Supplementary Information

Up-Regulating Pyocyanin Production by Amino Acid Addition for Early Electrochemical Identification of *Pseudomonas aeruginosa*

Hunter J. Sismaet, Thaddaeus A. Webster, Edgar D. Goluch*

Department of Chemical Engineering, Northeastern University, 360 Huntington Ave, 313 Snell Engineering, Boston, MA 02115 USA
* Corresponding Author: E.D.G., e.goluch@neu.edu, +1-617-373-3500

S1. Calibration curves for pyocyanin detection in M63 and TSB media

Known concentrations of pyocyanin (N-methylphenazin-1-one, CAS #85-66-5, Cayman Chemicals Cat. #10009594) were spiked in either M63 minimal media or trypticase soy broth to create a calibration curve. Concentrations of pyocyanin ranged from 0-50 μM. For each concentration, 100 μL of sample was spotted onto a disposable screen-printed carbon electrode connected to an external Ag/AgCl reference. Square-wave voltammetry was used to measure the electrochemical response of pyocyanin, scanning from −0.4 to 0.0 V at an amplitude voltage of 0.050 V and a frequency of 15 Hz. The sensitivity and limit of detection were calculated for each media type and are reported in Table S1. The sensitivity is the slope of the line calculated from the plot of the maximum current vs. concentration while the LOD was calculated as 3σ/sensitivity, where σ is the standard deviation of the blank solution current at the pyocyanin oxidation potential.

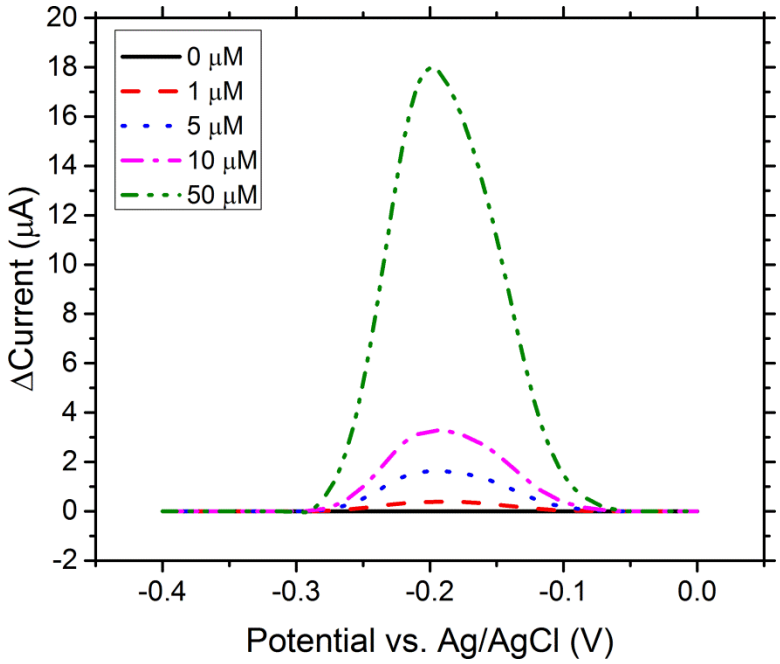

Fig. S1. Square-wave voltammograms of 0-50 μM pyocyanin in M63 minimal media.

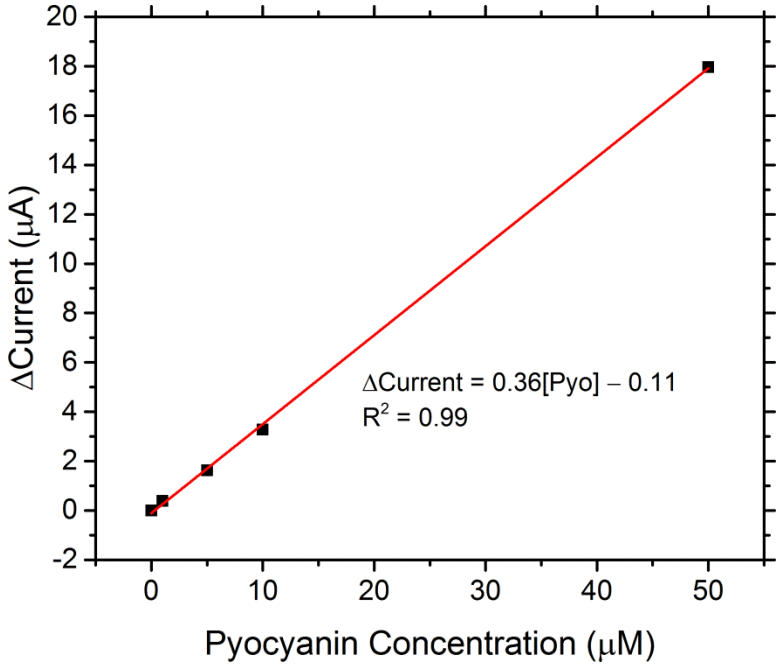

Fig. S2. Linear fit of 0-50 μM pyocyanin in M63 minimal media.

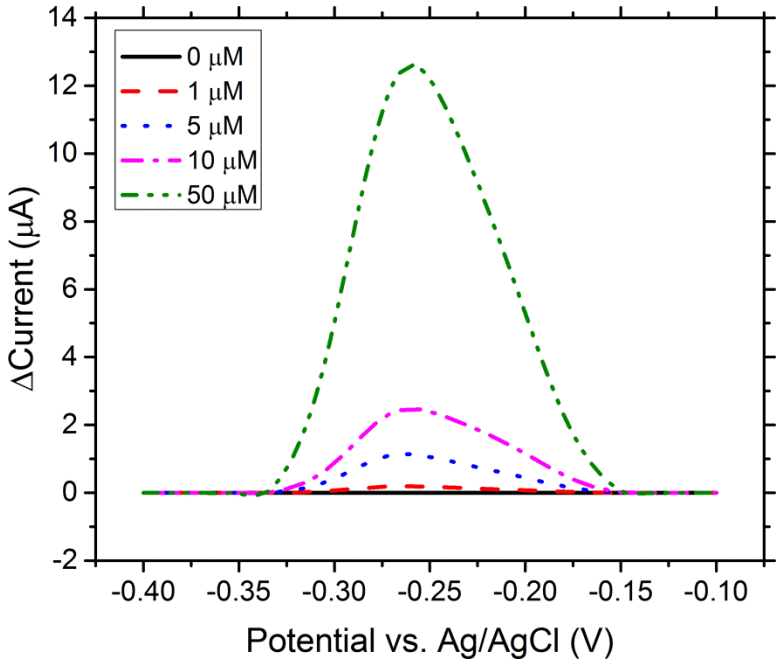

Fig. S3. Square-wave voltammograms of 0-50 µM pyocyanin in trypticase soy broth.

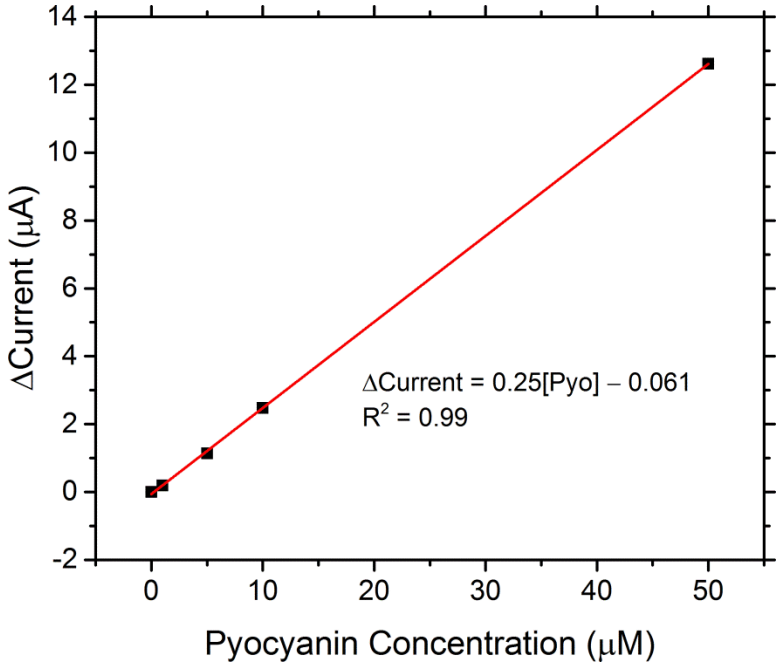

Fig. S4. Linear fit of 0-50 µM pyocyanin in trypticase soy broth.

Table S1. Pyocyanin sensitivity and limit of detection (LOD) for M63 and TSB.

Media Type	Sensitivity (µA/µM)	LOD (µM)	R^2
M63 Minimal Media	0.36	0.042	0.99
Trypticase Soy Broth	0.25	0.048	0.99

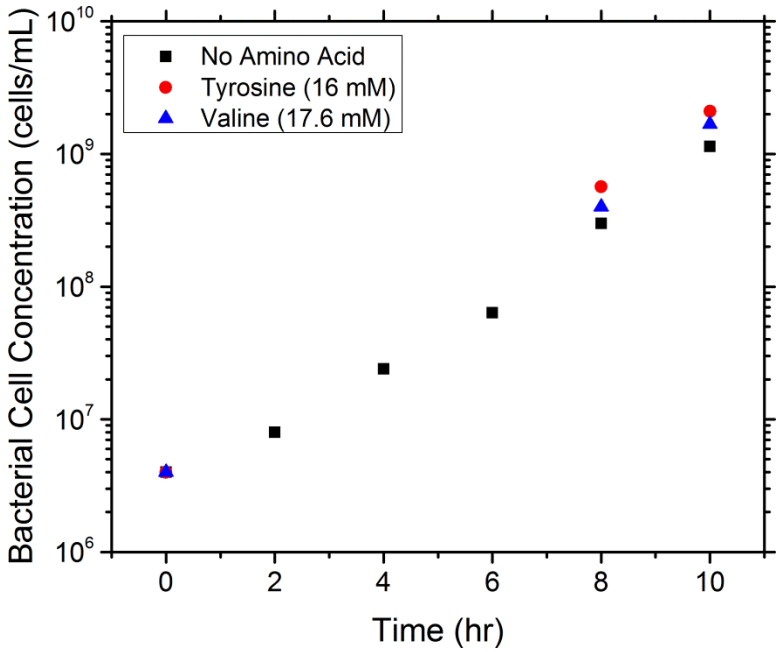

Fig. S5. Monitoring bacterial cell growth over the course of 10 hours in trypticase soy broth (TSB). A hemocytometer was used to determine cell density.

S2. Electrochemical detection of *P. aeruginosa* in M63 and TSB media

Pseudomonas aeruginosa was inoculated in either M63 minimal media or trypticase soy broth (TSB) in the presence of individual amino acids. These solutions were incubated over the course of a day with data points taken roughly every two hours. For each time point, 100 µL of sample was spotted onto a disposable screen-printed carbon electrode connected to an external Ag/AgCl reference. Square-wave voltammetry was used to measure the electrochemical response of pyocyanin production, scanning from −0.4 to −0.1 V at an amplitude voltage of 0.050 V and a frequency of 15 Hz. In the initial set of experiments, amino acids were used at the following concentrations (mM): proline (27.2), histidine (8), arginine (4.8), leucine (25.6), tyrosine (3.2) and valine (17.6). Figures S6 through S19 show results with these concentrations of amino acids in the respective growth media. We also tested each of the amino acids at a 5 mM concentration in TSB, as shown in Fig. S20.

