

Probing the Elasticity of Langmuir Polymer Films of High Molecular Weight Poly (γ -benzyl-L-glutamate)

Master thesis

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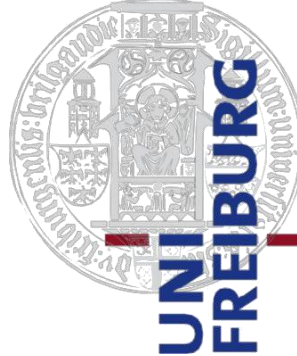
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Germany

July 2016



Einreichung der schriftlichen Masterarbeit

im Studiengang M.Sc. Sustainable Materials

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Acknowledgments

I would first like to thank Prof. Dr. Günter Reiter for giving me the opportunity to do my master project inside his group and for his scientific advices.

I am very grateful to Prof. Dr. Heiko Heerklotz for accepting to be my second reviewer.

I would like to express my sincere gratitude to Dr. Renate Reiter who led my project, for her continuous support, for her patience, and her guidance in all the time of research.

I would also like to thank Dr. Sivasurender Chandran, Mrs. Stefanie Dold and all the colleagues of Experimental Polymer Physics group for sharing their scientific knowledge and for their nice attitude.

At the end I would like to thank all the Professors, Doctors, members and colleagues of Freiburg and Strasbourg universities.

Abstract

Poly (γ -benzyl-L-glutamate) (PBLG) Langmuir Polymer films at high surface densities were compressed and expanded repeatedly. During expansion a very rapid initial drop of surface pressure occurs at areas per molecule which are smaller than expected for a closely packed array of helical rods of PBLG. This is followed by an extremely slow pressure decrease represented by a nearly horizontal line when the area is expanded to values even larger than the initial value obtained by material deposition. The same behavior of expansion curves is observed when the films are exposed to successive cycles of compression and expansion. However, with each cycle the shallow decreasing part of the curve is shifted to lower surface pressures, while essentially keeping the same slope. Even more surprising is the observation that re-compression traces show a seemingly negative compressibility i.e., a decrease in pressure during compression.

The present study focuses on understanding this surprising behavior.

Snapshots of the morphologies during these cycles were taken to correlate the information from the compression/expansion curves with structural information. Applying the Langmuir-Schäfer technique, we transferred the films to solid substrates at different surface densities during compression and also at different surface densities during expansion. We also changed the lateral position of the transferred film fragment with respect to the barriers and Wilhelmy device to investigate the lateral homogeneity of the film. The transferred film fragments were probed with Atomic Force Microscopy.

Our findings suggest the formation of a stable solidified layer at high surface pressures, which apparently did not disassemble upon expansion as the microscopic morphologies of compressed and expanded state resemble strongly when investigated at the microscale. However, probing the lateral homogeneity on the entire trough area shows, that the Langmuir film suffers local ruptures during expansion.

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Introduction

Insoluble Langmuir Polymer Films (LPFs) at the air water interface represent an important system for studying physical, chemical and biological phenomena in reduced dimensionality [1].

LPFs in dense states can exhibit a high degree of metastability [2]. Indeed surface pressure-area measurements recorded with constant rate compression might take the system out of equilibrium. This can be observed in particular when the time which elapses during the area reduction is shorter than the time needed to obtain an equilibrated organization of the molecules. The resulting film will consist of molecules with frozen conformations which are not able to optimize the coverage of the available surface and thus voids will be present. Interrupting the movement of the barriers during such an experiment will consequently show a decay of surface pressure with time as the molecules gradually rearrange and fill these voids.

Significant hysteresis between compression and expansion can also occur and depend strongly on the experimental protocol [3].

Polypeptide model-systems at well-defined interfaces constitute a useful means on studying the molecular orientation at interfaces and have a significant importance in biological and technological processes [4]. Indeed the surface pressure/area isotherms reflect the intermolecular interactions operating in the 2D array of molecules and provide information on the molecular packing [4].

The homo-polypeptide Poly (γ -benzyl-L-glutamate) (PBLG) can adopt helical conformation and is therefore considered as a suitable model for investigating the phase behavior of rod-like polymers at the air –water interface [5] [6].

In three dimensional systems, (PBLG) can exist in different phases (gels, liquid crystals...) [7]. One important question is if a similar behavior in quasi 2D systems will be observed.

The central subject of this thesis is the investigation of a totally unexpected hysteretic behavior when these Langmuir films are subject to expansion which was observed while studying the phase behavior of these Langmuir films.

1. PBLG Structure and Properties

The chemical structure of the Poly (γ -benzyl L-glutamate) (PBLG) is shown in figure 1.

PBLG is a molecule constituted of a polypeptide chain, with bulky and flexible side chains. Similar to natural polypeptides, synthetic polypeptides like PBLG can adopt α -helical conformation (figure 2), in helicogenic solvents [8].

The polymer backbone is stabilized by intra-molecular hydrogen bonds between the carbonyl function C=O of the i th residue and the amide group NH of the $i+4$ th residue [9].

In addition, the α -helical structure has a permanent electric dipole moment of 3,4D per repeating unit in direction of the helical axis [10]. PBLG has 18 monomers per 5 helical turns with a helical pitch of 0.54 nm [11]. The translation length along the helical axis per monomer is 0.15 nm.

PBLG has a basically hydrophobic character due to the helical structure and the presence of the benzene group. The molecule shows also a slight polarity due to the presence of the ester group in the side chains, but this hydrophilicity is very low and might only allow for a weak interaction with the water surface.

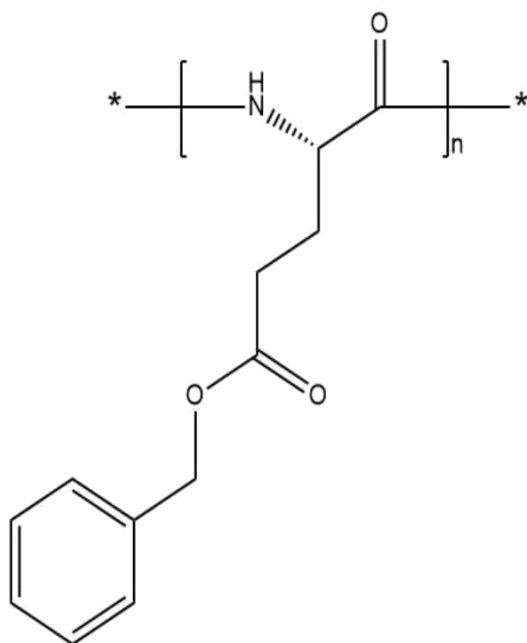


Figure 1: Schematic representation of poly (γ -benzyl-L-glutamate)

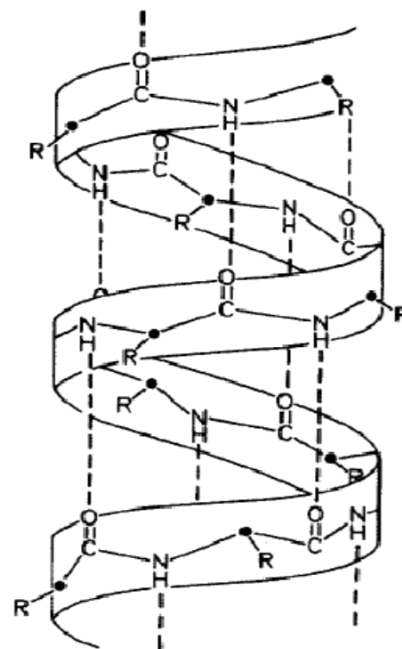


Figure 2: Schematic representation of α -helix with hydrogen bonds [12]

2. Surface Pressure and Wilhelmy Method

In studies of Langmuir films, usually the difference in surface tension between a pure liquid surface and the one covered with a film is measured [13].

