Supplemental Information

Electrochemical Detection of Pyocyanin in Nanochannels with Integrated Palladium Reference Electrodes

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Testing of the finished devices was performed using ferrocene dimethanol in concentrations ranging from 1-1000 µM dissolved in 1 M KCl solution (Figure S1, 1000 µM data removed for clarity). The test solutions were scanned from 0 to 0.5 V versus a Ag/AgCl (1 M KCl) reference electrode using cyclic voltammetry. The PDMS well was washed three times with blank KCl solution between each test to ensure no cross contamination (results not shown). For these tests, only the gold working electrode was utilized as a means of establishing that the device was functioning. Ferrocene dimethanol was chosen because its electrochemistry has been widely studied. The half wave potential or the point where the current is half of the limiting current is shown in Figure S1 to be about 250 mV which matches values reported in literature.

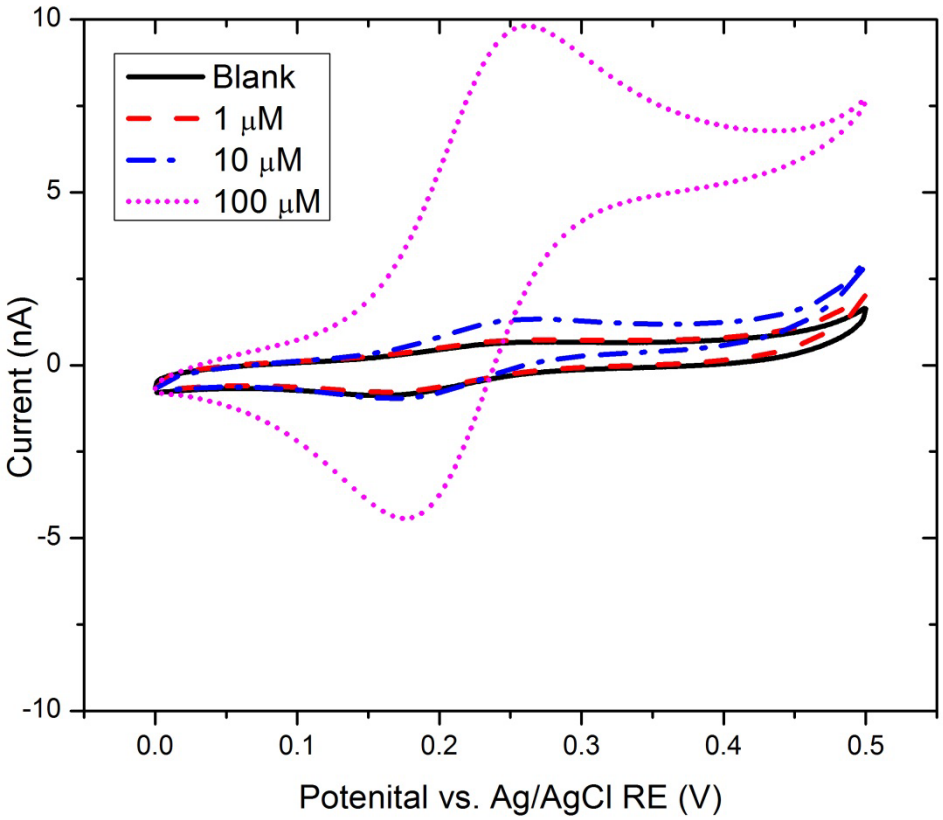

Figure S1: Cyclic voltammograms of 1-100 µM ferrocene dimethanol in 1 M KCl solution. Scan rate of 0.050 V/s from 0 to 0.750 V using a Ag/AgCl reference electrode.

In order to determine the stability of the constructed reference electrode with time, differential pulse voltammograms of ferrocene dimethanol were measured at different time intervals. Over a 4 day period of measurements the oxidation potential of ferrocene dimethanol vs. the PdH Re was found to be approximately 28 mV with a standard error between the measurements of only 2.2 mV. The expected oxidation potential is approximately 220 mV vs. a Ag/AgCl reference electrode. The measured potential difference is approximately 190 mV which agrees with the measured potential difference seen in pyocyanin measurements.

