Supporting Information

Bacteria-instructed synthesis of polymers for self-selective microbial binding and labelling

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Materials and methods

Tris(2-pyridylmethyl)amine (**TPA**),[1] O-Benzyl α-bromoester (**In2**),[2] 3-(trimethylsilyl)prop-2-yn-1-yl methacrylate (**TMS-PMA**),[3] (E)-N–(pyridine-2-ylmethylene)propan-1-amine (**IP1**) and (*E*)-N–(pyridine-2-ylmethylene)octan-1-amine (**IP2**),[4] and 2,3-dihydroxypropylmethacrylate (**DHPMA**)[5] were synthesized according to literature procedures. 3-Azo-7-hydroxycoumarin[6] was synthesised by modification of an existing procedure.

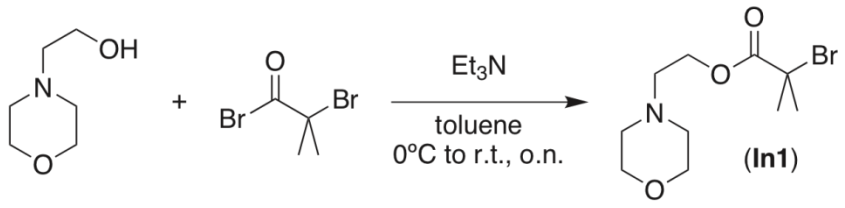
Nuclear Magnetic Resonance (NMR) spectra were recorded on a Bruker 400 MHz spectrometer. Chemical shifts are reported in ppm (δ units) downfield from internal tetramethylsilane (dmso-*d6*) or the -OD signal (D_2O). Mass spectra (MS) (TOF-ESI) were

recorded on a Waters 2795 separation module/micromass LCT platform, under positive scan mode with direct injection of the purified compounds. Gel Permeation Chromatography (GPC) was performed on a Polymer Laboratories GPC 50 with RI detector. Separations were performed on a pair of PLgel Mixed-D columns (300 × 7.8 mm, 5 µm bead size, Polymer Labs UK) fitted with a matching guard column (50 × 7.8 mm). The mobile phase was a 5% Et_3N solution in $CHCl_3$ at a flow rate of 1 mL/min. Molecular weights were calculated based on a standard calibration method using poly(styrene) narrow standards (EasiVial PS-M, Agilent Tech. Inc.). Samples were prepared at 1–5 mg/mL in the mobile phase and 100 µL injected onto the column. Molecular weight and polydispersity index were calculated using Polymer Labs Cirrus 3.0 Software. Cationic (acidic) aqueous GPC was performed on a Shimadzu Prominence UPLC system fitted with a differential refractive index detector. The eluent was 1 M acetic acid containing 0.3 M NaH_2PO_4 (pH 3) at a flow rate of 1 mL/min with column and detector cell temperatures maintained at 35 °C. The instrument was fitted with a Polymer Labs Aquagel-OH guard column (50 × 7.5 mm, 8 µm) followed by a pair of PL Aquagel-OH columns (30 and 40, 300 × 7.5 mm, 8 µm). Column calibration was achieved using narrow poly(2-vinyl pyridine) standards (Polymer Standards Service, Germany) of known molecular weight in the range 0.8-256 kDa. Molecular weights and dispersity values were calculated using Shimadzu LabSolutions software with GPC analysis add-on.

Bacterial cluster size was determined by laser diffraction using a Coulter LS230 particle size analyser (Beckman Coulter, High Wycombe, UK). A Beckman Coultier DU 800 UV spectrophotometer with a thermostat was used for turbidimetry measurements. A Nikon optical microscope equipped with a camera connected to a personal computer was used for optical microscopy studies.

C. difficile 630 Δ*erm* and *Y. pseudotuberculosis* YpIII pIB1 were grown in BHI-S medium and L broth Lennox, respectively. *C. difficile* cultures were maintained in an anaerobic work station (MG1000, Don Whitley Scientific) containing an atmosphere of 80% nitrogen, 10% hydrogen, and 10% carbon dioxide. *H. pylori* 26695 and *C. jejuni* NCTC 11168 were grown in brain–heart infusion (BHI) broth supplemented with 5% (v/v) foetal calf serum (BHI-FCS) and cultures were maintained in a MACS VA500 microaerobic workstation (Don Whitley Scientific) using a humidified atmosphere consisting of 6% oxygen, 3% hydrogen, 5% carbon dioxide, and 86% nitrogen. *E. coli* and *P. aeruginosa* strains were grown in standard LB medium. All bacterial cultures were incubated at 37 °C, except *Y. pseudotuberculosis* which was incubated at 30 °C. Cultures were harvested at late exponential or early stationary phase.

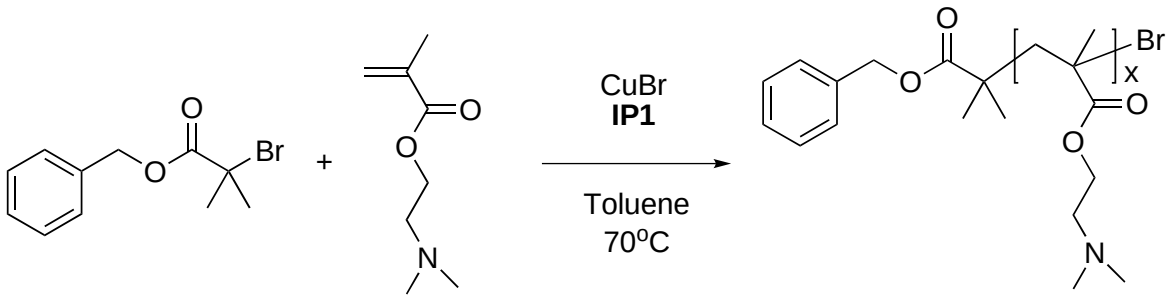
- **Synthesis of 2-(N-Morpholino)ethyl-2-bromobutyrate (In1)**



In a round bottomed flask, 8.6 mL (9.3 g, 71 mmol) of 4-(2-hydroxyethyl) morpholine and 15.3 mL (11.1 g, 109 mmol) of triethylamine were dissolved in 300 mL of toluene. Over ice, and under nitrogen, 13.2 mL (24.5 g, 107 mmol) of α-bromoisobutyryl bromide were added dropwise with a pressure equalising addition funnel over 3 hours. The reaction mixture was left to react overnight before filtering. The solid was disregarded and the filtrate was washed with 0.1 M sodium carbonate (3 x 100 mL) and *di*-water (3 x 100 mL) before being dried over magnesium sulphate. The toluene was removed under reduced pressure and

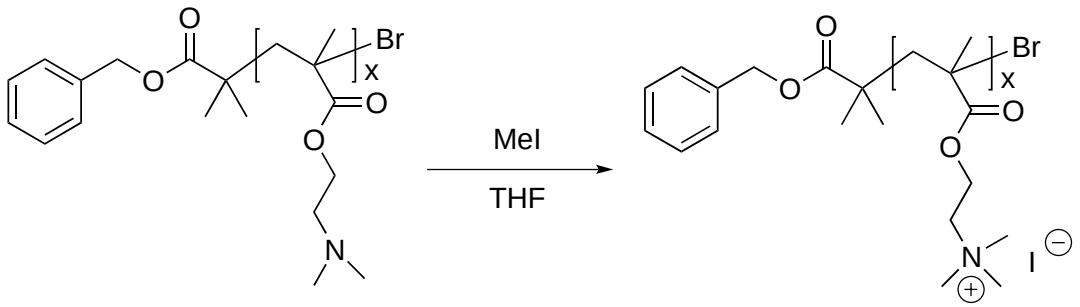
the brown liquid was purified using flash chromatography (dichloromethane:ethylacetate 4:1) to yield a yellow oil (**1**) (13.3 g, 47.5 mmol, Yield 66.9%). **^{1}H-NMR** ($CDCl_3$, 400 MHz) δ (ppm): 1.87 (s, 6H, CH_3), 2.47-2.50 (m, 4H, CH_2NCH_2), 2.63 (t, *J* = 5.75 Hz, 2H, NCH_2), 3.62-3.65 (*m*, 4H, CH_2OCH_2), 4.26 (t, *J* = 5.75 Hz, 2H, CH_2O). **^{13}C-NMR** ($CDCl_3$, 100 MHz) δ (ppm): 30.72 (CH_3), 53.56 (CBr), 55.63 (NCH_2), 56.52 (CH_2NCH_2), 63.06 (CH_2O), 66.67 (CH_2OCH_2), 171.34 (CO). **HRMS** (m/z): calculated for $C_{10}H_{18}BrNO_3$ 279.0470 (100.0%), 281.0450 (97.3%), found 279.0674.

- **Synthesis of boundary polymers: p(TMAEMA) and p(MEDSA)**
  - **<u>Poly(2-(N,N-dimethylamino)ethyl methacrylate) p(DMAEMA)</u>**



2-(Dimethylamino)ethyl methacrylate (**DMAEMA**) (20.0 g, 127 mmol), **IP1** (397 µL, 1.27 mmol), benzyl-2-bromo-2-methylpropanoate (327 mg, 1.27 mmol) were added to a large dry Schlenck tube along with toluene (20 mL) as solvent. The tube was sealed with a rubber septum and subjected to five freeze-pump-thaw cycles. At the end the mixture was left frozen, flushed with nitrogen, and copper (I) bromide (182 mg, 1.27 mmol) was added. The system was then subjected to three nitrogen/vacuum cycles with freezing and thawing, filled with nitrogen and the temperature adjusted to 70"C with constant stirring (t = 0). At the end of the polymerisation the reactor was opened and air was allowed to enter the system, causing the copper catalyst to oxidise to Cu(II) and effectively stopping the polymerisation reaction. During this process the flask was lifted from the bath and the temperature reduced to ambient. The mixture was then passed through two neutral alumina columns in order to remove residual Cu(II) salts present in the reaction mixture. The volume was reduced under vacuum and **p(DMAEMA)** obtained through precipitation into petroleum ether. Conversion 93%, **^{1}H-NMR** (400 MHz, $CDCl_3$) δ (ppm): 0.8-1.2 (m, 3H, $CH_{3\ backbone}$), 1.8-2.1 (m, 2H, $CH_{2\ backbone}$), 2.3 (bs, 6H, $N(CH_3)_2$), 2.6 (bs, 2H, CH_2N), 4.1 (bs, 2H, OCH_2). M_n (GPC, $CHCl_3$/Et_3N) = 17.1 kDa, PDI (GPC) = 1.18.

