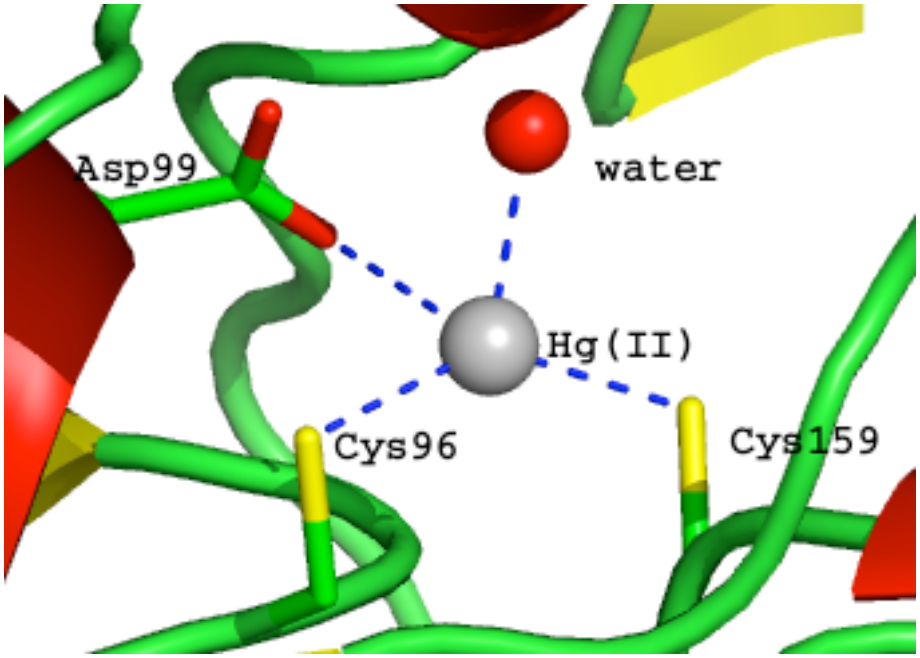
Quantum chemical studies of a bacterial mercury resistance enzyme: The demethylation mechanism of MerB



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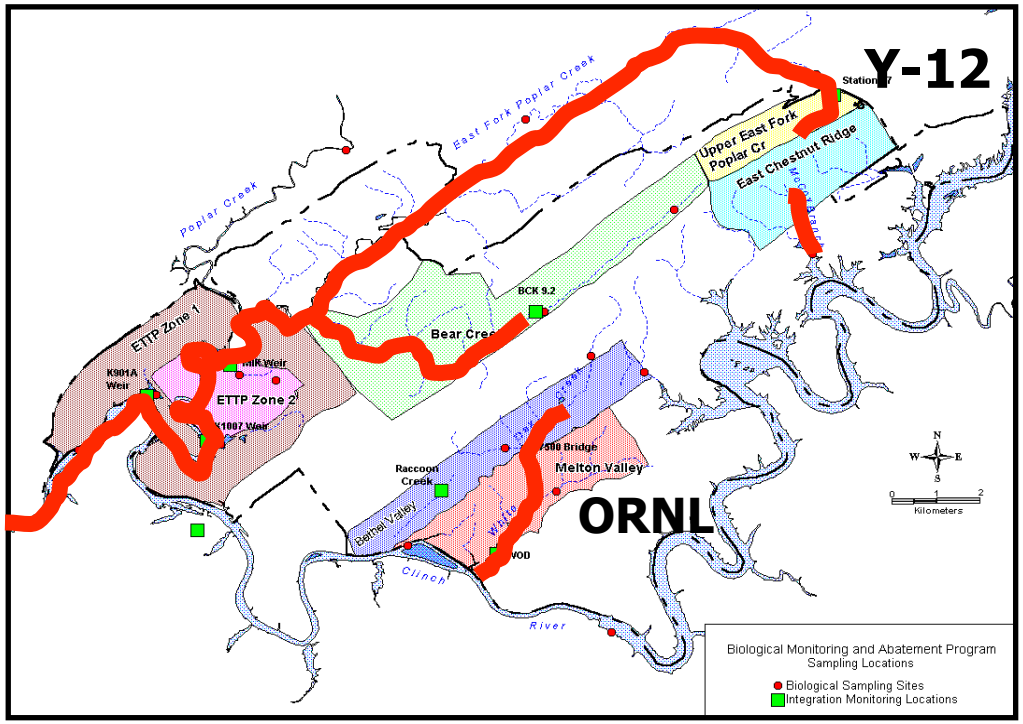
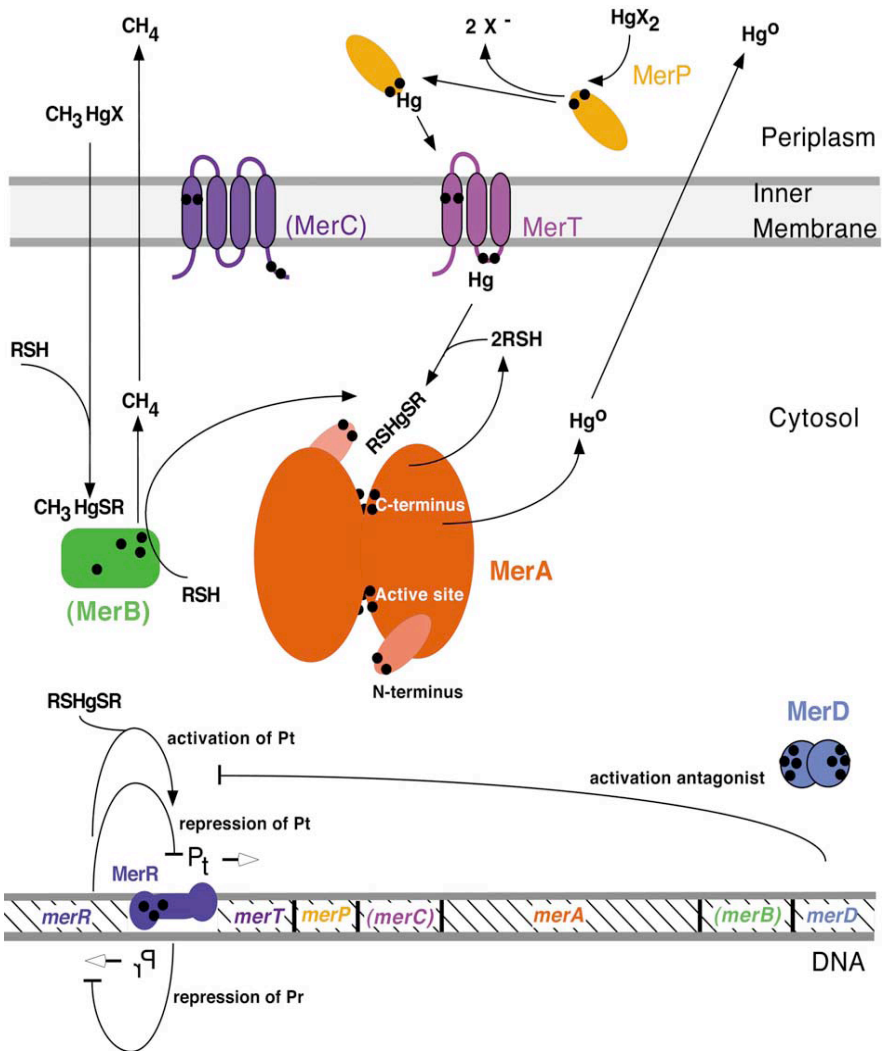
The Mercury Problem

