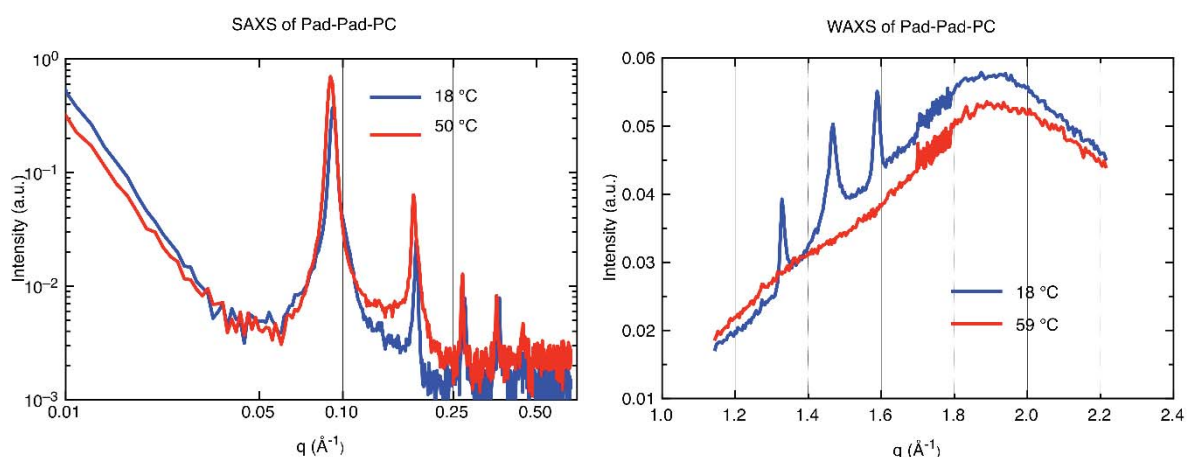


Figure SI 10: SAXS pattern of a Pad-Pad-PC (**1**) MLV suspension (10 wt% of Pad-Pad-PC (**1**) in MilliQ water) at 18 °C (blue) and 50 °C (red). The  $q$ -value for the first order scattering maximum translates into a  $d$ -distance of 6.85 nm (18 °C) and 6.96 nm (50 °C). The WAXS region is shown in the right figure at 18 °C (blue) and 59 °C (red).

Table SI 3: d-distance calculated from the SAXS peaks

	1st order (nm)	2nd order (nm)	3rd order (nm)	4th order (nm)	5th order (nm)	mean (nm)
18 °C	6.851	6.829	6.857	6.844	6.878	6.85
50 °C	6.961	6.993	6.967	7.096	7.036	6.99

