Identifying Mechanisms of Toxic Metal Stress with Global Proteomics

AO Summers[1], S LaVoie[1], L Olliff[1], MK Johnson[2], RA Scott[2], SM Miller[3], B Polacco[3], MS Lipton[4], SO Purvine[4], & EM Zink[4]. [1]Microbiology, Chemistry[2] UGA, [3]Pharm. Chem., UCSF, [4]EMSL-PNNL

$MeHg^+$ and Hg^{2+} directly inactivate proteins by binding to their cysteine or selenocysteine residues. As previously reported (Polacco, et al. MCP, 2011), we have devised a high-throughput global proteomics method using the 7 stable isotopes of Hg to identify proteins of *E.coli* most vulnerable to forming stable adducts of phenylmercury ($PhHg^+$; a proxy for methylmercury). Analysis of the dataset of ~1.62 million total observed MS2 spectra continues and here we report establishment of a MySQL database for these data, an overview of the biological findings, independent confirmation of the stability of Hg-peptide adducts under the LC-MS/MS conditions used, and initial work extending this method to the Hg^{2+}-methylating bacterium, *Desulfovibrio* ND132.

Hg EXPOSOME DATABASE: Unlike transcriptomic's MIAME standards, there is no agreed protocol for storage, sharing, and reporting of basic proteomics data, much less those with adducts. So, using the relational database freeware, MySQL, we built our own repository and analytical tools. Housed on a dedicated server at the University of Georgia, the database records every experimental aspect from cell culture thru spectra collection to identification and quality scoring with three widely used algorithms. It is available to all members of our collaboration by password.

OVERVIEW OF BIOLOGICAL FINDINGS: From three complete biological replicates (developed via five full scale pilot experiments) we identified by two or more peptides 1301 of *E.coli* MG1655's 4249 encoded proteins (WISC_ASAP); an additional 261 proteins were identified by multiple observations of a single high quality peptide for a total of 1562 rigorously identified proteins (37% of total). There are 3654 cysteine-containing proteins encoded by MG1655 (86% of total); they vary widely in abundance and conditions for expression. We observed 303 cysteine-containing proteins modified by Hg (19.4 % of identified proteins) and 85% of these have at least one cysteine that is highly modified (Def: >50% of observations have Hg-modification). These Hg-vulnerable proteins represent all metabolic functional groups, and especially energy generation, translation and amino acid biosynthesis (Zink et al, in preparation). Bulk cell properties altered by PhHg or Hg exposure include thiol homeostasis, electrolyte balance, and free iron (LaVoie et al, in preparation).

STABILITY OF Hg-PEPTIDES TO PROTEOMICS: The 4-fold lower recovery of cysteine containing proteins than expected has several possible physiological causes, including their differential occurrence under the aerobic growth conditions used and natural differential abundance. These can be resolved via ontological analysis underway; although absolute abundance is unknown for many proteins, it can be inferred from their functional class. Possible technical factors include differential stability to pre-column procedures, column conditions, and MS conditions. We're using our database to examine cysteine peptide/protein yield variation related to pre-MS variables. Experiments with pure Hg-adduct peptides show they are stable to the MS conditions.

Hg EXPOSOME OF A METHYLATING BACTERIUM: To identify proteins involved in Hg^{2+} uptake and methylation we've first grown *Desulfovibrio* ND132 facultatively without Hg^{2+} to test the efficacy of our modified proteomics method on it. The results (~1350 identified proteins of 3455 encoded) were slightly less than work on pyruvate or fumarate grown *Desulfovibrio* G20 (1900 identified proteins of 3258 encoded). Optimization of growth conditions for maximum methylation is underway and, since this project requires exposure to bivalent Hg^{2+}, we are adapting our Hg-proteomics method to identify the distinct peptides of pairs of Hg-crosslinked peptides. This work will also uncover cysteine peptides vulnerable to being missed because they are crosslinked.