Supporting Information for

A Multivalent Probe for AI-2 Quorum Sensing Receptors

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A. General Methods and Materials

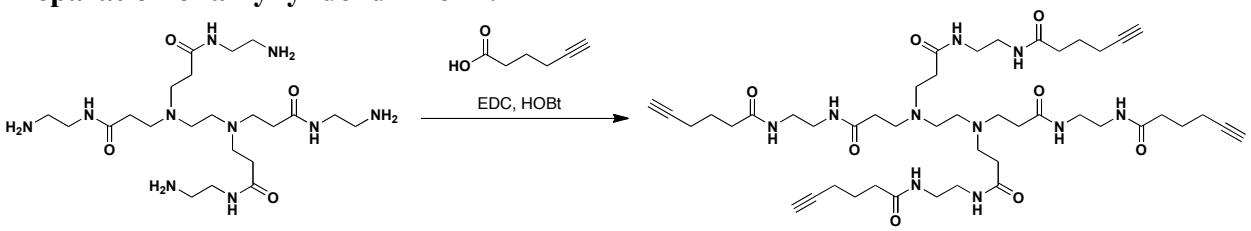
General chemistry methods: RP-HPLC was performed using binary gradients of solvents A and B, where A is 0.1% TFA in water and B is 0.09% TFA in acetonitrile. Analytical RP-HPLC was performed using a Vydac 218TP5415 column at a flow rate of 1 mL/min, with detection at 214 or 254 nm. Preparative RP-HPLC was performed using a custom-packed, 2-inch bore C18 column at a flow rate of 15 mL/min, with detection at 214 or 254 nm. In all cases, fractions were analyzed off-line using an ABI/Sciex 150EX single quadrupole mass spectrometer and judged for purity after a consistent summing of 50 scans in multichannel analysis (MCA) mode. For preparative purification purposes, fractions that contained no consistent charged species, which accounted for >10% of the total ion intensity were designated "pure" and pooled; the homogeneity of the pool was verified by analytical RP-HPLC and was >95%. LC-MS or MALDI-TOF were used to monitor reaction progress.

Data analysis: All data was analyzed using GraphPad Prism version 5.0a for Mac OS X (GraphPad Software, www.graphpad.com).

Materials: PAMAM dendrimer, ethylenediamine core, generation 0.0 solution (20 wt% in methanol) was purchased from Aldrich and used as received. 5-Hexynoic acid was purchased from Aldrich and used as received. Copper sulfate, sodium ascorbate and tris[(1-benzyl-1*H*-1,2,3-triazol-4-yl)methyl]amine (TBTA) were purchased from Aldrich and used as received.

B. Synthetic Procedures and Characterization Data

Preparation of alkynyl dendrimer 7.