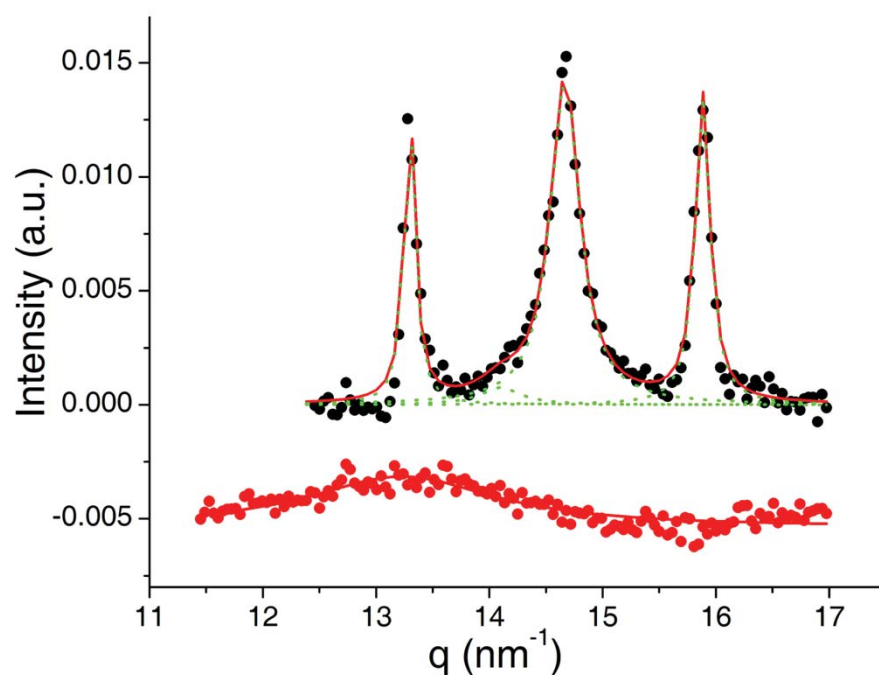


Figure SI 11. WAXS data corrected for the non-horizontal background. The traces at 18 °C (black) and 59 °C (red) are shifted vertically in order to allow easier data interpretation. At 59 °C a 'halo' for a molten chain is visible. The average packing is hexagonal ( $a=0.5488$  nm) with a chain area of  $0.261 \text{ nm}^2$ . At 18 °C more peaks are visible than in a normal gel phase. If the Bragg reflexes at  $14.67 \text{ nm}^{-1}$  is indexed as (02) and the reflex at  $15.88 \text{ nm}^{-1}$  as (11), a mean chain area of  $0.191 \text{ nm}^2$  is calculated. In this orthorhombic packing the (01) reflex should be at  $7.33 \text{ nm}^{-1}$  (beyond the field of measurement) and the (01) reflex should lie at  $14.08 \text{ nm}^{-1}$  (visible as reflex with small intensity) .

## Differential scanning calorimetry (DSC)

Differential scanning calorimetry experiments were performed using a Nano DSC (TA Instruments, USA). The unextruded MLV suspensions were formulated as described above (cryo-TEM), omitting the extrusion step. Prior to DSC measurements, samples were degassed for 30 minutes using a TA degassing station. Scans were recorded from 5 °C to 90 °C with a scanning speed of 0.5 °C/min. The experiment was performed twice, starting with new suspensions, in order to ensure reproducibility.

Four heating/cooling cycles were recorded for each sample. Raw data was base-line corrected and converted to molar heat capacity (MHC) using the NanoAnalyze software (TA Instruments, USA). The main phase transition and enthalpy were determined with the same software and verified using the OriginLab software (OriginLabs, USA).

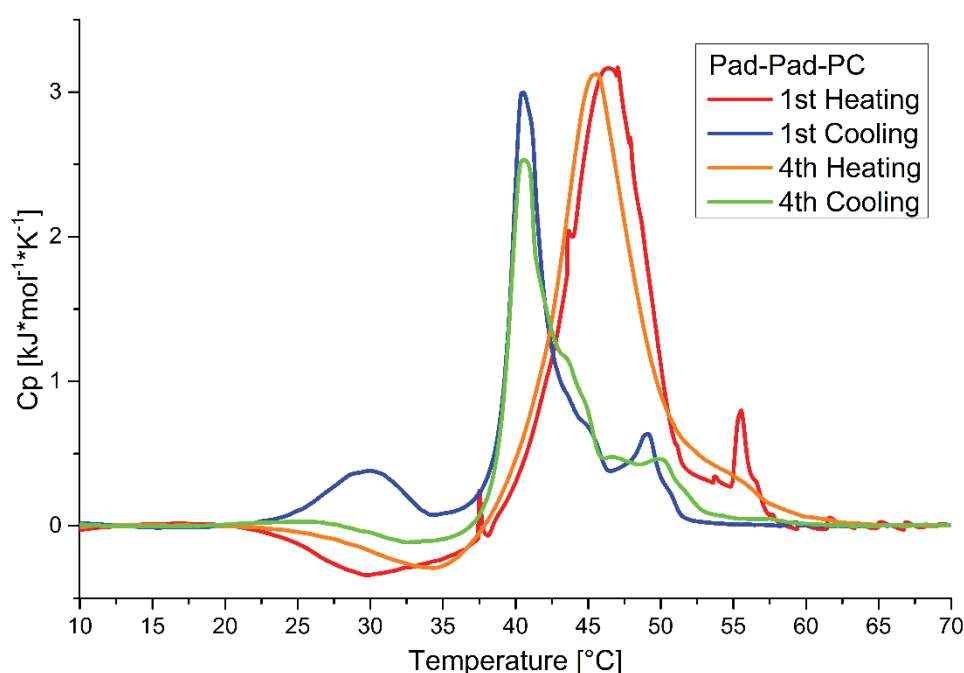


Figure SI 12: DSC traces of Pad-Pad-PC (1). The traces represent the complex melting behaviour of phospholipids forming intermolecular hydrogen bonds.



Table SI 4: Values taken from the DSC traces.

