Structural & Computational Analysis of MerR's Unique Allosteric Activation of *mer* Operon Transcription

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Mercury resistant (HgR) bacteria strongly influence the bioavailability of toxic methylmercury levels at Hg contaminated DOE sites, most notably the Y-12 NSC. Expression of HgR genes by the *mer* operon is transcriptionally controlled by the repressor-activator MerR [1] which binds its operator-promoter DNA (MerOP) at a dyad between the -10 and -35 RNA polymerase (RNAP) recognition sites [2]. Without Hg(II) MerR binds tightly to MerOP repressing transcription by holding RNAP inactive at the promoter. When Hg(II) appears it binds MerR strongly and, thus activated, MerR transmits its consequent allosteric movement to the DNA of MerOP, underwinding the promoter and enabling RNAP to begin transcription [3]. There are 3D structures for several activated MerR-family regulators, but not as yet for MerR itself, nor are there structures of the repressed form of any MerR-family protein. We have used MerR's homology to structures of other family members to construct a 3D model of activated Hg-MerR and also collected small-angle X-ray scattering (SAXS) data on MerR alone and Hg-MerR. SAXS showed activated Hg-MerR was more extended than MerR alone. Long timescale molecular dynamics simulations indicated high flexibility of the Hg-MerR[4]. Neutron Spin Echo experiments are performed on Hg-MerR to experimentally determine the internal dynamics, which are important to describe the allosteric transition mechanism. We have also prepared a complex of MerR with a 23bp MerOP and found by dynamic light scattering that this complex forms oligomeric structures in solution. To overcome such oligomerization we devised an alternative buffer that affords a monodisperse solution at high protein concentrations in physiologically relevant thiol buffer levels. Small-angle neutron scattering (SANS) allowed us to probe differences in the conformations of MerR and of DNA in the complex. We included a H_2O/D_2O contrast variation series to obtain complete data sets for the MerR/MerOP complex in the active and repressed conformations. Concurrently, we are optimizing our computational molecular model of the MerR-23bp-MerOP complex by including previous genetic and DNA footprinting data in order to describe the Hg(II)-induced transition from repression to activation.

[1] Summers, A. O. (2009). *Curr. Opin. Microbiol.* **12**, 138–144.
[2] Park, S. J. et al. (1992). *J. Bacteriol.* **174**, 2160–2171.
[3] Ansari, et al. (1992). *Nature*, **355**, 87–89.
[4] Guo et al. (2010). *J Mol Biol* **398**(4)**:** 555-568.