# Experimental report

29/11/2018

**Proposal:** **8-05-437**

**Council:** 4/2018

**Title:** Mucin Films under Mechanical Confinement

**Research area:** Soft condensed matter

**This proposal is a continuation of 9-13-604**


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**Samples:** mucin

| Instrument | Requested days | Allocated days | From | To |
|---|---|---|---|---|
| D22 | 1 | 1 | 06/10/2018 | 07/10/2018 |
| D17 | 3 | 0 | | |


**Abstract:**

Nowadays, a significant research effort is devoted to understand and mimic biological lubricants which, in contrast to most man-made lubricants, are based on water. It has been extensively shown that nature overcomes the poor lubricity of water with the addition of biological molecules, mostly proteins. Among these proteins, mucins are recognized as instrumental for biological lubrication. However, the molecular details of their lubricating properties are yet poorly understood. While structural studies of mucins at surfaces have given some insight into this aspect, the fact is that very little is known on the structure of confined mucin films, i.e. the really relevant system in the study of mucin lubrication. We propose to study this system by means of neutron reflectivity and grazing-incidence small-angle scattering employing a recently developed surface force type apparatus that allows the investigation of confined thin films.

Nowadays, a significant research effort is devoted to aqueous lubrication [1]. In contrast to man-made devices, which are mostly lubricated with oil, nature lubricates with water. Nature overcomes the poor lubricity of water by modifying the sheared surfaces with biological molecules. In the search for key components of biological lubricants, long glycoproteins like mucins seem to be a cornerstone [2]. While it has been proposed that this type of molecules provide efficient boundary lubrication due to a combination of entropic and hydration lubrication effects [2], the underlying molecular mechanisms are far from being understood. In this regard, Neutron Reflectometry (NR) is a promising tool for extracting structural information of boundary lubricant films under confinement and shear. Recently, a team partly formed by the applicants developed a sample environment for NR studies of mechanically confined thin soft films [3]. In this setup (Fig. 1), a flexible membrane (Melinex) that can conform to long range waviness or bend around any entrained dust is inflated against a solid hard surface. This setup has been successfully used for several NR studies at ILL [3-6]. However, GISANS studies have not been performed until now. In the general scope of the beamtime 8-05-437, the confinement cell has been tested for the first time using GISANS, proving that the setup can be used for different samples under confinement.

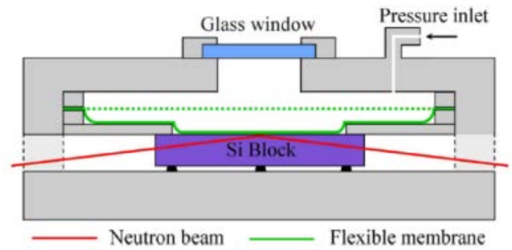


**Figure 1**. Currently available NR confinement cell [3].

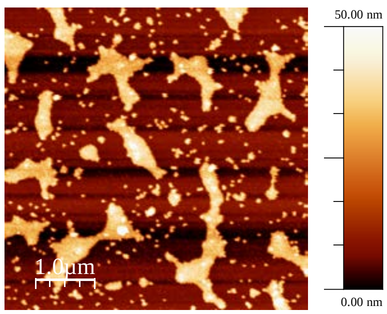


**Figure 2**. Topography image from the spin coated Si 111 surface (10 microns).

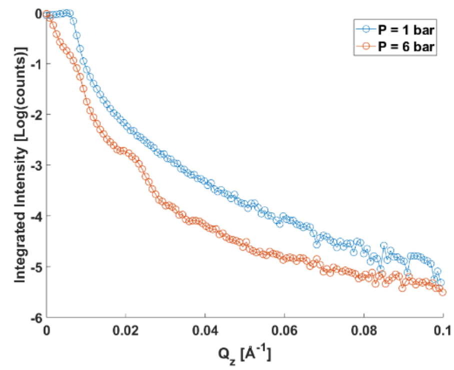


**Figure 3**. NR curve obtained on SuperADAM using the confinement cell at 1 and 6 bars, showing the presence of a silica particle layer.

In the proposal we applied for beam time at both D17 and D22 for characterizing mucin films under mechanical confinement both in NR and GISANS geometries. We expected that structural data of mucin films under mechanical confinement would have provided insight into the molecular mechanisms behind mucin lubrication. However, we only were awarded with one day on D22. As the confinement cell had not been tested before in a GISANS experiment, and as we would have lacked reliable NR data that would have helped us to evaluate GISANS data, we decided to test instead a more controlled system for the D22 experiment that would allow us to estimate the performance of the cell in this geometry. Therefore, we used for this experiment a sample with a known structure that was previously tested using AFM (Atomic Force Microscopy [7]) and NR. In a NR experiment on SuperADAM a silicon block (3 inches of diameter, 1 cm of thickness, Si 111) has been treated using a method proposed by Andrea Tummino and Philipp Gutfreund. In this method, silica nanoparticles [8] are spin coated on the active silica surface (cleaned with RCA and treated with UV). In the ideal case, the particles should form a well distributed monolayer covering an area large enough for the neutron footprint. Fig. 2 shows the AFM layer of nanoparticles attached to the cited surface, while Fig. 3 shows the NR curve for that configuration. The monolayer in this case was not compact enough.