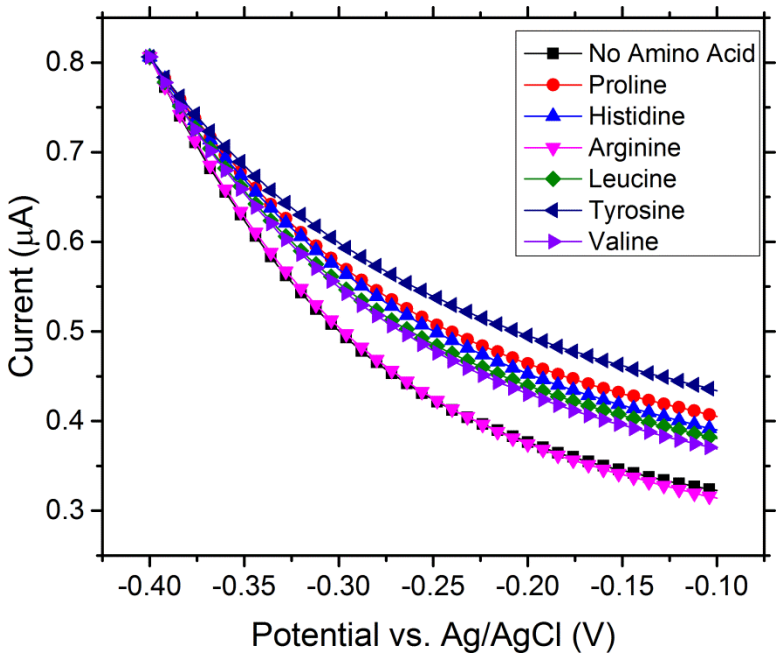

Fig. S6. Square-wave voltammograms of *P. aeruginosa* grown for 0 hours in the presence of individual amino acids in M63 minimal media.

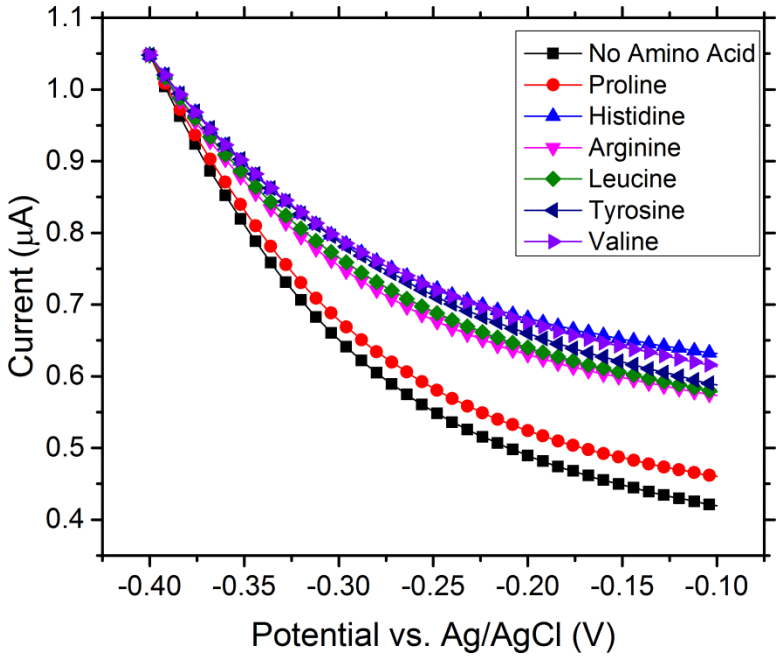

Fig. S7. Square-wave voltammograms of *P. aeruginosa* grown for 2 hours in the presence of individual amino acids in M63 minimal media.

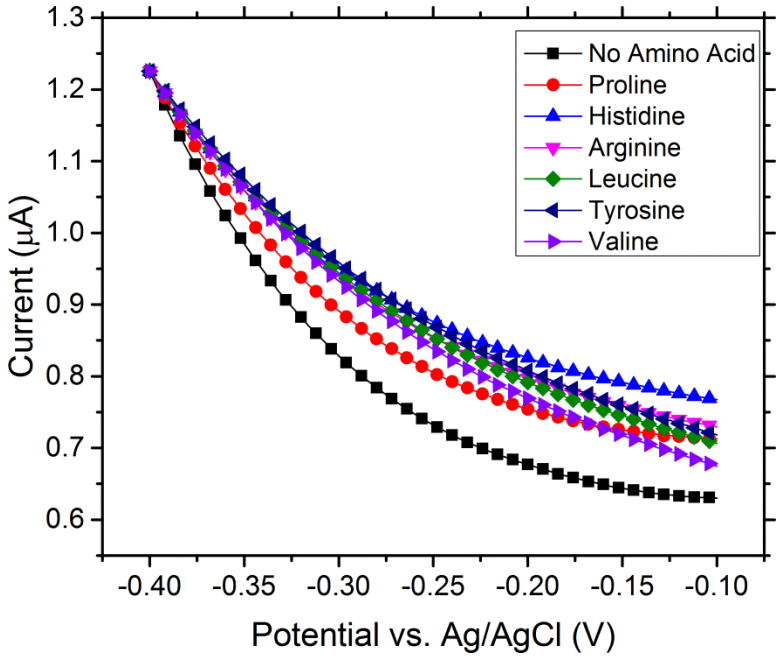

Fig. S8. Square-wave voltammograms of *P. aeruginosa* grown for 4 hours in the presence of individual amino acids in M63 minimal media.

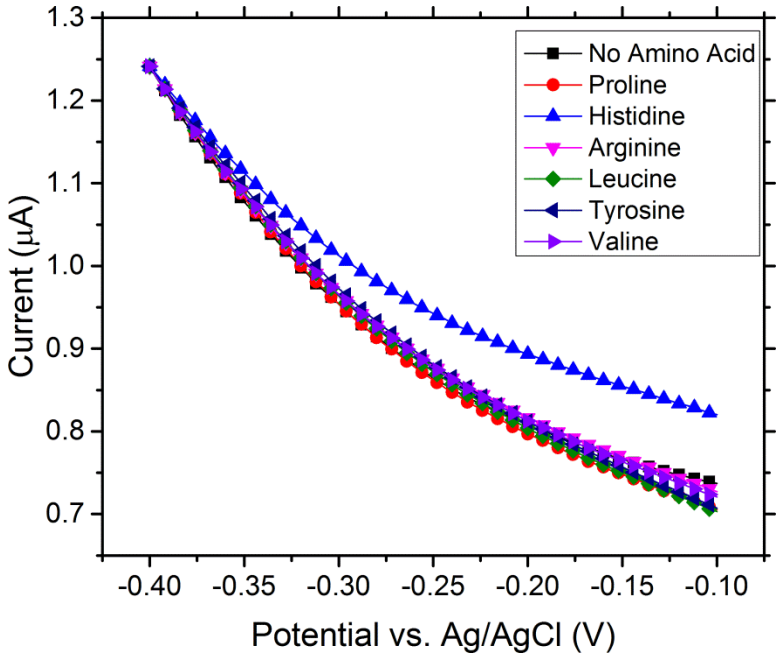

Fig. S9. Square-wave voltammograms of *P. aeruginosa* grown for 8 hours in the presence of individual amino acids in M63 minimal media.

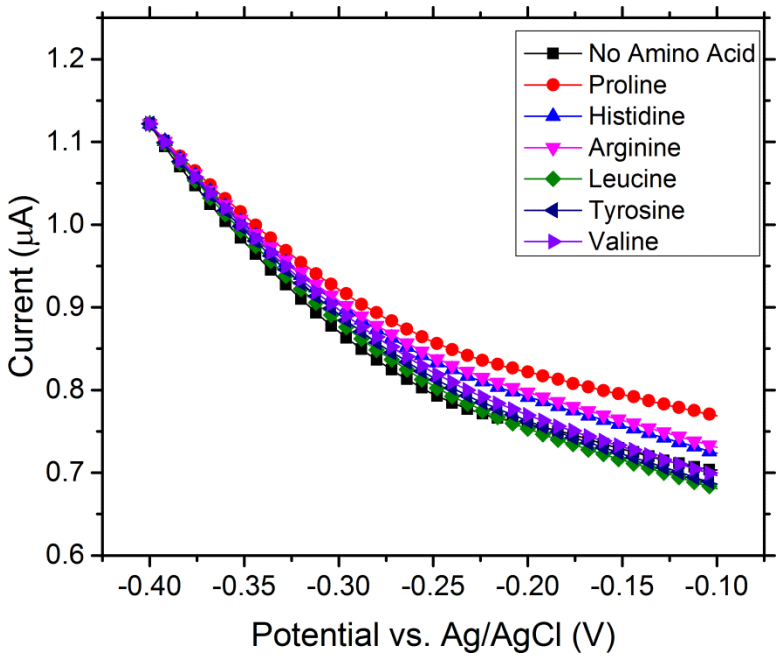

Fig. S10. Square-wave voltammograms of *P. aeruginosa* grown for 12 hours in the presence of individual amino acids in M63 minimal media.

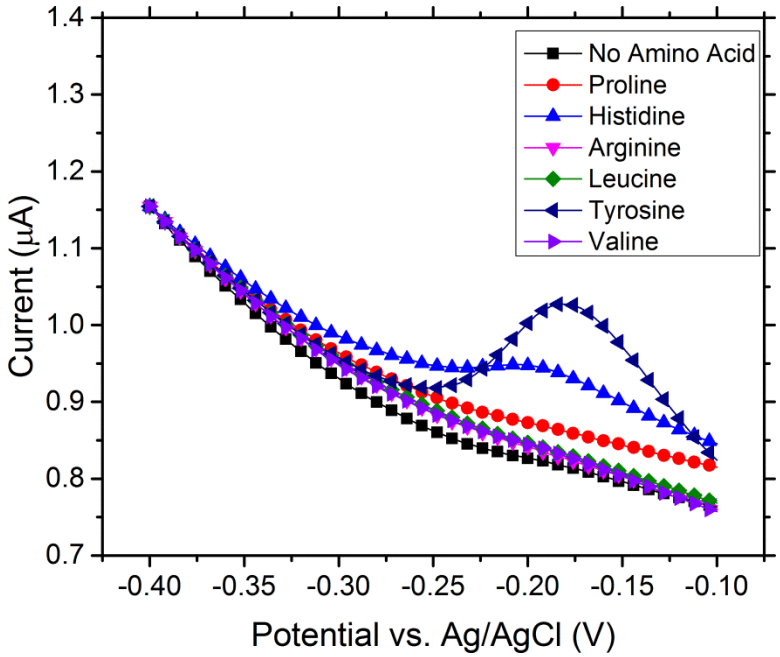

Fig. S11. Square-wave voltammograms of *P. aeruginosa* grown for 21 hours in the presence of individual amino acids in M63 minimal media.

