Two-Way Chemical Communication between Artificial and Natural Cells

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MATERIAL AND METHODS

**Bacterial strains and media.** Strains used in this study are listed in Table S4. *E. coli* and *P. aeruginosa* were grown in LB. *V. fischeri* was grown in LBS (10 g/L tryptone, 5 g/L yeast extract, 20 g/L NaCl, 50mM Tris-HCl, 0.3% glycerol) for experiments and either LBS or photobacterium broth (0.3 g/L NH₄Cl, 1 g/L CaCO₃, 5 g/L casein enzymatic hydrolysate, 0.01 g/L FeCl₃, 0.3 g/L MgSO₄•7H₂O, 3 g/L KH₂PO₄, 30 g/L NaCl, 23.5 g/L sodium glycerophosphate, 2.5 g/L yeast extract) to make glycerol cell stocks. *V. harveyi* was grown in marine broth supplemented with 2% casamino acids when glycerol cell stocks were made or in Autoinducer Bioassay (AB) media (17.5 g/L NaCl, 12.3 g/L MgSO₄•7H₂O, 2 g/L casamino acids, 10 mM potassium phosphate pH 7, 1 mM L-ariginine, 1% (v/v) glycerol) for the chemical communication experiments. When necessary, media were supplemented with antibiotic (100 µg/mL ampicillin, 50 µg/mL kanamycin, or 34 µg/mL chloramphenicol).

**Genetic constructs.** *lsrR, lsrK,* and the intergenic operon region of *lsr* were amplified from the genome of *E. coli* MG1655 by PCR. The gene coding for *Staphylococcus aureus* α-hemolysin (αHL) was synthesized by Genscript. T3 RNA polymerase (BBa_K346000), AiiA (BBa_C0160) and the following devices BBa_K575024, BBa_K575037, and BBa_T9002 were taken from the registry of standard biological parts. The gene encoding firefly luciferase was amplified from pBESTluc (Promega). Sequence information for the exploited constructs can be found in Table S5.

**In vitro transcription-translation.** The constructs encoding parts of the *E. coli* AI-2 quorum pathway were expressed with the PURE system (New England BioLabs). Each reaction contained
10 µL solution A, 7.5 µL solution B, 20 U RNase inhibitor, and 250 ng DNA, unless specifically stated otherwise. When needed, between 0 µM and 250 µM AI-2 or 0.5 mM of S-adenosyl-L-homocysteine (SAH) was added to the solution. The constructs designed to express parts of the quorum pathways of *P. aeruginosa* and *V. fischeri* used either a cell-free *E. coli* S30 extract for circular DNA (Promega) (20 µL premix, 15 µL S30 extract, 5 µL amino acids mix, 40 U of RNase inhibitor, and 2 µg of DNA) or the *E. coli* S30 T7 High Yield Protein Expression System (Promega) (20 µL S30 premix, 18 µL T7 S30 extract, 40 U of RNase inhibitor, and 1 µg of DNA). When needed, 10 µM of 3OC12 HSL, C8 HSL, 3OC6 HSL, or C4 HSL (Sigma Aldrich or Cayman Chemical) were added to induce expression, or 300 µM of acetyl coenzyme A and 0.5 mM of S-adenosyl-L-methionine (SAM) were added for the synthesis of *N*-Acyl homoserine lactones. Reactions were incubated at 37 °C for 4 h to 6 h. In vitro reactions assembled for the sensing of 3OC12 HSL were shaken (50 s orbital shaking, 1 mm orbital amplitude prior to fluorescence acquisition every min) in 384 microwell plates (781076 Greiner Bio One) during incubation in an Infinite m200 plate reader (Tecan). Negative controls were the same reactions in the absence of quorum molecules. Fluorescence was either measured with a Photon Technology International (PTI) QuantaMaster 40 UV–vis spectrofluorometer or a CFX96 Touch Real-Time PCR Detection System (Bio-Rad).

**Enzymatic production of AI-2.** AI-2 was enzymatically produced *in vitro* with 12 µM of the purified fusion protein HLPT\(^1\) and 5 mM of S-adenosyl-L-homocysteine. Reactions were incubated overnight at 37 °C, 220 RPM. The protein was then removed by two chloroform extractions. The aqueous phase contained AI-2. Since the enzymatic reaction produces in a 1:1 ratio AI-2 and homocysteine, AI-2 was indirectly quantified using 5,5'-dithio-bis(2-nitrobenzoic acid) (DTNB).
The solution for quantification contained 10 µL of sample, 100 µL of Tris-HCl, pH 8, 50 µL of 2 mM DTNB, 50 µM sodium acetate, and 840 µL of water. After 5 min of incubation at room temperature, the absorbance at 412 nm was measured and the concentration of homocysteine was calculated by using the molar extinction coefficient (13,600 M⁻¹ cm⁻¹) of the reaction product 5-thio-2-nitrobenzoic acid (TNB).

**Vesicle stability.** To test whether bacteria could break phospholipid vesicles, a dye leakage assay was performed. Dehydrated aliquots of 1:2 POPC:cholesterol vesicles were hydrated with 60 µL S30 *E. coli* extract for circular DNA template (Promega) supplemented with 4 µg of DNA (RL081A), 1 mM S-adenosyl methionine, 700 µM acetyl coenzyme A, and 80 mM calcein (Sigma). The vesicles were then extruded through a polycarbonate membrane with 1 µm pores (Whatman) with an Avanti mini-extruder and purified with a sepharose 4B (Sigma-Aldrich) column. Fluorescence was monitored with excitation and emission at 495 nm and 515 nm, respectively. Subsequently, 0.3% (v/v) Triton X-100 was added as a control to break the vesicles.

**The effect of cholesterol on chemical communication.** Dehydrated aliquots of POPC vesicles with either 0 mol%, 10 mol%, or 66 mol% cholesterol were hydrated with 50 µL S30 *E. coli* extract for circular DNA template (Promega) supplemented with 4 µg of DNA (NY013A), 1 mM S-adenosyl-L-methionine, and 700 µM acetyl coenzyme A. The experiments were run as described below in the cellular Turing test section.
**Artificial cells that sense 3OC6 and C8 HSL.** Aliquots of 1:2 POPC:cholesterol vesicles were formed as previously described. 100 µL aliquots were rehydrated with 50 µL of S30 *E. coli* extract containing 20 µL S30 premix, 15 µL S30 extract, 5 µL amino acids mixture, 40 U of RNase inhibitor, and 4 µg of DNA (RL082A, RL093A or RL094A, see Table S5 for sequences). Vesicles were diluted 1:1 with LB supplemented with 0.7 mg/mL proteinase K, 0.07 mg/mL RNase A, and 170 U/mL RNase T1 (Thermo Fisher Scientific) to remove any residual activity in the extravesicular solution. Reactions were incubated at 30 °C for 4 h. For sensing of externally added 3OC6 HSL, 1 µM of synthetic 3OC6 HSL or C8 HSL (Cayman Chemical) was added to the artificial cells. For the sensing of *V. fischeri*, bacteria were first grown from 200 µL of a glycerol stock at 30 °C in LBS until OD\text{600 nm} = 1.8. The bacteria were then pelleted, and the supernatant filtered through a 0.2 µm membrane (Sartorius). The supernatant (100 µL) was then mixed with 50 µL of artificial cells. LBS was used as a negative control in place of the *V. fischeri* supernatant. Samples were incubated at 30 °C for 4 h, then the artificial cells were collected and loaded into 96 well plates. 0.3% (v/v) Triton X-100 was added to break the vesicles, and 150 µL of the luciferase assay reagent (Promega) was added to the samples. Luminescence was recorded immediately with a plate reader (Tecan).