<b>Run</b>	<b>T<sub>m</sub>/°C</b>	<b>ΔH/ kJ/(mol*K)</b>
<b>1st heating</b>	47.02	19.37
<b>1st cooling</b>	40.51	15.81
<b>4th heating</b>	45.55	20.38
<b>4th cooling</b>	40.59	12.70

## Release experiments

The inner buffer (CF buffer) was prepared from 50 mM 5(6)-carboxyfluorescein (powder, Sigma-Aldrich) and 10 mM HEPES buffer (powder, Sigma-Aldrich) dissolved in ultra pure water ( $18.2 \text{ M}\Omega\cdot\text{cm}$ ). The pH was adjusted to 7.4 (NaOH) and the osmotic concentration to  $200 \text{ mOsmL}^{-1}$  (NaCl). The outer buffer was prepared from 10 mM HEPES buffer (powder, Sigma-Aldrich) dissolved in ultra pure water ( $18.2 \text{ M}\Omega\cdot\text{cm}$ ). The pH was adjusted to 7.4 (NaOH) and the osmotic concentration to  $200 \text{ mOsm}^{-1}$  (NaCl). Pad-PC-Pad (2 mg) was weighed into a round-bottomed flask (25 mL) and dissolved in  $\text{CHCl}_3$  (1 mL, amylene stabilized, Sigma-Aldrich, USA). The solvent was removed by low-pressure rotatory evaporation. The thin film was dried under high vacuum overnight, to ensure the removal of residual water and prevent cholesterol oxidation. Inner buffer (1 mL) was added to the round-bottomed flask and the film was hydrated for 30 minutes at  $65^\circ\text{C}$ . The film was subjected to five cycles of freeze/thaw using liquid nitrogen and a  $65^\circ\text{C}$  water bath. The resulting MLV suspension was extruded 11 times through a track-etched filter membrane at  $65^\circ\text{C}$  (100 nm, Whatman, USA) placed in a Mini Extruder (Avanti Polar Lipids, USA). The vesicles were left standing at room temperature overnight.

The residual non-encapsulated CF buffer, in the  $\text{LUVET}_{100}$  suspension, was exchanged with the outer buffer using size exclusion chromatography (PD-10 desalting columns, GE Healthcare, UK). The size exclusion chromatography was carried out after 24 hours storage, in the dark at room temperature, of the  $\text{LUVET}_{100}$  suspension.

The purified  $\text{LUVET}_{100}$  suspension was diluted, in a volumetric flask, to 100 mL using additional outer buffer. Six aliquots (2 mL) were separated into vials (5 mL, PE caps) and vortex mixed for different amounts of time (0, 5, 10, 20, 30, 60 s) at 2500 rpm. The 5(6)-carboxyfluorescein release was quantified using a fluorospectrometer (Sense 425-301, Hidex, Finland). For each sample, five microplate wells were filled with  $200 \mu\text{L}$  of the vortex

mixed vesicular suspension. The wavelengths used for measurements were 485 nm (excitation) and 535 nm (emission). As a control for the maximum dye release ( $F_{100}$ ), a Triton X-100 solution (2  $\mu$ L of a 10 vol% solution) was added to additional five microplate wells, filled with 200  $\mu$ L of vesicular suspension, for each sample. The release fraction at time X was calculated with the formula:

$$Release(\%) = \frac{F_X - F_0}{F_{100} - F_0}$$

where  $F_X$  is the fluorescence at time X,  $F_0$  the fluorescence at time zero and  $F_{100}$  the maximum fluorescence recorded after treatment with Triton X-100.

