

A Comprehensive study of the biogeochemical and molecular mechanisms on mercury transformation at a contaminated site in Oak Ridge, Tennessee, USA

Mercury Summit Presentation

October 22, 2009

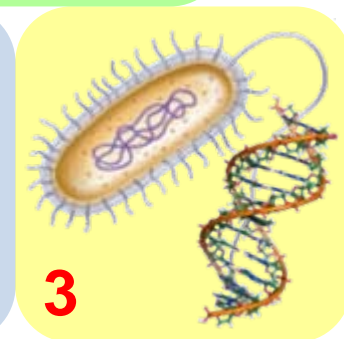
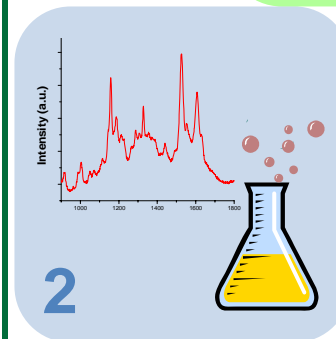
Vanderbilt University



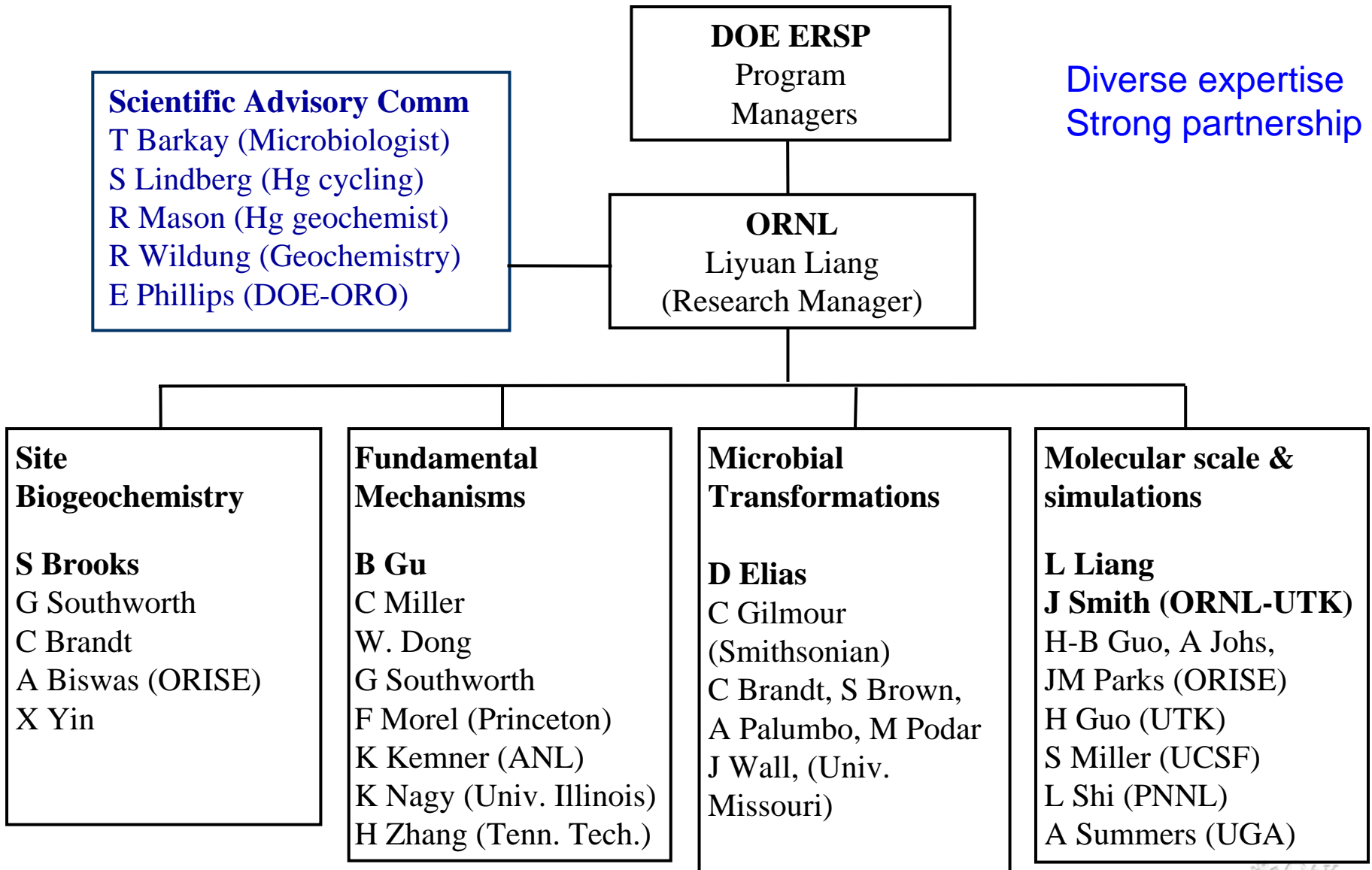
Oak Ridge National Laboratory
ERSP Science Focus Area (SFA)



