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:** Mechanism of Degradation of Frozen Proteins:Investigation of Protein-Ice and Protein-Solute Interactions by Monitoring Ice and Solute Crystallization using High-Resolution XRD	**Experiment number**: LS-2742
Beamline:	**Date of experiment**: from: 21 Jun 2018 to: 24 Feb 2018	**Date of report**: 21 Feb 2020
Shifts: 9	**Local contact(s)**: Mashikoane wilson Mogodi (email: **wilson.mogodi@uct.ac.za**)	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Dr. Bhatnagar Bakul*, Pfizer, Inc. BTx PharmSci Pharmaceutical Res and Dev One Burtt Road, K1007 US - 01810 ANDOVER

Dr. Shalaev Evgenyi, Allergan Inc Pharmaceutical Development 2525 Dupont Drive US - 92612 IRVINE

Prof. Boldyreva Elena, Institute of Solid State Chemistry SB RAS Kutateladze Street, 18 RU - 630128 NOVOSIBIRSK

Dr. Boris Zakharov*, Institute of Solid State Chemistry and Mechanochemistry, Department of Reactivity of solids, Kutateladze Str. 18, RUS - 630128 NOVOSIBIRSK

Prof. Fisyuk Alexander*, Omsk F.M. Dostoevsky State University Laboratory of Organic Synthesis Chemistry Department Prospect Mira 55a RU - 644077 OMSK

Prof. Iurii Seretkin*, Institute of Geology and Mineralogy RAS, Laboratory of Metamorphism, pr. ac. Koptyuga 3, RU – NOVOSIBIRSK

Report:

While proteins can often be stabilized by maintaining them in the frozen state, water-to-ice transformation can also lead to their degradation. The primary objective of the proposed study was to investigate solute crystallization in frozen, aqueous solutions containing protein (AFP and pharmaceutical proteins), buffer, and cryoprotectants. using protocols established in the previously completed ESRF study for evaluation of solute-ice interaction, specifically AFPs and pharmaceutical proteins (LS-2228, LS-2601). The previous work was extended to investigate the effect of proteins (AFP and pharmaceutical) on ice as well as solute-ice crystallization. In the latter case, a study of the solute-ice crystallization involved the phase behavior of ionic solutes (example: buffers and salt) and non-ionic solutes (examples: sugars and sugar alcohols). The list of performed experiments is presented in **Table 1.**

Based on the title and previous experiments a new method to study protein/ice interaction is presented, which is based on measuring the characteristic features of X-ray diffraction (XRD) patterns of hexagonal ice (Ih). The analysis demonstrates that two pharmaceutical proteins, recombinant human albumin (rHA) and monoclonal antibody (mAb), have a pronounced effect on the properties of ice crystals. In particular, the size of the crystalline domains is significantly smaller, and the microstrain is larger, in the solutions of the pharmaceutical proteins, when compared with a model protein (lysozyme), an antifreeze protein, and sucrose and histidine. Neither of the proteins studied exhibit preferred interaction with specific crystalline faces of Ih. The results are consistent with indirect interaction of the pharmaceutical proteins with ice, in which protein molecules are accumulated in the quasi-liquid layer next to growing ice crystallization front. Direct interaction would indicate a sorption of protein molecules on ice crystals, whereas "indirect interaction" terminology is used to describe any interference of proteins with ice crystals without sorption involved. Lysozyme molecules, on the other hand, do not exhibit any evidence of interaction (either direct or indirect) with ice crystals. This is the first report, to the best of our knowledge, of major difference in protein/ice interaction between different types of non-antifreeze proteins.

Selected results from the experiment were published in: Bhatnagar, B., Zakharov, B., Fisyuk, A., Wen, X., Karim, F., Lee, K., Seryotkin, Y., Mogodi, M., Fitch, A., Boldyreva, E., Kostyuchenko, A., Shalaev, E. Protein/Ice Interaction: High-Resolution Synchrotron X-ray Diffraction Differentiates Pharmaceutical Proteins from Lysozyme (2019) Journal of Physical Chemistry B, 123 (27), pp. 5690-5699. DOI: 10.1021/acs.jpcb.9b02443

Table 1. List of performed experiments

Expt. #	Sample description
1	20 mM Histidine buffer, pH 5.8
2	100 mg/mL monoclonal antibody + 20 mM Histidine buffer, pH 5.8
3	100 mg/mL monoclonal antibody + 20 mM Histidine buffer + 20 mM NaCl, pH 5.8
4	100 mg/mL monoclonal antibody + 20 mM Histidine buffer + 150 mM NaCl, pH 5.8
5	100 mg/mL monoclonal antibody + 20 mM Histidine buffer + 5% w/v Sucrose, pH 5.8
6	100 mg/mL monoclonal antibody + 20 mM Histidine buffer + 5% w/v Sucrose + 0.02% w/v surfactant, pH 5.8
7	1 mg/mL monoclonal antibody + 20 mM Histidine buffer, pH 5.8
8	1 mg/mL monoclonal antibody + 20 mM Histidine buffer + 20 mM NaCl, pH 5.8
9	1 mg/mL monoclonal antibody + 20 mM Histidine buffer + 150 mM NaCl, pH 5.8
10	100 mg/mL Lysozyme in water, pH 3.3
11	100 mg/mL Lysozyme + 20 mM Histidine buffer, pH 5.8
12	100 mg/mL Lysozyme + 20 mM Histidine buffer + 20 mM NaCl, pH 5.8
13	1 mg/mL antifreeze protein (AFP) in water
14	Sterile water for injection
15	20 mM Histidine buffer + 20 mM NaCl, pH 5.8
16	20 mM Histidine buffer + 150 mM NaCl, pH 5.8
17	0.02% w/v surfactant in water
18	20 mM Histidine buffer + 5% w/v Sucrose, pH 5.8
19	20 mM Histidine buffer + 0.02% w/v surfactant, pH 5.8