This difference is called surface pressure and is determined by this equation:

$\pi = \gamma_0 - \gamma$, where γ_0 is the surface tension of the pure liquid and γ is the tension of the film covered surface. This surface pressure is related to a force acting on the measuring plate [13].

We use the Wilhelmy method, which measures the force on a plate partially immersed in the liquid and suspended to the spring of an inductive displacement transducer. We used a small piece of a filter paper as a plate [13]. The forces acting on the plate consist of the gravitational and surface tension effects, downward, partially compensated by the buoyant force due to the weight of the displaced liquid [13]. As

shown in figure 3, for a rectangular plate of dimensions l , and of material of density ρ , the net downward force is given by this equation [13]:

$$F = \rho g l w t + 2\varphi(t + w)\cos\theta - \rho g l w h$$

Where φ is the surface tension of the liquid, θ is the contact angle of the liquid on the solid plate and g is the gravitational constant.

Here we assume a complete wetting and therefore the contact angle is 0, and the measured force is related directly to the surface tension of the liquid.

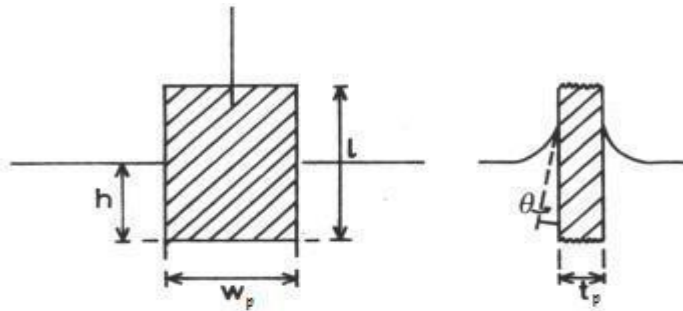


Figure 3: The Wilhelmy plate method [13]

3. Atomic Force Microscopy

Atomic Force Microscopy (AFM) has an important impact in different scientific fields. It is basically measuring the topography of a sample which should have certain rigidity to allow for scanning its surface to obtain an image.

AFM causes bending of a cantilever with a sharp tip in order to measure the forces acting between the sample surface and the tip.

The forces could be attractive or repulsive. The forces could be Van der Waals forces, adhesion and friction forces, electrostatic forces or magnetic forces.

Optical detection systems and micro-fabricated cantilevers can detect forces in AFM at a range down to the Pico Newton **[14]**.

Depending on the application and the required resolution there are different modes differing if the tip is mounted on the axe and the sample is scanned or vice versa. The AFM measures tip-sample displacement. The movement of the tip is performed by means of a piezo-drive, which consists of three mutually perpendicular piezoelectric transducers: x-piezo, y-piezo, and z-piezo. A piezoelectric transducer expands or contracts by applying a voltage. For instance by applying a voltage on the x- piezo and a voltage on the y-piezo, the tip scans on the xy-plane. Using the positioner and the z-piezo, the tip and the sample can approach to a few angstroms with respect to each other **[15]**.

The AFM can be operated either in static or dynamic mode **[16]**.

In the static mode, which is also called contact mode, the tip and the sample are brought into contact so that electronic orbitals of the atoms of the tip and the sample overlap. In contact mode, the tip scans over the surface, so the height of the cantilever does not change, this is known as constant height imaging.

In the dynamic mode, the tip is brought close to the sample (a few nanometers), but not into contact. We use this technique, in order to avoid any sample (or tip) crash or deformation. Indeed the normal pressure exerted at the interface is close to zero. This makes the non-contact mode preferable to the contact mode. By measuring the tip-sample distance we can have a topographic image of the sample surface.

Indeed the cantilever is an oscillator which will vibrate over the surface. We can record the oscillation response in frequency and amplitude for the frequency and the amplitude input we choose.

4. Material and Experimental Techniques

4.1 Preparation of the Solution

PBLG with an average number of 768 monomer units corresponding to a number average molar mass of 168100 g/mol was synthesized by ring opening polymerization of γ -benzyl-L-glutamate N-carboxy anhydride and purchased from Sigma Aldrich.

The PBLG molecule was dissolved in chloroform to a final concentration of 0.3 mg/ml. We choose this solvent because of its low boiling point and its low surface tension which facilitates the spreading of molecules on the surface of pure water and because of its helicogenic properties [17].

4.2 Preparation of the Langmuir Trough

PBLG Langmuir polymer films were formed by spreading the solution on the subphase using a Hamilton micro syringe. Double and de-ionized water was used as a subphase.

The isotherms were measured in a Teflon trough built by Riegler and Kirstein (R&K) having a total surface area of 192 cm²; equipped with a Wilhelmy system using filter paper (3mm \times 15mm) as a plate and two motor driven barriers.

The Wilhelmy plate was positioned in the middle between the two barriers. The temperature of the subphase was maintained at 20 °C by circulating thermostated water through the base plate of the trough.

The PBLG molecules on the water surface were compressed by the two moving barriers and the surface pressure was recorded as a function of area per molecule. The films were compressed at a rate of 5 cm²/min, which corresponds to 10 Å²/min per monomer, when 90 μ L of solution were spread on the trough.

The crucial step in preparing a Langmuir polymer film is the spreading onto the water surface.

Therefore, a careful procedure of depositing the solution was established by placing the droplets at well-defined positions and keeping their size as constant as possible.

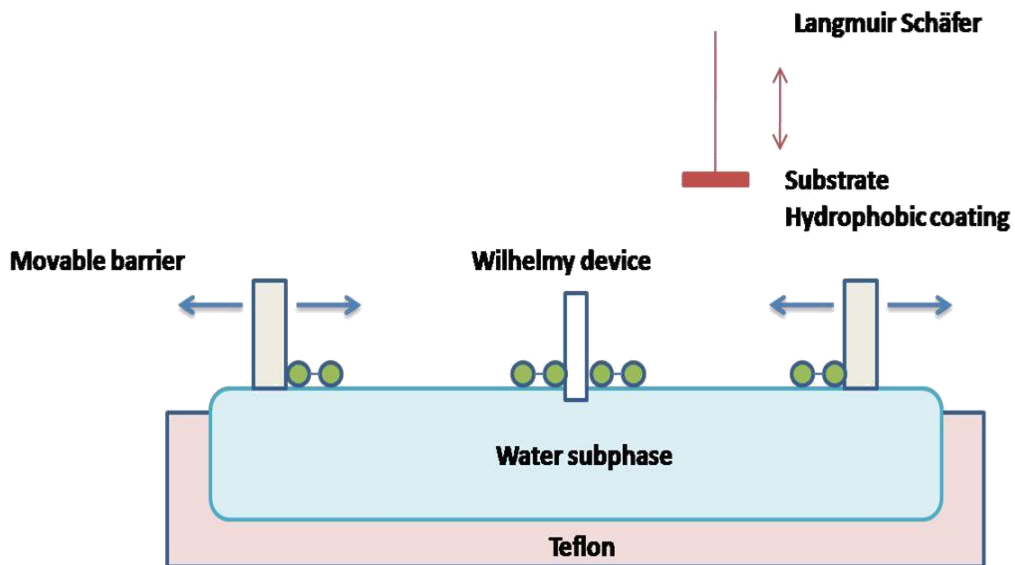


Figure 4: Schematic representation of the Langmuir Trough

4.3 Preparation of the Silicon Substrate

Silicon wafers from Silchem Handelsgesellschaft GmbH (Freiberg, Germany) were cut into small pieces (2cm×1,5cm) and coated by PDMS. First the silicon wafer was exposed to a Digital UV Ozone System by Novascan. The oxide layer is activated by UV irradiation in presence of distilled water for 60 minutes.

