Supporting Information

Single-Cell Imaging and Spectroscopic Analyses of Cr(VI) Reduction on the Surface of Bacterial Cells

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1. Significance of Raman spectroscopy and methods

Raman spectroscopy has been widely applied to chemical and biological molecular analyses. Raman signals are sensitive to specific molecular vibrational modes that are typically unique for specific chemical molecules and species, such as proteins, minerals, nano-materials, and bacterial cell components. There are a number of Raman spectroscopy approaches.[1] Nevertheless, in this work, we have used two types of Raman spectroscopic analyses: Resonant Raman spectroscopy and surface enhanced Raman spectroscopy. Resonance Raman spectroscopy probes the inherent Raman responses of molecules under specific excitation energy that matches with the chemical molecules' absorption resonance wavelengths. Surface enhanced Raman spectroscopy (SERS) utilizes the Au or Ag material surface chemical and electromagnetic field enhancements for often millions or much higher signal intensity and sensitivity in analyzing biological and chemical molecules on surfaces, such as the proteins on the bacterial outer membrane surfaces or chemical molecules on mineral surfaces. SERS, therefore, has a high selectivity and sensitivity to the surface-exposed molecules rather than molecules under the surfaces and in the bulk phases of materials, such as the inner membrane proteins in the bacterial cells. In this work, we applied Resonance Raman and SERS spectroscopy to characterize Hemin molecule and outer membrane proteins (OmcA and MtrC) of the *Schewanella* bacterial cells.

In our experiments, we prepared the Ag substrate for SERS. Hemin Chloride, $AgNO_3$, and Sodium citrate are purchased from Sigma Aldrich and used as received. Silver nanoparticles are synthesized by citrate reduction of $AgNO_3$ according to Lee-Meisel method.[1] NaCl is added to the Ag nanoparticle solution as activation component for SERS measurements. The average size of the Ag nanoparticles is ~50 nm identified by SEM.

2. SEM image of nanoparticles on the bacterial surface

HV | mag | WD | HFW | det | 8/30/2010 | ——— 1 μm ———
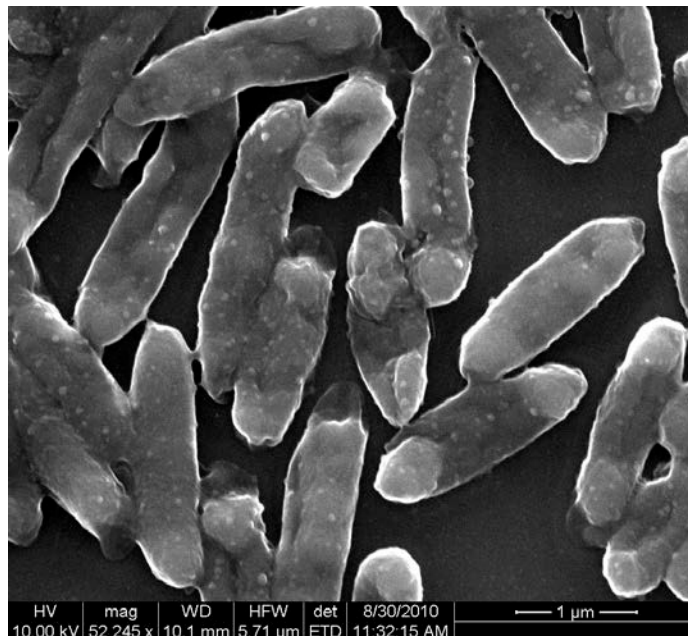10.00 kV | 52 245 x | 10.1 mm | 5.71 μm | ETD | 11:32:15 AM

Figure S1. SEM image of the wild-type *Shewanella oneidensis* MR-1 surface after the redox reaction experiment with Cr(VI). Nanoparticles are clearly shown on the bacterial surface. Both SERS and SEM/EDS analyses identified that the nanoparticles are Cr_2O_3, the reduced Cr(III) oxides.

3. The control experiments for identification of the Heme proteins on the wild-type *Shewanella oneidensis* MR-1 surface using surface-enhanced Raman spectroscopy[2]
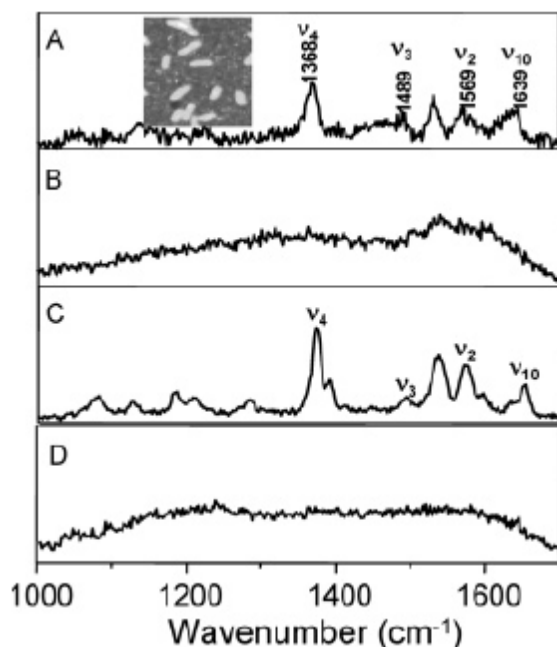

Figure S2. SERS spectra of (A) the cell surface of S. oneidensis grown anaerobically. The spectrum was taken from a single cell under a focused laser excitation. The size of the laser focus spot is about 300 nm diameter. (B) Identical sample as in (A) except that it was bubbled with O_2 for 5 s to relieve electron acceptor limitation before the cell was fixed. (C) Bovine heart cytochrome c. (D) a MR-1 gspD mutant cultivated under electron acceptor limited condition. The inset of (A) is a far-field Raman spectroscopic image of anaerobically grown cells, obtained by recording the Raman signals in the region from 1350 to 1600 cm^{-1}.[2]

References:

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(2) Lee, P. C.; Meisel, D. *J. Phys. Chem* **1982**, *86*, 3391-3395.

(3) Biju, V.; Pan, D.; Gorby, Y. A.; Fredrickson, J.; McLean, J.; Saffarini, D.; Lu, H. P. Combined Spectroscopic and Topographic Characterization of Nanoscale Domains and Their Distrubitions of a Redox Protein on Bacterial Cell Surfaces. *Langmuir* **2007**, *23*, 1333-1338.