BIOCHEMISTRY SEMINAR

Structural Characterization of the *Clostridioides difficile* Transferase Reveals Intermediates that Occur During Intoxication

Michael Sheedlo

Assistant Professor, University of Minnesota

Abstract: *Clostridioides difficile* is currently the leading cause of antibiotic-associated, hospital-acquired diarrhea in the United States. The symptoms accompanying *C. difficile* infection are triggered by the activity of up to three toxins secreted by the bacterium. One of these toxins, the *C. difficile* Transferase (CDT), is often found in clinical strains associated with severe symptoms and high rates of mortality, suggesting it may exacerbate the pathology related to infection. CDT is a member of the Iota family of binary toxins and is comprised of two proteins known as CDTa and CDTb. Under the accepted model of intoxication, CDTb acts as a delivery apparatus and injects CDTa into the cytoplasm of the host. Inside the host cell, CDTa modifies host cell proteins eventually leading to the disruption of the cytoskeleton. Despite its prevalence in problematic strains, little is known about how CDT enters cells to deliver its toxic cargo. We have used cryogenic electron microscopy (Cryo-EM) to generate a framework for understanding CDT function. These studies have led to the elucidation of several unique CDT structures which, we suggest, correspond to intermediates that occur during intoxication. Using these structures, we present a revised model of intoxication and highlight new mechanistic insight into how the toxin assembles into an oligomeric structure. We also provide additional biochemical evidence and propose a model to explain how CDTa is primed for delivery into host cells. We expect that these mutants will serve as valuable tools for future studies that seek to define the role CDT plays in evoking *C. difficile* infection related pathology.

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James Tarpo Jr. and Margaret Tarpo Department of Chemistry