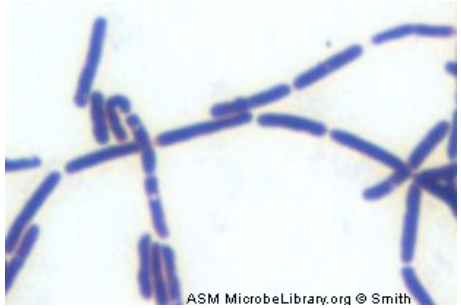
- Hg(0) - Highly reactive, volatile
- Hg(II) - high affinity for thiols
- Methylmercury - charged, amphiphilic
- Biotic/abiotic origin
- Bioaccumulation



Many bacteria are resistant to toxic mercury species

Bacterial Mercury Resistance – The *Mer* Operon

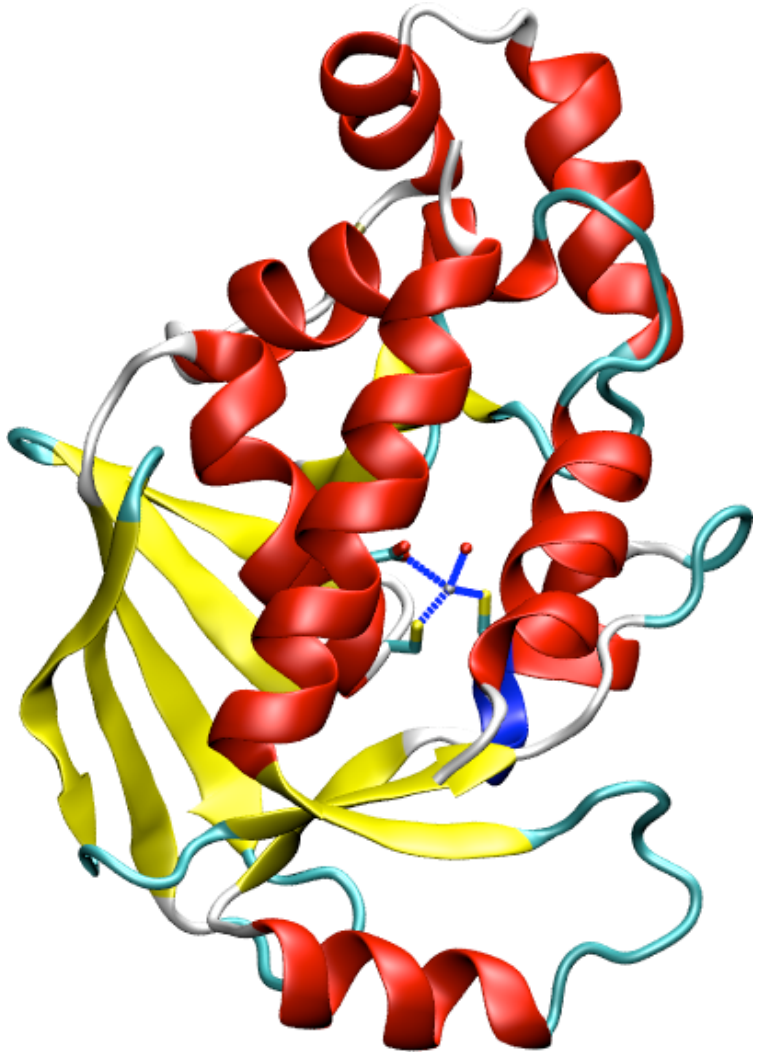




www.clfs.umd.edu/CBMG/faculty/asmith/smith2.html

- **MerR – regulation (transcriptional activator)**
- **MerB – organomercurial lyase**
- **MerA – mercuric reductase**