Oak Ridge Mercury Program



Research Team and Organization



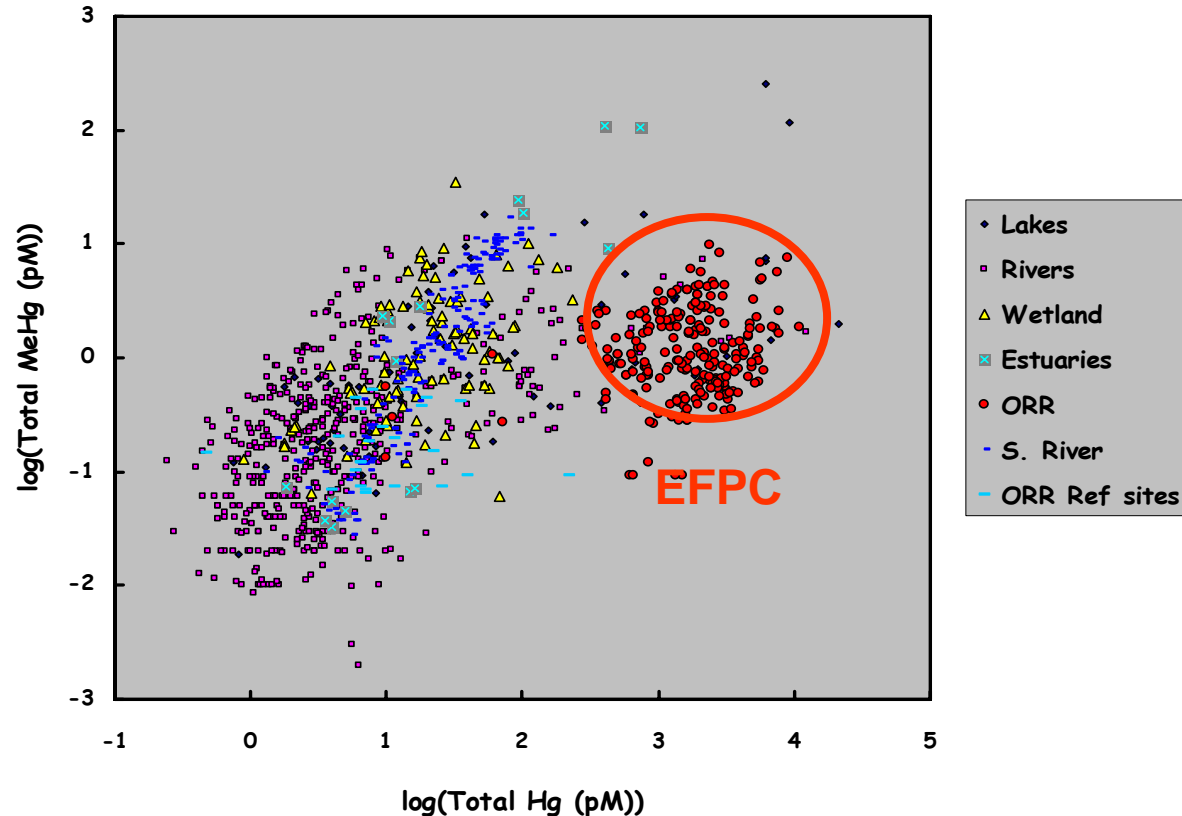
Research Goal

Provide scientific understanding of the rates and mechanisms of net methylmercury production in the environment

Scope and Objectives

- Elucidate the rates, mechanisms and controls of abiotic and microbial processes affecting Hg speciation and transformation
- Resolve what and how critical Hg precursors are produced and subsequently methylated
- Understand fundamental microbial subcellular mechanisms by studying the structure and dynamics of biological macromolecules involved in the acquisition, transport and transformation of major Hg species and MeHg
- Determine the net balance of methylation and demethylation

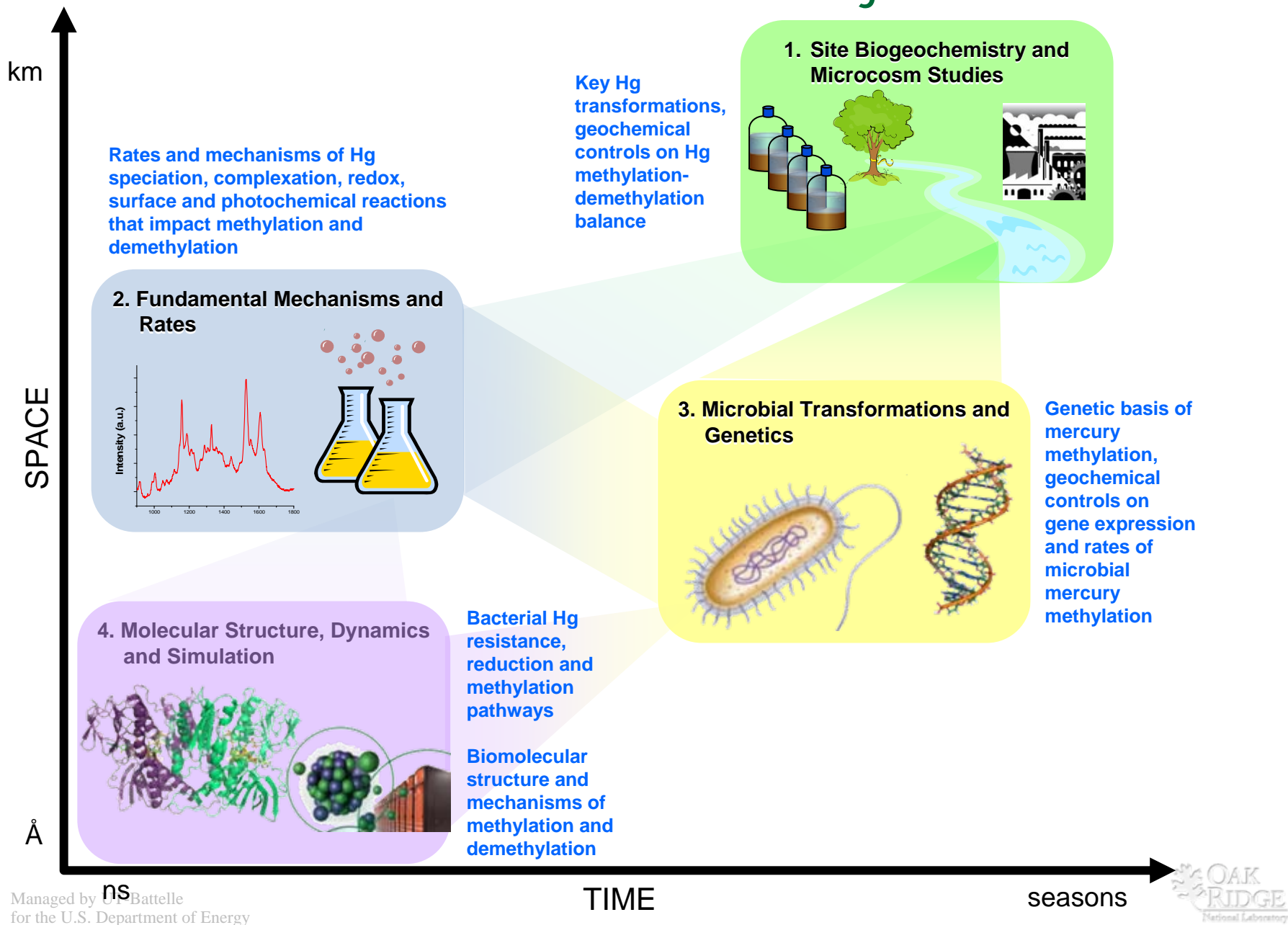
Oak Ridge Reservation offers a unique opportunity to explore what controls the level of methylmercury in the environment



- Relationship between total Hg and methylmercury in water
- EFPC MeHg is lower than might be expected
- Lowering Hg does not appear to lower MeHg

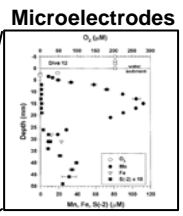
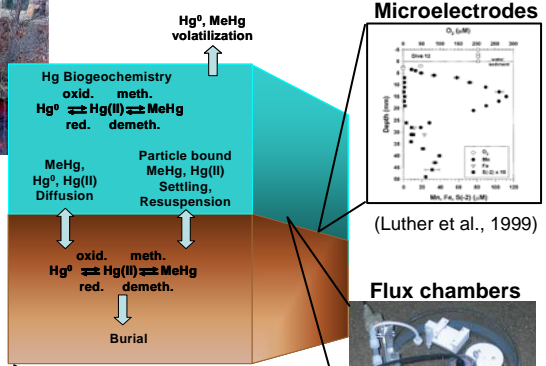
Is methylation inhibited?
Is demethylation enhanced?
What forms of Hg are bioavailable?

