Structure/Function Analysis of Protein/Protein Interactions and Role of Dynamic Motions in Mercuric Ion Reductase

$Hg(SR)_2 + NADPH + H^+ \text{--->} Hg(0) + NADP^+ + 2RSH$

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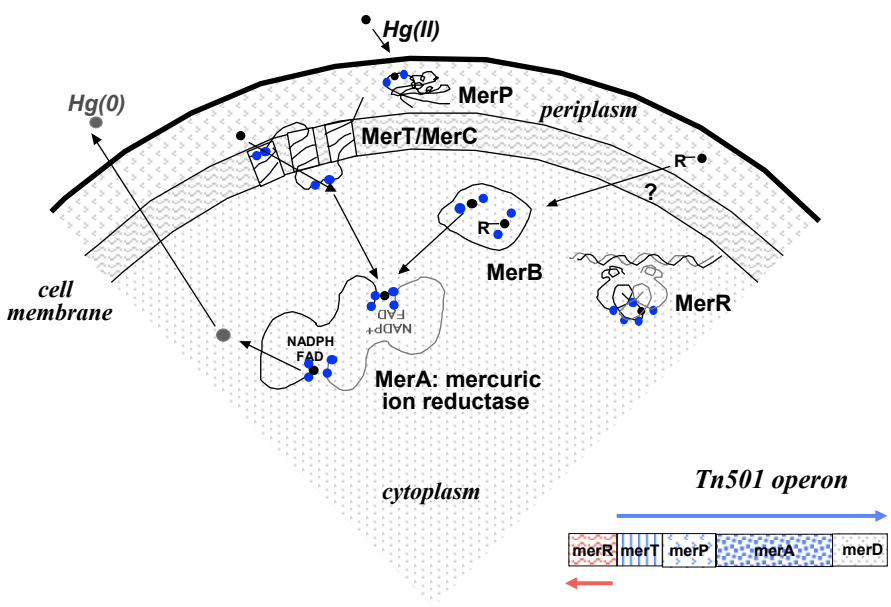
Emil Pai Lab — *U. Toronto*

Anne Summers Lab — *U. Georgia*

Bacterial Detoxification of Hg(II) and Organomercurials



Two MerA "Domains": Catalytic Core and NmerA

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HUM-GR  ------------------------------------------------------------
BacMerA MKKYRVNVQGMTCSGCEQHVAVALENMGAKAIEVDFRRGEAVFELPDDVKVEDAKNAI
501MerA ------------------------------------------------------------

HUM-GR  ------------------------------------------------------------
BacMerA HPGEAEEFQSEQKTNLLKKYRLNVEGMTCTGCEEHIAVALENA-GAKGIEVDFRRGEAL
501MerA -----------------MTHLKITGMTCDSCAAHVKEALEKVPGVQSALVSYPKGTAQLAIV

                                                                  ↓ start of
                                                                  catalytic core
HUM-GR  -------------------------------MACRQEPQPQGPPPAAGAVASYDYLVIGGGS
BacMerA YDVDIDIAKTAITDAQYQPGEAEEIQVQSEK---------RTDVSLNDEGNYDYDYIIIGSGG
501MerA PGTSPDALTAAVAGLGYKATLADAPLADNRVGLLDKVRGWMAAAEKHSGNEPPVQVA
                                  : .:*****

HUM-GR  GGLASARRAAELGARAAVVESHKLGGTCVNVGCVPKKVMWNTAVHSEFMHDH-ADYG
BacMerA AAFSSAIEAVALNAKVAMIERGTVGGTCVNVGCVPSKTLLRAGEINHLAKNN-PFVG...
501MerA AAMAAALKAVEQGAQVTLIERGTIGGTCVNVGCVPSKIMIRAAHIAHLRRESPFDGG...
     **:::**.**  .*:*::*****:************ :***. * ** :    *
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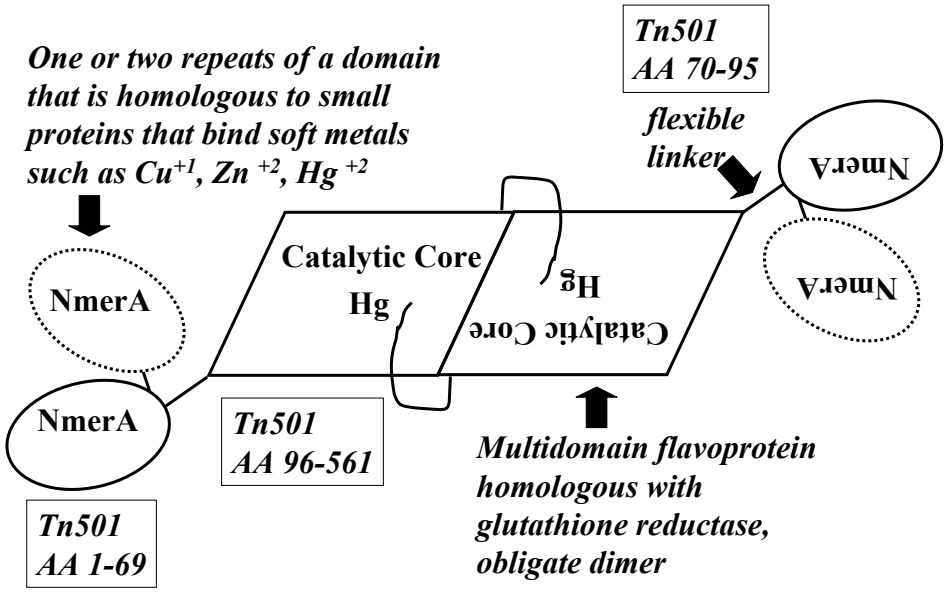
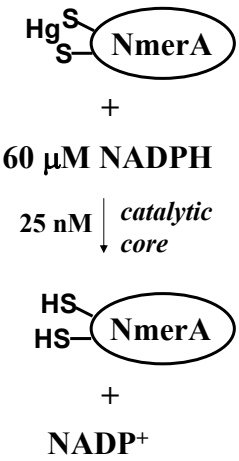
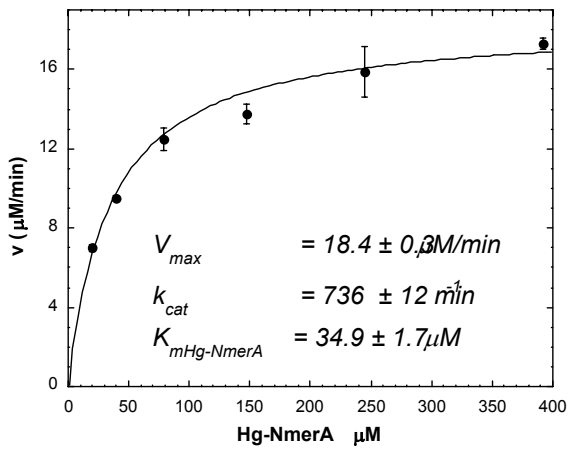
Typical Structural Components of MerA

