ABSTRACT: Methylmercury (MeHg) and inorganic Hg directly inactivate proteins by binding to their cysteine or selenocysteine residues. As recently 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) and are now also using this proteomics method to identify proteins involved in Hg methylation by Desulfovibrio ND132. The E.coli dataset of ~1.62 million total observed MS2 spectra from three complete biological replicates identified 1562 of E.coli MG1655's 4249 encoded proteins (37% of total) and 303 of these, in a wide variety of functional groups, had stable cysteine-Hg adducts (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).

PROTEOMIC LABEL-FREE QUANTIFICATION OF Hg PEPTIDES – Quantification of unique modified peptides can afford comparison of their relative detectability as a function of different iodoacetamide (IAM) treatments after phenylmercury acetate (PMA) exposure. We devised an indirect and a direct method for quantifying peptides modified by PMA. First, since existing signal collation methods are challenged by the 7 stable isotopes of Hg, we simply used current label-free AMT methods to quantify indirectly the PMA-provoked decline in iodoacetylation modifications (CAM-mods) of 428 robustly observed peptide cysteines. PMA-exposure decreased CAM-mods in essentially all detectable cysteines by ~5-fold (average). This PMA-provoked decrease per-cysteine was sufficiently reproducible to allow ranking each cysteine for likelihood of PhHg modification. Our second approach to quantifying Hg-modified peptide cysteines was achieved by a novel computational breakthrough in LC-MS feature detection allowing use of the broad isotope envelopes of Hg peptides and, thereby, the direct quantification of 369 Hg-modified peptide cysteines. With this latter tool we found Hg-modified peptides decreased in cells treated with 20 mM IAM but not with 10 mM IAM (vs. no IAM treatment) but in this case the decrease was only 1.7-fold, a difference too small to rank cysteines as more or less likely to suffer an Hg-adduct. While it is not yet clear why the indirect metric of CAM-mods per peptide cysteine display a wider range than the direct measure of the Hg-mods themselves, this latter new method will greatly expedite robust direct assessment of the modifiability of individual peptide cysteines.

STABILITY OF Hg-PEPTIDES TO PROTEOMICS: We find the abundance of Hg-modifiable peptides is lower than their unmodified form and the same is true of the Hg-modified tripeptide, glutathione (GSH). Possible technical factors include differential stability to pre-column procedures, column conditions, and MS conditions. Initial experiments with a pure Hg-adduct peptide showed it was stable to a broad range of MS inlet temperature and voltage conditions. We are extending this analysis to a more hydrophobic peptide and additional work with Hg- and CAM-modified GSH and lysozyme.

Hg EXPOSOME OF A METHYLATING BACTERIUM: To identify proteins involved in Hg²⁺ uptake and methylation we have first done a total proteome on Desulfovibrio ND132 growing facultatively without Hg²⁺ to test the efficacy of our modified proteomics method on it. We saw 3008 proteins of 3455 encoded (87%; based on 66,121 distinct peptides) considerably more than reported on pyruvate or fumarate grown Desulfovibrio G20 (1900 identified proteins of 3258 encoded), likely due to better recovery of cysteine-containing peptides with IAM treatment. Indeed, we observed 1851 cysteine-
containing proteins of 3071 encoded (60%). Preliminary analysis of an Hg-exposed ND132 proteome done with and without IAM treatment yielded 28,622 distinct peptides corresponding to just 2,535 proteins. Of these, 3,921 distinct peptides were modified with CAM and 308 with Hg. Differences with respect to the no-Hg ND132 proteome and with respect to the *E.coli* PMA-exposure proteome will be discussed. In related work, a double mutant in the genes (*hgcAB*) encoding the newly discovered Hg(II) transmethylase is only slightly less resistant to Hg(II) than its parent wild-type strain, showing definitively that the ability to methylate Hg does not enhance resistance to Hg, as had been earlier suggested by work with unrelated methylating and non-methylating field isolates of *Desulfovibrio*. 