Supporting Information

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SI Materials and Methods

Calibration Curve for Pyocyanin (PYO). Biofilm was prepared as described in the "Bacteria Culture and Biofilm Sample Preparation" section of *Materials and Methods*. After growing the *P. aeruginosa* Δ*phz*1/2 biofilm on a polycarbonate membrane in an anaerobic environment, it was carefully transferred on a 35-mm Petri dish (Falcon; catalog no. 351008). A PDMS stencil was carefully placed over the membrane, as shown in Fig. 1*A*. LB-MOPS (500 μL) was then added to the 1-cm diameter stencil chamber. The working (10-μm Pt), reference, and counter electrodes were arranged as shown in Fig. 1*A*.

Aliquots of 1 mM PYO were then added to 500 μL of LB-MOPS to make 6, 10, 20, 50, 70, and 100 μM PYO solutions. Square wave voltammetry (SWV) was recorded at each PYO concentration in triplicate by using a 10-μm Pt tip located in bulk solution. The parameters for SWV were as follows: initial potential: −0.35 V, final potential: −0.85 V, increment potential: 0.004 V, amplitude: 0.025 V, frequency: 5 Hz.

Measurement of Pyocyanin Production by Planktonic Bacteria and pH. One milliliter of an overnight culture of *P. aeruginosa* was washed twice in an equal volume of LB and the OD_{600} determined. Washed cells were diluted into 50 mL of LB or LB-MOPS in a 250-mL Erlenmeyer flash and incubated at 37 °C shaking at 250 RPM. One milliliter was removed each hour for absorbance, pH, and PYO measurements. Abs_{600} was used to quantify cell growth, and pH was determined by calomel electrode. PYO concentration was determined by the addition of 1 mL of culture supernatant to 500 μL of chloroform. The mixture was vortexed for 1 min and centrifuged for 5 min at 14,000 × *g*. The organic phase was transferred to a 3-mL glass vial, and chloroform was removed by an N_2 stream until no visible solvent remained. The dried contents of the vial were resuspended in 250 μL of MOPS medium (pH = 7), and the amount of PYO was determined by using the molar extinction coefficient of PYO at 690 nm (4,130 $M^{-1}{\cdot}cm^{-1}$).

SECM Biofilm Chamber Apparatus Assembly. Biofilm samples were prepared as described in *Materials and Methods*. Thereafter, biofilm-laden membranes were carefully removed from the agar surface and placed on a clean 35-mm plastic Petri dish. The second silicon stencil (Fig. S5) was placed over the membrane with the biofilm portion aligned in the middle of the chamber. The 35-mm Petri dish was attached to the bottom of a glass 100-mm Petri dish by double-sided tape. The entire assembly was then placed over copper heating plate, which was in turn placed over an insulating O-ring. The completed apparatus was then placed on the SECM stage as shown in Fig. S6 and Fig. 1.

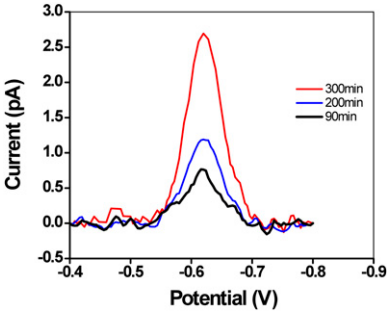

Fig. S1. SWV of PYO produced by a 5-mm *P. aeruginosa* biofilm in LB-MOPS at 36 °C. SWV were recorded by 10-μm Pt tip in real-time over 300 min at 20 μm distance from the biofilm surface. Counter and reference electrode used were 0.5-mm tungsten wire and Hg/Hg_2SO_4, respectively.

