

Towards an atomic-resolution functional study of the ClpXP machinery in action using cell free and NMR technologies

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The assembly of caseinolytic proteases P and X (ClpXP) is a supramolecular machine involved in protein quality control in bacteria and mitochondria. ClpXP is required for a wide range of cellular processes, from protein maintenance to bacterial virulence. It also participates in the regulation of cell division by degrading its principal regulator, FtsZ. ClpX is an ATPase associated with a variety of cellular activities, and self-assembles to form a ring of six subunits capable of unfolding and translocating client proteins into ClpP's 14-mer barrel pore for degradation. The large size of this machine (800 kDa), its low stability, the complexity of its biological substrates and structural rearrangements pose a series of logistical problems. Structural studies of such systems using X-ray crystallography or cryo-EM generally provide only a static image of the system, and rarely report the kinetic data needed for a complete, atomically-resolved understanding of the mode of action. In this project, we use state-of-the-art NMR spectroscopy, advanced isotope labeling and cell-free techniques to characterize the non-equilibrium dynamics of the ClpXP machinery. Cell-free technology is exploited to produce and stabilize the ClpX unfoldase by forming a homogeneous complex with the ClpP protease. Then, advanced isotope labeling methods are used to produce the perdeuterated particle, labeled with ¹³CH₃ probes on selected methyl groups of ClpP or ClpX. This strategy enabled the acquisition of high-quality 2D NMR spectra of the full-size ClpXP complex and the study of interaction with ATP and FtsZ. NMR allows us to detect the transient interaction with the client protein and the nucleotide-induced structural rearrangement of ClpXP as the molecular machine processes its substrate. These developments pave the way for future structural studies of the ClpXP mechanism.