Supporting Information

The Chemical Structure of Cationic Groups in Amphiphilic Polymethacrylates Modulates the Antimicrobial and Hemolytic Activities

Edmund F. Palermo and Kenichi Kuroda

Tables and Figures

Characterization of the Random Copolymers

Tables S1-S6 show the chemical structure of copolymers in each series and their ^{1}H NMR peak assignments. For some representative polymers, the NMR spectra are also shown (Figures S1-S3) and the analysis of peak integrations to find DP and f_{methyl} or f_{butyl} are described in detail.

In each case, the integration of peaks in the region 2.9-2.5 ppm, corresponding to protons in the polymer terminal groups (**h+g+e'+j** in the tables), was normalized to 7.00. Then, the peak integrations arising from the polymer side chains were compared to give the values of DP and f_{methyl} or f_{butyl}.

For example, Figure S1 shows the spectrum of copolymer PM$_{29}$

Table S1. Characterization of PM series

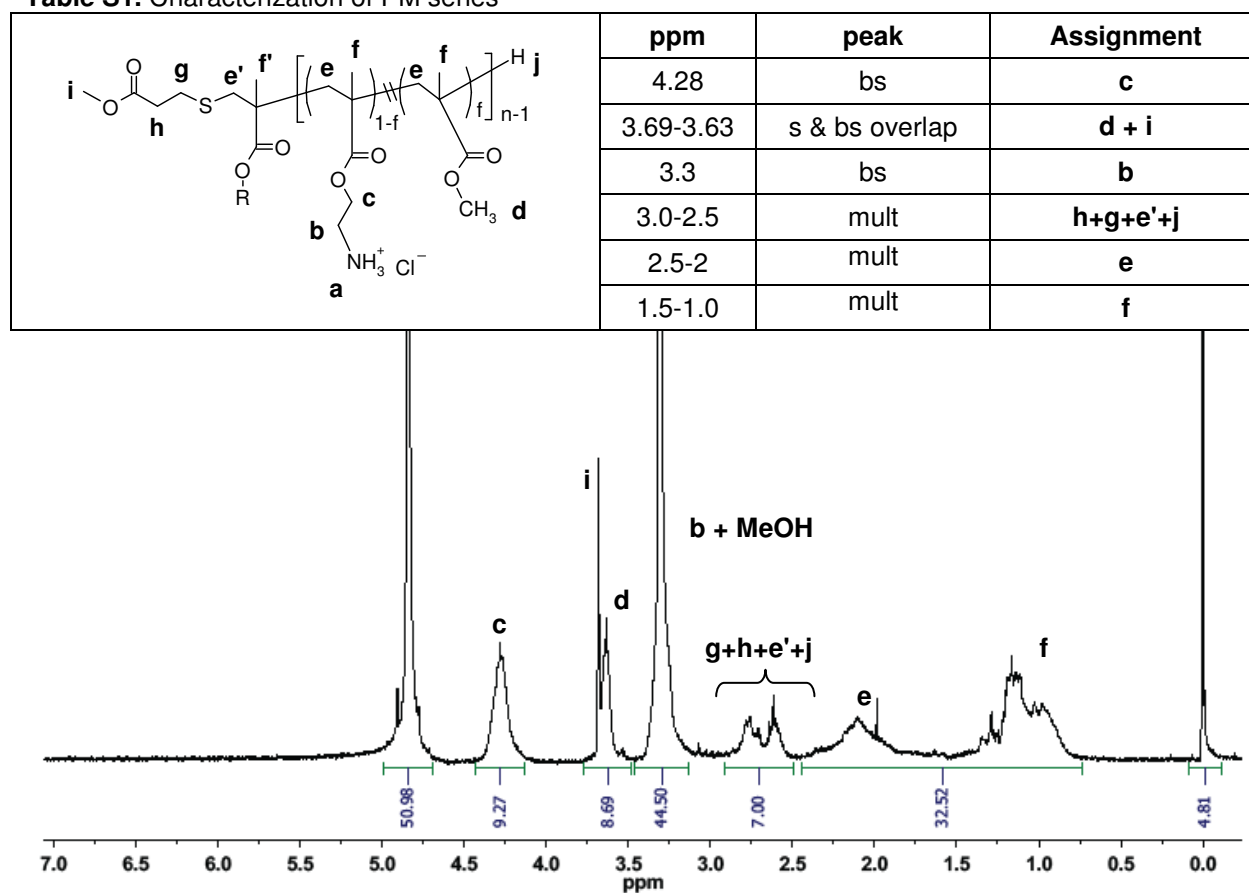| ppm	peak	Assignment
4.28	bs	**c**
3.69-3.63	s & bs overlap	**d + i**
3.3	bs	**b**
3.0-2.5	mult	**h+g+e'+j**
2.5-2	mult	**e**
1.5-1.0	mult	**f**

