SI Text

Biomineralization in killed and metabolically inert biofilms. Biomineralization experiment was performed in killed biofilms. 3-day PAO1 biofilms were completely killed by 200 μg/mL tobramycin continuously supplied for 48 hrs or 200 μg/mL NaClO for 1 hr at a flow rate of 10 mL/hr. Killed biofilms were stained with 60 μM PI for 15 min and visualized under confocal microscopy. Killed biofilms were then subjected to the saturated calcium carbonate medium used previously. Time series confocal images were taken for mineral and biomass at t = 0, 1, 4, and 24 hrs. To further understand the role of biofilm metabolism in carbonate biomineralization, we used chloramphenicol, a bacteriostatic agent, to shut down biofilm metabolism (1). Chloramphenicol, in contrast to antibiotics, does not lead to cell death in biofilms (2). We confirmed this by applying PI staining at the end of the experiment. Dead biomass was below 5% of the total biomass (results not shown). Biofilms were treated with 250 μg/mL chloramphenicol for 3 hours prior to the introduction of the precipitation medium. Chloramphenicol was also continuously supplied together with the precipitation medium at the concentration of 250 μg/mL to maintain the inert metabolism of the biofilms.

EPS staining in PAO1 biofilms with calcite precipitation. PAO1 biofilms after biomineralization were stained with TRITC conjugated *Hippeastrum* hybrid (amaryllis) (HHA) lectin (EY laboratories).

Biomineralization in PAO1 EPS mutant biofilms. We tested carbonate biomineralization in PAO1 *Δpel Δpsl* which is incapable of producing both pel and psl EPS component (3). PAO1 *Δpel Δpsl* biofilms were grown for 3 days in 1% TSB and then subjected to the oversaturated calcium carbonate medium described previously.

Due to the EPS production deficiency, PAO1 *Δpel Δpsl* forms significantly smaller cell clusters after 3 days of incubation (Fig. S10). After the precipitation medium was applied for 17 hrs, no mineral formation was observed in PAO1 *Δpel Δpsl* biofilms. Because PAO1 *Δpel Δpsl* biofilm morphology is significantly different from the wild type, the specific role of EPS in biomineralization is difficult to ascertain from this experiment. However, there is rich literature showing the critical role of biofilm EPS in inducing carbonate biomineralization (4-6).

pH change during biomineralization. Calcium carbonate precipitation is highly pH dependent and high pH facilitates carbonate biomineralization (6). Bacterial metabolism potentially changes the pH environment. Therefore we performed experiments to test bulk fluid pH changes during planktonic cell growth, biofilm development, and biomineralization. Bacterial growth in TSB is known to increase the medium pH, mainly because of the decomposition of protein components in TSB and the consequential ammonium production (7, 8). We confirmed this by culturing PAO1 planktonic cells in pure TSB at 37 °C and monitoring the pH of the cell culture. The cell culture pH increased from 7.21 to 7.92 during the incubation (Fig. S11). However, in the flow cell experiment, where we used 1% TSB in growing biofilms, we did not observe an increase of bulk pH. At day 3 of biofilm growth, the flow cell influent has a pH of 7.05; while the flow cell effluent has a pH of 6.88. We also monitored the pH of the flow cell effluent during biomineralization. pH in the effluent slightly decreased compared to the flow cell influent during biomineralization (Fig. S12).

These results show that *P. aeruginosa* cells could increase the alkalinity of the medium by metabolizing TSB components. However, under the biomineralization condition, PAO1 biofilms did not trigger a noticeable change of bulk pH. Therefore, the observed carbonate

biomineralization in biofilms does not result from a major pH change in bulk fluid but from the *in situ* processes within the biofilm.

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Figure S1.