**Artificial sender cells.** Dehydrated aliquots of 1:2 POPC:cholesterol vesicles were hydrated with 50 µL of S30 T7 High-Yield Protein Expression System supplemented with 2 µg of DNA encoding the corresponding synthase behind a T7 promoter (constructs MC001A, MC002A, MC003A, NY018A, NY019A and JF005A, Table S5). For the production of acyl homoserine lactones, 1 mM S-adenosyl-L-methionine and 700 µM acetyl coenzyme A were added. The
production of AI-2 required 1 mM S-adenosyl-L-homocysteine. For the experiment corresponding to Figure 2A, artificial cells were diluted 1:3 in buffer A (50 mM HEPES, 10 mM MgCl₂, 100 mM KCl, pH 7.6) plus 0.7 mg/mL proteinase K. Reactions were incubated at 37 °C for 6 h. In the meantime, the corresponding E. coli reporter strain was grown from one colony in LB supplemented with antibiotic until OD₆₀₀ nm = 0.5. Bacteria were pelleted, resuspended in fresh LB, and added to artificial cells to a final OD₆₀₀ nm = 0.1. Samples were incubated at 37 °C. Aliquots were removed every hour, diluted in PBS, and monitored by flow cytometry with a FACS canto A (BD biosciences). Positive controls contained 0.1 µM of the corresponding quorum molecule. LB was added in place of quorum molecules for the negative control. Parameters for each flow cytometry experiment were: Forward scatter (FSC) signal (Ex.: 488 nm, Type: Area, Voltage: 525); Side scatter (SSC) signal (Ex.: 488 nm, Em.: 488 +/- 10 nm, Type: Area, Width, Voltage: 403)’ Green channel (FITC) signal (Ex.: 488 nm, Em.: 530 +/- 30 nm, Type: Area, Voltage: 600); Threshold parameters (FCS: 200, SSC: 200, Threshold operator: And). For the experiment corresponding to Figure 2B, artificial sender cells were diluted 1:1 with V. fischeri MJ11 at OD₆₀₀=0.2-0.3 and 0.7 mg/mL Proteinase K, 0.07 mg/mL RNase A, and 170 U/mL RNase T1 (Thermo Fisher Scientific) were also added to avoid any residual activity of the S30 reactions outside the artificial cells. Samples were incubated at 30 °C in 96-well plates (Thermo Fischer Scientific, 216305) without shaking. Every hour luminescence was measured with an Infinite M200 plate reader (Tecan). After 3 h of incubation, 5 µL of each sample were serially diluted and 10 µL of the 10⁻⁵ fold dilution were plated on LBS agar following the “track dilution” method to enumerate the colony forming units (CFU) with one plating per sample per experiment. Pictures from luminescent bacteria colonies were captured in a dark room with an Olympus OM-D EM5 camera and a M-Zuiko ED
12-50 mm 1:3.5-6.3 EZ lens using an exposure of 40 s at F 5 and an ISO of 200. Negative control were liposomes encapsulating the S30 extract without DNA. For the experiment corresponding to Figure 2C, AI-2 was detected with V. harveyi BB170, a strain that can naturally sense AI-2 but has been engineered to not sense its own autoinducer N-(3-oxobutanoyl)homoserine lactone. The assay was performed as described by Vilchez et al. Briefly, V. harveyi was grown overnight from a 200 µL glycerol stock in AB medium supplemented with 50 µg/mL kanamycin at 30 °C, 220 RPM. The day after, bacteria were diluted to OD\textsuperscript{600 nm} = 0.7 and grown for 1.5 h (to OD\textsuperscript{600 nm} = 1.1) in AB medium. The culture was then diluted 1:5000 and 90 µL were loaded into a white 96-well plate (Nunc). 10 µL of sample was added to each well. AB medium was used as a blank, and 100 µM of enzymatically produced AI-2 was used as a positive control. AI-2 activity was calculated by dividing the sample value by the blank. Plates were incubated at 30 °C and luminescence was monitored with Infinite M200 plate reader plate reader (Tecan) after 3 h.

**Artificial cells that mediate communication with two different cell types.** E. coli reporter strains were grown to OD\textsuperscript{600 nm} = 0.5 from one colony in LB supplemented with antibiotic. Bacteria were pelleted and resuspended in fresh LB. Dehydrated aliquots of 1:2 POPC cholesterol vesicles were hydrated with 50 µL E. coli S30 extract for circular DNA supplemented with 4 µg of DNA encoding a 3OC6 HSL sensing device and the corresponding synthase (NY016A, RL079A, or RL080A, Table S5). 1 mM of S-adenosyl-L-methionine and 700 µM of acetyl coenzyme A were added for the synthesis of acyl homoserine lactones and 1 mM of SAH for the production of AI-2. When commercial 3OC6 HSL was used, 1 µM of 3OC6 HSL was added to 50 µL of artificial cells mixed with 50 µL of the corresponding E. coli reporter strain at a final OD\textsuperscript{600 nm} = 0.1. 0.7 mg/ mL
of proteinase K were added, and the samples were incubated at 37 °C. Aliquots were collected after every hour, diluted in PBS, and monitored by flow cytometry. When the presence of _V. fischeri_ 7744 was sensed, bacteria were grown at 28 °C in LBS supplemented with ampicillin until OD$_{600}$ = 1.2. Cells were harvested and the supernatant mixed with 50 µL of artificial cells and 50 µL of the _E. coli_ reporter strain. Samples were incubated at 37 °C. Aliquots were collected each hour, diluted in PBS, and monitored by flow cytometry. Positive controls contained 0.1 mM of 3OC12 HSL. Negative controls were in the absence of supplemental quorum molecules or _V. fischeri_ supernatant. Unspecific fluorescence was determined by adding the supernatant of _V. fischeri_ to the _E. coli_ reporter strain. Samples collected at 6 h were analyzed with BD FACSDiva software. For AI-2 synthesis, samples were mixed with _V. harveyi_ BB170, following the protocol described above for the _V. harveyi_ bioluminescence assay.

**Quorum quenching.** Dehydrated aliquots of 1:2 POPC:cholesterol vesicles were hydrated with 50 µL of S30 _E. coli_ extract supplemented with 4 µg of DNA encoding AiiA behind a tet promoter or a 3OC6-C8 HSL responsive-AiiA production device. 1 U of DNaseI (Thermo Fisher Scientific), 0.07 mg/mL RNaseA, and 170 U/mL of RNase T1 (Thermo Fisher Scientific) were added to the extravesicular solution to remove any residual activity of the S30 _E. coli_ extract outside of the artificial cells. When necessary, 1 µM of 3OC6 HSL and 1 µM of C8 HSL or an aliquot of a _V. fischeri_ culture at OD$_{600 \text{ nm}}$ = 0.2 were added to the outside of the vesicles. Reactions were incubated at 37 °C for 4 h. _P. aeruginosa_ was grown from 200 µL of a glycerol stock in LB until OD$_{600 \text{ nm}}$ = 0.3 and added to artificial cells in a 1:1 ratio. After 2 h of incubation at 37 °C at 220 RPM, cells were harvested and the supernatants mixed 1:20 with the 3OC12 HSL _E. coli_ sensor strain. Samples
were incubated at 37 °C. 2 μL were collected every hour, diluted 1:100 in PBS, and monitored by flow cytometry. Controls were performed by adding to the reporter strain a culture of *P. aeruginosa* grown in LB and with *V. fischeri* supernatant to monitor unspecific interaction. The positive control was the addition of 0.1 μM 3OC12 HSL to the *E. coli* reporter strain. Negative controls included samples without added quorum molecules. Samples were collected at 2 h and analyzed with BD FACSDiva software. The population distribution was analyzed and plotted with FlowJo software.

