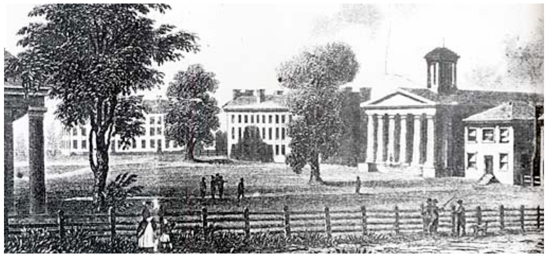
Integrating the Molecular Machines of Mercury Detoxification into Host Cell Biology

University of Georgia
Anne O. Summers
Bijal Patel
Lyn Olliff
Lingyun Song
Caran Cagle
John Brewer
Cory Momany
Rob Phillips
Bob Scott
Quincy Teng



Univ. of California-San Francisco
Susan M. Miller, UCSF
Xiaohua Feng
Ian Harwood
Rachel Nauss
Richard Ledwidge
Andrew Sandstrom
Robert Stroud

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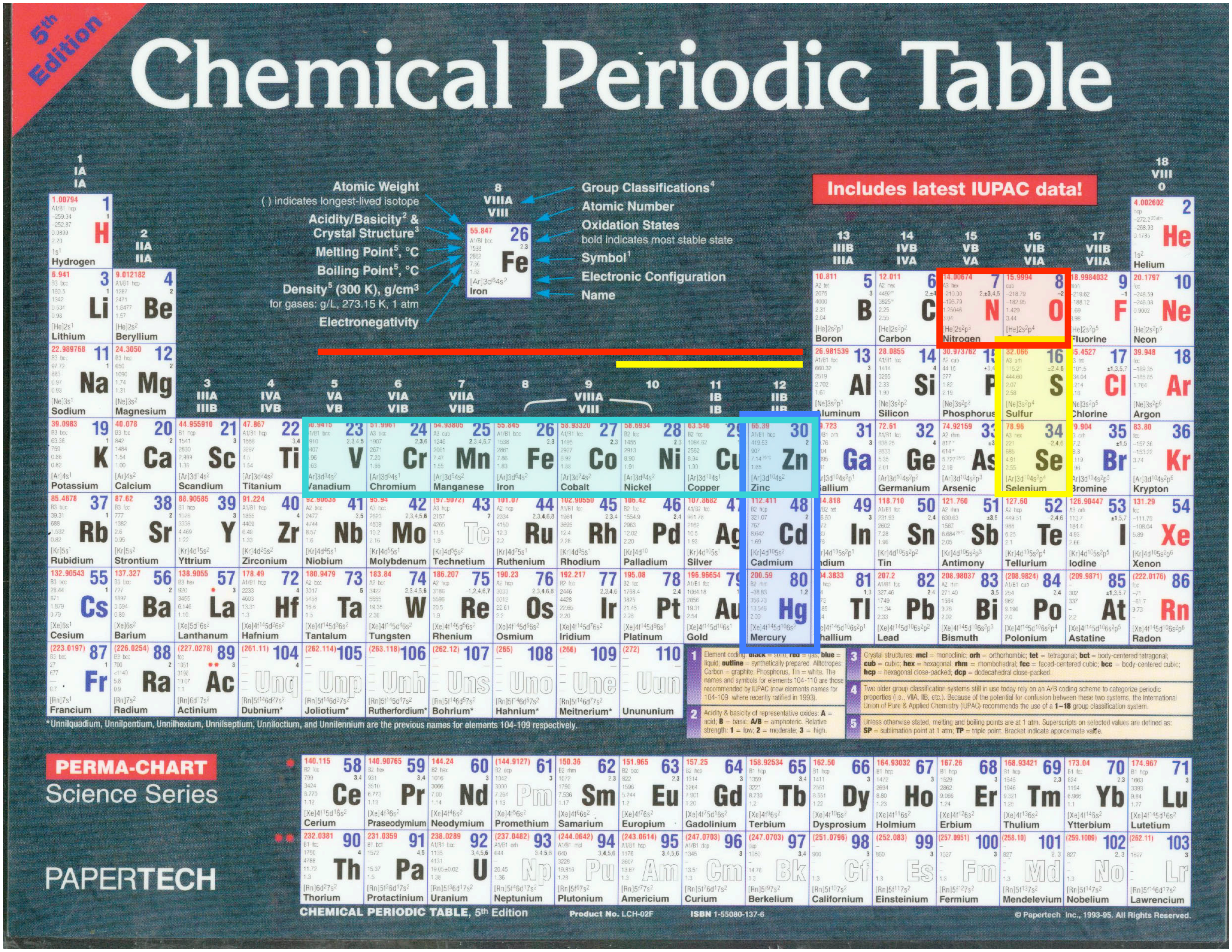
The University of Georgia

UCSF University of California San Francisco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Chemical Periodic Table
Includes latest IUPAC data!
Atomic Weight
( ) indicates longest-lived isotope
Acidity/Basicity & Crystal Structure
Melting Point, °C
Boiling Point, °C
Density (300 K), g/cm3
for gases: g/L, 273.15 K, 1 atm
Electronegativity
Group Classifications
Atomic Number
Oxidation States
bold indicates most stable state
Symbol
Electronic Configuration
Name
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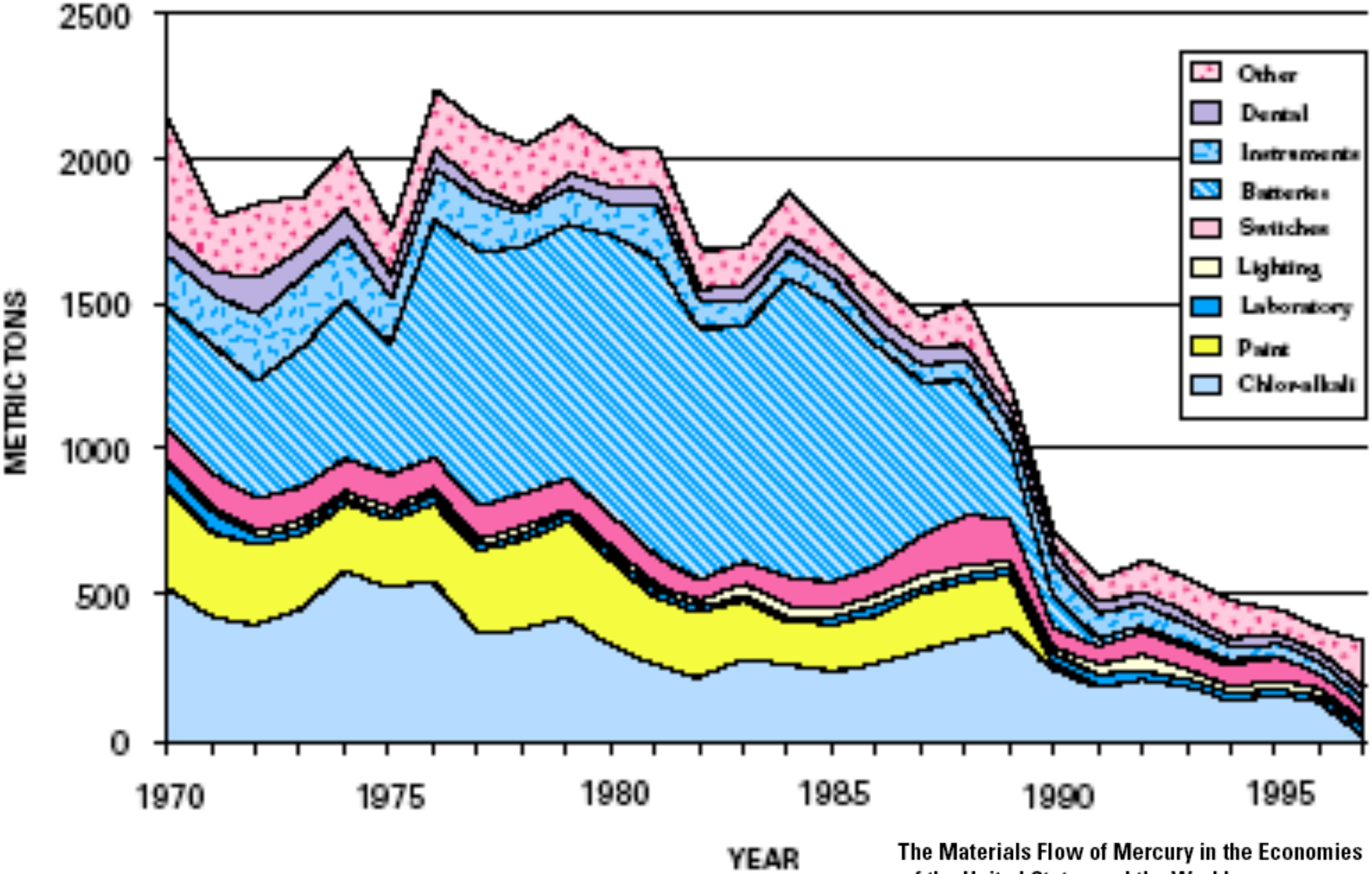
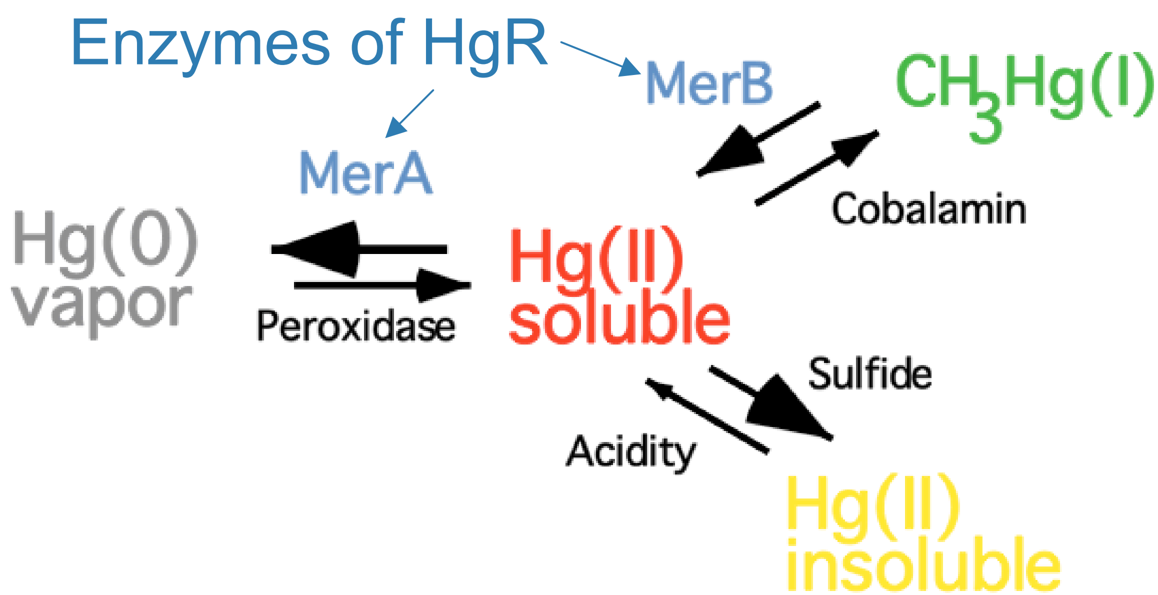
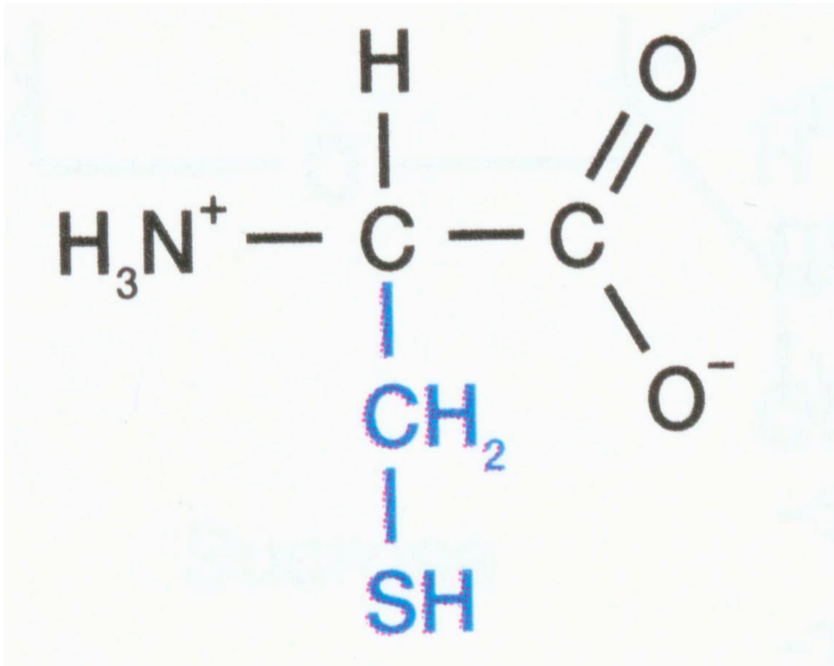


Figure 3. U.S. industrial reported consumption of mercury (1970–1997).

