ESRF

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

Deadlines for submission of Experimental Reports

Experimental reports must be submitted within the period of 3 months after the end of the experiment.

Experiment Report supporting a new proposal ("relevant report")

If you are submitting a proposal for a new project, or to continue a project for which you have previously been allocated beam time, you must submit a report on each of your previous measurement(s):

- even on those carried out close to the proposal submission deadline (it can be a *"preliminary report"),*
- even for experiments whose scientific area is different form the scientific area of the new proposal,
- carried out on CRG beamlines.

You must then register the report(s) as "relevant report(s)" in the new application form for beam time.

Deadlines for submitting a report supporting a new proposal

1st March Proposal Round - **5th March**
10th September Proposal Round - **13th September**

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.

Instructions for preparing your Report

- fill in a separate form for each project or series of measurements.
- type your report in English.
- include the experiment number 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: Studying Crowding Dynamics in Concentrated Protein Solutions With XPCS	**Experiment number**: SC 5275
Beamline: ID10	**Date of experiment**: from: 14/06.2022 to: 20/06/2022	**Date of report**:
Shifts: 18	Local contact(s): Federico Zontone	*Received at ESRF:* *15/09/2022*

Names and affiliations of applicants (* indicates experimentalists):

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Report:

The experiment SC5275 aimed at measuring the dynamics of proteins in crowded environments by means of X-ray Photon Correlation Spectroscopy (XPCS). The understanding of molecular interactions in crowded environments is crucial for biological processes. Molecular crowding can dramatically alter molecular motion and thus influence the biological function of proteins. Here, we focused on the globular ferritin protein in cryoprotected solution, i.e. solution of 50w% glycerol in water, which allowed us to explore the accessible time window by increasing the viscosity of the solvent and therefore slowing down the dynamics of the system, as well as to prevent freezing of the sample at cryogenic conditions.

During the experiment we measured different ferritin concentrations, from 60 to 120 mg/ml, ranging from room temperature down to 210 K, which was the focus of the preliminary analysis, as well as apoferritin solutions and ferritin solutions with alginate. We utilised a Eiger 500k detector located at a distance of 7.11 m from the sample, which was measured in quartz capillaries of 1.5 mm outer diameter at a photon energy of 9 keV. The geometry allowed us to access the q-range between 0.01-0.4 nm^{-1}. We analysed the flux dependence of the scattering pattern by varying the number of absorbers to establish the radiation damage threshold. Moreover, the radiation damage test was performed for different temperature conditions, from 280 down to 210 K.

The XPCS analysis of protein solutions is often complicated by the fact that the scattering signal at large momentum transfer q is low. However, the system used in this study allows us to obtain a significantly higher signal-to-noise ratio because of the presence of the iron core inside the protein. According to preliminary data analysis, the high signal allows us to compute intensity autocorrelation functions g_2 at a larger momentum transfer ($q \approx 0.4$ nm^{-1}) than ever achieved before for protein systems (Figure 1a, concentration of 60 mg/ml). The g_2 function is modelled by $g_2(\tau, q) = 1 + \beta \exp[-2(\Gamma(q)\,\tau)^{\alpha}]$, where α is the Kohlrausch-Williams-Watts exponent, indicative of the type of motion of the system, Γ the decay rate and β

the contrast. The two-time correlation function map shows that the measurements were performed in an equilibrium regime, which suggests that the dynamics are not influenced by the beam with driven dynamics or radiation damage (inset of Figure 1a).

In order to study the crowding effect, the measurements were repeated for the concentrations 60, 100, 120 mg/ml at variable temperatures. Preliminary g_2 functions for the most concentrated solution are shown in Figure 1b, where the colours correspond to the different temperatures (see legend) and we observe that the dynamics is slowed down upon cooling. A summary of the different measurements performed (concentrations, attenuations) is reported in Figure 1c, where the diffusion coefficient extracted from the fits is plotted as a function of temperature and compared to the estimated values based on the Stokes-Einstein relation (purple circles). Additionally, from the integrated scattering intensity, it is possible to observe the structure factor peak at q ≈ 0.3 nm^{-1}. A deeper analysis of the structure factor peak position for the different concentrations and temperatures can provide insight into the protein-protein interactions from the dilute to the more crowded solution.

The aim of the experiment was successfully achieved. We were able to measure the cryoprotected ferritin solutions under variable conditions, e.g. flux, concentration and temperature, according to the proposal. The preliminary analysis is very promising and the data are expected to lead to a publication. The beamline staff will be included in the publications as co-authors. The staff was always available during the beamtime and we appreciate their valuable support.

