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3D ultrastructure of the archaeon *Ignicoccus hospitalis*, analysed by different tomography techniques

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Cells of the hyperthermophilic, anaerobic archaeon *Ignicoccus hospitalis* thrive optimally at T=90°C, by chemolithoautotrophy; they gain energy by reducing elemental sulfur with molecular hydrogen. They also exhibit exclusive structural features, with their unique cell envelope lacking an S-layer but consisting of two membranes: they encase two cell compartments, a tightly packed cytoplasm, and a weakly stained intermembrane compartment (IMC) with a variable width of 20 to 1000 nm, containing round or elongated vesicles [1]. In addition, *I. hospitals* cells can serve as host in a unique biocoenosis, when cultivated together with *Nanoarchaeum equitans*: cells of *N. equitans* cannot be grown alone, but require cell-cell contact to host cells for being able to thrive, and they obtain at least their lipids and (some) amino acids from the host [2].

We are interested in analysing the ultrastructure of the membrane system and cell-cell interaction of these prokaryotes at high resolution. In particular, we want to identify the path and the interconnectivity of the membranes; for this, structural investigations with an isotropic resolution of about or better than 5 nm are a prerequisite.

The width of *I. hospitalis* cells of up to 3 µm makes them unsuitable for direct observation using cryo-TEM. In addition, we aimed for localizing proteins using immunolabeling on ultrathin sections. Therefore, we favour embedding the samples into resins, for detailed (S)TEM/SEM observation. The labile cell structure of *I. hospitalis* (due to the lack of a solid cell wall) and the fragile cell-cell interaction between *I. hospitalis* and *N. equitans* requires great care when preparing samples for electron microscopy. It turned out the cryo-processing is absolutely necessary. Our routing protocol includes the following steps: cultivation in capillary tubes, or enrichment of cells using ultrafiltration membranes; high-pressure freezing; freeze-substitution fixation in acetone/ glutaraldehyde/ Uranyl acetate/ water [3]; and embedding in a resin, usually Epon, and recently also Lowicryl. So far, the 3D analysis is done using different routes: serial ultrathin sections [6], also including serial section labelling; serial 200 nm sections and analysing each by TEM tomography at 200 kV; and FIB-SEM tomography [4]. In addition, we aim to analyse thicker sections (>500 nm) using either BF or HAADF STEM tomography [5].

Datasets obtained so far confirm earlier results [6], and highlight some exclusive structural features, in particular the polar, highly asymmetric organization of the cells; and the energized outer cellular membrane [1], which hosts the primary and secondary proton pumps / energy converting enzymes: the sulfur hydrogen oxidoreductase, and the A_OA_1 ATP synthase (Fig 1). The data also show that the vesicles in the IMC are not only round but can frequently be found elongated like a tubular system. They are likely to shed off from the cytoplasmic membrane, and can contain low amounts of the ATP synthase, probably in the course of transport from the cytoplasm to the outer cellular membrane.

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6. We kindly acknowledge the support of the DFG by a grant to HH and RR.

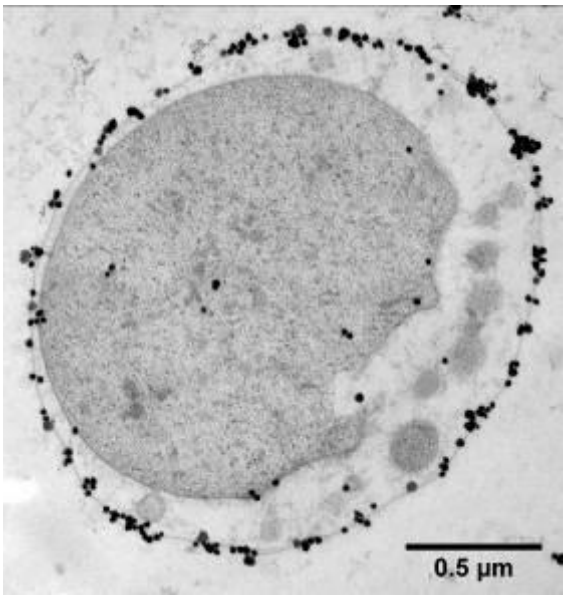

Figure 1. Ultrathin section of *I. hospitalis*; immuno-localization of the A_OA_1 ATP synthase (secondary antibody with US gold, plus silver enhancement).

