PolyProt: Protein-Repellent Polymers

Background and objectives: Protein adsorption to material surfaces causes problems in numerous technological and medical applications. A favored approach in order to prevent protein adsorption is to decorate surfaces with brushes of terminally anchored, neutral water soluble polymers (NWSP). But despite the great importance of NWSP-functionalization in medicine and technology, the interaction of proteins with NWSP is not fully understood. In particular, little is known about the mechanisms responsible for regularly observed "brush failure", where protein adsorption occurs despite NWSP functionalization. The project's objective was the detailed structural characterization of protein adsorption onto NWSP brushes using neutron and x-ray scattering techniques. Structural insight is an essential prerequisite for the rational design of protein-repellent surface functionalization based on NWSP brushes. The project in particular focused on protein adsorption mechanisms that contribute to brush failure *in vivo* and in medical and technological applications.

Work performed: The study concerned NWSP-decorated surfaces of defined characteristics (i.e., polymer length and grafting density) and two types of proteins: 1) Globular proteins whose undesired adsorption to polymer surfaces is believed to be driven by weak non-specific interactions. 2) Anti-NWSP proteins that adsorb because of strong specific binding. They are believed to have implications in the field of biocompatible functionalization. NWSP brushes of controlled polymer lengths and grafting densities were deposited onto planar surfaces by the Langmuir-Schaefer technique and subsequently incubated with aqueous solutions of defined protein concentrations. The density profiles of adsorbed proteins perpendicular to the sample plane were then determined using neutron reflectometry (NR) with contrast variation. This included the characterization of the brush density profiles as the initial analysis step. In parallel a standing-wave x-ray fluorescence (SWXF) technique was optimized and adapted for the localization of the light elements phosphorus (P) and sulfur (S). The measurements confirmed that a single protein monolayer at the solid/liquid interface can be structurally characterized via the proteins' sulfur content, suggesting a great potential of the method for the element-specific high-resolution structural characterization of biological and bio-mimetic systems at the solid/liquid interface

Results achieved: NR on the interaction of myoglobin with poly(ethylene glycol) (PEG) polymers grafted to hydrophobic polystyrene (PS) surfaces revealed significant primary adsorption, where the adsorbed protein amount decreases with increasing polymer amount per surface area. Closer inspection revealed that the primary adsorption involves two distinct sub-layers, one dense inner layer directly on top of the grafting surface and a second, thicker and more dilute outer layer on top of the inner layer (Fig. 1 left). The amount of protein adsorbed in the inner layer is independent of the polymer length N but varies with the brush grafting density σ, while for the outer layer it is correlated to the amount of grafted PEG and is thus sensitive to both N and σ. The experiments also revealed that anti-PEG antibodies interact with PEG brushes via the mechanism of specific ternary adsorption. The antibodies binding the methoxy-terminated PEG chain segment specifically adsorb onto PEG brushes grafted to lipid monolayers on a solid support. The antibodies adsorb at the outer edge of the brush suggesting an inverted "Y" orientation with two F_{AB} segments facing the brush (Fig. 1 right). The thickness and density of the adsorbed antibody layer as well as its separation from the grafting surface grow with increasing brush grafting density when most of the protein is excluded from the brush.

The different adsorption modes and the dependence of the adsorbed amount on the brush parameters were rationalized on a theoretical level. The results obtained for the various systems investigated allow envisaging optimal brush parameters that likely minimize undesired protein adsorption, that is, maximize brush performance.

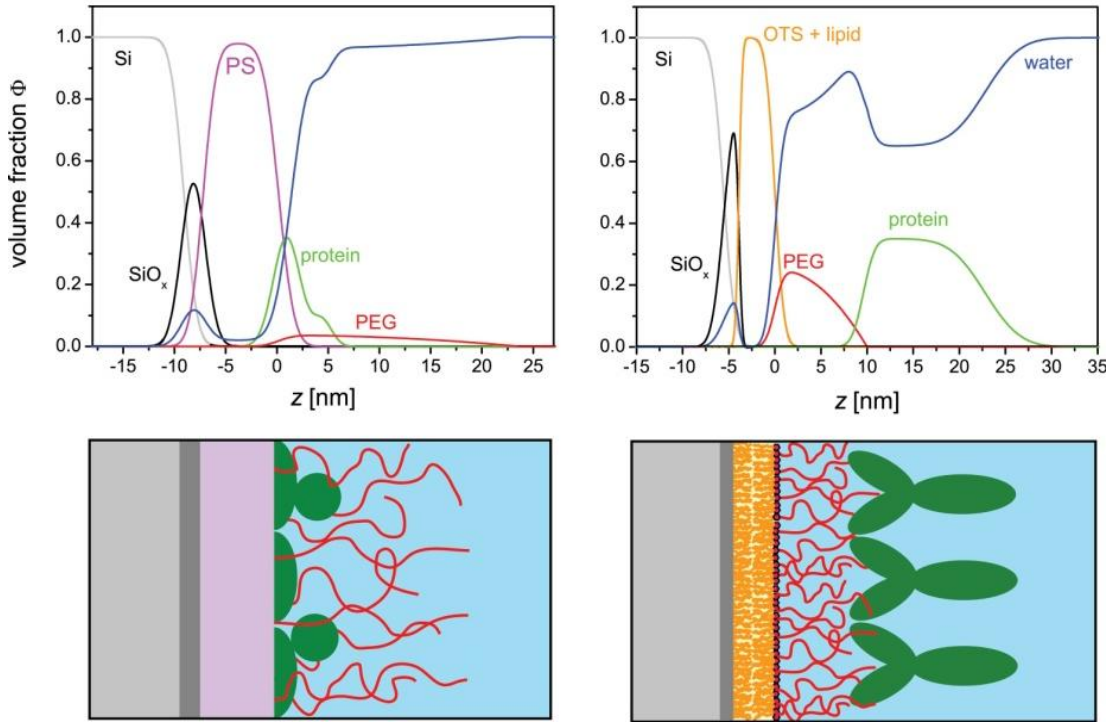

Figure 1: (top left) Volume fractions Φ of silicon (Si), silicon oxide (SiOx), polystyrene (PS), PEG, myoglobin (Mb), and water at a PEG-functionalized surface after incubation with myoglobin solution as determined by neutron reflectometry, plotted as functions of the distance *z* perpendicular to the sample plane. (bottom left) The interpretation of the concentration profiles involves two sublayers of adsorbed Mb, a dense inner layer and a dilute outer layer. (top right) Volume fractions Φ of various compounds at a PEG-functionalized surface after incubation with anti-PEG antibodies. (bottom right) The interpretation of the volume fraction profiles in terms of a dense layer of oriented antibodies.

Expected final results and their potential impact and use: Besides the substantial methodological progress made within the project, the key conclusions are the eminent roles of the grafting surface and the polymer antigenicity in the performance of protein repellent NWSP brushes. The insights gained within the project allow envisaging optimal brush parameters that likely minimize undesired protein adsorption under defined conditions. The following challenge is the transfer of these insights to more complex systems which are closer to medical applications. Based on the methodology established here, high-precision structural studies on protein adsorption from the relevant body liquids like blood serum or urine appear feasible. From that point on fruitful implementation of the fundamental research into medial studies and accompanying significant socio-economical impact may be expected.