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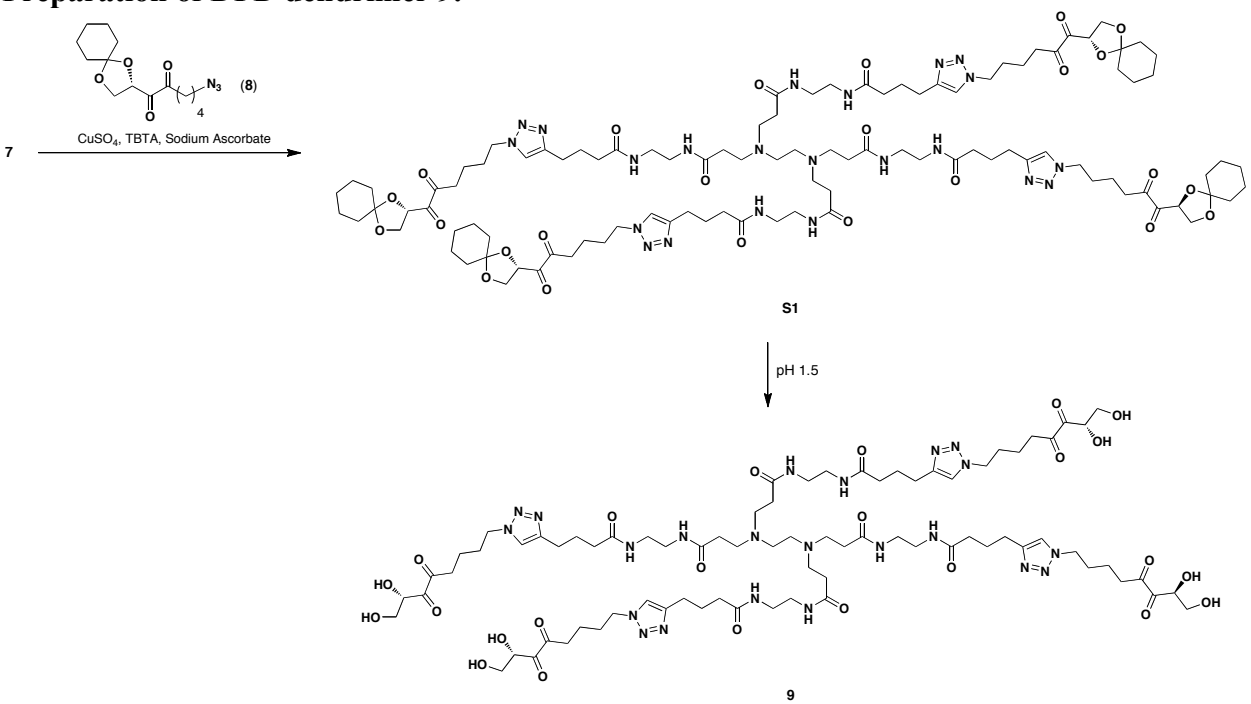
5-hexynoic acid (107 μL, 0.968 mmol), EDC (186 mg, 0.968 mmol), HOBt (131 mg, 0.968 mmol) and *N*,*N*-diisopropylethylamine (169 μL, 0.968 mmol) were added to a stirring solution of DMF (5 mL) at 25 °C. After 30 min at 25 °C, **5** (117 μL, 0.193 mmol) was added and the resulting mixture was stirred at 25 °C for 24 h. Reaction progress was monitored by LC-MS for formation of **7** until completion. Alkynyl dendrimer **7** was used as crude for the subsequent click reaction after removal of DMF and formation of an aqueous stock solution (10 mM in water). MALDI-TOF *m/z* calcd for $C_{46}H_{72}N_{10}O_8$ 892.553, found 893.126.

Preparation of DPD dendrimer 9.