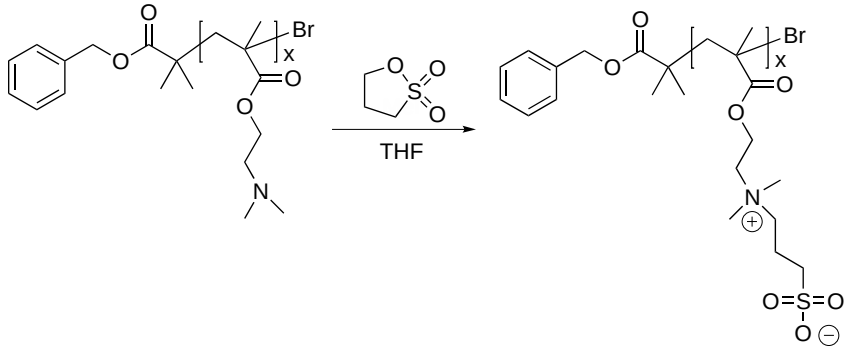
  - **<u>Poly[2-(methacryloyloxy)-N,N,N-trimethylethanaminium iodide] p(TMAEMA)</u>**



p(DMAEMA) (0.20 g, 1.27 mmol) was dissolved in THF (8.2 mL). Methyl iodide (79.2 µL, 1.27 mmol) was added under stirring and the reaction mixture allowed to react for 48 hours, upon which the polymer precipitated. The solvent was removed under vacuum, the polymers dissolved in deionised water and the resulting solutions freeze-dried to give the

desired **p(TMAEMA)**. The quaternisation of the polymer was confirmed using ^{1}H-NMR spectroscopy. **^{1}H-NMR** (400 MHz, $(CD_3)_2SO$) δ (ppm): 0.8-1.5 (m, 3H, $CH_{3\ backbone}$), 1.7-2.4 (m, 2H, $CH_{2\ backbone}$), 3.3 (bs, 9H, $N(CH_3)_3$), 3.9 (bs, 2H, CH_2N), 4.6 (bs, 2H, OCH_2).

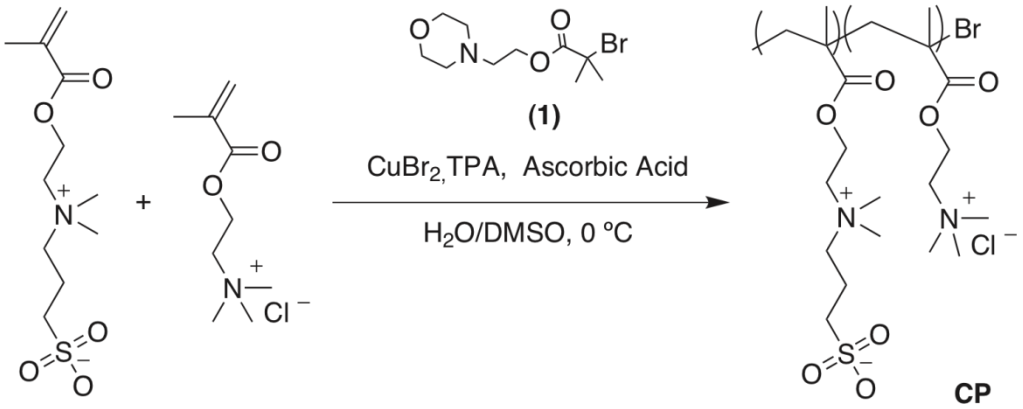
Poly[2-(N-3-sulfopropyl-N,N-dimethyl ammonium)ethyl methacrylate] p(MEDSA)



p(DMAEMA) (0.20 g, 1.27 mmol) was dissolved in THF (8.2 mL). 1,3-propane sultone (112 µL, 1.27 mmol) was added under stirring and the reaction mixture allowed to react for 16 hours, upon which the polymer precipitated. The solvent was removed under vacuum, and traces of unreacted 1,3-propane sultone were removed by washing the polymer with diethyl ether (3 x). The polymers dissolved in deionised water and the resulting solutions freeze-dried to give the desired **p(MEDSA)**[†]. The quaternisation of the polymer was confirmed using ^{1}H-NMR spectroscopy. **^{1}H-NMR** (400 MHz, $(CD_3)_2SO$) δ (ppm): 0.7-1.4 (m, 3H, $CH_{3\ backbone}$), 1.6-2.2 (m, 2H, $CH_{2\ backbone}$), 2.3 (bs, 2H, $C\underline{H_2}CH_2SO_3^-$), 3.0 (bs, 2H, $CH_2C\underline{H_2}SO_3^-$), 3.3 (bs, 6H, $N(CH_3)_2$), 3.6 (bs, 2H, $NC\underline{H_2}CH_2CH_2SO_3^-$), 3.9 (bs, 2H, $OCH_2C\underline{H_2}N$), 4.5 (bs, 2H, OCH_2).

Microbial templated polymers: p(TMAEMA-*co*-MEDSA)[†] (P1)

- **Control polymer by conventional AGET ATRP**



To a reaction flask, 144 mg (0.695 mmol) of **TMAEMA**, 194 mg (0.695 mmol) of **MEDSA**, 1.554 mg (5.6 µmol) of **In1**, 200 µL of a 0.069 M aqueous solution with $CuBr_2$ and **TPA**, and 50 µL of DMSO were added. This mixture was degassed for 30 minutes over ice after which 270 µL of a degassed 1 mg/mL solution of ascorbic acid were added to begin the polymerisation. The polymerisation was monitored by ^{1}H-NMR spectroscopy over time and when the desired conversion was reached (Table S01) the polymerisation was terminated by exposing to air. The polymers were obtained by dialysis against water for 3 days followed by freeze drying to yield a white amorphous solid (**CP**). **^{1}H-NMR** (D_2O, 400 MHz)

[†] poly[2-(methacryloyloxy)-N,N,N-trimethylethanaminium chloride]-co-[2-(N-3-sulfopropyl-N,N-dimethyl ammonium)ethyl methacrylate]

δ (ppm): 1.0-2.0 (m, 6H, CH_3), 2.28 (m, 2H, $C\underline{H}_2CH_2SO_3^-$), 2.98 (m, 2H, $CH_2SO_3^-$), 3.60 (m, 2H, $C\underline{H}_2CH_2CH_2SO_3$), 3.2 (m, 15H, $N(CH_3)$), 3.78 (m, 4H, NCH_2), 4.49 (m, 4H, $COCH_2$).

- **Microbial directed polymer synthesis by b-ATRP**

To a reaction flask, 144 mg (0.695 mmol) of **TMAEMA**, 194 mg (0.695 mmol) of **MEDSA**, 1.554 mg (5.6 µmol) of the morpholine initiator **In1** and 50 µL of DMSO were added. This mixture was mixed with bacteria as a 7 mL suspension with an optical density at 600 nm of 93.6 and degassed for 30 minutes over ice after which 200 µL of a degassed 0.69 mM aqueous solution with $CuBr_2$ and TPA were added to begin the polymerisation. The reaction was monitored by ^{1}H-NMR spectroscopy when the desired conversion was reached (Table S01) the polymerisation was terminated by exposing to air. Polymers were obtained from the reaction by first washing the cells with deionised water (**WTPs**) (3 x 5 mL) followed by washing with a saturated solution of sodium chloride (0.15M aq) (**STPs**) (3 x 5 mL). These two separated solutions were then dialysed against water for 3 days followed by freeze drying to obtain the polymers as a white amorphous solid. Typical unoptimised yields of polymers in these experiments were 10-12 mg (STP) and 50-100 mg (WTP).

Note: - Under these conditions, it was not possible to eliminate bacterial growth completely, but total yields of polymers for bacteria-mediated syntheses carried out in triplicate varied by ±26 %, and the yield recovered from the salt varied by ±14 %. The monomer compositions of the polymers prepared over three experimental replicates varied by < 4%.

- **Calculation of monomer ratios**

Monomer final composition was calculated by comparing the integral for the CH_2 adjacent to the quaternary amine (Figure S01, 3.8-3.9 ppm, signals a and b) which both monomers share, with the other CH_2 adjacent to the quaternary amine which is only found on the zwitterionic monomer **MEDSA** (Figure S01, 3.6-3.7 ppm, signal c). An example of the calculation for the control polymer can be seen in the Figure S01.

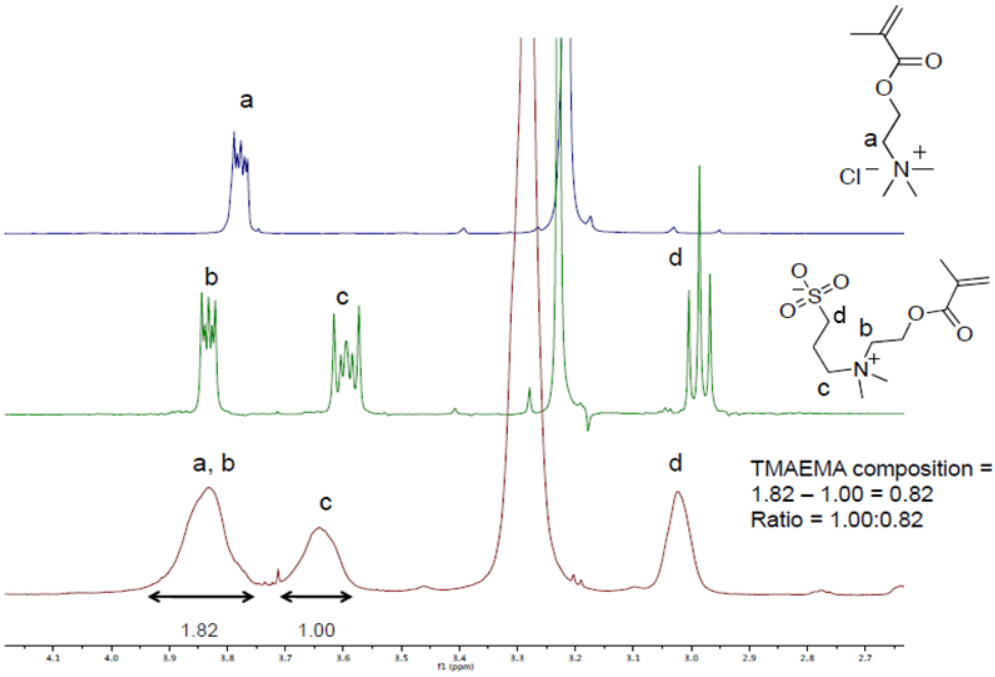


Figure S01: Representative ^{1}H-NMR spectra of **TMAEMA**, **MEDSA** and **CP**, relevant signals integrated and calculation of monomer composition.

Table S01: Monomer Feed ratio, conversion and final composition for diol containing polymers.

