

## Investigating cell and “organelle” division in anammox bacteria using electron tomography

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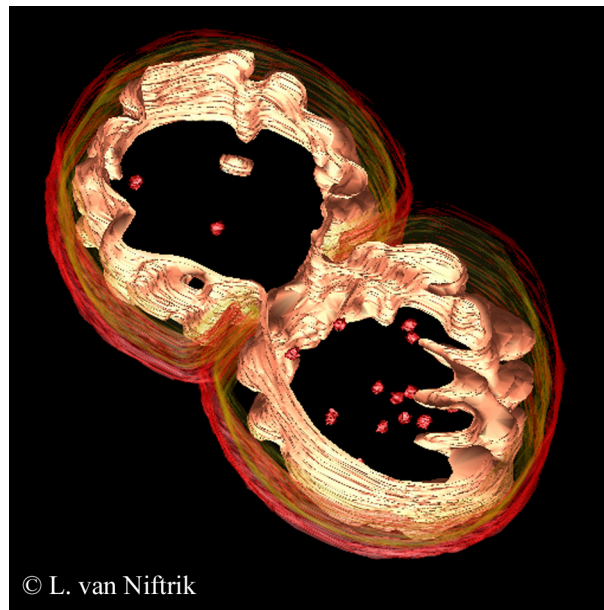
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The anaerobic oxidation of ammonium to dinitrogen gas (anammox) is a recently discovered pathway of the biological nitrogen cycle [1] and is currently estimated to be a major source of gaseous nitrogen on Earth [2]. Anammox is performed by a deep-branching clade of planctomycete bacteria, which are applied in wastewater treatment for the removal of ammonium. Anammox bacteria divide only once per two weeks at maximum speed and possess a “prokaryotic organelle”, the anammoxosome; a separate, intracytoplasmic compartment surrounded by a bilayer membrane. This major compartment is dedicated to anammox catabolism that involves the rocket fuel hydrazine as an intermediate. The anammoxosome as the locus of respiration in anammox bacteria is thus possibly analogous to the mitochondria of Eukaryotes. The anammoxosome membrane consists mainly of unique ladderane lipids [3], which make this membrane more impermeable to protons and the valuable intermediates of anammox catabolism. With their small size (< 1 µm) and intriguing cell biology and architecture [4], these prokaryotes are an ideal target to be investigated with transmission electron microscopy including immunolabeling, histochemical staining and electron tomography in order to correlate ultrastructure and function.

Different stages of the anammox cell cycle were investigated and compared using transmission electron microscopy, including electron tomography, to resolve how the anammox cell divides and passes on the anammoxosome to daughter cells (Figure 1). Electron tomography showed that the bacterial organelle was enlarged and divided equally among the daughter cells during the process of cell division. Further, a cell division ring was observed in the outermost compartment of dividing anammox cells. In general, GTP hydrolysis drives the tubulin-analogue FtsZ to assemble into a ring-like structure at the cell division site in bacteria where it functions as a scaffold for the molecular machinery that performs cell division. However, the genome of the anammox bacterium “*Candidatus Kuenenia stuttgartiensis*” [5] does not encode *ftsZ*. Genomic analysis of open reading frames with potential GTPase activity indicated a possible novel cell division ring gene, which was unrelated to *ftsZ*. An antibody was raised against a heterologously expressed part of this open reading frame. This antibody was used in immunogold localization to investigate the location of the putative anammox cell division ring protein in the cell.

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**Figure 1.** Snapshot of an electron tomography model showing a 300 nm thick section of a dividing “*Candidatus Kuenenia stuttgartiensis*” cell [6].