Supplementary materials

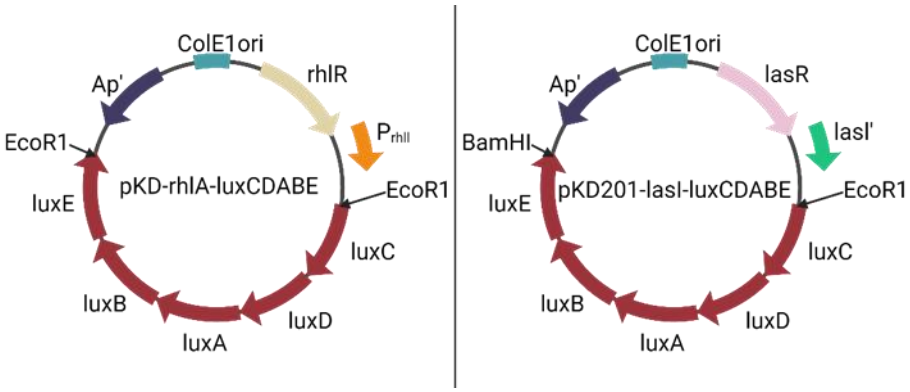

Figure S1: The plasmids used in the study.

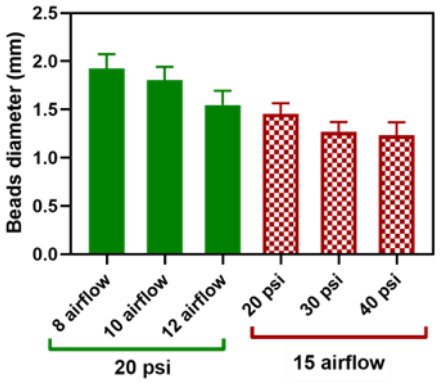

Figure S2: Bead sizes are a function of air pressure.

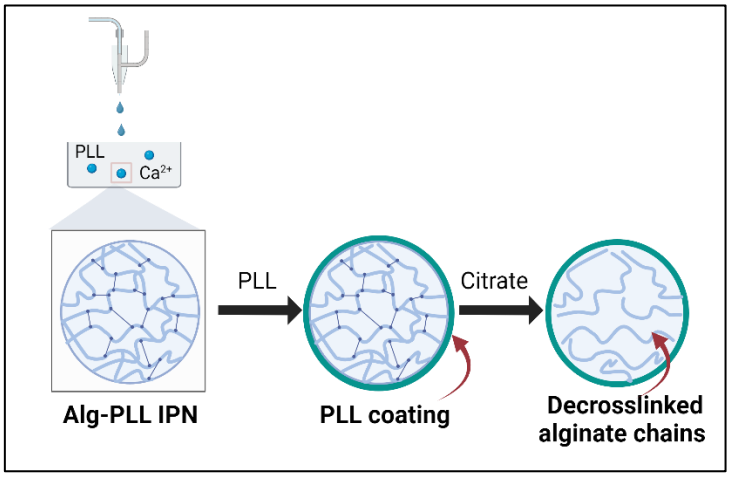

Figure S3: Alginate-PLL IPN showed enhanced stability chelators.

