Biomolecular Aspects of Mercury Transformations

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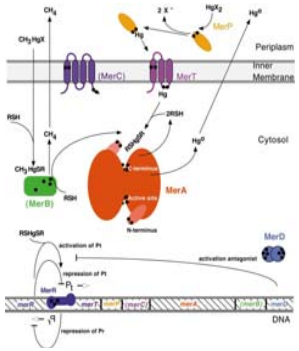
Introduction

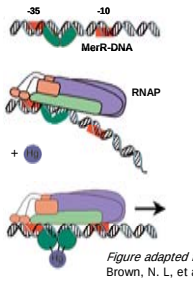
Bacteria participate significantly in mercury transformation in natural and industrial environments. Previous studies have shown that bacterial mercury resistance is mediated by the *mer* operon, typically located on transposons or plasmids. It encodes specific genes that facilitate uptake of mercury species, cleavage of organomercurials, and reduction of Hg(II) to Hg(0).

Expression of *mer* operon genes is regulated by MerR, a metal-responsive regulator protein on the level of transcription. *In vitro* studies have shown that MerR forms a non-transcribing pre-initiation complex with RNA polymerase and the promoter DNA. Binding of Hg(II) induces conformational changes in MerR and other components of the complex resulting in the transcription of *mer* operon genes.



T. Barkay, S.M. Miller, and A.O. Summers: FEMS Microbiol Rev, 2003. **27**(2-3): p. 355-84.

Transcriptional Regulation - MerR

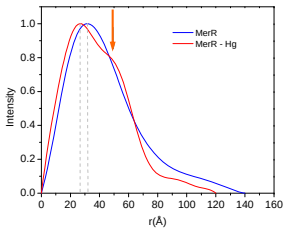


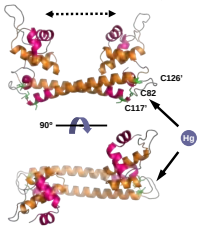
Dimeric MerR binds to a region of dyad symmetry between the -35 and -10 recognition sites of the DNA operator (MerOP). This results in the formation of an inactive preinitiation complex with RNA polymerase (RNAP).

Allosteric conformational changes in MerR/MerOP and the ternary MerR/MerOP/RNAP complex including the sigma70 subunit are induced by Hg(II) and are the cause for initiation of transcription of *mer* genes.

Contrast variation SANS can detect conformational changes in MerOP or specific deuterium labelled subunits, like sigma70, within the assembled complex.

Figure adapted from:
Brown, N. L, et al. FEMS Microbio. Rev. 27:145-163 (2003)





Preliminary small angle X-ray scattering data (left) reveals a distinct conformational change in the metal-dependent regulator MerR upon binding of Hg(II). Dimeric MerR makes a transition from a compact overall shape to a structure with two separate domains. This conformational state corresponds well to the homology model of MerR bound to Hg(II) (right), which is based on the homologous structures shown in the table below.

protein	*Sequence identity*	**E value**
CueR	36/129 (27%), Positives = 62/129 (48%), Gaps = 3/129 (2%)	3e-10
ZntR	34/95 (35%), Positives = 60/95 (63%), Gaps = 5/95 (5%)	1e-8
SoxR	40/126 (31%), Positives = 61/126 (48%), Gaps = 10/126 (7%)	5e-6
BmrR	27/107 (25%), Positives = 57/107 (53%), Gaps = 11/107 (10%)	4e-5
MtaN	20/61 (32%), Positives = 33/61 (54%), Gaps = 0/61 (0%)	2e-3

Small Angle Neutron and X-ray Scattering

Small angle scattering can be applied to obtain low resolution envelope shapes of proteins in monodisperse solutions at physiological pH, ionic strength and temperature. This enables the researcher to compare overall conformations in solution with structural models obtained from X-ray crystallography.

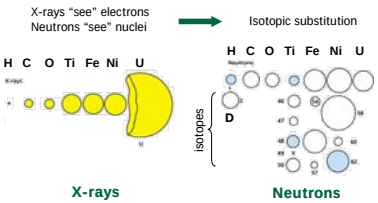
SAXS/SANS can be used to elucidate dynamic protein functional relationships and their alteration by assemblies, modifications, and metabolites

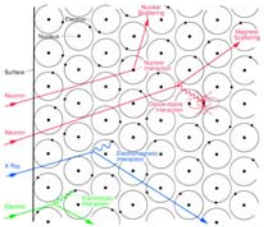
SANS with contrast variation offers a method to reveal the orientation and location of specific components, i.e. subunits, in large macromolecular complexes.

Neutron Scattering

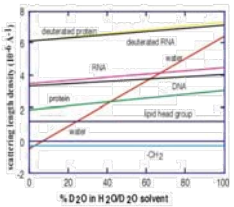
X-rays "see" electrons
Neutrons "see" nuclei

Isotopic substitution





Radii of spheres = *b* = scattering amplitude

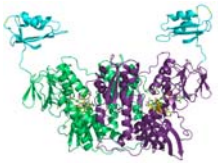


Coherent neutron scattering length density of biological macromolecules in D_2O/H_2O mixtures.