Research Tasks and Objectives



I. Site biogeochemical processes and microcosm studies

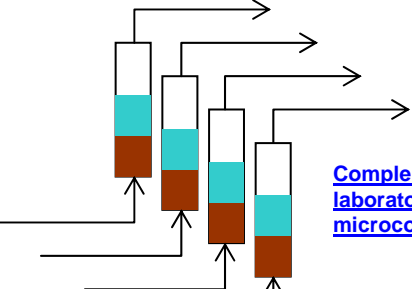
Field measurements



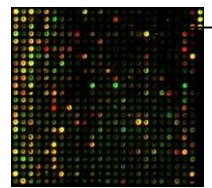
(Luther et al., 1999)



(Menheer, 2004)

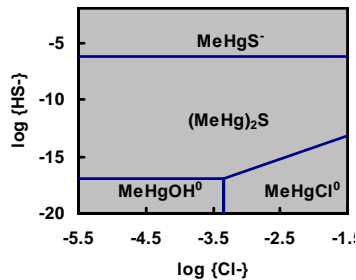
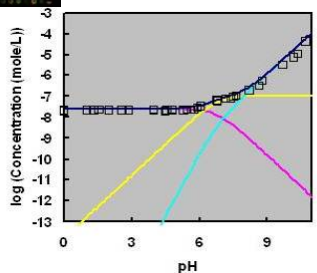


Complementary laboratory microcosms



Functional gene arrays

Geochemical modeling

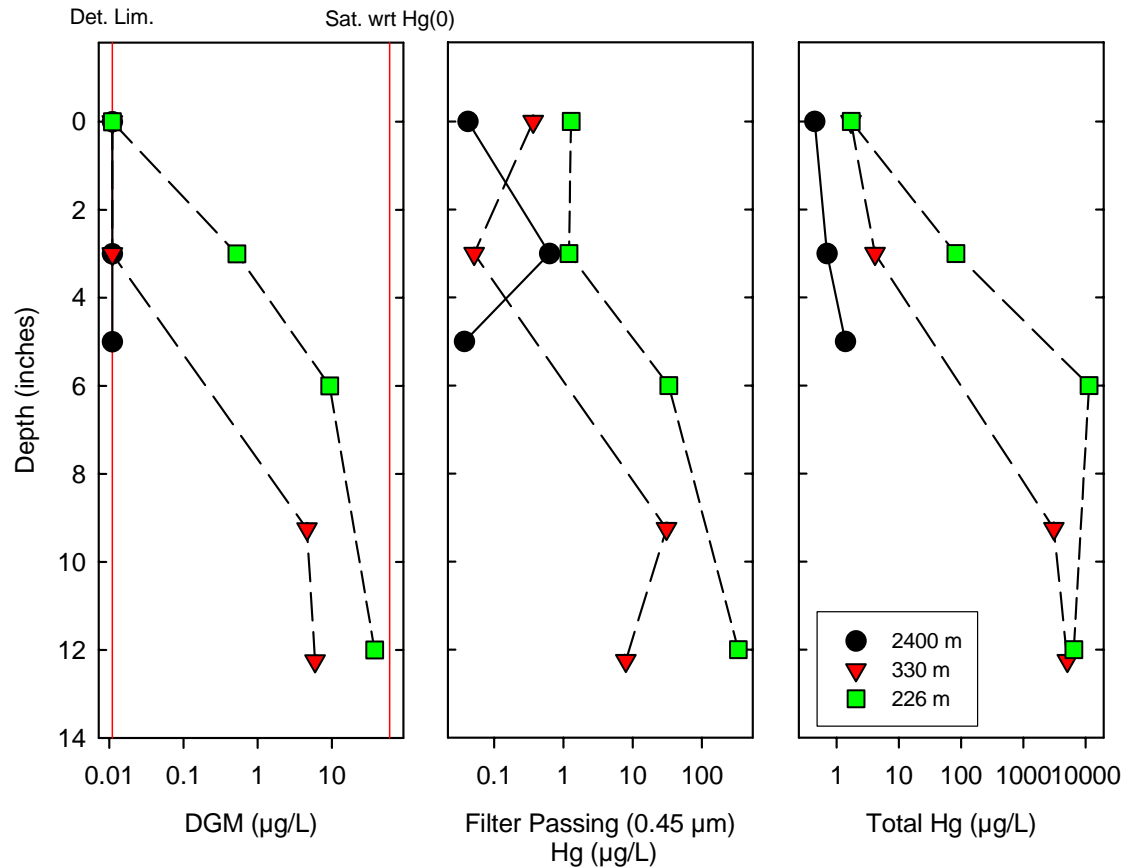


- Site Investigations
 - Chemical, physical, microbial data relevant to Hg transformations (Tasks 1-3)
- Hg reactivity
 - Correlating Hg reactivity with methylation potential
- Methylation Bioassay
 - Hg speciation and methylation rate
- Microcosm Studies
 - Methylation/ demethylation in ecologically intact system
- Enclosure Studies
 - key variables on net methylation in-stream
- Data Analysis, Geochemical Modeling, Site Conceptual and Numerical Model