Polymer	Feed Ratio (**DHPMA:TMAEMA**)	Conversion	Composition (NMR) (**DHPMA:TMAEMA**)
CP	1:1.49	37%	1:1.02
E. coli MG1655 **WTP**	1:1.49	38%	1:0.96
E. coli MG1655 **STP**	1:1.49	38%	1.086
P. aeruginosa PAKR76 **WTP**	1:1.49	29%	1:1.24
P. aeruginosa PAKR76 **STP**	1:1.49	29%	1:0.98

Polymer	Feed Ratio (**DHPMA**:**MEDSA**)	Conversion	Composition (NMR) (**DHPMA**:**MEDSA**)
CP	1:2.75	38%	1:1.80
E. coli MG1655 **WTP**	1:2.75	35%	1.2.29
E. coli MG1655 **STP**	1:2.75	35%	1:2.59
P. aeruginosa PAKR76 **WTP**	1:2.75	31%	1:1.24
P. aeruginosa PAKR76 **STP**	1:2.75	31%	1:1:87

- **Effect of incubation with bacteria over monomer composition of isolated polymers**

- The ability of the bacteria surface to select different populations of polymers as a function of their monomer composition was evaluated by incubating a batch of control polymer (CP) under the conditions used for the microbial directed polymer synthesis by b-ATRP (see above). In brief, 100 mg of CP were diluted with 7 mL of a bacteria suspension with an optical density at 600 nm of 93.6, and this mixture was allowed to react for 30 min. Polymer fractions were obtained from this mixture by first washing the cells with deionised water (**WTP**s) (3 x 5 mL) followed by washing with a saturated solution of sodium chloride (0.15M aq) (**STP**s) (3 x 5 mL). These two separated solutions were then dialysed against water for 3 days followed by freeze drying to obtain the polymers as a white amorphous solid. In all cases, there were no significant differences in monomer compositions when compared to the starting **CP** (Figures S02-S06).

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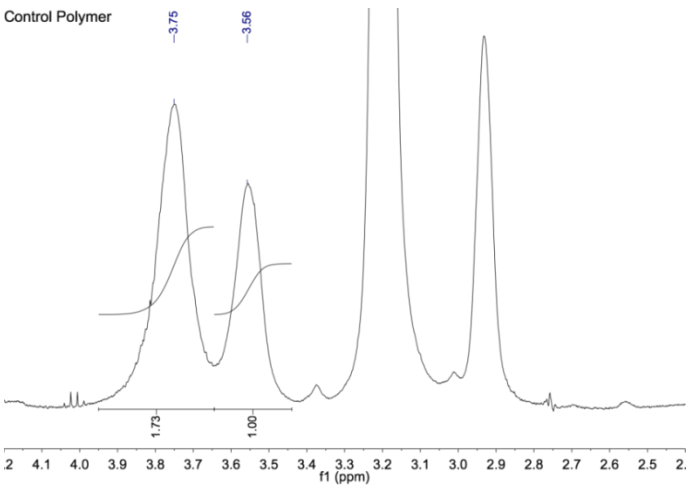

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Figure S02: ^{1}H-NMR spectra of starting CP with relevant signals integrated.

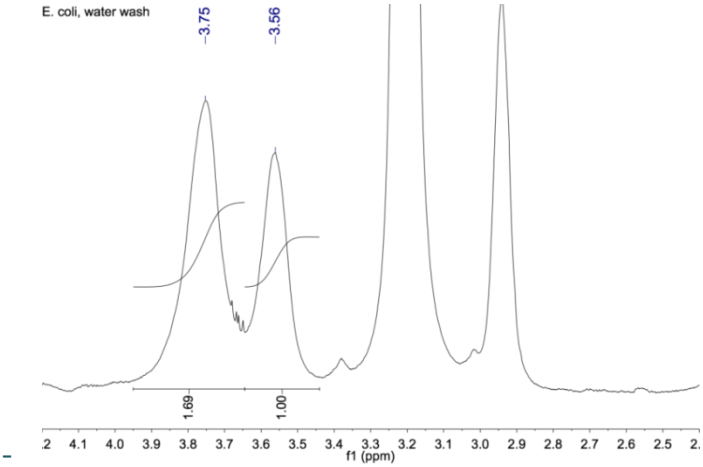


Figure S03: ^{1}H-NMR spectra of recovered CP after incubation with *E. coli* MG1665 and washes with water. Relevant signals have been integrated.

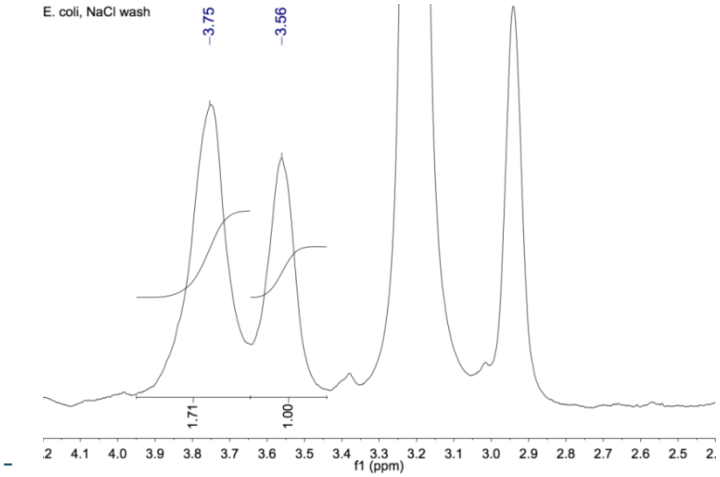


Figure S04: ^{1}H-NMR spectra of recovered CP after incubation with *E. coli* MG1665 and washes with NaCl. Relevant signals have been integrated.

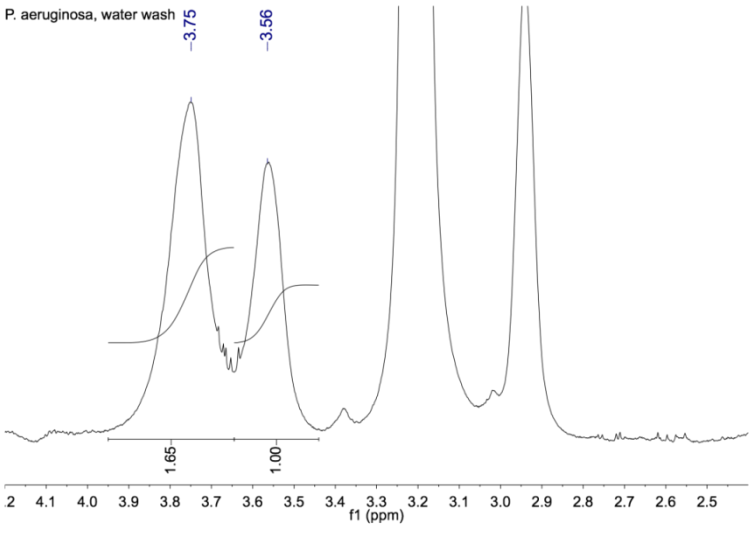


Figure S05: ^{1}H-NMR spectra of recovered CP after incubation with *P. aeruginosa* PAO1 and washes with water. Relevant signals have been integrated.

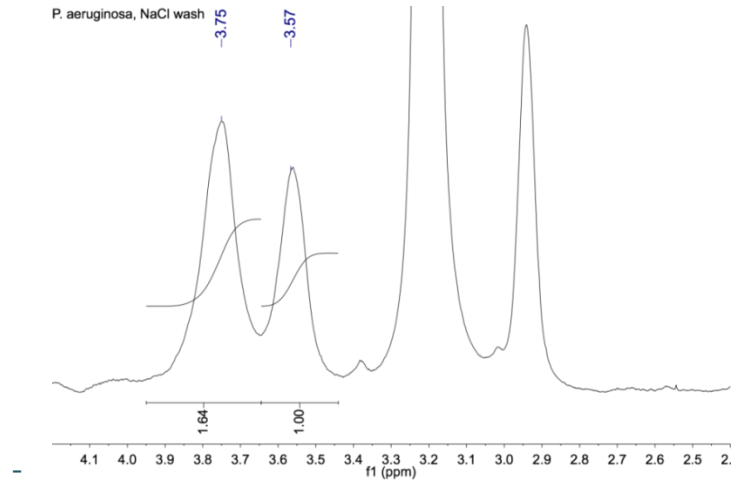


Figure S06: ^{1}H-NMR spectra of recovered CP after incubation with *P. aeruginosa* PAO1 and washes with NaCl. Relevant signals have been integrated.

- **Calculation of reactivity ratios**

The monomer reactivity ratios were calculated at low conversion (<10%) from the copolymer composition at various monomer feed ratios (85:15 → 98:2 **TMAEMA**:**MEDSA**; 4 ratios) using the error in variables model (EVM) method,[8,9] and the computer software package reactivity ratios error in variable model (RREVM).[10,11]

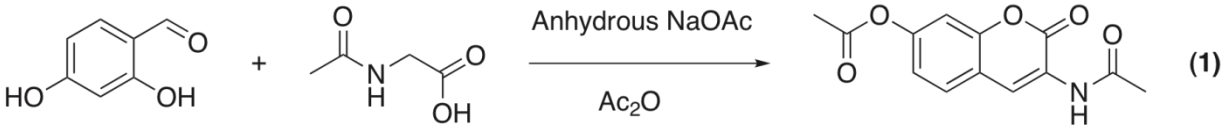
Table S02: Targeted molar compositions, conversion and final compositions for control polymer reactivity ratio experiments.

	TMAEMA:MEDSA	TMAEMA:MEDSA	TMAEMA:MEDSA	TMAEMA:MEDSA
Targeted molar composition	85:15	90:10	94:06	98:02
Conversion (%)	7	5.5	3.5	6
Composition by NMR	6.6:1.0	11.65:1.0	25.36:1.0	96.04:1
Percentage composition	87:13	92:08	96.2:3.8	99:01

We calculated the reactivity ratios by using the Mayo-Lewis instantaneous copolymer composition (Eq 1), where F_x are the experimental mole fractions of monomers (**TMAEMA** and **MEDSA**) that are incorporated into the copolymer after conversion; r_x are reactivity ratio values; and $[M_x]$ the molar feed ratios of monomers. The starting values for the reactivity ratios were chosen as 1, and the calculated values were: r_{TMAEMA} = 0.9988 and r_{MEDSA} = 1.0012.

