Electronic Supplementary Material (ESI) for Chemical Science.

Electrochemical Recognition and Quantification of Cytochrome c Expression in Bacillus subtilis and aerobe/anaerobe Escherichia coli using N,N,N',N'-tetramethyl-para-phenylene-diamine (TMPD)

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Supporting Information

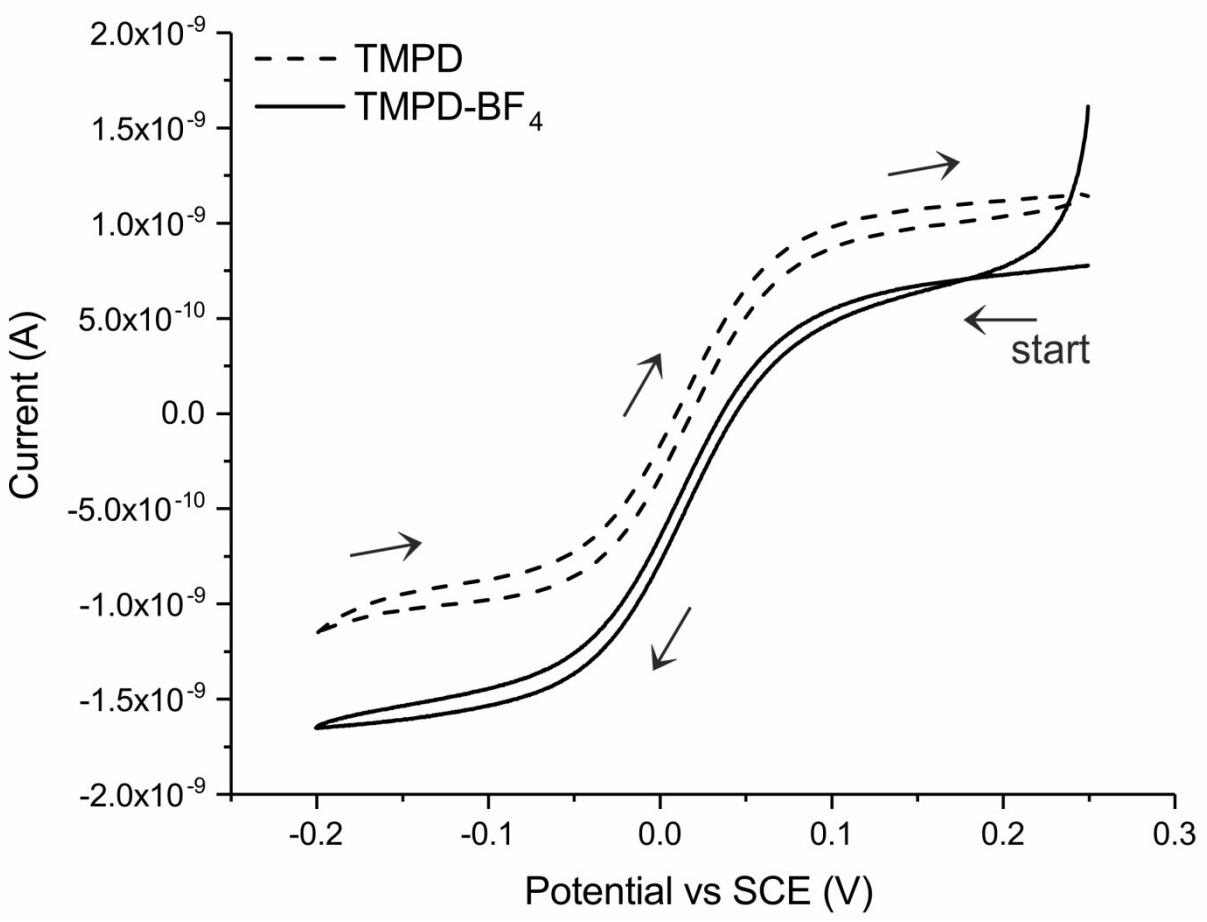

Figure S1: Cyclic voltammetry of 1 mM TMPD and TMPD-BF_4 using a 6.9 μm diameter gold electrode in PBS at pH 7.4.

Determination of species concentration

Concentrations of reduced and oxidized TMPD were determined, using the equation

$$C = \frac{I_{ss}}{4FDa} \quad (1)$$

where *C* is the concentration, *Iss* represents the steady state current, *F* is the Faraday constant, *D* is the diffusion coefficient and *a* the radius of the electroactive surface of the microelectrode.

