Experimental Report

15/01/2014

Proposal: **9-13-486** **Council:** 10/2012

Title: Structure of the "protein corona" for Human Serum Albumin-Silica nanoparticles

This proposal is continuation of: 9-13-360

Researh Area: Soft condensed matter

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Samples: C2918 H5691 O1473 N786 S41

Instrument	Req. Days	All. Days	From	To
D11	1	1	27/05/2013	28/05/2013
FIGARO Adsorption troug	2	2	29/05/2013	31/05/2013

Abstract:

The "protein corona" phenomenon has been invoked to explain the presence of cryptic epitopes - immunologically recognisable peptide strands - when proteins are mixed with nanoparticles. Its structure is not known. Our focus is the surface and bulk structure of complexes between human serum albumin and 20nm silica particles. On FIGARO, our previous experiments at the air-water interface showed that the silica-protein layer structure was well fitted by a three-layer model and by merging the bottom and the second layers the composition equations could be solved for mass concentrations of the three components. On D11, data have been fitted by a “beads on a string” model and the extent of protein “coating” of the nanoparticle (as proposed for the “corona”) is not yet proven. Here our aim is to add the missing contrasts needed to resolve the structure of the air-water interface (FIGARO) and resolve the lowest protein concentration required to induce aggregation of a small fraction of the silica (D11). This will be achieved because we will use fully deuterated human serum albumin (for the first time): its availability from the DLAB will allow us to write up the work for publication.

Report on Proposal 9-13-486
Structure of the "protein corona" for Human Serum Albumin – silica nanoparticles

Experiments were carried out in June 2013 on both FIGARO and D11 with the intention of adding the contrast from fully deuterated human serum albumen to the data from FIGARO and D11 collected in 2012. Shortly before the experiment it was found that the albumen had aggregated and this unexpected phenomenon is the subject of an accepted proposal DL-03-114 based on subsequent analysis of the problem. The 10% un-aggregated deuterated defatted albumin fraction was chromatographically separated, the reflectivity troughs remade for smaller volumes and successful, though limited experiments on both instruments done. Most of the material was needed for Figaro (the first experiment in line) and useful contrasts were obtained. The figure shows one example of the RQ^4 vs Q data and the corresponding real space profile:

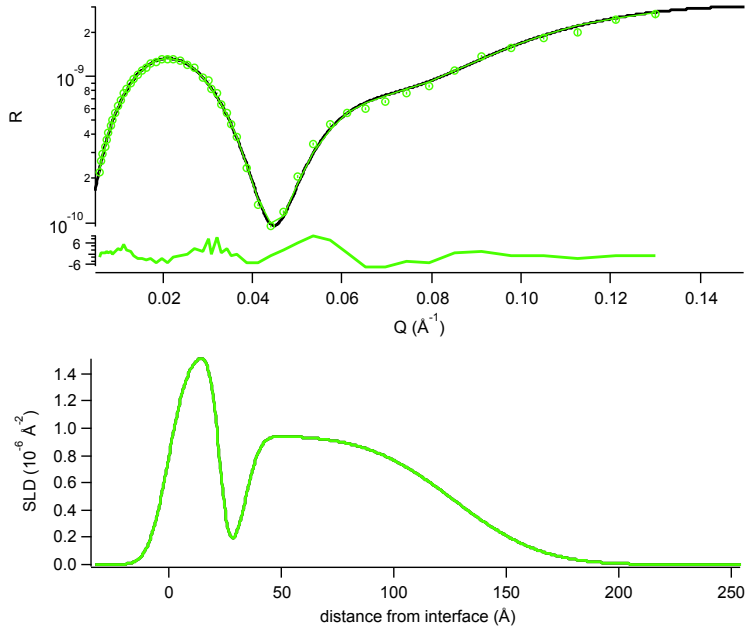

Figure 1 Reflectivity from perdeutero, defatted HSA on ACMW PBS (upper) and the real space profile showing the surface protein film and the attached silica particle

This work will lead to a publication – though must be followed up with the same measurement using perdeutero fatted HSA.

The remaining small quantity of deuterated defatted HSA enabled part of a contrast series for the small angle scattering to be determined. The small angle scattering is very important as the identification of the solution species is of high priority. This experiment needs to be repeated with perdeutero fatted HSA to be correlated with the work that we have done using the hydrogenous native material.

Figure 2 shows the data for an almost contrast matched silica with the deuterated protein.

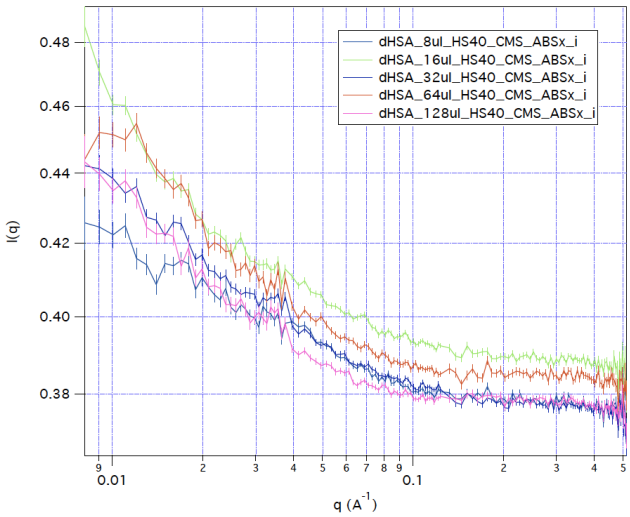

Figure 2 I(Q) vs Q Fully deuterated, defatted human serum albumin in 60% D_2O particle contrast matched.

We are still keen to give the classical model of the corona some credence though the scattering signatures from the native material – silica – interactions point towards protein-induced aggregation.

Two models fit the above data and native albumin-silica very well . They are "beads on a string" and "sticky spheres". To sort these out and fully test the symmetrical spherical model of the corona now needs data for wider Q range. The deuterated protein contrasts are essential. Figure 3 shows the sticky hard sphere model for the hydrogenous fatted HAS in ACMW.

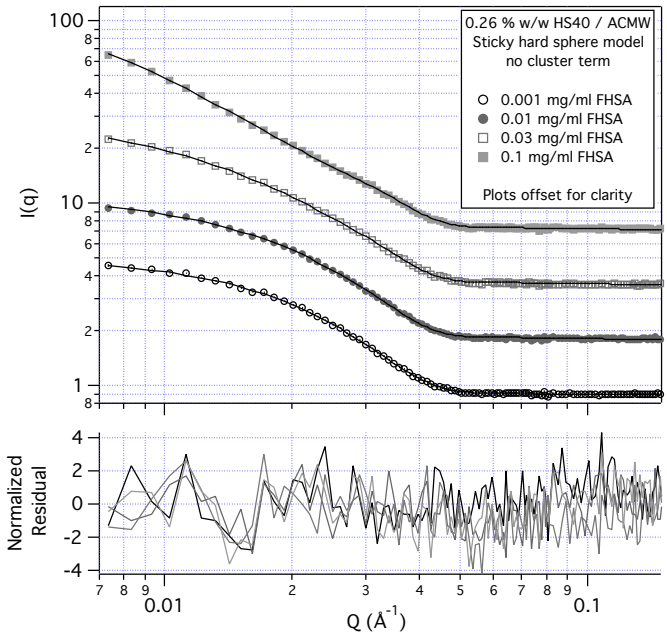

Figure 3 Fitted "sticky hard Sphere model to the low protein concentration D11 data