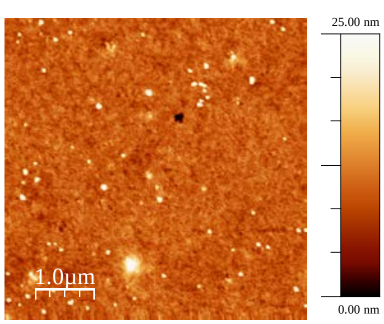


**Figure 4**. Topography image from the spin coated Si 100 surface (5 microns).

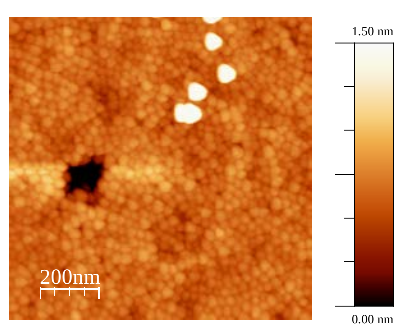


**Figure 5.** Zoom of the Si 100 spin coated surface (1 micron).

For the D22 experiment the Si 111 surface was replaced with a float-zone grown intrinsic Si 100 surface, due to its very low-defect density and hence reduced background scattering. Probably due to the changed crystal orientation the silica nanoparticles formed a dense monolayer on the Si surface in this case (Fig. 4 and Fig. 5). From the analysis of the AFM images, the distance between the center of particles was 30 ± 2 nm.

In the GISANS experiment, the specular reflectivity curve and a 2D GISANS plot were obtained (both obtained with a modified version of Grasp, from Dirk Honneker). Selecting the beam center and subtracting the background, the points of the reflectivity curve were obtained from a series of 110 images at varying incident angle (Fig. 6). The resulting data were fitted using Motofit with five layers (Table 1). Although the reflectivity curve is not ideally fitted, the values obtained for the different layers are in the expected range.

| Layer | Thickness (Å) | SLD (Å$^{-2}$) | Roughness (Å) |
|---|---|---|---|
| Silicon oxide | - | **2.07** | - |
| Silicon | 10.4 | **3.47** | 3.8 |
| Silica particles | 507.3 | 2.91 | 31.1 |
| Deuterated Polystere | 454.1 | 5.44 | 82.7 |
| Melinex | - | **2.6** | **15** |

**Table 1**. Parameters obtained (red) from the reflectivity curve fit using Motofit. The parameters in bold were known.

The 2D GISANS image (Fig. 7) was obtained after a measurement of ten hours (five images of 2 hours each one). The incident angle was set to 0.685º and the wavelength was 9 Å. The detector was 10 m away from the sample and the collimation distance was set to 17.6 m with a 20 mm diameter round aperture and a 20mm x 0.5 mm sample slit. The value for the distance between the center of the particles could also be obtained from the GISANS measurement: 28 nm. The resulting value is in accordance with the obtained for the AFM measurements.

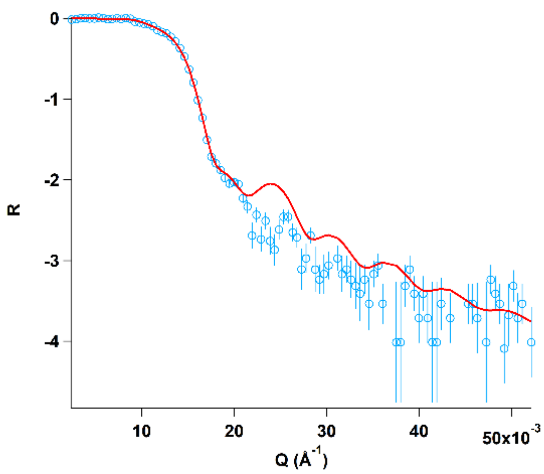


**Figure 6**. Reflectivity curve data (blue) and fit (red), in log-scale.

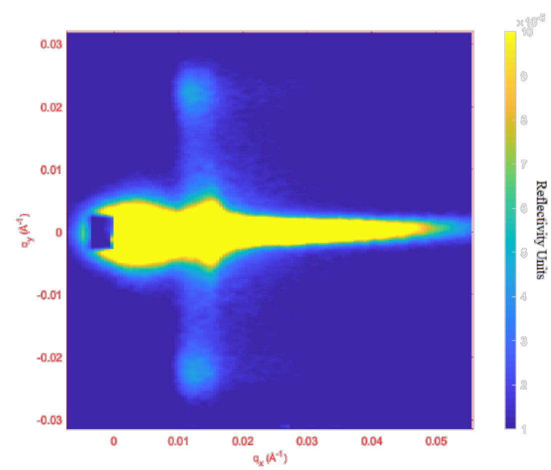


**Figure 7**. GISANS image from D22.

In conclusion, data obtained in this experiment proves that the confinement cell can be use in a GISANS configuration not only for the sample used but for other type of samples. Considering that the new confinement cell will include shear, the results from this experiment paves the road for future studies under *in situ* confinement and shear.


***References:*** [1] Aqueous lubrication: Natural and Biomimetic Approaches. 2014: World Scientific Publishing. [2] Curr. Opin. Colloid Interface Sci., 2010. 15: 406. [3] Rev. Sci. Instrum., 2012. 83: 113903. [4] Macromolecules, 2016, 49: 4349. [5] Macromolecules, 2014. 47: 3263. [6] Macromolecules, 2013. 46: 1027. [7] Cypher Asylum Research AFM instrument from ESRF microscopy group. [8] Lumox TM-50 Colloidal Silica, Sigma-Aldrich.