

ORNL SFA Task 4: Visualizing Intramolecular Interactions of the Mercuric Reductase (MerA) Catalytic Core with Its Hg(II) Shuttling Metallochaperone Domain (NmerA)

Alexander Johs¹, Ian M. Harwood², Jerry M. Parks¹, Rachel Nauss², Jeremy C. Smith^{1,3}, Susan M. Miller², Liyuan Liang¹.

¹Oak Ridge National Laboratory, Oak Ridge, TN, ²University of California San Francisco, San Francisco, CA, USA, ³University of Tennessee, Knoxville, TN

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. Many microbes possess the ability to deal with heavy metal toxicity through elaborate metal resistance mechanisms. One well known example, bacterial mercury resistance, is mediated by the *mer* operon, which encodes specific genes involved in the transfer and transformation of toxic Hg(II) species. The mercuric reductase MerA is a key component of the *mer* operon. MerA is an NADPH-dependent flavin-disulfide oxidoreductase and catalyzes the reduction of Hg(II) to Hg(0), which is relatively inert and passively diffuses from the bacterial cell. Here, we combine experimental biophysics and computer simulation techniques to investigate structural features important for Hg(II) transfer in MerA. All MerA proteins consist of a homodimeric catalytic core domain, and many also possess an N-terminal metallochaperone-like domain NmerA, which is tethered to the core by a ~30 amino acid linker of unknown fold. Prior studies using separately expressed NmerA and core domains showed that NmerA acquires Hg(II) from other *mer* proteins such as the organomercurial lyase, MerB, and the membrane transport protein, MerT, and delivers it to the MerA catalytic core for reduction. Here, we have applied small-angle X-ray scattering (SAXS), small-angle neutron scattering (SANS) and molecular dynamics simulations to characterize the interactions of NmerA and the core in full-length MerA in solution. Our data reveals the extent of spatial sampling of the two NmerA domains relative to the homodimeric catalytic core and identifies the inter-domain docking orientation that occurs during transient handoff of Hg(II) from a pair of cysteine residues on NmerA to a pair of cysteines on the C-terminus of the catalytic core.