Experimental Report

Proposal: **9-13-451** **Council:** 4/2012

Title: Interface characterization of mixed fibrinogen-hydrogenated and fluorinated surfactant films

This proposal is a new proposal

Researh Area: Soft condensed matter

Main proposer: **RUSO Juan**

Experimental Team: RUSO Juan
HASSAN Natalia
CAMPBELL Richard

Local Contact: CAMPBELL Richard
CAMPBELL Richard

Samples: bovine fibrinogen
sodium octanoate
sodium dodecanoate
sodium perfluorooctanoate

Instrument	Req. Days	All. Days	From	To
FIGARO Adsorption troug	3	3	09/11/2012	12/11/2012

Abstract:

Understanding the role of interactions between proteins and surfactants provides insight into the mechanism of molecular recognition and the role of binding cooperativity in the protein structure. Fibrinogen is interesting because it is a major inhibitor of lung surfactant function at the lining layer of the alveoli. Recently we have characterized the complexation of fibrinogen with three different surfactants: sodium octanoate, dodecanoate and perfluorooctanoate in the bulk and at the air-water interface. The differences in alkyl chain length, stiffness and hydrophobicity promote a variety of electrostatic, hydrophobic and steric hindrance forces which results in different stability and structural film patterns. To understand these systems better we now require a direct characerization of the composition and structure of the adsorbed layer in situ at the air-water interface. The logical next step in this project is therefore a set of neutron reflectivity measurements using isotopic contrast variation which here we propose to carry out on FIGARO.

Final Report for Experiment #9-13-451 on FIGARO (09/11/12 –12/11/12):
Interface characterization of mixed fibrinogen-hydrogenated and fluori nated surfactant films

Main proposer: Juan M. Ruso Local contact: Richard Campbell

Scientific background

For blood plasma proteins, such as fibrinogen, adsorption plays a key role in enhancing hemocompatibility. Also, the interactions between small molecules with proteins affect their respective biological functions and determine their stability. Understanding the role of these interactions must provide insights into the mechanism of molecular recognition and the role of binding cooperativity in the protein structure [1]. The interaction of fibrinogen is of particular interest, because fibrinogen is quite surface active and a major inhibitor of lung surfactant function at the lining layer of the alveoli.

Preliminary investigations

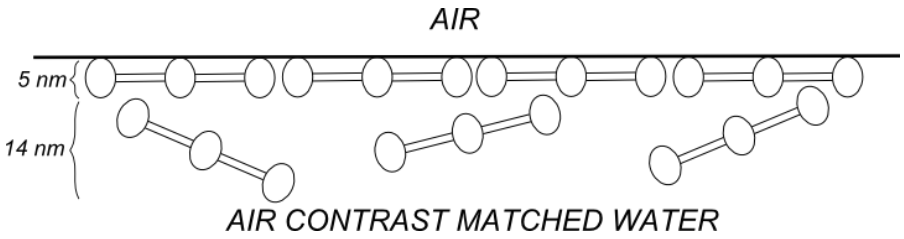
Recently we have studied the supramolecular assembly between bovine fibrinogen and sodium perfluorooctanoate, octanoate, and dodecanoate in buffer solution using experimental techniques such as DSC, CD, Raman, UV-vis and SAXS [2]. These surfactants were chosen to distinguish the effects of chain length and stiffness. We showed that the unfolding process of fibrinogen does not follow a two-state process but instead involves intermediate states. Increasing the surfactant concentration results in changes in secondary structure. However, neither the quaternary nor tertiary structure undergoes large variations, as can be inferred from UV–vis and Raman spectra. SAXS measurements have shown that pure fibrinogen exists as a paired-dimer in this medium. Such structure is unaltered in the presence of sodium octanoate or perfluoroctanoate, but sodium dodecanoate affects the protein conformation leading to complex formation. We then characterized the adsorption behavior at the air-water interface by a combination of surface tension measurements and, after transfer of the film onto mica sheets, atomic force microscopy (AFM) [3]. First, interfacial rheology showed that fibrinogen has a low dilatational modulus at the air-water interface when compared to other proteins, suggesting the formation of a weak surface network. These studies suggested that complexes formed between fibrinogen and fluorinated surfactants are more surface active than fibrinogen, while the absence of interaction between fibrinogen and hydrogenated surfactants results in compaction of the surface layer. However, currently we lack a direct determination of the composition and structure of the films *in situ* at the air/water interface.

In order to gain detailed insight into the adsorption process and intermolecular surface interactions of fibrinogen with sodium octanoate (system 1), sodium dodecanoate (system 2) and sodium perfluoroctanoate (system 3), we propose to measure the composition and structure with neutron reflectivity measurements at the air/water interface on FIGARO. This work will allow us to correlate the role of the different chain lengths, hydrophobicity and stiffness of the three surfactants with the different pre-characterized physical properties of the formed films. These systems are very important. First, they will be useful to match our previous works (references 2 to 4). Second, it allows comparing the effect that the substitution of H by F has on the properties of the films. And finally, for the best of our knowledge, there are not previous studies on fibrinogen fluorinated films.

