Supporting Information (SI) for

To Boost C-type Cytochromes Wire Efficiency of Electrogenic Bacteria with Fe_3O_4/Au Nanocomposites

Liu Deng, Shaojun Guo, Zuojia Liu, Dan Li, Ling Liu, Gaiping Li, Erkang Wang* and Shaojun Dong*

State Key Laboratory of Electroanalytical Chemistry, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, Changchun, Jilin,130022, China, and Graduate School of the Chinese Academy of Sciences, Beijing, 100039, China.
E-mail: dongsj@ciac.jl.cn, ekwang@ciac.jl.cn

1. Experimental

2. TEM of Fe_3O_4/Au nanocomposites
Figure S1

3. Electricity generation of *S. oneidensis* MR-1 on GC electrode in absence of Fe_3O_4/Au nanocomposites
Figure S2

4. Cyclic voltammograms of *S. oneidensis* MR-1 biofilm
Figure S3

5. Cyclic voltammograms of OmcA liposome at bare GC electrode
Figure S4

6. Comparison of current and specific electron transfer rate of *S. oneidensis* MR-1
Table S1

1. Experimental Section:

1.1 Synthesis Fe_3O_4/Au nanocomposite: a) The Fe_3O_4 nanospheres with high saturation magnetization were synthesized by a solvothermal method according to reference (17). b) For APTMS-functionalized Fe_3O_4 spheres, Fe_3O_4 nanospheres (20 mg) were added to ethanol (30 mL), followed by the addition of water (2 mL). Then, ammonium hydroxide (25%; 2 mL) and APTMS (200 mL) were added to the above solution. The resulting solution was sonicated for about 8 h. After four-step separation by means of an external magnetic field, the resulting product was dissolved in water (10 mL). c) 13 nm Au NPs were synthesized according to reference (18). Thus, a 1 mm $HAuCl_4$ solution (100 mL) was brought to reflux while stirring and then 38.8 mm trisodium citrate solution (10 mL) was added quickly, which resulted in a color change of the solution from pale yellow to deep red. The solution was then heated to reflux for an additional 15 min. The concentration of Au NPs was about 1 mm provided that $HAuCl_4$ was completely reduced. d) The Fe_3O_4/Au nanocomposite were prepared by mixing an aqueous solution of APTMS-functionalized Fe_3O_4 nanospheres (1 mL) with 10 mL of a solution of Au NPs (excess). The resulting products were collected by means of an external magnet and dissolved in 1 mL of water. Transmission electron microscopy (TEM) measurements were made on a HITACHI H-8100 EM with an accelerating voltage of 200 kV.

1.2 Organisms, Media, and Growth Conditions: *S. oneidensis* strain MR-1 were cultivated in minimal salts medium with 10 mM HEPES. Medium contained (per liter): 0.46 g NH_4Cl, 0.225 g K_2HPO_4, 0.117 g $MgSO_4 \cdot 7\ H_2O$, 0.225 $(NH_4)_2 \cdot SO_4$, plus 10 ml of a mineral mixture (containing per liter: 0.1 g $MnCl_2 \cdot 4H_2O$, 0.3 g $FeSO_4 \cdot 7H_2O$, 0.17 g $CoCl_2 \cdot 6H_2O$, 0.1 g $ZnCl_2$, 0.04 g $CuSO_4 \cdot 5H_2O$, 0.005 g H_3BO_3, 0.09 g Na_2MoO_4, 0.12 g $NiCl_2$, 0.02 g $NaWO_4 \cdot 2H_2O$, and 0.10 g Na_2SeO_4). Medium was adjusted to pH 7, saturated with N_2:CO_2=80:20, sealed with butyl stoppers and aluminum seals, and autoclaved. Cultures for each reactor were grown from frozen stocks, then transferred into anaerobic medium with 20 mM fumarate as the electron

acceptor until an OD of 0.4 and transferred into the electrochemical cell, and lactate was added (20 mM).

1.3 Microbial current measurements: All electrochemical experiments were carried out under potentiostatic control utilizing a three electrode arrangement, consisting of the glassy carbon working electrode (0.07 cm^2), a Ag/AgCl reference electrode (sat. KCl) and a counter electrode (platinum wire or). The working electrode was poised at a potential of +300 mV vs Ag/AgCl reference electrode. Experiments were conducted in a constant temperature room (30°C) in duplicate. The electrochemical measurements were performed with an EG&G 273A electrochemical system (Princeton Applied Research, USA). Sealed and thermostated vessels (100 mL) were served as electrochemical cells which hosted the fermentation medium and the electrodes. All experiments were performed under strictly anoxic conditions. Scanning electron microscopy (SEM) images were determined with a Philips XL-30 ESEM. The accelerating voltage was 20 kV.

