The Effect of Crystal Contact Forces on Protein Intramolecular Dynamics
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Increasingly time resolved X-ray crystallography and solid state NMR have been employed to characterize dynamics. In the advent of X-ray free electron sources at Stanford (LCLS), and Hamburg (European XFEL) there is a strong push to extend time-resolved measurements. A persistent question for these techniques however, is how the crystal contact forces may strongly perturb these dynamics from those in vivo. While some theoretical studies have indicated that the crystal contact perturbation is minor [1], other calculations suggest it is significant [2]. Surprisingly, there have been few studies to actually determine from the data what the effects are. Given the enormous effort currently underway for extending crystal phase dynamics measurements, it is imperative to determine how the crystal contact forces affect large scale motions necessary for function. Here we show how anisotropic optical measurements in the extreme infrared (10-100 cm⁻¹) using the technique of Crystalline Anisotropy Tenertz Microscopy (CATM) can quantify the effect [3], by measuring the perturbation of the global motions for a given symmetry group.

Chicken egg white lysozyme (CEWL) is used as a benchmarking model. Calculations and measurements are performed for tetragonal and monoclinic symmetry groups, for which B-factor measurements indicate that there is a significant difference in the motional constraint arising from the crystal geometry.


Thermodynamic and Dynamic Basis for the Broadened Ligand Specificity of a Tiam2 PDZ Domain Mutant

Biochemistry, University of Iowa, Iowa City, IA, USA.

PDZ (PSD-95/Dlg/ZO-1) domains are protein-protein interaction modules that typically recognize their binding partners through the use of two specificity pockets. Here we examine the consequence of mutating four residues in the Tiam2 PDZ domain specificity pockets to produce a quadruple mutant (QM). Equilibrium binding studies show that the specificity of the Tiam2 QM mutant is similar to that seen in the wild type Tiam1 PDZ domain. Isothermal titration calorimetry experiments show a large entropic contribution to ligand binding in the QM PDZ domain compared to the WT PDZ domain. Double-mutant cycle analysis uncovered cooperativity between residues in the two specificity pockets with respect to both ligand binding and protein folding. NMR-based HSCQ studies reveal that the wild type Tiam2 PDZ has severe line broadening in several loops, while the QM PDZ had additional regions of line broadening. However, peptide ligand binding dampens line broadening for both the Tiam2 WT and QM PDZ domains. Finally, CPMG dispersion experiments indicate that the number of residues experiencing micro to millisecond motions is significantly increased in the QM PDZ domain. We propose a model where enhanced dynamics alters the QM PDZ domain conformational ensemble allowing for broader ligand specificity relative to the WT PDZ domain.

Relative Mechanical Flexibility of Ubiquitin Family Proteins: A Study using Elastic Network Model

Chemical Sciences, Tata Institute of Fundamental Research, Mumbai, India.

The conformational flexibility of biomolecules is essential for their function. Elastic Network Model (ENM) is a class of harmonic models used to computationally describe the flexibility of biomolecules. Despite the simplicity of the underlying potential, ENMs show intriguing abilities to capture functionally relevant conformational changes in proteins, as seen in their crystallographic structures, through their low-frequency normal-mode displacements. We present an ENM based study of the mechanical flexibility of proteins having high structural similarity but low sequence homology. Single-molecule atomic force microscopic (AFM) measurements reveal that ubiquitin requires a higher unfolding force when pulled along N-C termini than the SUMO proteins. The higher mechanical stability of ubiquitin relative to the SUMOs is presumably a sequence effect, as the proteins have identical secondary structures. Our calculations at the atomistic resolution show a strong imprint of the experimentally observed disparity in stabilities of the ubiquitin-like proteins in their flexibilities. Spring constants for normal modes of ubiquitin are higher than that of the SUMOs, implying larger stiffness of ubiquitin over the latter. The residues on the clamp (terminal β-sheets) region of these proteins that govern their stabilities show mobility that is implicated in their flexibilities. We discuss physical considerations for extracting a reduced dimensional basis from ENM for the description of equilibrium flexibility of proteins. The large-amplitude normal modes that represent concerted protein motions additionally reveal the conformational changes taking place when ubiquitin and SUMOs bind with substrates, as observed in the complex crystallographic structures. The flexible SUMO proteins tend to be as stiff as ubiquitin on substrate-binding whereas, there seems to be no considerable enhancement in the rigidity of apo-ubiquitin. We elucidate this feature in our study in the light of spring constants of the slowest normal modes.

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