Figure S1. ^{1}H NMR spectrum of PM$_{29}$ with DP = 6.5

The spectrum contains a broad singlet centered at 4.28 ppm with integration equal to 9.27, which we assign to the methylene protons adjacent to the ester in the amine-functionalized repeat units (**c**). Hence,

$9.27 = n_{amine} * 2$
$n_{amine} = 4.635$

where n_{amine} is the average number of amine-functionalized repeat units per individual polymer chain.

The spectrum also shows a sharp singlet overlapping with a broad singlet near 3.6-3.7 ppm. The total integration equals 8.68. The sharp singlet is assigned to the three methyl protons on the polymer end group (**i**), whereas the broad feature represents the three protons of each methyl ester side chain (**d**). Hence,

$8.68 = 3 + n_{methyl} * 3$
$n_{methyl} = (8.68-3) / 3 = 1.893$

where n_{methyl} is the average number of methyl repeat units per individual polymer chain.

The number average degree of polymerization (DP) is the sum of n_{amine} and n_{methyl}. The mole fraction of methyl groups in the copolymer, f_{methyl}, equals the number of repeat units with methyl side chains divided by the DP. Hence,

$DP = n_{amine} + n_{methyl} = 6.528$
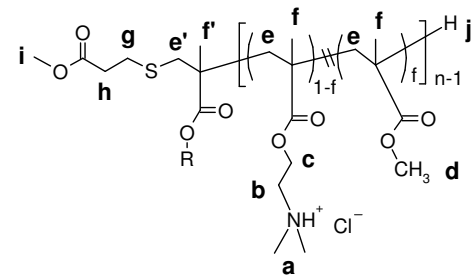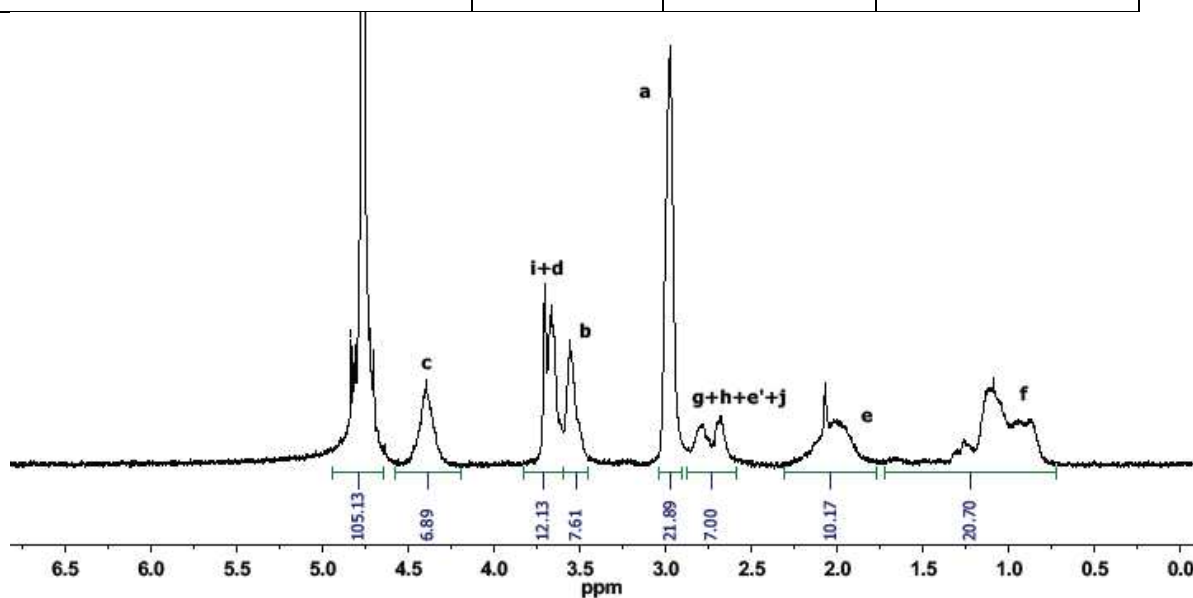$f_{methyl} = n_{methyl} / 6.2 = 0.29$

