ESRF	**Experiment title:** HERFD of Hg(II) coordination in bacterial samples	**Experiment number**: 16-01-787
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Report:

Anaerobic bacteria possessing the *hgcAB* gene cluster can transform Hg(II) into the potent neurotoxin methylmercury (MeHg)[1] and are the primary source of MeHg in the environment.[2] For Hg methylation to occur, Hg(II) must be internalized by the cell and hence interact with the cell surface. However, the uptake pathway and internalized Hg(II) species have yet to be discovered. To gain insight into Hg(II) uptake in Hg-methylating bacteria, our experiment (proposal reference number 77688) employed high energy resolution fluoresence detection (HERFD) to determine the coordination environment of Hg(II) in an actively Hg-methylating bacterium – *Geobacter sulfurreducens*. We exposed *G. sulfurreducens* to low concentrations of total Hg to mimic the conditions in Hg methylation assays.

Experiment: *Geobacter sulfurreducens* PCA was harvested in exponential growth phase and exposed to varying concentrations of total Hg(II) for 2 hours. Afterwards, the cells were washed 2 times in 0.1 M $NaClO_4$, collected on 0.2 μm cellulose nitrate filter paper, and sandwiched between 2 pieces of Kapton tape. All samples were plunged in liquid nitrogen and remained frozen throughout analysis. The samples were prepared at the home institute and shipped to the ESRF on dry ice. All samples were measured in HERFD mode with 7 spherically bent Si crystal analyzers (bending radius = 1 m, crystal diameter = 0.1 m). The Hg $L_{\alpha 1}$ fluoresence line was selected using the 555 reflection, and the diffracted fluorescence was measured with a silicon drift detector (SDD, Vortex EX-90). Data normalization and processing were performed with Athena.[3]

Results: Our previous HR-XANES results collected on *E. coli* that were exposed to 50 nM and 500 nM Hg(II) revealed mixtures of Hg(II) coordination environments associated with the bacterial cells (experiment number 30-02-1118). The Hg species included 2-coordinate

Hg-thiolate species (i.e., $Hg(SR)_2$), β-HgS-like species, and α-HgS-like species. *E. coli* exposed to higher Hg concentrations as well as cysteine, which is known to biodegrade into sulfide,[4] contained more β-HgS-like species at the expense of $Hg(SR)_2$. Surprisingly in this experiment on a Hg-methyalting organsim, the speciation of Hg(II) associated with the bacterial cells did not significantly change with total added Hg concentration (Figure 1). Each spectrum for *G. sulfurreducens* expsoed to 50 nM, 100 nM, and 200 nM Hg(II) contain a peak in the absorption edge indicative of Hg(II) coordinated to 2 thiols (Figure 1). When considering the standard deviation of each spectrum, the slight differences in peak height are insignificant.

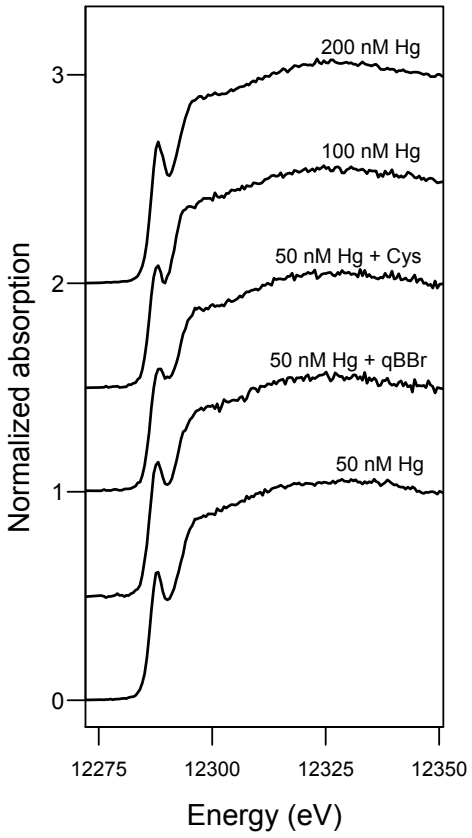

Figure 1: HR-XANES of actively methylating *G. sulfurreducens* exposed 50, 100, and 200 nM Hg with and without 100 µM cysteine (Cys) and 50 µM qBBr measured at the Hg L_{III}-edge.

In addition, we exposed cells to monobromo(trimethylammonio)bimane (qBBr), which is a compound that binds thiols via an S_N2 reaction. The newly formed C-S bond is not broken upon the addition of Hg(II). HR-XANES show that the addition of qBBr and consequent blockage of ~ 60% of the reactive cell surface thiols had no effect on Hg(II) coordination in the cell (Figure 1). In addition, we exposed *G. sulfurreducens* to 50 nM Hg(II) and 100 µM cysteine (Cys), conditions known to greatly enhance the amount of MeHg production.[5] With linear combination fitting, we determined that the spectrum can be decomposed into ~40% of a $Hg(SR)_2$ reference and ~60% of a β-$HgS_{(s)}$ reference. The relationship between the presence of β-HgS-like species in the cell and enhanced methylmercury production should be explored further.

References:

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3. Ravel, B.; Newville, M., Athena, Artemis, Hephaestus: Data analysis for X-ray absorption spectroscopy using IFEFFIT. *Journal of Synchrotron Radiation* **2005,** *12*, 537-541.
4. Thomas, S. A.; Gaillard, J.-F., Cysteine addition promotes sulfide production and 4-fold Hg(II)–S coordination in actively metabolizing *Escherichia coli*. *Environmental Science & Technology* **2017,** *51*, (8), 4642-4651.
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