**Cellular Turing test.** Dehydrated aliquots of 1:2 POPC:cholesterol vesicles³ were hydrated with 50 μL S30 *E. coli* extract for circular DNA template (Promega) supplemented with 4 μg of DNA, 1 mM S-adenosyl-L-methionine, and 700 μM acetyl coenzyme A. 200 μL glycerol stock of exponential phase *V. fischeri* MJ11 were grown in 5 mL of LBS (30 °C, 145 rpm) until OD⁶⁰₀nm = 0.2-0.3. Cells were undiluted or mixed in a 1:1 ratio with either functional artificial cells encapsulating DNA plasmids (RL078A, NY009A, NY013A, or NY014A) coding for the different versions of *luxR* and the *luxI* or nonfunctional artificial cells containing DNA plasmid (RL081A) coding for *luxR* and T7 RNA polymerase. Extravesicle solutions contained 0.7 mg/mL Proteinase K, 0.07 mg/mL RNase A, and 170 U/mL RNase T1 (Thermo Fisher Scientific) to avoid any residual activity of the S30 *E. coli* extract outside of the artificial cells. Samples were incubated at 30 °C in 96-well plates (Thermo Fischer Scientific, 216305) without shaking. Every hour luminescence was measured with an Infinite M200 plate reader (Tecan). After 3 h of incubation, 5 μL of each sample were serially diluted and 10 μL of the $10^{-5}$ fold dilution were plated on LBS agar following the “track dilution” method to enumerate the colony forming units (CFU)⁶ with one plating per
sample per experiment. Pictures from luminescent bacteria colonies were captured in a dark room with an Olympus OM-D EM5 camera and a M-Zuiko ED 12-50 mm 1:3.5-6.3 EZ lens using an exposure of 40 s at F 5 and an ISO of 200. Noise reduction and an exposure setting of +1.0 EV were applied on the JPEG image files in Adobe Photoshop lightroom CC 2016 prior the cell counting. A crop of one of the resulting images is shown in (Figure S10). Single colonies were then counted manually. Subsequently, the rest of the samples were collected for RNA extraction. Total RNA was isolated with the GeneJET RNA Purification Kit (Thermo Fischer Scientific), and 500 ng of RNA was retro transcribed using the RevertAid Reverse Transcriptase kit (Thermo Fischer Scientific). 5 ng of cDNA was mixed with the iQ SYBR Green supermix (Bio-Rad) and supplemented with the appropriate primers. 10 µL reactions were loaded in 96-well plates (HSP9655 Bio-Rad), and the cDNA was quantified with a CFX96 Touch real-time PCR (Bio-Rad) with SYBR green detection. The real-time PCR run protocol was one initial cycle of denaturation at 95 °C for 3 min followed by 40 cycles of denaturation (95 °C, 10 s) and annealing + extension (60 °C, 30 s) followed by one melt curve cycle (55-95 °C with 0.5 °C, 40 s). The primers to quantify the gene expression of luxA and luxB were luxA FW: 5'-cagagtgttctttcacgggaaat-3' (150 nM), luxA REV: 5'-gggtgctgtcggaataac-3' (150 nM), luxB FW: 5'-attaccacccatccctgt-3' (250 nM), luxB REV: 5'-gtcactaaacaagaatgaagcg-3' (250 nM). Gene expression was normalized to the expression of the malate dehydrogenase (mdh) housekeeping gene that was amplified with the following primers mdh FW: 5'-cactctgtgttatcttaacctct-3' and mdh REV: 5'-acttctgttccgcattttgg-3' (300 nM). Primers were designed with Primer3 software.
For RNA-seq analysis, total RNA was treated with DNase (RapidOut DNA Removal kit, Thermoscientific) prior to RNA quantification with a spectrofluorometric detection method using the Quantum-iT RiboGreen RNA assay kit (Life-Technologies). Library preparation and sequencing were performed at Edinburgh Genomics (Ashworth laboratories, University of Edinburgh). Briefly, libraries were prepared using the TruSeq stranded total RNA-seq kit (Illumina) and the depletion of ribosomal RNAs was accomplished with the RiboZero rRNA removal kit for Gram negative bacteria (Illumina). Libraries were then sequenced on one lane of an Illumina HiSeq2500 in high output mode with v4 chemistry to a length of 125 base paired end. The quality of the raw sequence data was assessed with FastQC\textsuperscript{7}. The average number of reads were 10,397,486, 11,006,173, and 11,077,471 for samples containing functional artificial cells, nonfunctional artificial cells, and no artificial cells, respectively. Reads in FASTQ format were mapped to the \textit{V. fischeri} MJ11 genome reference sequence using bowtie\textsuperscript{8}. Transcripts were assembled with cufflink and cuffmerge and the quantification of isoforms was with cuffdiff\textsuperscript{9–11}. The sample size for the RNA-seq experiments was chosen based on the average number of reads per sample (10M), read length (200bp), preliminary results, and prior reports\textsuperscript{12} showing an effect size of at least two for \textit{lux} operon gene expression from activation by quorum sensing. Therefore, to ensure a statistical power of at least 0.8 at a significance level of 0.05 for a standard two-tailed t-test, the sample size was set to six. Differences in the mean between groups were assessed using an unpaired two-tailed standard t-test. Standard deviations are shown in the bar plots as a measure of variability. RNA-seq differentially expressed genes are determined by cufflinks/cuffdiff after \textit{p} value adjustment for multiple comparisons using FDR (False Discovery Rate). The functional and
clusterization analysis for the three sets of differently expressed coding sequences was with DAVID\textsuperscript{13}. The Benjamini test threshold was set to $10^{-2}$. 
Figure S1. In vitro sensing of quorum molecules. (a) A schematic illustration of the genetic constructs for the sensing of 3OC6 HSL, 3OC6 HSL and C8 HSL, and C8 HSL is illustrated. (b) LuxR controlled in vitro expression of GFP was monitored by fluorescence spectroscopy for the genetic
constructs shown in panel a (n=3 biological replicates, mean ± s.d.). (c) DNA encoding \textit{lasR} for the sensing of 3OC12 HSL controlled the expression of GFP (n=3 biological replicates, mean ± s.d.). (d) The C4 HSL responsive, \textit{rhlI} encoding construct was expressed \textit{in vitro} (n=2 technical replicates). (e) The genetic circuit for sensing AI-2 was expressed with the PURE system (n=2 technical replicates). (f) CRP was added to the AI-2 sensing genetic circuit (n=2 technical replicates). RFU (Relative Fluorescence Units).

\textbf{Figure S2. Artificial cells sense quorum molecules.} Artificial cells carrying genetic constructs for the sensing of quorum molecules (a) were incubated with the corresponding HSL and monitored by luminescence (b) (n=3 technical replicates, mean ± s.d.). RLU/CFU (Relative luminescence Units/ Colony Forming Units per milliliter).
Figure S3. *In vitro* production of quorum sensing molecules. (a) Genetic constructs for the synthesis of 3OC12 HSL, 3OC6 HSL, and C4 HSL were expressed *in vitro*. After 6 h, samples were incubated with *E. coli* sensor strains and quantified by flow cytometry (*n*=3 technical replicates, mean ± s.d.). (b) Genetic constructs expressing the AI-2 synthesizing, HLPT fusion protein were expressed *in vitro*. After 6 h, samples were incubated with *V. harveyi* BB170 and the luminescence (referred to as AI-2 activity) was measured. Samples were normalized against reactions without a DNA template (*n*=3 technical replicates, mean ± s.d.).
Figure S4. Artificial cell leakage assay. (a) Artificial cells supplemented with the self-quenching fluorophore calcein were incubated with different bacteria and monitored by fluorescence spectroscopy for 6 h. (b) E. coli, V. fischeri, and V. harveyi did not degrade the artificial cells under the same test conditions used for the chemical communication experiments, whereas the presence of P. aeruginosa compromised the integrity of the membrane. * indicates the addition of 0.3% (v/v) Triton X-100. RFU (Relative Fluorescence Units).
Figure S5. Artificial cells failed to produce AI-2 in response to quorum sensing molecules. (a) Artificial cells carrying a genetic construct to produce AI-2 in response to 3OC6 HSL and C8 HSL were incubated at 30 °C for 6 h. (b) Artificial cells were then mixed with V. harveyi BB170 and luminescence was monitored after 3 h (n=3 technical replicates, mean ± s.d.).
Figure S6. Artificial cells quench *P. aeruginosa* quorum sensing. (a) *In vitro* expressed AiiA was sufficient to degrade the 3OC12 HSL released by *P. aeruginosa*. 3OC12 HSL levels were assessed by flow cytometry of an *E. coli* reporter strain (n=3 technical replicates, mean ± s.d.). (b) Artificial cells carrying the same construct in panel a were capable of degrading *P. aeruginosa* secreted 3OC12 HSL (n=3 biological replicates, mean ± s.d.).
Figure S7. Screening of genetic constructs for the cellular Turing test. (a) A schematic illustration of the four genetic constructs tested for their ability to sense and produce 3OC6 HSL. (b) Luminescence data were acquired after 3 h of incubation of artificial cells with V. fischeri (n=1). (c) The number of viable cells per sample was determined by measuring the colony forming units (n=1). RLU/CFU (Relative Luminescence Units/Colony Forming Units per milliliter), CFU/mL (Colony Forming Units per milliliter).
Figure S8. Influence of cholesterol on chemical communication. Artificial cells with different membrane compositions (POPC, 10:1 POPC:cholesterol, 1:2 POPC:cholesterol) containing DNA encoding LuxR* and LuxI* (NY013A) was incubated with _V. fischeri_. (a) The luminescent response per single cell of _V. fischeri_ after 3 h showed a clear dependence on the concentration of cholesterol. (b) The number of viable _V. fischeri_ cells per sample was determined by measuring the colony forming units. The negative control was an unencapsulated S30 reaction containing the same DNA and necessary components for transcription-translation. RLU/CFU (Relative Luminescence Units/Colony Forming Units per milliliter), CFU/mL (Colony Forming Units per milliliter).
Figure S9. RNA sequencing data. (a) The correlation of *V. fischeri* gene expression in response to nonfunctional and functional artificial cells was highly correlated, $r=0.99$. Blue dots depict the genes falling off the correlation trend, including six out of the seven genes of the *lux* operon. (b) Distribution of the difference in FPKM per coding sequence between *V. fischeri*–*V. fischeri* with *V. fischeri*–functional artificial cells (green) and *V. fischeri*–*V. fischeri* with *V. fischeri*–nonfunctional artificial cells (black). FPKM (fragments per kilobase of transcript per million mapped reads), CDS (Coding DNA Sequences).
Figure S10. Determining the viable *V. fischeri* count for the cellular Turing test experiments. (a) A schematic illustration of the samples used for CFU enumeration. The number of cells was determined by track dilution with one plating per sample per experiment. (b) A representative picture of a plated sample of bacterial colonies used to calculate CFU. (c) No significant differences in number of viable cells were observed among the samples. (*n*=6 biological replicates, mean ± s.d.). CFU/mL (Colony Forming Units per milliliter).
SUPPORTING TABLES

