

Laboratory dissection of the complementary pathways used by iron-reducing *Geobacter* bacteria to finely tune environmental cues to the mineralization of toxic metals

MORGEN M CLARK¹, KAZEM KASHEFI¹ AND GEMMA REGUERA²

¹Michigan State University

²Department of Microbiology, Genetics, and Immunology,
Michigan State University

Presenting Author: clarkmo9@msu.edu

Geobacter bacteria rely on abundant *c*-cytochrome complexes and retractable protein nanowires of Type IVa pilus class to reductively dissolve Fe(III) (oxyhydr)oxides and soluble mineral-bound metals. Specialized motifs on the nanowires then bind and reductively precipitate the soluble metals outside of the cell to prevent their permeation and cytotoxicity. As an added layer of protection, the cells produce a rough (no O-antigen) lipopolysaccharide (LPS) to bind the metal cations and vesiculate to release the metal-saturated LPS and regenerate the LPS coat. The uranyl cation ($[\text{UO}_2]^{2+}$) is bound with high affinity by both the nanowire traps and the rough LPS of the laboratory strain *Geobacter sulfurreducens* PCA. Using this experimental system, we show that 75% of the U(VI) in the uranyl cation was reductively precipitated to a mononuclear U(IV) mineral by the nanowires, while the remaining 25% was immobilized, but not reduced, by the LPS layer. Vesiculation and nanowire production were however sensitive to subtle differences in sodium content (as low as 10 mM) in the growth medium. Furthermore, an inverse relationship existed between nanowire assembly and vesiculation in response to uranium exposure in the tested media. Yet, the minor differences in salt during cultivation did not impact growth, making their impacts on cell envelope chemistry and dynamics easily overlooked. These findings warn about the importance of *in vitro* cultivation on metal-reducing phenotypes in *Geobacter*. With careful media formulation, it was possible to reproducibly dissect the contribution of the nanowire and LPS-vesiculation pathways to the respiration and immobilization of the uranyl cation by *G. sulfurreducens*. Notably, the results reveal a high plasticity in the metal detoxification responses that allow *Geobacter* bacteria to finely tune the composition and chemistry of the cell envelope to the surrounding environment and inform strategies for the reclamation of toxic metals from environmental contaminants and wastes.