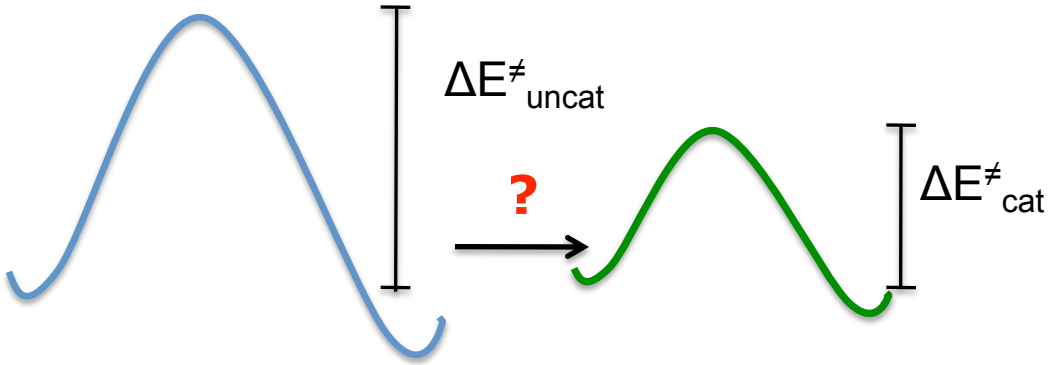
Mercury Demethylation: MerB



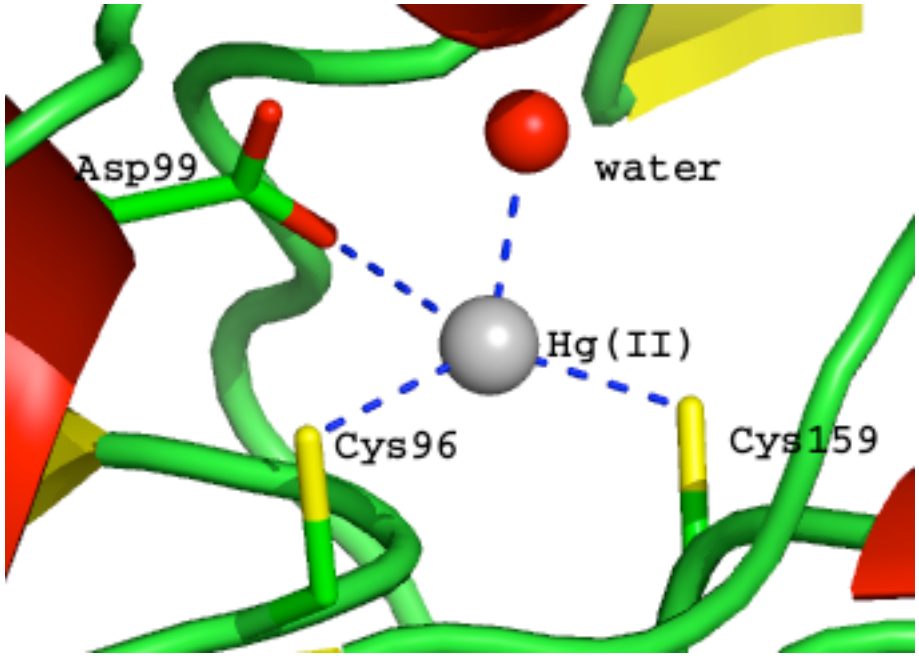
Lafrance-Vanasse *J. Biol. Chem*. 2009

The organomercurial lyase, MerB, catalyzes the cleavage of Hg-C bonds in organomercurial substrates.

$$[\text{R-Hg(II)}]^{+} \xrightarrow{\text{MerB}} [\text{Hg(II)}]^{2+} + \text{R-H}$$