Table S1. Enrichment analysis of the 81 differently expressed coding sequences that were commonly found for *V. fischeri* + functional artificial cells and *V. fischeri* + nonfunctional artificial cells with respect to *V. fischeri* + *V. fischeri*. Highlighted in grey are the significantly enriched ten gene sets with $FDR < 10^{-2}$. 
| Category       | Term                  | Count | %     | PValue        | Genes                                                                 | List Total | Pop Hits | Pop Total | Fold Enrichment | Bonferroni | Benjamini | FDR     |
|----------------|-----------------------|-------|-------|---------------|----------------------------------------------------------------------|------------|----------|-----------|-----------------|------------|------------|---------|
| GOTERM_BP_FAT  | GO:00065 25-arginine metabolic process | 8     | 10    | 3.3E-09       | 5096830, 5094984, 5098318, 5095180, 5093155, 5097146, 5095530, 5098218 | 46         | 17       | 2757      | 28.2            | 4.92E-07   | 4.92E-07   | 3.91E-06 |
| GOTERM_BP_FAT  | GO:000001 03-sulfate assimilation | 6     | 7.5   | 4.99E-08      | 5096365, 5094445, 5099740, 5100074, 5099138, 5096397                 | 46         | 8        | 2757      | 44.95           | 7.48E-06   | 3.74E-06   | 5.96E-05 |
| KEGG_PATHWAY  | vfm00920 :Sulfur metabolism | 7     | 8.75  | 3.251E-07     | 5096365, 5094445, 5096432, 5099740, 5099138, 5096397                 | 40         | 14       | 1634      | 20.43           | 1.69E-05   | 1.69E-05   | 3.16E-04 |
| SP_PIR_KEY_WORDS | amino acid biosynthesis | 9     | 11.2  | 7.588E-07     | 5099805, 5096830, 5098318, 5097146, 5096342, 5099740, 5099138, 5096397 | 80         | 68       | 7033      | 11.64           | 4.25E-05   | 4.25E-05   | 7.49E-04 |
| KEGG_PATHWAY  | vfm00330 :Arginine and proline metabolism | 9     | 11.2  | 9.292E-07     | 5096830, 5094984, 5098318, 5095180, 5093155, 5097146, 5095530, 5098218 | 40         | 36       | 1634      | 10.21           | 4.83E-05   | 2.42E-05   | 9.03E-04 |
| SP_PIR_KEY_WORDS | arginine biosynthesis | 5     | 6.25  | 1.781E-06     | 5096830, 5098318, 5097146, 5095530, 5098218                         | 80         | 9        | 7033      | 48.84           | 9.97E-05   | 4.99E-05   | 1.76E-03 |
| KEGG_PATHWAY  | vfm00330 :Arginine and proline metabolism | 9     | 11.2  | 1.794E-06     | 5096830, 5094984, 5098318, 5095180, 5093155, 5097146, 5095530, 5098218 | 40         | 39       | 1634      | 9.43            | 9.33E-05   | 3.11E-05   | 1.74E-03 |
| GOTERM_BP_FAT  | GO:00090 64-glutamine family amino acid metabolic process | 8     | 10    | 3.955E-06     | 5096830, 5094984, 5098318, 5095180, 5093155, 5097146, 5095530, 5098218 | 46         | 43       | 2757      | 11.15           | 5.93E-04   | 1.98E-04   | 4.72E-03 |
| GOTERM_BP_FAT  | GO:00065 | 5     | 6.25  | 4.139E-06     | 5096830, 5094984, 5098318, 5095180, 5093155, 5097146, 5095530, 5098218 | 46         | 8        | 2757      | 37.46           | 6.21E-04   | 1.55E-04   | 4.94E-03 |
| Category     | Term                                      | Count | %     | PValue  | Genes | List | Pop Hits | Pop Total | Fold Enrichment | Bonferroni | Benjamini | FDR    |
|--------------|-------------------------------------------|-------|-------|---------|-------|------|----------|-----------|-----------------|------------|------------|--------|
| _FAT         | 26-arginine biosynthetic process           |       |       |         | 5098318, 5097146, 5095530, 5098218 | 46    | 57   | 2757     | 8.41     | 4.09E-03 | 8.20E-04 | 3.26E-02 |
| GOTERM_BP    | GO:000067 90-sulfur metabolic process      | 8     | 10    | 2.73E-05| 5099805, 5096365, 5094445, 5096432, 5099740, 5100074, 5099138, 5096397 | 46    | 110  | 2757     | 5.45     | 7.33E-03 | 1.22E-03 | 5.85E-02 |
| GOTERM_BP    | GO:000866 52-cellular amino acid biosynthetic process | 10    | 12.5  | 4.90E-05| 5099805, 5096830, 5098318, 5094445, 5097146, 5096432, 5095530, 5098218, 5099138, 5096397 | 46    | 113  | 2757     | 5.3      | 9.07E-03 | 1.30E-03 | 7.25E-02 |
| GOTERM_BP    | GO:00093 09-amine biosynthetic process     | 10    | 12.5  | 6.08E-05| 5099805, 5096830, 5098318, 5094445, 5097146, 5096432, 5095530, 5098218, 5099138, 5096397 | 46    | 113  | 2757     | 5.3      | 9.07E-03 | 1.30E-03 | 7.25E-02 |
| SP_PIR_KEY   | oxidoreductase                            | 13    | 16.2  | 1.11E-04| 5099805, 5094200, 5100277, 5095180, 5094467, 5099138, 5097770, 5096140, 5098116, 5094445, 5095530, 5096397, 5093448 | 40    | 299  | 7033     | 3.82     | 6.18E-03 | 2.07E-03 | 1.09E-01 |
| KEGG_PATH    | vfm00020 Citrate cycle (TCA cycle)        | 6     | 7.5   | 1.17E-04| 5094200, 5093157, 5100277, 5097920, 5096543, 5096140 | 40    | 22   | 1634     | 11.14    | 6.05E-03 | 1.52E-03 | 1.13E-01 |
| GOTERM_BP    | GO:00090 84-glutamine family amino acid biosynthetic process | 5     | 6.25  | 1.26E-04| 5096830, 5098318, 5097146, 5095530, 5098218 | 46    | 17   | 2757     | 17.63    | 1.88E-02 | 2.37E-03 | 1.51E-01 |
| GOTERM_BP    | GO:00193 44-cysteine biosynthetic process | 4     | 5     | 2.15E-04| 5094445, 5096432, 5099138, 5096397 | 46    | 8    | 2757     | 29.97    | 3.17E-02 | 3.58E-03 | 2.56E-01 |
| Category          | Term                      | Count | %    | PValue   | Genes                          | List Total | Pop Hits | Pop Total | Fold Enrichment | Bonferroni | Benjamini | FDR          |
|------------------|---------------------------|-------|------|----------|--------------------------------|------------|----------|-----------|-----------------|------------|------------|--------------|
| KEGG_PATH        | vfm00920: Sulfur metabolism | 5     | 6.25 | 2.34E-04| 5094445, 5096432, 5099740, 5100074, 5096397 | 40         | 14       | 1634      | 14.59           | 1.21E-02  | 2.43E-03  | 2.27E-01    |
| GOTERM_BP_FAT    | GO:000000: 97--sulfur amino acid biosynthetic process | 5     | 6.25 | 3.03E-04| 5099805, 5094445, 5096432, 5099138, 5096397 | 46         | 21       | 2757      | 14.27           | 4.44E-02  | 4.53E-03  | 3.61E-01    |
| GOTERM_BP_FAT    | GO:00463: 94--carboxylic acid biosynthetic process | 10    | 12.5 | 3.58E-04| 5099805, 5096830, 5098318, 5094445, 5097146, 5096432, 5095530, 5098218, 5099138, 5096397 | 46         | 142      | 2757      | 4.22            | 5.22E-02  | 4.87E-03  | 4.26E-01    |
| GOTERM_BP_FAT    | GO:00160: 53--organic acid biosynthetic process | 10    | 12.5 | 3.77E-04| 5099805, 5096830, 5098318, 5094445, 5097146, 5096432, 5095530, 5098218, 5099138, 5096397 | 46         | 143      | 2757      | 4.19            | 5.50E-02  | 4.70E-03  | 4.49E-01    |
| GOTERM_BP_FAT    | GO:000000: 96--sulfur amino acid metabolic process | 5     | 6.25 | 4.38E-04| 5099805, 5094445, 5096432, 5099138, 5096397 | 46         | 23       | 2757      | 13.03           | 6.35E-02  | 5.04E-03  | 5.21E-01    |
| GOTERM_BP_FAT    | GO:00065: 34--cysteine metabolic process | 4     | 5    | 4.50E-04| 5094445, 5096432, 5099138, 5096397 | 46         | 10       | 2757      | 23.97           | 6.53E-02  | 4.81E-03  | 5.36E-01    |
| GOTERM_BP_FAT    | GO:00090: 70--serine family amino acid biosynthetic process | 4     | 5    | 8.07E-04| 5094445, 5096432, 5099138, 5096397 | 46         | 12       | 2757      | 19.98           | 1.14E-01  | 8.04E-03  | 9.59E-01    |
| KEGG_PATH        | vfm00650: Butanoate metabolism | 5     | 6.25 | 8.31E-04| 5099833, 5094200, 5100277, 5097920, 5096543 | 40         | 19       | 1634      | 10.75           | 4.23E-02  | 7.18E-03  | 8.05E-01    |
| KEGG_PATH        | vfm00620: Pyruvate metabolism | 6     | 7.5  | 1.31E-03| 5099833, 5093157, 5100277, 5098542, 5093596, 5096140 | 40         | 36       | 1634      | 6.81            | 6.57E-02  | 9.66E-03  | 1.26E+00    |
| SP_PIR_KEY_WORDS | Cysteine biosynthesis | 3     | 3.75 | 1.82E-03| 5096432, 5099138, 5096397 | 80         | 6        | 7033      | 43.96           | 9.67E-02  | 2.51E-02  | 1.78E+00    |
| GOTERM_BP        | GO:00551:  | 14    | 17.5 | 2.07E-03| 5099805, 5099138, 5096397 | 46         | 338      | 2757      | 2.48            | 2.67E-01  | 1.92E-02  | 2.45E+00    |
| Category   | Term                                                | Count | %     | PValue   | Genes                                                                 | List Total | Pop Hits | Pop Total | Fold Enrichment | Bonferroni | Benjamini | FDR      |
|------------|-----------------------------------------------------|-------|-------|----------|----------------------------------------------------------------------|------------|----------|-----------|------------------|------------|------------|----------|
| _FAT       | 14-oxidation reduction                              |       |       |          | 5094200, 5100277, 5095180, 5094467, 5099138, 5097770, 5094788, 5098116, 5094445, 5094744, 5095530, 5096397, 5093448 | 5096365, 5096432, 5099740, 5100074 | 40       | 12       | 1634      | 13.62            | 1.17E-01 | 1.54E-02 | 2.29E+00 |
| KEGG_PATH  | vfm00450 :Selenoamino acid metabolism               | 4     | 5     | 2.38E-03 | 5096636, 5096432, 5099740, 5100074                                      | 5099635, 5100277, 5098542, 5093596, 5096140 | 46       | 21       | 2757      | 11.42            | 4.84E-01 | 3.82E-02 | 5.13E+00 |
| GOTERM_BP  | GO:00090 69-serine family amino acid metabolic process | 4     | 5     | 4.40E-03 | 5094445, 5096432, 5099138, 5096397                                      | 5093157, 5100277, 5098542, 5093596, 5096140 | 40       | 30       | 1634      | 6.81             | 2.24E-01 | 2.78E-02 | 4.62E+00 |
| KEGG_PATH  | vfm00010 :Glycolysis / Glucose metabolism            | 5     | 6.25  | 4.86E-03 | 5093157, 5100277, 5098542, 5093596, 5096140                                      | 5099635, 5100277, 5098542, 5093596, 5096140 | 40       | 30       | 1634      | 6.81             | 2.24E-01 | 2.78E-02 | 4.62E+00 |
| KEGG_PATH  | vfm00230 :Purine metabolism                         | 7     | 8.75  | 6.97E-03 | 5093686, 5096365, 5099740, 5098542, 5093596, 5096140                                      | 5099635, 5100277, 5098542, 5093596, 5096140 | 40       | 74       | 1634      | 3.86             | 3.05E-01 | 3.57E-02 | 6.57E+00 |
| GOTERM_BP  | GO:00442 72-sulfur compound biosynthetic process    | 5     | 6.25  | 7.14E-03 | 5099805, 5094445, 5096432, 5099138, 5096397                                      | 5093157, 5100277, 5098542, 5093596, 5096140 | 46       | 48       | 2757      | 6.24             | 6.59E-01 | 5.80E-02 | 8.20E+00 |