The Biotic Hg Cycle



All forms of Hg are biologically available.



Cysteine (Cys, C)

Potential Human Targets for Interaction with Hg(II)

System	Protein/Process	Molecular Target
Signal transduction		
	Protein tyrosine phosphatase	Invariant Cys215
	Zinc Finger Proteins	Multiple Cysteines
	LIM proteins	Multiple Cys-His domains
Metal Homeostasis		
	Metallothionein	Multiple Cysteines
	Menkes Disease (Cu) "	"
	Wilson's Disease (Cu) "	"
Renal transport	CHIP28 Water Channel	Cys 189
Growth Factors	Trefoil, EGF-like, Cystine Knot	Three clustered cystine bridges
CNS	Membrane Cysteine String Proteins (synaptic vesicles and termini)	Cysteine rich proteins
Cardiovascular	apolipoprotein(a)	Cys 4057 - important for assembly
Viruses	HIV Tat protein	Cysteine-rich protein
Oncogenes	RAS	Thioether farnesyl linkage

Why study Hg resistance?

Only naturally occurring system that biotransform a toxic metal in bulk

Handles inorganic and organic Hg(II)

Widely found in eubacteria and archaea that are the major Hg transformers in highly contaminated settings.

Transposable and laterally transferrable in proteobacteria.

Highly conserved mecahnistically - I.e pump Hg(II) in and reduce to volatile Hg(0)

Illuminates some basic biology of enzymology, gene regulation, redox metabolism

Employed in paradigm example of engineered metallophytoremediation

Transgenic *merA* tobacco plants survive transplantation to contaminated soils and detoxify Hg(II) to less toxic Hg(0)

Hg(II) 0 ppm 100 ppm 500 ppm

WT ***merA*** **WT** ***merA*** **WT** ***merA***

GA Piedmont 2% organic

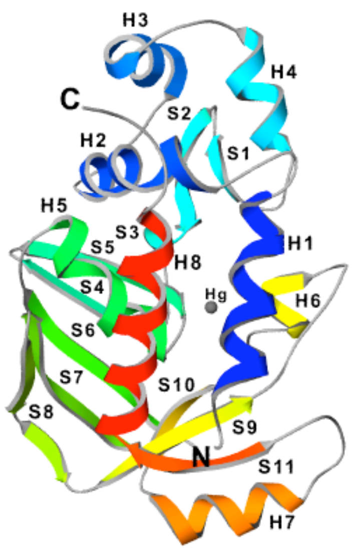
GA Coastal 2% organic

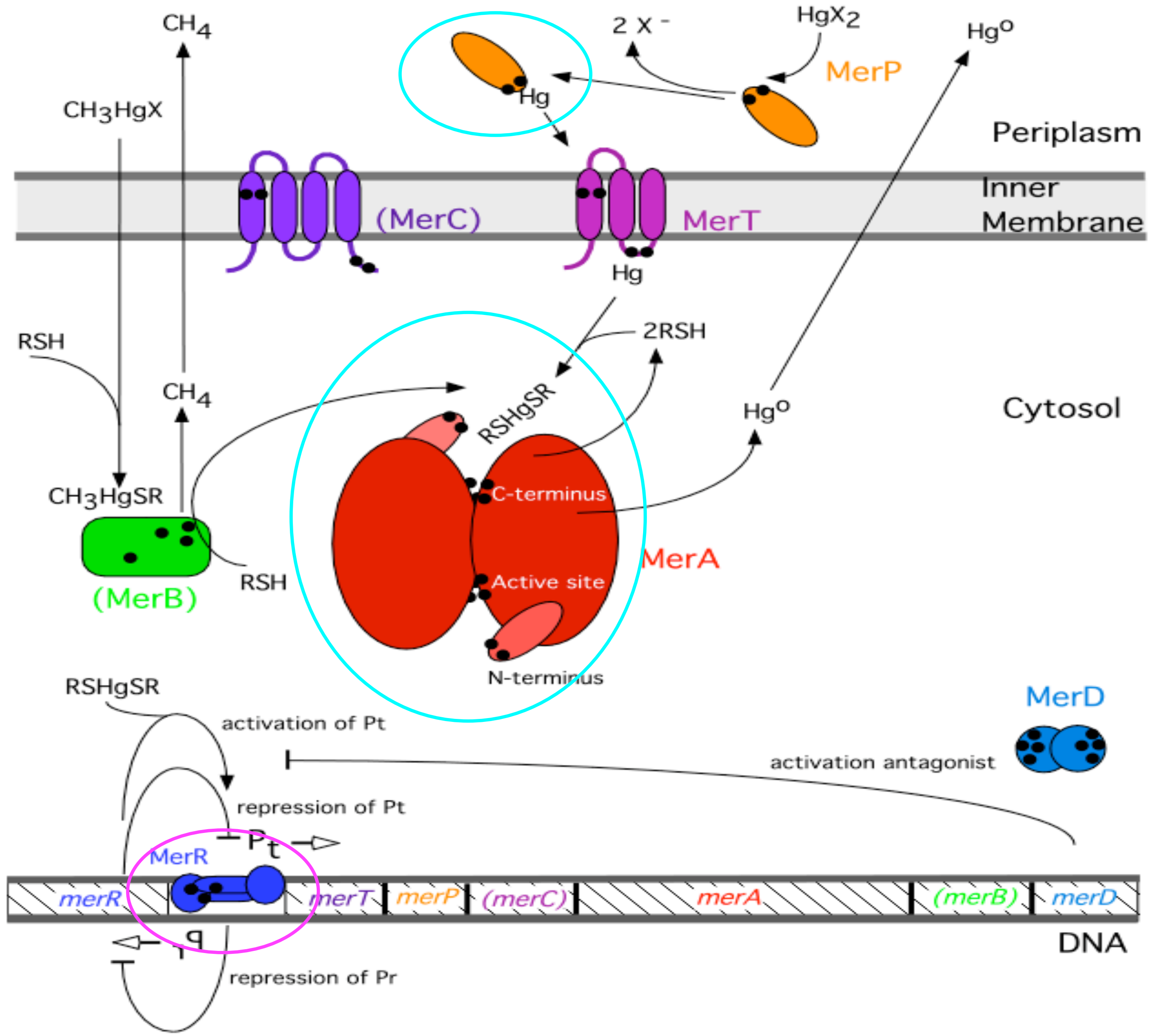
Heaton et al. (1998) Hort. Sci., Meagher (2000) Cur Op Plant Sci