Mechanism of MerB: Experimental Data



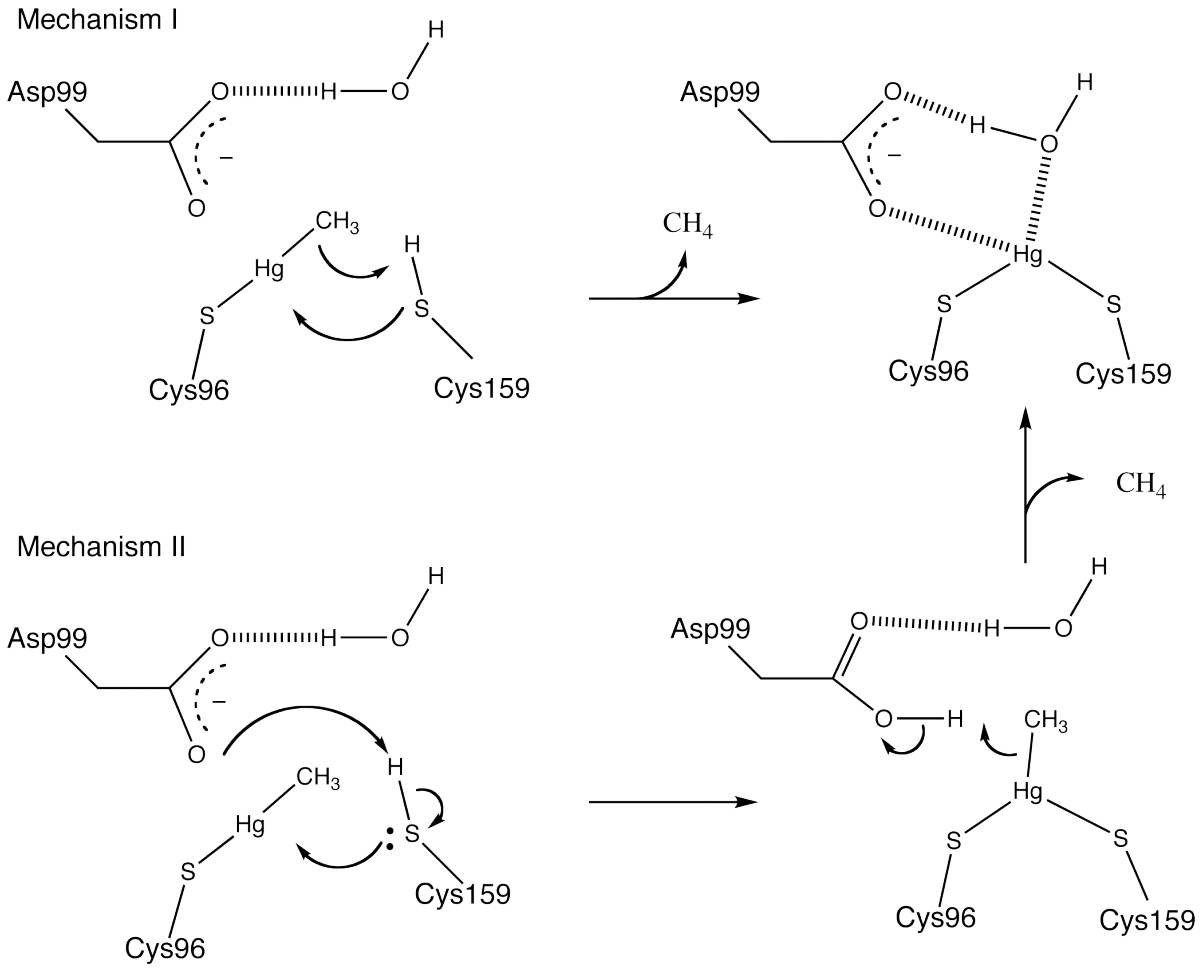
Lafrance-Vanasse et al. *J. Biol. Chem*. 2009

$k_{cat} = k_B T/h \exp[-\Delta G^{\neq}/RT]$

Eyring Transition State Theory

- **Recent X-ray structures for apo and Hg(II)-bound MerB**
- **Cys96, Cys159, Asp99 required for activity**
- **Electrophilic substitution (S_E2) mechanism proposed**
- **Experimental reaction rates (k_{cat}) known for several organomercurial substrates**

Mechanism of MerB: Proposed Mechanisms



Mechanism I:

Direct protonation of the hydrocarbon leaving group by Cys159 or Cys96

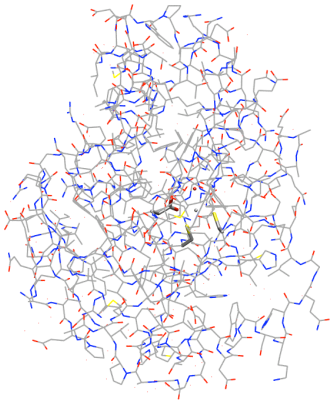
Mechanism II:

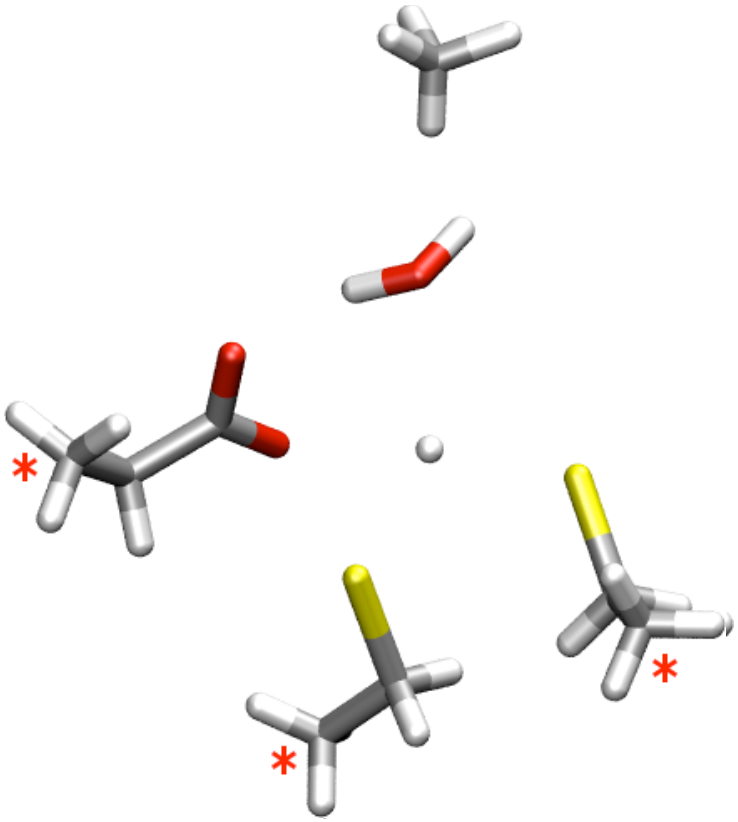
Protonation of the leaving group by Asp99