This is the copolymer PM_{29} with DP = 6.5 given in Table 1 of the text.

All of the copolymers in this study were characterized by a similar procedure. As additional examples, the ^{1}H NMR spectra of TM_{46} (Figure S2) and QB_{39} (Figure S3) are also shown and the detail of the peak integration analyses are described.

Notably, no signals appear in the 5.5-6.5 ppm region, indicating that unreacted monomer was completely removed by the purification procedure (size exclusion of Boc-protected polymer). Also, the peak arising from the *t*-butyl ester protons of the Boc protecting groups (1.4 ppm) is not observed in any of the spectra, indicating quantitative removal of Boc was achieved within the detection limit of the NMR.

Table S2. Characterization of TM series

ppm	peak	Assignment
4.44	bs	**c**
3.70-3.67	s & bs	**d + i**
3.59	bs	**b**
3.01	bs	**a**
2.85-2.6	mult	**h+g+e'+j**
2.4-1.9	mult	**e**
1.4-0.9	mult	**f**

Figure S2. ^{1}H NMR spectrum of TM$_{46}$ with DP = 6.7

The integration of the peak at 4.39 ppm (**c**) and the peak at 3.56 ppm (**b**) each correspond to twice the number of amine side chains per polymer. The integration of the peak at 2.98 ppm corresponds to six times the number of amine side chains per polymer. We calculate n_{amine} based on each of these peaks and take the average.

n_{amine} = 6.89/2 = 3.445 *or* 21.89/6 = 3.648.
The average n_{amine} = 3.63

The broad singlet and sharp singlet overlapping near 3.6-3.7 ppm are the methyl ester end group of the polymer and the methyl ester side chains.

n_{methyl} = (12.13-3)/3 = 3.04

DP = n_{amine} + n_{methyl} = 6.67
f_{methyl} = n_{methyl}/DP = 0.46

Table S3. Characterization of QM series

ppm	peak	Assignment
4.55	bs	**c**
3.85	bs	**b**
3.74-3.70	s & bs	**d + i**
3.30	bs	**a**
2.9-2.6	mult	**h+g+e'+j**
2.4-1.9	mult	**e**
1.4-0.9	mult	**f**

Table S4. Characterization of PB series

ppm	peak	Assignment
4.28	bs	**c**
3.99	bs	**d**
3.68	s	**i**
2.8-2.5	mult	**h+g+e'+j**
2.4-1.9	mult	**e**
1.7-0.8	mult	**f+k+l+m**

Table S5. Characterization of TB series

ppm	peak	Assignment
4.38	bs	**c**
4.01	bs	**d**
3.68	s	**i**
3.54	bs	**b**
3.03	bs	**a**
2.8-2.5	mult	**h+g+e'+j**
2.3-1.9	mult	**e**
1.7-0.8	mult	**f+k+l+m**

