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Supporting Information

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Antibacterial and Hemolytic Activities of Pyridinium Polymers as a Function of the Spatial Relationship Between the Positive Charge and the Pendant Alkyl Tail

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1. Materials and Instrumentation

4-Vinylpyridine and methyl methacrylate were purchased from Aldrich. Butyl methacrylate and hexyl methacrylate were purchased from Acros. Ethyl methacrylate and propyl methacrylate were purchased from Alfa Aesar. The monomers were purified by distillation under reduced pressure prior to use. 2,2'-Azobis-(isobutyronitrile) AIBN (Acros), iodomethane (Acros), 1-iodoethane (Aldrich), 1-iodopropane (Aldrich), 1-iodobutane (Aldrich) and 1-iodohexane (Aldrich), all 99%+ purity were used as received. HPLC grade methanol and chloroform (Aldrich) were used in free radical polymerization reactions. ACS grade nitromethane (Acros) was used in quaternization reactions. ACS grade ethyl ether (EMD chemicals) was used for polymer precipitation. Bacterial growth media and agar were purchased from Difco. 5-(Iodoacetamido) fluorescein was purchased from Sigma. A Perkin Elmer HTS 7000 plate reader was used for measuring bacterial Optical Density values. ^{1}H and ^{13}C NMR spectra were recorded on a Brucker DPX-400 or a CDPX-300 instrument. GPC were recorded on a Shimadzu instrument. Freshly drawn human red blood cells were purchased from Innovative Research, Southfield, MI (Cat # IPLA-WB3). An Olympus Fluoview 1000 confocal laser scanning microscope was used for confocal imaging.

2. General Procedure for Synthesis of Copolymers in Series A, B, C, P and Q

Representative general synthetic procedures of one polymer each from series **A**, **B**, **P** and **Q** are describe below.

2.1 Synthesis of Polymers in Series A: Specific Example A_4

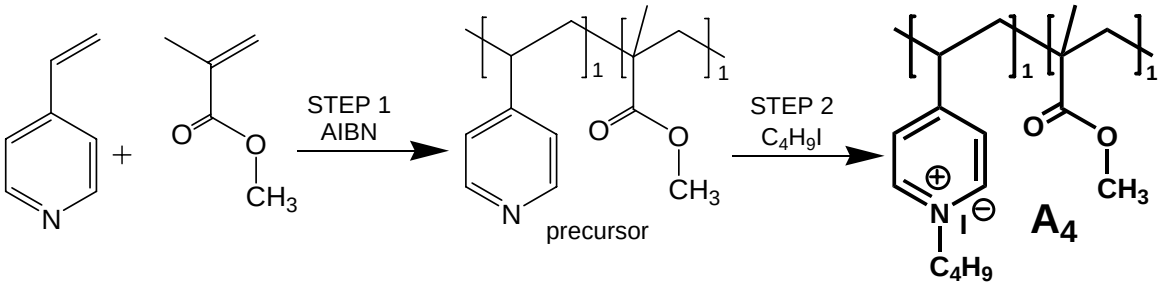

Figure S**1**: Schematics of synthesis of vinylpyridine/methylmethacrylate copolymer with butyl tail on same center as the positive charge, viz. polymer A_4 from series A.

Step 1: 4-Vinylpyridine (3.512 g) and methyl methacrylate (3.313 g) were added to a RB flask. AIBN (0.218 g; 50:1 molar monomer: initiator ratio) was dissolved in 10 mL anhydrous $CHCl_3$ and was added to the flask. The flask was immediately put under N_2 and was cooled in liq. N_2 bath. The contents of the flask were then deoxygenated by three freeze-pump-thaw cycles. The reaction mixture was then stirred under N_2 at 65°C in a preheated oil bath for 4 h. The contents were then added dropwise to a stirring solution of diethyl ether, which caused the polymer to precipitate. The precipitated polymer was washed with ether and was separated by suction filtration. The polymer was finally dried at RT under vacuum for 24 h.

Step 2: 0.329 g of the pure dry polymer from step 1 above was dissolved in 10 mL nitromethane. A slight excess of 1-iodobutane (0.4 g) was added and the contents were heated at 65^{o}C for 24h. The quarternized polymer was precipitated by dropwise addition to diethyl ether. The precipitated polymer was collected by centrifugation and was dried under vacuum for 24 h.

NMR Characterization of A_4:

Step 1: Radical Polymerization

^{1}H NMR plot with peak assignments is shown in Figure S**2** below. The –OCH_3 peaks c are split between 2.6 and 3.6 ppm based on the sequence distribution of VP/MMA units along the polymer chain. This effect has been previously described by other researchers, and is due to the screening effect of the aromatic nucleus.[**1**] Copolymer composition was established by peak integration between the pyridine protons a & b, and the backbone protons d. In the copolymer, let the fraction of VP units be x. Hence fraction of MMA units = 1-x. Integrating peak areas of aromatics/backbone (there are 4 H from VP aromatics / 3H from VP backbone and 5H from MMA backbone), (4x)/ 3x + 5(1-x) = (a+b)/d = 2/3.50. Solving for x, fraction of VP units= 0.55, and fraction of MMA units = 0.45. Hence backbone ratio *BR* = fraction of VP units/ fraction of MMA units = 0.55/0.45 = 1.22.

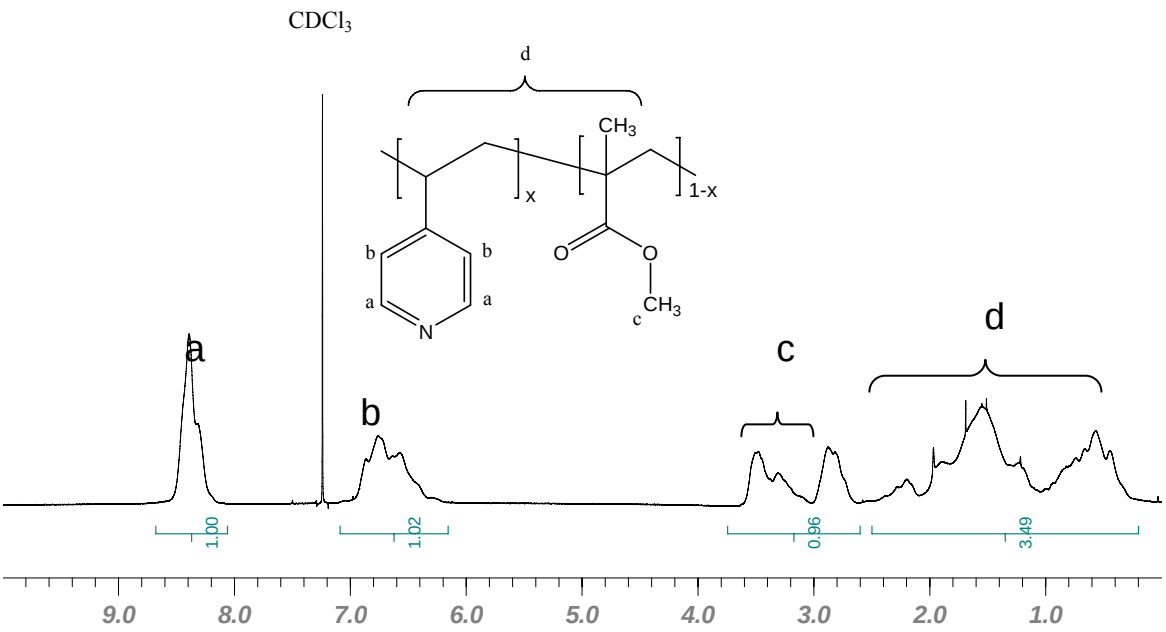

Figure S**2**: ^{1}H NMR (400 MHz, $CDCl_3$, ppm) of VP-MMA copolymer i.e. precursor to polymer A_4.

Step 2: N-Alkylation by 1-iodobutane

All the pyridine units of the precursor copolymer were N-alkylated with butyl groups by heating the precursor copolymer with 1-iodobutane. ^{1}H NMR plot with peak assignments is shown in Figure S**3** below. From the NMR it can be seen that all the pyridines have been N-alkylated viz. both pyridine peaks shift from 6.8 and 8.3 ppm to 8.1 and 9.1 ppm respectively. Since all the pyridines have been N-alkylated, amphiphilicity ratio *AR* = moles positive charge/moles of tail = 1. Hence polymer A_4 has *BR* of 1.2 and *AR* of 1.