Methods

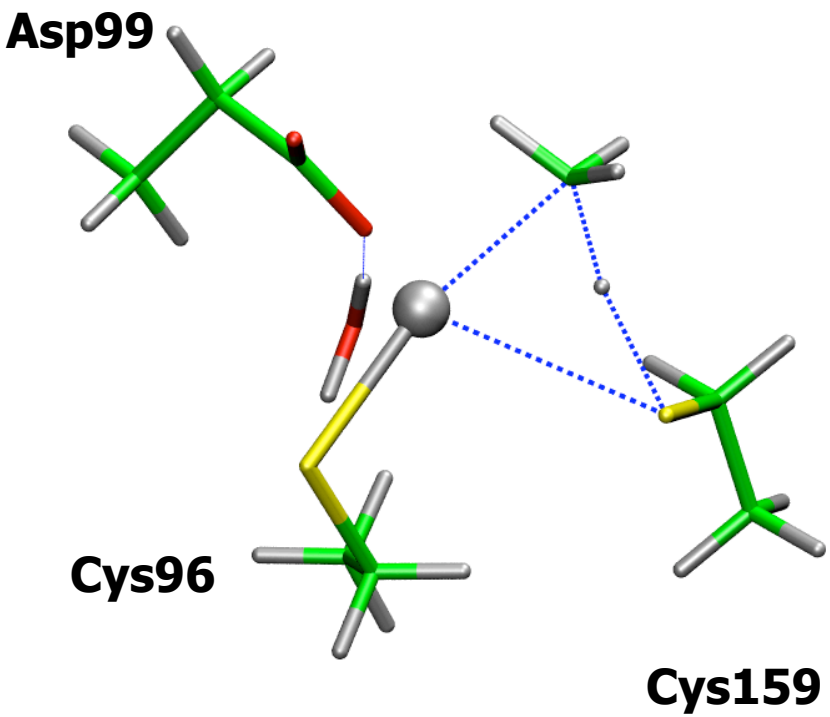
Quantum chemical cluster model of MerB active site

- **Extract coordinates from X-ray**
- **Add hydrogen atoms**
- **Add methane**
- **Constrain 3 alpha carbons**
- **Geometry optimization**
- **Solvation and high-level energy correction**





Results: Mechanism I

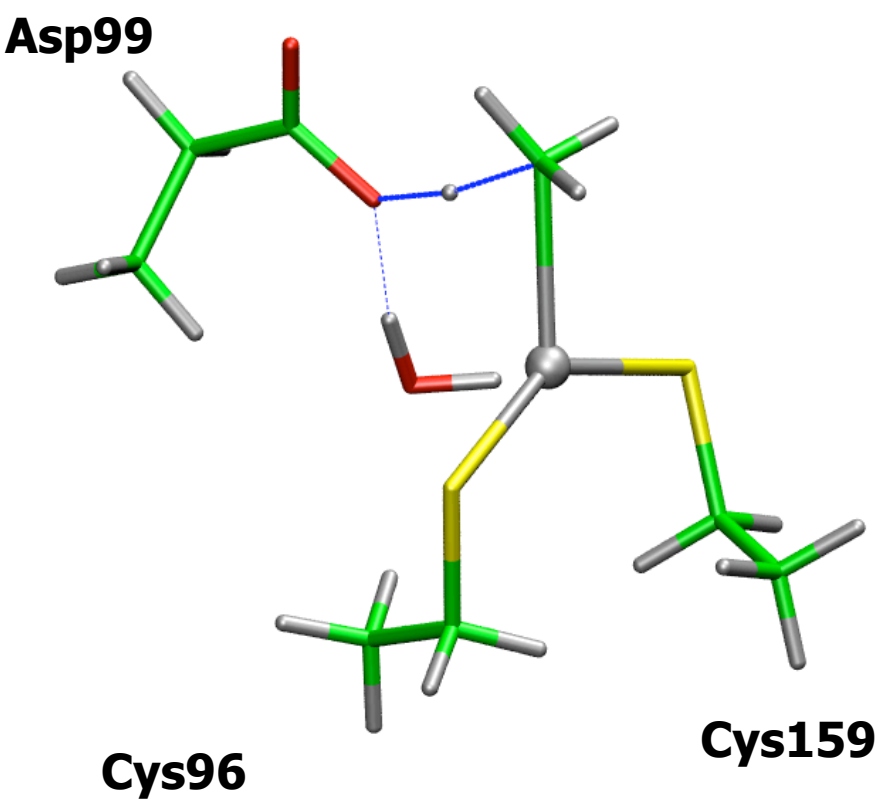


- **Direct protonation of the leaving group by Cys159**

$\Delta E^{\neq}$ = **35.6** kcal/mol

Cys159 is *not* coordinated With Hg at the TS.

