Synthetic biohybrid peptidoglycan oligomers enable pan-bacteria-specific labeling and imaging : in vitro and in vivo

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2020

He, J.-X., Hoang, K. L. M., Kho, S. H., Guo, Z., Zhong, W., Thappeta, K. R. V., . . . Liu, X.-W. (2020). Synthetic biohybrid peptidoglycan oligomers enable pan-bacteria-specific labeling and imaging : in vitro and in vivo. Chemical Science, 11(12), 3171-3179. doi:10.1039/c9sc06345e

https://hdl.handle.net/10356/138576

https://doi.org/10.1039/c9sc06345e

Supporting Information

Synthetic Biohybrid Peptidoglycan Oligomers Enable Pan-Bacteria-Specific Labeling and Imaging: *In Vitro* and *In Vivo*

Jing-Xi He, Dr. Kim Le Mai Hoang, Shu Hui Kho, Dr. Zhong Guo, Wenbin Zhong, Dr. Kishore Reddy Venkata Thappeta, Dr. Rubí Zamudio-Vázquez, Sin Ni Hoo, Qirong Xiong, Prof. Hongwei Duan, Prof. Liang Yang, Prof. Mary B. Chan-Park* and Prof. Xue-Wei Liu*

Outline

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Materials

Chitosan (Mw ≤ 3000 Da, degree of deacetylation > 85%) was purchased from Carbosynth Ltd. (Berkshire, UK). *N*-(3-Dimethylaminopropyl)-*N′*-ethylcarbodiimide hydrochloride (EDCI), 1-[Bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-*b*]pyridinium 3-oxid hexafluorophosphate (HATU), 3*H*-[1,2,3]-Triazolo[4,5-*b*]pyridin-3-ol (HOAt) and all amino acids used in synthesis were purchased from GL Biochem Ltd. (Shanghai, China). Membrane dye FM 1-43fx was purchased from Thermo Fisher Scientific Inc. (Waltham, USA). All other chemicals used in synthesis were purchased from Sigma-Aldrich Co. LLC. (St. Louis, USA). Bacterial strains (*Escherichia coli* ATCC 29425, ATCC958, ATCC8739, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC BAA-40, ATCC 1556, ATCC29213 *Enterococcus faecalis* ATCC 700802, and *Bacillus Subtilis* ATCC 6633) were purchased from the American Type Culture Collection (Manassas, USA) and stored at -80 ^{o}C. Mueller-Hinton broth (MHB, Difco), brain heart infusion broth (BHI, Difco) and trypticase soy broth (TSB, Difco) were purchased from Beckton, Dickinson and company (Franklin Lakes, USA). Enzyme was purchased from Cusabio (Texas, USA). Dialysis tubing was purchased from Spectra/Por (Singapore).

The reactions were all performed under nitrogen atmosphere. Starting materials and reagents were all purchased commercially and used as received. Solvents used in reactions were all purified according to standard procedures in literature. Thin layer chromatography (TLC) with Merck TLC silica gel 60 F254 plate was used to check reaction progress. UV, or potassium permanganate staining if necessary, was used to visualize compounds on TLC plates. Flash column chromatography with silica gel 60 (0.010-0.063 mm) and gradient solvent system was

used to isolate products. ^{1}H and ^{13}C NMR spectra were obtained using 400 MHz Bruker AVIII 400 spectrometer or 500 MHz Bruker AV 500 spectrometer. Tetramethylsilane (TMS) was used as internal standard in measurement of chemical shifts (ppm). Multiplicities were reported as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet or unsolved), br s (broad singlet) or dd (doublet of doublets). The number of protons (n) corresponding to a resonance signal was indicated as nH and coupling constants were reported as J values in units of Hz. Characterization data for known compounds were checked in comparison with literature for consistency and not presented in this report. Polymeric substrates were purified by dialysis using a dialysis tubing cellulose membrane (3.5 kDa MWCO) for 2 days. Shimadzu LCsolution and Kromasil 100-5C8 reverse phase column was used for HPLC analysis with deuterium lamp at 280 nm.

Experimental for Chemical Synthesis

Protection of chitosan was done following protocols similar to those reported by Ifuku *et al* and Gagnon *et al*.[1] Coupling with peptide, glycosylation and deprotection followed protocols similar to those reported by Kahne *et al* and Wong *et al*.[2] Whereas substrates were prepared following to literature, ^{1}H NMR for substrates were attached below for reference. In addition, ^{13}C NMR and HRMS were given for newly prepared peptides. Calculations are based on repeating monosaccharide units on the oligomers.

Synthesis of polymer **2**.

Chitosan **1** (2.0 g, 12.5 mmol) was dissolved in 100 mL mixture of AcOH/H_2O (v/v, 1:9). Phthalic anhydride (5.6 g, 37.5 mmol) was then added and the solution was stirred at 120 ^{o}C for 24 hours before cooling down to room temperature. The solvent was removed under reduced pressure and residue washed with ethanol and diethyl ether to give product **2** (3.3 g, 88%) as an off white solid.

Synthesis of polymer **3**

Polymer **2** (1.5 g, 5 mmol) was dissolved in 100 mL DMF. Imidazole (2.7 g, 40 mmol) was added, followed by triisopropylsilyl chloride (6.8 g, 35 mmol) dropwise at 0 ℃. The reaction mixture was slowly warmly up to room temperature and stirred for 48 hours before solvent was removed under reduced pressure. The residue was washed with ethanol and diethyl ether to give product **3** (1.7 g, 71%) as a yellow solid.

Synthesis of polymer **4**

Polymer **3** (0.96 g, 2 mmol) was dissolved in 20 mL DMF at 0 ℃. Sodium hydride (200 mg, 5 mmol) was added portionwise and then (*S*)-(−)-2-Bromopropionic acid (153 mg, 1 mmol)

was added dropwise. The reaction mixture was slowly warmed up to room temperature and stirred for 48 hours before quenching with methanol. The solvent was removed under reduced pressure and residue washed with water and ethanol consecutively to give product **4** (0.69 g, 69%) as a yellow solid.

Synthesis of polymer **5**

Polymer **4** (0.50 g, 1 mmol) and 4-dimethylaminopyridine (244 mg, 2 mmol) were dissolved in 20 mL pyridine at 0 ℃. Acetic anhydride (510 mg, 5 mmol) was added to the solution dropwise with stirring. The reaction was slowly warmed up to room temperature and stirred for 48 hours. Then solvent was removed under reduced pressure and the residue was washed with saturated ammonium chloride solution, followed by water to give product **5** (0.46 g, 92%) as an off white solid.

Synthesis of polymer **6**

To a solution of pentapeptide **12** (91 mg, 0.1 mmol) in 20 mL CH_2Cl_2 was added 4 mL 2.0 M HCl in Et_2O and the mixture was stirred at room temperature for 4 hours. After checking full consumption of **12** by TLC, the solvent was removed *in vacuo* and the crude H-Ala-D-*iso*-Glu(OBn)-Lys(Fmoc)-D-Ala-D-Ala-OMe ·HCl **13** was used without further purification. Polymer **5** (100 mg, 0.2 mmol) and DIPEA (52 mg, 0.4 mmol) were dissolved in 25 mL DMF at room temperature. To the stirring solution was added HATU (190 mg, 0.5 mmol) and HOAt (68 mg, 0.5 mmol). After 5 min, H-Ala-D-*iso*-Glu(OBn)-Lys(Fmoc)-D-Ala-D-Ala-OMe ·HCl (170 mg, 0.2 mmol) was added and the reaction mixture was left stirring overnight. After removing solvent under reduced pressure, the residue was washed with saturated ammonium chloride solution and water to give product **6** (146 mg, 79%) as a brown solid.

Synthesis of polymer **7**

Polymer **6** (92 mg, 0.1 mmol) was dissolved in 30 mL methanol. Acetic acid (120 mg, 2 mmol), tetrabutylammonium fluoride (1.0 M THF solution, 2.0 mL) and hydrazine (64 mg, 2 mmol) were added consecutively. The mixture was stirred at room temperature for 48 hours before solvent was removed under reduced pressure. The residue was washed with saturated ammonium chloride solution and water, then dried and redissolved in pyridine together with 4-dimethylaminopyridine (25 mg, 0.2 mmol). Acetic anhydride (51 mg, 0.5 mmol) was added to the solution dropwise at 0 ℃ with stirring. The reaction was slowly warmed up to room temperature and stirred for 48 h. Then solvent was removed under reduced pressure and the residue was washed with saturated ammonium chloride solution, followed by water to give product **7** as a yellow oil. The crude oil was used without further purification.

Synthesis of polymer **8**

Crude polymer **7** was dissolved in 15 mL THF at 0 ℃. Methylamine (1.0 M THF solution, 0.3 mL) was added dropwise and the mixture was slowly warmed up to room temperature with stirring. After 24 hours, the solvent was removed under reduced pressure. The residue was washed with saturated ammonium chloride solution and water, then evaporated to dryness and dissolved in 30 mL dichloromethane. To this solution was added 1*H*-tetrazole (21 mg, 0.3 mmol) and dibenzyl *N*,*N*-diisopropylphosphoramidite (70 mg, 0.2 mmol) at 0 ℃. The mixture was warmed up and stirred at room temperature for 5 hours before cooling to -50 ℃. Then *tert*-butyl hydroperoxide (70%, 1 mL) was added and the mixture was left stirring overnight. After removing solvent under reduced pressure, the residue was washed with saturated ammonium chloride solution, saturated sodium bicarbonate solution and water to give product **8** as a yellow

oil. The crude was used for next step without further purification.

Synthesis of polymer **9**

Tetradecyl monophosphate (59 mg, 0.2 mmol) was dissolved in 10 mL mixture of DMF and THF (v/v, 1:1) under room temperature. Then CDI (162 mg, 1 mmol) was added and the solution was stirred for 2 h before 1 mL dried methanol was added. The mixture was stirred for another 1 hour and dried to give activated tetradecyl phosphoroimidazolidate (C_{14}PIm). To a solution of crude polymer **8** in 10 mL MeOH was added 8 mg Pd on activated charcoal. The suspension was stirred under H_2 atmosphere at room temperature overnight before filtration through a pad of celite. The solution was dried, and redissoved in 10 mL DMF before transferring to C_{14}PIm. Subsequently, 1*H*-tetrazole (14 mg, 0.2 mmol) was added and the mixture was stirred for 24 hours before evaporation to dryness. Then the residue was dispersed in 20 mL mixture of methanol and water (v/v, 1:1) and LiOH (1 M aqueous solution, 1 mL) was added. The mixture was stirred for 2 hours before dialysis and lyophilization to give the final product **9** (50 mg, 50% for three steps) as a yellow solid. HPLC analysis was performed using NH_4OH/MeOH from 0/100 to 10/90 in 60 min and **9** had a retention time of 7 min. Tagging by sulforhodamine B was done following literature procedures.[3] Generally, 10 mg **9** was dissolved in 2 mL carbonate buffer (0.1 M, pH = 9) and a solution of sulforhodamine B acid chloride in DMF (2 mg/mL, 100 μL) was added. The mixture was left to stir in dark at room temperature for 2 hours and dialyzed afterwards to give PGOs-rhodamine.

The pentapeptide **13** for coupling to chitosan backbone was synthesized by a condensation reaction between Boc-Ala-D-*iso*-Glu(OBn)-OH and H-Lys(Fmoc)-D-Ala-D-Ala-OMe followed by Boc removal with hydrogen chloride according to methods reported in literature.[4]

Synthesis of compound **11**, Boc-Lys(Fmoc)-D-Ala-D-Ala-OMe

H-D-Ala-D-Ala-OH (320 mg, 2.00 mmol) was dissolved in 20 mL MeOH at 0 ℃ and acetyl chloride (785 mg, 10.0 mmol) was added dropwise. The reaction was stirred for 15 minutes before slowly warming up to room temperature and stirring overnight. After removing solvents *in vacuo*, crude H-D-Ala-D-Ala-OMe **10** was dissolved in 10 mL anhydrous DMF followed by addition of DIPEA (646 mg, 5.00 mmol). Subsequently, Boc-Lys(Fmoc)-OH (937 mg, 2.00 mmol), HOAt (408 mg, 3.00 mmol) and EDCI (575 mg, 3.00 mmol) were added and the mixture was stirred for 2 hours before pouring into 50 mL water. Then EtOAc (30 mL x 2) was used for extraction and the combined organic layer was washed with water (50 mL x 5), brine (50 mL) and dried with Na_2SO_4. The crude was purified by flash column chromatography (50% CH_2Cl_2/EtOAc) to give compound **11** as a white solid (0.99 g, 79%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.18 (d, *J* = 7.2 Hz, 1H), 7.99 (d, *J* = 7.9 Hz, 1H), 7.88 (d, *J* = 7.5 Hz, 2H), 7.68 (d, *J* = 7.5 Hz, 2H), 7.41 (t, *J* = 7.5 Hz, 2H), 7.33 (t, *J* = 7.5 Hz, 2H), 7.25 (t, *J* = 5.9 Hz, 1H), 6.92 (d, *J* = 7.3 Hz, 1H), 4.40 – 4.15 (m, 6H), 3.60 (s, 3H), 2.95 (q, *J* = 6.6 Hz, 2H), 1.63 – 1.06 (m, 21H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 173.22, 172.44, 172.24, 156.52, 155.94, 144.39, 141.19, 128.04, 127.49, 125.58, 120.56, 78.60, 65.65, 54.93, 52.31, 48.00, 47.92, 47.24, 32.56, 31.79, 30.06, 29.52, 28.61, 23.18, 18.64, 17.23. HRMS (ESI) calcd. for C33H45N4O8 [M+H]: 625.3237, found: 625.3237.

Synthesis of compound **12**, Boc-Ala-D-*iso*-Glu(OBn)-Lys(Fmoc)-D-Ala-D-Ala-OMe.

To a solution of **11** (624 mg, 1.00 mmol) in 30 mL CH_2Cl_2 was added 5 mL 2.0 M HCl in Et_2O and the mixture was stirred at room temperature for 4 hours. After checking full consumption of **11** by TLC, the solvent was removed *in vacuo* and the crude H-Lys(Fmoc)-D-

Ala-D-Ala-OMe·HCl was used without further purification. Boc-Ala-OSu (286 mg, 1.00 mmol) and H-D-Glu(OH)-OBn (237 mg, 1.00 mmol) were dissolved in 10 mL DMF and 2 mL saturated $NaHCO_3$ (aq.) solution was added to the mixture. After stirring at room temperature overnight, 30 mL water was added and pH of the solution was adjusted to 2 by careful addition of HCl. The solution was extracted with EtOAc (20 mL x 2) and the combined organic layer was washed with 1 mM aq. HCl (30 mL x 2), water (30 mL x 2) and brine (30 mL). After drying over Na_2SO_4, the solvent was removed *in vacuo* and the crude Boc-Ala-D-*iso*-Glu(OBn)-OH was dissolved in 20 mL DMF. To the solution was added DIPEA (388 mg, 3.00 mmol), crude H-Lys(Fmoc)-D-Ala-D-Ala-OMe·HCl, HATU (760 mg, 2.00 mmol) and HOAt (272 mg, 2.00 mmol). The mixture was stirred at room temperature overnight before 60 mL water was added. Then it was extracted with EtOAc (50 mL x 2) and the combined organic layer was washed with water (80 mL x 5) and brine (80 mL). After removing solvent *in vacuo*, the crude was purified by flash column chromatography (60% CH_2Cl_2/Acetone) to give compound **12** (730 mg, 77%) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.27 (d, *J* = 7.8 Hz, 1H), 8.18 (d, *J* = 7.5 Hz, 2H), 8.02 (d, *J* = 7.2 Hz, 1H), 7.88 (d, *J* = 7.5 Hz, 2H), 7.84 (d, *J* = 7.5 Hz, 2H), 7.41 (t, *J* = 7.5 Hz, 2H), 7.38 – 7.24 (m, 8H), 6.86 (d, *J* = 7.7 Hz, 1H), 6.60 (t, *J* = 5.9 Hz, 1H), 6.28 (d, *J* = 1.5 Hz, 2H), 5.11 (s, 2H), 4.27 (dh, 3H), 4.15 (q, *J* = 7.2 Hz, 1H), 4.02 (p, *J* = 7.2 Hz, 1H), 3.59 (s, 3H), 2.88 (q, *J* = 6.6 Hz, 2H), 2.19 (q, *J* = 7.7 Hz, 2H), 1.97 (h, *J* = 7.3, 6.6 Hz, 1H), 1.83 (dq, *J* = 15.0, 8.4, 7.6 Hz, 1H), 1.65 – 0.97 (m, 24H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 173.49, 173.28, 172.52, 171.99, 157.77, 155.44, 143.03, 139.87, 137.88, 136.35, 129.38, 128.84, 128.43, 128.18, 127.74, 121.83, 120.48, 110.19, 78.54, 66.39, 53.50, 52.25, 52.04, 50.13, 48.07, 48.02, 31.85, 31.67, 30.06, 29.84, 28.63, 27.37, 23.11, 18.92, 18.40, 17.22.

HRMS (ESI) calcd. for C48H63N6O12 [M+H]: 915.4504, found: 915.4513.

Rhodamine labeled pentapeptide **14** was synthesized by replacing Fmoc on lysine side chain with rhodamine B, followed by removal of Boc with hydrogen chloride using the same procedure as **13** and removal of OBn and OMe with LiOH using the same procedure as **9**. The product was dialyzed using 100-500 MWCO dialysis tubing and lyophilized without further purification.

Synthesis of compound **15**, Boc-Ala-D-*iso*-Glu(OBn)-Lys(Rhodamine)-D-Ala-D-Ala-OMe.

To a solution of **12** (91.4 mg, 0.10 mmol) in 10 mL DMF was added 2 mL diethylamine and the mixture was stirred at room temperature for 1 hour. After checking full consumption of **12** by TLC, diethylamine was removed *in vacuo* and rhodamine B (71.9 mg, 0.15 mmol), HOAt (20.4 mg, 0.15 mmol) and EDCI (28.8 mg, 0.15 mmol) were added. Then DIPEA (38.8 mg, 0.30 mmol) was added into the solution and it was left to stir at room temperature overnight before 30 mL water was added. Then it was extracted with EtOAc (30 mL x 2) and the combined organic layer was washed with water (50 mL x 5) and brine (50 mL). After removing solvent *in vacuo*, the crude was purified by flash column chromatography (40% CH_2Cl_2/Acetone) to give compound **15** (73.6 mg, 64%) as a red solid. ^{1}H NMR (500 MHz, Chloroform-d) δ 8.34 (d, J = 7.9 Hz, 1H), 7.82 (s, 1H), 7.74 (s, 3H), 7.55 (q, J = 3.8 Hz, 2H), 7.33 (dd, J = 14.2, 5.8 Hz, 4H), 7.24 (d, J = 15.1 Hz, 2H), 7.09 – 6.99 (m, 4H), 6.85 (d, J = 9.8 Hz, 2H), 6.77 (s, 2H), 5.01 (s, 2H), 4.24 (t, J = 7.2 Hz, 3H), 3.66 (q, J = 7.5 Hz, 10H), 3.38 – 3.31 (m, 1H), 2.94 (d, J = 37.7, 35.0 Hz, 1H), 2.33 (s, 1H), 2.14 (s, 1H), 2.02 (s, 2H), 1.74 – 1.60 (m, 4H), 1.48 – 1.40 (m, 10H), 1.39 – 1.33 (m, 23H), 1.32 – 1.23 (m, 18H), 1.22 – 1.15 (m, 4H), 0.97 – 0.82 (m,

16H). ^{13}C NMR (101 MHz, Chloroform-d) δ 167.88, 165.14, 157.75, 155.61, 134.57, 133.46, 133.23, 132.60, 131.58, 131.33, 131.00, 130.51, 130.38, 128.93, 128.68, 128.65, 128.58, 128.40, 114.23, 113.65, 96.59, 68.30, 67.65, 46.27, 38.89, 30.51, 29.83, 29.07, 28.52, 23.90, 23.11, 14.17, 12.81, 11.09, 1.15.