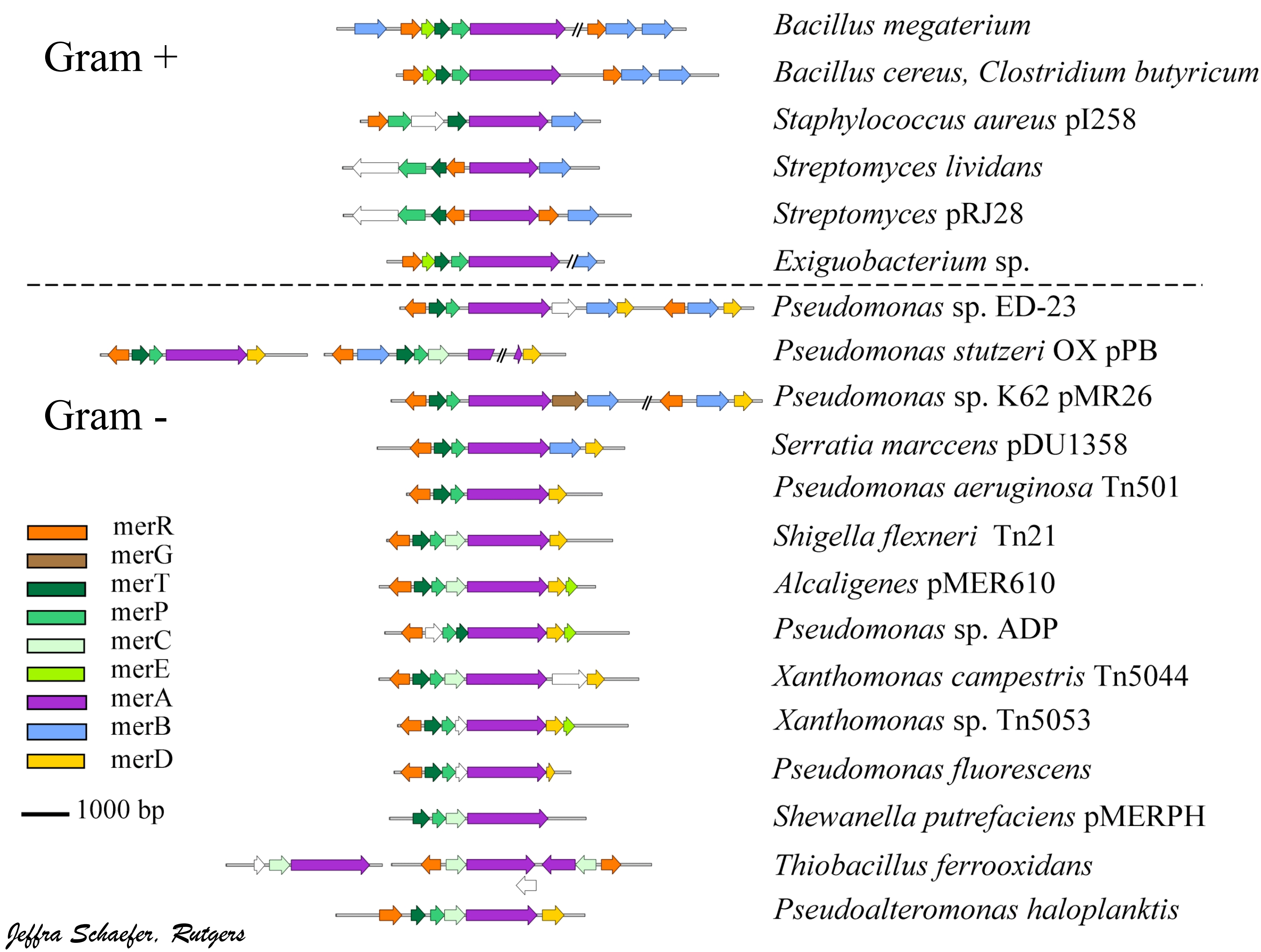
Poster, Weds night

The Bacterial Mercury Resistance Locus

MerB

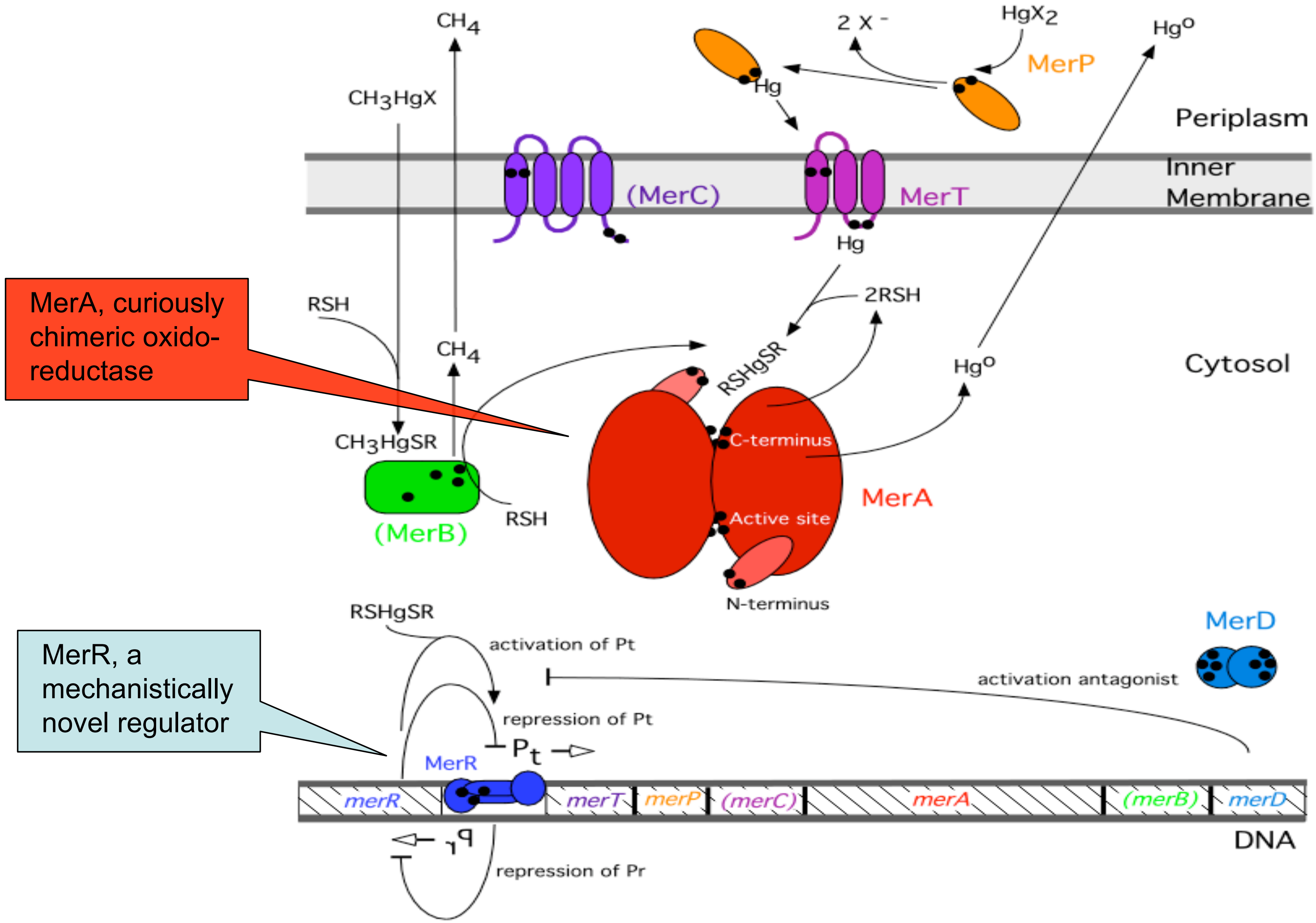




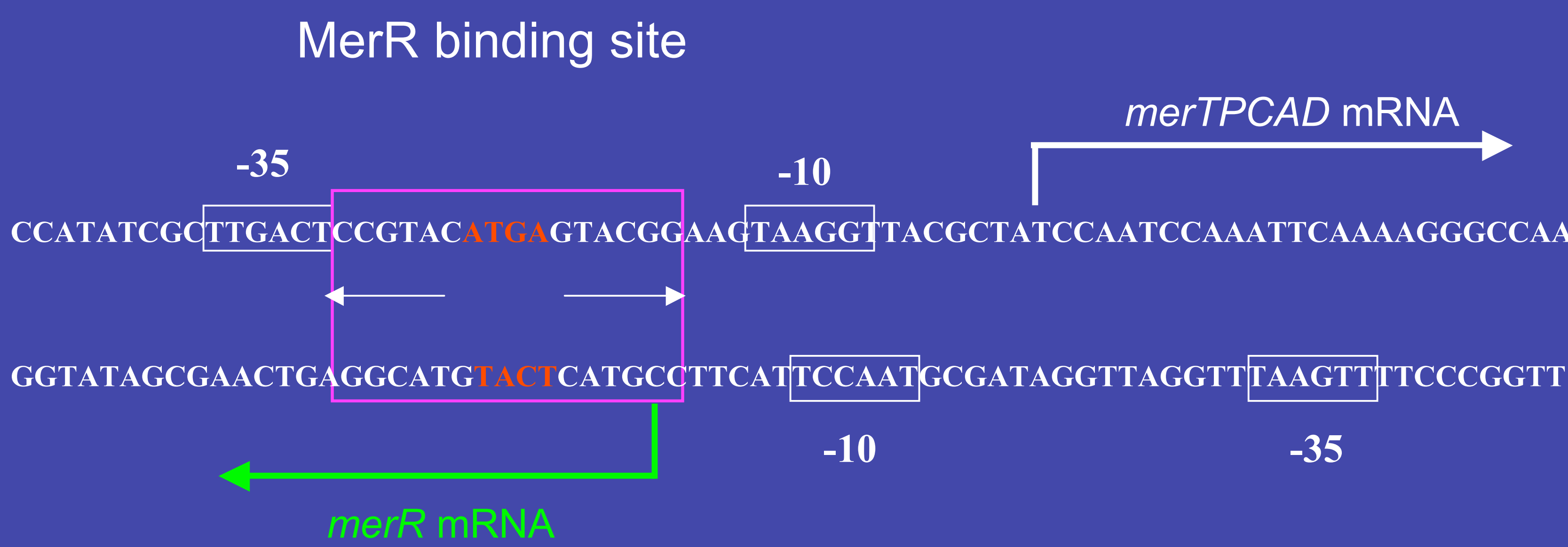


Gram +
Gram -
Bacillus megaterium
Bacillus cereus, Clostridium butyricum
Staphylococcus aureus pI258
Streptomyces lividans
Streptomyces pRJ28
Exiguobacterium sp.
Pseudomonas sp. ED-23
Pseudomonas stutzeri OX pPB
Pseudomonas sp. K62 pMR26
Serratia marccens pDU1358
Pseudomonas aeruginosa Tn501
Shigella flexneri Tn21
Alcaligenes pMER610
Pseudomonas sp. ADP
Xanthomonas campestris Tn5044
Xanthomonas sp. Tn5053
Pseudomonas fluorescens
Shewanella putrefaciens pMERPH
Thiobacillus ferrooxidans
Pseudoalteromonas haloplanktis
merR
merG
merT
merP
merC
merE
merA
merB
merD
1000 bp
Jeffra Schaefer, Rutgers

