Exploration of bacterial cell architecture by cryo-EM: S-layers and mini microtubules

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Many prokaryotic cells are encapsulated by a surface layer (S-layer) that consists of repeating units of S-layer proteins (SLPs). SLPs are a diverse class of molecules found in Gram-positive and Gram-negative bacteria and most archaea 1-5. S-layers protect cells from the outside, provide mechanical stability, and also play roles in pathogenicity. In situ structural information on this highly abundant class of proteins is scarce, therefore atomic details of how S-layers are arranged on the surface of cells have remained elusive. Here, using purified C. crescentus' sole SLP RsaA we obtained a 2.7 Å X-ray structure that shows the hexameric S-layer lattice. Next, we solved a 7.4 Å structure of the S-layer through electron cryotomography (cryo-ET) and sub-tomogram averaging of cell stalks. The X-ray structure was docked unambiguously into the cryo-ET map, resulting in a pseudo-atomic level description of the in vivo S-layer, which agrees completely with the atomic X-ray lattice model. The cellular S-layer atomic structure shows that the S-layer is porous, with the largest gap dimension being 27 Å, and is stabilised by multiple Ca2+ ions bound near interfaces. This study spans different spatial scales from atoms to cells by combining X-ray crystallography with cryo-ET and sub-nanometre resolution sub-tomogramaveraging.

Before presenting the S-layer structure, I will briefly present the structure of bacterial mini microtubules and how their diverged architecture still leads to strong dynamic instability, the hallmark of eukaryotic microtubules.