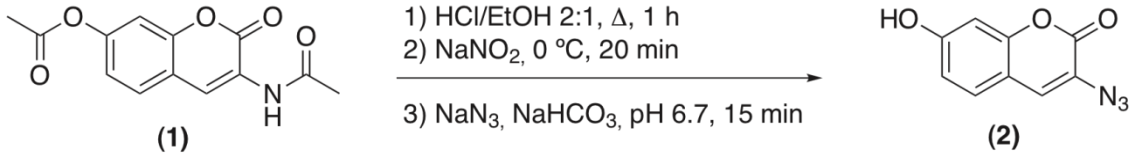
$$\frac{F_1}{F_2} = \frac{r_1 \cdot [M_1]^2 + [M_2][M_1]}{r_2 \cdot [M_2]^2 + [M_1][M_2]} \; ; Eq\,1$$

- **Synthesis of 3-acetamido-2-oxo-2H-chromen-7-yl acetate (1)**



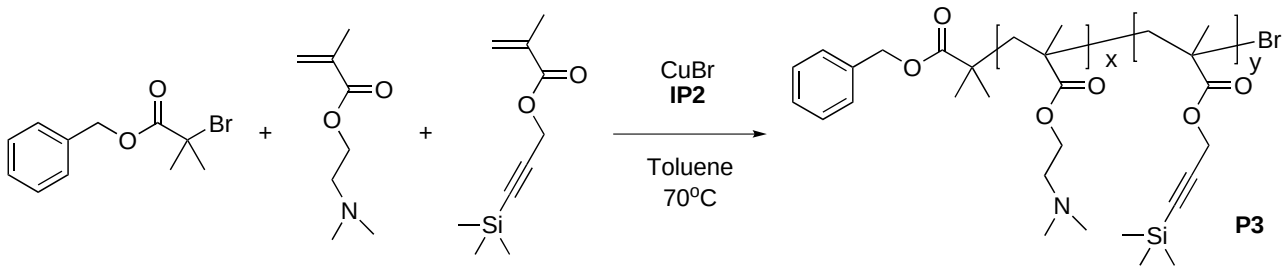
A mixture of 2,4-dihydroxy benzaldehyde (2.8 g, 20 mmol), N-acetylglycine (2.3 g, 20 mmol) and anhydrous sodium acetate (4.9 g, 60 mmol) were added to 100 mL of acetic anhydride in a round bottomed flask. To the flask a reflux apparatus was attached and the mixture heated with stirring until reflux occurred. The mixture was then left to reflux for 4 hours until the reaction had completed and a colour change from light yellow to red was observed. After cooling ice was added to the mixture and it was left overnight to produce a highly crystalline yellow solid **1** (2.70 g, 10.4 mmol Yield 51%). **IR** (KBr) υ (cm^{-1}): 3342, 1760, 1720, 1682, 1536, 1373, 1252, 1209, 1157, 916. **^{1}H-NMR** (400 MHz, $CDCl_3$) δ (ppm): 2.25 (s, 3H, (NHCO), 2.34 (s, 3H, ($OCOCH_3$), 7.06-7.09 (dd, *J* = 2.23 Hz, 1H, CHCN), 7.13 (d, *J* = 2.27 Hz, 1H, CHCO), 7.50-7.52 (d, *J* = 8.47 Hz, 1H, COCH), 8.02 (s, 1H, (NH), 8.67 (s, 1H, CHC). **^{13}C-NMR** (100 MHz, $CDCl_3$) δ (ppm): 21.11 (CH_3), 24.74 (CH_3), 110.08 (CH), 117.63 (C), 119.63 (CH), 122.72 (CH), 123.56 (CH), 128.37 (C), 150.12 (C), 151.35 (C), 158.48 (CO), 168.86 (CO), 169.35 (CO). **HRMS** (m/z): calculated for $C_{13}H_{11}NO_5$) [M-H^+]: 261.2314, found 260.9206

- **Synthesis of 3-azo-7-hydroxycoumarin (2)**



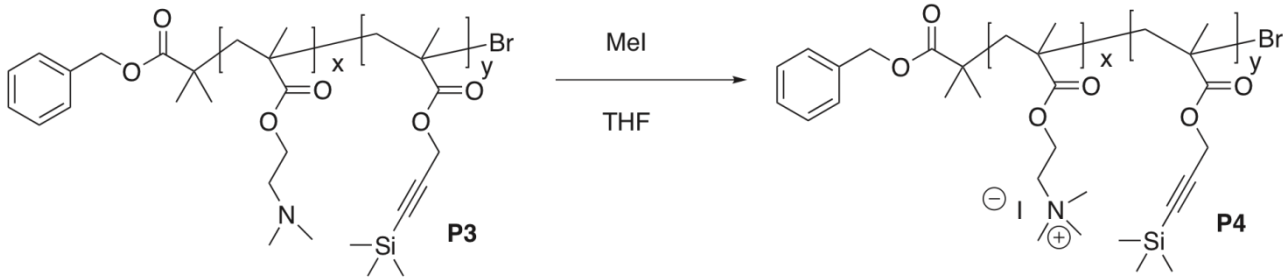
The protected coumarin **1** (2.65 g, 10.1 mmol) was dissolved in a mixture of concentrated hydrochloric acid and absolute ethanol (30 mL) in a ratio of 2:1. To the flask, a reflux apparatus was attached. The mixture was heated with stirring until reflux occurred and the mixture was left to reflux for 60 minutes. Ice water (40 mL) was then added followed by sodium nitrite (2.76 g, 40.0 mmol). This mixture was left to react for 20 minutes before the pH was adjusted to 6.7 in an ice bath with frequent addition of ice to the bath. This pH adjustment was done to prevent the evolution of hydrazoic acid (HN_3) gas which is both toxic and explosive. Once the pH had reached a safe value of 6.7, sodium azide (3.90 g, 60.0 mmol) was added very slowly in small portions before the mixture was then left to react for a further 15 minutes. The crude product was extracted with ethylacetate (200 mL x 6) before being purified by flash chromatography (SiO_2, gradient ethyl acetate to petroleum ether) to yield the product **2** (180 mg, 0.886 mmol, Yield: 8.73%) **IR** (KBr) υ (cm^{-1}): 3425 (broad), 2922, 2120, 1680, 1623, 1319, 1343, 1259, 1224. **^{1}H-NMR** (400 MHz, $CDCl_3$) δ (ppm): 6.71 (d, *J* = 2.1 Hz, 1H, CHCOH), 6.78 (dd, *J* = 8.5 Hz, 2.3, 1H, CH), 7.35 (d, *J* = 3.4 Hz, 1H, CH), 7.37 (s, 1H, CHN_3). **^{13}C-NMR** (100 MHz, $CDCl_3$) δ (ppm): 102.50 (CH), 111.80 (CH), 114.25 (C), 121.61 (CN_3), 128.32 (C), 129.56 (CH), 153.22 (CO), 157.77 (C), 160.73 (COH).

- **Synthesis of bacterial pro-fluorescent marker: p(TMAEMA-co-PMA) (P2)**
  - **poly[(2-(N,N-dimethylamino)ethyl methacrylate)-co-(3-(trimethylsilyl)prop-2-yn-1-yl methacrylate)] p(DMAEMA-co-TMS-PMA) (P3)**



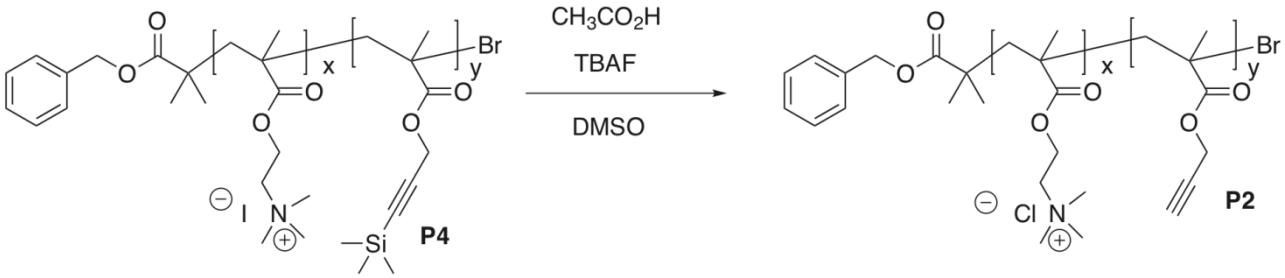
DMAEMA (9.33 g, 59.3 mmol), **TMS-PMA** (1.1 g, 5.6 mmol), **IP2** (546 µL, 2.37 mmol), benzyl-2-bromo-2-methylpropanoate (305 mg, 1.19 mmol) were added to a large dry Schlenck tube along with toluene (10 mL) as solvent. The tube was sealed with a rubber septum and subjected to five freeze-pump-thaw cycles. At the end the mixture was left frozen, flushed with nitrogen, and copper (I) bromide (170 mg, 1.19 mmol) was added. The system was then subjected to three nitrogen/vacuum cycles with freezing and thawing, filled with nitrogen and the temperature adjusted to 70"C with constant stirring (t = 0). At the end of the polymerisation the reactor was opened and air was allowed to enter the system, causing the copper catalyst to oxidise to Cu(II) and effectively stopping the polymerisation reaction. During this process the flask was lifted from the bath and the temperature reduced to ambient. The mixture was then passed through two neutral alumina columns in order to remove residual Cu(II) salts present in the reaction mixture. The volume was reduced under vacuum and **P3** obtained through precipitation into petroleum ether. Conversion 21%, **^{1}H-NMR** (400 MHz, $(CD_3)_2SO$) δ (ppm): 0.37 (m, 9H, $Si(CH_3)_3$), 1.06-1.44 (m, 4H, CH_3), 3.52 (m, 9H, $N(CH_3)_2$), 4.13 (m, 2H, CH_2), 4.61 (m, 4H, CH_2O), 7.33-7.47 (m, 5H, ArH). Mn (GPC, $CHCl_3$/Et_3N) = 9.2 kDa, PDI (GPC) = 1.28.

  - **poly[(2-(methacryloyloxy)-N,N,N-trimethylethanaminium chloride)-co-(3-(trimethylsilyl)prop-2-yn-1-yl methacrylate)] p(TMAEMA-co-TMS-PMA) (P4)**



In a round bottom flask with a magnetic stirring bar, **P4** (1.8 g) was dissolved in tetrahydrofuran (50 mL). The flask was sealed with a rubber septum and methyliodide (914 µL, 14.69 mmol) was added. The mixture was left to react for 48 hours. After this time the flask was opened and the solvent removed under reduced pressure to yield a yellow solid (poly((TMAEMA)-co-(TMS-PMA)) (**P4**). The quaternisation of the polymer was confirmed using ^{1}H-NMR spectroscopy. **^{1}H-NMR** (400 MHz, D_2O) δ (ppm): 0.14-0.24 (m, 9H, $Si(CH_3)_3$, 1.08-2.09 (m. 6H, CH_3), 3.36 (m, 9H, $N(CH_3)_3$), 3.93 (m, 2H, NCH_2), 4.57 (m, 4H, CH_2CO).