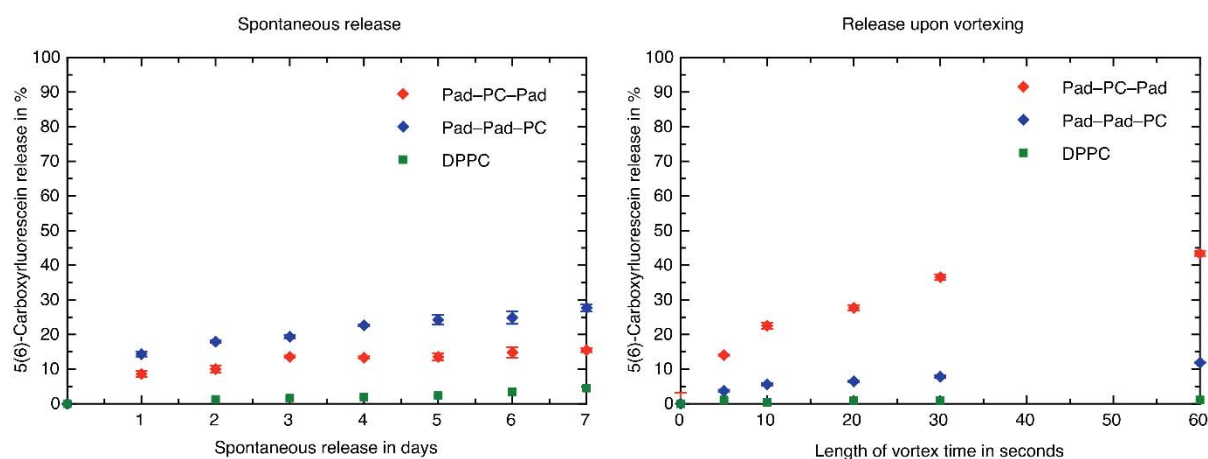


Figure SI 13: Release data from long-term and short-term release experiments (under mechanical stress) performed with LUVs formulated with Pad-PC-Pad (2), Pad-Pad-PC (1) and DPPC (3). Compared to the other two phospholipid vesicles, Pad-Pad-PC vesicles show an intermediate mechanoresponsiveness.

## Hydrogen-bonding network

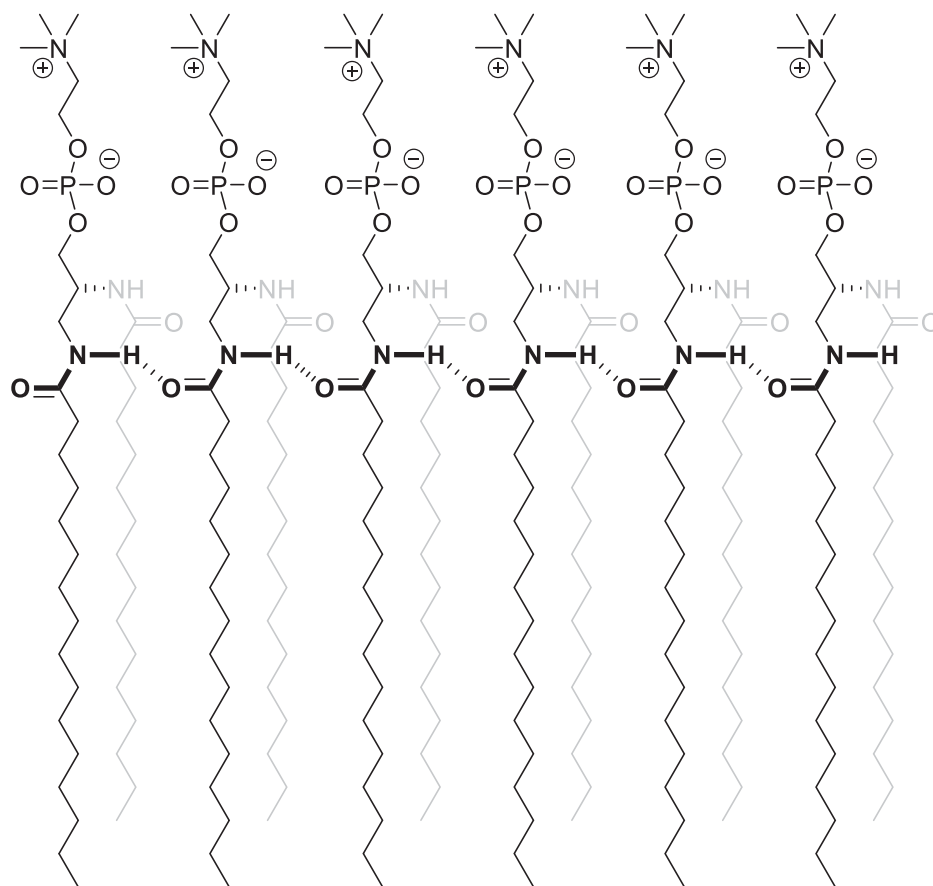


Figure SI 14: Artist rendering of the possible intermolecular hydrogen-bonds in a Pad-Pad-PC (**1**) monolayer.

