Supplementary Information for

Interaction between *in vivo* bioluminescence and extracellular electron transfer in *Shewanella woodyi* via charge and discharge

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Experimental

Preparation of chemicals. Analytical grade reagents used in this work were purchased from Sinopharm Chemical Reagents Co. Ltd (Shanghai, China; NaCl, $Na_2HPO_4 \cdot 12H_2O$, KH_2PO_4, sodium lactate) or Aladdin (Shanghai, China; FMN, RF and FAD). *Shewanella* marine agar (SMA) broth (pH 7.6 ± 0.2) contained peptone (5 $g \cdot L^{-1}$), yeast extract (1 $g \cdot L^{-1}$) and 1 × sea salts (5× stock solution containing 2.58 M NaCl, 0.125 M $MgCl_2$, 0.125 M $MgSO_4$ and 0.1 M KC1).[1]

Culturing and characterization of microorganisms. *S. woodyi* MS32 (Strain No. DSMZ-12036) was purchased from Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ) and was activated aerobically in 2216E medium at 25 ºC with shaking. When the optical density at 600 nm (OD_{600}) reached ~1.2, 1 mL of cell suspension was inoculated into 100 mL SMA broth and growth recorded every four hours by measuring the OD_{600}. Meanwhile, bacterial bioluminescence was simultaneously measured using an *in vivo* imaging & analysis system (IVIS Lumina II, Caliper Life Sciences, USA). After culturing for 32 h, the bacterial suspension was directly used for electrochemical bioluminescence (EBL) experiments. Prior to electrochemical redox measurements, cultures were centrifuged at 5000 rpm for 5 min and resuspended in 50 mM PB solution twice, and the resultant cell pellet was placed on the ITO electrode (1.1 mm thickness, <17 ohm/sq).

Electrochemical measurements. The redox properties of *S. woodyi* were investigated using cyclic voltammetry and potentiostatic tests. Electrochemical experiments were

performed with a standard three-electrode cell on a CHI660D workstation (Shanghai Chenhua Apparatus Corporation, China). A 3 M Ag/AgCl and Pt slice were used as reference and the auxiliary electrodes, respectively, and the *S. woodyi*-coated ITO electrode was used as the working electrode. Sample solutions were purged with high-purity nitrogen for at least 10 min to remove oxygen prior to electrochemical experiments. EBL experiments were performed on an IFFM-E instrument (Xianruimai analytical instruments Co. Ltd., China) combined with the CHI660D workstation, and the polyethylene terephthalate (PET)-ITO electrode (0.125 mm thickness, 35 ± 5 ohm/sq) was used as the working electrode. Electrochemical information and bioluminescence responses were recorded simultaneously. Both the ITO and PET-ITO electrodes were purchased from Zhuhai Kavio Electronic Components Co., Ltd (Zhuhai, Guangzhou, China).

Qualitative and quantitative analysis of FMN(H_2) using HPLC-FLD. Flavin mononucleotide (FMN) and Riboflavin (RF) can be secreted from microbial cells. The concentration of FMN and RF were determined by following a method reported previously.[2, 3] Culture supernatants were collected and filtered through a 0.22 μm membrane prior to HPLC analysis. Supernatant (20 μL) was injected onto a C18 column, and HPLC was performed at 25 ºC with a mobile phase consisting of methanol and 4% glacial acetic acid in ultrapure water at a flow rate of 1 mL/min. Flavins were detected by the fluorescence detector at an excitation wavelength of 445 nm and an emission wavelength of 525 nm (Fig. S1). To obtain a calibration curve, standard solutions of FMN and RF were prepared in SMA broth at concentrations ranging from