Determination of the TMPD-BF_4 diffusion coefficient

A diffusion coefficient ($D_{TMPD\text{-}BF4}$) of 1x 10^{-5} cm^2 s^{-1} was found following the reversible Randles-Ševčík method. Cyclic voltammetry in 2.1 mM TMPD-BF_4 in PBS buffer solution (pH 7.4) was performed using a 3 mm gold working electrode at scan rates ranging from 30 to 300 mV. A linear fit of the peak current values as a function of scan rate reveals a linear relationship with a slope of 1.3 x 10^{-4} A $s^{1/2}$ $V^{-1/2}$. The diffusion coefficient was determined, using the equation

$$D = \left(\frac{m}{268600 \frac{As}{V^{1/2} mol} A\, C} \right)^2 \quad (2)$$

where *D* is the diffusion coefficient, *m* represents the slope, *A* is the electrode surface area, and *C* is the concentration.

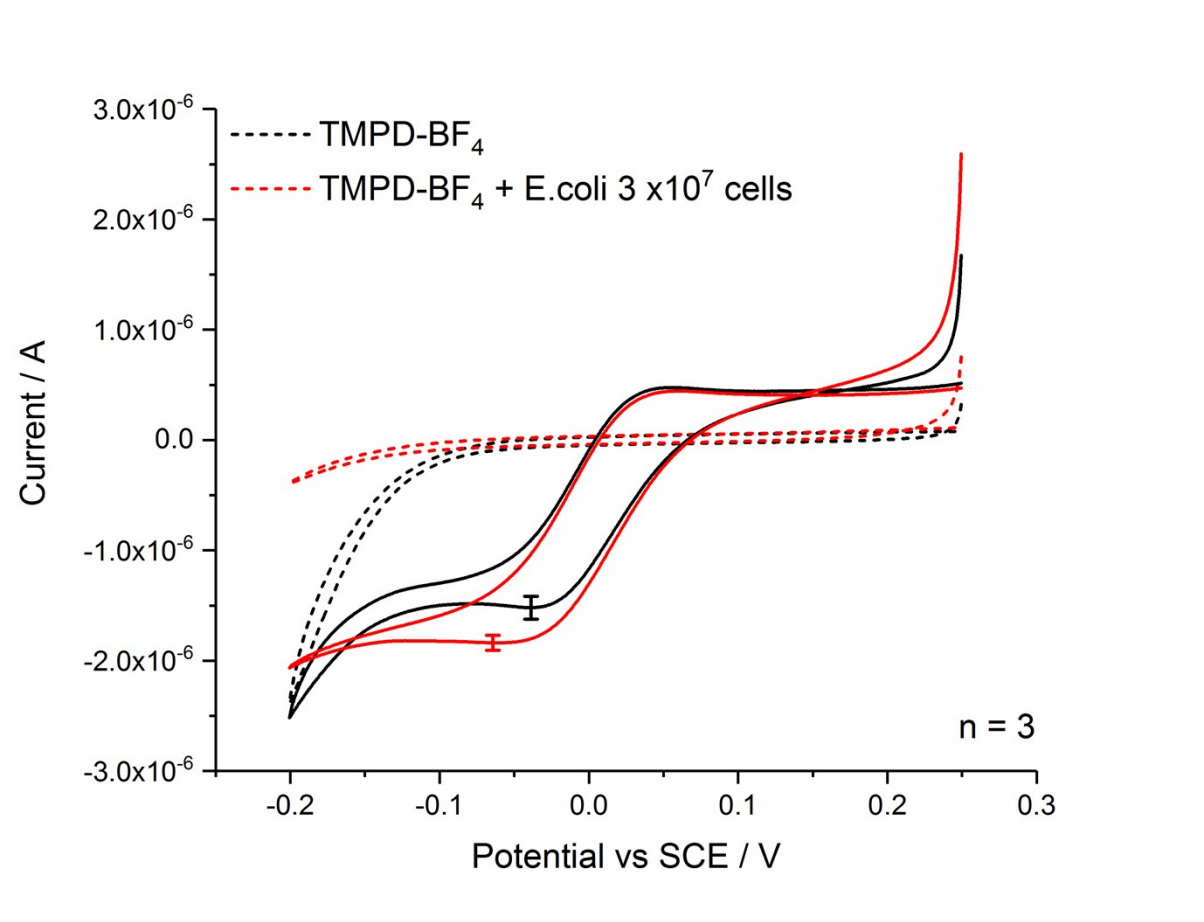

Figure S2: Cyclic voltammetry in 0.5 mM TMPD-BF_4 in PBS (full lines) in the presence (red) and the absence (black) of *E.coli* bacteria, dropcasted onto a 3 mm gold electrode. Dotted lines represent cyclic voltammetry in PBS (pH 7.4) only. The consumption of oxygen by the bacteria can be seen in the reduced reduction signal in PBS and an increase in TMPD reduction was observed.