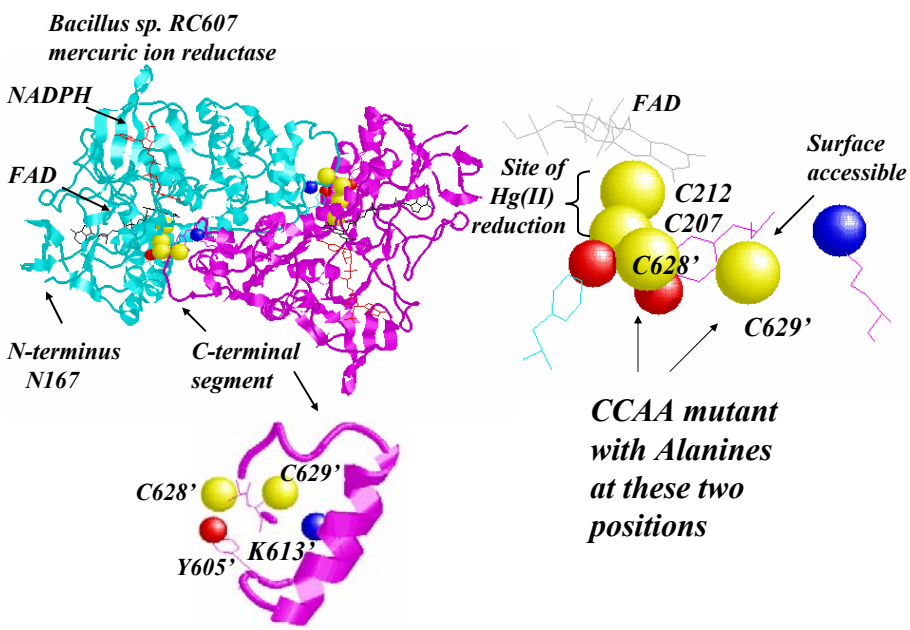


NmerA does bind Hg(II) and donate it to the catalytic core!

