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:** Physicochemical properties and self-assembly of phosvitin	**Experiment number**: 26-02-925
Beamline:	**Date of experiment**: from: April 2021 to: May 2021	**Date of report**: November 29th 2021
Shifts:	**Local contact(s)**: Dr. Daniel Hermida Merino	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

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Report:

1. Aim of the experiment

Phosvitin is one of the main proteins in egg yolk and can be found in many egg-derived food emulsions.[1] Because phosvitin contains a large number of phosphoserines, these phosphoric acid groups are negatively charged at pH > pI (≈ 2).[2] Due to the negatively charged groups, phosvitin binds to biologically essential multivalent cations such as Ca^{2+}, Mg^{2+}, and Fe^{3+} ions.[3] Furthermore, its amphiphilic structure provides excellent emulsifying properties.[4,5] Although a lot of research has been done to study functional properties of phosvitin as metal chelator or emulsifier, the fundamental physicochemical characteristics of phosvitin such as size and aggregation behavior with cations are not well-understood. In the beamtime experiment, we aimed to investigate the structure of phosvitin complexes with metal ions, especially Ca^{2+} and Fe^{3+}.

In a previous experiment at ID02 (SC 4997), the effect of pH, salt concentration, and the type of salts (Mg^{2+}, Ca^{2+}, and Fe^{3+}) on the size and assembly behavior of phosvitin was investigated and we obtained high quality data and insightful results. (A manuscript about those results was recently submitted to a journal and is currently under review.) However, we observed the formation of insoluble iron phosphate and calcium phosphate precipitates in the solution due to the potassium phosphate buffer we used and these hampered interpretation of the SAXS scattered intensity profiles.

Therefore, with the BM26 beamline experiment we aimed to circumvent these problems using phosvitin solutions that were prepared with Tris-HCl buffer instead of potassium phosphate buffer to avoid the precipitate formation in order to further characterize aggregation behavior of phosvitin with multivalent ions.

2. Experimental and Results

While we initially aimed for an on-site experiment, it was carried out remotely due to the pandemic. The samples were prepared in our laboratory in 2 mm quartz capillaries and immediately frozen after preparation. Two batches of samples were prepared and sent to the beamline in frozen state packed with dry ice. As these proteins, like many biological samples, are sensitive towards degradation, the samples were supposed to be stored in a freezer until just before the experiment; defrosting each batch at room temperature before starting the measurement. These storage requirements were clearly mentioned in the sample sheets.

However, 1 month after we had sent the samples, we were told by the local contact that there was no freezer available at the beamline so the protein samples were not stored in a freezer, contrasting our instructions. (We know from experience that there are sufficient freezers at ESRF.) This effectively ruined our highly precious samples of which we had no further stock (and preparation of a new batch would take months). For a reliable study of the assembly behavior of phosvitin in aqueous solution sample storage in a freezer was crucial. Therefore, we couldn't get good and useful information from these measurements.

Furthermore, it was totally unclear when our experiment would be performed (at one point we got an email that the experiment would be performed the next day; how we are supposed to prepare and be available on such short notice is not clear to us). Communication with the local contact was next to impossible and several attempts to have preparatory meetings yielded no result. In the end some experiments were performed on the degraded samples although the data quality seems highly questionable. Afterwards we did not succeed in getting any help from the local contact regarding data processing, e.g. to get the 2D-data integrated or background subtracted. It is an understatement to say that we are strongly disappointed by this experience that are not up to the high standards that we are used to from ESRF.

1. Samaraweera H, Zhang WG, Lee EJ, Ahn DU. Egg Yolk Phosvitin and Functional Phosphopeptides-Review. *J Food Sci*. 2011;76(7). doi:10.1111/j.1750-3841.2011.02291.x
2. Taborsky G. Optical Rotatory Dispersion of Phosvitin at Low pH Circular Dichroism. *J Biol Chem*. 1968;243(22):6014-6020.
3. Huopalahti R, López-Fandiño R, Anton M, Schade R. *Bioactive Egg Compounds*. 1st ed. Springer-Verlag Berlin Heidelberg; 2007. https://link.springer.com/content/pdf/10.1007/978-3-540-37885-3.pdf.
4. Castellani O, Belhomme C, David-Briand E, Guérin-Dubiard C, Anton M. The role of metal ions in emulsion characteristics and flocculation behaviour of phosvitin-stabilised emulsions. *Food Hydrocoll*. 2008;22(7):1243-1253. doi:10.1016/j.foodhyd.2007.08.005
5. Kato A, Miyazaki S, Kawamoto A, Kobayashi K. Effects of Phosphate Residues on the Excellent Emulsifying Properties of Phosphoglycoprotein Phosvitin. *Agric Biol Chem*. 1987;51(11):2989-2994.