Synthesis of polymer **16**, chitosan-pentapeptide conjugate with rhodamine tag

To a solution of pentapeptide **15** (23.0 mg, 0.02 mmol) in 20 mL CH_2Cl_2 was added 4 mL 2.0 M HCl in Et_2O and the mixture was stirred at room temperature for 4 hours. After checking full consumption of **15** by TLC, the solvent was removed *in vacuo* and DIPEA (12.9 mg, 0.1 mmol) in 20 mL DMF was added at room temperature. Then polymer **5** (10.0 mg, 0.02 mmol), HATU (19.0 mg, 0.05 mmol) and HOAt (6.8 mg, 0.05 mmol) were added into the solution and the reaction was left to stir overnight. After removing solvent under reduced pressure, the residue was washed with saturated ammonium chloride solution and water to give a crude of protected chitosan-pentapeptide conjugate. The crude was redispersed in 10 mL MeOH, followed by addition of tetrabutylammonium fluoride (261 mg, 1 mmol), hydrazine (32 mg, 1 mmol) and LiOH (1 M aqueous solution, 0.1 mL). The mixture was stirred for another 12 hours before dialysis and lyophilization to give the final product **16** as a red solid.

Synthesis of inhibitor **17**

Crude polymer **7** was dissolved in 15 mL THF at 0 ℃. Methylamine (1.0 M THF solution, 0.3 mL) was added dropwise and the mixture was slowly warmed up to room temperature with stirring. After 24 hours, the solvent was removed under reduced pressure. The residue was washed with saturated ammonium chloride solution and water, then evaporated to dryness and dissolved in 30 mL dichloromethane. To this solution was added 1*H*-tetrazole (21 mg, 0.3 mmol)

and methyl (2R)-3-(((benzyloxy)(diisopropylamino)phosphanyl)oxy)-2-(dodecyloxy)propanoate[5] (102 mg, 0.2 mmol) at 0 ℃. The mixture was warmed up and stirred at room temperature for 5 hours before cooling to -50 ℃. Then *tert*-butyl hydroperoxide (70%, 1 mL) was added and the mixture was left stirring overnight. After removing solvent under reduced pressure, the residue was washed with saturated ammonium chloride solution, saturated sodium bicarbonate solution and water to give crude product **17**. Then the residue was dispersed in 20 mL mixture of methanol and water (v/v, 1:1) and LiOH (1 M aqueous solution, 1 mL) was added. The mixture was stirred for 2 hours before dialysis and lyophilization to give the final product **17** (70 mg, 70% for two steps) as a yellow solid.

Figure S1 | ^{1}H NMR spectrum for **3** (400 MHz, DMSO)

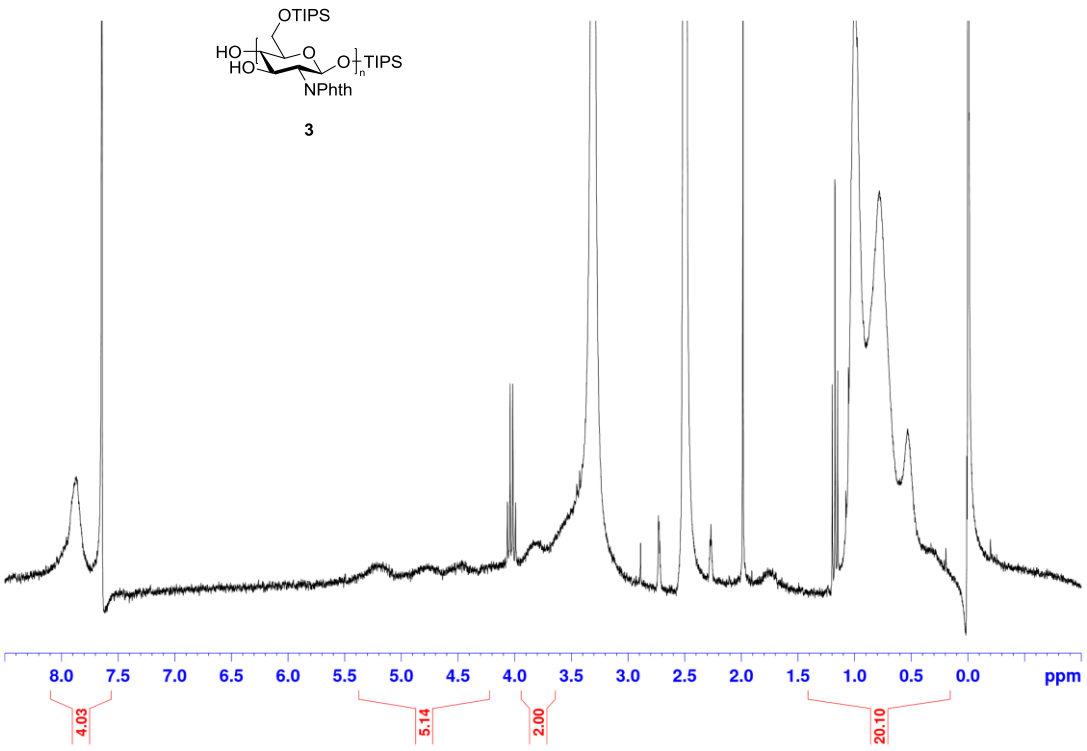


Figure S2 | ^{1}H NMR spectrum for **4** (400 MHz, DMSO)

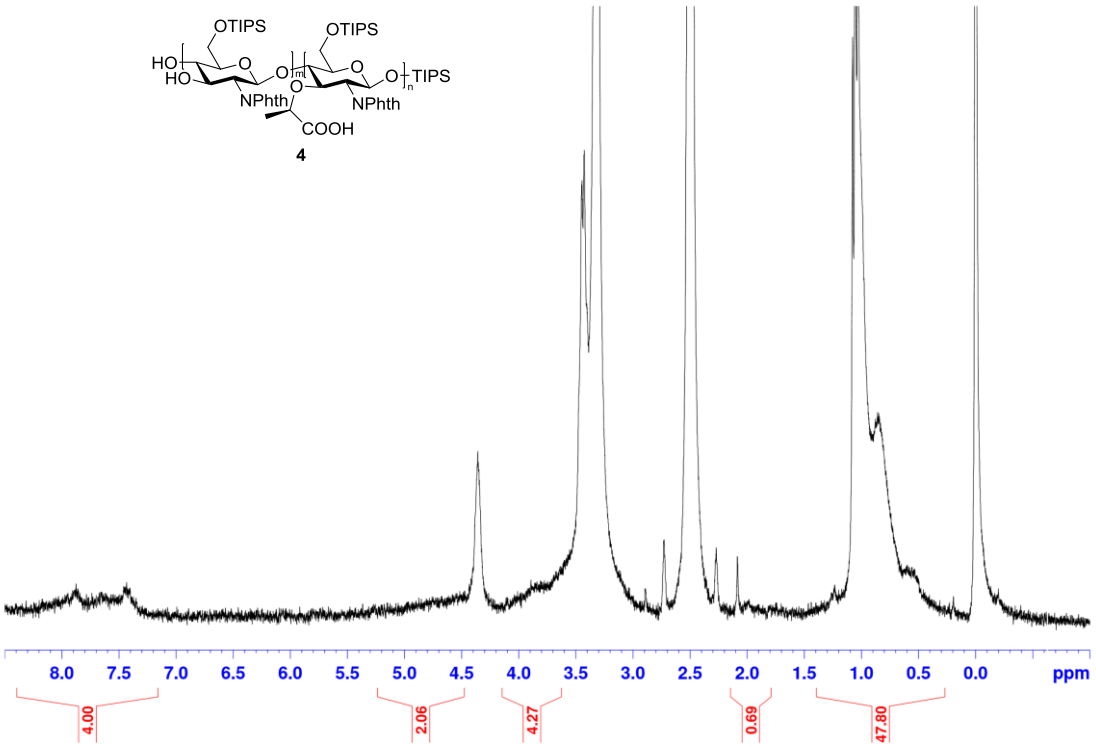


Figure S3 | ^{1}H NMR spectrum for **5** (400 MHz, DMSO)

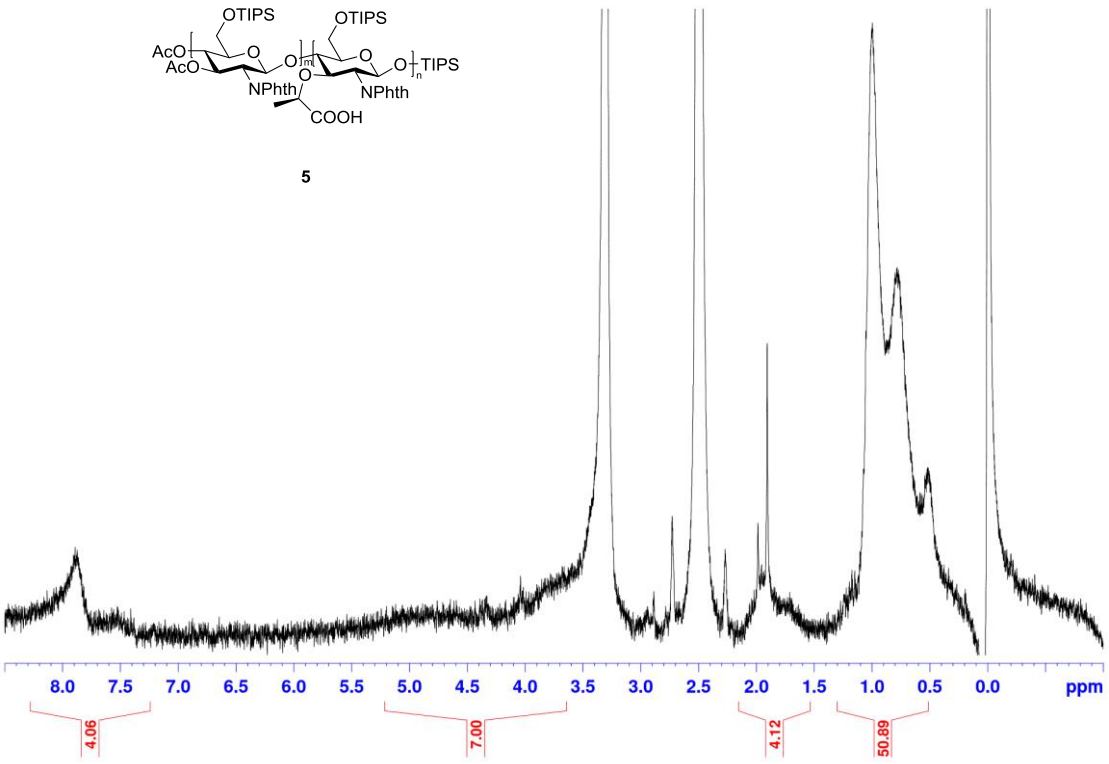


Figure S4 | ^{1}H NMR spectrum for **6** (400 MHz, DMSO)

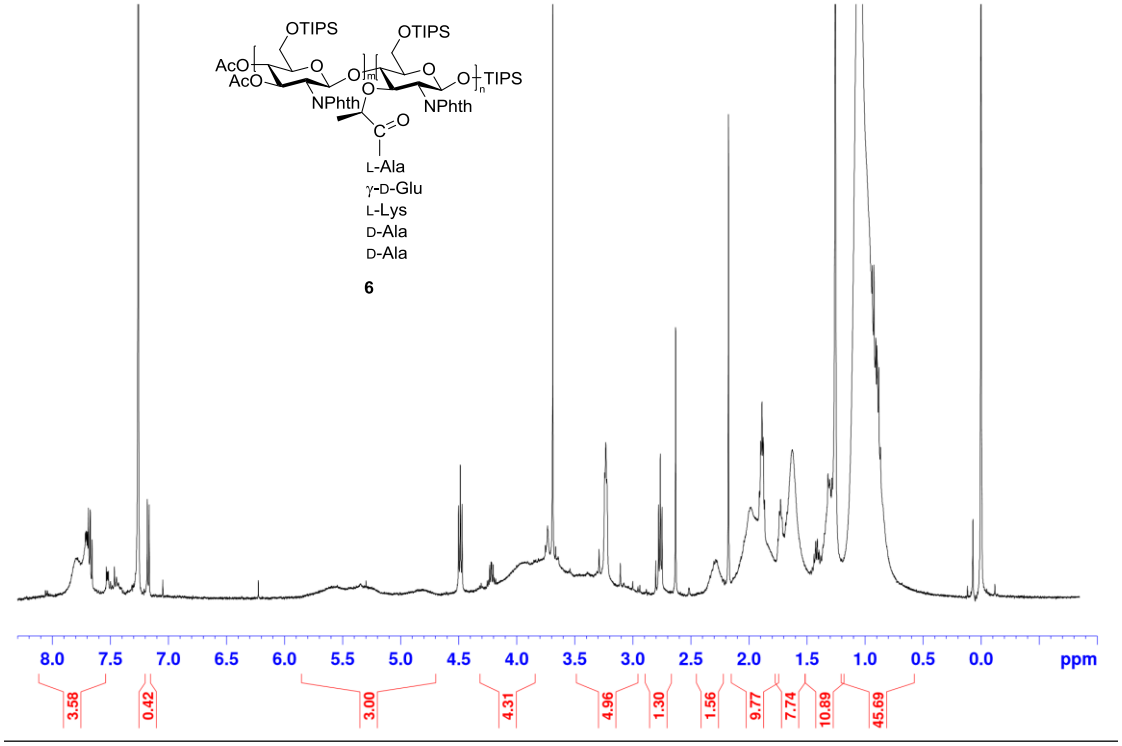
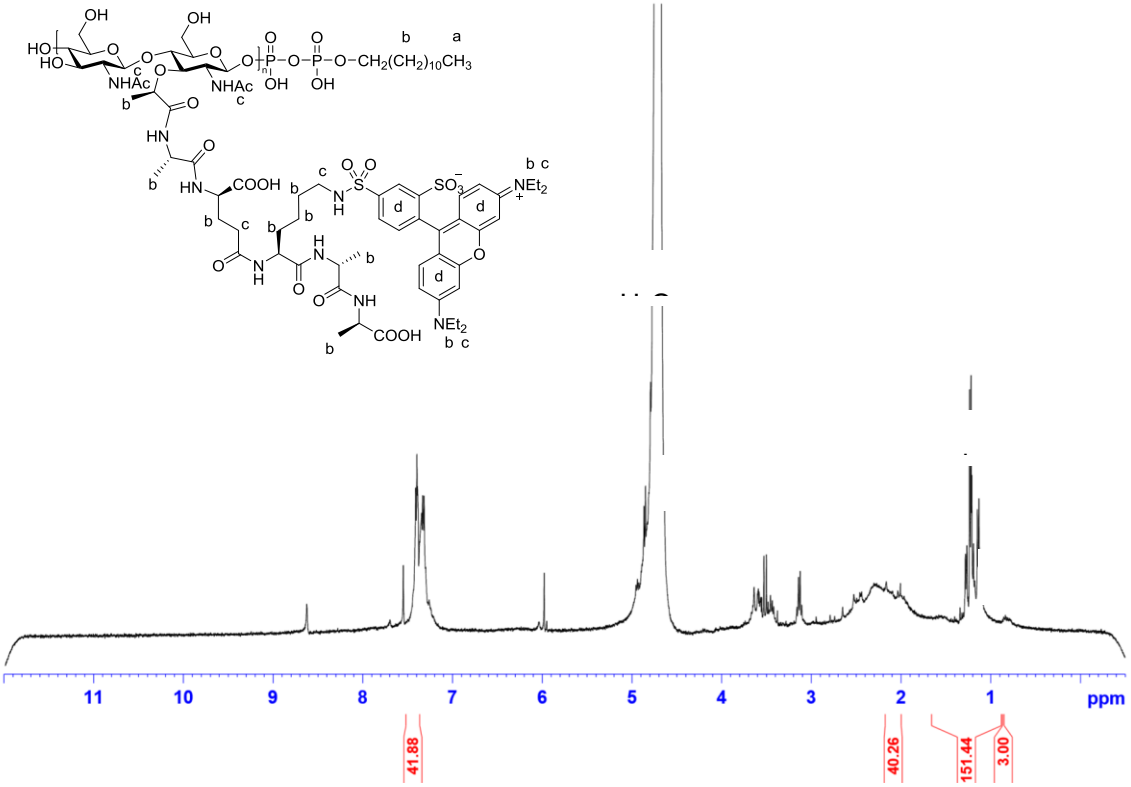


Figure S5 | ^{1}H NMR spectrum of fluorescently labeled **9** used for STED confocal microscopy (400 MHz, D_2O)



The methyl end of lipid moiety (0.8-0.9, **a**) has been calibrated to be 3 protons (1 lipid as base for calculation)

Aromatic protons (7-8, **d**) were only from rhodamine (9 per molecule), and 42 protons in total matched 4.6 rhodamine moieties per polymer on average.

Alkyl protons (0.9-1.5, **b**) were from lipid (24 per molecule), rhodamine (6 per molecule) and peptide (20 per molecule), and 151 protons matched 5.0 peptide moieties per polymer on average

Acetyl protons (2-2.2, **c**) were from NAG/NAM (3 per molecule) and glutamate on peptide (2 per molecule), and 40 protons matched 10.0 sugar moieties per polymer on average.

