Supporting Information

Electrochemical Surface Enhanced Raman Spectroscopy of Pyocyanin Secreted by *Pseudomonas aeruginosa* Communities

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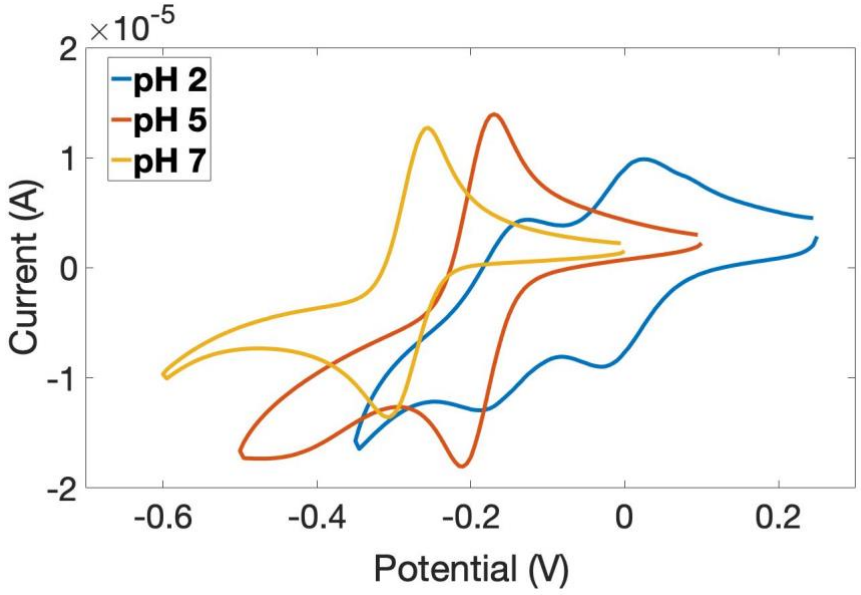

Figure S1. Cyclic voltammetry of PYO at pH 2, 5 and 7. CVs acquired from 500 µM PYO in 60 mM aqueous KNO_3 on a screen-printed Au working electrode. Scan rate was 100 mV s^{-1}.

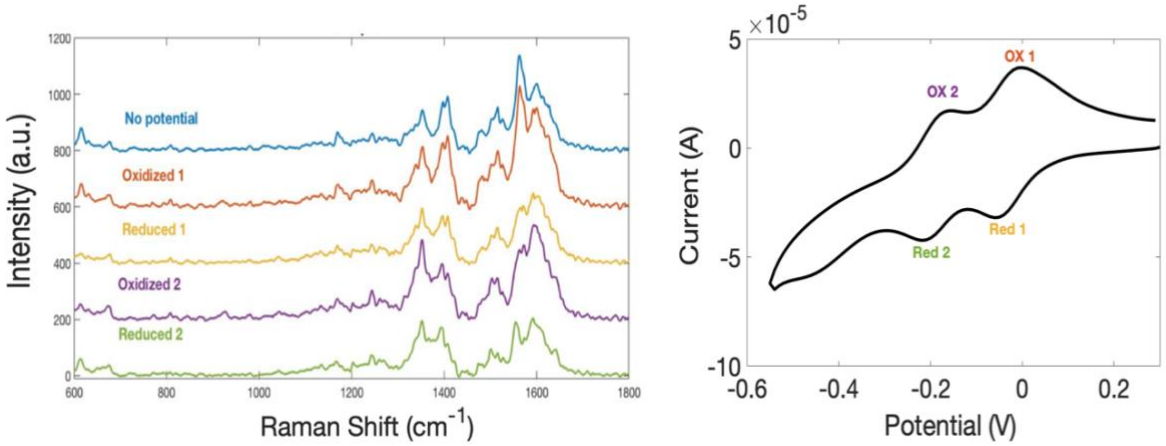

Figure S2. (*Left*) SERS spectra acquired at pH 2.4 at potentials ranging from the fully oxidized to the fully reduced forms, as deduced from the pH 2.4 CV (*Right*).