The activated wafer was then washed few times with heptane prior to coat it with PDMS (1 wt. % in heptane, simultaneously heated at 60 °C) at 2000 rpm for 2 minutes. The coated films were kept at the temperature of 170 °C for 6 hours to remove the excess solvent and enhance the adsorption of siloxane groups to silicon substrate. This reaction eventually leads to a irreversible adsorption of PDMS on the activated Si-wafer. The substrates are then washed in a bath of heptane for 60min to remove all the

unabsorbed PDMS monomer. At the end we obtain a hydrophobic layer which can undergo hydrophobic interaction with PBLG.

4.4 Transfer of Polymer Films

The films were transferred from the water surface to the PDMS coated silicon substrates through the Langmuir-Schäfer method.

The substrate was positioned parallel to the water surface. The substrate was approached to the water surface at very low speed to avoid large perturbations of the water surface, which could create modifications of the morphology of the formed Langmuir polymer films [17]. The wafer never immersed in the water and after a contact time of 60 s the substrate was retracted. This time interval is sufficient to account for a damping of the surface waves created through the approaching wafer and should therefore reduce possible artifacts due to the transfer protocol [17].

The obtained films showed high stability for periods of several months.

5. Results and Discussion

5.1 PBLG Isotherm

The isotherms provide information about the thermodynamic properties of molecules at the air-water interface [18].

They constitute a fundamental step in any subsequent study of a Langmuir film; it is basically the measurement at constant temperature of the surface pressure as a function of area per repeating unit [18]. Isotherms can reveal the existence of several phases with transitions between them which appear as changes in slope and sometimes the existence of a plateau. This corresponds to discontinuities in the free energy landscape of the film [18].

The first step in preparing a Langmuir polymer film is the spreading of the dilute polymer solution on the surface of water. After evaporation of the solvent, the water surface is populated with insoluble polymer molecules. The γ_0 surface tension of water decreases when the film is formed thereby exerting a surface pressure $\pi = \gamma_0 - \gamma$, where γ the surface tension of water covered by the polymer molecules [13].

Figure 5 shows a Brewster angle micrograph directly after spreading and allowing for the evaporation of the solvent. No movement of the barriers has been performed.

Here we can observe the presence of large compact islands of material separated by smaller dispersed material patches, which are locally interconnected through fibrous elements. The observed structures were indeed the result of the spreading procedure.

This indicates that already in the gaseous phase, cohesion and locally dense packing of the rod molecules takes place. Evidently the system has the property to form aggregates even without applying an external pressure.



Figure 5*: BAM Image of a Langmuir PBLG film in the gaseous phase, before moving the barriers Image size 510*485 μm^2

*BAM image made by Ahmed Eltawarji.

Figure 6 shows the isotherm of PBLG (768) performed under the following experimental conditions; a concentration of 0.3 mg / ml, a volume of 90 μ l of the solution deposited on the water surface and a compression rate of the order of 5cm² / min.

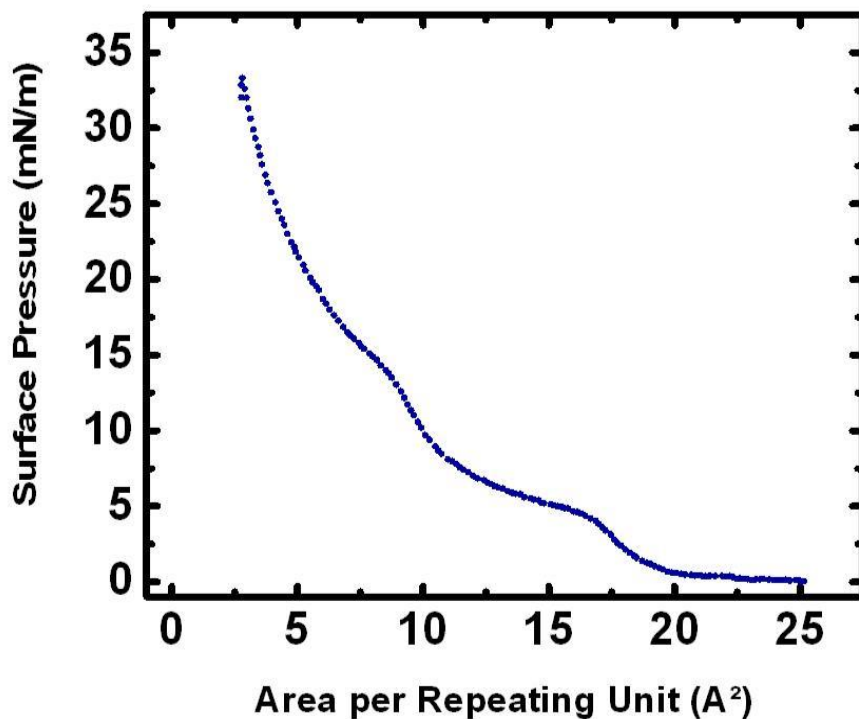


Figure 6: A characteristic isotherm, surface pressure versus area per monomer unit of PBLG (768)

The surface pressure remains initially very low at large specific areas even when reducing the area per monomer by compressing the film. This indicates that the water surface is not completely covered with molecules.

The surface pressure increases more or less rapidly when the film is compressed to the point where the macromolecules themselves occupy most of the available surface. With increasing compression the film becomes more compact as the surface area per

monomer gets smaller and thus the rearrangement of the molecules is constrained because of the limiting space. The molecules will be in metastable states after compressing the polymeric film at a constant rate up to high packing densities. This is concluded from the observation that the surface pressure starts to decay when the movement of the barriers is stopped and can be explained by the fact that the molecules cannot reorient and reorganize their conformations fast enough to attain their minimum in free energy.

From this isotherm, we also notice the presence of slope changes which tell us about the presence of zones of transition.

Additional information is needed for a detailed understanding and a molecular interpretation of the isotherm. This will be discussed later by the investigation of the morphological features of PBLG molecules by means of the Atomic Force Microscopy.

5.2 Cycle of Compression-Expansion-Recompression

The measurements of hysteresis cycles provide information on the reversibility of the transitions of the film and its characteristics [2].

Performing several compression and expansion cycles consecutive allows us to obtain a better understanding of the process governing the hysteresis [2].

At this experiment we initially perform a compression to the maximum values of pressure that we can reach with these experimental conditions in order to achieve the condensed phases and then we expand the system. The obtained results are shown in figure 7.