- **poly[(2-(methacryloyloxy)-N,N,N-trimethylethanaminium chloride)-co-(prop-2-yn-1-yl methacrylate)] p(TMAEMA-co-PMA) (P2)**



In a round bottom flask with a magnetic stirring bar, **P4** (1.3 g) was dissolved in water (20 mL). To this mixture 500 µL (8.73 mmol) of glacial acetic acid were added followed by 8 mL of a 1 M solution of tetra-n-butylammonium fluoride. The reaction mixture was stirred with continual monitoring by ^{1}H-NMR for the disappearance of the TMS protecting group. After 16 hours the polymer was completely deprotected. To the mixture, water (10 mL) was added and the solution dialysed against sodium chloride for 3 days followed by 3 days of deionised water before freeze drying, to yield **P2**. **^{1}H-NMR** (400 MHz, D_2O) δ (ppm): 1.05-2.03 (m, 6H, CH_3), 3.29 (m, 9H, $N(CH_3)_3$), 3.84 (m, 2H, CH_2), 4.53 (m, 4H, CH_2O).

Aggregation Experiments

- **Bacterial aggregation by turbidimetry**

The ability of the polymers to aggregate bacteria was initially evaluated by turbidimetry experiments. Briefly, polymer solutions were prepared at a concentration of 1 mg/mL in sterile deionised water. Bacteria were grown to an optical density at 600 nm (OD_{600}) such that they were still in the exponential phase of their growth curve (OD_{600} around 0.4), at which point, they were washed once with PBS and twice with sterile deionised water. The cells were finally resuspended to a cell density such that when they were mixed with the polymer solutions they had an $OD_{600} \approx 1.9$. This way, 0.5 mL of a polymer solution were added to a UV cuvette followed by 1 mL of the bacteria suspension. The OD_{600} was quickly recorded (t0) and the change in OD_{600} was monitored with time.

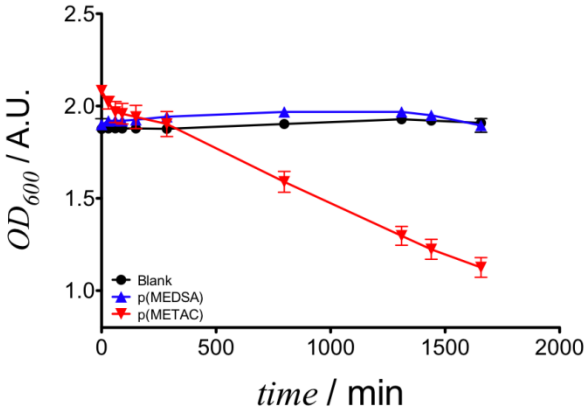


Figure S06: Aggregation as measured by turbidimetry for *E. coli* MG1665 in the absence (●) and presence of p(MEDSA) (▲) and p(TMAEMA) (▼).

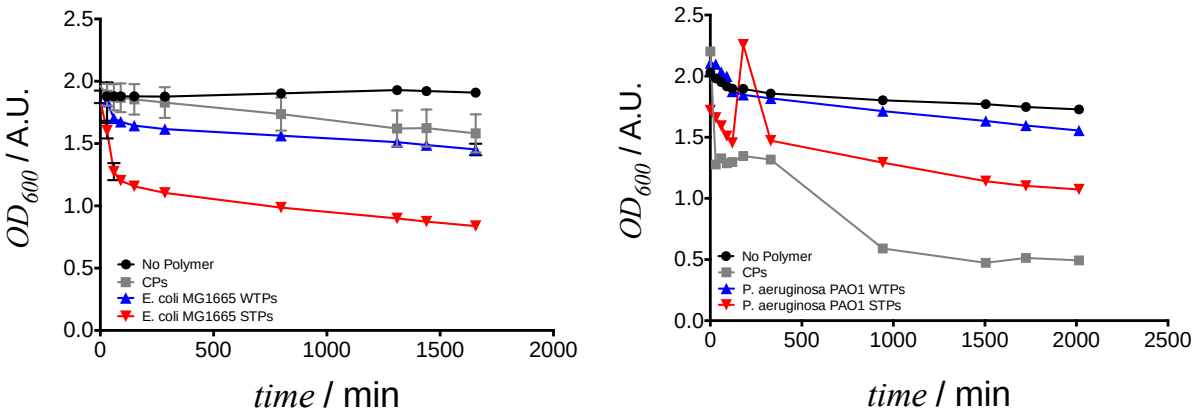


Figure S07: Aggregation as measured by turbidimetry for *E. coli* MG1665 (left) and *P. aeruginosa* PAO1 (right) in the absence (●) and presence of CPs (■), WTPs (▲) and STPs (▼).

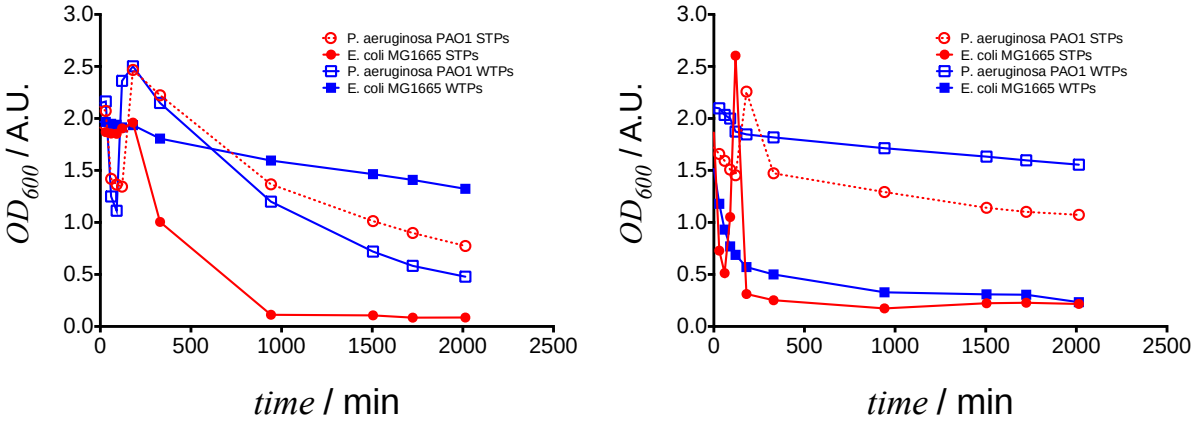


Figure S08: Templating as measured by turbidimetry for *E. coli* MG1665 (left) and *P. aeruginosa* PAO1 (right) in the presence of WTPs (■ *E. coli*, □ *P. aeruginosa*) and STPs (● *E. coli*, ◦ *P. aeruginosa*).

- **Measurement of polymer-bacteria clusters**

Size distributions of bacterial clusters were determined under moderate stirring (default speed 5 setting) to the required concentration as indicated by the in-built display software. Particle size ranges were defined using PSS-Duke standards (Polymer Standard Service, Kromatek Ltd, Dunmow, UK). Particle size distribution was then determined as a function of the particle diffraction using the Coulter software (version 2.11a) and plotted as a function of the percentage of distribution volume.

In a typical experiment, 200 µL of a bacterial suspension with an OD_{600} of 1.9 were added to the flow cell (~ 14 mL) to obtain an obscuration of 8-12%. At this point the t0 population distribution was recorded with constant mixing. Then 100 µL of a 1 mg/mL polymer solution were added, the mixture was allowed to equilibrate and the population distribution was recorded after 15 and 30 minutes.

In order to determine the relative populations of individual bacteria, dimers and clusters, particle size distributions were deconvoluted using the peakfit.m command (http://terpconnect.umd.edu/~toh/spectrum/InteractivePeakFitter.htm#command) in MATLAB® R2012a package. The size of the clusters was then normalized to a single bacteria size (~ 1.5 µm), so that the relative population of unimers (~ 1.5 µm), dimers (~ 3 µm) and clusters (≥ 4.5 µm) could be plotted as a function of time.

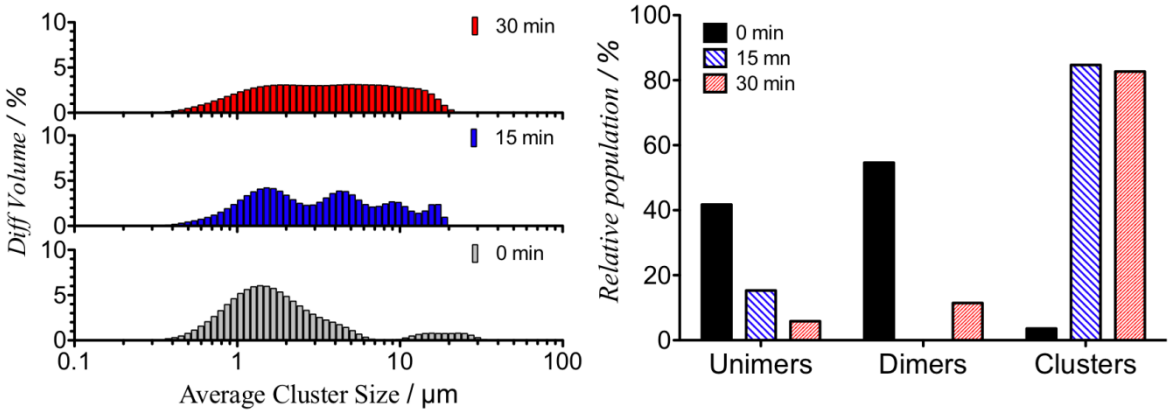


Figure S09: Evolution with time of the size distribution of *E. coli* MG1665 clusters in suspension in the absence (0 min) and presence of **CPs** (1 mg·mL^{-1}) (left), and relative population of unimers, dimers and clusters at each time point (right).

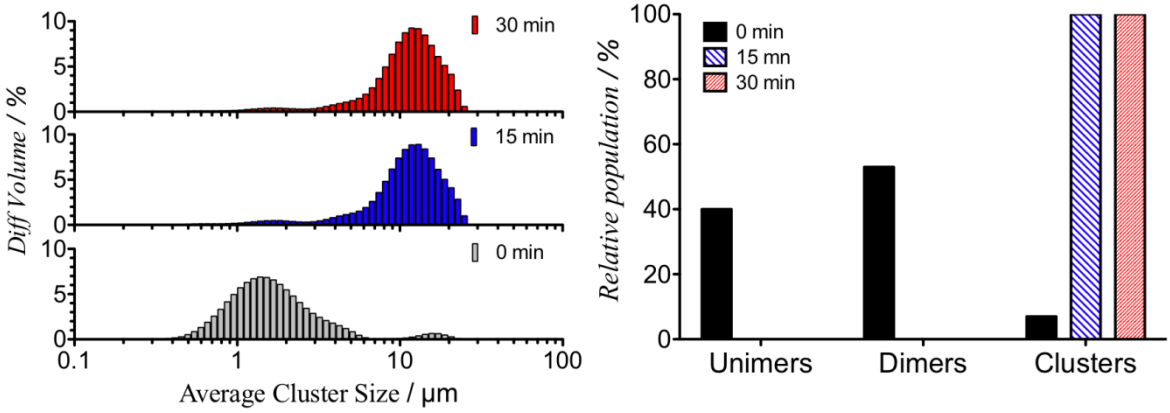


Figure S10: Evolution with time of the size distribution of *E. coli* MG1665 clusters in suspension in the absence (0 min) and presence of *E. coli* MG1665 **NTPs** (1 mg·mL^{-1}) (left), and relative population of unimers, dimers and clusters at each time point (right).