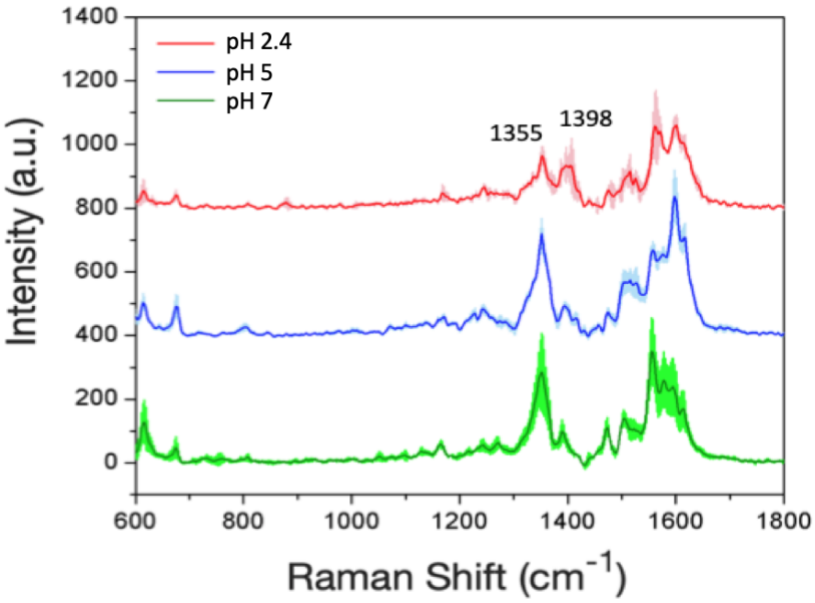

Figure S3. Replicate SERS spectra of PYO at pH 2.4, 5, and 7. The solid lines represent the mean spectrum under each condition, and shaded area indicates the standard deviation at each spectral position.

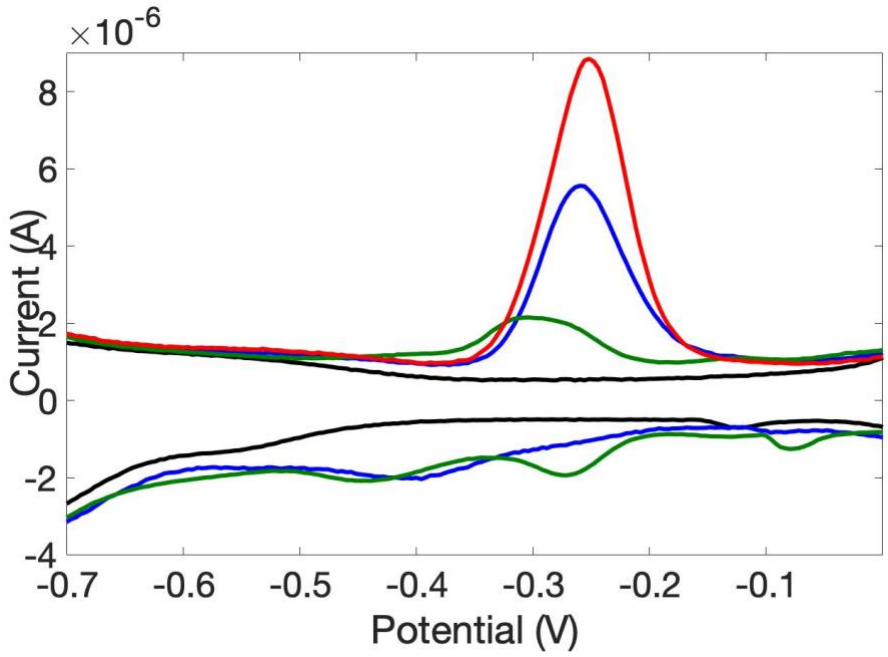

Figure S4. Square wave voltammetry performed on various *P. aeruginosa* strains: PA14 *wt* (blue), FRD1 (green), PA14 Δphz mutant (black) pellicle biofilms, and PA14 *wt* pellicle biofilm with 100 µM PYO added (red).