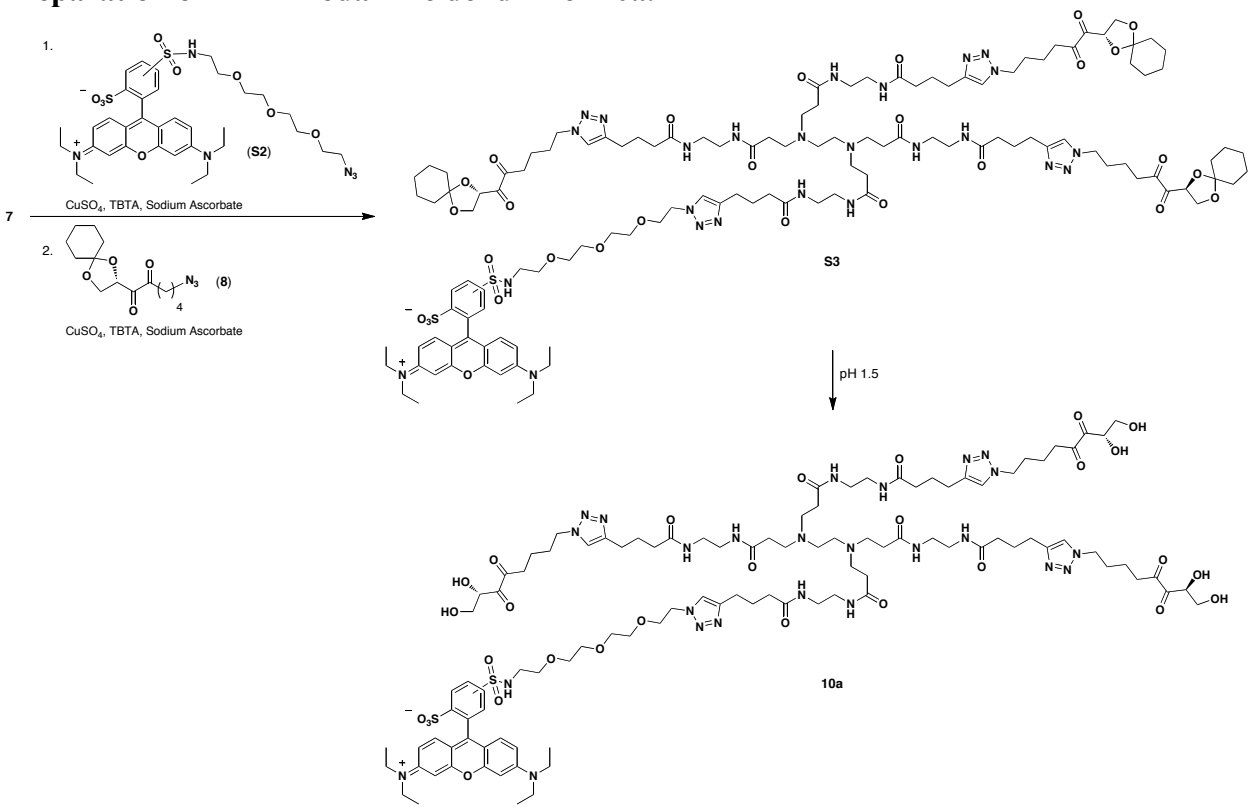


A stirring solution of THF/water (1:2, 1.5 mL) at 25 °C was charged with **7** (10 mg, 0.011 mmol), **8** (15 mg, 0.05 mmol), copper sulfate (1.0 mg, 0.0055 mmol), TBTA (3.0 mg, 0.0055 mmol) and sodium ascorbate (2.0 mg, 0.011 mmol). The resulting mixture was stirred at 25 °C for 24 h. Reaction progress was monitored for formation of **S1** by MALDI-TOF. The mixture was concentrated *in vacuo* to remove THF. Residual copper was then removed using Deloxan® MP Metal Scavenger (thio-functionalized polysiloxane, Strem Chemicals) according to the manufacturer's protocol. After removal of the metal scavenger resin, **S1** was purified via dialysis from water. Following lyophilization, **S1** was obtained as a white solid. MALDI-TOF *m/z* calcd

for $C_{102}H_{156}N_{22}O_{24}$ 2073.966, found 2074.463.

To obtain DPD dendrimer **9**, 5.0 mg of **S1** was treated with pH 1.5 buffer as previously described.[1] MALDI-TOF *m/z* calcd for $C_{78}H_{124}N_{22}O_{24}$ 1752.916, found 1753.952.

Preparation of DPD-rhodamine dendrimer 10a.

