ESRF	**Experiment title:** Time-resolved SAXS study of the adsorption of proteins on spherical polyelectrolyte brushes	**Experiment number**: Sc-1641
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Report:

We investigated the adsorption of the protein bovine serum albumin (BSA) on colloidal spherical polyelectrolyte brushes (SPB) by time resolved small-angle x-ray scattering at low ionic strength. The spherical polyelectrolyte brushes consist of a poly(styrene) core onto which long chains of poly(acrylic acid) (PAA) or poly(styrene sulfonic acid) (PSS) are grafted. Static SAXS experiments conducted previously [1] have demonstrated that the proteins penetrate fully into the surface of the brush layer (fig. 1).

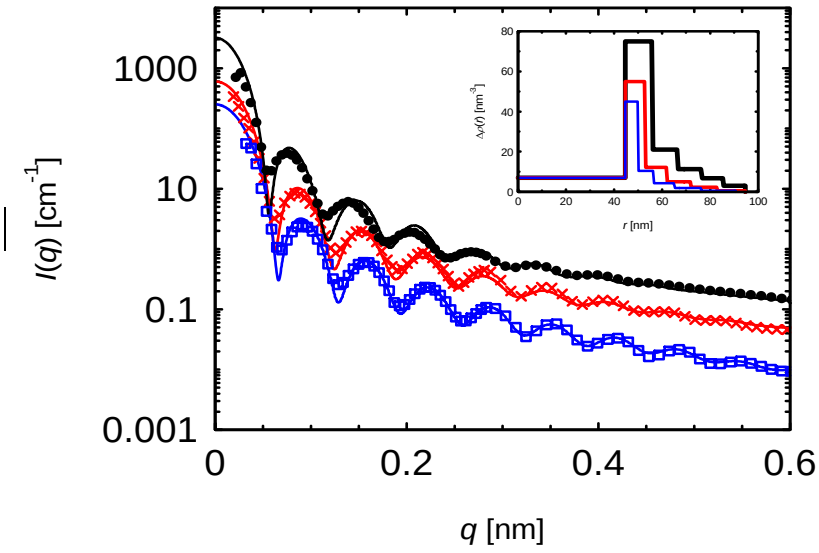

Fig. 1:

Adsorption of bovine serum albumin BSA onto a spherical polyelectrolyte brush (PAA brush) monitored by a Kratky-Compact-Camera.
Open squares: scattering intensity of the unloaded brush, crosses: 296mg BSA/g carrier particles, circles: 1116mg BSA/g carrier particles. [1]

In order to investigate the kinetics of this process (protein adsorption) we conducted time-resolved studies on these systems. Solutions of brush and protein were rapidly mixed in a stopped flow apparatus. The *pH*-value of both components were adjusted by a 10 mM MES buffer containing 2mM NaN_3 to prevent bacterial growth. SAXS-data were recorded in smallest possible time steps (100ms) using a CCD detector equipped with an image intensifier. Moreover, both components alone were measured.

It is well known that proteins may degrade which subjected to strong radiation. Fig. 2 shows the scattering intensity of the pure brush solution after an exposure time of 0.1s and 2s. The scattering intensities differ in the absolute scale. The different slope of the intensity curves correspond to a change in the radius of gyration from 2.9nm to 3.3nm. Therefore we recorded the scattering intensities of the protein solution with the same time resolution as used in the mixing experiments. The exposure time was set to 0.1s. In this case no significant change of the scattering intensities of the pure BSA solution was detected.

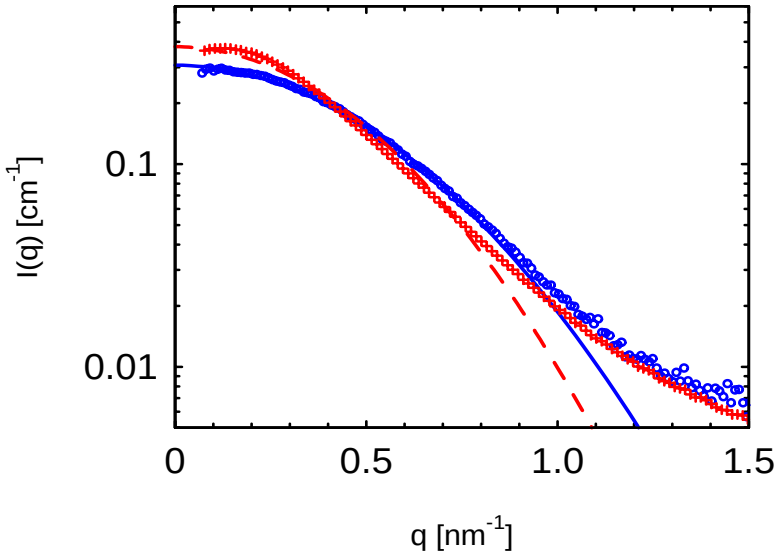

Fig. 2:

Degradation of BSA in the X-ray beam: Scattering intensities of the protein solution after an exposure time of 0.1s (circles) and 2s (crosses) with corresponding Gaussian fit (4 g/L bovine serum albumin in MES-buffer).

