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Structure determination of high-energy states in a dynamic protein ensemble

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Macromolecular function frequently requires that proteins change conformation into high-energy states; 1-4; . However, methods for solving the structures of these functionally essential, lowly populated states are lacking. Here we develop a method for high-resolution structure determination of minorly populated states by coupling NMR spectroscopy-derived pseudocontact shifts; 5; (PCSs) with Carr-Purcell-Meiboom-Gill (CPMG) relaxation dispersion; 6; (PCS-CPMG). Our approach additionally defines the corresponding kinetics and thermodynamics of high-energy excursions, thereby characterizing the entire free-energy landscape. Using a large set of simulated data for adenylate kinase (Adk), calmodulin and Src kinase, we find that high-energy PCSs accurately determine high-energy structures (with a root mean squared deviation of less than 3.5 angström). Applying our methodology to Adk during catalysis, we find that the high-energy excursion involves surprisingly small openings of the AMP and ATP lids. This previously unresolved high-energy structure solves a longstanding controversy about conformational interconversions that are rate-limiting for catalysis. Primed for either substrate binding or product release, the high-energy structure of Adk suggests a two-step mechanism combining conformational selection to this state, followed by an induced-fit step into a fully closed state for catalysis of the phosphoryl-transfer reaction. Unlike other methods for resolving high-energy states, such as cryo-electron microscopy and X-ray crystallography, our solution PCS-CPMG approach excels in cases involving domain rearrangements of smaller systems (less than 60 kDa) and populations as low as 0.5%, and enables the simultaneous determination of protein structure, kinetics and thermodynamics while proteins perform their function.
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