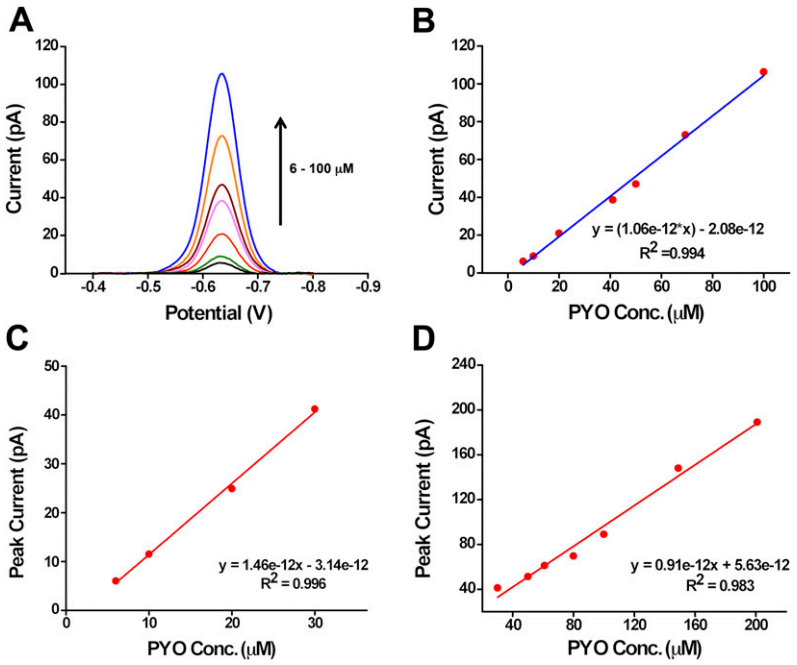

Fig. S2. (*A* and *B*) SWV and calibration curve at multiple PYO concentrations (6–100 μM) in LB-MOPS. (*C*) Calibration curve at varying concentrations of PYO in the range of 6–30 μM in LB-MOPS. (*D*) Calibration curve at varying concentrations of PYO in the range of 30–200 μM in LB-MOPS. Working electrode: 10 μm Pt; Counter: 0.5 mm tungsten wire; Reference: Hg/Hg_2SO_4.

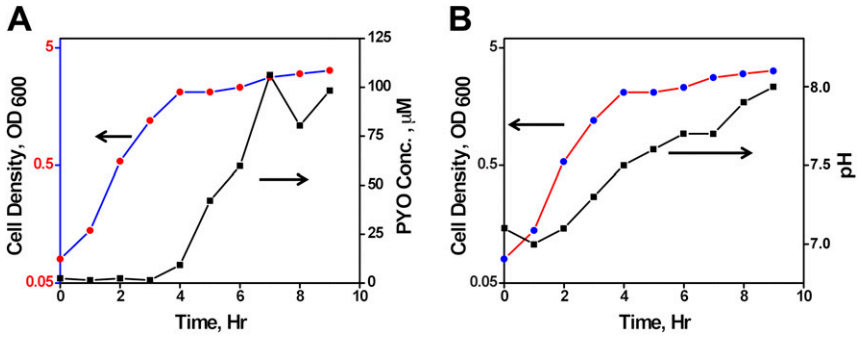

Fig. S3. PYO concentration (squares, solid black line) (*A*) and pH (squares, solid black line) (*B*) from planktonic *P. aeruginosa* PA14 cultures over time (h). For each graph, cell density on a logarithmic scale is also plotted. Arrows indicate the appropriate *y* axis for each dataset.

