Tuberculosis (TB) is an infectious disease caused by *Mycobacterium tuberculosis* (*Mtb*). TB is the second largest cause of mortality from an infectious agent worldwide. The lack of an effective vaccine and the continued spread of multidrug-resistant (MDR) strains threaten to undermine the progress made against TB. This calls for fundamental research to develop novel narrow-spectrum antimicrobials against MDR-*Mtb*. A unique protein degradation system in *Mtb*, called the Pup-proteasome system (PPS), is essential for the pathogenesis and survival of the pathogen in the host. Analogous to the E3 ubiquitin ligase enzyme in eukaryotes, the *Mtb* Proteasome Accessory Factor A (PafA) attaches Prokaryotic Ubiquitin-like Protein (Pup) to substrates and targets them for degradation. While thousands of E3 ligases regulate substrate selection in eukaryotes, PafA is the sole Pup ligase in *Mtb* and pupylates over 200 substrates. The mechanisms by which PafA can recruit and accommodate a multitude of substrates remains elusive. My presentation will highlight how a combination of biochemical and structural biology tools can be used to gain a mechanistic understanding of substrate recruitment in the PPS. Using recombinantly expressed proteins, I will i) establish the pupylation pathway *in vitro*; and ii) use H/D exchange coupled to mass spectrometry to study the role of conformational dynamics in the substrate promiscuity of PafA. Our structural and biochemical insights into PafA-substrate interactions could provide clues important for the development of chemical probes to understand the mechanism of action of the PPS, and as leads for a future therapeutic strategy against TB.