ORNL Mercury SFA Annual Report

July 2015

Biogeochemical and Molecular Mechanisms Controlling Contaminant Transformation in the Environment

Mercury Program Overview

Anthropogenic releases and changing environmental conditions profoundly affect the biogeochemical cycling of trace metals, such as mercury (Hg). Mercury can be methylated to form methylmercury (MeHg), a neurotoxin that bioaccumulates in the food web, endangering humans and other biota. While mercury contamination in natural environments results mostly from atmospheric processes,[1–4] mining and industrial processes can lead to severe local pollution. On the Oak Ridge Reservation (ORR), for example, mercury pollution in the East Fork Poplar Creek (EFPC) watershed is caused by historical mercury use at the Y-12 National Security Complex where large quantities of mercury were lost to the environment during the 1950s and 1960s.

Since its inception 6 years ago, the Mercury Science Focus Area (SFA) led by Oak Ridge National Laboratory (ORNL)—formally known as the *Biogeochemical and Molecular Mechanisms Controlling Contaminant Transformation in the Environment* project—has made substantial progress in fulfilling its overarching research aim:

> ***Elucidating the mechanisms by which inorganic mercury is transformed into methylmercury at the sediment-water interface and the processes that determine net methylmercury production at contaminated sites.***

Contents

Distinguishing attributes of the ORNL Mercury SFA include the range of spatiotemporal scales studied—spanning from molecules to watersheds and from picoseconds to seasonal variations—and the effective integration of diverse technical expertise and disciplines, including hydrology, geochemistry, microbiology, biomolecular sciences, high-performance computer simulations, and neutron science. This unique combination of scales and skills has resulted in a number of groundbreaking insights, including the discovery of the mercury methylation genes (*hgcAB*) and their organismal and environmental distribution.[5,6] SFA research has also provided new understanding of the dual functional role of dissolved organic matter (DOM) in mercury redox transformation[7] and knowledge that certain anaerobic microorganisms are capable of using dissolved elemental Hg(0) for methylation.[8] Furthermore, SFA scientists constructed a computational framework for understanding subcellular mercury processes and used it to determine that mercury methylation occurs via a novel biochemical mechanism.[9] In parallel, field-relevant geochemical and microbial data established that DOM dominates aqueous mercury speciation in EFPC[10,11] and that both iron (Fe)- and sulfate-reducing bacteria are prevalent in the system.[12-14] Laboratory studies established that mercury methylation is strongly coupled to mercury redox transformation and its sorption or binding at cell surfaces[15,16] and that mercury uptake in certain bacteria (e.g., *Geobacter sulfurreducens*) is an energy-dependent process influenced by the chemical characteristics of complexing thiols.[17] Lastly, our research team identified important roles of DOM and carbonates in

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Sponsor: The ORNL Mercury SFA is sponsored by the Subsurface Biogeochemical Research (SBR) program within the U.S. Department of Energy's Office of Biological and Environmental Research. Contact Paul Bayer, SBR Program Manager, at paul.bayer@science.doe.gov.

the photodegradation of methylmercury and photoredox transformation of mercury in natural waters.[18,19] (See sidebar, BBC Program Spotlights ORNL Mercury Research, this page, for project achievements featured in a special news series on reversing global pollution.)

This annual report summarizes these and other ORNL Mercury SFA accomplishments from July 2014 to June 2015, a period representing the third year following the program's triennial peer review in May 2012 by the U.S. Department of Energy's (DOE) Office of Biological and Environmental Research.

Achieving the goals of the renewed SFA program would enable the development of a long-term, targeted strategy to significantly mitigate the adverse impacts of mercury contamination at DOE sites and across the nation.

Scientific Progress and Select Research Highlights

This section summarizes the technical progress made since the last SFA annual report in July 2014 and concludes with four select highlights from recent research. The accomplishments described here contribute to the program's overarching research aim and four objectives:

- **Objective 1** — Examining site biogeochemical processes of the East Fork Poplar Creek watershed and identifying key methylation source areas.
- **Objective 2** — Investigating key mechanisms and geochemical controls on mercury and methylmercury species transformation and mercury reactivity leading to its uptake and methylation.
- **Objective 3** — Identifying microbial species and their biochemical pathways responsible for biological methylation and demethylation.
- **Objective 4** — Understanding biogeochemical transformations within and outside the microbial cell using structural biology and computational chemistry.

Additional Accomplishments

See Appendices B–D, pp. 25–38, for details on other progress made from July 2014 to June 2015, including:

- 11 papers published or in press
- 17 presentations, abstracts, or posters delivered or accepted
- 2 invited talks

These scientific objectives are aligned to four integrated research tasks that have well-developed milestones, outcomes, and schedules:

- **Task 1** — Site Biogeochemical Processes
- **Task 2** — Fundamental Mechanisms
- **Task 3** — Microbial Transformations and Genetics
- **Task 4** — Molecular Structure, Function, and Mechanisms

Details regarding task accomplishments and publications are provided in the following sections.

Task 1: Site Biogeochemical Processes

Task 1 research examines the biogeochemical controls on mercury methylation and demethylation within the context of the flowing creek system and its connection with the surrounding watershed. Emphasis is on field-based investigations with supporting laboratory work to elucidate mechanisms. Overarching objectives of Task 1 are to:

- Identify the ecosystem compartment(s) (e.g., channel margin, floodplain, and periphyton) and hydro-biogeochemical conditions that govern net methylmercury concentration in EFPC.
- Study total mercury (HgT) and methylmercury dynamics and relationships to organic matter under

BBC Program Spotlights ORNL Mercury Research

The British Broadcasting Company (BBC) recently featured ORNL mercury research as part of its series on Pollution Solutions: Reversing the Effects of Pollution. SFA accomplishments—including discovery of the two-gene cluster responsible for microbial mercury methylation—were highlighted, along with other mercury-focused research within ORNL's Environmental Sciences Division, such as fish-sample collection to assess methylmercury bioaccumulation in the food web.

Senior Scientist Eric Pierce noted the potentially far-reaching effects of basic research to understand the biochemical mechanisms of methylmercury production. "The knowledge . . . as well as the technology that we develop out of these various projects solves mercury problems not only here, but really globally," he said. "The work that we're doing provides knowledge for mercury challenges worldwide."

The BBC video, titled Episode 6: Fishing for Mercury, can be viewed at: **www.bbc.com/specialfeatures/horizonsbusiness/seriesfive/episode-6-pollution-solutions/?vid=p02tg625**. For other media spotlights of Mercury SFA research, see Appendix D, p. 38.

high-flow when EFPC is more hydrologically connected to the watershed.

FY14 – FY15 Accomplishments

Over the past 12 months, researchers within Task 1 made significant progress toward milestones and published two papers. One paper, in collaboration with Task 2, described the interactions between dissolved organic matter (DOM) and methylmercury and the mechanisms by which these interactions can promote photo-demethylation of methylmercury in creek water.[20] In collaboration with scientists at Harvard University and the Woods Hole Oceanographic Institute, SFA researchers also report on interfacial biogeochemical reactions between bacteria and the surfaces of sulfide minerals they colonize.[21] Using a combination of field deployments, laboratory experiments, and high-resolution surface spectroscopy, we show that commonly occurring chemotrophic bacteria of the genus *Thiobacillus* enhance metacinnabar (ß-HgS) solubility under circumneutral pH, resulting in the subsequent reduction of the released Hg(II) to Hg(0). These results demonstrate a novel pathway for Hg(0) formation in aquatic environments. Other milestone progress is summarized below.

Role of Periphyton in EFPC Mercury Cycling

Dissolved methylmercury concentration in EFPC has shown diel variability over multiple sampling events, and this variability appears to be correlated with the daily photocycle. These observations, coupled with the results of storm event sampling, imply that key controls on net mercury methylation occur within the stream or on the stream bed and include factors such as small-scale temperature changes in the water column and the photosynthetic activity of stream biofilms.

We began initial assessments of the role of stream periphyton biofilms in methylmercury generation. Periphyton biofilms are complex assemblages comprising algae, bacteria, fungi, diatoms, extracellular polymers, invertebrates, detritus, and mineral particles. Due to the diversity of their components, these biofilms can be important in the biogeochemical cycling of many elements. Moreover, their structure and activity can promote the development of the biogeochemical conditions associated with mercury methylation (e.g., iron-reducing, sulfate-reducing, and fermentative conditions). Periphyton samples collected monthly along the length of the creek are analyzed for total mercury and methylmercury content to assess spatial and seasonal effects. The methylmercury content of the biofilms is higher than that in the surrounding creek sediment and increases with distance downstream. Biofilm samples grown on artificial substrates deployed in the creek have methylmercury content similar to natural biofilms.

Samples of intact periphyton biofilms grown in the field and returned to the lab are used in mercury methylation and methylmercury demethylation assays. These assays use enriched stable isotopes of mercury to distinguish *de novo* activity from the ambient mercury and methylmercury present in the samples. Initial experiments demonstrate that both mercury methylation and methylmercury demethylation occur in the biofilms, but the estimated rate of methylation outpaced the rate of demethylation by a factor of ~2.5 (see Fig.1a, p. 4). During our February 2015 tests, methylmercury produced in the biofilms could account for 30% to 40% of the methylmercury flux, as estimated at our downstream monitoring station (EFK 5.4). Periphyton biofilms thus appear to play a central role in net methylmercury generation in EFPC.

Redox Zonation Within Periphyton Biofilms

We acquired a potentiostat to conduct electrochemical measurements within the periphyton biofilms. Using the correct combination of microelectrodes and micromanipulators allows us to directly quantify important redox couples at high spatial resolution, including dissolved oxygen, manganese, Fe(II), S(II), and iron sulfide. The development of iron- and sulfate-reducing conditions is particularly important because such conditions are necessary for mercury-methylating microorganisms. With this new equipment, we demonstrated that over a distance of ~2 mm, conditions changed from aerobic above the biofilm to sulfate-reducing at its base (see Fig. 1b, p. 4). These findings are thus consistent with the methylmercury-generation results mentioned above.

Shallow Groundwater

Groundwater sampled from shallow wells at two sites has a distinct chemical signature from the surface water, as evidenced by lower pH (1–1.5 units), absence of dissolved oxygen and nitrate, lower sulfate concentration, and elevated concentrations of Fe(II) and sulfide. The lower pH is likely due to organic acid production from fermentative microorganisms, and the other parameters are indicative of active anaerobic microbial activity. Dissolved methylmercury concentration in the groundwater is comparable to or up to 10 times greater than the surface water, suggesting that the groundwater may be negatively impacting surface water quality. Further studies including collaboration with Task 2 and 3 are planned.

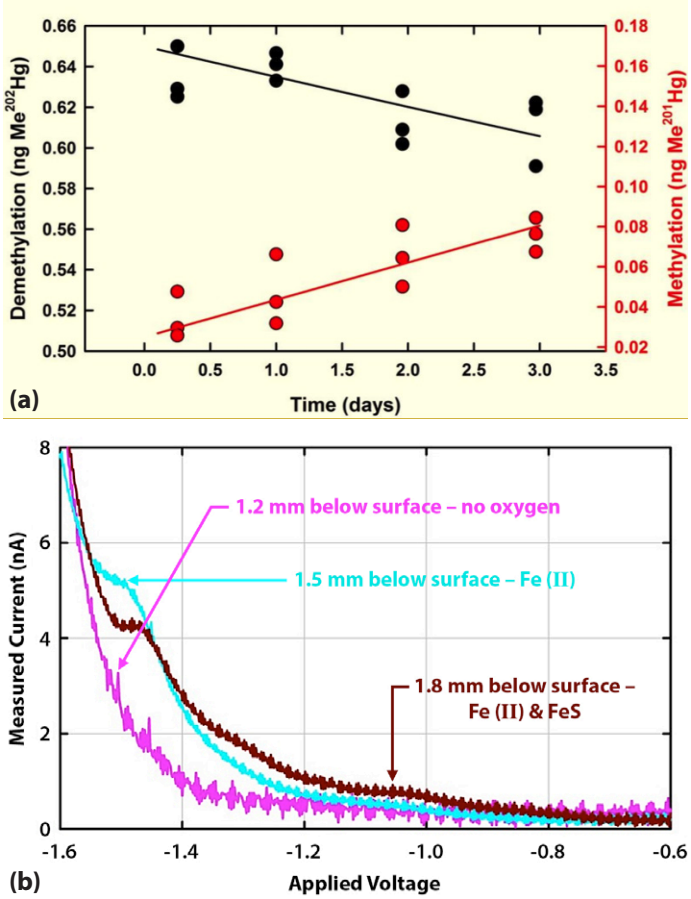

Fig. 1. (a) Methylmercury demethylation (black) and mercury methylation (red) in periphyton biofilms. Lines represent pseudo first-order decay and production curves fit to the data by adjusting the value of the rate constant. **(b) Voltammograms** collected from intact periphyton samples demonstrate a steep gradient in geochemical conditions from aerobic to sulfate-reducing over a distance of less than 2 mm.

Creek Response to Changes in Flow Management Practices

Since the mid-1990s, the flow in EFPC had been managed via the addition of lake water at the rate of 5 million gallons per day at the headwaters of the creek (this constituted ~65% of baseflow as measured at the point where EFPC exits Y-12). By order of the Tennessee Department of Environment and Conservation, this practice was ceased on April 30, 2014. We took this opportunity to begin a sampling campaign to document creek response to the decreased flow. Notable among the changes are a decrease in total suspended solids (TSS) and an increase in the fraction of total mercury and dissolved methylmercury (<0.2 μm). On average, dissolved methylmercury concentration in the creek was 1.6 times greater in EFPC during summer 2014 relative to conditions during flow management. To the extent that dissolved methylmercury is more bioavailable than particle-associated methylmercury, this change in flow management and the consequent changes in mercury and methylmercury speciation could have important implications for mercury bioaccumulation in EFPC.

Manuscripts

Published or In Press

Qian, Y., X. Yin, H. Lin, B. Rao, S. Brooks, L. Liang, and B. Gu. 2014. "Why dissolved organic matter enhances photodegradation of methylmercury." *Environ. Sci. Technol. Lett.* **1**(10): 426–431. [DOI:10.1021/ez500254z]

Vázquez-Rodríguez, A. I., C. M. Hansel, T. Zhang, C. H. Lamborg, C. M. Santelli, S. M. Webb, and S. C. Brooks. 2015. "Microbial- and thiosulfate-mediated dissolution of mercury sulfide minerals and transformation to gaseous mercury." *Frontiers Microbiol.* **6**: 1–11.

In Preparation

Riscassi, A. L., C. L. Miller, and S. C. Brooks. In review. "Seasonal and flow-driven dynamics of unfiltered and filtered mercury and methylmercury in a stream impacted by an industrial mercury source." *Environmental Toxicology and Chemistry*

Riscassi, A. L., C. Miller, and S. Brooks. "Diel mercury-concentration variations in a mercury impacted stream."

Brooks, S. C., C. L. Miller, D. Kocman, A. L. Riscassi, X. Yin, K. Lowe, T. Lowe, and M. A. Bogle. "Effect of flow management changes on Hg dynamics in a Hg-contaminated creek."

Task 2: Fundamental Mechanisms

The primary objective of Task 2 is to provide a fundamental understanding of the key geochemical and biochemical mechanisms controlling mercury sorption, uptake, and transformation at the interfaces between microbes, fluids, and particulate minerals.

FY14 – FY15 Accomplishments

SFA research previously found that microbial methylation is strongly influenced by specific thiol ligands and that the uptake process is energy dependent. We also identified the strain-specific, bacterially mediated oxidation and methylation of dissolved elemental Hg(0) under anoxic incubations and the unexpected effects of gene deletion on mercury interactions with the methylation-deficient

mutant *ΔhgcAB*. Our subsequent studies (FY2014) focused on coupled mercury reduction, oxidation, cell-surface adsorption, and methylation by *G. sulfurreducens* PCA. We found that mercury methylation was positively correlated with mercury sorption but negatively correlated with mercury reduction. These reactions depended on the ratio of mercury to cellular thiols: Increasing this ratio shifts the major reaction from oxidation to reduction, and vice versa. We also found that the *c*-cytochrome deletion mutant, *ΔomcBESTZ*, decreases mercury reduction but increases methylation. Additionally, we recently reported the influences of cysteine on time-dependent Hg(II) reduction, sorption, and methylation by the wild-type (WT) strain of PCA and its mutant *ΔomcBESTZ* (see research highlight, *Cysteine Inhibits Production of Methylmercury Neurotoxin by Geobacter Mutant ΔomcBESTZ*, p. 10). Without cysteine, the mutant methylated twice as much Hg(II) as the WT, but the addition of cysteine inhibited mercury methylation regardless of reaction time or cysteine concentration. These results contrast with the common belief that cysteine increases mercury uptake and methylation and suggest that the role of cysteine in microbial mercury methylation is more complicated than previously thought.

We also investigated the rates and mechanisms of methylmercury photodegradation and Hg(0) photo-oxidation. All DOM and organic ligands are found to increase methylmercury photolysis under solar irradiation, but the first-order rate constants vary depending on DOM oxidation state and the type and concentration of the complexing ligands[20]. Compounds containing both thiol and aromatic moieties within the same molecule (e.g., thiosalicylate and reduced DOM) increased methylmercury photodegradation rates far greater than those containing only aromatics or thiols (e.g., salicylate, glutathione, or their combinations). In other words, the synergistic effects of thiols and aromatics in DOM can lead to greatly enhanced methylmercury photodegradation. The SFA research team also identified a new mechanism for direct energy transfer from an excited triplet state of DOM to break the mercury–carbon bond in methylmercury, thus answering a long-standing question about why DOM enhances methylmercury photodegradation. Additionally, we identified a new pathway of Hg(0) photo-oxidation by carbonate radicals ($CO_3^{\bullet -}$). Photo-oxidation of Hg(0) is affected by reactive ionic species (e.g., DOM, carbonate, and nitrate). Using scavengers and enhancers for singlet oxygen ($^1O_2$) and hydroxyl ($HO^\bullet$) radicals, as well as electron paramagnetic resonance spectroscopy, we showed that carbonate radicals primarily drive the Hg(0) photo-oxidation in EFPC water.[18] These findings are of great significance in understanding mercury chemical speciation, transformation, and transfer at the water-air interface in the environment.

Published or Submitted Manuscripts

Lin, H., X. Lu, L. Liang, and B. Gu. 2015. "Cysteine inhibits mercury methylation by *Geobacter Sulfurreducens* PCA mutant *ΔomcBESTZ*." *Environ. Sci. Technol. Lett.* **2**: 144–48.

He, F., W. Zhao, L. Liang, and B. Gu. 2014. "Photochemical oxidation of dissolved elemental mercury by carbonate radicals in water." *Environ. Sci. Technol. Lett.* **1**: 499–503.

Lin, H., J. L. Morrell-Falvey, B. Rao, L. Liang, and B. Gu. 2014. "Coupled mercury–cell sorption, reduction, and oxidation on methylmercury production by *Geobacter sulfurreducens* PCA." *Environ. Sci. Technol.* **48**(20): 11969–11976.

Qian, Y., X. Yin, H. Lin, L. Rao, S. C. Brooks, L. Liang, and B. Gu. 2014. "Why dissolved organic matter enhances photodegradation of methylmercury." *Environ. Sci. Technol. Lett.* **1**: 426–431.

