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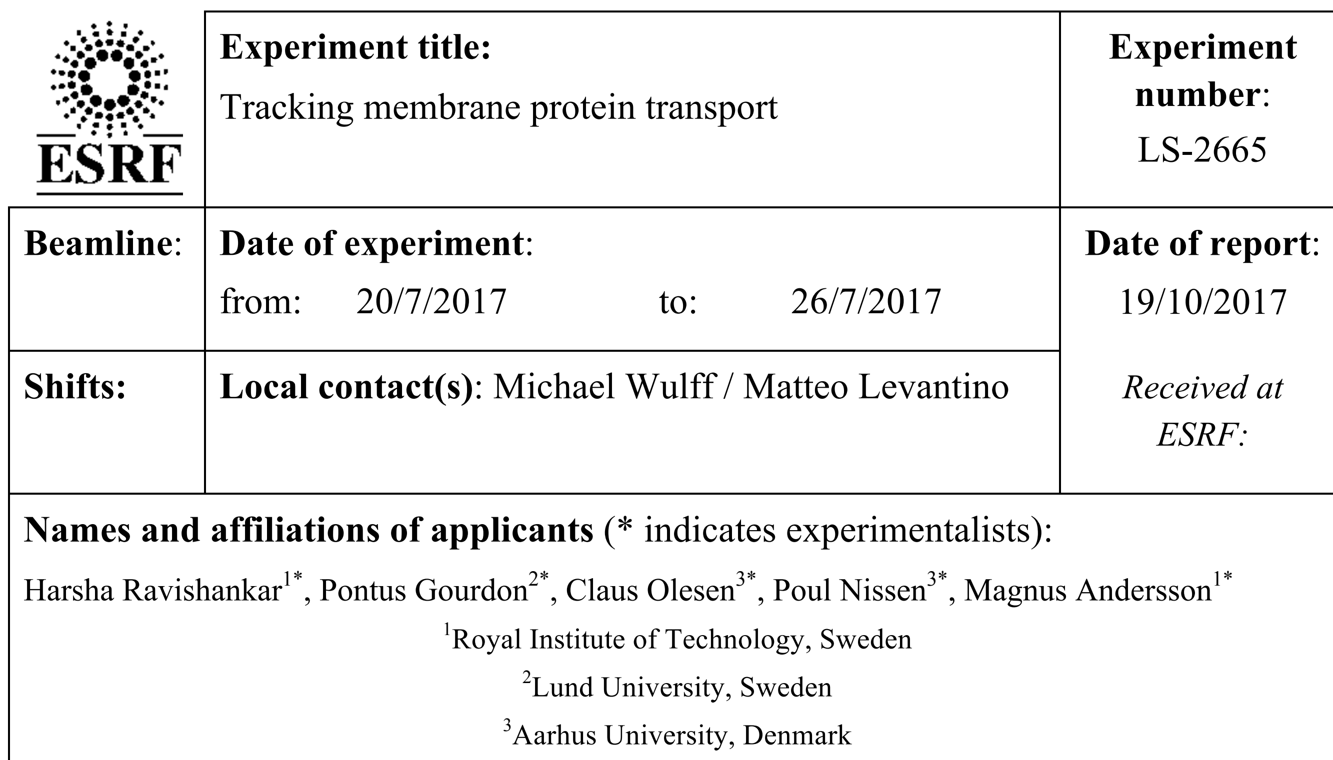
Experiment Report Form



ESRF	**Experiment title:** Tracking membrane protein transport	**Experiment number**: LS-2665
Beamline:	**Date of experiment**: from: 20/7/2017 to: 26/7/2017	**Date of report**: 19/10/2017
Shifts:	**Local contact(s)**: Michael Wulff / Matteo Levantino	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Harsha Ravishankar[1*], Pontus Gourdon[2*], Claus Olesen[3*], Poul Nissen[3*], Magnus Andersson[1*]

[1]Royal Institute of Technology, Sweden

[2]Lund University, Sweden

[3]Aarhus University, Denmark

Report:

The sarcoplasmic reticulum Ca^{2+}ATPase (SERCA) is a membrane protein that governs muscle contraction in the presence of ATP. It is by far the best-characterized member of the P-type ATPase family of membrane protein transporters and several intermediates have been crystallized [1]. However, transition paths between intermediates are unknown and it remains to validate the major structural findings in condensed phase without the restraints of a crystal lattice, at room temperature. The bacterial Ca^{2+}ATPase LMCA1 was recently characterized with single-molecule FRET [2] and could potentially offer more constraints in the computational refinement. The Zn^{2+} (ZntA) and Cu^{+} (CopA) ATPases belong to the same superfamily of proteins (P-type ATPases) as the Ca^{2+} transporters, but are much less well characterized with crystal structures limited to two ion-free states [3-5]. Mutations in the human CopA protein cause hereditary disease and ZntA is prevalent in pathogenic bacteria but not in human, which makes it a highly attractive target for new antibiotics to fight multidrug resistance.