The Bacterial Mercury Resistance Locus



MerR’s “muscular” transcriptional control

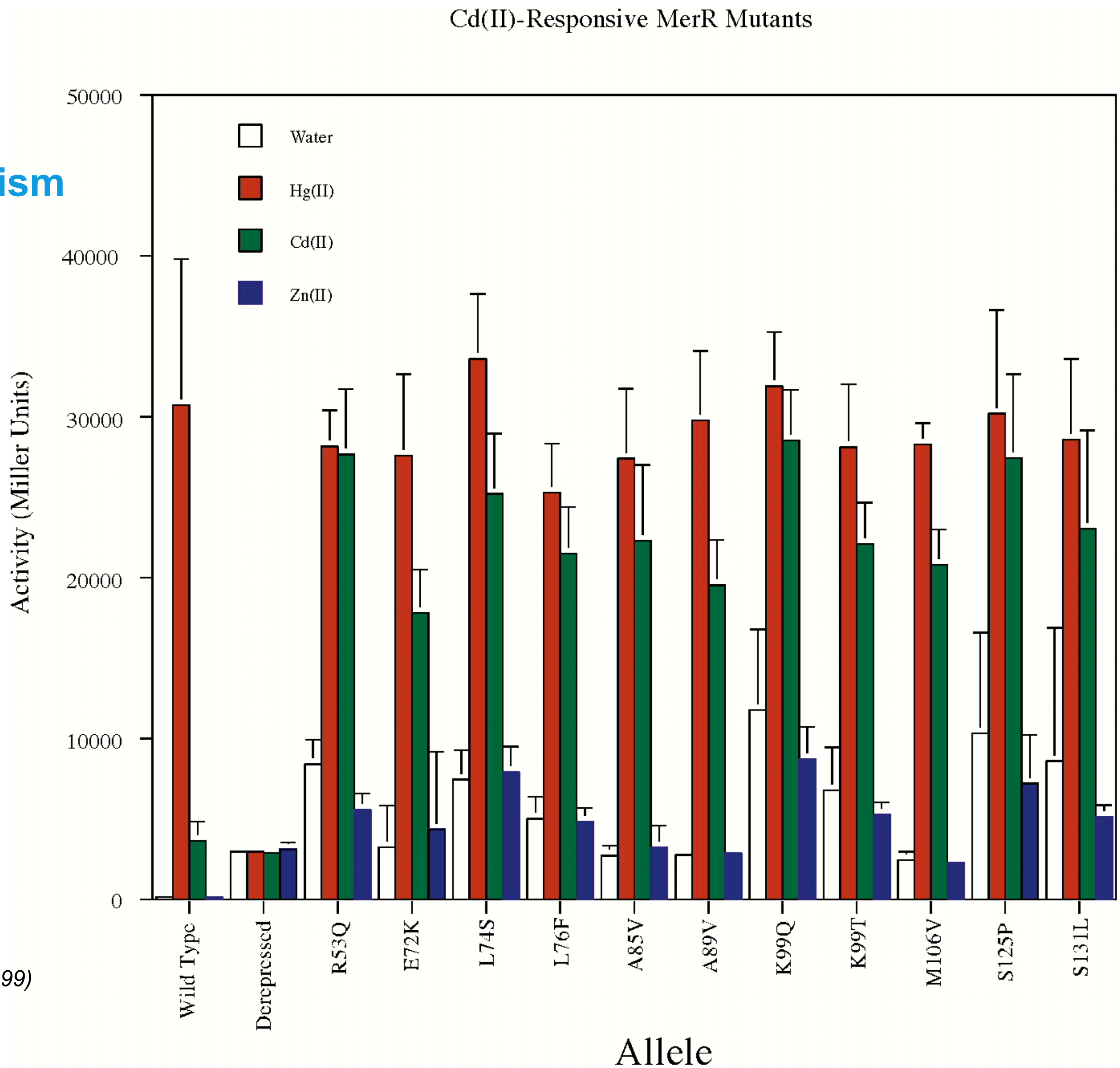


Hg(II) provokes MerR to underwind

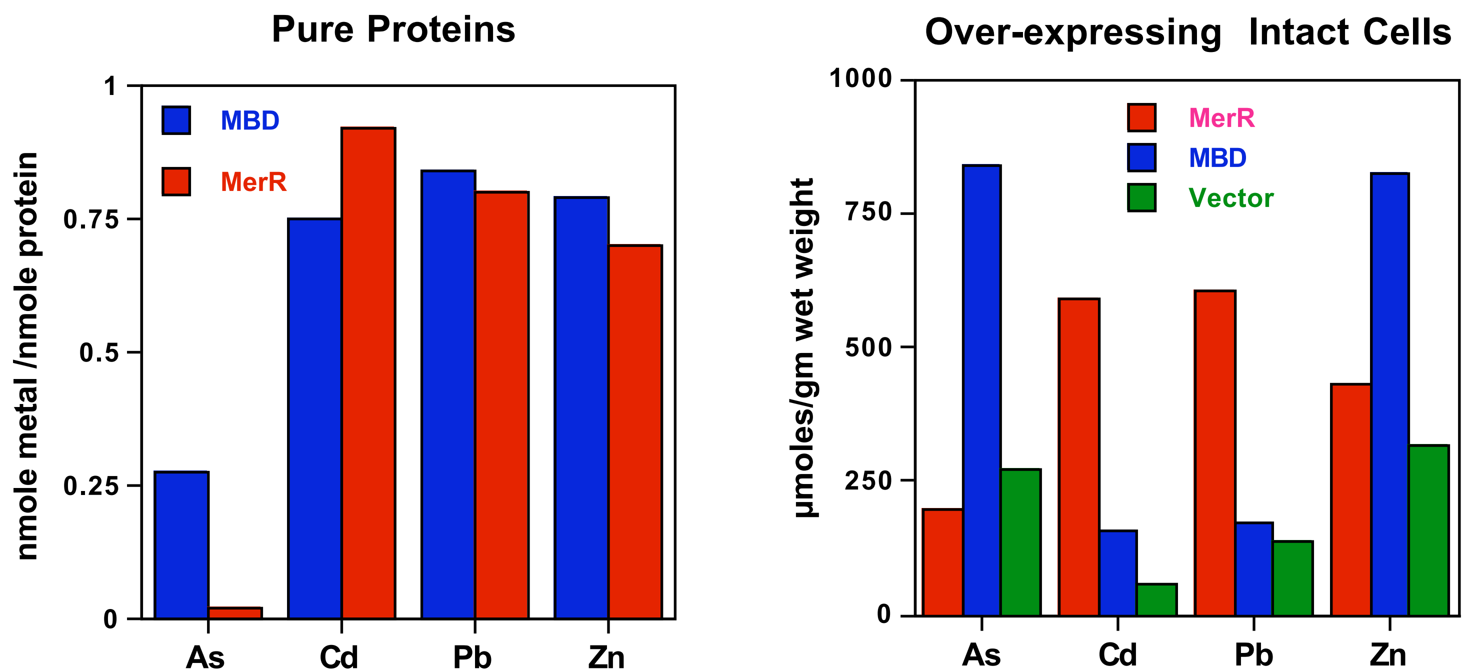
the MerO dyad center

Heltzel et al, Biochem, 1990, Frantz et al, Biochem. 1989, Lee, Livrelli, JBC 1993

Using mutants to dissect the mechanism of metal specificity



Caguiat et al. J. Bacteriol181 (1999)



MerR and MBD bind metals other than Hg in vitro and in vivo, possibly with differing specificities

MerR binds other thiophilic metals in vivo and in vitro so its specificity as a transcriptional activator must lie in more than just metal binding….

Possibilities:

Other metals do not provoke DNA distortion

YES, Chuan He, U. Chicago, JACS 2004

Other metals don't bind MerR when it is bound to DNA

NO, Song et al., JMB 2007, *in press*

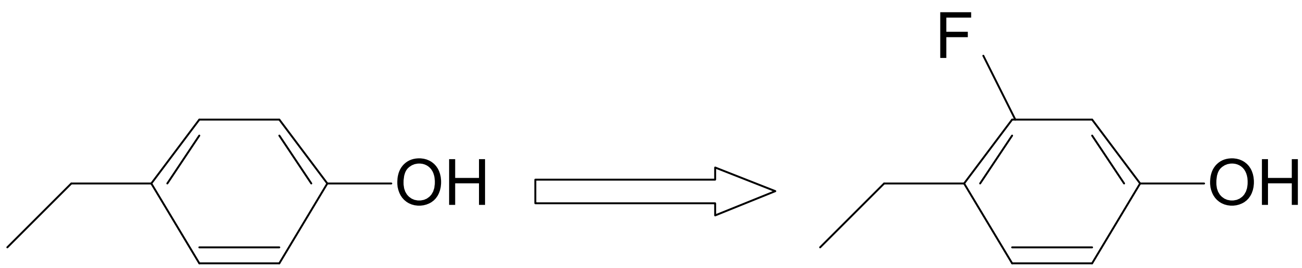
Does Hg(II) provoke a conformational change distinct from that of non-inducers?

^{19}F NMR: Watching MerR's Tyrosines

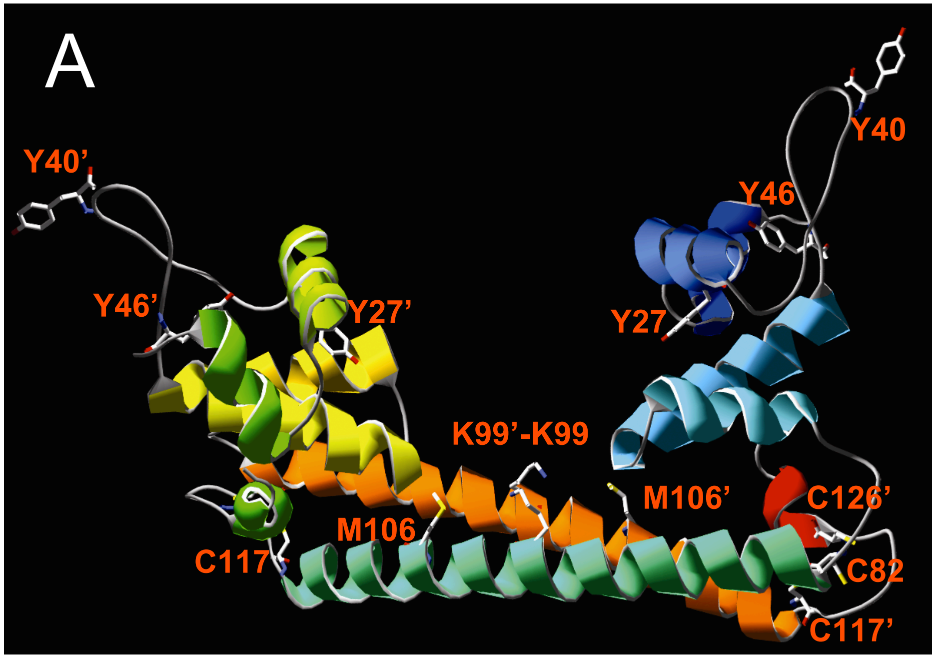
Y27 (Conserved) Y40 (not conserved)

Y46 (conserved)

```
MENNLENLTIGVFAKAAGVNVETIRFYQRKGLLREPDKPYGSIR
RYGEADVVRVKFVKSAQRLGFSLDEIAELLRLDDGTHCEEASSL
AEHKLKDVREKMADLARMETVLSELVCACHARKGNVSCPLIASL
QGEAGLARSAMPSAWSHPQFEK
```