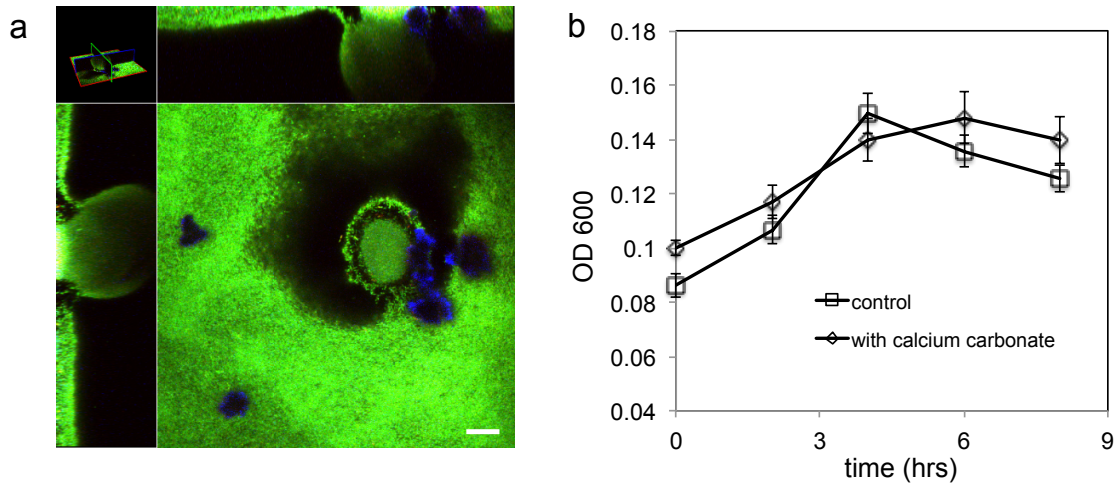

Fig. S1. Calcium carbonate medium is non-toxic to *P. aeruginosa* cells in biofilm or batch culture. Biofilm after 12 hrs calcium carbonate medium treatment was stained by PI (red). The cross-sectional image is an overlay of reflectance, gfp and PI channels (a). The PI signal (red) was barely detected. Scale bar = 20μm. In batch experiment, cell growths showed the same trend with and without the addition of calcium carbonate in the medium (b).

Figure S2.

