ESRF	Polymer-Functionalized Surfaces in Contact with Protein Solutions: Quantifying Different Modes of Protein Adsorption	**Experiment number**: **SC- 3699**
Beamline: ID03	**Date of experiment**: from: 11.05.2013 to: 14.05.2013	**Date of report**: 28.08.2013
Shifts: 9	**Local contact(s)**: Dr. Roberto FELICI	*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). Such specific structural information would be a valuable basis for the "rational design" of surfaces for medical and technological applications that rely on the control or suppression of protein adsorption.

The SWXF technique is based on the standing wave created at the solid/liquid interface by interference of the incident wave with the wave reflected from the substrate. The proteins are localized by exciting the characteristic fluorescence of their sulphur (S) content with the standing wave whose shape depends on the incident angle. When it comes to the localization of the relatively light but biologically important element S at the solid/liquid interface, the application of SWXF requires a compromise between fluorescence yield and undesired beam absorption: Decreasing the beam energy increases the fluorescence yield but also increases absorption. In addition, the chemical purity of all illuminated materials is crucial. In a previous test beamtime at ID03 we tested a number of substrates for the generation of a tunable standing wave, and found that the substrate composition is the strongest limitation to the sensitivity of SWXF in localizing S atoms.

During the recent first regular beamtime (SC-3699), we demonstrated that high-purity sapphire-supported Ni/Al multilayers with 10 nm period are well-suited substrates. Fig. 1b shows the measured x-ray reflectivity curve of such a substrate. Standing waves with long periods are generated at incident angles below the critical angle of total reflection, while standing waves with short periods are generated around the strong Bragg peak. The substrates, thus, combine a wide *z*-range with a high *z*-resolution for the localization of S atoms.
During the same beam time we also confirmed that a dry monolayer of human serum albumin (HSA) proteins adsorbed on the substrate surface can be detected from the S fluorescence signals. Fig. 1c shows an x-ray fluorescence spectrum exhibiting the characteristic lines of aluminum (Al), zirconium (Zr), sulphur (S), chlorine (Cl), and argon (Ar) on top of a background and a number of weaker lines. To extract the amount of S fluorescence, the spectra are comprehensively modeled by a procedure that is based on physically realistic peak shapes [10, 11]. Fig. 1d shows the S fluorescence intensity as a function of the incident angle around the Bragg peak. The pronounced intensity variation in the vicinity of the Bragg angle corresponds to the narrow S distribution in the protein monolayer adsorbed to the substrate surface.

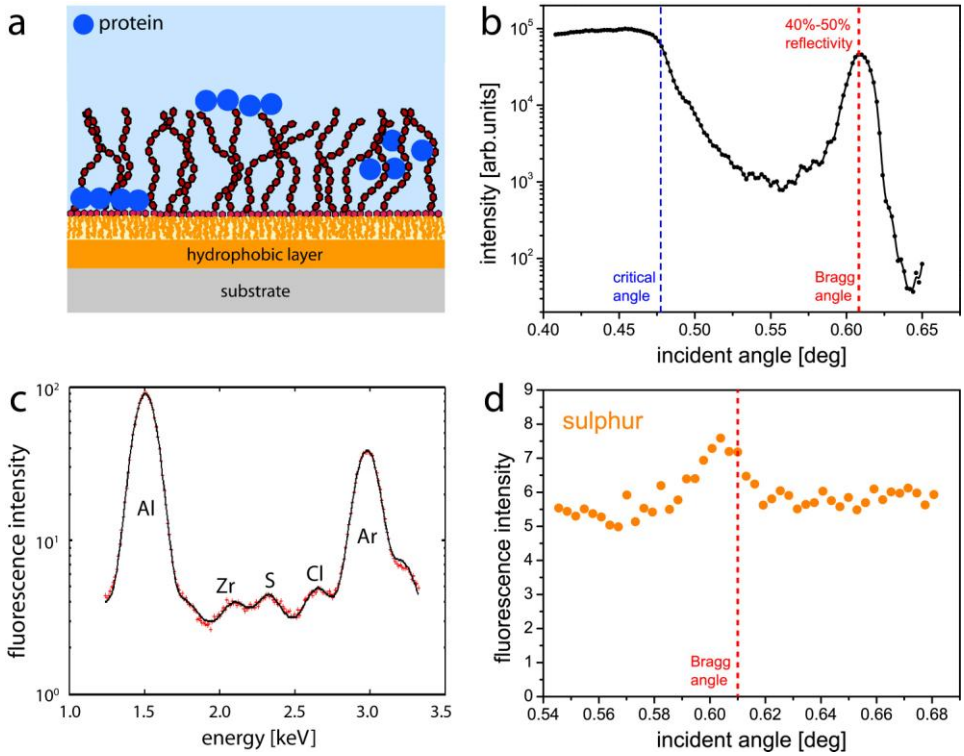

Figure 1: (a) Sketch of proteins interacting with polymer functionalized surfaces in various ways. (b) X-ray reflectivity of sapphire-supported Ni/Al multilayers with 10 nm period. (c) X-ray fluorescence spectrum exhibiting the characteristic sulphur (S) line originating from a protein monolayer. (d) S fluorescence intensity as a function of the incident angle around the Bragg peak.

We also carried out measurements on a HSA monolayer at the solid/liquid interface using a dedicated liquid cell, but were not able to collect fluorescence signals with sufficient statistics, due to problems with the sample envronment: There was undesired background fluorescence due to unexpected S and Cl (chlorine) impurities in a thin foil used and in the aqueous medium. These limitations can be easily avoided in the future, by the choice of optimal materials and high-purity chemicals.