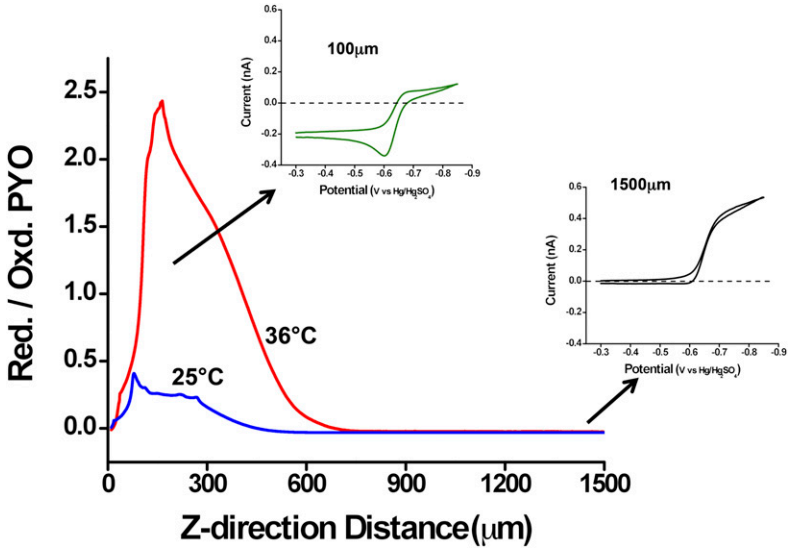

Fig. S4. *Z* direction reduced PYO profile above a *P. aeruginosa* Δ*phz1/2* biofilm at 25 °C (blue line) and 36 °C (red line). Cyclic voltammograms were taken at 1,500 μm (black) and at 100 μm (green) at 36 °C. The *y* axis was normalized to the concentration of oxidized PYO (200 μM) added to each biofilm. PYO was oxidizing at the tip while the tip was approaching the biofilm.

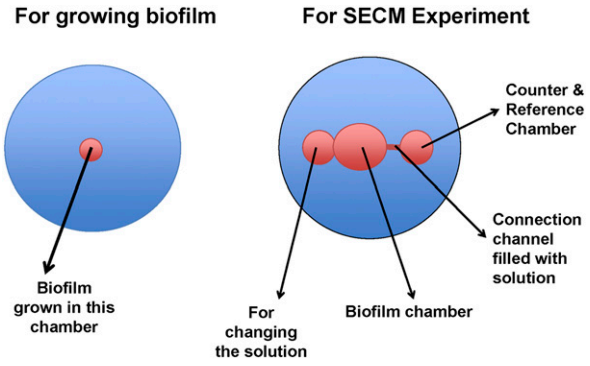

Fig. S5. Schematic representation of 35 mM PDMS molds (red) used for biofilm culturing (*Left*) and the SECM apparatus (*Right*) detailed in Fig. 1 and Fig. S6. Both molds were cast in 35-mm polycarbonate Petri dishes (blue circles).

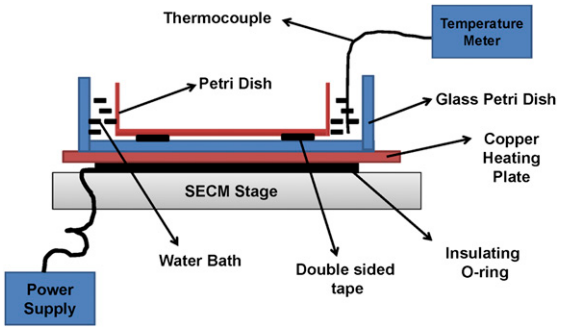

Fig. S6. Schematic of the temperature regulatory elements of the biofilm/SECM apparatus. See Fig. 1 for comparison with the overall assembly including the biofilm chamber and sample.

Table S1. Strains used in this study

Strain	Genotype	Ref.
P. aeruginosa PA14	Wild-type	—
P. aeruginosa Δ*phzA1/2*	PA14 Δ*phzA1*, Δ*phzA2*	1
P. aeruginosa napA^{-}	PA14 *napA*::Mar2xT7 (Gm^{r})	2
P. aeruginosa fbcC^{-}	PA14 *fbcC*::Mar2xT7 (Gm^{r})	2

1. Dietrich LE, Price-Whelan A, Petersen A, Whiteley M, Newman DK (2006) The phenazine pyocyanin is a terminal signalling factor in the quorum sensing network of Pseudomonas aeruginosa. *Mol Microbiol* 61:1308–1321.
2. Liberati NT, et al. (2006) An ordered, nonredundant library of Pseudomonas aeruginosa strain PA14 transposon insertion mutants. *Proc Natl Acad Sci USA* 103:2833–2838.