Type III secretion needles studied by solid-state NMR

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Gram-negative bacteria use a molecular machine called the type three secretion system (T3SS) to deliver effector proteins to host cells. Our research group has recently solved an atomic model of the extracellular T3SS needle of *Salmonella typhimurium* (Loquet et al., *Nature*, 2012). Concurrently, a high-resolution cryo-electron microscopy density map of the T3SS needle of *Shigella flexneri* was obtained by Fujii et al. (*PNAS*, 2012). Modeling of the *Shigella* needle subunit protein to fit the EM density produced a model incompatible with the atomic model of the *Salmonella* needle in terms of secondary structure and subunit orientation. We then determined directly the secondary structure of the *Shigella* needle subunit using solid-state NMR, and its orientation using *in vitro* and *in vivo* immunogold labeling in functional needles. We found that *Shigella* subunits adopt the same secondary structure and orientation as in the atomic model of *Salmonella*, and we generated a homology model of the *Shigella* needle consistent with the EM density (Demers et al., *PLOS Pathogens*, 2013). Here, we will discuss our recent efforts in obtaining higher resolution structures of *Shigella* and *Salmonella* needles. We will also present proton-detected solid-state NMR experiments on perdeuterated T3SS needles. A set of five 3D correlation experiments allowed for the unambiguous assignment of the backbone resonances. Finally, we will show first results of DNP experiments on uniformly ^{13}C-labeled needles.