EUROPEAN SYNCHROTRON RADIATION FACILITY

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ESRF

Experiment Report Form

The double page inside this form is to be filled in by all users or groups of users who have had access to beam time for measurements at the ESRF.

Once completed, the report should be submitted electronically to the User Office via the User Portal:

https://wwws.esrf.fr/misapps/SMISWebClient/protected/welcome.do

Reports supporting requests for additional beam time

Reports can be submitted independently of new proposals – it is necessary simply to indicate the number of the report(s) supporting a new proposal on the proposal form.

The Review Committees reserve the right to reject new proposals from groups who have not reported on the use of beam time allocated previously.

Reports on experiments relating to long term projects

Proposers awarded beam time for a long term project are required to submit an interim report at the end of each year, irrespective of the number of shifts of beam time they have used.

Published papers

All users must give proper credit to ESRF staff members and proper mention to ESRF facilities which were essential for the results described in any ensuing publication. Further, they are obliged to send to the Joint ESRF/ ILL library the complete reference and the abstract of all papers appearing in print, and resulting from the use of the ESRF.

Should you wish to make more general comments on the experiment, please note them on the User Evaluation Form, and send both the Report and the Evaluation Form to the User Office.

Deadlines for submission of Experimental Reports

- 1st March for experiments carried out up until June of the previous year;
- 1st September for experiments carried out up until January of the same year.

Instructions for preparing your Report

- fill in a separate form for each project or series of measurements.
- type your report, in English.
- include the reference number of the proposal to which the report refers.
- make sure that the text, tables and figures fit into the space available.
- if your work is published or is in press, you may prefer to paste in the abstract, and add full reference details. If the abstract is in a language other than English, please include an English translation.

ESRF	**Experiment title:** In-situ monitoring of protein adsorption layer thickness during protein-A chromatography using SAXS	**Experiment number**: MX-1875
Beamline: BM29	**Date of experiment**: from: 17 February 2017 to: 19 February 2017	**Date of report**:
Shifts: 6	**Local contact(s)**: Petra Pernot	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

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Report:

The aim of this experiment was to investigate the thickness of the protein adsorption layer during protein-A chromatography and the impact of adsorption on the protein structure in-situ. The experiment employed SAXS for in-situ monitoring of the adsorption layer during protein loading and elution of a SAXS column.

Specially prepared SAXS column comprising of ø 1.5 mm capillary (10 µm glass thickness) glued in metal framework and attached to HPLC adapters for connection to chromatography system was installed in-line BM29 beamline and connected to Schimadzu SPD-M20A HPLC system (Figure 1).

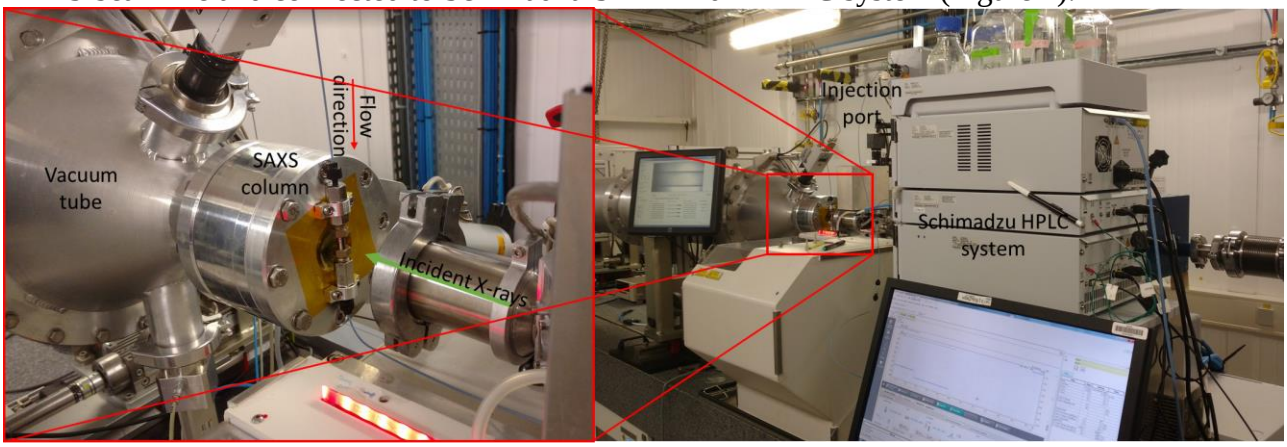

Figure 1 **Picture of hardware layout at BM-29. Due to fixed position of HPLC system used tubing for connection of SAXS column was longer than expected, however the dilution problems was solved by using injection port closer to the column position and tubing of 1/16" diameter.**