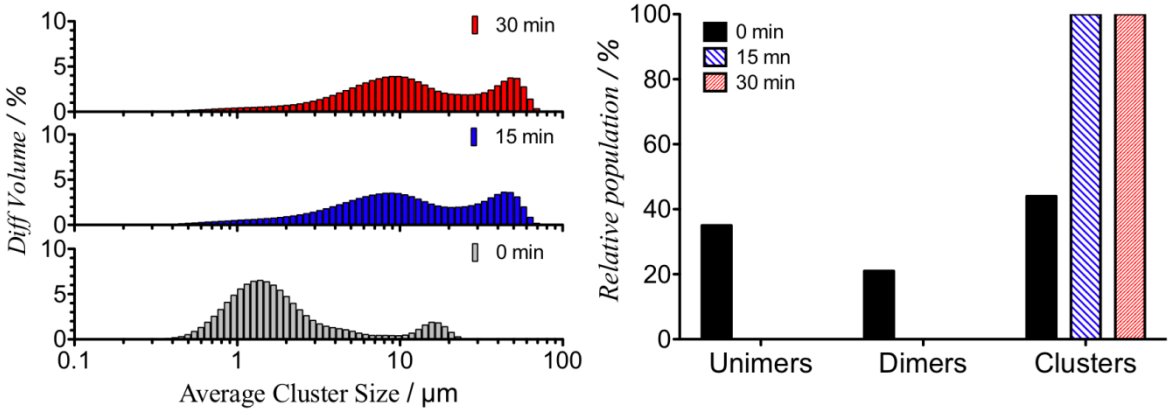


Figure S11: Evolution with time of the size distribution of *E. coli* MG1665 clusters in suspension in the absence (0 min) and presence of *E. coli* MG1665 **TPs** (1 mg·mL^{-1}) (left), and relative population of unimers, dimers and clusters at each time point (right).

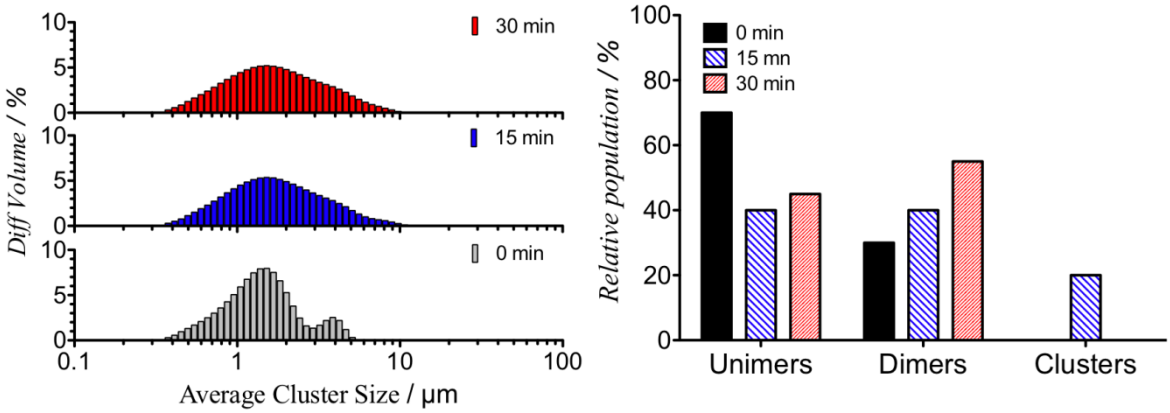


Figure S12: Evolution with time of the size distribution of *E. coli* MG1665 clusters in suspension in the absence (0 min) and presence of *P. aeruginosa* PAO1 **NTPs** (1 mg·mL^{-1}) (left), and relative population of unimers, dimers and clusters at each time point (right).

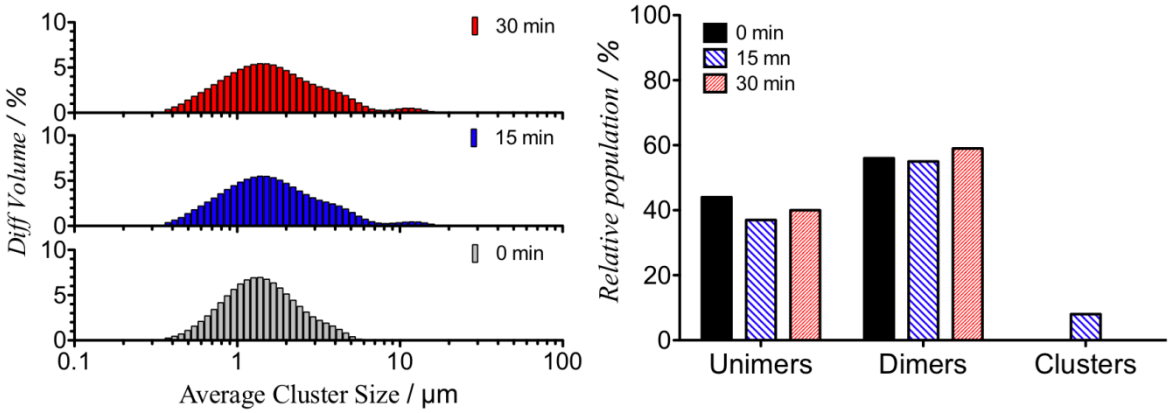


Figure S13: Evolution with time of the size distribution of *E. coli* MG1665 clusters in suspension in the absence (0 min) and presence of *P. aeruginosa* PAO1 **TPs** (1 mg·mL^{-1}) (left), and relative population of unimers, dimers and clusters at each time point (right).

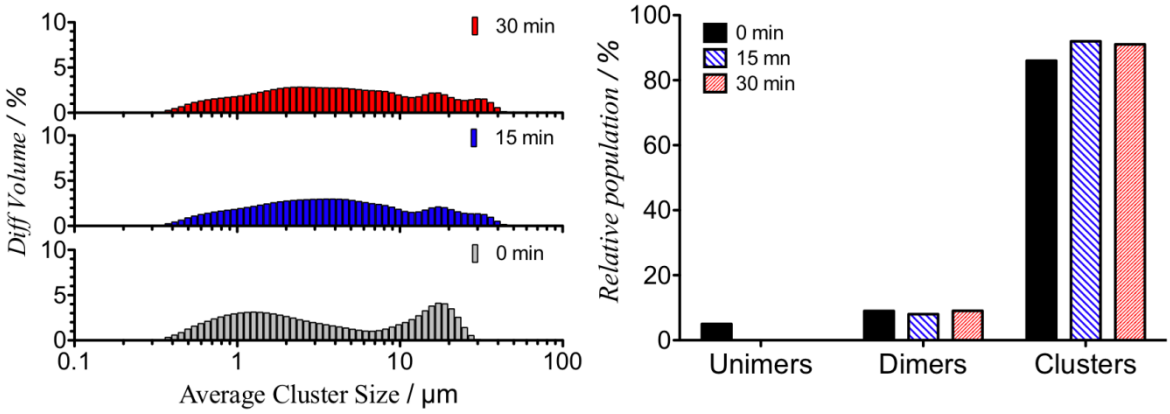


Figure S14: Evolution with time of the size distribution of *P. aeruginosa* PAO1 clusters in suspension in the absence (0 min) and presence of **CPs** (1 mg·mL^{-1}) (left), and relative population of unimers, dimers and clusters at each time point (right).

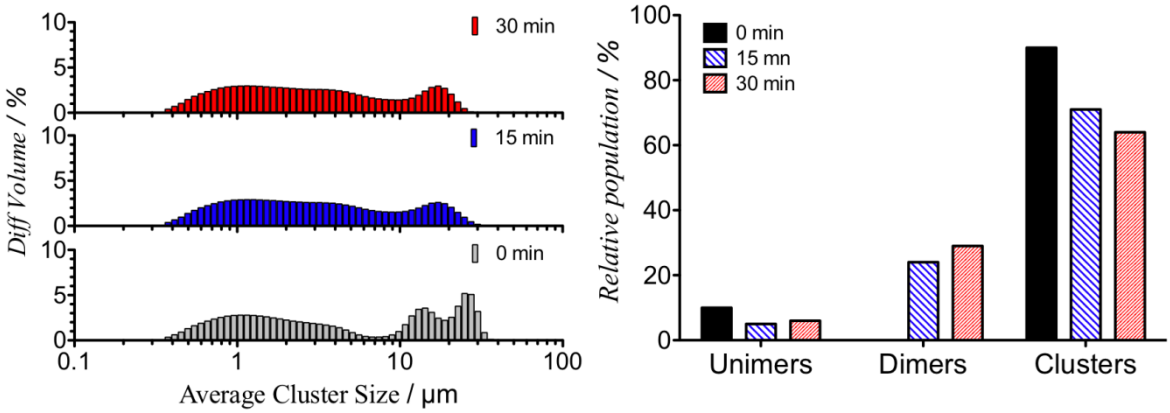


Figure S15: Evolution with time of the size distribution of *P. aeruginosa* PAO1 clusters in suspension in the absence (0 min) and presence of *P. aeruginosa* PAO1 **WTPs** (1 mg·mL^{-1}) (left), and relative population of unimers, dimers and clusters at each time point (right).