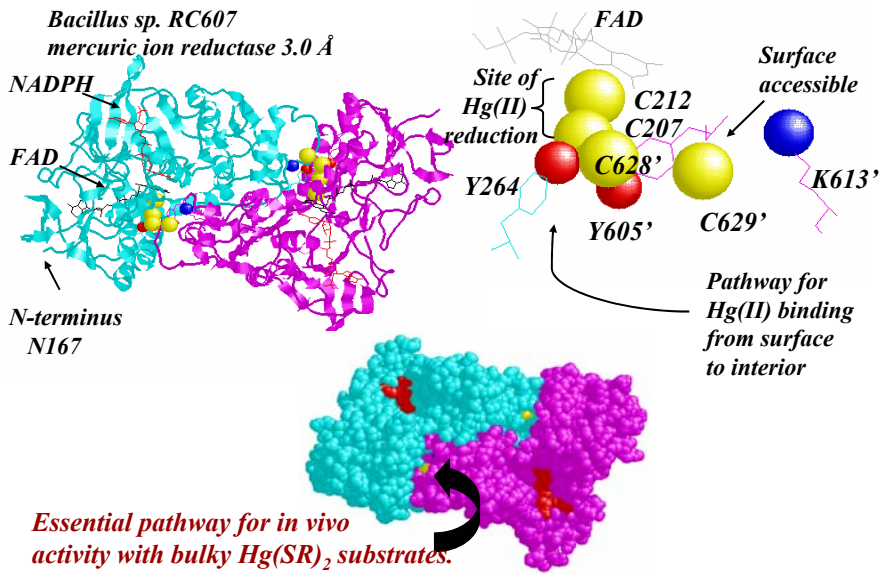




Catalytic Core of MerA

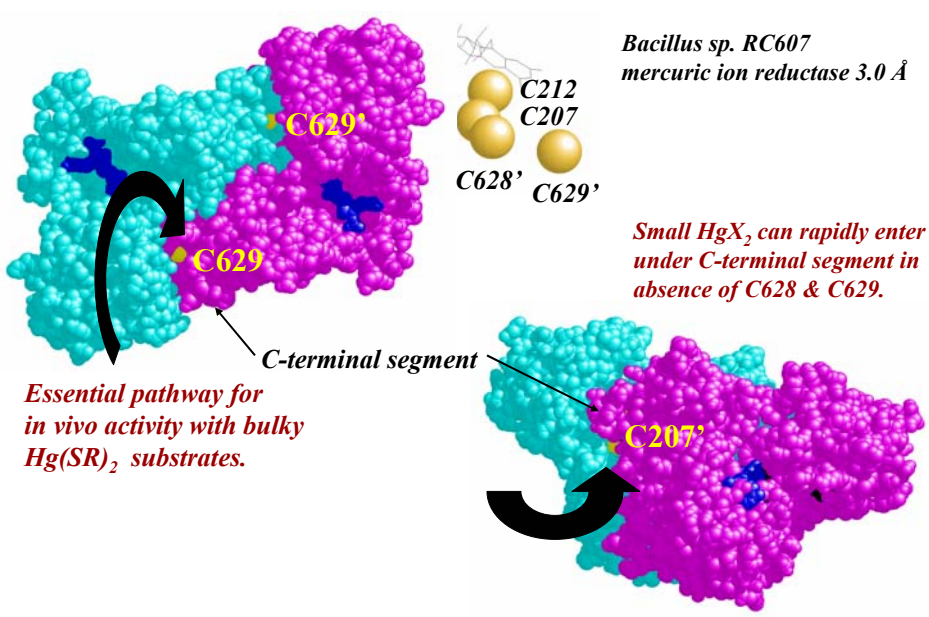


Catalytic Core of MerA



Essential pathway for in vivo activity with bulky $Hg(SR)_2$ substrates.

Entryways for Hg(II) in MerA

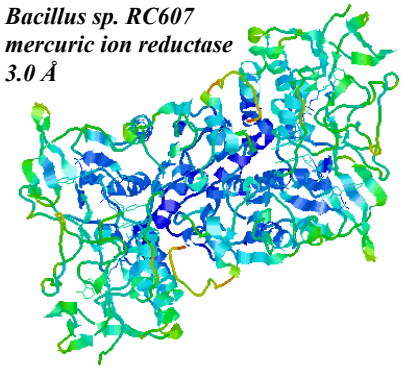


Small HgX_2 can rapidly enter under C-terminal segment in absence of C628 & C629.

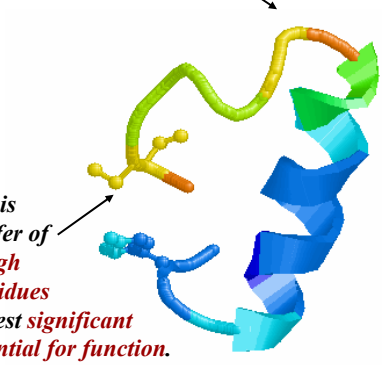
Essential pathway for in vivo activity with bulky $Hg(SR)_2$ substrates.

Dynamic Motion of C-Terminal Segment May be Critical for Rapid Transfer of Hg(II) into Active Site and for Release of Hg(0)

Bacillus sp. RC607
mercuric ion reductase
3.0 Å



High B-factors at beginning of C-terminal segment suggest a pivot point for rapid segmental motion that may be critical for binding of Hg(II) &/or release of Hg(0).

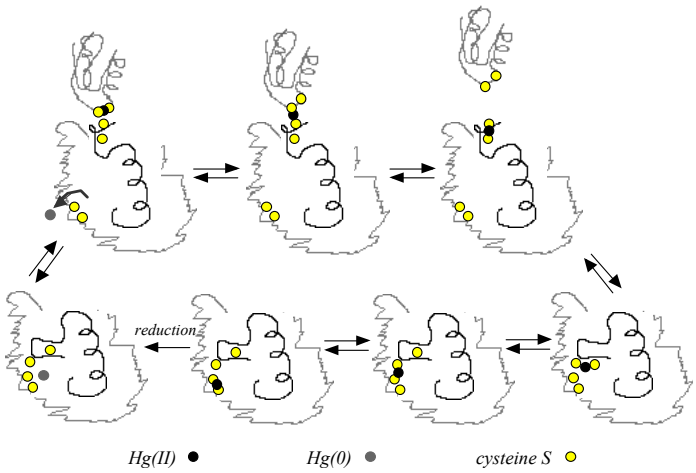


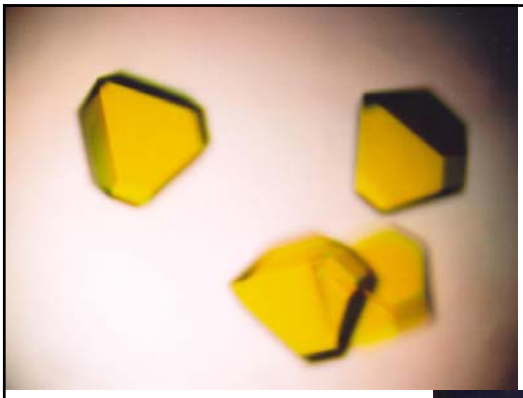
flexible

rigid

Orientation of C628 & C629 is inconsistent with rapid transfer of Hg(II) into active site, but high B-factors of C-terminal 4 residues including C628 & C629suggest significant flexibility is present and essential for function.