Oxidation waves near -0.25 V *vs.* Ag/AgCl from FRD1 (green) and PA14 *wt* (blue) are assigned to PYO, as confirmed by the addition of 100 µM PYO to the PA14 *wt* pellicle biofilm (red). The FRD1 strain (green) displays measurable waves upon both reduction and oxidation. These additional waves may arise from other phenazines. For example, the reduction wave around -0.4 V may arise from either phenazine-1-carboxamide (PCN) or phenazine-1-carboxylic acid (PCA) [21], and the wave near -0.10V may from 5-methylphenazine-1-carboxylic acid (5-MCA) [21]. None of these electrochemical waves are observed in the Δphz mutant strain (black).

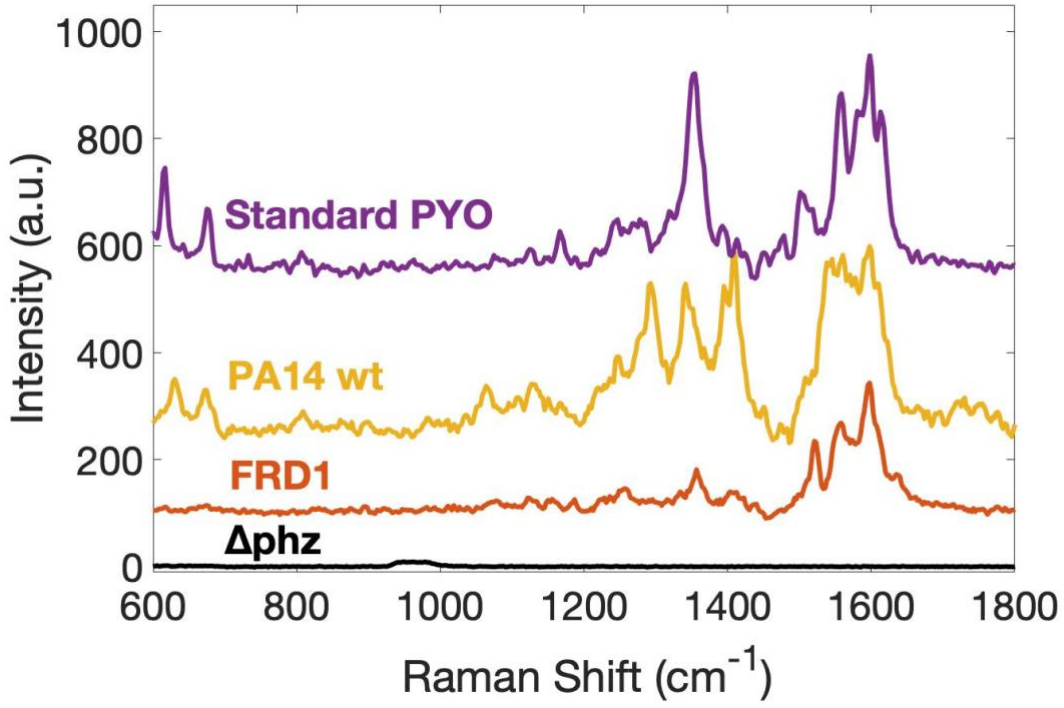

Figure S5. Comparison of SERS spectra obtained from PYO standard at pH 7 and *P. aeruginosa* pellicle biofilms obtained from FRD1 (yellow), PA14 *wt* (red), and PA14 Δphz (black) strains. The distinct PYO peaks at 1355 cm^{-1} (combined C-C/C-N stretch) and 1598 cm^{-1} (ring deformation) confirm the presence of PYO.