Gu, B., B. Mishra, C. Miller, W. Wang, B. Lai, K. M. Kemner, and L. Liang. 2014. "X-ray fluorescence mapping of mercury on suspended mineral particles and diatoms in a contaminated freshwater system." *Biogeosci.* **11**: 5259–5267.

Luo, H., X. Lu, W. Zhang, H. Lin, H. Yu, L. Liang, G. Sheng, and B. Gu. In review. "Decreased biological methylation upon photochemical reactions between mercury and natural organic matter." *Nature Geosci.*

Task 3: Microbial Transformations and Genetics

The overarching goals of Task 3 are twofold: (1) Determine the breadth and depth of mercury-methylating species and (2) determine the native biochemical function(s) of HgcA and HgcB and their participation in other cellular biochemical pathways. Our research is designed to resolve three specific questions:

- How widespread is the ability to methylate mercury, and what are the relative contributions to the overall net pool of methylmercury generated?
- What is the native biochemical function of HgcA and HgcB, and in which biochemical pathways do they participate?
- Under what conditions are the expression of HgcA and HgcB increased or decreased?

FY14 – FY15 Accomplishments

In the past year, we have made considerable progress in understanding the processes governing microbially mediated mercury transformations and the physicochemical factors that influence these processes across a range of scales. A summary of this progress is presented in the following two sections.

Microbial Cellular Mercury Methylation

Based on the discovery of the mercury-methylation genes *hgcAB* and their ability to be used as biomarkers for mercury methylation, we have collaborated with Task 4. At the molecular level, we determined the cysteine residues that are essential to methylation to understand how Hg(II) is transferred and bound to the methyl group to form methylmercury (see research highlight, *Key Amino Acid Residues for Mercury Methylation Confirmed*, p. 11). Through targeted codon substitutions, we found that many residues in and out of the active site are necessary for mercury methylation. At the biochemical level, we are determining HgcAB native function (i.e., what these proteins do in the absence of mercury) and are mapping the path of the methyl group for methylmercury (i.e., identifying the methyl donor).

As an independent task that will benefit the overall project, we have completed development of degenerate primers for the detection, identification, and quantification of *hgcAB* from all environments. Three other reports exist but are incomplete. Two reports determine and quantify only *hgcA*, which we have shown cannot methylate without *hgcB*, suggesting that false positives may occur.[22,23] The third report uses primers for *hgcAB* and suggests that *Firmicutes* are the dominant mercury methylators.[24] However, the Bae et al. 2014 [24] study did not quantify *hgcA*. We tested all three methods using their protocols and materials and found that the first two reports yielded amplification of two to five pure cultures out of the 28 tested that spanned all four clades, while the latter amplified only *Firmicutes*. These findings suggest that Liu et al. 2014[22] and Schaefer et al. 2014[23] captured only a small fraction of the mercury-methylating community. The Bae et al.[24] study led to incorrect conclusions that *Firmicutes* are the major methylators in the Florida Everglades because, according to our results, they preferentially amplified those organisms. Our latest results show that we have both a universal set of degenerate primers that amplify *hgcAB* from 26 of the 28 tested organisms and clade-specific quantitative polymerase chain reaction (qPCR) primers for the *Deltaproteobacteria, Firmicutes,* and the *Archaea*. These primers will enable the overall capture and identification of organisms containing *hgcAB* while yielding quantitative *hgcA* values that are clade specific, thus providing data that tie community metabolism to mercury methylation.

Microbial Ecology of Mercury Methylation

Investigations into the microbial ecology and physiology of mercury methylation have yielded an increased understanding of methylmercury production at the community level and the geochemical influences on its generation. We have investigated the role of *Deltaproteobacteria* in mercury methylation on the Oak Ridge Reservation (ORR) and found that they are likely the major methylators. While working with Task 2 to understand cell surface interactions, Task 3 researchers have been determining methylation kinetics and the role of DOM in mercury methylation during sulfate reduction. These findings will be tested in the proposed work using model (synthetic) microbial communities with results that can be extrapolated to and tested in the field.

We have taken this knowledge a step further and surveyed >3,500 publicly available microbial metagenomes (with >1.6 billion genes) across various databases. This survey yielded a global picture of mercury methylation potential in many environments (see research highlight, *Global Prevalence and Distribution of hgcA, a Gene Encoding Microbial Mercury Methylation*, p. 12). We found that while *hgcAB* was absent from mammalian microbiomes and the open oceans, it was prevalent in coastal dead zones, contaminated sites, engineered sites, and Arctic permafrost. We also discovered associations of methylating and nonmethylating organisms that co-occur in nature. These findings are important in understanding the metabolic associations of key types of organisms and their impact on mercury methylation. For example, we have determined that some fermenters (e.g., *Clostridium* spp.) frequently associate with selected sulfate-reducers and methanogens. These symbiotic relationships are known to increase nutrient sharing and thus create more efficient lifestyles. Similar relationships may transfer to more efficient mercury methylation potentials than have been observed in monocultures in the laboratory. This knowledge will guide the types of organisms used in synthetic communities.

Published or Submitted Manuscripts

Smith, S. D., R. Bridou, A. Johs, J. M. Parks, D. A. Elias, R. A. Hurt Jr., S. D. Brown, M. Podar, and J. D. Wall. 2015. "Site-directed mutagenesis of HgcA and HgcB reveals amino acid residues important for mercury methylation." *Appl. Environ. Microbiol.* **81**(9): 3205–3217.

Hurt, R. A. Jr., J. G. Moberly, M. Shakya, T. A. Vishnivetskaya, B. Gu, and D. A. Elias. 2014. "Improved yield of high molecular weight DNA coincides with increased microbial diversity access from iron oxide cemented

sub-surface clay environments." *PLoS ONE* **9**(7): e102826. [DOI:10.1371/journal.pone.0102826]

Podar, M., C. C. Gilmour, C. C. Brandt, A. Soren, S. D. Brown, B. R. Crable, A. V. Palumbo, A. C. Somenahally, and D. A. Elias. Accepted. "Global prevalence and distribution of genes and microorganisms involved in mercury methylation." *Science Advances*.

Task 4: Molecular Structure, Function, and Mechanisms

The overarching objective of Task 4 is to understand at the molecular scale how mercury interacts with and is transformed by the species it encounters in natural and contaminated environments. Since our discovery of the mercury methylation genes, we have focused primarily on characterizing the structure and function of HgcA but have also continued work on mercury-ligand interactions and bacterial mercury resistance.

FY14 – FY15 Accomplishments

Since July 2014, we have published four papers. Jointly with Task 3 and Judy Wall's lab (University of Missouri), we have identified several amino acids in HgcA and HgcB that play important roles in mercury methylation.[25] We have also published two papers on mercuric ion reductase (MerA),[26,27] which is involved in bacterial mercury resistance, and another paper describing computational methods for studying mercury interactions with natural organic matter (NOM) and proteins.[28]

In the last year, we have made significant progress toward characterizing HgcA. We have developed a protocol to express the cobalamin-binding domain (CBD) of HgcA heterologously in *Escherichia coli* as an N-terminal maltose binding protein (MBP) fusion (see Fig. 2, this page), which greatly improves protein solubility and production yields. Fusion constructs were gifts from Steve Ragsdale (University of Michigan). We can now readily produce sufficient protein for detailed spectroscopic, structural, and functional characterization. Reconstitution with hydroxocobalamin (Cbl) yielded ~0.9 to 0.95 mol of Cbl per mol of purified protein. All manipulations from cell lysis to protein purification and cofactor reconstitution are now performed under strictly anaerobic conditions (<1 ppm O_2) in an anaerobic chamber. With the availability of sufficient cofactor-loaded $HgcA_{CBD}$, we are now in a position to characterize HgcA by various spectroscopic and biophysical techniques. Experimental characterization is complemented by computational approaches. Confirming the proposed, unprecedented "Cys-on" coordination in HgcA, elucidating its effects on redox chemistry and reactivity, and working toward developing an *in vitro* enzymatic mercury methylation assay are of particular interest.

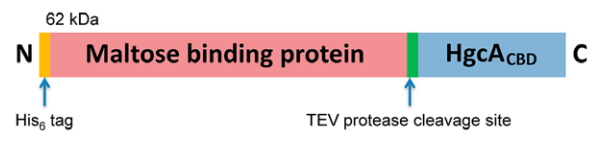

Fig. 2. Biophysical and biochemical characterization of HgcA.

In addition to ultraviolet–visible (UV/Vis) spectroscopy (see Fig. 3, this page), we are working closely with the Ragsdale lab (University of Michigan, unfunded collaborator) to characterize HgcA with electron paramagnetic resonance (EPR), magnetic circular dichroism (MCD), and resonance Raman spectroscopy. We are also working with Graham George (University of Saskatchewan and Canadian Light Source) to study the coordination of cobalt (Co) in HgcA with extended X-ray absorption fine structure (EXAFS) spectroscopy. EXAFS data for $HgcA_{CBD}$ have revealed evidence for the predicted "Cys-on" coordination (manuscript in preparation; see research highlight, *Structural Details of Key Protein in Mercury Methylation Reveal Insights into Its Reactivity*, p. 13). Density functional theory (DFT) calculations were found to be indispensable in generating three-dimensional structural models for interpreting the EXAFS data. We are also using cyclic voltammetry (CV) to measure the midpoint reduction potentials of the cofactor in HgcA, and we have found that E^0 for the Co(II/I) couple is -560 mV. All spectroscopic and electrochemical experiments are being performed with complementary quantum chemical approaches.

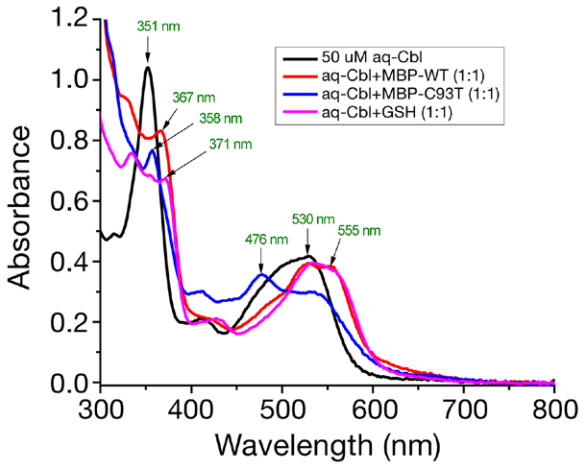

Fig. 3. Ultraviolet–visible spectra of Aqua-Cbl, GS-Cbl, and recombinantly expressed and reconstituted HgcA.

Structure Determination of HgcA$_{CBD}$

Through a user proposal with the DOE Environmental Molecular Sciences Laboratory (EMSL; Proposal 48393), we are working to determine the structure of HgcA$_{CBD}$ by nuclear magnetic resonance (NMR) spectroscopy. An NMR structure would provide direct evidence for the Rossmann fold, cap helix motif, and "Cys-on" coordination proposed for HgcA$_{CBD}$. We have prepared ^{15}N-labeled protein, and preliminary data from ^{15}N–HSQC NMR (heteronuclear single quantum coherence NMR) revealed a limited number of well-dispersed but broad resonances that indicate sample polydispersity (see Fig. 4, this page). HgcA from *Desulfovibrio desulfuricans* ND132 contains two nonconserved cysteine residues, Cys47 and Cys142, which can form intermolecular disulfides. Dynamic light scattering and SDS-PAGE (sodium dodecyl sulfate–polyacrylamide gel electrophoresis) under reducing and nonreducing conditions revealed disulfide bond formation through Cys crosslinking, which results in high molecular weight oligomers. To overcome this issue, we have obtained a modified expression vector encoding a C47S/C142S double mutant in which only Cys93 is retained. In other work, we have heterologously expressed and purified native (i.e., untagged, full-length) HgcA, which includes its predicted transmembrane domain, and have solubilized it in the detergent *n*-dodecylmaltoside.

During the last fiscal year, we used DFT calculations to investigate methyl transfer to Hg(II) in a model of HgcA.[9] We predicted that a mutant of HgcA with Cys93 replaced by His may still be able to methylate mercury *in vivo*. This prediction was subsequently verified experimentally.[25] We are now expanding upon that work by computing Co(III/II) and Co(II/I) standard reduction potentials, UV–visible absorption and Co(II) EPR spectra, and other molecular properties that will be used to help interpret experimental data and to make experimentally testable predictions.

Mercury-Ligand Interactions

In Task 4.2, we investigate mercury-ligand interactions in collaboration with Task 2. We have developed software that facilitates efficient workflows for computational chemistry of these interactions.

Bacterial Mercury Resistance

Task 4.3 investigates the molecular basis of mercury resistance mechanisms, specifically MerA. To accomplish Hg(II) reduction to Hg(0), MerA transfers Hg(II) from a pair of cysteines at its solvent-exposed C-terminus to another pair of cysteines in its active site. We have

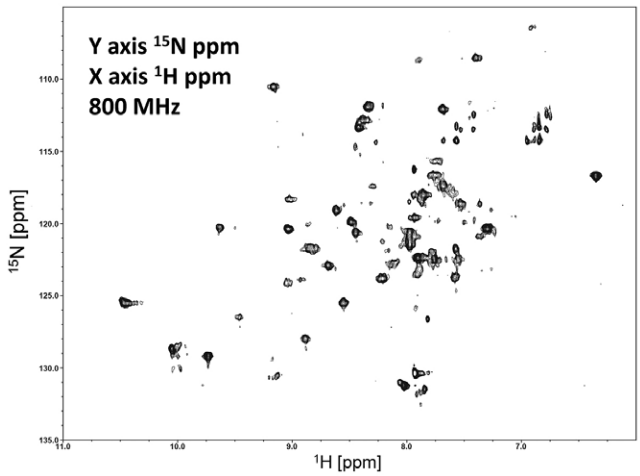

Fig. 4. Preliminary two-dimensional ^{15}N–HSQC NMR spectra of CbI/^{15}N-labeled HgcA$_{CBD}$.

combined molecular dynamics simulation on ORNL's Titan supercomputer with neutron scattering experiments to probe the dynamics of a compact state of MerA.[26] We have also combined X-ray crystallography, quantum mechanics, and molecular mechanics calculations to study the mechanism of intramolecular Hg(II) transfer in the catalytic core of MerA.[27] This work was performed jointly by ORNL, the University of California–San Francisco (Susan Miller), and the University of Tennessee (UTK) as part of our previous, university-led mercury project funded by the Subsurface Biogeochemical Research (SBR) program within DOE's Office of Biological and Environmental Research.

FY14 – FY15 Accomplishments

Task 4 published four manuscripts in the past year (one led by Task 3); several others are in preparation. Below is a list of key results.

- Identified several amino acid residues in HgcA and HgcB that are important for mercury methylation (with Task 3).
- Heterologously expressed and reconstituted HgcA$_{CBD}$ with cobalamin under strictly anaerobic conditions.
- Developed computational tools for studying mercury-ligand and mercury-protein interactions.
- Characterized cofactor binding to HgcA$_{CBD}$ by UV/Vis spectroscopy.
- Computed reduction potentials for several cobalamins.
- Observed "Cys-on" coordination in HgcA$_{CBD}$ with EXAFS spectroscopy.

- Measured midpoint reduction potentials for $HgcA_{CBD}$ with CV.
- Prepared ^{15}N-labeled $HgcA_{CBD}$ for NMR spectroscopy and collected preliminary spectra as part of a user proposal to EMSL.
- Heterologously expressed and purified native (i.e., untagged, full-length) HgcA.

Published or Submitted Manuscripts

Smith, S. D., R. Bridou, A. Johs, J. M. Parks, D. A. Elias, R. A. Hurt Jr., S. D. Brown, M. Podar, and J. D. Wall. 2015. "Site-directed mutagenesis of HgcA and HgcB reveals amino acid residues important for mercury methylation." *Appl. Environ. Microbiol.* **81**: 3205–3217.

Riccardi, D., J. M. Parks, A. Johs, and J. C. Smith. 2015. "HackaMol: An objected-oriented Modern Perl library for molecular hacking on multiple scales." *J. Chem. Inf. Model*. **55**: 721–726.

Lian, P., H.-B. Guo, D. Riccardi, A. Dong, J. M. Parks, Q. Xu, E. Pai, S. M. Miller, D.-Q. Wei, J. C. Smith, and H. Guo. 2014. "X-ray structure of a Hg^{2+} complex of mercuric reductase (MerA) and QM/MM study of Hg^{2+} transfer between the C-terminal and buried catalytic site cysteine pairs." *Biochemistry* **53**: 7211–7222.

Hong, L., M. A. Sharp, S. Poblete, R. Biehl, M. Zamponi, N. Szekely, M.-S. Appavou, R. G. Winkler, R. Nauss, A. Johs, J. M. Parks, Z. Yi, X. Cheng, L. Liang, M. Ohl, S. M. Miller, D. Richter, G. Gompper, and J. C. Smith. 2014. "Structure and dynamics of a compact state of a multi-domain protein, the mercuric ion reductase." *Biophys. J.* **107**: 393–400.

Manuscripts in Preparation

"Co–S coordination by a conserved cysteine in the corrinoid protein HgcA revealed by EXAFS and DFT."

"Evaluation of density functional approximations for computing reduction potentials of cobalamins and cobinamides."

ORNL Mercury SFA Research Highlight

Cysteine Inhibits Production of Methylmercury Neurotoxin by *Geobacter* Mutant *ΔomcBESTZ*

Study shows that cysteine significantly affects microbial mercury methylation and highlights the need for further insight into reaction mechanisms.

The Science

Microbial conversion of inorganic mercury (Hg) to a neurotoxic form called methylmercury in the environment is influenced by many factors, including pH, sulfide, and complexing organic ligands and thiols. Some thiol compounds such as cysteine have previously been found to enhance Hg(II) uptake and methylation by the wild-type (WT) strain of the bacterium *Geobacter sulfurreducens* PCA. The prevalence of this enhancement in other strains is not clear. To better understand the complex roles of cysteine in microbial mercury uptake and methylation, we systematically examine how cysteine concentration and reaction time affect methylmercury production by *G. sulfurreducens* PCA and its c-cytochrome-deficient mutant, *ΔomcBESTZ*.

The Impact

In contrast to previous findings, our results show that cysteine does not always increase mercury uptake and methylation in anaerobic microbes but, in fact, inhibits these processes in certain strains such as *ΔomcBESTZ*. These results provide improved understanding on how competitive mercury interactions between microbial cells and complexing thiol ligands in solution may control microbial uptake of mercury and production of methylmercury, a global pollutant.

Summary

Results demonstrate the time-dependent effect of cysteine concentration on mercury methylation by *G. sulfurreducens* PCA cells: addition of cysteine alters Hg(II) speciation, decreasing Hg(II) reduction and sorption but increasing the initial Hg(II) aqueous concentration (see Fig. 5, this page). These interactions are kinetically limiting and may thus control the rate of mercury uptake and methylation by both the mutant and the WT. The demonstrated significant effect of cysteine on mercury methylation by anaerobic bacteria underscores the need to further examine the effects of complexing ligands such as thiols or thiol groups in natural organic matter on methylmercury production in the environment.