Neutron scattering techniques can provide additional information on the structure of supramolecular complexes. Light elements dominate the composition of biological macromolecules and neutrons are particularly sensitive to hydrogen atoms.

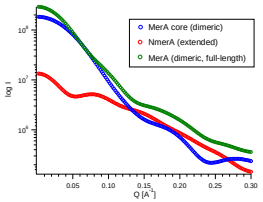
The significant difference in the scattering lengths of hydrogen (^1H or H) and deuterium (^2H or D) can be used to selectively highlight components in a macromolecular complex. This allows the observation of quaternary structure or the spatial characterization of interacting macromolecules.

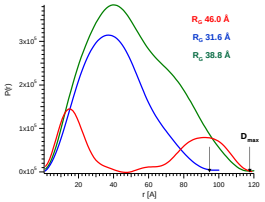
Mercuric Reductase - MerA



Small angle scattering data of dimeric full-length MerA in solution can help clarify the conformation and functional role of N-terminal domains in the dimeric complex. Flexibility of putative hinge regions between NmerA and MerA core can be assessed.

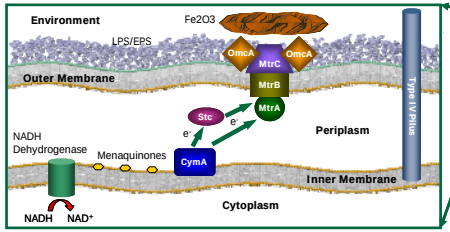
Using SANS in combination with contrast variation and deuterium labelled NmerA, the interaction sites of NmerA with the dimeric catalytic core can be obtained by selective deuteration of single Cys mutants.

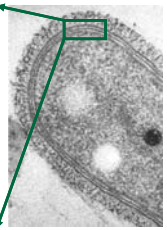




Hypothetical model of MerA (top) based on crystal structure of dimeric core with N-terminal NmerA connected by modeled linkers. Simulated scattering profiles (left) and calculated distance distribution functions (right) at three different levels of solvent contrast matching either core, NmerA or none.

Molecular Basis for Dissimilatory Metal Reduction



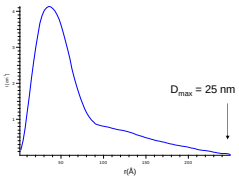


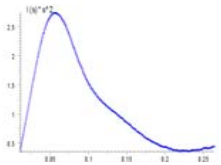
L. Liang, R. Rinaldi, H. Schober(Eds.): Neutron Applications in Earth, Energy and Environmental Sciences, 16, 461-486, Springer, 2009.

Gram-negative bacterial cell

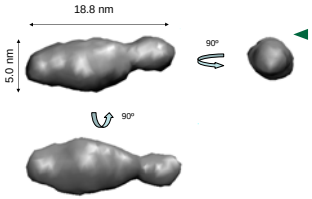
The genome of *Shewanella oneidensis* MR-1 contains 42 possible *c*-type cytochrome coding sequences. The decaheme outer membrane cytochrome OmcA is the terminal electron donor transferring electrons to mineral surfaces and may also be responsible for the reduction of Hg(II) at concentrations too low to induce *mer* operon functions.

Molecular Structure of OmcA



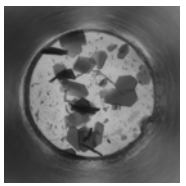


Distance distribution function (left) of OmcA in solution indicating an elongated molecular shape with a radius of gyration of 50Å and a maximum diameter of 250Å. The Kratky plot (right) indicates that the protein is properly folded.



Average envelope shape of OmcA derived from ten independent *ab initio* models using the programs DAMMIN, SUBCOMB and DAMAVER.

To reveal details of protein function, high resolution structure information is required. Crystallization conditions for OmcA were obtained and we are currently working to obtain a crystal structure of OmcA.



Summary

- Mercury resistance in bacteria is mediated by a set of highly specialized enzymes that require unique interactions to hand over Hg(II). Small angle neutron scattering can provide structural information on protein-protein interactions that is critical to understand transport and handover of toxic Hg(II) inside the cell. Conformational changes of macromolecules can be observed directly at physiological conditions and with high sensitivity.
- The expression of the *mer* operon is regulated on the level of transcription by a Hg(II)-dependent regulatory protein MerR. Our data shows that Hg(II) causes a significant change in the conformation of the MerR dimer. This change is propagated to the DNA operator and allows RNA polymerase to initiate transcription.
- Dissimilatory reduction processes can reduce Hg(II) to Hg(0) at levels too low to induce *mer* operon functions. Structural information was obtained for a key component, the decaheme outer membrane cytochrome OmcA.

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