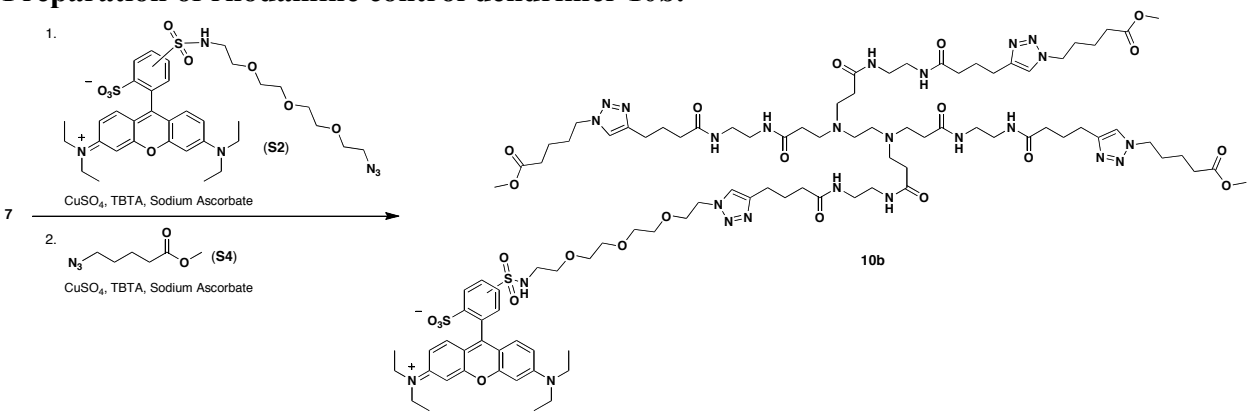


A stirring solution of THF/water (1:2, 1.5 mL) at 25 °C was charged with **7** (10 mg, 0.011 mmol), **S2** (8.3 mg, 0.011 mmol), copper sulfate (1.0 mg, 0.0055 mmol), TBTA (3.0 mg, 0.0055 mmol) and sodium ascorbate (2.0 mg, 0.011 mmol). The resulting mixture was stirred at 25 °C for 24 h. Reaction progress was monitored by LC-MS. The mixture was concentrated *in vacuo* to remove THF. Following lyophilization, the rhodamine dendrimer was dissolved in 50% AcOH and purified by RP-HPLC (95% A/5% B–50% A/50% B). MALDI-TOF *m/z* calcd for $C_{83}H_{126}N_{16}O_{17}S_2$ 1682.226, found 1682.115.

A stirring solution of THF/water (1:2, 1.5 mL) at 25 °C was charged with rhodamine dendrimer (10 mg, 0.011 mmol), **8** (15 mg, 0.05 mmol), copper sulfate (1.0 mg, 0.0055 mmol), TBTA (3.0 mg, 0.0055 mmol) and sodium ascorbate (2.0 mg, 0.011 mmol). The resulting mixture was stirred at 25 °C for 24 h. Reaction progress was monitored for formation of **S3** by MALDI-TOF. The mixture was concentrated *in vacuo* to remove THF. Residual copper was then removed using Deloxan® MP Metal Scavenger (thio-functionalized polysiloxane, Strem Chemicals) according to the manufacturer's protocol. After removal of the metal scavenger resin, **S3** was lyophilized, dissolved in 50% AcOH and purified by RP-HPLC (95% A/5% B–50% A/50% B). MALDI-TOF *m/z* calcd for $C_{125}H_{189}N_{25}O_{29}S_2$ 2568.352, found 2570.118.

To obtain DPD-rhodamine dendrimer **10a**, 5.0 mg of **S3** was treated with pH 1.5 buffer as previously described.[1] MALDI-TOF *m/z* calcd for $C_{107}H_{165}N_{25}O_{29}S_2$ 2328.165, found 2329.735.

Preparation of rhodamine control dendrimer 10b.



A stirring solution of THF/water (1:2, 1.5 mL) at 25 °C was charged with **7** (10 mg, 0.011 mmol), **S2** (8.3 mg, 0.011 mmol), copper sulfate (1.0 mg, 0.0055 mmol), TBTA (3.0 mg, 0.0055 mmol) and sodium ascorbate (2.0 mg, 0.011 mmol). The resulting mixture was stirred at 25 °C for 24 h. Reaction progress was monitored by LC-MS. The mixture was concentrated *in vacuo* to remove THF. Following lyophilization, the rhodamine dendrimer was dissolved in 50% AcOH and purified by RP-HPLC (95% A/5% B–50% A/50% B). MALDI-TOF *m/z* calcd for $C_{83}H_{126}N_{16}O_{17}S_2$ 1682.226, found 1682.115.

