Turning Up the Heat on Molecular Machines with Multi-Temperature and Temperature-Jump X-ray Scattering Experiments

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Molecular machines are oligomeric protein complexes that convert thermal or chemical energy into mechanical or biochemical work in the cell. Despite their fundamental biological importance, studying the mechanisms of molecular machines is challenging. Typically, they are large and dynamic macromolecules that undergo complex conformational cycles with motions that cover length scales ranging from Ångströms to tens of nanometers, and time scales ranging from nanoseconds to seconds. One family of molecular machines with rich structural dynamics over multiple length and time scales are Heat Shock Protein 90 (Hsp90) chaperones. These large homodimers use ATP hydrolysis and thermal energy to unfold misfolded proteins, acting to help maintain protein homeostasis. While much is known about the large-scale motions that constitute the Hsp90 functional cycle, important questions remain relating to the spatial and temporal coupling of the conformational changes inferred from static structures. Specifically, we lack knowledge of the allosteric communication network that couples local conformational changes, induced by substrate binding at the ATPase active site, to large scale conformational changes that occur over length scales of multiple nanometers. In this presentation, I will describe how we are using multi-temperature and temperature-jump X-ray crystallography and solution scattering to map the conformational landscape of an Hsp90 family member, human TNF receptor-associated protein 1 (TRAP1), by measuring its structural dynamics over broad length and time scales.