*** Critical understanding of Hg flux, biogeochemical controls, and microbial determinants**

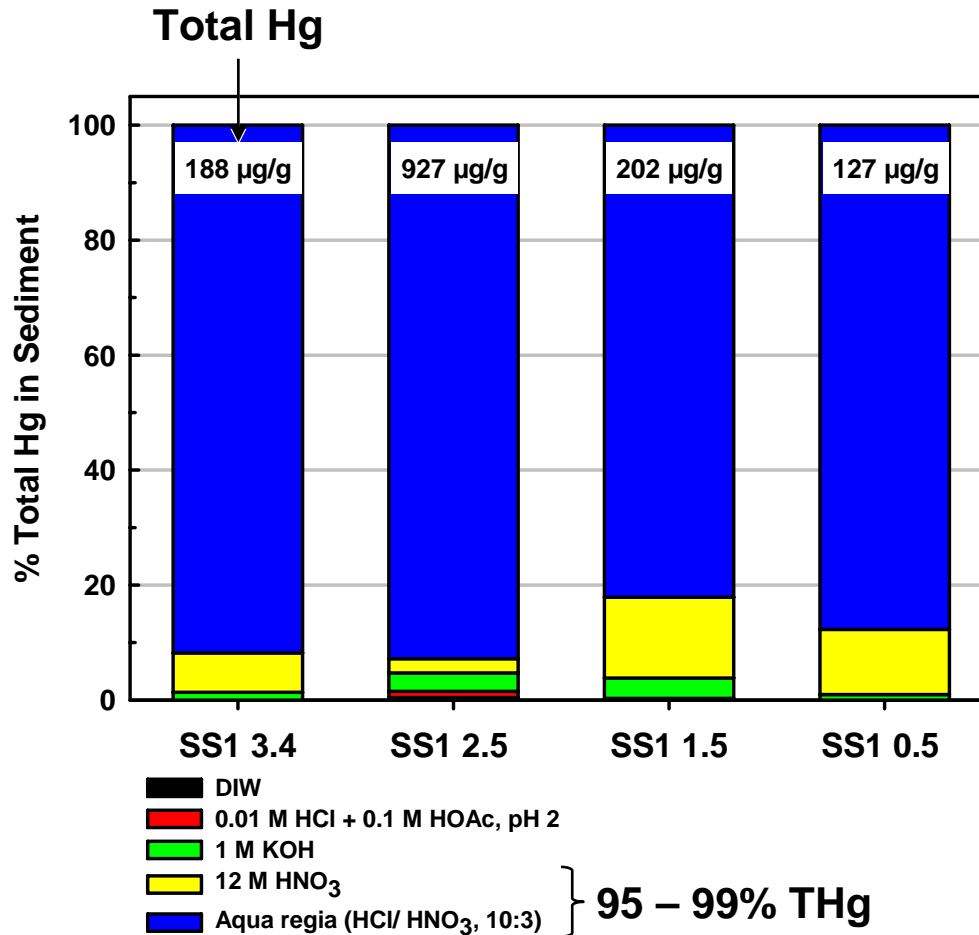


Examples of Current Research: Vertical profiling of water-sediment geochemical parameters



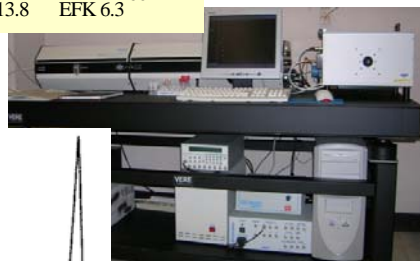
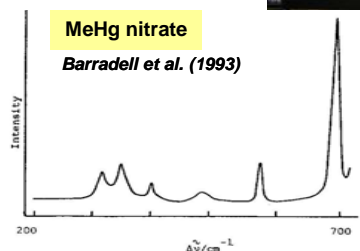
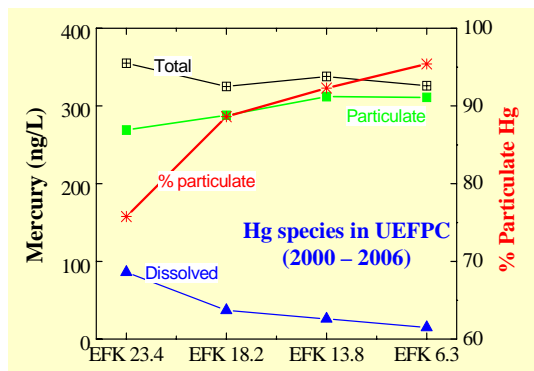
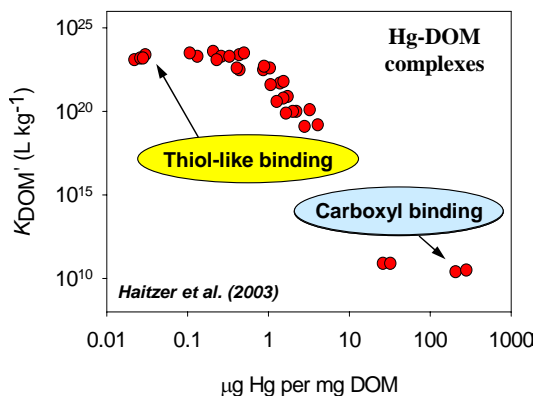
➤ **Vertical profile (mercury species concentration as a function of depth) in stream bed sediment for UEFPC. Data collected 14 October 2008**

Examples of Current Research



- Lateral transect at SS1
- top ~2 cm
- < 125 µm size fraction
- 5 step sequential extraction procedure (Bloom et al., 2003)
- THg = sum of fractions, i.e., 100% mass recovery
- Majority of Hg strongly bound
 - Sequestered or labile/exchangeable?

II. Fundamental mechanisms and transformations

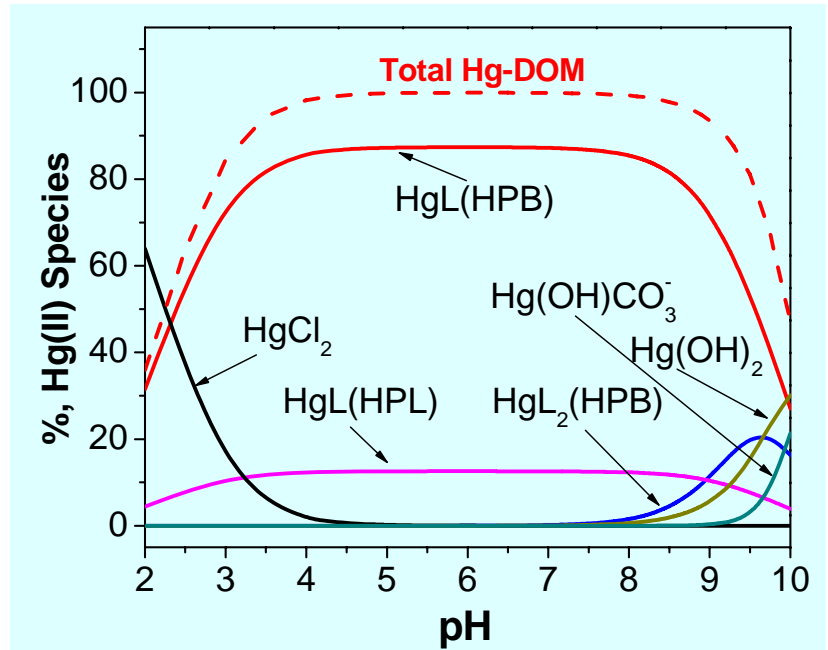
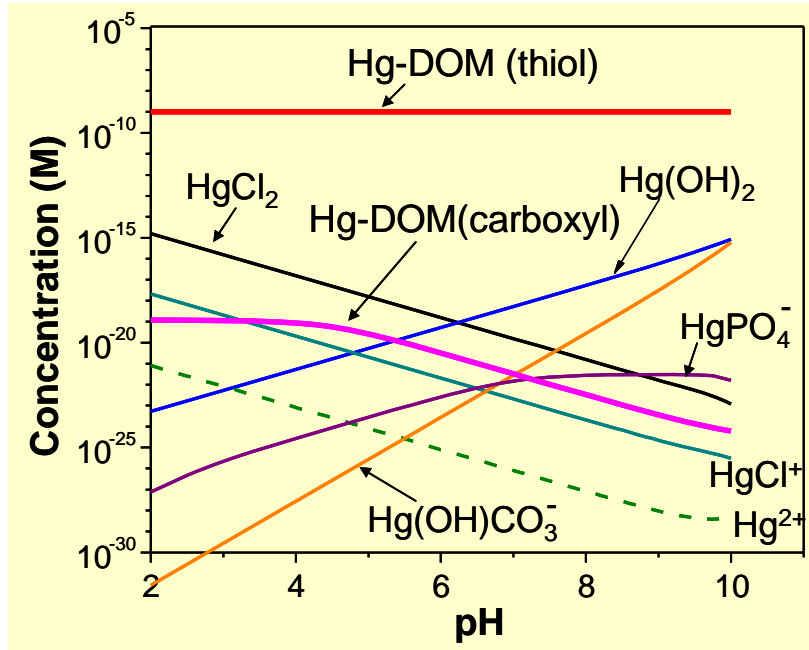


