Electronic Supplementary Information

Feedback stabilization involving redox states of *c*-type cytochromes in living bacteria

Huan Liu[a], Shoichi Matsuda[b], Tomoyuki Kawai[b], Kazuhito Hashimoto[a,b,c,*], Shuji Nakanishi[a,c]

Methods

Microbe preparation. *Shewanella loihica* PV-4 (wild type and mutants lacking major *c*-Cyt genes *shew*2525(the homolog of MtrC) and *shew*2522 and *shew*2524 (the homologs of OmcA)) as well as *S. oneidensis* MR-1 were cultured aerobically in 10 mL of marine broth (MB) (20 g/l) at 30 °C for 24 h. Method for the mutants preparation can be found in elsewhere.[s1] Cells were collected by centrifugation, washed three times with defined media (DM) ($NaHCO_3$ (2.5 g), $CaCl_2{\cdot}2H_2O$ (0.08 g), NH_4Cl (1.0 g), $MgCl_2{\cdot}6H_2O$ (0.2 g), NaCl (10 g), and HEPES (7.2 g) per litre) and then further cultivated at 30 °C for 24 h in DM supplemented with 10 mM lactate as a carbon source. The cell suspensions were then centrifuged for 20 min at 5,000 x *g* and the collected cells were used for electrochemical characterization. The concentration of the cell suspension in the electrochemical cell was determined by measuring the optical density (OD) at 600 nm.

Ref. S1: G. J. Newton, S. Mori, R. Nakamura, K. Hashimoto, K. Watanabe, *Appl. Environ. Microbiol.* **2009**, *75*, 7674-7681.

Electrochemical characterization. A single chamber, three-electrode system was used to monitor the electrochemical behaviour of the microbes. A tin-doped In_2O_3 (ITO) glass substrate with a surface area of 3.2 cm^2 was used as the working electrode and was mounted on the bottom of the reactor. An Ag/AgCl (sat. KCl) and a platinum wire were used as the reference and counter electrodes, respectively. DM containing 10 mM lactate was used as an electrolyte and was deaerated with N_2 bubbling for 30 min (remaining O_2 was ca. 0.1 ppm) prior to injection of the cell suspension into the reactor. The temperature of the reactor was maintained at 30 °C by water circulation and no agitation was made during measurements. Suspensions containing a high density of *Shewanella* cells (~10^8 cell/ml) were subjected to electrochemical characterizations. The microbes were first aerobically cultured in DM media for one day, and further cultured electrochemically either at +200 mV (E_{cul} = +200 mV) or –200 mV (E_{cul} = –200 mV) for 10 h, using lactate as a carbon and electron source. Then, cyclic voltammetries (CVs) were performed with a scan rate of 50 V/s (Fig. 1). In order to confirm the observed phenomenon is not

specific to the ITO electrode, the fluorine-doped Sn_2O_3 (FTO) and graphite was also used as electrodes in this study.

Supporting result

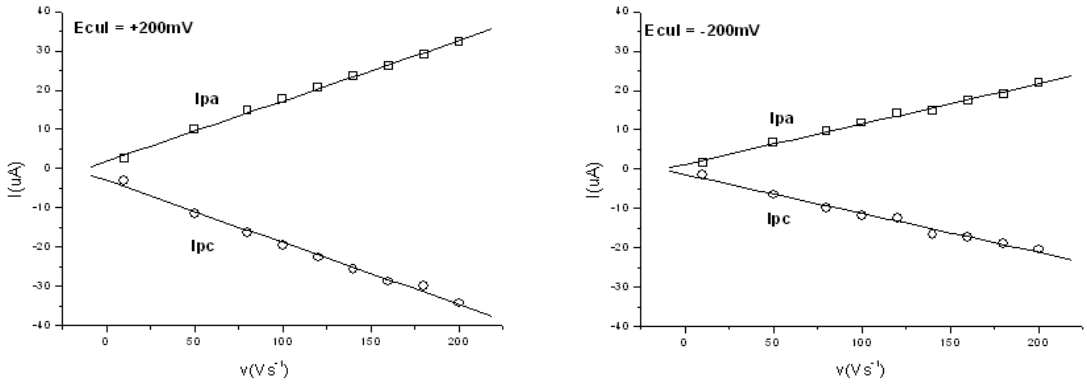

Figure S1. Plot of an anodic (Ipa) and a cathodic (Ipc) peak current as a function of a scan rate at (left) E_{cul} = +200mV and (right) E_{cul} = -200mV.

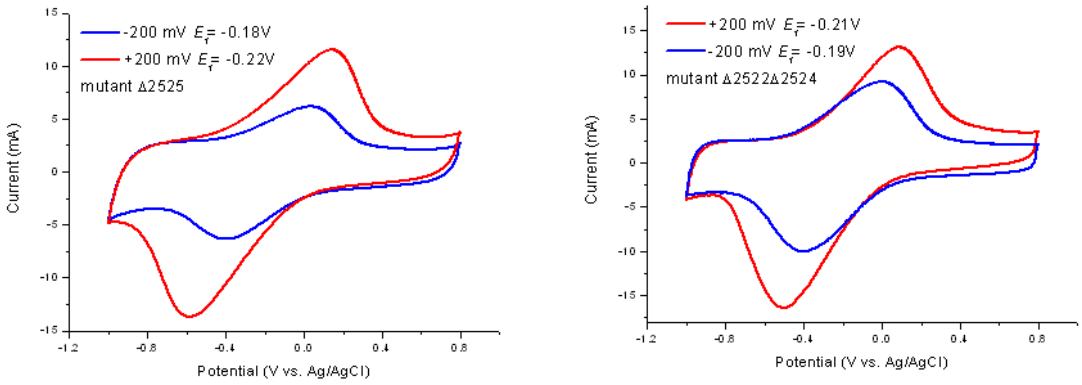

Figure S2. Whole-cell cyclic voltammograms for mutant PV-4 strains lacking major *c*-Cyt genes *shew*2525 (left) and *shew*2522 and *shew*2524 (right) electrochemically cultured at +200 mV (red) and -200 mV (blue). The scan rate of the potential was 50 V/s.