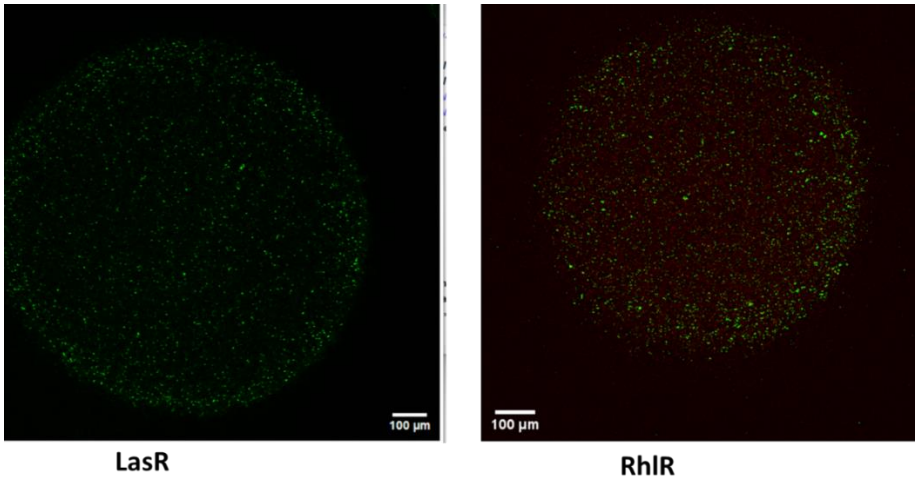

Figure S4: The micrograph of live/dead stained bioreporter beads. Microcapsules containing LasR and RhlR strains were placed in a sterile 24-well plate containing LB-broth and were mildly shaken for about 25 hours in an orbital shaker in a hot room maintained at 30 °C. For live/dead staining, capsules were washed with saline solution (0.9% w/v NaCl) three times. After washing, capsules were incubated at room temperature for 15 min in a saline solution containing propidium iodide (dead) and SYTO™ 9 (live) stain washed with saline and transferred to the ibid 12-well plate for confocal microscope imaging.

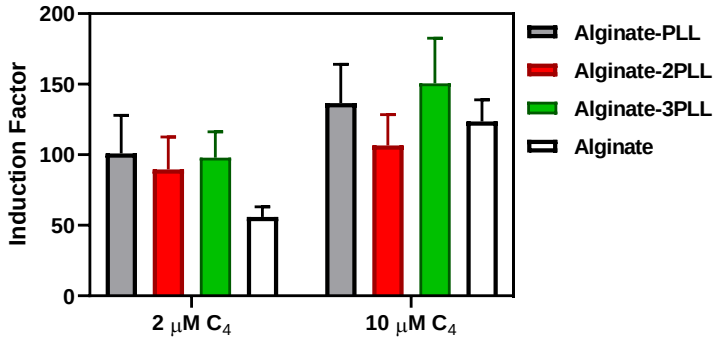

Figure S5: Different layers of the microbeads Performance. The different poly-lysine coating layers were prepared and tested for bioluminescent activity. For the sake of structural strength, all experiments were carried out using 3 layers of poly-lysine coating, as shown in the main text.

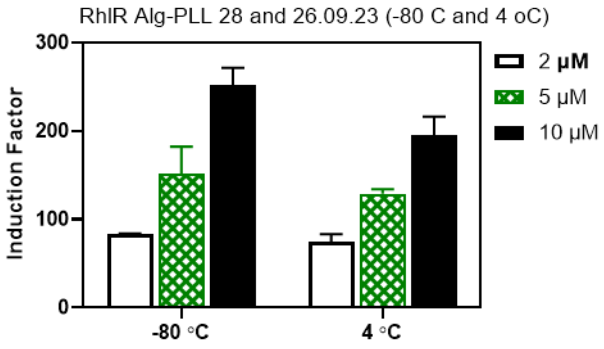

Figure S6: Storage 48 h refrigerator and freezer.

The activities of the reporter bacteria stored at 4 °C and -80 °C (48 h) were determined and compared (figure 6).

***Protocol S1**: The detection of AHLs in the biofilm effluent obtained in the flow cell experiment.*

The biofilm experiment was carried out under the flow conditions using a standard method [1], as shown in the following scheme (Figure 4). Briefly, to initiate biofilm growth, overnight cultures of *P. aeruginosa*, *P. baumanni*, or *E. coli* were diluted with saline solution (0.9% NaCl) to an OD_{600} of about 0.1 and 250 µL of the diluted cultures were injected into separate static Ibidi flow cell (µ-slide sizes of 17 × 3.8 × 0.4 mm). After 1 h of incubation under static conditions to allow bacterial adhesion, the flow was introduced with a constant flow rate of 3 mL h^{-1} (Masterflex L/S peristaltic pump, Cole-Parmer, Vernon Hills, IL, USA) of 10% tryptic soy broth (TSB) supplemented with 0.1% glucose. The flow was allowed to continue undisturbed for 12, 24, 48, and 72 h, and the flow through (effluent) at the determined time was collected, filtered using 0.22 µm membrane filter, and stored at -80 °C until required. The flow-through was used as the source of AHLs for the biosensors testing. The resulting biofilms were macroscopically visualized using CLSM and 3D processed by IMARIS software, as shown in the next sections.

