



UMEÅ UNIVERSITY

Characterizing ATP-dependent protein structural dynamics in solution

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Academic dissertation

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Abstract

Proteins are dynamic molecules whose function depends on structural changes that occur over a broad range of timescales. Protein motions can be linked to important functionalities such as ligand binding, catalysis, transport and regulation. To understand such processes, it is necessary to go beyond determination of static structures and follow protein conformational changes in time. The work presented in this thesis focuses on ATP-dependent protein dynamics in solution using time-resolved X-ray solution scattering (TR-XSS), combined with molecular dynamics-based structural refinement and ensemble analysis.

A detector readout-based TR-XSS setup was established using adenylate kinase (AdK) as a model system (

Paper I). AdK is a soluble protein that carries out the interconversion of ATP, AMP and ADP, helping the cell balance adenine nucleotide levels. Using laser-induced release of caged-ATP, we collected time-resolved scattering data at a general-purpose synchrotron beamline, and the radiation damage, and data quality was evaluated. Although the temporal resolution was lower than what can be achieved at dedicated time-resolved beamlines, the setup enabled detection of structural changes on the millisecond timescale and provided a promising and accessible workflow for TR-XSS measurements.

Then we proceeded to investigate conformational heterogeneity and ATP-induced domain motions in AdK (

Paper III). Ensemble based refinement was applied to time-resolved difference scattering data, showing that AdK exists as a heterogeneous conformational ensemble in solution. This ensemble shifts towards catalytically active conformations after ATP release. In later work (

Paper IV), an application of an improved ATP releasing strategy, combined with TR-XSS and metadynamics-derived structure pools enabled us to resolve conformational changes in the microsecond range. These results showed that the substrate binding domains of AdK do not close simultaneously but follow a defined sequence of events and reach the closed state of the enzyme on a sub-millisecond timescale. The final part of the work focused on a bacterial Ca^{2+} ATPase (LMCA1). We used TR-XSS combined with targeted molecular dynamics to investigate ATP-dependent structural changes in LMCA1 (

Paper II). The results identified that phosphorylation is the rate-limiting step of the Ca^{2+} transport cycle.

Overall, this thesis demonstrates that TR-XSS, when combined with simulation-based structural refinement, is a powerful methodology for the study of structural dynamics in solution and makes a contribution to the characterization of ATP-driven protein function.

Keywords: TR-XSS, molecular dynamics, ensemble optimisation method, adenylate kinase, P-type ATPase, ATP, calcium transport

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