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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 using the **Electronic Report Submission Application:**

http://193.49.43.2:8080/smis/servlet/UserUtils?start

Reports supporting requests for additional beam time

Reports can now 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

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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 structural study of iron-storage proteins and of its nano-ironcore.	**Experiment number:** SC-1957
Beamline: ID02	**Date of experiment:** from: 10 March2006 to: 13 March2006	**Date of report:**
Shifts: 9	**Local contact(s):** Dr. Stephanie FINET (e-mail: finet@esrf.fr)	*Received at ESRF:*

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Our aim was to study three different proteins from the iron-storage protein superfamily: a hemoferritin from a sulphate reducing bacteria *Desulfovibrio desulfuricans (Dd)* and two Dps proteins from the radiation resistance bacterium *Deinococcus radiodurans (Dr)*, Dps-1 and Dps-2. The crystal structures of these three proteins were already been determined. Our aim on this proposal was to determine the conformational changes during the iron incorporation for the three protein targets using SAXS, and also to determine the structure of iron mineral core formed inside of the cavity of those proteins.

- **Experimental method:**

The three proteins proposed to be studied were previously purified, concentrate and dialyzed to a proper buffer: pH 6.0, 7.0 or 8.0. Protein concentration ranges from 2-8 mg/ml. Protein test stability was performed on both *Dr*Dps in order to check protein samples stability for radiation damage resistance in both apo and iron loaded states, therefore tests of up to 0.5 seconds with an X-ray intensity of 2.1 x 10^{12} photons/s were used. For each protein sample measurement a proper blank (without protein) was measured in the same ionic strength and pH as the protein sample. Each sample was injected into a motorized flow-through quartz capillary for exposure to the X-ray beam with diameter of 1.7 mm. The detector was positioned at 1 and 5 meters from the sample.

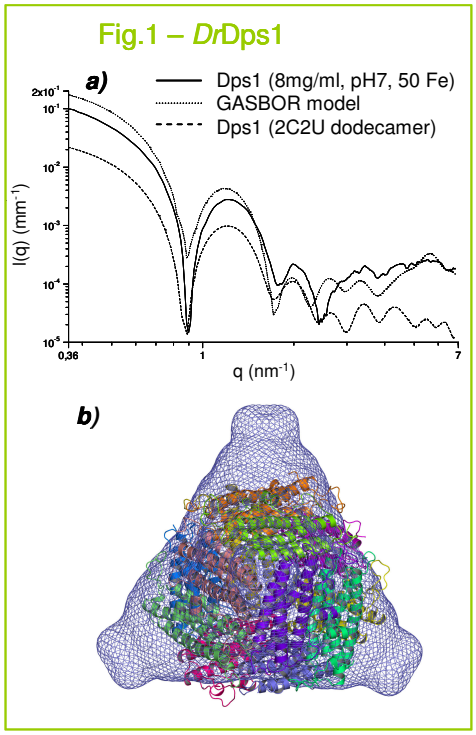
Static SAXS experiments were performed with the following protein samples: ***Dd* hemoferritin**: native and upon addition of iron 24 Fe/24-mer; ***Dr*Dps1**: at different pH 6.0, 7.0, 8.0, native, in the presence of glycerol, upon the addition of iron (12 – 1000 Fe/dodecamer), DNA and calcium; ***Dr*Dps2**: at pH8.0, native, with glycerol, upon addition of iron (12-100 Fe/dodecamer), DNA and calcium.

Stopped Flow SAXS experiments were carried out using the apparatus SFM-3, Bio-Logic, Pont de Claix – France. For these measurements the ratio of protein to iron was 1:1 (v/v), which correspond to a final protein concentration of 4mg/ml and loaded with 50 Fe/dodecamer for both *Dr*Dps.

- **Results:**

No SAXS patterns were obtained for the *Dd* hemoferritin, the spectra indicate that the samples tested were polydisperse, which indicated that the protein in solution was in a heterogenous form, therefore no further SAXS experiments were done with this protein.

