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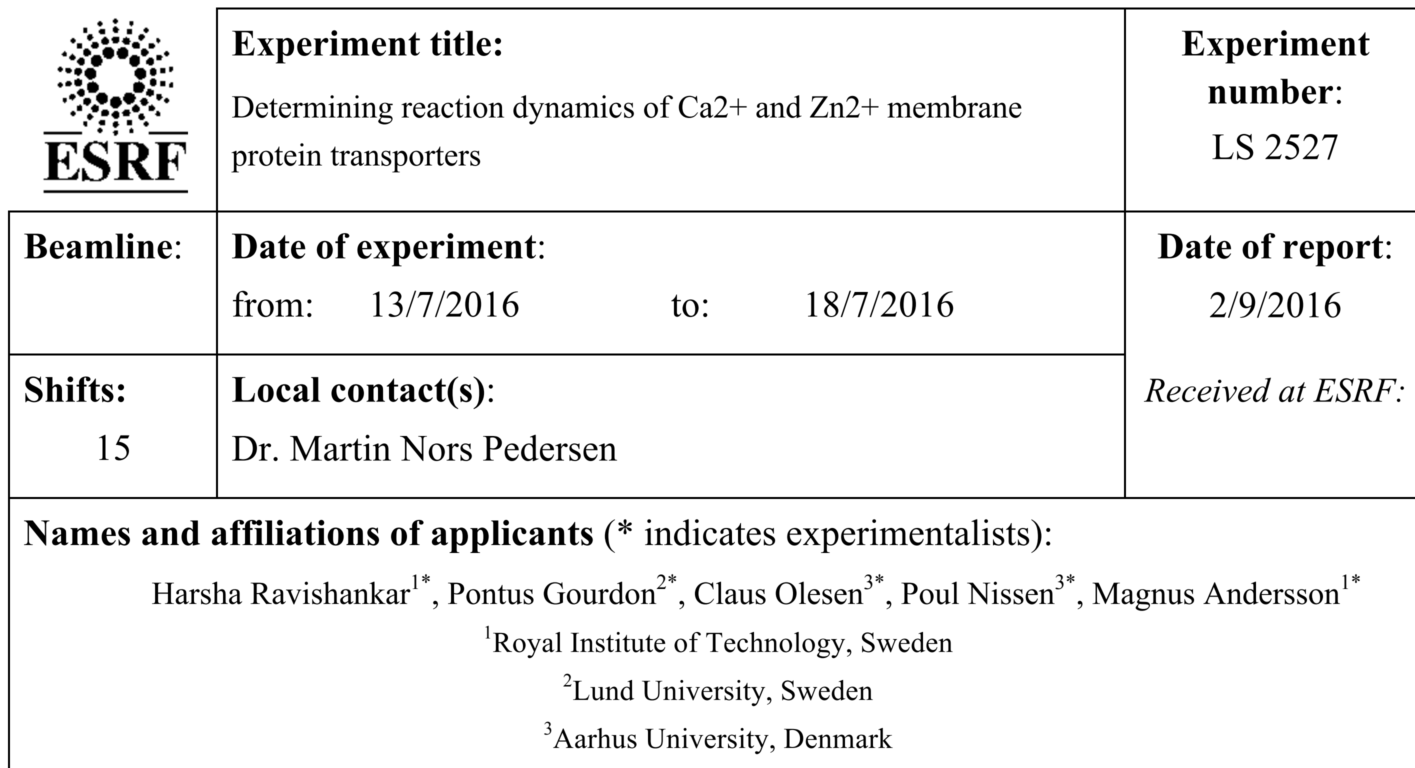
Experiment Report Form



ESRF	**Experiment title:** Determining reaction dynamics of Ca2+ and Zn2+ membrane protein transporters	**Experiment number**: LS 2527
Beamline:	**Date of experiment**: from: 13/7/2016 to: 18/7/2016	**Date of report**: 2/9/2016
Shifts: 15	**Local contact(s)**: Dr. Martin Nors Pedersen	*Received at ESRF:*

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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 Zn^{2+} ATPase (ZntA) belongs to the same superfamily of proteins (P-type ATPases) as SERCA, but is much less well characterized with only two crystal structures presented in 2014 [2]. Because ZntA is prevalent in pathogenic bacteria but not in human, it is an highly attractive target for new antibiotics to fight multidrug resistance.