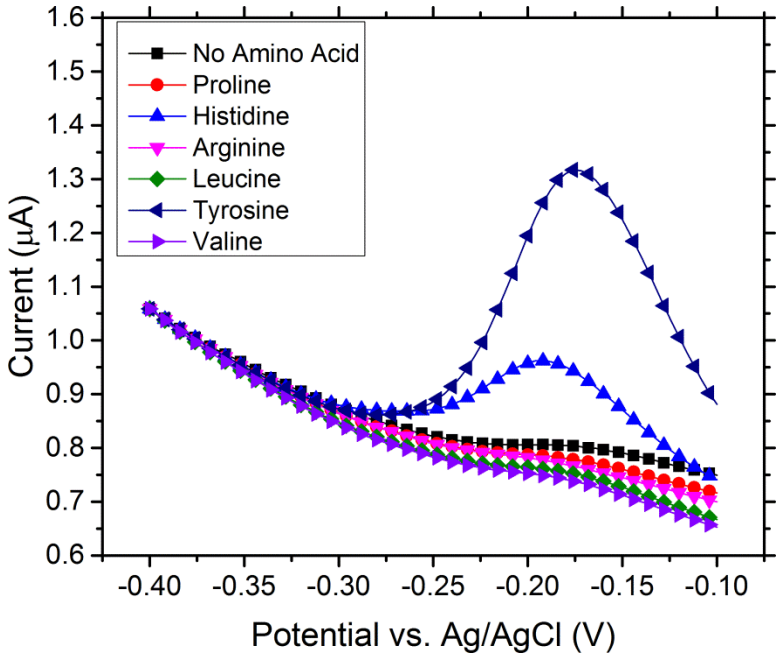

Fig. S12. Square-wave voltammograms of *P. aeruginosa* grown for 24 hours in the presence of individual amino acids in M63 minimal media.

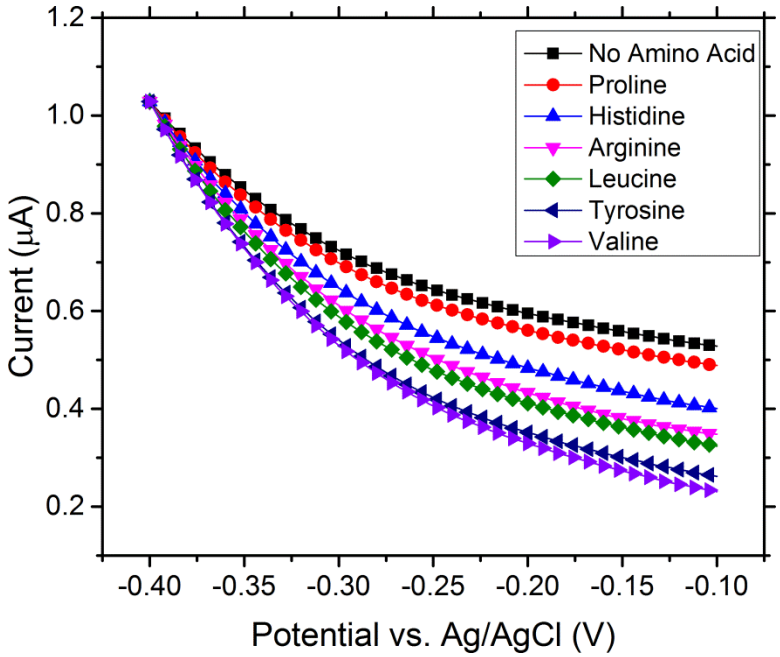

Fig. S13. Square-wave voltammograms of *P. aeruginosa* grown for 0 hours in the presence of individual amino acids in trypticase soy broth.

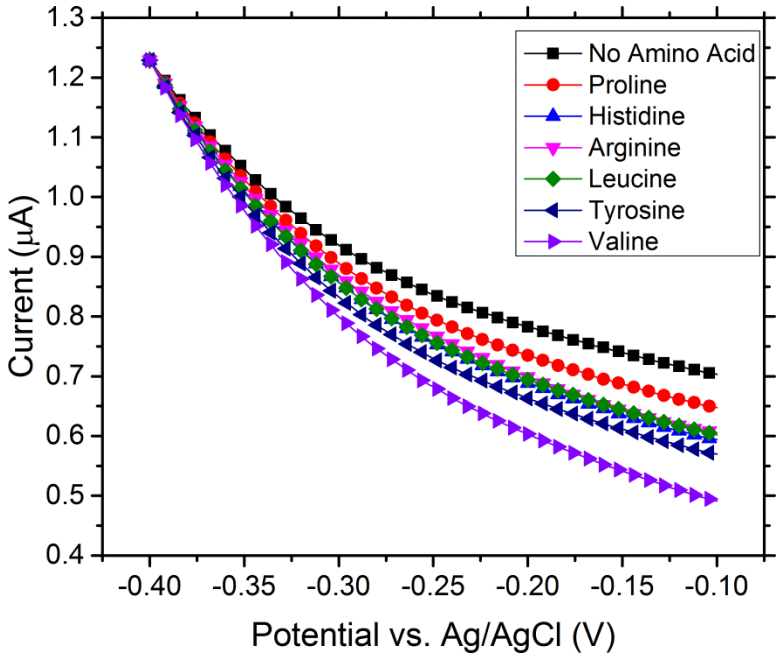

Fig. S14. Square-wave voltammograms of *P. aeruginosa* grown for 2 hours in the presence of individual amino acids in trypticase soy broth.

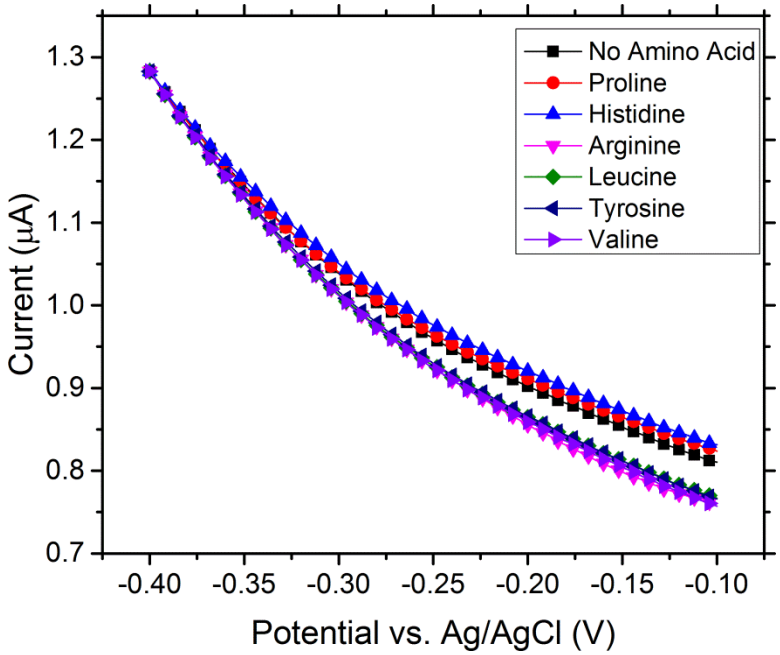

Fig. S15. Square-wave voltammograms of *P. aeruginosa* grown for 4 hours in the presence of individual amino acids in trypticase soy broth.

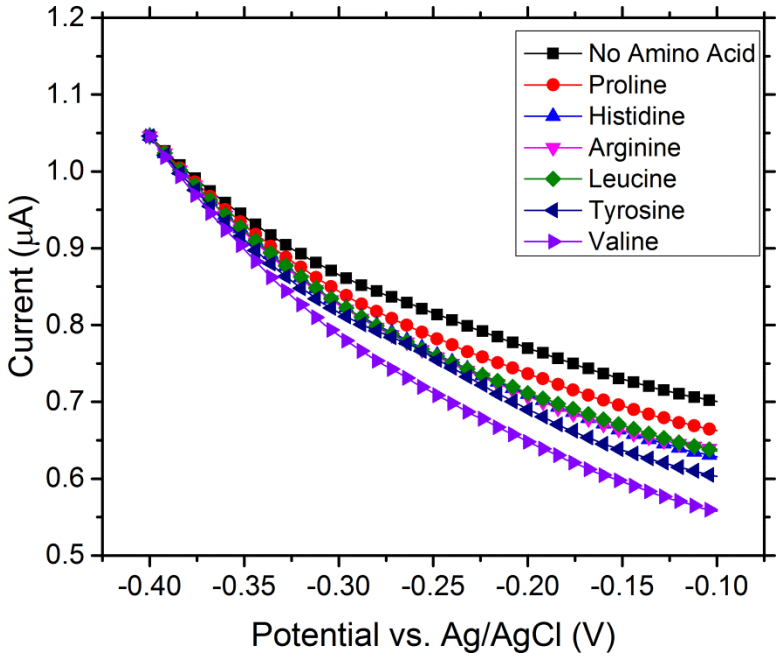

Fig. S16. Square-wave voltammograms of *P. aeruginosa* grown for 8 hours in the presence of individual amino acids in trypticase soy broth.

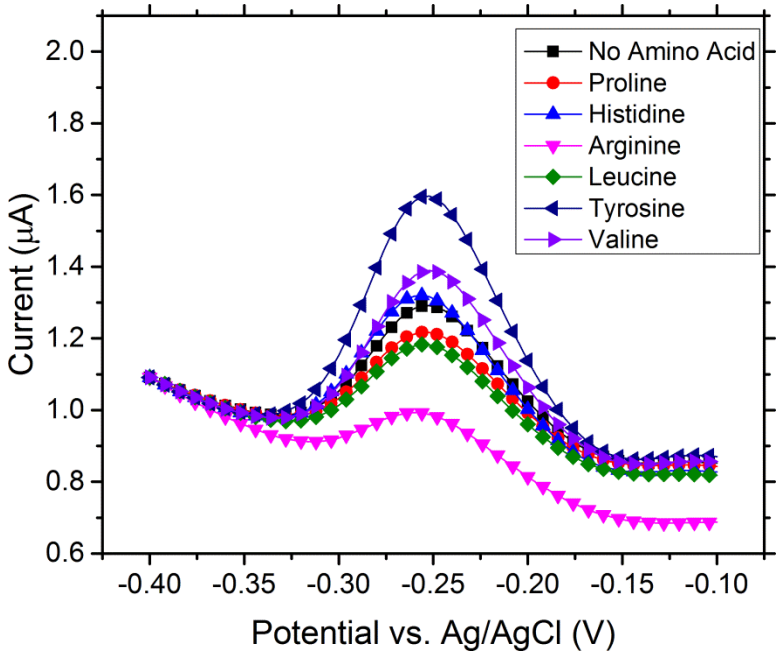

Fig. S17. Square-wave voltammograms of *P. aeruginosa* grown for 12 hours in the presence of individual amino acids in trypticase soy broth.

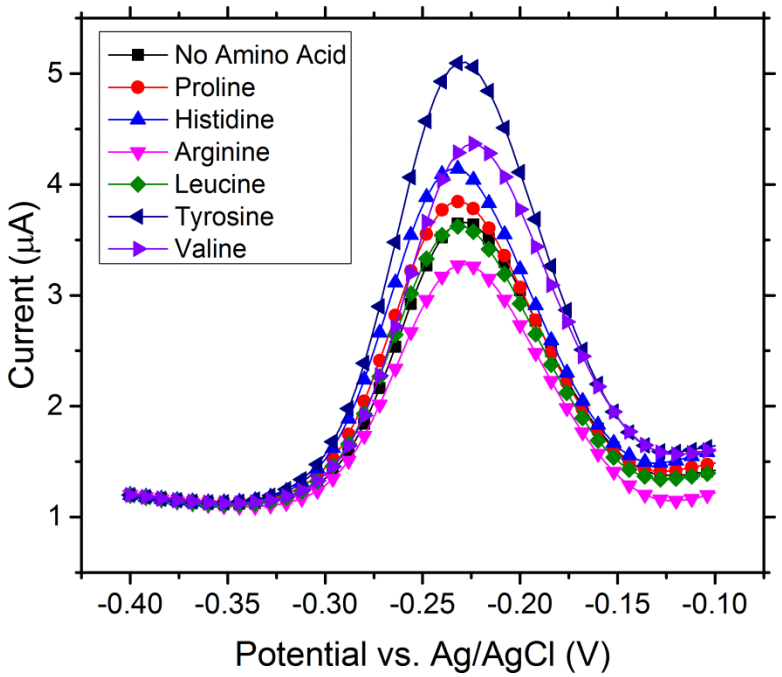

Fig. S18. Square-wave voltammograms of *P. aeruginosa* grown for 21 hours in the presence of individual amino acids in trypticase soy broth.