Publication

Lin, H., X. Lu, L. Liang, and B. Gu. 2015. "Cysteine inhibits mercury methylation by *Geobacter sulfurreducens* PCA mutant *ΔomcBESTZ*." *Environ. Sci. Technol. Lett.* **2**: 144–48. [DOI:10.1021/acs.estlett.5b00068]

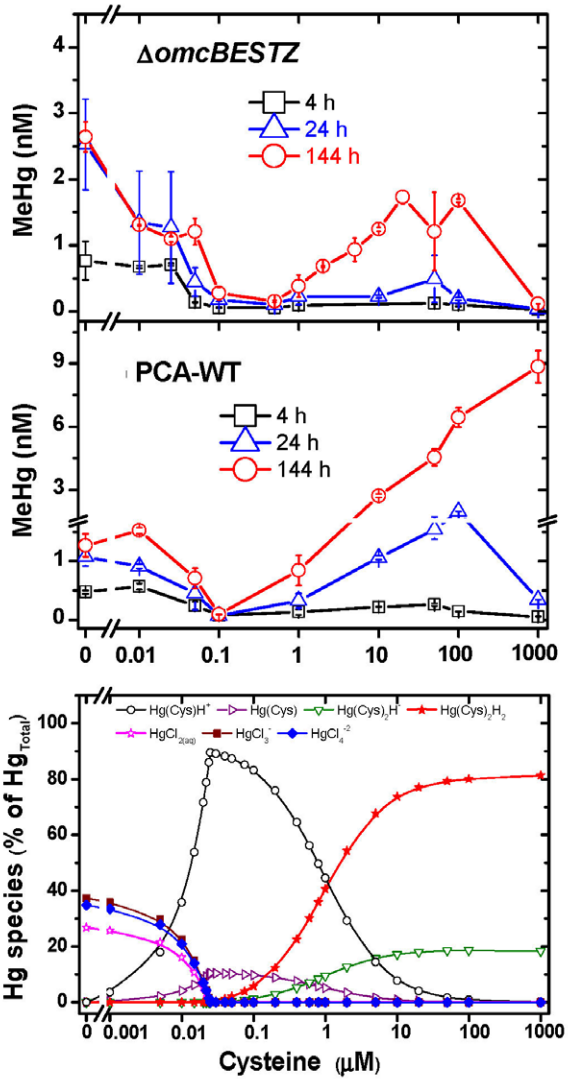

Fig. 5. Production of methylmercury (MeHg) by a *Geobacter sulfurreducens* PCA mutant (top) and wild-type strain (middle) as a function of cysteine concentration. Bottom graph shows mercury (Hg) speciation calculations using Minteq computer code at cysteine concentrations of 0–1000 μM in phosphate-buffered saline.

ORNL Mercury SFA Research Highlight

Key Amino Acid Residues for Mercury Methylation Confirmed

Team constructs site-directed mutants of HgcA and HgcB proteins and examines the effect on production of methylmercury pollutant.

The Science

Methylmercury is a potent neurotoxin produced from inorganic mercury by anaerobic microbes in natural environments worldwide. Once produced, methylmercury accumulates in the aquatic food chain, thus posing a risk to human health in many regions. Recently, the two genes necessary for microbial production of methylmercury were identified: *hgcA* encoding a corrinoid protein and *hgcB* encoding a ferredoxin. To date, all microbes possessing orthologs of these genes have been found to be capable of methylating mercury, whereas organisms lacking these genes are not. Although these findings firmly establish the significance of these proteins in mercury methylation, mechanistic details about their predicted roles in the process require further study. In this research, the proteins were explored to determine whether amino acid residues predicted to be important in methylmercury formation could be mutated and how these mutations affect methylmercury production.

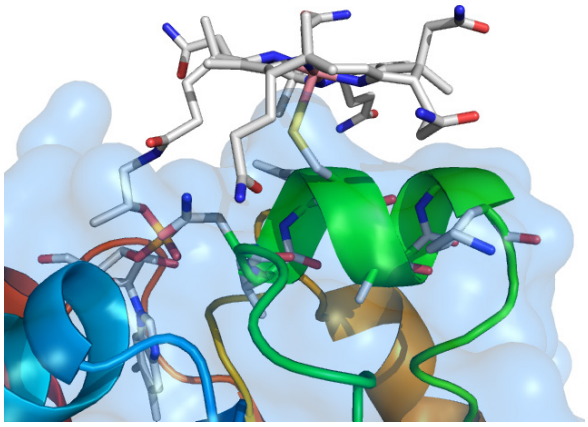

Fig. 6. Homology model of the corrinoid-binding domain of HgcA from *Desulfovibrio desulfuricans* ND132. The corrinoid cofactor is illustrated at the top of the figure with the cobalt shown in pink. The proximity of the strictly conserved Cys (sulfur in yellow) to cobalt in the corrinoid suggests a unique "base-off" "Cys-on" configuration and thus a possible functional role for this interaction in the methylation reaction. The "cap-helix" is shown as a green ribbon (N90 – K97). Amino acid side chains are represented as stick models. Additional elements are oxygen, red; nitrogen, blue; and carbon, gray.

The Impact

This research reveals new insight into mercury methylation by anaerobic microorganisms. Results support the previously predicted importance of an amino acid residue (Cys93) in HgcA and reveal additional residues in both proteins that facilitate methylmercury production. In addition, these structural details may help identify alternative physiological functions of these proteins that could point to evolutionary drivers for maintaining the capacity of anaerobes to methylate mercury.

Summary

The deduced amino acid sequence of HgcA was threaded onto the crystal structure of the corrinoid iron-sulfur protein (CFeSP), revealing a cysteine (Cys93) to be within liganding distance of the cobalt of the corrinoid of HgcA. This finding supported the predicted transfer of a carbanion or methyl radical from the corrinoid to the mercuric ion leading to methylmercury formation. Mutation of Cys93 to Ala93 or Thr93 completely eliminated the methylation capacity, whereas His93 retained a small methylation capacity. Thus, the previously predicted importance of the strictly conserved Cys93 in HgcA was confirmed, as was the "cap helix" orienting the Cys93 to the cobalt (see Fig. 6, this page). Surprisingly, HgcB could not be compensated by other ferredoxins apparently encoded in the genome.

Publication

Smith, S. D., R. Bridou, A. Johs, J. M. Parks, D. A. Elias, R. A. Hurt Jr., S. D. Brown, M. Podar, and J. D. Wall. 2015. "Site-directed mutagenesis of HgcA and HgcB reveals amino acid residues important for mercury methylation." *Appl. Environ. Microbiol.* **81**: 3205–17. [DOI:10.1128/AEM.00217-15]

ORNL Mercury SFA Research Highlight

Global Prevalence and Distribution of *hgcA*, a Gene Encoding Microbial Mercury Methylation

The gene is effectively absent in ~1500 human microbiomes, suggesting a low risk of in-body production of the neurotoxin.

The Science

Most natural and anthropogenic mercury exists as inorganic Hg^{2+} that can be transformed into toxic methylmercury by anaerobic microbes. Recent discovery of the two genes responsible for this transformation—*hgcA* and *hgcB*—provides a genetic tool to identify the capacity and distribution of mercury-methylating bacteria across the globe. This knowledge would in turn help improve insights into the potential cycling and health impacts of environmental mercury. To gain an understanding of the global distribution and abundance of *hgcAB*, we queried the diversity and distribution of these genes in >3,500 publicly available microbial metagenomes encompassing a broad range of environments (see Fig. 7, this page).

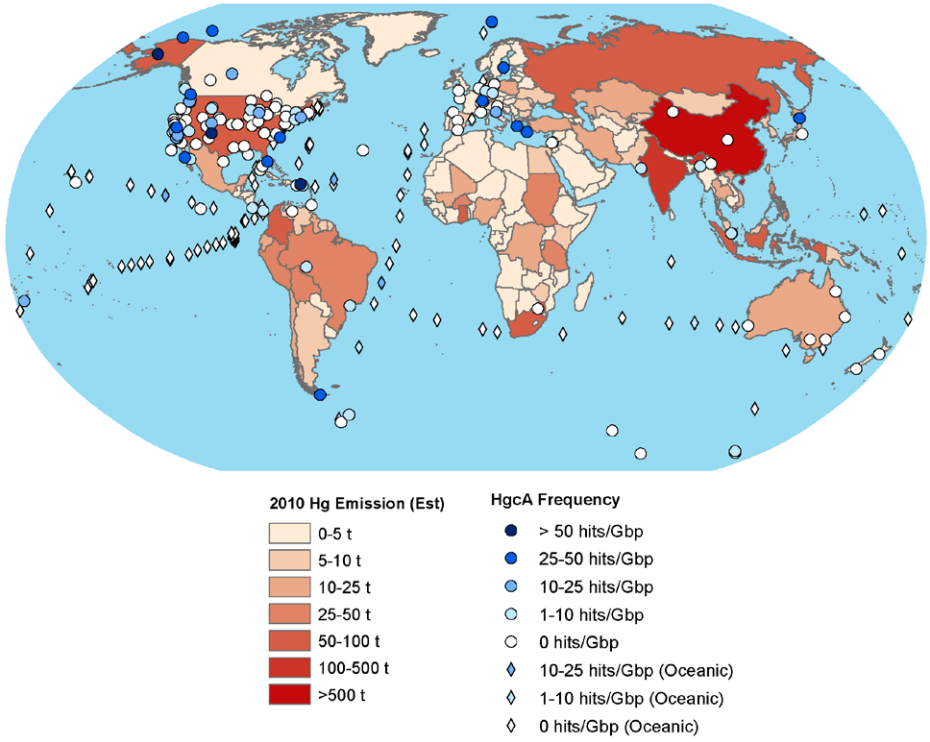

Fig. 7. Global relative frequency of HgcA based on metagenomic projects. Overlay is the estimated continental emission of mercury (in tons), based on the United Nations Environment Programme Global Mercury Assessment 2013 report.[29] Diamonds represent pelagic ocean water samples, and circles represent all other samples.

The Impact

Results suggest that *in situ* or in-body mercury methylation is extremely rare (if not absent) in all mammals including humans, indicating that human methylmercury poisoning probably only occurs through ingestion of the toxin. New potential methylation habitats also were identified, including invertebrate guts, thawing permafrost, coastal "dead zones," soils, sediments, and extreme environments, suggesting multiple routes for methylmercury entry into food webs. In addition, this study begins to address long-standing evolutionary questions about mercury methylation while generating a new global view of its potential.

Summary

The *hgcAB* genes were found in nearly all anaerobic, but not aerobic, environments including oxygenated layers of the open ocean. Critically, *hgcAB* was effectively absent in ~1500 human microbiomes, suggesting a low risk of endogenous methylmercury production. Several new taxonomic groups capable of mercury methylation emerged, including lineages having no cultured representatives.

Publication

Podar, M., C. C. Gilmour, C. C. Brandt, A. Soren, S. D. Brown, B. R. Crable, A. V. Palumbo, A. C. Somenahally, and D. A. Elias. Accepted. "Global prevalence and distribution of genes and microorganisms involved in mercury-methylation." *Science Advances*.

ORNL Mercury SFA Research Highlight

Structural Details of Key Protein in Mercury Methylation Reveal Insights into Its Reactivity

Spectroscopy and computation provide evidence of unique "Cys-on" coordination in the corrinoid protein HgcA.

The Science

The pathway for microbial conversion of inorganic mercury to toxic methylmercury in the environment was recently found to involve a two-gene cluster consisting of *hgcA* and *hgcB*. The *hgcA* gene encodes a corrinoid protein (HgcA) with a strictly conserved cysteine (Cys). To better understand this protein's reactivity and its role in mercury methylation, greater details of its structure are needed. In this study, extended X-ray absorption fine structure (EXAFS) spectroscopy and density functional theory (DFT) calculations were used to examine the protein structure (see Fig. 8, this page). Specifically investigated was whether the central cobalt in the corrinoid cofactor of HgcA is coordinated to the strictly conserved Cys residue (i.e., "Cys-on" coordination) in HgcA.

The Impact

Results provide key evidence for the unique coordination of the corrinoid cofactor in HgcA by a conserved cysteine. Never before observed in any corrinoid protein, this "Cys-on" coordination mode critically influences the reactivity of the protein's methyl group, thus enabling methyl transfer to mercury substrates to form highly toxic methylmercury.

Summary

To characterize the structure of HgcA, we heterologously expressed its corrinoid-binding domain as a maltose-binding protein (MBP) fusion in *Escherichia coli* and reconstituted it with aquacobalamin. Three-dimensional structural models were generated using DFT calculations. These models were indispensable for interpreting the EXAFS data, which provided direct evidence for the previously predicted "Cys-on" coordination in HgcA.

Publication

Manuscript in preparation.

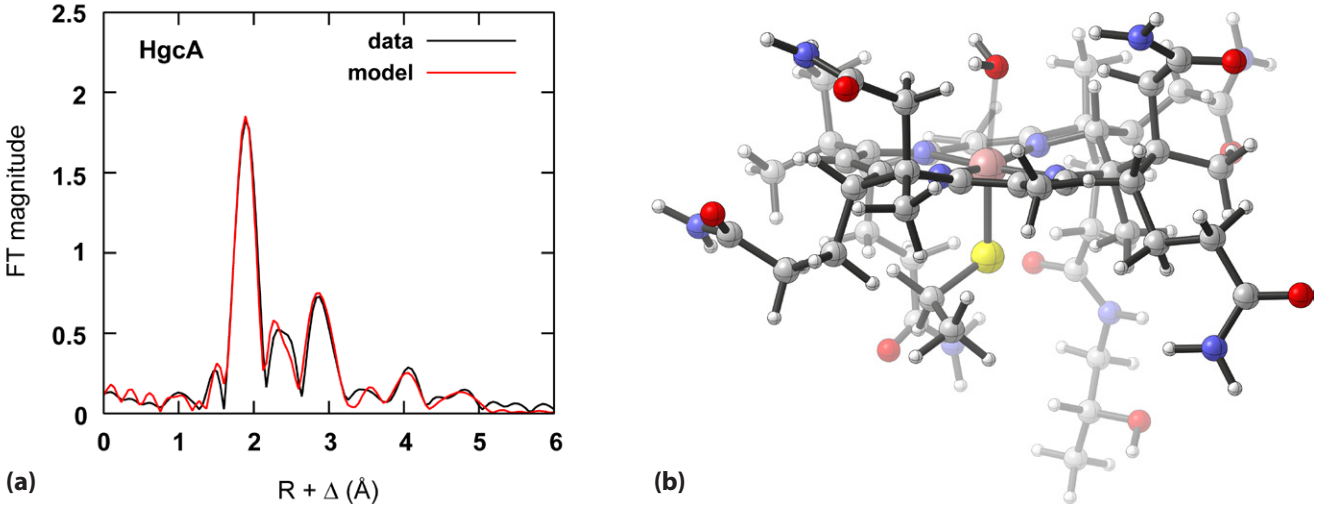

Fig. 8. (a) Cobalt K-edge extended X-ray absorption fine structure data in R space and fit to a model calculated with density functional theory (DFT). (b) DFT model of "Cys-on" coordination in HgcA.

National and International Impact

ORNL Mercury SFA team members attend strategic conferences in the United States and abroad to gain insights into the state of the science, share project findings and strategies with the broader mercury research community, and identify collaborative opportunities. From July 2014 to June 2015, SFA scientists delivered or published 17 presentations, abstracts, or posters and gave two invited talks (see Appendix C, p. 29, for details). Described below are team members' contributions to the 2015 International Conference on Mercury as a Global Pollutant (ICMGP).

ICMGP 2015 Meeting

Every 2 years, since the first meeting in Gävle, Sweden, in 1990, the ICMGP convenes researchers, policy-makers, and industrial organizations in diverse locations around the world to discuss important advances in mercury research and facilitate international collaborations. ICMGP 2015 was held in Jeju, Republic of Korea, on June 14–19 and represented the first meeting since the 2013 signing of the Minamata Convention on Mercury, a global treaty to protect human health and the environment from the adverse effects of mercury.

Session Led by SFA Researchers

ORNL scientists Baohua Gu and Alexander Johs attended the meeting and hosted a special session (along with Tamar Barkay at Rutgers University) titled *Effects of Coupled Microbiological and Geochemical Interactions on Mercury Speciation, Uptake, and Methylation*. Description of the session follows:

"Both biological and geochemical processes determine the rate of net methylmercury production in the environment. Global mercury (Hg) cycling and its impacts on human and environmental health are determined by a complex interplay between abiotic reactions, microbial transformations, and bioaccumulation. A multidisciplinary approach is required to identify key factors that control these processes. This session focuses on coupled microbiological and geochemical interactions affecting Hg chemical speciation, mobility, microbial uptake, and methylation in both natural and contaminated marine and freshwater ecosystems. Topics may include, but are not limited to, recent advances in biogeochemical transformations of mercury (e.g., redox reactions, methylation, and demethylation), biochemical pathways and mechanisms, biomolecular and genetic research, marine and freshwater bioaccumulation of Hg, and novel analytical tools including modeling approaches from molecular to global scales."

The keynote presentation for the session was given by Ulf Skyllberg from the Swedish University of Agricultural Sciences. Other speakers included Alexandre Poulain (University of Ottawa); Nourredine Bousserrhine (EES-Paris. UPEC; Equipe DIIM); Sofi Jonsson (University of Connecticut); Dr. Gu (ORNL); and graduate student Daniel Steven Grégoire (University of Ottawa), who received an award from the conference committee for his presentation.

Opportunities for Collaboration

There was significant interest in the mercury research conducted at ORNL, specifically with respect to identification and characterization of the methylation genes *hgcA* and *hgcB*, abiotic mercury transformations, and mercury interactions with cell surfaces (see sidebar, Presentations by ORNL Mercury SFA Researchers at ICMGP 2015, p. 15). Drs. Gu and Johs discussed collaborative opportunities with numerous researchers, most notably, keynote speaker Ulf Skyllberg, Lars-Eric Heimbürger (University of Bremen, Germany), Xinbin Feng (State Key Laboratory of Environmental Geochemistry, China), Alexandre Poulain (University of Ottawa, Canada), Thomas Giegerich (Karlsruhe Institute of Technology, Germany), Erik Bjorn (Umea University, Sweden), Shuxiao Wang and Jiming Hao (Tsinghua University, China), Dang Fei (Chinese Academy of Sciences), and Huang Zhong (Nanjing University, China). Discussion topics included:

- Production and distribution of methylmercury in oceans.
- Uptake and allocation of methylmercury in rice.
- Mercury speciation and DOM.
- Thiol analysis and characterization by Fourier transform ion cyclotron resonance–mass spectrometry (FTICR–MS) and liquid chromatography–mass spectrometry (LC–MS).
- Phylogeny and evolution of mercury resistance genes.
- Technology development to limit mercury emissions.

In addition to fundamental scientific research on mercury biogeochemistry and mercury control technologies, the implementation of the Minamata Convention on Mercury and impacts of mercury exposure on humans were central topics of the conference. A delegation from the United Nations Environmental Programme (UNEP) discussed environmental policies that aim to reduce global mercury emissions and solicited scientific contributions and institutional partnerships to support convention goals.

Ongoing Collaborative Research Activities

The ORNL Mercury SFA continues to engage a number of key collaborators in the project (see Fig. 9, this page). In FY15, we have actively collaborated with the DuPont-sponsored South River Science Team, enabling us to compare similar mercury cycling studies in South River, Virginia. Scott Brooks is a co-lead on their Remedial Options Team — Lab and Small Scale Field Testing. Baohua Gu continues work with university-funded research projects, including (1) Princeton University (François Morel and Jeffra Schaefer) to study the uptake and transport mechanisms of Hg(II) into bacteria cells and (2) the University of Michigan (Joel Blum) on source identification of methylmercury through stable isotope fractionation studies. We also continue to work with Dr. Blum in support of field sampling efforts. Cynthia Gilmour (Smithsonian Research Institute) and Judy Wall (University of Missouri) are conducting globally diverse environmental studies to ascertain native functions of the genes *hgcA and hgcB*, respectively. The Task 4 team will continue to collaborate closely with Task 3 and Dr. Wall in the characterization of proteins and enzymes relevant for mercury methylation. Nonfunded collaborators include Graham George at the Canadian Light Source to investigate cobalt-ligand binding environments and Steve Ragsdale at the University of Michigan for his expertise in corrinoid protein structure and function. Task 4 will continue collaborating with Task 2 on elucidating mechanistic aspects of abiotic processes and cell surface interactions.