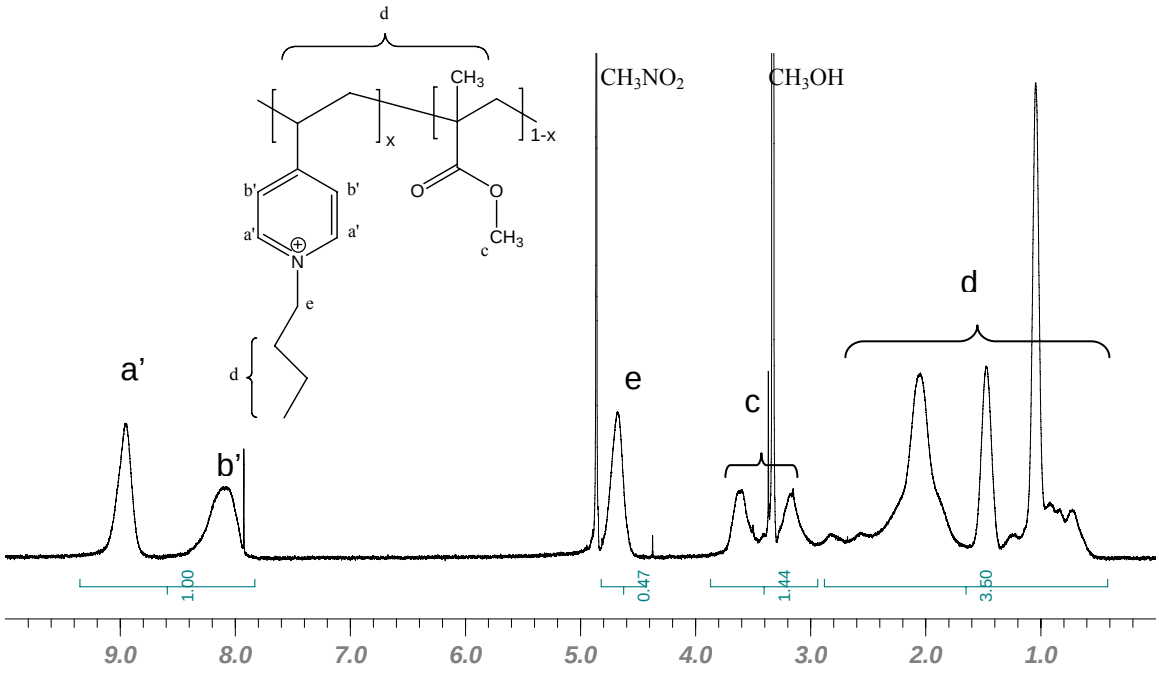

Figure S**3**: ^{1}H NMR (400 MHz, MeOH-d_4, ppm) of VP-MMA copolymer A_4.

^{13}C NMR (75 MHz, MeOH-d_4, ppm) of polymer A_4: 177.0, 163.5, 145.1, 128.5, 61.5, 48.1, 45.6, 40.3, 33.5, 19.6, 13.1.

2.2 Synthesis of Polymer from Series B: Specific Example B_4

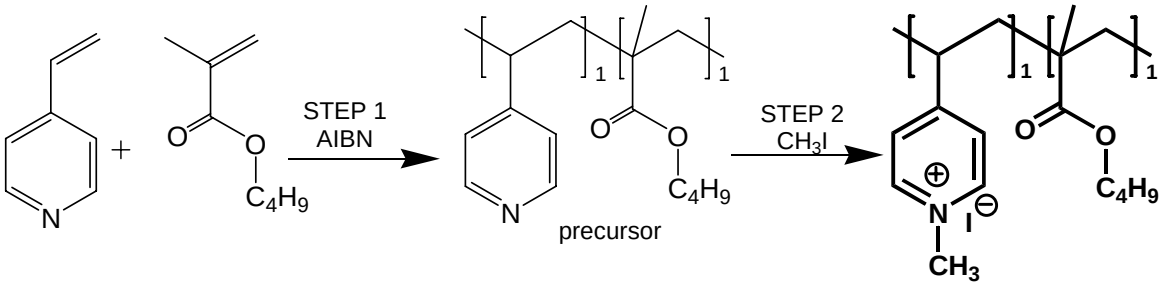

Figure S**4**: Schematics of synthesis of vinylpyridine/butylmethacrylate copolymer with butyl tail on separate center as the positive charge viz. polymer B_4 from series **B**.

Step 1: 4-Vinylpyridine (2.858 g) and butyl methacrylate (4.257 g) were added to a RB flask. AIBN (0.187g; 50:1 molar monomer: initiator ratio) was dissolved in 10 mL anhydrous $CHCl_3$ and was added to the flask. The flask was immediately put under N_2 and was cooled in liq. N_2 bath. The contents of the flask were then deoxygenated by three freeze-pump-thaw cycles. The reaction mixture was then stirred under N_2 at 65°C in a preheated oil bath for 4 h. The contents were then added dropwise to a stirring solution of diethyl ether, which caused the polymer to precipitate. The precipitated polymer was washed with ether and was separated by suction filtration. The polymer was finally dried at RT under vacuum for 24 h.

Step 2: 0.632 g of the pure, dry polymer from step 1 above was dissolved in 10 mL nitromethane. A slight excess of 1-iodomethane (0.7 g) was added and the contents were stirred at RT for 24h. The quarternized polymer was precipitated by dropwise addition to diethyl ether. The precipitated polymer was collected by centrifugation and was dried under vacuum for 24 h.

NMR Characterization of B_4:

Step 1: Radical Polymerization

^{1}H NMR spectrum with peak assignments is shown in Figure S**5** below. The –OCH_2- peaks c are split between 3 and 4 ppm based on the sequence distribution of VP/BMA units along the polymer chain. This effect has been previously described by other researchers, and is due to the screening effect of the aromatic nucleus.[**1**] In the copolymer, let the fraction of VP units be x. Hence fraction of BMA units = 1-x. Integrating peak areas of aromatics/backbone + butyl tail (there are 4 H from aromatic VP / 3H from VP backbone, 5H from BMA backbone and 7H from butyl tail), (4x)/ 3x +1 2(1-x) = (a+b)/d = 2/7.00. Solving for x, fraction of VP units = 0.52, fraction of BMA units = 0.48. Hence *BR* = fraction of VP units/ fraction of BMA units = 0.52/0.48 = 1.08.

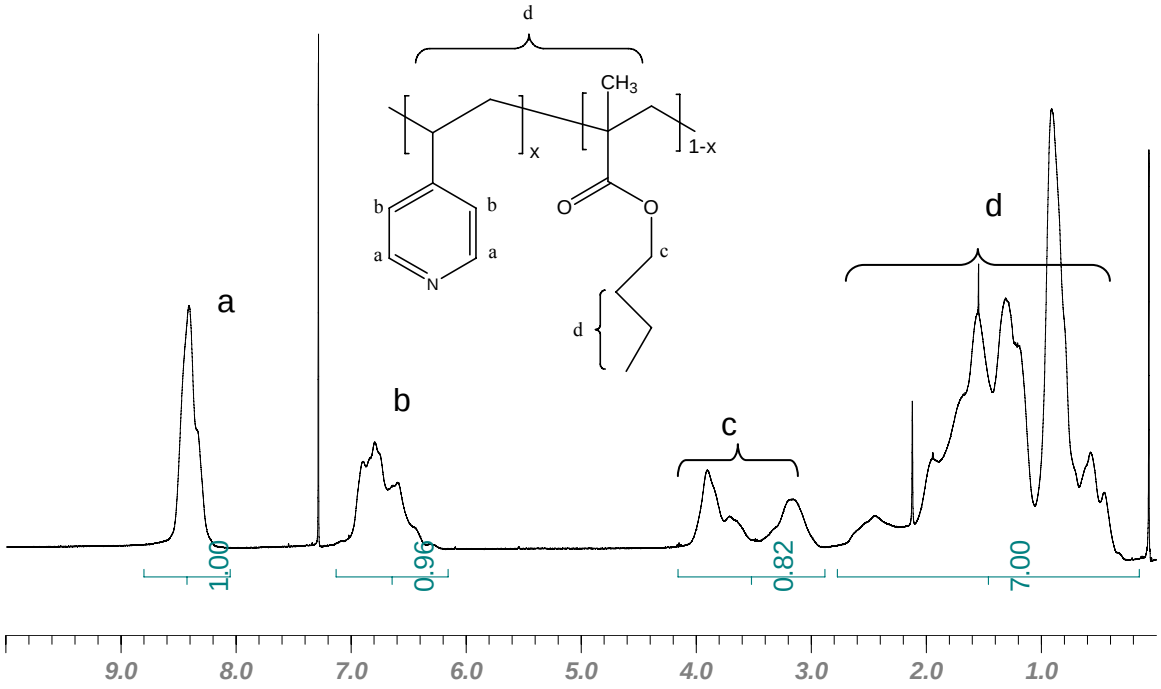

Figure S**5**: ^{1}H NMR (400 MHz, $CDCl_3$, ppm) of VP-BMA copolymer, i.e. precursor to polymer B_4.