A stirring solution of THF/water (1:2, 1.5 mL) at 25 °C was charged with rhodamine dendrimer (10 mg, 0.011 mmol), **S4** (7.8 mg, 0.05 mmol), copper sulfate (1.0 mg, 0.0055 mmol), TBTA (3.0 mg, 0.0055 mmol) and sodium ascorbate (2.0 mg, 0.011 mmol). The resulting mixture was stirred at 25 °C for 24 h. Reaction progress was monitored for formation of **10b** by MALDI-TOF. The mixture was concentrated *in vacuo* to remove THF. Residual copper was then removed using Deloxan® MP Metal Scavenger (thio-functionalized polysiloxane, Strem Chemicals) according to the manufacturer's protocol. After removal of the metal scavenger resin, **10b** was lyophilized, dissolved in 50% AcOH and purified by RP-HPLC (95% A/5% B–50% A/50% B). MALDI-TOF *m/z* calcd for $C_{101}H_{159}N_{25}O_{23}S_2$ 2154.148, found 2155.627.

C. β-Galactosidase Assay for LsrB Antagonism in *Salmonella typhimurium*[1,2]

Evaluation of dendrimers **9** and **10a** was performed in *S. typhimurium* by measuring β-galactosidase activity as follows: an overnight culture was diluted 1:100 in LB broth and incubated with dendrimers **9** and **10a** (0.05% DMSO) and 50 μM DPD at 37 °C for 4 h. After incubation, the cells were centrifuged, resuspended in Z-buffer, and an aliquot was added to a 96-well plate to measure OD_{600}. 1% SDS and chloroform were added to solubilize the remaining cells, and aliquots of differing volumes were added to the plate. β-Mercaptoethanol dissolved in Z-buffer was added to bring the final volume to 200 μL per well, at which point 50 μL of substrate (*o*-nitrophenyl-β-D-galactopyranoside) in Z-buffer was added. The OD_{420} was read every 5 min for 1 h. β-Galactosidase activity was calculated according to the following equation: Activity = $[OD_{420}/(OD_{600} \times \text{time} \times \text{cells})] \times (1 \text{ nmol}/0.0045 \text{ mL/cm}) \times (\text{total volume})$ (time =

reaction time in minutes, cells = volume of bacterial cell culture used in mL).

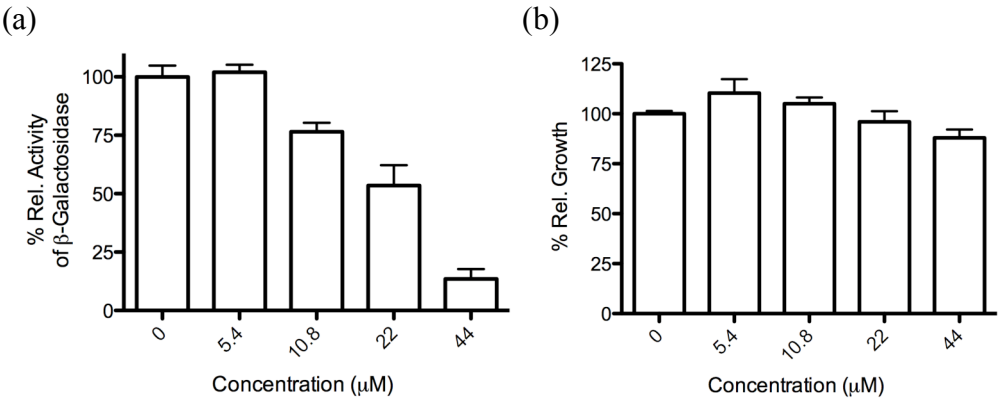


Figure S1. Quorum sensing modulation (a) and growth impact (b) by DPD-rhodamine dendrimer **10a**.