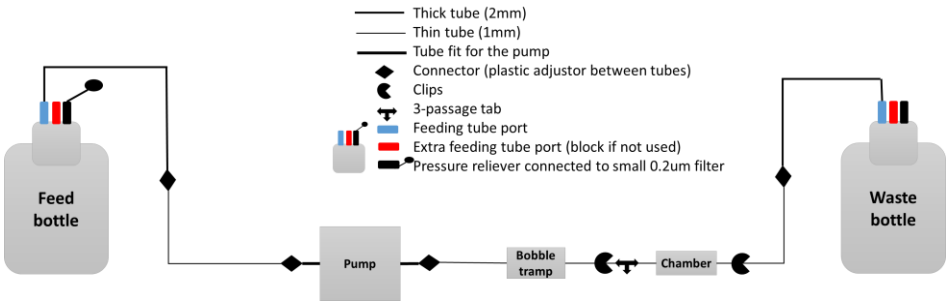

Figure S7: Flow-cell biofilm experiment.

***Protocol S2**: Biofilm Visualization*

The biofilm's thickness and architecture were acquired using a Confocal Laser Scanning Microscope (CLSM) manufactured by Olympus in Tokyo, Japan. The microscope was equipped with a 60 × 1.35NA lens. To detect the green-live SYTO 9 dye, an excitation wavelength of 488 nm was used, and the emission wavelengths of 515 nm were collected. Similarly, for detecting the red-dead PI stain, excitation was done at 530 nm, and the emission was recorded at 617 nm. Using the Easy 3D function of the IMARIS software, three-dimensional projections of biofilm structures were reconstructed while incorporating quantitative structural parameters of the biofilms recorded as biovolume.

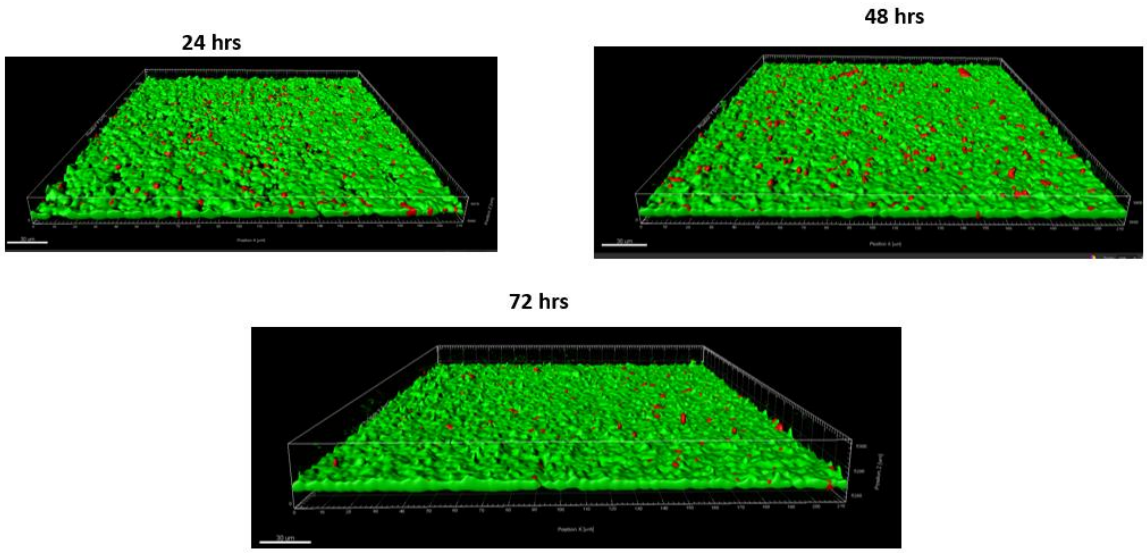

Figure S8: Typical visualization of biofilm after 24, 48 and 72 hrs.