As we can see during the expansion cycle, a very rapid initial drop of surface pressure occurs at areas which are smaller than expected for a closely packed array of helical rods of PBLG. This is followed by an extremely slow pressure decrease represented by a nearly horizontal line when the area is increased.

The re-compression trace shows a seemingly negative compressibility i.e., a decrease in pressure with compression. The variation of the surface pressure as a function of time shown in figure 8 indicates a linear decrease of the surface pressure, which is quite

surprising. We performed the same experiment several times and the same behavior occurred in a strongly reproducible manner.

Therefore a systematic study to shed light on this strange hysteretic behavior was initiated. We aimed at detecting structural elements in the Langmuir layers that are capable to bear elasticity.

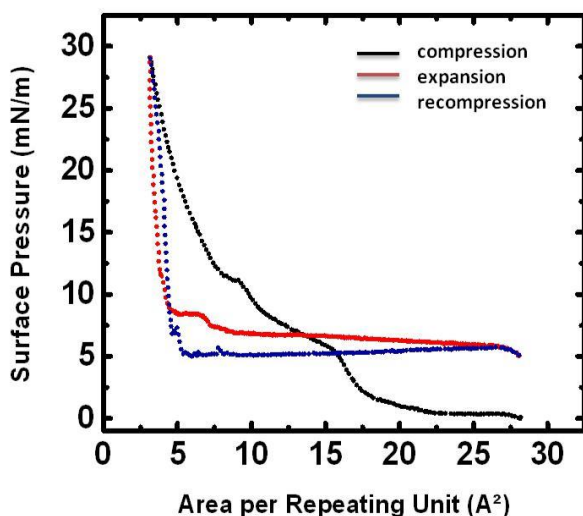


Figure 7: Cycle of compression-expansion-recompression

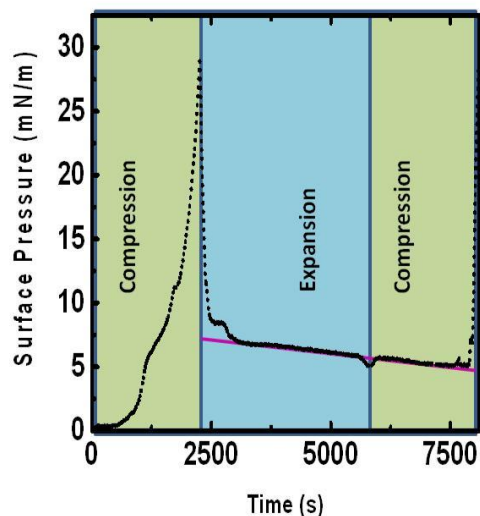


Figure 8: Variation of surface pressure as a function of time showing the linear decrease of the pressure

5.3 Successive Cycles of Compression-Expansion

Poly (γ -benzyl-L-glutamate) Langmuir polymer films at high surface densities were compressed and expanded repeatedly. On complete expansion we observed a non-zero pressure, which decreased when increasing the number of compression and

expansion cycles. After each cycle, the very shallow decreasing part of the curve is shifted to lower surface pressures while essentially keeping the same slope.

Even more surprising, the re-compression traces show a seemingly negative compressibility, i.e., a decrease in pressure with compression, as shown in figure 9.

In order to understand this behavior, we will investigate the influence of compression – expansion forces on the morphological features of Langmuir Polymer films of PBLG by means of Atomic Force Microscopy.

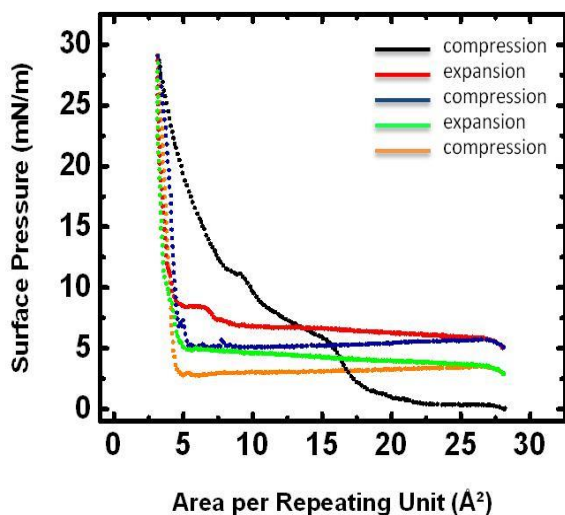


Figure 9: Successive cycles of compression-expansion

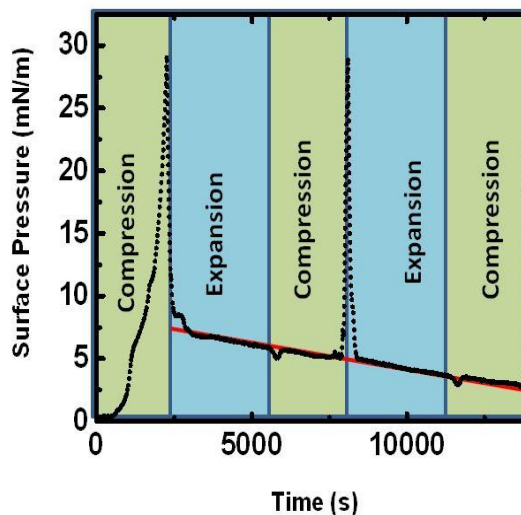


Figure 10: Variation of surface pressure as a function of time showing the linear decrease of the pressure

5.4 AFM Measurements of PBLG Langmuir Polymer Films

The films were transferred through the Langmuir Schafer method at different surface pressures and accordingly at different packing densities along the isotherm.

Figure 11 indicates the precise isotherm positions of the sample transfers.

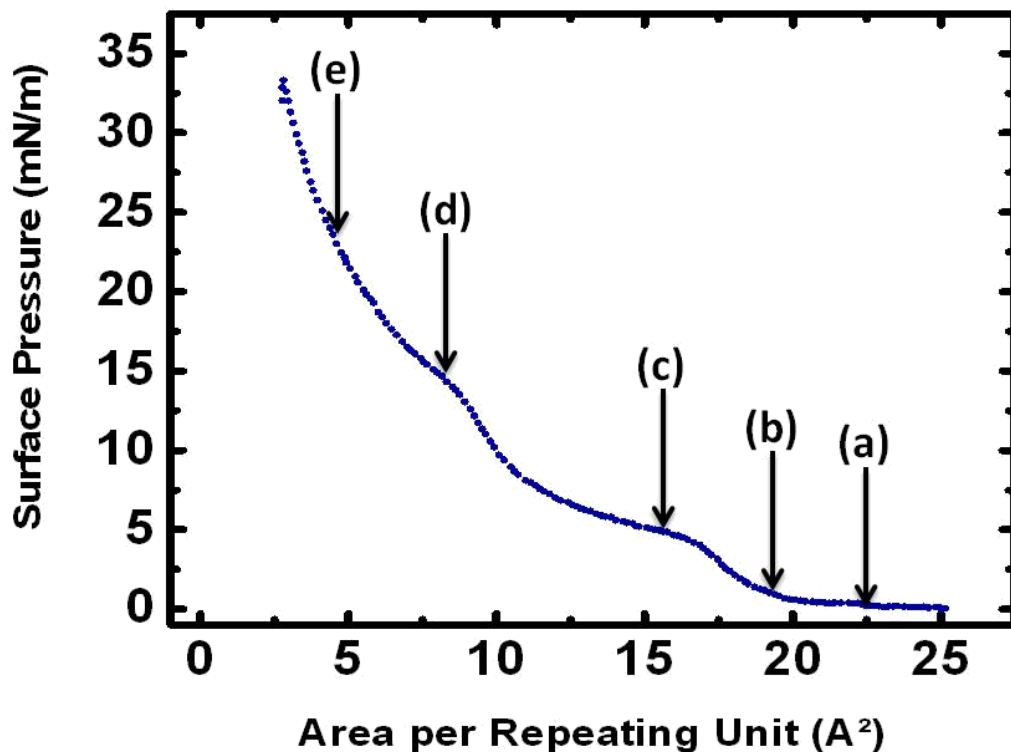


Figure 11: Surface pressure-area isotherm of PBLG (768) measured at 20°C: The arrows indicate the positions, where AFM images of the morphology of the films were taken.