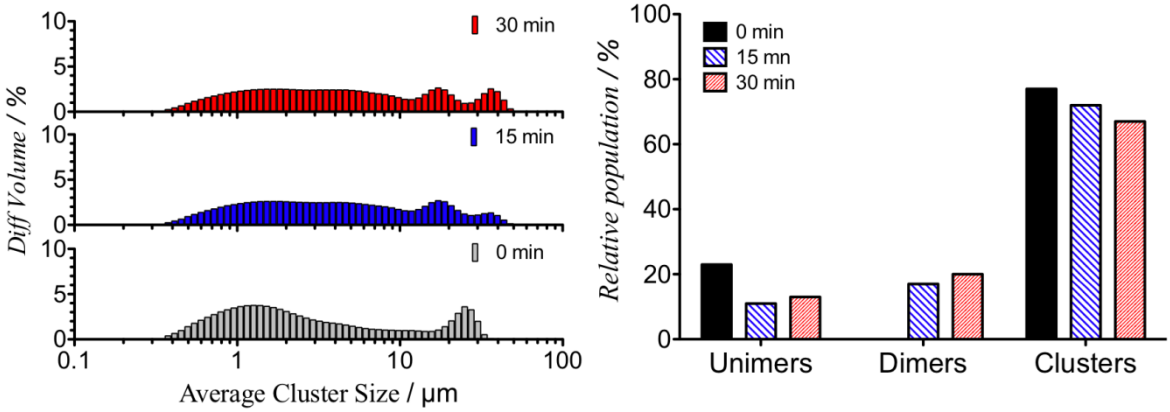


Figure S16: Evolution with time of the size distribution of *P. aeruginosa* PAO1 clusters in suspension in the absence (0 min) and presence of *P. aeruginosa* PAO1 **STPs** (1 mg·mL^{-1}) (left), and relative population of unimers, dimers and clusters at each time point (right).

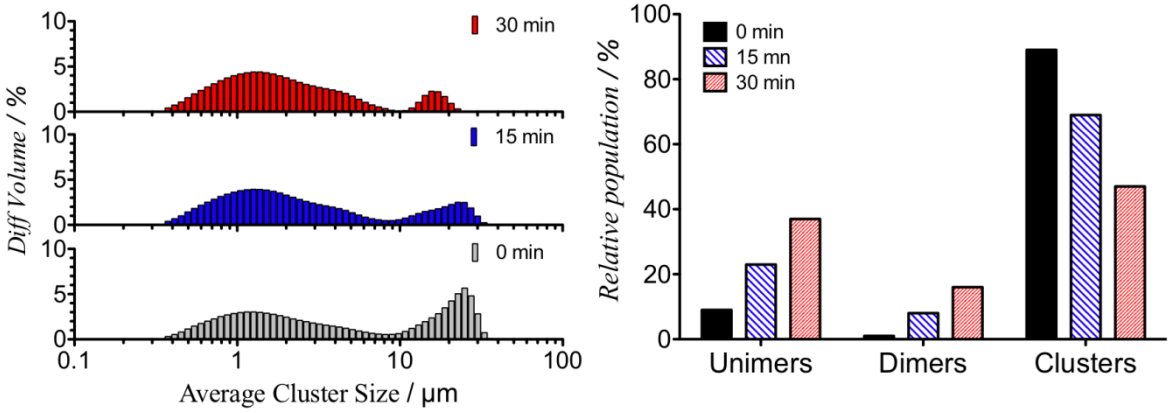


Figure S17: Evolution with time of the size distribution of *P. aeruginosa* PAO1 clusters in suspension in the absence (0 min) and presence of *E. coli* MG1665 **WTPs** (1 mg·mL^{-1}) (left), and relative population of unimers, dimers and clusters at each time point (right).

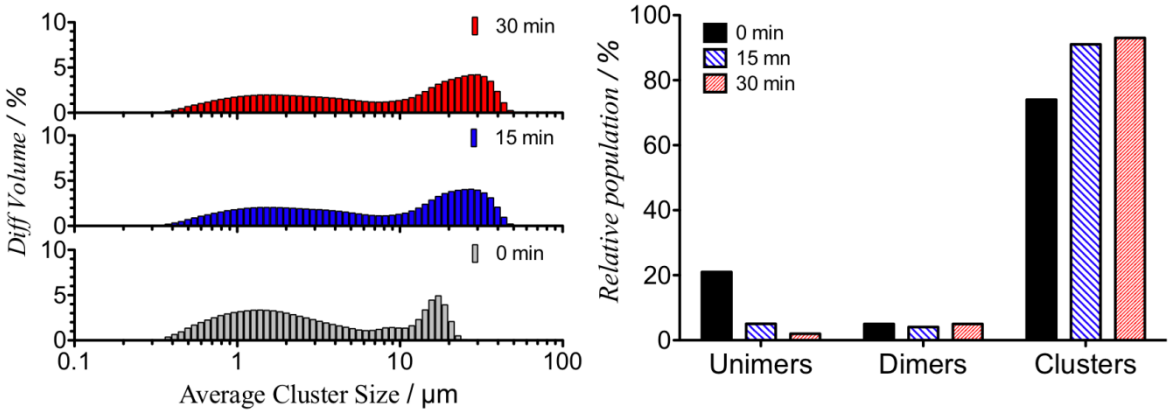


Figure S18: Evolution with time of the size distribution of *P. aeruginosa* PAO1 clusters in suspension in the absence (0 min) and presence of *E. coli* MG1665 **STPs** (1 mg·mL^{-1}) (left), and relative population of unimers, dimers and clusters at each time point (right).

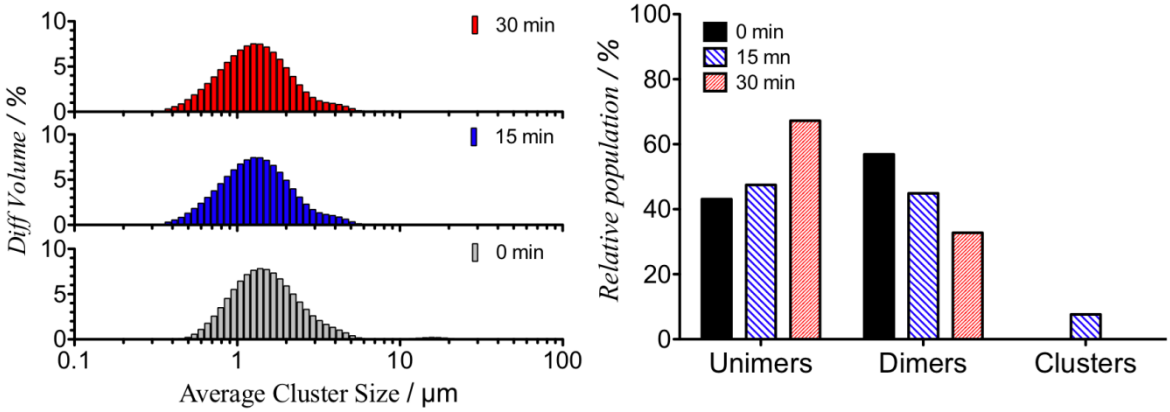


Figure S19: Evolution with time of the size distribution of *E. coli 536* clusters in suspension in the absence (0 min) and presence of **CPs** (1 mg·mL^{-1}) (left), and relative population of unimers, dimers and clusters at each time point (right).

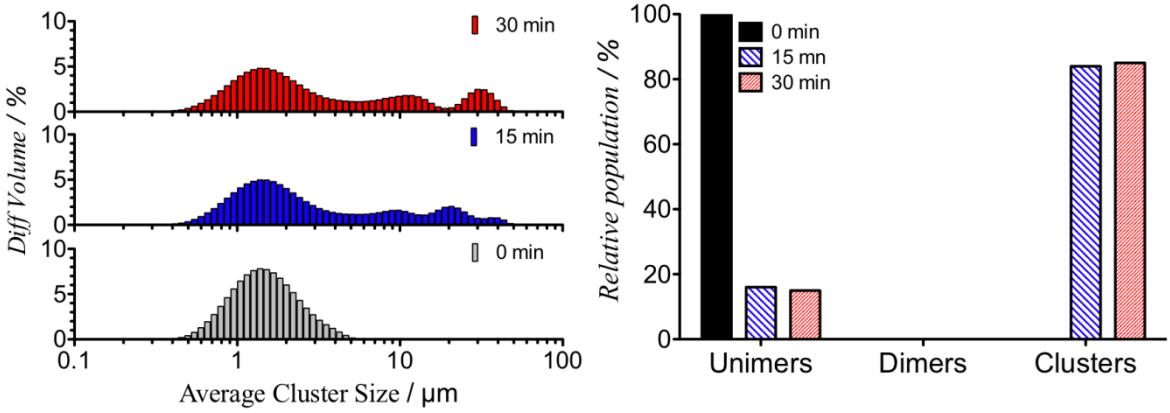


Figure S20: Evolution with time of the size distribution of *E. coli 536* clusters in suspension in the absence (0 min) and presence of *E. coli 536* **WTPs** (1 mg·mL^{-1}) (left), and relative population of unimers, dimers and clusters at each time point (right).

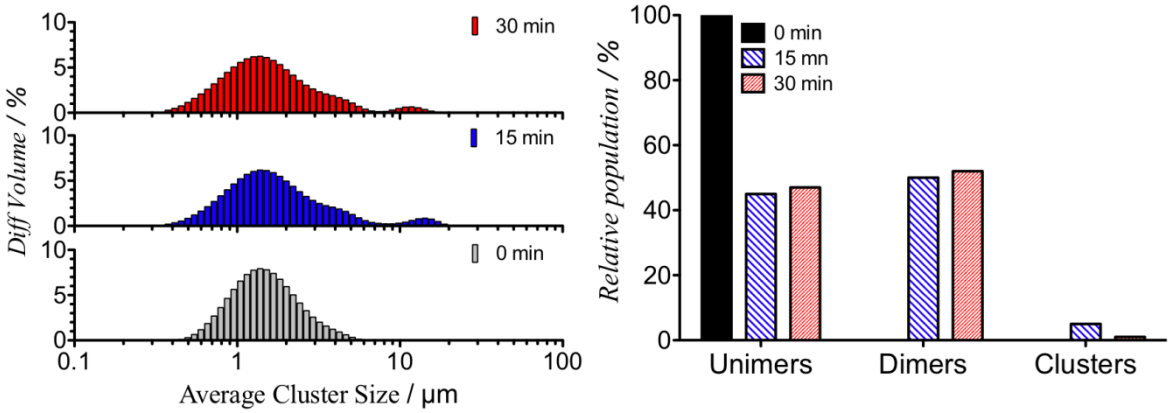
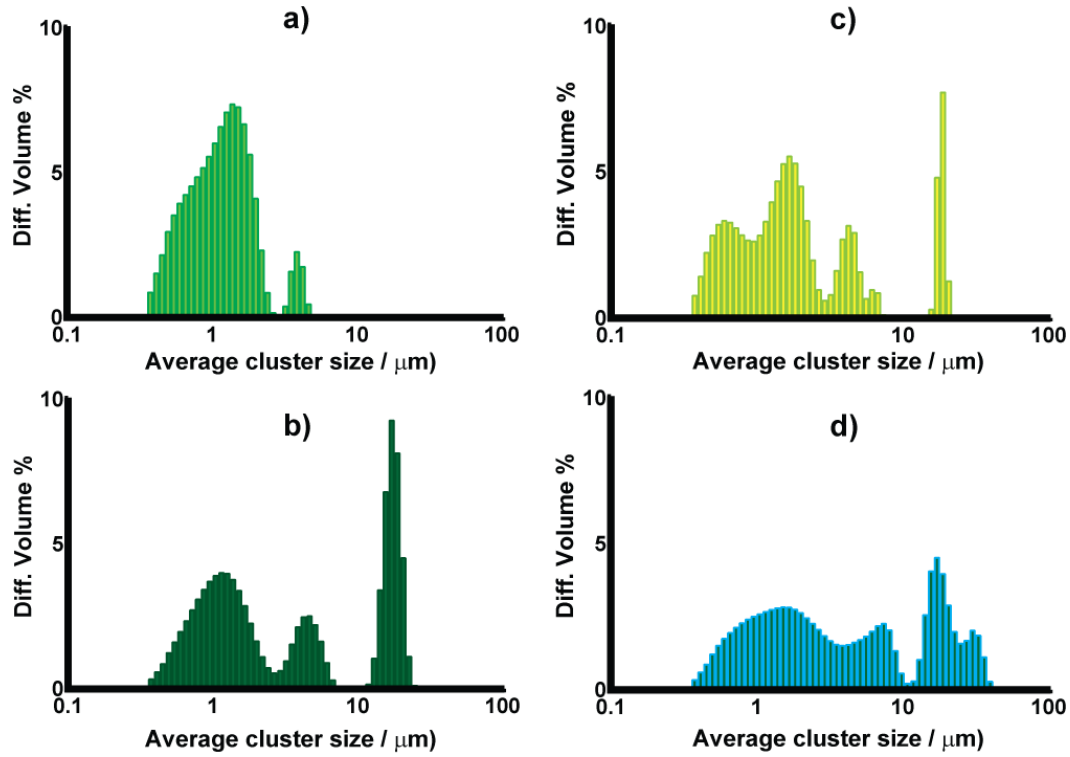


