Electronic Supplementary Material (ESI) for Chemical Communications

Bioinspired, Releasable Quorum Sensing Modulators

Supplemental Information

José Gomes,[a] Alexander Grunau,[b] Adrien K. Lawrence,[c] Leo Eberl,[b,*] and Karl Gademann[a,c,*]

[a] Department of Chemistry, NCCR Chemical Biology, University of Basel,
St. Johanns-Ring 19, 4056 Basel, Switzerland.
E-mail: karl.gademann@unibas.ch
[b] Department of Microbiology, Institute of Plant Biology, University of Zurich,
Zollikerstrasse 107, 8008 Zurich, Switzerland.
[c] Chemical Synthesis Laboratory, Swiss Federal Institute of Technology (EPFL), 1015 Lausanne,
Switzerland.

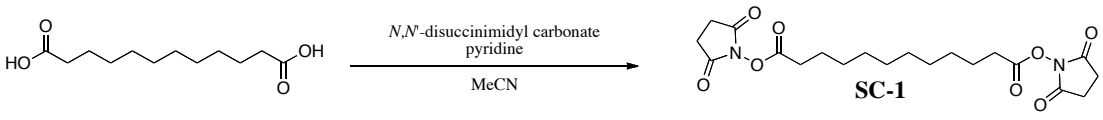
SUPPORTING INFORMATION

1. General Information

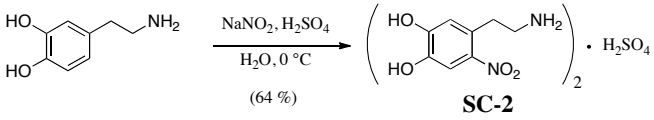
All glassware used for surface modification was cleaned in "piranha solution" (H_2SO_4/H_2O_2 = 7:3; Fluka/Sigma-Aldrich) and extensively rinsed with ultrapure water (sterile filtered, Sigma). Unless otherwise stated, chemicals were purchased from Sigma-Aldrich, Acros, Alfa Aesar, or Fluka and used without further purification. Solvents for work-up and chromatography were distilled from technical quality. Solvents used for chemical transformations were either puriss quality or dried by filtration through activated aluminium oxide under argon or nitrogen (H_2O content < 30 ppm, *Karl-Fischer* titration). All non-aqueous reactions were run in oven-dried or flame dried glassware under a positive pressure of argon or nitrogen. Concentration under reduced pressure was performed by rotary evaporation at 40 °C (unless otherwise specified). Yields refer to purified, dried and spectroscopically pure compounds. Analytical thin layer chromatography (TLC) was performed on Merck silica gel 60 F_{254} plates (0.25 mm thickness) precoated with fluorescent indicator. The developed plates were examined under UV light and stained with ceric ammonium molybdate followed by heating. Flash chromatography was performed using silica gel 60 (230-240 mesh) from Fluka using a forced flow eluant at 0.3-0.5 bar pressure. All ^1H and ^{13}C NMR spectra were recorded using either Varian Gemini 300 MHz (^1H) or 75 MHz (^{13}C), Varian Mercury 300 MHz (^1H) or 75 MHz (^{13}C), Bruker DRX 500 MHz (^1H) or 125 MHz (^{13}C), Bruker DRX 400 MHz (^1H) or 100 MHz (^{13}C), Bruker DRX 600 MHz (^1H) or 150 MHz (^{13}C), Bruker Advance 800 MHz (^1H) or 200 MHz (^{13}C) FT spectrometers at room temperature. Chemical shifts δ are reported in ppm, multiplicity is reported as follows: s = singlet, d = doublet, t = triplet, q = quartet, quint. = quintet, sext. = sextet, sept. = septet, m = multiplet or unresolved and coupling constant *J* in Hz. IR spectra were recorded using a *Varian 2000 FT-IR ATR Spectrometer* or *Varian 800 FT-IR ATR Spectrometer*. The absorption is reported in cm^{-1} and the IR bands were assigned as *s* (strong), *m* (medium) or *w* (weak). Optical rotation $[\alpha]^T_D$ were measured at the sodium D line using a 1 mL cell with a 1 dm path length on a Jasco 2000 digital polarimeter and the concentration *c* is given in g/100 mL and the used solvent is $CHCl_3$, MeOH or H_2O. Melting points (M.p.) were determined using a Büchi B-545 apparatus in open capillaries and are uncorrected. Accurate mass determinations using