The black arrows represented at the left sides of the AFM images indicate the direction of compressing by the barriers.

The resulting AFM images in figure 12 obtained at an area per repeating unit equal to 22,5 Å² in the region labeled with (a) in the isotherm in figure 11 show the presence of randomly distributed domains of fiber-like network structure domains. We notice as well the curvature of the fibers (Figure 12 b) which was not observed for smaller molecules in previous studies [17].

For such large areas, the observation of a network of interconnected fibers is quite surprising. In the literature, molecularly dispersed rods were postulated for the gaseous phase.

This indicates that such networks form already during the spreading process because of the attractive forces between PBLG rods.

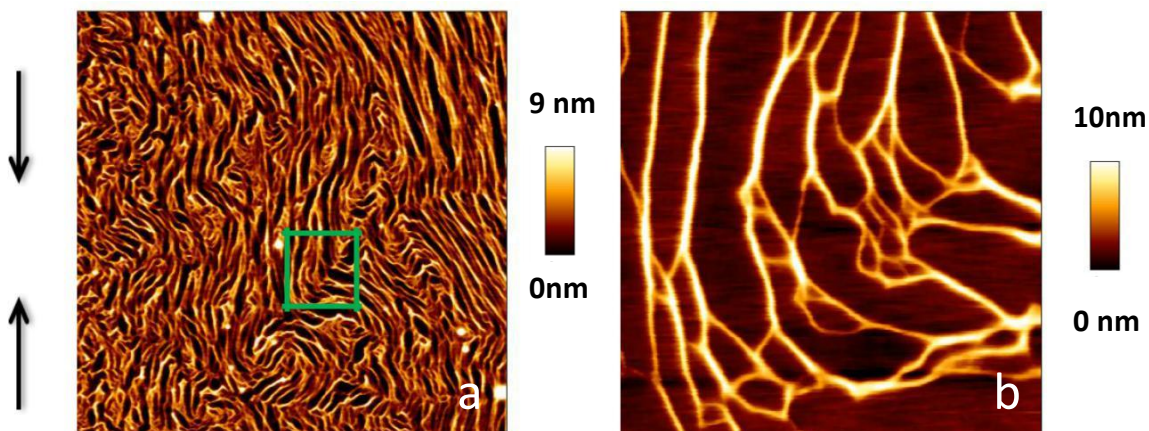


Figure 12: a) AFM height image taken at position **(a)** in the isotherm of Figure 11 Image size: $8 \times 8 \mu\text{m}^2$. b) Corresponding AFM height image of a zoomed region of a) Image size: $1 \times 1 \mu\text{m}^2$

In the following, we examine the morphology in the region labeled with (b) in the isotherm in figure 11 where the surface pressure was slightly increased. The molecules are brought closer together by continuous rate compression until a pressure of 2 mN/m is reached. We could notice that increasing the compression intensified the local shear forces, and caused local changes in the orientation of the fibers.

This can be seen in the AFM image in figure 13. In comparison to the state at large area per repeating unit, which consisted of a randomly oriented network structures, the fibers here have the tendency to align with respect to the compression direction which is indicated by two black arrows on the left side of figures.

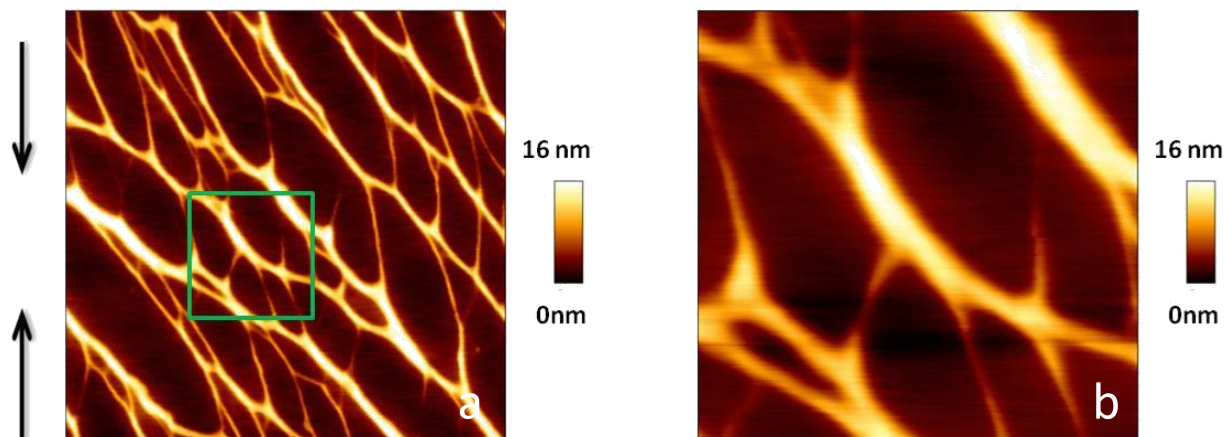


Figure 13: a) AFM height image taken at position **(b)** in the isotherm of Figure 11 Image size: $4 \times 4 \mu\text{m}^2$
 b) Corresponding AFM height image of a zoomed region of a) Image size: $600 \times 600 \text{ nm}^2$

We continued to increase the surface pressure and then checked the morphology of our PBLG films at position (c) in the isotherm of figure 11 which is located at 6A^2 .

At this state as shown in figure 14, we can observe several domains with fibers which are oriented in different directions. At the boundaries of these domains, we observe a local coarsening of the fibers (figure 14c) as they show increased height and width.