Figure S6 | ^{1}H NMR spectrum for **11** (400 MHz, DMSO)

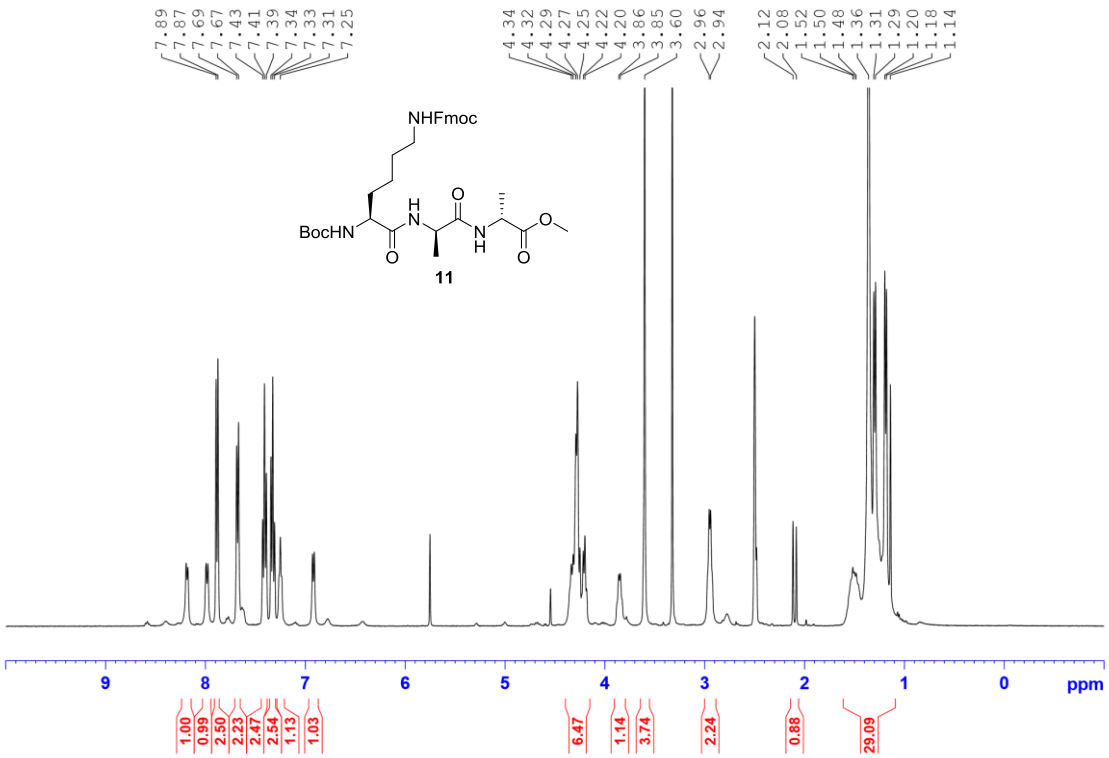


Figure S7 | ^{13}C NMR spectrum for **11** (101 MHz, DMSO)

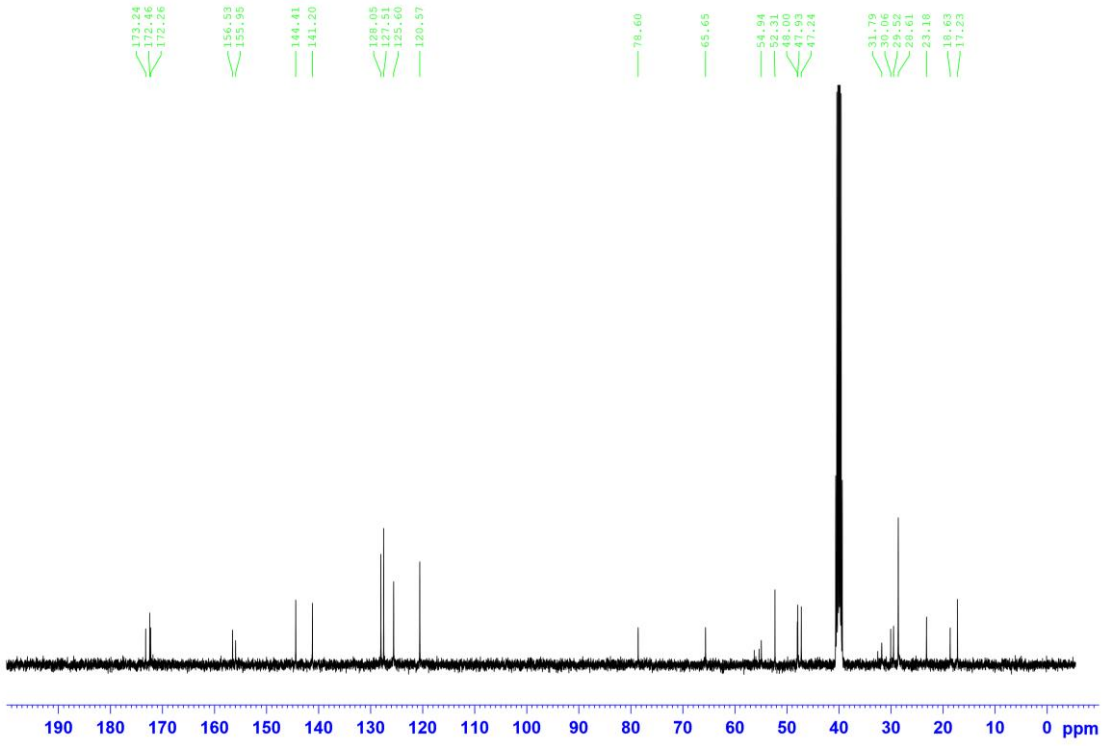


Figure S8 | ^{1}H NMR spectrum for **12** (400 MHz, DMSO)

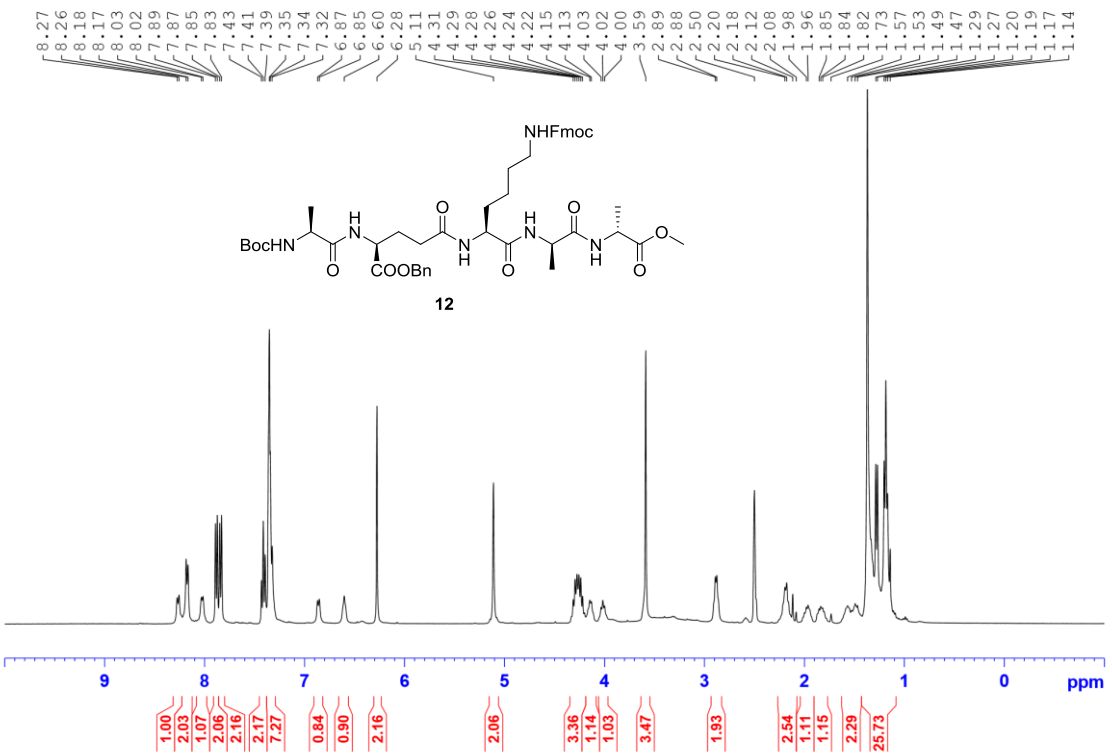


Figure S9 | ^{13}C NMR spectrum for **12** (101 MHz, DMSO)

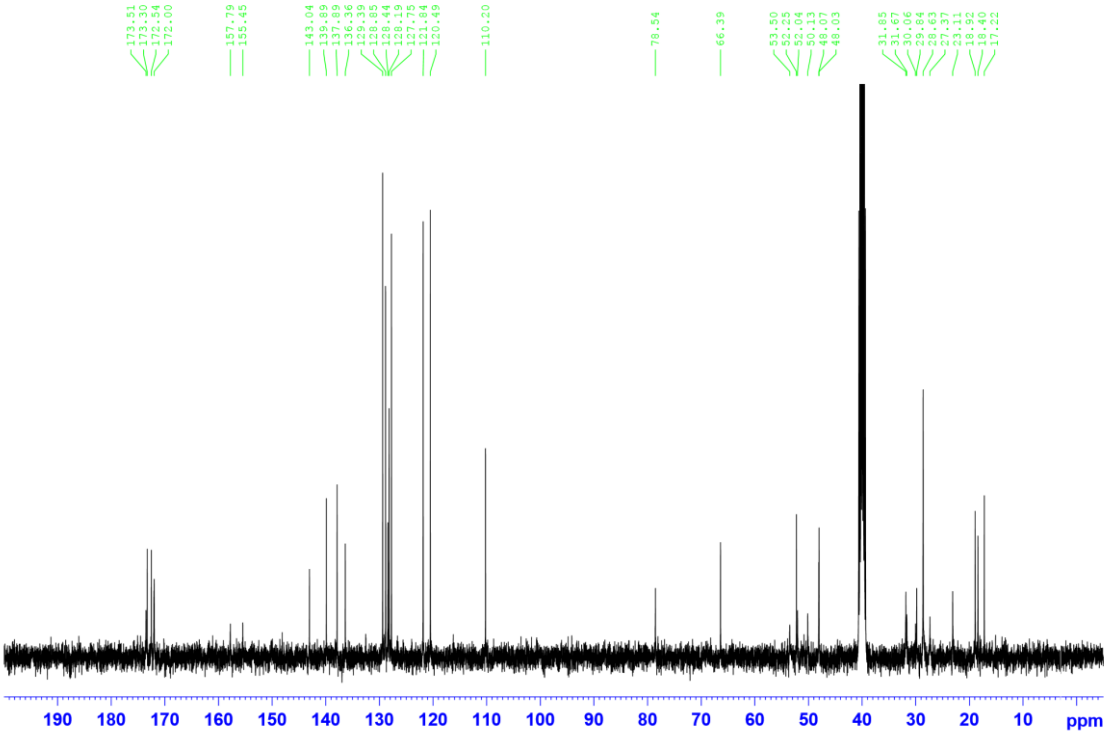


Figure S10 | ^{1}H NMR spectrum for **15** (500 MHz, Chloroform-*d*)

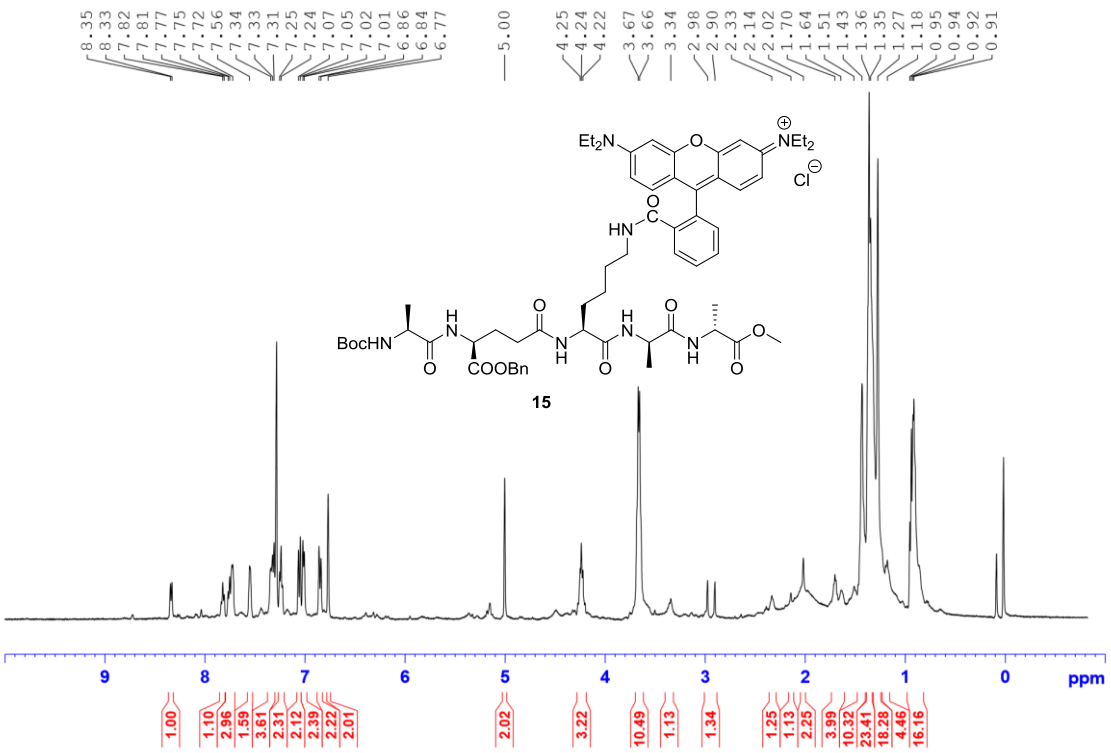


Figure S11 | ^{13}C NMR spectrum for **15** (101 MHz, Chloroform-*d*)

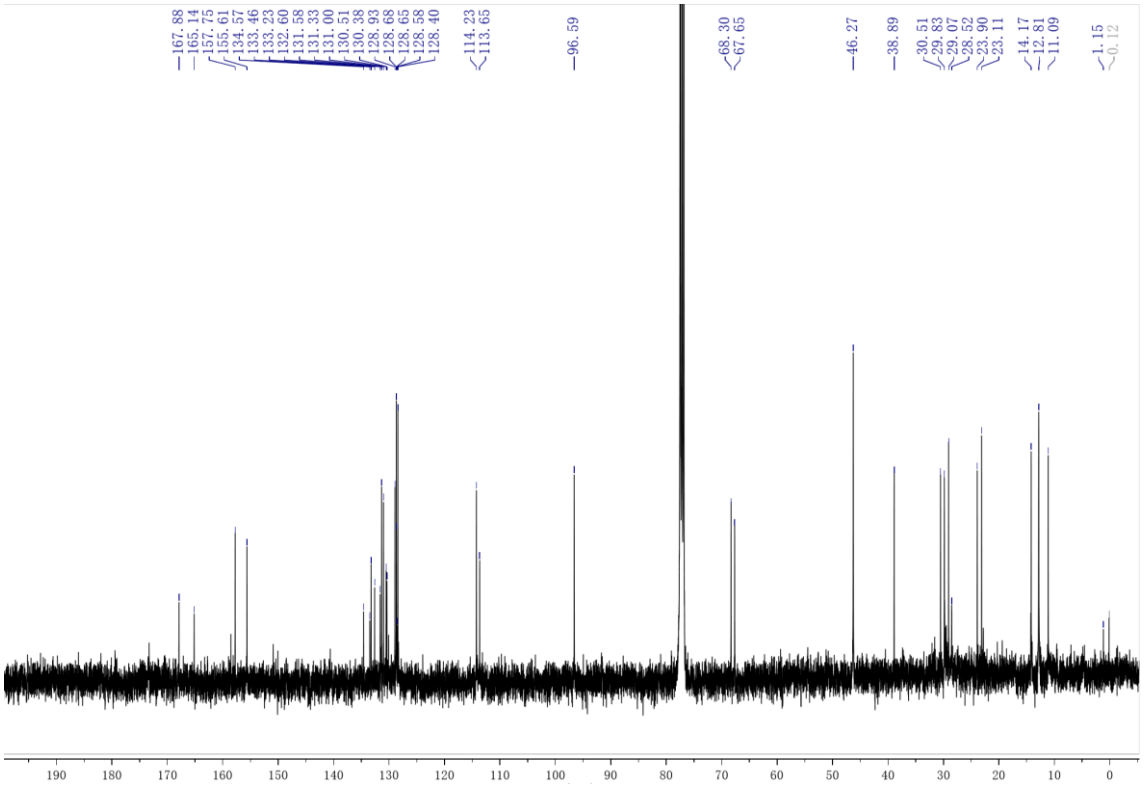


Figure S12 | ^{1}H NMR spectrum for rhodamine labeled Chitosan-peptide **16** (500 MHz, D_2O)

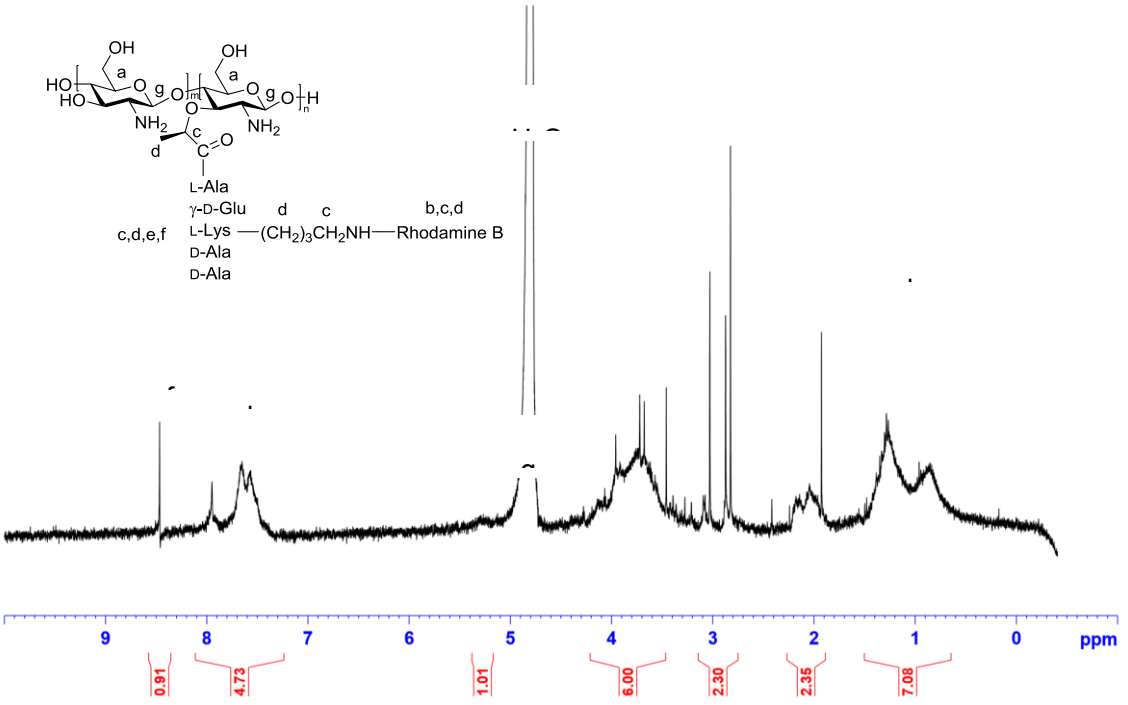
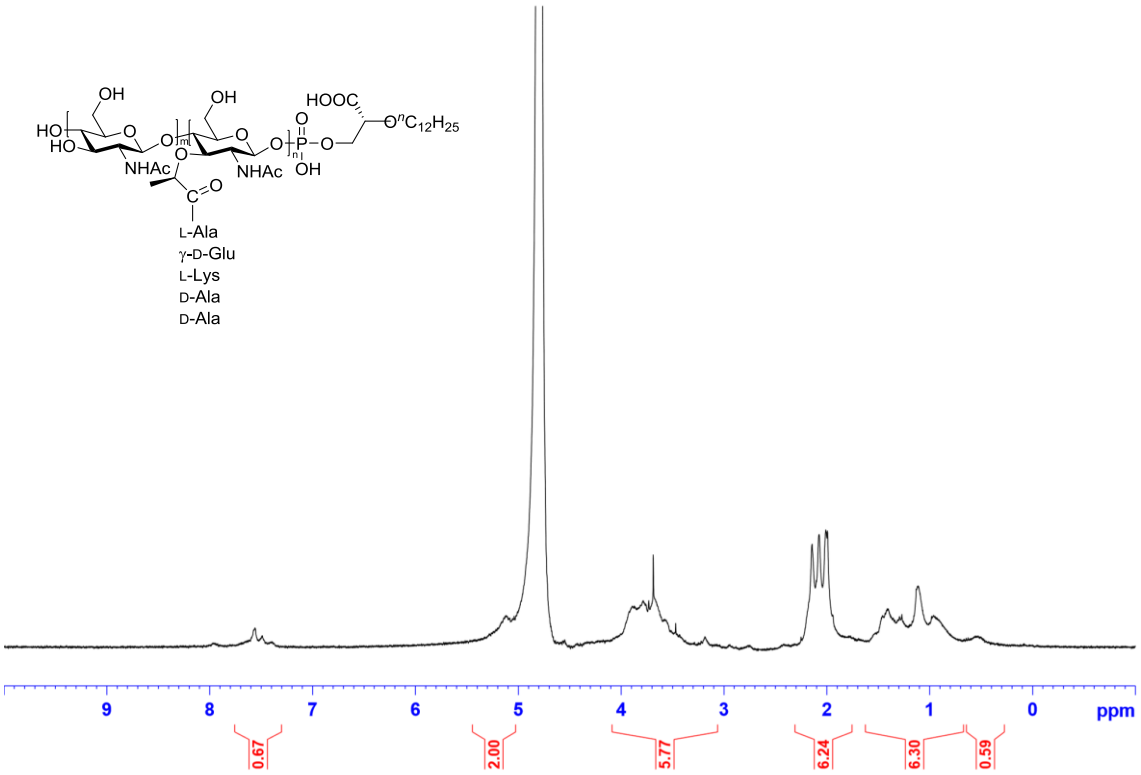