Data was collected using MabSelect SuRe and Toyopearl AF-Protein A resins and Herceptin ® (Trastuzumab) from Roche – dialysed into running buffer 0.01M NaPO4 + 0.15M NaCl pH 7.4 and concentrated to 16 mg/mL. During chromatographic run 4 mg of Herceptin was loaded on the column ensuring saturation of the column followed by protein elution and subsequent column regeneration. Data was collected in the effective q range of 0.03-2.45 nm^{-1} using 8 keV X-rays every second, automatically integrated and normalized on the transmission. Futher data analysis was done by semiautomated algorithm written in Mathematica that characterize each scattering curve by means of:

- **Rc** - Radius of gyration for cylinders: where intensity I(q) is dependent on q^{-1} ($-q^2rc^2/2$) where rc is radius of gyration for infinitely long cylinder L>>R. The cross-sectional radius of gyration for the rod like scatterer was chosen based on the assumption that internal network of porous resin could be regarded as cylinders (confirmed by SEM images).
- **Rg** - Radius of gyration for the shoulders visible in double logarithmic plot
- **d0** - Initial slope that gives information if the scatterer is a 1D, 2D or 3D object
- **d1** - Porod slope that indicates if the surface of scatterer is smooth or not,
- **d2** - Protein slope that indicates presence of protein attached to resin or in the bulk.

Exemplary data: MabSelect SuRe resin + 4 mg Herceptin at 0.2 ml/min flowrate

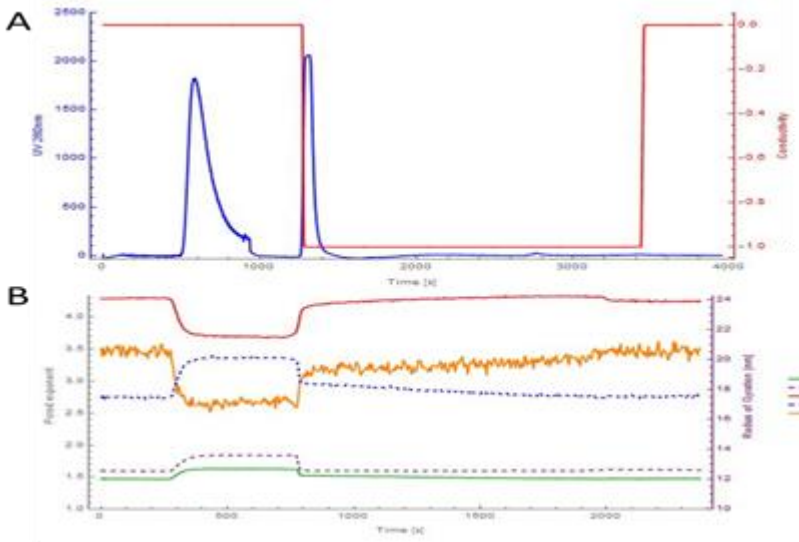

Figure 2 **A - a chromatogram from protein-A run. First UV peak (blue curve) indicates overloading of the SAXS column with protein, whereas second peak is an elution peak at the breakthrough of buffers (indicated by conductivity change (red). B – fitting analysis of scattering profiles. Red indicates Porod constant, yellow – protein presence, green – initial slope, blue dotted – Rg and violet dotted – radius of gyration of cyliner.**

As stated before, column was overloaded with Herceptin protein solution to achieve full saturation of all available binding sites within column which is indicated by the presence of first UV peak around 400-800s. Presence of the bound protein is visible in the global parameters Figure 2 A where around 400s all the parameters start to gradually change. The unbound protein was washed with running buffer in washing step. The elution was carried with 100 mM Glycine-HCl, pH 3.5 buffer. Since no pH probe was installed, the change of buffer was indicated by lower conductivity signal compared to running buffer (20 mM Na-phosphate, 150 mM NaCl, pH 7.4). The elution happened at the breakthrough of conductivity curve as expected. It is also represented by sudden come back of global SAXS parameters to their initial values. After the elution, the resin was once more equilibrated with running buffer and prepared for next run.

The global SAXS derived parameters Figure 2 B present that the response to the loading of protein on the column correlate with the first UV peak from the chromatogram. The values for Porod exponent (red) indicate change from smooth surface (values around 4) to surface fractal (values around 3.5). Also the values for protein slope (orange) indicate presence of protein (drop of values). It also shows that Herceptin is actually not instantly washed away from the column with elution buffer as the initial values are reached at the end of the run (hence the prolong washing step resulted in retrieving the initial "resin" signal). The values for radius of gyration (a measure of size of analyzed system) also responded to the presence of protein bound to the resin. Rg gradually changed from 11 to 14 nm and Rc (radius of gyration for cylinders from 17 to 20 nm). The in-depth analysis of those results and correlating them to the real space has still to be done. Nevertheless, the increase in those values certainly indicates the increase in the size within analyzed system (due to attachment of Herceptin to chromatographic resin).

Similar behavior was observed for other tested resins and chromatographic parameters.