Fig. 9. Key ORNL Mercury SFA partners.

Although the SFA's primary objective is fundamental science, it is important that project personnel know how their science can support broader DOE programmatic objectives, such as environmental remediation and long-term stewardship of the Oak Ridge Reservation. As DOE's Oak Ridge Office of Environmental Management (OREM) embarks on its legacy cleanup mission at the Y-12 National Security Complex and ORNL, understanding how mercury may be transformed under transient biogeochemical conditions is essential. Such needs have been outlined in two recent OREM reports: *Strategic Plan for Mercury Remediation at the Y-12 National Security Complex* and *Mercury Technology Development Plan for Remediation of the Y-12 Plant and East Fork Poplar Creek*.

Eric Pierce is ORNL's point of contact for the DOE Office of Environmental Management (EM) headquarters and the OREM applied research and technology development program; he also actively participated in both mercury strategy reports published by OREM. The goal of these programs is to develop new or adapt existing remediation approaches and technologies to enable efficient cleanup of the nation's legacy waste sites. In this role, Dr. Pierce and others will have the opportunity to translate scientific discovery into information relevant to OREM and the broader DOE complex. Regular interactions will occur with local DOE staff (Elizabeth Phillips and Laura Wilkerson) and site-specific advisory panels to ensure information exchange.

Presentations by ORNL Mercury SFA Researchers at ICMGP 2015

Special Session

- *Effects of Coupled Microbiological and Geochemical Interactions on Mercury Speciation, Uptake, and Methylation* — Hosted by Baohua Gu and Alexander Johs

Oral Presentations

- *Coupled Mercury Cell Sorption, Reduction, and Oxidation on Microbial Mercury Uptake and Methylation* — B. Gu
- *Site-Directed Mutagenesis of HgcA and HgcB Reveals Key Cysteines Necessary for Mercury Methylation* — A. Johs (co-presenter with Steven Smith, University of Missouri–Columbia)
- *The Role of Natural Organic Matter and Carbonate in Mercury Photochemical Reduction and Oxidation in Water* — B. Gu, as part of the special session *Photochemistry of Mercury in Aquatic Environments: Importance and Mechanisms*

Poster

- *The Biomolecular Origins of Methylmercury: Characterization of HgcA* — A. Johs

Program Structure and Advisory Committee

Organization and Leadership

The scientific objectives of the Mercury SFA are aligned to the four integrated research tasks of Site Biogeochemical Processes (Task 1); Fundamental Mechanisms (Task 2); Microbial Transformations and Genetics (Task 3); and Molecular Structure, Function, and Mechanisms (Task 4). These tasks are managed across the SFA as an integrated team effort.

Eric Pierce assumed the role of Laboratory Research Manager (LRM) of the SFA program in July 2014 from Scott Brooks who served as interim LRM following the departure of Liyuan Liang in August 2013. Dr. Pierce oversees the research program and manages the SFA budget that has been approved by DOE. He is the point of contact with DOE Subsurface Biogeochemical Research program managers and speaks to Paul Bayer biweekly on SFA progress and potential issues.

Task leaders are Scott Brooks, Baohua Gu, Dwayne Elias, and Jeremy Smith, who lead Tasks 1–4, respectively (see Fig. 10, this page). These leaders meet triweekly to discuss research directions, staffing, budget, and cost issues. SFA staff also meets triweekly to discuss task progress.

Scientific Advisory Committee
D. Krabbenhoft (USGS/ Chair)
A. MacKerell (U. Maryland)
R. Sparling (U. Manitoba)
E. Phillips (DOE-ORO)
TBA
J. Blum (U. Michigan)*
T. Barkay (Rutgers)*
S. Lindberg*
R. Mason (U. Connecticut)*
R. Wildung (PNNL)*

BER SBR
Paul Bayer
Program Manager

ORNL
Eric M. Pierce
(Research Manager)

	Task 1: Site Biogeochemical Processes	Task 2: Fundamental Mechanisms	Task 3: Microbial Transformations and Genetics	Task 4: Molecular Structure, Function and Mechanisms
Staff	**S. Brooks** C. Brandt X. Yin K. Lowe C. Miller* G. Southworth*	**B. Gu** X. Yin R. Hettich E. Pierce F. He* G. Southworth*	**D. Elias** C. Brandt S. Brown M. Podar A. Palumbo*	**J. Smith** A. Johs J. Parks L. Liang
Postdocs and students	T. Olsen TBA A. Riscassi* D. Kocman* A. Biswas*	H. Chen X. Lu K. Neupane H. Lou* H. Lin* B. Mann* B. A. Rao* C. Miller*‡ W. Zheng* H. Hu* Y. Bian* W. Dong*	B. Crable G. Christensen A. King S. Smith R. Bridou* A. Graham* R. Hurt* A. Somenahaly* J. Moberly* J. Mosher* T. Vishnivetskaya* I. Porat*	S. Tomanicek Jing Zhou (student) D. Riccardi* H.-B. Guo* H. Guo* A. Johs*‡ J. Parks*‡
Subcontractors	Y. Qian* (UTK) M. A. Bogle* R. Landis† (DuPont) J. Dyer† (DuPont)	Y. Qian* (UTK) F. Morel* (PU) J. Schaefer* (PU) K. Kemner* (ANL) B. Mishra* (ANL) H. Zhang* (Tenn. Tech)	C. Gilmour (Smithsonian) J. Wall (U. Missouri)	S. Miller* (UCSF) A. Summers* (U Ga) L. Shi* (PNNL)

Task Leader; * Term finished or left the SFA; † unfunded collaborator; ‡ became ORNL staff

Fig. 10. SFA organization and personnel. Postdoctoral researchers are hired through ORNL and the University of Tennessee–Knoxville.

Scientific Advisory Committee

The SFA team meets with its external Scientific Advisory Committee (SAC) each year to report research progress and obtain input on program directions. The 2015 meeting was held April 9–10 and served as a practice run for the DOE Triennial Review (see Fig. 11, this page, and the SAC meeting agenda in Appendix E, p. 39). SAC members provided valuable input to the project's LRM and task leads through review of presentation material, proposed plans, and progress. Currently, there is a vacancy on the SAC because Joel Blum rotated off the committee last year. We are actively trying to fill this role and will continue to stagger committee members, asking them to serve 2 to 3 years. Additional details on the SAC are included in the charter.

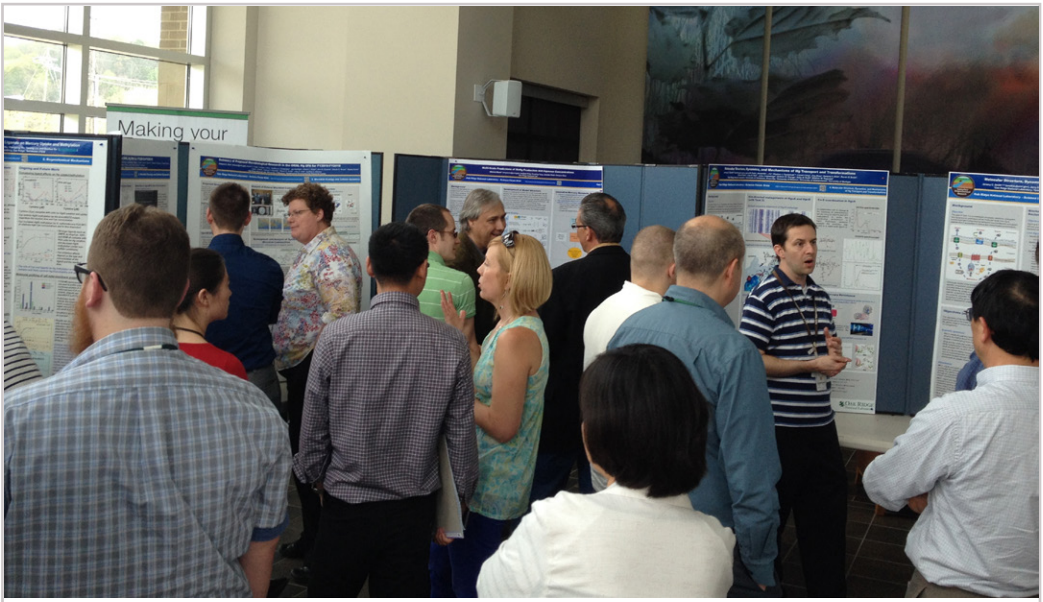

Fig. 11. Poster session at the 2015 ORNL Mercury Scientific Advisory Committee Meeting.

Staffing

Personnel Actions and Procedures

In FY15, several postdoctoral students departed to pursue new job opportunities, including Yun Qian (mercury and methylmercury analysis), Hui Lin and Balaji A. Rao (isotope chemistry and redox reactions), Benjamin Mann (mass spectrometry), Anil Somenahally (microbiology), and Sabornie Chatterjee (organic chemistry).

We have replaced these skills and expertise with the following new postdocs and students:

Todd Olsen. Field mercury speciation and characterization, mercury and methylmercury analysis

Hongmei Chen. Organic chemistry, mass spectrometry

Lindou Zhao. Environmental biogeochemistry, transmission electron microscopy

Yurong Liu. Environmental microbiology (visiting scholar from China)

Xia Lu. Physical chemistry and biochemistry (visiting student from China)

Jeremy Eskelsen. Physical chemistry, atomic force microscopy

Bryan Crable. Microbiology, C1 metabolism

Geoffrey Christensen. Molecular microbiology, genetics

Andrew King. Microbial genetics, bioinformatics

Ryne Johnston. Quantum chemistry

Jing Zhou. Computational chemistry (UTK graduate student)

Angela Belic. Biochemistry (UTK undergraduate)

No additional staff were hired in FY15. With the departure of Carrie Miller (mercury environmental biogeochemistry) in FY15, field technician Kenneth Lowe is now providing field characterization expertise and sampling support. Several staff members hired in the first phase of the study have continued their careers at ORNL, including Dwayne Elias, Alexander Johs, and Jerry Parks. In the future, our post-doc hires will be brought in through the new ORNL-administered program. Our staff scientists' contributions to the program include not only their research, but also their role in communicating our results to external scientific communities.

Staff Awards

Baohua Gu received the 2014 UT-Battelle Researcher of the Year Award for sustained, significant contributions to advancing fundamental knowledge of the biogeochemical cycling of metals, other pollutants, and carbon in the environment and for related technological achievements. Dr. Gu also received the *Environmental Science & Technology* Excellent in Review Award from the journal's editor (Jerald L. Schnoor) and associate editors. This award recognizes significant contributions made to *Environmental Science & Technology* by reviewers for 2014.

Postgraduate Spotlight

A key goal of the Mercury SFA and ORNL is to train the next generation of scientists and engineers. To this end, the SFA has maintained a number of outstanding graduate and postgraduate researchers since its inception 6 years ago (see Table 1, p. 20). As part of this year's report, we highlight three outstanding postgraduate researchers—Romain Bridou, Hui Lin, and Jing Zhou—who have contributed significantly to the overall SFA goals and objectives.

Romain Bridou

Romain Bridou obtained his bachelor degree in Environmental Sciences from Toulouse, Paul Sabatier University in France. He received his master's degree in Environmental and Technical Sciences and Ph.D. in Physiology and Biogeochemistry with honors from the University of Pau, France, in 2010. His Ph.D. dissertation reported on "Anaerobic Microbial Activities and Their Effects on the Transfer and Transformation of Metals and Organometals in the Environment: The Case of Tin and Mercury." His research demonstrated demethylation of methylmercury by sulfate-reducing bacteria (SRB) and established a unique set of references for the natural isotopic fractionation of mercury during its methylation by SRB. After his graduation, he was appointed a postdoctoral fellow under the mentoring of Judy Wall at the University of Missouri and Dwayne Elias, SFA Task Leader at ORNL. Dr. Bridou developed genetic tools for the mercury-methylating SRB *D. desulfuricans* ND132. As a result, he was part of the team that established that the two-gene cluster, *hgcA* and *hgcB,* was required for mercury methylation in apparently all mercury-methylating

organisms. This noteworthy finding was published in *Science*. Dr. Bridou also contributed to a research article, published in *Applied and Environmental Microbiology*, which explored the special chemistry of the HgcA corrinoid center. He was recruited by the University of Pau, France, to fulfill a teaching and research position and is currently developing the genetics of bacterial model systems to study the transfer and transformation of metals at the subcellular level. He enjoys reading books, wood crafting, fishing, and "courir les bois" in the Pyrenees Mountains.

Hui Lin

Hui Lin obtained her bachelor and master's degree with honor in Environmental Sciences from Peking University in China and earned her Ph.D. in Environmental Geochemistry from Georgia Institute of Technology in 2012. Her Ph.D. dissertation was on "Anaerobic Respiration of Manganese Oxides and its Effects on Carbon and Nitrogen Cycles." After graduation, she joined a multidisciplinary research team at ORNL as a postdoctoral research associate in the Environmental Sciences Division under Baohua Gu. Her work focused mostly on the mechanisms and geochemical controls on mercury transformation leading to its uptake and methylation in the environment. As a result of this work, Dr. Lin published three research articles (as the first author) in *Environmental Science & Technology* and co-authored more than four additional publications. Dow Chemical recently recruited her as a chemist in the Environment, Health, & Safety analytical support segment. Dr. Lin's hobbies include playing tennis and reading.

Jing Zhou

Jing Zhou is currently a fourth-year graduate student in the Genome Sciences and Technology Graduate Program at the University of Tennessee under the supervision of Jeremy Smith and Jerry Parks. She is part of a multidisciplinary research team at ORNL, where her current research is focused on performing quantum chemical calculations to understand redox properties and methyl transfer mechanisms of the protein HgcA, which plays a key role in mercury methylation. She has published one first-author publication in *Inorganic Chemistry* and has co-authored two additional publications in *Environmental Science & Technology*.

Table 1. Placement of Postgraduate Researchers Following ORNL SFA Work	
Task 1	
Abir Biswas	Faculty, Evergreen State University
David Kocman	Scientist, Jožef Stefan Institute
Ami Riscassi	Faculty, University of Virginia Chief Scientist, Shenandoah Watershed Program Recipient of the 2014 GSA Subaru Outstanding Woman in Science Award
Carrie Miller	Assistant Professor, Troy University
George Southworth	Retired
Mary Anna Bogle	Retired
Task 2	
Feng He	Assistant Professor, Zhejiang University of Technology
Wang Zheng	Canadian MAGNET Fellowship
Haiyan Hu	Staff Scientist, Geochemistry Institute, Chinese Academy of Sciences
Yongrong Bian	Staff Scientist, Nanjing Institute of Soil Science, Chinese Academy of Sciences
Wenming Dong	Staff Scientist, Lawrence Berkeley National Laboratory
Balaji Rao	Postdoc Fellow, Texas Tech University
Benjamin Mann	Chemist, Merck
Hui Lin	Research Scientist, Dow Chemical
Task 3	
Richard Hurt	Research Faculty, University of Tennessee
Anil Somenahally	Faculty, Texas A&M University AgriLife Research and Extension Center
James Moberly	Faculty, University of Idaho
Jennifer Mosher	Faculty, Marshall University
Tatiana Vishnivetskaya	Research Faculty, University of Tennessee
Iris Porat	Director of R&D, Global Future Solutions US
Task 4	
Hao-Bo Guo	Postdoc, University of Tennessee–Knoxville
Alexander Johs	ORNL Staff Scientist 2013 Recipient Stanley Auerbach Award for Excellence in Environmental Sciences
Jerry Parks	Staff Scientist, Oak Ridge National Laboratory
Katherine Rush	Ph.D. Student, University of Michigan
Demian Riccardi	Visiting Assistant Professor, Earlham College
M. Sawnhey	Medical Student, University of Memphis

National Laboratory Investments

ORNL is committed institutionally to the success of the Mercury SFA program. In FY15, ORNL initiated a Laboratory Directed Research and Development project titled "Functional Domains in Model Membranes and Protocells Probed with High-Performance Simulation and Neutron Scattering." This 3-year, $1.2M project is expected to support the SFA by developing a modular platform to study structures at biomembrane interfaces, providing critical information about molecular processes at cell membranes using unique tools. The aim is to investigate dynamic changes in membrane organization

related to the transport of biomolecules and solutes such as mercury and their interactions.

Additionally, ORNL has invested in new equipment, specifically the MicroCal PEAQ-ITC, to support the SFA in making fundamental thermochemical measurements of mercury-ligand interactions. These measurements are critical to understanding biochemical mechanisms, changes to mercury speciation in environmental systems, and the dynamic interactions that occur between mercury and cell surfaces.

Equipment Investments

In FY15, resources from the Mercury SFA project were used to purchase a LECO TruMac CN Series Elemental Analyzer to replace our broken instrument. This tool is key in delineating relationships among sediment organic carbon content and mercury and methylmercury content and in assessing mercury methylation potentials.

Also, resources from other research projects were used to acquire a new Fourier transform infrared (FTIR) spectrometer from Bruker to replace our aging (>5 years old) and obsolete Nicolet FTIR system. The new tool is invaluable and needed for molecular and structural characterization of various organic and inorganic complexes, solid- and aqueous-phase chemical speciation, and metal-organic complexation such as reactions between natural organic matter and mercury or minerals and particulates.

Capital Equipment Needs

A super-resolution fluorescence imaging spectrometer is requested to support direct characterization of cell surface functionalities and interactions with mercury or mercury-ligand complexes, cell-cell interactions in biofilms, and mercury-particulate and cell-particulate interactions. The estimated cost of the system is about $150,000.

Summary and Conclusions

As highlighted in this report, the Mercury SFA led by ORNL has made substantial progress in fulfilling its overarching research aim during its previous two 3-year phases (FY2009–15):

> ***Elucidating the mechanisms by which inorganic mercury is transformed into methylmercury at the sediment-water interface and the processes that determine net methylmercury production at contaminated sites.***

These advances have transformed the approaches used to conduct mercury research globally. For example, Krabbenhoft and Sunderland 2013[30] stated that "New and rapidly developing scientific tools such as . . . genetic markers for the capacity to methylate mercury will help to improve understanding of the cycling and health impacts of environmental mercury." ORNL Mercury SFA research findings from the past 6 years have led to the realization that exchange and feedback processes occurring at critical interfaces are key factors limiting the predictive understanding of net methylmercury production in environmental systems. Critical interfaces connect different ecosystem compartments and subsystem components enabling exchange, feedback, and co-evolution (see Fig. 12, p. 22).

The SFA's next 3-year phase (FY2016–18) aims to generate new knowledge in three thrust areas:

1. Microbial community dynamics
2. Fundamental biogeochemical processes
3. Molecular-scale interactions that control mercury speciation, bioavailability, and transformations at critical interfaces along the hydrologic transport pathway.

Accomplishing these objectives will contribute to the SFA's 3-year goal of determining the fundamental mechanisms and environmental factors that control mercury biogeochemical transformations at critical interfaces in terrestrial and aquatic ecosystems (see Fig. 13, p. 22).