Step 2: N-Alkylation by iodomethane

All the pyridine units of the precursor copolymer were N-methylated by stirring the precursor copolymer with iodomethane in methanol. ^{1}H NMR plot with peak assignments is shown in Figure S**6** below. From the NMR it can be seen that all the pyridines have been N-alkylated viz. both pyridine peaks shift upfield from 6.8 and 8.4 ppm to 8.1 and 9.0 ppm. Since all the pyridines have been N-alkylated, *AR* = moles positive charge/moles of tail (moles VP/moles BMA) = 1.08. Hence polymer B_4 has *BR* of 1.08 and *AR* of 1.08.

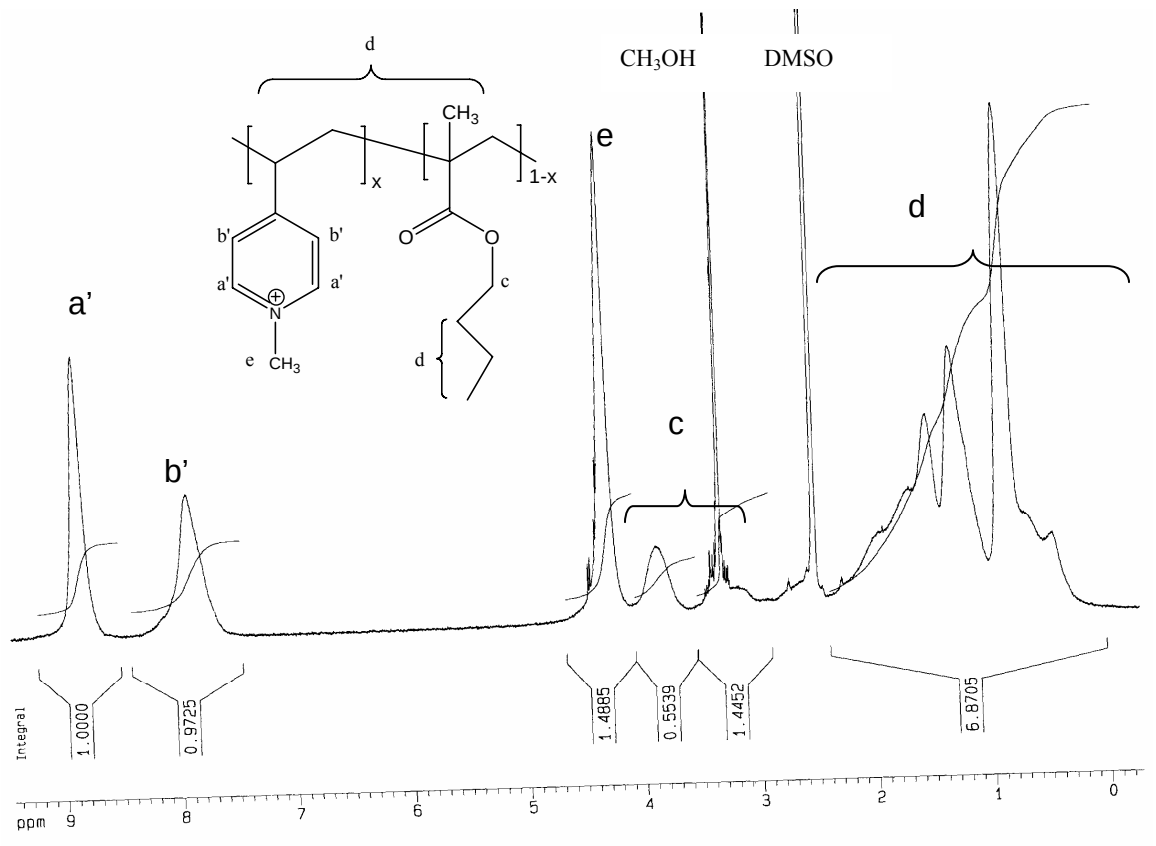

Figure S**6**: ^{1}H NMR (400 MHz, DMSO-d_6, ppm) of polymer B_4.

^{13}C NMR (75 MHz, MeOH-d_4, ppm) of polymer B_4: 177.2, 162.9, 145.9, 128.2, 65.4, 48.3, 44.9, 40.1, 30.5, 19.5, 13.5.

2.3 Synthesis of Polymer from Series C: Specific Example C_4

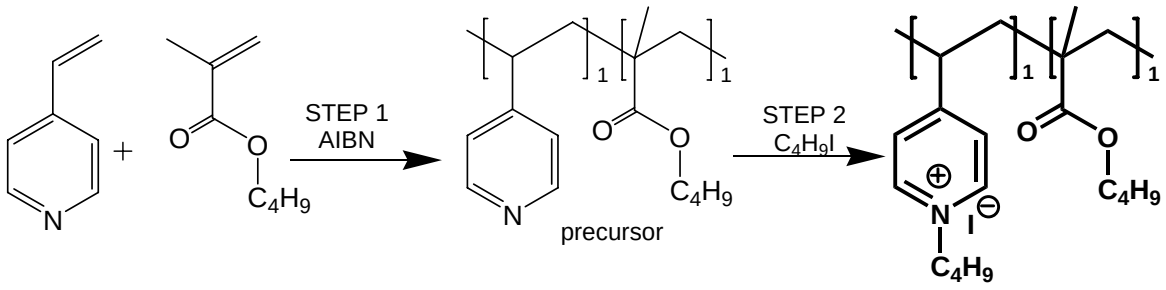

Figure S**7**: Schematics of synthesis of vinylpyridine/butylmethacrylate copolymer with butyl tail on both the positive center as well as a separate center viz. polymer **C_4** from series **C**.

Copolymer of 4-vinylpyridine and butyl methacrylate was prepared as described previously in section 2.2 of supporting information. Purified precursor was heated with excess of 1-iodobutane in nitromethane for 24h at 65^{o}C to N-alkylate all the pyridine nitrogen with butyl tails. Polymer purification and NMR characterization was done as described previously.

2.4 Synthesis of Hydrophilic Derivative of A_4

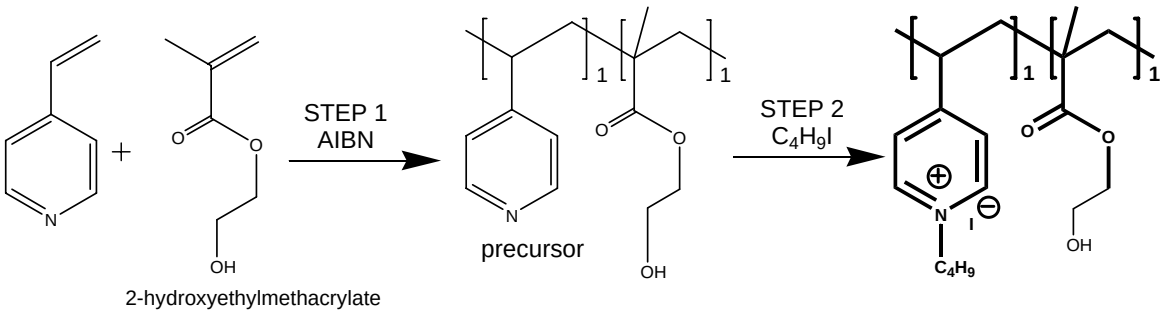

Figure S**8**: Schematics of synthesis of the hydrophilic derivative of polymer $\mathbf{A_4}$.

Step 1: 4-Vinylpyridine (3.512 g) and 2-hydroxyethyl methacrylate (HEMA) (4.310 g) were added to a RB flask. AIBN (0.21g; 50:1 molar monomer: initiator ratio) was dissolved in 20 mL anhydrous methanol and was added to the flask. The flask was immediately put under N_2 and was cooled in liq. N_2 bath. The contents of the flask were then deoxygenated by three freeze-pump-thaw cycles. The reaction mixture was then stirred under N_2 at 65°C in a preheated oil bath for 4 h. The contents were then added dropwise to a stirring solution of diethyl ether, which caused the polymer to precipitate. The precipitated polymer was washed with ether and was separated by suction filtration. The polymer was finally dried at RT under vacuum for 24 h. ^{1}H NMR plot with peak assignments is shown in Figure S**3** below. The copolymer composition BR = (moles of VP/moles of HEMA) = 1.2. Thus the copolymer composition was similar to that of precursor to polymer A_4.

Step 2: N-Alkylation by 1-iodobutane

All the pyridine units of the precursor copolymer were N-alkylated with butyl groups by heating the precursor copolymer with 1-iodobutane. ^{1}H NMR indicated that all the pyridines have been N-alkylated viz. both pyridine peaks shift from 7.0 and 8.3 ppm to 8.2 and 9.1 ppm, respectively. Since all the pyridines have been N-alkylated,

amphiphilicity ratio *AR* = moles positive charge/moles of tail = 1. Hence this derivative of polymer A_4 has *BR* of 1.2 and *AR* of 1.

