Surface pressure and elasticity of hydrophobin HFBII layers on the air – water interface: rheology vs structure detected by AFM imaging

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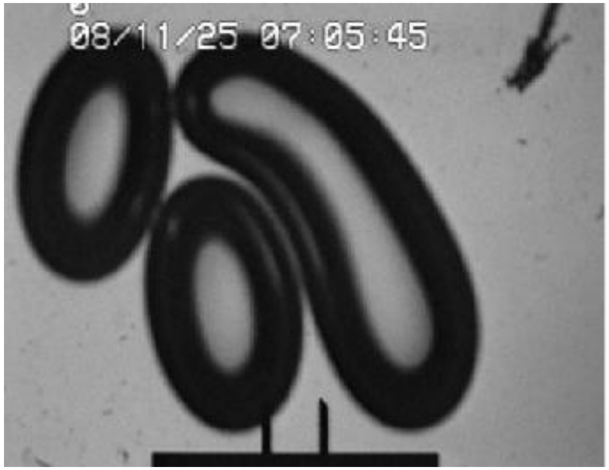
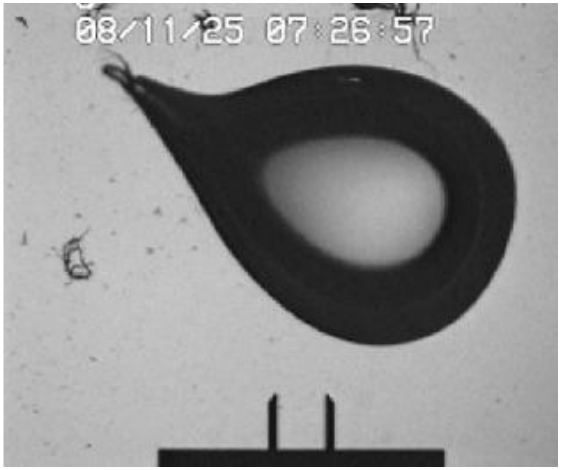
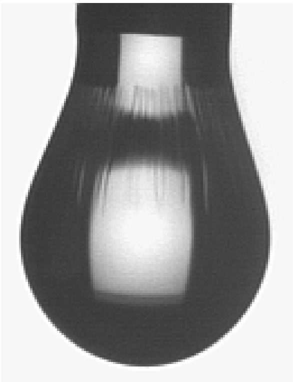
Motivation of the study

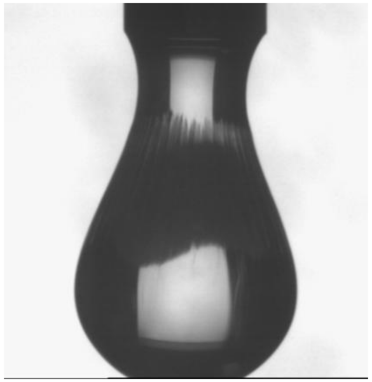




“equilibrium” millimeter-sized bubbles in HFBII solution

Basheva et al.
Langmuir 27 **(2011) 2382**

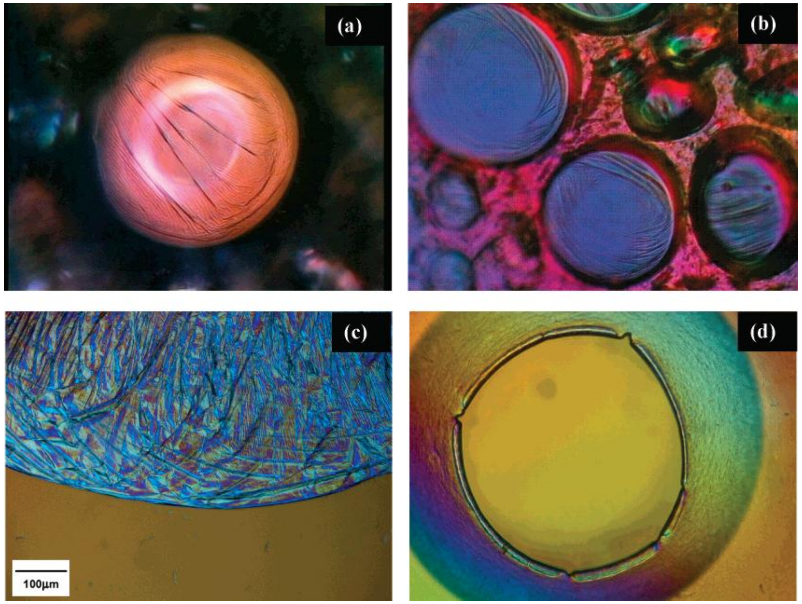




Solidified pendant drop of HFBII solution

Alexandrov et al., ***J. Colloid Interface Sci.*** **376 (2012) 296**

HFBII stabilized bubbles



Cox et al., Langmuir **2007,** ***23,*** **7995-8002**

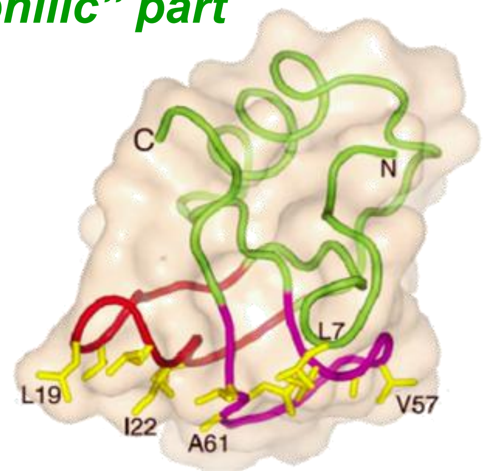
Aims of the Study

- To characterize the mesoscopic structure of the HFBII layers as a function of the surface pressure.
- To correlate and explain the relation between the structure and the surface rheology.

Protein HFBII

Hydrophobin of class II

"Hydrophilic" part



Hydrophobic part

Small (M_w = 7200 g/mol) globular protein isolated from fungus *Trichoderma reesei.*

The molecule contains ~ 70 amino acids and 4 disulfide bonds.

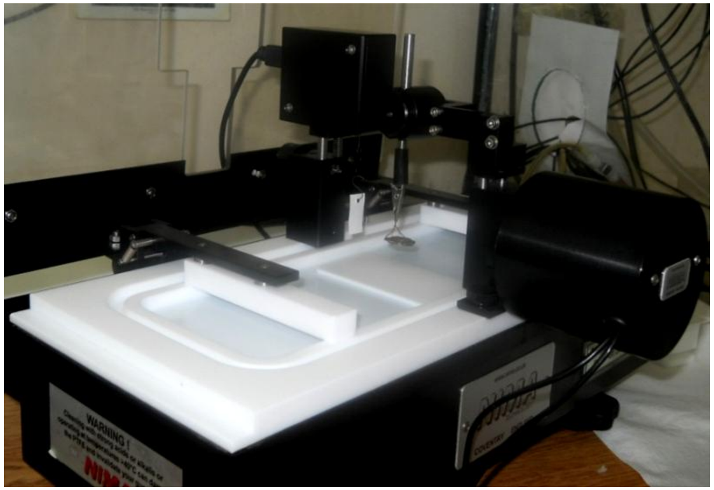
Dimensions: 24 × 27 × 30 Å and thickness of the adsorption monolayer ~ 2.5 – 3 nm.