| SP_PIR_KEY | heme                                                | 3     | 3.75  | 8.97E-03 | 5094788, 5096397, 5093448                                               | 5094788, 5096397, 5093448 | 80       | 13       | 7033      | 20.29            | 3.96E-01 | 9.60E-02 | 8.51E+00 |
| GOTERM_BP  | GO:00442 72-nitrogen compound biosynthetic process  | 11    | 13.7  | 9.37E-03 | 5093686, 5099805, 5096830, 5098318, 5094445, 5097146, 5096432, 5095530, 5098218, 5099138, 5096397 | 5099805, 5094445, 5096432, 5099138, 5096397 | 46       | 268      | 2757      | 2.46             | 7.56E-01 | 7.16E-02 | 1.06E+01 |
| SMART      | SM00116: CBS                                        | 3     | 3.75  | 1.03E-02 | 509786, 5098938, 5096473                                               | 509786, 5098938, 5096473 | 7        | 31       | 1123      | 15.53            | 6.04E-02 | 6.04E-02 | 4.85E+00 |
| GOTERM_M   | GO:00480 37-cofactor or binding                     | 9     | 11.2  | 1.55E-02 | 5094984, 5098116, 5094200                                               | 5094984, 5098116, 5094200 | 48       | 224      | 3173      | 2.66             | 8.96E-01 | 8.96E-01 | 1.69E+01 |
| Category | Term | Count | %    | PValue | Genes                                                                 | List Total | Pop Hits | Pop Total | Fold Enrichment | Bonferroni | Benjamini | FDR    |
|----------|------|-------|------|--------|----------------------------------------------------------------------|------------|----------|-----------|-----------------|------------|------------|--------|
| GOTERM_BP_FAT | GO:00060 | 6     | 7.5  | 1.72E-02 | 5093157, 5096432, 5095530, 5099138, 5096397, 5096140 | 46         | 94       | 2757      | 3.83            | 9.26E-01  | 1.22E-01  | 1.87E+01 |
| SP_PIR_KEY WORDS | Acyltransf erase | 5     | 6.25 | 1.96E-02 | 5096830, 5096397, 5093157, 5093155, 5097146 | 80         | 92       | 7033      | 4.78            | 6.69E-01  | 1.68E-01  | 1.77E+01 |
| KEGG_PATHWAY | vfn00250: Alanine, aspartate and glutamate metabolism | 4     | 5    | 2.24E-02 | 5094788, 5094200, 5099357, 5096397, 5096140 | 40         | 26       | 1634      | 6.28            | 6.92E-01  | 1.01E-01  | 1.97E+01 |
| KEGG_PATHWAY | vfn00190: Oxidative phosphorylation | 4     | 5    | 2.48E-02 | 5096830, 5096397, 5093157, 5093155, 5097146 | 40         | 27       | 1634      | 6.05            | 7.28E-01  | 1.03E-01  | 2.16E+01 |
| KEGG_PATHWAY | vfn00682: Benzoate degradation via CoA ligation | 3     | 3.75 | 2.67E-02 | 5094788, 5094200, 5097920, 5096543 | 40         | 11       | 1634      | 11.14           | 7.55E-01  | 1.02E-01  | 2.31E+01 |
| KEGG_PATHWAY | vfn00250: Alanine, aspartate and glutamate metabolism | 4     | 5    | 2.73E-02 | 5094788, 5094200, 5097920, 5096543 | 40         | 28       | 1634      | 5.84            | 7.63E-01  | 9.76E-02  | 2.36E+01 |
| GOTERM_MF_FAT | GO:005066: Coenzyme binding | 7     | 8.75 | 2.89E-02 | 5098116, 5094200, 5093157, 5095530, 5099138, 5096397, 5096140 | 48         | 160      | 3173      | 2.89            | 9.86E-01  | 8.81E-01  | 2.94E+01 |
| SP_PIR_KEY WORDS | nadp | 3     | 3.75 | 2.93E-02 | 5095530, 5099138, 5096397 | 80         | 24       | 7033      | 10.99           | 8.11E-01  | 2.12E-01  | 2.55E+01 |
| GOTERM_MF_FAT | GO:001641: 07-acetyltransferase activity | 5     | 6.25 | 2.94E-02 | 5096114, 5096830, 5099331, 5093157, 5097146 | 48         | 80       | 3173      | 4.13            | 9.87E-01  | 7.64E-01  | 2.98E+01 |
| GOTERM_MF_FAT | GO:001666: 07-oxidoreductase activity, acting on | 4     | 5    | 3.13E-02 | 5094445, 5094467, 5099138, 5096397 | 48         | 47       | 3173      | 5.63            | 9.90E-01  | 6.85E-01  | 3.15E+01 |
| Category    | Term                          | Count | %     | PValue     | Genes                                                                 |
|-------------|-------------------------------|-------|-------|------------|------------------------------------------------------------------------|
|             | sulfur group of donors        |       |       |            |                                                                        |
| GOTERM_M    | GO:00090 55--electron carrier activity | 7     | 8.75  | 3.22E-02  | Genes: 5094788, 5094200, 5094467, 5094744, 5099138, 5096397, 5096140 |
| F_FAT       | SP_PIR_KEYWORDS pyruvate      | 3     | 3.75  | 3.40E-02  | Genes: 5093157, 5100277, 5098542                                      |
|             | GOTERM_M F_FAT GO:00162 09--antioxidant activity | 3     | 3.75  | 3.44E-02  | Genes: 5094467, 5093448, 5097770                                     |
|             | GOTERM_BF_BP FAT GO:00093 10--amine catabolic process | 3     | 3.75  | 3.74E-02  | Genes: 5095180, 5093155, 5097935                                    |
|             | GOTERM_BF_BP FAT GO:00090 63--cellular amino acid catabolic process | 3     | 3.75  | 3.74E-02  | Genes: 5095180, 5093155, 5097935                                    |
|             | INTERPRO IPR:00250 0:Phosphoadenosine phosphosulfate reductase | 2     | 2.5   | 3.75E-02  | Genes: 5096365, 5094445                                             |
|             | INTERPRO IPR:00036 2:Fumarate lyase | 2     | 2.5   | 3.75E-02  | Genes: 5096830, 5099257                                             |
|             | SP_PIR_KEYWORDS transferase 15 | 18.7  | 4.32E-02 | 1.73       | Genes: 5096114, 5093157, 5093155, 5097146, 5096432, 5099740, 5097432, 5093686, 5096830, 5099833, 5094984, 5096365, 5098542, 5096830, 5099833, 5094984, 5096365, 5098542, 50180074, 50982187 |
|             | GOTERM_M F_FAT GO:00047 83--sulfite reductase (NADPH) activity | 2     | 2.5   | 4.38E-02  | Genes: 5099138, 5096397                                             |
|             | GOTERM_M F_FAT GO:00047 79--sulfate adenylyltranferase activity | 2     | 2.5   | 4.38E-02  | Genes: 5096365, 5100074                                             |
| Category          | Term                                                                 | Count | %    | PValue        | Genes                      | List Total | Pop Hits | Pop Total | Fold Enrichment | Bonferroni | Benjamini | FDR  |
|-------------------|----------------------------------------------------------------------|-------|------|---------------|----------------------------|------------|----------|-----------|-----------------|------------|------------|------|
| GOTERM_MF_FAT     | GO:00047 81-sulfate adenylyltransferase (ATP) activity               | 2     | 2.5  | 4.38E-02      | 5096365, 5100074           | 48         | 3        | 3173      | 44.07           | 9.98E-01  | 6.05E-01  | 4.12E+01 |
| COG_ONTOLOGY      | Amino acid transport and metabolism / Coenzyme metabolism            | 2     | 2.5  | 4.39E-02      | 5096365, 5094445           | 15         | 4        | 1257      | 41.9            | 3.61E-01  | 3.61E-01  | 2.36E+01 |
| UP_SEQ_FEATURE    | domain:N-acetyltransferase                                           | 2     | 2.5  | 4.39E-02      | 5096830, 5097146           | 16         | 2        | 676       | 42.25           | 8.26E-01  | 8.26E-01  | 3.35E+01 |
| GOTERM_BP_FAT     | GO:00194 19-sulfate reduction                                        | 2     | 2.5  | 4.82E-02      | 5096365, 5094445           | 46         | 3        | 2757      | 39.96           | 9.99E-01  | 2.86E-01  | 4.46E+01 |
| GOTERM_BP_FAT     | GO:00160 54-organische acid catabolic process                         | 3     | 3.75 | 4.89E-02      | 5095180, 5093155, 5097935  | 46         | 22       | 2757      | 8.17            | 9.99E-01  | 2.79E-01  | 4.51E+01 |
| GOTERM_BP_FAT     | GO:00463 95-carboxylic acid catabolic process                        | 3     | 3.75 | 4.89E-02      | 5095180, 5093155, 5097935  | 46         | 22       | 2757      | 8.17            | 9.99E-01  | 2.79E-01  | 4.51E+01 |
| SP_PIR_KEYWORDS   | arginine metabolism                                                  | 2     | 2.5  | 5.49E-02      | 5095180, 5093155           | 80         | 5        | 7033      | 35.17           | 9.58E-01  | 2.71E-01  | 4.28E+01 |
| INTERPRO          | IPR00064 4:Cystathionine beta-synthase, core                         | 3     | 3.75 | 5.81E-02      | 5099786, 5098938, 5096473  | 71         | 31       | 5525      | 7.53            | 1.00E+00  | 9.95E-01  | 5.22E+01 |
| GOTERM_BP_FAT     | GO:00090 66-aspartate family amino acid metabolic process             | 3     | 3.75 | 7.07E-02      | 5099805, 5099257, 5093650  | 46         | 27       | 2757      | 6.66            | 1.00E+00  | 3.67E-01  | 5.83E+01 |
| GOTERM_MF_FAT     | GO:00164 10-N-acetyltransferase activity                             | 4     | 5    | 7.55E-02      | 5096114, 5096830, 5093155, 5097146 | 48         | 67       | 3173      | 3.95            | 1.00E+00  | 7.59E-01  | 6.06E+01 |
| KEGG_PATHWAY     | vf00450: Selenoamino acid metabolism                                 | 3     | 3.75 | 8.05E-02      | 5096432, 5099740, 5100074  | 40         | 20       | 1634      | 6.13            | 9.87E-01  | 2.52E-01  | 5.58E+01 |
| GOTERM_BP_FAT     | GO:00453 33-cellular respiration                                     | 3     | 3.75 | 8.49E-02      | 5094788, 5094200, 5099257  | 46         | 30       | 2757      | 5.99            | 1.00E+00  | 4.13E-01  | 6.54E+01 |
| Category      | Term          | Count | PValue     | Genes            | List Total | Pop Hits | Pop Total | Fold Enrichment | Bonferroni | Benjamini | FDR       |
|---------------|---------------|-------|------------|------------------|------------|----------|-----------|-----------------|------------|-----------|-----------|
| INTERPRO      | IPR000089     | 2     | 8.54E-02   | 5093157, 5095222 | 71         | 7        | 5525      | 22.23           | 1.00E+00   | 9.95E-01  | 6.67E+01  |
| SMART         | SM00421:HTH_LU| 2     | 9.26E-02   | 5097659, 5097215 | 7          | 18       | 1123      | 17.83           | 4.42E-01   | 2.53E-01  | 3.72E+01  |
| GOTERM_BP_FAT | GO:0006527    | 2     | 9.41E-02   | 5095180, 5093155 | 46         | 6        | 2757      | 19.98           | 1.00E+00   | 4.35E-01  | 6.93E+01  |
Table S2. Enrichment analysis of the 94 differently expressed coding sequences for *V. fischeri* + nonfunctional artificial cells with respect to *V. fischeri* + *V. fischeri*.

