Metallomics: A Multidisciplinary Approach for the Measurement of Metalloproteins for Health and Disease Markers

The emerging science of "metallomics" requires versatile measurement tools that allow for trace detection, identification, and quantitation of covalently bound metals in proteins or metal-protein complexes. NIST is pioneering advanced metalloprotein measurement capabilities involving protein separation by multidimensional liquid chromatography and/or 2D gel electrophoresis interfaced to inductively coupled plasma mass spectrometry (ICP-MS). New technology is expected to enable innovation of new ways to monitor health status and diagnose diseases.

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There have been estimates that over 30% of known proteins require metal cofactors for proper functionality. Since their discovery, metalloproteins have been the focus of research in biology and medicine because of their various functions in connection with protein folding and neurodegenerative diseases, metalloenzyme activity, and detoxification through transport and storage mechanisms. For example, quantitative determination of Zn, Cu, and Fe in brain proteins is of interest for studying Alzheimer's and Parkinson's diseases.

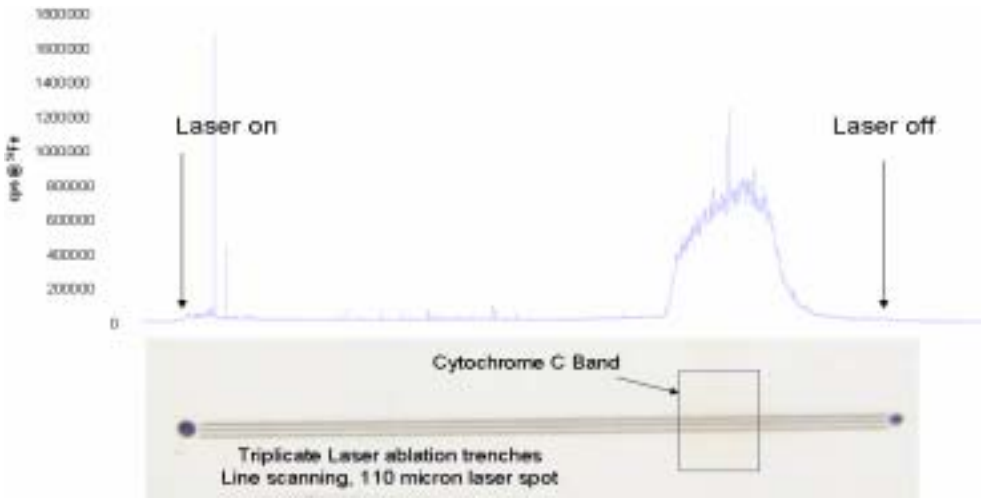
It is difficult to identify and quantify a metalloprotein in a complex biological matrix with a single analytical technique. The isolation, quantitation, and structural determination of a metalloprotein requires the use of numerous complementary biological processing, instrumental separation, detection technologies. Protein separation by multidimensional liquid chromatography and/or 2D gel electrophoresis interfaced to inductively coupled plasma mass spectrometry (ICP-MS) may provide for rapid, metal-selective screening of target proteins, their subsequent quantitation at low concentration levels, and finally, structure elucidation with the assistance of organic mass spectrometry.

NIST researchers are working to combine multidimensional separation techniques with mass spectrometry to provide metal-selective screening, quantitation, and structural information for metalloproteins.

We are investigating the use of laser ablation (LA) ICP-MS for the multi-elemental screening of separated proteins on polyacrylamide gels. The analysis is relatively rapid, allowing mapping of whole gels in less than two hours; is amenable to multi-element data collection including the physiologically important elements P and S; and affords detection limits for important trace elements like Cu and Zn at the nanogram of protein level. This technique is useful in identifying areas of elemental localization as a precursor to more in-depth studies using organic mass spectrometry.