Fig 3. displays a typical result of TR-SAXS by plotting the intensities measured after different mixing times. Similar results have been detected in most of runs regardless of the mixing volume, mixing ratio and mixing time. During the evaluation of the data we found out, that the data at time ranges near 0s could only be explained by assuming a mixing ratio differing from the one adjusted in the stopped-flow experiment. This may be due to a mixing process not achieved at this time scale. For small time scales, where none or low adsorption is expected, the total scattering intensity at low *q*-value was lower then half of the mixed concentration of the brush solution. At intermediate q-range the scattering of the proteins dominate the total scattering, the measured intensity was significant higher than half of the concentration of the proteins. Additional mixing experiments confirmed that the total scattering intensity at times near 0s can indeed be explained by adding up the intensities of the components using half of original concentration (inset fig. 3).

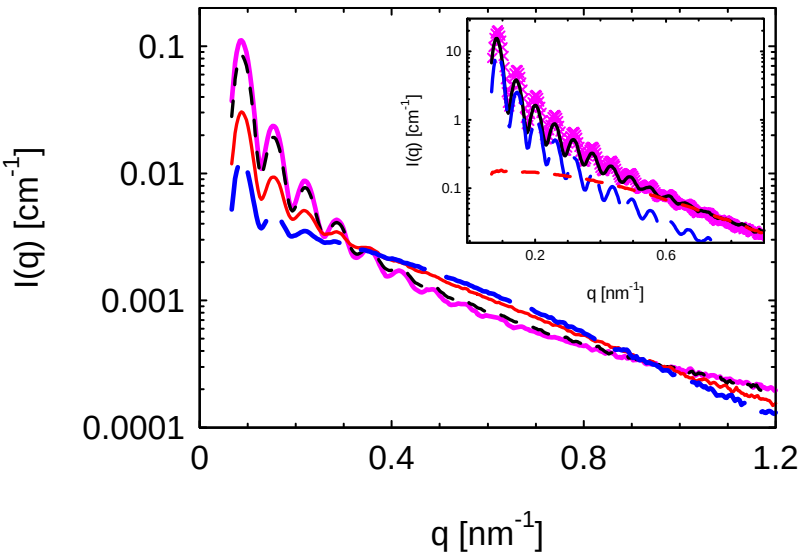

Fig. 3:
Time resolved SAXS of adsorption of protein on spherical polyelectrolyte brushes. Mixing of 75µl PSS brush (1 wt-%) and 75µl BSA (5g/l) in 25 ms. Scattering intensities after 0.1s, 10.9s, 323.1s and 1026s (bottom (blue) to top (pink)).

<u>Inset:</u>
Intensities after mixing 2ml of BSA (4g/l) with 2ml PAA brush by hand and measuring the mixed solution as fast as possible (line: after about 40s, crosses: after 6h). Additonally the scattering intensities of free brush (dashed line) and free BSA (dotted line) assuming half concentration after mixing are shown.

For the interpretation of the time resolved data we assumed no significant change in the number density due to mixing problems or diffusion processes after about 5s. To get the number density of the system we fitted the height of the first maximum of the measured scattering intensity after 5s using the parameters of the pure brush. For a longer time the number density was set to this value. In fig. 4 the experimental scattering intensity of the adsorbed system is shown after 5.3s and 65.9s including the corresponding fits. The fits describe the experimental data up to q values about 0.9nm^{-1}. The deviations for higher scattering angles can be explained by the approximation of scattering intensity of the adsorped protein by a Gaussian. From the fits the amount of adsorbed protein was calculated to 11% after 5.3s and to 65% after 65.9s.

