Understanding Adenylate Kinase's Structure and Dynamics through Protein Crystallography

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Adenylate kinase (AK), an enzyme that catalyzes the reversible conversion between ATP/AMP and two ADP molecules, is a model enzyme for protein dynamics. Enzymes, including AK, have various conformational states that are sampled through their dynamic processes. The structures of AK's bound and unbound static conformations can be solved through the formation of AK macrocrystals, in apo form and co-crystallized with its ligands. Despite the current knowledge of AK's static conformational structures, its dynamics should be further characterized. Prior to crystallization, affinity and size exclusion chromatography were utilized to extract and purify AK expressed in *E. coli* bacterial cell cultures. Our results demonstrate that AK macrocrystals can be successfully formed in several conditions. They show that the methods used are an effective means of achieving extensive single protein crystal growth. Potential promising conditions to form microcrystals were found, which could be optimized for future work. Using macrocrystals from the three different AK conformations (apo, AMP, and AMP-PNP), diffraction pattern data will be collected using a synchrotron to solve those structures. Ultimately, we anticipate to be able to successfully produce AK microcrystals to map out its enzyme catalysis with structures and time stamps using an X-ray free electron laser through mix-and-inject serial crystallography. The data collected will be processed to produce a complete molecular movie of AK's catalysis. Understanding an enzyme's conformations paired with their time scales provides us with a thorough understanding of enzyme catalysis and has broad applications for protein and ligand design.