Results: Mechanism II

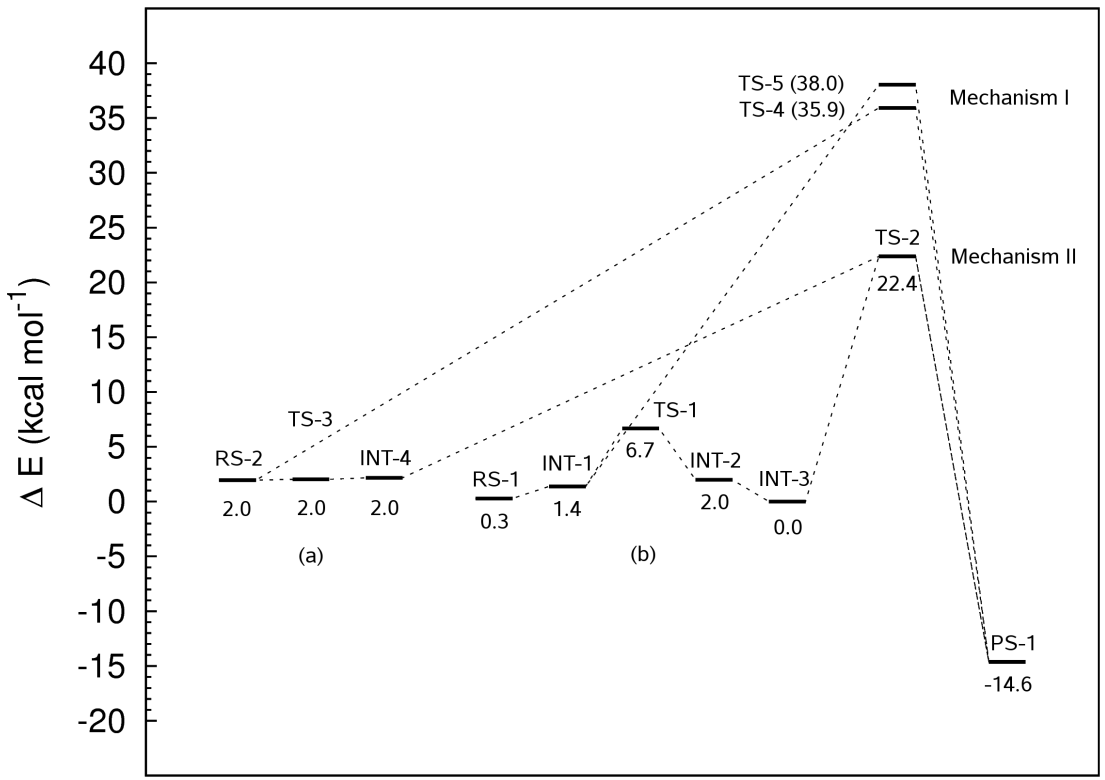


- **Proton transfer from Cys159 to Asp99**
- **Asp99 protonates the leaving group**

$\Delta E^{\neq}$ = **22.4** kcal/mol

At the TS: Both Cys96 and Cys159 are coordinated with Hg.

Mechanism of MerB: Simulation vs Experiment



$[CH_3Hg(II)]^+$ substrate

$\Delta G^{\neq}_{exp}$ = 20.1 kcal/mol

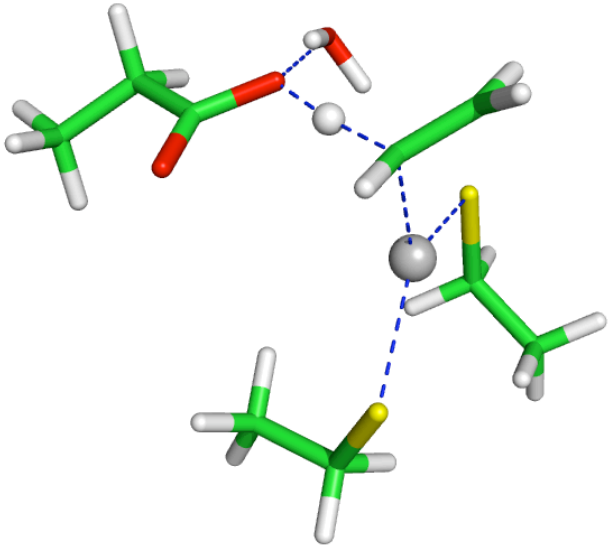
$\Delta E^{\neq}_{Mech\ I}$ = 35.6 kcal/mol

$\Delta E^{\neq}_{Mech\ II\text{-}a}$ = 20.4 kcal/mol

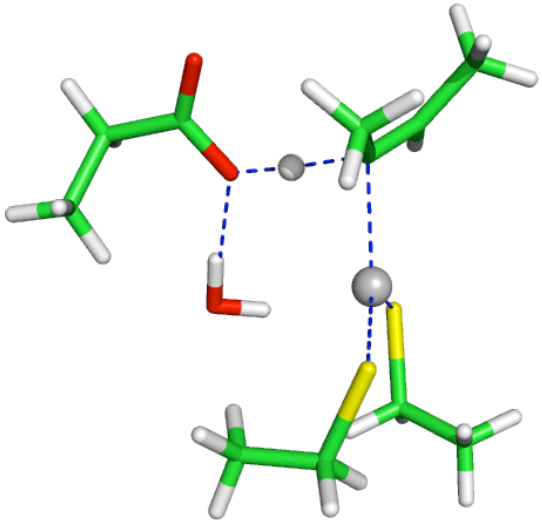
$\Delta E^{\neq}_{Mech\ II\text{-}b}$ = 22.4 kcal/mol

Participation of **Asp99** as the **catalytic base/acid** (**Mechanism II**) is clearly favored over direct protonation of the leaving group by Cys (Mechanism I).