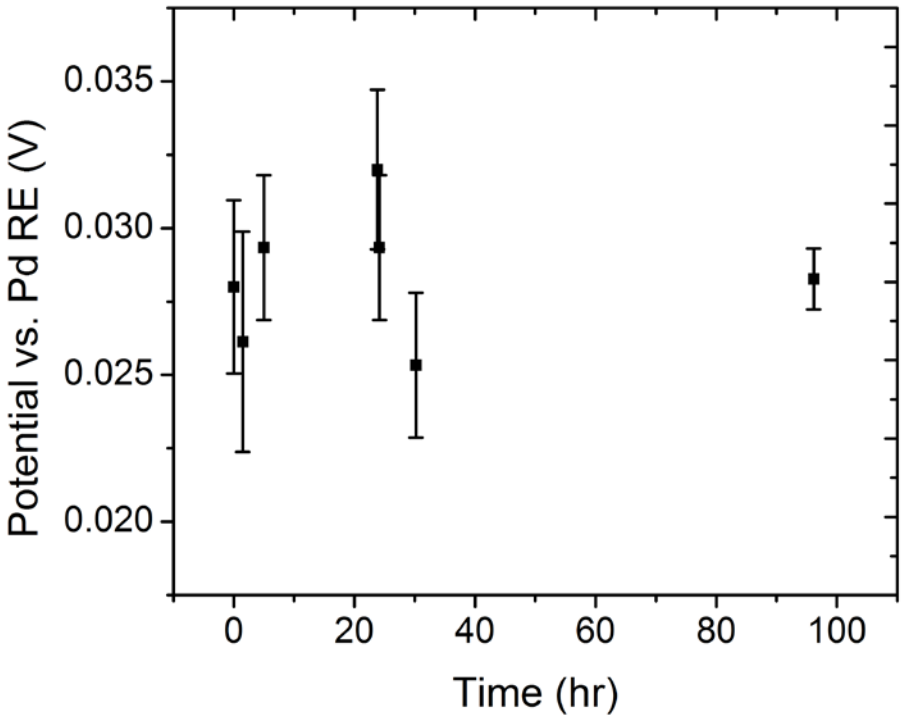

Figure S2: Oxidation potential of ferrocene dimethanol vs. a semi-charged PdH Re

The measurements in Figure 3 compare the detection of pyocyanin using the gold working electrode inside of a microfabricated NEA. In the first case, an external, commercially available Ag/AgCl (1M KCl) Reference Electrode is used. In the second case, the microfabricated palladium hydride reference electrode inside the NEA was used. In Figure S3, two different NEA devices are compared. The data in the graph on the left side of Figure S3 was obtained using a NEA with a gold working electrode and a semi-charged PdH reference electrode. The data in the right graph of Figure S3 was obtained using NEA with both a gold working electrode and an integrated gold reference electrode. The bottom electrode was used as the reference and the top was used as the working electrode. A platinum wire was used as a counter electrode in all cases.

