EUROPEAN SYNCHROTRON RADIATION FACILITY
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Experiment Report Form

	Experiment title: VISUALIZATION OF CALCIUM-TRANSPORT REGULATORY MECHANISMS	**Experiment number**: LS-3302
Beamline:	**Date of experiment**: from: 26/09/2023 to: 02/10/2023	**Date of report**: 24/10/2023
Shifts:	**Local contact(s)**: Kevin Pounot	*Received at ESRF:*

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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-3302 experiment, we continued our efforts to characterize regulatory effects from lipids and pH. To avoid the problems with random, extensive, low-q fluctuations observed in earlier experiments (see Report LS-3158) – we optimized experimental conditions with a benchmark protein (adenylate kinase, AdK), performed live tracking of beam drift, optimized laser power, and deleted drift images before downstream data analyses. These precautions resulted in stable and reliable data collection with minimal disturbances from low-q fluctuations.

To determine the kinetics and structural dynamics involved in pH regulation of the bacterial calcium transporter LMCA1, we collected datasets at pH 7 (Fig. 1B) and pH 8 (Fig. 1C) covering all time points necessary for kinetic and structural analyses, which are ongoing. Tracking of the positive q-feature at $0.07\ Å^{-1} < q < 0.08\ Å^{-1}$, after compensating for the longer release time of caged ATP at higher pH, shows a faster reaction at higher pH (Fig. 1D), which corroborates with pH optimum at pH 8 for the *Listeria monocytogenes* bacteria from which the protein originates.

Mutational studies have assigned the unusually high pH optimum for LMCA1 to Arg795. We therefore performed TR-XSS experiments at pH 7 and pH 8 on a Arg795Gln mutant, which lacked the Arg charge. The data showed clear differences to the wild-type data in both kinetics and structural q-shifts (data not shown) and analyses are ongoing.

We also collected a dataset of LMCA1 inserted into nanodiscs with a controlled POPG lipid composition (Fig. 1E). Here, we optimized protein concentration and laser power without significant improvements. However, we added more time points in addition to those collected earlier (LS-3158) to enable kinetic and structural analysis despite the weaker signal. The data collected in POPG lipids (optimal lipid environment) will be compared to the earlier data in (suboptimal) POPC lipids to address how membrane proteins have evolved to function in a specific lipid chemistry.

We also collected TR-XSS data on adenylate kinase (AdK) with (Fig. 1F) and without (Fig. 1G) AMP. How the protein responds structurally to the presence of ATP (from the cage) in absence of AMP is not known. The TR-XSS data show clearly the lack of concerted structural changes in the absence of AMP. We then also added AMP to the sample and regained the signal. These results build on our earlier work on AdK where we addressed cooperativity in the reaction mechanism [2].

In summary, a series of optimization steps eliminated low-q fluctuations that prevented us from obtaining data in the earlier experiment (LS-3158) and allowed for successful data collection. We anticipate that the obtained data will be concluded in three manuscripts.

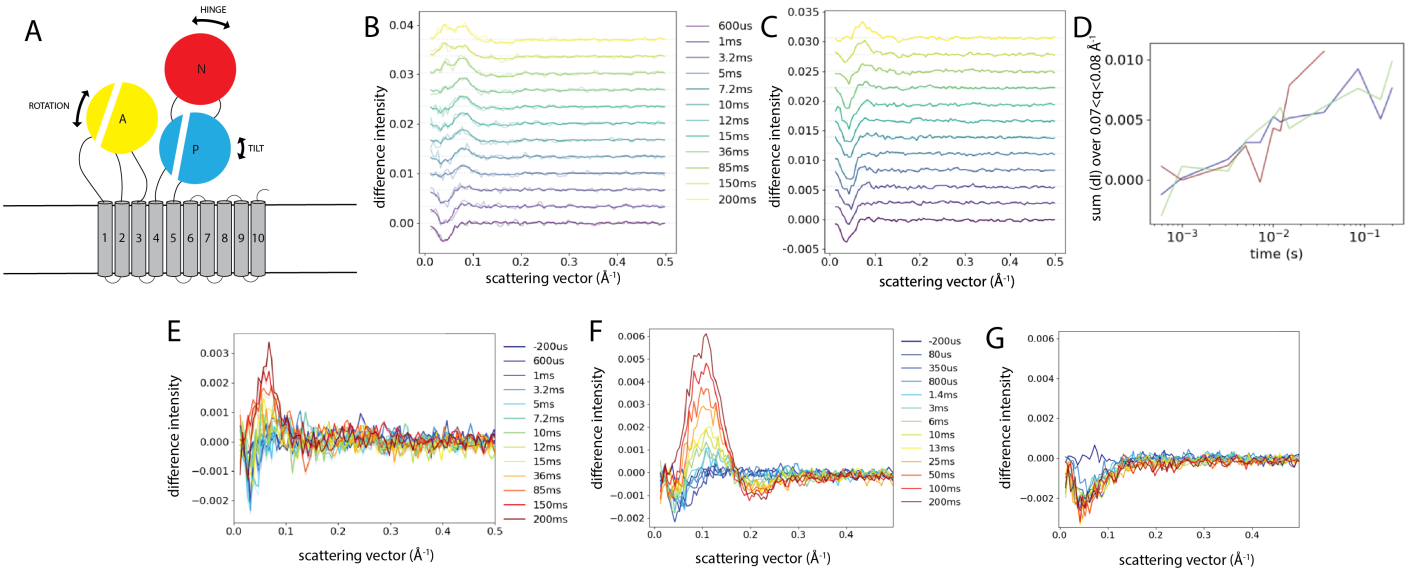

Figure 1. (A) Schematic of P-type ATPase protein dynamics that can be resolved with TR-XSS. Datasets of LMCA in detergent micelles at **(B)** pH 7 and **(C)** pH 8 and the time evolution of the dataset 1 (blue) and 2 (green) at pH 7 and at pH 8 (red). **(E)** Dataset of LMCA1 in POPG nanodiscs. Adenylate kinase datasets **(F)** with and **(G)** without AMP.

References

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