Results and discussion

All experiments were performed in 50 mM glycine NaOH buffer pH 8.5. First of all, we have performed kinetic measurement of the adsorption of pure fibrinogen at the interfaces. For this

purpose four different concentrations were chosen. 0.1, 1, 3 and 10 gl^{-1}. The adsorption process was checked for three hours. Previous ellipsometry measurement at 633 nm revealed that the adsorbed fibrinogen layers had refractive indices from 1.35 to 1.39, indicating high water content, as expected from the non-compact molecular structure of the protein. The surface layer thicknesses ranged from 14 to 46 nm, and the surface densities ranged from 3 to 16 mg m^{-2}, indicating that if the surface layer is a monolayer, then its average orientation increases with increasing FB concentration [4]. However, based on neutron reflectometry measurements we have found that the best model that fit our data is a two layer model. The first layers will be formed by protein molecules placed parallel to the interface with a thickness of about 5 nm which correlates perfectly with the size of the fibrinogen molecule. The second layer will be composed by protein molecules randomly oriented with little density. The thickness of this layer is about 14 nm (see scheme).



***Scheme**. Model of two layers proposed for fibrinogen adsorption at the interface*

We have checked the time evolution of these two layers. The two layers are formed almost immediately and its dimensions do not vary over time. However, the protein concentration in each layer is more susceptible to time. In the first layer a slight increase in coverage from 40 % (for t = 0) to 45 % (for t = 2 hours) have been observed. This seems to indicate a very stable monolayer with no reversible adsorption. In the second layer the change in coverage is more pronounced, from 5 % to 12 %. This fact it could be related with a dynamical exchange of protein molecules between the layer and the bulk, see figure 1.

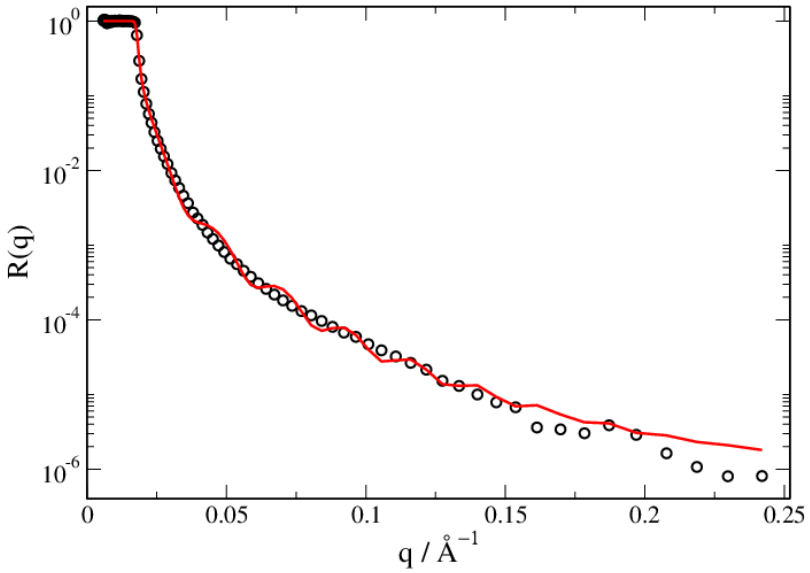


Figure 1. Reflectivity curve of the pure fibrinogen 1 gl^{-1} system in D_2O. The red line corresponds to the best fit to a two layer model.

In the second part of our work we have performed measurement of the systems fibrinogen-surfactant: system 1, system 2 and system 3. We used 4 contrasts per sample: fibrinogen with (1) d-surfactant/D_2O, (2) d-surfactant/ACMW, (3) h-surfactant/D_2O and (4) h-surfactant/ACMW. Naturally we cannot deuterate a fluorinated surfactant. The fibrinogen concentration was kept constant at a value of 1 gl^{-1}. The surfactant concentrations used were: 5, 10, 15, 20 and 30 mM for sodium octanoate; 0.1, 0.5, 1, 2.5 and 5 mM for sodium dodecanoate and 15 mM for sodium perlfluorooctanoate. At this time we have not finished of analyzed this part of the proyect.

References

1. Zhong,D.; Douhal, A.; Zewail, A.H. *Proc. Natl. Acad. Sci*. 97 (**2000**) 14056.
2. Hassan, N.; Barbosa, L. R. S.; Itri, R.; Ruso, J. M. *J. Colloid Interface Sci.* 362 (**2011**) 118.
3. Hassan, N.; Maldonado-Valderrama, J.; Gunning, A. P.; Morris, V. J.; Ruso, J. M. *J. Phys. Chem. B* (**2011**) 115, 6304.
4. Hernandez, E. M.; Franses, E. I. Colloids and Surfaces A: Physicochem. Eng. Aspects 214 (2003) 249