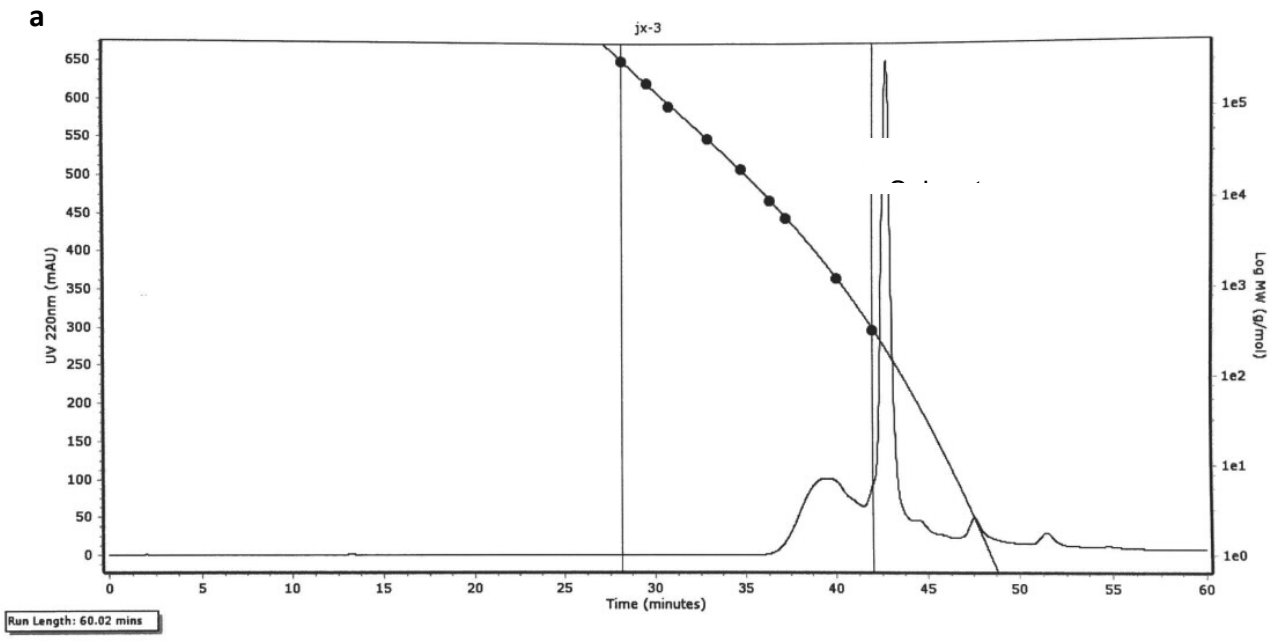
Figure S13 | ^{1}H NMR spectrum for **17** (500 MHz, D_2O)

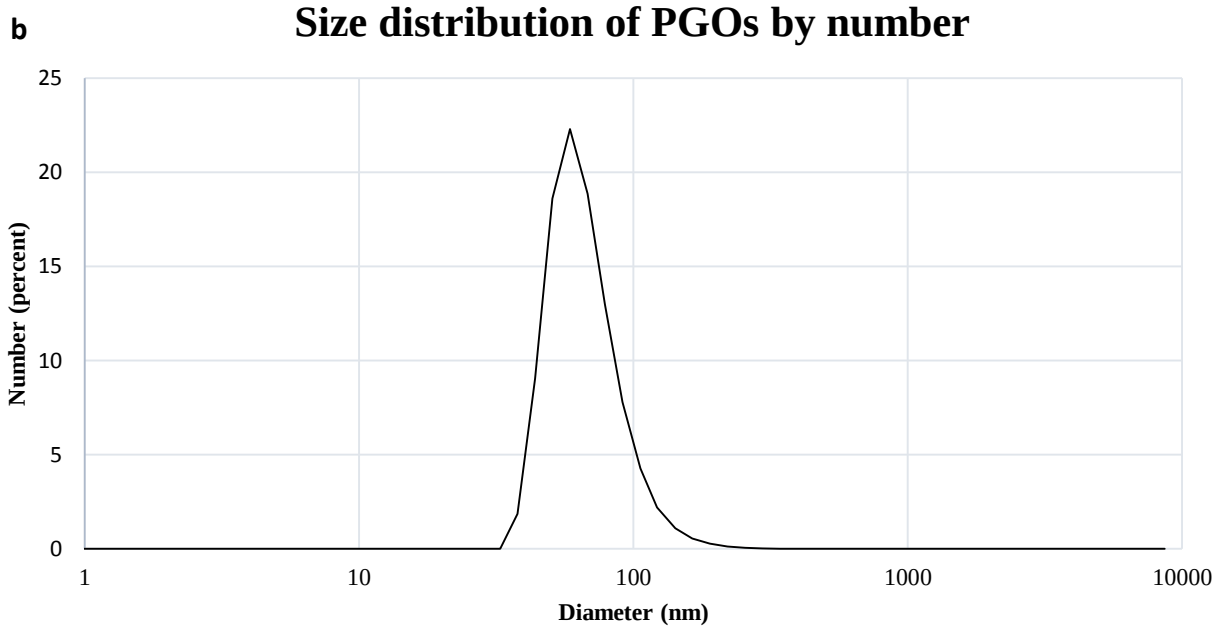


Gel Permeation Chromatography (GPC) and Dynamic Light Scattering (DLS)

Shodex SB-803 HQ and SB-805 HQ columns (Showa Denko, Tokyo, Japan) were connected in series for GPC in Agilent 1260 infinity system (Agilent, CA, USA). Samples were eluted at 0.5 mL/s through columns. Light scattering was done using Malvern zetasizer (Malvern Instruments Ltd, Malvern, UK). Secondary structures existed in the solutions of PGOs to broaden and shift their peak in GPC, which was confirmed by DLS.

Figure S14 | (a) Gel permeation chromatography results of PGOs eluted with 0.05 M NaCl in deionized water. Peak broadening was from various self-assemblies of the polymer molecules. (b) Size distribution of 100 μg/mL PGOs in deionized water measured by dynamic light scattering. Results indicated spontaneous self-assembly of PGOs, probably due to the amphiphilic nature from co-existence of sugar, peptide and lipid moieties in the substrate.

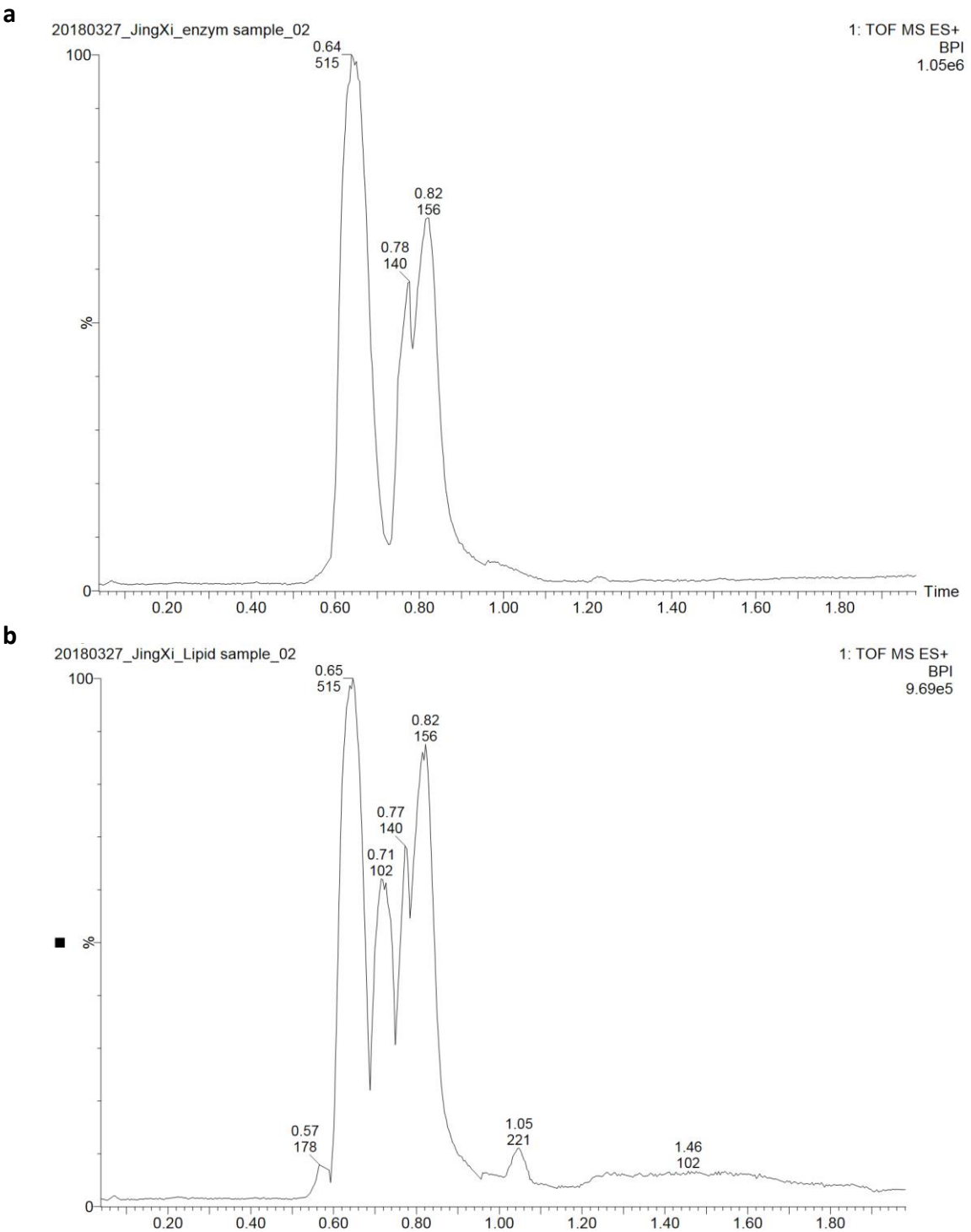




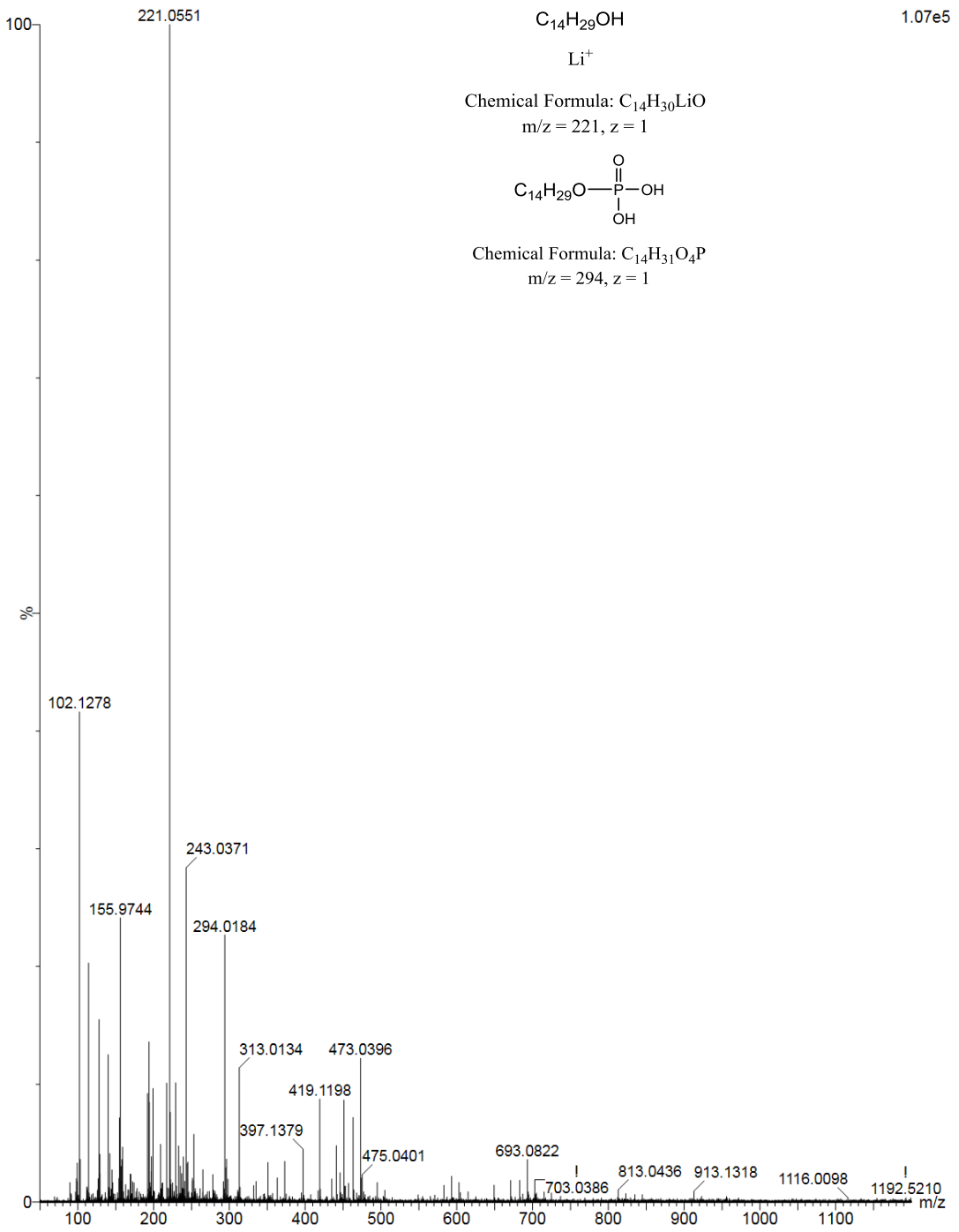
Lysozyme Degradation Assay

To prepare crude for mass analysis, 2 mg substrate **9** was dissolved in 0.2 mL 10 mM acetate buffer (pH = 5.0) before addition of 0.4 mg lysozyme and incubation at 38 ℃ for 24 hours. Then the enzyme was pelleted by centrifugation at 1,500 X *g* for 5 min, and the solution of crude metabolites was collected, diluted to 2 mL with 0.1 % formic acid in deionized water and analyzed by ESI-TOF. A signal of the PG building block, NAG-NAM with pendant pentapeptide, was observed with control experiments shown in Figure S10. The presence of this fragment supported our hypothesis during design of synthetic route, that our polymeric substrate **9** had an overall alternating NAM-NAG pattern. The resultant structure is similar to that found from natural bacterial cell wall, which is assembled from Lipid II.

Figure S15 | ESI-TOF analysis of (a) the crude enzyme buffer and (b) phospholipid in buffer. The spectrum of signal at 1 min (c) matched with tetradecanyl phosphate under different ionization and fragmentation pattern. The protocol for LCMS was the same as the lysozyme degraded PGOs.



c


221.0551
$C_{14}H_{29}OH$
1.07e5
100
Li^+
Chemical Formula: $C_{14}H_{30}LiO$
m/z = 221, z = 1
$C_{14}H_{29}O$—P(=O)(OH)—OH
Chemical Formula: $C_{14}H_{31}O_4P$
m/z = 294, z = 1
%
102.1278
243.0371
155.9744
294.0184
313.0134
473.0396
419.1198
397.1379
475.0401
693.0822
703.0386
813.0436
913.1318
1116.0098
1192.5210
0
100
200
300
400
500
600
700
800
900
1000
1100
m/z

Stimulated Emission Depletion Microscopy (STED)

To prepare samples for super resolution STED microscopy, overnight broth cultures were subsequently grown in 5 ml of fresh culture broth (1:100 dilution) to prepare logarithmic phase cultures after incubation at 37 ℃ for 4 hours in a shaking incubator (225 rpm). Then, bacteria cells were pelleted by centrifugation at 1,500 X *g* for 5 min, suspended in culture media at a concentration of 10^8 CFU ml^{-1} and incubated for 1 hour in the dark in the presence of 100 μg/mL of rhodamine-labeled derivative of **9** at 37 ℃ with agitation (225 rpm). Bacterial cells were next incubated with the membrane stain FM1-43FX (Life Technologies) at a final concentration of 5 μg/ml for 5 min, as suggested by the manufacturer, and subsequently washed three times with PBS and resuspended in a fixative solution of 2% paraformaldehyde in PBS (pH 7.0). Cells were fixed for 1 hour at 37 ℃ in a shaking incubator (225 rpm), washed three times in PBS and applied to a sterile glass bottom collagen coated dish (MatTek Corporation). STED super resolution microscopy was performed on a Leica TCS SP8 STED-3X microscope (Leica Microsystems, Wetzlar, Germany) at SingHealth Advanced Biomaging Core. 479 nm and 556 nm lasers were used for fluorescence excitation, while 660 nm STED laser was used for depletion. In order to achieve maximum lateral resolution, all images were acquired in 2D STED mode. Further image processing required deconvolution, which was done using Huygens Professional software (Scientific Volume Imaging, Hilversum, Netherlands). ImageJ was utilized for further image processing.

Preparation of L-form Enterococcus and TIRF Microscopy

L-forms were generated using DM3 agar by modified methods from reported protocol.[6] DM3 medium consists of 1.2 % agar, 0.5 % Tryptone, 0.5 % yeast extract, 1 M Succinate(pH 7.3), 3.5 % K_2HPO_4 and 1.5 % KH_2PO_4, 20% Glucose, 1 M $MgCl_2$ AND 2% BSA. Parental strain *E.faecalis* OG1RF was grown overnight at 37 ℃ in DM3 broth. 100 μL of an overnight culture was directly plated on DM3 agar plates supplemented with 200 μg/ml penicillin G. The plates were incubated at 37 ℃. Small fried egg-like shaped colonies appeared after 5 days. The colonies were restreaked on DM3 agar with 200 μg/mL penicillin G for a few times to get pure colonies, and serial passaging of pure colonies in DM3 agar with decreasing penicillin G concentrations to generate stable L-forms. The stable L-forms were stored at -80 ℃ in 20 % glycerol.

Fluorescence microscopy was performed on Nikon TIRF microscope (Nikon instruments, NY, USA). BODIPY FL and sulforhodamine B were excited at 488 and 560 nm and emitted at 512 and 580 nm respectively. Three days old L-forms grown in DM3 broth was washed and incubated with 1 μL of Polymyxin B or Boc-FL (1 mg/mL) and 2 μL substrate (2 mg/mL) for 30 min at 37 ℃. After 30 min, cells were washed thrice with 1 mL of liquid DM3. The final pellet was suspended in 30 μL liquid DM3. 5 μL of cells were placed on poly-lysine coated slides and observed under TIRF microscope (Figure S16). Image processing was done using MetaMorph Microscopy Automation & Image Analysis Software (Photometrics, AZ, USA). ImageJ was utilized for further image processing.

For FRET studies, 488 nm laser was used for fluorescence excitation, while emissions from both BODIPY FL (512 nm) and sulforhodamine B (580 nm) were collected simultaneously.

Samples were photobleached with FRAP device using 561 nm laser for 1 second while fluorescence intensity was monitored for both channels for 100 seconds in total (Figure S17).

Figure S16 | TIRF images and FRET results (a) Wild type and L-form cells of *E.faecalis* OG1RF grown in DM3 medium were incubated with PGOs-rhodamine and membrane dye Polymyxin B-BODIPY FL. Whereas wild type cell envelope demonstrated a comprehensible enhancement at septum compared to cell surface, only a few discrete spots of substrate binding were seen on the L-form surface. Scale bars, 1 μm and 10 μm for wild type and L-forms respectively. (b) Fluorescence intensity change of the two fluorophores during and after photobleaching. The alignment of change was marked with dashed lines in the combined graph.