Figure S21: Evolution with time of the size distribution of *E. coli 536* clusters in suspension in the absence (0 min) and presence of *E. coli 536* **STPs** (1 mg·mL^{-1}) (left), and relative population of unimers, dimers and clusters at each time point (right).

Figure S22: Comparison of cell clustering of *P. aeruginosa* PA01 by (a) control polymer (DHPMA:MEDSA = 1:1.80) and b) *P. aeruginosa* PA01-templated polymer (DHPMA:MEDSA = 1:1.87) after 15 minutes incubation time. In (c) and (d) are shown the binding of *P. aeruginosa* PA01 by control polymer and *P. aeruginosa* PA01-templated polymer after 30 minutes.



Area under the curve (AUC) for *P. aeruginosa*-templated polymer is 5.4 fold greater compared to control polymer at 15 minutes, with 82 % of the AUC in the *P. aeruginosa*-templated polymer derived from the larger (> 10 µm) clusters (which are absent in the suspensions of *P. aeruginosa* and control polymer). Effect was retained over 30 minutes (after which polymer-cell clusters begin to precipitate) at which point the AUC of the *P. aeruginosa*-templated polymer-cell clusters was > 2-fold higher than the AUC for *P. aeruginosa* and control polymer.

- **Optical Microscopy**

Aliquots (10μL) of the samples used to measure average cluster size were collected after 60 min, mounted on a glass slide with a cover slip on top and examined with an optical microscope.

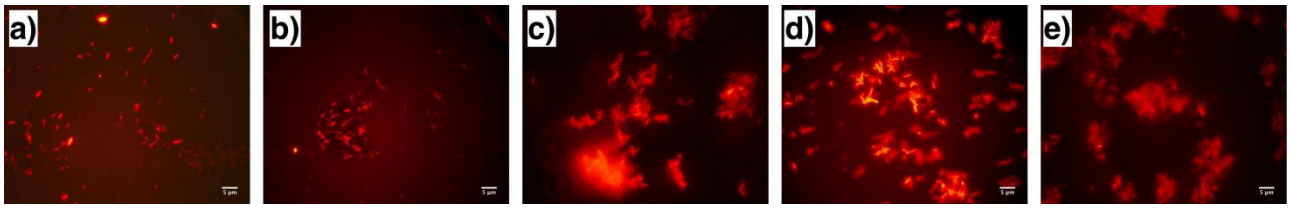


Figure S23: Representative examples of bacteria-polymer aggregates, as seen by optical microscopy, for *E. coli* MG1665 in the presence of **CP** (a), *E. coli* MG1665 **WTPs** (b) and **STPs** (c), *P. aeruginosa* PAO1 **WTPs** (d) and **STPs** (e).

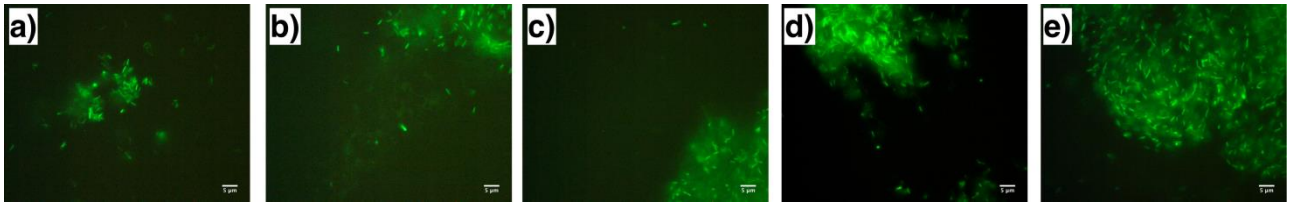


Figure S24: Representative examples of bacteria-polymer aggregates, as seen by optical microscopy, for *P. aeruginosa* PAO1 in the presence of **CP** (a), *E. coli* MG1665 **WTPs** (b) and **STPs** (c), *P. aeruginosa* PAO1 **WTPs** (d) and **STPs** (e).

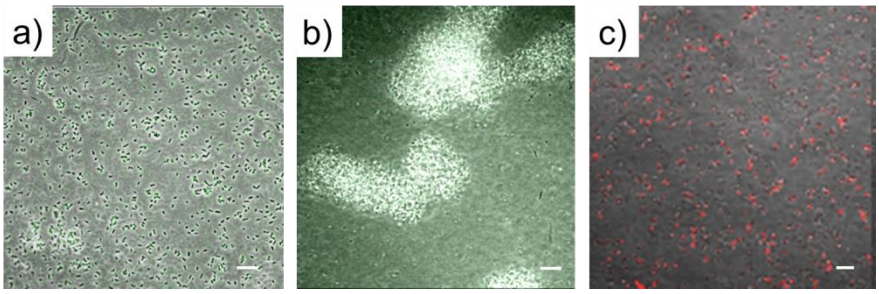


Figure S25: Representative examples of bacteria-polymer aggregates, as seen by combined phase and confocal microscopy, for *P. aeruginosa* PAO1 (a), *P. aeruginosa* PAO1 in the presence of *P. aeruginosa* PAO1 **STP** prepared from DHPMA and MEDSA monomers (b); and *E. coli* MG1665 in the presence of *P. aeruginosa* PAO1 **STP** (poly (DHPMA-co-MEDSA)) (c). Scale bar = 5 μm.

- **Predicted polymer properties**

  - Table S03: Predicted molar masses and chain lengths of control and templated polymers (NMR).

Polymer	M_n / kDa (NMR[a])	MEDSA: TMAEMA ratio	D.P.	Extended chain length / nm	Min End-to end distance / nm
Stat co-polymer	21.78	1:0.82	87	13.4	1.44
E. coli MG1655 WTP	21.4	1:0.27	79	12.2	1.37
E. coli MG1655 STP	18.2	1:1.06	79	12.2	1.37
P. aeruginosa PA01 WTP	29.7	1:0.35	112	17.2	1.63
P. aeruginosa PA01 STP	27.0	1:1.05	112	17.2	1.63

[a]- based on overall monomer conversion.

References

1. Xia, J. & Matyjaszewski, K. Controlled/'Living' Radical Polymerization. Atom Transfer Radical Polymerization Catalyzed by Copper(I) and Picolylamine Complexes. *Macromolecules* **32**, 2434–2437 (1999).

2. Hovestad, N. J., van Koten, G., Bon, S. A. F. & Haddleton, D. M. Copper(I) Bromide/ N-(n-Octyl)-2-pyridylmethanimine−Mediated Living-Radical Polymerization of Methyl Methacrylate Using Carbosilane Dendritic Initiators. *Macromolecules* **33**, 4048–4052 (2000).

3. Ladmiral, V. *et al.* Synthesis of Neoglycopolymers by a Combination of 'Click Chemistry' and Living Radical Polymerization. *J. Am. Chem. Soc.* **128**, 4823–4830 (2006).

4. Haddleton, D. M., Jasieczek, C. B., Hannon, M. J. & Shooter, A. J. Atom transfer radical polymerization of methyl methacrylate initiated by alkyl bromide and 2-pyridinecarbaldehyde imine copper(I) complexes. *Macromolecules* **30**, 2190–2193 (1997).

5. Compañ, V. *et al.* A potentiostatic study of oxygen transport through poly(2-ethoxyethyl methacrylate-co-2,3-dihydroxypropylmethacrylate) hydrogel membranes. *Biomaterials* **26**, 3783–3791 (2005).

6. Sivakumar, K. *et al.* A fluorogenic 1,3-dipolar cycloaddition reaction of 3-azidocoumarins and acetylenes. *Org. Lett.* **6**, 4603–4606 (2004).

7. Shaner, N. C. *et al.* Improved monomeric red, orange and yellow fluorescent proteins derived from Discosoma sp. red fluorescent protein. *Nat. Biotechnol.* **22**, 1567–1572 (2004).

8. Van Der Meer, R., Linssen, H. N. & German, A. L. Improved methods of estimating monomer reactivity ratios in copolymerization by considering experimental errors in both variables. *J. Polym. Sci. Polym. Chem. Ed.* **16**, 2915–2930 (1978).

9. Patino-Leal, H., Reilly, P. M. & O'Driscoll, K. F. On the estimation of reactivity ratios. *J. Polym. Sci. B Polym. Lett. Ed.* **18**, 219–227 (1980).

10. Dube, M., Sanayei, R. A., Penlidis, A., O'Driscoll, K. F. & Reilly, P. M. A microcomputer program for estimation of copolymerization reactivity ratios. *J. Polym. Sci., Part A: Polym. Chem.* **29**, 703–708 (1991).

11. Polic, A. L., Duever, T. A. & Penlidis, A. Case studies and literature review on the estimation of copolymerization reactivity ratios. *J. Polym. Sci., Part A: Polym. Chem.* **36**, 813–822 (2000).