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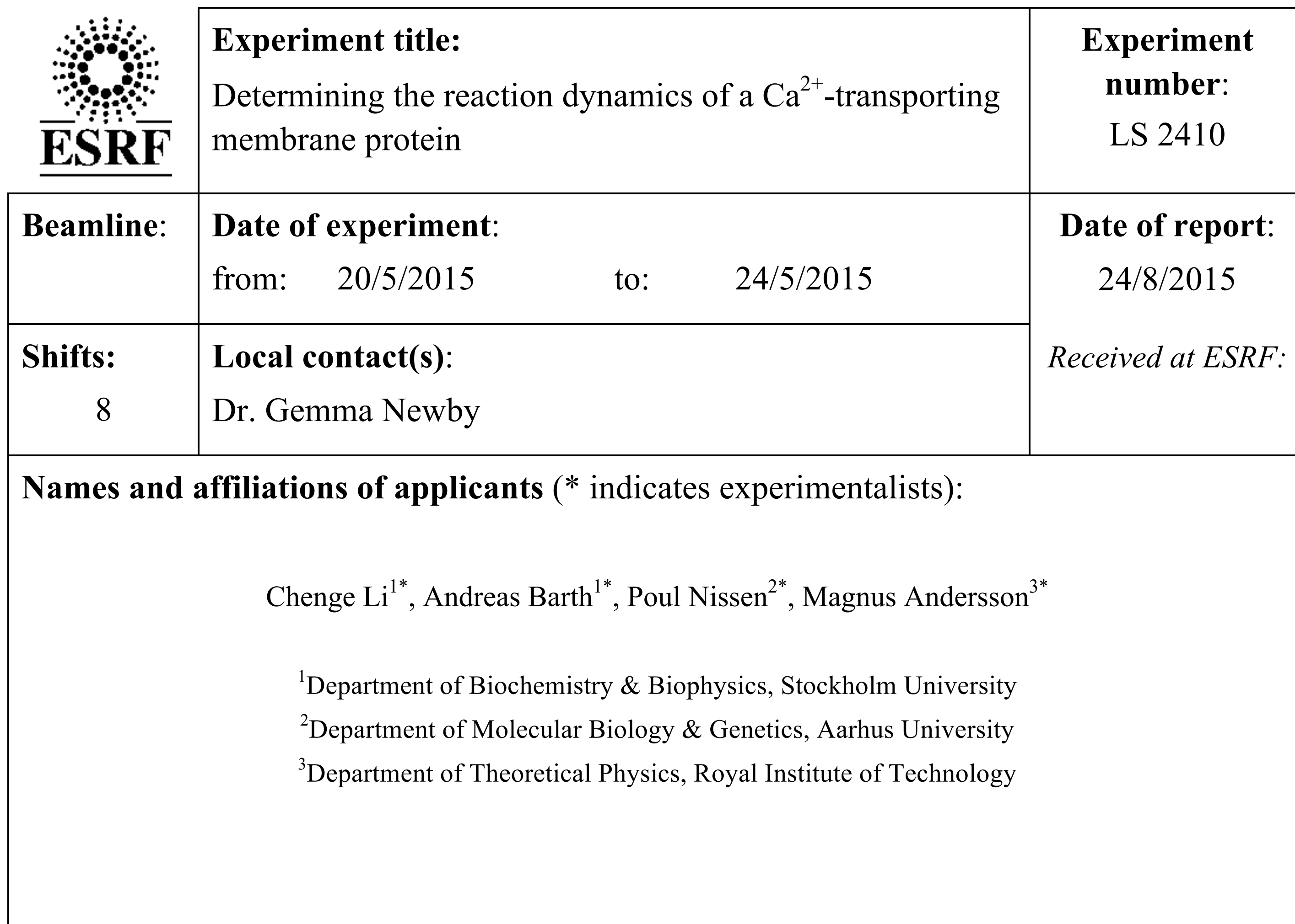
Experiment Report Form



ESRF	**Experiment title:** Determining the reaction dynamics of a Ca^{2+}-transporting membrane protein	**Experiment number**: LS 2410
Beamline:	**Date of experiment**: from: 20/5/2015 to: 24/5/2015	**Date of report**: 24/8/2015
Shifts: 8	**Local contact(s)**: Dr. Gemma Newby	*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.

This experiment aimed to track conformational changes of SERCA in real time at the dedicated time-resolved wide-angle X-ray scattering (TR-WAXS) beamline ID09B at ESRF. This experiment builds on earlier work on light-sensitive membrane proteins [2,3] to include triggering of a caged compound (caged ATP). Given an ever-increasing number of caged compounds (ions, neurotransmittors etc), such an extension is extremely valuable since it would enable an entirely new range of biological targets (outside of the light-sensitive proteins).