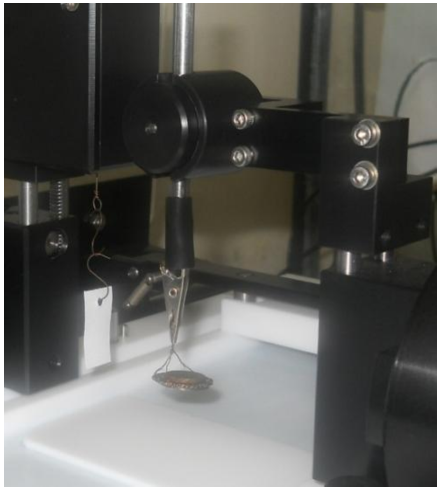
Hydrophobin molecules form dimers, tetramers and larger aggregates in the bulk of aqueous solutions.

Solution is sonicated in an ultrasound bath for 5 min before its use.

Methods

<u>Langmuir Trough</u> (Nima Technology Ltd, UK)
+ dipper mechanism





Substrate: pure water, 120 cm^2 initial area.

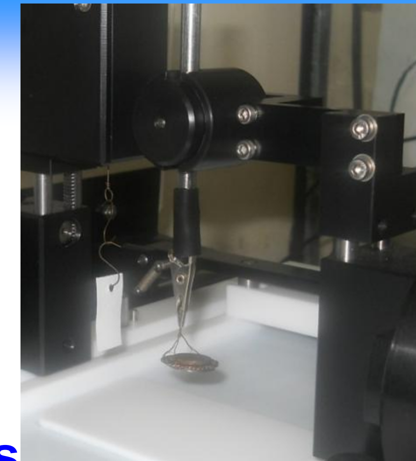
HFBII spread from a concentrated solution (32.9 μL 0.34 %)

Layers characterized from $\Pi(A)$ dependencies upon

- <u>slow compression</u> (d*A*/d*t* = 4 cm^2/min) vs.

- <u>oscillations</u> (d*A*/d*t* = 10 cm^2/min, ΔA = 2.4 %).

Methods



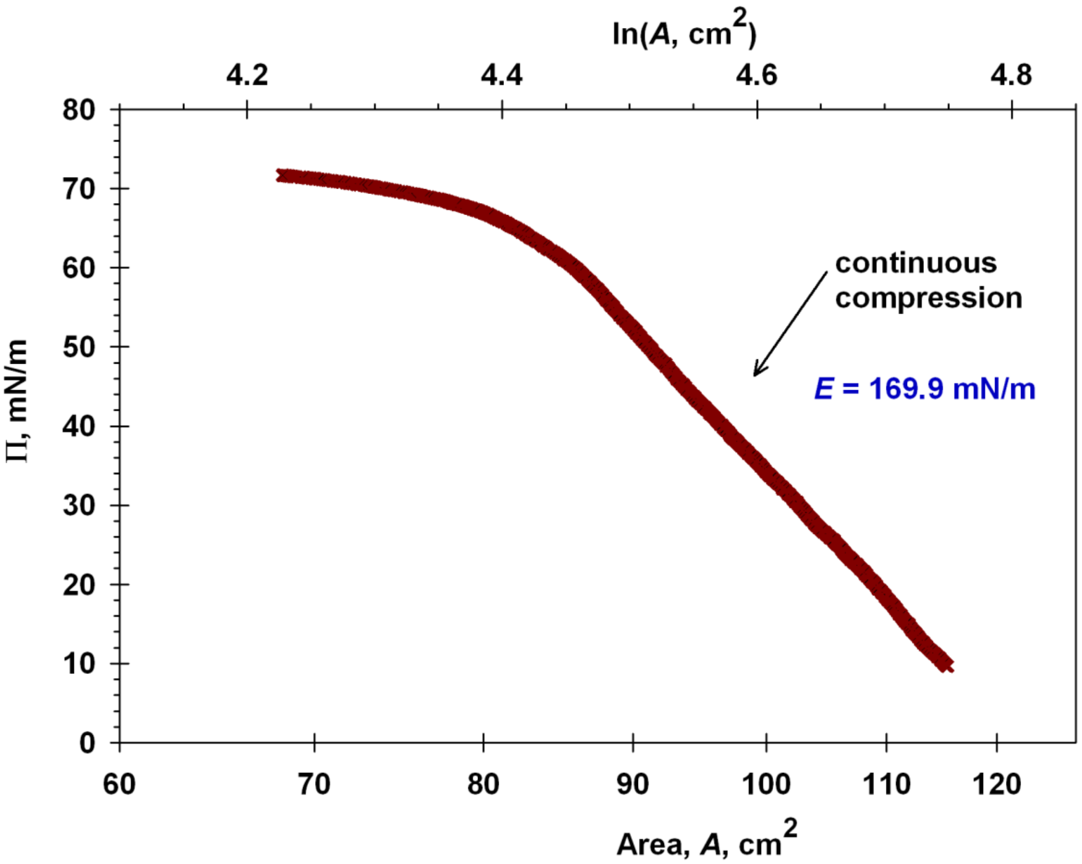
AFM sample preparation:

- Freshly cleaved mica - hydrophobized in HMDS vapors.
- Dry hydrophobic mica, attached to the dipper, is touched to the spread HFBII layer from above (Langmuir - Schaefer method).
- Spread HFBII transfers on the mica after a few minutes in contact.

Atomic force microscopy AFM
Nano Scope Multi Mode V
Bruker Inc., Germany

AFM imaging performed in Tapping mode.

Surface pressure isotherm



Continuous compression of the spread layer.

– Π increases with an almost constant slope up to Π = 60 mN/m, i.e. *E* is constant.