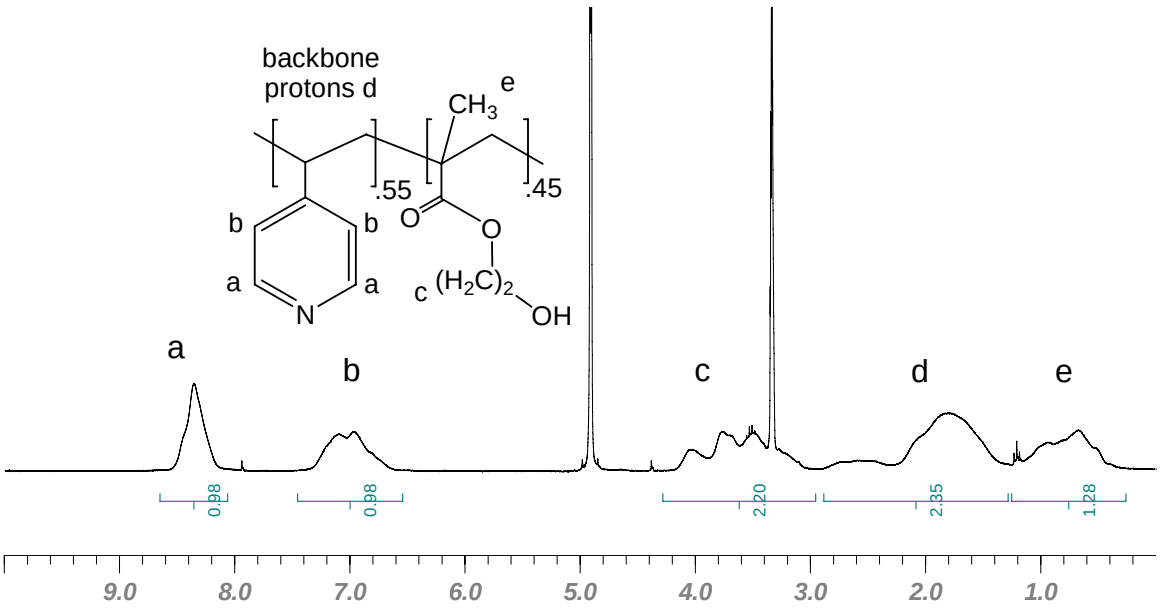

Figure S**9**: ^{1}H NMR (400 MHz, DMSO-d_6, ppm) of VP-HEMA copolymer.

All other polymers from series **A**, **B and C** were synthesized and characterized as describe above. Different methacrylate monomers viz. ethyl methacrylate, propyl methacrylate and hexyl methacrylate were copolymerized with required amounts of vinylpyridine to yield random polymer precursors. N-alkylation was performed with the respective iodoalkane to yield the final polymer. Copolymers were characterized using ^{1}H NMR peak integrations. The amphiphilicity ratios of the polymers from ^{1}H NMR peak integrations is given in (Table S**1**) below.

Table S**1**: AR values of polymers from series **A** and **B** as determined by ^{1}H NMR peak integrations. Subscripts denote the length of the alkyl tail.

Series A	**AR_a**	**Series B**	**AR_b**
A_2	1.00	**B_2**	1.15
A_3	1.00	**B_3**	1.12
A_4	1.00	**B_4**	1.08
A_6	1.00	**B_6**	1.13
A_8	1.00	**B_8**	1.03
A_{10}	1.00	**B_{10}**	1.03

2.5 Synthesis of Polymers from Series P: Specific Example $P_{2.3}$

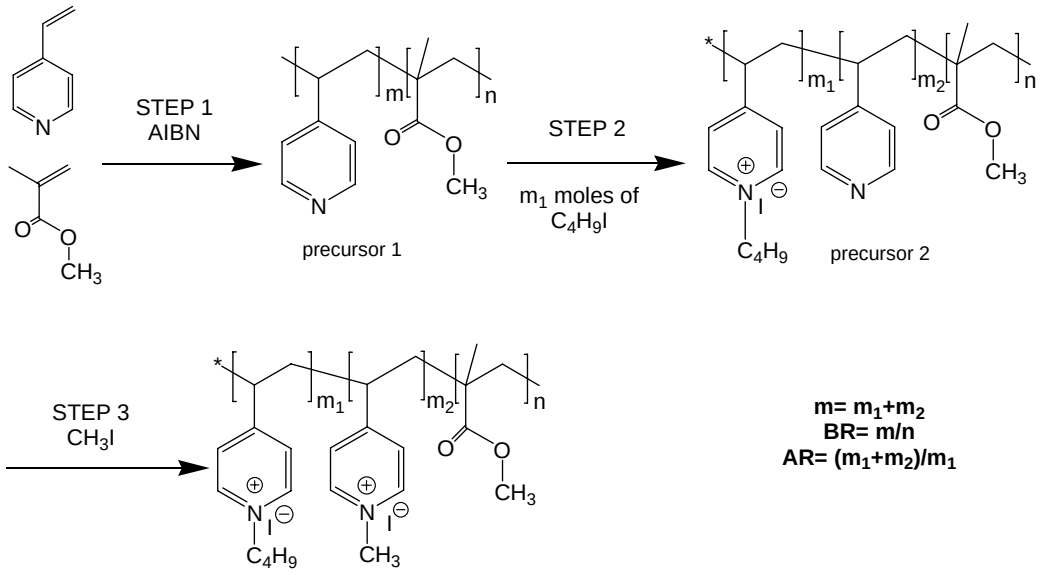

Figure S**10**: Schematics of synthesis of different vinylpyridine/methylmethacrylate copolymers with butyl tail on same center as the positive charge viz. polymer $P_{2.3}$ from series P having an AR (moles charge/moles tail) of 2.3.

Step 1: 4-Vinylpyridine (3.133 g) and methyl methacrylate (1.657 g) were added to a RB flask. AIBN (0.152g; 50:1 molar monomer: initiator ratio) was dissolved in 5 mL anhydrous methanol and 10 mL anhydrous $CHCl_3$, and was added to the flask. The flask was immediately put under N_2 and was cooled in liq N_2 bath. The contents of the flask were then deoxygenated by three freeze-pump-thaw cycles. The reaction mixture was stirred under N_2 at 65°C in a preheated oil bath for 4 h. The contents were then added dropwise to a stirring solution of ethyl ether, which caused the polymer to precipitate. The precipitated polymer was washed with ether and was separated by suction filtration. The polymer was finally dried at RT under vacuum for 24 h.

Step 2: 0.5347 g of the purified polymer from step 1 was dissolved in 10 mL nitromethane. It was then heated with 0.291 g of iodobutane at 65 ^oC for 24 h to yield the desired intermediate polymer having partially N- alkylated pyridine groups.

Step 3: Excess iodomethane was added to the above solution, and the solution was stirred at RT for 24 h to yield the final polymer. The polymer was precipitated in diethyl ether and was collected by centrifugation. It was then dried under vacuum for 24 h.

NMR Characterization of $P_{2.3}$:

Step 1: Radical Polymerization

^1H NMR plot with peak assignments is shown in Figure S**8** below. In the copolymer, let the fraction of VP units be x. Hence fraction of MMA units = 1-x. Integrating peak area of aromatics/backbone (there are 4 H from aromatics and 3H from VP backbone and 5H from MMA backbone), (4x)/ 3x + 5(1-x) = (a+b)/d = 2/2.67. Solving for x, fraction of VP units = 0.69, fraction of MMA units= 0.31. Hence *BR* = fraction of VP units/fraction of MMA units = 0.69/0.31 = 2.22