This system science program integrates hydrology, geochemistry, microbiology, and computational science, including molecular simulations, to investigate mercury behavior in environmental systems. The multidisciplinary and multi-institutional program is supported by ORNL's core expertise in geochemistry and microbiology from the field-to-laboratory scale and its world-class neutron source and high-performance computing capabilities. Newly generated tools and knowledge will enable a deeper understanding of mercury speciation and flux in stream systems locally and globally. This foundational information is particularly important for addressing a key environmental challenge facing the Department of Energy, the United States, the state of Tennessee, and the city of Oak Ridge: namely remediating mercury contamination on the Oak Ridge Reservation. Although this program uses mercury as a model element and East Fork Poplar Creek as model stream system, the information generated and the integrated, multiscale approach pioneered by ORNL will impact subsurface biogeochemical research beyond these models. SFA results will enable a transformational paradigm for understanding biogeochemical processes that affect the fate, toxicity, and fluxes of not only mercury, but other trace metals (e.g., radionuclides) in complex, heterogeneous, and multiscale environmental systems.

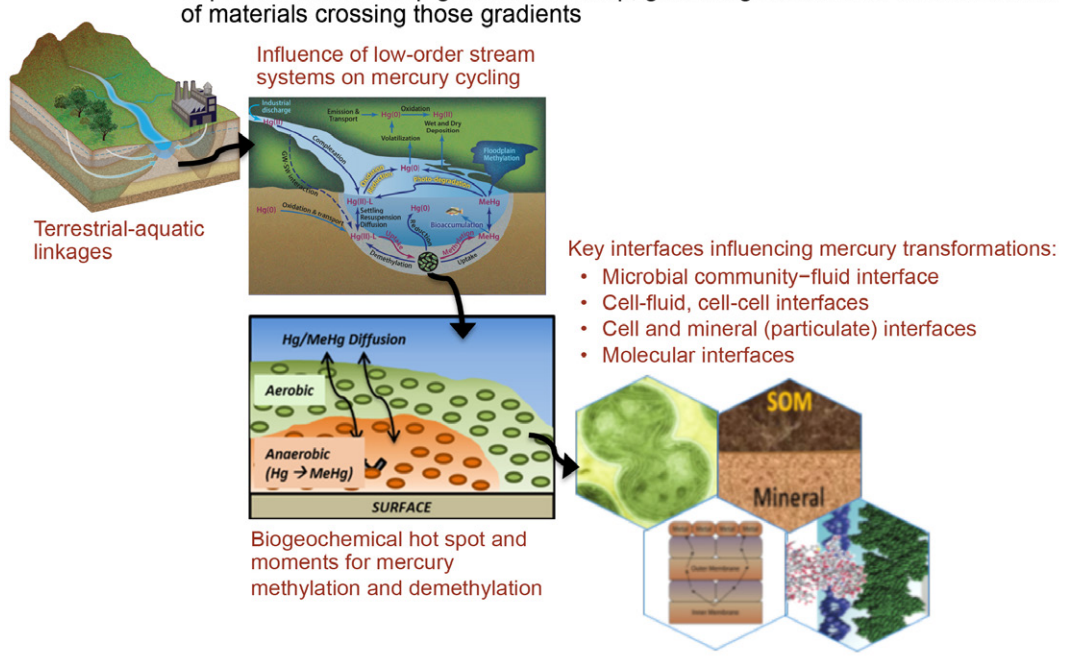

Fig. 12. Key biogeochemical interfaces.

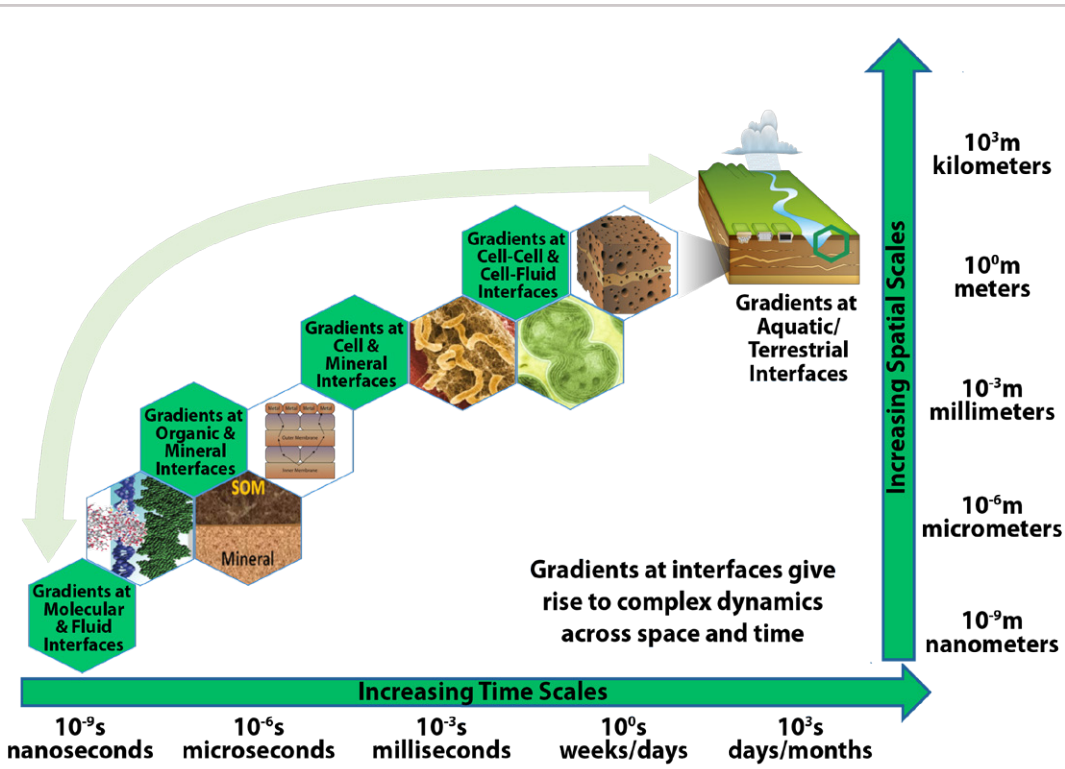

Fig. 13. Illustration of key interfaces that affect biogeochemical transformations.

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Appendix B. SFA Publications, Patents, and Invention Disclosures

A total of 59 peer-reviewed publications have been produced by the ORNL Mercury SFA. Of these publications, 44 are the result of new mercury research; 15 represent DOE Environmental Remediation Sciences Program projects that were completed with partial SFA funding.

Published or Accepted Manuscripts

2015

1. Vázquez-Rodríguez, A. I., C. M. Hansel, T. Zhang, C. H. Lamborg, C. M. Santelli, S. M. Webb, and S. C. Brooks. 2015. "Microbial- and thiosulfate-mediated dissolution of mercury sulfide minerals and transformation to gaseous mercury." *Frontiers Microbiol*. **6**: 1–11.
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(As these projects were wrapping up, the SFA contributed some funding in FY08–09 to complete them. The publications resulted from partial SFA funding.)

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Submitted Manuscripts

1. Podar, M., C. C. Gilmour, C. C. Brandt, A. Soren, S. D. Brown, B. R. Crable, A. V. Palumbo, A. C. Somenahally, and D. A. Elias. Accepted. "Global prevalence and distribution of genes and microorganisms involved in mercury methylation." *Science Advances*.

2. Luo, H., X. Lu, W. Zhang, Lin, H., H. Yu, L. Liang, G. Sheng, and B. Gu. "Decreased biological methylation upon photochemical reactions between mercury and natural organic matter." In review. *Nature Geosci.*

3. Riscassi, A. L., C. L. Miller, and S. C. Brooks. In review. "Seasonal and flow-driven dynamics of unfiltered and filtered mercury and methylmercury in a stream impacted by an industrial mercury source." *Environ. Toxicol. Chem.*

Manuscripts in Preparation (wtih Target Journal and Date)

1. Smith S. D., R. B. Bridou, M. E. Taga, D. A. Elias, J. D. Wall. "A methyltransferase (THF-MeTr), a nicotinate-nucleotide—dimethylbenzimidazole phosphoribosyltransferase (CobT), and a rare cobamide cofactor essential for maximal mercury methylation in the sulfate-reducing bacteria *D.Desulfuricans* ND132." Target submission: Fall 2015 to *PNAS*.

2. Riscassi, A. L., C. L. Miller, S. C. Brooks. "Diel mercury-concentration variations in a mercury impacted stream." Target submission: By September 2015, journal TBD.
3. Lin, H., B. Gu, et al. "Thiol-facilitated methylmercury export in anaerobic microbial methylation." Target submission by September 2015 to *Environ. Sci. Technol. Lett.*
4. Brooks, S. C., C. L. Miller, D. Kocman, A. L. Riscassi, M. A. Bogle, and X. Yin. "Mercury biogeochemistry in contaminated sediments of East Fork Poplar Creek, Tenn." Target submission: By December 2015, journal TBD.
5. Somenahally A. C., J. G. Moberly, R. A. Hurt Jr., S. D. Brown, M. Podar, C. C. Brandt, A. V. Palumbo, and D. A. Elias. "Mercury methylation and microbial community composition in river sediments amended with carbon substrates." Target submission: By December 2015 to *Nature Geosci.*
6. Miller, C., S. Brooks, A. Riscassi, D. Kocman, and X. Yin. "Factors influencing sediment methylmercury concentrations in a mercury-contaminated creek." Target submission: By December 2015, journal TBD.
7. Miller, C. L., A. L. Riscassi, S. C. Brooks, and X. Yin. "Longitudinal gradients in Hg cycling and associated geochemical parameters in a mercury-contaminated creek." Target submission: By January 2016, journal TBD.
8. Hurt Jr., R. A., A. C. Somenahally, K. L. Bailey, K. S. Bender, A. Wymore, S. D. Brown, M. Podar, A. V. Palumbo, C. C. Brandt, and D. A. Elias. "Development of clade specific molecular probes for the detection and quantification of mercury methylating genes." Target submission: March 2016 to *Environ. Sci. Technol.*
9. Crable, B. R., R. Guyoneaud, J. D. Wall, C. C. Gilmour, and D. A. Elias. "Mercury and mercury methylation: Where we have been, where we are and what is next." Target submission: March 2016 to *Trends Microbiol.*
10. Mann, B., H. Chen, B. Gu, et al. "Molecular profiling of natural organic matter and cell surface interactions with mercury." Target submission: March 2016 to *J. Proteomics*.
11. Bender, K. S., R. A. Hurt Jr., C. L. Miller, S. C. Brooks, A. C. Somenahally, K. L. Bailey, M. W. Fields, A. Wymore, S. D. Brown, M. Podar, A. V. Palumbo, C. C. Brandt, and D. A. Elias. "Systems biology investigation of the mercury methylating genes." Target submission: By May 2016 to *Nature Geosci*.
12. Vishnivetskaya, T. A., B. Gu, H. Hu, R. A. Hurt Jr., and D. A. Elias. "A combined molecular and geochemical assessment of mercury methylation in rice paddies in China." Target submission: May 2016 to *Nature Geosci.*
13. Brooks, S. C., C. L. Miller, D. Kocman, A. L. Riscassi, X. Yin, K. Lowe, T. Lowe, and M. A. Bogle. "Effect of flow management changes on Hg dynamics in a Hg-contaminated creek." Target submission: July 2016.
14. "Co–S coordination by a conserved cysteine in the corrinoid protein HgcA revealed by EXAFS and DFT."
15. "Evaluation of density functional approximations for computing reduction potentials of cobalamins and cobinamides."

Published Reports

1. Peterson, M., S. Brooks, T. Mathews, M. Bevelhimer, S. Bhaskar, C. Miller, A. Riscassi, and G. Southworth. 2014. *Evaluation of Lower East Fork Poplar Creek Mercury Sources.* ORNL/TM-2014/474, Oak Ridge National Laboratory, Oak Ridge, Tenn.
2. Southworth, G. R., M. A. Bogle, S. Brooks, L. Liang, M. Peterson, B. Spalding, D. Watson, F. Zhang, and T. J. Abraham. 2010. *Evaluation of New Options for Reducing Mercury Release at the Y-12 National Security Complex.* ORNL/TM-2010/33, Oak Ridge National Laboratory, Oak Ridge, Tenn.
3. Southworth, G. R., S. C. Brooks, M. Peterson, M. A. Bogle, C. Miller, M. Elliott, and L. Liang. 2009. *Controlling Mercury Release from Source Zones to Surface Water: Initial Results of Pilot Tests at the Y-12 National Security Complex*. ORNL/TM-2009/035, Oak Ridge National Laboratory, Oak Ridge, Tenn.

Edited Book

1. *Neutron Application in Earth, Energy, and Environmental Sciences*. Eds. L. Liang, R. Rinaldi, H. Schober. Springer, 2009. ISBN: 978-0-387-09415-1.

Ph.D. Thesis

1. Smith, Steven D. 2015. *Protein components of the microbial mercury methylation pathway*. University of Missouri– Columbia.

Patents Filed

1. Mercury Methylation Genes in Bacteria and Archaea. (U.S. patent application 14/132,906). 2013 J. M. Parks and A. Johs.
2. Method of Making Gold Thiolate and Photochemically Functionalized Microcantilevers. Boiadjiev, V., L. A. Pinnaduage, G. M. Brown, and T. Thundat (U.S. Patent application filed).

Invention Disclosures

1. 2011. R. A. Hurt, Jr., and D. A. Elias. "Recovery of Nucleic Acid from Iron Oxide Complexed Clay Environments."
2. 2011. R. A. Hurt, Jr., and D. A. Elias. "Sequencing Intractable DNA to Close Microbial Genomes."

Appendix C. Presentations and Conferences

The 110 presentations and abstracts listed below represent the mercury-focused study exclusively. SFA staff also gave 44 invited presentations and participated in leadership activities in the research community, including organizing 10 scientific conference sessions.

Published or Accepted Conference Abstracts or Presentations

2015

1. B. R. Crable, S. D. Brown, S. D. Smith, R. Bridou, J. D. Wall and D. A. Elias. "Elucidating the physiological function of HgcAB: metabolic characterization of *Desulfovibrio desulfuricans* strain ND132 and associated mutants." Applied and Environmental Microbiology, Gordon Research Conference. July 12–17, 2015. Mount Holyoke College, South Hadley, Mass.
2. B. Gu. "Coupled mercury cell sorption, reduction, and oxidation on microbial Hg uptake and methylation." 12th International Conference on Mercury as a Global Pollutant. June 14–19, 2015. Jeju, Korea.
3. F. He, H. Luo, G. Sheng, L. Liang, and B. Gu. "The role of natural organic matter and carbonate in mercury photochemical reduction and oxidation in water." 12th International Conference on Mercury as a Global Pollutant. June 14–19, 2015. Jeju, Korea.
4. S. J. Tomanicek, K. Neupane, J. Zhou, K. W. Rush, A. Belic, J. M. Parks, S. W. Ragsdale, L. Liang, J. Smith, and A. Johs. "The biomolecular origins of methylmercury: Characterization of HgcA." 12th International Conference on Mercury as a Global Pollutant Conference. June 14–19, 2015. Jeju, Korea.
5. S. D. Smith, R. Bridou, J. M. Parks, A. Johs, D. A. Elias, and J. D. Wall. "Site directed mutagenesis of HgcA and HgcB reveals key cysteines necessary for mercury methylation." 12th International Conference on Mercury as a Global Pollutant. June 14–19, 2015. Jeju, South Korea.
6. B. R. Crable, S. D. Brown, S. D. Smith, R. Bridou, J. D. Wall, and D. A. Elias. "Elucidating the native function of the Hg methylating genes *hgcAB*: metabolic characterization of *Desulfovibrio desulfuricans* ND132 WT, Δ*hgcAB* and Δ*hgcAB*::*hgcAB*." 115th General Meeting of the American Society for Microbiology. May 30–June 2, 2015. New Orleans.
7. G. A. Christensen, A.M. Wymore, A. King, M. Podar, R. A. Hurt Jr., S.D. Brown, A.V. Palumbo, K.S. Bender, M. W. Fields, C. Gilmour, J. Santillan, C. C. Brandt, and D. A. Elias. "The connection between *hgcA* expression and mercury methylation rates in the environment."115th General Meeting of the American Society for Microbiology. May 30–June 2, 2015. New Orleans.
8. A. M. Wymore, G. A. Christensen, A. J. King, M. Podar, R. A. Hurt Jr., C. Gilmour, E. Santillan, S. D. Brown, and D. A. Elias. "Study of mercury methylation, analysis of *hgcA* in pure culture by qPCR." 115th General Meeting of the American Society for Microbiology. May 30–June 2, 2015. New Orleans.
9. E. M. Pierce, S. C. Brooks, B. Gu, D. Elias, J. Parks, A. Johs, C. Brandt, M. Podar, S. Brown, C. Gilmour, J. Wall, and J. C. Smith. "Biogeochemical and molecular mechanisms controlling contaminant transformation in the environment." Third Annual DOE Joint Terrestrial Ecosystem Science (TES)/Subsurface Biogeochemical Research (SBR) Investigators Meeting. April 27–29, 2015. Potomac, Md.
10. T. A. Olsen and S. C. Brooks. "Periphyton biofilms generate methylmercury in a contaminated creek system." Third Annual DOE Joint TES/SBR Investigators Meeting. April 27–29, 2015. Potomac, Md.
11. B. Gu, H. Luo, F. He, Y. Qian, L. Liang, and E. Pierce. "Mechanisms and factors controlling photochemical transformation of mercury and net methylmercury formation in the aquatic environment." Third Annual DOE Joint TES/SBR Investigators Meeting. April 27–29, 2015. Potomac, Md.
12. K. P. Neupane, H. Lin, X. Lu, W. Fang, L. Liang, and B. Gu. "Complex organic ligands affecting mercury uptake and methylation." Third Annual DOE Joint TES/SBR Investigators Meeting. April 27–29, 2015. Potomac, Md.
13. D. A. Elias, C. C. Gilmour, M. Podar, K. S. Bender, C. C. Brandt, J. Santillan, A. M. Wymore, G. A. Christensen, A. J. King, A. Soren, R. A. Hurt Jr., S. D. Brown, B. R. Crable, A.V. Palumbo, A. C. Somenahally, and E. M. Pierce. "Development of molecular tools for the accurate assessment of mercury methylation potentials in any environment." Third Annual DOE Joint TES/SBR Investigators Meeting. April 27–29, 2015. Potomac, Md.
14. A. Johs, A. Belic, S. J. Tomanicek, K. Neupane, K. W. Rush, S. W. Ragsdale, J. M. Parks, and J. C. Smith. "Reconstitution and spectroscopic characterization of the corrinoid protein HgcA." Third Annual DOE Joint TES/SBR Investigators Meeting. April 27–29, 2015. Potomac, Md.
15. J. M. Parks, A. Johs, K. Neupane, J. C. Smith, S. J. Tomanicek, and J. Zhou. "Toward understanding the biomolecular mechanism of HgcA." Third Annual DOE Joint TES/SBR Investigators Meeting. April 27–29, 2015. Potomac, Md.
16. D. A. Elias, R. A. Hurt Jr., G. A. Christensen, A. M. Wymore, A. King, M. Podar, S. D. Brown, A. V. Palumbo, J. Santillan, A. Soren, and C. C. Gilmour. "Determining microbial methylmercury production through the use of molecular probes: Insights into *hgcAB* expression and distribution in the environment." Joint Assembly of the American Geophysical Union. March 3–7, 2015. Montreal.
17. C. C. Gilmour, J. Santillan, A. Soren, R. A. Hurt Jr., G. A. Christensen, A. M. Wymore, A. King, M. Podar, S. D. Brown, A. V. Palumbo, and D. A. Elias "Mercury methylation by *hgcAB*+ organisms." Joint Assembly of the American Geophysical Union. March 3–7, 2015. Montreal.