This experiment aimed to track conformational changes in real time of ATP-dependent SERCA, LMCA1, ZntA, and CopA transporters using laser-induced caged ATP activation at the dedicated time-resolved wide-angle X-ray scattering (TR-WAXS) beamline ID09B at ESRF. This experiment was a continuation of experiment LS-2527 (in 2016) where we collected several timepoints for the SERCA and ZntA proteins. This experiment in turn was enabled by developments performed in the first LS-2410 (in 2015) experiment. Now (LS-2665), we recorded data with improved signal-to-noise for SERCA, which have allowed us to resolve distinct kinetic intermediates. The corresponding signal for the recombinant proteins (LMCA1, CopA, and ZntA) were not as strong, which could potentially limit the subsequent kinetic and structural modeling. For upcoming experiments, the protein concentration should therefore match that of SERCA (25 mg/mL).

We triggered the reaction with caged ATP after calibrating the flow rate with dye (to save protein sample) through the capillary with the peristaltic pump at the ID09B beam station. To improve signal-to-noise

compared to the LS-2527 experiment, we used 20 µs X-ray pulse length (instead of 10 µs). In addition, we collected data using a 1 Hz repetition rate to allow reaction cycles in the order of tens-to-hundreds of milliseconds to complete (in the LS-2527 experiment we focused on the faster dynamics using a 10 Hz repetition rate). We optimized the laser intensity to maximize the yield of caged ATP release. This allowed us to collect time points: 20 µs, 1 ms, 2 ms, 5 ms, 10 ms, 20 ms, 50 ms, 100 ms, and 200 ms with excellent signal-to-noise for the SERCA protein (Fig. 1A, black lines). Spectral decomposition based on a three-state model with buildup of the final intermediate resulted in good fits to the experimental data (Fig. 1A, red lines). The corresponding time-independent basis spectra showed significant similarities to scattering profiles calculated from available crystal structures (Fig. 1B) and structural refinement is ongoing using our developed metadynamics tool [6]. Further, the evolution of transient populations yielded rate constants k(early-to-intermediate) = 1.5 ms and k(intermediate-to-late) = 13 ms (Fig. 1C), which are in agreement with a SERCA reaction cycle of ~50 ms. The ongoing structural refinement will result in intermediate SERCA structures in the native environment along with the relative timing of the structural events. We believe that the SERCA results obtained in this experiment (and subsequent structural refinement) has the potential to make significant contribution to the structural biology field.

In this experiment we also collected time-resolved X-ray data of recombinant proteins (ZntA, LMCA1, CopA). While the CopA data suffered from low signal-to-noise, we obtained time-dependent signals both for ZntA (Fig. 1D) and LMCA1 (Fig. 1E), which therefore constitute first direct measurements of reaction cycle dynamics for these proteins. We are now performing kinetic modeling, which resolves transient intermediates and their rate constants. In addition, structural refinement using existing X-ray structures and our structural models can potentially provide a first view of inward-facing states. However, we note that at wider angles the signal-to-noise was reduced compared to SERCA. We believe that this originated from lower protein concentration (15 mg/mL) compared to SERCA (~25 mg/mL). This will limit our structural interpretation. Therefore, to prepare for upcoming experiements, we will optimize conditions to enable higher (>25 mg/mL) concentration of the recombinant detergent-solubilized proteins. The low signal-to-noise in the recombinant protein experiments required extensive sampling (averging over significant number of experiments), which prevented us from exploring faster dynamics using the fast-release pHP-caged ATP (lack of time).

In conclusion, we collected SERCA data with excellent signal-to-noise that will enable a full kinetic and structural characterization. We also collected satisfactory data of ZntA and LMCA1 dynamics, which will pave way for further characterization of recombinant proteins – and hence significantly increase the number of potential biological targets amendable with time-resolved experiments at the ID09B beam station.

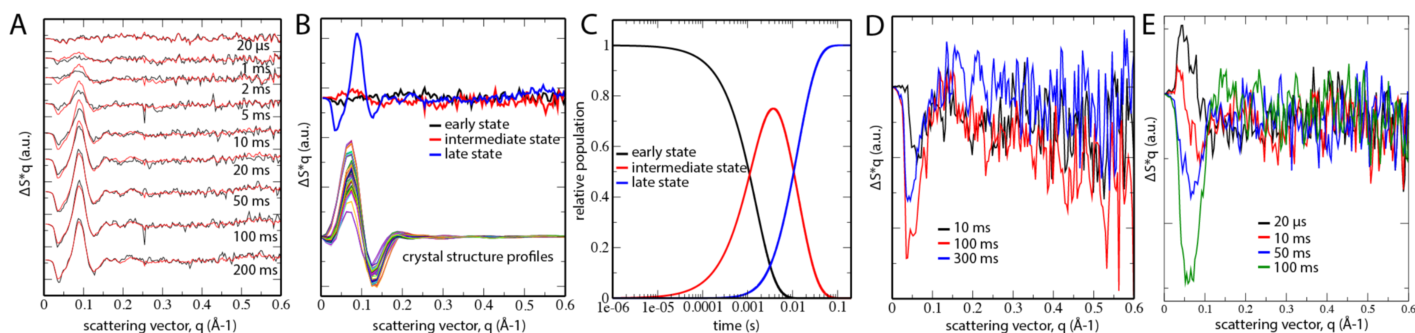


Figure 1. (A) Time-resolved X-ray scattering data for SERCA (black lines) and corresponding fits from kinetic modeling (red lines) **(B)** Time-independent basis spectra (upper) and X-ray scattering profiles calculated from available SERCA crystal structures (lower) **(C)** Transient populations of basis spectra. Time-resolved X-ray scattering data for **(D)** ZntA and **(E)** LMCA1.

References

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