Proposed Domain Interactions and Dynamic Motion During Catalysis





← **CCAA**

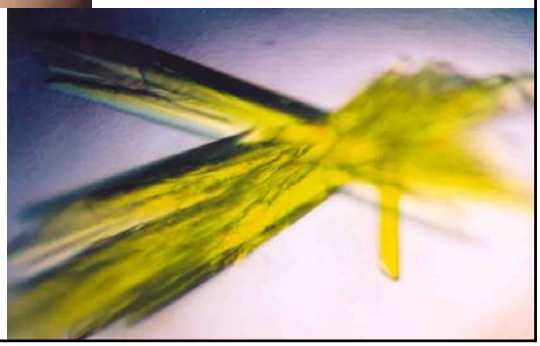
0.1 M Tris, pH 8.5
+ 2 M $(NH_4)_2SO_4$

First data set to 1.6 Å, but another set to <u>at least</u> 1.3 Å

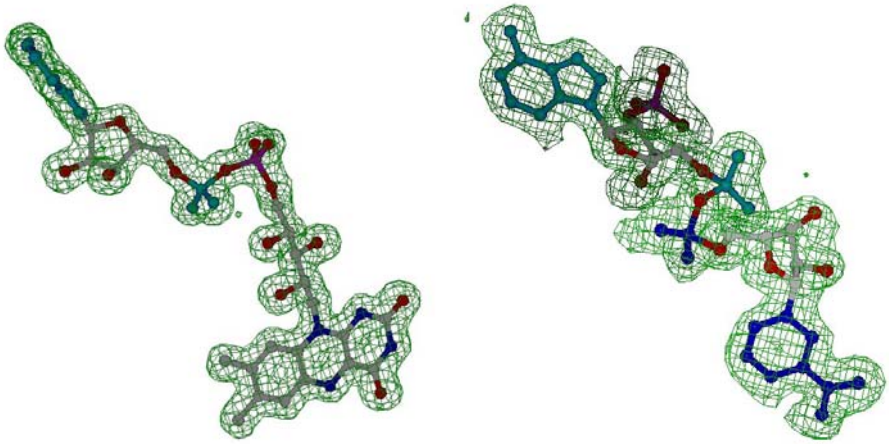
CCCC →

0.1 M NaAc, pH 4.4
+ 14% PEG

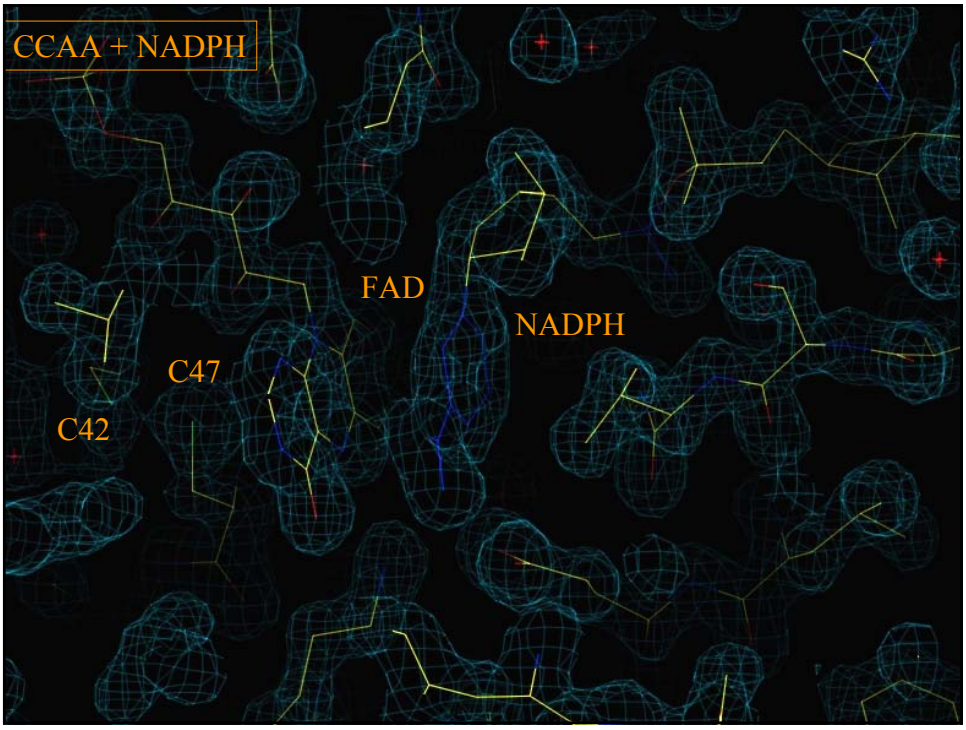
First data set to 1.9 Å, but expect better at a different light source.

