Over the last 3 years, the ORNL CI-SFA program has made substantial progress in fulfilling its Phase I (FY 2016–2018) objective of determining the fundamental mechanisms and environmental factors that control Hg biogeochemical transformations at critical interfaces in terrestrial and aquatic ecosystems. Unique attributes of the CI-SFA include the range of spatiotemporal scales studied—spanning from molecular to watershed length scales and from picoseconds to seasonal variations in time—and the effective integration of technical expertise from widely varying science 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 and discoveries, including the determination of importance of periphyton biofilms on net methylmercury (MeHg) production in East Fork Poplar Creek (EFPC) (Olsen et al., 2016; Olsen et al., in press). Although previous studies confirmed the prevalence of iron- and sulfate-reducing bacteria in EFPC sediments, our recent lab investigations demonstrated that changes to the carbon source impact microbial community composition and ultimately Hg methylation potential in EFPC sediment samples (Christensen et al., 2017). We have also determined computationally the permeability of Hg complexes through biomembranes (Zhou et al., 2017). We have discovered that certain methanotrophs and iron-reducing bacteria (e.g., Geobacter bemidjiensis Bem) are capable of degrading MeHg (Lu et al., 2017; Lu et al., 2016). Further, building on the discovery of the Hg methylation genes (hgcAB), we computationally probed the reaction mechanism for HgcA in MeHg formation (Johnston et al., 2016), designed hgcAB biomarkers (Christensen et al., 2016), and applied these probes to EFPC and to a range of other environmental systems in collaboration with US and international groups conducting Hg research. Leveraging our previous studies which established that dissolved organic matter (DOM) dominates aqueous Hg speciation in EFPC, we conducted the first-ever application of high-resolution mass spectrometry combined with quantum chemical calculations to confirm the molecular composition and structure of specific Hg-DOM complexes in EFPC (Chen et al., 2017) and evaluated the influence of EFPC derived DOM on Hg methylation by anaerobic bacteria (Zhao et al., 2017). Lastly, we have made substantial progress in developing our biogeochemical modeling framework for predicting Hg transformations in EFPC (Painter, submitted).