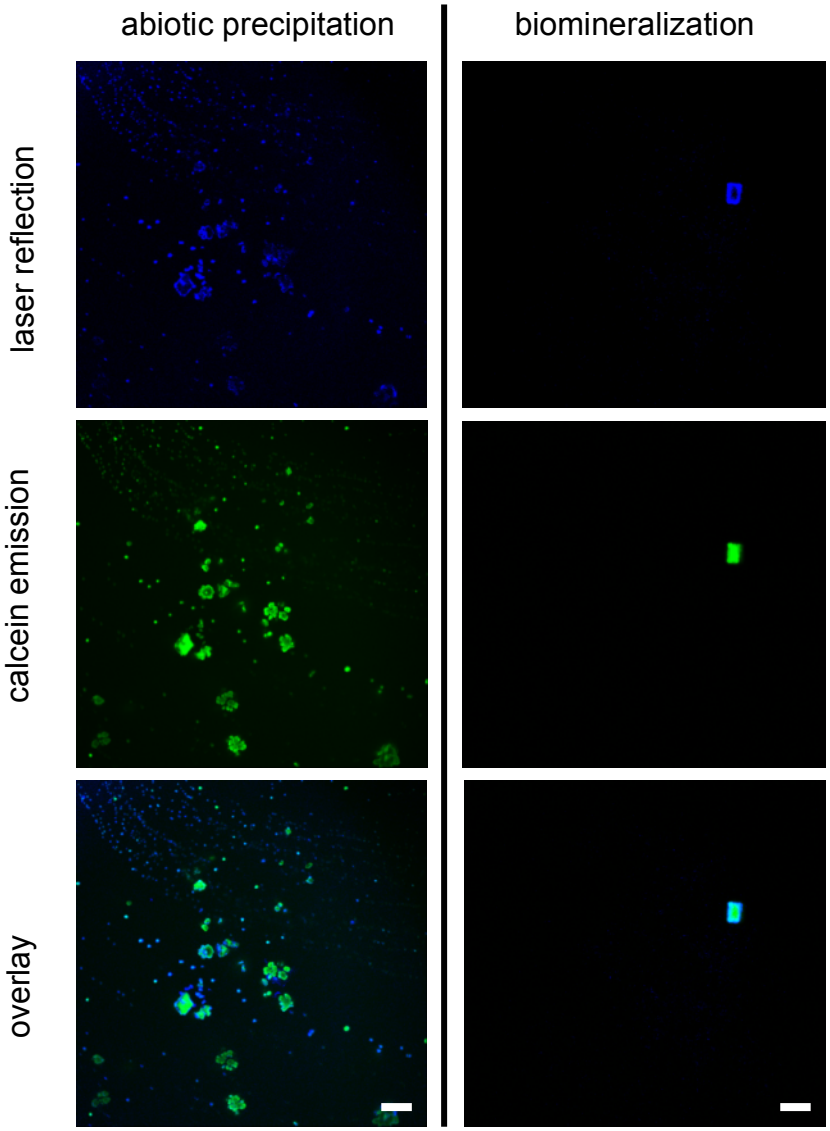

Fig. S2. Laser reflection and calcein emission signals are highly co-localized in both abiotic precipitation (left) and in situ biomineralization (right) in biofilms. Calcite minerals were imaged simultaneously by laser reflection (blue) and calcein emission (green). Biofilm biomass is not shown. Scale bar = 20μm.

Figure S3.