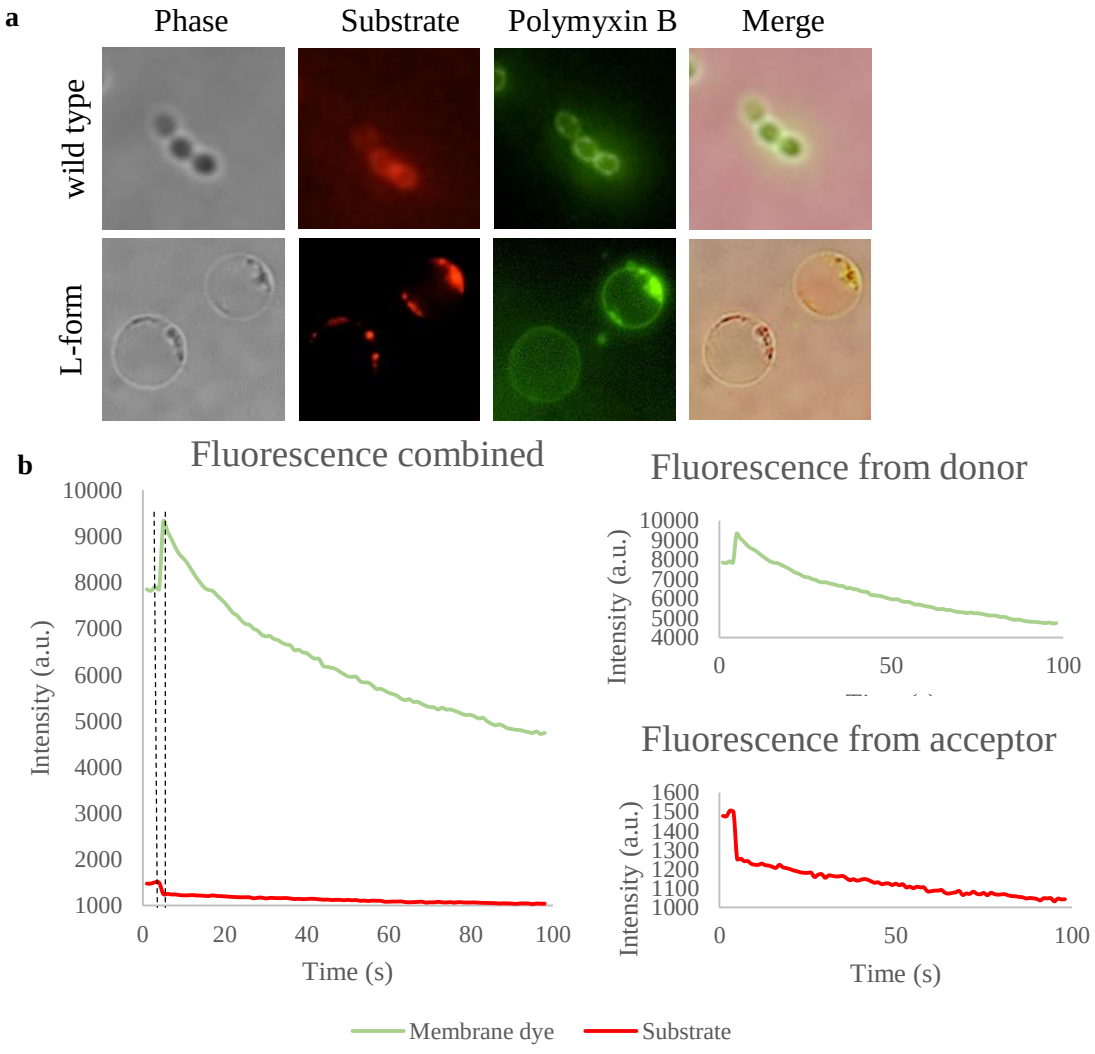
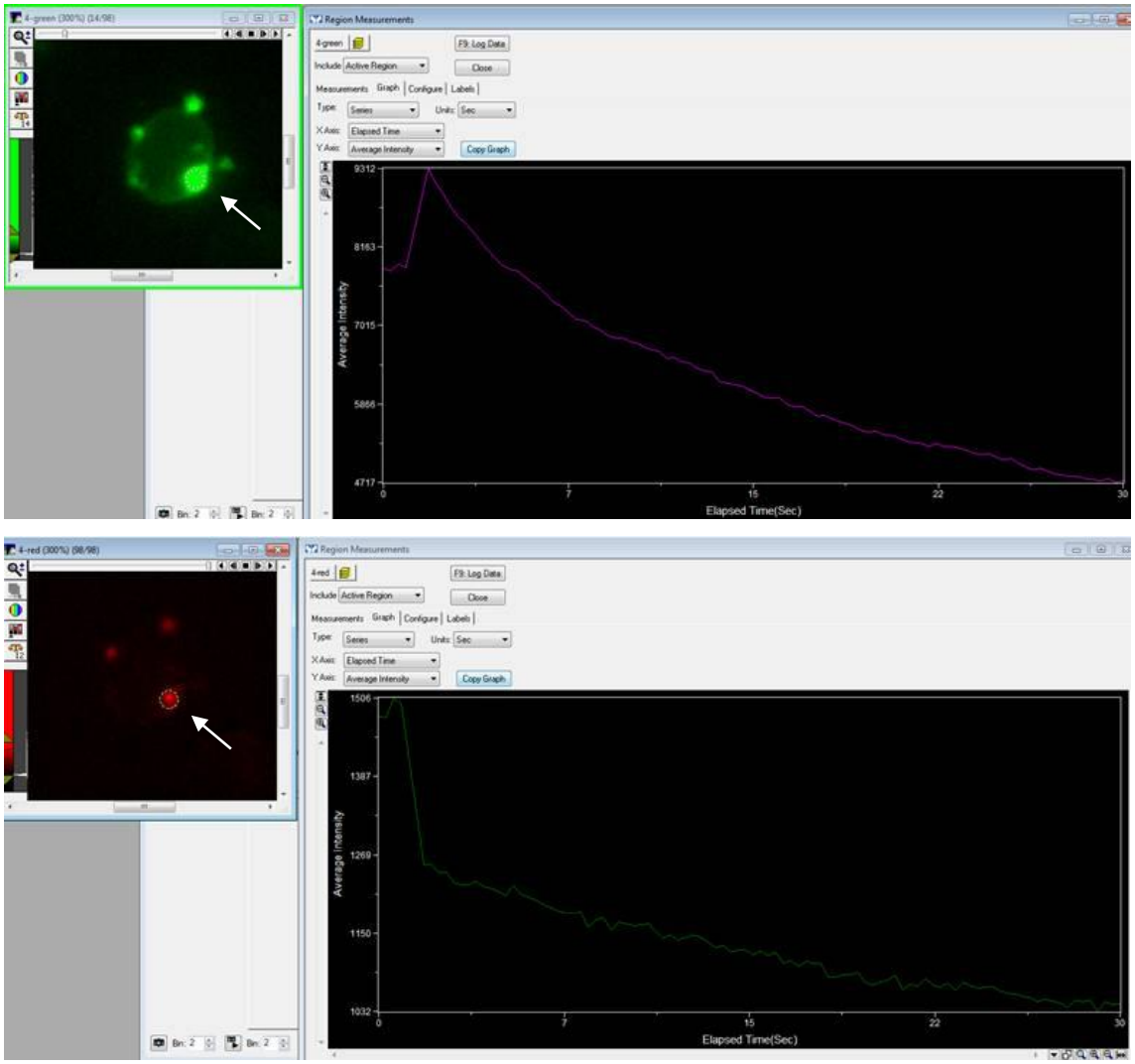


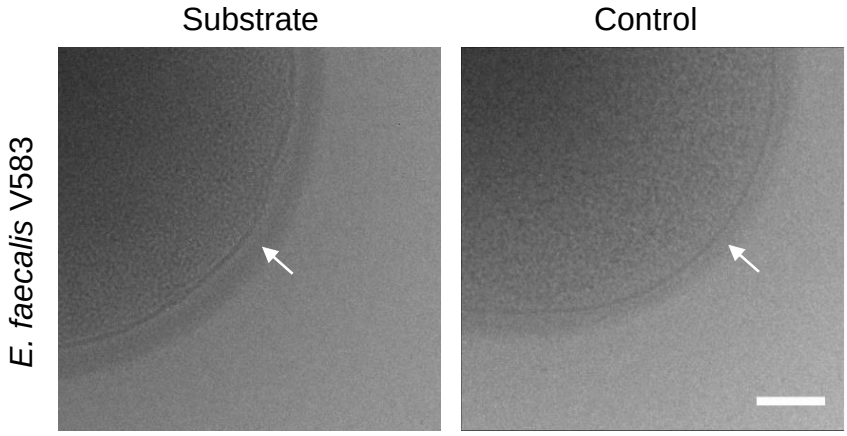
Figure S17 | Schematic illustration of photobleaching process. White arrow marked the area illuminated by 561 nm laser and right panel showed the monitored fluorescence intensity change of Bodipy FL (top) and sulforhodamine B (bottom) respectively.



Cryo-Transition Electron Microscopy (cryo-TEM)

To prepare samples for cryo-TEM, overnight TSB cultures were subsequently grown in 5 ml of fresh culture broth (1:100 dilution) to prepare logarithmic phase cultures after incubation at 37 ℃ for 4 hours in a shaking incubator (225 rpm). Then, *E. faecalis* bacteria cells were pelleted by centrifugation at 1,500 X *g* for 5 min, suspended in TSB culture media at a concentration of 10^8 CFU ml^{-1} and incubated for 2 h in the dark in the presence of 1 mg/mL of PGOs-rhodamine at 37 ℃ with agitation (225 rpm). The cells were subsequently washed three times with PBS and frozen onto copper grid by liquid nitrogen. Cryo-TEM was performed using FEI Titan Krios (300kV, FEG, Falcon II direct detector, and Gatan Tridiem GIF with 2k x 2k post-GIF Gatan CCD) at NUS Centre for BioImaging Sciences. The images were taken at 14,000x magnification and processed subsequently by ImageJ.

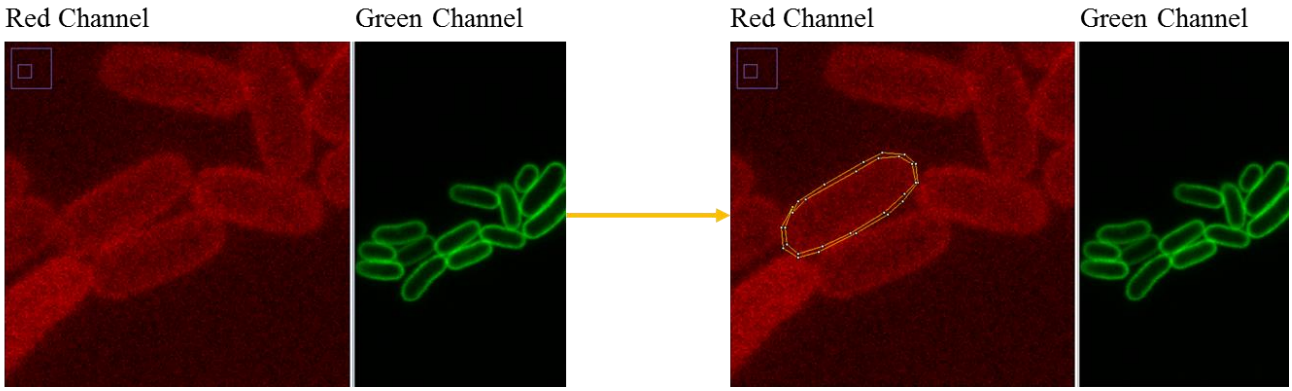
Figure S18 | Cryo-TEM images of *E. faecalis* sample, scale bar = 100 nm. Dark line represented cell membrane and white arrow marks where low density of PG was observed.



Measurement of Fluorescence Intensity

Calculation was done using Fiji ImageJ according to the procedure reported by Burgess.[7] Both the laser power and STED power were kept constant throughout the experiments for the acquisition of the images for calculation purposes. To minimize crosstalk, the excitation wavelength of the red channel was set at 570 nm, with the emission photons collected from 580 nm to 620 nm. Only the cells that were in focus were taken into account for calculation, and the channels of the images (not processed by deconvolution) were split prior to calculation; calculating only those from the red channel. The area with fluorescence on each bacterial cell surface was drawn (see image below) and the total intensity was normalized by the number of pixels found in the area (mean). A total of hundred or more cells per bacterial strain were computed, and the average was taken for comparison.

Figure S19 | Schematic illustration of intensity calculation method. Only the surface part of bacterial cells was measured to compare the amount of substrate incorporated onto cell wall.



The images used for intensity calculation are listed below:

Figure S20 | Images of *P. aeruginosa* O1 used for calculation

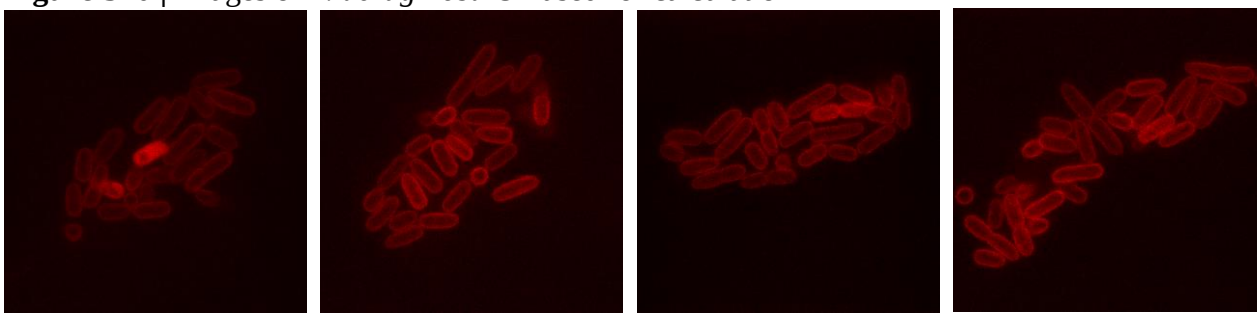


Figure S21 | Images of *E. coli* K12 used for calculation

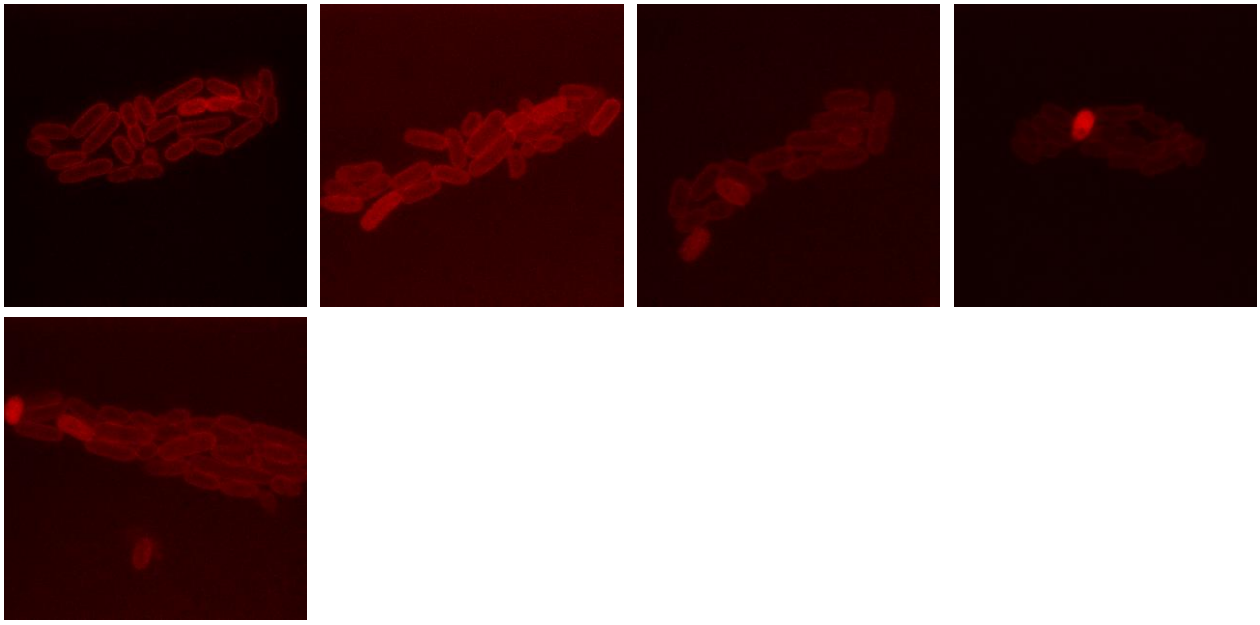


Figure S22 | Images of *B. subtilis* 6633 used for calculation

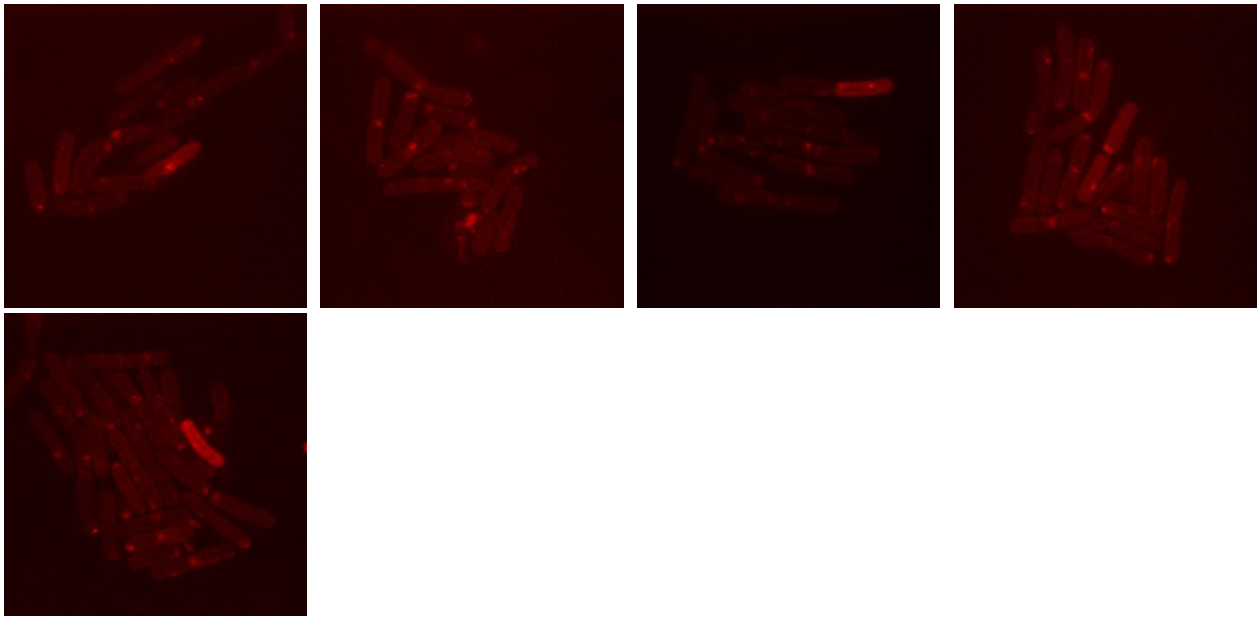


Figure S23 | Images of *E. faecalis* V583 used for calculation

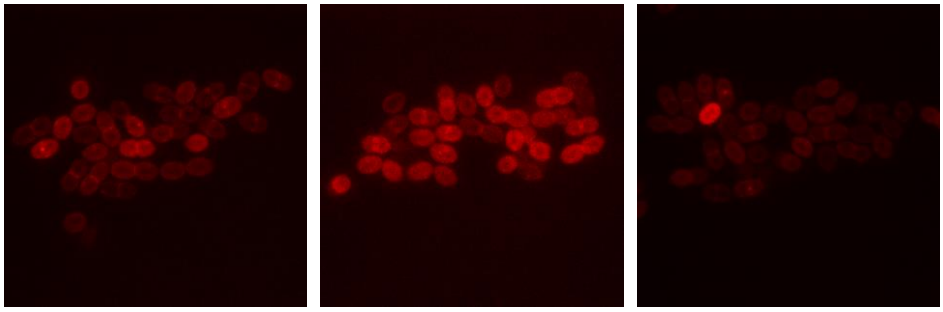


Figure S24 | Images of *S. aureus* BAA40 used for calculation

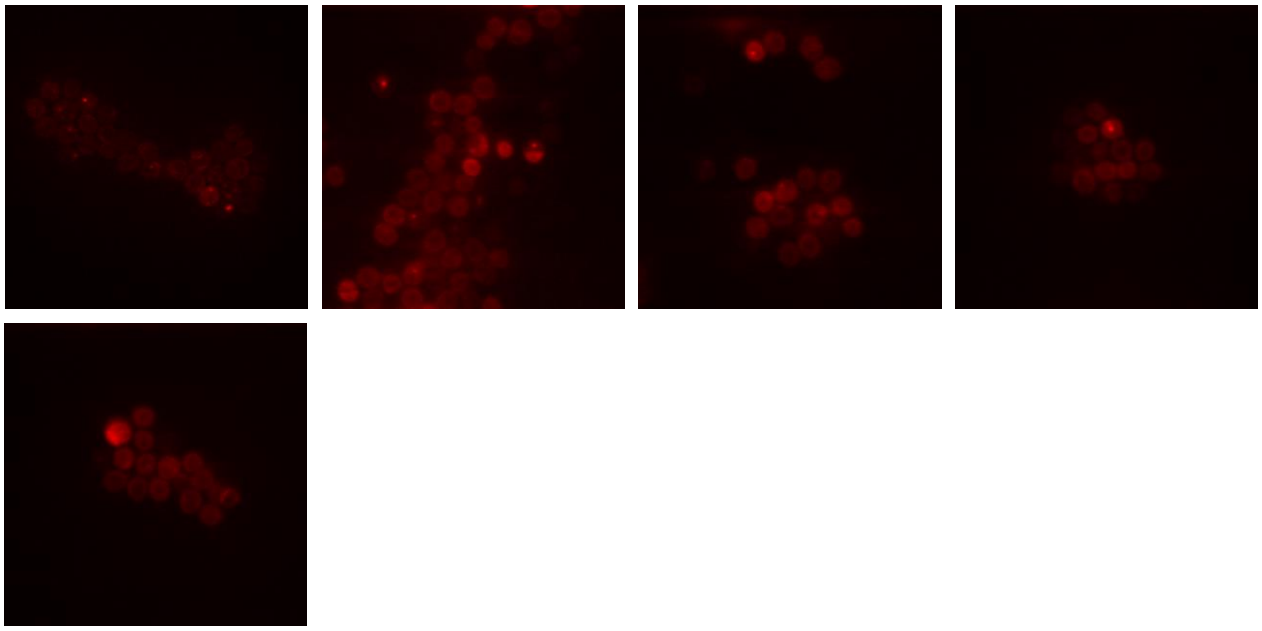


Figure S25 | Images of *S. aureus* USA300 used for calculation

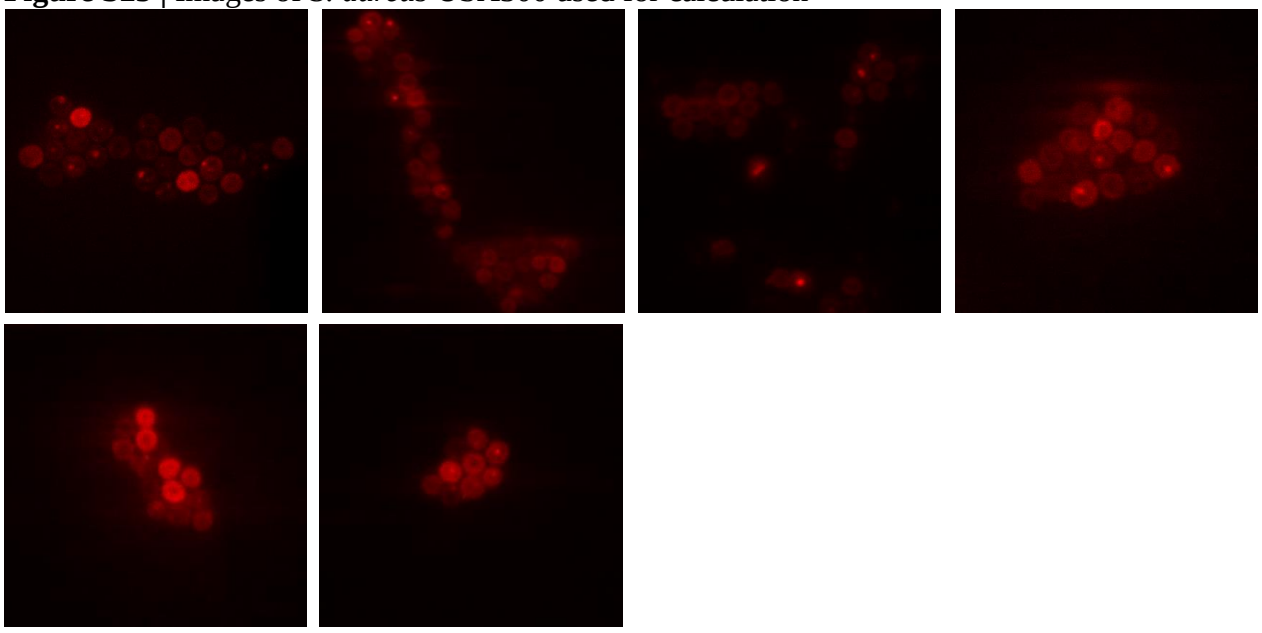


Table used for computation is shown as follows:

Table S1 | Intensity of *P. aeruginosa* O1 (au) and area normalization

PA01		Red Channel			
Series01		Area	Mean	IntDen	RawIntDen
Cell	1	0.1	17.618	1.758	2537
	2	0.207	17.759	3.68	5310
	3	0.203	20.034	4.068	5870
	4	0.215	15.823	3.399	4905
	5	0.247	23.298	5.748	8294
	6	0.104	31.387	3.263	4708
	7	0.204	18.337	3.736	5391
	8	0.211	20.489	4.331	6249
	9	0.108	22.481	2.43	3507
	10	0.109	17.57	1.924	2776
	11	0.101	24.644	2.493	3598
	12	0.111	18.594	2.062	2975
	13	0.216	37.01	8.002	11547
	14	0.259	19.021	4.93	7114
	15	0.261	19.78	5.168	7457
	16	0.26	20.237	5.259	7589
	17	0.198	16.839	3.326	4799
	18	0.291	20.343	5.921	8544
	19	0.1	18.278	1.824	2632
	20	0.179	18.236	3.261	4705
	21	0.169	18.697	3.161	4562
	22	0.264	15.302	4.04	5830
	23	0.268	21.398	5.739	8281
Avg (-background):			14.63372609		
Std Dev:			4.917777026		
Std Error:			1.025427395		
BG	1	0.115	5.446	0.626	904
	2	0.15	6.088	0.911	1315
	3	0.15	5.958	0.892	1287
	4	0.15	5.875	0.879	1269
	5	0.15	5.764	0.863	1245
	6	0.15	6.144	0.92	1327
	7	0.15	6.736	1.008	1455
	8	0.15	5.472	0.819	1182
	9	0.15	6.491	0.972	1402
	10	0.15	5.417	0.811	1170
Avg:			5.9391		

PA01		Red Channel			
Series02		Area	Mean	IntDen	RawIntDen
Cell	1	0.175	17.793	3.11	3861
	2	0.228	16.76	3.82	4743
	3	0.198	17.467	3.461	4297
	4	0.255	16.237	4.146	5147
	5	0.23	20.561	4.72	5860
	6	0.242	24.711	5.991	7438
	7	0.228	14.314	3.263	4051
	8	0.176	19.128	3.359	4170
	9	0.231	23.118	5.344	6635
	10	0.215	20.449	4.398	5460
	11	0.193	18.803	3.62	4494
	12	0.147	26.098	3.847	4776
	13	0.201	26.333	5.282	6557
	14	0.203	19.175	3.892	4832
	15	0.165	25.776	4.256	5284
	16	0.437	15.994	6.996	8685
	17	0.255	15.64	3.994	4958
	18	0.238	14.24	3.395	4215
	19	0.159	23.645	3.752	4658
	20	0.264	19.009	5.022	6235
	21	0.222	22.764	5.061	6283
	22	0.238	16.537	3.943	4895
	23	0.263	21.483	5.659	7025
Avg (-background):			15.7658087		
Std Dev:			3.801236171		
Std Error:			0.792612533		
BG	1	0.184	4.259	0.782	971
	2	0.184	4.158	0.764	948
	3	0.184	4.224	0.776	963
	4	0.184	3.833	0.704	874
	5	0.184	4.096	0.752	934
	6	0.184	3.706	0.681	845
	7	0.184	3.75	0.689	855
	8	0.184	3.895	0.715	888
	9	0.184	4.368	0.802	996
	10	0.184	4.329	0.795	987
Avg:			4.0618		

PA01		Red Channel			
Series03		Area	Mean	IntDen	RawIntDen

PA01		Red Channel			
Series04		Area	Mean	IntDen	RawIntDen

Cell	1	0.234	13.341	3.116	3869
	2	0.202	12.554	2.538	3151
	3	0.244	16.089	3.927	4875
	4	0.284	15.127	4.301	5340
	5	0.303	17.019	5.154	6399
	6	0.241	17	4.094	5083
	7	0.266	20.185	5.365	6661
	8	0.183	17.916	3.276	4067
	9	0.184	14.43	2.65	3290
	10	0.174	14.685	2.555	3172
	11	0.138	17.409	2.398	2977
	12	0.22	17.597	3.87	4804
	13	0.214	18.729	4.013	4982
	14	0.286	18.563	5.308	6590
	15	0.177	24.923	4.417	5483
	16	0.291	16.277	4.733	5876
	17	0.208	18.229	3.788	4703
	18	0.215	15.569	3.348	4157
	19	0.268	14.694	3.941	4893
	20	0.18	20.359	3.657	4540
	21	0.205	22.392	4.599	5710
	22	0.16	18	2.885	3582
	23	0.172	15.014	2.588	3213
	24	0.171	13.778	2.353	2921
Avg (-background):			14.65429167		
Std Dev:			2.905052009		
Std Error:			0.592991258		
BG	1	0.155	2.651	0.41	509
	2	0.155	2.812	0.435	540
	3	0.155	2.516	0.389	483
	4	0.155	1.917	0.296	368
	5	0.155	2.609	0.404	501
	6	0.155	2.365	0.366	454
	7	0.155	1.958	0.303	376
	8	0.155	2.448	0.379	470
	9	0.155	2.25	0.348	432
	10	0.155	2.714	0.42	521
Avg:			2.424		

Cell	1	0.21	17.18	3.612	4484
	2	0.304	16.889	5.142	6384
	3	0.219	16.757	3.671	4558
	4	0.221	16.011	3.534	4387
	5	0.241	16.803	4.047	5024
	6	0.258	17.328	4.467	5545
	7	0.239	20.502	4.905	6089
	8	0.221	23.026	5.082	6309
	9	0.259	20.131	5.205	6462
	10	0.241	18.97	4.569	5672
	11	0.226	14.256	3.227	4006
	12	0.126	21.75	2.733	3393
	13	0.255	14.633	3.725	4624
	14	0.257	12.856	3.303	4101
	15	0.244	15.31	3.737	4639
	16	0.184	15.576	2.873	3567
	17	0.214	19.733	4.228	5249
	18	0.158	22.209	3.506	4353
	19	0.271	21.507	5.838	7248
	20	0.198	17.089	3.386	4204
	21	0.277	23.608	6.541	8121
	22	0.143	21.921	3.143	3902
	23	0.18	20.987	3.787	4701
	24	0.207	21.381	4.426	5495
	25	0.121	14.053	1.698	2108
	26	0.168	14.76	2.473	3070
	27	0.11	17.577	1.94	2408
	28	0.166	19.223	3.19	3960
	29	0.178	20.113	3.58	4445
	30	0.184	19.332	3.566	4427
Avg (-background):			15.23216667		
Std Dev:			2.970623231		
Std Error:			0.542359118		
BG	1	0.143	2.74	0.391	485
	2	0.143	3.056	0.436	541
	3	0.143	3.531	0.503	625
	4	0.143	3.209	0.458	568
	5	0.143	3.215	0.458	569
	6	0.143	3.395	0.484	601
	7	0.143	3.316	0.473	587
	8	0.143	2.944	0.42	521
	9	0.143	2.825	0.403	500
	10	0.143	3.271	0.466	579

		Avg:	3.1502

Table S2 | Intensity of *E. coli* K12 (au) and area normalization

***E. coli* K-12**		Red Channel					***E. coli* K-12**		Red Channel			
Series01		Area	Mean	IntDen	RawIntDen		**Series02**		Area	Mean	IntDen	RawIntDen
Cell	1	0.25	23.108	5.781	8342		Cell	1	0.283	21.721	6.141	8862
	2	0.103	28.311	2.904	4190			2	0.207	22.505	4.663	6729
	3	0.244	25.435	6.204	8953			3	0.247	24.272	5.988	8641
	4	0.201	25.569	5.139	7415			4	0.243	23.66	5.739	8281
	5	0.286	24.421	6.99	10086			5	0.285	24.998	7.12	10274
	6	0.225	23.96	5.396	7787			6	0.241	24.71	5.959	8599
	7	0.255	23.965	6.111	8819			7	0.259	25.083	6.501	9381
	8	0.207	25.768	5.321	7679			8	0.236	25.188	5.935	8564
	9	0.24	23.087	5.536	7988			9	0.249	20.953	5.227	7543
	10	0.257	26.949	6.929	9998			10	0.225	24.731	5.553	8013
	11	0.22	26.16	5.765	8319			11	0.168	23.44	3.947	5696
	12	0.143	24.869	3.55	5123			12	0.291	28.076	8.172	11792
	13	0.256	27.293	6.979	10071			13	0.304	26.872	8.157	11770
	14	0.201	46.862	9.418	13590			14	0.162	21.607	3.504	5056
	15	0.175	44.866	7.866	11351			15	0.095	25.029	2.376	3429
	16	0.185	28.869	5.342	7708			16	0.169	25.779	4.359	6290
	17	0.209	27.056	5.662	8171			17	0.238	26.933	6.421	9265
	18	0.103	31.642	3.245	4683			18	0.148	30.582	4.514	6514
	19	0.244	28.727	7.008	10112			19	0.104	23.12	2.403	3468
	20	0.236	23.094	5.441	7852			20	0.231	28.018	6.466	9330
	21	0.091	21.076	1.913	2761			21	0.213	24.655	5.245	7569
	22	0.27	20.139	5.429	7834			22	0.252	21.333	5.367	7744
	23	0.26	21.984	5.713	8244			23	0.245	27.59	6.768	9767
Avg (-background):			16.77838696				Avg (-background):			14.20218261		
Std Dev:			6.511262508				Std Dev:			2.435198311		
Std Error:			1.357692087				Std Error:			0.507773949		
BG	1	0.906	9.528	8.636	12462		BG	1	0.291	11.631	3.385	4885
	2	0.906	10.346	9.378	13533			2	0.291	10.895	3.171	4576
	3	0.906	10.338	9.371	13522			3	0.291	9.94	2.893	4175
	4	0.906	10.718	9.715	14019			4	0.291	10.529	3.064	4422
	5	0.906	10.489	9.507	13719			5	0.291	10.286	2.994	4320
	6	0.906	10.056	9.115	13153			6	0.291	10.764	3.133	4521
	7	0.906	10.037	9.098	13129			7	0.291	10.5	3.056	4410
	8	0.906	10.644	9.649	13923			8	0.291	10.593	3.083	4449
	9	0.906	10.161	9.21	13290			9	0.291	10.586	3.081	4446
	10	0.906	10.86	9.844	14205			10	0.291	10.452	3.042	4390
Avg:			10.3177				Avg:			10.6176		

***E. coli* K-12**		Red Channel			
Series03		Area	Mean	IntDen	RawIntDen
Cell	1	0.25	25.895	6.478	9348
	2	0.214	23.793	5.095	7352
	3	0.169	24.082	4.072	5876
	4	0.126	29.571	3.73	5382
	5	0.272	26.407	7.192	10378
	6	0.253	26.753	6.767	9765
	7	0.258	28.199	7.269	10490
	8	0.216	25.763	5.57	8038
	9	0.22	25.899	5.707	8236
	10	0.235	26.785	6.292	9080
	11	0.195	31.655	6.164	8895
	12	0.179	24.209	4.328	6246
	13	0.245	26.89	6.597	9519
	14	0.2	23.436	4.694	6773
	15	0.192	25.56	4.906	7080
	16	0.222	26.75	5.932	8560
Avg (-background):		12.9667375			
Std Dev:		2.14288679			
Std Error:		0.535721698			
BG	1	0.292	13.176	3.844	5547
	2	0.292	13.102	3.823	5516
	3	0.292	13.622	3.974	5735
	4	0.292	13.822	4.033	5819
	5	0.292	14.107	4.116	5939
	6	0.292	12.9	3.764	5431
	7	0.292	13.031	3.802	5486
	8	0.292	13.249	3.866	5578
	9	0.292	13.43	3.918	5654
	10	0.292	13.423	3.916	5651
Avg:		13.3862			

***E. coli* K-12**		Red Channel			
Series04		Area	Mean	IntDen	RawIntDen
Cell	1	0.213	23.642	5.03	7258
	2	0.193	25.273	4.869	7026
	3	0.15	26.602	3.982	5746
	4	0.252	27.755	7.001	10103
	5	0.199	28.296	5.628	8121
	6	0.252	54.959	13.825	19950
	7	0.21	27.554	5.786	8349
	8	0.227	27.341	6.215	8968
	9	0.262	29.235	7.658	11051
	10	0.172	25.419	4.369	6304
	11	0.188	26.812	5.035	7266
	12	0.239	24.435	5.842	8430
	13	0.191	24.58	4.701	6784
	14	0.191	29.855	5.71	8240
	15	0.281	26.202	7.354	10612
	16	0.108	32.455	3.509	5063
	17	0.157	25.257	3.956	5708
Avg (-background):		17.16754118			
Std Dev:		7.152348718			
Std Error:		1.734699367			
BG	1	0.277	11.14	3.088	4456
	2	0.277	11.307	3.134	4523
	3	0.277	11.812	3.274	4725
	4	0.277	11.273	3.125	4509
	5	0.277	11.322	3.139	4529
	6	0.277	11.39	3.157	4556
	7	0.277	11.367	3.151	4547
	8	0.277	11.393	3.158	4557
	9	0.277	11.758	3.259	4703
	10	0.277	11.252	3.119	4501
Avg:		11.4014			

***E. coli* K-12**		Red Channel			
Series05		Area	Mean	IntDen	RawIntDen
Cell	1	0.216	31.788	6.873	9918
	2	0.229	25.109	5.759	8311
	3	0.261	23.691	6.173	8908
	4	0.243	23.889	5.811	8385
	5	0.23	35.084	8.072	11648
	6	0.213	22.623	4.829	6968
	7	0.168	25.252	4.235	6111