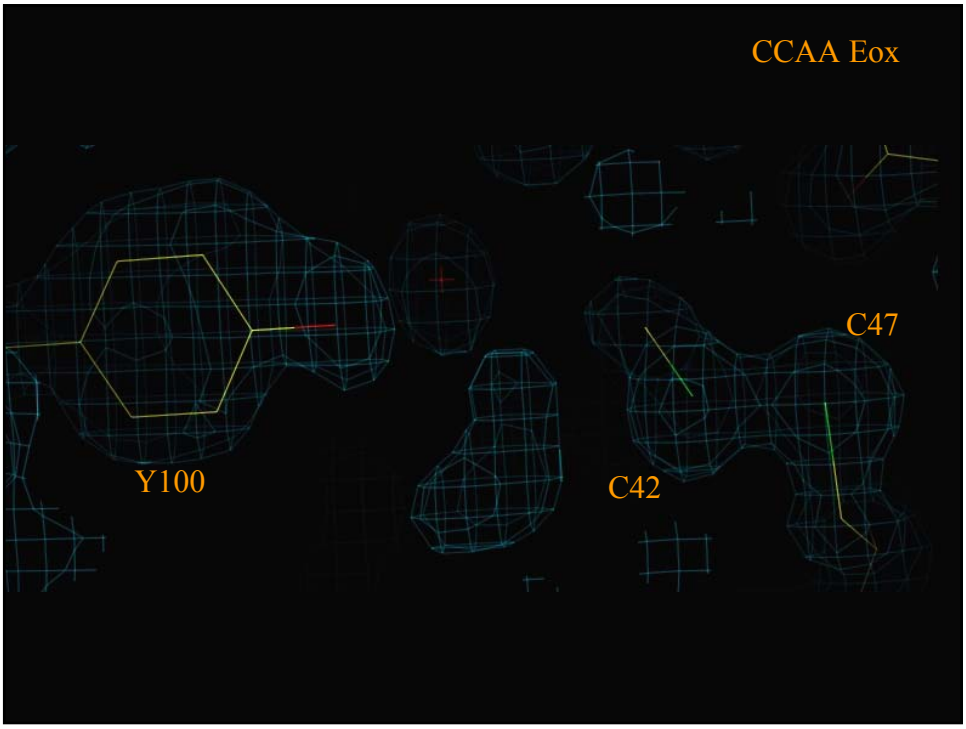


Electron Density for FAD and NADPH in CCAA



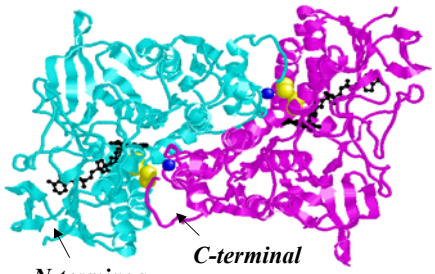
From structure with 1.6 Å resolution. Density at 0.7s.
FAD is tightly bound cofactor, NADPH is substrate.


CCAA + NADPH
FAD
NADPH
C47
C42


CCAA Eox
C47
Y100
C42

C-Terminal Segment of Tn501 Core May Be Even More Flexible...

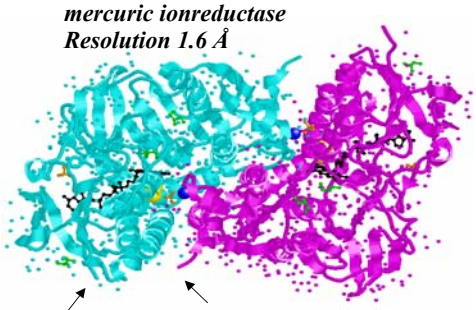
Bacillus sp. RC607
mercuric ionreductase
Resolution 3.0 Å



N-terminus
N167

C-terminal
segment

Tn501 CCAA core
mercuric ionreductase
Resolution 1.6 Å

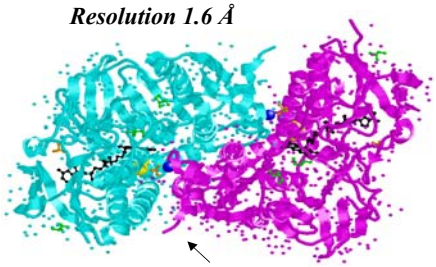


N-terminus
M1

Last visible residue:
458 instead of 467.

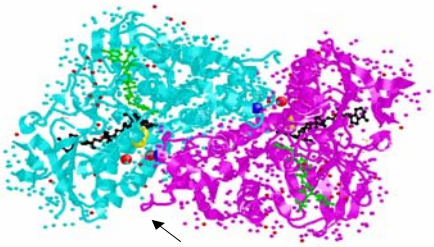
…but Less Flexible In the Reduced Protein

Tn501 oxidized CCAA core
mercuric ionreductase
Resolution 1.6 Å



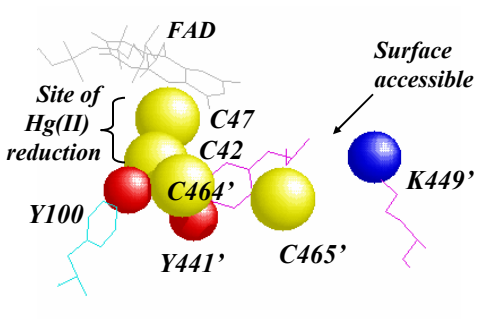
Last visible residue:
458 instead of 467.

