

Time-resolved and Multi-temperature Crystallography of PEPCK Allows Observation of Previously Unobserved Dynamics and Structural States

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Phosphoenolpyruvate carboxykinase (PEPCK), an essential enzyme that converts oxaloacetate to phosphoenolpyruvate and gates the gluconeogenesis pathway has recently been found to be upregulated in certain cancers.(1,2) Utilizing MMQX (Millisecond Mix-and-Quench Crystallography) we collected time-resolved crystallography data at timepoints of 40ms, 120ms, and 200ms. These datasets were able to capture PEPCK motions associated with substrate binding and catalysis as well as binding positions of the phosphoenolpyruvate and carbon dioxide products.(3) In addition to time-resolved crystallography, we also performed multi-temperature crystallography of PEPCK to better understand the energy landscape in steady-state conditions. These experiments captured the opening of the omega active site gating loop in PEPCK. Taken together, these experiments greatly improve our understanding of PEPCK's structural fluctuations.

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