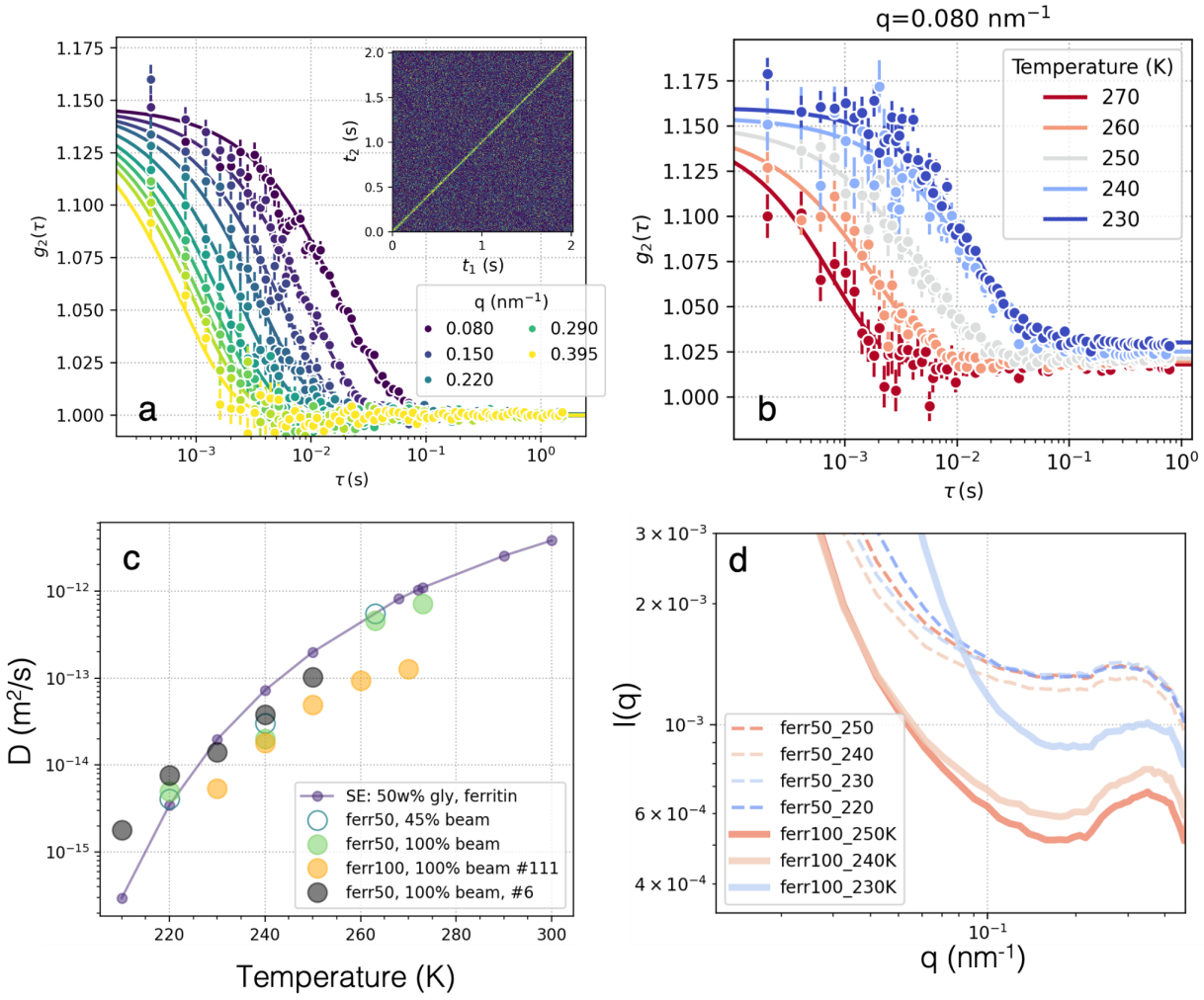

Figure 1: (a) Intensity autocorrelation functions g_2 of 60 mg/ml ferritin in glycerol 50w% solution, shown for the momentum transfer q=0.08-0.4 nm^{-1} at 240 K. Inset: two-time correlation function at q=0.11 nm^{-1} suggesting that the experimental conditions were optimised to mitigate the radiation induced effect. (b) Intensity autocorrelation functions g_2 of the concentrated ferritin solution (120 mg/ml) in glycerol 50w% at q=0.8 nm^{-1} for temperatures in the range 230-270 K. (c) Diffusion coefficient of different ferritin solutions under different conditions, as reported in the legend), shown as a function of temperature. The diffusion coefficients are extracted from the fit of the correlation functions ($\Gamma = Dq^2$) and are compared to the estimations based on the Stokes-Einstein relation. (d) Static scattering signal of different ferritin solutions, as reported in the legend, for the temperatures 250, 240, and 230 K, showing the structure factor peak at q≈0.3 nm^{-1}.