EUROPEAN SYNCHROTRON RADIATION FACILITY

INSTALLATION EUROPEENNE DE RAYONNEMENT SYNCHROTRON

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.

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

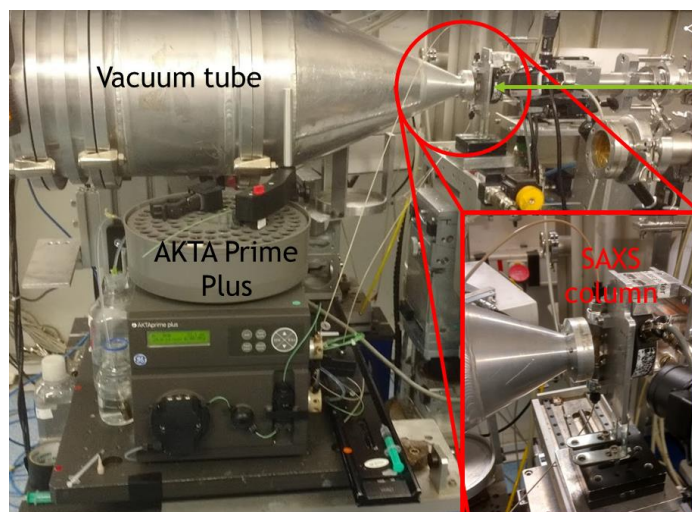in-line BM29 beamline and connected to AKTA prime plus HPLC system (Figure 1).

Figure 1 Picture of hardware layout at BM-26B. AKTA prime Plus was integrated into beamline system and connected with software outside hutch. The advantage of the set-up was motorized sample holder in both horizontal and vertical direction allowing scans of the column throughout the experiment.

Data was collected using MabSelect SuRe resin and Herceptin ® (Trastuzumab) from Roche – dialyzed into running buffer 0.01M NaPO4 + 0.15M NaCl pH 7.4 and concentrated to 13 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 12 keV X-rays every second, automatically integrated and normalized on the transmission. Futher data analysis was done by semiautomated algorithm written in Mathematica.

To analyze the data-set from ESRF a previously establish method called broken-rod modelling was used. This method requires a simplification of MabSelect SuRe inner network into solid rods (cylinders) representing strands of agarose and junctions between them as visible in the Figure 2. The choice of model was based on previous considerations of other organogels like Ca-alginate gels, plus from the visual examinations of SEM images of MabSelect SuRe, where for some distance strands of agarose (blue rectangles) seem like solid rods, whereas the junctions are less consistent in shape, but still cylinders as a model give more flexibility than e.g. sphere (radius restriction, which would not work with flat or elongated objects). Using
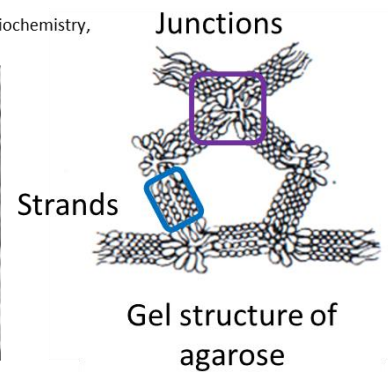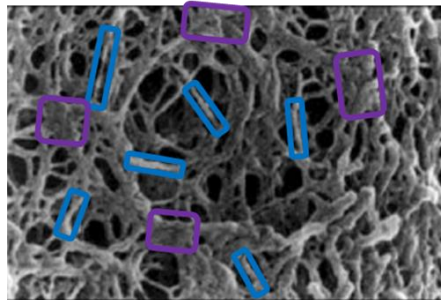

Figure 2 Explanation of broken rod model on the example of MabSelect SuRe micrographs on the left and structure of cross-linked gel on the right. On short distances (yet long enough compared to size of x-ray beam size) agarose strands could be approximated with solid rods (blue brackets), whereas junctions which lacks the consistency in shape could potentially be also approximated with cylinders, as cylinder model has more flexibility than e.g. sphere which would not work with elongated or flat objects

this approach, we assume that the protein layer thickness is homogenous around the agarose strand, which is of course not true. But, since SAXS scattering profile is nevertheless an average of illuminated volume, such assumption should still be valid. According to this model, the size of the cylinders (representing strands and junctions of MabSelect SuRe resin) should gradually increase in radii size with loading of antibodies on the column and then decrease to initial values after elution as binding of the protein to the resin physically increases protein layer thickness.

As presented in the Figure 3, size of the cylinder radii for strand (blue) and junctions (purple) increases with the UV peak indicating oversaturation of the column and decreases to initial values after elution. The results indicate that the initial radii of the strand are 11.8 +/- 3.1 nm in size (23.6 nm in diameter), which is in a good agreement with both literature and visual examination of SEM micrographs. 40s after injection, the size of radii starts increasing and plateau around 200 s after injection at the size of 17.3 +/4.9 nm (diameter of 34.6 nm). Around 1100 s after injection the radii size decreases to 12.0+/-3.2 nm (diameter of 24
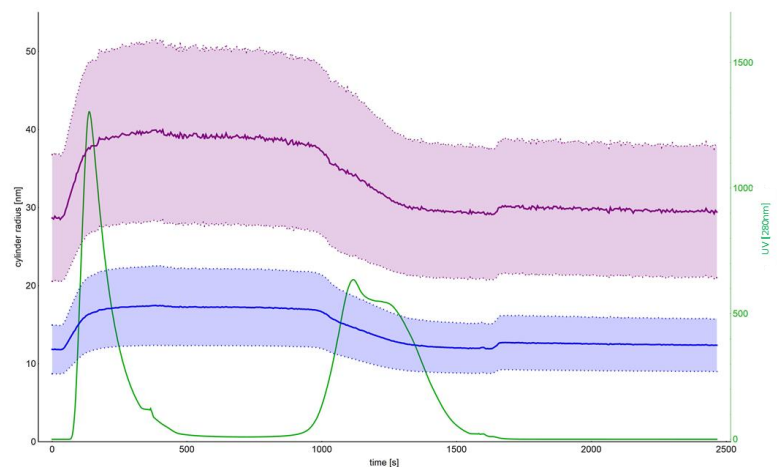
Figure 3 An overlay of a UV chromatography for protein-A overload run and results from broken-rod modelling indicated as a cylinder radii for strand (in blue) and junctions (in purple) with corresponding standard deviations. The first sharp UV peak represents overloading of the SAXS column ensuring full saturation of all biding sites within column, whereas the second, broader peak represents an elution peak.

nm) which correlates in time with elution peak. This indicates that the protein layer thickness for fully saturated MabSelect SuRe resin is 5.5+/-5.8 nm. For the junctions the initial radius starts at 28.7+/-8.1 nm and tops at 39.1+/-11.3 nm giving protein layer thickness of 10.5+/-13.9 nm.

Collected data supports the claim that with SAXS we are able to monitor protein-A chromatography process and also model the parameters like protein layer thickness in-situ during the experiment. Presented data trend is thrue for all tested chromatographic parameters (flow-rate etc) and will be soon published.