	Tyrosine	2-Fluorotyrosine (2-FY)
pKa	10.05 ± 0.04	9.04 ± 0.03



A Candidate Allosteric Signalling Pathway in MerR

B

α1 α2

MENNLENL **TIGVFAKAA** GVN **VETIRF Y QR** KGLLRE

α3

PDKP **Y** GSIRR **Y** GEAD **VVRVKFVKS AQRL** GFS **LDE**

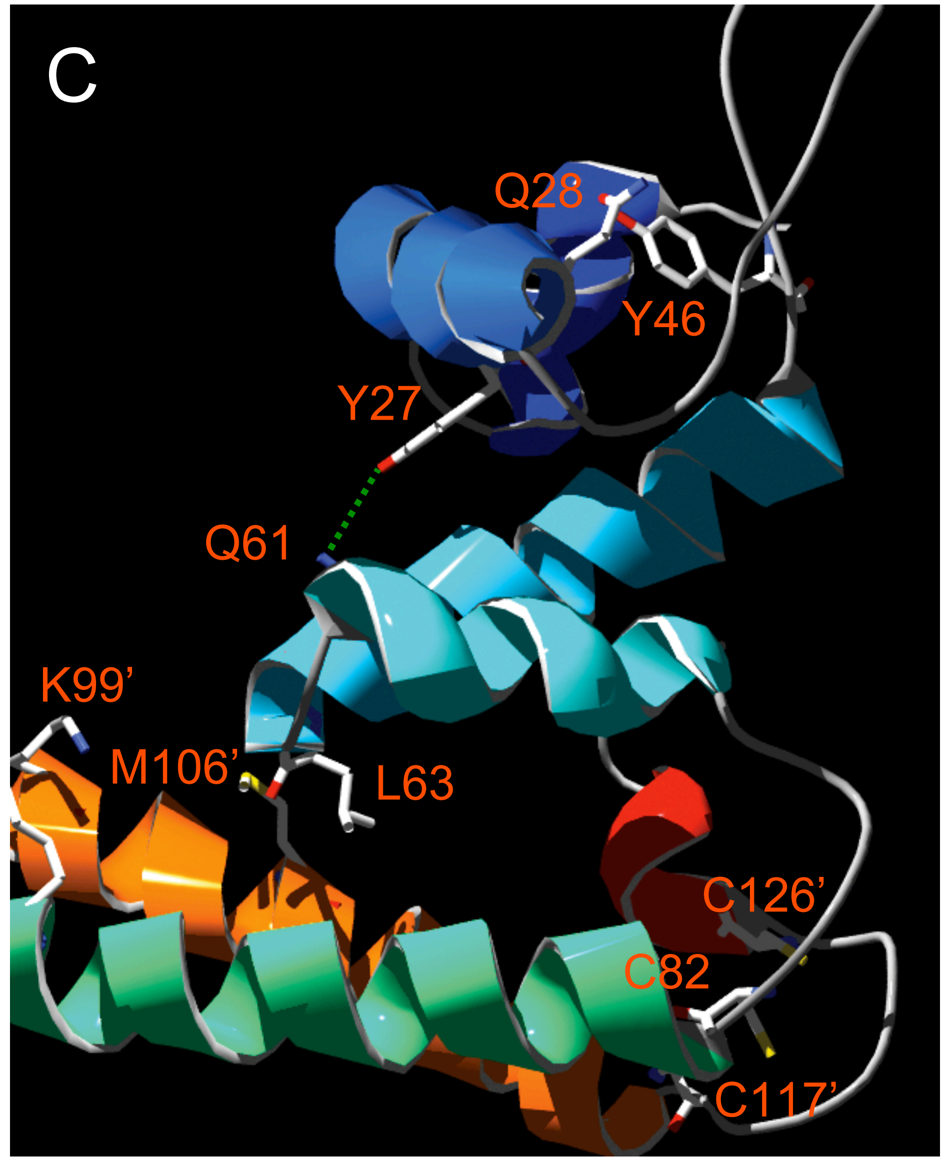
α4 α5

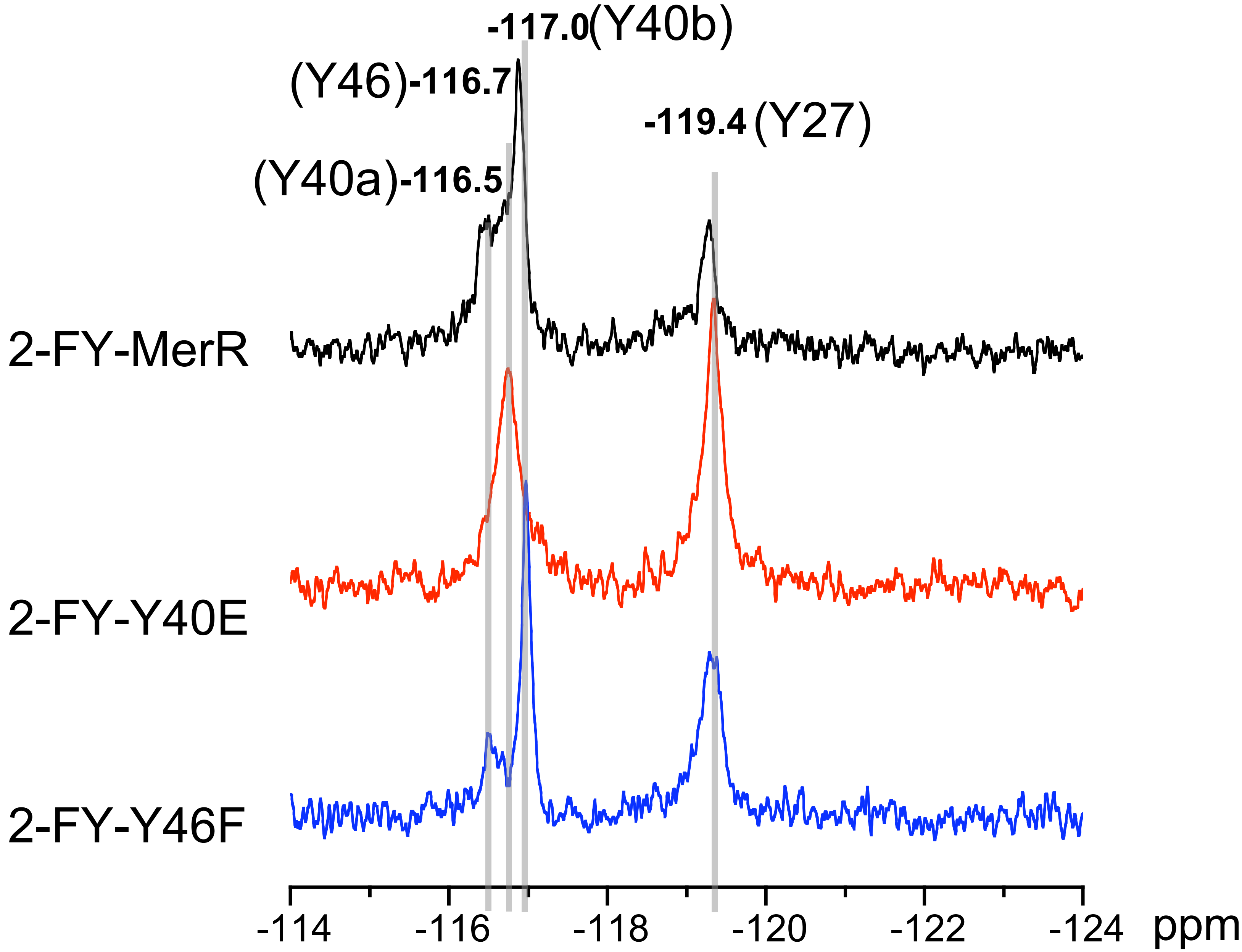
IAELLRL DDGTH **C EEASSLAEHKLKDVRE K MADL**

α5 α6

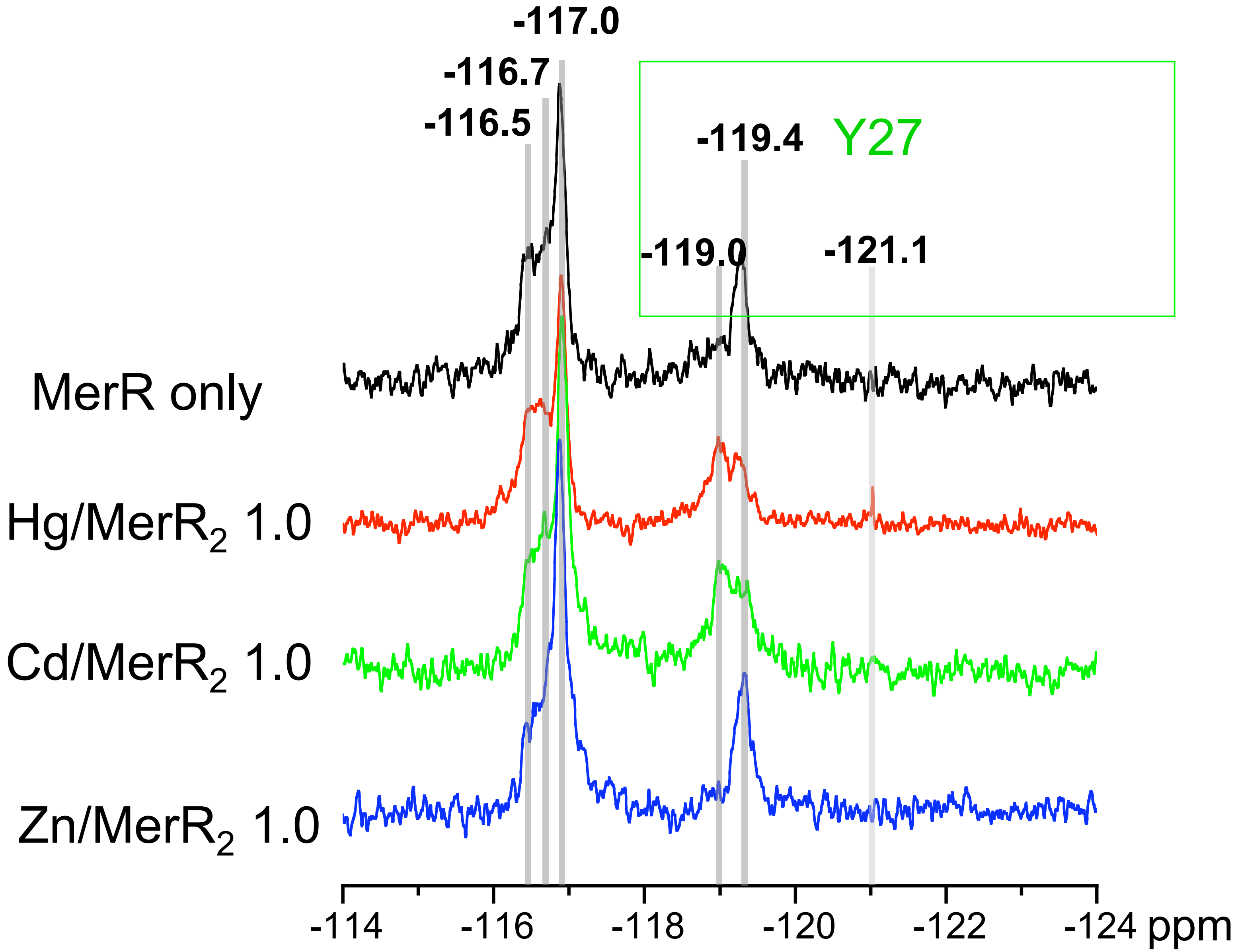
AR M ETVLSELVCA C HARKGNVS **C** P **LIAS** LQGEA

GLARSAMP



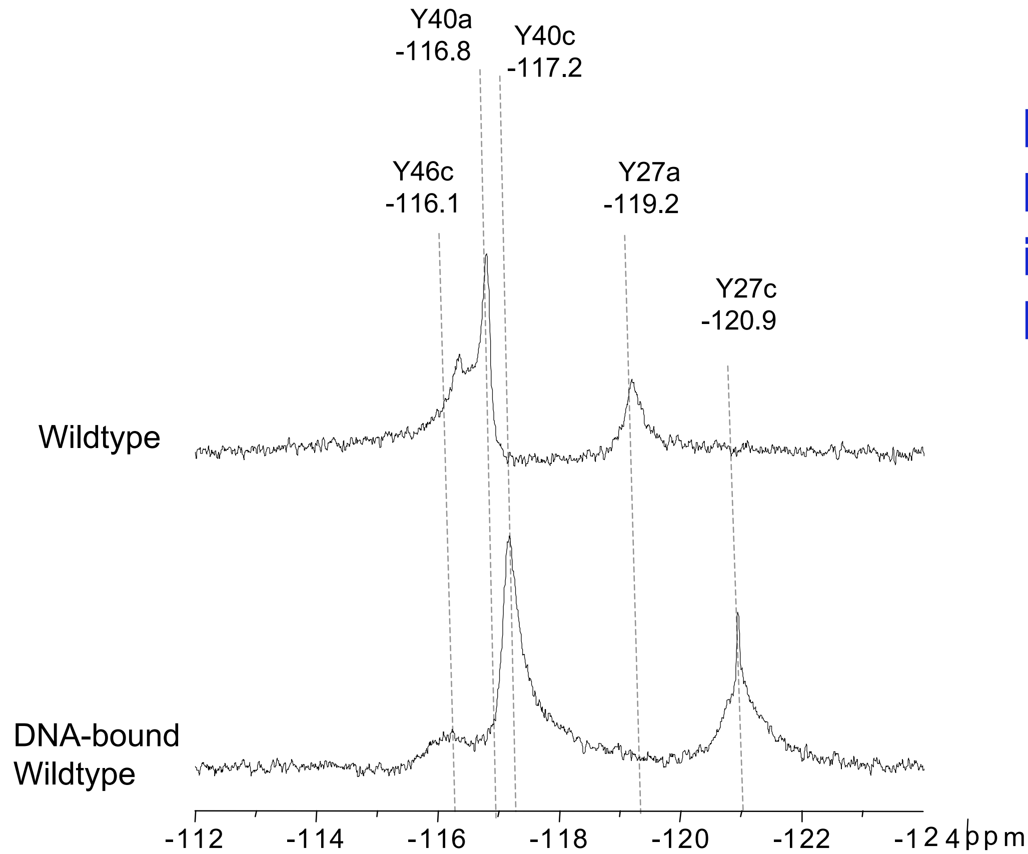


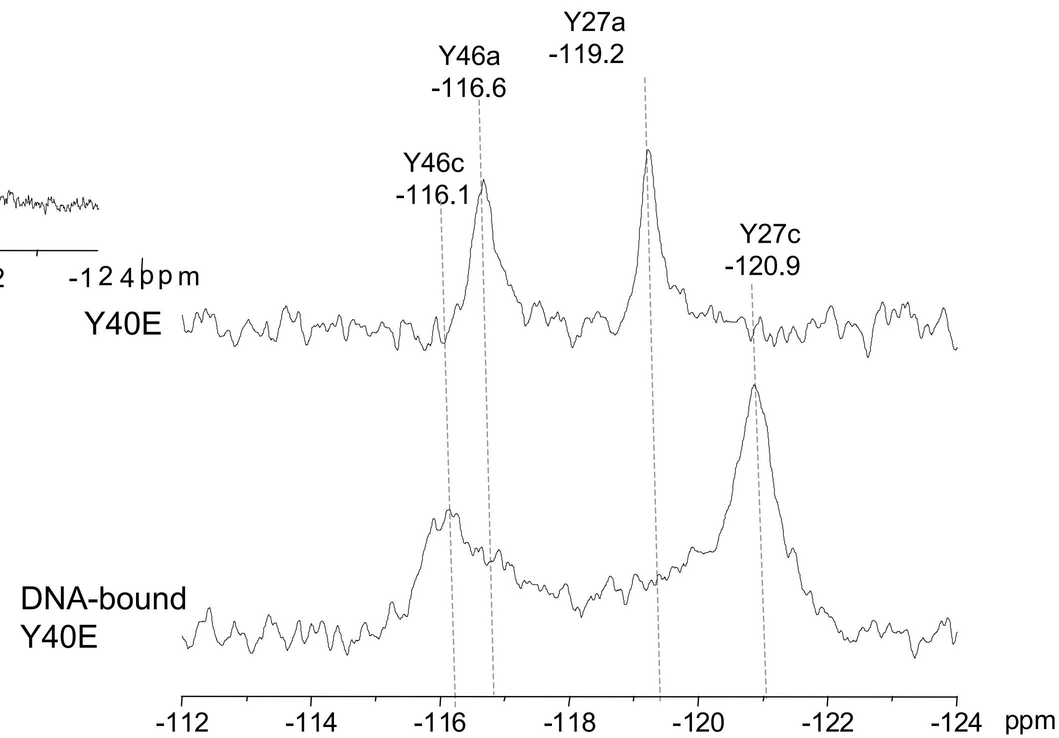
Using substitution mutants to assign resonances



