Tracking Ca^{2+} ATPase intermediates in real-time by X-ray solution scattering

Magnus Andersson

Department of Chemistry, Umeå University, Umeå, Sweden,
magnus.p.andersson@umu.se

The Ca^{2+}-transporting sarcoplasmic reticulum ATPase (SERCA) is a membrane protein transporter involved in calcium signaling by active Ca^{2+} reuptake to internal stores. Several of the structural transitions associated with transport have been characterized by X-ray crystallography, but critical intermediates of the inward-outward switching are missing. We combined time-resolved X-ray solution scattering (TR-XSS) experiments at beamline ID09, ESRF, and molecular dynamics (MD) simulations to characterize SERCA activation, phosphorylation and calcium release in real-time in the native membrane. Two transient intermediates were identified, one with a 1.5 ms rise-time that showed closing of the cytosolic domains typical of Ca^{2+}- and ATP-bound SERCA states. The subsequent 13 ms intermediate, however, showed a completely novel arrangement of the catalytic domains that exposed the ADP-binding site, which remains buried in crystal structures. This conformational arrangement is consistent with the elusive ADP-sensitive, Ca^{2+}-bound state in mid-transition between known inward-facing and outward-facing states. Hence, this time-resolved scattering approach enables identification and structural characterization of transient intermediates in irreversible membrane protein reactions, and therefore significantly increases the number of possible targets beyond the light-sensitive proteins.