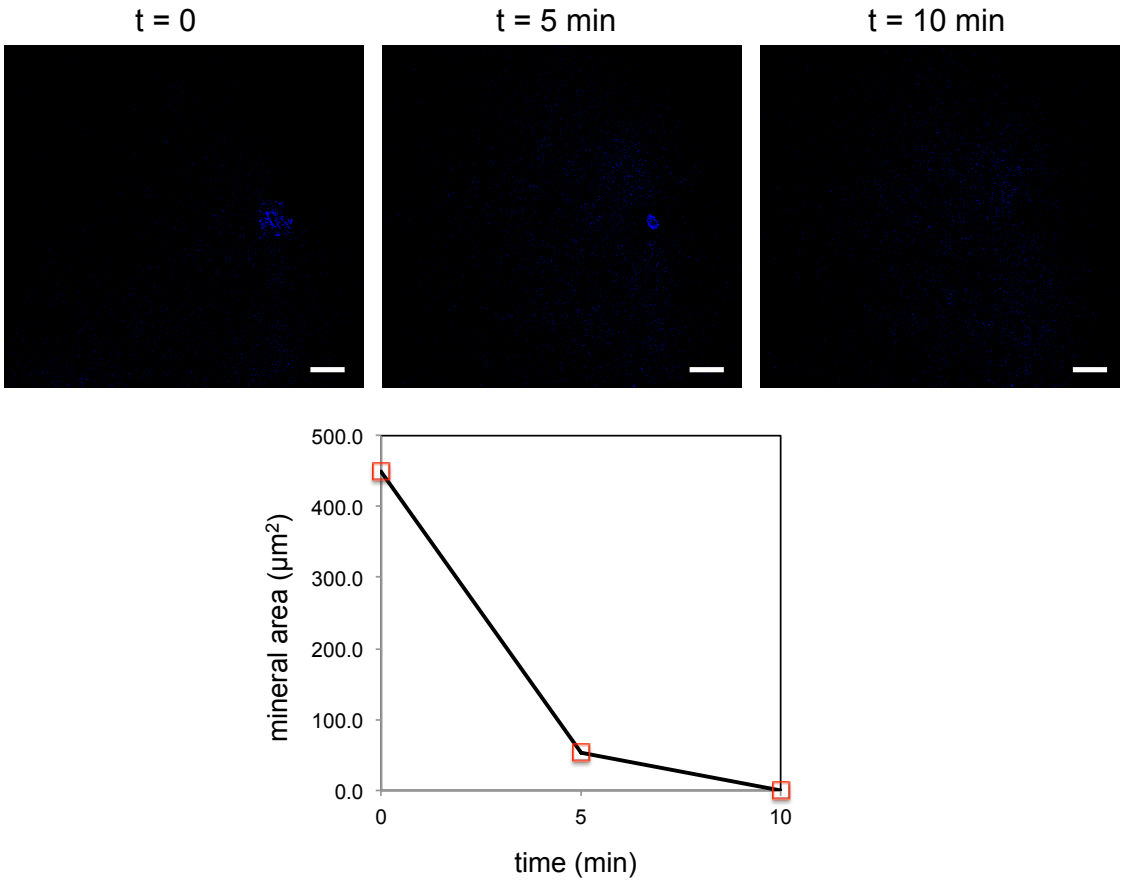

Fig. S3. Biomineralized deposits in biofilms dissolve when exposed to acidic conditions. Time-series images of the mineral deposits in biofilms (blue) after the introduction of 0.01 M HCl. The mineral dissolved over time and completely disappeared after 10 min. Scale bar = 20μm.