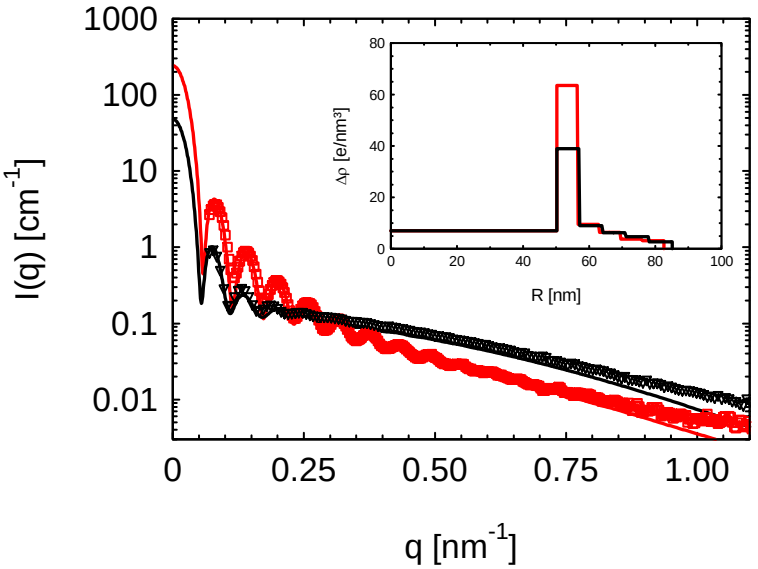

Fig. 4:
Time resolved SAXS of adsorption of protein on spherical polyelectrolyte brushes. Mixing of 50µl PAA brush (1 wt-%) and 50µl BSA (2g/l). Scattering intensities after 5.3s (black triangles) and 65.9s (red crosses) with corresponding fits.

<u>Inset:</u> Electron density distributions obtained from the analysis of the data.

The result of the evaluation of the mixing experiment by hand is shown in fig. 5. In this case the number density of the system was not a free parameter. The adsorption of the model leads to an amount of adsorbed protein of 90% after about 40s and 100% after 6h. To explain the small differences between experiment and theory seen in fig. 5 the used model must be improved.

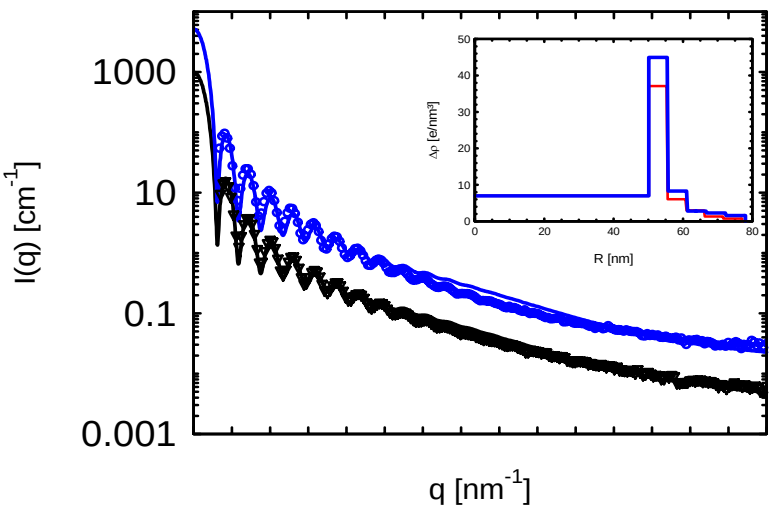

Fig. 5:

Scattering intensities of the mixture PAA brush and BSA after about 40s (black triangles), 6h (blue circles) and corresponding fits. For the sake of clarity the scattering intensity after 6h is multiplied by factor 10.

Inset: Electron density distribution of pure brush and of brush with adsorbed protein after 6h.

Conclusion

We have performed first TR-SAXS measurements on the adsorption of proteins onto spherical polyelectrolyte brushes. In these experiments we encountered problems with the stopped flow apparatus which precluded a quantitative analysis of the data so far. However, we obtained first clear evidence that the process of adsorption is much slower than expected from diffusion: A spherical particle with radius 3nm in water needs 4.9ms to diffuse over a length of 50nm. The above experiments indicate that even after about 40s the total amount of proteins was still not adsorbed. We assign this finding to electrostatic repulsion between the polyelectrolyte chains and the proteins. We plan to elucidate this point in further experiments.

Literature

[1] Rosenfeldt, S; Wittemann, A; Ballauff, M; Breininger, E; Bolze, J; Dingenouts, N: Interaction of proteins with spherical polyelectrolyte brushes in solution as studied by small-angle X-ray scattering, Physical Review E, **70**, 061403 (2004)