electrospray ionization (HRMS-ESI) were performed on a Sciex QSTAR Pulsar mass spectrometer. Analytical, reversed-phase UPLC was performed with an Agilent 1290 Infinity LC system on an Eclipse plus C18 (1.8 μm, 50 x 2.1 mm) column eluting with a linear gradient of CH_3CN containing 0.1% trifluoroacetic acid (TFA) in H_2O containing 0.1% TFA and 1% CH_3CN at a flow rate of 1.0 mL min^{-1}. Preparation of MOPS buffer: 2.09 g *N*-morpholinopropanesulphonic acid (≥99.5 %, Sigma), 3.52 g sodium chloride (≥99.5 %, Sigma-Aldrich), and 10.46 g potassium sulfate (≥99.0 %, Sigma-Aldrich) were dissolved in 100 mL ultrapure water (sterile filtered, Sigma).

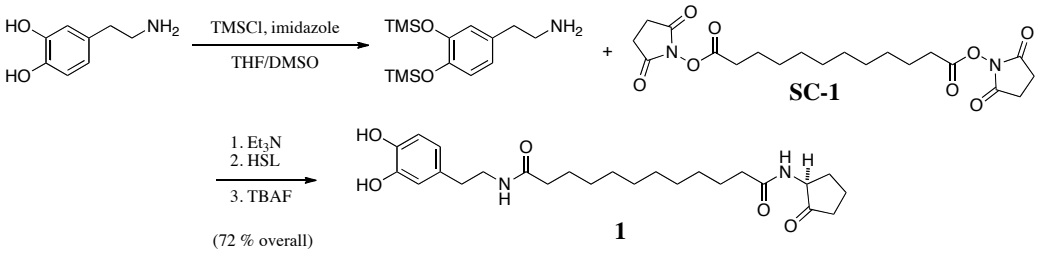
2. Preparation of Compounds



2.1 Dodecanedioic acid bis(2,5-dioxopyrrolidin-1-yl) ester SC-1: Following the procedure reported in reference [1]. Dodecandioic acid (235.0 mg, 1.0 mmol) was dissolved in CH_3CN (3 mL) and pyridine (0.16 mL, 2.0 mmol, 2 equiv) and *N*,*N*'-disuccinimidyl carbonate (539.0 mg, 2.0 mmol, 2 equiv) were added at room temperature. After 7 h, the solvent was removed by rotary evaporation. The residue was poured into a 1N-HCl aqueous solution and extracted with DCM. The organic layer was evaporated to yield the desired product (423.7 mg, 1.0 mmol, 100 %). White solid. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 2.84 (s, 8 H), 2.60 (t, J = 7.54 Hz, 4 H), 1.79-1.69 (m, 4 H), 1.40-1.30 (m, 12 H). ^{13}C-NMR (100.6 MHz, $CDCl_3$) δ 169.1, 168.5, 31.0, 29.2, 29.0, 28.8, 25.7, 24.6. The data is in agreement with the literature.[1]



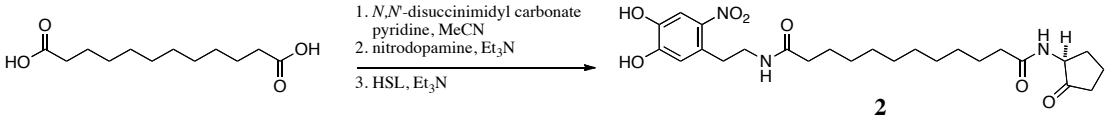
2.2 Nitro-dopamine SC-2: Following the procedure reported in reference [2]. 3-Hydroxytyramine hydrochloride (946.6 mg, 5.0 mmol) and sodium nitrite (757.5 mg, 11 mmol) were dissolved in water (13 mL) and cooled down to 0 °C. Sulfuric acid (8.7 mmol in 5 mL of water) was added slowly to the mixture, and a yellow precipitate was formed. After stirring at RT overnight, the precipitate was filtered and recrystallized from water. The product was dried under high vacuum yielding nitrodopamine as the hemisulfate salt (641.1 mg, 1.6 mmol, 64 %). Brown solid. ^{1}H-NMR (400 MHz, D_2O) δ 7.69 (s, 1 H), 6.85 (s, 1 H), 3.30 (t, *J* = 7.0 Hz, 2 H), 3.19 (t, *J* = 7.0 Hz, 2 H). The data is in agreement with the literature.[2]