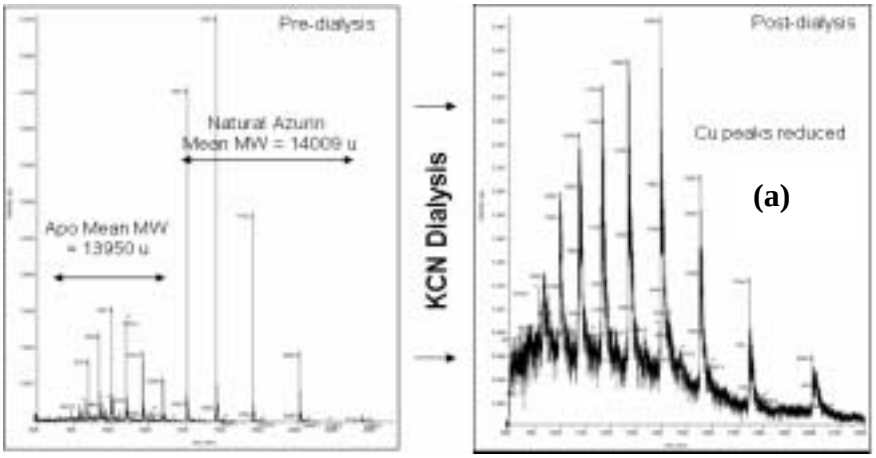
To date our research has concentrated on development of methods for separation of proteins and their subsequent transfer onto polyvinylidene fluoride (PVDF) membranes that are amenable to LA-ICP-MS (see example in Figure 1), and assessment of metal response as a function of metalloprotein concentration.

Figure 1. ***Laser ablation trenches and corresponding ICPMS $^{56}Fe^{+}$ response across a cytochrome c protein band separated by SDS-PAGE and transferred to PVDF.***



We are developing new methods for quantitation of metalloproteins by measuring the associated metal. To this end, proof-of-principle experiments have been performed to synthesize isotopic metal analogs of metalloproteins through dialytic exchange and employ them as calibrants. This requires monitoring the exchange of the metal using both electrospray and ICP mass spectrometry (Figure 2a and 2b).

Impact:
Development of qualitative and quantitative tools for metalloprotein metrology will help benchmark measurements in biological systems and identify new classes of quantifiable biomarkers.



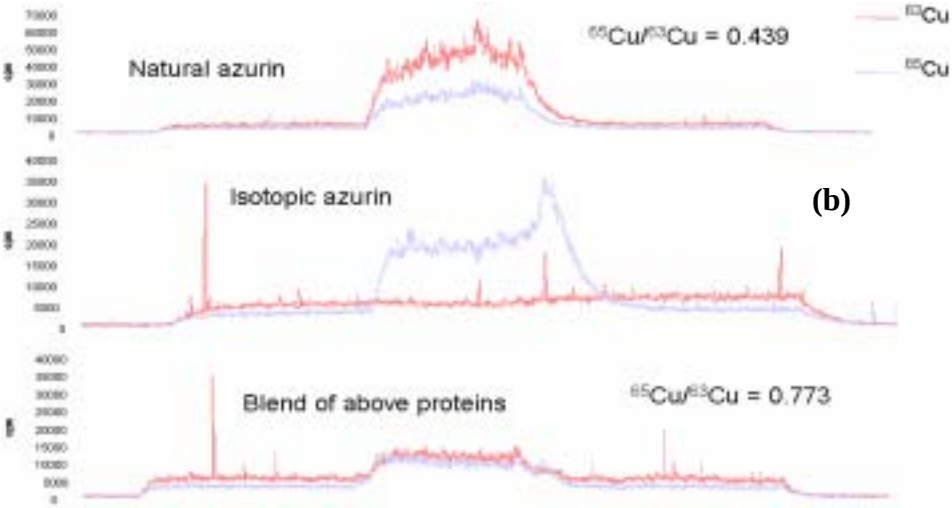


Figure 2.
(a) Monitoring the extraction of natural copper from Azurin to form the apoprotein.

(b) LA-ICP-MS isotopic tracking of Cu isotopes in a mixture of natural and isotopic Azurin; protein bands are blotted onto a PVDF membrane.