Electronic supplementary information to

Detection of *Escherichia coli* Bacteria by Impact Electrochemistry

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Experimental

Chemicals and reagents

All chemicals were purchased from Sigma-Aldrich at the highest purity available if not indicated otherwise. The radical cation salt TMPD-BF_4 was synthesized following the method proposed by Yamauchi *et al*[1]. Briefly, TMPD was dissolved in 18 mL ultrapure H_2O (resistivity not less than 18.2 MΩ cm; Millipore) and 24 mL methanol, containing 9 g sodium tetrafluoroborate (Alfa Aesar, UK). 32 mL aqueous bromine solution was added dropwise to the cooled TMPD solution (-10 °C). Resulting crystals were washed repeatedly with ice-cold methanol, followed by dry ether, and were recrystallized from methanol. Crystals appeared brownish purple, in accordance with literature[1]. The product composition was examined electrochemically (Figure S1). Phosphate buffered saline (PBS) solution (pH = 7.4) consists of 137 mM sodium chloride, 2.7 mM potassium chloride, 10 mM sodium phosphate dibasic and 1.8 mM potassium phosphate dibasic. Fresh TMPD-BF_4 stock solution was prepared right before the impact experiment its concentration was determined electrochemically.

Bacteria culture

E. coli cells were obtained from Prof F.A. Armstrong's group, University of Oxford. The number of bacteria in solution was determined by measuring the optical density (OD) at a wavelength of 600 nm (OD_{600} of 1.0 = 8 x 10^8 cells mL^{-1}) until an OD_{600} between 1.0 and 1.3 was reached. Culture medium was then removed by centrifugation (Centrifuge 5702, Eppendorf, UK) for 15 min at 3000 rcf and bacteria were re-suspended in pre-warmed (37°C) PBS, used as *E. coli* stock solution.

Electrochemical procedures

Electrochemical experiments were performed at a thermostatted (37.0 ± 0.5 °C) Faraday cage with a standard three-electrode system using a μAutolab II potentiostat (Metrohm-Autolab BV, Netherlands). A gold macroelectrode (diameter 3.0 mm, CH Instrument) was used to determine the diffusion coefficient of TMPD-BF_4 at 37°C by cyclic voltammetry. A carbon microdisc electrode (IJ Cambria Scientific Ltd, UK) was employed to determine the concentration of TMPD-BF_4 stock solution for the impact experiment by cyclic voltammetry. The diameter of the carbon *micro*disc electrode was electrochemically calibrated as 44.4 μm by analysing the steady state voltammetry of 1.0 mM hexaammineruthenium(III) chloride in aqueous solution containing 0.1 M KCl, using a diffusion coefficient for $[Ru(NH_3)_6]^{3+}$ of 8.43 $\times$ 10^{-10} m^2s^{-1} at 298 K[2]. Prior to each measurement, the working electrodes were polished using alumina of decreasing particle size (1.0, 0.3 and 0.05 μm, Buehler, IL, UK) followed by drying with nitrogen. In all experiments, a standard calomel electrode (SCE, ALS distributed by BASi, Tokyo, Japan) and a graphite rod were employed as reference and counter electrodes, respectively.

For the electrochemical measurements of the single *E. coli*, the same carbon *micro*disc electrode was used as the working electrode. 4 mL of TMPD-BF_4 stock solution was heated to 37.0 ± 0.5 °C and 2 mL of *E. coli* stock solution was then slowly added, followed by manually gentle stirring with the pipette tip to get an even suspension. Chronoamperometry was carried out immediately at a reductive potential of -0.15 V vs SCE for 50s. Only first fifteen scans were used in data analysis. The experiment was repeated until a large sample size was obtained at each E. coli concentration. The experimental impact frequency was determined by dividing the number of impact spikes over total chronoamperometric scan time. A homemade low noise potentiostat was used, as described previously[3]. A 4 kHz preamplier was used and filtered digitally (4-pole Bessel) to 25 Hz using a scriptwritten in Python 3.5.