	8	0.164	24.92	4.093	5906
	9	0.306	30.893	9.441	13624
	10	0.277	28.018	7.747	11179
	11	0.139	27.463	3.825	5520
	12	0.168	26.453	4.455	6428
	13	0.294	28.854	8.478	12234
	14	0.164	25.844	4.245	6125
	15	0.109	24.49	2.665	3845
	16	0.157	27.132	4.268	6159
	17	0.201	26.121	5.249	7575
	18	0.161	27.515	4.443	6411
	19	0.35	26.794	9.377	13531
	20	0.175	26.818	4.702	6785
	21	0.202	24.712	5.001	7216
	22	0.091	20.364	1.863	2688
	23	0.158	25.009	3.951	5702
	24	0.233	29.622	6.897	9953
	25	0.087	24.736	2.143	3092
	26	0.335	26.377	8.829	12740
	27	0.169	26.287	4.445	6414
Avg (-background):			13.29215926		
Std Dev:			2.95911578		
Std Error:			0.569482097		
BG	1	0.265	11.527	3.06	4415
	2	0.265	13.337	3.54	5108
	3	0.265	13.358	3.545	5116
	4	0.265	14.713	3.905	5635
	5	0.265	13.389	3.554	5128
	6	0.265	14.616	3.879	5598
	7	0.265	13.984	3.712	5356
	8	0.265	11.911	3.161	4562
	9	0.265	12.433	3.3	4762
	10	0.265	12.943	3.435	4957
Avg:			13.2211		

Table S3 | Intensity of *B. subtilis* 6633 (au) and area normalization

***B. subtilis* 6633**			Red Channel				***B. subtilis* 6633**			Red Channel		
Series01		Area	Mean	IntDen	RawIntDen		**Series02**		Area	Mean	IntDen	RawIntDen
Cell	1	0.267	12.033	3.208	3983		Cell	1	0.34	13.239	4.495	6487
	2	0.493	11.538	5.688	7061			2	0.336	14.682	4.935	7121
	3	0.383	12.412	4.759	5908			3	0.363	14.635	5.315	7669
	4	0.243	11.358	2.763	3430			4	0.387	13.806	5.339	7704
	5	0.352	14.471	5.094	6324			5	0.301	12.938	3.9	5628

	6	0.268	11.282	3.026	3757
	7	0.282	16.123	4.545	5643
	8	0.242	12.814	3.107	3857
	9	0.425	20.4	8.676	10771
	10	0.43	14.455	6.218	7719
	11	0.424	14.072	5.974	7416
	12	0.415	16.355	6.785	8423
	13	0.292	15.096	4.414	5480
	14	0.435	12.622	5.49	6816
Avg (-background):			7.091285714		
Std Dev:			2.522312681		
Std Error:			0.674116419		
BG	1	0.184	7.855	1.443	1791
	2	0.184	6.908	1.269	1575
	3	0.184	6.864	1.261	1565
	4	0.184	6.553	1.203	1494
	5	0.184	6.009	1.104	1370
	6	0.184	6.842	1.257	1560
	7	0.184	7.004	1.286	1597
	8	0.184	7.044	1.294	1606
	9	0.184	6.702	1.231	1528
	10	0.184	6.614	1.215	1508
Avg:			6.8395		

	6	0.277	14.148	3.922	5659
	7	0.27	13.841	3.741	5398
	8	0.282	18.002	5.078	7327
	9	0.305	13.152	4.01	5787
	10	0.29	14.048	4.079	5886
	11	0.271	13.169	3.568	5149
	12	0.76	12.55	9.54	13767
	13	0.483	14.861	7.178	10358
	14	0.431	13.638	5.879	8483
	15	0.306	12.548	3.843	5546
Avg (-background):			9.938566667		
Std Dev:			1.339895351		
Std Error:			0.345959492		
BG	1	0.496	3.486	1.73	2496
	2	0.399	4.082	1.629	2351
	3	0.496	4.043	2.006	2895
	4	0.496	3.722	1.847	2665
	5	0.496	3.966	1.968	2840
	6	0.496	3.711	1.841	2657
	7	0.496	4.383	2.175	3138
	8	0.496	4.413	2.19	3160
	9	0.496	4.439	2.202	3178
	10	0.496	3.874	1.922	2774
Avg:			4.0119		

***B. subtilis* 6633**			Red Channel		
Series03		Area	Mean	IntDen	RawIntDen
Cell	1	0.325	10.631	3.455	4986
	2	0.292	11.423	3.333	4809
	3	0.247	11.317	2.8	4040
	4	0.34	13.573	4.609	6651
	5	0.356	11.207	3.984	5749
	6	0.367	13.885	5.1	7359
	7	0.311	13.488	4.197	6056
	8	0.347	14.467	5.023	7248
	9	0.331	16.207	5.369	7747
	10	0.318	13.322	4.238	6115
	11	0.39	14.943	5.83	8413
	12	0.394	15.023	5.924	8548
	13	0.568	13.532	7.68	11083
	14	0.325	12.141	3.946	5694
	15	0.358	12.01	4.294	6197
	16	0.339	12.951	4.389	6333

***B. subtilis* 6633**			Red Channel		
Series04		Area	Mean	IntDen	RawIntDen
Cell	1	0.371	11.35	4.208	6072
	2	0.36	10.398	3.747	5407
	3	0.358	12.198	4.362	6294
	4	0.34	11.996	4.082	5890
	5	0.346	15.006	5.189	7488
	6	0.362	13.125	4.748	6851
	7	0.444	13.752	6.099	8801
	8	0.405	15.259	6.175	8911
	9	0.696	11.228	7.812	11273
	10	0.615	11.717	7.202	10393
	11	0.322	14.049	4.527	6533
	12	0.369	15.146	5.595	8073
	13	0.358	17.872	6.391	9222
	14	0.359	17.11	6.142	8863
	15	0.313	13.721	4.298	6202
	16	0.408	14.217	5.803	8374

	17	0.344	14.546	5	7215
	18	0.352	20.398	7.181	10362
Avg (-background):			8.160666667		
Std Dev:			2.278128489		
Std Error:			0.536960034		
BG	1	0.277	5.505	1.526	2202
	2	0.277	5.555	1.54	2222
	3	0.277	5.598	1.552	2239
	4	0.139	5.285	0.732	1057
	5	0.182	5.198	0.947	1367
	6	0.08	5.207	0.419	604
	7	0.277	5.218	1.446	2087
	8	0.277	5.65	1.566	2260
	9	0.277	5.695	1.579	2278
	10	0.153	5.629	0.862	1244
Avg:			5.454		

	17	0.369	14.403	5.32	7677
	18	0.312	12.738	3.972	5732
	19	0.332	14.781	4.906	7080
	20	0.383	14.043	5.372	7752
	21	0.368	11.842	4.358	6288
	22	0.311	12.038	3.746	5405
	23	0.321	11.227	3.602	5198
	24	0.321	14.205	4.558	6577
	25	0.332	13.843	4.595	6631
Avg (-background):			7.91946		
Std Dev:			1.846469832		
Std Error:			0.369293966		
BG	1	0.4	5.53	2.211	3191
	2	0.4	5.558	2.222	3207
	3	0.4	5.575	2.229	3217
	4	0.4	5.47	2.187	3156
	5	0.4	5.53	2.211	3191
	6	0.4	5.66	2.263	3266
	7	0.4	5.636	2.254	3252
	8	0.4	5.461	2.184	3151
	9	0.4	5.65	2.259	3260
	10	0.4	5.641	2.256	3255
Avg:			5.5711		

***B. subtilis* 6633**			Red Channel		
Series05		Area	Mean	IntDen	RawIntDen
Cell	1	0.485	10.52	5.103	7364
	2	0.451	10.625	4.793	6917
	3	0.353	12.006	4.243	6123
	4	0.421	9.758	4.112	5933
	5	0.109	15.614	1.71	2467
	6	0.623	10.046	6.258	9031
	7	0.446	11.616	5.176	7469
	8	0.45	10.963	4.938	7126
	9	0.57	12.062	6.871	9915
	10	0.407	13.032	5.31	7663
	11	0.397	14.262	5.663	8172
	12	0.398	13.471	5.368	7746
	13	0.477	12.168	5.81	8384
	14	0.536	15.658	8.388	12104
	15	0.388	11.421	4.432	6396
	16	0.374	13.978	5.221	7534
	17	0.369	14.133	5.22	7533

	18	0.332	12.432	4.127	5955
	19	0.344	11.133	3.834	5533
	20	0.374	11.223	4.192	6049
	21	0.383	12.031	4.602	6641
	22	0.346	11.94	4.137	5970
	23	0.374	14.409	5.392	7781
	24	0.488	12.224	5.964	8606
	25	0.34	12.163	4.13	5960
	26	0.36	13.452	4.847	6995
	27	0.441	12.625	5.573	8042
	28	0.36	11.046	3.973	5733
	29	0.376	12.853	4.836	6979
	30	0.537	12.406	6.663	9615
	31	0.509	23.74	12.075	17425
	32	0.214	10.084	2.159	3116
	33	0.183	10.583	1.936	2794
Avg (-background):			7.444172727		
Std Dev:			2.507908258		
Std Error:			0.436570791		
BG	1	0.218	5.362	1.17	1689
	2	0.218	4.978	1.087	1568
	3	0.218	5.117	1.117	1612
	4	0.218	5.292	1.155	1667
	5	0.179	5.043	0.902	1301
	6	0.218	5.184	1.132	1633
	7	0.218	5.076	1.108	1599
	8	0.218	5.13	1.12	1616
	9	0.218	5.044	1.101	1589
	10	0.218	5.295	1.156	1668
Avg:			5.1521		

Table S4 | Intensity of *E. faecalis* V583 (au) and area normalization

***E. faecalis* V583**			Red Channel				***E. faecalis* V583**			Red Channel		
Series01		Area	Mean	IntDen	RawIntDen		**Series02**		Area	Mean	IntDen	RawIntDen
Cell	1	0.311	28.245	8.789	12682		Cell	1	0.104	34.413	3.577	5162
	2	0.268	52.106	13.974	20165			2	0.192	32.657	6.269	9046
	3	0.156	45.653	7.118	10272			3	0.182	30.825	5.618	8107
	4	0.113	48.73	5.504	7943			4	0.123	33.864	4.154	5994
	5	0.193	37.627	7.275	10498			5	0.125	39.667	4.948	7140
	6	0.199	27.537	5.477	7903			6	0.223	22.22	4.958	7155
	7	0.19	36.369	6.906	9965			7	0.142	18.888	2.683	3872
	8	0.203	31.597	6.416	9258			8	0.249	30.287	7.535	10873
	9	0.258	37.016	9.568	13807			9	0.203	29.898	6.071	8760

	10	0.119	28.715	3.423	4939
	11	0.277	23.609	6.528	9420
	12	0.204	39.241	7.995	11537
	13	0.29	39.038	11.335	16357
	14	0.19	27.894	5.297	7643
	15	0.177	47.762	8.473	12227
	16	0.305	56.468	17.218	24846
	17	0.178	32.008	5.701	8226
	18	0.171	31.486	5.389	7777
	19	0.185	30.165	5.581	8054
	20	0.179	49.838	8.945	12908
	21	0.105	28	2.949	4256
	22	0.142	36.439	5.177	7470
	23	0.281	26.569	7.475	10787
	24	0.161	27.082	4.354	6283
	25	0.151	29.202	4.412	6366
	26	0.195	34.408	6.724	9703
	27	0.278	29.741	8.265	11926
	28	0.221	31.614	6.989	10085
	29	0.245	46.824	11.454	16529
	30	0.261	28.215	7.352	10609
	31	0.264	34.625	9.142	13192
	32	0.266	26.557	7.067	10198
Avg (-background):			26.066875		
Std Dev:			8.75256418		
Std Error:			1.547249371		
BG	1	0.123	8.847	1.085	1566
	2	0.123	9.514	1.167	1684
	3	0.123	9.723	1.193	1721
	4	0.123	9.35	1.147	1655
	5	0.123	8.932	1.096	1581
	6	0.123	9.475	1.162	1677
	7	0.123	8.542	1.048	1512
	8	0.123	9.305	1.141	1647
	9	0.123	9.175	1.125	1624
	10	0.123	9.712	1.191	1719
Avg:					9.2575

	10	0.157	27.379	4.307	6215
	11	0.149	25.307	3.771	5441
	12	0.211	34.397	7.27	10491
	13	0.203	37.075	7.528	10863
	14	0.125	35.21	4.416	6373
	15	0.13	30.75	4.006	5781
	16	0.155	24.567	3.814	5503
	17	0.15	23.189	3.487	5032
	18	0.144	23.091	3.328	4803
	19	0.125	34.95	4.36	6291
	20	0.139	33.194	4.624	6672
	21	0.131	20.873	2.734	3945
	22	0.143	35.246	5.056	7296
	23	0.151	39.904	6.028	8699
	24	0.108	31.981	3.457	4989
	25	0.102	21.898	2.231	3219
	26	0.119	37.07	4.393	6339
	27	0.15	30.281	4.554	6571
	28	0.257	36.779	9.456	13645
	29	0.184	19.545	3.603	5199
	30	0.172	17.395	2.99	4314
	31	0.164	27.161	4.442	6410
	32	0.269	33.75	9.075	13095
	33	0.17	38.398	6.546	9446
	34	0.166	34.658	5.764	8318
Avg (-background):			23.11712941		
Std Dev:			6.419002639		
Std Error:			1.100849871		
BG	1	0.218	7.359	1.606	2318
	2	0.218	7.238	1.58	2280
	3	0.218	6.721	1.467	2117
	4	0.218	6.781	1.48	2136
	5	0.218	7.349	1.604	2315
	6	0.218	8.273	1.806	2606
	7	0.218	7.679	1.676	2419
	8	0.218	6.159	1.344	1940
	9	0.218	6.949	1.517	2189
	10	0.218	6.311	1.378	1988
Avg:					7.0819

***E. faecalis* V583**			Red Channel		
Series03		Area	Mean	IntDen	RawIntDen

	1	0.146	29.474	4.31	6219
	2	0.155	33.272	5.165	7453
	3	0.182	32.416	5.886	8493
	4	0.103	37.765	3.899	5627
	5	0.1	39.965	3.988	5755
	6	0.174	75.45	13.124	18938
	7	0.122	42.705	5.209	7516
	8	0.119	43.907	5.233	7552
	9	0.123	33.904	4.159	6001
	10	0.141	39.642	5.604	8087
	11	0.1	30.959	3.111	4489
	12	0.098	31.606	3.11	4488
	13	0.128	36.027	4.619	6665
	14	0.177	24.867	4.394	6341
	15	0.172	37.161	6.387	9216
	16	0.118	28.782	3.391	4893
	17	0.092	41.797	3.852	5559
	18	0.097	40.643	3.943	5690
	19	0.137	37.707	5.174	7466
Cell	20	0.105	30.311	3.172	4577
	21	0.144	34.106	4.916	7094
	22	0.135	43.631	5.896	8508
	23	0.114	34.878	3.964	5720
	24	0.114	23.994	2.727	3935
	25	0.101	34.466	3.487	5032
	26	0.125	37.171	4.662	6728
	27	0.187	39.963	7.477	10790
	28	0.141	26.814	3.791	5470
	29	0.146	36.281	5.28	7619
	30	0.139	34.91	4.863	7017
	31	0.152	34.909	5.298	7645
	32	0.139	24.285	3.366	4857
	33	0.146	24.576	3.577	5161
	34	0.13	32.25	4.202	6063
	35	0.112	29.571	3.299	4761
	36	0.128	29.076	3.708	5350
	37	0.127	22.568	2.862	4130
	38	0.106	22.471	2.383	3438
	39	0.104	39.353	4.091	5903
Avg (-background):			27.35383846		
Std Dev:			8.988189762		
Std Error:			1.439262233		
BG	1	0.132	7.479	0.985	1421

	2	0.132	6.795	0.895	1291
	3	0.132	7.737	1.019	1470
	4	0.132	7.205	0.949	1369
	5	0.132	7.205	0.949	1369
	6	0.132	7.753	1.021	1473
	7	0.132	7.805	1.028	1483
	8	0.132	6.937	0.913	1318
	9	0.1	7.347	0.733	1058
	10	0.132	7.284	0.959	1384
Avg:			7.3547		

Table S5 | Intensity of *S. aureus* BAA40 and USA300 (au) and area normalization

MRSA-BAA40			Red Channel				**MRSA-BAA40**			Red Channel		
Series01		Area	Mean	IntDen	RawIntDen		**Series02**		Area	Mean	IntDen	RawIntDen
Cell	1	0.452	16.765	7.576	9405		Cell	1	0.115	22.933	2.648	5137
	2	0.349	23.573	8.222	10207			2	0.211	22.954	4.84	9388
	3	0.349	35.206	12.279	15244			3	0.106	22.834	2.413	4681
	4	0.349	28.739	10.024	12444			4	0.081	21.108	1.708	3314
	5	0.349	27.746	9.677	12014			5	0.132	23.191	3.061	5937
	6	0.349	22.506	7.85	9745			6	0.171	25.885	4.417	8568
	7	0.349	32.478	11.328	14063			7	0.125	28.124	3.509	6806
	8	0.349	26.956	9.402	11672			8	0.126	21.657	2.735	5306
	9	0.349	18.501	6.453	8011			9	0.101	27.658	2.795	5421
	10	0.349	30.166	10.521	13062			10	0.125	24.252	3.026	5869
	11	0.349	23.882	8.33	10341			11	0.206	37.902	7.796	15123
	12	0.349	18.975	6.618	8216			12	0.084	36.871	3.098	6010
	13	0.349	24.134	8.417	10450			13	0.174	31.855	5.551	10767
	14	0.349	23.781	8.294	10297			14	0.115	24.621	2.843	5515
	15	0.349	33.704	11.755	14594			15	0.123	37.017	4.542	8810
	16	0.349	26.575	9.269	11507			16	0.113	24.068	2.73	5295
	17	0.349	39.744	13.862	17209			17	0.087	22.13	1.928	3740
	18	0.349	36.081	12.584	15623			18	0.117	21.894	2.551	4948
	19	0.349	37.727	13.159	16336			19	0.104	21.289	2.206	4279
	20	0.349	57.497	20.054	24896			20	0.126	28.739	3.63	7041
	21	0.362	32.029	11.584	14381			21	0.164	29.028	4.759	9231
	22	0.362	16.88	6.105	7579			22	0.119	24.9	2.952	5727
	23	0.362	32.107	11.612	14416			23	0.113	25.918	2.926	5676
	24	0.362	31.857	11.522	14304			24	0.123	20.854	2.569	4984
	25	0.362	38.67	13.986	17363			25	0.123	22.454	2.755	5344
	26	0.362	25.739	9.309	11557			26	0.11	19.315	2.121	4114
	27	0.362	25.842	9.346	11603			27	0.116	23.929	2.776	5384
	28	0.362	26.078	9.432	11709			28	0.18	32.157	5.802	11255
	29	0.362	15.43	5.581	6928			29	0.119	31.515	3.753	7280

	30	0.362	19.027	6.881	8543
	31	0.421	36.201	15.251	18933
Avg (-background):			26.63205484		
Std Dev:			8.706465933		
Std Error:			1.563727443		
BG	1	0.421	1.677	0.706	877
	2	0.421	2.193	0.924	1147
	3	0.421	2.447	1.031	1280
	4	0.421	2.034	0.857	1064
	5	0.421	1.792	0.755	937
	6	0.421	1.57	0.661	821
	7	0.421	2.096	0.883	1096
	8	0.421	1.95	0.822	1020
	9	0.421	1.553	0.654	812
	10	0.421	1.721	0.725	900
Avg:			1.9033		