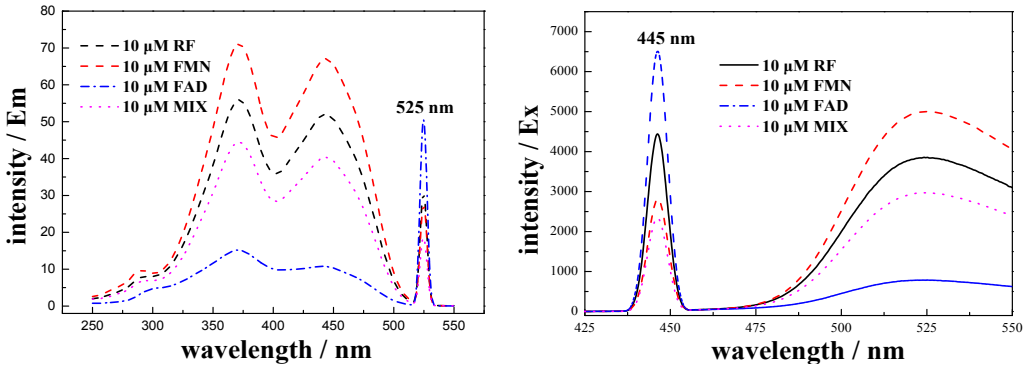
0.1 to 5 μM. Concentrations of FMN and RF in samples were determined by comparing the integrated area of the corresponding peak to the area of standard peaks.

Table S1.

Time and mobile phase data for gradient elution during HPLC-FLD.

Time / min	Mobile phase A (%) (methanol)	Mobile phase B (%) (4% glacial acetic acid)
0.0	9.0	91.0
1.0	9.0	91.0
8.0	89.0	11.0
11.0	89.0	11.0
11.1	9.0	91.0
16.0	9.0	91.0

Fig. S1



(a) **(b)**

Fig. S1. Fluorescence spectra of culture media containing 10 μM FMN, 10 μM FAD, 10 μM RF, and a mixture of all three. (a) Fluorescence spectra for measure the maximum emission wavelength (Em). (b) Fluorescence spectra to measure the maximum excitation wavelength (Ex).

These three flavins, i.e., FMN, RF, and FAD, have fluorescence properties and can be detected by the fluorescence spectrophotometer. In order to get the max emission and excitation wavelengths for following HPLC experiments, the fluorescence spectra of culture media containing FMN, FAD and RF was shown in Fig. S1. The Maximum emission wavelength (Em) of flavins was 525nm which can be read from Fig. S1(a) , and the maximum excitation wavelength (Ex) was 445 nm which was shown in Fig.S1(b)

