Mercury occurs largely as elemental Hg^0 (a liquid or monoatomic vapor) or the oxidized, mercuric ion, $Hg^{2+,}$ with both forms undergoing redox changes in biotic and abiotic processes. Relatively unreactive, Hg^0 is oxidized to $Hg^{2+,}$ which is highly reactive, neurotoxic, nephrotoxic, hepatotoxic and immunotoxic. In addition, Hg^{2+} is the most immediate substrate for microbiological synthesis of neurotoxic monomethylmercury. Consequently, the World Health Organization recommends phasing out all use of mercury and sequestering existing stocks, although existing anthropogenic dissemination of thousands of metric tonnes of Hg and its natural release from volcanoes and deep sea vents will continue to impact terrestrial and oceanic biota.

Quantification of the factors underlying differences in the affinities of diverse partners for Hg^{2+} is central to understanding biotic and abiotic mercury trafficking in the environment. Metal ligand binding affinities are qualitatively rationalized in terms of hard and soft acids and bases (HSAB) theory. From HSAB theory, the relative affinities of metals for different ligands follows the general pattern of hard metals interacting more strongly with hard ligands and soft metals with soft ligands.

To furnish a theoretical basis for Hg^{2+} speciation, we used quantum chemistry to calculate the aqueous binding free energy for a series of environmentally relevant chalcogenide and halide ligands[1]. We systematically investigated hydration effects using gas phase calculations and a cluster- continuum model that that has been extensively validated for group 12 divalent cations and anions.

The experimental aqueous phase binding affinity trend of $Hg(SH)_2 > Hg(OH)_2 > HgBr_2 \simeq Hg(OH)Cl > HgCl_2$ was correctly captured by the calculations. After adding as few as two water molecules to each gas-phase species, the binding affinity switches from favoring $Hg(OH)_2$ to favoring $Hg(SH)_2$. The driver of this switch is the much larger interaction energy between OH^- and the local H_2O molecules compared to that of SH^-.

Understanding the factors that drive mercury speciation is critical for alleviating its damaging effects on the environment and on human health. We provided for the first time the quantitative physicochemical underpinnings of the binding free energy differences between environmentally-relevant mercury complexes. Our examination of the experimental binding and hydration free energies revealed a surprising increase in local interaction strength between Hg^{2+} and two negatively charged ions with increasing hardness of the ions. We verified this observation using quantum chemical calculations and showed how differences in proton affinity and interactions with a very small number of local water molecules recover the experimentally well-known increasing affinity for softer ligands consistent with HSAB concepts. Analysis of the change in binding free energy from binding one ligand to two ligands allowed the source of the phase dependence to be identified as large differences in reactivity of the HgL^+ complexes.

The analytical framework and systematic treatment of hydration provides a molecular explanation of the experimentally-observed, robust preference of Hg^{2+} for soft ligands such as thiols. This framework is applicable to interactions of organic mercurials and of

other metal cations with inorganic and organic anionic ligands, including those of cysteine and selenocysteine essential to living organisms.

Mercuric reductase, MerA, is a key enzyme in bacterial mercury resistance. This homodimeric enzyme captures and reduces toxic Hg^{2+} to Hg^{0}, which is relatively unreactive and can exit the cell passively. Prior to reduction the Hg^{2+} is transferred from a pair of cysteines (C558' and C559' using Tn*501* numbering) at the C-terminus of one monomer to another pair of cysteines (C136 and C141) in the catalytic site of the other monomer. In the second phase of the research we presented the X-ray structure of the C-terminal Hg^{2+} complex of the C136A/C141A double mutant of the Tn*501* MerA catalytic core, and explored the molecular mechanism of this Hg transfer with a quantum mechanical/molecular mechanical (QM/MM) simulation approach [2].