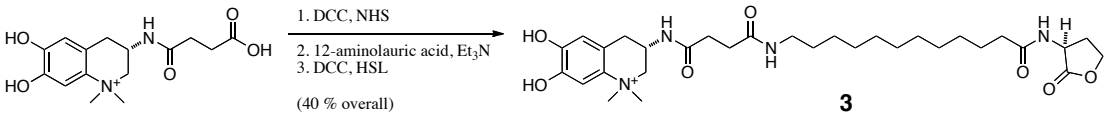
2.3 Hybrid 1: 3-Hydroxytyramine hydrochloride (56.9 mg, 0.3 mmol) was dissolved in THF/DMSO (7.0 mL/0.4 mL). Imidazole (54.5 mg, 0.8 mmol, 2.7 equiv) and TMSCl (108.5 mg, 0.7 mmol, 2.3 equiv) were added at RT and the mixture was stirred for 22 h. Saturated aqueous NH_4Cl solution (10 mL) was added and the resulting mixture was extracted with EtOAc (3 x). The combined organic layers were washed with brine, dried over Na_2SO_4 and evaporated by rotary evaporation. Protected dopamine was used without further purification.

To a solution of dodecanedioic acid (69.1 mg, 0.3 mmol) in THF/DMSO (7.0 mL/0.4 mL) were added EDC (115.0 mg, 0.6 mmol, 2.0 equiv) and NHS (69.1 mg, 0.6 mmol, 2.0 equiv). The resulting mixture was stirred overnight at RT. Protected amine and Et_3N (some drops) were then added and stirred for 20 h before addition of HSL hydrobromide (54.6 mg, 0.3 mmol, 1.0 equiv). After another 24 h, the mixture was filtrated and concentrated *in vacuo*. The crude was extracted with EtOAc and water (2 x). Organic layer was subsequently washed with brine, dried over Na_2SO_4 and concentrated. Crude was dissolved in 5 mL THF and 1 M TBAF (0.75 mL, 0.75 mmol, 2.5 equiv) was added at RT. Mixture was stirred for 1.5 h, quenched with saturated aqueous NH_4Cl solution and extracted with EtOAc (3 x). Combined organic layers were washed with brine, dried over Na_2SO_4, filtered, concentrated in vacuo, and purified by C_{18} reverse phase chromatography (water) to afford **1** (110.4 mg, 0.25 mmol, 82 %). Brown solid. Optical rotation $[\alpha]^{24}_{D}$ (2.5 mg/mL, MeOH) = -13.6°. ^{1}H-NMR (400 MHz, CD_3OD) δ 6.70 (m, 1 H), 6.66 (d, J = 2 Hz, 1 H), 6.54 (dd, J_1 = 8.0 Hz, J_2 = 1.7 Hz, 1 H), 4.61 (dd, J_1 = 10.9 Hz, J_2 = 9.2 Hz, 1 H), 4.45 (td, J_1 = 9.0 Hz, J_2 = 1.8 Hz, 1 H), 4.31 (m, 1 H), 3.36 (m, 2 H), 3.25 (m, 2 H), 2.64 (m, 2 H), 2.58-2.49 (m, 1 H), 2.27 (m, 5 H), 2.16 (m, 2 H), 1.67 (m, 4 H), 1.33 (m, 12 H). ^{13}C-NMR (100.6 MHz, CD_3OD) δ 176.4, 174.9, 170.5, 144.9, 143.4, 130.7, 119.7, 115.5, 115.0, 65.8, 35.8, 35.4, 34.6, 33.6, 30.2, 29.1, 28.8, 28.3, 25.4, 25.1, 24.7, 23.4, 19.5, 12.6. FTIR ν 3314*w*, 2925*m*, 2853*m*, 1776*m*, 1641*s*, 1547*s*, 1448*m*, 1378*m*, 1260*s*, 1173*s*,

1113*s*, 1015*s* cm^{-1}. HRMS (+*ESI*) calcd. for $[C_{24}H_{36}N_2O_6]^+$: 449.2652. Found: 449.2638.



