

Cryo-EM Reveals New Species-specific Proteins and Symmetry Elements in the *Legionella pneumophila* Dot/Icm T4SS

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Type IV secretion systems are bacterial weapons used to facilitate the transfer of protein or DNA substrates into host cells. These double membrane spanning complexes can mediate the conjugative transfer of plasmid DNA, as in the case of *Escherichia coli* and *Agrobacterium tumefaciens*, or they can transfer virulence proteins as in the case of *Helicobacter pylori*, *Neisseria gonorrhoeae*, and *Legionella pneumophila* (*L. pneumophila*).¹ The prototypical T4SS contains 12 components whose architecture can be divided into four main features: the outer membrane cap (OMC), the inner membrane complex, a complex of cytosolic ATPases, and in some cases, an extracellular pilus. (1-3) Although many features are conserved between species, the architecture varies between systems. Biochemical and *in vivo* cryo-electron tomography (cryo-ET) studies of *L. pneumophila* and *H. pylori* T4SSs show these complexes have features unique from previously characterized T4SSs, most notably a larger and more intricately organized OMC (4-8). The *L. pneumophila* T4SS is one of the most elaborate known (9), requiring 26 identified genes named *dot* (defect in organelle trafficking) or *icm* (intracellular multiplication) and translocating as many as 300 different protein substrates (10,11). These 300 protein substrates among other roles inhibit phagosome fusion with the lysosome, providing the bacteria with a replicative niche within the host cell.

We recently biochemically purified the *L. pneumophila* T4SS, subjected it to single particle CryoEM, and described parts of the structures and positions of DotC, DotD, and DotH, as well as two additional proteins that associate with the OMC. (9,12,16) However, several features in this map could not be unambiguously identified, including the PR, three chains within the OMC, and a low-resolution ‘dome’ positioned in the center of the OMC (9). We have now determined sub-3.0 Å resolution maps of the *L. pneumophila* T4SS core complex by single particle cryoEM. Here we can identify two distinct areas of symmetry mismatch; one connecting the C13 outer membrane cap (OMC) with the C16 dome, and one connecting the C13 OMC with the C18 periplasmic ring (PR). Unexpectedly, the protein facilitating the connection between the PR and OMC is DotH, with five copies at the interface of the OMC and PR. The high-resolution structural analysis has allowed us to identify two additional proteins encoded outside the Dot/ICM genetic locus that contribute to the core T4SS structure: Dis2 and Dis3. Additionally, a higher resolution structure of the outer membrane cap (OMC) allows for the assignment of putative lipidation

sites where proteins DotC, DotD, and DotK interact with the outer membrane and visualization of the pore that facilitates the transfer of cargo. Finally, we observe multiple conformations in the reconstructions which indicate flexibility between the 16 copies of DotG and the rest of the OMC disk [14].

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