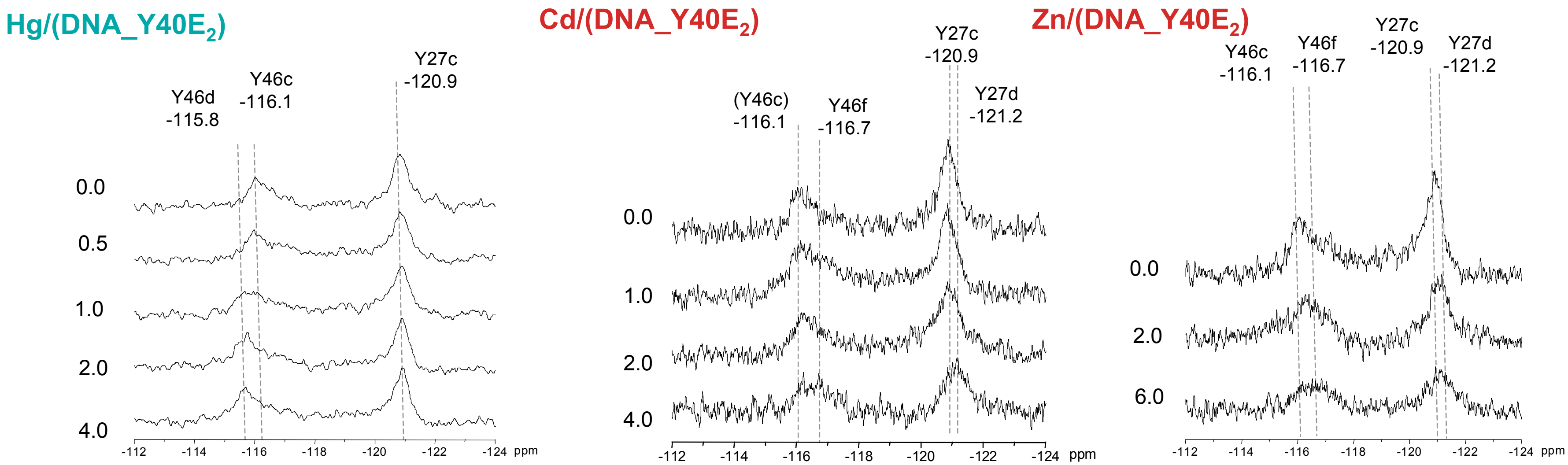
-117.0
-116.7
-116.5
-119.4
Y27
-119.0
-121.1
MerR only
Hg/MerR2 1.0
Cd/MerR2 1.0
Zn/MerR2 1.0
-114
-116
-118
-120
-122
-124 ppm

MerOP DNA binding produces large chemical shift changes in Y27 and Y46 of wildtype MerR and mutant Y40E





Metal-specific changes occur at Y27 and Y46 when MerR is bound to MerOP



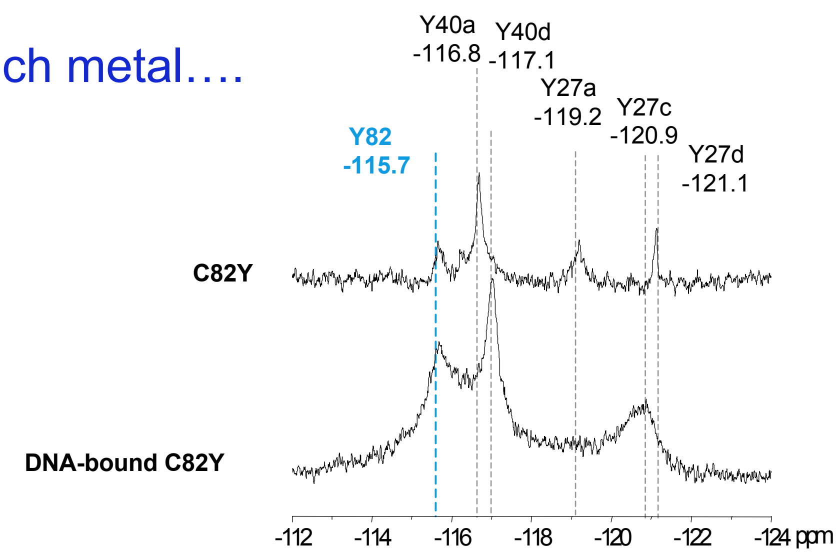
Y46, downfield
Y27, no change

Y46, upfield
Y27, slight upfield

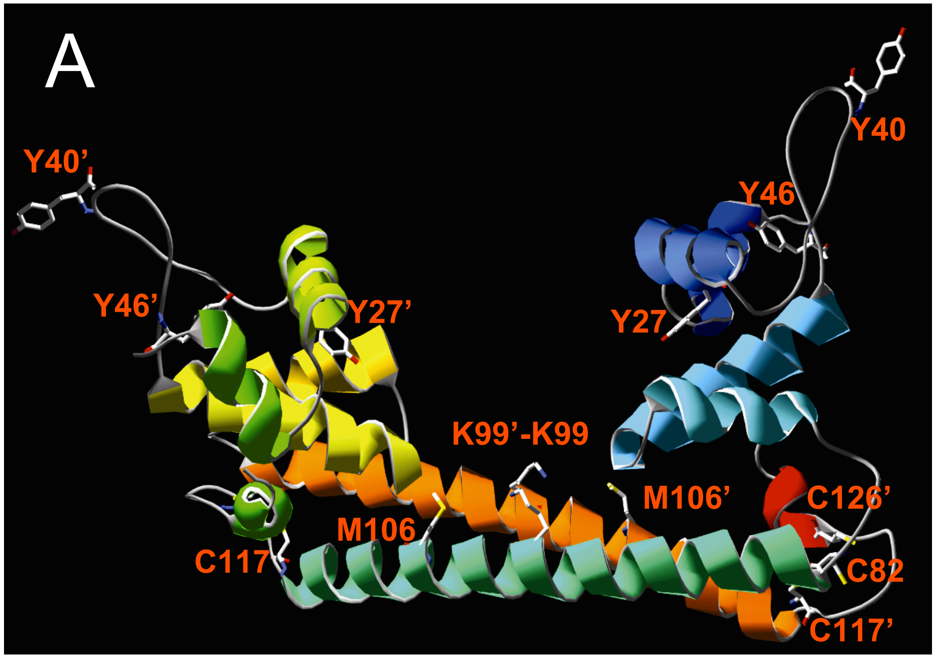
Y46, upfield
Y27, slight upfield

So DNA constrains MerR's response to each metal….

…and C82Y in the metal-binding site 'notices' DNA binding.