Determination of the synthesized TMPD-BF_4 composition

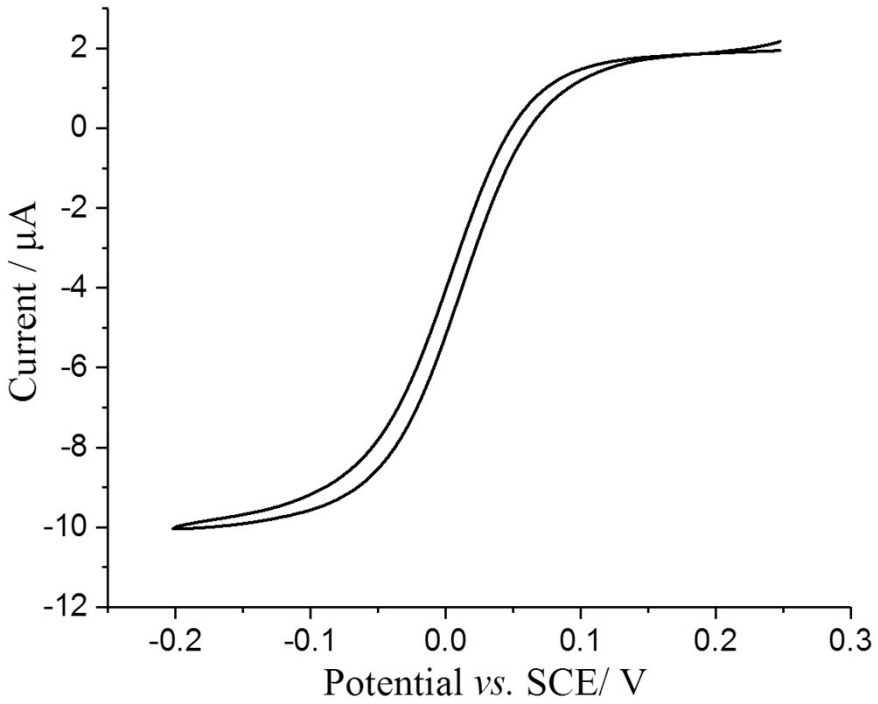

Figure S1: Cyclic voltammetry of 1.5 mM TMPD-BF_4 using a 44.4 µm diameter carbon microdisc electrode in PBS at pH 7.4.

A product composition of *ca.* 80% TMPD-BF_4 and *ca.* 20% TMPD in solution was determined.

Determination of the TMPD-BF_4 diffusion coefficient

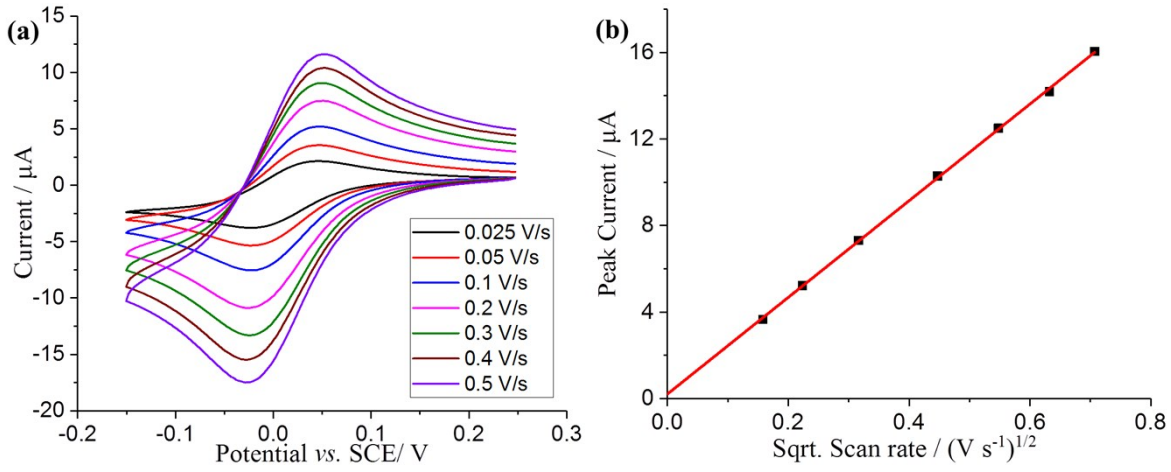

Figure S2: (a) Cyclic voltammetry of 1.5 mM TMPD-BF_4 using a 3.0 mm diameter bare gold macroelectrode in PBS at pH 7.4 recorded as a function of scan rate at 37 °C. (b) The plot of peak current as a function of the square root of the scan rate from 25 mV s^{-1} to 500 mV s^{-1}.