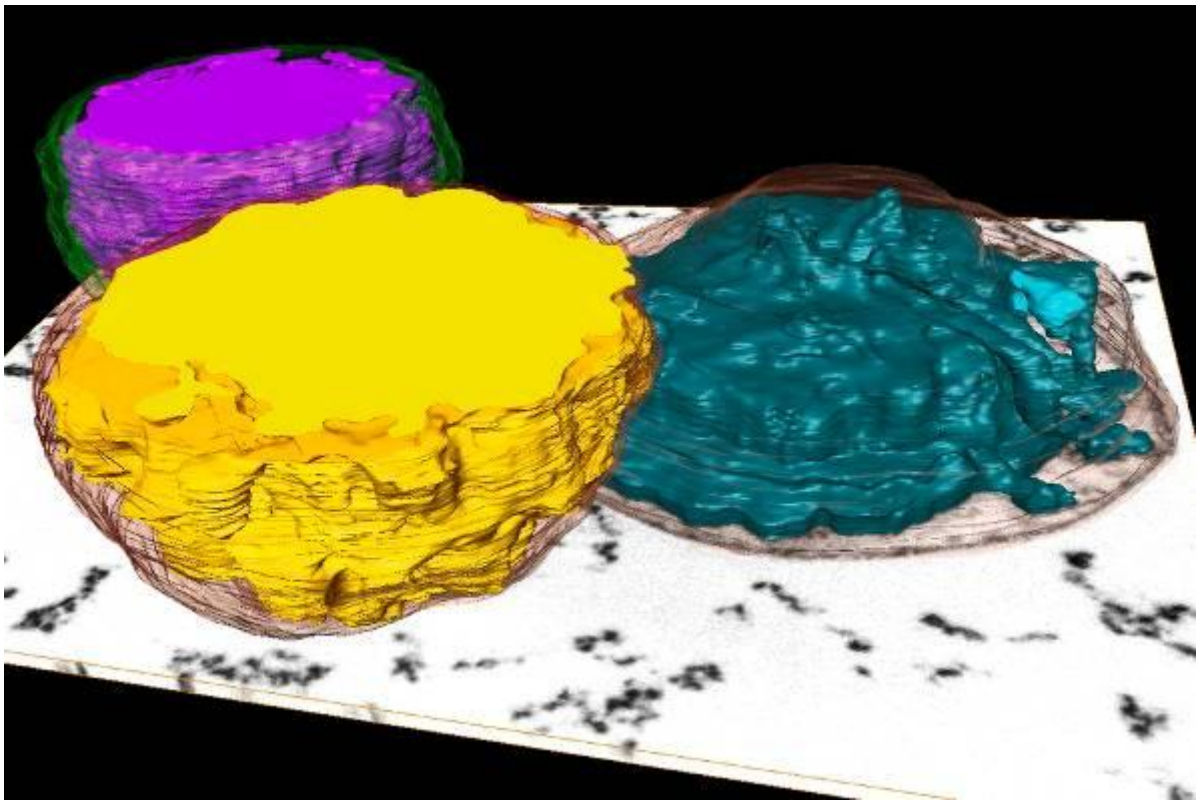

Figure 2. 3D visualization of three cells of *I. hospitalis* after HPF, FSF, Epon embedding, and FIB-SEM data recording (1024 x 768 x 299 voxel; each about 5 nm). Data were aligned and visualized using AMIRA. Scale: one single cell is about 2.0 µm in diameter.