Figure S4.

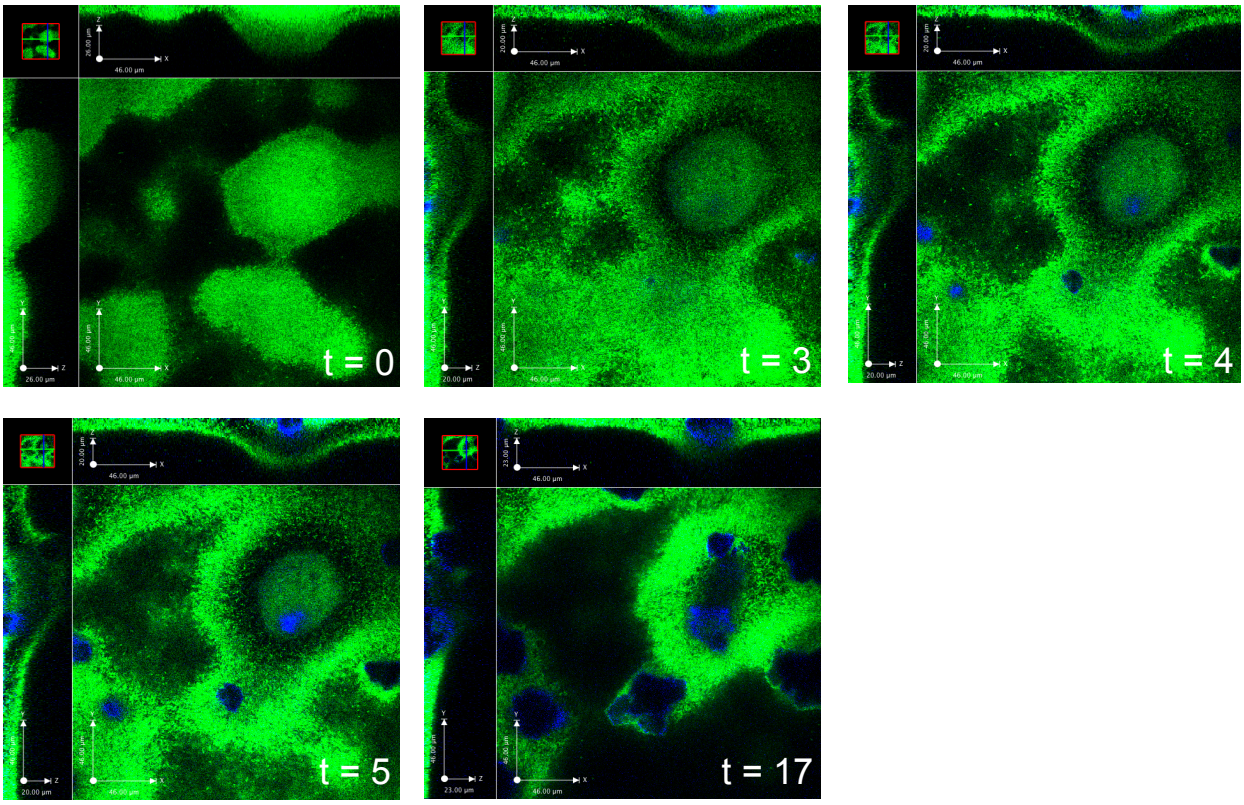

Fig. S4. Time series of cross-sectional images of biomineralization at t = 0, 3, 4, 5, and 17 hours after introduction of calcium carbonate medium. Calcite (blue) and P. aeruginosa cells (green) are overlaid in these images.

