Structural biology of the sequestration and transport of heavy metal toxins:NMR structure determination of proteins containing the –Cys-X-Y-Cys- metal binding motif.

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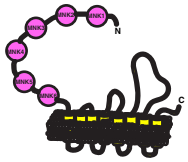
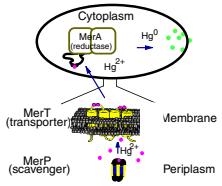
The overall goal of the research is to utilize the methods and ideas of structural biology that have been successfully applied to many biomedical research problems to address an environmental problem that has significant consequences for human health. Proteins from the bacterial mercury detoxification system and related polypeptides are the principal subjects of the research. There is a direct molecular connection between the mechanism by which bacteria detoxify heavy metals and a number of diseases because of the high levels of homology among many proteins responsible for binding and transporting heavy metals in bacteria and humans. The structures of proteins that bind and transport heavy metals will be determined by NMR spectroscopy. These structures will provide insights needed to develop molecular systems capable of removing heavy metals from the environment as well as ameliorating symptoms of toxicity in humans resulting from environmental exposure or disease states. There are two principal aspects to the planned structural studies. We will continue our structural studies of peptides and proteins containing the –Cys-X-Y-Cys- metal binding motif, building upon our success in determining the structures of MerP, the periplasmic metal binding protein in bacteria; MNK1, the first metal binding domain of the ATPase associated with Menkes disease; and several 18-residues peptides corresponding to the metal binding loops of these proteins, and then design and prepare related polypeptides with variants of this metal binding motif. We will also continue our structural studies of the membrane proteins that transport heavy metals into the cell. The ultimate goal is to combine the information from both aspects of the research to obtain a detailed chemical and structural understanding of how both prokaryotes and eukaryotes deal with heavy metals, and then utilize this molecular information to develop methods for ameliorating the environmental problems and human diseases that result from the presence of heavy metal toxins.

Human copper transporting ATPase.

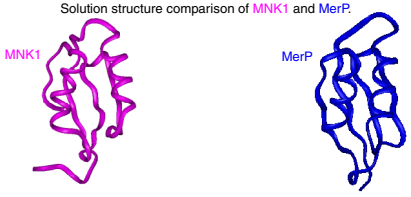


The protein encoded by the Menkes gene is a copper transporting ATPase which contains six cytoplasmic metal-binding domains. These domains are characterized by a heavy metal associated sequence (GMTCXXC).

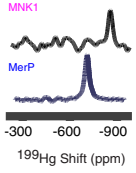
Bacterial mercury detoxification system.



Solution structure comparison of MNK1 and MerP.

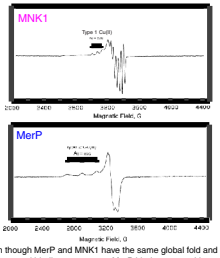


Comparison of ^{199}Hg NMR spectra.



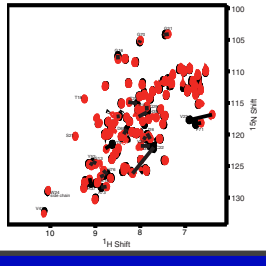
The coordination geometry of 199Hg can be probed by NMR spectroscopy as demonstrated by O'Halloran et.al, 1993. The chemical shift of bound 199Hg to MNK1 and MerP is within the range observed for linear bicoordinate compounds.

Comparison of Cu(II) EPR spectra.



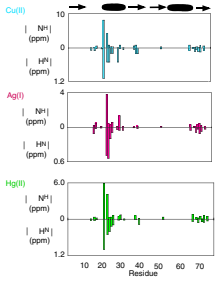
Even though MerP and MNK1 have the same global fold and similar metal binding sequences, MerP binds copper with type 2 coordination and MNK1 binds copper with type 1 coordination.

Hsqc of uniformly ^{15}N labeled MNK1 with and without Cu(II) bound.

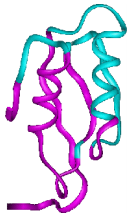


Peaks in black represent the spectrum of MNK1 with no metal bound. Peaks in red represent the spectrum of MNK1 after addition of 1.5 molar excess of copper. The backbone amide resonances of T18 and S21 are not observable in the apo state but are detected in the metal bound state.

Chemical Shift changes upon addition of different metals to MNK1.



Structure comparison of reduced and Cu(II) bound MNK1.