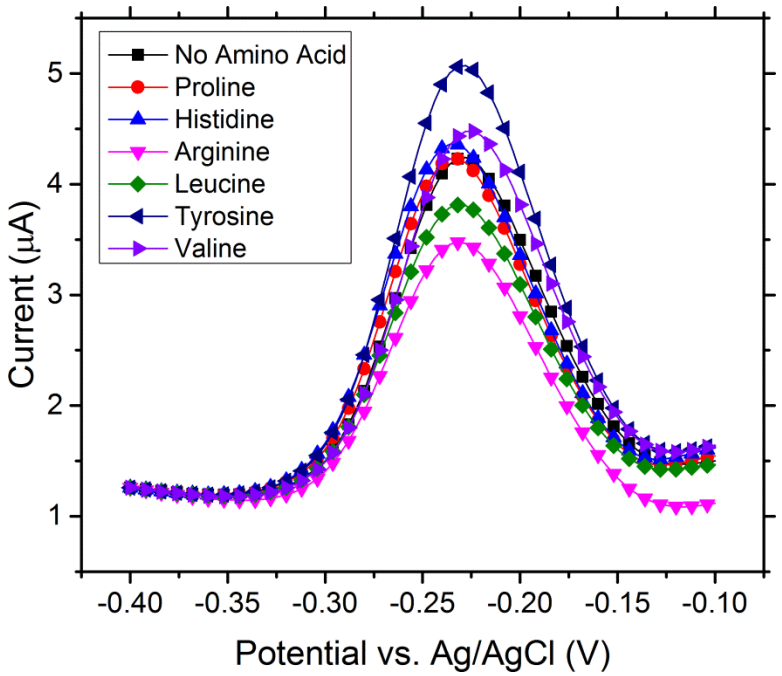

Fig. S19. Square-wave voltammograms of *P. aeruginosa* grown for 24 hours in the presence of individual amino acids in trypticase soy broth.

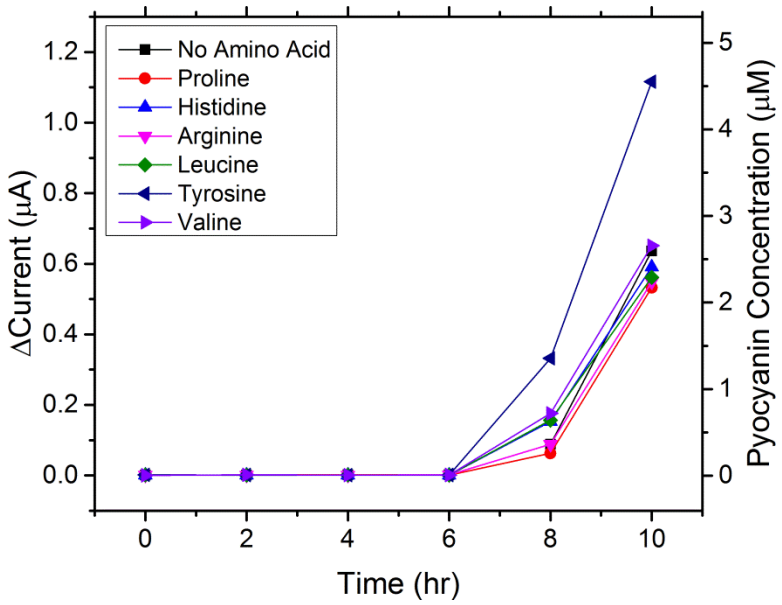

Fig. S20. Monitoring *P. aeruginosa*'s production of pyocyanin over the course of 10 hours in TSB cultures containing individual amino acids. Final concentrations of each amino acid was 5 mM.

S3. Electrochemical detection of *P. aeruginosa* by varying tyrosine and valine concentration

Pseudomonas aeruginosa was inoculated in trypticase soy broth liquid media in the presence of either tyrosine or valine. These solutions were incubated over the course of 10 hours with data points taken every two hours. For each time point, 100 µL of sample was spotted onto a disposable screen-printed carbon electrode connected to an external Ag/AgCl reference. Square-wave voltammetry was used to measure the electrochemical response of pyocyanin production, scanning from −0.4 to −0.1 V at an amplitude voltage of 0.050 V and a frequency of 15 Hz. The two amino acids were created at various concentrations ranging from those quantified in typical cystic fibrosis infection levels (tyrosine: 0.2 mM, valine: 1.1 mM) all the way to an 80-fold increase.

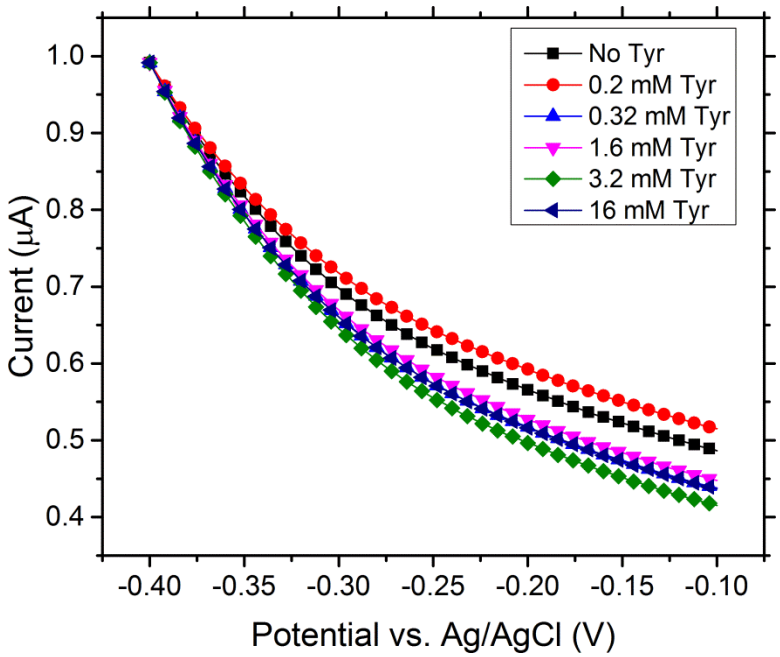

Fig. S21. Square-wave voltammograms of *P. aeruginosa* grown in trypticase soy broth for 0 hours in the presence of tyrosine at various concentrations.

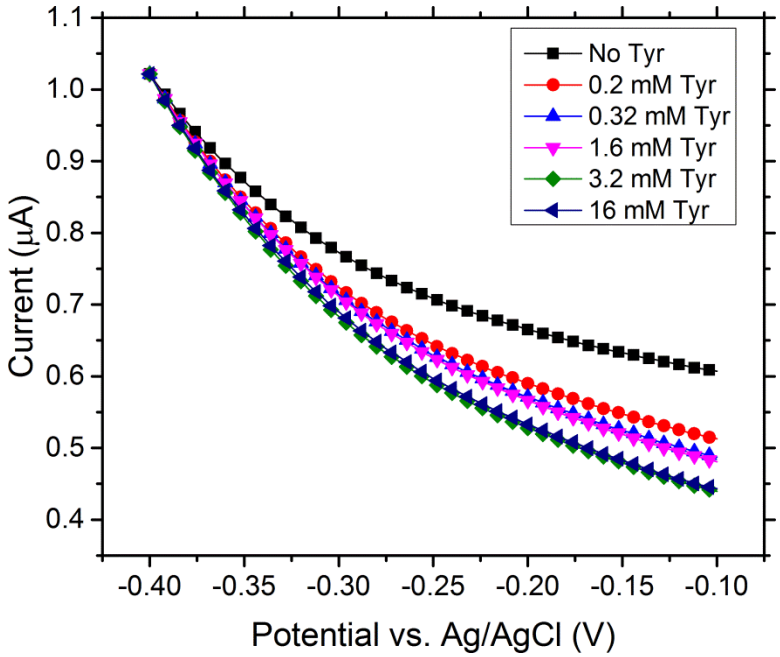

Fig. S22. Square-wave voltammograms of *P. aeruginosa* grown in trypticase soy broth for 2 hours in the presence of tyrosine at various concentrations.

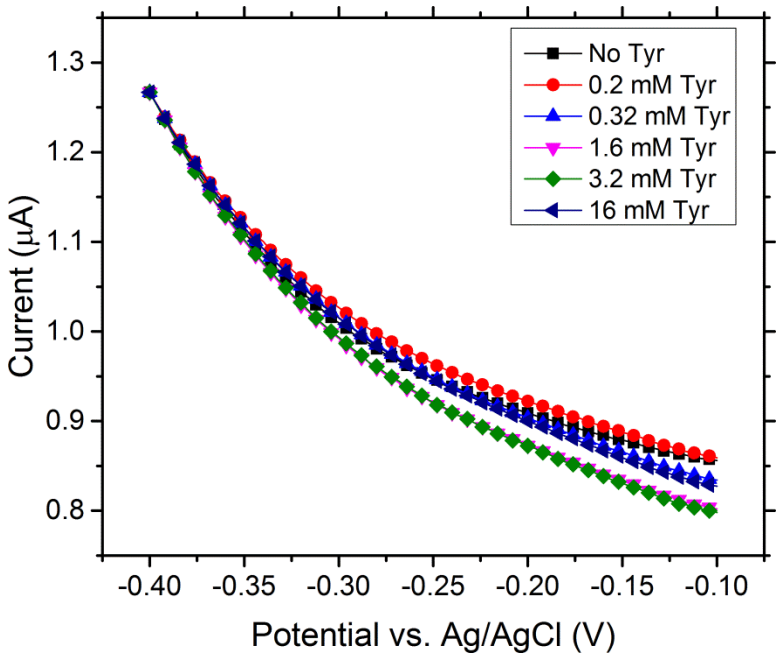

Fig. S23. Square-wave voltammograms of *P. aeruginosa* grown in trypticase soy broth for 4 hours in the presence of tyrosine at various concentrations.

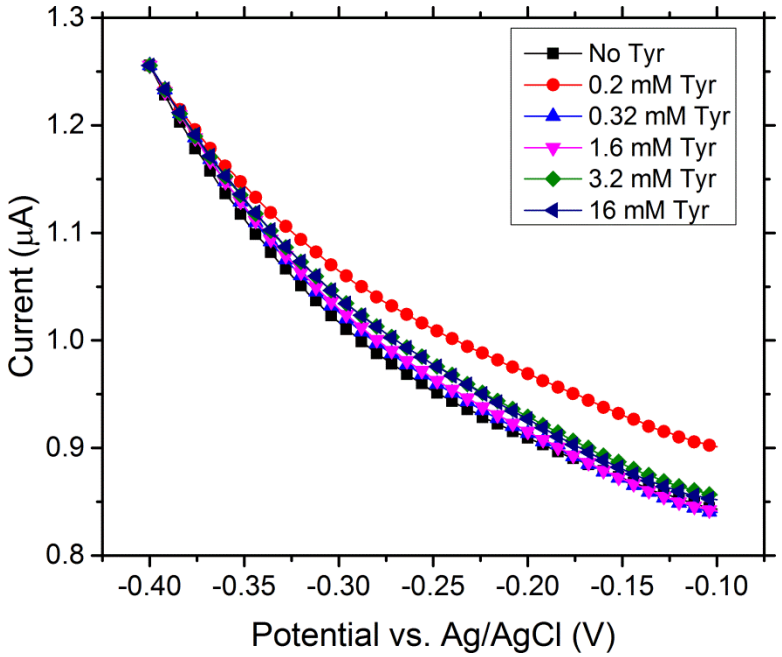

Fig. S24. Square-wave voltammograms of *P. aeruginosa* grown in trypticase soy broth for 6 hours in the presence of tyrosine at various concentrations.