MerB: Other substrates



vinylmercury



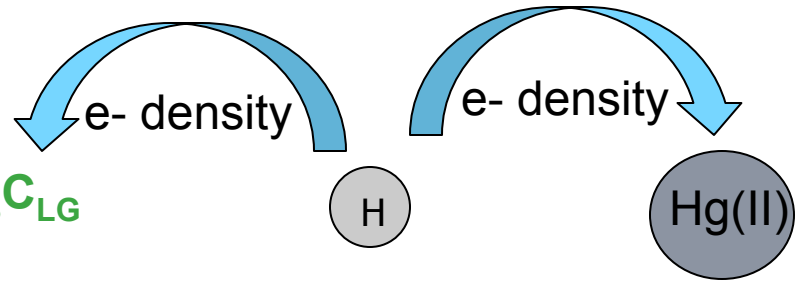
***cis*-2-butenyl-2-mercury**

MerB: Simulation vs. Experiment

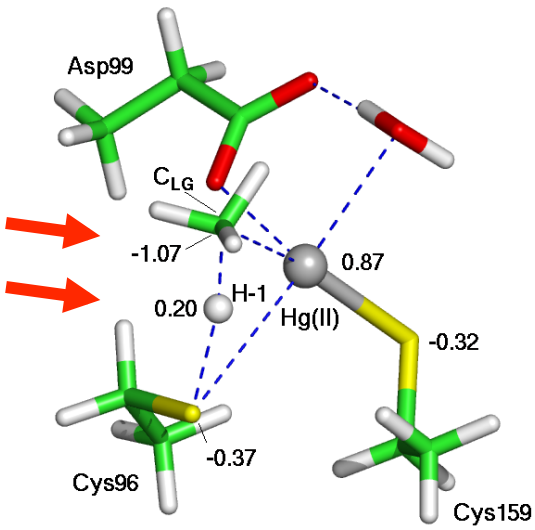
Substrate	Turnover no. (min^{-1})	$\Delta E^{\neq}_{exp}$	$\Delta E^{\neq}_{calc}$
$[CH_3Hg(II)]^+$	0.7	20.1	20.4/22.4
$[CH_2{=}CH\text{-}Hg(II)]^+$	12	18.4	18.3/18.4
[*cis*-2-butenyl-2-Hg(II)]$^+$	240	16.6	15.6/16.4

Experimental data from Begley, et al. 1986, *Biochem.*, 25, 7192.

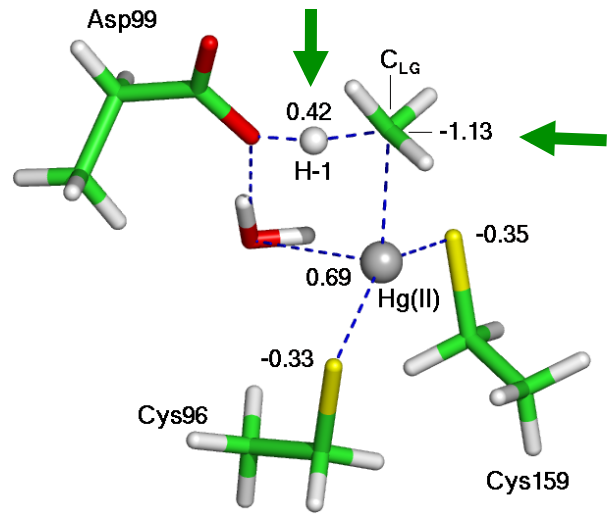
Mechanism of MerB: Transition States



	H_3C_{LG}	H	Hg(II)
Mechanism I	-1.07	+0.20	+0.87
Mechanism II	-1.13	**+0.42**	+0.69



Mechanism I



Mechanism II

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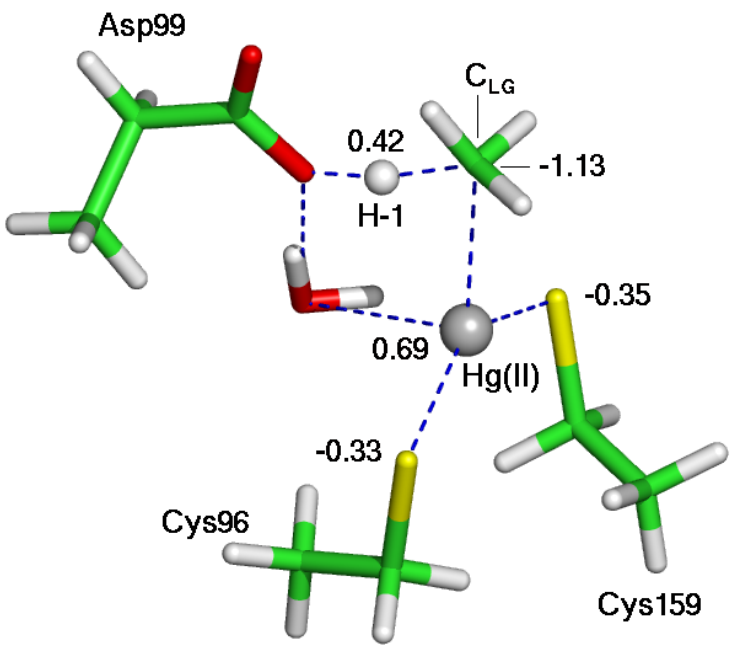
Mechanism of MerB: Summary



- **Quantum chemical active site model is sufficient to describe the Hg-C protonolysis reaction in MerB**
- **Calculated energy barriers are consistent with experimental k_{cat} data**
- **Coordination of Hg by two cysteines is necessary and sufficient to activate Hg-C bond**
- **Verified Asp99 as the catalytic base/acid in MerB (Lafrance-Vanasse, et al. 2009)**

Parks, Guo, Momany, Liang, Miller, Summers, and Smith, *J. Am. Chem. Soc.*, Accepted.