2.4 Hybrid 2: To a solution of dodecanedioic acid (69.2 mg, 0.3 mmol) in THF/DMSO (7.0 mL/0.4 mL) were added EDC (116.6 mg, 0.6 mmol, 2.0 equiv) and NHS (69.6 mg, 0.6 mmol, 2.0 equiv). The resulting mixture was stirred overnight at RT. The hemisulfate salt **SC-2** (74.9 mg, 0.15 mmol, 0.5 equiv) and Et_3N (0.13 mL, 0.9 mmol) were then added and stirred for 20 h before addition of HSL hydrobromide (54.6 mg, 0.3 mmol, 1.0 equiv). After another 24 h, the mixture was filtrated and concentrated *in vacuo*. The crude was extracted with EtOAc and water (3x). The organic layer was subsequently washed with brine, dried over Na_2SO_4 and concentrated *in vacuo*. The crude product was purified by chromatography on Sephadex LH-20 (MeOH) giving **2** (107.2 mg, 0.2 mmol, 72 %). Orange solid. Optical rotation $[\alpha]^{24}_D$ (4.9 mg/mL, MeOH) = -7.3°. ^{1}H-NMR (400 MHz, CD_3OD) δ 7.54 (s, 1 H), 6.71 (s, 1 H), 4.58 (t, J = 10.0 Hz, 1 H), 4.43 (td, J_1 = 9.0 Hz, J_2 = 1.8 Hz, 1 H), 4.29 (ddd, J_1 = 10.5 Hz, J_2 = 9.0 Hz, J_3 = 6.5 Hz, 1 H), 3.43 (t, J = 7.0 Hz, 2 H), 3.31 (dt, J_1 = 3.3 Hz, J_2 = 1.6 Hz, 2 H), 3.02 (t, J = 6.9 Hz, 2 H), 2.58-2.49 (m, 1 H), 2.30-2.19 (m, 5 H), 2.13 (t, J = 7.6 Hz, 2 H), 1.66-1.50 (m, 4 H), 1.39-1.22 (m, 12 H). ^{13}C-NMR (100.6 MHz, CD_3OD) δ 177.7, 176.3, 174.9, 152.2, 145.3, 141.7, 129.3, 119.3, 113.4, 67.1, 49.9, 40.7, 37.2, 36.7, 34.9, 34.2, 30.5, 30.4, 30.3, 30.2, 30.1, 29.7, 27.0, 26.8, 26.3, 26.1. FTIR ν 2921*m*, 2852*m*, 2488*w*, 2437*w*, 1776*m*, 1697*m*, 1636*s*, 1526*m*, 1467*m*, 1287*s*, 1222*m*, 1173*s*, 1007*s*, 946*m* cm^{-1}. HRMS (+*ESI*) calcd. for $[C_{24}H_{36}N_3O_8]^+$: 494.2497. Found: 494.2487.



