

## **Correlative High Resolution Imaging and Spectroscopy to Characterize the Structure and Biogeochemical Function of Biofilm EPS**

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We have examined the biomolecular mechanisms of radionuclide transformation by *Shewanella oneidensis* MR-1 and demonstrated that the redox-active proteins involved in these biotransformations are found associated with two sub-cellular locations: the periplasmic space and exterior to the cell outer membrane. These studies have demonstrated the utility of electron microscopy (EM) for studying how microorganisms interact with minerals and soluble contaminants in the environment. Although EM imaging provides excellent high-resolution structural analysis, the amount of chemical information that can be collected during EM analysis is limited to techniques such as energy dispersive spectroscopy (EDS), electron energy loss spectroscopy (EELS) and selected area electron diffraction (SAED). To further our chemical and mechanistic understandings of EM-generated images, the correlated use of synchrotron-based chemical analysis can be employed to investigate the local chemical composition of elements in samples and produce high-sensitivity, element-specific distributions which correspond to EM images. Using a multi-faceted approach of high-resolution EM, synchrotron-based, hard X-ray absorption spectroscopy (XAS), micro X-ray fluorescence ( $\mu$ -XRF) imaging, and high-resolution immuno-EM, we have shown that biogenic extracellular uraninite ( $\text{UO}_{2(s)}$ ) nanoparticles are associated with a complex extracellular polymeric substance (EPS) containing redox-active proteins. We hypothesize that the redox-active proteins in EPS may directly transfer electrons to U(VI). These studies emphasize the need to develop additional high-resolution imaging techniques which can be correlated with synchrotron-based chemical analysis at high spatial resolution.

On going studies in our lab focus on the role of microenvironments in controlling the fate and transport of contaminant migration in the subsurface. We have shown that EPS (the principal extracellular component of biofilms) is a complex organic matrix containing polysaccharide, lipid, protein, DNA and large quantities of water. Understanding how biofilm EPS functions and interacts with inorganic substrates such as metal ions and mineral surfaces connects molecular-scale biogeochemical processes to those at the microorganism-level, and provides insight to how microorganisms influence larger, pore-scale biogeochemical processes. Because of the high water content, the most significant limitation to high-resolution imaging for understanding how hydrated biofilm EPS interacts with minerals and metal ions has been a requirement for drying and/or dehydration of samples before vacuum-based imaging. This process can cause substantial ultrastructural damage to samples due to the constriction of delicate structures such as cellular membranes and EPS and can ultimately disrupt the true interaction with mineral surfaces. To minimize these artifacts while providing high-resolution detail, we have used cryogenic (cryo) sample preparation in which cells are flash-frozen in amorphous (vitrified) ice and imaged under vacuum at cryogenic temperatures. The preparation of frozen-hydrated samples is the emerging method for visualizing biological material in its closest-to-natural, fully hydrated state since these can be generated and visualized entirely in vitreous ice without dehydration steps. In addition, soft X-ray

microscopy techniques are now being applied to fully hydrated biological samples for imaging in their close-to-native state<sup>1,2</sup>. XAS information at small spatial scales (*i.e.*, grain coatings, weather mineral clasts, within biofilms) would be an extremely valuable compliment to these analyses. However, there remains a disconnect between the preservation of delicate hydrated biological samples and additional synchrotron-based spectroscopies to provide chemical and elemental analysis with high spatial resolution on these fragile, hydrated biological samples. Additionally, the *in situ* concentrations uranium in these microenvironments is typically at concentrations too low for good spectroscopy. Hence, there is a need for novel XAS technologies with high spatial resolution, improved sensitivity and the ability to obtain chemical information from hydrated biological samples.

1 McDermott, G., et al. *Trends Cell Biol* **19**, 587-595 (2009).

2 Ogura, T. *Biochem. Biophys. Res. Commun.* **391**, 198-202 (2010).