The reversible Randles–Sevcik equation[4] for a one electron transfer reaction is expressed as:

$$I_p = 0.446FAC_{bulk}\sqrt{\frac{FDv}{RT}}$$

where F is the Faraday constant, A is the electrode geometry area, C_{bulk} is the bulk concentration of the reactant, D is the diffusion coefficient of the reactant, v is the scan rate and T is the temperature. Using the I_p-$v^{1/2}$ relationship measured in the experiment (Figure S2b), the diffusion coefficient of TMPD-BF_4 at 37 °C was found to be $(7.6 \pm 0.2) \times 10^{-10}$ m^2/s.

Determination of TMPD-BF_4 stock solution

Concentrations of TMPD-BF4 stock solution was determined, using the equation

$$C = \frac{I_{ss}}{4nFDr}$$

where C is the concentration, I_{ss} represents the steady state limiting current, F is the Faraday constant, n = 1 (the number of electrons transferred), D is the diffusion coefficient and r the radius of the electroactive surface of the microelectrode.

Representative samples of current profile in each E. coli concentrations

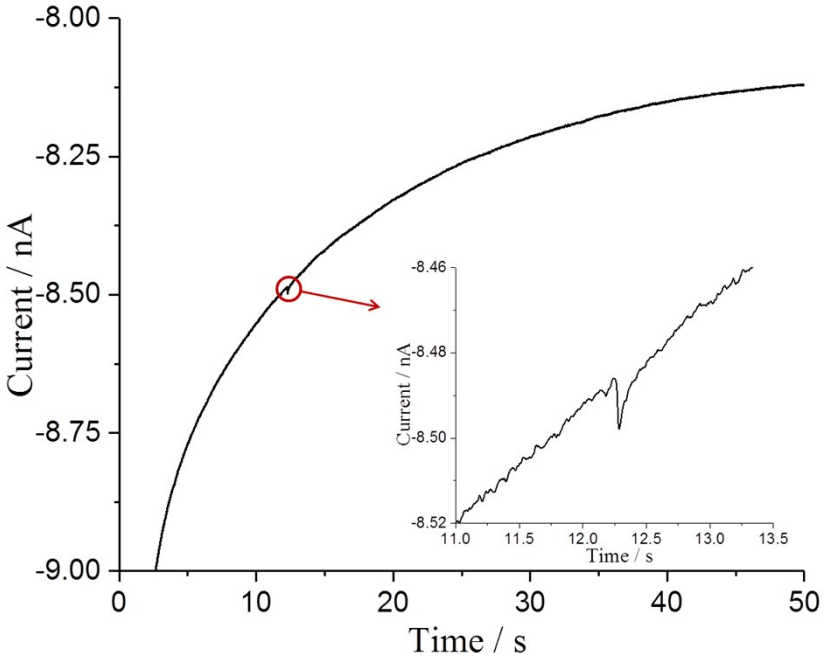

Figure S3: Representative current profile during chronoamperometry recorded in 0.869 mM TMPD-BF_4 in PBS solution (pH 7.4) containing 3 x 10^8 *E. coli* bacteria mL^{-1}.