Metal ions play important functional roles in biological systems, but can also be significant environmental pollutants. A detailed understanding of the mechanisms of speciation and transfer of heavy metals in biological and environmental systems is thus of both fundamental and practical interest. Among those chemical mechanisms involving heavy metals, of particular biological importance are ion transfers in proteins.[1]

Some microorganisms are able to overcome high concentrations of toxic heavy metals, and can directly biotransform contaminants to innocuous or immobile forms. A key example is mercury resistance in bacteria conferred by the *mer* operon, which encodes a suite of proteins that carry out the transport and reduction of Hg^{2+} to transform this toxic ion into less toxic, elemental Hg^{0}. *Mer* loci have been discovered in many different species, underscoring the ubiquitous nature of this mode of mercury detoxification among bacterial communities.

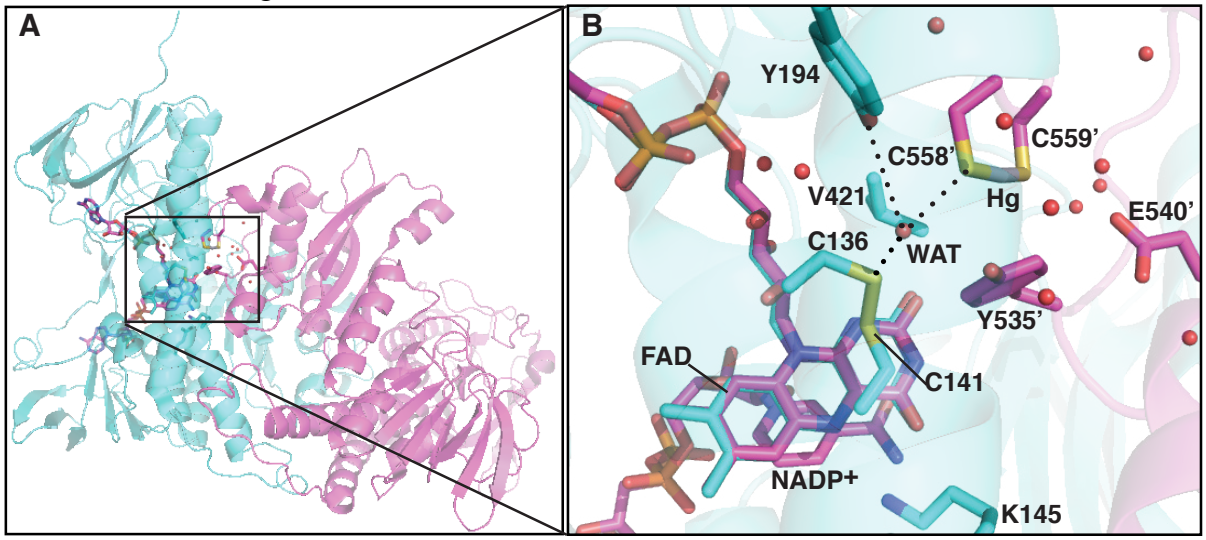

Figure 1. Panel A: Structure of the MerA homodimeric catalytic core with monomer chains in cyan and magenta. Panel B: Close-up of the interfacial active site. FAD, $NADP^{+}$, and residues with cyan carbons are from the left monomer. Amino acid residues with magenta carbons are from the right monomer. Several ordered water molecules are shown as red spheres. Structure

was constructed from overlay of the AACC[†] Hg^{2+}/$NADP^+$ complex (PDB 4K7Z, magenta) and the oxidized wild type (CCCC) enzyme (PDB 1ZK7, cyan) with only one chain from each shown for clarity. FAD molecules from the left monomer in both structures are shown to highlight the close alignment of the two structures. Heteroatoms are shown in standard CPK colors.