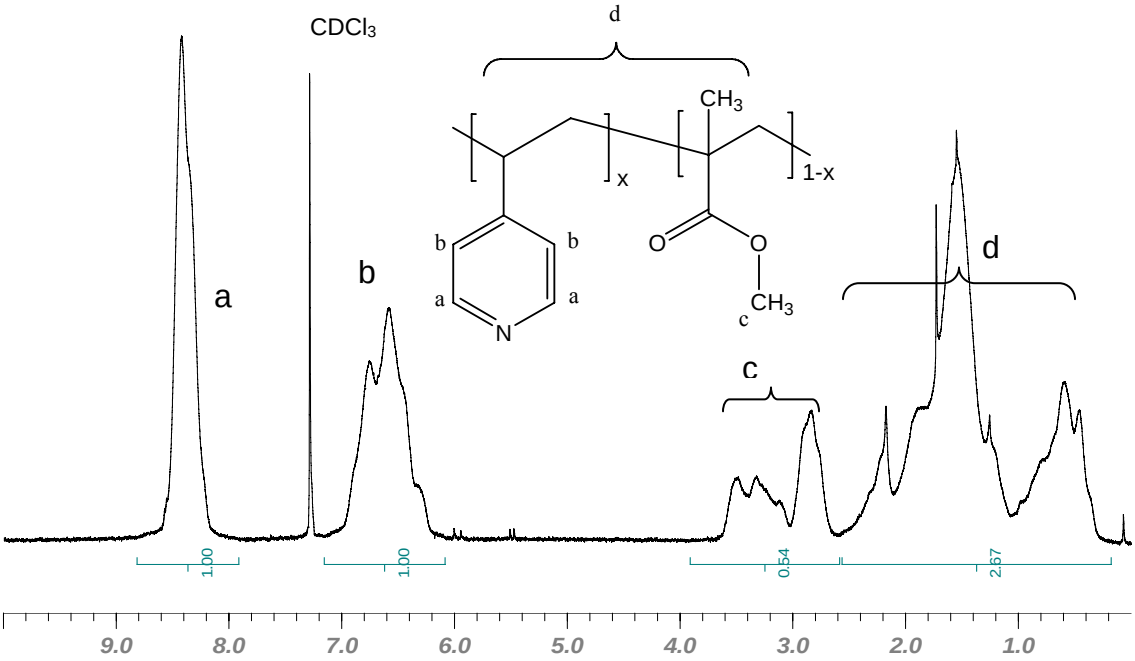

Figure S**11**: ^{1}H NMR (400 MHz, $CDCl_3$, ppm) of VP-MMA copolymer i.e. precursor 1 to polymer $P_{2.3}$.

Step 2: Partial N-alkylation with iodobutane

Purified precursor 1 was treated with calculated amount of iodobutane to yield the precursor 2 in which only some pyridines were N-alkylated by butyl tails. ^{1}H NMR plot with peak assignments is shown in Figure S**9** below. From the previous ^{1}H NMR, (Figure S**8**) we know that the total fraction of pyridine units in the copolymer is 0.69. The two pyridine peaks, a and b from step 1, split into 4 peaks a, a', b and b' depending on the charge on nitrogen. This was used to calculate the fraction of alkylated and free pyridines. From the NMR for the free pyridines, b = 2H = 1. Hence each pyridine proton = 0.5. From the N-alkylated pyridine, b' = 2H = 0.78. Hence each pyridinium proton = 0.39. Hence dividing pyridinium by pyridine, x/0.69-x = 0.39/0.5. Solving for x, pyridiniums with butyl tails = 0.30, and non-alkylated pyridines = 0.39.

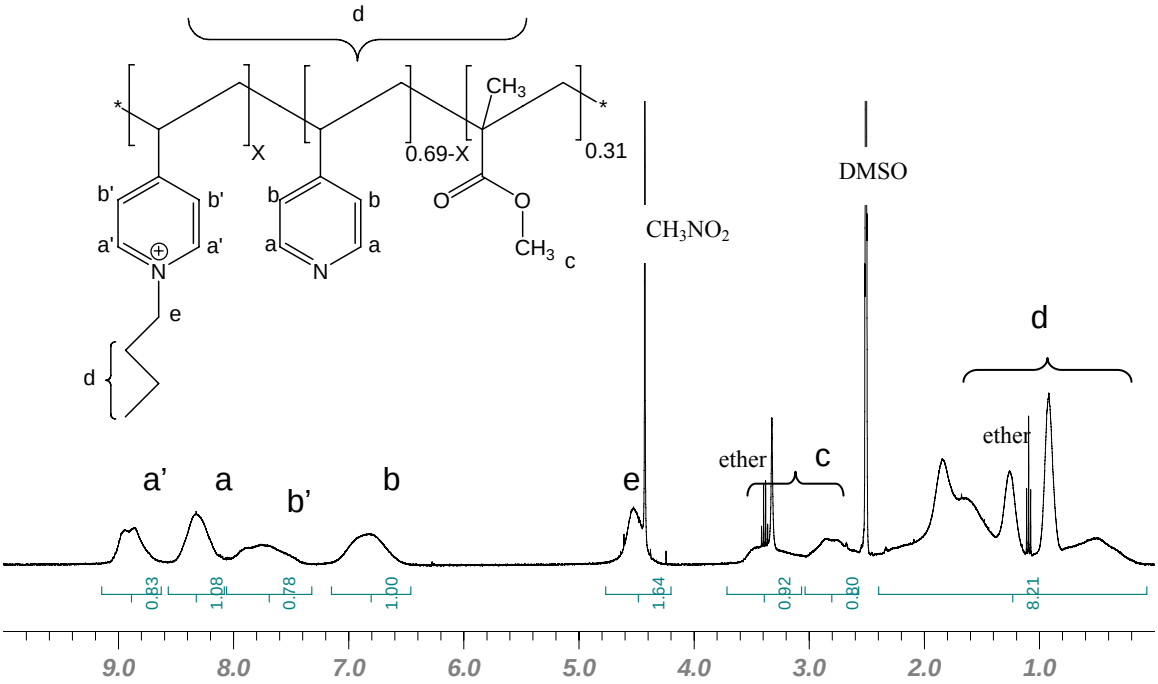

Figure S**12**: ^{1}H NMR (400 MHz, DMSO-d_6, ppm) of VP-MMA copolymer i.e. precursor 2 to polymer $P_{2.3}$.

Step3: Complete N- alkylation with iodomethane

Excess iodomethane was added to precursor 2 to completely N-methylate all the remaining free pyridines. As can be seen from the ^{1}H NMR in Figure S**10** below, all the non alkylated pyridines have been methylated i.e. non-alkylated pyridine peaks a and b disappear. Hence *AR* = total moles of positive charge/moles of tail = 0.69/0.30 = 2.3.

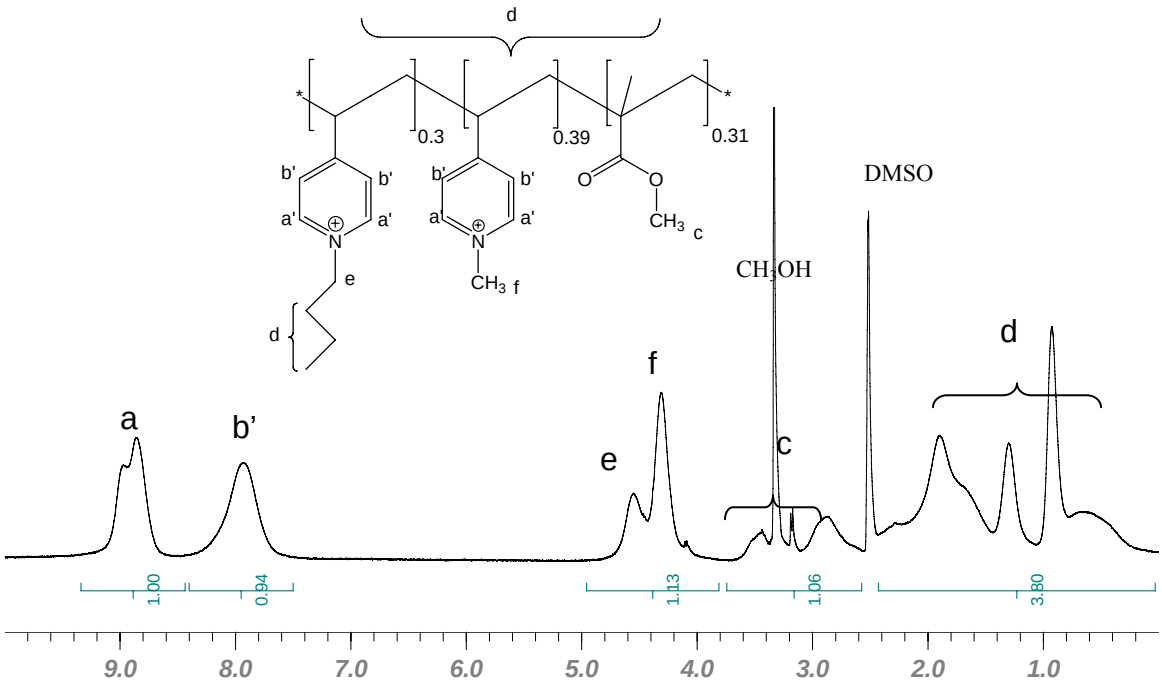

Figure S**13**: ^{1}H NMR (400 MHz, DMSO-d_6, ppm) of VP-MMA copolymer $P_{2.3}$.

^{13}C NMR (75 MHz, DMSO-d_6, ppm) of polymer $P_{2.3}$: 176.5, 164.3, 143.9, 127.8, 62.4, 48.7, 44.3, 40.7, 32.1, 18.9, 12.5.