D. Real-Time PCR

Primers	Forward	Reverse
Downregulated:		
LsrK	AGAATGCCGCCATTGTTTCC	ATCCTCCGCCAGCAAAAACC
LsrB	CTGACGCTAACGCTTTACC	GCCAAACTCTTTAACAGTGCC
LsrF	ACCTCCGGCAATCAATAAAC	AGCAGCACAGCTATTCAAC
invF	GCAGGATTAGTGGACACGAC	TTTACGATCTTGCCAAATAGCG
Control:		
rpsA 30S ribosomal protein	ACCAACAAAAACATCCACCC	CCAGAACCATCACTTCCACTAC

Overnight-cultured *S. typhimurium* (LT2; ATCC 700720) was diluted 1:100 in fresh LB medium (1 mL) and grown for 4 h in the presence of **9** (25 μM) at 37 °C with vigorous shaking. Cells were then harvested by centrifugation and total RNA was isolated using the RNAeasy Mini Kit (Qiagen). Reverse transcription and target amplification were performed using the SuperScript III Platinum SYBR Green One-Step qRT-PCR Kit (Invitrogen) using a DNA Engine Thermo Cycler equipped with a Chromo4 detector (Bio-Rad). Relative quantification analysis was performed using an Opticon Monitor v3.1 (Bio-Rad).

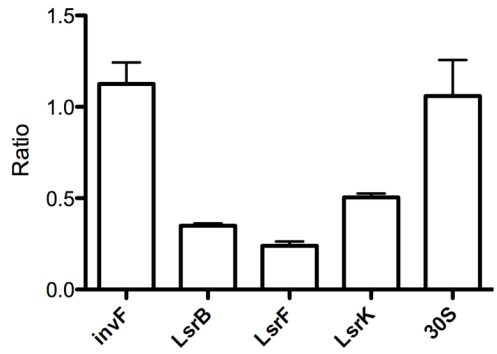


Figure S2. RT-PCR analysis with DPD dendrimer **9**. Ratio is $E_{target}\Delta C_{T\ target}/E_{ref}\Delta C_{T\ ref}$.

E. Confocal Microscopy

S. typhimurium (LT2; ATCC 700720) was grown overnight in 5 mL LB Broth at 37 °C, 250 rpm. Cells were then harvested by centrifugation (3600 rpm) post 14 h growth and resuspended in a 5 mL solution of either **10a** or **10b** (50 μM) with Hoescht 33342 (50 μM) in PBS (pH 7.4 without $MgCl_2$ and $CaCl_2$) at 4 °C. Cells were then incubated at 37 °C for 2 h, pelleted, and washed two times with PBS (pH 7.4 without $MgCl_2$ and $CaCl_2$) at 4 °C. Cells were fixed with 4% formaldehyde in PBS for 20 min at 25 °C. 40 μL of each fixed sample was coated on separate Poly-L-Lysine coated slides (Lab Scientific, Inc., Livingston, NJ), dried at 37 °C and sealed using Immuno-Fluore mounting medium (MP Biomedicals, Inc., Solon, OH). Similar procedures were used for the imaging of *V. harveyi* and *B. cereus*.

Images were obtained using a Zeiss LSM 710 laser scanning confocal microscope (LSCM) attached to a Zeiss Observer Z1 microscope equipped with a 63x oil Plan Apo, 1.4na infinity corrected optics. A 488 nm argon laser was utilized for continuous spectral detection. Image analysis was achieved through Image Pro Plus 7(Media Cybernetics, Inc., Bethesda, MD).

F. Complete Reference 31

Johansson, E. M. V.; Crusz, S. A.; Kolomiets, E.; Buts, L.; Kadam, R. U.; Cacciarini, M.; Bartels, K.-M.; Diggle, S. P.; Camara, M.; Williams, P.; Loris, R.; Nativi, C.; Rosenau, F.; Jaeger, K.-E.; Darbre, T.; Reymond, J.-L. *Chem. Biol.* **2008**, *15*, 1249.

G. References

1. Lowery, C. A.; Park, J.; Kaufmann, G. F.; Janda, K. D. *J. Am. Chem. Soc.* **2008**, *130*, 9200.
2. Taga, M. E.; Miller, S. T.; Bassler, B. L. *Mol. Microbiol.* **2003**, *50*, 1411.