Experimental report

25/07/2016

Proposal: 9-13-577 **Council:** 10/2014

Title: Neutron Reflection Study of Proteins Adsorbed at the Oil/Water Interface

Research area: Soft condensed matter

This proposal is a new proposal

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Samples: Proteins
DOPC
DOPG
18-MER ANTIBACTERIAL PEPTIDE

Instrument	Requested days	Allocated days	From	To
FIGARO	4	4	16/07/2015	20/07/2015

Abstract:

New experiment exploration for oil/water interface measurements, focusing on the adsorption of proteins with different globular stability.

Neutron Reflection Study of Proteins Adsorbed at the Oil/Water Interface

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We have measured the adsorption of model proteins at the oil/water interface by making extensive use of contrast variation of solvent. In this work, we have investigated the effect of denaturants on both the adsorbed amount adsorbed at the interface and the interfacial structure formed. This is important as some proteins tend to increase their ability to stabilise emulsion after treatment with denaturants, and the difference in performance must reflect their different interfacial structure. In this study, we used lysozyme as it has been extensively characterised in a number of systems. We have compared its interfacial structure before and after treatment with Dithiothreitol (DTT), which is known for its ability to reduce disulphide bridges in proteins. It has already been reported that lysozyme increases its surface activity after DTT treatment because of the loss of its globular conformation. This work examines the structural difference of the layers formed at the oil/water interface by native and denatured lysozyme for the first time.

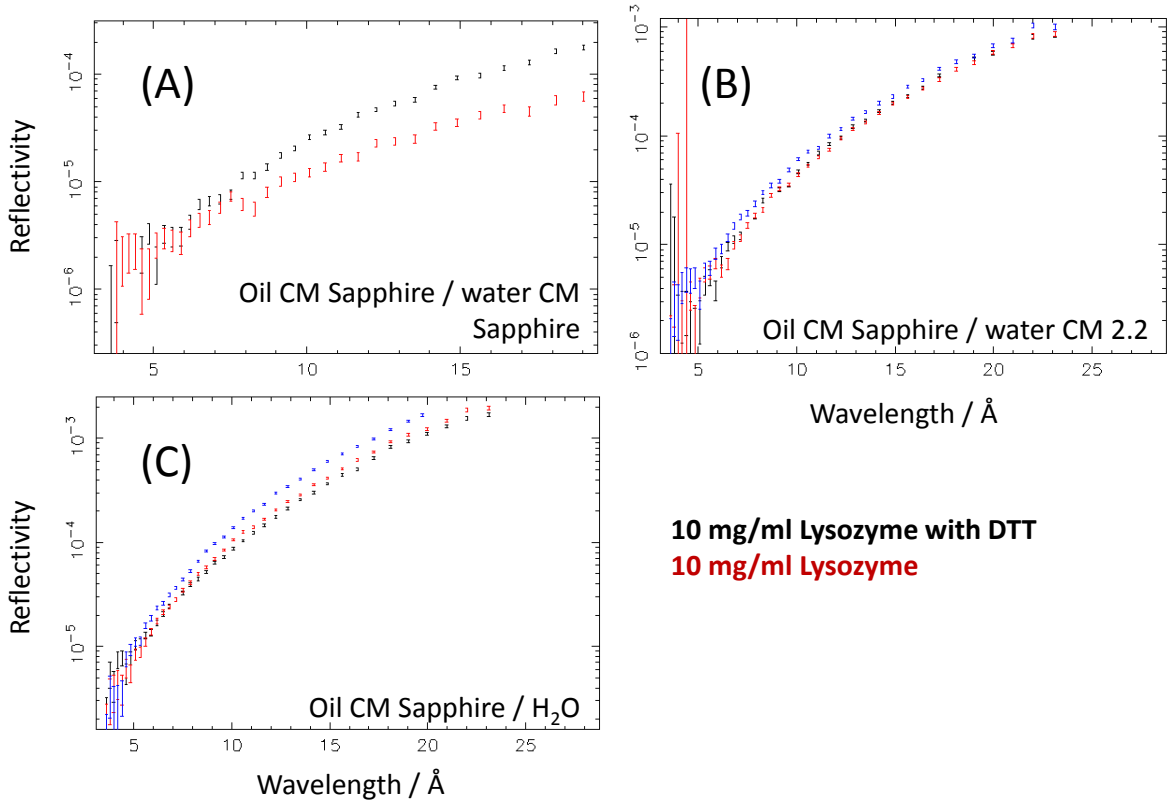

Figure 1: reflectivity profiles for 10 mg/ml lysozyme before (red) and after (black) treatment with DTT. The bare interface, when present, is shown as the blue data. The oil used was always contrast matched to sapphire to minimise the reflectivity

contribution from the solid/oil interface. The subphases used were, respectively, CM sapphire (A), CM 2.2 (B) and H_2O (C).

In Figure 1 we summarise the main results measured from the main solvent contrasts. In all cases, the concentration of lysozyme was kept at 10 mg/ml. At this concentration, the surface adsorption tends to plateau. Figure 1A shows the results when both oil and water were contrast matched to sapphire. Under these circumstances, the total reflectivity is a function of the adsorbed amount Γ at the interface. We found that Γ increases from 1.41 to 2.42 mg/m^2 when lysozyme was treated with DTT, showing a clear trend of increased adsorption as a result of structural unfolding.

Figure 1B shows the results measured at the oil CM sapphire / water CM protein interface. Under these circumstances, the reflectivity arises only from the layer immersed in the oil phase. This contrast is thus sensitive to the oil distribution across the interface as a result of protein or polypeptide distribution. No differences could be found, suggesting that no major differences occurred on the oil side of the interface. This implies that the difference must be sought on the aqueous side of the interface.

This was indeed confirmed when using H_2O as subphase (Figure 1C). In this case the sensitivity is extended to the overall interfacial structure: with addition of DTT the reflectivity decreases, consistent with more material adsorbed at the interface.

We are currently finalising the fitting and combining these results with previous results obtained on the INTER reflectometer at ISIS. We believe we have enough data for a publication and should start the write-up process soon.