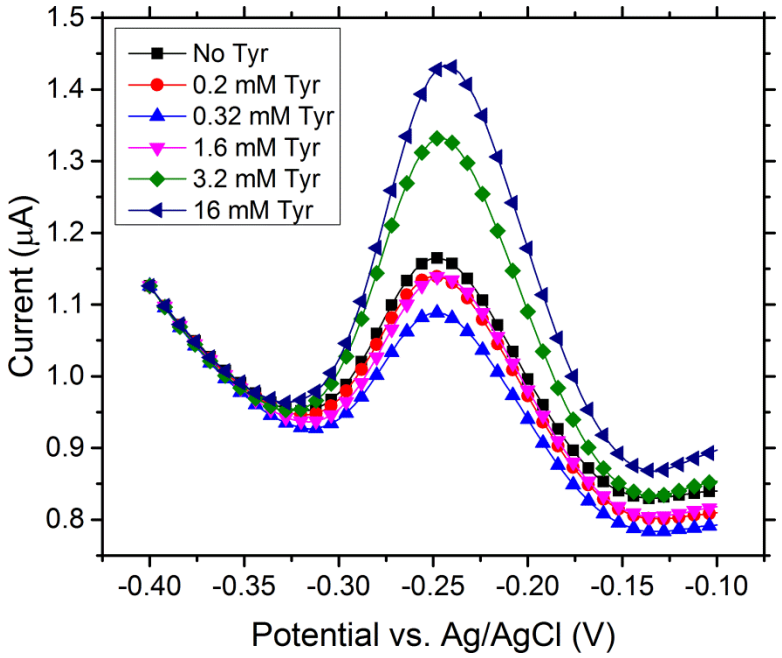

Fig. S25. Square-wave voltammograms of *P. aeruginosa* grown in trypticase soy broth for 10 hours in the presence of tyrosine at various concentrations.

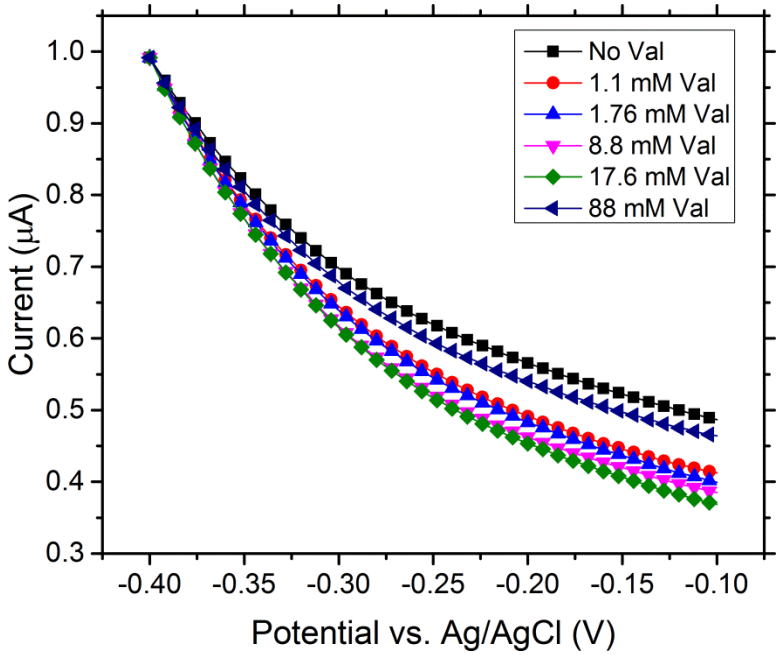

Fig. S26. Square-wave voltammograms of *P. aeruginosa* grown in trypticase soy broth for 0 hours in the presence of valine at various concentrations.

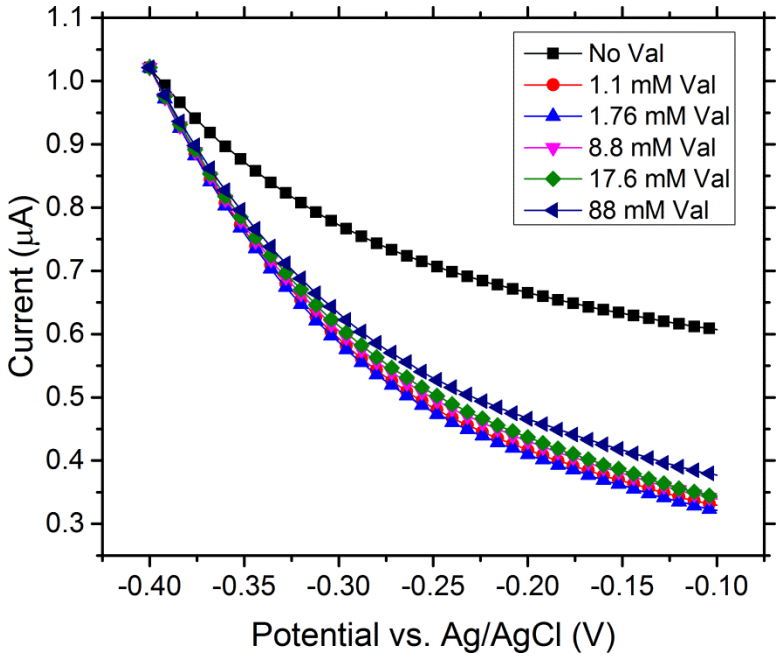

Fig. S27. Square-wave voltammograms of *P. aeruginosa* grown in trypticase soy broth for 2 hours in the presence of valine at various concentrations.

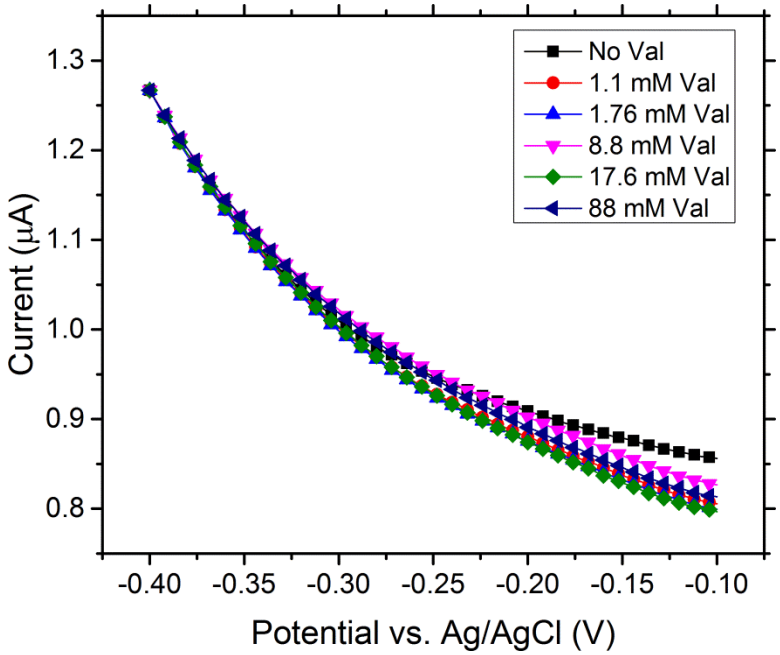

Fig. S28. Square-wave voltammograms of *P. aeruginosa* grown in trypticase soy broth for 4 hours in the presence of valine at various concentrations.

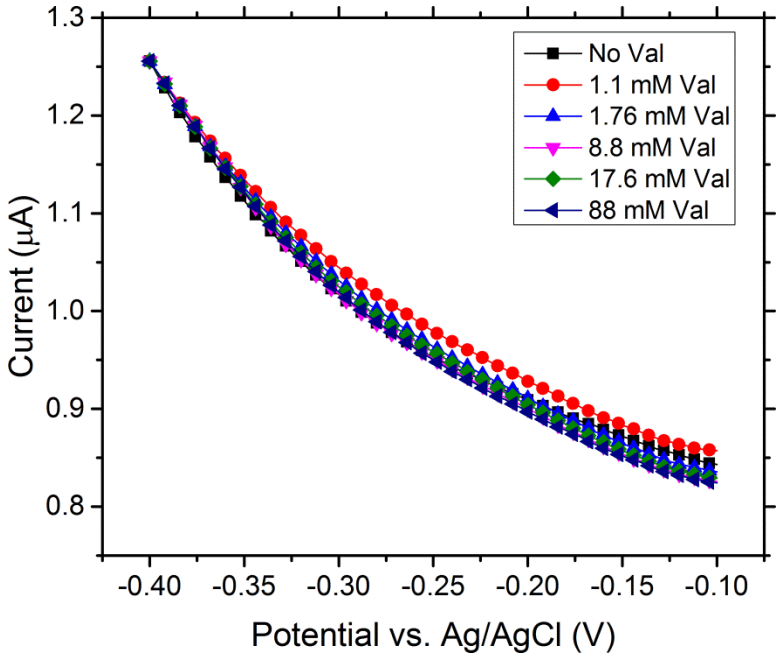

Fig. S29. Square-wave voltammograms of *P. aeruginosa* grown in trypticase soy broth for 6 hours in the presence of valine at various concentrations.

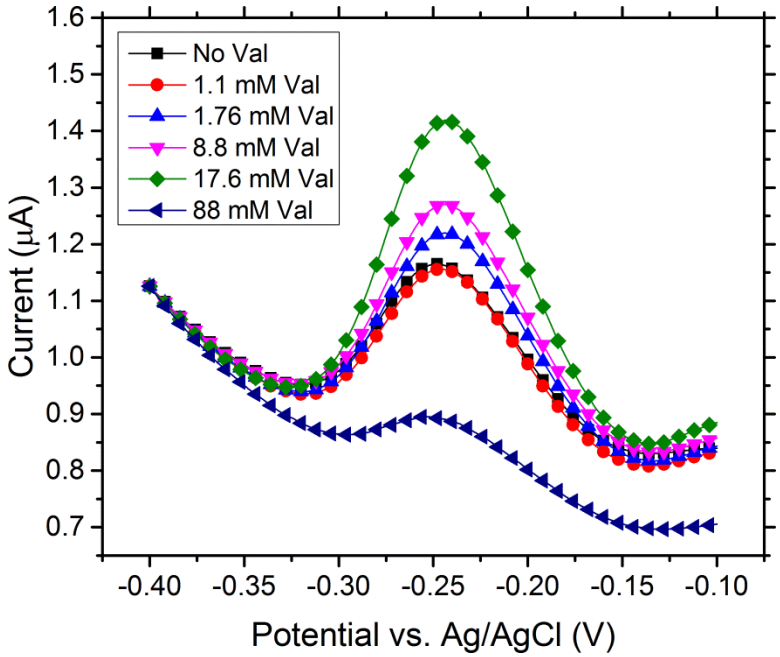

Fig. S30. Square-wave voltammograms of *P. aeruginosa* grown in trypticase soy broth for 10 hours in the presence of valine at various concentrations.