Figure S5.

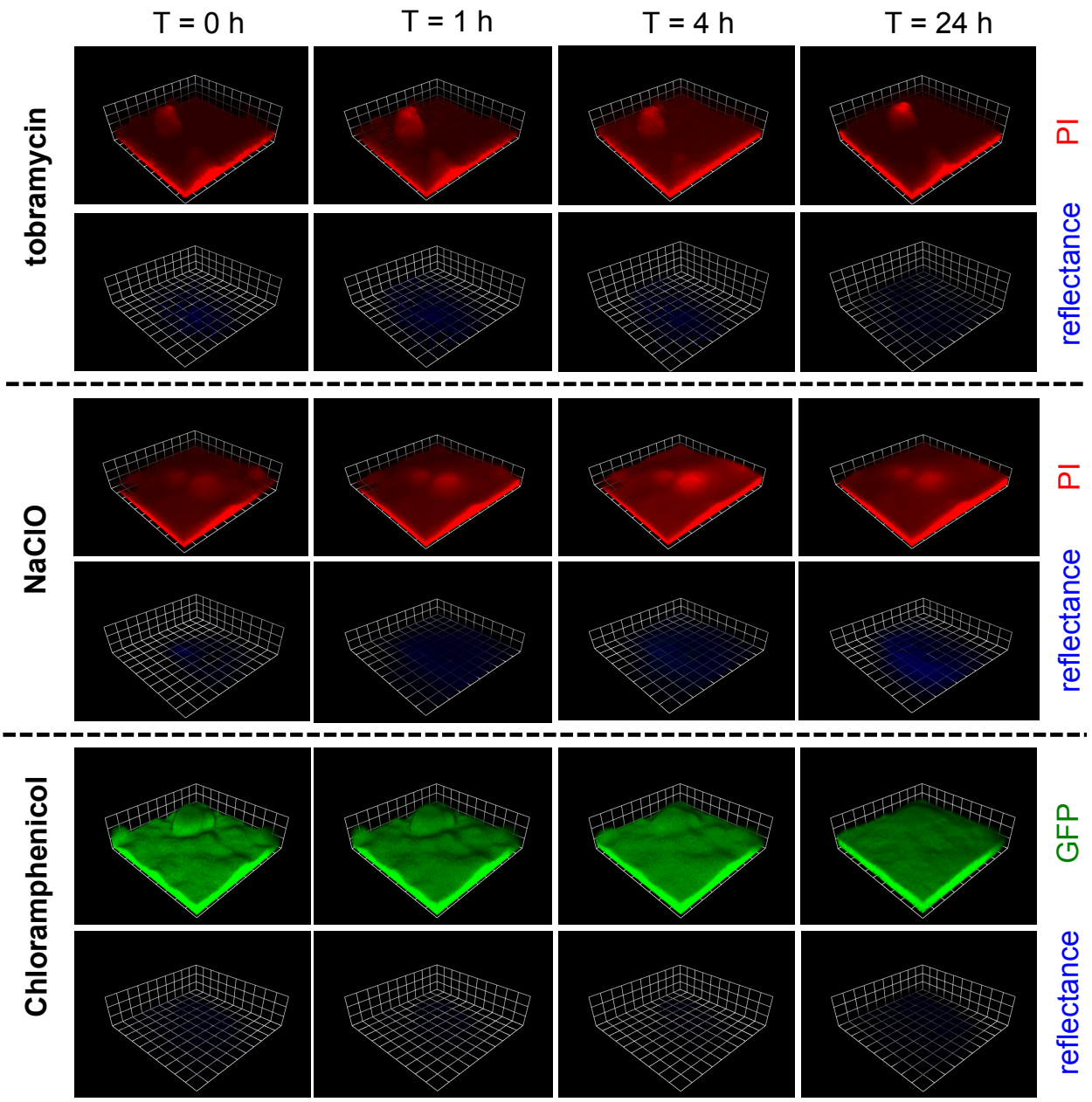

Fig. S5. Killed or metabolically inert biofilms do not precipitate calcium carbonate. Biofilm in the upper panel was killed by tobramycin and in the middle panel by NaClO. Dead biofilm was stained by propidium iodide (red). The metabolism of the biofilm in the lower panel was shut down by treating the biofilms with chloramphenicol. Time-series images were then obtained of

biofilm (red) and mineral reflectance (blue) 0, 1, 4, and 24 hours after introduction of the saturated calcium carbonate medium to the killed biofilms. Calcite biomineralization did not occur during the 24 hours of treatment (note lack of deposits in the reflectance channel). Scale grid = 23 μm.

Figure S6.

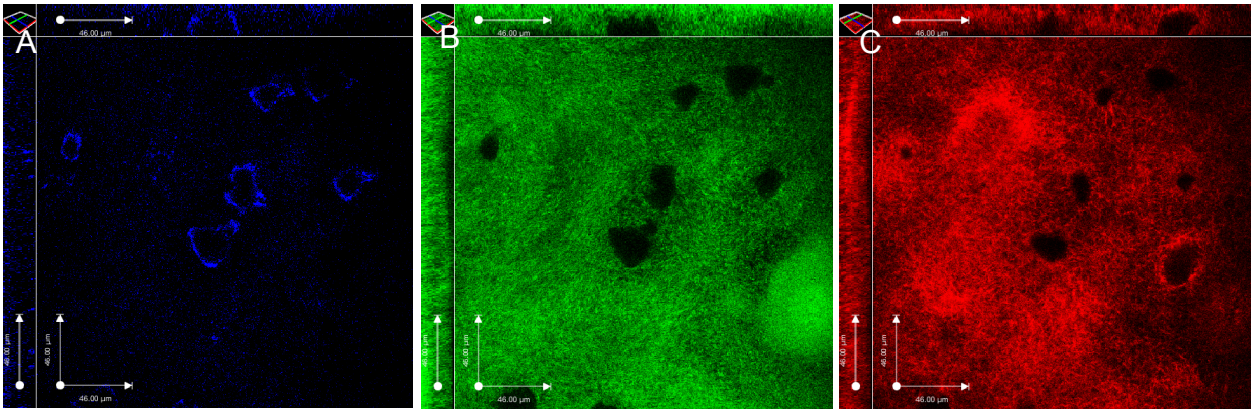

Figure S6. EPS staining in biofilms with calcite precipitation. Biofilm EPS stained by HHA appear in red (C). Mineral and biomass are shown in (A) and (B), respectively. Mineral formation locations do not have a clear correlation with EPS density.

Figure S7.