The major challenge in the experiment was sample delivery. With the light-sensitive proteins (that returns back to the ground state after excitation), we could simply seal the protein sample within a capillary that were

translated through the pump-and-probe window [2,3]. With caged compounds, however, the sample needed to be renewed at each pump-and-probe cycle, hence we needed a continuous sample flow through the capillary. Such experimental setup is complex and needs careful optimization.

In this experiment, we first build a delivery system (syringe pump, capillary holder and waste collector) with a minimum dead volume to save protein sample (Fig. 1A). We then optimized the flow rate using the heating signal in water as a proxy (to save protein sample). Because no heating signal should be observed at negative time delays, we used this as a tool to optimize the flow rate. This optimization procedure was complicated by the fact that the relative timing between the laser and x-rays was off by 80 µs. The issue was eventually identified and rectified by the staff scientists.

Once we turned to the protein sample, we optimized the concentrations of protein and caged ATP and collected high-quality data at 8 ms after laser initiation and a control measurement before the laser pulse (Fig. 1B). This is a critical time point in the reaction cycle where the SERCA protein transitions between inward-facing and outward-facing conformations. Since such protein transition states cannot be captured by x-ray crystallography, the recorded structural data is of great interest to the structural biology field. After switching the UV for an IR laser, we also recorded the heating signal. By alignment of the heating signals, the deposited heat in the experiment was determined to be 0.6 K, which is in the range of earlier experiment [2-3]. The signal at low angles was observed only in the presence of the UV laser, which shows that the observed intensity differences indeed stems from protein rearrangements (Fig. 1B).

Comparing the scattering profile at 8 ms to the predicted scattering resulting from SERCA crystal structures shows significant differences suggesting that the protein strucural changes in solution is quite different from those in the crystalline phase (Fig. 1C). Similar differences between the crystalline and condensed phases were observed for bacteriorhodopsin [2]. However, the main features are present and also the offset in the scattering angle will be reduced in the refinement procedure (due to compensation of the unavoidable error introduced in removal of the heating signal [2]). The data is currently modeled using our recently published metadynamics refinement approach aimed at SAXS data [4], recoded for time-resolved WAXS data.

Despite very encouraging results the following improvements will be proposed in future applications; Becuase the optimized flow rate was significantly higher than predicted, we used our caged compund faster than expected and therefore focused on recording good signal-to-noise data for a critical time point. Similar recordings of time points 5 ms, 15 ms, 25 ms, 35 ms and 45 ms would cover the full reaction cycle of SERCA. In addition, 10 µs x-ray pulses instead of 1 µs used in this experiment (to further improve signal-to-noise) and to record data at a lower scattering vector of 0.01-0.02 Å^{-1} (the predicted scattering profiles show significant features also in this range). Also, an even higher flow rate would reduce the features at low scattering angles in the control (Fig. 1B).

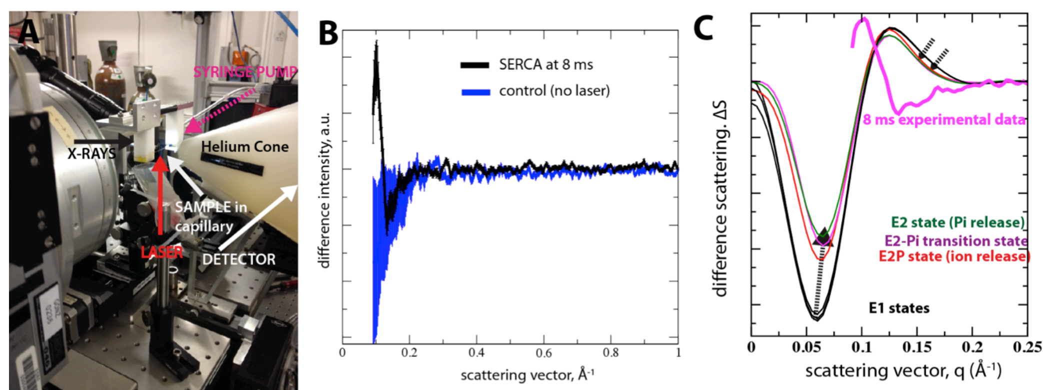

Figure 1. (A) Experimental setup **(B)** Time-resolved X-ray Scattering data for SERCA at 8 ms and control recording (no laser). **(C)** Predicted x-ray scattering from SERCA crystal structures and the 8 ms difference data.

References
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