Table S6. Characterization of QB series

ppm	peak	Assignment
4.58	bs	**c**
4.11	bs	**d**
3.85	bs	**b**
3.75	s	**i**
3.31	bs	**a**
2.9-2.6	mult	**h+g+e'+j**
2.2-1.8	mult	**e**
1.8-0.8	mult	**f+k+l+m**
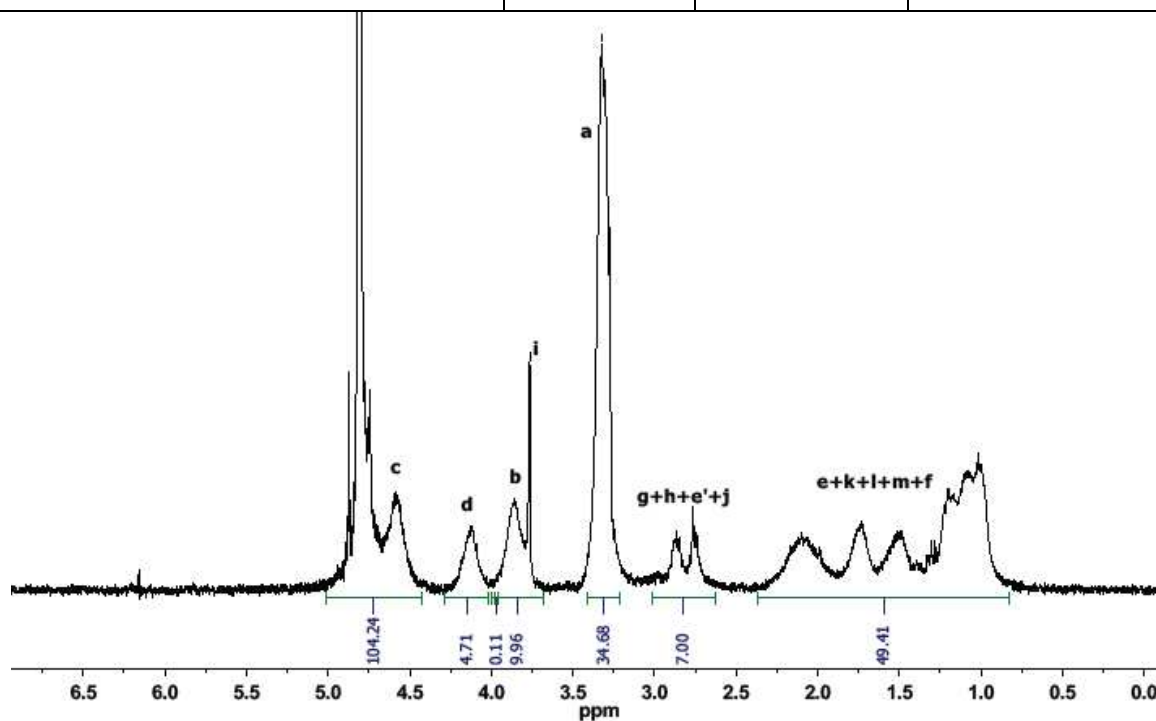
Figure S3. ^{1}H NMR spectrum of QB$_{39}$ with DP = 6.0

The integration of the peak at 3.31 ppm (**a**) corresponds to nine times the number of amine side chains per polymer. The integration of the peaks near 3.7 ppm correspond to the methyl group of the polymer terminal (**i**) plus the methylene protons adjacent to the QAS groups (**b**).

$34.68 = 9 * n_{amine}$ *or* $9.96 = 3 + 2 * n_{amine}$
The average $n_{amine} = 3.667$

The integration of the peak at 4.11 ppm (**d**) corresponds to twice the number of butyl side chains per polymer.

