Structure of the *Legionella* Dot/Icm type IV secretion system *in situ* by electron cryotomography

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SI Figure 1. Western blot analysis of expression of core components.

Western blot showing that the Lp02 strain expresses all the core component proteins (DotFGHCD) (left lane), the dot/icm super-deletion strain (SΔ) does not express any of the core-complex proteins (middle lane) and the reconstituted core complex strain express all the core complex proteins at comparable levels to the Lp02 strain (right lane). The unrelated cytoplasmic protein isocitrate dehydrogenase (ICDH) is used as loading control.
SI Figure 2. Flexibility within Dot/Icm particles.

(A) When a mask is used to focus alignment on the densities near the outer membrane, little density is seen around the inner membrane. (B) Similarly, when the mask is moved near the inner membrane part, densities near the outer membrane complex are barely visible. (C) Composite model generated by juxtaposing the outer membrane and inner membrane averages. (D) Relative translations found between the outer-membrane-aligned and inner-membrane-aligned regions (green dots). Dotted yellow lines indicate where the outer membrane average is merged with the inner membrane average to generate the composite model. Scale bar 10 nm.
SI Figure 3. Resolution and difference maps.

(A) Local resolution of the subtomogram average calculated by ResMap (Kucukelbir et al., 2014). (B) Difference map between the WT complex and the reconstituted core complex. Densities missing in the reconstituted complex are colored yellow, additional densities in red. (C) Outline of the core complex (orange dotted line) superimposed on the Dot/Icm structure. Scale bar 10 nm.
SI Figure 4. Genetic organization of T4ASSs and T4BSSs.

(A) Organization of the T4ASS (VirB type) and T4BSS (Dot/Icm type) genes. The T4BSS has a more elaborate and complex genetic organization. Genes colored in blue are ATPases. (B) VirB10 and DotG show clear sequence homology. Both proteins have a conserved TrbI domain in their C-terminal region.
SI Movie: 1. Cryotomogram of an intact *L. pneumophila* (Lp02) cell. Dot/Icm particles are visible in different slices (see arrows) with the characteristic shape of a “Wi-Fi” symbol. Scale bar 100 nm.

SI Movie: 2. Slices through subtomogram average of Dot/Icm particles. The outline of VirB10 density from the crystal structure 3JQO matches well with the hat density of the Dot/Icm structure. Scale bar 10 nm.