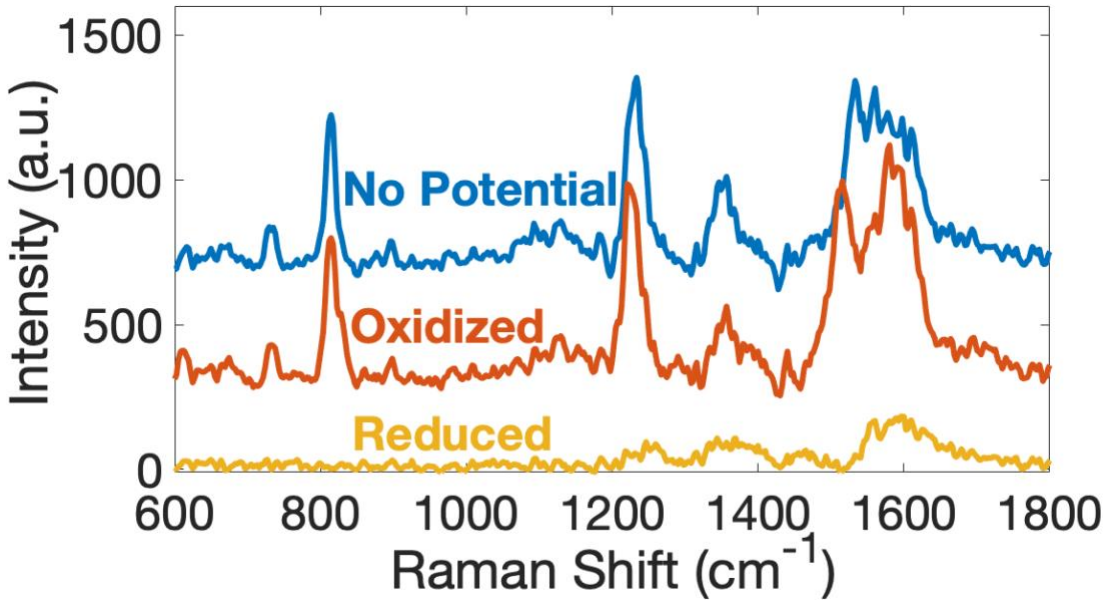

Figure S6. SERS spectra of a 48 h FRD1 pellicle biofilm as a function of applied potential. 0 V and -0.7 V *vs*. Ag/AgCl were used as the oxidation and reduction potentials, respectively.

The spectrum obtained in the absence of applied potential contains not only PYO but also other components, such as a tyrosine-associated peak at ~815 cm^{-1} and the amide III peak around 1230 cm^{-1}. The data indicate that the initial form of the biofilm is close to that obtained under oxidizing conditions and similar to the behavior of the PA14 *wt* pellicle biofilm, shown in **Figure 5**, with intensities decreasing substantially under reducing conditions.

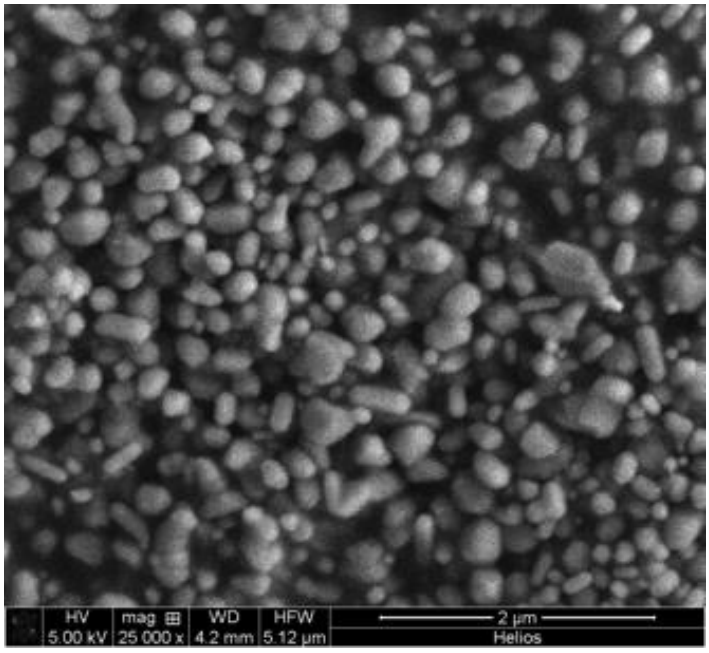

Figure S7. Scanning electron microscope (SEM) image of concentrated 40nm AgNP deposition on gold paste cured working electrode, SERS substrate.

In order to assess the spatial heterogeneity of the AgNP deposits, AgNPs were added to the surface in multiple 10 μL aliquots and allowed to dry, similar to the process used to produce the SERS-active surfaces in the main text. SEM images show that while the AgNPs exhibit some aggregation, the distribution of nanoparticles is homogeneous over the typical 2 μm lengthscale corresponding to the size of a *P. aeruginosa* cell.