1.4 Protein Purification and Analysis: The cell protein attached on the electrode surface was quantified as the same way as previously described (19). For protein quantification, cells were collected by flushing and scraping the biofilm with a pipettor from the electrode surface with 1mL of sterile isotonic wash buffer, then freezedried and quantified. OmcA was purified from anaerobically grown *S. oneidensis* MR-1 in defined media containing 30 mM lactate and 50 mM ferric citrate (20). The OM fraction was treated with 2% Triton X-100 and loaded onto a Q-Sepharose anion-exchange column and equilibrated with 50 mM HEPES, pH 7.5, and 5% Triton X-100. Fractions were eluted with a gradient of 0 to 0.5 M NaCl over 160 ml at 1 ml/min. The OmcA liposome was prepared as the same protocol (5g) without the dye addition.

1.5 Preparation of OmcA/Fe_3O_4/Au NCs/GC electrode and OmcA/Fe_3O_4 NPs GC electrode: For preparation of OmcA/Fe_3O_4/Au NCs/GC electrode, 5μL of Fe_3O_4/Au NCs suspension (10 mg mL^{-1}) was spread evenly onto the GC electrode surface with syringe. Then 5μL of the OmcA liposome (5 mg mL^{-1}) was spread onto the Fe_3O_4/Au NCs/GC electrode surface. Finally, the modified electrode was allowed to dry for 24 h

at 4 ◦C. The resulted OmcA/Fe3O4/Au NCs/GC was washed thoroughly with water and stored at 4 ◦C when not in use. The preparation of OmcA/Fe_3O_4 NPs/GC electrode was almost under the same way, except 5μL of Fe_3O_4 NPs suspension (10 mg mL^{-1}) was used to prepare the Fe_3O_4 NPs/GC electrode.

References:

(17) Deng, H.; Li, X. L.; Peng, Q.; Wang, X.; Chen, J. P.; Li, Y. D. *Angew. Chem.* **2005**, 117, 2842-2845.

(18) Deng, L.; Wang, Y. Z.; Shang, L.; Wen, D.; Wang, F. A.; Dong, S. J. *Biosens. Bioelectron*. **2008**, 24, 951–957

(19) Richter, H.; McCarthy, K.; Nevin, K. P.; Johnson, J. P.; Rotello, V. M.; Lovley, D. R. *Langmuir*, **2008**, 24, 4376-4379

(20) Ross, D. E.; Ruebush, S. S.; Brantley, S. L.; Hartshorne, R.S.; Clarke, T. A.; Richardson, D. J.; Tien, M. *Appl Environ Microbiol.* **2007**, 73, 5797–5808

2. TEM of Fe_3O_4/Au nanocomposites

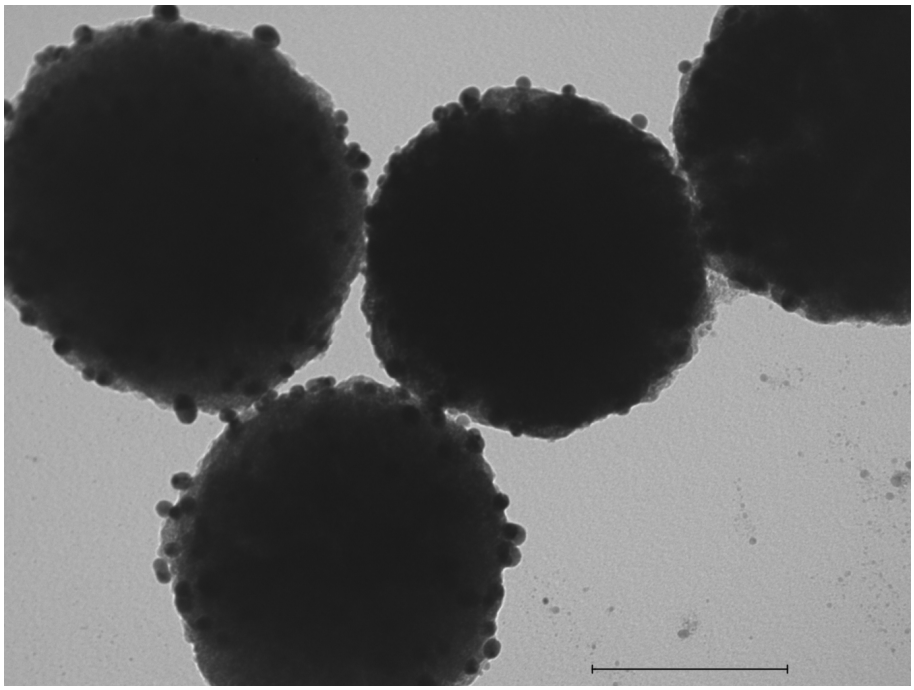

Figure S1 The TEM image of the prepared Fe_3O_4/Au nanocomposites.

