ESRF	Polymer-Functionalized Surfaces in Contact with Protein Solutions: Quantifying Different Modes of Protein Adsorption	**Experiment number**: **SC- 3699-2**
Beamline: ID03	**Date of experiment**: from: 21.09.2013 to: 32.09.2013	**Date of report**: 14.10.2013
Shifts: 6	**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 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.

During the very recent beamtime (SC-3699-2) we carried out measurements on a HSA monolayer adsorbed to the substrate from the aqueous phase using a dedicated liquid cell covered with a thin *ultralene* foil of high chemical purity. The system is illustrated in Fig. 1c. Fig. 1d shows x-ray fluorescence spectra measured with the bare substrate in air (black), with the bare substrate covered by the foil (red), and with the substrate with adsorbed protein monolayer in a thin water layer covered by the foil (green, see Fig. 1c). The spectra are scaled such that the relative

peaks intensities can be visually compared on a qualitative level. The sulfur (S) line emitted by the S atoms in the HSA monolayer is clearly identified on top of the well-charactzerized fluorescence background created by substrate and foil. These preliminary results suggest that a single protein monolayer at the solid/aqueous interface can be characterized by SWXF via the characteristic fluorescence of sulfur atoms. Careful analysis of the angle-dependence of the recorded fluorescence spectra, accounting for the background created by substrate and foil, will allow for the reconstruction of the proteins' sulphur distribution normal to the surface.

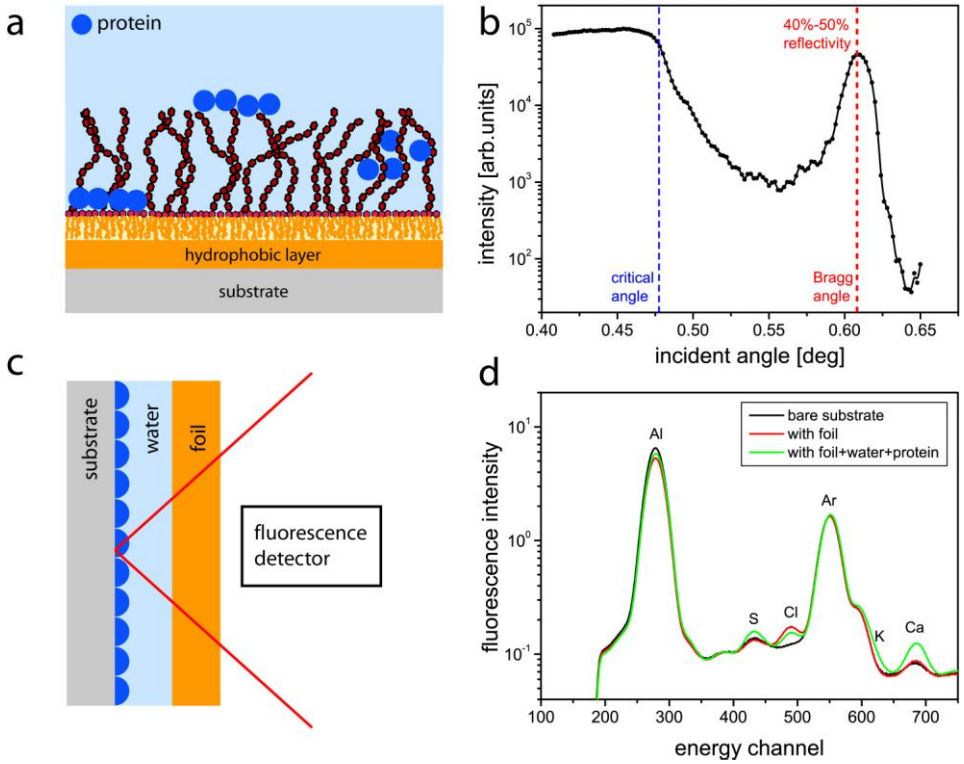

Figure 1: (a) Sketch of proteins interacting with polymer functionalized surfaces in various ways. (b) X-ray reflectivity of sapphire-supported Ni/Al multilayers with 10 nm period. (c) SWXF measurements on a HSA protein monolayer adsorbed to the substrate from the aqueous phase using a dedicated liquid cell covered with a thin foil. (d) Fluorescence spectra of bare substrate (black), substrate with foil (red), and substrate with adsorbed protein monolayer in a thin water layer covered by the foil (green).