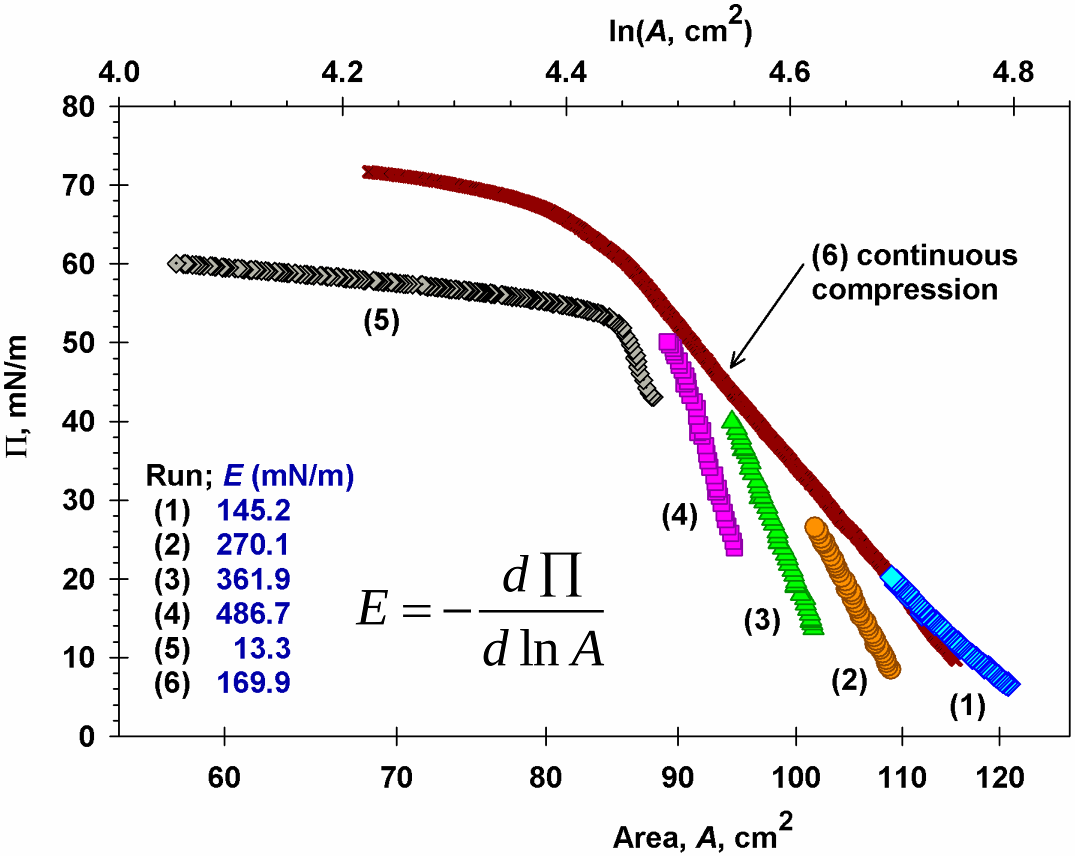
– HFBII does not desorb.

$$E = -\frac{d\,\Pi}{d\ln A}$$

Note: The layers solidify at Π > 22 mN/m

Alexandrov et al., *J. Colloid Interface Sci.* 376 (2012) 296.

Surface pressure isotherms



The upper curve (6) – continuous compression of the spread layer.

The lower curves, i.e. Stages (1) – (5) – partial compressions.

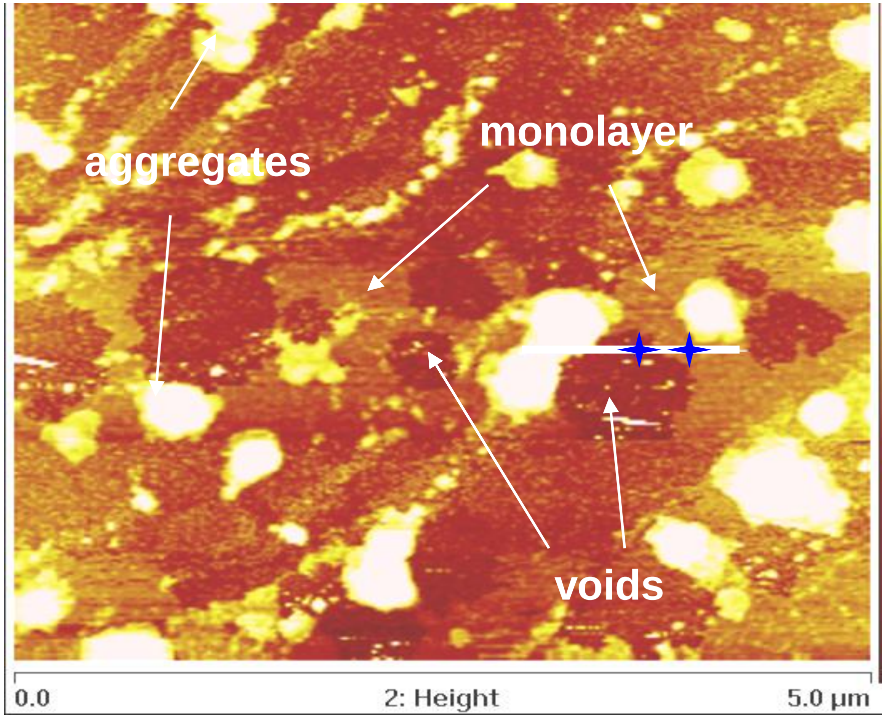
After each stage - oscillations of relatively small amplitude were performed.

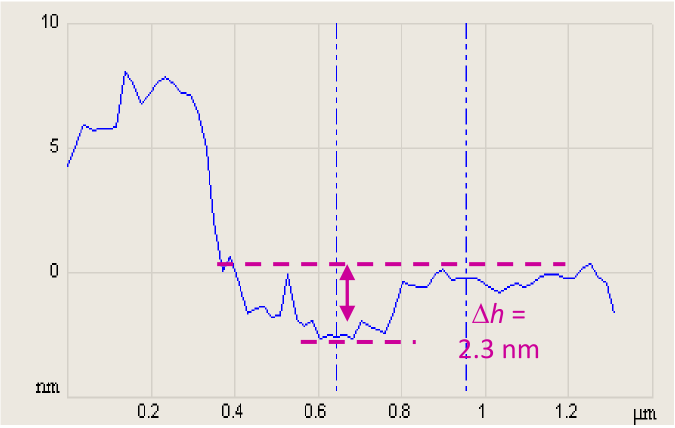
- The slopes for stages 2, 3 and 4 significantly increase, i.e. *E* also increases with Π (up to *E* = 486.7 mN/m).

The increase of *E* can be explained with a compaction of the protein layer during the area oscillations,

→ check by AFM.

