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Three-dimensional (3D) bacteria-mineral associations within the deep sea hydrothermal vent shrimp *Rimicaris exoculata*.

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The chemical and temperature conditions around deep sea hydrothermal vents are both dynamic and extreme, yet the shrimp *Rimicaris exoculata* flourishes around these environments on the Mid-Atlantic Ridge (MAR). Epibiotic bacteria and minerals found within the branchial chamber of the shrimp are of great interest in the search for a chemical model for the Rainbow MAR hydrothermal vent site. Here we examine the close three-dimensional (3D) relationship between bacteria (on inner surface of the branchial chamber wall) and the minerals that surround them. The morphology and chemistry of the minerals were analysed by Energy-filtering Transmission Electron Microscopy (EFTEM, LEO 912 Omega) and X-ray Nanoanalysis (XN, JEOL-2010 FEG) respectively, and the 3D organisation was determined by Transmission Electron Tomography (TOMO).

Samples of *Rimicaris exoculata* were collected from the Rainbow site (36º 13' N, 2320m depth). The cuticle of the branchial chamber was conventionally dehydrated and impregnated in resin. Consecutive thin and semi-thin sections of 80nm (for EFTEM and XN) and 150nm – 200nm (for TOMO) were cut and mounted on standard microscope grids. Thin-section grids were observed initially for morphology, to find broad relationships between bacteria and minerals, and also as a tool to find areas for EFTEM, XN and TOMO. The 3D TOMO reconstruction volume was produced from a tilted series, comprising images taken at one degree increments between -55° and +55°. Tilted series were obtained using the ESIvision program (Version 3.0, Soft-Imaging Software, SIS GmbH, D-49153 Münster, Germany) with additional in-house scripts for automated acquisition.

In many cases the minerals exhibit a sharp boundary against the bacteria, often with a substantial void between bacterial membrane/cell wall and mineral boundary. Mineral layering and areas of elemental zoning are also observed. Iron is the most prevalent element, with a close association to the bacteria and displays an inverse relationship to oxygen. In areas of heavy mineralization one can see a trend of increasing relative iron percentage towards the bacteria and decreasing oxygen. This pattern is not observed in areas of true interface. Future work will combine the elemental data obtained by EFTEM with tomography (EFTET) to produce a 3D elemental map of the minerals surrounding the bacteria, focusing particularly on the bacteria-mineral interface using recently developed EFTET-J software (http://www.snv.jussieu.fr/∼wboudier/softs.html).