ESRF	Density profiles of proteins interacting with functionalized surfaces	**Experiment number**: **SC- 3802**
Beamline: ID03	**Date of experiment**: from: 23.05.2014 to: 26.05.2014	**Date of report**: 03.10.2016
Shifts: 9	**Local contact(s)**: Dr. Jakub DRNEC	*Received at ESRF:*

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The goal of this beamtime was the determination of density profiles of proteins interacting with functionalized substrates using standing-wave x-ray fluorescence (SWXF) targeting the proteins' sulfur (S) atoms. Such specific structural information is a valuable basis for the "rational design" of surfaces for medical and technological applications that rely on the control or suppression of protein adsorption [1-3].

The SWXF technique is based on the standing wave created at the solid/liquid interface by interference of the incident electromagnetic wave with the wave reflected from the substrate under Bragg reflection conditions generated with dedicated nanometric multilayers [4, 5]. Fig. 1C schematically illustrates the measurement geometry. During a scan of the angle of incidence θ around the Bragg condition the maxima of the standing wave move along the surface normal (z). The standing wave is used to excite the specific fluorescence of the chemical elements of interest. From the angle-dependent fluorescence intensity the element distribution can then be reconstructed. Earlier SWXF studies have dealt with the localization of heavy chemical elements, often used as labels for biological systems [4, 5].

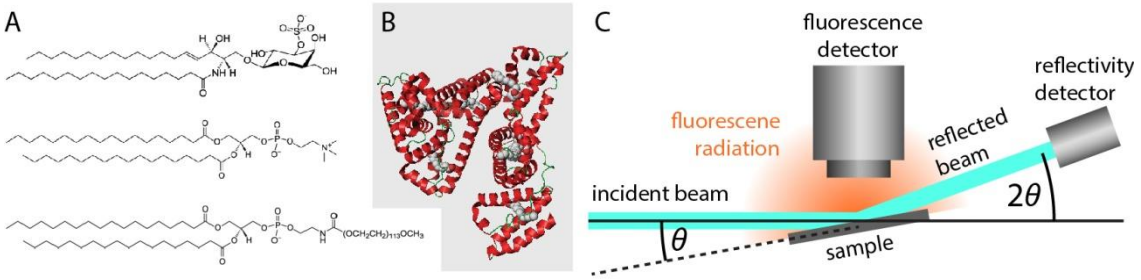

Figure 1: (A) Chemical structures of SGS (top), DSPC (middle), and PEG-lipid (bottom). (B) Structure of the protein HSA. (C) Setup and measurement geometry (top view) of the SWXF experiments. The x-ray beam illuminates the sample surface at an incident angle θ. At each incident angle fluorescence spectra are recorded with an energy-sensitive fluorescence detector oriented perpendicular to the incident beam and close to the sample surface.

During beamtime SC-3802 we extended the SWXF approach to the comparatively light yet biologically very important chemical elements S and P. This opens up new possibilities for the label-free application of SWXF to biomolecular samples, yielding element-specific density profiles at atom scale resolution.
During that beamtime we worked with lipid and protein molecules as illustrated in Fig. 1 A and B. In particular, we carried out measurements of the S distribution in human serum albumin (HSA) monolayers adsorbed to hydrophilic and hydrophobic substrates from the aqueous phase using a dedicated liquid cell. The angle-dependent S fluorescence for the different conditions is shown in Fig. 2A. Solid lines indicate the simulated intensities according to the best matching madol parameters for the protein configurations (schematically illustrated in Fig. 2B). The results of this beamtime (on proteins but also on lipid systems) were published in the interdisciplinary journal *Proceedings of the National Academy of Sciences of the USA* [6]. More recently, we have shown in a beamtime at ID10 that even for the light elements S and P, the sensitivity of the method is sufficient to characterize layers with only one atom per 5-10 square nanometers [7].

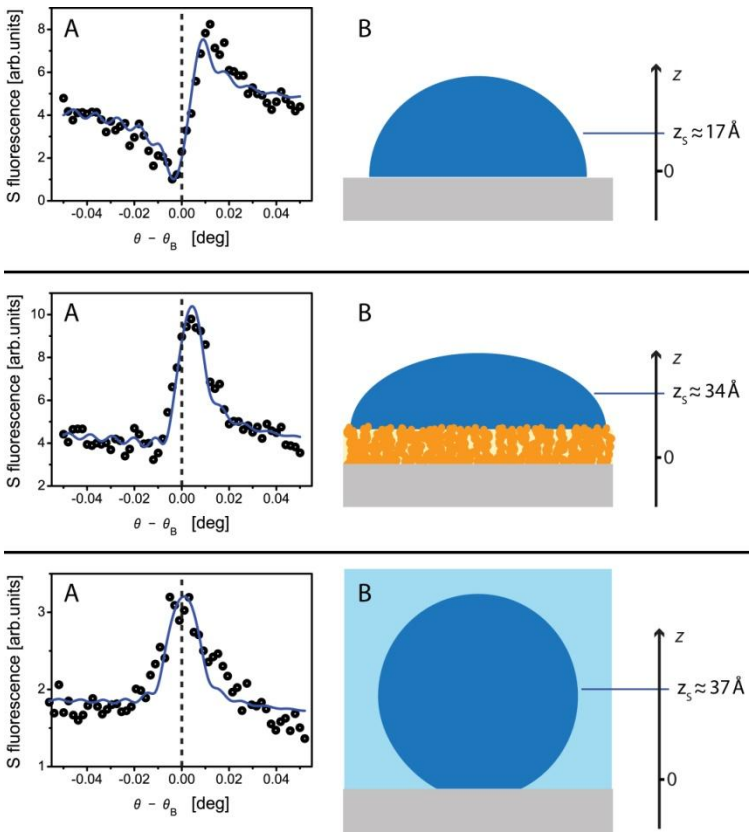

Figure 2: Experimental results on surface-adsorbed protein layers: HSA adsorbed to bare Al oxide (top), to OTS functionalized Al oxide (middle) and to bare Al oxide at full hydration under water (bottom). (A) Measured angle-dependent S fluorescence intensities (symbols) together with the simulated intensities (solid lines) corresponding to the best-matching models. Vertical dashed lines indicate the Bragg condition. (B) Sketches of the protein layers configurations. The axis perpendicular to the sample surface is denoted with *z*.

References

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