2014

18. S. Brooks, A. L. Riscassi, and C. Miller. "Diel mercury-concentration variations in a mercury impacted stream." 126th Annual Meeting of the Geological Society of America. Oct.19–22, 2014. Vancouver, Canada.
19. A. Riscassi, C. Miller, and S. Brooks. "Diel mercury-concentration variations in a mercury impacted stream." 23rd V. M. Goldschmidt International Conference. June 8–13, 2014. Sacramento, Calif.
20. H. Lin et al. "The coupling between mercury-cell surface interactions and mercury uptake and methylation." 23rd V. M. Goldschmidt International Conference, Session 19f. June 8–13, 2014. Sacramento, Calif.
21. R. Bridou et al. "The toxicogenomic response of *Desulfovibrio vulgaris* Hildenborough to sub- and inhibiting concentrations of Hg(II)." 23rd V. M. Goldschmidt International Conference, Session 19g. June 8–13, 2014. Sacramento, Calif.
22. A. Johs, S. J. Tomanicek, A. Belic, K. Rush, J. M. Parks, D. Riccardi, and J. C. Smith. "Biophysical characterization of HgcA, a protein required for the biosynthesis of methylmercury." 23rd V. M. Goldschmidt International Conference. June 8–13, 2014. Sacramento, Calif.
23. A. Johs, S. J. Tomanicek, A. Belic, K. Rush, J. M. Parks, D. Riccardi, and J. C. Smith. "The molecular basis of mercury methylation: Characterization of the corrinoid protein HgcA." Symposium Frontiers in Metallobiochemistry III. June 4–7, 2014. Pennsylvania State University, College Park, Pa.
24. S. Smith et al. "Site directed mutations of *hgcA* and *hgcB* from *Desulfovibrio desulfuricans* ND132 reveal residues or structures critical for mercury methylation." 114th General American Society for Microbiology Meeting. May 17–20, 2014. Boston.
25. A. L. Riscassi, C. L. Miller, and S. C. Brooks. 2014. "Diel mercury-concentration variations in a mercury impacted stream." Second Annual DOE Joint Terrestrial Ecosystem Science (TES)/Subsurface Biogeochemical Research (SBR) Investigators Meeting. May 6–7, 2014. Potomac, Md.
26. D. A. Elias, S. Brooks, R. A. Hurt Jr., A. C. Somenahally, R. Bridou, S. D. Smith, M. Podar, S. D. Brown, C. C. Brandt, A. V. Palumbo, J. D. Wall, and C. C. Gilmour. "Organismal and environmental level investigations of the mercury methylating genes *hgcAB*." Second Annual DOE Joint TES/SBR Investigators Meeting. May 6–7, 2014. Potomac, Md.
27. D. A. Elias, S. Brooks, R. A. Hurt Jr., A. C. Somenahally, R. Bridou, S. D. Smith, M. Podar, S. D. Brown, C. C. Brandt, A. V. Palumbo, J. D. Wall, and C. C. Gilmour. "Molecular biology level investigations of the mercury methylating genes *hgcAB*." Second Annual DOE Joint TES/SBR Investigators Meeting. May 6–7, 2014. Potomac, Md.
28. A. Johs, S. Brooks, D. Riccardi, A. Belic, S. J. Tomanicek , R. Bridou, S. D. Smith, J. D. Wall, J. M. Parks, D. A. Elias, and J. C. Smith. "The molecular basis of mercury methylation: expression, purification, and characterization of HgcA." Second Annual DOE Joint TES/SBR Investigators Meeting. May 6–7, 2014. Potomac, Md.
29. J. C. Smith, S. C. Brooks, A. Beste, H.-B. Guo, H. Guo, A. Johs, S. M. Miller, J. M. Parks, D. Riccardi, A. O. Summers, S. J. Tomanicek, and J. Zhou. 2014. "Determining mechanisms of Hg methylation by HgcA and intramolecular Hg transfer by MerA at the atomic scale." Second Annual DOE Joint TES/SBR Investigators Meeting. May 6–7, 2014. Potomac, Md.
30. Hui, L. et al. "Microbial cell surface interactions and biogeochemical controls on mercury (Hg) redox transformation and methylation." Second Annual DOE Joint TES/SBR Investigators Meeting. May 6–7, 2014. Potomac, Md.

2013

31. A. L. Riscassi, S. Brooks, C. Miller, and X. Yin. 2013. "Storm dynamics of Hg and MeHg in an industrially contaminated creek: What can it tell us about source areas of Hg and MeHg within the catchment?" Society of Environmental Toxicology and Chemistry (SETAC) North America 34th Annual Meeting. Nov. 17–21, 2013. Nashville, Tenn.
32. C. Miller, A. Riscassi, X. Yin, and S. Brooks. "Comparison of Hg and MeHg cycling in a contaminated creek with uncontaminated systems." SETAC North America 34th Annual Meeting. Nov. 17–21, 2013. Nashville, Tenn.
33. Y. Qian, B. Gu, B. Rao, and L. Liang. "Methylmercury photodegradation affected by the presence of organic ligands." SETAC North America 34th Annual Meeting. Nov. 17–21, 2013. Nashville, Tenn.
34. B. F. Mann, L. Liang, and B. Gu. "Characterization of mercury–natural organic matter interactions by measuring the transformations of individual molecular species in the complex mixtures." SETAC North America 34th Annual Meeting. Nov. 17–21, 2013. Nashville, Tenn.
35. D. A. Elias, M. Podar, J. M. Parks, A. Johs, R. Bridou, R. A. Hurt Jr., S. D. Smith, S. J. Tomanicek, Y. Qian, S. D. Brown, C. C. Brandt, A. V. Palumbo, J. C. Smith, J. D. Wall, and L. Liang. "The genetic basis for mercury methylation: Discovery of the mercury methylation genes and implications for mercury research." 11th International Conference on Mercury as a Global Pollutant. July 28–Aug. 2, 2013. Edinburgh, Scotland.
36. S. D. Smith, R. B. Bridou, R. A. Hurt Jr., L. Lang, D. A. Elias, A. O. Summers, and J. D. Wall. "Biochemistry and regulation of mercury methylation by sulfate reducing bacteria." 11th International Conference on Mercury as a Global Pollutant. July 28–Aug. 2, 2013. Edinburgh, Scotland.
37. R. Bridou, S. D. Smith, A. Kucken, S. Fels, J. M. Parks, A. Johs, R. Hurt Jr., M. Podar, C. C. Gilmour, L. Liang, D. A. Elias, and J. D. Wall. "Biochemistry and regulation of mercury methylation by sulfate reducing bacteria." 11th International Conference on Mercury as a Global Pollutant. July 28–Aug. 2, 2013. Edinburgh, Scotland.
38. C. C. Gilmour, A. M. Graham, A. L. Bullock, A. C. Maizel, A. C. Somenahally, A. Johs, J. M. Parks, M. Podar, J. C. Smith, and D. A. Elias. "Phylogenetic distribution of the ability to methylate Hg among bacteria and archaea." 11th International Conference on Mercury as a Global Pollutant. July 28–Aug. 2, 2013. Edinburgh, Scotland.

39. A. Riscassi, S. Brooks, C. Miller, and X. Yin. "Storm dynamics of Hg and MeHg in a contaminated creek: What can it tell us about transport and source areas within the catchment?" 11th International Conference on Mercury as a Global Pollutant. July 28–Aug. 2, 2013. Edinburgh, Scotland.

40. C. Miller, S. Brooks, A. Riscassi, D. Kocman, and X. Yin. "Factors influencing sediment methylmercury concentrations in a mercury contaminated creek." 11th International Conference on Mercury as a Global Pollutant. July 28–Aug. 2, 2013. Edinburgh, Scotland.

41. A. I. Vazquez-Rodriguez, T. Zhang, C. M. Santelli, S. C. Brooks, C. H. Lamborg, and C. M. Hansel. "Thiobacillus species and thiosulfate implicated in HgS mobilization." 11th International Conference on Mercury as a Global Pollutant. July 28–Aug. 2, 2013. Edinburgh, Scotland.

42. B. Gu, H. Hu, Y. Bian, W. Zheng, X. Feng, D. A. Elias, and L. Liang. "Complex interactions between Hg and natural organic matter and microorganisms on Hg redox transformation in anoxic environments." 11th International Conference on Mercury as a Global Pollutant. July 28–Aug. 2, 2013. Edinburgh, Scotland.

43. L. Liang, J. M. Parks, A. Johs, M. Podar, R. Bridou, R. A. Hurt, S. D. Smith, S. J. Tomanicek, Y. Qian, S. D. Brown, C. C. Brandt, A. V. Palumbo, J. C. Smith, J. D. Wall, and D. A. Elias. "New approaches for determining the molecular basis of bacterial mercury methylation." 11th International Conference on Mercury as a Global Pollutant. July 28–Aug. 2, 2013. Edinburgh, Scotland.

44. H. Hu, X. Feng, D. A. Elias, L. Liang, and B. Gu. "The reduction, oxidation, and methylation of mercury by anaerobic microorganisms." 11th International Conference on Mercury as a Global Pollutant. July 28–Aug. 2, 2013. Edinburgh, Scotland.

45. L. Liang, F. He, Y. Qian, and B. Gu. "Photolytic redox transformation of mercury and degradation of methylmercury as influenced by complexing organic ligands." 11th International Conference on Mercury as a Global Pollutant. July 28–Aug. 2, 2013. Edinburgh, Scotland.

46. D. Riccardi, H.-B. Guo, J. M. Parks, B. Gu, L. Liang, and J. C. Smith. "Quantum chemical studies of the hydration of Hg^{2+} with comparisons to other divalent metal cations." 11th International Conference on Mercury as a Global Pollutant. July 28–Aug. 2, 2013. Edinburgh, Scotland.

47. A. Johs, J. M. Parks, M. Podar, R. Bridou, R. A. Hurt, S. D. Smith, S. J. Tomanicek, Y. Qian, S. D. Brown, C. C. Brandt, A. V. Palumbo, J. C. Smith, J. D. Wall, D. A. Elias, and L. Liang. "How do bacteria methylate mercury?" 29th International Conference of the Society for Environmental Geochemistry and Health. July 8–12, 2013. Toulouse, France (invited keynote).

48. L. Liang, A. Johs, F. He, H.-B. Guo, Y. Qian, and B. Gu. "Effect of organic ligands on photolytic redox transformations of mercury." 29th International Conference of the Society for Environmental Geochemistry and Health. July 8–12, 2013. Toulouse, France.

49. A. C. Somenahally, J. G. Moberly, R. A. Hurt, M. Podar, S. D. Brown, A. V. Palumbo, and D. A. Elias. "Microbial community response to carbon substrates amendment in mercury impacted sediments: Implications on microbial methylation of mercury." 113th American Society for Microbiology General Meeting. May 18–21, 2013. Denver.

50. B. Gu, H. Lin, H. Hu, B. Rao, B. Mann, W. Zheng, Y. Qian, and L. Liang. "Microbial cell surface interactions and biogeochemical controls on mercury (Hg) redox transformation and methylation." First Annual DOE Joint Terrestrial Ecosystem Science (TES)/Subsurface Biogeochemical Research (SBR) Investigators Meeting. May 14–15, 2013. Potomac, Md.

51. D. A. Elias, R. A. Hurt, A. C. Somenahally, R. Bridou, S. D. Smith, M. Podar, S. D. Brown, C. C. Brandt, A. V. Palumbo, J. D. Wall, and C. C. Gilmour. "Mercury methylation: Microbial species and communities involved in Hg transformations." First Annual DOE Joint TES/SBR Investigators Meeting. May 14–15, 2013. Potomac, Md.

52. C. Miller, S. Brooks, A. Riscassi, D. Kocman, and X. Yin. "Factors influencing sediment methylmercury concentrations in a mercury contaminated creek." First Annual DOE Joint TES/SBR Investigators Meeting. May 14–15, 2013. Potomac, Md.

53. J. M. Parks. Invited plenary presentation: "The genetic basis of bacterial mercury methylation." First Annual DOE Joint TES/SBR Investigators Meeting. May 14–15, 2013. Potomac, Md.

54. J. M. Parks, A. Johs, M. Podar, R. Bridou, R. A. Hurt, S. D. Smith, S. J. Tomanicek, Y. Qian, S. D. Brown, C. C. Brandt, A. V. Palumbo, J. C. Smith, J. D. Wall, D. A. Elias, and L. Liang. "The biomolecular and genetic basis of mercury methylation." First Annual DOE Joint TES/SBR Investigators Meeting. May 14–15, 2013. Potomac, Md.

55. B. Rao, B. Mann, H. Lin, L. Liang, and B. Gu. "Profiling mercury binding functional groups in natural organic matter and methylating bacteria." First Annual DOE Joint TES/SBR Investigators Meeting, May 14–15, 2013, Potomac, Md.

56. A. Riscassi, S. Brooks, C. Miller, and X. Yin. "Storm dynamics of Hg and MeHg in East Fork Poplar Creek: What can it tell us about transport and source areas of Hg and MeHg within the catchment?" First Annual DOE Joint TES/SBR Investigators Meeting. May 14–15, 2013. Potomac, Md.

57. J. C. Smith, B. Gu, H.-B. Guo, H. Guo, P. Lian, L. Liang, S. M. Miller, J. M. Parks, D. M. Riccardi, A. O. Summers, and Q. Xu. "Origin of strong affinity of mercury for soft ligands and mechanism of the mercuric ion reductase MerA." First Annual DOE Joint TES/SBR Investigators Meeting. May 14–15, 2013. Potomac, Md.

58. H. Lin, J. Schaefer, L. Liang, and B. Gu. "Effects of mercury–microbial cell surface interactions on mercury redox transformation." American Chemical Society Spring 2013 National Meeting and Exposition. April 2–11, 2013. New Orleans.

59. L. Liang, J. Gulledge, A. V. Palumbo, and A. Johs attended a meeting with program managers from the DOE Office of Biological and Environmental Research and Office of Environmental Management at DOE headquarters to present SFA mercury research featured in Parks, J. M., et al. 2013. "The genetic basis for bacterial mercury methylation," *Science* **339**(6125), 1332–1335. Feb. 11–12, 2013. Germantown, Md.

60. A. Johs. "Bridging the gap between sequence and function." *Biophysical J.* **104**(2): 230A–231A. 57th Annual Meeting of the Biophysical Society. Feb. 2–6, 2013. Philadelphia.

2012

61. C. Miller, S. Brooks, A. Riscassi, D. Kocman, and X. Yin. "Factors influencing sediment methylmercury concentrations in a mercury contaminated creek." Geological Society of America Annual Meeting. Nov. 4–7, 2012. Charlotte, N.C.

62. A. Riscassi, S. Brooks, and C. Miller. "Using high-frequency *in situ* optical sensors to understand seasonal and event-driven variability in mercury transport and transformations in a heavily contaminated creek." Geological Society of America Annual Meeting. Nov. 4–7, 2012. Charlotte, N.C.

63. B. Gu. "Roles of natural humic substances in biogeochemical transformation of mercury in the environment." Ninth International Symposium on Persistent Toxic Substances. Oct. 23–27, 2012. Miami.

64. H. Hu, W. Zheng, J. Schaefer, X. Feng, L. Liang, D. Elias, and B. Gu. "The reduction and surface complexation of mercury by anaerobic microorganisms." American Chemical Society Fall 2012 National Meeting and Exposition. Aug.19–23, 2012. Philadelphia.

65. K. W. Rush, S. J. Tomanicek, A. Johs, A. O. Summers, and L. Liang. "Solubility optimization and structural characterization of the Hg(II)-specific transcriptional regulator MerR in complex with its operator DNA." American Crystallographic Association Annual Meeting. July 28–Aug. 1, 2012. Boston.

66. S. J. Tomanicek, A. Johs, M. S. Sawhney, K. W. Rush, R. E. Nauss, S. M. Miller, and L. Liang. "X-ray crystallographic structural studies of the metallochaperone-like N-terminal domain (NmerA) of the mercuric ion reductase MerA." American Crystallographic Association Annual Meeting. July 28–Aug. 1, 2012. Boston.

67. L. Liang, D. Watson, C. Miller, J. Howe, F. He, and E. Pierce, "The fate of mercury at the contaminated site." 22nd V. M. Goldschmidt International Conference. June 24–29, 2012. Montreal.

68. H. Hu, W. Zheng, J. Schaefer, X. Feng, L. Liang, D. Elias, and B. Gu. "The reduction and surface complexation of mercury by *Geobacter sulfurreducens* PCA." 22nd V. M. Goldschmidt International Conference. June 24–29, 2012. Montreal.

69. A. Johs, S. Tomanicek, H.-B. Guo, A. Summers, and L. Liang. "Neutron scattering reveals conformations of the transcriptional regulator MerR in complex with its operator DNA." 22nd V. M. Goldschmidt International Conference. June 24–29, 2012. Montreal.

70. S. Brooks, D. Kocman, C. Miller, X. Yin, and A. Riscassi. "Biogeochemistry of mercury in contaminated sediments of East Fork Poplar Creek." 22nd V. M. Goldschmidt International Conference. June 24–29, 2012. Montreal, Canada.

71. Wang Zheng, Liyuan Liang, and Baohua Gu. "Natural organic matter and thiol compounds affect Hg redox cycling in anoxic environments." 22nd V. M. Goldschmidt International Conference. June 24–29, 2012. Montreal.

72. S. Tomanicek, A. Johs, M. Sawhney, K. W. Rush, R. E. Nauss, S. M. Miller, and L. Liang. "X-ray crystallographic structural studies of the metallochaperone-like N-terminal domain NmerA." 22nd V. M. Goldschmidt International Conference. June 24–29, 2012. Montreal.

73. J. Moberly, C. Miller, R. Hurt, Jr., S. Brown, S. Brooks, C. Brandt, M. Podar, A. V. Palumbo, and D. Elias. "GOLIATH: A systems biology, geochemical and physiological approach to discern microbial transformations of mercury and methylmercury." 22nd V. M. Goldschmidt International Conference. June 24–29, 2012. Montreal.

74. A. Vazquez-Rodriguez, C. Santelli, C. Kim, S. Brooks, and C. Hansel. "*In situ* colonization of HgS mineral by sulfur-oxidizing bacteria and the enhancement of HgS weathering." 22nd V. M. Goldschmidt International Conference. June 24–29, 2012. Montreal.

75. A. D. Campiglia, F. E. Hernandez, E. C. Heider, W. Chemnasiry, K. Trieu, C. Diaz, V. Diaz, A. F. Moore, and S. C. Brooks. "Field-deployable nanosensing approach for real-time detection of free mercury speciation and quantification in surface stream waters and groundwater samples at the U.S. DOE Contaminated Sites." DOE Subsurface Biogeochemical Research Program Annual Contractor-Grantee Workshop. April 30–May 2, 2012. Washington, D.C.