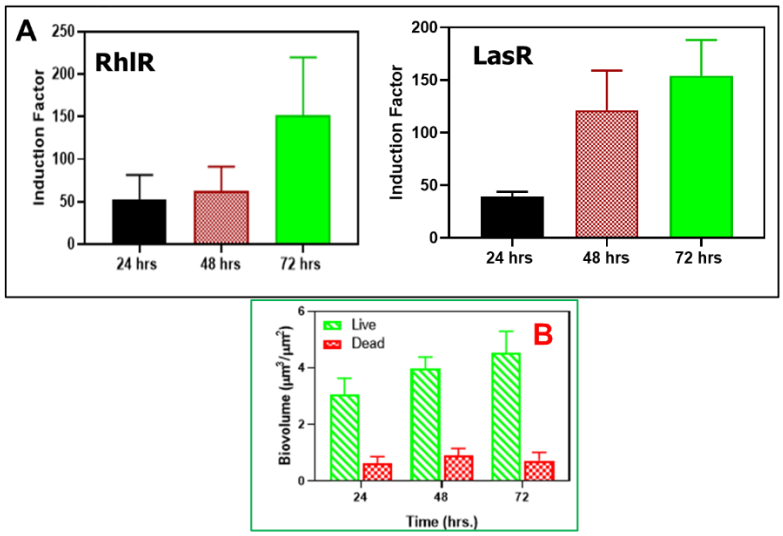

Figure S9: Bioluminescent response of the bioreporters to biofilm effluents (Panel A) and biovolume determined from the confocal microscopy (Panel B)

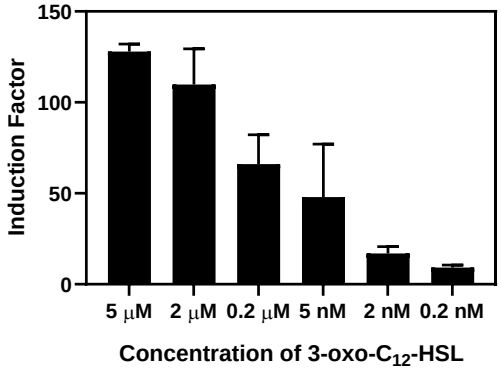

Figure S10: Dose-dependent response of LasR bioreporter carried out as described in section 2.5.2 in the main text.

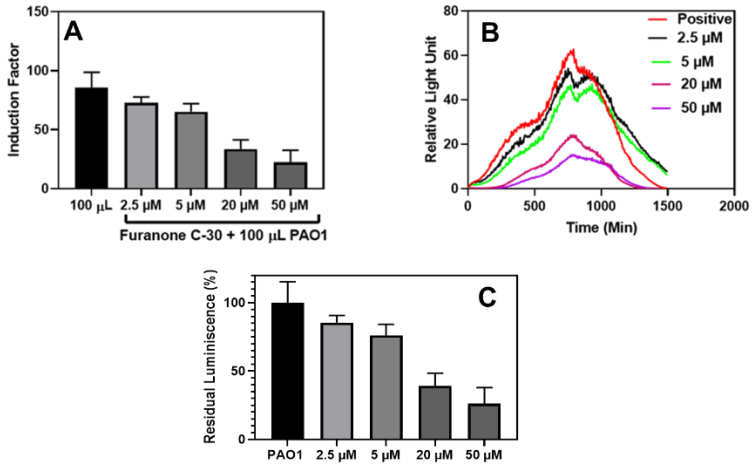

Figure S11: Inhibitory effect of furanone C-30 (FC30) on the activity of RhlR in the presence of cell-free supernatant of *P. aeruginosa* PAO1 (100 μL), Expressed as induction factor (A), spectrum (B), and percentage (C).

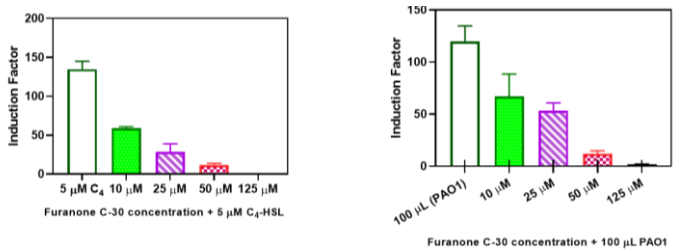

Figure S12: At higher concentrations of F-C30, the bioluminescence of the RhlR reporter was almost quenched.

