Experimental report

26/10/2016

Proposal: 9-13-604 **Council:** 4/2015

Title: Structure of Confined Mucin Films

Research area: Soft condensed matter

This proposal is a new proposal

Main proposer: Javier SOTRES

Experimental team: Javier SOTRES
Marite CARDENAS
Stuart PRESCOTT
Tania LIND
Robert M. RICHARDSON

Local contacts: Philipp GUTFREUND
Robert BARKER

Samples: MUC5B (Oral Mucin Protein)

Instrument	Requested days	Allocated days	From	To
D17	3	3	28/11/2015	01/12/2015

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 experiments employing a recently developed surface force type apparatus that allows the investigation of confined thin films.

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In the reported experiment, we investigated the structure of mechanically confined mucin films. Mucins are long glycoproteins which play a major role in both the lubrication and hydration of biological surfaces. Mucin films cover all epithelium surfaces serving as a lubricious barrier. At the same time, the prevent dehydration of these surfaces. Inspired from their biological functions, mucins have also attracted interest as coatings for biomedical implants and devices [1-3]. In these systems, mucin films are expose to mechanical forces/confinement. How they respond to these situations is of high importance for their function. To investigate this, we employed a confinement cell for Neutron Reflectometry (NR) studies developed recently by researches from Bristol University [4]. In this set-up (Fig. 1), an inflated flexible surface (e.g. a Melinex film) approaches a rigid one (e.g. a silicon block). The elasticity of the flexible surface allows the adjustment to the waviness of the rigid surface making the approach very suitable to study the compression of thin soft layers.

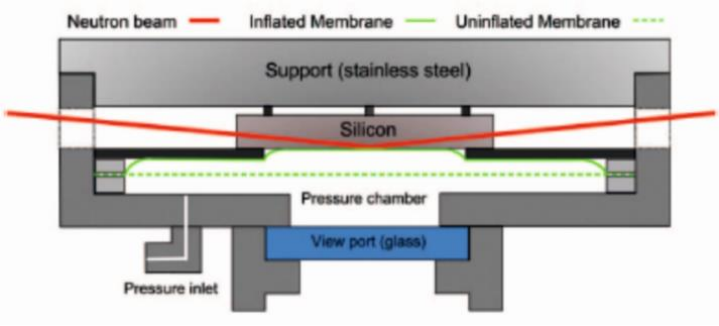

Figure 1. Schematic illustration of the NR Bristol confinement cell [4].

Experiments were performed at D17 reflectometer, for which the Bristol confinement cell has been optimized. For the experiments, mucins were adsorbed on both the rigid (silicon) and the flexible (Melinex) surfaces. Mucins films are known to be more resistant when formed on hydrophobic surfaces. Therefore, both the silicon and the Melinex were hydrophobized. Specifically, the silicon block was methylated with dichlorodimethylsilane, whereas a film of polystyrene was spin-coated on the Melinex. Then, a 0.2 mg/ml solution of oral mucins was let to adsorb on both surfaces for 1h and subsequently rinsed.

Initially, we characterized the unconfined mucin films on the hydrophobized silicon blocks (Fig. 2) both in H2O and D2O. Analysis of these data provided a thickness of 39.7 nm and a hydration of 93% for the mucin films, values which are in agreement with those obtained by the investigators by means of other techniques [5].

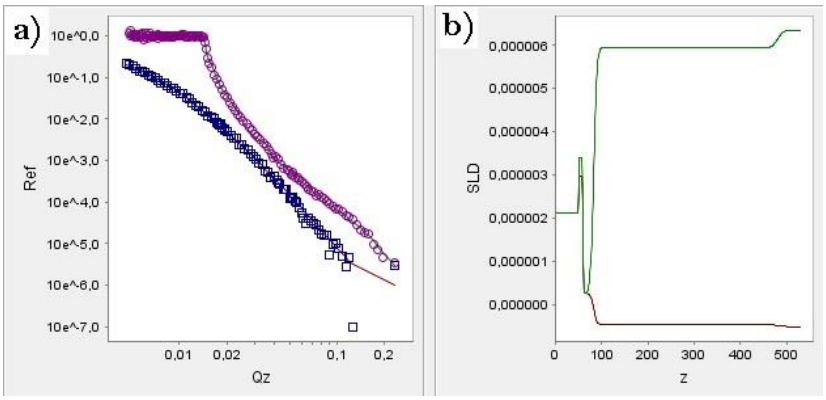

Figure 2. a) Neutron reflectometry data and corresponding fits for mucin films adsorbed on hydrophobized silicon blocks in H2O (blue) and D2O (purple). **b)** Calculated SLD profile for the mucins films in H2O (red) and D2O (green).

Subsequently, we investigated by means of NR the structure of the mucin films adsorbed on the hydrophobized silicon confined by mucin films adsorbed on the polystyrene (PS) coated Melinex under different pressures. Specifically, we investigated different contrasts: 1) D2O and hydrogenated PS (Fig. 3a), 2) H2O and hydrogenated PS (Fig. 3b) and 3) D2O and deuterated PS (Fig. 3c).