Tn501 reduced CCAA core
mercuric ionreductase
Resolution 1.6 Å



Last visible residue:
462 instead of 467.

"Proton" Mutants of MerA



Bacillus = Tn501 = core

Y264 = Y193 = Y100
Y605 = Y534 = Y441
K613 = K542 = K449

$Hg(SR)_2 + NADPH + H^+ \xrightarrow{xs\ Cys} Hg(0) + NADP^+ + 2RSH$

Preliminary Steady-state Results

Y441F — k_{cat} & k_{cat}/K_m ↓ 5-10-fold

Y100F — k_{cat} & k_{cat}/K_m ↓ at least 100-fold

K449A — High oxidase but no measurable Hg(II) reductase activity

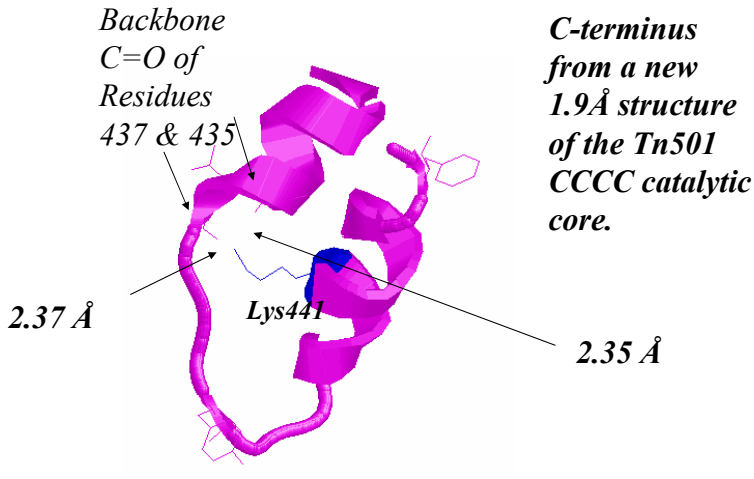
Preliminary Results from Presteady-state Kinetic Studies

EH_2•NADPH
+ 1 eq NADPH

varied xs $Hg(Cys)_2$

	Hg(II) binding	Hg(II) reduction
WT	fast	5 s^{-1}
Y441F	fast or faster	5 - 7 s^{-1}
Y100F	very slow	0.05 s^{-1}
K449A	Slow similar to CCAA	?

Does the Lysine Have a Structural Rather than Chemical Role



C-terminus from a new 1.9Å structure of the Tn501 CCCC catalytic core.

