EUROPEAN SYNCHROTRON RADIATION FACILITY
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Experiment Report Form

	Experiment title: TRACKING ATP-DEPENDENT PROTEIN DYNAMICS	**Experiment number**: LS-2999
Beamline:	**Date of experiment**: from: 30/09/2021 to: 05/10/2021	**Date of report**: 01/03/2022
Shifts:	**Local contact(s)**: Matteo Levantino	*Received at ESRF:*

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Report:

P-type ATPase proteins are found in biological membranes and execute active transport by means of ATP hydrolysis to transport (mostly ions) against a concentration gradient. These membrane protein transporters are critical to several important biological processes, such as the muscle contraction-relaxation cycle, cellular homeostasis of transition metals, and upholding membrane potential. The sarcoplasmic reticulum Ca^{2+}ATPase (SERCA) governs muscle relaxation and is by far the best-characterized member of the P-type ATPase family with several crystallized intermediates [1]. However, not all transient intermediate states are amendable to trapping techniques and we therefore developed and used a time-resolved X-ray solution scattering (TR-XSS) approach to determine the structure and timing of two transient intermediate states [2]. We also determined the level of cooperativity and identifed a trigger mechanism in the adenylate kinase reaction [3].

Having established a TR-XSS experimental design and MD simulation-based structural refinement protocol for P-type ATPase activation, we now seek to explore other targets in the family. SERCA is a special case due to its very high presence in membranes from rabbit skeletal muscle. Therefore, to study other P-type ATPases would require performing TR-XSS experiments on recombinant proteins. In the LS-2999 experiment, we collected two high-quality datasets a bacterial Ca_{2+} ATPase (LMCA1) (Fig. 1A) [4] and the human Ca^{2+} ATPase (SERCA2b) (Fig. 1B) [5] - both in detergent micelles, which will enable structural refinement (ongoing). Importantly, we also collected TR-XSS data on the LMCA1 protein inserted into nanodiscs, which provide a controlled uniform surrounding – much like the native membrane. While we were able to obtain good signal-to-noise for one timepoint (Fig. 1C), the full dataset suffered from technical problems due to unforseen experimental drift (described below). We encountered similar problems when measuring on the SERCA1a transporter (data not shown).

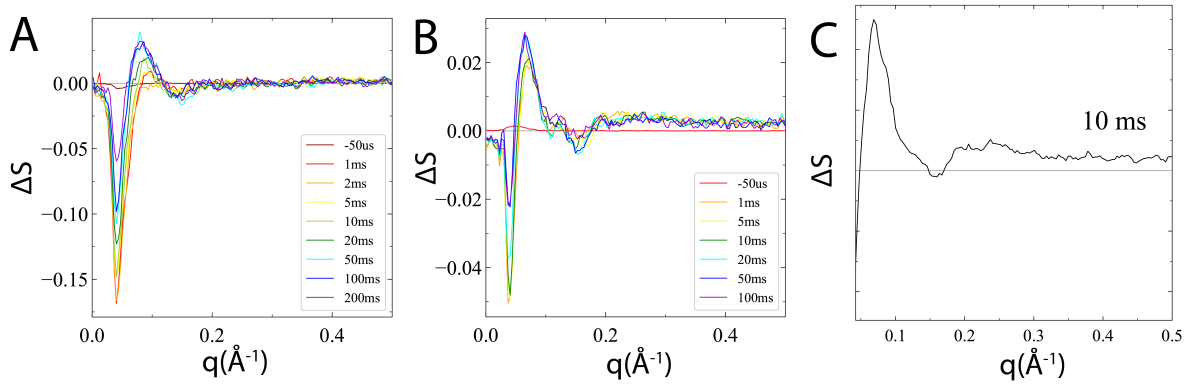

Figure 1. Time-resolved X-ray scattering data for **(A)** SERCA2b and **(B)** LMCA1 in detergent micelles. **(C)** A single 10-ms timepoint for LMCA1 inserted into DOPC-filled nanodiscs.

For the experimental drift (which typically manifested itself as in Fig. 2A), we were able to track its progression by counting the sum of the intensity at low- and high-q regions (Fig. 2B-C). At some point, the X-ray beam was 26 µm off along the vertical with respect to the sample, which obscured the measurement. We managed to compensate for the drift by frequent alignments and negative time delays. In future experiments, we will also increase the capillary diameter from 300 µm to 800 µm, which should render the measurements considerably less sensitive to this experimental drift.

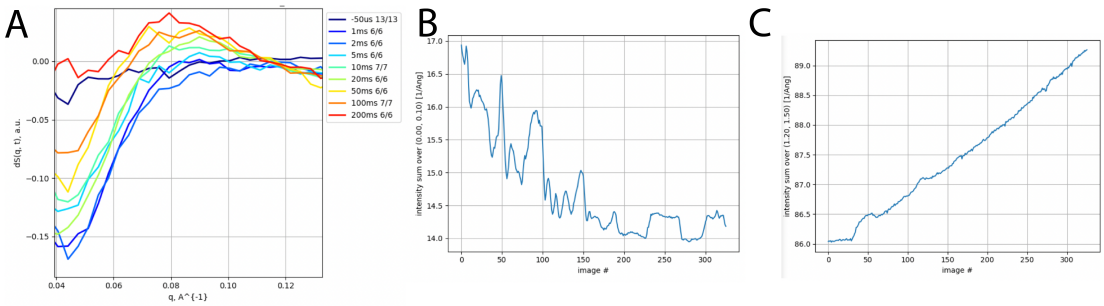

Figure 2. (A) Drift-affected time-resolved X-ray scattering data for SERCA2b. Summed X-ray intensity at **(B)** low and **(C)** high q regions across consecutive detector images.

In conclusion, we have established an experimental and modeling protocol for laser-induced ATP activation of P-type ATPase proteins – and have now capitalized on this progress by including recombinant proteins, which significantly enhance then number of putative target proteins amendable to TR-XSS characterization. We have also established proof-of-principle of nanodisc-inserted recombinant proteins, which will enable characterization of how lipid chemistry regulates functional protein dynamics. Together these results showcase the broad applicability of our developed approach and the versatility of the ID09B beam station.

References

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