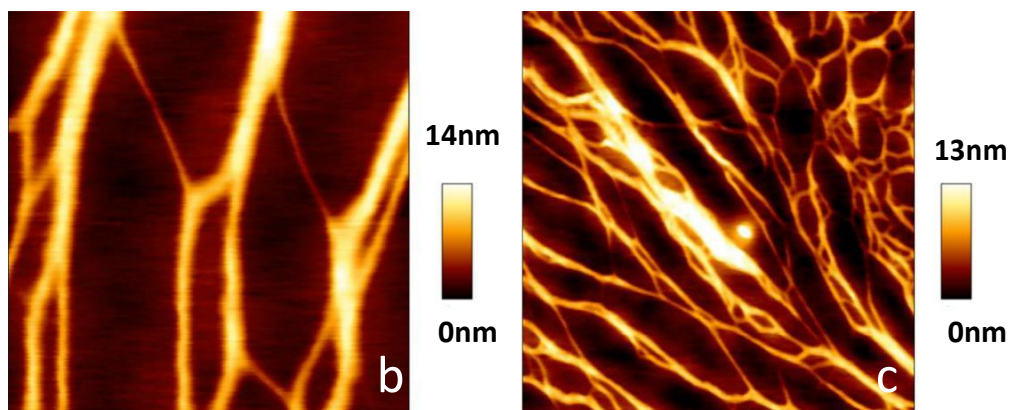
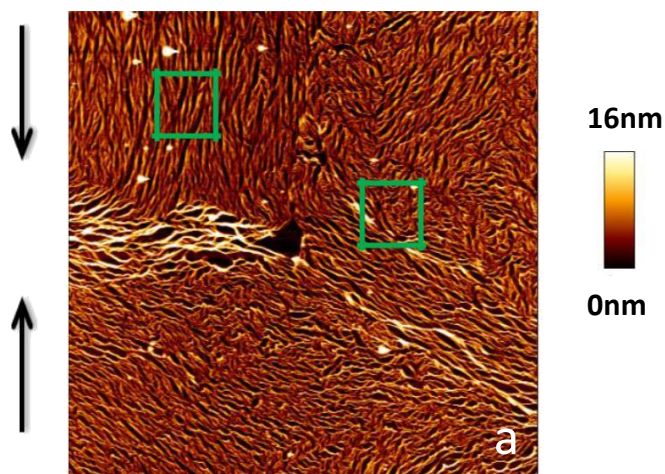


Figure14: a) AFM height image taken at position **(c)** in the isotherm of Figure 11 Image size: 20x20 μm^2 , b) Corresponding AFM height image of a zoomed region of a) Image size: 600x600 nm², c) Corresponding AFM height of a zoomed region of a) Image size: 2x2 μm^2

Upon further compression to position (d) in the isotherm, lateral coalescence of fibers continued. The AFM image presented in figure 15 shows a large empty space between the wider fibers. We can as well notice an enhanced orientation of the fibers with respect to the direction of compression as they show an almost perpendicular orientation now.

In terms of height, we observe an increased value compared to the previous pressures. This is indicative of a subsequent coarsening process leading to still enlarged fibers through increasing the surface pressure.

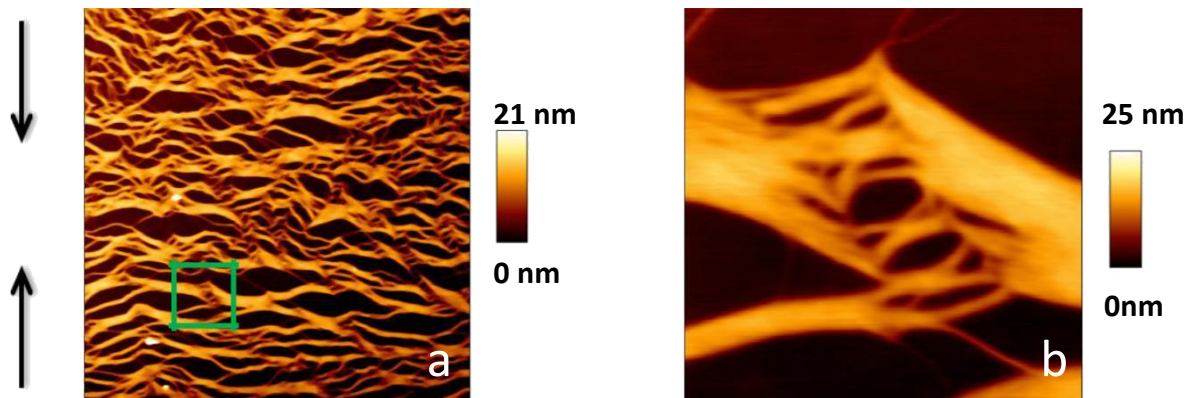


Figure 15: a) AFM height image taken at position **(d)** in the isotherm of Figure 11 Image size: $10 \times 10 \mu\text{m}^2$ b) Corresponding AFM height of a zoomed region of a) Image size: $1 \times 1 \mu\text{m}^2$

Finally, we present an AFM image in figure 16 taken at position **(e)** in the high density region of the isotherm shown in figure 11.

There the area per repeating unit had a value of approximately $5A^2$ which is considered too small to have enough free space for all helical rods within the plane.

The AFM image showed a large empty space between the wider fibers, and a highly compacted structure. This indicates that the area was more covered compared with the previous transfer at the position **(d)** in the isotherm. However we didn't notice any difference in the height of the fibers.

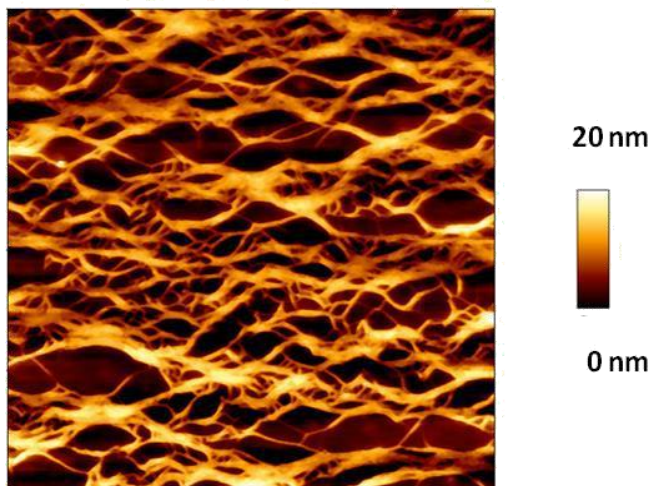


Figure16: AFM height image taken at position **(e)** in the isotherm of Figure11
Image size: $4 \times 4 \mu\text{m}^2$

From the AFM measurements realized at those specific surface pressures in the isotherm of figure 11, we can conclude that Langmuir films of PBLG at high surface densities are a result of a concerted orientation with respect to the flow during compression and a subsequent coalescence of fibers.

We proceeded as well performing transfers through the Langmuir Schäfer method at the state of expansion. We examined first the morphology of our PBLG molecules in the region labeled with (f) in the cycle of compression-expansion in figure 17, which corresponds to a very low area per monomer.