## Lipid tail packing density

Table SI 5: Lipid tail packing density.<sup>5</sup>

Lipid	$\Pi/\text{mNm}^{-1}$	T/°C	$A_{xy}/\text{\AA}^2$ per lipid tail	$A_0/\text{\AA}^2$ per lipid tail	tilt angle
3	45	15	22.4 <sup>6</sup>	20.3	25° <sup>6</sup>
C16:0-Dietherlipid	41	15	21.8 <sup>6</sup>	20.3	21° <sup>6</sup>
2	35	5	23.7	20.2	31.5°
1	25	15	20.1	20.1	0°

<sup>5</sup> H. Möhwald, H. Baltes, M. Schwendler, C. A. Helm, G. Brezesinski, H. Haas, *Jpn. J. Appl. Phys.* **1995**, 34, 3906–3913.

<sup>6</sup> G. Brezesinski, A. Dietrich, B. Struth, C. Böhm, W. G. Bouwman, K. Kjaer, H. Möhwald, *Chem. Phys. Lipids* **1995**, 76, 145–157.



## Monolayer phase diagram

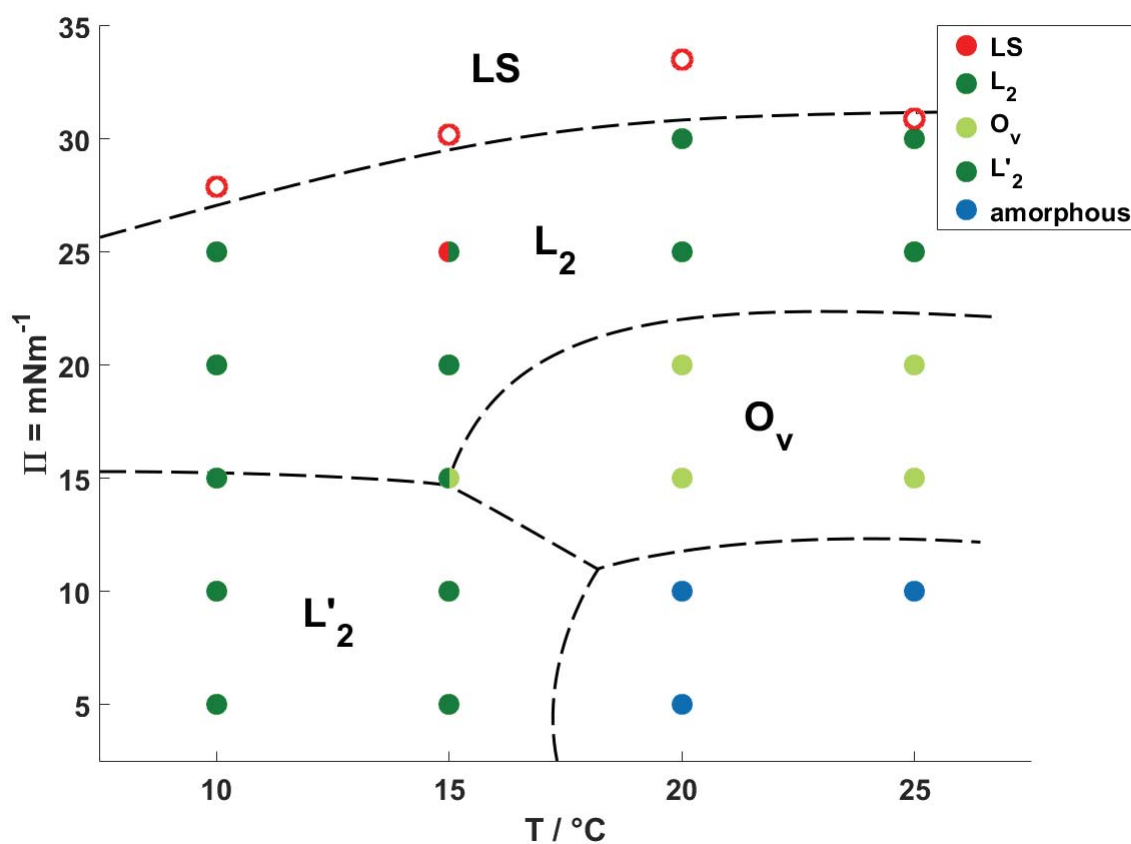


Figure SI 15: Phase diagram for Pad-Pad-PC (1) in the monolayer at the air/water interface.

The dashed lines were set as guides for the eye. The filled circles represent GIXD measurements, the open circles are the onset of the LS phase in the molecular area/surface pressure diagram Figure SI 5.