2.5 Hybrid 3: To a solution of anachelin chromophore (16.4 mg, 0.053 mmol) in DMSO (0.6 mL) were added NHS (11.1 mg, 0.095 mmol, 1.8 equiv) and DCC (15.5 mg, 0.075 mmol, 1.4 equiv). The resulting mixture was stirred overnight at RT. 12-Aminolauric acid (12.0 mg, 0.056 mmol, 1.1 equiv) and Et_3N (5 drops) were then added and the reaction mixture was stirred for 24 h. DCC (33.0 mg, 0.160 mmol, 3.0 equiv) and HSL (42 mg, 0.231 mmol, 4.4 equiv) were added and the resulting mixture was stirred for further 24 h. Concentration in vacuo and purification by C18 reverse phase chromatography (water) yielded **3** (22.3 mg, 0.021 mmol, 40 %). Orange solid. Optical rotation $[\alpha]^{24}_{D}$ (1.25 mg/mL, MeOH) = -3.6°. ^{1}H-NMR (400 MHz, CD_3OD) δ 7.20 (s, 1 H), 6.64 (s, 1 H), 4.59 (m, 1 H), 4.46 (m, 2 H), 4.33 (m, 3 H), 3.69 (m, 6 H), 3.22 (m, 3 H), 2.54 (m, 5 H), 2.31 (m, 3 H), 2.26 (m, 6 H), 2.07 (m, 6 H), 1.88 (m, 6 H), 1.69 (m, 3 H), 1.46 (m, 2 H). ^{13}C-NMR (100.6 MHz, CD_3OD) δ 179.1, 174.8, 174.1, 156.0, 119.1, 115.3, 106.8, 66.0, 57.7, 56.6, 55.4, 54.0, 53.3, 51.3, 46.6, 39.1, 32.6, 31.8, 29.0, 28.4, 27.9, 25.2, 24.9, 24.7, 7.9. FTIR ν 3510, 3387, 3032, 2940, 2855, 2596, 2291, 1759, 1705, 1647, 1555, 1508, 1308, 1211, 1018 cm^{-1}. HRMS (+*ESI*) calcd. for $[C_{31}H_{49}N_4O_7]^+$: 589.3601. Found: 589.3619.

3. Functionalized Beads Ti-2

The particles were cleaned using toluene (HPLC quality), 2 x 10 min ultrasonication and 2-propanol (HPLC quality), 2 x 7 min ultrasonication, dried under a nitrogen stream and finally put overnight in an oven at 120 °C. 50 mg cleaned particles (99.6 %, ~325 mesh powder, Alfa Aesar) were placed into glass vials with 3 mL of MOPS buffer and 1.5 mg of the hybrid **2**. After 4 h incubation at 50 °C, the resulting particles were centrifuged for 5.5 min at 4'500 rpm, and the aqueous layer was decanted. The resulting particles were washed with water and dried with a stream of nitrogen. Further washing with water was carried out the same way (particles suspended in H_2O for 5-20 min, and centrifugation 10-30 min at 4'500 rpm).

4. Biological Evaluation

4.1 Sensor strains

Pseudomonas putida **Iso F117** (*putI*-knockout) **pAS-C8**;[3] highly sensitive towards OHL (*N*-octanoyl-L-homoserine lactone), less sensitive for DHL (*N*-decanoyl-L-homoserine lactone), almost no senstitivity for BHL (*N*-butyryl-L-homoserine lactone). The quorum-sensing regulated promoter p_{cepI} is from *Burkholderia cenocepacia*, and controls expression of green-fluorescent protein (GFP). Plasmid-encoded resistance to gentamycin.

Pseudomonas putida **Iso F117** (*putI*-knockout) **pKR-C12**;[3] highly sensitive towards Od-DHL (*N*-(3-oxo-decanoyl)-L-homoserine lactone), almost no senstitivity for shorter AHLs. The quorum-sensing regulated promoter p_{lasB} is from *Pseudomonas aeruginosa*, and controls expression of GFP. Plasmid-encoded resistance to gentamycin.

Culture conditions: The sensor strains were initially cultivated on LB-agar plates with gentamycin (20ug/ml). Single colonies were suspended in 5ml LB (Luria-Bertani broth) for overnight cultivation (with gentamycin). These stationary phase starter cultures were diluted 1:100 into fresh LB and grown to mid logarithmic phase (OD600nm = 0.8) at 30°C with shaking (Infors HT incubator, Bottmingen, Switzerland). At this point, cultures were used for inoculation with test compounds.

4.2 Assessment of the biological activity of functionalyzed AHLs (assay / microscopy)

The TiO_2-conjugated AHLs were suspended as a 10% slurry (w/v) in 1:1 DMSO:H_2O. Initial sensor activation experiments were conducted with a final concentration range of 0.05% - 0.25% conjugated beads added to mid-log phase sensor cultures (a total of 200ml culture volume). After these initial tests, a final conjugated beads concentration of 0.1% was used throughout for further activation assays and for microscopy.