| GOTERM_ BP_FAT       | GO                  | FDR   | DEG  | DEG  | P-Value  | FDR1  | FDR2  | FDR3  |
|----------------------|---------------------|-------|------|------|----------|-------|-------|-------|
| ~'de novo' IMP biosynthetic process | GO:0006189         | 4.26  | 8.40E-05 | 5097940, 5095472, 5093469, 5098195 | 6      | 2757   | 39.11 | 1.62E-02 | 1.62E-02 | 1.05E-01 |
| ~IMP biosynthetic process | GO:0006188         | 4.26  | 2.30E-04 | 5097940, 5095472, 5093469, 5098195 | 8      | 2757   | 29.33 | 4.36E-02 | 2.20E-02 | 2.86E-01 |
| ~IMP metabolic process | GO:0046040         | 4.26  | 6.53E-04 | 5097940, 5095472, 5093469, 5098195 | 11     | 2757   | 21.33 | 1.19E-01 | 4.14E-02 | 8.12E-01 |
| ~purine ribonucleoside monophosphate biosynthetic process | GO:0009168         | 4.26  | 6.53E-04 | 5097940, 5095472, 5093469, 5098195 | 11     | 2757   | 21.33 | 1.19E-01 | 4.14E-02 | 8.12E-01 |
| ~purine nucleoside monophosphate metabolic process | GO:0009126         | 4.26  | 6.53E-04 | 5097940, 5095472, 5093469, 5098195 | 11     | 2757   | 21.33 | 1.19E-01 | 4.14E-02 | 8.12E-01 |
| ~purine nucleoside monophosphate metabolic process | GO:0009127         | 4.26  | 6.53E-04 | 5097940, 5095472, 5093469, 5098195 | 11     | 2757   | 21.33 | 1.19E-01 | 4.14E-02 | 8.12E-01 |
| ~purine nucleoside monophosphate metabolic process | GO:0009161         | 4.26  | 6.53E-04 | 5097940, 5095472, 5093469, 5098195 | 14     | 2757   | 16.76 | 2.37E-01 | 6.53E-02 | 1.72E+00 |
| ~ribonucleoside monophosphate metabolic process | GO:0009156         | 4.26  | 6.53E-04 | 5097940, 5095472, 5093469, 5098195 | 14     | 2757   | 16.76 | 2.37E-01 | 6.53E-02 | 1.72E+00 |
| ~nitrogen compound | GO:0044271         | 12.7  | 3.59E-03 | 5097940, 5096595, 5099333 | 7      | 268    | 2.63  | 5.03E-01 | 1.30E-01 | 4.39E+00 |
| Pathway | Description | Score | P-Value | fold change | q-value | Top Enters | KEGG Pathway IDs |
|---------|-------------|-------|---------|-------------|---------|------------|-----------------|
| vfm00230 | Biosynthetic Process of Urine Metabolism | 7.45 | 4.01E-03 | 4.29 | 2.01E-01 | 2.01E-01 | 5097940, 5099350, 5099487, 5097435, 5097146, 5099025, 5095472, 5093469, 5100143, 5098195, 5096166 |
| GO:0009124 | Nucleoside Monophosphate Biosynthetic Process | 4.26 | 4.06E-03 | 11.73 | 5.46E-01 | 1.23E-01 | 5097940, 5095472, 5093469, 5098195 |
| GO:0009123 | Nucleoside Monophosphate metabolic Process | 4.26 | 5.36E-03 | 10.67 | 6.48E-01 | 1.38E-01 | 5097940, 5095472, 5093469, 5098195 |
| GO:0034404 | Nucleobase, Nucleoside and Nucleotide Biosynthetic Process | 6.38 | 7.48E-03 | 4.69 | 7.67E-01 | 1.66E-01 | 5097940, 5096595, 5095472, 5093469, 5098195 |
| GO:0034654 | Nucleobase, Nucleoside, Nucleotide and Nucleic Acid Biosynthetic Process | 6.38 | 7.48E-03 | 4.69 | 7.67E-01 | 1.66E-01 | 5097940, 5096595, 5095472, 5093469, 5098195 |
| GO:0009152 | Purine Ribonucleotide Biosynthetic Process | 4.26 | 1.17E-02 | 8.09 | 8.99E-01 | 2.25E-01 | 5097940, 5095472, 5093469, 5098195 |
| GO:0009150 | Purine Ribonucleotide metabolic Process | 4.26 | 1.41E-02 | 7.57 | 9.36E-01 | 2.41E-01 | 5097940, 5095472, 5093469, 5098195 |
| GO:0009260 | Ribonucleotide Biosynthetic Process | 4.26 | 1.54E-02 | 7.33 | 9.51E-01 | 2.39E-01 | 5097940, 5095472, 5093469, 5098195 |
| SP_PIR_KEWORDS | Cytoplasm | 10.6 | 1.59E-02 | 2.53 | 6.24E-01 | 1.49E+01 | 5099350, 5094741, 5099487, 5093882, 5096673, 5096854, 5099676, 5098195 |
| SP_PIR_KEYWORDS | purine biosynthesis | 3 | 1.62E-02 | 3.93E-01 | 1.52E+01 | 5097146, 5094544, 5097559, 5100143, 5098195 |
| GOTERM_BP_FAT | GO:0009259 | ~ribonucleotide metabolic process | 4 | 1.81E-02 | 5097940, 5095472, 5093469, 5098195 |
| GOTERM_BP_FAT | GO:0009165 | ~nucleotide biosynthetic process | 5 | 1.97E-02 | 5097940, 5096595, 5093469, 5098195 |
| KEGG_PATHWAY | vft00230: Purine metabolism | 6 | 2.66E-02 | 3.36 | 7.79E-01 | 5.30E-01 | 2.34E+01 |
| GOTERM_BP_FAT | GO:0006399 | ~tRNA metabolic process | 5 | 2.92E-02 | 4.13 | 9.97E-01 | 3.37E-01 | 3.09E+01 |
| INTERPRO | IPR014729: Rossmann-like alpha/beta/alpha sandwich fold | 4 | 3.03E-02 | 5.79 | 9.98E-01 | 9.98E-01 | 3.20E+01 |
| GOTERM_BP_FAT | GO:0009264 | ~deoxyribonucleotide catabolic process | 2 | 3.31E-02 | 58.66 | 9.99E-01 | 3.53E-01 | 3.43E+01 |
| GOTERM_BP_FAT | GO:0006163 | ~purine nucleotide biosynthetic process | 4 | 3.57E-02 | 5.33 | 9.99E-01 | 3.57E-01 | 3.65E+01 |
| GOTERM_BP_FAT | GO:0004638 | ~phosphoribosylaminomimidazole carboxylase activity | 2 | 4.93E-02 | 39.17 | 9.99E-01 | 9.99E-01 | 4.52E+01 |
| GOTERM_BP_FAT | GO:0006164 | ~purine nucleotide metabolic process | 4 | 4.94E-02 | 4.69 | 1.00E+00 | 4.39E-01 | 4.68E+01 |
| GOTO_BYPARSER | amino-acid biosynthesis | 4 | 6.06E-02 | 4.4 | 9.78E-01 | 7.20E-01 | 4.67E+01 |
| GOTO_BYPARSER | 5 | 7.62E-02 | 3.02 | 1.00E+00 | 5.75E-01 | 6.28E+01 |
| Gene Ontology | Description | GO Term | p-value | q-value | FDR | Significance | p-value | q-value | FDR |
|---------------|-------------|---------|---------|---------|-----|--------------|---------|---------|-----|
| BP_FAT        | ~ncRNA metabolic process | GO:0016042 | 2.13    | 8.07E-02 | 20  | 2.13         | 8.07E-02 | 5      | 2757 | 0.00E+00 | 5.77E-01 | 6.50E+01 |
| BP_FAT        | ~l lipid catabolic process | GO:0009262 | 2.13    | 8.07E-02 | 47  | 2.13         | 8.07E-02 | 5      | 2757 | 0.00E+00 | 5.77E-01 | 6.50E+01 |
| BP_FAT        | ~d deoxyribo nucleotid metabolic process | GO:0009308 | 2.13    | 9.94E-02 | 83  | 2.13         | 9.94E-02 | 7      | 5525 | 0.00E+00 | 1.00E+00 | 7.31E+01 |
| BP_FAT        | ~deoxyribo nucleotid metabolic process | GO:0009308 | 2.13    | 9.94E-02 | 83  | 2.13         | 9.94E-02 | 7      | 5525 | 0.00E+00 | 1.00E+00 | 7.31E+01 |
| BP_FAT        | ~f deoxyribo nucleotid metabolic process | GO:0009308 | 2.13    | 9.94E-02 | 83  | 2.13         | 9.94E-02 | 7      | 5525 | 0.00E+00 | 1.00E+00 | 7.31E+01 |
| BP_FAT        | ~g deoxyribo nucleotid metabolic process | GO:0009308 | 2.13    | 9.94E-02 | 83  | 2.13         | 9.94E-02 | 7      | 5525 | 0.00E+00 | 1.00E+00 | 7.31E+01 |
**Table S3.** Enrichment analysis of the 26 differently expressed coding sequences for *V. fischeri* + functional artificial cells with respect to *V. fischeri* + *V. fischeri*. Highlighted in grey are the significantly enriched five gene sets with FDR <10^{-2}.