The experimental scattering pattern of native *Dr*Dps1 at pH7.0 fits well with the curve simulated by CRYSOL using the dodecameric assembly of the deposited pdb coordinates from the previously determined X-ray crystal structure and also with the GASBOR model generated from the experimental scattering pattern (*Fig.1a*). The local maxima of the experimental fringes observed fit with the simulated curve, only the simulated inflexion around at 3 nm^{-1} is missing and the oscillations fade above 5 nm^{-1} on the experimental curve. These differences could be due to the fact that in *Dr*Dps1 crystal structure the N-terminal extensions are missing, probably due disorder. The radius of gyration obtained from the crystal structure and the experimental scattering patterns using Guinier fits implemented in the program CRYSOL are respectively 3.67 versus 3.80 nm. This indicates that *Dr*Dps1 have in solution a diameter similar to the one determined from the crystal structure dodecameric assembly of this protein and the small difference could be due to the fact that in solution the N-terminal extension can be organized in such a way that can be detected by SAXS. The models generated from the experimental scattering functions (*Fig.1a*) shows that the docked *Dr*Dps1 crystal structure fits the central SAXS central spherical envelope with some extra density with could account for the N-terminal extension not observed on the crystal structure (*Fig.1b*). The *Dr*Dps1 SAXS data analysis at different pH suggests that the protein may be in different multimeric forms at different pH. In order to confirm this hypothesis and to have a higher resolution of the extra density observed (*Fig.1b*) it will be extremely essential to performed more experiments.



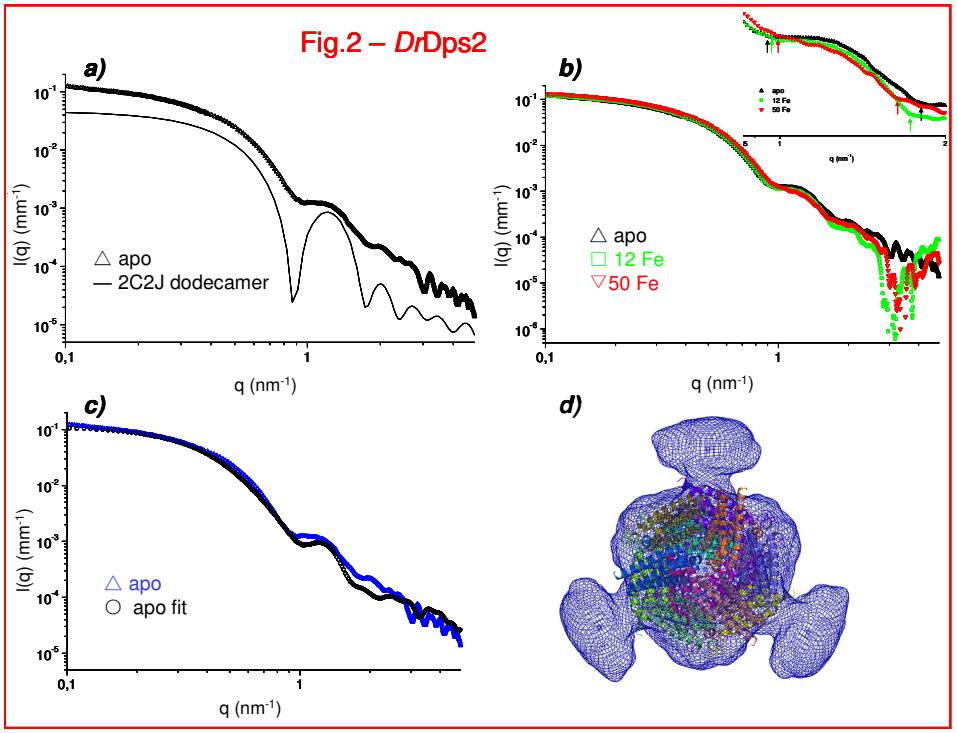


Figure 1 – *Dr*Dps1 at 8mg/ml, pH7 *a)* Scattering patterns from the experimental data (line), modelled with GASBOR (dashed line) and computed with CRYSOL (thick line) using the pdb coordinate of the X-ray crystal structure. *b)* Model generated from the SAXS scattering function showing a dense core corresponding to the dodecameric form of the protein with some extra density that may correspond to the N-terminal extensions not observed on the crystal structure previously determined.