S4. Electrochemical detection of *P. aeruginosa* by varying initial bacterial concentration

Pseudomonas aeruginosa was inoculated in trypticase soy broth liquid media in the presence of either tyrosine (16 mM), valine (17.6 mM), or a combination of both amino acids at those respective concentrations. The initial concentration of *P. aeruginosa* inoculated in the liquid samples was varied (4, 20, 40, and 400 million cells per mL). Two control experiments were also included: one with an initial *P. aeruginosa* concentration of 4 million cells per mL without additional amino acids, and the second with the amino acids added but no bacteria. These solutions were incubated over the course of 10 hours with data points taken every 2 hours. For each time point, 100 µL of sample was spotted onto a disposable screen-printed carbon electrode connected to an external Ag/AgCl reference. Square-wave voltammetry was used to measure the electrochemical response of pyocyanin production, scanning from −0.4 to −0.1 V at an amplitude voltage of 0.050 V and a frequency of 15 Hz.

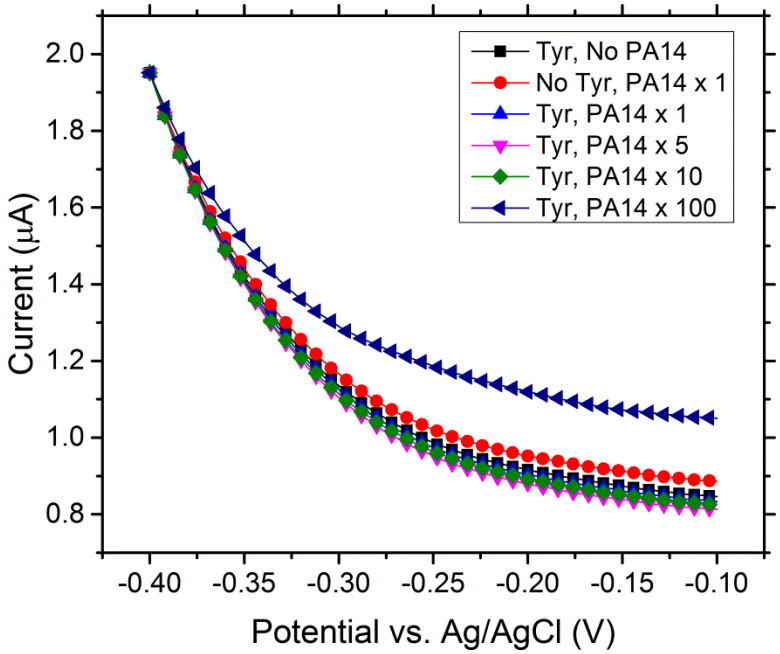

Fig. S31. Square-wave voltammograms of *P. aeruginosa* grown in trypticase soy broth for 0 hours in the presence of tyrosine (16 mM). *P. aeruginosa* was used at the following concentrations (million cells per mL): 4, 20, 40, and 400 respectively.

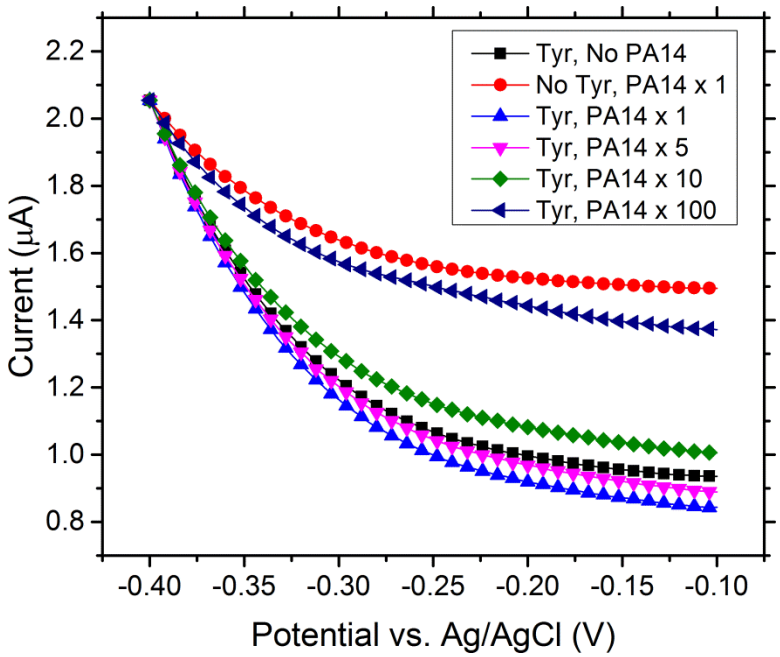

Fig. S32. Square-wave voltammograms of *P. aeruginosa* grown in trypticase soy broth for 2 hours in the presence of tyrosine (16 mM). *P. aeruginosa* was used at the following concentrations (million cells per mL): 4, 20, 40, and 400 respectively.

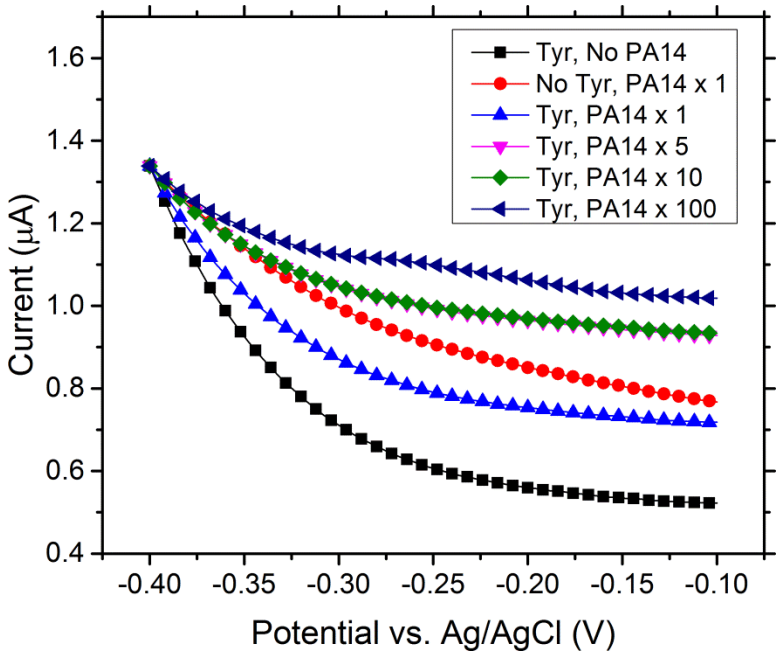

Fig. S33. Square-wave voltammograms of *P. aeruginosa* grown in trypticase soy broth for 4 hours in the presence of tyrosine (16 mM). *P. aeruginosa* was used at the following concentrations (million cells per mL): 4, 20, 40, and 400 respectively.

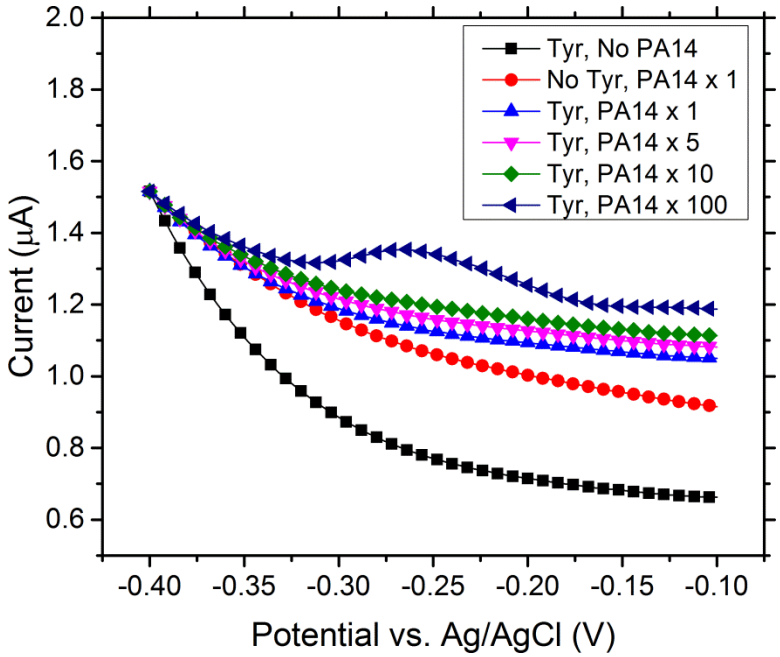

Fig. S34. Square-wave voltammograms of *P. aeruginosa* grown in trypticase soy broth for 6 hours in the presence of tyrosine (16 mM). *P. aeruginosa* was used at the following concentrations (million cells per mL): 4, 20, 40, and 400 respectively.

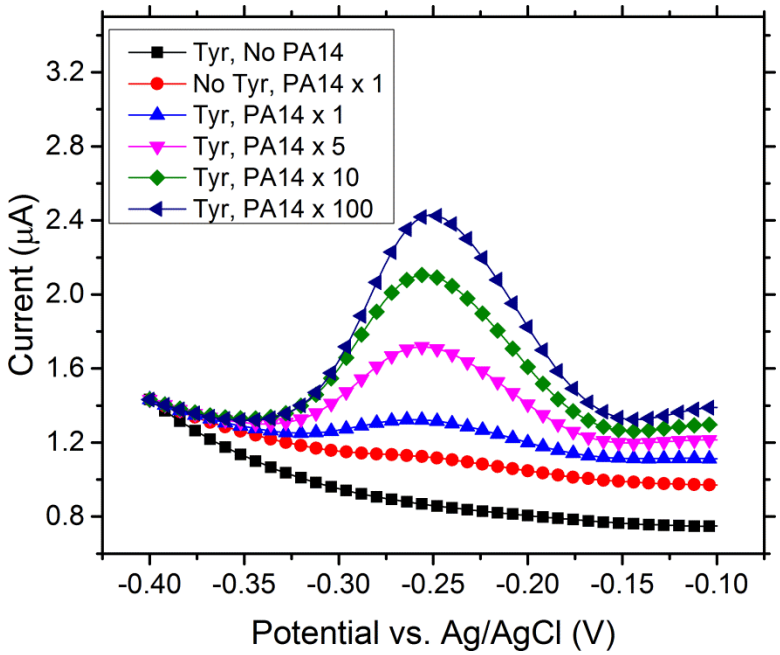

Fig. S35. Square-wave voltammograms of *P. aeruginosa* grown in trypticase soy broth for 10 hours in the presence of tyrosine (16 mM). *P. aeruginosa* was used at the following concentrations (million cells per mL): 4, 20, 40, and 400 respectively.