This experiment aimed to track conformational changes of ATP-dependent SERCA and ZntA transporters using laser-induced caged ATP activation in real time at the dedicated time-resolved wide-angle X-ray scattering (TR-WAXS) beamline ID09B at ESRF. This experiment was a continuation of the experiment LS-2410 (in 2015) in which we developed a sample delivery system, optimized flow rates, caged ATP and protein concentrations, and collected data at 8 ms. Now (LS-2527), we successfully recorded data for several time points spanning the full reaction cycles of both SERCA and ZntA. By successfully activating proteins using a caged compound, we have now shown that the potential biological targets amendable to TR-WAXS characterization is not limited to light-sensitive proteins (that carry an inherent reaction trigger [3-4]). Given an ever-increasing number of caged compounds (ions, neurotransmittors etc), this extension can potentially be extremely valuable.

The key difference between activating light-sensitive proteins and triggering by means of a caged compound is sample delivery. The caged compound trigger requires a continuous sample flow through the capillary. Because we had experienced problems maintaining a steady flow rate with the syringe pump sample delivery (LS-2410), we now tried replacing it with a newly purchased peristaltic pump at the ID09B beam station.

Calibration showed that this was a much better option.

Due to a problem in the data collection software there was a drift in the laser/X-ray timing and we did not start measuring data until after 2 days of beamtime (20160715, 10.30 am) when the problem was resolved by ESRF engineers.

We first optimized the flow rate using the scattering from a dye (to save protein sample) to make sure that the capillary window was completely replenished before the onset of a new experiment without wasting unnecessary sample (Fig. 1A). We first used a repetition rate of 10 Hz, which enabled monitoring reaction dynamics of SERCA for up to 30 ms. The length of the X-ray pulses were 10 μs which resulted in much better signal-to-noise compared to the 1 μs used in LS-2410 and we further optimized concentration of caged ATP and laser intensity. This allowed us to collect time points: 2 ms, 5 ms, 10 ms, 20 ms, and 30 ms with excellent signal-to-noise (Fig. 1B). We note that the signal is prevailing at > 30 ms, which corroborates with the SERCA reaction cycle length of ~50 ms. Importantly, we performed a number of negative controls (i.e. caged compound only, protein only, and buffer only), which showed no low-q features. Initial comparison to theoretical difference scattering profiles generated from existing SERCA crystal structures show that the main features are reproduced (Fig. 1C). While the low-q negative peak is more or less identical to X-ray crystallography, the rest of the TR-WAXS features are shifted to higher-q and are less pronounced. This is similar to that observed for archaeal rhodopsins [3]. We have now initiated computational simulation refinement of this data, which is based on a protocol that we have recently published [5].

We then changed into a 1 Hz repetition rate to allow monitoring of the ZntA protein, which is an order of magnitude slower than SERCA and collected data at 10 ms, 100 ms, 200 ms, 300 ms, and 750 ms (Fig. 1D). The obtained data show a clear signal, although not as strong as for SERCA, which is expected since ZntA was expressed recombinantly and resides in a detergent micelle / lipid mixture rather than the native membrane. Nevertheless, the acquired data provide a first direct measurement of the reaction cycle of the ZntA protein including the elusive inward-facing states. We have now created a model of an inward-facing ZntA state based on structural homology to SERCA and will include this in the molecular dynamics based refinement procedure.

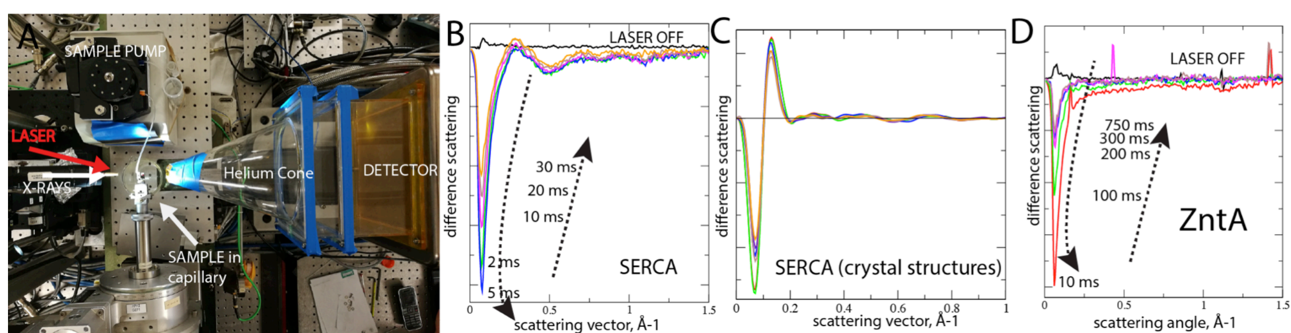

Figure 1. (A) Experimental setup **(B)** Time-resolved X-ray Scattering data for SERCA **(C)** Predicted X-ray scattering from SERCA crystal structures **(D)** Time-resolved X-ray Scattering data for ZntA.

References

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