

Architecture and host interface of environmental chlamydiae revealed by electron cryotomography

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Supplementary Information

Figure S1. *Simkania*, *Parachlamydia* and *Protochlamydia* do not recruit mitochondria to their inclusions.

MitoTracker staining of mitochondria (red) was combined with detection of chlamydiae (green) and the amoeba host (blue) by fluorescence *in situ* hybridization. No significant clustering of mitochondria around inclusions of *Simkania* (A), *Parachlamydia* (B) or *Protochlamydia* (C) was detected in *A. castellanii*. Bars 10 μm .

Figure S2. More examples of putative *Simkania* T3S structures with sheared-off needles.

Shown are slices through cryotomograms of purified *Simkania*. Often, a widening of the periplasm and basal body-like densities were observed in the absence of a T3S needle, suggesting some needles are sheared off during purification. Bar 100 nm.

Movie S1. Crescent bodies are not seen in projection images of plunge-frozen *Parachlamydia* cells.

Movie S2. Cryotomogram and 3D model of the *Simkania* inclusion from Figure 3D.

Movie S3. Cryotomogram and 3D model of the *Parachlamydia* inclusion from Figure 3L.

Movie S4. Cryotomogram and 3D model of the *Protochlamydia* inclusion from Figure 4C.