Culture/ beads suspensions were incubated in black lidded 96-well microtiter plates (Nunc, Denmark), in the dark at 30°C for two hours, and induction of GFP fluorescence was assessed by spectrofluorimetry, with excitation and emission wavelengths at 485nm at 520nm, respectively (Microplate reader, BioTek Instruments, VT, USA) or by fluorescence microscopy (DM6000B, Leica, Germany).

Positive control: OHL (10nM – 1mM, diluted from 10mM stock solutions in ethylacetate) was incubated with the sensor cultures in the same way.

Negative control: Sensor cultures with corresponding aliquots of 1:1 DMSO:H_2O or ethylacetate.

4.3 Sequential washing of the beads and biological activity evaluation

The beads were washed up to 10 times sequentially as described in 3 above. The resulting washed beads were tested for their biological activity as described in 4.2.

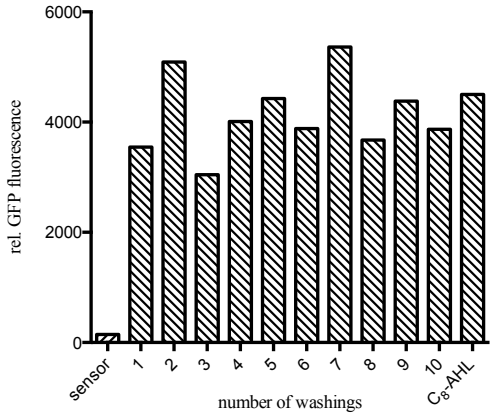


Fig. S1 Activity of incubated beads **Ti-2** (9.5 mg/mL) after washing with water compared with C8-AHL (0.1 µM).

4.4 Investigation of slow release (dialysis experiments)

Dialysis experiments were conducted to assess the chemical stability of the conjugated TiO_2-beads in the presence and absence of *P. putida* pAS-C8 sensor cells. Briefly, 1% suspensions of conjugated beads were prepared in either i) 1ml PBS pH 6.5, or ii) phosphate-bufferd LB medium containing 10^5 - 10^6 CFU/ml sensor cells and placed inside 1ml-capacity dialysis tubes (SpectraPor „Float-a-lyzer“, 1ml, 3.5-5kDa MWCO). The dialysis tubes were tightly capped and were then allowed to float freely inside culture flasks containing 30ml sterile LB supplemented with 20mg/ml gentamycin. As a positive control, 10mM OHL in 1ml PBS pH6.5 was dialysed under the same conditions.

The culture flasks were placed in a slow-shaking incubator in the dark at 30°C. Aliquots were removed from the LB dialysis medium at various time intervals during incubation and stored at -20°C. After the final dialysis period of 16 hours, the frozen aliquots were thawed and used to each resuspend ca. 10^8 CFU/ml uninduced sensor cells. Induction of GFP fluorescence was subsequently assayed as described in 4.2 above.

4.4.1 Positive control

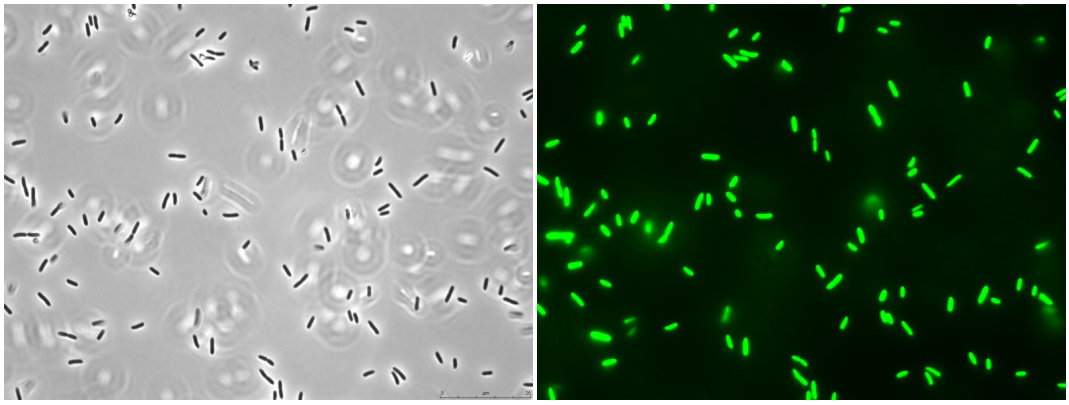


Fig. S2 Phase contrast (left) and GFP fluorescence (right) of the sensor dialyzed with C8-AHL (0.3 µM).