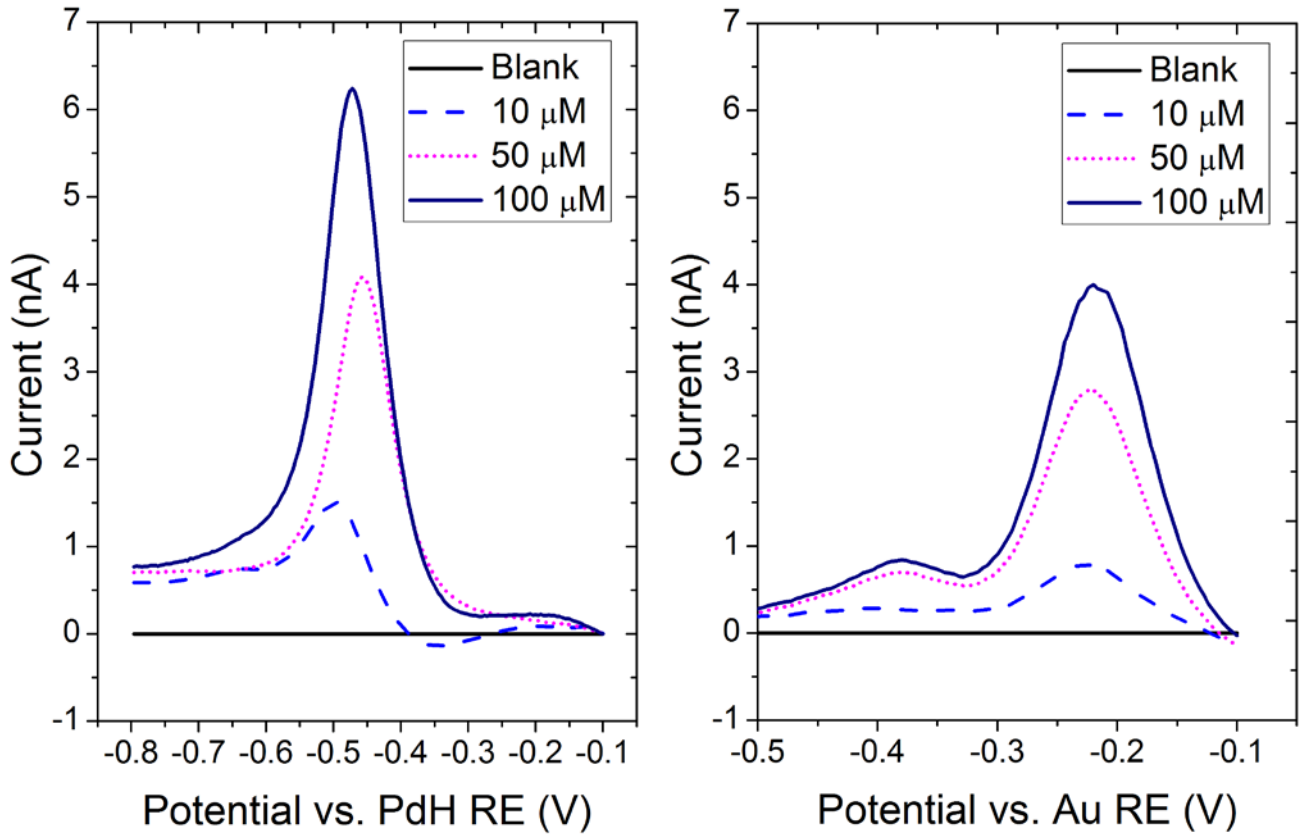

Figure S3: A) Differential pulse voltammograms of 0 - 100 µM pyocyanin in 100 mM PB from -0.800 to -0.100 V vs. PdH reference electrode. B) Differential pulse voltammograms of 0 - 100 µM pyocyanin in 100 mM PB from -0.500 to -0.100 V vs. Au reference electrode. All scans were performed at an amplitude voltage of 0.05 volts and a frequency of 15 Hz.

A significant shift in the oxidation potential was observed when pyocyanin was dissolved in phosphate buffer versus pyocyanin produced by wild type *Pseudomonas aeruginosa* in trypticase soy broth when using a Pd reference electrode. The shift in oxidation potential that was observed was believed to be linked to components in the trypticase soy broth. Scans of 100 µM pyocyanin in phosphate buffer and trypticase soy broth were performed. Systematically changing the composition of the solution revealed a gradual shift in oxidation potential for pyocyanin as shown in Figure S2. All cell culture experiments were performed with trypticase at a concentration of 30 g/L accounting for the observed peak shift. The underlying cause is not yet understood, but it is believed that one or more of the chemicals present in the trypticase soy broth interact with the palladium electrode. Given the data presented in Figure 2D in the main text, it can be stated that the addition of NaCl, one of the main components of tyrpticase soy broth, is not the cause the observed shift.