	30	0.1	30.263	3.027	5871
	31	0.137	26.272	3.589	6962
	32	0.114	22.611	2.576	4997
	33	0.148	32.618	4.843	9394
Avg (-background):			21.58455758		
Std Dev:			5.028379082		
Std Error:			0.875328444		
BG	1	0.107	4.937	0.527	1022
	2	0.107	4.986	0.532	1032
	3	0.104	4.297	0.447	868
	4	0.107	4.213	0.45	872
	5	0.107	4.357	0.465	902
	6	0.107	4.961	0.529	1027
	7	0.107	4.836	0.516	1001
	8	0.107	4.7	0.502	973
	9	0.107	4.952	0.528	1025
	10	0.107	5.193	0.554	1075
Avg:			4.7432		

MRSA-BAA40			Red Channel		
Series03		Area	Mean	IntDen	RawIntDen
Cell	1	0.144	36.804	5.312	10305
	2	0.139	21.637	3.012	5842
	3	0.213	21.075	4.498	8725
	4	0.241	30.45	7.331	14220
	5	0.163	27.82	4.532	8791
	6	0.139	46.226	6.434	12481
	7	0.229	30.649	7.031	13639
	8	0.16	24.379	3.909	7582
	9	0.239	30.054	7.189	13945
	10	0.111	18.386	2.038	3953
	11	0.178	19.272	3.428	6649
	12	0.126	28.498	3.599	6982
	13	0.131	22.752	2.979	5779
	14	0.212	30.167	6.407	12429
	15	0.141	21.157	2.988	5797
	16	0.112	16.76	1.875	3637
Avg (-background):			23.108175		
Std Dev:			7.62787086		
Std Error:			1.906967715		
BG	1	0.123	3.416	0.419	813
	2	0.123	3.218	0.395	766
	3	0.123	3.324	0.408	791

MRSA-BAA40			Red Channel		
Series04		Area	Mean	IntDen	RawIntDen
Cell	1	0.103	25.575	2.637	5115
	2	0.123	29.444	3.628	7037
	3	0.116	39.582	4.591	8906
	4	0.127	30.502	3.884	7534
	5	0.131	40.193	5.263	10209
	6	0.113	25.836	2.93	5684
	7	0.112	18.064	2.03	8015
	8	0.128	32.189	4.132	5158
	9	0.105	25.409	2.659	9320
	10	0.124	38.672	4.805	5856
	11	0.129	23.424	3.019	9530
	12	0.156	31.452	4.913	3595
	13	0.107	17.367	1.853	5733
	14	0.13	22.75	2.955	3591
	15	0.12	15.478	1.851	3938
Avg (-background):			22.65013333		
Std Dev:			7.872994247		
Std Error:			2.032798373		
BG	1	0.123	5.412	0.664	1288
	2	0.123	5.13	0.629	1221
	3	0.123	5.525	0.678	1315
	4	0.123	5.601	0.687	1333

	4	0.123	3.651	0.448	869			5	0.123	4.866	0.597	1158
	5	0.123	4.008	0.492	954			6	0.123	4.298	0.527	1023
	6	0.123	3.962	0.486	943			7	0.123	4.483	0.55	1067
	7	0.123	3.399	0.417	809			8	0.123	4.975	0.61	1184
	8	0.123	4.038	0.495	961			9	0.123	5.143	0.631	1224
	9	0.123	2.748	0.337	654			10	0.123	5.357	0.657	1275
	10	0.123	3.458	0.424	823			Avg:		5.079		
Avg:			3.5222									

MRSA-BAA40			Red Channel		
Series05		Area	Mean	IntDen	RawIntDen
Cell	1	0.114	34.095	3.902	7569
	2	0.144	60.039	8.635	16751
	3	0.119	32.333	3.85	7469
	4	0.121	35.615	4.296	8334
	5	0.105	34.235	3.6	6984
	6	0.134	23.112	3.086	5986
	7	0.13	29.306	3.807	7385
	8	0.131	32.638	4.274	8290
	9	0.188	45	8.444	16380
	10	0.121	36.628	4.419	8571
	11	0.114	34.045	3.896	7558
	12	0.126	31.074	3.909	7582
	13	0.106	28.597	3.037	5891
	14	0.148	34.436	5.095	9883
Avg (-background):			26.40105714		
Std Dev:			8.661895073		
Std Error:			2.314988834		
BG	1	0.132	9.332	1.232	2389
	2	0.132	9.777	1.29	2503
	3	0.132	8.188	1.081	2096
	4	0.132	8.516	1.124	2180
	5	0.132	7.301	0.964	1869
	6	0.132	7.824	1.033	2003
	7	0.132	8.484	1.12	2172
	8	0.132	9.102	1.201	2330
	9	0.132	9.488	1.252	2429
	10	0.132	8.801	1.161	2253
Avg:			8.6813		

MRSA-USA300			Red Channel				**MRSA-USA300**			Red Channel		
Series01		Area	Mean	IntDen	RawIntDen		**Series02**		Area	Mean	IntDen	RawIntDen
Cell	1	0.188	23.433	4.398	5460		Cell	1	0.342	35.141	12.03	14935

	2	0.171	26.208	4.475	5556
	3	0.155	20.663	3.212	3988
	4	0.28	29.107	8.136	10100
	5	0.242	24.697	5.968	7409
	6	0.248	21.448	5.321	6606
	7	0.268	35.559	9.538	11841
	8	0.293	20.044	5.877	7296
	9	0.138	23.088	3.18	3948
	10	0.151	17.481	2.633	3269
	11	0.242	18.127	4.38	5438
	12	0.249	16.32	4.062	5043
	13	0.262	27.557	7.214	8956
	14	0.227	23.674	5.378	6676
	15	0.159	22.409	3.574	4437
	16	0.179	19.396	3.468	4306
	17	0.19	16.775	3.189	3959
	18	0.331	21.275	7.043	8744
	19	0.377	43.235	16.299	20234
	20	0.212	19.084	4.043	5019
	21	0.319	28.321	9.034	11215
	22	0.183	15.868	2.901	3602
	23	0.136	17.746	2.416	2999
	24	0.173	26.484	4.587	5694
	25	0.191	16.245	3.101	3850
	26	0.163	19.01	3.093	3840
Avg (-background):			20.87866154		
Std Dev:			6.338790176		
Std Error:			1.243139031		
BG	1	0.35	2.558	0.894	1110
	2	0.35	1.544	0.54	670
	3	0.35	0.226	0.079	98
	4	0.35	0.15	0.052	65
	5	0.35	2.804	0.98	1217
	6	0.35	2.749	0.961	1193
	7	0.35	0.316	0.11	137
	8	0.35	2.926	1.023	1270
	9	0.35	2.878	1.006	1249
	10	0.35	3.237	1.132	1405
Avg:			1.9388		

	2	0.141	30.697	4.327	5372
	3	0.172	23.953	4.11	5102
	4	0.176	23.009	4.04	5016
	5	0.184	28.465	5.228	6490
	6	0.17	36.18	6.149	7634
	7	0.176	22.886	4.037	5012
	8	0.176	18.699	3.299	4095
	9	0.278	19.426	5.398	6702
	10	0.146	22.89	3.337	4143
	11	0.226	20.411	4.603	5715
	12	0.236	32.635	7.702	9562
	13	0.271	20.582	5.587	6936
	14	0.147	21.76	3.208	3982
	15	0.162	27.239	4.41	5475
	16	0.144	25.944	3.741	4644
	17	0.132	26.915	3.555	4414
	18	0.147	26.219	3.865	4798
	19	0.182	21.204	3.86	4792
	20	0.209	17.723	3.712	4608
	21	0.194	18.734	3.637	4515
	22	0.143	20.472	2.935	3644
	23	0.136	31.361	4.269	5300
	24	0.178	23.729	4.224	5244
	25	0.28	19.635	5.504	6833
	26	0.19	35.441	6.737	8364
	27	0.176	24.516	4.325	5369
	28	0.171	22.708	3.878	4814
Avg (-background):			22.63727143		
Std Dev:			5.408819752		
Std Error:			1.022170854		
BG	1	0.514	2.13	1.095	1359
	2	0.514	2.053	1.055	1310
	3	0.514	2.082	1.07	1328
	4	0.514	2.649	1.361	1690
	5	0.514	2.741	1.409	1749
	6	0.514	2.188	1.124	1396
	7	0.514	2.375	1.22	1515
	8	0.514	2.335	1.2	1490
	9	0.514	1.994	1.025	1272
	10	0.514	2.571	1.321	1640
Avg:			2.3118		

MRSA-USA300	Red Channel	**MRSA-USA300**	Red Channel

Series03		Area	Mean	IntDen	RawIntDen
Cell	1	0.122	27.349	3.348	4157
	2	0.124	22.273	2.763	3430
	3	0.118	27.212	3.2	3973
	4	0.101	24.392	2.456	3049
	5	0.13	31.441	4.077	5062
	6	0.113	23.443	2.644	3282
	7	0.111	23.63	2.627	3261
	8	0.1	22.887	2.286	2838
	9	0.121	17.78	2.148	2667
	10	0.104	22.465	2.334	2898
	11	0.118	21.644	2.545	3160
	12	0.133	22.103	2.938	3647
	13	0.121	20.427	2.468	3064
	14	0.114	19.475	2.212	2746
	15	0.116	24.5	2.842	3528
	16	0.109	24.711	2.687	3336
	17	0.128	27.264	3.492	4335
	18	0.118	20.075	2.377	2951
	19	0.108	20.022	2.161	2683
Avg (-background):			21.26998421		
Std Dev:			3.325245673		
Std Error:			0.762863676		
BG	1	0.11	1.838	0.201	250
	2	0.11	2.316	0.254	315
	3	0.11	1.706	0.187	232
	4	0.11	1.713	0.188	233
	5	0.11	2.096	0.23	285
	6	0.11	1.721	0.188	234
	7	0.11	2.243	0.246	305
	8	0.11	2.544	0.279	346
	9	0.11	2.279	0.25	310
	10	0.11	2.051	0.225	279
Avg:			2.0507		

Series04		Area	Mean	IntDen	RawIntDen
Cell	1	0.191	16.482	3.152	6115
	2	0.276	26.062	7.188	13943
	3	0.172	14.895	2.565	4975
	4	0.149	23.536	3.507	6802
	5	0.168	23.843	3.995	7749
	6	0.215	27.368	5.898	11440
	7	0.276	22.297	6.15	11929
	8	0.221	16.801	3.707	7191
	9	0.163	22.823	3.718	7212
	10	0.219	25.519	5.578	10820
	11	0.165	18.442	3.052	5920
	12	0.201	23.138	4.652	9024
Avg (-background):			19.78246667		
Std Dev:			4.106346902		
Std Error:			1.185400245		
BG	1	0.386	1.981	0.765	1484
	2	0.386	1.629	0.629	1220
	3	0.386	2.007	0.775	1503
	4	0.386	1.825	0.705	1367
	5	0.386	2.053	0.793	1538
	6	0.386	2.152	0.831	1612
	7	0.386	1.802	0.696	1350
	8	0.386	2.166	0.836	1622
	9	0.386	2.053	0.793	1538
	10	0.386	2.179	0.841	1632
Avg:			1.9847		

MRSA-USA300			Red Channel		
Series05		Area	Mean	IntDen	RawIntDen
Cell	1	0.219	38.856	8.493	16475
	2	0.211	19.443	4.099	7952
	3	0.203	23.567	4.775	9262
	4	0.216	39.095	8.445	16381
	5	0.196	38.969	7.654	14847
	6	0.176	31.692	5.571	10807

MRSA-USA300			Red Channel		
Series06		Area	Mean	IntDen	RawIntDen
Cell	1	0.156	23.139	3.614	7011
	2	0.125	25.634	3.211	6229
	3	0.206	25.325	5.222	10130
	4	0.168	28.485	4.787	9286
	5	0.195	34.639	6.768	13128
	6	0.152	19.2	2.92	5664

	7	0.199	41.391	8.236	15977
	8	0.192	21.614	4.156	8062
	9	0.224	20.278	4.547	8821
	10	0.234	19.725	4.616	8955
	11	0.175	20.558	3.593	6969
Avg (-background):			26.70185455		
Std Dev:			9.30949216		
Std Error:			2.806917498		
BG	1	0.727	2.172	1.579	3062
	2	0.727	1.825	1.326	2573
	3	0.727	2.323	1.688	3275
	4	0.727	1.862	1.354	2626
	5	0.727	1.987	1.444	2802
	6	0.727	1.733	1.259	2443
	7	0.19	2.084	0.395	767
	8	0.727	1.733	1.26	2444
	9	0.727	1.547	1.124	2181
	10	0.716	2.25	1.61	3123
Avg:			1.9516		

	7	0.125	17.407	2.181	4230
	8	0.125	16.819	2.107	4087
Avg (-background):			21.7166		
Std Dev:			6.051167184		
Std Error:			2.139410675		
BG	1	0.232	2.355	0.547	1062
	2	0.232	2.716	0.632	1225
	3	0.232	1.659	0.386	748
	4	0.232	2.82	0.656	1272
	5	0.232	2.16	0.502	974
	6	0.232	2.614	0.608	1179
	7	0.232	1.437	0.334	648
	8	0.232	2.106	0.49	950
	9	0.232	1.539	0.358	694
	10	0.232	1.738	0.404	784
Avg:			2.1144		

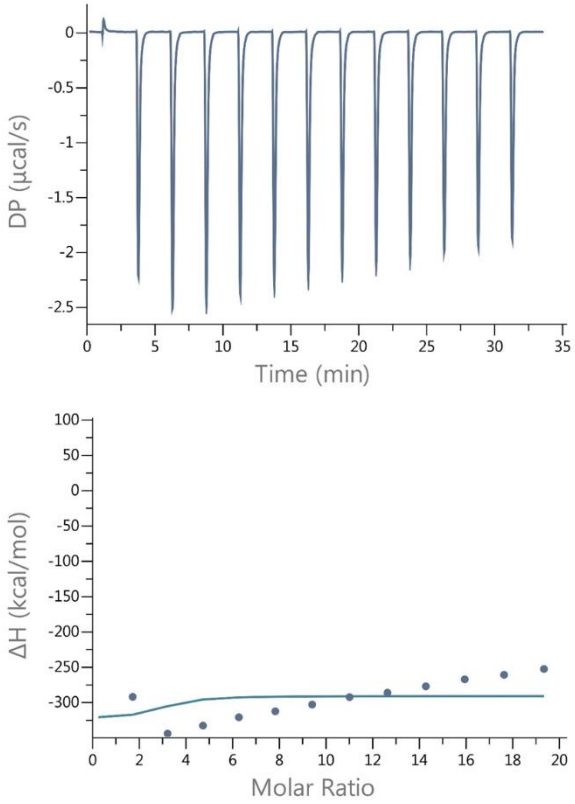
Overall values used for chart:

	MRSA-BAA40	**MRSA-USA300**	***E. faecalis* V583**	***B. subtilis* 6633**	***E. coli* K-12**	***P. aeruginosa* PA01**
	26.63205484	20.87866154	26.066875	7.091285714	16.778387	14.63372609
	21.58455758	22.63727143	23.11712941	9.938566667	14.202183	15.7658087
	23.108175	21.26998421	27.35383846	8.160666667	12.966738	14.65429167
	22.65013333	19.78246667		7.91946	17.167541	15.23216667
	26.40105714	21.7166		7.444172727	13.292159	
		26.70185455				
Average	**24.07519558**	**22.16447306**	**25.51261429**	**8.110830355**	**14.881402**	**15.07149828**
No of cells	109	104	105	105	106	100
Std Dev	7.654065019	5.960115982	8.286341025	2.316132294	4.8666858	3.652046134
Std Error (±)	**0.366563233**	**0.292218728**	**0.404332041**	**0.113015684**	**0.2363472**	**0.182602307**

Isothermal Titration Calorimetry (ITC)

ITC experiments were performed using Microcal PEAQ-ITC instrument (Malvern Instruments Ltd, Malvern, UK). The solutions of PGOs (30 μM) and PBP1a (300 nM) in deionized water were prepared fresh before each experiment and three replicates were performed for each setting. 0.4 μL of PGOs solution was titrated into 300 μL *E. coli* PBP1a solution followed by twelve 3-μL injections at 150 seconds intervals. The reaction cell was stirred at 750 rpm and reference was set at 10 μcal/s. The data were all obtained and analysed using Microcal softwares.

Figure S26 | ITC plot of PGOs and PBP1a



Minimal inhibition concentration (MIC) determination

Bacteria cells were grown overnight at 37 ℃ in Mueller−Hinton broth (MHB) to mid log phase and diluted to 10^5-10^6 CFU mL^{-1} in MHB. A 2-fold dilution series of 100 μL of polymer

solution in medium was made in 96-well microplates, followed by the addition of 100 μL of the bacterial suspension (10^5 - 10^6 CFU mL^{-1}). The plates were incubated at 37 ℃ for 18−24 h, and the absorbance at 600 nm was measured with a microplate reader (BIO-RAD Benchmark Plus). A positive control with 1 μg/mL vancomycin, a negative control without polymer, and a blank without bacteria were included. MICs were determined as the lowest concentration that inhibited cell growth by more than 90%. **17** had an MIC of 32 mg/mL against MRSA USA300 and *S. aureus* ATCC29213.

Cytotoxicity evaluation

NIH 3T3 cells (1×10^4 cells/well) were seeded with complete medium on a 96-well plate and cultured overnight. The old medium was replaced with fresh medium containing polymer for 24 hours. Then, 10 μL MTT solutions were added to the media. After incubation for 2 hours, the OD_{450nm} of the media was measured by using a microplate reader. The cell viability was calculated using the formula: % cell viability = (AbsTest - AbsBlank) / (AbsControl - AbsBlank) ×100%. **17** had an IC_{50} of > 2048 μg/mL against 3T3 cell line.

Bacterial detection with PGOs

For limit of detection, 1 mL *E. coli* EC958 was prepared at different concentrations each, and PGOs-rhodamine (200 μg) was added for metabolic labeling for 1 hour at 37 ℃. All the bacteria were harvested by centrifugation at 5000 rpm for 15 min and washed with PBS for 3 times. The bacteria pellet was finally dispersed in 1ml PBS for fluorescence analysis with fluorospectrometer.

For resistant strain detection, 1 mL of drug-sensitive and drug-resistant bacteria (10^6 CFU/mL) were treated with different concentration of antibiotics (Penicillin G sodium salt) ranging from 0 to 1000 μg/ml for 2 hours. PGOs-rhodamine (50 μg) were then added for metabolic labeling for 1 hour at 37 ℃. All the bacteria were harvested by centrifugation at 5000 rpm for 15 minutes and washed with PBS for 3 times. The bacteria pellet was finally dispersed in 1mL PBS for fluorescence analysis with fluorospectrometer.

Imaging of fluorescence *in vivo*

PGOs-Cy7.5 was prepared according to protocols given by the supplier of sulfo-Cyanine7.5 NHS ester (Lumiprobe, USA) with minor modifications. Generally, a solution of 1 mg NIR dye in DMF (300 μL) was added to a solution of 10 mg PGOs in 0.1 M aqueous $NaHCO_3$ (2.7 mL). The reaction vessel was shielded from light and the mixture was dialyzed, lyophilized after overnight stirring to give the product PGOs-Cy7.5.

S. aureus (ATCC29213) was intraperitoneally injected into mice to develop bacterial infection in most organs of mice, including liver and kidney. At 2 hours post-infection, 5 mg/kg of PGOs-Cy7.5 was intravenously administrated to non-infected and infected mice. Non-invasive image was taken at varied time point using IVIS SpectrumCT (PerkinElmer, USA) to track fluorescence difference. At 10 hours post-infection, mice were euthanized and dissected, and fluorescence intensity of varied organs were imaged and quantified for the fluorescence intensity. Eventually, organs were homogenized and serial diluted to get the exact bacterial CFU count.

This study was performed in strict accordance with the guidelines for the care and use of

laboratory animals from Nanyang Technological University (NTU) and was approved by the Institutional Animal Care and Use Committee (IACUC) of NTU (Singapore).

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