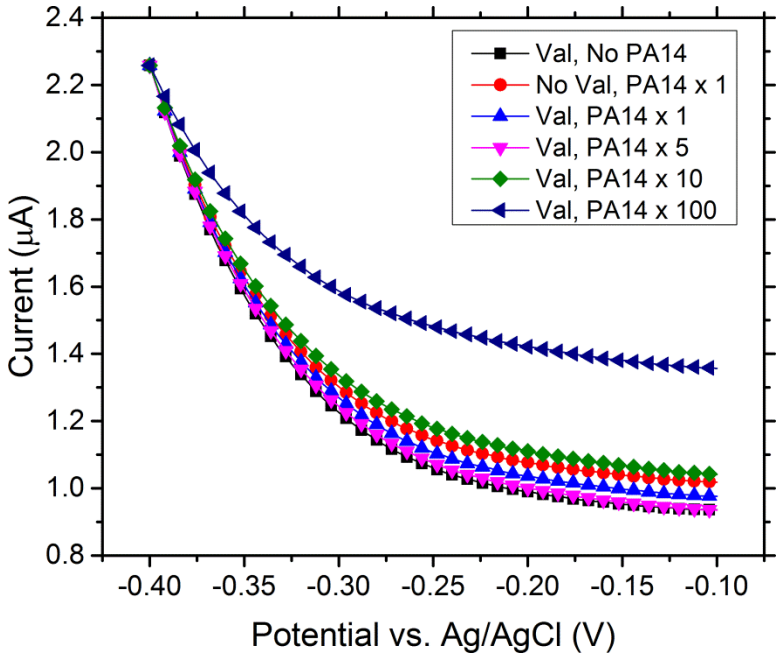

Fig. S36. Square-wave voltammograms of *P. aeruginosa* grown in trypticase soy broth for 0 hours in the presence of valine (17.6 mM). *P. aeruginosa* was used at the following concentrations (million cells per mL): 4, 20, 40, and 400 respectively.

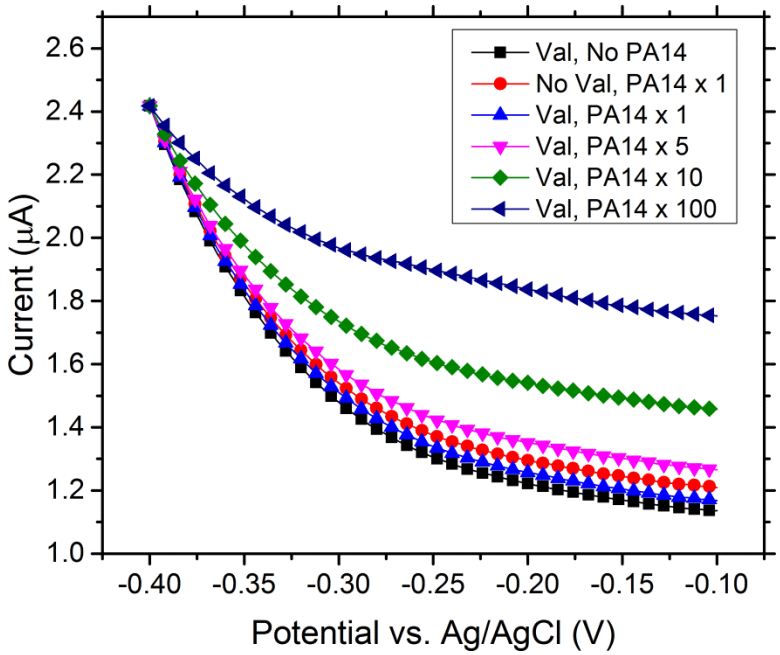

Fig. S37. Square-wave voltammograms of *P. aeruginosa* grown in trypticase soy broth for 2 hours in the presence of valine (17.6 mM). *P. aeruginosa* was used at the following concentrations (million cells per mL): 4, 20, 40, and 400 respectively.

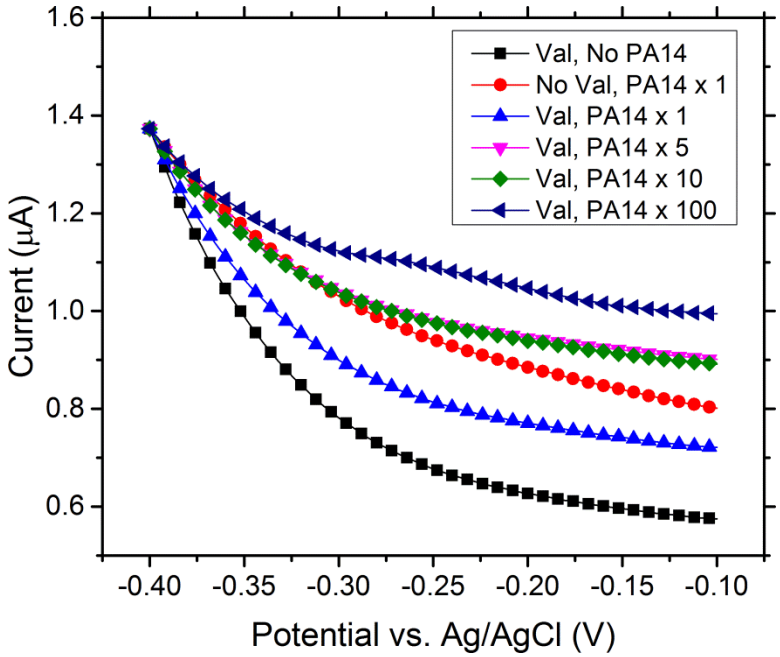

Fig. S38. Square-wave voltammograms of *P. aeruginosa* grown in trypticase soy broth for 4 hours in the presence of valine (17.6 mM). *P. aeruginosa* was used at the following concentrations (million cells per mL): 4, 20, 40, and 400 respectively.

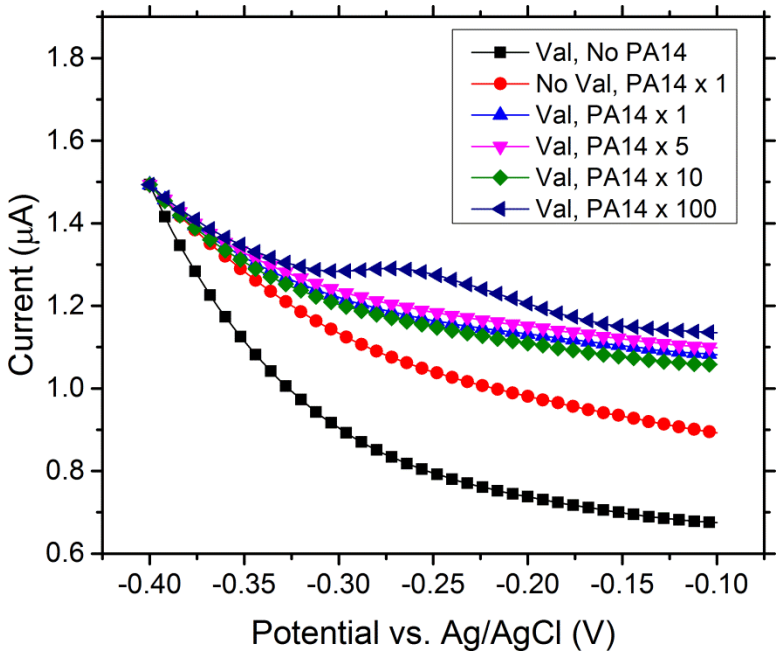

Fig. S39. Square-wave voltammograms of *P. aeruginosa* grown in trypticase soy broth for 6 hours in the presence of valine (17.6 mM). *P. aeruginosa* was used at the following concentrations (million cells per mL): 4, 20, 40, and 400 respectively.

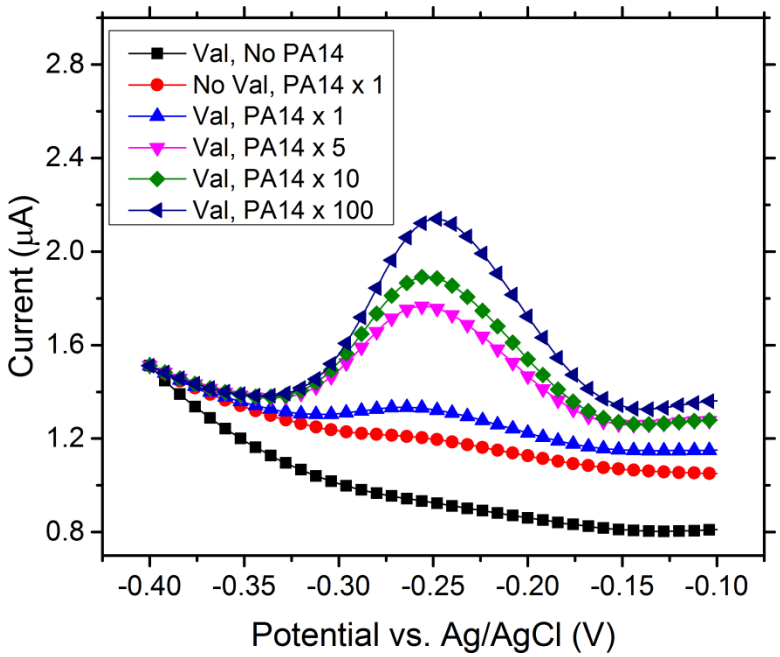

Fig. S40. Square-wave voltammograms of *P. aeruginosa* grown in trypticase soy broth for 10 hours in the presence of valine (17.6 mM). *P. aeruginosa* was used at the following concentrations (million cells per mL): 4, 20, 40, and 400 respectively.

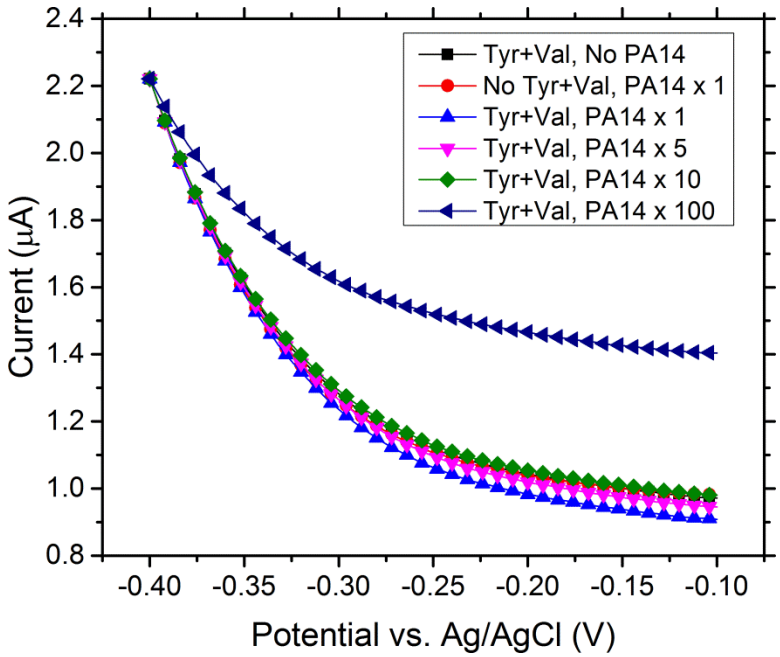

Fig. S41. Square-wave voltammograms of *P. aeruginosa* grown in trypticase soy broth for 0 hours in the presence of tyrosine (16 mM) and valine (17.6 mM). *P. aeruginosa* was used at the following concentrations (million cells per mL): 4, 20, 40, and 400 respectively.