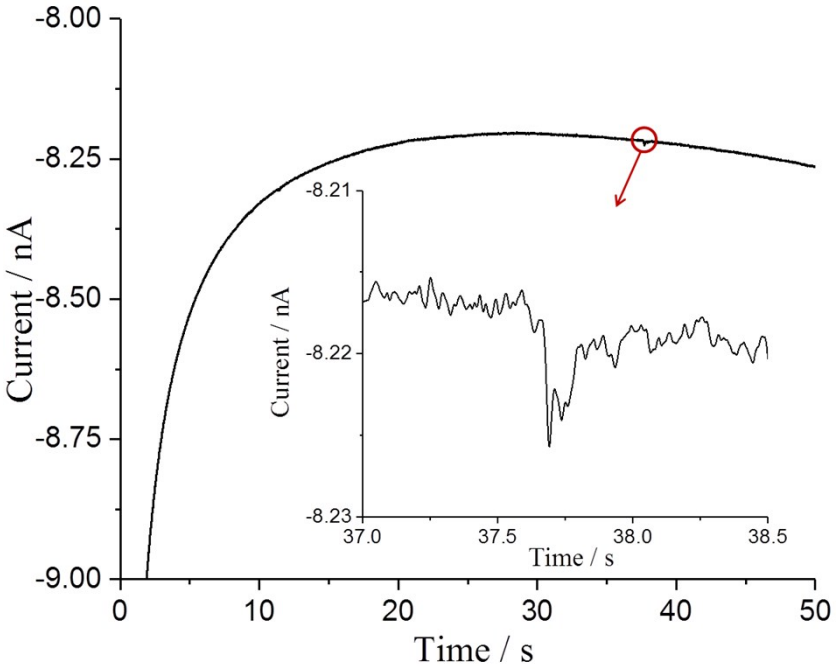

Figure S4: Representative current profile during chronoamperometry recorded in 0.880 mM TMPD-BF_4 in PBS solution (pH 7.4) containing 4.5 x 10^8 *E. coli* bacteria mL^{-1}.

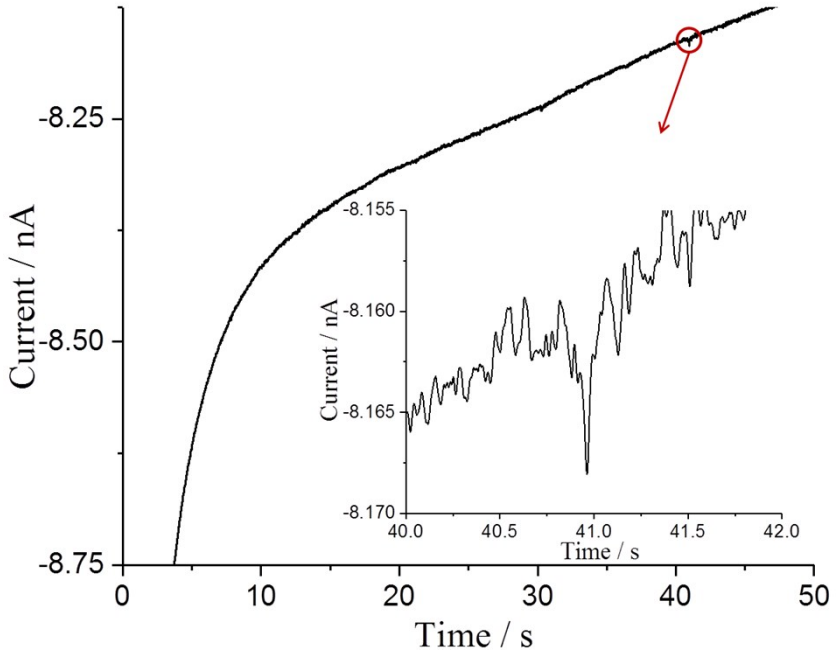

Figure S5: Representative current profile during chronoamperometry recorded in 0.875 mM TMPD-BF_4 in PBS solution (pH 7.4) containing 6 x 10^8 *E. coli* bacteria mL^{-1}.

References

1. J. Yamauchi and H. Fujita, *Bulletin of the Chemical Society of Japan*, 1990, **63**, 2928-2932.
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3. C. Batchelor-McAuley, J. Ellison, K. Tschulik, P. L. Hurst, R. Boldt and R. G. Compton, *Analyst*, 2015, **140**, 5048-5054.
4. R. G. Compton and C. E. Banks, *Understanding Voltammetry*, Imperial College Press, 2nd edn., 2011.