Title: Molecular Mechanisms of Bacterial Mercury Transformation

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Recipient Organization: University of California, San Francisco

DOE Award No: DE-SC0004919 (also denoted ER65063), Project ID: 0016628; (This award was made to the UCSF PI Miller as a collaboration with overall PI of the project Jeremy Smith from the University of Tennessee whose award # is ER65062.)

Researchers working on project at UCSF:

Name	Position	Current position
Susan Miller	Professor	Professor
Rachel Nauss	Staff Research Associate	same
Russell Goodman	Graduate Student	Graduated with MS
Gurnimrat Sidhu	Summer student intern	Peace Corps

Executive Summary

The bacterial mercury resistance (*mer*) operon functions in Hg biogeochemistry and bioremediation by converting reactive inorganic Hg(II) and organic $[RHg(II)]^{1+}$ mercurials to relatively inert monoatomic mercury vapor, Hg(0). Its genes regulate operon expression (MerR, MerD, MerOP), import Hg(II) (MerT, MerP, and MerC), and demethylate (MerB) and reduce (MerA) mercurials. We focus on how these components interact with each other and with the host cell to allow cells to survive and detoxify Hg compounds. Understanding how this ubiquitous detoxification system fits into the biology and ecology of its bacterial host is essential to guide interventions that support and enhance Hg remediation. In the current overall project we focused on two aspects of this system: *(1)* investigations of the energetics of Hg(II)-ligand binding interactions, and *(2)* both experimental and computational approaches to investigating the molecular mechanisms of Hg(II) acquisition by MerA and intramolecular transfer of Hg(II) prior to reduction within the MerA enzyme active site. Computational work was led by Prof. Jeremy Smith and took place at the University of Tennessee, while experimental work on MerA was led by Prof. Susan Miller and took place at the University of California San Francisco.

Accomplishments

Contributions to computational work at UT

Energetics of Hg(II)-ligand binding interactions – PI Miller contributed significantly to the discussion and interpretation of computational results on the energetics of Hg(II)-ligand

interactions, providing context from an experimentalist's perspective, and also contributed significantly to writing the accepted manuscript on the work:

Riccardi D, Guo H-B, Parks JM, Gu B, Summers AO, Miller SM, Liang L, Smith JC. (2013) Why mercury prefers soft ligands. *J Phys Chem Lett* 4, 2317-2322.

Molecular mechanisms of Hg(II) transfers and reduction in MerA – As the leading expert on structural and mechanistic studies of the enzyme mercuric ion reductase (MerA), PI Miller proposed mechanistic hypotheses based on both published and unpublished experimental data (from the Miller lab) to be tested in the computational studies. The major computational effort focused on evaluation of the pathway for Hg(II) transfer from the C-terminal pair of cysteines, which form the entry point for Hg(II) onto the MerA protein, to the inner pair of cysteines that form the binding site for Hg(II) where it undergoes reduction by electrons transferred through the flavin cofactor from the co-substrate NADPH. Mechanistic variations for the Hg(II) transfers were postulated on the basis of the experimentally established roles for the active site cysteines and the presence of conserved amino acid residues that lie in the vicinity of the Hg-binding cysteines. A lowest energy pathway has been identified and analyzed in the context of X-ray structural information obtained on a Hg(II)-complex of the protein obtained by Miller and Pai under another DOE award (ER63087). The manuscript on this work has been submitted for review:

Lian P, Guo H-B, Riccardi D, Dong, A, Parks JM, Xu Q, Pai, EF, Miller SM, Wei D-Q, Smith JC, Guo H. X-ray Structure of a Hg^{2+} Complex of Mercuric Reductase and QM/MM Study of Hg^{2+} Transfer between the C-terminal and Buried Catalytic Site Cysteine Pairs. *Submitted*.

Experimental work in the Miller lab

NSE contribution – A portion of the funds from this grant supported efforts in the Miller lab to produce a special construct of the full-length MerA enzyme that was used in a separately-funded combined experimental and computational study using Neutron Spin Echo and Small Angle Neutron Scattering experiments and both coarse-grained and atomic scale molecular dynamics computational methods to evaluate the dynamic behavior of the NmerA domains in full length MerA. A manuscript on this work is under revision.

Role of Glu446 in Hg(II) transfer pathway in MerA – Tyrosine 100 (Tyr100) and glutamate 446 (Glu446) (numbering of Tn501 MerA Core construct) are two completely conserved residues that lie deep in the Hg(II) binding cavity in positions where they could potentially participate as acid/base catalysts in proton transfers to and from the various cysteine thiolates and thiols as they dissociate from and attack Hg(II) during the successive transfer steps. Mutation of Tyr100 to phenylalanine has previously been shown to dramatically lower the rate of Hg(II) reduction, and has been evaluated in the computational studies as a participant in the mechanism. As mutation of Glu446 had not previously been examined, the Miller lab performed experimental studies to examine the effects of mutation of Glu446 to Glutamine (Gln) on several aspects of MerA catalysis including (1) effects on the steady-state kinetic behavior with $Hg(S\text{-glutathione})_2$ ($Hg(SG)_2$) as substrate in the presence of varied glutathione, (2) effects of the rate of Hg(II) transfer from $Hg(SG)_2$ to the C-terminal cysteines in the absence of free GSH, and (3) effects on apparent pK_a values of residues in the active site. A manuscript on this work is in preparation. (R Goodman, G Sidhu, SM Miller).

Posters, Seminars, Publications

RC Goodman, R Soinski, SM Miller. Thermodynamics and Kinetics of a Flexible C-terminal Tail are Modulated by an Electrostatic Interaction. Poster Presentation at the Joint Chemistry & Chemical Biology and Integrated Program in Quantitative Biology Graduate Program Symposium, Monterey, CA (2012)

RC Goodman, JS Mugridge, R Soinski, SM Miller. Investigation of the Structural Features Controlling the Kinetics and Thermodynamics of the Mercuric Ion Reductase Carboxy-Terminal Tail. Poster Presentation at the Biophysics Graduate Program Symposium (June 2013)

RC Goodman, JS Mugridge, R Soinski, SM Miller. Investigation of the Structural Features Controlling the Kinetics and Thermodynamics of the Mercuric Ion Reductase Carboxy-Terminal Tail. Poster Presentation at the Gordon Research Conference on Enzymes, Coenzymes and Metabolic Pathways (July 2013)

SM Miller. Investigation of the Structural Features Controlling the Kinetics and Thermodynamics of the Mercuric Ion Reductase Carboxy-Terminal Tail. Invited talk at the upcoming EMBO Enzyme Mechanisms by Biological Systems Conference (June 2014)

Riccardi D, Guo H-B, Parks JM, Gu B, Summers AO, Miller SM, Liang L, Smith JC. (2013) Why mercury prefers soft ligands. *J Phys Chem Lett* 4, 2317-2322.

Lian P, Guo H-B, Riccardi D, Dong, A, Parks JM, Xu Q, Pai, EF, Miller SM, Wei D-Q, Smith JC, Guo H. X-ray Structure of a Hg^{2+} Complex of Mercuric Reductase and QM/MM Study of Hg^{2+} Transfer between the C-terminal and Buried Catalytic Site Cysteine Pairs. *Submitted*.