ESRF	**Experiment title:** Time-resolved SAXS study on the release of proteins on spherical polyelectrolyte brushes	**Experiment number**: SC 2495
Beamline: ID02	**Date of experiment**: from: 07/11/2008 to: 11/11/2008	**Date of report**: 25/02/2009
Shifts: 12	**Local contact(s)**: Dr. A. Martel, Dr. T. Narayanan	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

S. Rosenfeldt *, K. Henzler*, B. Haupt *, M. Skarba*, B. Ballauff, University of Bayreuth, Germany.

A. Martel*, T. Narayanan, ESRF, France.

Report:

The adsorption and immobilization of proteins from aqueous solutions onto solid surfaces is a topic of considerable interest. For various potential applications some knowledge of the mechanism of the release of the proteins is needed. We investigated the adsorption and release of the proteins onto colloidal spherical polyelectrolyte brushes (SPB) by time resolved small-angle x-ray scattering (TR-SAXS) at different ionic strengths (0.007-1M). Figure 1 shows the adsorption process of the protein β-lactoglobulin (BLG) onto a SPB consisting of a polystyrene core with densely attached polystyrenesulfonate chain. Solutions of 1.8 wt% brush and 15 g/L protein were rapidly mixed in a stopped flow apparatus. The *pH*-value of both components were adjusted by a 10 mM MES buffer. The final state of the adsorption process was reached after 9.8 ms, thus we follow a rapid kinetics. The data were analyzed after reference [1-2]. It turned out that about 745 mg BLG/g SPB was adsorbed. This result is corroborated by ITC measurements. Furthermore, we have first evidence from the fits, that the BLG exist partionaly as tetramer in the adsorbed state inside the brush layer. This may be explained by the different pH-values inside the brush layer.

Moreover, we investigated the reverse process, the desorption of bovine serum albumin (BSA) from a brush. The adsorption process has already been studied in Ref. [2]. For the desorption experiments, solutions of SPBs with adsorbed BSA and buffer solution with additional salt were rapidly mixed and the desorption of BSA was monitored by measuring the SAXS signal in smallest time steps. Fig. 2 shows the typical time scale for the desorption of BSA from a brush consisting of a polystyrene (PS) core onto which poly(acrylic acid) (PAA) chains are densely grafted. We find that the main change in the scattering intensity happened directly after the mixing process. The final state of the adsorption was reached after 880 ms. Similar results were obtained for different ionic strengths. Further evaluation is currently in progress.

