Experimental Report

Proposal: **9-13-535** **Council:** 4/2014

Title: Protein-surfactant co-adsorption at the oil/water interface: exploring neutron reflection measurements

This proposal is a new proposal

Researh Area: Soft condensed matter

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Samples: Eggshell membrane polypeptides, d- and h-C16 oils and d- and h-SDS

Instrument	Req. Days	All. Days	From	To
FIGARO	4	4	15/09/2014	19/09/2014

Abstract:

The proposal seeks to develop Figaro as a neutron reflection instrument to undertake the oil-water interface studies using polypeptide-surfactant mixtures as an object of study. As the start, the interfacial tension values are modest, so that the interfacial structures are relatively easy to determine. The ultimate technical challenge lies in the ability to unravel meaningful information when the interfacial tension values are getting lower. As before, we will also test how the combined use of deuterium substitution to the surfactant and solvent help define the interfacial structures.

Relevant publications are:

Chen, C.X.; Hu, J.; Zhang, S.Z.; Zhou, P.; Zhao, X.C.; Xu H.; Zhao, X.B.; Yaseen, M.; Lu, J.R., Biomaterials 2012, 33, 592-603.
Han, S.; Xu, W.; Cao M.; Wang J. Xia, D.; Xu, H.; Zhao, X.; Lu, J.R., Soft Matter 2011, 8, 645-652 .
Han, S.; Cao, S.; Wang, Y.; Wang, J.; Xia, D.; Xu, H.; Zhao, X.; Lu, J.R., Chem. Euro. J. 2011, 17, 13095-13102.
Wang, S.J.; Ge, X.; Xue, J.Y.; Fan, H.M.; Mu, L.J.; Li, Y.P.; Xu, H.; Lu, J.R., Chem. Mater. 2011, 23, 2466-2474.

Adsorption of Globular Proteins at the Oil/Water Interface

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With recent advance in neutron reflection and instrumental development by Zarbakhsh et al, it is now becoming feasible to undertake the neutron reflection measurement at the oil-water interface. Following our previous work on the adsorption of BSA at the hexadecane/water interface (the draft paper is under submission), this report summarises the more recent adsorption work of two other proteins: lysozyme and β-Casein.

Initially the solution scattering length densities *Nb* were matched to the proteins: this would ensure the maximum sensitivity to the protein segment layer immersed in the oil phase, with no contribution to the reflectivity originating from the aqueous side of the protein interfacial region (isotopic contrast matching out).

Lysozyme solutions

At very low lysozyme concentration of lysozyme (0.01 mg/ml) the reflectivity was modelled with a single layer with a thickness of 25 Å. No change in the reflectivity profile was observed with concentration increasing up to 4 mg/ml, suggesting that this layer saturates already at very low protein concentration. This is similar to what observed at the oil/water interface for a series of BSA, where the layer in the oil phase had reached full coverage at low [BSA]. The structure of the adsorbed layer was studied using H_2O subphase, thus enabling sensitivity to the layer immersed in the aqueous phase. The thickness of the layer in the aqueous side was consistent with a single protein layer adsorbed along the long axis.

β-Casein

β-Casein solutions are more surface active if prepared in phosphate buffer than in pure water. In this first part of the experiment solutions were prepared in 10 mM NaCl, experiments in buffer will follow in later allocated beam times. It was found that β-Casein adsorbed at the oil/water interface forming a 20 Å layer submerged in the oil phase from the first set measurements of contrast matching to the protein. This layer did not change in thickness nor

in *Nb* with increasing protein concentration. As this was already observed for BSA and lysozyme, it may be indicative of the existence of a pattern in protein adsorption at the oil/water interface. By using H_2O as subphase, it was found that the aqueous side of the interface can be best modelled using 2 layers. The first layer, adjacent to the oil phase, has a relatively high protein volume fraction. The second layer is a diffuse water-rich region. For both layers the protein volume fraction increased with concentration.

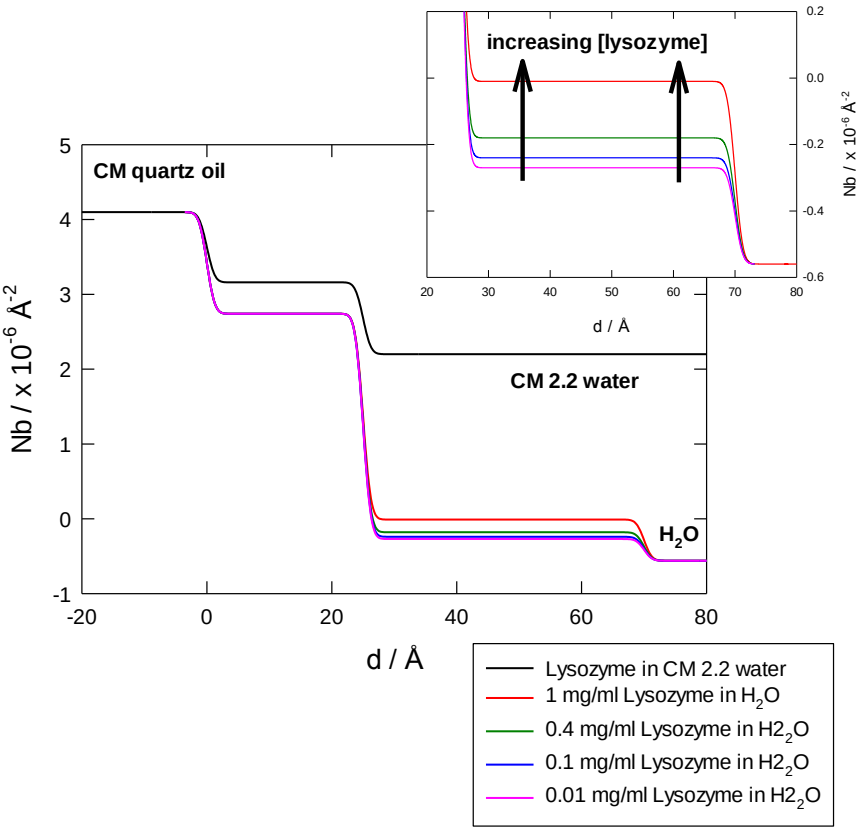

Figure 1. *Nb* profiles for a series of lysozyme solutions at the oil (contrast matched to the quartz substrate)/water interface. When the water is contrast matched to the protein (CM 2.2) the sensitivity is limited to the layer immersed in the oil phase (see black line). The full structural resolution was achieved by using H_2O subphase (se coloured lines). The small differences with concentration are highlighted in Figure insert.

We are still in the process of studying β-casein adsorption from buffer solutions to get useful insight of the impact of interfacial tension changes. Once this information is available we will be a step closer to submission for publication.