Structural and functional analysis of the *Legionella pneumophila Dot/Icm* Type IV secretion system

Jacquelyn Roberts[1,2], Arwen E. Frick-Cheng[1], Henry Styron[3], Clarissa Durie[4], Louise Chang[1], and Melanie D. Ohi[1,5]

[1]Life Sciences Institute, University of Michigan, Ann Arbor, MI USA.

[2]Department of Biological Chemistry, University of Michigan, Ann Arbor, MI USA.

[3]Department of Molecular Cellular and Developmental Biology, University of Michigan, Ann Arbor, MI USA.

[4]Department of Biochemistry, University of Missouri, Columbia, MO USA.

[5]Department of Cell and Developmental Biology, University of Michigan, Ann Arbor, MI USA.

Legionella pneumophila, the etiological agent of Legionnaires' disease, commandeers host cellular processes via the Dot/Icm Type IV Secretion System. This secretion system is required for injecting effector proteins into host cells, enabling bacterial colonization. Central to the T4SS is the outer membrane core complex (OMCC), comprised of an outer membrane cap (OMC) and periplasmic ring (PR). Additionally, the T4SS is anchored by a stalk that extends from the OMCC to the bacterial inner membrane, and an inner membrane complex (IMC) of cytoplasmic ATPases. While atomic resolution structures of the OMCC subassembly have been instrumental in understanding the composition of Legionella-specific T4SS elements, the full mechanistic role of the T4SS has remained elusive, primarily due to the absence of a high-resolution structure of the entire T4SS. Here, we investigated the structural and functional contributions of two previously uncharacterized OMCC proteins and employed a novel engineered substrate protein to isolate an additional subcomplex of the Dot/Icm T4SS. By highlighting the discrete contributions of individual T4SS components and subassemblies, this study provides structural insights that pave the way for better understanding the diverse class of type IV secretion systems.