A Candidate Allosteric Signalling Pathway in MerR



B

α1 α2

MENNLENL **TIGVFAKAA** GVN **VETIRF Y QR** KGLLRE

α3

PDKP **Y** GSIRR **Y** GEAD **VVRVKFVKS AQRL** GFS **LDE**

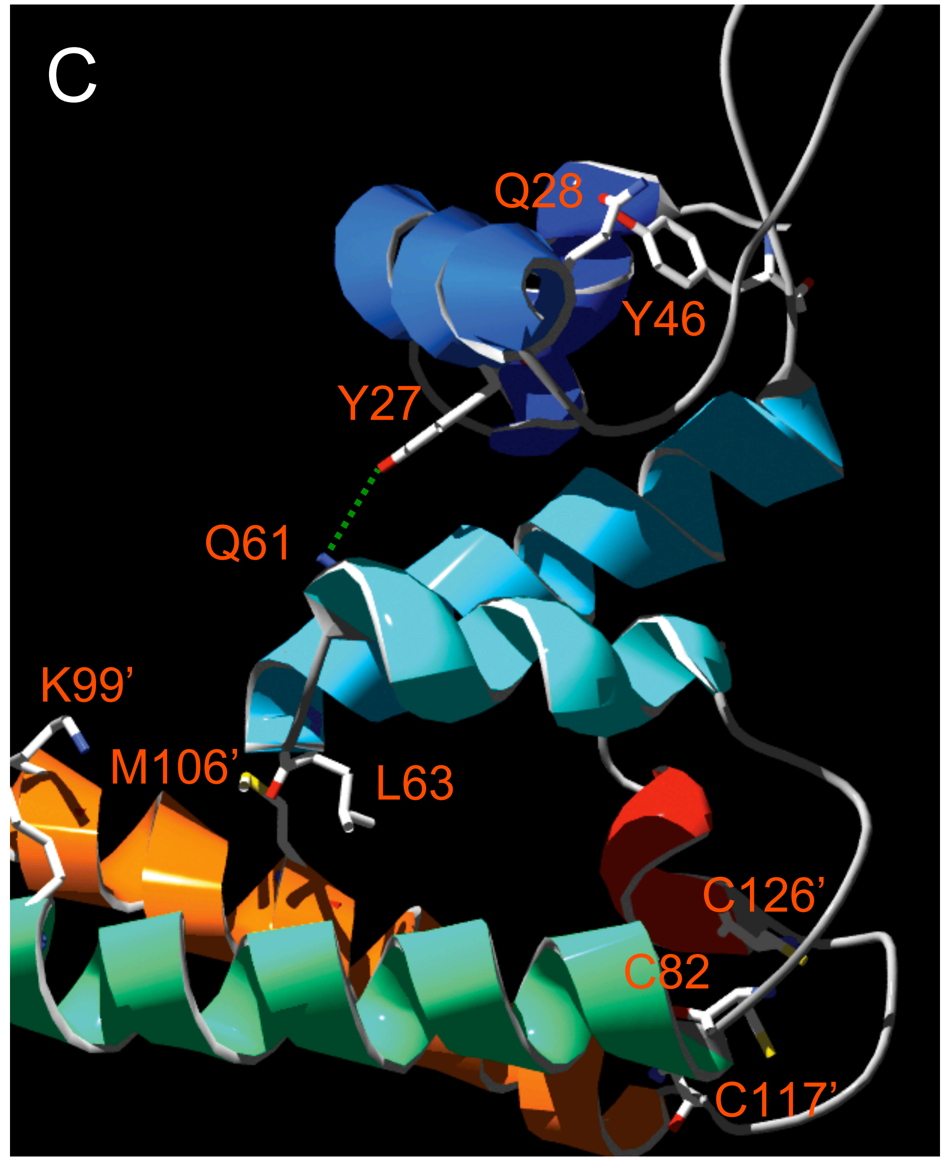
α4 α5

IAELLRL DDGTH **C EEASSLAEHKLKDVRE KMADL**

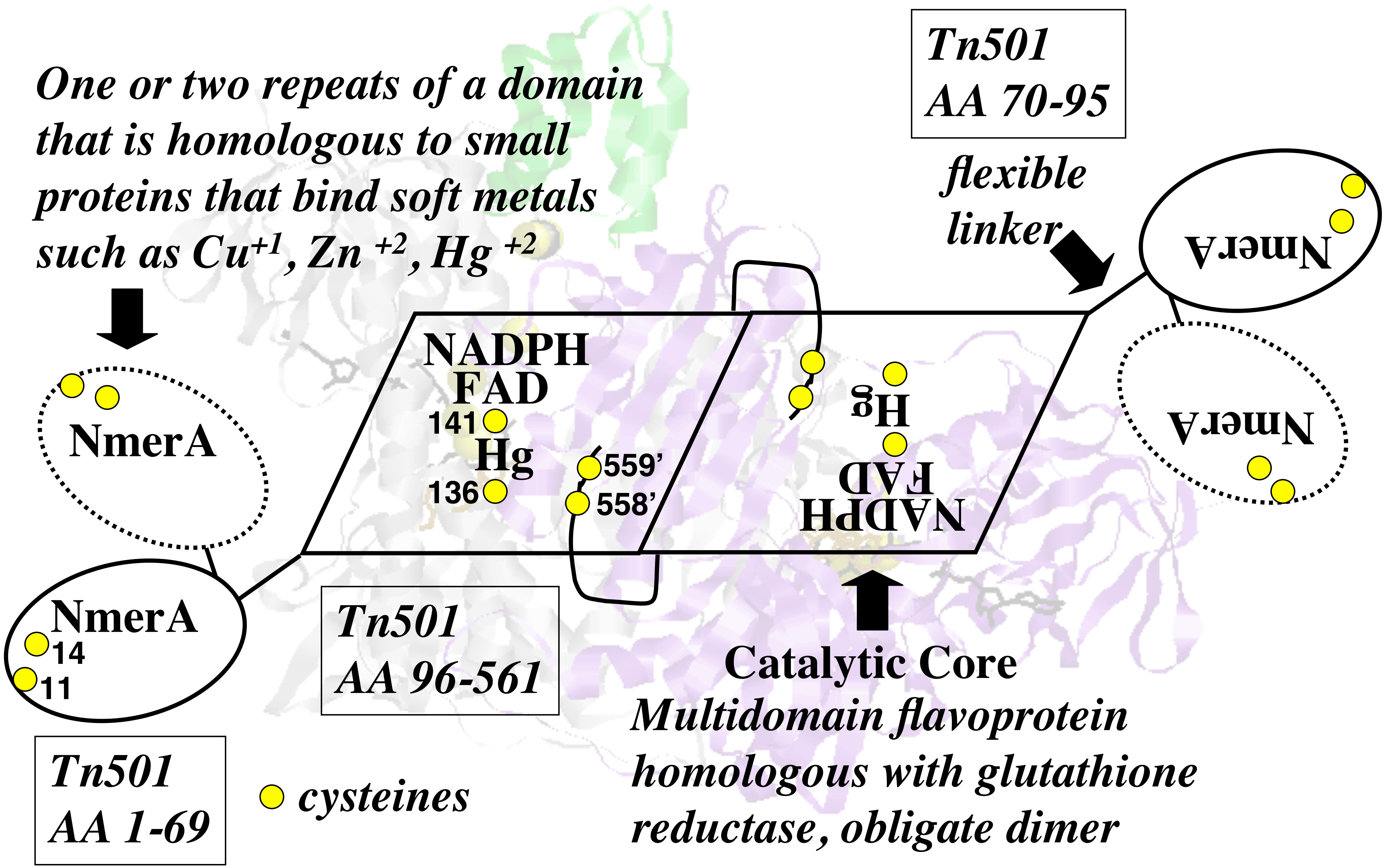
α5 α6

ARMETVLSELVCA C HARKGNVS **C** P **LIAS** LQGEA

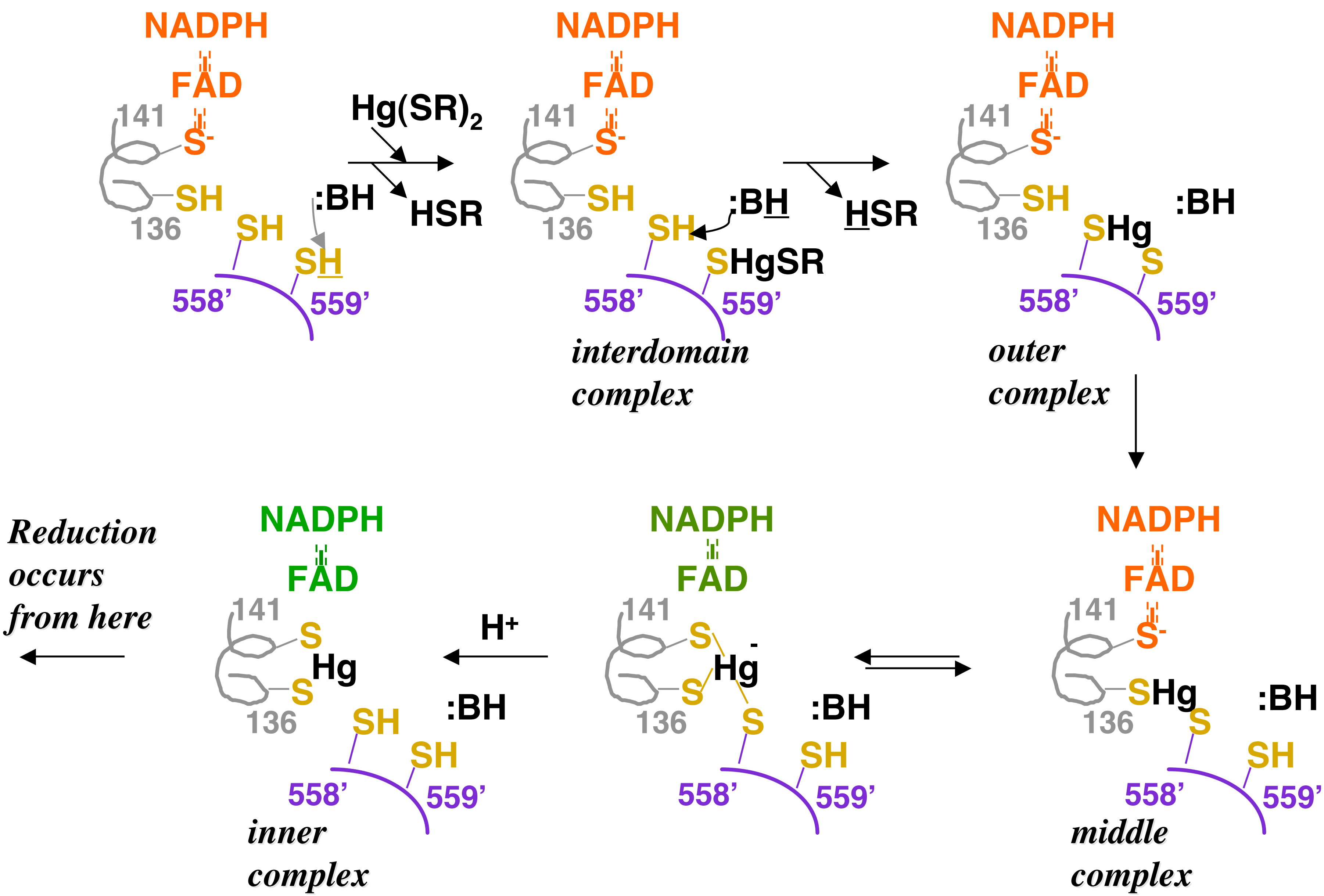
GLARSAMP



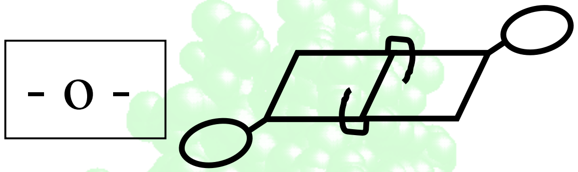
Typical Structural Components of MerA



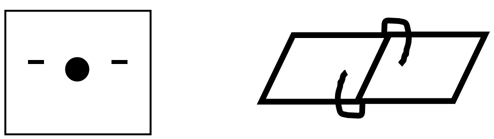
C-terminal CC Remove High Affinity RS^- Ligands



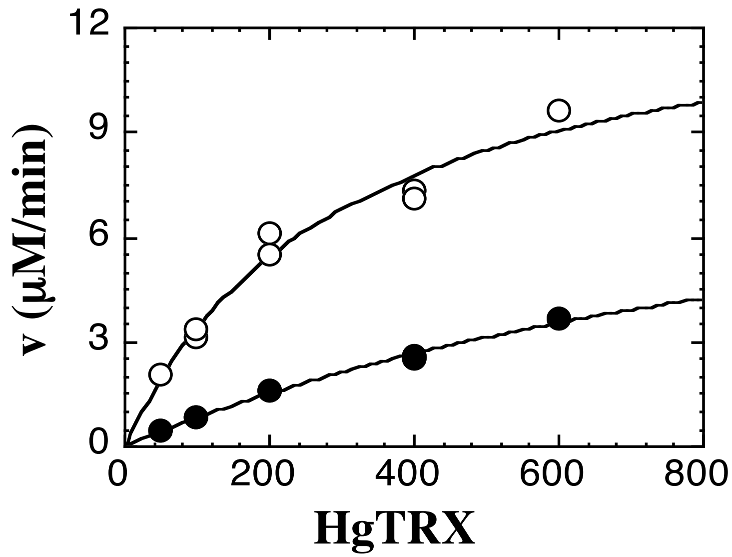
NmerA Facilitates Transfer from Hg-Thioredoxin in vitro



$k_{cat}/K_{MHg\text{-}TRX}$ =
$3.0 \times 10^{4}\ M^{-1}\ s^{-1}$
$K_{MHg\text{-}TRX} \sim 300\ \mu M$

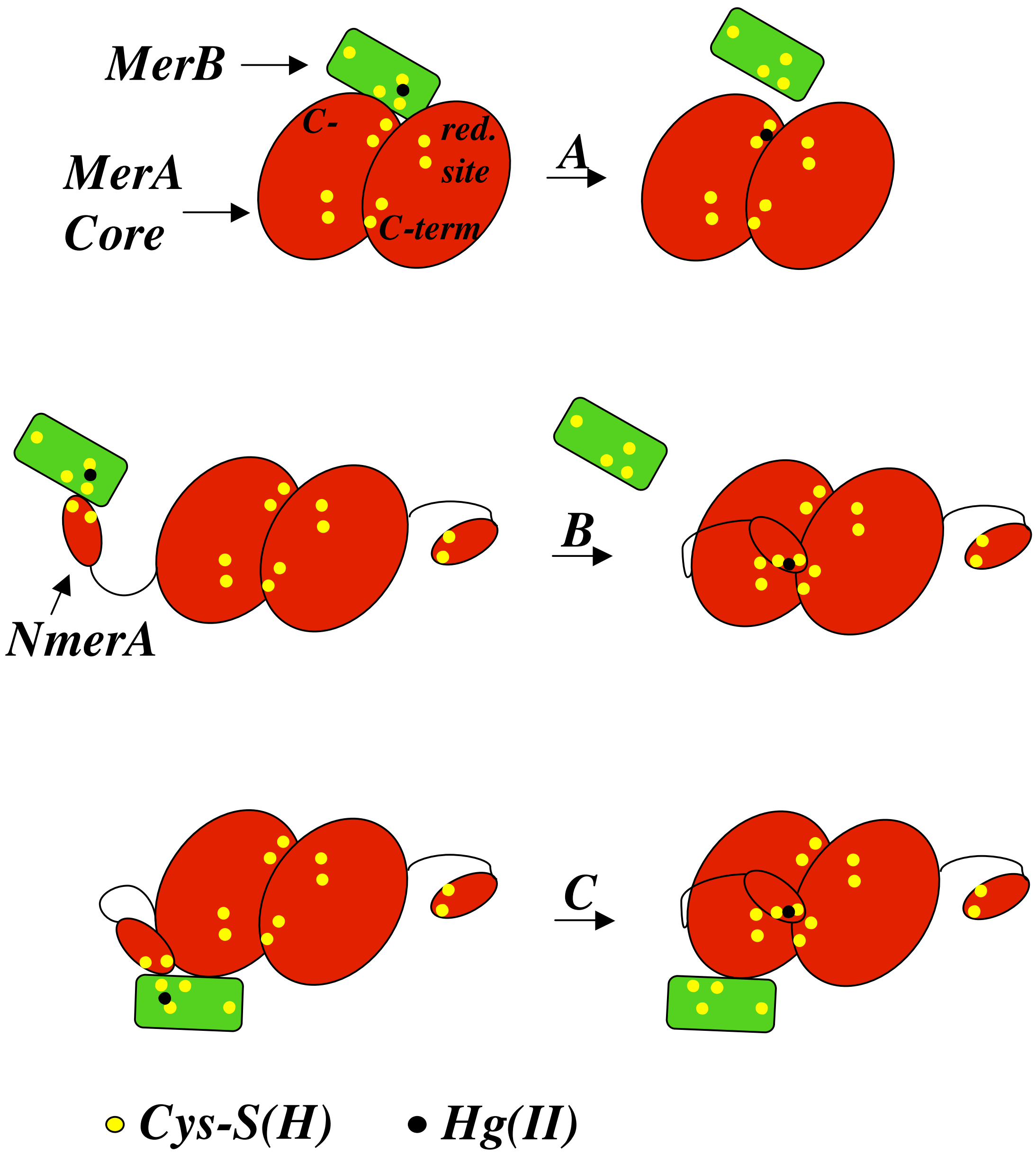


$k_{cat}/K_{MHg\text{-}TRX}$ =
$6.0 \times 10^{3}\ M^{-1}\ s^{-1}$
$K_{MHg\text{-}TRX} \sim 1200\ \mu M$