In Fig. 3a and 3b, the critical angle for the silicon/D2O interface at zero applied pressure can be clearly observed. Interestingly, when increasing the applied pressure, this critical angle is still present, though, shifted towards lower Q values, until it disappears when a pressure in the 3-5 bar range is applied. This suggests that a significant amount of solvent is retained by the confined mucin films up to these pressures. When deuterated PS was used (Fig. 3c) the critical angle corresponding to the silicon/ deuterated PS could also be observed for the lowest applied pressure (0.5 bar), suggesting as well contact between both confined surfaces. Finally, data at higher Q also suggest a clear structural change in the mucin films taking place in the 1-3 bar range.

At present data analysis is being performed and future experiments are being planned in order to understand the observed behavior of confined mucin films, which indicates that they can retain a significant amount of solvent up to significant higher pressures than previously studied polymer brushes.

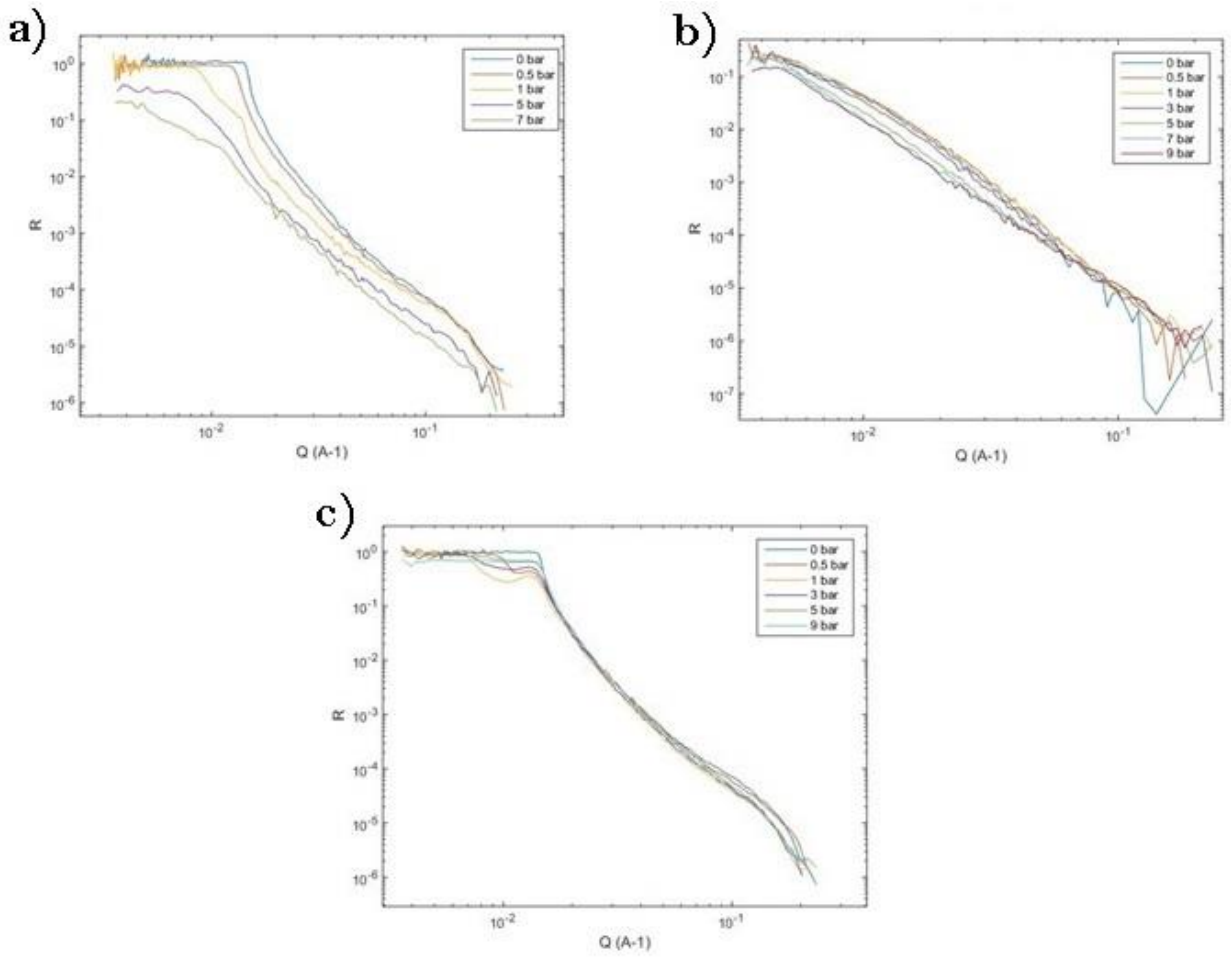

Figure 3. Neutron reflectometry data for mucin films adsorbed on hydrophobized silicon blocks confined against mucin films adsorbed on **a)** polystyrene in D2O, **b)** polystyrene in H2O and **c)** deuterated polystyrene in D2O.

References

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