4.4.2 Dialysed hybrid beads Ti-2

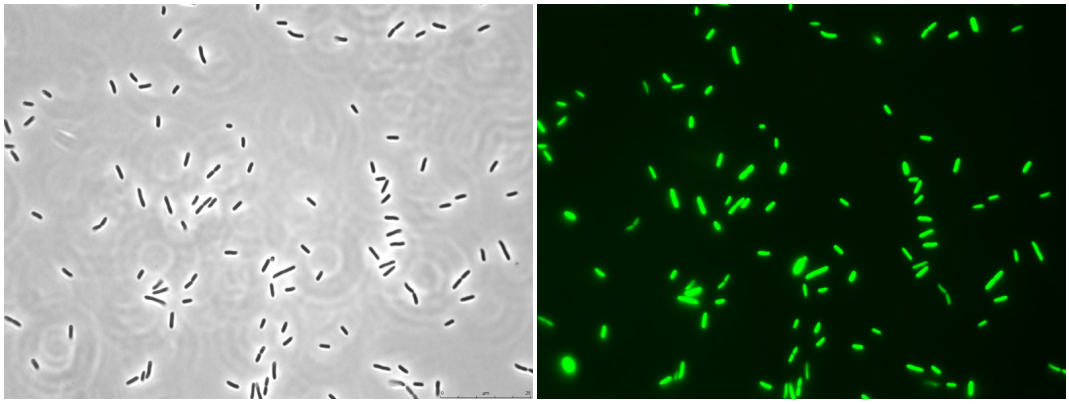


Fig. S3 Phase contrast (left) and GFP fluorescence (right) of the sensor dialyzed with TiO_2 beads (0.6 mg/mL).

4.4.3 Negative control

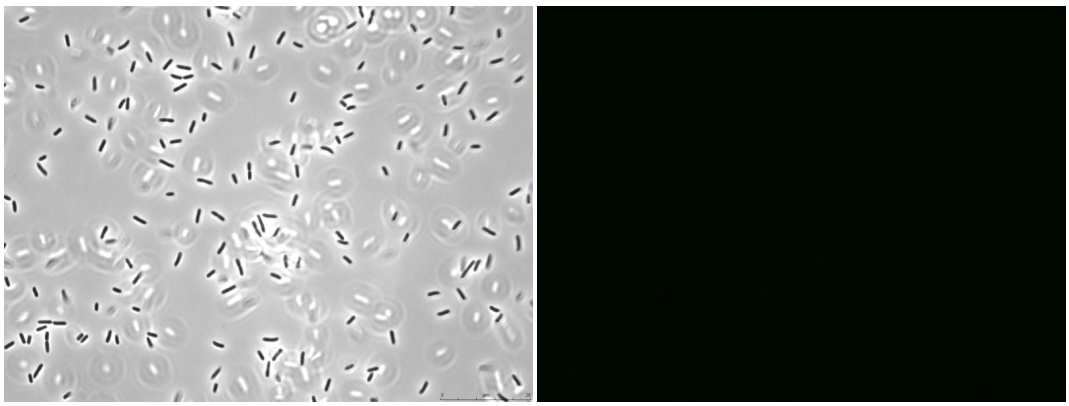


Fig. S4 Phase contrast (left) and GFP fluorescence (right) of the sensor alone.

5. Quantitative Experiments

5.1 Dependence of P. putida pAS-C8 fluorescence on C8-AHL concentration

Induction of GFP fluorescence was assayed as described in 4.2 using different concentrations of C8-AHL. The concentration dependent fluorescence is plotted in a logarithmic (upper) and linear (lower) scale. Each data point is an average of four measurements.

