3D Structure of the Archaea *Ignicoccus* and *Nanoarchaeum*, as determined by serial section electron microscopy

T. Heimerl[1], N. Wasserburger[1], B. Junglas[1], C. Meyer[1], R. Wirth[2], H. Huber[2], and R. Rachel[1]

1. University of Regensburg, Centre for Electron Microscopy, Universitätsstr. 31, D-93053 Regensburg, Germany
2. University of Regensburg, Dept. of Microbiology, Universitätsstr. 31, D-93053 Regensburg, Germany

Thomas.heimerl@biologie.uni-regensburg.de, reinhard.rachel@biologie.uni-regensburg.de
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The hyperthermophilic Archaeon *Ignicoccus hospitalis* has a unique ultrastructure, including a huge periplasmic space and an outer membrane (2,6,7). *I. hospitalis* cells also have a novel CO_2 fixation pathway (1) and are the only known host for the smallest Archaeon, *Nanoarchaeum equitans* (2). These two Archaea form an intimate association; it is not clear yet to which extent *N. equitans* cells have damaging effects to their host cells (3). *N. equitans* is not able to synthesize lipids, amino acids, cofactors, and nucleotides, according to physiological and genome studies (4). Therefore, the interaction of these cells with *I. hospitalis* is of high interest, in order to understand, how cells specifically recognize each other and how the transport of metabolites is organized. Labelling studies showed that archaeal lipids (5) and amino acids are transported from *I. hospitalis* to *N. equitans* (3).

In order to achieve the best preservation of the delicate contact site, we used cultivation in capillary tubes, cryo-fixation by high-pressure freezing, freeze-substitution and resin embedding; serial sections were imaged by transmission electron microscopy, and data aligned and visualized as 3D stacks (6). In almost all 3D data sets analyzed so far, the interpretation of the structures is greatly facilitated, compared to images of single sections only. In particular, the *I. hospitalis* cytoplasmic membrane shows an unusual high tendency to form membrane vesicles and invaginations. The vesicles are often still in contact with the cytoplasmic membrane or with other vesicles. The physiological role of this membrane vesicle system is unknown, yet. – In about 75% of the cells, the cytoplasm of *I. hospitalis* cells is involved in the interaction with *N. equitans*; in about 20%, periplasmic vesicles appear to be unloaded at the contact site, possibly releasing 'cargo' to the *N. equitans* cell. In 5% of the imaged cells, the contact site showed structures which cannot be interpreted yet. Immuno-localisation confirms that the Ihomp1 protein of the *I. hospitalis* outer membrane (7) and the S-layer of *N. equitans* are present at and involved in the contact site. Biochemical studies helped to identify further proteins which might be relevant for cell-cell interaction and / or metabolite transport, like components of ABC transporters (8). They are in the focus of ongoing studies on the contact site.

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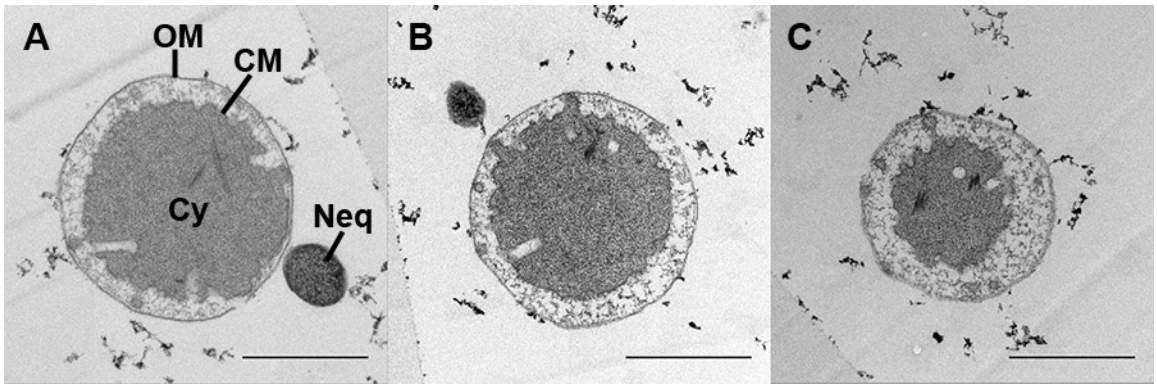

Figure 1. Transmission electron micrographs of ultrathin sections of *I. hospitalis* and *N. equitans*; displayed are three sections from a series of 20. OM: outer membrane; CM: cytoplasmic membrane; Cy: cytoplasm; Neq: *N. equitans*. Bars: 1 µm.

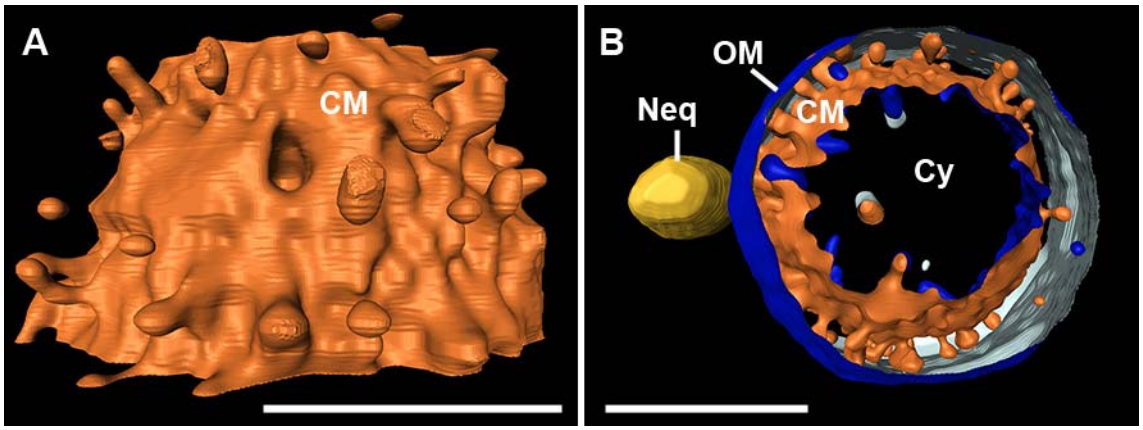

Figure 2. 3D reconstructions from serial ultrathin sections of *I. hospitalis* and *N. equitans*. Orange (shown in A): surface of the cytoplasmic membrane, with numerous vesicles and invaginations. Blue: outer membrane. Yellow (small sphere on the left in B): *N. equitans*. Abbreviations as in Fig. 1. Bars: 1 µm.

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