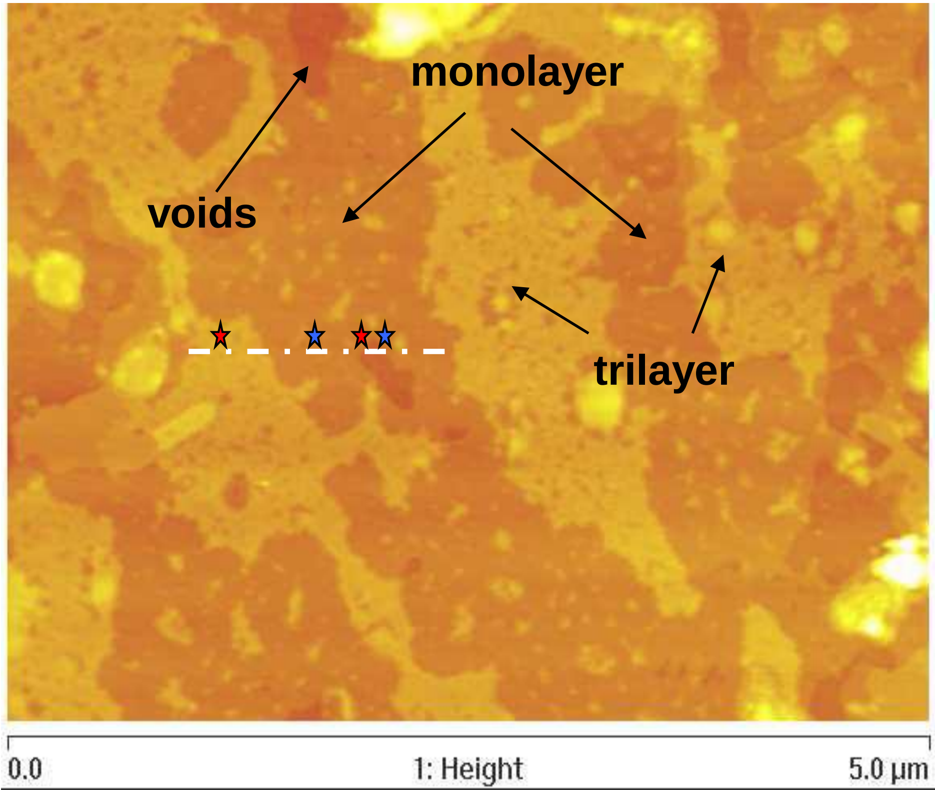
AFM of Spread Layers at Π = 25 mN/m

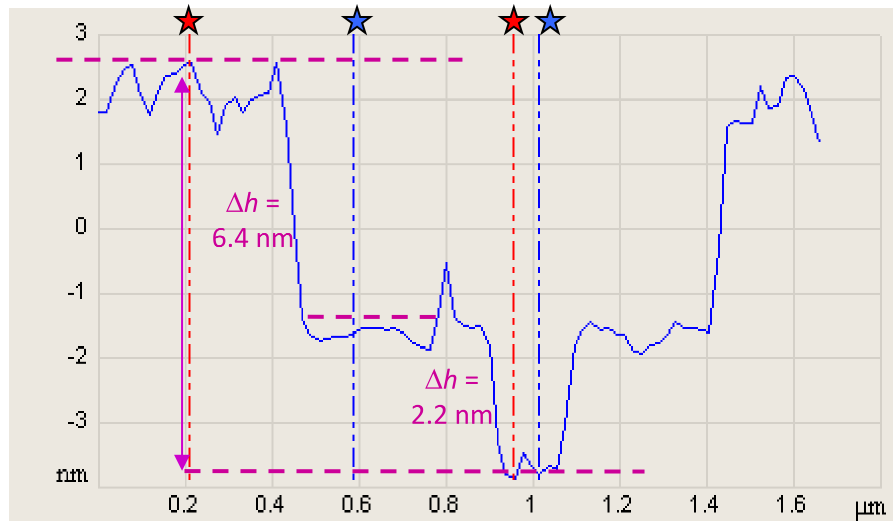




- The spread HFBII layer at Π = 25 mN/m is rather inhomogeneous;
- The layer contains voids, monolayer and bigger aggregates;
- Monolayer (Δ*h* = 2.3 nm) covers larger area.

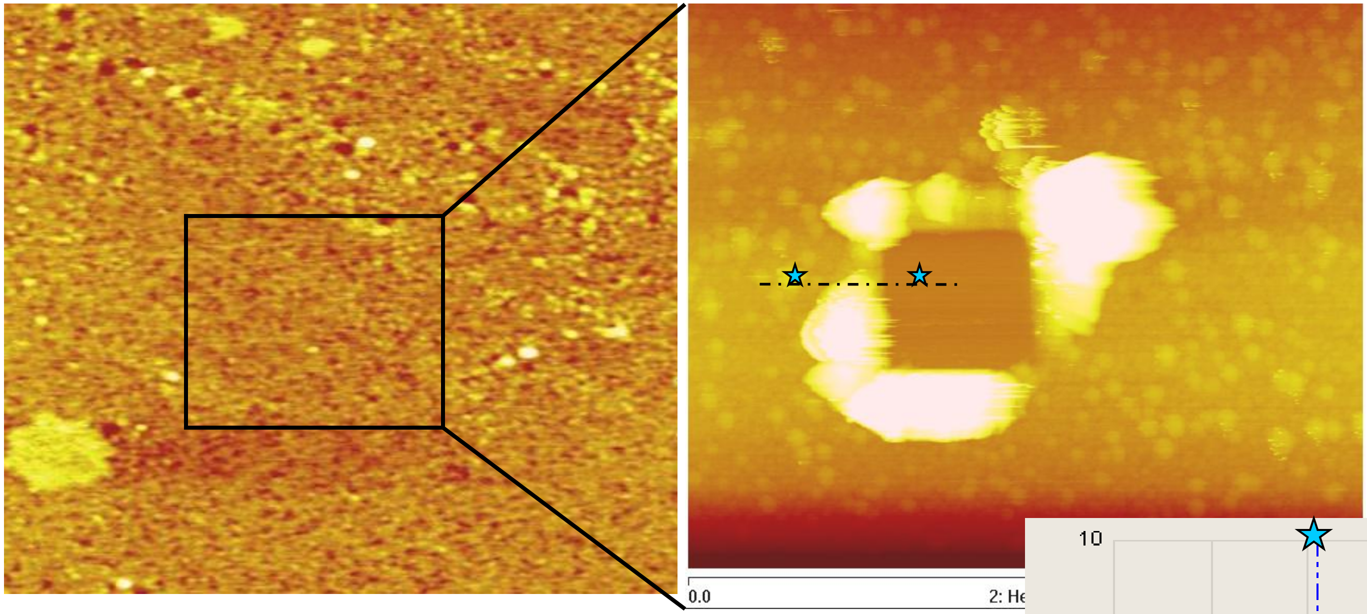
Spread Layers – Structure at Π = 48 mN/m





- **The layer is denser than that one at Π = 25 mN/m (compaction).**
- **The area fraction of the voids is relatively small.**
- **Co-existence of <u>monolayer</u> (darker zones) and <u>trilayer</u> (brighter) in large areas.**