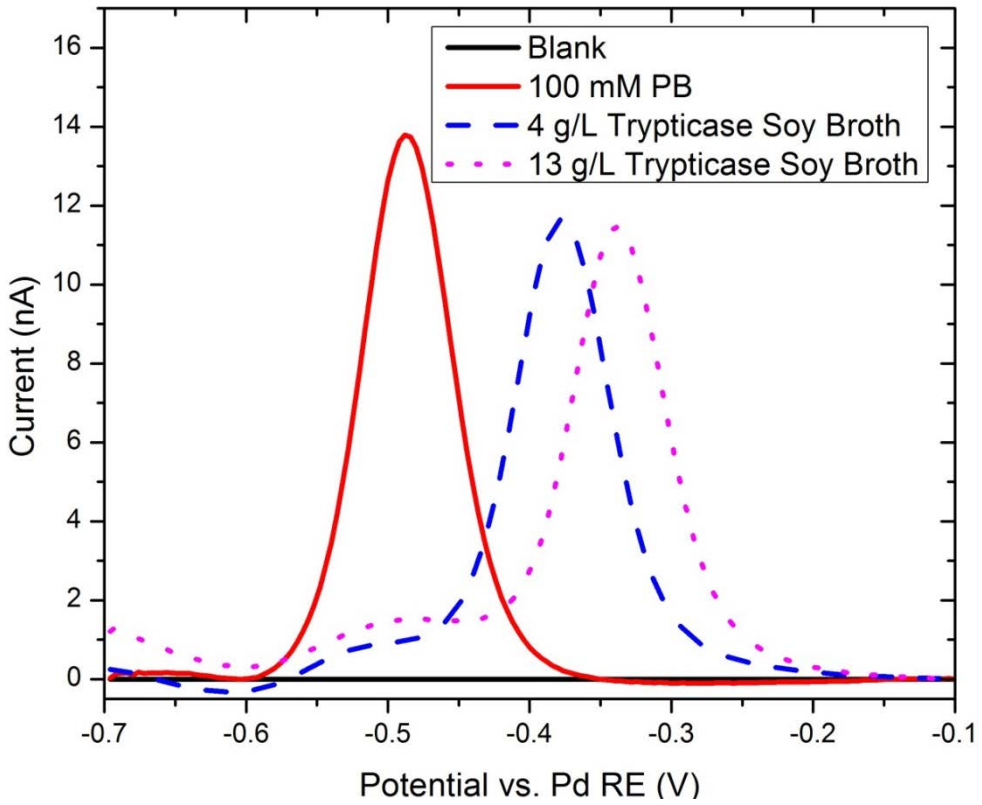

Figure S4: Differential pulse voltammograms of 100 µM pyocyanin in Phosphate Buffer (Red Line) and in 4 g/L (Blue Dashed Line) and 13 g/L (Pink Dotted Line) Trypticase Soy Broth. Scanned at an amplitude voltage of 0.05 V and a frequency of 15 Hz.

A goal of the paper is to selectively detect the production of pyocyanin by *Pseudomonas aeruginosa* . As such, mutant strains that were incapable of producing pyocyanin were used as controls. A peak was observed in the selected scan range for all strains. In the case of phzS, the size of the peak was significantly smaller than the pyocyanin peak produced by the wild type strain throughout the duration of the experiments and its maximum was shifted approximately 0.075 V. The strain *phz*S should be incapable of making pyocyanin, and from figure S2 it appears that the redox molecule detected around -0.33 V is not pyocyanin but a derivative or precursor. Furthermore, as more positive potentials are scanned another peak appears which is not present in the WT culture at -0.1 V (data not shown). Mixtures of several different phenazines known to be produced by *Pseudomonas aeruginosa* will be scanned in the future to confirm the above results. Similar results were obtained for the *phz*M strain.