76. A. M. Graham, R. Bridou, R.A. Hurt Jr., S. D. Smith, S. D. Brown, M. Podar, C. C. Brandt, A. V. Palumbo, J. D. Wall, C. C. Gilmour, and D. E. Elias. "Mercury methylation: Genetics and physiology of methylmercury production and degradation." DOE Subsurface Biogeochemical Research Program Annual Contractor-Grantee Workshop. April 30–May 2, 2012. Washington, D.C.

77. L. Liang, C. Brandt, S. Brooks, S. Brown, D. Elias, B. Gu, F. He, A. Johs, C. Miller, M. Podar, J. Parks, C. Gilmour, S. Miller, J. C. Smith, A. Summers, and J. D. Wall. "Biogeochemical and molecular mechanisms controlling contaminant transformation in the environment." DOE Subsurface Biogeochemical Research Program Annual Contractor-Grantee Workshop. April 30–May 2, 2012. Washington, D.C.

78. S. C. Brooks, C. Miller, C. Brandt, D. Kocman, A. Riscassi, X. Yin, Y. Qian, R. Landis, and J. Dyer. "Biogeochemical processes and Hg cycling in contaminated sediments of East Fork Poplar Creek, Oak Ridge, Tenn. (Hg SFA at ORNL)." DOE Subsurface Biogeochemical Research Program Annual Contractor-Grantee Workshop. April 30–May 2, 2012. Washington, D.C.

79. C. Miller, S. C. Brooks, D. Kocman, A. Riscassi, X. Yin, and Y. Qian. "Spatial and seasonal relationships between surface water total and methylmercury, dissolved organic matter and particulates in East Fork Poplar Creek, Oak Ridge, Tenn." DOE Subsurface Biogeochemical Research Program Annual Contractor-Grantee Workshop. April 30–May 2, 2012. Washington, D.C.

2011

80. C. Miller, S. Brooks, D. Kocman, X. Yin, and M. A. Bogle. "Influence of redox processes and organic carbon on mercury and methylmercury cycling in East Fork Poplar Creek, Tennessee." Abstracts of the 2011 American Geophysical Union Fall Meeting. Dec. 5–9, 2011. San Francisco.

81. A. I. Vazquez-Rodriguez, C. M. Santelli, S. C. Brooks, and C. M. Hansel. "Bacterial and fungal communities colonizing mercury sulfide surfaces." 21st V. M. Goldschmidt International Conference. Aug. 14–19, 2011. Prague.

82. A. K. Kucken, S. D. Smith, S. D. Brown, D. E. Elias, J. D. Wall. "Genetic approaches for mercury methylation enzymology in *Desulfovibrio desulfuricans* ND132." 10th International Conference on Mercury as a Global Pollutant. July 24–29, 2011. Halifax, Nova Scotia, Canada.

83. S. C. Brooks, D. Kocman, C. Miller, X. Yin, and M. A. Bogle. "Biogeochemistry of mercury in contaminated sediments of East Fork Poplar Creek." 10th International Conference on Mercury as a Global Pollutant. July 24–29, 2011. Halifax, Nova Scotia, Canada.

84. D. Elias, J. Mosher, T. Vishnivetskaya, S. Brown, C. Brandt, S. Brooks, M. Podar, C. Gilmour, A. Kucken, J. Wall, and A. Palumbo. "A multipronged approach ranging from pure cultures to microbial communities to determine the mechanism of mercury methylation." 10th International Conference on Mercury as a Global Pollutant. July 24–29, 2011. Halifax, Nova Scotia, Canada.

85. A. K. Kucken, S. D. Smith, D. E. Elias, J. D. Wall. "Genetic and biochemical approaches to identify mercury methylation enzymes in *Desulfovibrio desulfuricans* ND132." 111th General Meeting of the American Society for Microbiology. May 21–24, 2011. New Orleans.

86. S. C. Brooks, C. Miller, C. Brandt, M. A. Bogle, D. Kocman, X. Yin, Y. Qian, R. Landis, and J. Dyer. "Site biogeochemical processes and microcosm studies (Hg SFA at ORNL)." Sixth Annual DOE Subsurface Biogeochemical Research Program Contractor-Grantee Workshop. April 26–28, 2011. Washington, D.C.

2010

87. L. Liang, B. Gu, S. C. Brooks, C. L. Miller, F. He, D. Elias, D. B. Watson, and M. J. Peterson. "Challenges and opportunities of mercury remediation in East Fork Poplar Creek, Oak Ridge, Tennessee." Abstracts of the American Geophysical Union 2010 Fall Meeting. Dec. 13–17, 2010. San Francisco.

88. A. Biswas, S. Brooks, C. Miller, G. Southworth, J. Mosher, M. Drake, and X. Yin. "Methylmercury production by *Desulfovibrio desulfuricans* ND132: Influences of natural organic matter and growth stage." 20th V. M. Goldschmidt International Conference. June 14–18, 2010. Knoxville, Tenn.

89. T. A. Vishnivetskaya, J. J. Mosher, A. V. Palumbo, M. Podar, S. D. Brown, S. C. Brooks, M. M. Drake, C. C. Brandt, D. A. Elias. "Does bacterial community structure depend on geochemistry and mercury contamination level in low-order Tennessee streams?" Abstracts of the 110th General Meeting, American Society for Microbiology. May 23–27, 2010. San Diego.

90. J. J. Mosher, T. A. Vishnivetskaya, C. C. Brandt, S. C. Brooks, M. Podar, M. M. Drake, J. D. Wall, S. D. Brown, D. A. Elias, and A. V. Palumbo. "Deltaproteobacteria in surface water sediments in low order streams." Abstracts of the 110th General Meeting, American Society for Microbiology. May 23–27, 2010. San Diego.

91. S. C. Brooks, G. R. Southworth, X. Yin, A. Biswas, C. Miller, D. Elias, M. M. Drake, and J. J. Mosher. "Site biogeochemical processes and microcosm studies (Hg SFA at ORNL)." Fifth Annual DOE Environmental Remediation Sciences Program Principal Investigators Meeting. March 29–31, 2010. Washington, D.C.

92. D. A. Elias, T. A. Vishnivetskaya, J. D. Mosher, M. M. Drake, S. D. Brown, C. C. Brandt, S. C. Brooks, C. C. Gilmour, A. M. Kucken, J. D. Wall, and A. V. Palumbo. "Mercury methylation: genes and communities involved in Hg transformations (Hg SFA at ORNL, Microbial Genetic Study Task)." Fifth Annual DOE Environmental Remediation Sciences Program Principal Investigators Meeting. March 29–31, 2010. Washington, D.C.

93. L. Liang, Y. Bian, C. Brandt, A. Biswas, S. Brooks, S. Brown, M. Drake, W. Dong, D. Elias, B. Gu, H.-B. Guo, A. Johs, C. Miller, A. V. Palumbo, J. Parks, J. Smith, G. Southworth, and T. Vishnivetskaya. "ORNL SFA: Biogeochemical and molecular mechanisms controlling mercury transformation at a contaminated site in Oak Ridge, Tennessee." Fifth Annual DOE Environmental Remediation Sciences Program Principal Investigators Meeting. March 29–31, 2010. Washington, D.C.

94. A. Biswas, S. C. Brooks, C. Miller, M. Drake, and X. Yin. "Effects of natural organic matter on methylmercury production by pure cultures of *Desulfovibrio desulfuricans* ND-132." 239th American Chemical Society National Meeting. March 22–25, 2010. San Francisco.

2009

95. S. C. Brooks, M. A. Bogle, L. Liang, C. Miller, M. Peterson, G. Southworth, and B. Spalding. "Flow alteration and chemical reduction: air stripping to lessen subsurface discharges of mercury to surface water." Abstracts of the 2009 Fall Meeting, American Geophysical Union. Dec. 14–18, 2009. San Francisco.
96. C. L. Miller, B. Gu, G. Southworth, S. C. Brooks, and L. Liang. "Kinetic controls on the formation of complexes between mercury and DOM in a contaminated environment." 238th American Chemical Society National Meeting. August 16–20, 2009. Washington, D.C.
97. G. R. Southworth, S. C. Brooks, C. L. Miller, L. Liang, M. Peterson, and M. A. Bogle. "Flow reduction to reduce mercury flux from contaminated sediments to surface water." 238th American Chemical Society National Meeting. Aug. 16–20, 2009. Washington, D.C.
98. A. V. Palumbo, S. D. Brown, T. A. Vishnivetskaya, M. M. Drake, M. K. Kerley, S. C. Brooks, L. A. Fagan, B. Gu, M. Podar, L. Liang, M. Rodriguez, Jr., and C. C. Brandt. "Microbial community structure and function related to geochemistry in mercury contaminated stream sediments." 238th American Chemical Society National Meeting. Aug. 16–20, 2009. Washington, D.C.
99. G. Southworth, S. Brooks, M. A. Bogle, and M. Peterson. "Flow reduction to reduce mercury flux to surface water from contaminated sediments." Ninth International Conference on Mercury as a Global Pollutant. June 7–12, 2009. Guiyang, China.
100. M. J. Peterson, G. R. Southworth, L. Liang, and S. C. Brooks. "Mercury bioaccumulation remains unresponsive to point-source mercury remediation: Investigating factors and potential new approaches to the problem." Ninth International Conference on Mercury as a Global Pollutant. June 7–12, 2009. Guiyang, China.
101. L. Liang, S. Brooks, W. Dong, B. Gu, A. Johs, C. Miller, A. V. Palumbo, J. C. Smith, and G. Southworth. "A comprehensive study of the biogeochemical and molecular mechanisms on mercury transformation at a contaminated site in Oak Ridge, Tennessee." Ninth International Conference on Mercury as a Global Pollutant. June 7–12, 2009. Guiyang, China.
102. C. Miller, B. Gu, Scott C. Brooks, George Southworth, and Liyuan Liang. "Kinetic controls on the interaction of mercury with dissolved organic matter." Ninth International Conference on Mercury as a Global Pollutant. June 7–12, 2009. Guiyang, China.
103. A. V. Palumbo, T. A. Vishnivetskaya, S. D. Brown, G. R. Southworth, M. M. Drake, M. K. Kerley, M. Rodriguez, Z. K. Yang, C. W. Schadt, S. C. Brooks, C. L. Miller, and C. C. Brandt. "Phylogenetic and functional community characteristics in impacted streams." 109th General Meeting of the American Society for Microbiology. May 17–21, 2009. Philadelphia.
104. S. C. Brooks. "Overview of mercury contamination at the Oak Ridge Y-12 Site: History and hydrogeochemical setting." Fourth Annual DOE Environmental Remediation Sciences Program Principal Investigators Meeting. April 20–23, 2009. Lansdowne, Va.
105. A. V. Palumbo, T. A. Vishnivetskaya, T. Yan, C. C. Brandt, M. Podar, M. Drake, L. Liang, S. C. Brooks, S. D. Brown. "Mercury methylation: Genes and communities (Hg SFA at ORNL, Task 3)." Fourth Annual DOE Environmental Remediation Sciences Program Principal Investigators Meeting. April 20–23, 2009. Lansdowne, Va.
106. A. V. Palumbo, S. D. Brown, T. A. Vishnivetskaya, M. Drake, M. K. Kerley, S. C. Brooks, L. A. Fagan, B. Gu, M. Rodriguez, and C. C. Brandt. "Microbial community structure and function related to geochemistry in mercury contaminated stream sediments." Association for the Sciences of Limnology and Oceanography (ASLO) Aquatic Sciences Meeting. Jan. 25–30, 2009. Nice, France.

2008

107. S. C. Brooks, G. R. Southworth, R. R. Turner, and R. Jensen. "Comparison of two mercury contaminated surface water bodies." Abstracts of the 2008 Fall Meeting, American Geophysical Union. Dec. 15–19, 2008. San Francisco.
108. C. Miller, B. Gu, S. C. Brooks, and G. R. Southworth. "The influence of kinetics on the formation of complexes between mercury and dissolved organic matter." Abstracts of the 2008 Fall Meeting, American Geophysical Union. Dec. 15–19, 2008. San Francisco.
109. A. V. Palumbo, S. D. Brown, T. A. Vishnivetskaya, M. Drake, M. K. Kerley, S. C. Brooks, L. A. Fagan, B. Gu, M. Rodriguez, and C. C. Brandt. "An evaluation of microbial community structure and function in mercury contaminated stream sediments." Abstracts of the 2008 Fall Meeting, American Geophysical Union. Dec. 15–19, 2008. San Francisco.
110. L. Liang, S. C. Brooks, B. Gu, A. Palumbo, G. Southworth, J. Smith, A. Johs, C. Miller, and T. Phelps. "Oak Ridge Scientific Focus Area: Biogeochemical and molecular mechanisms controlling contaminant transformation in the environment." Third Annual DOE Environmental Remediation Sciences Program. April 7–9, 2008. Lansdowne, Va.

Invited Presentations

2015

1. B. R. Crable, S. D. Brown, S. D. Smith, R. Bridou, J. D. Wall, and D. A. Elias. "Elucidating the native function of the Hg methylating genes *hgcAB*: Metabolic characterization of *Desulfovibrio desulfuricans* ND132 WT, Δ*hgcAB* and Δ*hgcAB*::*hgcAB*." 115th General Meeting of the American Society of Microbiology. May 30–June 2, 2015. New Orleans.
2. J. Smith. "Computational science: curing disease and saving the environment." Keynote lecture at the Tschira Foundation 20th Anniversary Symposium. Jan. 23, 2015. Heidelberg, Germany.

2014

3. A. Johs. "Methylmercury: The biomolecular origins of a potent neurotoxin." Dec. 2014. Karl-Franzens-Universität Graz, Graz, Austria.
4. J. Smith. "Computational science: curing disease and saving the environment." Keynote lecture at the National Institute for Mathematics in Biology. Dec. 2014. Knoxville, Tenn.
5. J. Smith "Computer simulation for energy, the environment, and health." Pregl Lecture at the National Institute of Chemistry. Oct. 2014. Ljubljana, Slovenia.
6. J. Parks. "Discovery and DFT characterization of HgcA, a new class of corrinoid protein." Southeastern Regional Meeting of the American Chemical Society 2014. Oct. 18, 2014. Nashville, Tenn.
7. J. Smith. "Computer simulation for energy, the environment, and health." Keynote lecture at the Annual Conference of the Oak Ridge Leadership Computing Facility. July 2014.
8. J. Smith. "Computer simulation for energy, the environment, and health." Departmental Chemistry Colloquium. July 17, 2014. Florida State University.
9. Gilmour, C. C., et al. "Mercury methylation by *hgcAB*+ methanogens." Goldschmidt 2014 International Conference, Session 19f. June 8–13, 2014. Sacramento, Calif.
10. B. Gu "Complex interactions between metal ions, dissolved organic matter, and microbes on metal transformation and trafficking in the environment." Goldschmidt 2014 International Conference. June 8–13, 2014. Sacramento, Calif.
11. D. A. Elias et al. "Development of new tools and approaches for determining mercury methylation in the environment." Goldschmidt 2014 International Conference, Session 19f. June 8–13, 2014. Sacramento, Calif.
12. A. Johs. "The interactions of biological macromolecules with metals revealed by neutron and x-ray scattering techniques." 2014 Joint National Synchrotron Light Source and Center for Functional Nanomaterials Users' Meeting. May 19–21, 2014. Brookhaven National Laboratory.
13. J. Parks. "The genetic and biomolecular basis of mercury methylation." Florida Annual Meeting and Expo (FAME 2014) of the American Chemical Society Florida Section. May 8–10, 2014. Tampa, Fla.
14. J. Smith. "Mad as hatters: Albert Einstein, Maggie Thatcher, and Mercury." Keynote lecture at the Southeast Enzyme Conference. April 2014. Georgia State University.
15. J. Smith. "Mad as hatters: Albert Einstein, Maggie Thatcher, and Mercury." Genome Sciences and Technology Symposium. March 2014. Knoxville, Tenn.
16. J. Smith "Computer simulation for energy, the environment, and health." Karcher Lecture. Feb. 14, 2014. University of Oklahoma.
17. J. Smith. "Molecular simulation in energy and environmental science." Presentation to DOE Office of Biological and Environmental Research staff. Feb. 2014.

2013

18. J. Smith. "The life, loves, and death of Hg^{2+}." 2013 Annual U.S.-China Environment Symposium. Nov. 19, 2013. Gatlinburg, Tenn.
19. B. Gu. "Biogeochemical transformation of mercury as a global pollutant in the environment." 2013 Annual U.S.-China Environment Symposium. Nov. 18–19, 2013. Gatlinburg, Tenn.
20. J. Smith. "The life, loves, and death of Hg^{2+}." Society of Environmental Toxicology and Chemistry. Nov. 18, 2013. Nashville, Tenn.
21. J. D. Wall. "Microbial methylation of mercury: A solution to the mystery of neurotoxin production." Sept. 28, 2013. Saturday Morning Science, University of Missouri–Columbia.
22. S. Brooks. "Mercury use at ORNL and its environmental legacy: What we knew, what we know now, what we are learning, and what we have to learn." East Tennessee Health Physics Society meeting. Sept. 17, 2013.
23. J. Smith. "Neutrons in Biology." Keynote lecture at the International Conference on Neutron Scattering. July 12, 2013. Edinburgh, Scotland.
24. L. Liang, A. Johs, and J. Parks. "The ORNL mercury research program and the story of methylation gene discovery. Friends of Oak Ridge National Laboratory at the University of Tennessee Resource Center. June 11, 2013. Oak Ridge, Tenn.
25. J. Parks. "The genetic basis of bacterial mercury methylation at the DOE Joint Terrestrial Ecosystem Science/Subsurface Biogeochemical Research Investigators Meeting. May 14–15, 2013. Potomac, Md.
26. J. D. Wall. "The genetic basis for bacterial mercury methylation." Plant & Microbial Biology, Koshland Hall, University of California–Berkeley. April 31, 2013.
27. J. Smith. Presentation about ORNL's advances in mercury research. East Tennessee Economic Council. March 1, 2013. Oak Ridge, Tenn.
28. J. D. Wall. "The genetic basis for bacterial mercury methylation." Biological and Environmental Research Advisory Committee (BERAC) meeting. Feb. 21, 2013. Washington, D.C.
29. J. Smith. "Computer simulation for energy, the environment, and health." Physics Department Colloquium. Feb. 11, 2013. University of Illinois, Urbana-Champaign.
30. J. Smith. Neutrons in Biology Symposium. Jan. 2013. University of California–San Diego.

2012

31. L. Liang and A. Johs. "Mechanisms controlling mercury speciation in a contaminated environment: Role of naturally dissolved organic matter." Canadian Light Sources. Dec. 12, 2012. Saskatoon, Canada.
32. B. Gu. "Mercury contamination and transformation in the environment." Department of Biosystems Engineering & Soil Science. Dec. 10, 2012. University of Tennessee–Knoxville.

33. J. Smith. "Computer simulation for energy, the environment, and health." Physics Department Colloquium. Nov. 28, 2012. Arizona State University.
34. B. Gu. "Natural humic substances in biogeochemical transformation of mercury in the environment." 9th International Symposium on Persistent Toxic Substances. Oct. 23–27, 2012. Miami.
35. J. Smith "Computer simulation for energy, the environment, and health." Chemistry Department Seminar. Sept. 24, 2012. Imperial College, London.
36. J. Smith. "Neutrons in Biology." European Spallation Source Symposium. Sept. 17, 2012. Copenhagen.
37. L. Liang, D. Watson, C. Miller, J. Howe, F. He, and E. Pierce. "The fate of mercury at the contaminated site." 22nd V. M. Goldschmidt Conference. June 24–29, 2012. Montreal.
38. S. C. Brooks. "History of Hg use at the Y-12 Plant and its environmental legacy." Oak Ridge Institute for Continued Learning. July 12, 2012. Oak Ridge, Tenn.