Credits

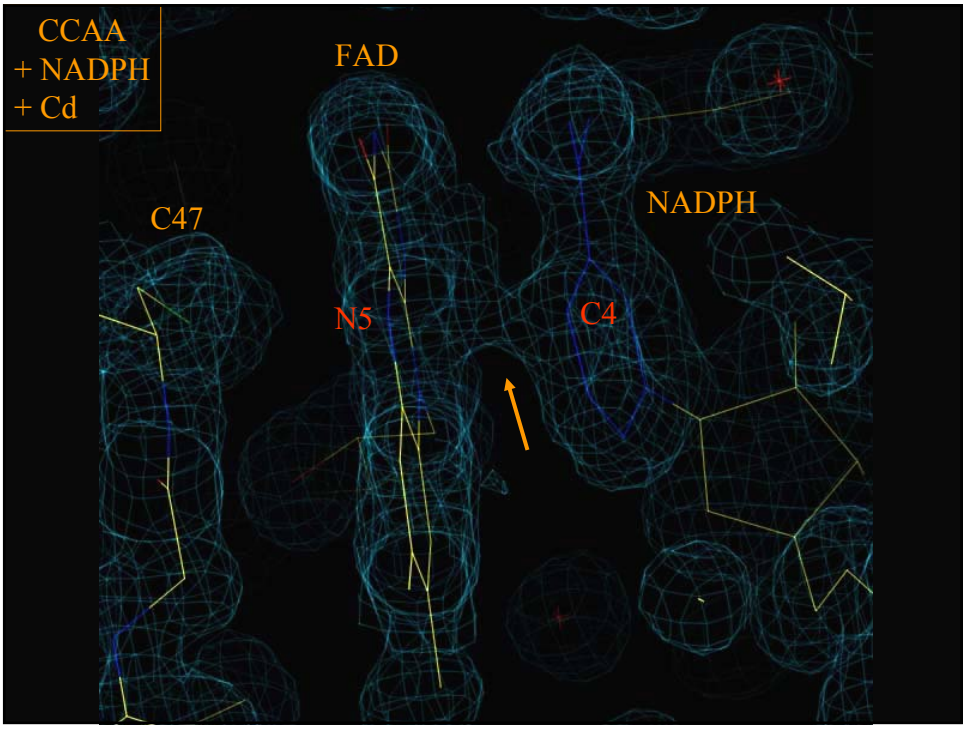
Mercuric Ion Reductase

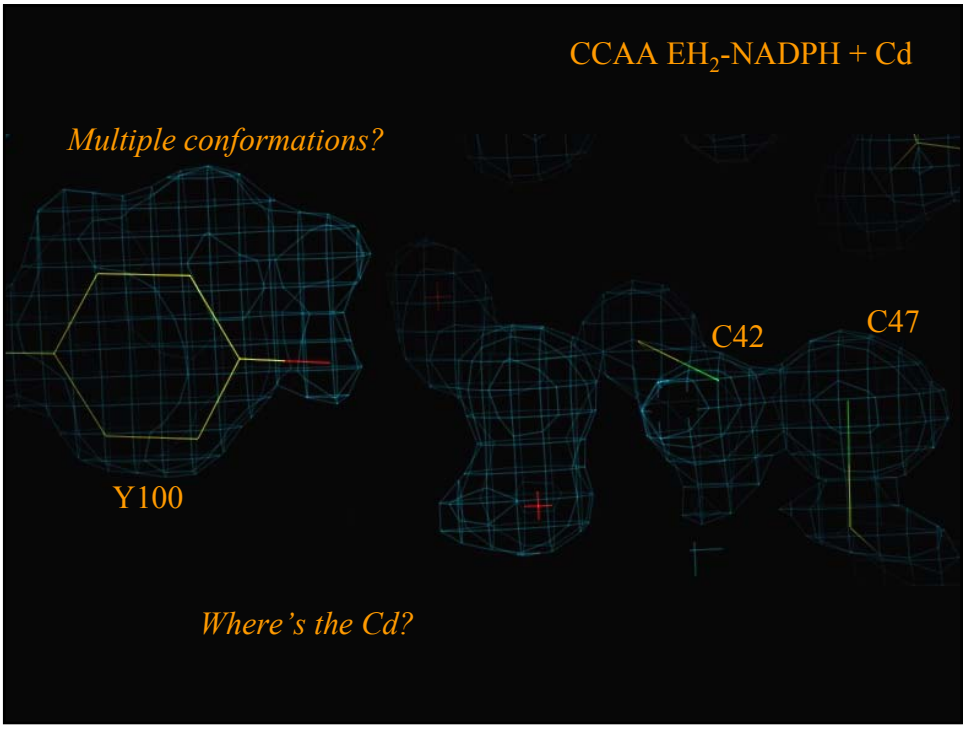
Miller Lab-UCSF
Mat Falkowski
Richard Ledwidge
Melissa Malone
Lisa Van Hoozer
Stefan Engst
Lisa Kim-Shapiro

U. Toronto
Collab. Emil Pai
Aiping Dong

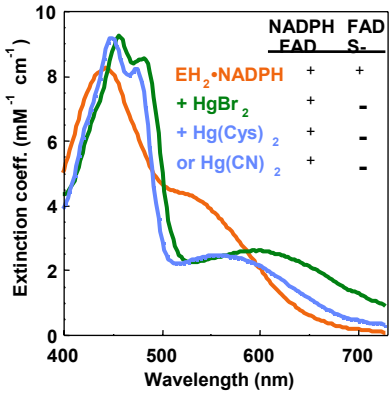
U. Georgia
Collab. Anne Summers
Jane Zelikova

DOE, NSF


CCAA
+ NADPH
+ Cd
FAD
C47
NADPH
N5
C4


CCAA EH_2-NADPH + Cd
Multiple conformations?
C42
C47
Y100
Where's the Cd?

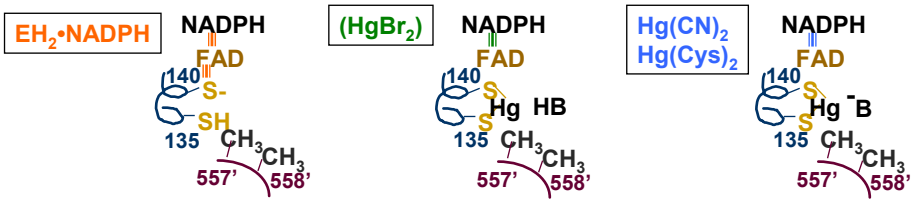
The CT λ_{max} is a Sensor of Active Site Charge



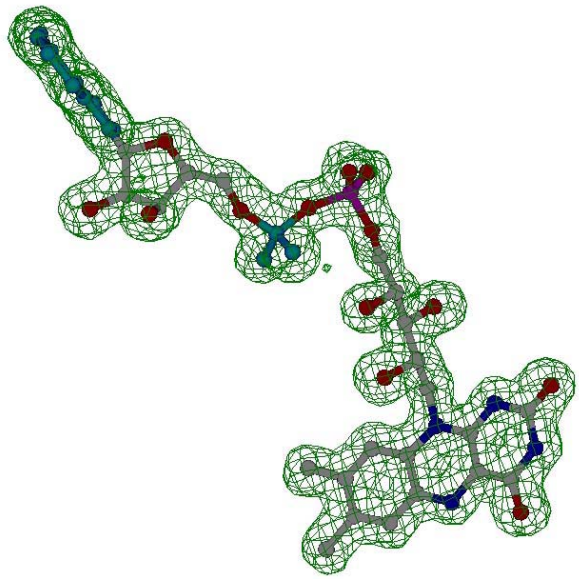
Results with CCAA mutant

Reducible!

Not reducible!