3. Electricity generation of *S. oneidensis* MR-1 on GC electrode in absence of Fe_3O_4/Au nanocomposites

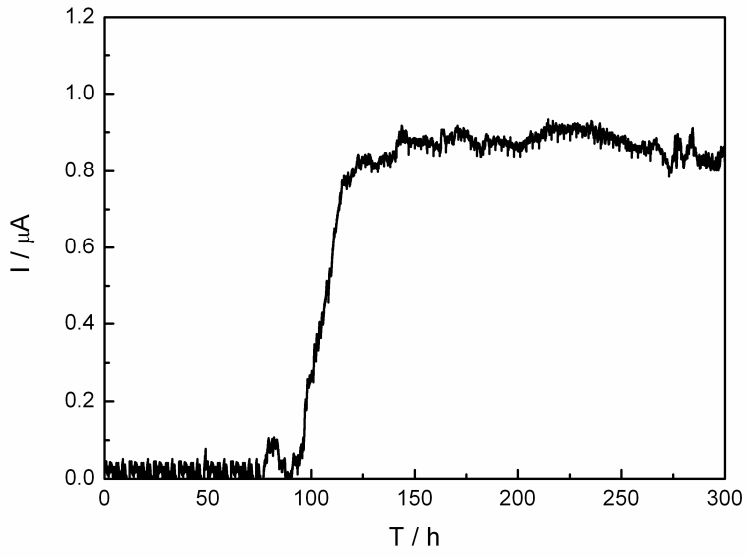

Figure S2 Current generation by *S. oneidensis* MR-1 on GC electrode poised at a potential of +200 mV vs Ag/AgCl reference electrode.

4. Cyclic voltammograms of *S. oneidensis* MR-1 biofilm

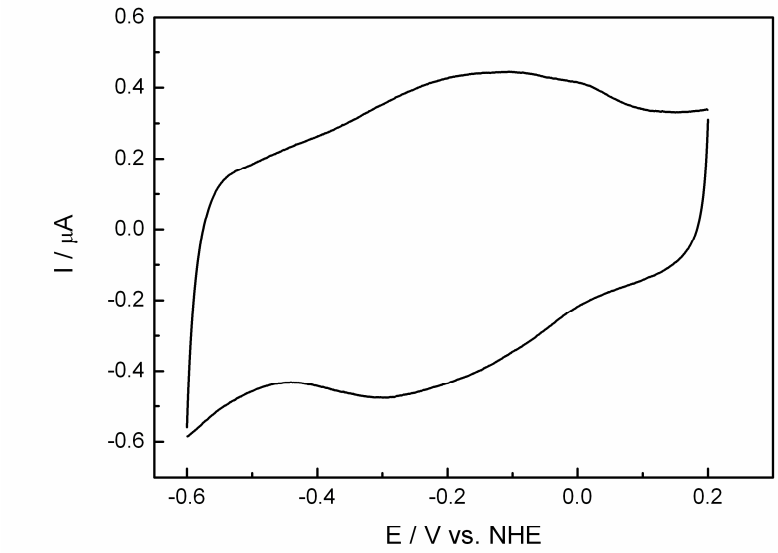

Figure S3 Cyclic voltammograms of *S. oneidensis* MR-1 biofilm on GC electrode, scan rate: 1mV/s. The voltammograms were recorded in a substrate depleted culture medium.

5. Cyclic voltammograms of OmcA liposome at bare GC electrode

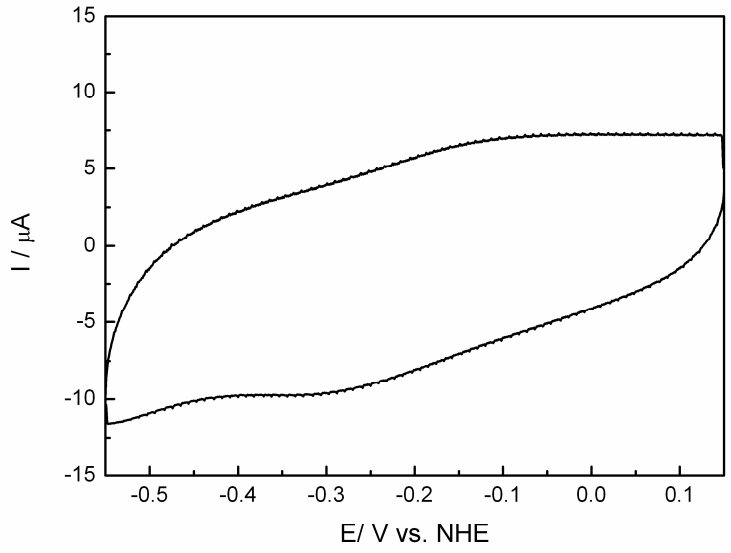

Figure S4 Cyclic voltammograms of OmcA liposome at bare GC electrode at a scan rate of 50 mV/s in 0.1 M PBS (pH=7.2) under a N_2-saturated atmosphere.

6. Comparison of current and specific electron transfer rate of *S. oneidensis* MR-1

Experimental condition	Current (μA)	ET rate (μmol/min.g)	Current density (mA/m^2)
a	0.87	237	125
b	19.78	702	2826

Table S1 Comparison of current, specific electron transfer (ET) rate in μmol of electrons per minute and gram cell protein attached of *S. oneidensis* MR-1 on GC electrodes in absence of Fe_3O_4/Au nanocomposites (a) and presence of Fe_3O_4/Au nanocomposites (b)