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.

ESRF	**Experiment title:** Probing pH-Dependent Conformational Transitions of von Willebrand Factor	**Experiment number**: MX-1704
Beamline: BM29	**Date of experiment**: from: 02/03/15 to: 03/03/15	**Date of report**: 29/10/15
Shifts: 3	**Local contact(s)**: Adam Round	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Prof. Dr. Jan Lipfert, LMU Munich, Chair of Biophysics and Applied Materials

*Linda Bruetzel, LMU Munich, Chair of Biophysics and Applied Materials

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Report:

The beamtime allocated to proposal MX 1704 was used to perfrom solution SAXS measurements on the large dimeric glycoprotein Von Willebrand factor (VWF), in order to probe the conformational states under varying pH and salt conditions. VWF plays an important role in thrombosis and hemostasis. Table I gives an overview of the tested samples. On each sample we performed concentration series of 0.25 mg/ml, 0.5 mg/ml and 1mg/ml. We performed 10 runs in 'flow' mode using the automated sample roboter installed at BM29. Sample profiles were analyzed for radiation damage and matching profiles were averaged. Appropriate buffer profiles were averaged and subtracted from the sample profiles. Figure 1 shows an example of SAXS data of VWF dimers at a pH of 6.2 for the three different concentrations scaled to each concentration. At low q-values the profiles exhibit deviations and possible aggregation effects. This behaviour could be seen for all pH conditions. Thus, an adequate Guinier analysis could not be performed. However, when performing dynamic light (DLS) measurements, aggregation could not be observed. When comparing scattering profiles of dimeric VWFs at different pH and salt conditions deviations in the scattering profiles can be detected, indicating a pH and divalent ion dependent conformational change of the dimers. At a pH of 6.2 the SAXS profile in Kratky representation assumes a rather plateau-like shape implying an ensemble of mostly folded (closed) dimer conformations (Figure 2). A closed conformation of the dimer was also observed by AFM imaging measurements that we performed in parallel. The conformation of the dimers changes when

changing the pH to 7.4. At this pH and in the absence of divalent ions the Kratky plot exhibits a non-parabolic diverging shape for higher q-values, indicating that the dimers adopt a more flexible ensemble (Figure 2). We succeeded in getting highly purified samples of VWF dimers, which we measured under the same solution conditions at the P12 BioSAXS beamline in Hamburg. The obtained SAXS data confirmed the results from BM12 and a Guinier analysis was possible. A paper including the data is now under review at PNAS.

Table I pH and salt conditions for SAXS measurements on VWF dimers.

pH	Divalent ion concentration
6.2	1 mM $CaCl_2$, 1mM $MgCl_2$
7.4	1 mM $CaCl_2$, 1mM $MgCl_2$
7.4	20 mM EDTA

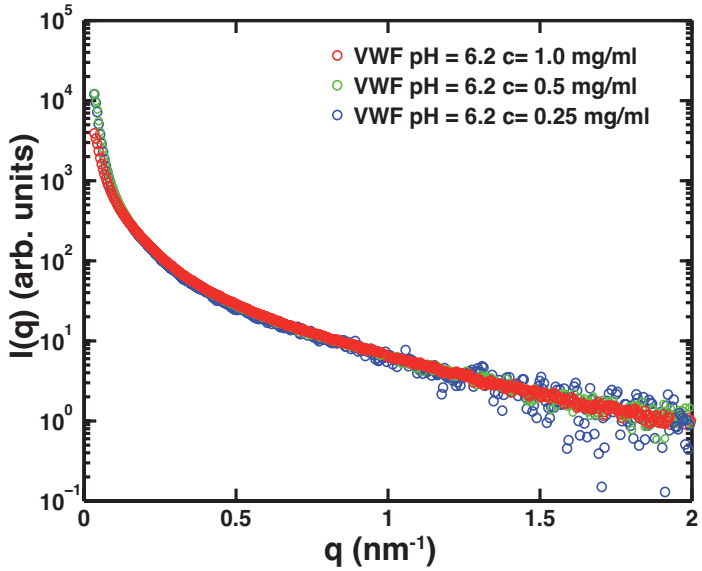

Fig. 1 Scattering profiles of VWF dimers at a pH of 6.2 and in the presence of divalent ions for varying concentrations. Profiles are scaled to their concentrations.

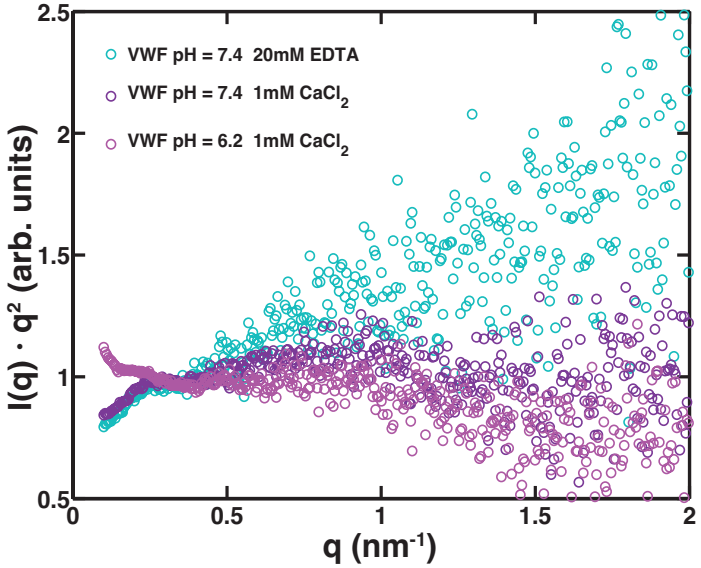

Fig. 2 Kratky representation of VWF dimers at a pH of 6.2 and a pH of 7.4 in the presence and absence of divalent ions. Data are scaled with a constant factor.