Mycobacterium tuberculosis (Mtb) is the causative pathogen of tuberculosis (TB), the leading cause of death from a single infectious agent worldwide. Mtb relies on a unique proteasome system to resist the human immune system. Therefore, obtaining a detailed and mechanistic understanding of the structure and function of the Mtb proteasome system and its regulatory particles will better equip us with insights that can be exploited by novel therapeutics. The 20S proteasome core particle (20S CP) has a barrel-like structure that sequesters fourteen catalytic sites. Substrate access to the active sites is regulated via gated narrow pores to ensure minimal spurious degradation occurs. Regulatory particles induce proteasome gate opening and are essential for maintaining regulated substrate degradation. In Mtb, this task is partly handled by the ATP-independent bacterial proteasome activator (Bpa). The mechanism in which Bpa binds and translocates partially folded protein substrates for proteolysis is poorly understood. I will use a host of structural biology and biochemical tools to uncover the molecular mechanisms of Bpa substrate recruitment and translocation. Using hydrogen/deuterium exchange mass spectrometry (HDX-MS), I will localize the sites of interaction between native and model substrates with Bpa. Additionally, I will characterize the conformational changes in both substrates and Bpa upon binding using HDX-MS. My structural data will be complemented and validated using single-point mutations and functional assays. I anticipate that the insights obtained from my project will inform rational design of antitubercular molecules that target the Mtb proteasome machinery.