- Speciation and geochemical controls
 - Rates and mechanisms, oxidation/reduction
 - Single reactant to multi-component systems
 - Real-time spectroscopic analysis coupled with CVAA or CVAFS analysis
- Roles of DOM and POM in Hg methylation, demethylation, and redox transformations
 - Specific moieties and functional groups
 - EXAFS analysis, speciation and coordination chemistry
 - Species, models, and effects on bioavailability
- Catalyzed surface and photochemical reactions
 - Roles in Hg reactivity and demethylation
 - Sorbed species and reactions
 - Labeled stable isotope studies

* Critical understanding of dominant Hg species, its bioavailability, and biogeochemical controls on rates and mechanisms of Hg methylation and demethylation

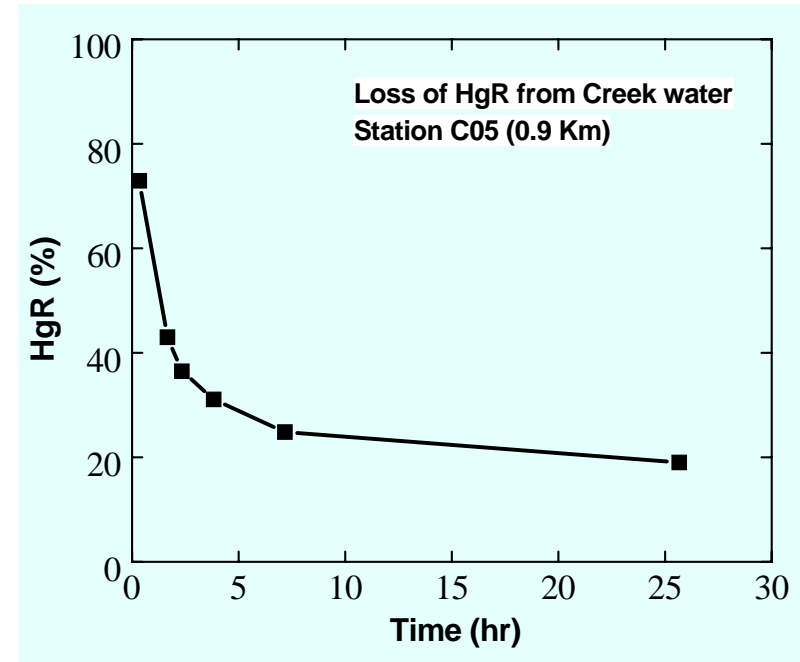
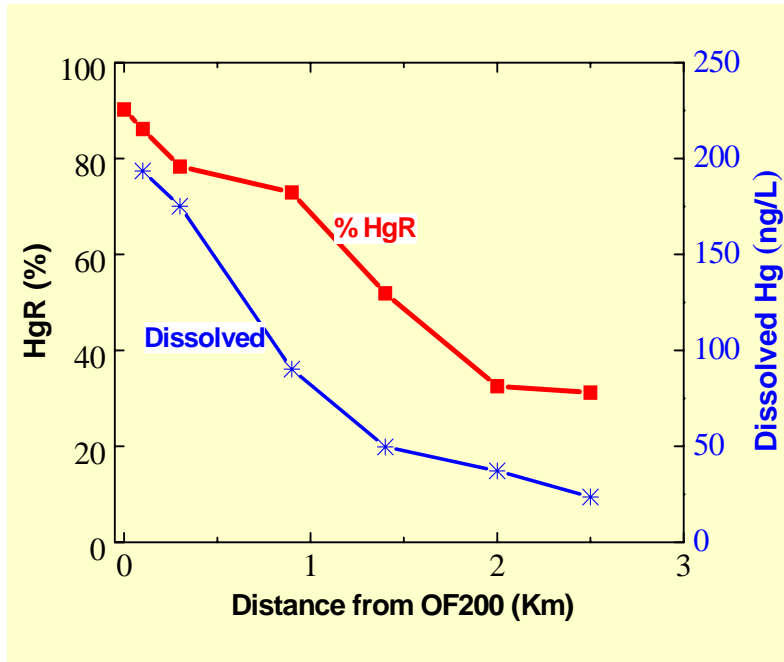
Examples of Current Research

