ESRF	Self-assembly of hydrophobin proteins at air/water interface	**Experiment number:** SC-1563
Beamline: ID10B	**Date of experiment:** from: 17.11.2004 to: 23.11.2004	**Date of report:** 24.2.2005
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Report:

Hydrophobins are small fungal proteins, which are among the strongest biosurfactants known. They have diverse roles in fungal life, such as adhesion to different surfaces, formation of coatings and lowering of surface tension, which they achieve by self-assembling at hydrophobic/hydrophilic interface [1].

The self-assembly of hydrophobins HFBI and HFBII from *Trichoderma reesei* [2] was studied directly at the air/water interface at the surface diffraction beamline ID10B. The incoming beam was monochromatized using a diamond (111) double crystal monochromator to the energy of 7.99 keV. For the grazing incidence diffraction measurements the angle of incidence was chosen to be 0.12°, 80 % of the critical angle of water. The intensity was measured using a linear position sensitive detector equipped with a Soller collimator.

The protein solution was injected to a Langmuir trough containing the water subphase and allowed to settle before the measurements. The measurements were conducted in series of increasing surface pressure from $\Pi = 0$ mN/m in steps of 5 mN/m to 20 or 30 mN/m depending on the sample. Finally the samples were lifted to a Si-substrate and measured on the substrate. All the measurements were conducted under helium athmosphere.

On air/water interface the hydrophobins self-assemble already at very small concentrations into hexagonal crystallites (Figure 1a). For both HFBI and HFBII the structure is similar with a = b = 55 Å. Because of the similarity of their amino acid sequences (69 %) [2] their folded structures may resemble each other closely allowing the same packing. The structure is the same as that of a multilayer LB film studied previously [3]. Also dimerized HFBI formed a structure very similar to HFBI and HFBII.

Increasing the pressure does not affect the ordering (figure 1a), but decreases the unit cell dimensions slightly. This is assumed to be due to the formation of small crystallites, which come closer to each other as the available area is reduced.

The structure of the protein layer remains the same after lifting it to a solid substrate and drying (Figure 1b). This shows that the structure is relatively stable and could be used as a template for a tailored regularly spaced functionality.

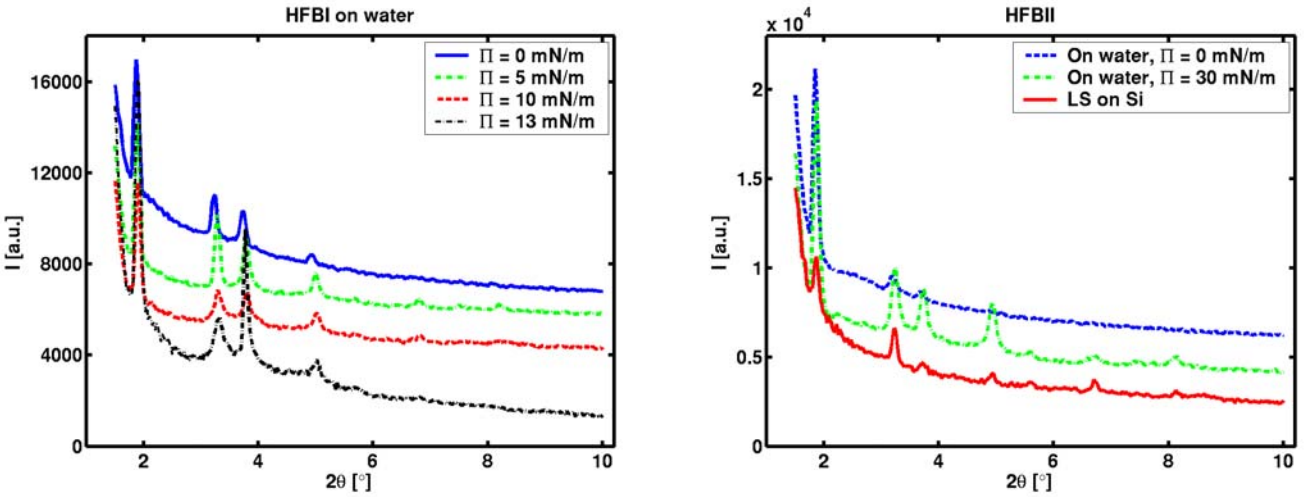

Figure 1: The diffraction pattern of a) HFBI on air/water interface at different pressures and b) HFBII on water/air interface and after lifting on a silicon substrate.

References

[1] H.A.B. Wösten, *Annu. Rev. Microbiol.* 55 (2001) 625.
[2] T. Nakari-Setälä, N. Aro, M. Ilmén, G. Muñoz, N. Kalkkinen and M. Penttilä, *Eur. J. Biochem.* 248 (1997) 415.
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