Supporting Information

Okamoto et al. 10.1073/pnas.1220823110



Fig. S1. (*A*) Confocal fluorescent images of GFP-labeled MR-1 cells attached on an indium tin-oxide (ITO) electrode surface in the presence of 0, 2, or 16 μ M flavin mononucleotide (FMN) (*left, center,* and *right* panels in *A*). The number of cells attached on the surface did not change, even 1 h after the addition of 16.0 μ M FMN. (*B*) Differential pulse (DP) voltammograms for monolayer biofilms of *Shewanella oneidensis* MR-1 on an ITO electrode surface in the presence of 4, 8, 12, 16, 20, 28, 36, 44, and 52 μ M FMN. (*C*) Plot of the peak potential (*E_p*) of FMN against added FMN concentration. As the FMN concentration increased, the *E_p* of FMN shifted to that of free-form FMN. SHE, standard hydrogen electrode.



Fig. S2. DP voltammograms for monolayer biofilms of *Shewanella oneidensis* MR-1 on an ITO electrode surface in the presence of 2.0 μ M FMN (a) before and (b) after the addition of 1.0 mM tocopherol. Current was normalized by the redox peak current of outer-membrane *c*-type cytochromes (OM *c*-Cyts) at $E_p = +50$ mV.



Fig. S3. DP voltammograms of a monolayer biofilm of strains $\Delta omcA$ (A) and Δ SO3177 (B) in the presence of 2.0 μ M flavin mononucleotide.



Fig. 54. (*A*) Current vs. time measurements of microbial current generation for strain MR-1 cells on an ITO electrode surface at +200 mV (vs. Ag/AgCl KCl saturated) in a reactor containing 10 mM lactate and 16.0 μ M flavin mononucleotide (FMN). DP voltammetry was performed (*B*) before and (*C*) after lactate oxidation current decrease. (*D*) Current vs. time measurements of microbial current generation for strain MR-1 cells on an ITO electrode surface at +200 mV in a reactor containing 10 mM lactate and 4.0 μ M FMN. DP voltammetry was performed (*E*) before and (*F*) after the lactate oxidation current decrease by the addition of 1 mM 2-heptyl 4 hydroxyquinoline *N*-oxide (HQNO). HQNO (>99% purity; Santa Cruz Biotech) was added as aliquots from a stock solution of 50 mM in ethanol/water (50/50, vol/vol). SHE, standard hydrogen electrode.



Fig. S5. DP voltammograms of a monolayer biofilm of (A) WT, (B) ΔmtrC, and (C) ΔomcA in the presence of 2.0 μM riboflavin.

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Fig. S6. Schematic illustration for the interaction between OM c-Cyts and flavins. (A) In the presence of lactate, the extracellular electron-transport (EET) process is activated by semiquinone (Sq) formation in the reduced OM c-Cyts scaffolds. (B) In the absence of lactate, the dissociation of flavin from oxidized OM c-Cyts impairs EET processes. Mtr, metal reduction.



Fig. 57. (A) The ratio of k_f [oxidized flavin/semiquinone (Ox/Sq)] to k_f (Ox/Hq) was plotted as a function of the redox potential of a heme molecule (E_{heme}) with $\alpha = 0.4$, 0.5, and 0.6. Rate constants were calculated based on the Arrhenius equation (Eq. 3) using reported k_{05} for the flavin electrode reactions. (B) The microbial current density (J) estimated by the Eq. 5 was plotted on the y axis as a function of the concentration ratio of C_O to C_R . The J value was calculated by using k_f (Ox/Sq) and k_b (Ox/Sq) values at E_{heme} of +80 mV assuming that the sum of the heme concentration was 100 pmol.



Fig. S8. Energy diagram and schematic illustration for the interconnection between metabolic activity and extracellular electron transfer (EET) mediated by flavin molecules. (*A*) In the presence of lactate, intracellular matrix turns to be reductive, and MtrC protein with reduced hemes promotes semiquinone (Sq) formation to activate the EET process to dump excessive electrons effectively. (*B*) In the absence of lactate, the cellular metabolic reactions are impaired and the matrix is oxidative and impairs EET processes by the destabilization of Sq. In both cases, electron input and output for MR-1 cell are coupled via the oxidation state of heme in MtrC protein scaffolds.

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