➤ Hg(II) speciation analysis in EFPC at equilibrium



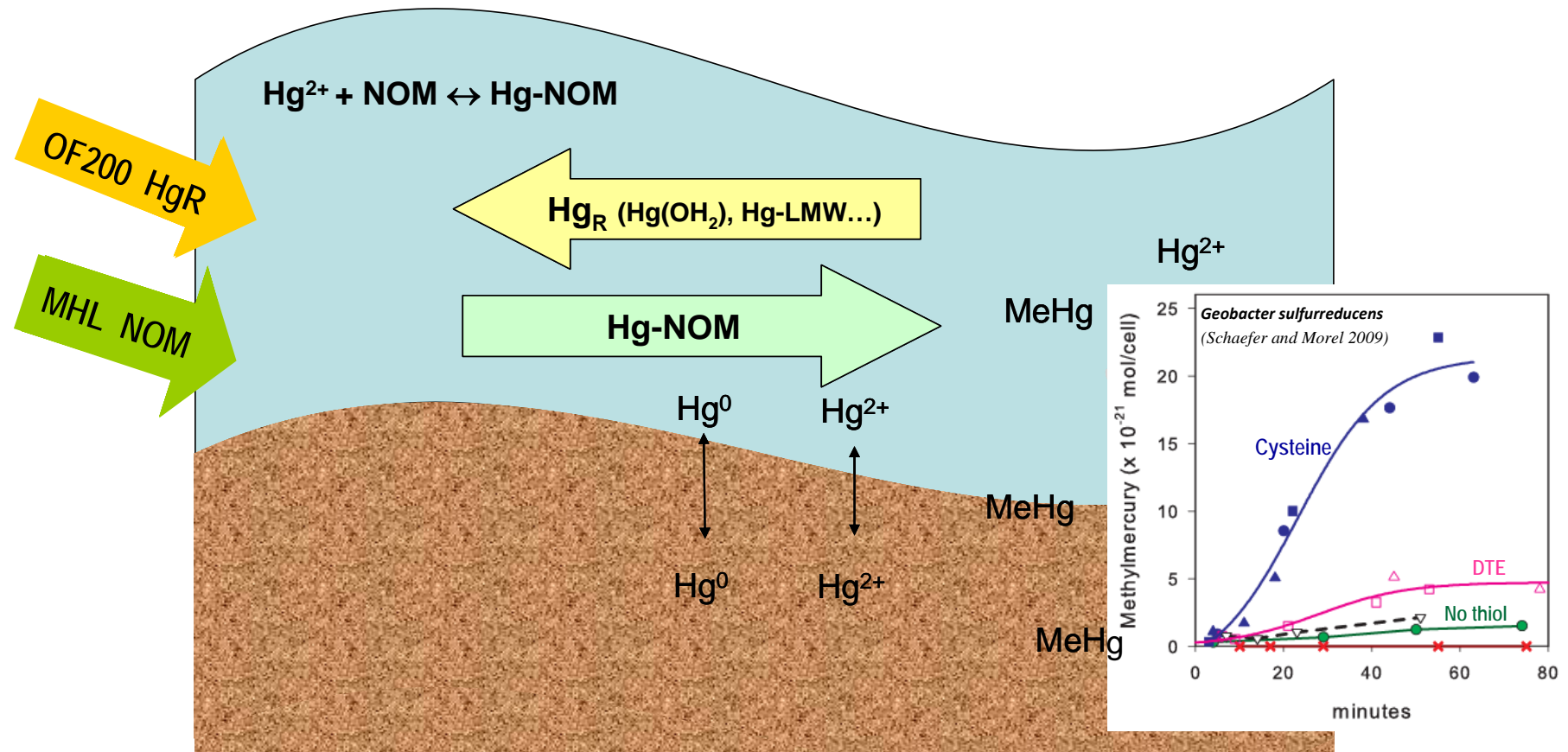
- Hg(II)-DOM complexes dominate in the EFPC water (using Haitzer et al. Log K = 28.7 for 1:2 thiols and Log K = 10 for 1:1 carboxyl complexes)
- DOM isolates (hydrophobic and hydrophilic fractions) obtained from EFPC; Log K's determined (22-24)
- Hg(II)-HPB DOM complexes dominate within pH 4 and 8 at [HPB] = 1.1 ppm and [HPL] = 0.6 mg/L

Examples of Current Research: Hg(II)-DOM formation kinetics in EFPC creek



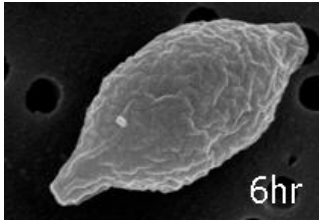
- Equilibrium Hg-DOM complexation is not established in UEFPC 2.5 km
- Kinetic limitation is further supported by the loss of HgR from the creek water
- Kinetics of Hg(II)-DOM complexation should not be overlooked

Implications of Hg-DOM reaction kinetics

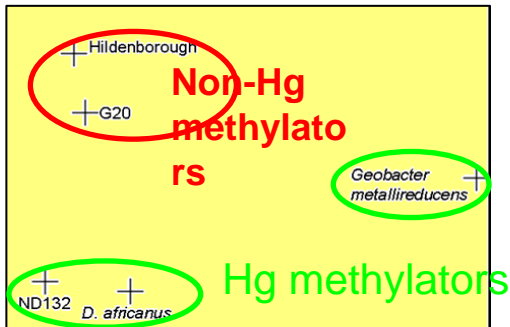


- Impact on species analysis and modeling (task 1)
- Impact on bioavailable Hg(II) species and Hg(II) uptake?
- Methylation and demethylation rates?

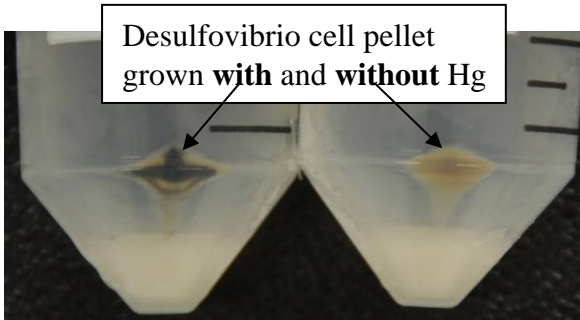
III. Microbial and genetic controls on mercury methylation and demethylation



Desulfovibrio africanus
(SEM by Dwayne Elias, U of Missouri)



Genome wide PCA of COGs



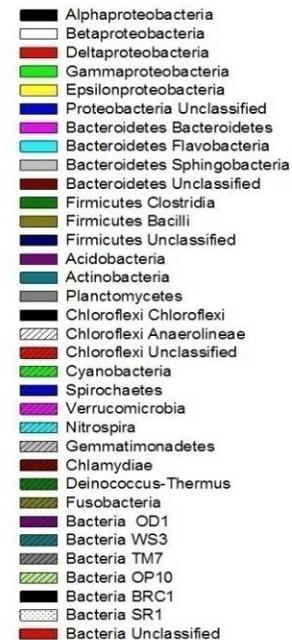
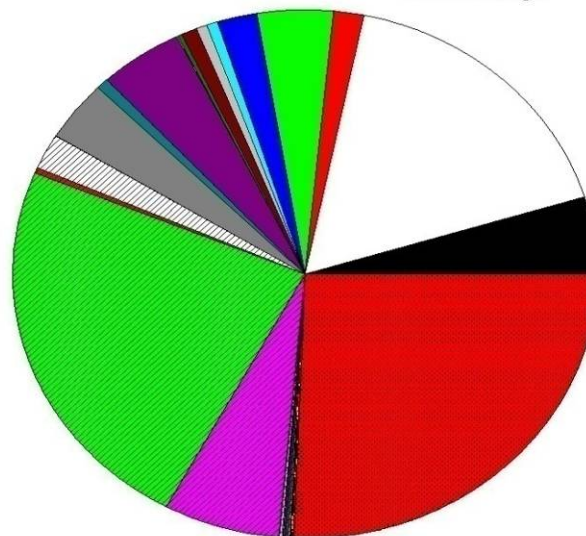
- Elucidate the genetic determinants of methyl Hg production and regulation
 - Comparative gene expression, mutagenesis, and complementation
- Determine the effect of geochemical factors on gene regulatory networks for mercury methylation
 - Use whole genome microarrays to examine both biotic and abiotic effects on the methylating and nonmethylating *Desulfovibrio* transcriptomic profiles
- Examine relationships among community structure, geochemical conditions, and methyl Hg production in sediments collected from Hg-contaminated sites
 - Functional gene arrays
 - 16S rRNA gene clone library analysis

***Critical understanding of the genetic basis of the methylation and demethylation processes and the geochemical controls on microbial transformation.**