Figure 2 – *Dr*Dps2 at 5 mg/ml, pH 8.0 in 50% glycerol *a)* Scattering patterns in apo state (triangles) and X-ray solution scattering curve computed by CRYSOL from the apo *Dr*Dps2 crystal structure (line). *b)* Scattering patterns in the apo state (black line), with 12 (green) and 50 (red) iron equivalents per dodecamer, *Inset*: Zoom of the graph in 0.8-2 nm^{-1} region showing the two first fringes, the arrows indicate the inflexion points. *c)* Scattering function in the apo state of the experimental data (triangles) and modelled using GASBOR (circles) to fit the experimental data. *d)* Model generated from the SAXS scattering function with the modelled and experimental scattering functions (*Fig2b*) for apo state, note the docked crystal structure is contained within the contour of the modelled envelope expect some extra density which may accommodate the N-terminal extensions not observed on the crystal structure previously determined.

The *Dr*Dps2 X-ray crystal structure of the native and iron loaded consists in a hollow roughly spherical shell but lacking the electron density for the N-terminal extension probably due to disorder as in the case of *Dr*Dps1. The curve simulated by CRYSOL using the dodecameric assembly of the deposited pdb coordinates is shown in Figure *2a*. The most important difference observed comes from polydispersity which could be caused by the presence in the *Dr*Dps2 sample of disordered and flexible N-terminal extensions protruding outside the protein dodecamer. The result of the simulation from the crystal structure of *Dr*Dps2 correspond to the local minima and maxima of the experimental scattering curve suggesting that the structure of the protein in solution is similar to that of the crystal structure even if the N-terminal extensions are missing in the latter. Therefore, in the absence of protein polydispersity (not flexible N-termini), an experimental curve with better defined minima should be obtained at lower angles (q values) because the entire protein has a larger radius than the incomplete dodecamer from the crystal structure.

The values of the radii of gyration between those determined from the experimental data using GNOM is 4.86 nm and those calculated form the crystal structure is 3.69±0.1 nm. The difference between the computed and experimental values for the radius of gyration suggests that in solution the protein structure extends beyond the protein shell observed in the crystal structure.

The addition of iron to the native *Dr*Dps2 with the ratios of 12 or 50 irons per dodecamer induced modifications to the scattering patterns characterised principally by smeared fringe minimum and shifts of their minima (*Fig2b* and *Inset*). This could be due to an increase of polydispersity from the protein shell which is observed upon addition of iron. The entrapment of an iron core within a protein shell can be simplified and seen as a core-shell model taking into account the contribution of two different particle sizes: inner core and outer protein shell, which gives rise to a complex scattering function minima. Therefore the contrast changes due to the iron loading of the protein cavity can be taken into account in the model. The position of the first and second minima in the curves generated from the core-shell model is consistent with the appearance of an iron core. The radii of gyration calculated from the experimental scattering patterns of the iron loaded (12 and 50 Fe/dodecamer) samples using the program GNOM is similar to the value determined for the apo form of the protein, corresponding to 4.86±0.1 nm^{-1}. However, the Guinier approximation of the radius of gyration shows a decrease from 5.95 to 5.85 ±0.1 nm, consistently with the decrease of the radius of gyration obtained with CRYSOL from the apo and iron soaked crystal structures. The occurrence of an iron core within the protein could have an incidence on the radius of gyration as expected by a modification of the centre of gravity of the electron shell by the presence of iron in the centre of the dodecamer. The incorporation of iron by the protein has been investigated using the stopped-flow small angle X-ray scattering technique and reveals that the modifications of the scattering patterns due to the mix of iron with the protein solution occurs within a second.

The scattering functions computed by GASBOR fit the experimental data however the oscillations do not perfectly match the experimental data and small differences of the minima are observed (*Fig.2c*). These scattering patterns were used to performed envelope calculations with the program GASBOR, which shows that there is a central dense core fitting the dodecameric assembly of the protein, however there is presence some density which may correspond to the N-terminal extensions not observed on the two forms of crystal structure determined.

Conclusions:

Using static and stopped-flow SAXS technique, the arrangement of these N-terminal extensions, protein conformational changes that occur due to iron incorporation, and information about the occurrence of a biomineral core formed inside of the cavity have been deduced. More experiments need to be performed to investigate the systematic variation of the iron core size with the various stages of iron loading. Experiments on the N-terminal truncated form of the protein are necessary to confirm unambiguously the results obtained above the N-terminal locations.

The recent enhancements to the ESRF beamline ID02, notably with a detector able to give better signal to noise ratio, is a strong advantage for more advanced future studies. Indeed, a detailed analysis of the system under study has been performed and could be completed by SAXS measurements with a wider range of protein concentrations and higher iron loadings. This study underlines the complementarily of both crystal X-ray diffraction and protein structure analysis in solution by SAXS.