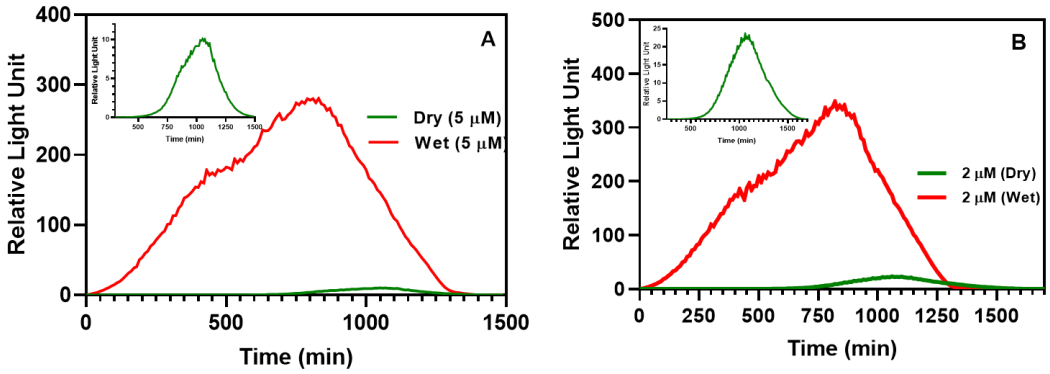

Figure S13: Activity of LasR beads Lyophilized. (A) after 4 hours of lyophilization and (B) after 2 hours of lyophilization. The green line represents the luminescence obtained from lyophilized samples, while the red line shows the activity of the fresh beads stored in the refrigerator prior to the bioluminescent assay. The spectra lines showed the average values of 3 beads per well and 4 replicate experiments.

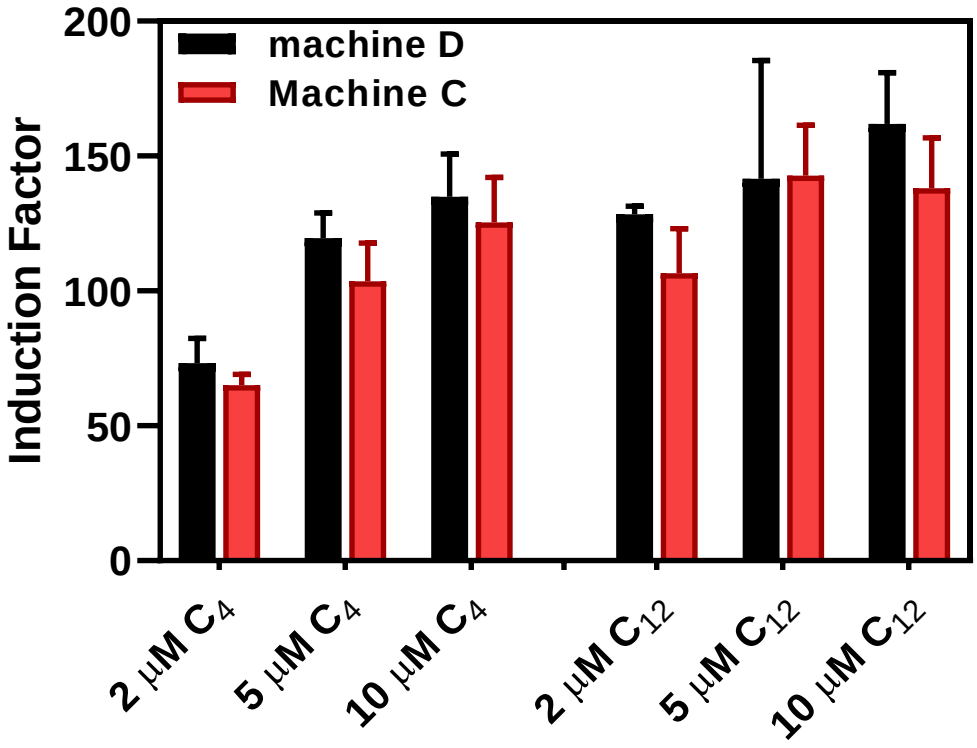

Figure S14: The intermachine differences. The intermachine differences. After 38 days of storage at 4 °C, two identical plates were prepared consisting of encapsulated RhlR strain incubated with different concentrations of autoinducers. The bioluminescent responses of each plate were recorded simultaneously using two different luminometers. The intermachine differences between each concentration tested were not significantly different ($p < 0.05$).

1. Golberg, K.; Markus, V.; Kagan, B.-e.; Barzanizan, S.; Yaniv, K.; Teralı, K.; Kramarsky-Winter, E.; Marks, R.S.; Kushmaro, A.J.P. Anti-Virulence Activity of 3, 3'-Diindolylmethane (DIM): A Bioactive Cruciferous Phytochemical with Accelerated Wound Healing Benefits. **2022**, *14*, 967.