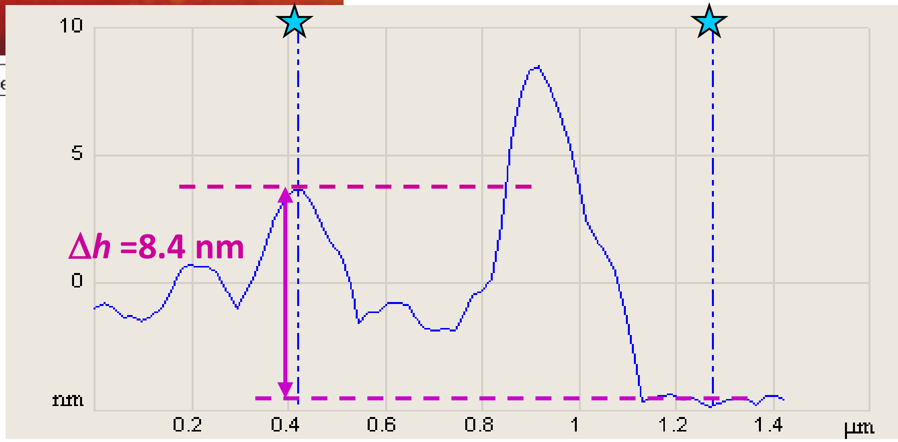
Spread Layers – Structure at Π = 62 mN/m



5 x 5 µm

To determine the absolute height of the dense layer we scratch the layer to reach the bare mica.

10 x 10 µm



- **Grainy structure.**

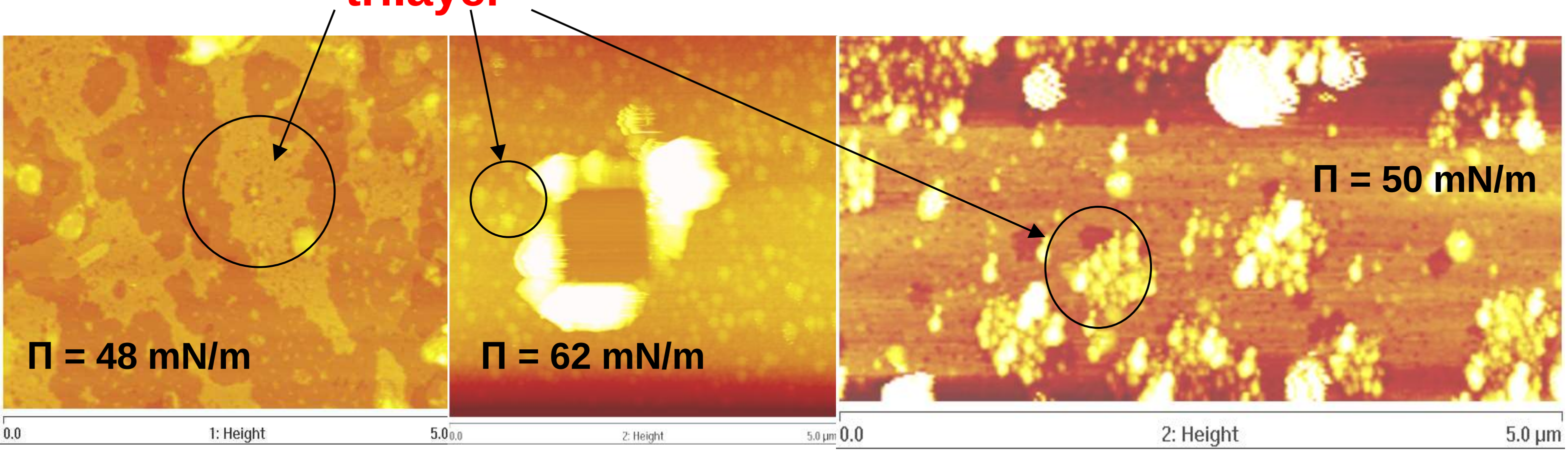
The tip cannot enter small holes.

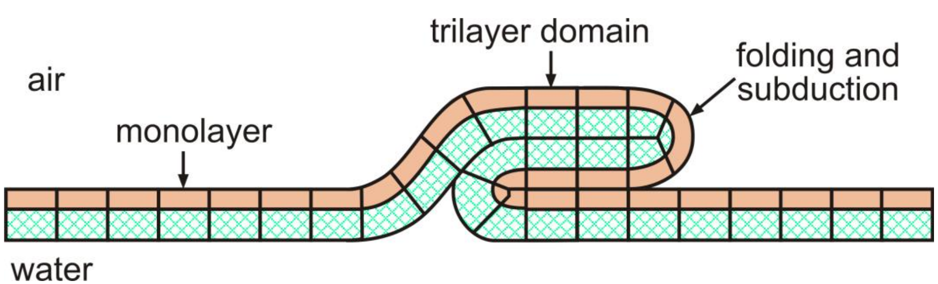
The dark areas are not bare mica.

- **The average height corresponds to a <u>trilayer</u> structure, covering almost fully the surface.**

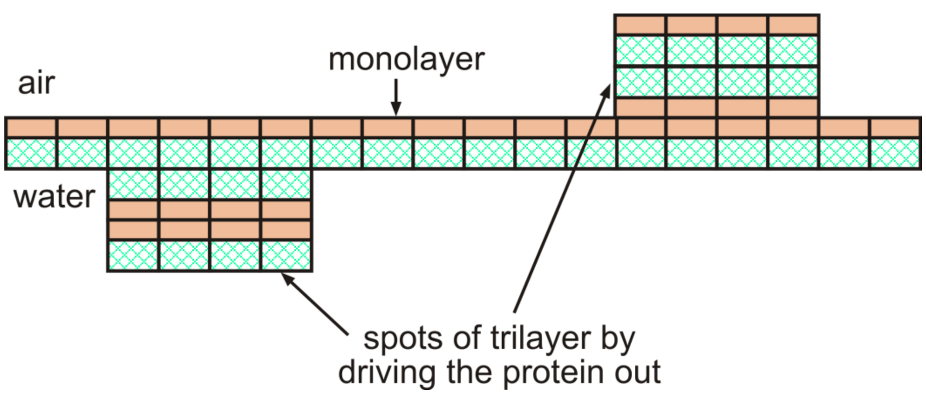
Mechanisms of trilayer formation

Two patterns of trilayer formation: (1) in the form of large surface domains; (2) in the form of numerous pimples.



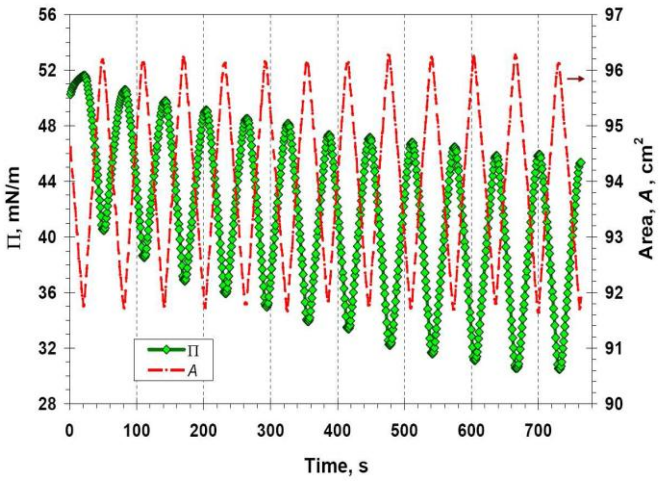


(1) Large trilayer domains can be formed from the monolayer by folding and subduction by continuous deformation (similarly to the lipids).