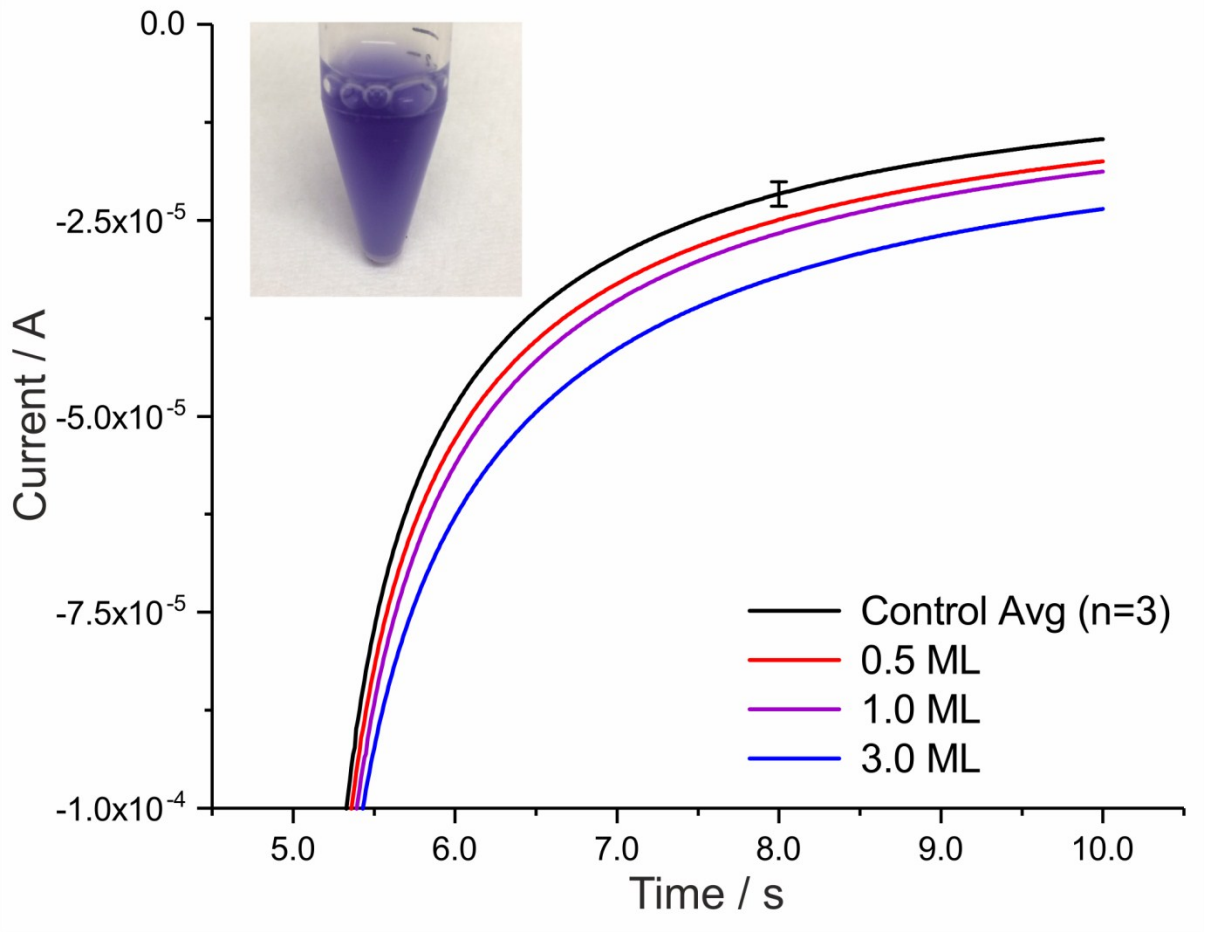

Figure S3: Chronoamperometry in 2.0 mM TMPD-BF_4 in the presence of different concentrations of *E.coli* bacteria (grown to stationary phase), dropcasted onto a 3 mm (diameter) gold electrode surface.