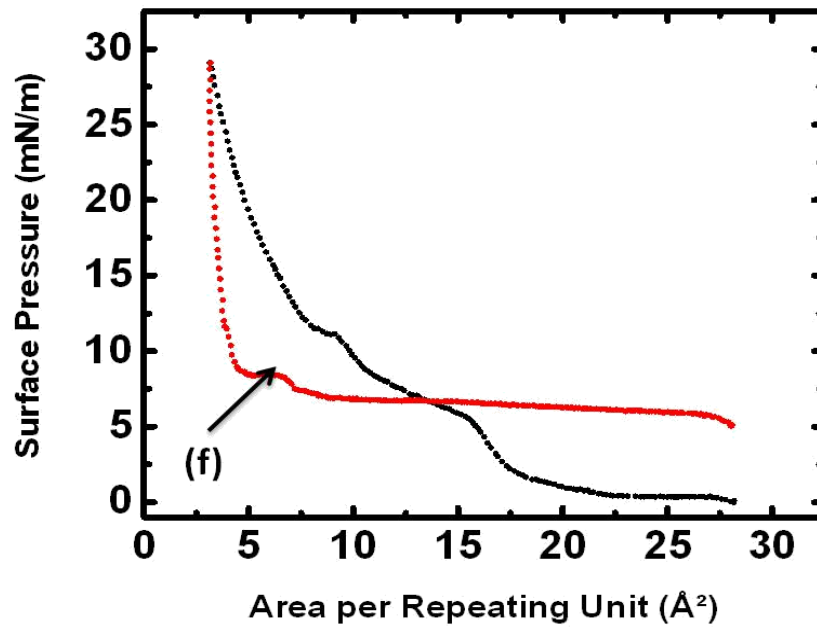


Figure17: LS transfer at expansion state at a reduced area per repeating unit ($6,5\text{\AA}^2$) close to the Wilhelmy plate indicated by the red arrow

The AFM image taken at this position is represented in the figure 18. It showed a structure with large empty space between wider fibers, and a highly compacted structure.

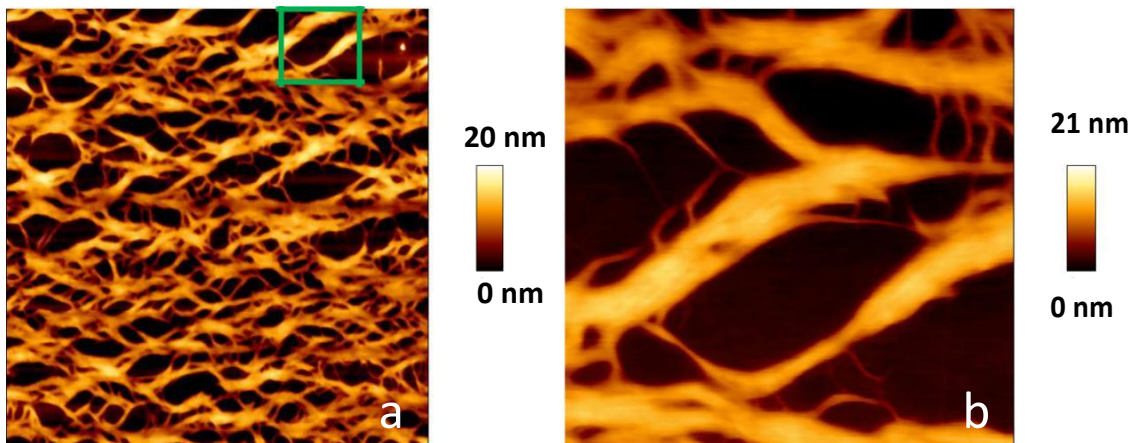


Figure18: a) AFM height image taken at position (f) in cycle of compression-expansion of Figure16 Image size: $4\times 4\mu\text{m}^2$ b) Corresponding AFM height of a zoomed region of a) Image size: $1\times 1\mu\text{m}^2$

If we make a comparison between the two structures as shown in figure 19, we can notice the similarity between them in terms of morphology (compact structure), height and surface coverage (66% for the transfer realized at compression and 62% for the transfer realized at expansion; here we used imageJ software to analyse the area coverage by using threshold parameters).

This is indicative that the structure remained the same after expansion of the barriers.

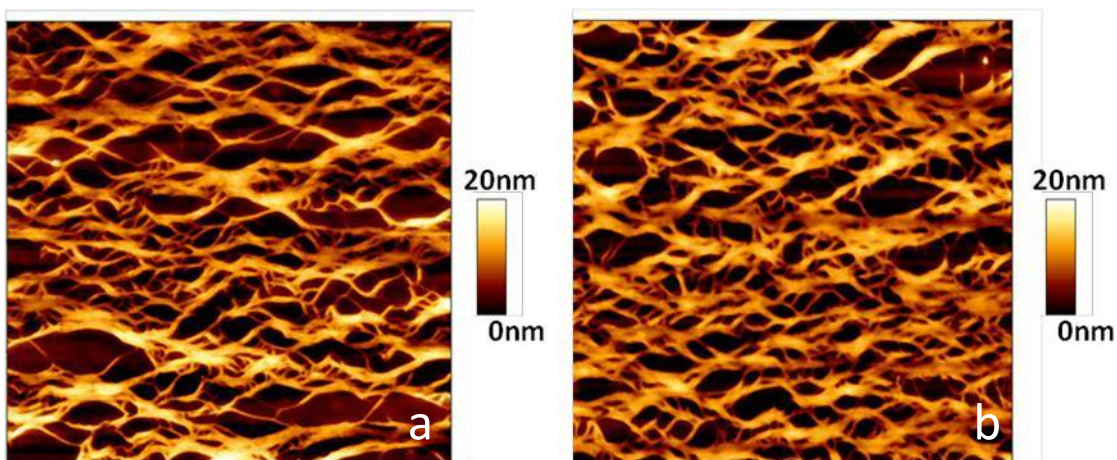


Figure19: Comparison between a) AFM height image taken at position **(e)** in the isotherm of Figure11 Image size: $4 \times 4 \mu\text{m}^2$ b) AFM height image taken at position **(f)** in the isotherm of Figure 11 Image size: $4 \times 4 \mu\text{m}^2$

We proceeded by realizing the transfer of the film at more larger area per repeating unit at the expansion state (Figure 20).

After checking with the Optical Microscope and the Atomic Force Micoscope, we didn't find any presence of a PBLG film transferred to the substrate, even after reproducing the experiment several times.

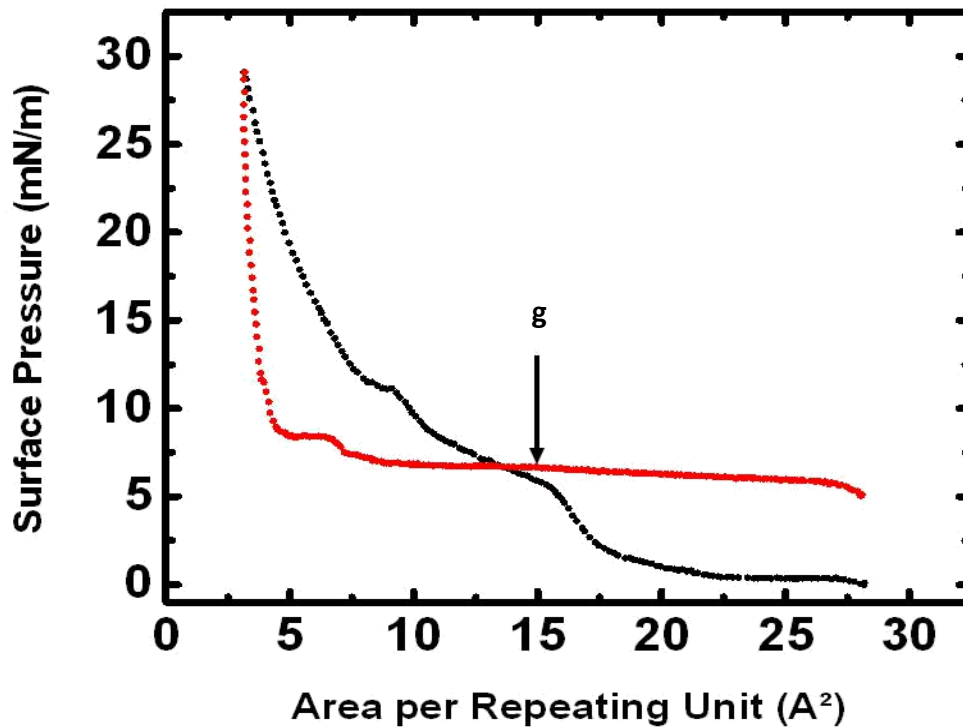


Figure 20: LS transfer at the expanded state at a large area per repeating unit (15 A^2) after moving the barriers indicated by the red arrow

Figure 21 shows such an experiment which consisted in a transfer at a certain small area.