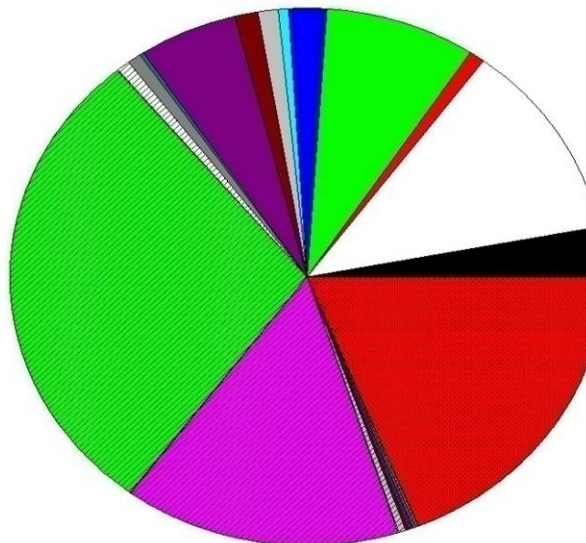
Taxonomic Diversity Analysis Using 454 Pyrosequencing

- Completed year long study of microbial community structure with 454 sequencing
 - 6 sites
 - Seasonal samples
 - Duplicates
- Two 454 runs to get the phylogenetic data for 59 samples
 - > 500,000 16s sequences in 59 samples
- Found much higher diversity than with clone libraries
- First sequencing of methylators in *Desulfovibrio* enabling comparative genomics
- Will examine relationships between microbial community structure to geochemistry, FGA

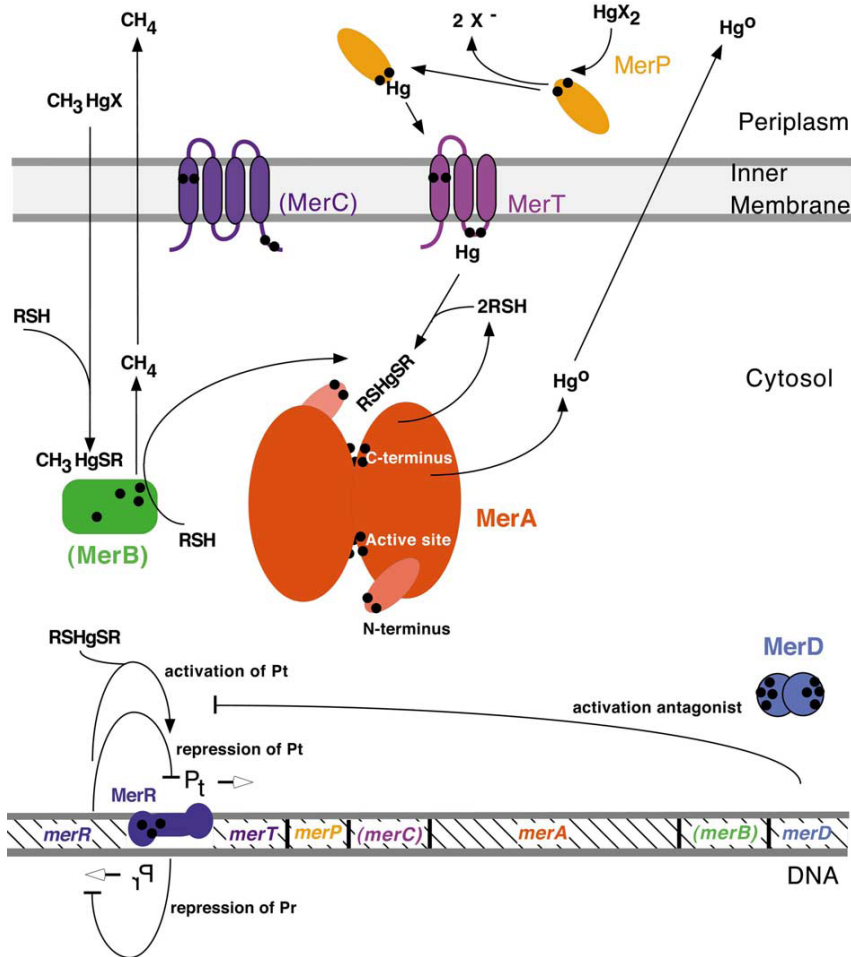
HC March 2008,
454 Average



EFPK 23.4
March 2008,
454 Average



IV. Molecular structure, dynamics and simulations



- Establish biochemical pathways in bacterial demethylation
- Obtain structure of protein/protein and protein/DNA complexes
 - Apply small angle neutron scattering to reveal structure-function relationships
- Reveal enzymatic mechanisms to understand the processes of demethylation and reduction
 - Use quantum mechanical/molecular mechanical simulations

***Critical understanding of the biochemical and biophysical mechanisms in Hg transformation (demethylation and methylation) by developing and validating subcellular models and investigation of structure-function relationships**

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

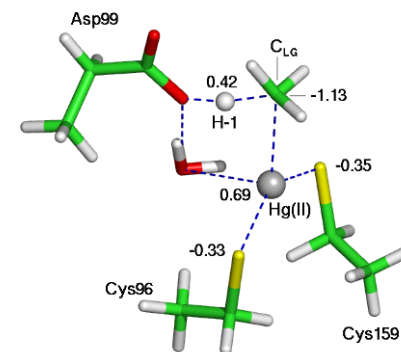
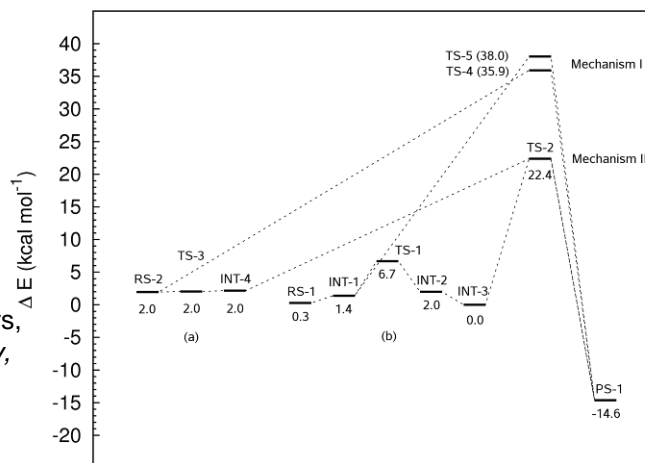
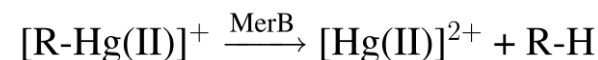
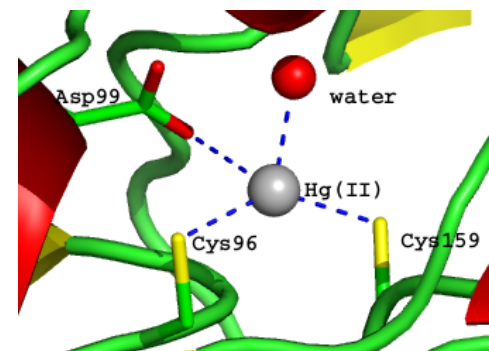
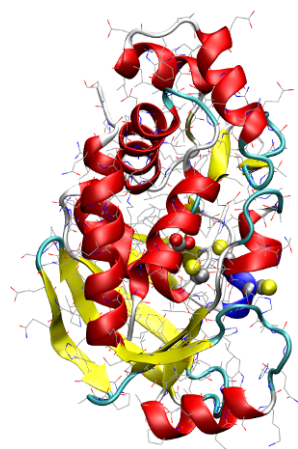
Examples of Current Research: Mechanism of Hg-C Protonolysis in Bacterial Organomercurial Lyase MerB