2011

39. S. C. Brooks. "The effects of groundwater–surface water interactions on the fate and transport of uranium and mercury at the Oak Ridge site." Sixth Annual DOE Subsurface Biogeochemical Research Principal Investigators Meeting. April 26–28, 2011. Washington, D.C.

2010

40. S. C. Brooks. "Overview of mercury contamination at the Oak Ridge Y-12 Site: History and hydrogeochemical setting." Department of Earth and Environmental Sciences. April 23, 2010. Vanderbilt University.
41. J. M. Parks. "The mechanism of mercury-carbon bond cleavage by MerB." Biochemistry and Cellular and Molecular Biology Departmental Colloquium. 2010. University of Tennessee– Knoxville.

2009

42. S. C. Brooks. "Brief history of mercury use at the Oak Ridge Y-12 Plant." Mercury Challenges in the Environment: A Technical Summit, sponsored by the DOE Office of Groundwater and Soil Remediation. Oct. 22–23, 2009. Vanderbilt University, Nashville, Tenn.
43. S.C. Brooks. "Mercury cycling in the environment: The complicated story of a complex system." DOE Environmental Remediation Sciences Program Strategic Planning Workshop. Aug. 2–5, 2009. Gaithersburg, Md.
44. S. C. Brooks. G. R. Southworth, R. R. Turner, and R. Jensen. "Comparison of two mercury contaminated surface water bodies." Fourth Annual DOE Environmental Remediation Sciences Program Principal Investigators Meeting. April 20–23, 2009. Lansdowne, Va.

National and International Conferences or Workshops Attended

1. Applied and Environmental Microbiology, Gordon Research Conference. July 12–17, 2015. Mount Holyoke College, South Hadley, Mass.
2. 12th International Conference on Mercury as a Global Pollutant. June 14–19, 2015. Jeju, Korea.
3. 115th General Meeting of the American Society for Microbiology. May 30–June 2, 2015. New Orleans.
4. Third Annual DOE Joint Terrestrial Ecosystem Science/ Subsurface Biogeochemical Research Investigators Meeting. April 27–29, 2015. Potomac, Md.
5. Joint Assembly of the American Geophysical Union. March 3–7, 2015. Montreal.
6. 126th Annual Meeting of the Geological Society of America. Oct.19–22, 2014. Vancouver, Canada.
7. 23rd V. M. Goldschmidt International Conference. June –13, 2014. Sacramento, Calif.
8. Symposium Frontiers in Metallobiochemistry III. June 4–7, 2014. Pennsylvania State University, College Park, Pa.
9. 114th General American Society for Microbiology Meeting. May 17–20, 2014. Boston.
10. Second Annual DOE Joint Terrestrial Ecosystem Science/ Subsurface Biogeochemical Research Investigators Meeting. May 6–7, 2014. Potomac, Md.
11. Society of Environmental Toxicology and Chemistry (SETAC) North America 34th Annual Meeting. Nov. 17–21, 2013. Nashville, Tenn.
12. 11th International Conference on Mercury as a Global Pollutant. July 28–Aug. 2, 2013. Edinburgh, Scotland.
13. 29th International Conference of the Society for Environmental Geochemistry and Health. July 8–12, 2013. Toulouse, France.
14. 113th American Society for Microbiology General Meeting. May 18–21, 2013. Denver.
15. First Annual DOE Joint Terrestrial Ecosystem Science/ Subsurface Biogeochemical Research Investigators Meeting. May 14–15, 2013. Potomac, Md.
16. American Chemical Society Spring 2013 National Meeting and Exposition. April 2–11, 2013. New Orleans.
17. 57th Annual Meeting of the Biophysical Society. Feb. 2–6, 2013. Philadelphia.
18. Geological Society of America Annual Meeting. Nov. 4–7, 2012. Charlotte, N.C.
19. Ninth International Symposium on Persistent Toxic Substances. Oct. 23–27, 2012. Miami.
20. 113th American Society for Microbiology General Meeting. May 18–21. Denver.
21. American Chemical Society Fall 2012 National Meeting and Exposition. Aug.19–23, 2012. Philadelphia.
22. American Crystallographic Association 2012 Annual Meeting. July 28–Aug. 1, 2012. Boston.
23. 22nd V. M. Goldschmidt International Conference. June 24–29, 2012. Montreal.
24. DOE Subsurface Biogeochemical Research Program Annual Contractor-Grantee Workshop. April 30–May 2, 2012. Washington, D.C.

25. 2011 American Geophysical Union Fall Meeting. Dec. 5–9, 2011. San Francisco.
26. 21st V. M. Goldschmidt International Conference. Aug. 14–19, 2011. Prague.
27. 10th International Conference on Mercury as a Global Pollutant. July 24–29, 2011. Halifax, Nova Scotia, Canada.
28. 111th General Meeting of the American Society for Microbiology. May 21–24, 2011. New Orleans.
29. Sixth Annual DOE Subsurface Biogeochemical Research Program Contractor-Grantee Workshop. April 26–28, 2011. Washington, D.C.
30. American Geophysical Union 2010 Fall Meeting. Dec. 13–17, 2010. San Francisco.
31. 20th V. M. Goldschmidt International Conference. June 14–18, 2010. Knoxville, Tenn.
32. 110th General Meeting, American Society for Microbiology. May 23–27, 2010. San Diego.
33. Fifth Annual DOE Environmental Remediation Sciences Program Principal Investigators Meeting. March 29–31, 2010. Washington, D.C.
34. American Chemical Society National Meeting. March 22–25, 2010. San Francisco.
35. 2009 Fall Meeting, American Geophysical Union. Dec. 14–18, 2009. San Francisco.
36. American Chemical Society National Meeting. August 16–20, 2009. Washington, D.C.
37. Ninth International Conference on Mercury as a Global Pollutant. June 7–12, 2009. Guiyang, China.
38. 109th General Meeting of the American Society for Microbiology. May 17–21, 2009. Philadelphia.
39. Fourth Annual DOE Environmental Remediation Sciences Program Principal Investigators Meeting. April 20–23, 2009. Lansdowne, Va.
40. Association for the Sciences of Limnology and Oceanography (ASLO) Aquatic Sciences Meeting. Jan. 25–30, 2009. Nice, France.
41. 2008 Fall Meeting, American Geophysical Union. Dec. 15–19, 2008. San Francisco.
42. Third Annual DOE Environmental Remediation Sciences Program. April 7–9, 2008. Lansdowne, Va.

National and International Leadership Activities — Conferences or Sessions Organized or Approved

1. B. Gu, T. Barkay, and A. Johs co-convened a session on "Effects of coupled microbiological and geochemical interactions on Hg speciation, uptake, and methylation" at the 12th International Conference on Mercury as a Global Pollutant. June 14–19, 2015. Jeju, Korea.
2. H. Lin, J. Bargar, M. Keiluweit, and B. Gu co-convened a symposium session on "Coupled cycling of biogeochemically critical elements and contaminants" at the 249th American Chemical Society National Meeting and Exposition. March 22–26, 2015. Denver.
3. H. Lin, A. Johs, Y. Yang, and J. Sonke co-convened a symposium session on "Complex interactions between metal ions, dissolved organic matter, and microbes on metal transformation and trafficking in the environment" at the 23rd V. M. Goldschmidt International Conference. June 8–13, 2014. Sacramento, Calif.
4. S. Brooks and T. Mathews co-convened a symposium session on "Mercury characterization and contaminated site remediation: Methods, challenges, and lessons learned" at the 2013 Society of Environmental Toxicology and Chemistry. Nov. 17–21, 2013. Nashville, Tenn.
5. R. Guyoneaud, D. Elias, D. Amouroux, and C. Gilmour co-convened a session on "Organisms, pathways, and regulations behind biological methylmercury production, from the cell to the ecosystem" at the 2013 International Conference on Mercury as a Global Pollutant. July 28–Aug. 2, 2013. Edinburgh, Scotland, UK. Additionally, Dr. Elias co-led a roundtable discussion following the session.
6. S. Brooks, T. Barkay, and N. Yee organized a working group meeting of mercury researchers funded by the DOE Subsurface Biogeochemical Research (SBR) program, along with other interested scientists at the 2014 Annual DOE Joint Terrestrial Ecosystem Science/SBR Investigators Meeting. May 6–7, 2014. Potomac, Md.
7. S. Brooks co-leads the South River Science Team Remedial Options Group: Laboratory and Small-Scale Field Testing team and is a participating member on the South River Science Team Long-Term Monitoring Committee, 2012–present.
8. L. Liang and N. Yee organized a breakout session titled "Emerging Challenges and Opportunities in Hg Research" at the 2013 DOE Joint Terrestrial Ecosystem Science/Subsurface Biogeochemical Research Investigators Meeting. May 14–15, 2013. Potomac, Md. The session included presentations by A. Johs on "The molecular basis of microbial mercury methylation," J. Schaefer on "Uptake pathways and Hg methylation," B. Gu and S. Myneni on "Mercury redox and cell surface–Hg interaction processes," and A. Summers on "What we don't know about Hg in the environment and how knowing could make a difference."
9. D. Elias co-chaired with P. Bayer and R. Guyoneaud a scientific session on "Geochemical influences on Hg bioavailability and biogeochemical transformations" at the 22nd V. M. Goldschmidt International Conference. June 24–29, 2012. Montreal.
10. F. He co-chaired with K. Skubal and E. Pierce a scientific session on "Soil and sediment remediation" at the 22nd V. M. Goldschmidt International Conference. June 24–29, 2012. Montreal.

Appendix D. Press, Outreach, and User Proposals

Television, News, and Radio Interviews or Releases

1. British Broadcasting Company program on ORNL mercury research, June 12, 2015. The segment highlighted many SFA accomplishments and other mercury-focused research within ORNL's Environmental Sciences Division — www.bbc.com/specialfeatures/horizonsbusiness/seriesfive/episode-6-pollution-solutions/?vid=p02tg625.
2. Joint news release from Oak Ridge National Laboratory and the Smithsonian Environmental Research Center on the confirmation of novel methylators greatly expanding the phylogenetic and environmental diversity of methylating organisms — www.ornl.gov/ornl/news/news-releases/2013/toxic-methylmercury-producing-microbes-more-widespread-than-realized.
3. Oak Ridge National Laboratory news release on the discovery of the two-gene cluster in bacteria responsible for mercury methylation that was published in *Science* — www.ornl.gov/ornl/news/news-releases/2013/ornl-scientists-solve-mercury-mystery--science-reports.
4. *Science* podcast — www.sciencemag.org/content/339/6120/718.2.full.
5. *Knoxville News Sentinel* article on the discovery of the *hgcA* and *hgcB* genes — www.knoxnews.com/news/2013/feb/07/ornl-led-team-hails-breakthrough-in-mercury/.
6. F1000Prime Article Recommendation, "The Genetic Basis for Bacterial Mercury Methylation" — f1000.com/prime/717979517.
7. DOE Discovery & Innovation article — science.energy.gov/discovery-and-innovation/stories/2013/127038/.
8. European Commission in a Science for Environmental Policy report — ec.europa.eu/environment/integration/research/newsalert/pdf/325na1_en.pdf.
9. *Knoxville News Sentinel* article on a Friends of Oak Ridge National Laboratory presentation given by Liyuan Liang, Alex Johs, and Jerry Parks on June 11, 2013 — knoxblogs.com/atomiccity/2013/06/04/mercury_researchers–at_fornl/.
10. Invited presentation about ORNL advances in mercury research at the East Tennessee Economic Council in Oak Ridge on March 1, 2013, by Jeremy Smith, Jerry Parks, and Alex Johs — eteconline.org/speakers/march-1-2013/.

Other Outreach Activities

1. Judy Wall gave a presentation titled "The genetic basis for bacterial mercury methylation" as part of the Mathematics in Life Sciences (MLS) Proseminar (George Smith, facilitator) at the University of Missouri–Columbia on April 18, 2013. There were 20 MLS freshmen distributed among 8 different science and engineering majors. The program consisted of dinner with students and a one-hour presentation concerning research.
2. Ami Riscassi participated in a citizen scientist project working with local high school students sampling dragonfly larvae for mercury analysis in the Great Smoky Mountains National Park during fall and spring 2012–13. The project is run by the National Park Service and University of Maine. Riscassi also attended a breakfast and networking event for the Girls in STEM program at the L&N STEM academy on March 20, 2013. The event encourages female high school students to pursue careers in science, technology, engineering, and math (STEM).
3. Carrie Miller collaborated with Brooks Rand Instruments on a project funded by the Small Business Innovation Research program to develop an *in situ* mercury analyzer for analyzing mercury in water. Brooks Rand field-tested the instrument prototype at ORNL in October 2012. This collaboration continued in 2014, with ORNL acting as a beta tester for the instrument.
4. Jerry Parks helped to mentor a high school student preparing for a science fair. Parks received a letter of thanks from the student, who earned several awards at the fair, including a $20,000 scholarship to a university and the Stockholm Junior Water Prize award.
5. Alex Johs served as a scientific judge at the Tennessee Science Bowl 2013 at Pellissippi State Community College, Blount County Campus, in Friendsville, Tenn., on February 23, 2013.
6. Carrie Miller and Scott Brooks attended the Expert Panel Meeting of the South River Science Team on October 9–10, 2013, in Harrisonburg, Va., to interact with mercury researchers from DuPont.
7. Baohua Gu hosted Marian Alicia, a summer visiting faculty member, from June–August 2012, and a visiting scholar, Weihua Zhang, for a year.
8. Carrie Miller attended the Expert Panel Meeting of the South River Science Team during October 23–25, 2012, in Harrisonburg, Va., to interact with mercury researchers from DuPont.

User Proposals Submitted

1. Baohua Gu and Benjamin Mann submitted a user proposal to the DOE Environmental Molecular Sciences Laboratory (EMSL) in January 2014. Title: "Identification of mercury-reactive species in natural organic matter."
2. A proposal was submitted to the DOE Joint Genome Institute's 2014 Community Sequencing Program. Title: "Microbial ecology and genetic drivers for mercury transformations in diverse environments."
3. A general EMSL user proposal to obtain preliminary nuclear magnetic resonance data on $HgcA_{CBD}$ was submitted and approved. Title: "Nuclear magnetic resonance spectroscopy structure determination of the cobalamin binding protein HgcA required for bacterial mercury methylation."
4. A proposal in response to the EMSL Science Theme Call was submitted and approved. Title: "Nuclear magnetic resonance (NMR) structure determination of the cobalamin binding protein HgcA and the ferredoxin-like protein HgcB, which are required for bacterial mercury methylation."

Appendix E. Agenda for 2015 Scientific Advisory Committee Visit

2015 Oak Ridge National Laboratory SFA Advisory Committee Site Visit

April 9–10, 2015

Bldg. 1520, Rm. 202
Meeting Host/Point of Contact: Eric Pierce, 865.574.9968

ORNL Team: Craig Brandt, Scott Brooks, Dwayne Elias, Baohua Gu, Alex Johs, Liyuan Liang, Melanie Mayes, Jerry Parks, Jeremy Smith, Guoping Tang, and Xiangping Yin

Post-Doctoral Researchers/Graduates: Hongmei Chen, Geoffrey Christensen, Andrew King, Kosh Neupane, and Todd Olsen

SFA Advisory Committee Members: David Krabbenhoft, Alex MacKerell, Elizabeth Phillips, and Richard Sparling

Schedule	Topic	Participants
8:00 a.m.–9:00 a.m.	Badging and reviewers' orientation	All
9:00 a.m.–9:30 a.m.	Start ORNL SFA overview presentation	Eric Pierce
9:30 a.m.–9:40 a.m.	Questions and discussion	All
9:40 a.m.– 10:00 a.m.	Task 1 overview presentation	Scott Brooks and Todd Olsen
10:00 a.m.–10:10 a.m.	Questions and discussion	All
10:10 a.m.–10:30 a.m.	Task 2 overview presentation	Baohua Gu, Kosh Neupane, and Hongmei Chen
10:30 a.m.–10:40 a.m.	Questions and discussion	All
10:40 a.m.–10:55 a.m.	Break	All
10:55 a.m.–11:15 a.m.	Task 3 overview presentation	Dwayne Elias and Geoffrey Christensen
11:15 a.m.–11:25 a.m.	Questions and discussion	All
11:25 a.m.–11:45 a.m.	Task 4 overview presentation	Jeremy Smith, Alex Johs, and Jerry Parks
11:45 a.m.–11:55 a.m.	Questions and discussion	All
11:55 a.m. – 12:10 p.m.	Break	All
12:10 p.m.–12:30 p.m.	Task 5 overview presentation	Melanie Mayes, Guoping Tang, and Andrew King
12:30 p.m.–12:40 p.m.	Questions and discussion	All
12:40 p.m.–12:55 p.m.	Management overview presentation	Eric Pierce
12:55 p.m.–1:05 p.m.	Questions and discussion	All
1:05 p.m.–1:20 p.m.	Break	All
1:20 p.m.–3:20 p.m.	Lunch and poster session	All
3:20 p.m.– 3:35 p.m.	Break	All
3:35 p.m.–5:00 p.m.	Final questions and discussion	All
5:00 p.m.–8:00 p.m.	Dinner at Riverside Grill, 100 Melton Lake Peninsula, Oak Ridge, TN 37830	All

Acronyms and Abbreviations

BBC	British Broadcasting Company
CBD	cobalamin-binding domain
CbI	hydroxocobalamin
CFeSP	corrinoid iron-sulfur protein
Co	cobalt
CV	cyclic voltammetry
DFT	density functional theory
DOE	U.S. Department of Energy
DOM	dissolved organic matter
EFPC	East Fork Poplar Creek
EM	DOE Office of Environmental Management
EMSL	DOE Environmental Molecular Sciences Laboratory
EPR	electron paramagnetic resonance
EXAFS	extended X-ray absorption fine structure spectroscopy
Fe	iron
FTIR	Fourier transform infrared
FTICR-MS	Fourier transform ion cyclotron resonance–mass spectrometry
Hg	mercury
HgT	total mercury
HSQC	heteronuclear single quantum coherence
ICMGP	International Conference on Mercury as a Global Pollutant
KBase	DOE Systems Biology Knowledgebase
LC–MS	liquid chromatography–mass spectrometry
LRM	Laboratory Research Manager
MBP	maltose binding protein
MCD	magnetic circular dichroism
MeHg	methylmercury
MerA	mercuric ion reductase
MLS	Mathematics in Life Sciences
MS	mass spectrometry
NMR	nuclear magnetic resonance
NOM	natural organic matter
ORNL	Oak Ridge National Laboratory
OREM	Oak Ridge Office of Environmental Management
ORISE	Oak Ridge Institute for Science and Education
ORR	Oak Ridge Reservation
PLFA	phospholipid fatty acid
qPCR	quantitative polymerase chain reaction
S	sulfur
SAC	Scientific Advisory Committee
SBR	DOE Subsurface Biogeochemical Research program
SDS-PAGE	sodium dodecyl sulfate–polyacrylamide gel electrophoresis
SFA	Science Focus Area
SRB	sulfate-reducing bacteria
STEM	science, technology, engineering, and math
TSS	total suspended solids
UNEP	United Nations Environmental Programme
UTK	University of Tennessee–Knoxville
UV/Vis	ultraviolet–visible spectroscopy
WT	wild type