In situ spectroscopy on intact *Leptospirillum ferrooxidans* reveals that reduced cytochrome 579 is an intermediate in the iron respiratory chain

Robert C. Blake II[1]* (rblake@xula.edu) and Megan N. Griff[1]

[1]Xavier University of Louisiana, New Orleans, LA

Project Goals: The practical goal is to develop a new and powerful approach to quantitatively examine the dynamic behavior of bacterial electron transport systems at the microbe-mineral interface, an interaction that has heretofore been difficult to probe directly. We will accomplish this goal by refining and exploiting a novel integrating cavity absorption meter (ICAM) where the sample observation cell is also a spherical reflecting cavity. Light scattering losses due to sample turbidity will be eliminated or minimized by multiple reflections at the cavity wall that will prevent the scattered light from escaping the detector. The utility of this novel ICAM to study *in situ* bacterial electron transfer reactions will be demonstrated in real time equilibrium and kinetic measurements of electron transfer reactions in intact bacteria that respire by exchanging electrons with soluble and insoluble extracellular iron. The ability to conduct accurate real-time absorbance measurements in live organisms will permit any biological process that involves colored biomolecules to be studied in new ways.

This abstract introduces a new means to study respiratory electron transfer reactions *in situ* in intact bacteria under physiological solution conditions. The premise is that accurate UV-visible spectroscopy of electron transfer reactions among colored biomolecules can be conducted in highly turbid suspensions if the live bacteria are irradiated in an isotropic homogeneous field of incident measuring light. Under those conditions, the absorbed radiant power is independent of scattering effects. We conducted equilibrium and kinetic studies on the Fe(II)-dependent reduction and O_2-dependent oxidation of cytochromes in intact *Leptospirillum ferrooxidans* at pH 1.7. We used a novel integrating cavity absorption meter where the cuvette comprised a reflecting cavity completely filled with the absorbing suspension. *L. ferrooxidans* was selected because it is only known to respire on one substrate, reduced iron.

The aerobic iron respiratory chain of *Leptospirillum ferrooxidans* was dominated by the redox status of an abundant cellular cytochrome with an absorbance peak at 579 nanometers in the reduced state. Intracellular cytochrome 579 was reduced within the time that it took to mix a suspension of the bacteria with soluble ferrous iron at pH 1.7. Subsequent oxidation of the reduced cytochrome appeared to be the rate-limiting step in the overall aerobic respiratory process. Steady state turnover experiments were conducted where the concentration of ferrous iron was less than or equal to that of the oxygen concentration. The concentration of the reduced cytochrome 579 at any time point was directly proportional to the velocity of product ferric ion formation. Further, the integral of the area of the reduced cytochrome accumulated over time was also directly proportional to the total concentration of ferrous iron in each reaction mixture. These kinetic data obtained using whole cells were consistent with the hypothesis that reduced cytochrome 579 is an obligatory steady state intermediate in the iron respiratory chain of this bacterium.

The direct and accurate observation of absorbance changes *in situ* in intact organisms is a useful complement to traditional reductionist approaches and recent advances in proteomic and transcriptomic studies. The colored prosthetic groups of most electron transport proteins comprise intrinsic spectrophotometric probes whereby transient changes in the oxidation-reduction state of the proteins may be monitored with great sensitivity. There is no better means to establish physiological relevance in a metabolic function than to directly observe the function as it occurs in the intact bacterium. The movement of electrons through electron transfer complexes is central to energy production in all living cells. The ability to conduct direct spectrophotometric studies under noninvasive physiological conditions represents a new and powerful approach to examine the extents and rates of biological events *in situ* without disrupting the complexity of the live cellular environment. Studies such as these should increase our fundamental understanding of biological energy transduction.

This research was supported by the Office of Science (BER), U. S. Department of Energy.