ESRF	**Experiment Title:** Competitive inhibition of iron storage in apoferritin solutions studied by SAXS and ASAXS	**Experiment number**: SC-3396
Beamline: ID2	**Date of experiment**: from: 16th May 2012 to: 20th May 2012	**Date of report**: 1st Aug. 2012
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Report:

Ferritin is a widespread iron storage and detoxification protein that stores iron and releases it through channels in a controlled fashion [1]. The unique structure of ferritin forms a spherical shell in which large amounts of iron are "stored" as a hydrous ferric oxide mineral core. The hollow protein shell-apoferritin is comprised of 24 H- and/or L- type subunits that self assemble to form a 12 nm spherical protein with an 8 nm diameter cavity. Including six 4-fold, eight 3-fold channels exist in apoferritin as the potential pathways for Fe^{2+} entry in to the protein cavity [2-4]. The solvated iron, $Fe(H_2O)_6^{2+}$ can enter through the channels and be oxidized at the ferroxidase centers.

Recently studies focus on the understanding of the detailed process of iron storage and the role of the protein coat in the oxidation and sequestration of ferrous iron [3]. It has been found that iron uptake in ferritin could be blocked by presenting of Zn^{2+}, Tb^{3+} and Cr(TREN) [4-6]. X-ray crystallographic analysis suggests that these ions bind at both the Fe^{2+} passing channels (the Glu-130 and Asp-127 side chains in the 3-fold channels) and the ferroxidase centers [4-6]. However, little is known about the dynamic procedure: whether the competitive binding by blocking the channels or the binding to the ferroxidase center being more decisive is still a matter of debate.

The aim of the project is to study iron mineralization in ferritin cage and the competitive inhibition mechanism using in-situ SAXS and ASAXS methods. In this context, we have performed optical (time resolution UV-visible spectroscopy) measurements on the proposed systems, which give a time dependent absorption changes before and after Fe^{2+} binding with apoferritin with and without Zn^{2+}. The pH, concentrations of Zn^{2+} and Fe^{2+} in the presence of O_2 have significant impacts on the ferroxidase, mineral core growth and phase behavior in solutions. The optimized conditions have been selected for the time resolution SAXS/ASAXS experiments.

In this beamtime, we have first measured apoferritin solutions (0.5, 1, 2, 5 mg/mL) by normal solution SAXS in order to obtain the form factor of the protein. We then measured the apoferritin solution in the presence Zn^{2+} as a as a function of salt concentration (0 to 5 mM). Protein solutions were filled into 2 mm

quartz capillaries. The same buffer solution without protein was measured as the background, in exactly the same way as the protein solutions and was subtracted from the sample scattering. All measurements were carried out at room temperature. The raw data were corrected for transmission, fluctuation of primary beam intensity, exposure time, and the response of the detector. When increase the concentration of Zn^{2+}, the interaction between the apoferritin changes from repulsive to attractive (Fig. 1). Further increasing the salts concentration leads to protein aggregation.

Iron mineralization has been investigated by time resolve SAXS as a function of Fe^{2+} and Zn^{2+} concentrations. In this step, a mixed salt ion solution with different molar ratio of Zn^{2+} and Fe^{2+} have been used to study the competition effect. Figure 2 presents the typical time-resolved SAXS data of iron mineralization with and without zinc. Without zinc ions, irons nucleated within the hollow cage of apoferritin, the SAXS profiles have a shoulder at q~0.4 nm^{-1} corresponding to the size of apoferritin. With 3 mM Zn^{2+}, irons are mainly mineralized outside of the apoferritin shell.

We have also performed some test ASAXS measurements for the iron mineralization around K-edge of iron (7.124keV). Data analysis is under way.

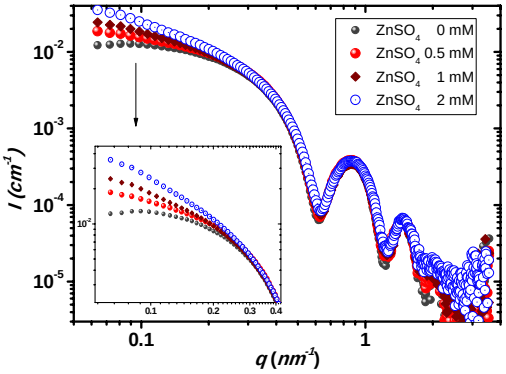

Fig. 1 SAXS curves of apoferritin 2 mg/ml in the presence of different concentration Zn^{2+} in HEPES buffer solutions (50 mM).

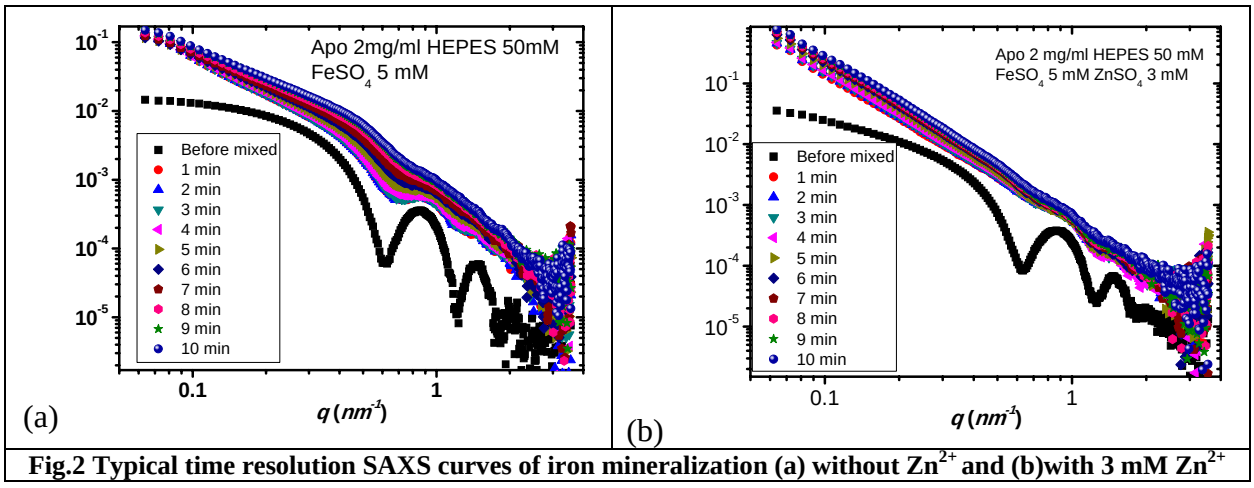

Fig.2 Typical time resolution SAXS curves of iron mineralization (a) without Zn^{2+} and (b)with 3 mM Zn^{2+}

References

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