| GOTERM_MF_FAT | GO:00161 | 51–nickel ion binding | 17 | 10 | 3173 | 93.32 | 4.63E-06 | 4.63E-06 | 8.65E-05 |
|---------------|-----------|-----------------------|-----|-----|-------|--------|-----------|-----------|-----------|
| SP_PIR_KEYWORDS | Chaperone | 6 | 23.08 | 9.64E-08 | 5100389, 5097037, 5098484, 5100141, 5097212 | 26 | 34 | 7033 | 47.74 | 3.28E-06 | 3.28E-06 | 8.48E-05 |
| GOTERM_MF_FAT | GO:00431 | 67–ion binding | 17 | 459 | 3173 | 4.88 | 5.94E-05 | 2.97E-05 | 1.11E-03 |
| GOTERM_MF_FAT | GO:00431 | 69–cation binding | 17 | 459 | 3173 | 4.88 | 5.94E-05 | 2.97E-05 | 1.11E-03 |
| SP_PIR_KEYWORDS | cytoplasm | 9 | 34.62 | 5.18E-06 | 5100389, 5099958, 5097037, 5098484, 5098990, 5095327, 5099644, 5099334, 5099244, 5097212 | 26 | 296 | 7033 | 8.22 | 1.76E-04 | 8.80E-05 | 4.55E-03 |
| GOTERM_MF_FAT | GO:00468 | 72–metal ion binding | 17 | 430 | 3173 | 4.77 | 3.68E-04 | 1.23E-04 | 6.87E-03 |
| GOTERM_BP_FAT | GO:000649 | 9 | 34.62 | 4.412E-05 | 5100389, 5097037, 5098484, 5098990, 5095327, 5099644, 5099334, 5100141, 5097212 | 17 | 306 | 317 | 5.49 | 2.29E-03 | 5.73E-04 | 4.29E-02 |
| SP_PIR_KEYWORDS | Nickel insertion | 3 | 11.54 | 1.21E-04 | 5100389, 5097037, 5100141 | 26 | 5 | 7033 | 162.3 | 4.09E-03 | 1.37E-03 | 1.06E-01 |
| SP_PIR_KEYWORDS | zinc | 4 | 15.38 | 3.13E-03 | 5098990, 5095327, 5099644, 5099334 | 26 | 84 | 7033 | 12.88 | 1.01E-01 | 2.63E-02 | 2.72E+00 |
| KEGG_PATHWAY | vfn00641 | 2 | 7.69 | 1.46E-02 | 5095327, 5099334 | 9 | 3 | 1634 | 121.04 | 2.66E-01 | 2.66E-01 | 1.08E+01 |
| KEGG_PATHWAY | vfn00624 | 2 | 7.69 | 1.46E-02 | 5095327, 5099334 | 9 | 3 | 1634 | 121.04 | 2.66E-01 | 2.66E-01 | 1.08E+01 |
| KEGG_PATHWAY | GO:000649 | 3 | 11.54 | 1.55E-02 | 5099958, 5098484, 5095327 | 13 | 45 | 2757 | 14.14 | 6.95E-01 | 6.95E-01 | 1.52E+01 |
| GO:00343 | GO:000649 | 08-monohydric alcohol metabolic process | 2 | 7.69 | 1.73E-02 | 5095327, 5099334 | 13 | 4 | 2757 | 106.04 | 7.35E-01 | 4.85E-01 | 1.68E+01 |
| GO:00196 | GO:000649 | 27-urea metabolic process | 2 | 7.69 | 1.73E-02 | 5098484, 5097212 | 13 | 4 | 2757 | 106.04 | 7.35E-01 | 4.85E-01 | 1.68E+01 |
| GO:00060 | GO:000649 | 69-ethanol oxidation | 2 | 7.69 | 1.73E-02 | 5095327, 5099334 | 13 | 4 | 2757 | 106.04 | 7.35E-01 | 4.85E-01 | 1.68E+01 |
| GO:00060 | GO:000649 | 67-ethanol metabolic process | 2 | 7.69 | 1.73E-02 | 5095327, 5099334 | 13 | 4 | 2757 | 106.04 | 7.35E-01 | 4.85E-01 | 1.68E+01 |
| INTERPRO | IPR01418 | 3:Alcohol dehydrogenase class III/S-(hydroxy methyl)glutathione dehydrogenase | 2 | 7.69 | 1.80E-02 | 5095327, 5099334 | 26 | 4 | 5525 | 106.25 | 7.19E-01 | 7.19E-01 | 1.71E+01 |
| KEGG_PATHWAY | vfn00980 | GO:000649 | 2 | 7.69 | 1.95E-02 | 5095327, 5099334 | 9 | 4 | 1634 | 90.78 | 3.38E-01 | 1.86E-01 | 1.41E+01 |
| KEGG PATHWAY | Gene | Description | E-value | ID1 | ID2 | ID3 | ID4 | ID5 | Proportion | MetaScore | p-value | q-value |
|--------------|------|-------------|---------|-----|-----|-----|-----|-----|------------|------------|---------|---------|
| vfn00350 | 2 | 3.38E-02 | 5095327, 5099334 | 9 | 7 | 1634 | 51.87 | 5.15E-01 | 2.14E-01 | 2.33E+01 |
| metal-binding | 4 | 4.09E-02 | 5098990, 5095327, 5099644, 5099334 | 26 | 218 | 7033 | 4.96 | 7.58E-01 | 2.47E-01 | 3.07E+01 |
| vfn00680 | 2 | 4.80E-02 | 5095327, 5099334 | 9 | 10 | 1634 | 36.31 | 6.44E-01 | 2.28E-01 | 3.16E+01 |
| vfn00071 | 2 | 5.27E-02 | 5095327, 5099334 | 9 | 11 | 1634 | 33.01 | 6.79E-01 | 2.03E-01 | 3.42E+01 |
| IPR01314 | 2 | 5.30E-02 | 5095327, 5099334 | 26 | 12 | 5525 | 35.42 | 9.78E-01 | 7.19E-01 | 4.31E+01 |
| IPR00208 | 2 | 5.30E-02 | 5095327, 5099334 | 26 | 12 | 5525 | 35.42 | 9.78E-01 | 7.19E-01 | 4.31E+01 |
| IPR01315 | 2 | 5.30E-02 | 5095327, 5099334 | 26 | 12 | 5525 | 35.42 | 9.78E-01 | 7.19E-01 | 4.31E+01 |
| GO:00510 | 82-unfolded protein binding | 2 | 7.69 | 6.85E-02 | 5099958, 5098484 | 17 | 14 | 3173 | 26.66 | 9.75E-01 | 3.69E-01 | 4.98E+01 |
| SP_PIR_KEYWORDS | nucleotide-binding | 5 | 19.23 | 6.96E-02 | 5096094, 5099958, 5097037, 5099244, 5094048 | 26 | 445 | 7033 | 3.04 | 9.14E-01 | 3.36E-01 | 4.70E+01 |
| GO:000065 | 47-histidine metabolic process | 2 | 7.69 | 8.38E-02 | 5098990, 5094537 | 13 | 20 | 2757 | 21.21 | 9.99E-01 | 8.91E-01 | 6.02E+01 |
| GO:000090 | 75-histidine family amino acid metabolic process | 2 | 7.69 | 8.38E-02 | 5098990, 5094537 | 13 | 20 | 2757 | 21.21 | 9.99E-01 | 8.91E-01 | 6.02E+01 |
### Table S4. Bacterial strains used in this study.