↪ The chemical mechanism of Hg-C bond cleavage in organomercurials by the enzyme MerB was investigated using a quantum chemical cluster model of the active site.

↪ Verified the mechanism recently proposed by Lafrance-Vanasse et al., 2009

↪ Showed quantitatively how methylmercury coordination by two cysteines enables Hg-C bond cleavage

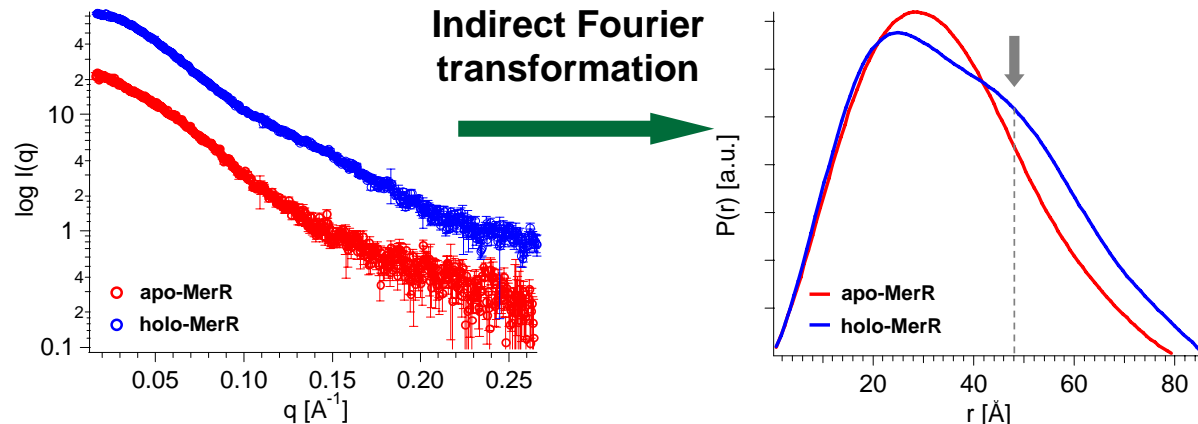
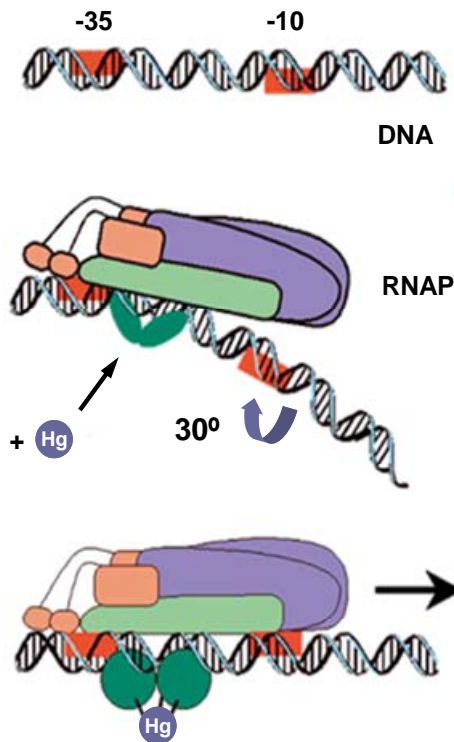
↪ Showed how an aspartic acid residue acts as a base and then an acid to deliver a proton to the hydrocarbon leaving group



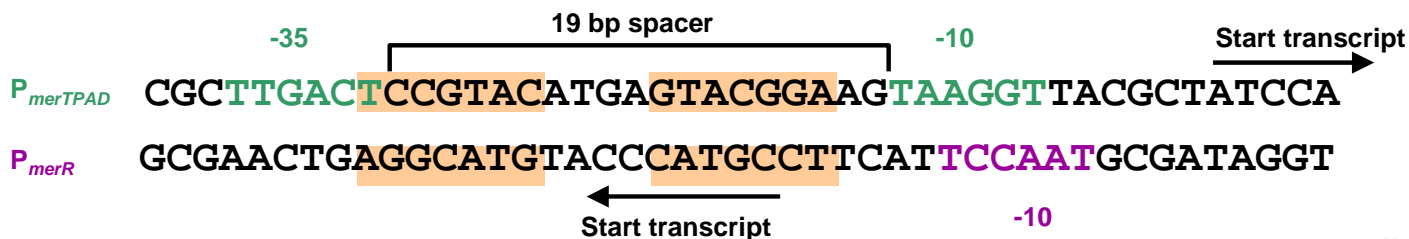
Parks, J.M., Guo, H., Momany, C., Liang, L., Miller, S.M., Summers, A.O., Smith, J.C. 2009. *Journal of the American Chemical Society*, 131:13278-13285

Examples of Current Research

- Studies of conformational change of homodimeric MerR by small angle scattering



- The allosteric effector Hg(II) induces a conformational change from a compact structure to a conformation with two separate domains
- $\Delta R_G = 1.7 \pm 0.6 \text{ Å}$.



SUMMARY

- Oak Ridge Mercury Program established to provide fundamental understanding of Hg transformation and mechanisms
- **SITE INVESTIGATION:** understanding of Hg flux, biogeochemical controls, and microbial determinants
- **CHEMISTRY:** understanding of dominant Hg species AND biogeochemical controls on rates and mechanisms of Hg methylation and demethylation
- **BIOLOGICAL PROCESSES:** understanding of microbial transformation and the genetic basis of the methylation and demethylation processes
- **SUBCELLULAR MECHANISMS:** understanding of the biochemical and biophysical mechanisms in Hg transformation and subcellular structure-function relationships

Parks, J.M., Guo, H., Momany, C., Liang, L., Miller, S.M., Summers, A.O., Smith, J.C. (2009) *J. Amer. Chem. Soc.*, 131, 13278-13285.

Miller, C.; Southworth, G.; Brooks, S. C.; Liang, L.; Gu, B. (2009) *Environ. Sci. Technol.*, 43, (in press).



Spallation Neutron Source at ORNL