Molecular Structure and Dynamics of Mercury Biotransformations

ORNL SFA (Laboratory Research Manager: Liyuan Liang)

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As an integral part of the ORNL SFA, this task investigates subcellular processes, including mechanisms of mercury trafficking, mercury-ligand interactions as well as enzyme-catalyzed reactions involved in bacterial mercury resistance and methylation. The initial focus has been on the biomolecular structure and function of several key proteins and enzymes that confer mercury resistance in bacteria and impact mercury speciation and bioavailability in the environment.

The expression of mercury resistance genes in the *mer* operon is controlled by the metalloregulator MerR at the level of transcription. Prior *in vivo* and *in vitro* biochemical work showed that Hg(II) binding by MerR induces a large conformational change that underwinds the operator-promoter DNA leading to transcriptional initiation. Although structures exist for several activated MerR-family regulators, there is no structure for MerR itself nor for any non-activated MerR-family protein. Using small-angle X-ray scattering (SAXS) and molecular dynamics simulations, we showed (Guo et al., JMB, 2010) that Hg(II) binding transforms apo-MerR from a flattened, compact state into an elongated barbell. MD simulations indicate large amplitude motions of the DNA-binding domains on the nanosecond time scale, which might propagate allosteric changes near the MerR metal-binding site to the DNA-binding site. We constructed a model of MerR bound to its operator-promoter DNA, MerOP, and are doing MD simulations to optimize this model in light of genetic and DNA footprinting data. The optimized model will be used for MD to obtain kinetic and thermodynamic parameters of the Hg(II)-induced transition from repression to activation and depict how Hg-binding site occupancy is communicated over a distance of 28Å to the DNA-binding sites. We have overcome previous solubility issues with MerR by screening many conditions including pH, salts, and other additives (see accompanying poster by Smith et al.). Optimized solubility resulted in successful crystal growth of Hg(II)-MerR and Hg-free-MerR for X-ray diffraction data collection at APS.

The mercuric reductase MerA reduces Hg(II) to Hg(0). Using SAXS and molecular dynamics simulations, we studied the structure and dynamics of full-length MerA to examine the behavior of its N-terminal metallochaperone domain relevant to Hg(II) transfer from *mer* pathway proteins or from Hg(II)-damaged cellular proteins directly to the MerA active site for reduction. (see accompanying poster by Johs et al.)

Bacterial Hg(II) reduction also occurs at a low level as a side reaction of dissimilatory iron reduction in Fe-reducing bacteria such as *Shewanella* and *Geobacter*. We hypothesize that this reduction is done by outer-membrane multiheme cytochromes whose natural role is in moving electrons to external Fe(III). We purified the decaheme outer-membrane cytochrome OmcA from *Shewanella oneidensis* MR-1 and determined its solution structure using SAXS. Neutron reflectometry also revealed it assembled as a monomolecular layer on a hematite surface (Johs et al., *Biophys. J.*, 2010). Reduction *in vitro* showed direct electron transfer from

OmcA to Hg(II). Ongoing studies measure the Hg(II) reducing capacity of OmcA *in vivo*. As a membrane protein OmcA is a challenge for X-ray diffraction structure determination. We improved protein solubility with a low ionic strength buffer, and explored several crystallization techniques including capillary counter-diffusion, vapor diffusion, seeding, and cofactor/additive screening. By applying these techniques, we aim to overcome previous issues with crystal morphology. Diffraction data collection and efforts to solve the structure of OmcA are currently underway.

Because of cost, the most mechanistically explicit quantum chemical density functional theory (DFT) calculations can only be applied to small Hg(II)-ligand complexes to yield quantitative understanding of Hg(II) speciation and ligand-exchange reactions. While presently applying these to the *mer* locus proteins, we are also moving to apply them to acellular components of NOM to describe Hg(II) exchange from NOM to surface ligands of any cells including Hg(II) methylating bacteria. Future efforts of this SFA Task will use structural biology and computer simulations to define macromolecular dynamics, enzyme reaction pathways, and routes of mercury transfer to the cell to understand biomolecular Hg(II) trafficking in impacted ecosystems.