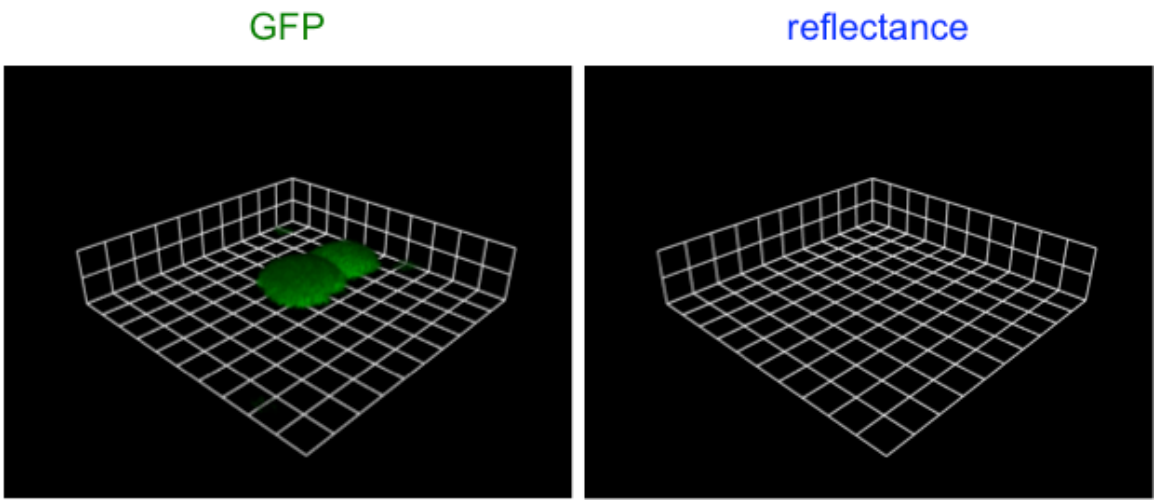

Fig. S7. PAO1 *Δpel Δpsl* biofilms do not precipitate calcium carbonate. Confocal images were taken after 17 hrs treatment of the precipitation medium. Note that no deposit shows up in the reflectance channel. Scale grid = 23 μm.

Figure S8.

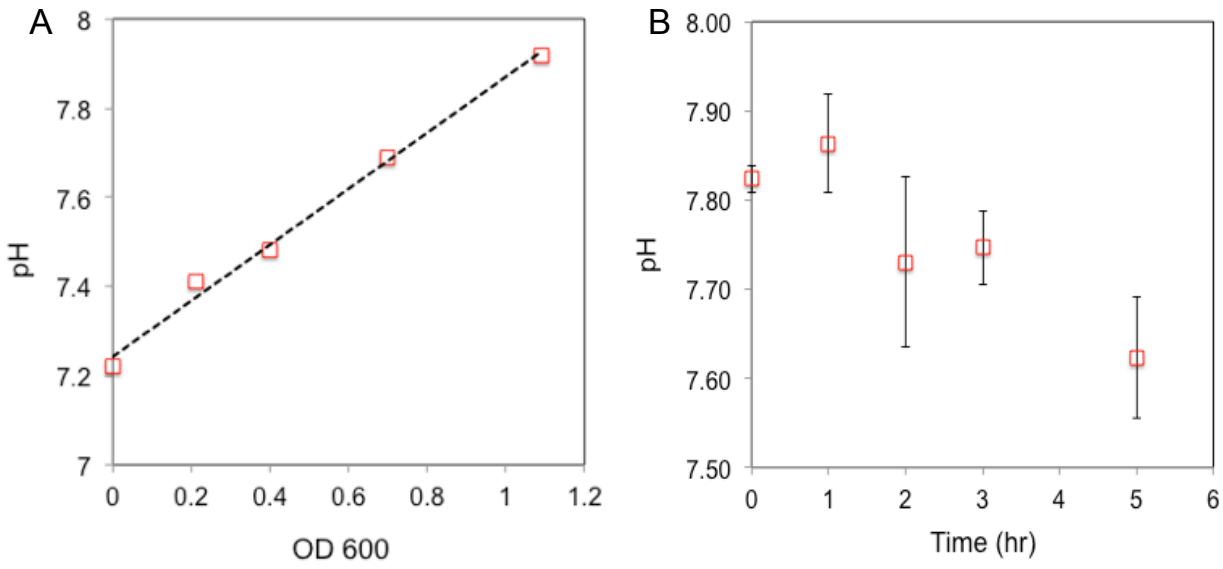

Fig. S8. (A): pH increases in PAO1 planktonic cell culture during incubation. pH increases linearly with cell density in planktonic culture. (B): pH of flow cell effluent during biomineralization. pH in the flow cell effluent was monitored at 0, 1, 2, 3, 5 hrs after the introduction of the biomineralization medium. pH at the flow cell inlet was 7.86 ± 0.06. The effluent pH slightly decreased compared to the influent pH and the decrease was within 0.2 pH unit. Error bars were obtained from triplicated experiments.