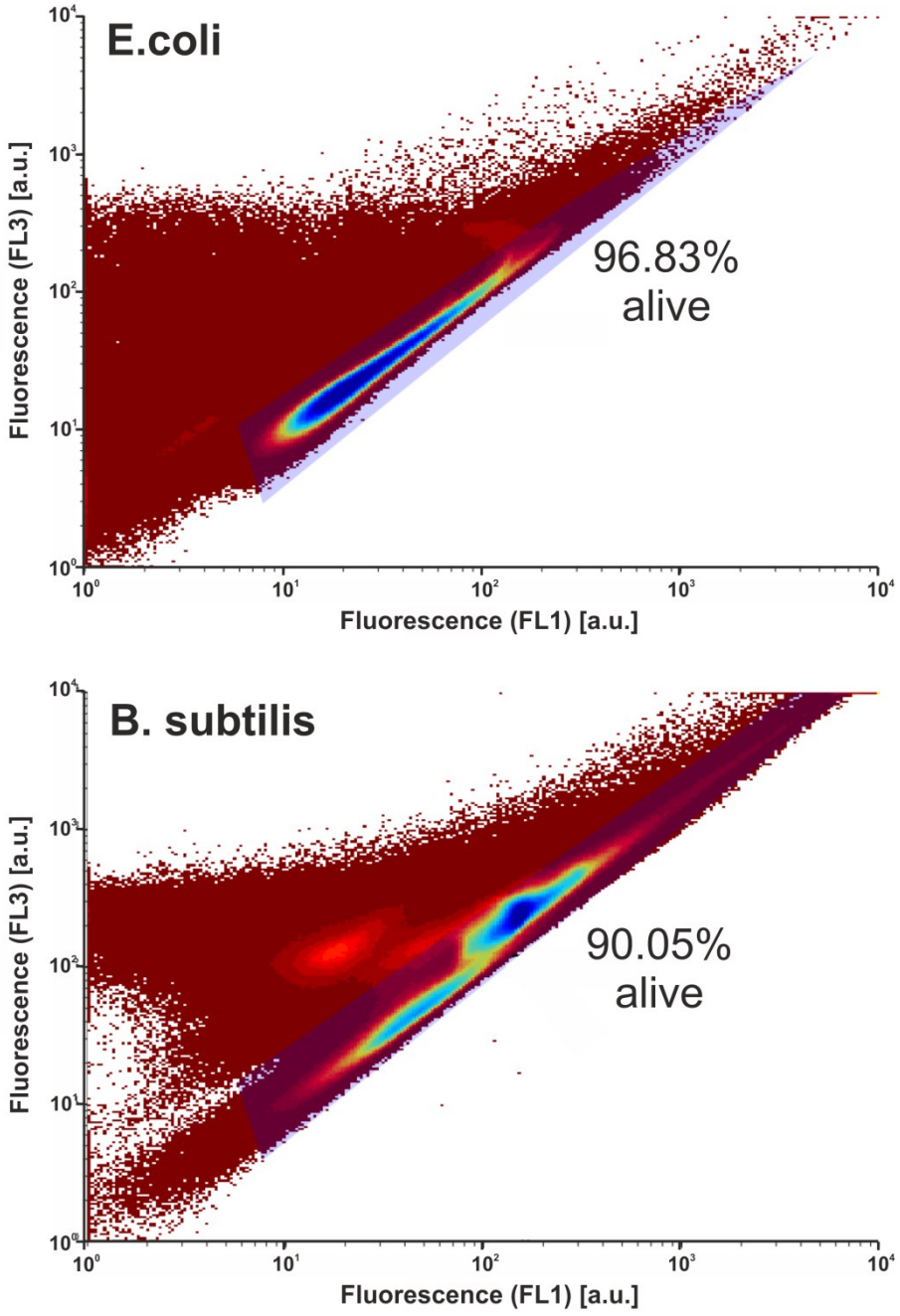

Figure S4: Flow cytometry measurements revealing information about cell viability in solution.

Experimental procedure

After harvesting, 1 ml bacteria suspension was transferred into an Eppendorf tube and stained with syto9 (1:1000) propidium iodide (1:200) to gain information about life and dead cells in

solution, respectively. Cells were incubated 5 min at room temperature, followed by centrifugation for 3 min at 3000 rcf. Bacteria were resuspended in 1 mL PBS, followed by another centrifugation step for 3 min at 3000 rcf. Cells were resuspended in PBS and analysed using a BioRad S3e FACS instrument (standard configuration, 488 nm and 651 nm lasers, autogimbal 100 μm nozzle, BioRad, UK) and BioRad ProSort 1.5 software.