$n_{butyl} = 4.71/2 = 2.355$

$DP = n_{amine} + n_{butyl} = 6.02$
$f_{butyl} = n_{butyl} / DP = 0.39$

Hemolysis Raw Data

Lines represent best fit to the equation $H = 1/(1+(HC_{50}/[\text{polymer}])^n)$ where HC_{50} and n are the curve fitting parameters. Curve fitting was not performed on polymers that failed to reach 50% hemolysis at the highest polymer concentration tested, 2000 ug/mL.
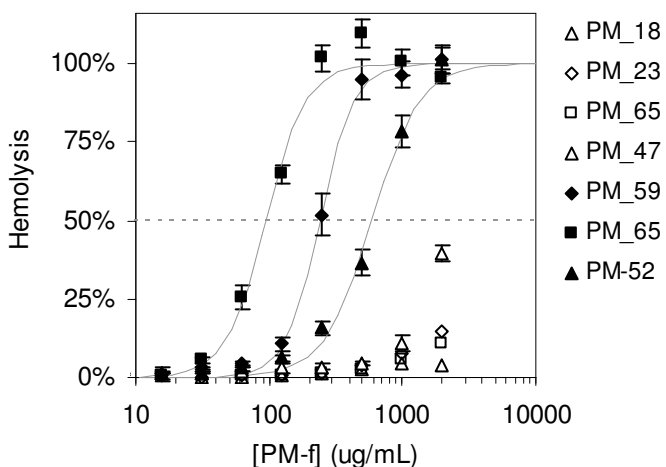

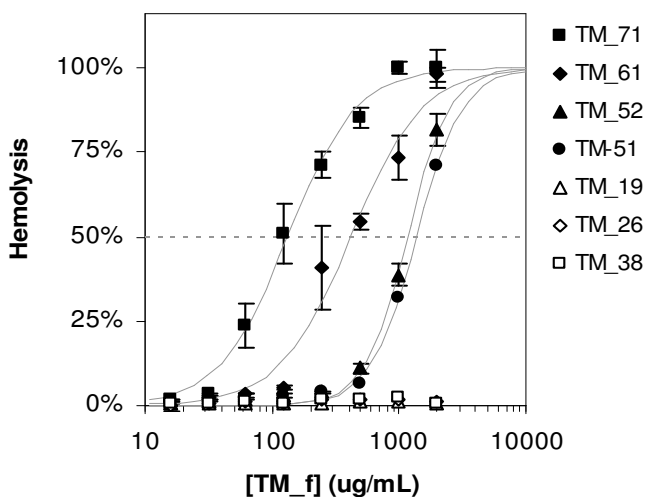**Figure S4.** Hemolysis dose-response curves for PM-polymer series.

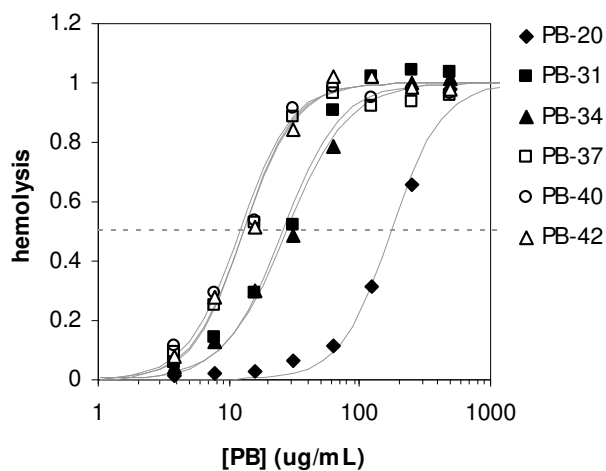**Figure S5.** Hemolysis dose-response curves for TM-polymer series.

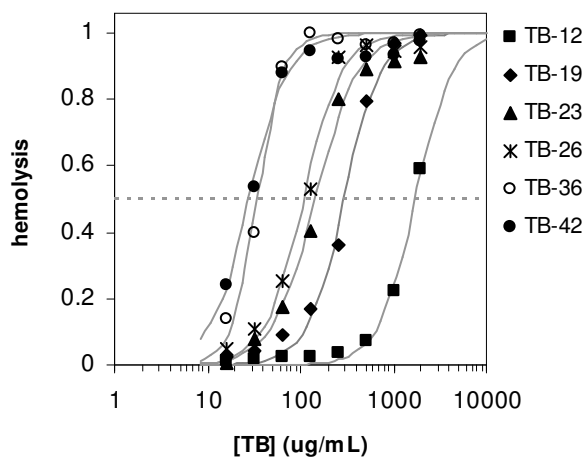**Figure S6.** Hemolysis dose-response curves for PB-polymer series.