One of the key enzymes in the *mer* system is mercuric reductase, MerA, which catalyzes the reduction of Hg^{2+} to Hg^0 within the bacterial cytoplasm. The active form of MerA is a homodimer in which the two active sites within the catalytic core domain are found at the dimer interface and comprise residues from both monomers (Fig.1). To compete with other cellular thiols for its Hg^{2+} substrate, each chain of the MerA dimer contains three pairs of strictly conserved cysteines that serve as binding sites for Hg^{2+} transfer and provide a platform for Hg^{2+} reduction. NmerA binds and delivers Hg^{2+} to the C-terminal cysteine pair, C558' and C559', of the other monomer near the surface of the MerA dimer interface. After Hg^{2+} binds to the C558'/C559' cysteine pair, the flexible C-terminal tail must change conformations to move the complex from the surface to the protein interior where Hg^{2+} is transferred to the active site cysteine pair, C136 and C141, located adjacent to the isoalloxazine ring of the flavin adenine dinucleotide (FAD) cofactor (Fig. 1B). From the opposite face of FAD, the other substrate, dihydronicotinamide adenine dinucleotide phosphate (NADPH), transfers hydride to FAD, yielding the two-electron reduced $FADH^-$ and oxidized $NADP^+$. Subsequently, $FADH^-$ reduces the C141-S-Hg(II)-S-C136 complex to yield Hg^0.

How the enzyme catalyzes these efficient transfers is one of the fundamental questions of interest in understanding the overall catalytic mechanism of MerA.
To gain more insight into intramolecular Hg^{2+} transfer in MerA, we determined the X-ray structure of the C-terminal Hg^{2+} complex of a double active-site mutant of the Tn*501* MerA core and performed DFT-based QM/MM simulations to explore Hg^{2+} transfer pathways from the C-terminal cysteine pair to the active site. Based on the mechanistic principles noted above and the X-ray structure (Fig. 1), we proposed a potential pathway for Hg^{2+} transfer in the MerA core and determine the corresponding potential energy profile. The results from the calculations provide a detailed picture of Hg^{2+} transfer likely to be of general applicability in biological systems.

The overall QM/MM MEP is shown in Fig. 2, and the geometries and energetics of each step of the path are described in detail below. We then analyze the energetics in terms of the overall contributions from the enzyme with comparisons with gas-phase and polarizable continuum calculations.

The Hg^{2+} transfer is modeled with a QM/MM MEP calculated using four distinct steps that capture representative configurations of the system during the Hg^{2+} transfer (Scheme 2). The net result of the pathway is that, as the Hg^{2+} is transferred from the C-terminal cysteine pair (C558':C559') to the active site cysteine pair (C136:C141), a proton is transferred in the opposite direction and a negative charge is transferred over a distance of ~7.5 Å, from the interior cysteine C141 to C558'. The presence of a C558' thiolate anion in the PS complex is consistent with the observation of an apparent pK_a of

[†] Abbreviations: CCCC, wild type MerA; CCAA, Cys136 Cys141 Ala558 Ala559 Tn*501* MerA; AACC, Ala136 Ala141 Cys558 Cys559 Tn*501* MerA; NADPH, dihydronicotinamide adenine dinucleotide phosphate; FAD, flavin adenine dinucleotide

~6.5 for C558' in the oxidized enzyme in which C141 and C136 form a neutral disulfide similar to the neutral C141-Hg-C136 complex.[12]

In this reaction path, as the Hg^{2+} is transferred inward the proton is shuttled outward from the active site through the ordered water molecule (WAT) observed in X-ray crystal structures of the wild type (PDB 1ZK7) and the AACC mutant (PDB 4K7Z) used for the simulation. Specifically, the proton is transferred from C136 to C558' (through WAT) and then from C558' to C559', each proton transfer being used to drive the transfer of Hg^{2+} toward the active site by destabilizing the interactions between the targeted thiolate and Hg^{2+}.

