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Probing the Organic/Inorganic Interface of the Ferritin Protein using Atom Probe Tomography

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The ability to image biointerfaces over nanometer to micrometer length scales is fundamental to correlating biological composition and structure to physiological function, and is aided by a multimodal approach using advanced complementary microscopic and spectroscopic characterization techniques. The regular application of atom probe tomography (APT) to soft biological materials is lacking in large part due to difficulties in specimen preparation and inabilities to yield meaningful tomographic reconstructions that produce atomic scale compositional distributions as no other technique currently can. Here we describe advancement in the atomic-scale tomographic analysis of biological materials using APT that is facilitated by an advanced focused ion beam based approach. A novel specimen preparation strategy is used in the analysis of horse spleen ferritin protein embedded in an organic polymer resin which provides chemical contrast to distinguish the inorganic-organic interface of the ferrihydrite mineral core and protein shell of the ferritin protein, as well as the organic-organic interface between the ferritin protein shell and embedding resin. Given the complex mass spectra that result from the pulsed-laser assisted field evaporation of organic polymer compounds using APT [1], the detection of multiple species having overlapping mass/charge ratio (m/z) can complicate or prevent a definitive identification of the nitrogen and iron species of interest. A systematic analysis of the pure resin at varying laser energies indicates that at 450 pJ laser energy, the contribution of organic species at 14 Da (i.e. CH_2^+) is eliminated to allow detection of N^+ from resin specimens containing the ferritin protein. Individual ferritin cores surrounded by carbon are observed in the ferritin embedded resin specimen (Fig. 1a). Using proximity histogram analysis, we are able to map the relative composition profiles of Fe, C, N, and P. Results are corroborated with the analysis of similarly prepared specimens of Fe_2O_3 nanoparticles embedded in the same resin. The results demonstrate a viable application of APT analysis to study the complex biological organic/inorganic interfaces with an extension of the specimen preparation technique to further enhance the study of organic and inorganic nanomaterials relevant to energy and the environment. In addition, progress in the analysis of cryo-prepared specimens will be discussed.

References:

[1] TJ Prosa, SK Keeney & TF Kelly, J. Microsc. **237**(2010), 155-167.
[2] The research was performed at the Environmental Molecular Sciences Laboratory; a national scientific user facility sponsored by the Department of Energy's Office of Biological and Environmental Research located at Pacific Northwest National Laboratory, and was supported by the Chemical Imaging Initiative Laboratory Research & Development program.

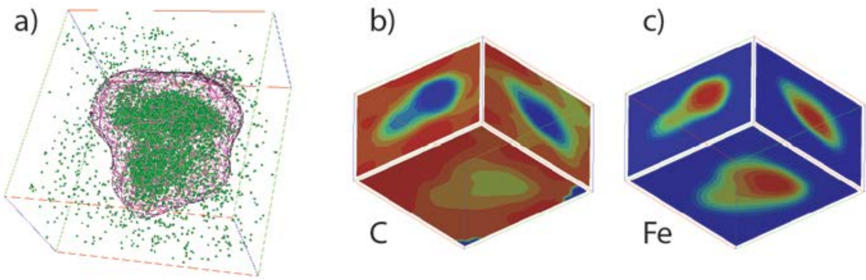

Figure 1: a) 3D compositional map of Fe from the iron-rich ferrihydrite mineral core of a ferritin molecule. Purple mesh surface is a 15% Fe isoconcentration surface. 2D composition maps of b) carbon and c) iron.