2.6 Synthesis of Polymers from Series Q: Specific Example $Q_{2.2}$

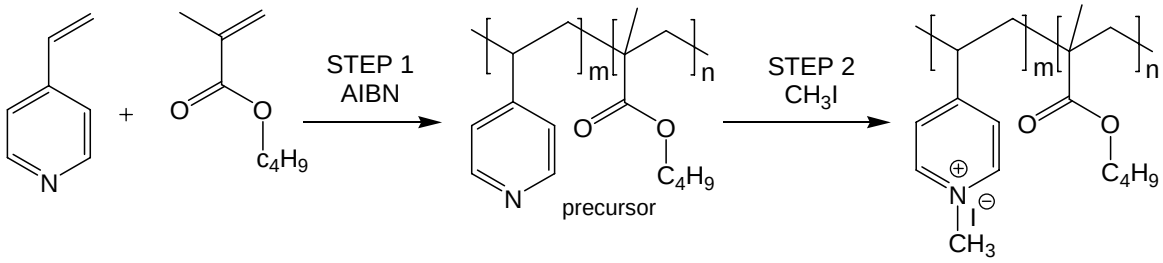

Figure S**14**: Schematics of synthesis of different vinylpyridine/butylmethacrylate copolymers with butyl tail on separate center from the positive charge viz. polymer $Q_{2.23}$ from series Q having an AR (moles charge/moles tail) of 2.2.

Different copolymers of 4-vinylpyridine (VP) and n-butylmethacrylate (BMA) were synthesized as described in section 2.2. Starting feed ratios of VP and BMA were tailored to get copolymers with increasing amounts of VP units. All the VP units were methylated with excess of iodomethane to get the final polymers. Polymers were characterized by ^{1}H and ^{13}C NMR as described previously.

All other polymers from series **P** and **Q** were synthesized and characterized as described above. Different feed ratios of methacrylate monomers with required amounts of vinylpyridine gave polymers with different *BR* and *AR*. N-alkylation was performed with the respective iodoalkane to yield the final polymer. Copolymers were characterized using ^{1}H NMR peak integrations. The amphiphilicity ratios of the polymers from ^{1}H NMR peak integrations is given in (Table S**2**) below.

Table S**2**: *BR* and *AR* values of polymers from series **P** and **Q** calculated using ^{1}H NMR peak integration. Subscripts denote the *AR* value.

Series P	BR	AR	Series Q	BR	AR
			$\mathbf{Q_{0.5}}$	0.49	0.49
$\mathbf{P_{1.0}}$	1	1.00	$\mathbf{Q_{1.1}}$	1.08	1.08
$\mathbf{P_{2.3}}$	2.22	2.30	$\mathbf{Q_{2.2}}$	2.23	2.23
$\mathbf{P_{3.1}}$	3.56	3.12	$\mathbf{Q_{3.4}}$	3.44	3.44
$\mathbf{P_{4.1}}$	4.24	4.13	$\mathbf{Q_{4.3}}$	4.28	4.28

3. Molecular Weight Determination

Molecular weights and polydispersities were determined on a Shimadzu gel permeation chromatograph (GPC) containing a Waters *Styragel* HR4E linear mixed bed column (7.8mm I.D. x 300 mm – Part No. WAT044240). The detectors were a Shimadzu RDI-10A differential refractometer and a Shimadzu SPD-10A tunable UV-Vis absorbance detector (254 nm). GPC samples were run in tetrahydrofuran having a 50 mM concentration of tetrabutylammonium bromide as eluent (flow rate of 1mL/min). Analysis was done using EZSTART 7.2 software. Three narrow molecular weight polystyrene standards obtained from Polymer Laboratories (EasiVial PS Standards – part no. 2010-0201) were used for calibration of the GPC. The range of calibration range was from 162 to 3,053,000 g/mol. The molecular weights reported are relative to these standards.

The final cationic polymers were not soluble in THF, so we obtained the molecular weights of the precursor copolymers. Molecular weight of the precursor polymers from series **A**, **B**, **P** and **Q** are reported in Table S**3** below. Single peaks without shoulders were observed on both UV-Vis and RID detectors for all polymers, thereby indicating that copolymers were present, rather than a mixture of two homopolymers.

Table S**3**: Molecular weights of various copolymers from Series A, B, P and Q from Gel Permeation Chromatography.

***Precursor* to Polymer-**	**Precursor Number Average M_n (g/mol)**	**Polydispersity Index**
A_2,A_3,A_4,A_6	27,698	1.3
B_2	28,444	1.3
B_3	29,577	1.3
B_4	33,156	1.3
B_6	27,7771	1.4
A_4 -higher MW	42,085	1.4
B_4 -higher MW	45,621	1.5
$P_{0.5}$	39,000	1.4
P_1	27,698	1.3
$P_{2.3}$	38,863	1.3
$P_{3.1}$	43,765	1.3
$Q_{0.5}$	39,000	1.4
$Q_{1.1}$	33,156	1.3
$Q_{2.2}$	45,520	1.4
$Q_{3.4}$	55,947	1.4

The molecular weights of corresponding precursor polymers from series **A** and **P** (same center) were lower than those of series **B** and **Q** (separate center). This was to be expected since these precursor polymers do not have the alkyl tail (ethyl, propyl, butyl and hexyl) added on them. However, the final polymers from series **A** and **P** would have the tail added by N-alkylation, and we expect their molecular weights to rise, becoming approximately equal to those from series **B** and **Q** respectively.

4. Antibacterial Testing

Escherichia coli DH5-α (Clontech) were grown at 37 °C and maintained on LB plates (Luria-Bertani broth, Lennox modification, with 1.5% agar). The relation between absorbance at 590 nm (OD_{590}) and colony forming units (CFU) per mL was determined using the plate count method as described by Herigstad et. al.[**2**] This allowed the standardization of assay inoculums by measurements of OD_{590} on a Perkin Elmer HTS 7000 plate reader. Bacteria were cultured for 16-18 h in LB broth (Luria-Bertani broth, Lennox modification), and cell counts were quantified by OD_{590} measurement. The cultures were then diluted to approximately 5×10^5 CFU/ml in LB broth. A standard testing protocol as described elsewhere was used for finding the Minimum Inhibitory Concentration (MIC) values of the polymers. [**3**]

All antibacterial tests were done in triplicate, and were also repeated on at least two different days. Polymers from series being compared (**A** vs. **B**, and **P** vs. **Q**) were tested on same days, and with the same bacterial solutions to rule out any inconsistencies in bacterial concentrations. All polymers being tested were purified by dialysis using a Spectrum cellulose dialysis membrane with a molecular weight cutoff of 3000. Dialysis was performed for 24 h in methanol to remove small molecule impurities that may have formed during the synthetic procedure. The polymers were then precipitated in ether and dried under vacuum for 1 d. They were then freeze-dried for an additional day. Polymer stock solutions were prepared in 80% methanol-20% water to a concentration of 1mg/300μL.

Calculated volumes (30-250 μL) of polymer solutions were then pipetted into sterile 15 mL Falcon tubes. Methanol was evaporated to yield the solid polymer. *E. coli* suspension (5×10^5 CFU/ml) in LB broth was then added to each tube to yield tubes containing various polymers at concentrations of 10-1500 μg/mL (MIC's of 10-1500). Negative control tubes contained only inoculated broth. The tubes were incubated at 37 °C with shaking at 250 rpm for 18-20 h. The visual turbidity of the tubes was noted both before and after incubation. Turbidity after incubation indicated bacterial growth. Aliquots from tubes (100 μl), including those that showed no turbidity (little or no cell

growth) were plated on LB agar plates to distinguish between bacteriostatic or bactericidal effects. These plates were incubated at 37 °C for 16-20 h and then colonies counted. If a particular polymer killed the *E. coli* cells at the tested concentration, no/few colonies were observed particular on the agar plate.

Bacterial growth was also studied by the optical density (OD_{590} method. Different polymer stock solutions were added to a 96 well plate to set up panel of polymer MIC concentrations (MIC = 10-1500 µg/mL). The solvent was evaporated overnight under sterile conditions to yield solid polymers at the bottom of the wells. 200 µL of LB broth containing *E. coli* (5×10^5 CFU/ml) was then added to each well, and the plate was incubated at 37 °C for 16-20 h. Bacterial growth was detected by measuring the optical density OD_{590} of the solutions, and comparing OD_{590} to controls consisting of LB broth and LB-*E.coli*. The results from both the plating method and OD_{590} method were identical. The MIC values for the different polymers is reported below. (Table S**4** - S**6**)

Antibacterial assays against gram positive *Bacillus. subtilis* (wt strain PLBS338) **[7]** were carried out as described for *E. coli*, with the exception that *B. subtilis* was grown and maintained at 30°C.

Table S**4**: Antibacterial activities of polymers from series **A**, **B** and **C** towards gram negative *E. coli*.