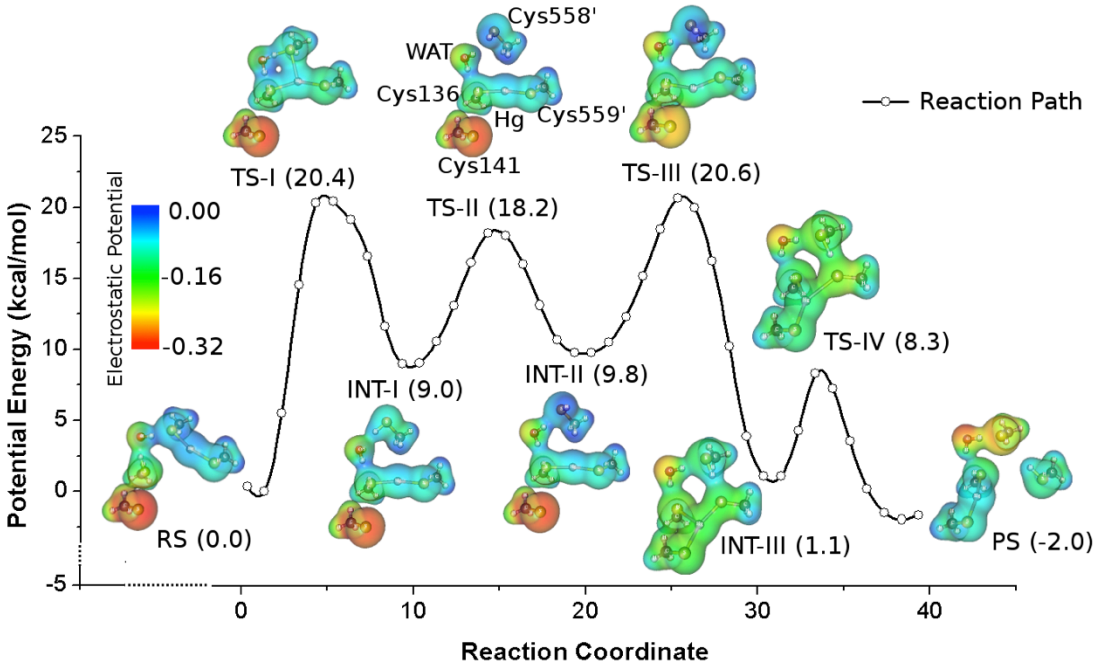

Figure 2. QM/MM minimum energy pathway and charge distributions at the stationary points. At each step, the electrostatic potential is mapped onto the electron density isosurface. Energies (relative to RS) are shown in parentheses.

Building on our previous studies detailing the modes of transfer of Hg^{2+} between the N-terminal domain (NmerA) and the C-terminal cysteine pair (C588' and C589') of MerA, the X-ray structure and QM/MM calculations presented provide molecular-scale insight into how Hg^{2+} is transferred from the C-terminal cysteines into the MerA core for reduction to Hg^0. The transfer was found to be nearly thermoneutral and to pass through a stable tri-coordinated intermediate that is marginally less stable than the two end states. For the overall process, Hg^{2+} is always paired with at least two thiolates, and thus is present at both the C-terminal and catalytic binding sites as a neutral complex. Prior to Hg^{2+} transfer, C141 is negatively charged. As Hg^{2+} is transferred into the catalytic site, a

proton is transferred from C136 to C559' while C558' becomes negatively charged, resulting in the net transfer of a negative charge over a distance of ~7.5 Å. Thus, the transport of this soft divalent cation is made energetically feasible by pairing a competition between multiple Cys thiolates for Hg^{2+} with a competition between the Hg^{2+} and protons for the thiolates.

MerA orchestrates the transport of this soft divalent cation by pairing a competition between Cys thiolates and Hg^{2+} with a corresponding competition between Hg^{2+} and protons for the Cys thiolates. From our analysis of the underlying quantum mechanical energetics of computed reaction pathways, we found that MerA makes the Hg^{2+} transfers feasible by electrostatic stabilization of the reactant and product states relative to the intermediates, preventing the system from being trapped in deep potential wells. These findings are of general relevance for understanding the mechanisms of heavy metal trafficking in biological systems.

1. D. RICCARDI, H-B. GUO, J.M.PARKS, B.GU, A. O. SUMMERS, S. MILLER, L. LIANG & J.C. SMITH. Why Mercury Prefers Soft Ligands. **Journal of Physical Chemistry Letters**, 4 (14), 2317–2322 (2013)
2. P. LIAN, H-B. GUO, D. RICCARDI, A. DONG, J. M. PARKS, Q. XU, E. F. PAI, S. M. MILLER, D.-Q. WEI, J. C. SMITH, & H. GUO. 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 to **Structure.**