

## **Taking the pulse of the seafloor biosphere, strategies for studying microbial activity and slow growth**

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The developing picture of life in the deep seafloor is one of minimal energy availability, low cell concentrations, and slow growth. Microbial generation times have been inferred using bulk porewater geochemical profiles (e.g. D'hondt et al 2004, Jørgensen and Marshall, 2016) and amino acid racemization (Lomstein et al 2012), with estimated in situ turnover times that span 100-100,000 years. To extend our understanding of the anabolic activity of indigenous microorganisms in the deep biosphere and other energy limited communities, we have optimized single cell targeted methods including a fluorescence-based technique known as BONCAT (Hatzenpichler et al., 2014) that enables the direct fluorescent tagging of newly synthesized proteins in a cell, and nanoSIMS combined with passive isotopic tracers (e.g. deuterated water) and nutrient-based amendments (<sup>15</sup>N or <sup>13</sup>C-labeled substrates). Successful application of these complementary approaches to samples of deep seafloor coal and shale collected during IODP Expedition 337 off of the Shimokita Peninsula confirmed the existence of a low abundance, slow growing microbial assemblage and yielded direct cell-specific data on the generation times and metabolic potential of microbial life residing >1900 m below the seafloor.

D'Hondt S, Jørgensen BB, Miller DJ, Batzke A, Blake R, et al. 2004. *Science* 306:2216–21

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Lomstein, B et al (2012) *Nature* 484:101–4

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