Series A	MIC (µg/mL)	Series B	MIC (µg/mL)	Series C	MIC (µg/mL)
$\mathbf{A_2}$	600	$\mathbf{B_2}$	350	$\mathbf{C_2}$	350
$\mathbf{A_3}$	200	$\mathbf{B_3}$	100	$\mathbf{C_3}$	175
$\mathbf{A_4}$	50	$\mathbf{B_4}$	15	$\mathbf{C_4}$	175
$\mathbf{A_6}$	100	$\mathbf{B_6}$	50	$\mathbf{C_6}$	300
$\mathbf{A_8}$	450	$\mathbf{B_8}$	125		
$\mathbf{A_{10}}$	1100	$\mathbf{B_{10}}$	650		

Table S**5**: Antibacterial activities of polymers from series **A**, **B** and **C** towards gram positive *B. subtilis*.

Series A	MIC (µg/mL)	Series B	MIC (µg/mL)	Series C	MIC (µg/mL)
$\mathbf{A_2}$	300	$\mathbf{B_2}$	300	$\mathbf{C_2}$	350
$\mathbf{A_3}$	100	$\mathbf{B_3}$	50	$\mathbf{C_3}$	25
$\mathbf{A_4}$	30	$\mathbf{B_4}$	15	$\mathbf{C_4}$	25
$\mathbf{A_6}$	25	$\mathbf{B_6}$	10	$\mathbf{C_6}$	100
$\mathbf{A_8}$	150	$\mathbf{B_8}$	40		
$\mathbf{A_{10}}$	300	$\mathbf{B_{10}}$	100		

Table S**6**: Antibacterial activities of polymers with increasing *AR* values from series **P** and **Q** towards gram negative *E. coli* and gram positive *B. subtilis*.

Series P	MIC (µg/mL) *E. coli*	MIC (µg/mL) *B. subtilis*	Series Q	MIC (µg/mL) *E. coli*	MIC (µg/mL) *B. subtilis*
			$\mathbf{Q_{0.5}}$	200	50
$\mathbf{P_{1.0}}$	50	30	$\mathbf{Q_{1.1}}$	15	15
$\mathbf{P_{2.3}}$	300	150	$\mathbf{Q_{2.2}}$	200	100
$\mathbf{P_{3.1}}$	400	250	$\mathbf{Q_{3.4}}$	400	250
$\mathbf{P_{4.1}}$	900	600	$\mathbf{Q_{4.3}}$	800	500

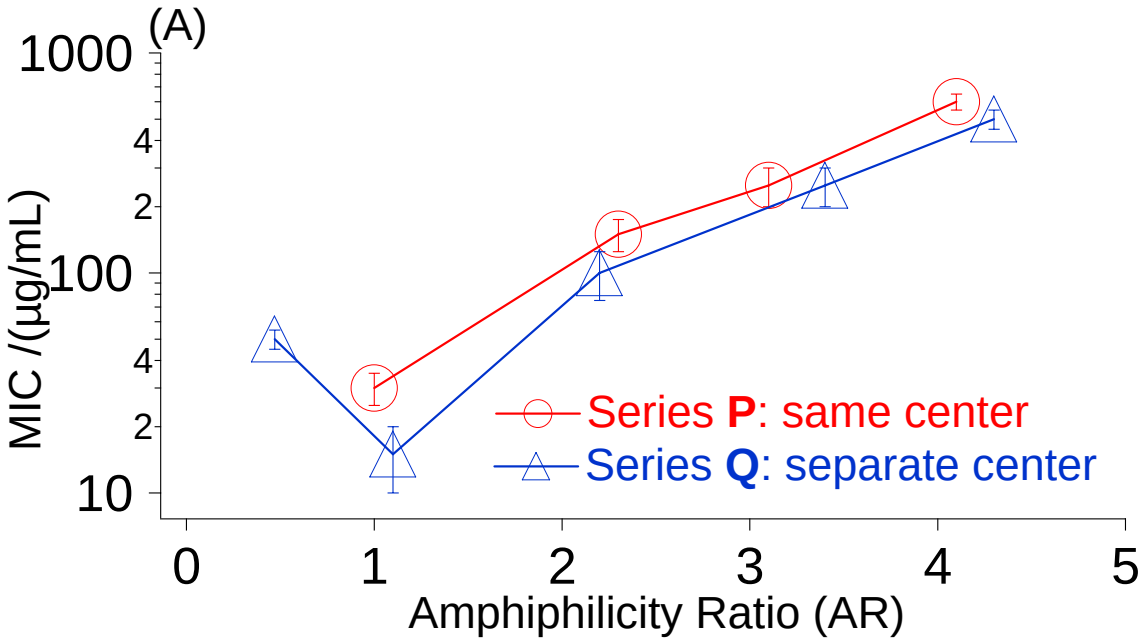

Figure S**15**: Plots of antibacterial activity of polymers from series P and Q towards gram positive *B. subtilis.*

Note that the error bars for the antibacterial activity values shown in Figures 1 and 2 in the paper represent the least count of the antibacterial assay measurements. MIC assays are carried out by testing serially diluted discreet incremental polymer concentrations e.g. polymer $\mathbf{B_4}$ (having MIC of 15 µg/mL) was tested at 5, 10, 15, 20…µg/mL concentration (least count of 5 µg/mL). The error bar for this polymer represents that it killed >99% of the bacteria at a concentration of 15±5 µg/mL. We feel this least count error reporting is more valid than reporting standard deviations. The standard deviations obtained were always smaller than the least count of the measurements, and hence we have reported the larger source of uncertainty in measurements.

5. Hemolytic Testing

Hemolytic assays were performed with a slight modification of procedures described elsewhere. [**4, 5, 6**]. Freshly drawn human red blood cells were purchased from Innovative Research, Southfield, MI (Cat # IPLA-WB3). The anticoagulant was citrate dextrose. RBC's were filtered through Pall 0.2 micron Acrodisc filter. 60µL of these filtered RBC's were washed three times with 10 mL of TRIS buffer saline (TBS) (10mM TRIS, 150 mM NaCl, pH 7.2) by centrifuging at 1500 rpm for 5 minutes and removing the supernatant. The RBC's were finally suspended in 30 mL TBS to yield RBC stock solution of 0.2 % v/v.

Polymer solutions of different concentrations (25 mg/mL to 1 µg/mL) were prepared in mixture of TBS/DMSO = 1/1, and were filtered through 0.2 micron filter. 25 µL of each polymer solution (different polymers at different concentrations) were pipetted into wells of a 96 well plate. 175 µL of RBC stock in TBS was then pipetted into each well containing the polymer solution. The final polymer concentrations in 200 µL volume in the wells were 3000 to 0.1 µg/mL. The plate was incubated in a shaker at 37^{o}C for 1 h. The plate was then centrifuged at 3900 rpm for 10 min to settle the unruptured RBC's and 100 µL of supernatant from each well was pipetted out into a new 96 well plate. Hemolysis was monitored by measuring the absorbance of the released hemoglobin in the supernatant at 420 nm in a Perkin Elmer HTS 7000 plate reader. Since the polymer had a comparatively small absorbance at 420 nm (typical polymer OD of 0.050 vs. polymer + hemoglobin OD of 0.450), to get accurate absorbance of hemoglobin released due to cell lysis, the absorbance of the polymer (well containing 25 µL polymer + 175 µL TBS) was subtracted from absorbance of the hemoglobin + polymer (well containing 25 µL polymer + 175 µL RBC).

100% hemolysis (positive control) was obtained by adding 25 µL of 1 % TRITON-X surfactant solution in TBS/DMSO to 175 µL of RBC stock solution to achieve complete lysis of the blood cells. Negative control (0% hemolysis) was obtained by adding 25 µL of TBS/DMSO to175 µL of RBC stock solution. Percentage of hemolysis was calculated using the following equation:

% hemolysis = [OD_{420}(polymer) – OD_{420}(negative control)/[OD_{420}(TRITON-X)-OD_{420}(negative control)] x 100

The percentage of hemolysis was plotted against polymer concentration, and the concentration of polymer required to cause 50% hemolysis (HC_{50}) was estimated by curve fitting. The HC_{50} values reported are averages of at least 2 different experiments with each set being done in triplicate. The average HC_{50} values are given in Table S7 and Table S8 below.

Table S7: Hemolytic activities (average HC_{50} values with standard deviations) of polymers with different tail lengths from series **A**, **B and C** towards human red blood cells.