In a next step the lateral homogeneity of these expanded films was probed on a larger scale by transferring samples at different positions at the surface of the trough with respect to the center where we usually transfer (Figure 21).

The film was not expanding when increasing the surface area. In fact it was breaking and attached one fragment to the Wilhelmy plate and other fragments to the barriers. The observed negative pressure was probably resulting from the additional weight of the film fragments hanging on the filter paper.

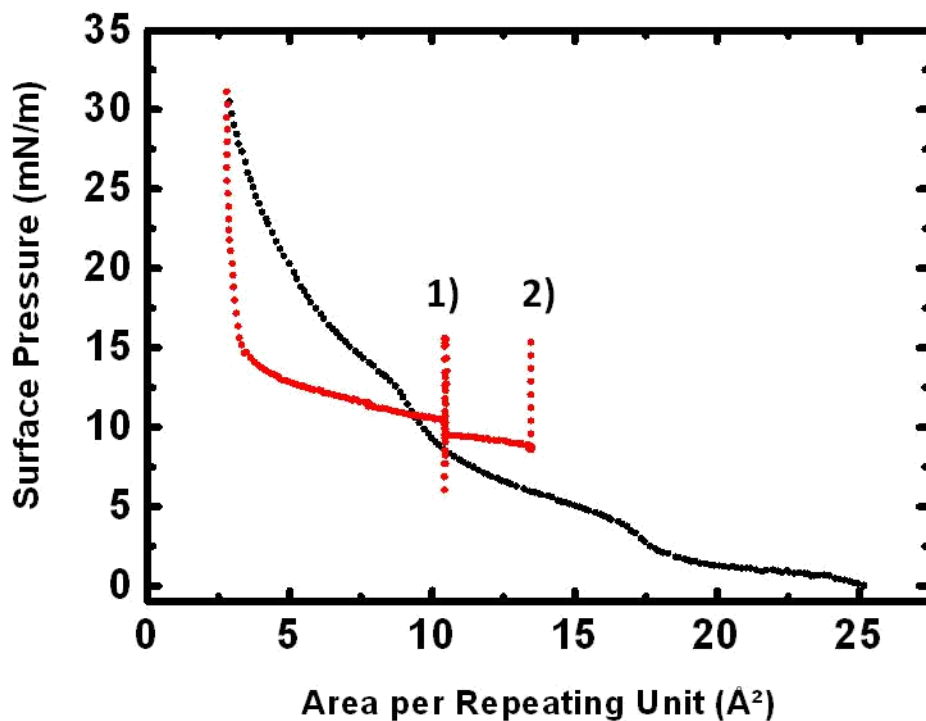


Figure21: LS transfer at expanded state after moving the barriers at large area per repeating unit

The obtained results are shown in figure 22. In the case of both transfers, the Optical Microscope images showed one part which was covered by the film and one part was showing only the presence of the PDMS-Si substrate.

This explains why we were not able to obtain the film in the previous experiment, where we made the transfer at large area per repeating unit far from the Wilhelmy plate.

At high pressures, a stable solid-like film was formed, which did not disassemble upon expansion. The PBLG molecules were indeed attached to the Wilhelmy plate and to the barriers and the attempts to transfer in the middle of the trough failed.

From this we can understand as well the origin of the negative pressure observed during the hysteresis experiments; Indeed the negative values of surface pressure indicate that a film fragment was attached to the Wilhelmy plate after reaching a

pressure of certain height, and pulling it lower. If the pulling force is more, the surface tension of the film is increasing, and will have a higher value than the surface tension of pure water. As a result the surface pressure will exhibit a negative value according to this equation $\pi = \gamma_0 - \gamma$.

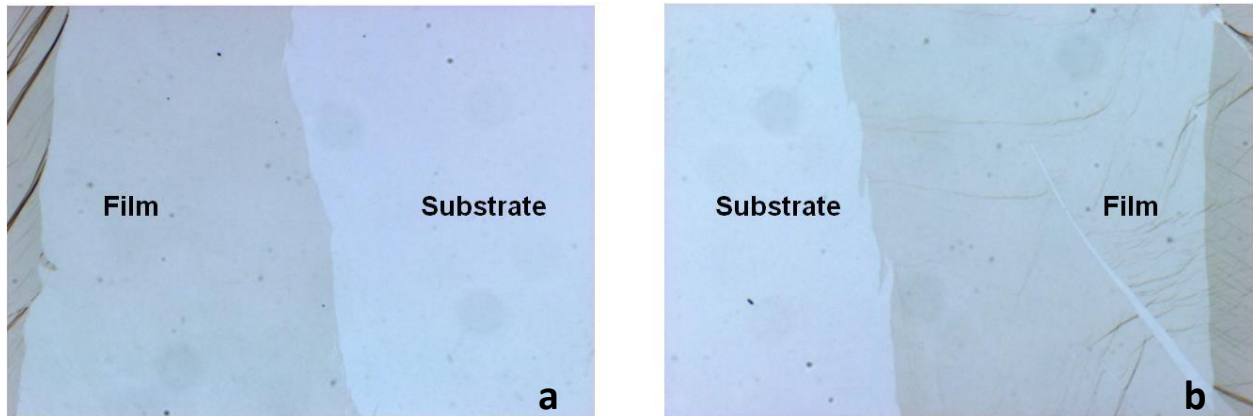


Figure 22: Representative Optical Microscope images showing the transfers done at the areas indicated in the isotherm of Figure: 21, a) close to the Wilhelmy plate, b) close to the barriers
Images sizes 648x486 μm^2

The BAM images shown in figures 23 and 24 confirm the previous observations.

Figure 23 shows the BAM image of a film with homogeneous regions and some defects probably at the boundaries of the domains.

Upon expansion, we observe the rupture of the film as shown in figure 24 in two big blocks of accumulated material separated by very small material patches floating at the water surface.



Figure 23**: Compact film during compression $510 \times 485 \mu\text{m}^2$



Figure 24**: Film rupture during expansion $510 \times 485 \mu\text{m}^2$

**BAM images made by Sabrina Kraus

Conclusion

According to our transfer results we observed cohesion between the rod like molecules which already occurred in the “gaseous” phase and led to the formation of a fiber-like network.

In addition a coalescence of these fibers occurs when they are aligned parallel and packed densely. With increasing surface pressure the coarsening gets more pronounced.

Our findings suggest the formation of a stable solid-like layer at high pressures observed which did not allow for disassembling upon expansion.

Furthermore the surface pressure-area curve during expansion, results from film rupture and the accumulation of the material at the Wilhelmy plate.

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