Fig. S2

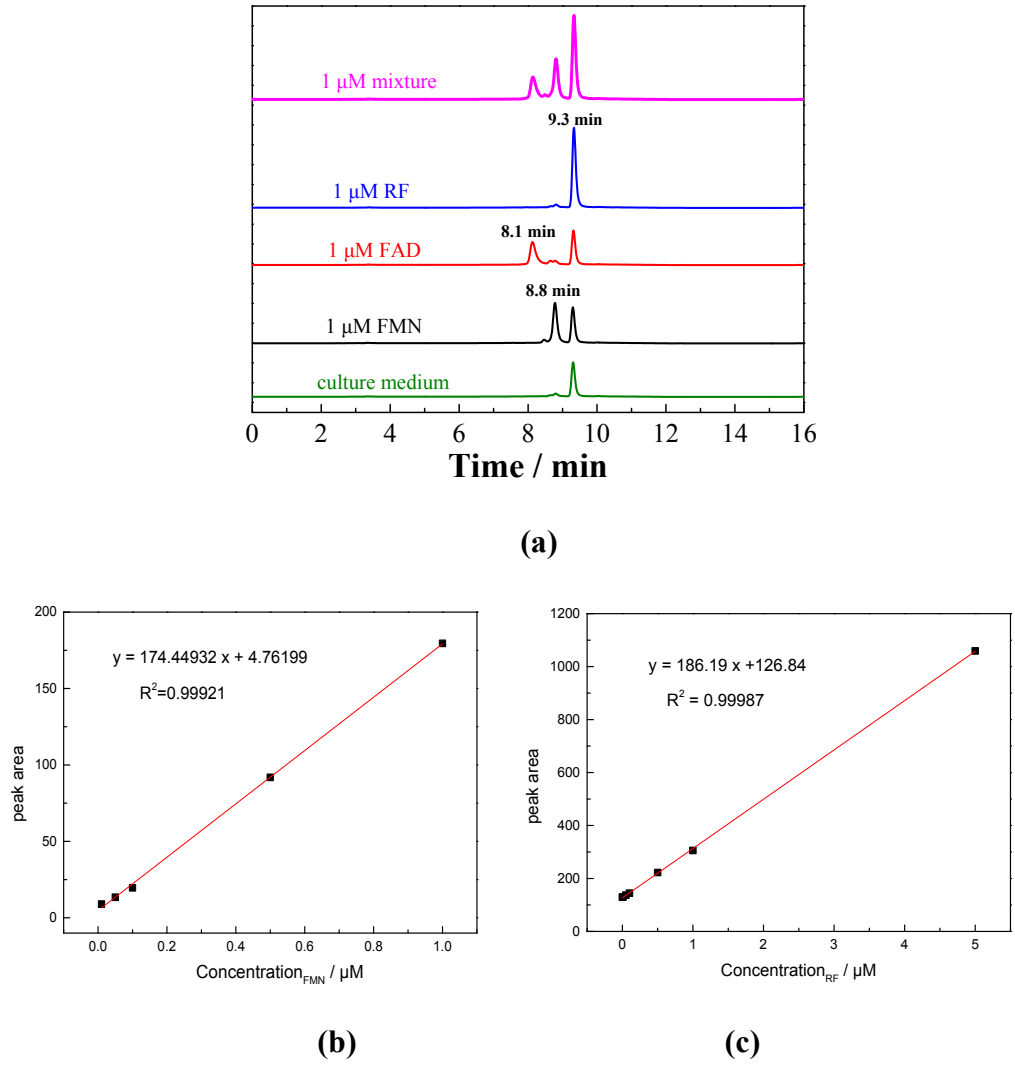


Fig. S2. (a) High performance liquid chromatography-fluorescence detection (HPLC-FLD) traces of culture media containing 1 μM FMN, 1 μM FAD, 1 μM RF and a mixture of all three. (b) Peak area-concentration curves of FMN and (c) RF standards.

Chromatographic peaks of RF, FMN and FAD were separated using HPLC-FLD as shown in Fig. S2(a). Retention times for RF, FMN and FAD were 9.3 min, 8.8 min and 8.1 min, respectively. Resolution was calculated using formula 1:

Resolution $$R_s = \frac{2(t_{R2} - t_{R1})}{W_{b2} + W_{b1}}$$ formula (1)

The resolution for RF and FMN was 4.1, and for FMN and FAD it was 4.0. Both values are > 1.5, confirming that RF, FMN and FAD can be completely separated and quantified under these chromatographic conditions. These results were used for qualitative and quantitative analysis of FMN in *S. woodyi* culture media.