| Strain | Plasmid | Use |
|--------|---------|-----|
| 3OC12 HSL *E. coli* TOP10 reporter | K575024 | *E. coli* strain used to sense 3OC12 HSL |
| 3OC12 HSL *E. coli* NEBExpress reporter | K575024 | *E. coli* strain used to sense 3OC12 HSL |
| 3OC6 HSL *E. coli* TOP10 reporter | T9002 | *E. coli* strain used to sense 3OC6 HSL and leakage experiments |
| C4 HSL *E. coli* TOP10 reporter | K575037 | *E. coli* strain used to sense C4SL |
| *V. fischeri* MJ11 (ATCC BAA-1741) | | Strain used to perform the cellular Turing test, quorum quenching experiments, to sense 3OC6 HSL, and leakage experiments |
| *V. fischeri* 7744 (ATCC 7744) | | Strain used to activate artificial cells able to produce 3OC12 HSL |
| *V. harveyi* BB170 (ATCC BAA-1117) | | Strain used to sense AI-2 and leakage experiments |
| *P. aeruginosa* PT5 PAO1 wild-type* | | Strain used for quorum sensing experiments and leakage experiments |

### Table S5. DNA sequences used in this study.

| NAME | NOTE | SEQUENCE* |
|------|------|-----------|
| JF005A | PT7- RBS- HLPT- T7 term | TAATAGGACACTCACATAGGGGAATTTGAGGCGAATACATTTCCCTTCGATTAATATTTTTGGTTTAACATTAAAGAAGGAGATATACATATG |
| pLasB | | TACTACGACGTGACGCTGTAAGCTGCAAGGTGAGCGGATAACAATTCCCCTCTAGAAATAATTGGTTTAACTTTGATGTTGTCGGGAA |
| K575024 | pLasB B0030 Bb_a_ J2311- 3b Bb_a_ B0034- lasR | GGGCTGCTGTAGAGATGGAAGGCTGCTGCTGCCACCGCTGAGCAATAACTAGCATAACCCCTTGGGGCCTCTAAACGGGTCTTGAGGGGTATTTTTTGATAGGAGCAGGTTACACGCTGCTGGGCTTCCTCTTATTAGAGCAGGAGGTTGCTGACGCGCAGGATTGCTAAGAATGGCAAAAGGAGATATACATATG |

*Note: All sequences are designed for specific applications in biological experiments.*
CTGCCGTACCTAAACGAGAAGACGACACTACAGTACCGGCTCCGGTGATGTAGATCGAGGAGAAT
CTGCCGTACCTAAACGAGAAGACGACACTACAGTACCGGCTCCGGTGAT
GTTGAAGAACAAAAAACGAGAAGGCTGCTGAGCTGCGTGATG
GAGCAGGCAAATGAGCCGCTCTGCTGAGCTGCGTGATG
CTTGAGTACGAGACAGTACACAGCCGAGTGCTGAGCAGAGGAAAGAGAG
CTTGAGTACGAGACAGTACACAGCCGAGTGCTGAGCAGAGGAAAGAGAG

RL078A

TAAATACGACTCACTATAGAG

GCTACACGAGACCCAACTGGACAAGATGCCTCCGCTTCCGAAGAAAGGAAACCTGAACCTG

RL078A

TCCCATATGGATTTTCTACAGGTTTACGCAAGAAAATGGTTTGTTAT

TCCCATATGGATTTTCTACAGGTTTACGCAAGAAAATGGTTTGTTAT
RL09C

```plaintext
RL092A

```

RL092A

```plaintext
RL092A

```

49
GAAGTCGTCTTAATGTATAGATTTGAAGAAGAGCTGTTTTTACGATCCCTTCAGGATTACAAAATTCAAAGTGCGTTGCTAGTACCAACCCTATTTTCATTCTTCGCCAAAAGCACTCTGATTGACAAATACGATTTATCTAATTTACACGAAATTGCTTCTGGGGGCGCACCTCTTTCGAAAGAAGTCGGGGAAGCGGTTGCAAAACGGTGAGTTAAGCGCATTGCCTATGATTTCAAGGCTCTAAAACGGCGCGTAGCTTCCATCTTCCAGGGATACGACAAGGATATGGGCTCACTGAGACTACATCAGCTATTCTGATTACACCCGAGGGGGATGATAAACCGGGCGCGGTCGGTAAAGTTGTTCCATTTTTTGAAAGCGAAGGTTGTGGATCTGGATACCGGGAAAACGCTGGGCCTTAATCAGAGAGGCAGAATTATGTGTCAGAGGACCTATGATTATGTCCGGTTATGTAAACAATCCGGAAGCGACCAACGCCTTGATTGACAAGGATGGATGGCTACATTCTGGAGACATAGCTTACTGGGACGAAGACGAACACTTCTTCATAGTTGACCGCTTGAAGTCTTTAATTAAATACAAAGGATATCAGGTAATGAAGATTTTTACATGCACACACGCTACAATACCTGTAGGTGGCCCCCGCTGAATTGGGGATCGATATTGTTACAACACCCCAACATCTTCGACGCGGGCGTGGCAGGTCTTCCCGACGATGA

T9002

| pTEt- | BbA_ |
|-------|------|
| B0034-| luxR- |
| B0015-| BbA_ |
| B0032-| GFPmut |
| b- | BbA_ |
| B0015 |

*Promoters are underlined, start and stop codons are in bold, the RBS is in italics, and linker sequences of encoded fusion proteins are in lowercase. The lrs intergenic region is in red.
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