EUROPEAN SYNCHROTRON RADIATION FACILITY

INSTALLATION EUROPEENNE DE RAYONNEMENT SYNCHROTRON

Experiment Report Form

	Experiment title: TRACKING ATP-DEPENDENT PROTEIN DYNAMICS	**Experiment number**: LS-3158
Beamline:	**Date of experiment**: from: 14/11/2022 to: 20/11/2022	**Date of report**: 09/09/2023
Shifts:	**Local contact(s)**: Matteo Levantino	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Fredrik Orädd[1*], Konstantinos Magkakis[1*], Irfan Parabudiansyah[1*], Magnus Andersson[1*]

[1]Umeå University, Sweden

Report:

We have developed a time-resolved X-ray solution scattering (TR-XSS) approach at beamline ID09 to determine the structure and timing in ATP-dependent biological processes. The main focus is on P-type ATPase proteins that are found in biological membranes and execute active transport by means of ATP hydrolysis to transport (mostly) ions against a concentration gradient (see schematic representation in Fig. 1A). 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. We have used the TR-XSS method to determine structural intermediate states of SERCA1a in solution [1] and of cooperative protein dynamics in adenylate kinase [2]. *Having established a TR-XSS experimental design and MD simulation-based structural refinement protocol for P-type ATPase activation, we now seek to understand regulation of the transport reaction, which constitutes a new frontline in structural biology.*

In the LS-3158 experiment, we aimed to characterize regulatory effects from lipids, pH, and an activating compound. Throughout this experiment, we observed random, extensive, low-q fluctuations (Fig. 1B) - that we have not encountered before in ID09 experiments (since 2015). Because the protein signal overlaps with this low-q region, the occurrence of fluctuations prevented us from data collection. Hence, significant time was spent trying to identify the source of the fluctuations. We observed the fluctuations primarily at 1 Hz, while the 10 Hz repetition rate showed higher stability and at any laser power (which rules out sample effects). We made several notes of extensive beam drift, which is a likely source for the observed fluctuations. Here, we got excellent support from the beamline to install live compensation of beam drift, although the problem persisted. Despite this reoccurring problem, we collected three high-quality datasets (described below) during the periods when the beam was stable. In the new proposal, we will use the adenylate kinase protein to test for beam stability since it is significantly less delicate and expensive than the "real" protein targets.

To determine the kinetics and structural dynamics involved in pH regulation of the bacterial calcium transporter LMCA1, we collected datasets at pH 7 (Fig. 1C) and pH 8 (Fig. 1D). Preliminary kinetic analysis shows that the datasets differ, but more time points need to be collected at 1 Hz for the pH 8 condition (see new proposal).

We also collected a dataset of LMCA1 inserted into nanodiscs with a controlled POPG lipid composition (Fig. 1E). The signal was weaker compared to an earlier dataset despite being higher in protein concentration, which indicates a more complex relationship between the difference signal and protein concentration. We have now proposed to optimize by screening a concentration range of empty and filled nanodiscs.

In earlier experiments aimed at characterization of internal regulation in Zn^{2+} ATPases, we have observed that the protein signal was obscured by what we determined to be a signal originating from a polymer-forming oxidative reaction caused by cysteine, which functions to deliver Zn^{2+} to the protein. In this experiment, we explored glutathione and a mix of cysteine and glutathione as alternative ways of Zn^{2+} delivery. We observed no obscuring signal in these experiments, which shows that glutathione paves way for characterization of internal regulation in Zn^{2+} ATPases. However, the low-q fluctuations prevented us from data collection.

In summary, we encountered unexpected low-q fluctuations that prevented us from meeting all the aims from the proposal. However, we developed methods to find good collection conditions. Since this optimization required large amounts of protein, we will now propose to bring a cheaper protein sample (adenylate kinase) in large quantities.

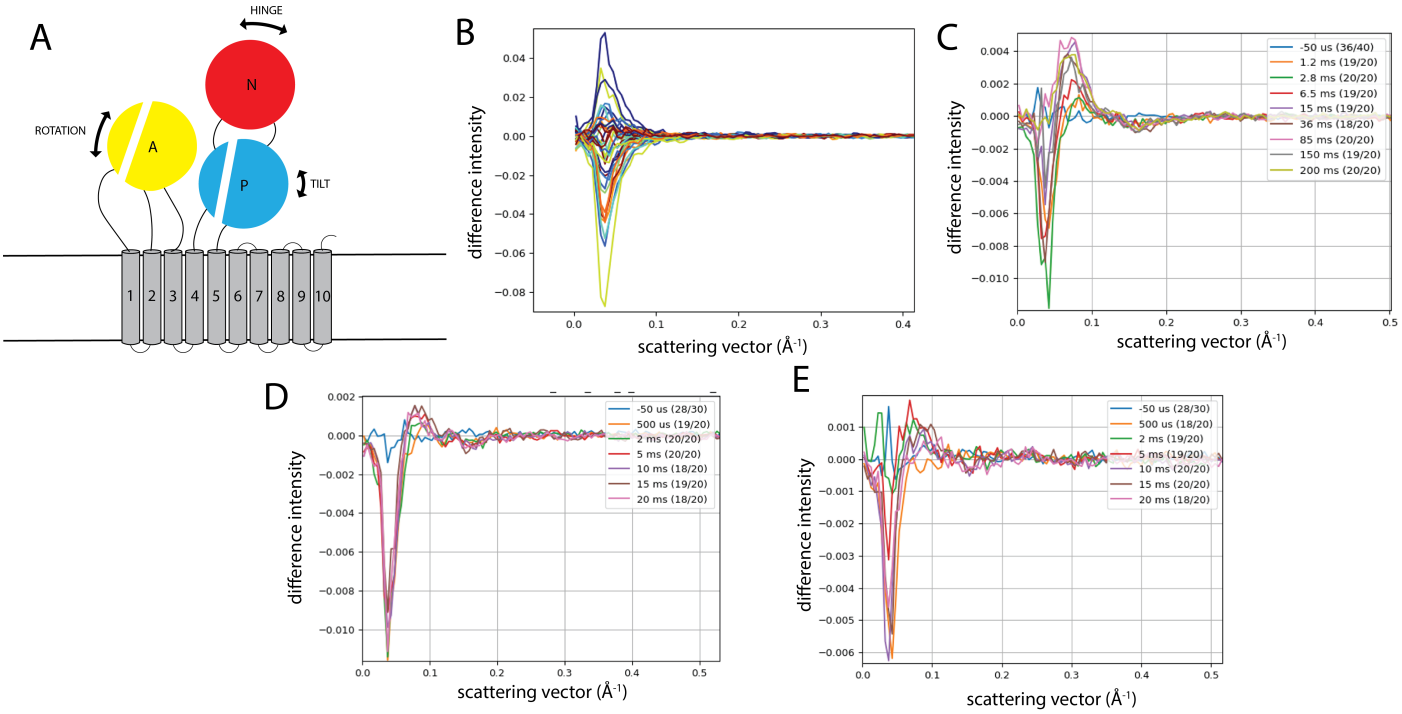

Figure 1. (A) Schematic of P-type ATPase protein dynamics that can be resolved with TR-XSS. **(B)** The low-q fluctuations caused by beam drift. Datasets of LMCA in detergent micelles at **(C)** pH 7 and **(D)** pH 8. **(E)** Dataset of LMCA1 in POPG nanodiscs.

References

[1] Ravishankar *et al.*, Science Advances. 6(12): eaaz0981 (2020) [2] Orädd *et al.*, Science Advances. 7(47): eabi5514 (2021)