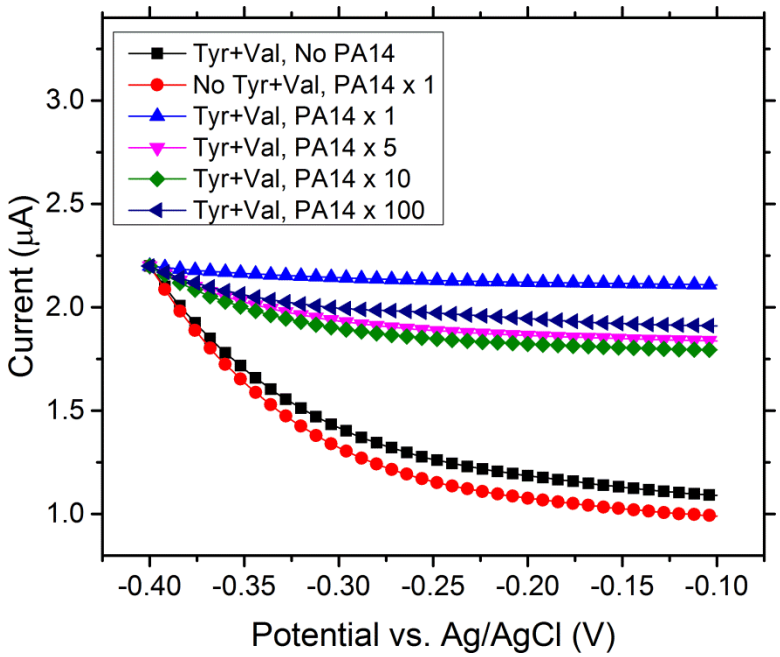

Fig. S42. Square-wave voltammograms of *P. aeruginosa* grown in trypticase soy broth for 2 hours in the presence of tyrosine (16 mM) and valine (17.6 mM). *P. aeruginosa* was used at the following concentrations (million cells per mL): 4, 20, 40, and 400 respectively.

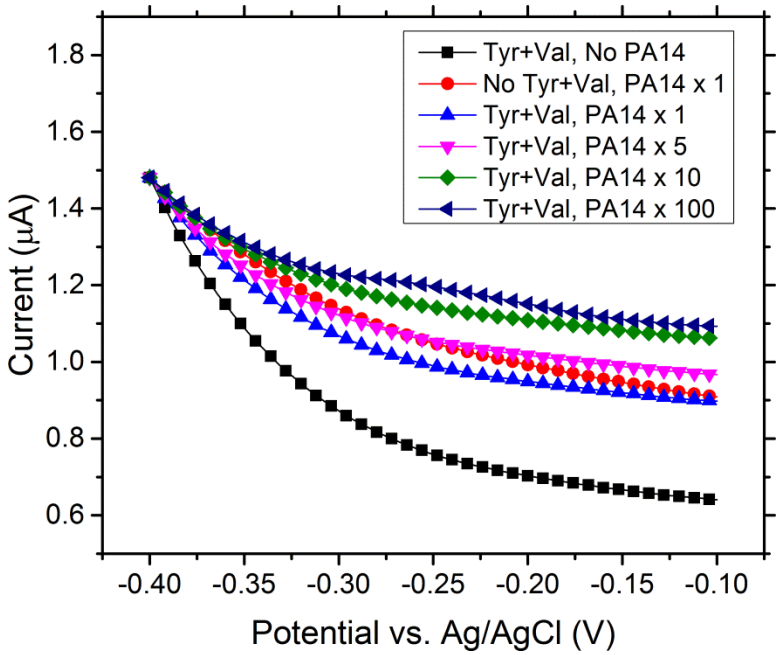

Fig. S43. Square-wave voltammograms of *P. aeruginosa* grown in trypticase soy broth for 4 hours in the presence of tyrosine (16 mM) and valine (17.6 mM). *P. aeruginosa* was used at the following concentrations (million cells per mL): 4, 20, 40, and 400 respectively.

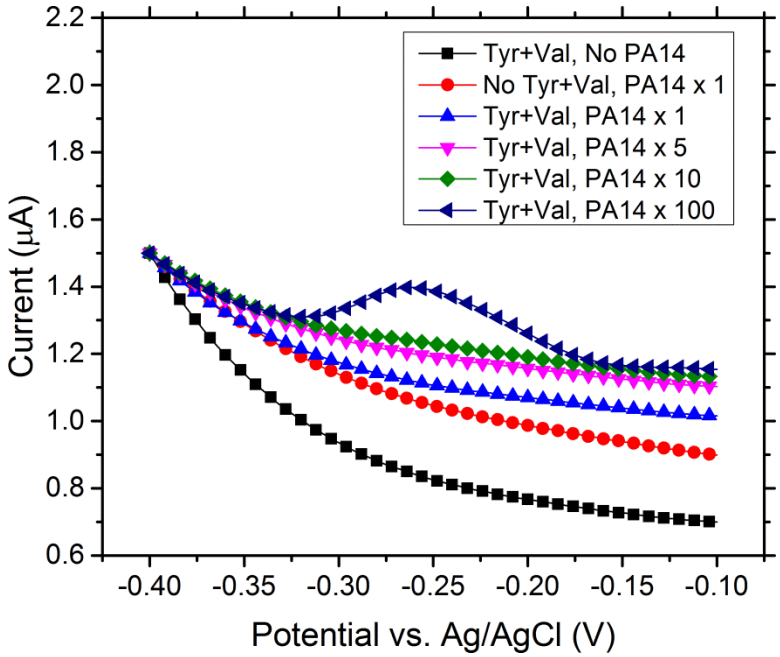

Fig. S44. Square-wave voltammograms of *P. aeruginosa* grown in trypticase soy broth for 6 hours in the presence of tyrosine (16 mM) and valine (17.6 mM). *P. aeruginosa* was used at the following concentrations (million cells per mL): 4, 20, 40, and 400 respectively.

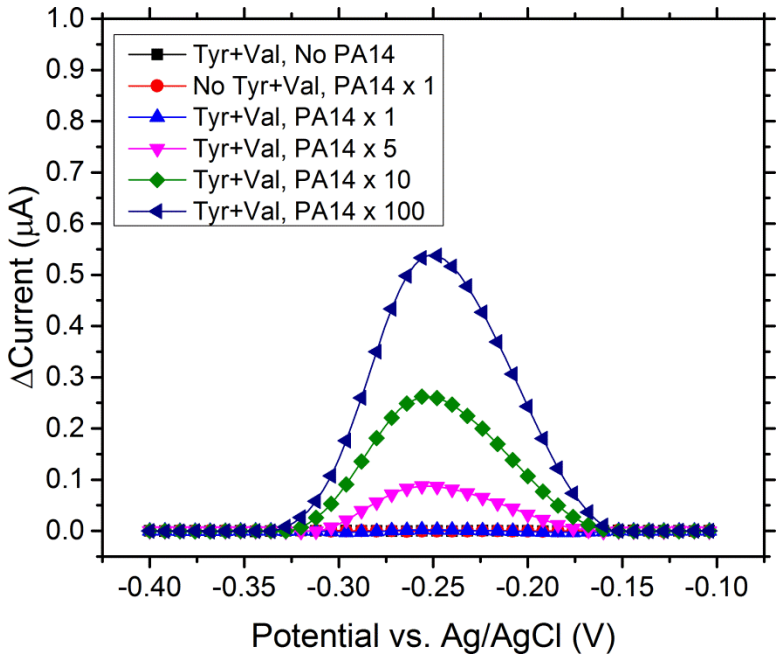

Fig. S45. Square-wave voltammograms of *P. aeruginosa* grown in trypticase soy broth for 8 hours in the presence of tyrosine (16 mM) and valine (17.6 mM). *P. aeruginosa* was used at the following concentrations (million cells per mL): 4, 20, 40, and 400 respectively. Baselines were created for this data set using spline interpolation with 8 base points. The resulting baseline was then subtracted from the raw data.

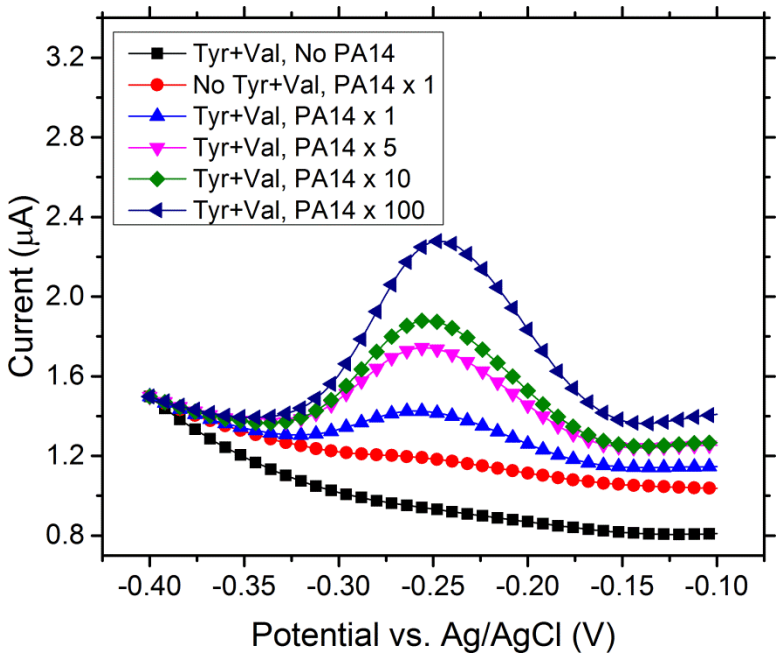

Fig. S46. Square-wave voltammograms of *P. aeruginosa* grown in trypticase soy broth for 10 hours in the presence of tyrosine (16 mM) and valine (17.6 mM). *P. aeruginosa* was used at the following concentrations (million cells per mL): 4, 20, 40, and 400 respectively.

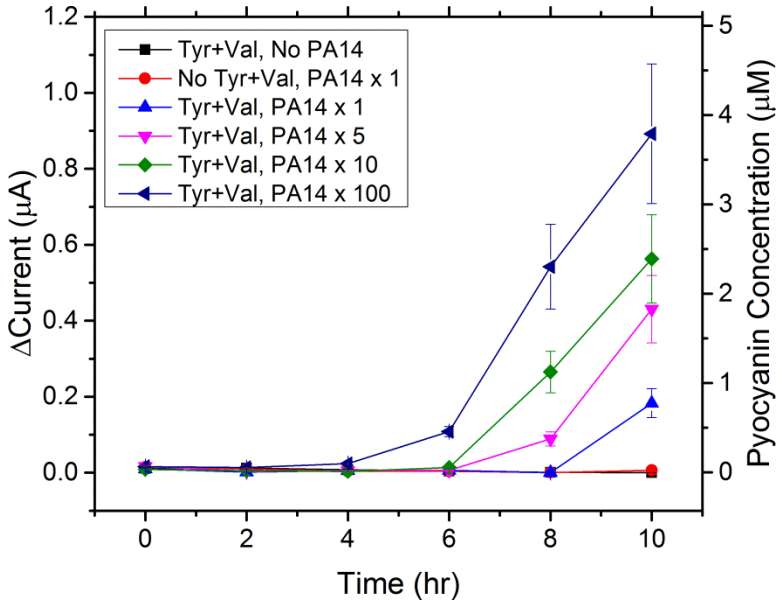

Fig. S47. Monitoring *P. aeruginosa*'s production of pyocyanin over the course of 10 hours in TSB cultures containing both tyrosine (16 mM) and valine (17.6 mM). *P. aeruginosa* was used at the following concentrations (million cells per mL): 4, 20, 40, and 400 respectively. Control experiments contained 4 million cells per mL without additional amino acids added and sterile growth media with added amino acids. Combining the two amino acids in the same media, surprisingly, lowers the pyocyanin production rate slightly below the maximum obtained using only 16 mM tyrosine. The underlying cause of this result has not yet been identified.