Figure S7. Hemolysis dose-response curves for TB-polymer series.
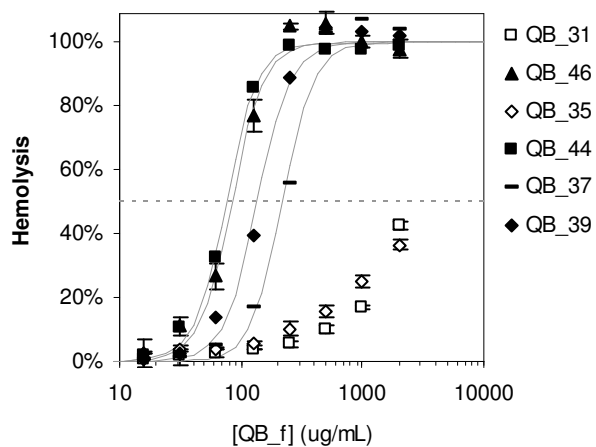

Figure S8. Hemolysis dose-response curves for QB-polymer series.
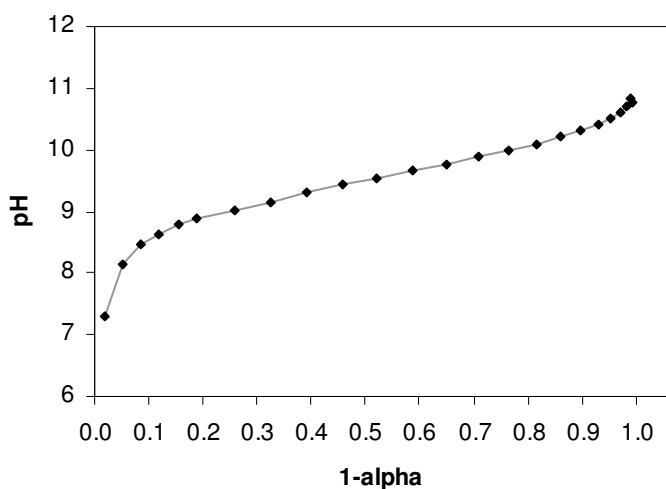
Potentiometric Titration Quality Assurance

Figure S9. Potentiometric titration of ethanolamine, pKa = 9.48 (accepted value = 9.5).

Potentiometric Titration of P_0

We performed forward-titration with NaOH (filled symbols) and back-titration with HCl (empty symbols) of the cationic homopolymer containing primary amine groups, P_0. As alpha approached 0.8, the back-titration data significantly deviated from the forward titration data. This deviation is likely due to chemical changes in the polymer side chains that occur in basic conditions, such as isomerization to hydroxyethylmethacrylamide linkages.

When the titration was limited to pH 8, back-titration data closely matched forward-titration data points (figure 2 panel A, in the text).
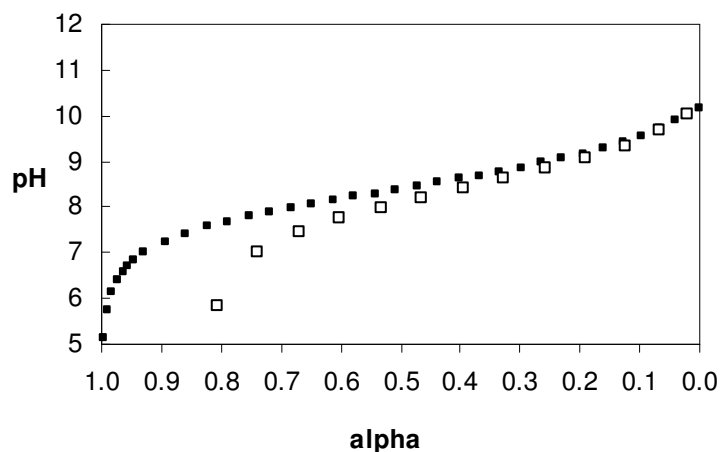

Figure S10. Potentiometric titration data of the cationic homopolymer Po, showing disagreement between forward (filled symbols) and back (empty symbols) titration curves.