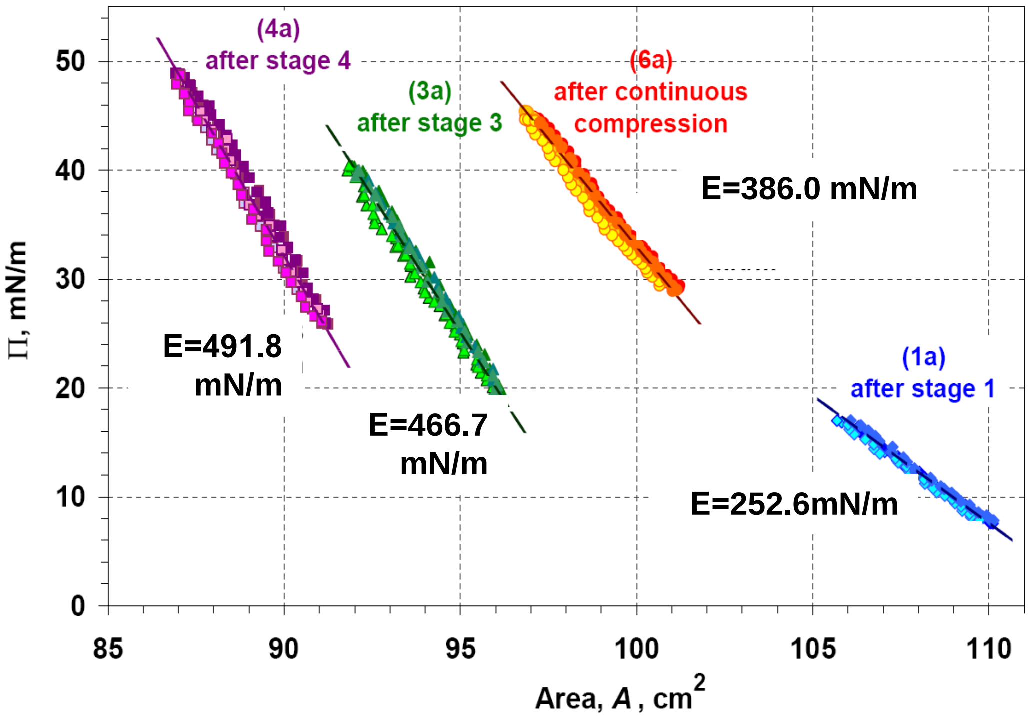


(2) Trilayer spots can be formed by squeezing of protein molecules out of the compressed monolayer upon fast oscillations. The displaced molecules spread on the monolayer and form two additional layers.

Elasticity from oscillatory experiments



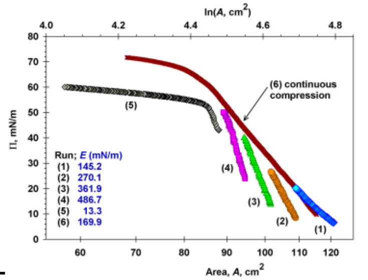
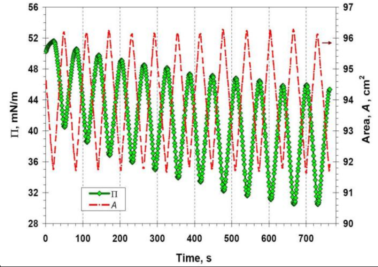
$$E = -\frac{d\prod}{d\ln A}$$



Dilatational elasticity *E* (i.e. slopes of the segments) increase with compression.

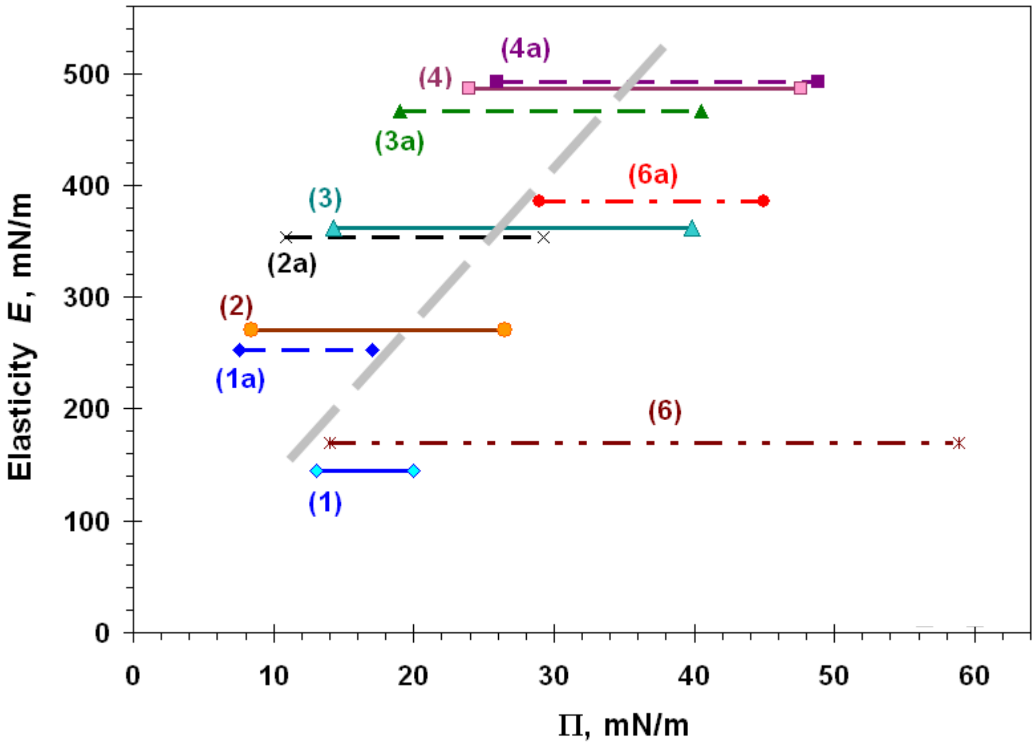
⇒ The compression of the layer leads to compaction and increase of dilatational elasticity *E*.