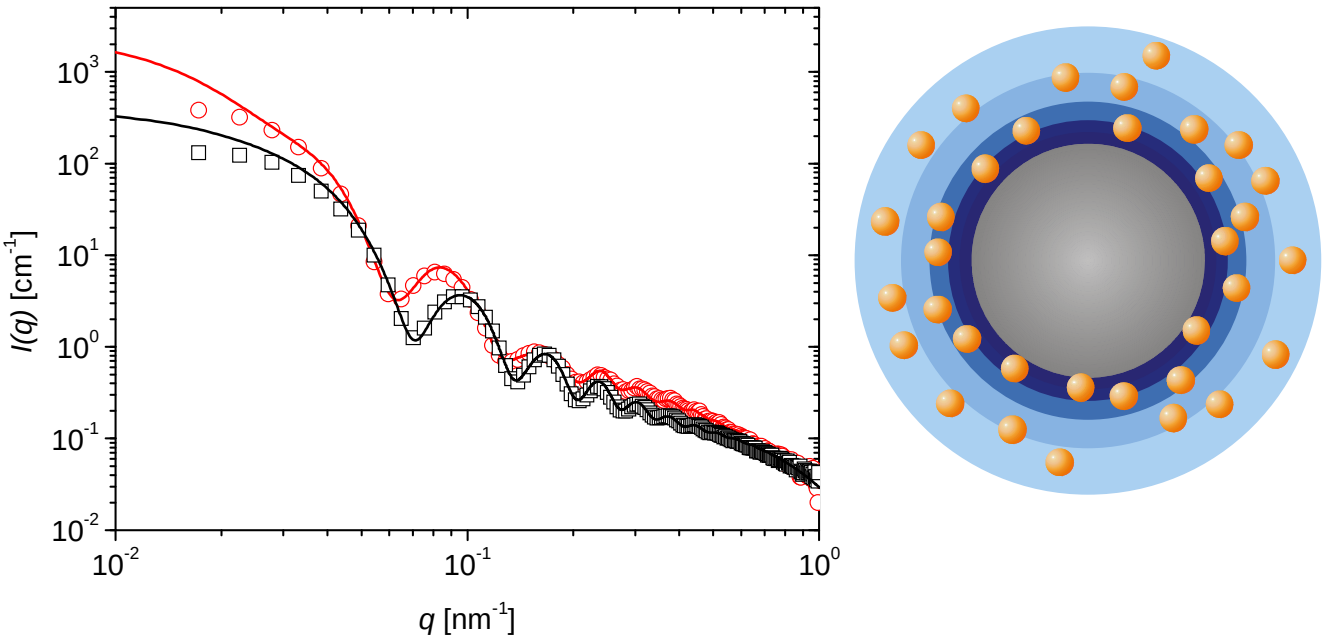

Fig. 1: a: Adsorption of BLG onto the SPB after 9.8 ms. SAXS intensities of the unloaded (black line) and the loaded (red line) SPB. b: Schematic representation of the multi-shell sphere model that was used for the calculation of the scattering intensities. The smaller spheres represent the proteins.

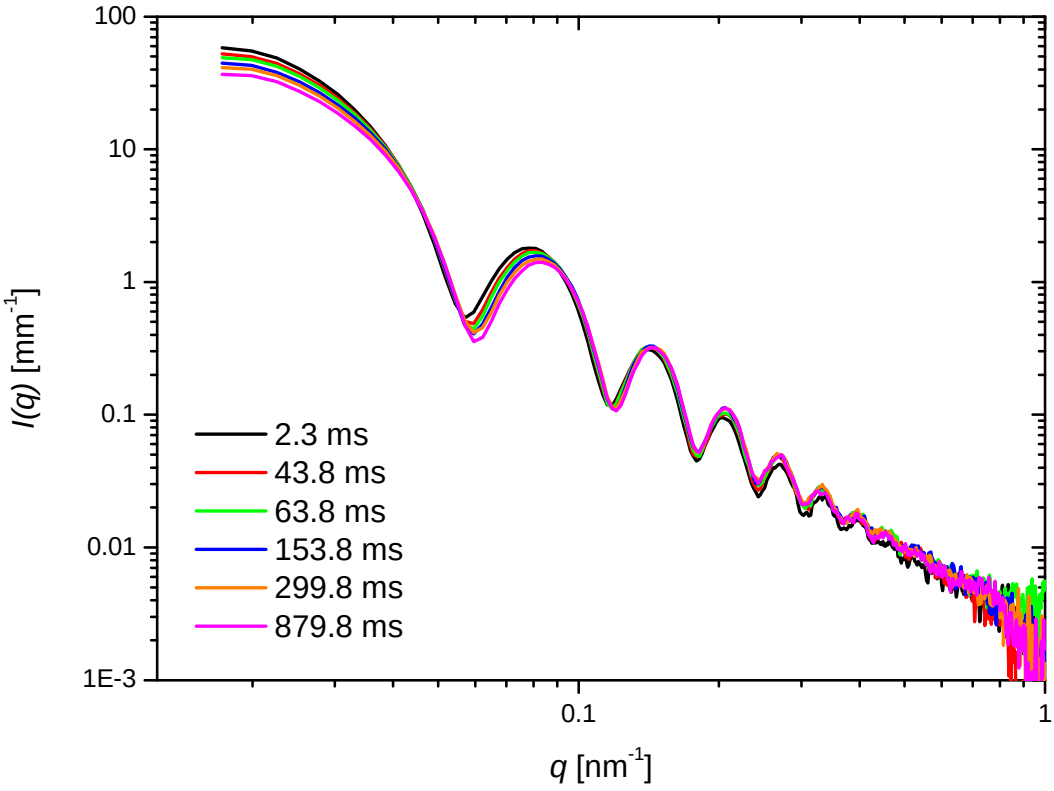

Fig. 2: Desorption of BSA onto PS/PAA-brush mixed with a buffer solution containing 100mM $NaNO_3$. SAXS intensities as function of time t.

Literature

[1] K. Henzler, A. Wittemann, E. Breininger, M. Ballauff, S. Rosenfeldt, Biomacromolecules 2007, 8, 3674-3681.

[2] K. Henzler, S. Rosenfeldt, A. Wittemann, L. Harnau, S. Finet, T. Narayanan, M. Ballauff, PRL 2008, 100, 158301.