Acknowledgments

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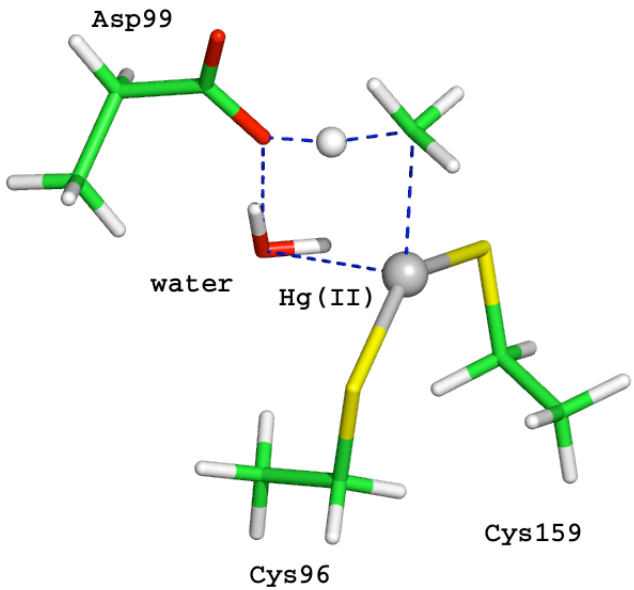
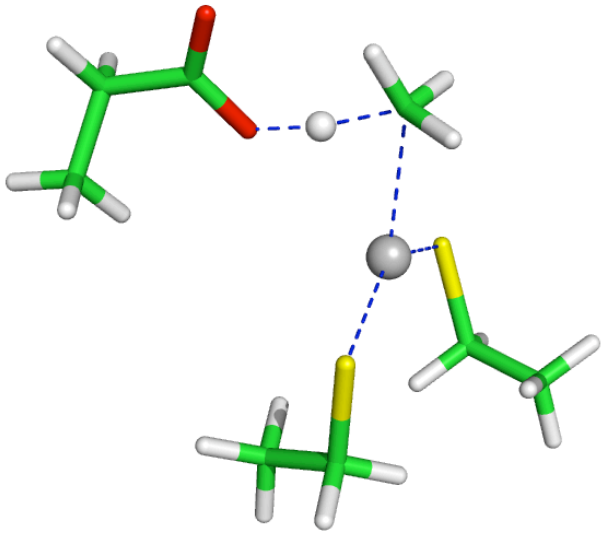
- Alex Johs
- ORNL Mercury SFA Team
- TeraGrid
- US DOE, Office of Science, Office of Biological and Environmental Research; Environmental Remediation Science Program

MerB: Explicit water contributions to catalysis





$\Delta\Delta E^{\neq}$ = 1.2-3.2 kcal/mol

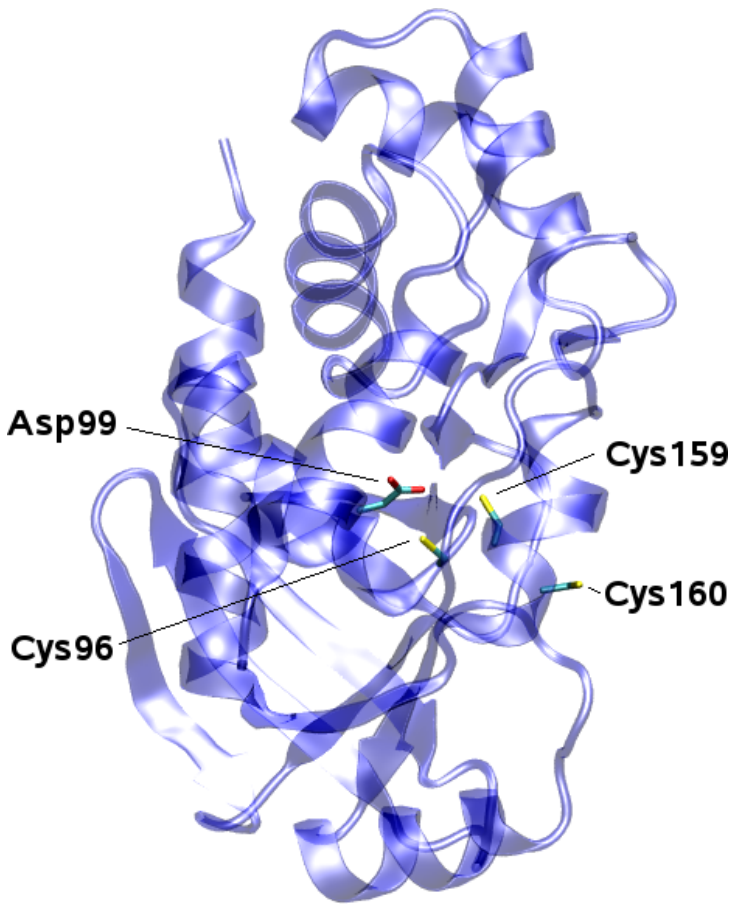
Asp99-water H-bonding: Relatively minor contributor to catalysis

pK_a values: Where are the protons?

PROPKA Predictions

Residue	pK_a (calc)
Cys96	11.4
Asp99	6.6
Cys159	14.0

Li, Robertson and Jensen, Proteins, 2005



Mechanism of MerB: Methods/Details

Hybrid Density Functional Theory (adiabatic connection functional) + polarized continuum solvation

1. **B3PW91/6-31G(d) gas-phase geometry optimization**

2. **C-PCM (ε = 4.0) solvation correction**

3. **B3PW91/6-311++G(3df,2p) single point energy**

Quadratic Synchronous Transit (QST3) Transition state optimization

Natural Population Analysis for atomic charges

All calculations performed with Gaussian 03

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Methods: Why should this approach work?

Quantum chemical cluster model of MerB active site

- **Limited conformational change expected during chemical step**
- **Short-range (first-sphere ligand) interactions dominate**
- **Density functional theory (DFT) reliable for Hg-S chemistry**
- **Systematic errors (3-5 kcal/mol) – Error cancellation**
- **Gas-phase vs enzymatic pKas – Same trend**
- **Hydrophobic active site**

Natural Population Analysis

- **Orbital transformation into a localized, orthonormal set**
- **Well-defined, intuitive representation of electron density**
- **Orbital occupancies + nuclear charge = NPA charge**

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pK_a values

$$pK_a = -\log_{10} K_a \qquad K_a = [A^-][H^+]/[AH]$$

$$pK_a = \Delta G°/2.303RT$$

- **Error: 1.4 kcal/mol = 1 p*K* unit**
- **Thermodynamic cycles require experimental $\Delta G_{solv}(H+)$, $G(H^+_{gas})$**
- **pK_as of neighboring residues are often coupled**

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