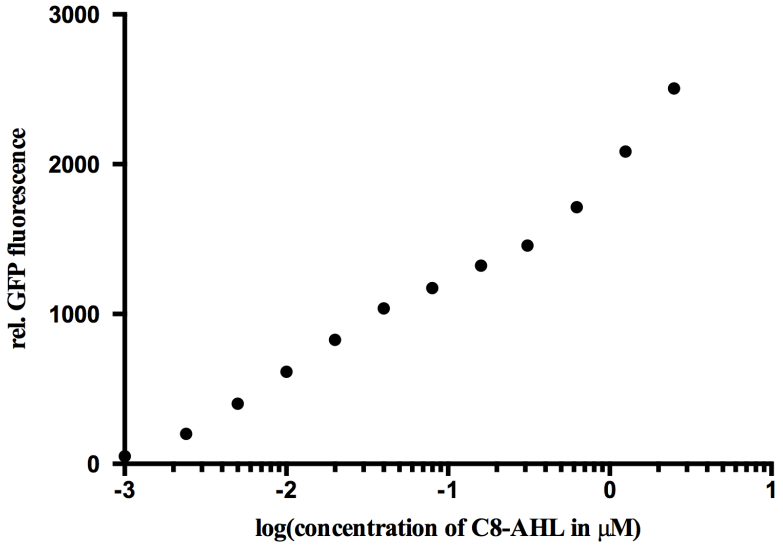


Fig. S5 Correlation between GFP-activity and concentration of C8-AHL (logarithmic scale).

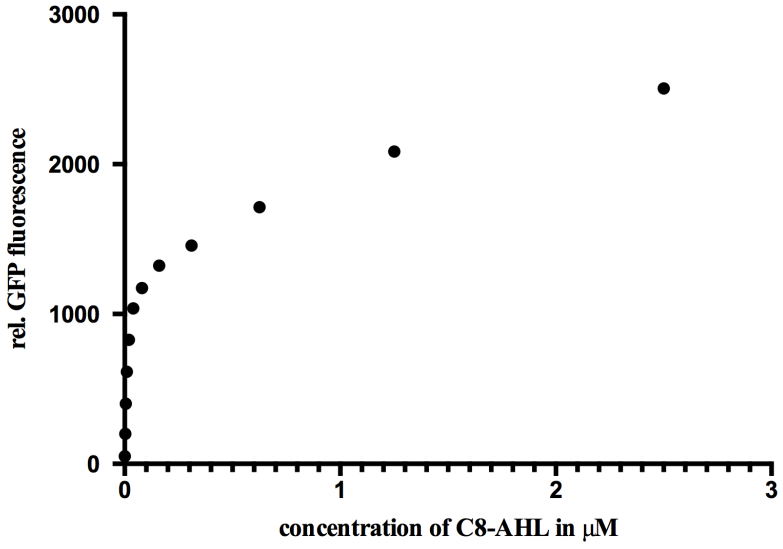


Fig. S6 Correlation between GFP-activity and concentration of C8-AHL (linear scale).

5.2 Calculated concentrations during dialysis assays

Time	0 h	1 h	2 h	4 h	5 h	6 h	8 h	16 h
Concentration	2.5 nM	3.8 nM	4.4 nM	5.6 nM	8.8 nM	9.5 nM	15.1 nM	270.4 nM

The assay was performed following the conditions described in 4.3 and quantification was determined using the calibrating results described in 5.1.

6. References

[1] K. Fujimoto, N. Oimoto, K. Katsuno, M., Inouye, *Chem. Comm*. 2004, **11**, 1280-1281.

[2] S. Knaggs, H. Malkin, H. M. I. Osborn, N. A. O. Williams, P. Yaqoob, *Org. Biomol. Chem*. 2005, **3**, 4002-4010.

[3] A. Steidle, K. Sigl, R. Schuhegger, A. Ihring, M. Schmid, S. Gantner, M. Stoffels, K. Riedel, M. Givskov, A. Hartmann, C. Langebartels, L. Eberl, *Appl. Environ. Microbiol.* 2001, **67**, 5761-5770.