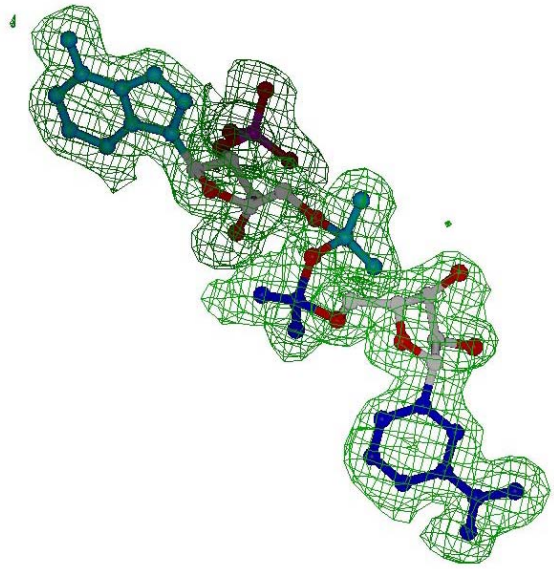


Electron Density for FAD in CCAA

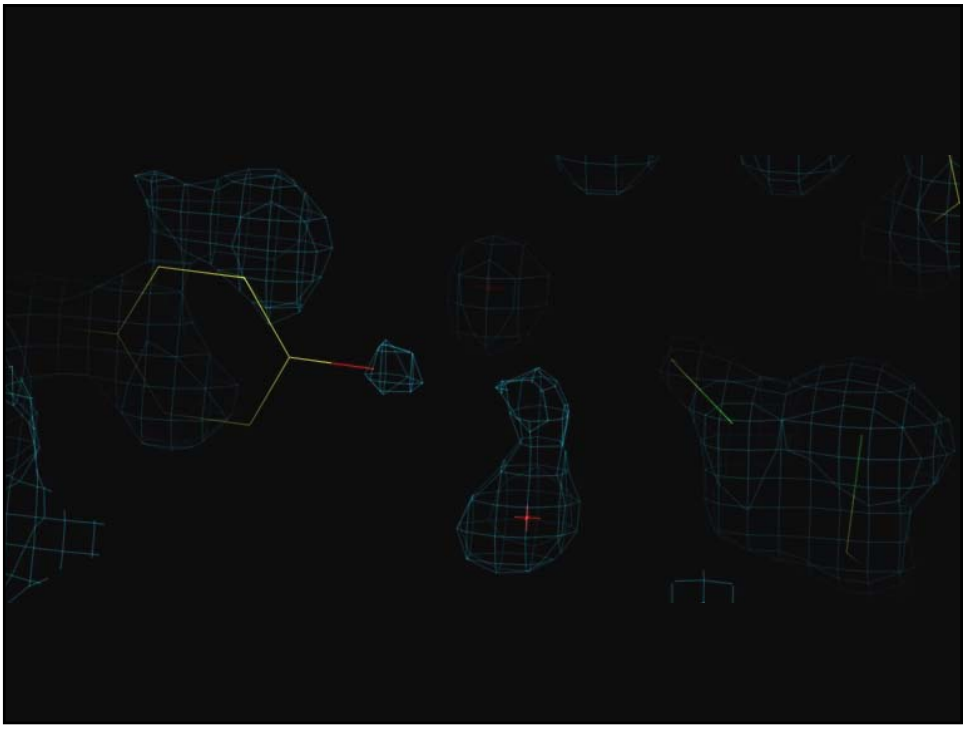
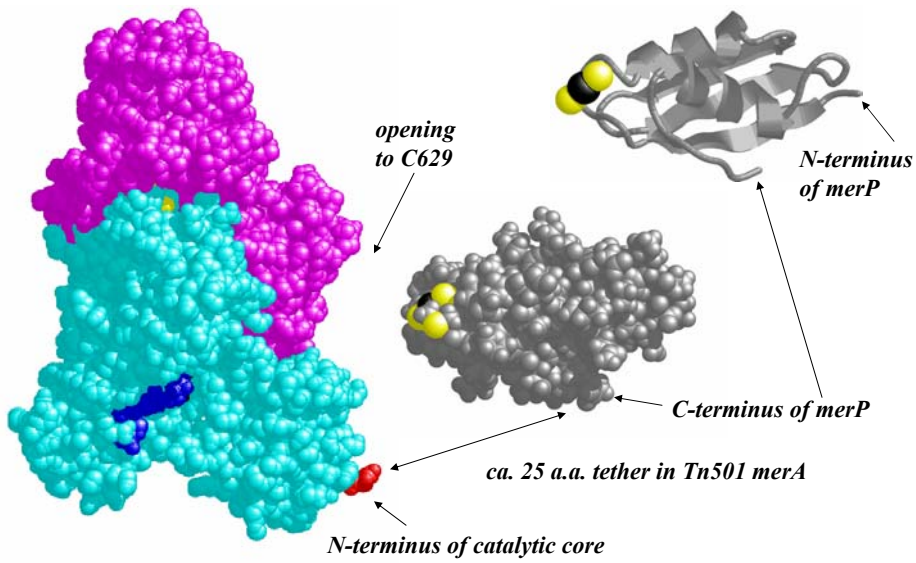


(0.7σ)

Electron Density for NADPH bound to CCAA



(0.7σ)



Is the metal binding domain, NmerA the "real" substrate?