Elasticity values measured in different regimes

<table>
<tr><th rowspan="2">Π, mN/m</th><th>Compressions</th><th>Oscillations</th></tr>
<tr><th>E, mN/m

</th><th>E, mN/m

</th></tr>
<tr><td>15</td><td>145.2</td><td>252.6</td></tr>
<tr><td>25</td><td>270.1</td><td>348.7</td></tr>
<tr><td>35</td><td>361.9</td><td>466.7</td></tr>
<tr><td>45</td><td>486.7</td><td>491.8</td></tr>
</table>

- **Larger elasticity, *E*, from oscillations as compared to the one upon slow compression.**
- **The oscillations additionally compact the protein layer.**

Elasticity vs. surface pressure for solidified HFBII layers



1, 2…6 - *E* values obtained from slow compression stages

1a, 2a…6a - *E* values obtained in oscillatory regime

Elasticity, *E*, increases up to 500 mN/m with the increasing of Π (i.e. with the layers compaction).

- **The elasticity of solidified HFBII layers depends on the prehistory.**

In view of the AFM images, the higher *E* can be explained with the ability of this protein to form compact and elastic interfacial layers, which are thicker than monolayer (e.g. trilayers).

Conclusions

- The mesocopic structure of the spread layers is rather inhomogeneous: voids, monolayer and multillayer domains are observed.
- A continuous compression of the layer leads to filling the voids and to the transformation of a part of the monolayer into trilayer.
- Two different trilayer patterns are formed:
  - Large domains by folding and subduction;
  - Spots (Pimples) by forcing HFBII molecules out of the monolayer.
- The elasticity of the solidified layers measured by oscillations is higher than the one determined from slopes of slow compression stages.
  The protein layer compacts faster during oscillations.

Acknowledgements

Reference:

R.D. Stanimirova, T.D. Gurkov, P.A. Kralchevsky, K.T. Balashev, S.D. Stoyanov, E.G. Pelan, *Langmuir* 29 (2013) 6053-6067.

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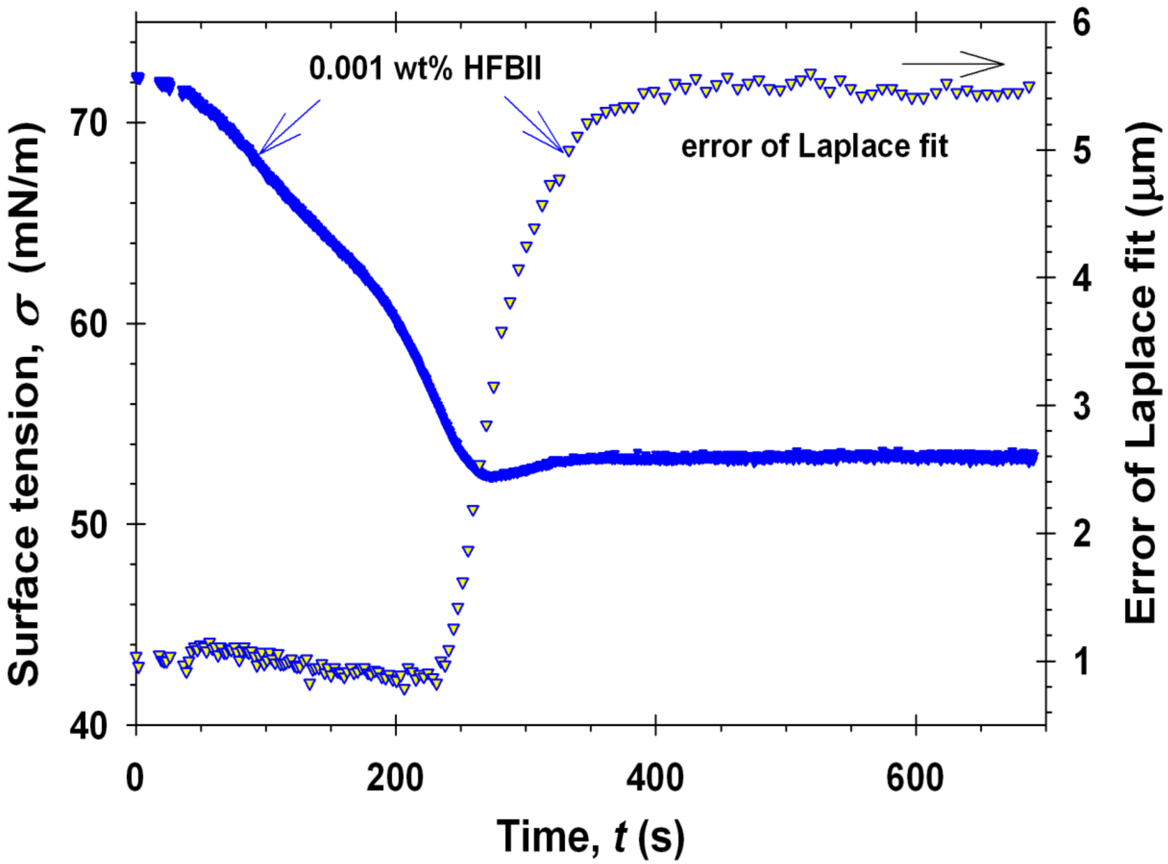
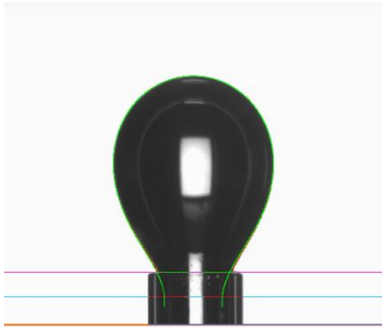
"Establishment of interdisciplinary teams of young scientists in the field of fundamental and applied research relevant to medical practice“

The project is implemented with financial support of the operative program "Human Resources Development" financed by the European Social Fund of the European Union. Sofia University “St. Kliment Ohridski”– Faculty of Medicine bears full responsibility for the content of this document and in no circumstances can be regarded as official position of the European Union or the Bulgarian Ministry of Education and Science.