	HC_{50} (µg/mL)	**s.d.**		**HC_{50} (µg/mL)**	**s.d.**		**HC_{50} (µg/mL)**	**s.d.**
A_2	2393	396.55	**B_2**	1147	192.54	**X_2**	868	167
A_3	1897	399.28	**B_3**	108	24.02	**X_3**	1.47	0.65
A_4	1709	276.28	**B_4**	0.23	0.26	**X_4**	0.46	0.47
A_6	351	47.79	**B_6**	0.15	0.15	**X_6**	3.40	0.82
A_8	229	49.96	**B_8**	0.11	0.08			
A_{10}	393	54.11	**B_{10}**	0.83	0.30			

Table S**8**: Hemolytic activities (average HC_{50} values with standard deviations) of polymers with increasing *AR* values from series **P** and **Q** towards human red blood cells

Series P	**HC_{50} (μg/mL)**	**s.d.**	**Series Q**	**HC_{50} (μg/mL)**	**s.d.**
			$Q_{0.5}$	1.1	0.77
$P_{1.0}$	1709	276.28	**$Q_{1.1}$**	0.23	0.26
$P_{2.3}$	2159	461.39	**$Q_{2.2}$**	81	79.1
$P_{3.1}$	4319	1765.31	**$Q_{3.4}$**	1518	805.7
$P_{4.1}$	4977	1651.9	**$Q_{4.3}$**	1723	1101.6

6. Fluorescent Labelling and Confocal Microscopy

Fluorescently labeled polymers **A_4** , **B_4** and **C_4** were prepared as shown in Figure S**16** below. The precursors to polymers **A_4** and **B_4** were heated with 1.5 mol% (w.r.t. moles of pyridine units) of 5-(iodoacetamido) fluorescein dye (λ_{ex} 492nm, λ_{em} 515nm) in DMF. The resulting labeled intermediate was then heated with slight excess of the respective iodoalkane (iodomethane or 1-iodobutane) to completely N-alkylate the remaining pyridine units. The final resulting labeled polymers i.e. **A_4** , **B_4** and **C_4** were purified by dialyzing against methanol for 24 h using a Spectrum cellulose dialysis membrane with a molecular weight cutoff of 3000. The extent of dye labeling was determined using ^{1}H NMR, UV absorption spectroscopy and fluorimetric analysis. Approximately equal extent of dye labeling i.e. 1 mol % was obtained for the polymers. The labeled polymers had the same MIC and HC_{50} values as unlabelled polymers in antibacterial and hemolytic assays.

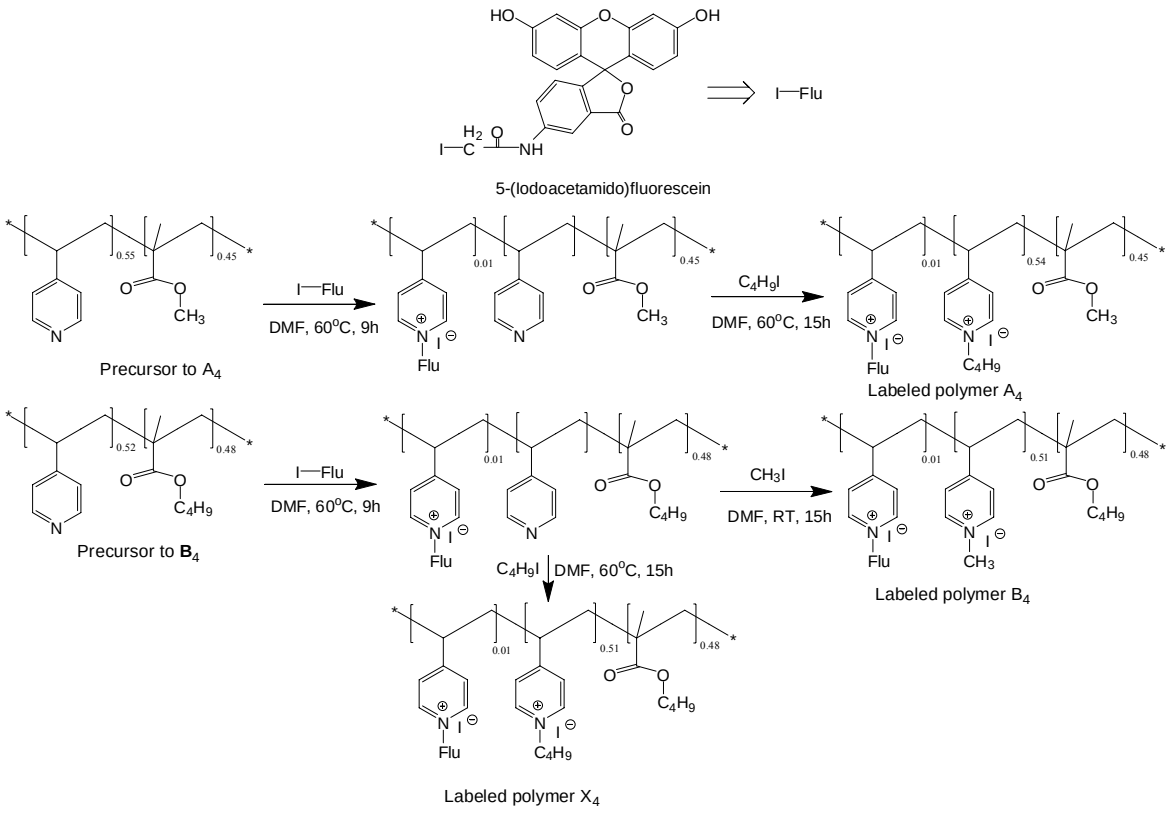

Figure S**16**: Schematics of synthesis of polymers labeled with the fluorescent dye 5-(iodoacetamido) fluorescein.

Freshly drawn human red blood cells were purchased from Innovative Research, Southfield, MI (Cat # IPLA-WB3). The anticoagulant was citrate dextrose. RBC's were filtered through Pall 0.2 micron Acrodisc filter. 120µL of these filtered RBC's were washed three times with 10 mL of TRIS buffer saline (TBS) (10mM TRIS, 150 mM NaCl, pH 7.2) by centrifuging at 1500 rpm for 5 min and removing the supernatant. The RBC's were finally suspended in 10 mL TBS to yield RBC stock solution of 1.2 % v/v. Note that the RBC concentration was 0.2% v/v for hemolytic testing. Higher

concentration of RBC were used to enable imaging a higher ensemble of cells in microscopy. The labeled polymers were dissolved in 1/1 DMSO/buffer to yield solution of concentration 40µg/mL. 50 µL of this polymer solution was added to 350 µL of RBC solution (final polymer concentration of 5 µg/mL) in an Eppendorf tube. The polymer/RBC were incubated at room temperature for 5 min, and were then placed on a glass coverslip and slide with silicone spacer and imaged under an Olympus Fluoview 1000 Confocal Laser Scanning Microscope (60x oil immersion objective, excitation 488nm laser at10mW). Confocal experiments were repeated on at least 2 different days to ensure reproducibility.

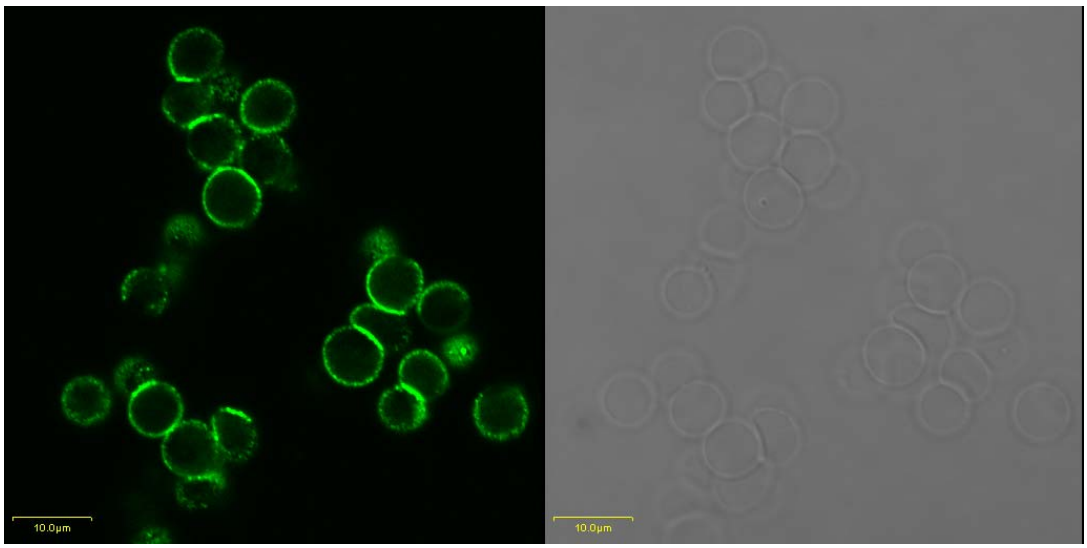

Figure S**17**: Confocal laser scanning microscopy images of human erythrocytes treated with dye labelled polymers **C_4** showing cellular agglutination, polymer binding and internalization into RBC.

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