Fig. S3

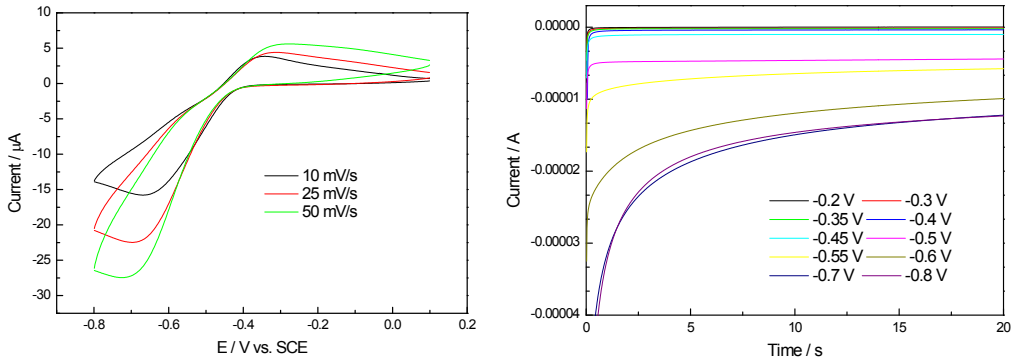


Fig. S3. (a) Cyclic voltammograms of data collected using an ITO electrode in buffer containing 1 mM FMN + 0.1 M PB solution at different scan rates. (b) Chronoamperometric plot of data collected using an ITO electrode in 1 mM FMN + 0.1 M PB solution at different electrode potentials. To investigate the electrochemical redox properties of FMN at the ITO electrode, FMN standards were used to interpret the cyclic voltammetry results. A clear reduction peak appeared at -0.6 V. When the constant potential was held between -0.5 and -0.7 V, the reduction current increased to -0.5, -0.6 and -0.7 V, respectively. When the potential was > -0.7 V, the current remained stable.

Fig. S4

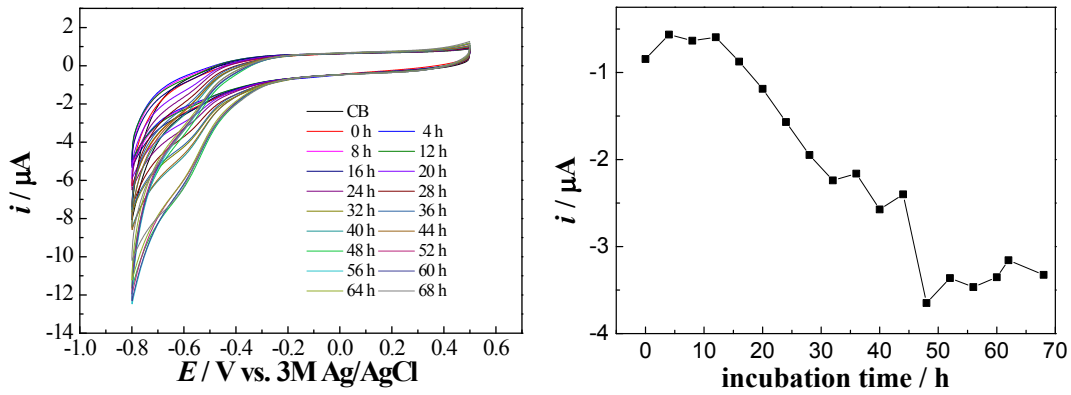


Fig. S4. (a) Cyclic voltammograms obtained using an ITO electrode in the supernatant of *S. woodyi*. Data were recorded every 4 h. (b) Plot of reduction current vs. time at a potential of -0.6 V.

Cyclic voltammetry was used to determine whether molecules secreted by *S. woodyi* were electrochemically active. An ITO electrode was used to apply an electrochemical potential in the supernatant of an *S. woodyi* culture, and the scan rate was 50 mV/s. For incubation, CV curves were recorded every 4 h (a), the reduction current began to increase from -0.4 V, and its value at -0.6V was plotted. The reduction current clearly increased with increasing incubation time. These results confirm that *S. woodyi* is able to secrete electrochemically active substance into the growth medium, and the concentration of electrochemically active substances increases with increasing incubation time.

Reference:

1. Makemson, J. C.; Fulayfil, N. R.; Landry, W.; Van Ert, L. M.; Wimpee, C. F.; Widder, E. A.; Case, J. F. *Int. J. Syst. Bacteriol.* **1997,** *47* (4), 1034-1039.

2. von Canstein, H.; Ogawa, J.; Shimizu, S.; Lloyd, J. R. *Appl. Environ. Microb* **2008,** *74* (3), 615-623.

3. Wu, C.; Cheng, Y.-Y.; Li, B.-B.; Li, W.-W.; Li, D.-B.; Yu, H.-Q. *Bioresource Technol.* **2013,** *136* (0), 711-714.