Magenta indicates those residues whose chemical shifts did not change upon addition of Cu(II). Blue indicates regions of the protein that did change upon Cu(II) binding.

Sequence comparison of MNK1 and MerP motifs.

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                    10        20        30        40        50        60        70
MNK1    MDPSMGVNSVTISV EGMTCNSCVW TIEQQIGKVN GVHH KVSLE EKNATIIYDP KLQTPKTLQE AIDDMGFDAV IHNPD

MerP          ATQTVTLAV PGMTCAACPI TVKKALSKVE GVTKVDVGFE KREAVVTFDD TKASVQKLTK ATADAGYPSS VKQ
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Peptides of MerP

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CAAC                TLAV PGMT     CAACPI TVKK
CACA                     TLAV PGMT     CACAPI TVKK
CCAA                     TLAV PGMT     CCAAPI TVKK
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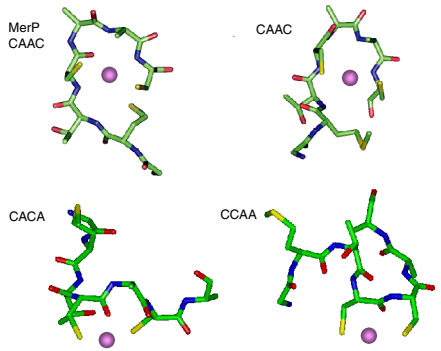
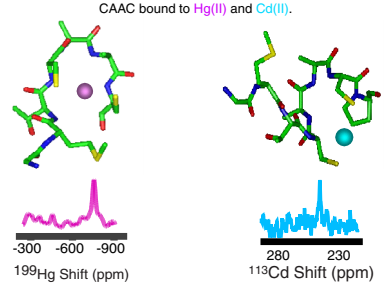
Comparison of Hg(II) bound structures of MerP and peptides.



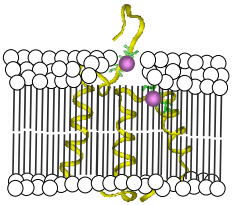
CAAC bound to Hg(II) and Cd(II).



Dissociation Constants

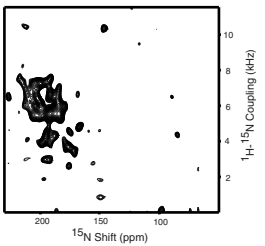
Metal	MerP Kd (M)	CAAC Kd (M)	CACA Kd (M)	CCAA Kd (M)	MNK1 Kd (M)
Hg(II)	2.8	90	207	78	4.4
Cu(II)	5.2	120	47	$>10^5$	1.8
Ni(II)	20	180	100	$>10^5$	2.3
Zn(II)	4	350	105	$>10^5$	---
Cd(II)	18	430	43	$>10^5$	3.6
Ag(I)	13	430	100	$>10^5$	6.8
Ca(II)	$>10^5$	$>10^5$	$>10^5$	$>10^5$	$>10^5$

Three-dimensional structrue of MerF determined in micelles b y solution NMR spectroscopy.



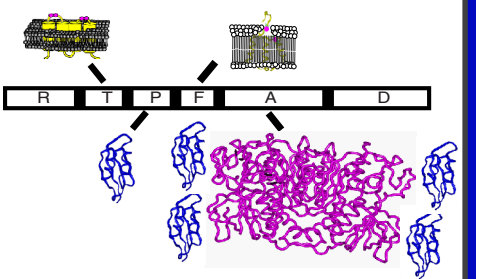
MerF has two pairs of vicinal cysteines, represented in green. Each pair of vincinal cysteines binds one mercury ion, represented in pink.

MerF in magnetically aligned bicelles.



Two-dimensional PISEMA spectrum of MerF in bicelles. MerF is an 81 amino acid polytopic membrane protein found in the mer operon of some mercury resistant bacteria.

Structural biology of the *mer* operon.



Conclusions:

- The metal binding loops of MerP and MNK1 are flexible to accommodate multiple bicoordinate geometries.
- The structure of the metal-binding motif GMTCAAC is similar in an 18-residue linear peptide and the mercury binding protein MerP.
- There is a 1-2 order of magnitude difference in metal-binding affinity between the peptides and the proteins.
- The preliminary PISEMA spectrum of merF in bicelles is consistant with the transmembrane topology determined by solution NMR.