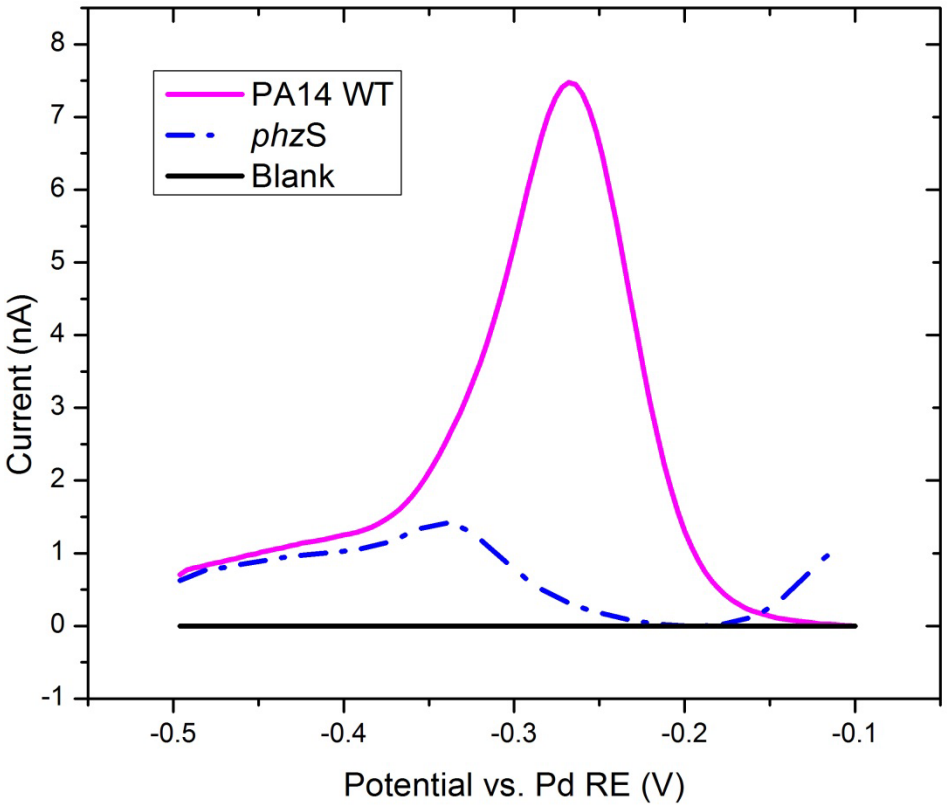

Figure S5: Differential pulse voltammogram of wild type PA14 and *phz*S after 6 days of growth at 37 °C. Scans were performed from -0.5 to -0.1 V at an amplitude voltage of 0.05 V and a frequency of 15 Hz.

A shift of only 0.020 V was observed in the oxidation peak of pyocyanin produced by wild type bacteria over the course of 6 days.

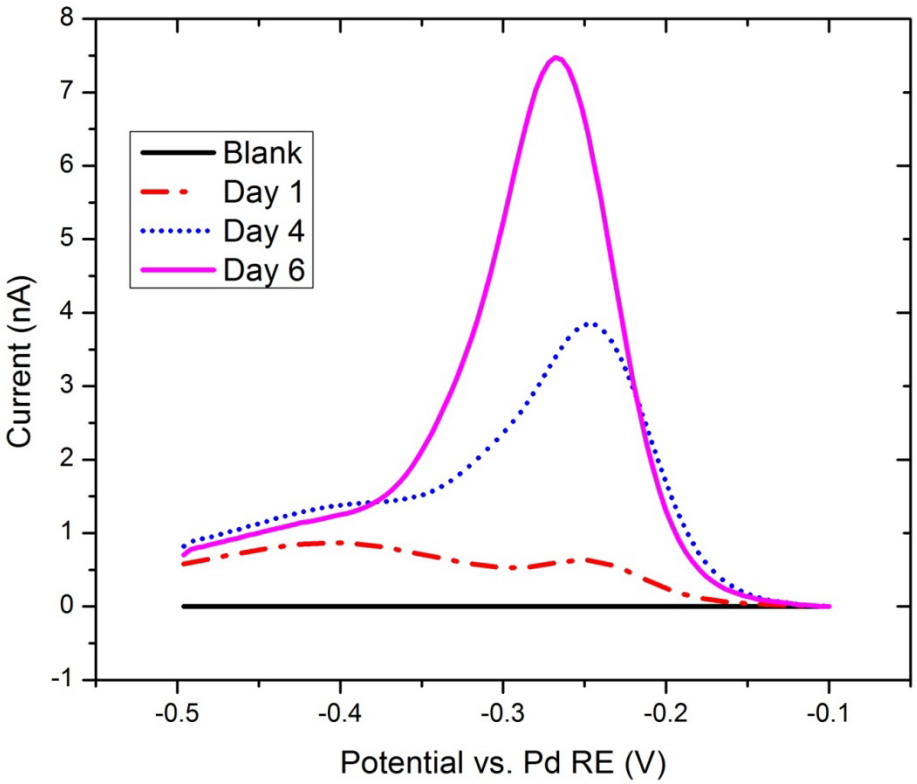

Figure S6: Differential pulse voltammograms of PA14 wild type supernatants over a 6 day period. Scans were performed from -0.5 to -0.1 V, at an amplitude voltage of 0.05 volts and a frequency of 15 Hz.