Biofilm Imaging: From Protein Complexes To Intact Microbial Communities

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Project Goals:

The ENIGMA (Ecosystems and Networks Integrated with Genes and Molecular Assemblies) project aims to elucidate the mechanisms and key processes that enable microorganisms and their communities to function in metal-contaminated soil sites. The ENIGMA Biotechnology Component focuses its efforts on providing cross-cutting technologies that will support all other ENIGMA components with a particular focus on biological imaging at different scales of bacteria and microbial communities. These data will help to develop models of microbial community activity and principles of community organization in an effort to predict the role that microbial species and their interactions play in the dynamics of geochemical transformations in a changing environment.

Abstract:

Microbial physiology is inherently a multiscale biological process that coordinates complex processes such as extracellular metal reduction and response to environmental stresses and competing species. Bacteria often assemble into sustainable communities that allow individual bacteria to coordinate their respective behavior and thus optimizing the efficiency of biological processes, which may enhance the chances for species survival.

ENIGMA is addressing the complexity of multiscale spatiotemporal biofilm organization through a combination of expertise in traditional structural biology and modern multimodal imaging. SAXS (Rambo *et al.* 2010) and single particle cryo-EM are proven technologies to determine protein complex stoichiometry and shape, allowing the fitting of high-resolution structures into the intermediate resolution density envelope (Han *et al.* 2009). Cryo-electron tomography of bacterial whole mount samples can detect intra- and extracelluar specializations e.g. those important for metal reduction. Cryo-EM analysis is complemented by widefield 2D section TEM and advanced 3D SEM imaging approaches (FIB/SEM & SBF/SEM) of cryo-preserved, freeze substituted and resin-embedded samples. With these novel EM imaging approaches, we have begun to examine large areas and volumes of biofilms in DvH and other soil bacteria. We have found outer membrane vesicles, vesicle chains and cell-cell connections (Palsdottir *et al.* 2009, Remis *et al*. 2010, Remis *et al.* submitted), as well as compartmentalization of metal

precipitation (Auer, unpublished observation). These observations suggest a an intricate set of interactions and possibly coordination of function between community members. X-ray and EM-based imaging approaches are complemented by tag-based labeling of proteins both at the light and electron microscopy level, and allow the study of cell-to-cell variations in protein abundance and protein localization (Chabra *et al.* 2010). Advanced optical super-resolution imaging methods (including PALM and STORM) allow high precision localization and counting (Betzig *et al* 2006). Further integration of small molecule mass spectrometry imaging, while at a somewhat larger size scale, promises to link structural observation and protein localization with metabolic activity of biofilm regions. Through the integrated application of these imaging modalities ENIGMA is deconstructing a mechanistic understanding of biofilm function.

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