HgTRX + NADPH ----------->
Hg(0) + NADP^{+} + TRX

Potential Modes of MerB/MerA Interactions

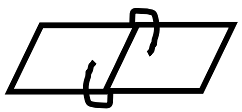


A) Transient interaction with direct transfer to Core C-terminal cysteines

B) Transient interaction and transfer to NmerA only

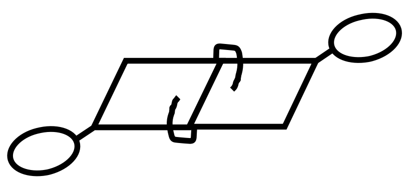
C) Stable complex with Core but transfer facilitated by NmerA

NmerA Facilitates Transfer from Hg-MerB

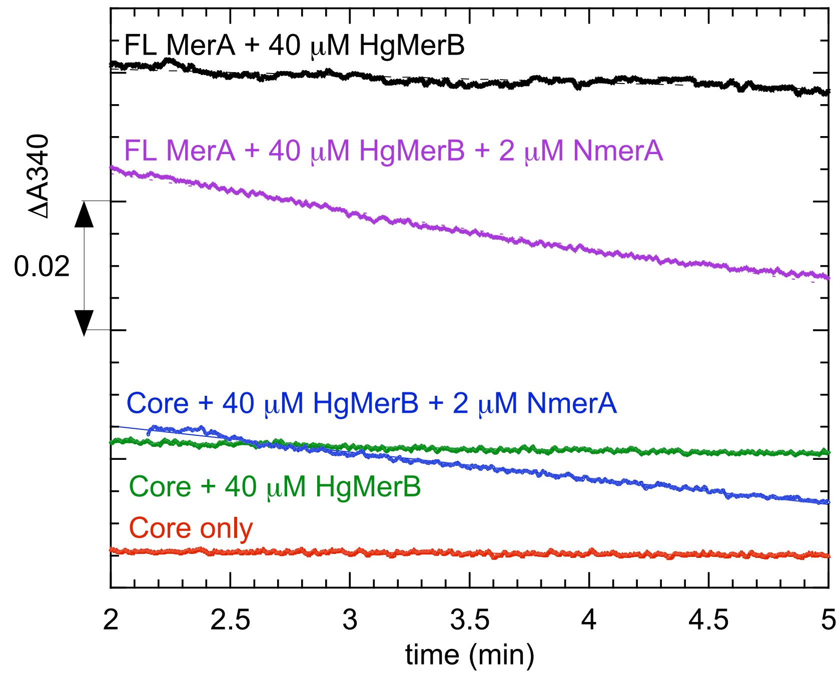


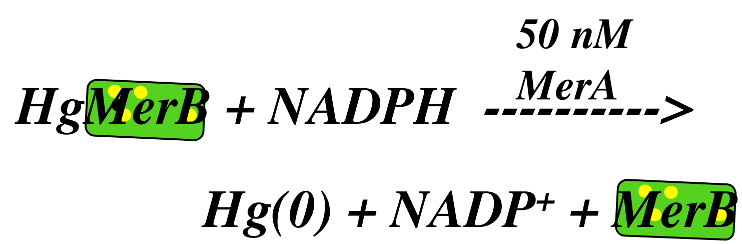
	0	*2 µM*
v_{rel}	*1**	*9.5*

**activity only 2-fold above background oxidase rate*



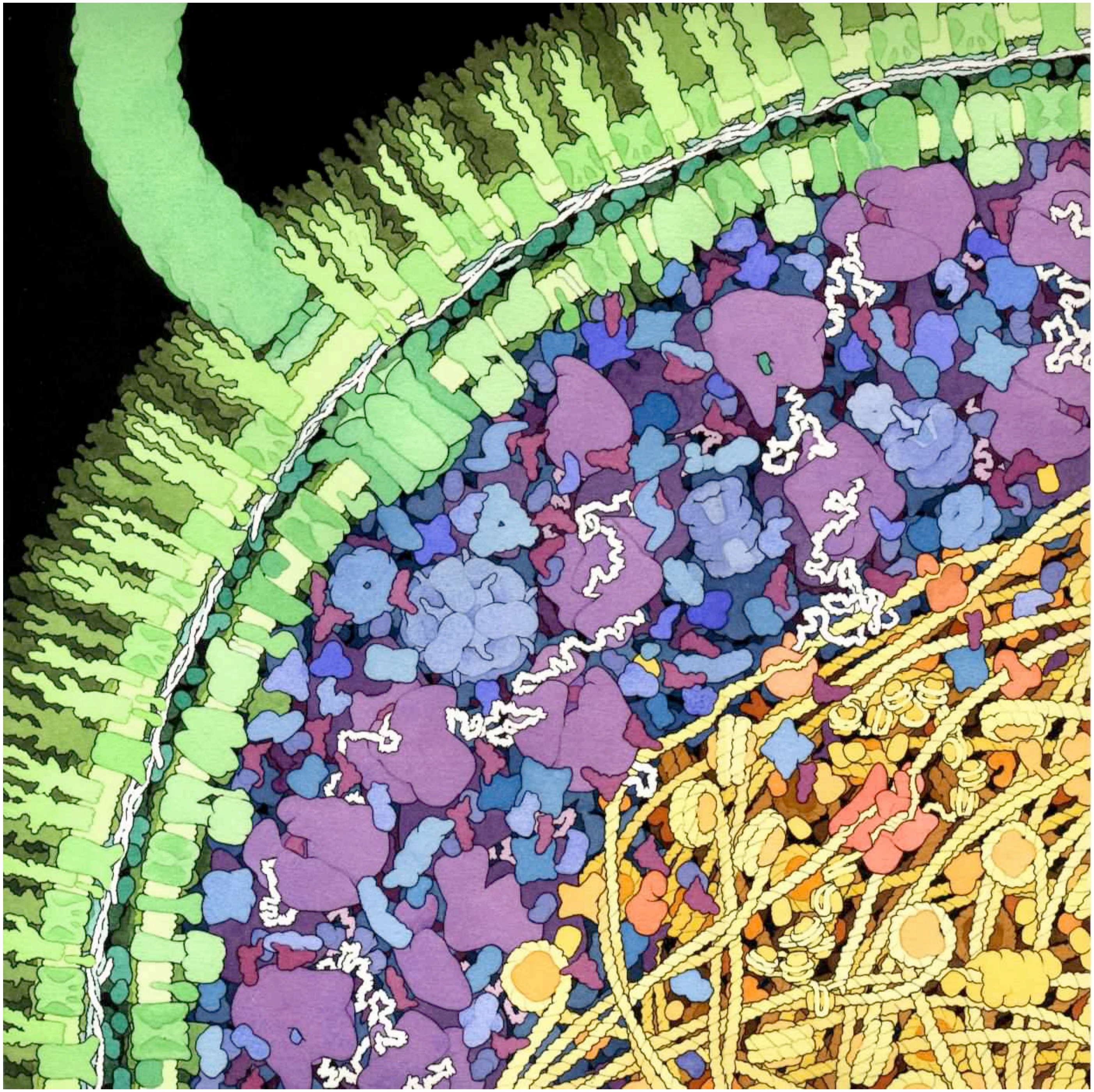
	0	*2 µM*
v_{rel}	*2*	*13.6*



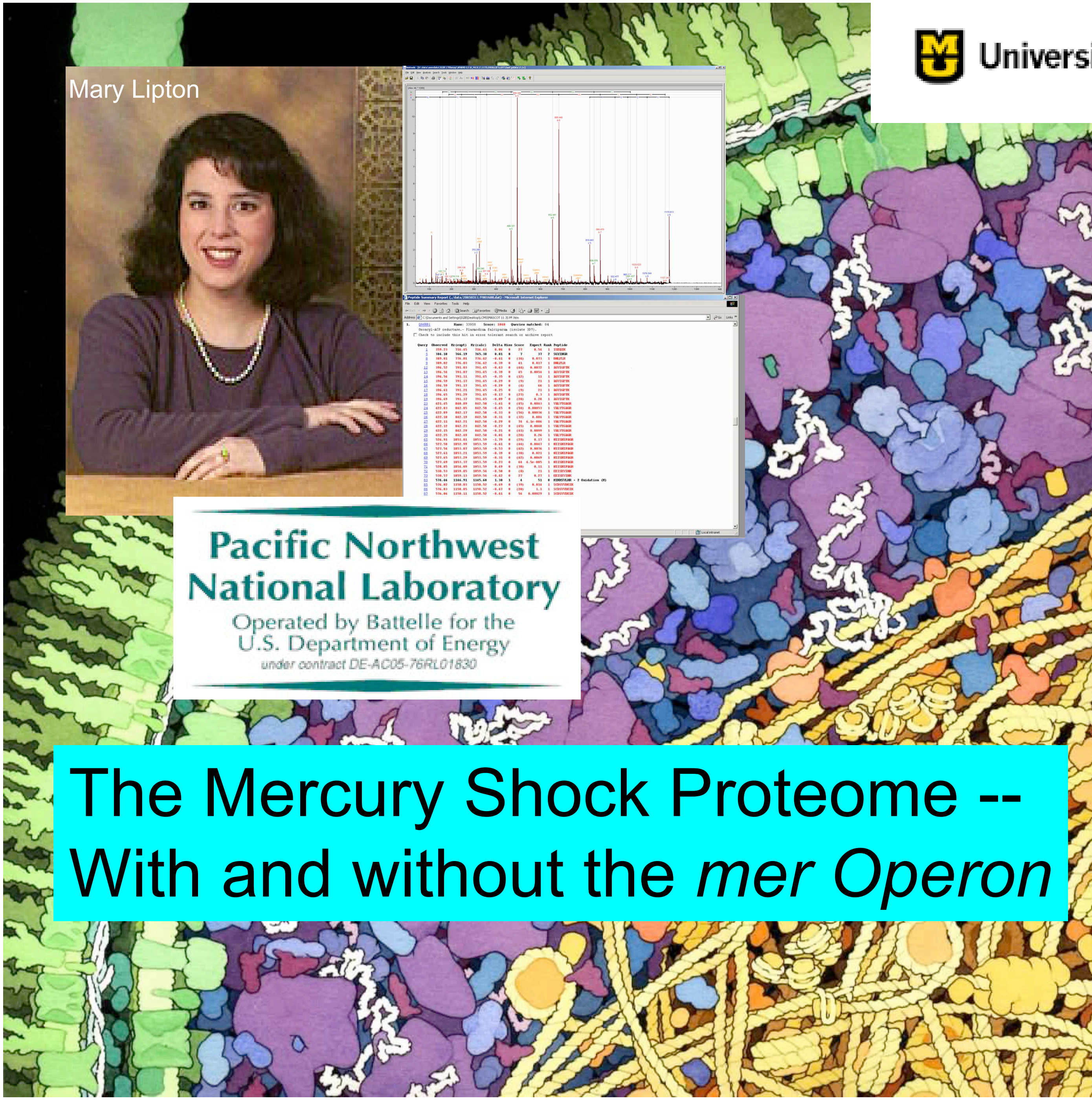


Consistent with Models B &/or C

Coming Attractions !!



Bacterial cell contents to scale.



Pacific Northwest National Laboratory

Operated by Battelle for the U.S. Department of Energy

under contract DE-AC05-76RL01830

The Mercury Shock Proteome -- With and without the *mer Operon*

University of Missouri-Columbia

Judy Wall, *Desulfovibrio*

Tom DiChristina, *Shewanella*

Georgia Tech