Surface tension and solidification of HFBII adsorption layers



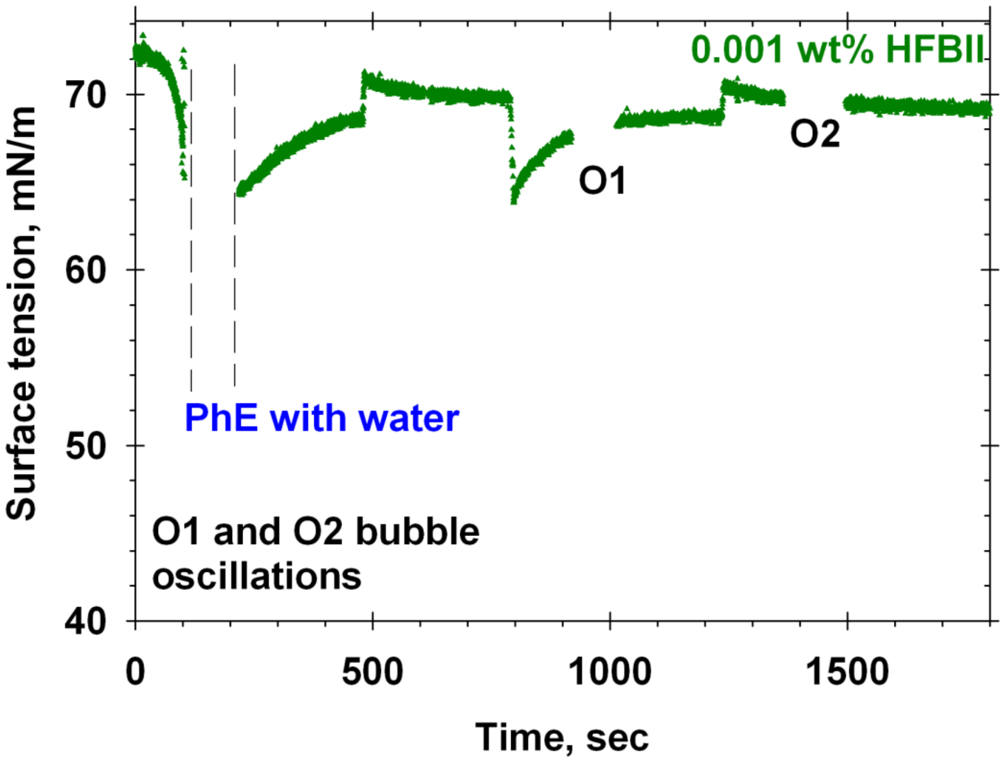


DSA 100R, Krüss GmbH, Hamburg, Germany

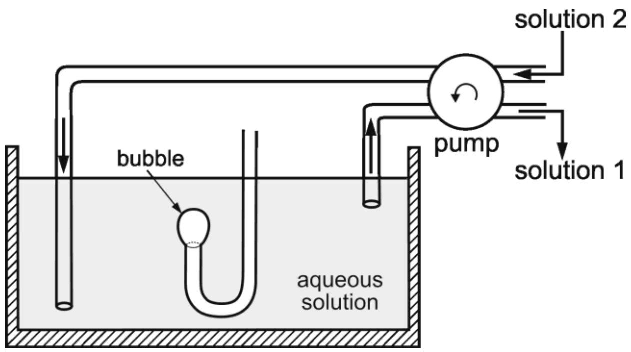
Slow kinetics by 0.001 wt% HFBII

The increase of error of Laplace fit is an indication solidifying of the HFBII adsorption layer.

Irreversibly adsorption of HFBII



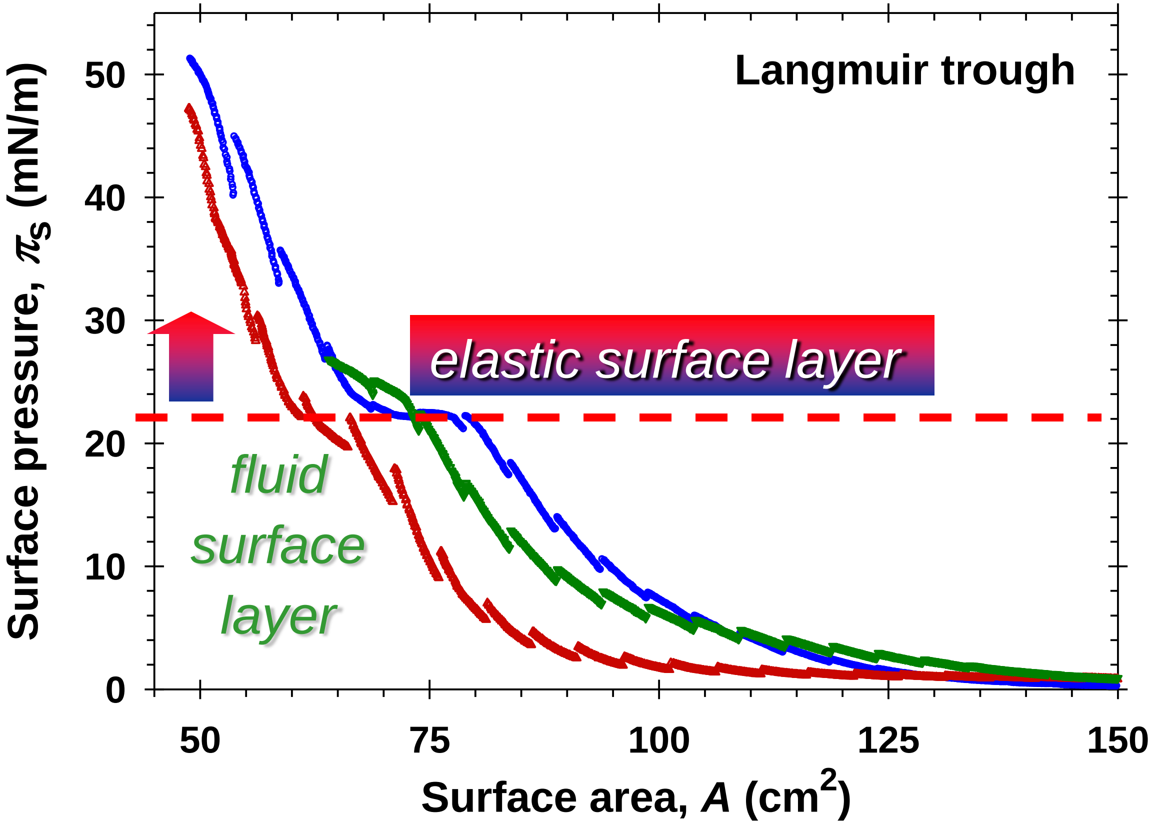
Phase exchange (PhE) cell

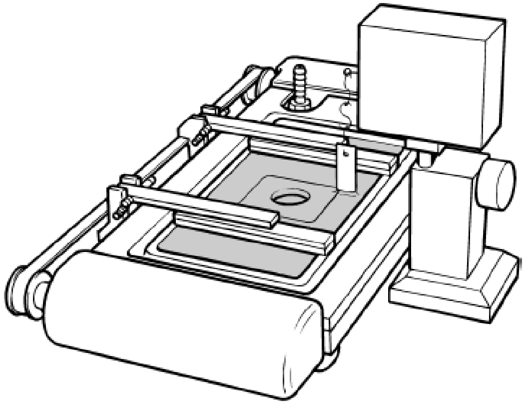


HFBII molecules irreversibly adsorb on air – water surface.

0.001 wt% HFBII	σ, mN/m	*E'*, mN/m	*E"*, mN/m
Bubble oscillation (O1)	67.6	122.5	10.2
Bubble oscillation (O2)	69.2	103.8	9.5

$\pi_s(A)$ – spread HFBII in the Langmuir trough





$\pi_s(A)$ curves change their slope at π_s ~ 22 mN/m

→ indication for a phase transition!

Applications:

The dense HFBII adsorption layers block the Ostwald ripening in foams and emulsions.

The low surface tension and high surface elasticity favor the production of stable foams with fine bubbles, *d* = 5–10 μm.