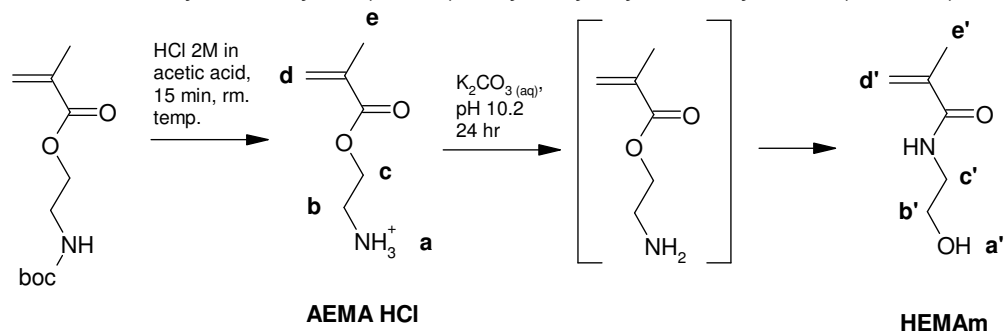*Isomerization of aminoethylmethacrylate (AEMA) to hydroxyethylmethacrylamide (HEMAm)*

Figure S11. Base-induced isomerization of the monomer containing a primary amine

Boc-AEMA was deprotected to give AEMA HCl. The cationic monomer was precipitated from methanol into diethylether. The precipitate was collected by centrifugation and then lyophilized.
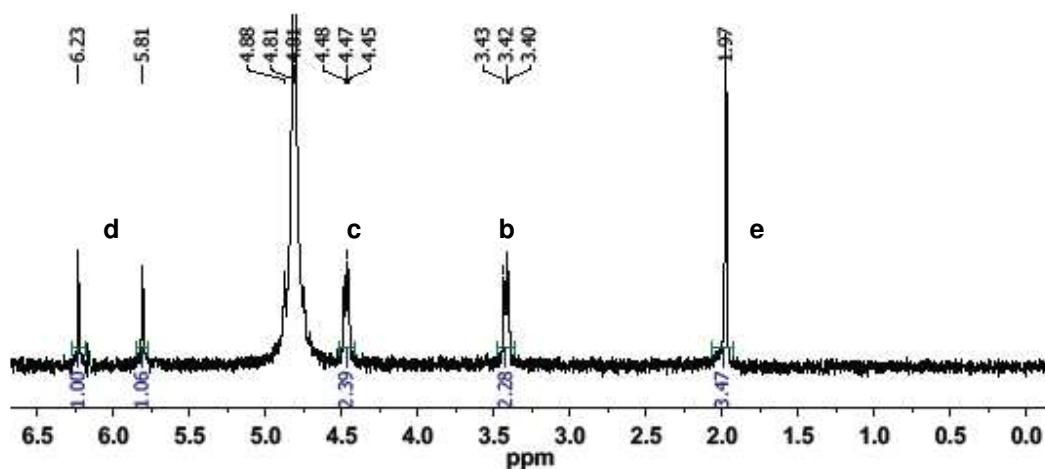

Figure S12. ^{1}H NMR of AEMA HCl (300 MHz, D$_2$O)

AEMA HCl was then dissolved in 10% K$_2$CO$_3$(aq), pH 10.2, room temp for 24 hr. The lyophilized powder was added to methanol-d$_4$ and filtered. NMR was performed on the clear filtrate.

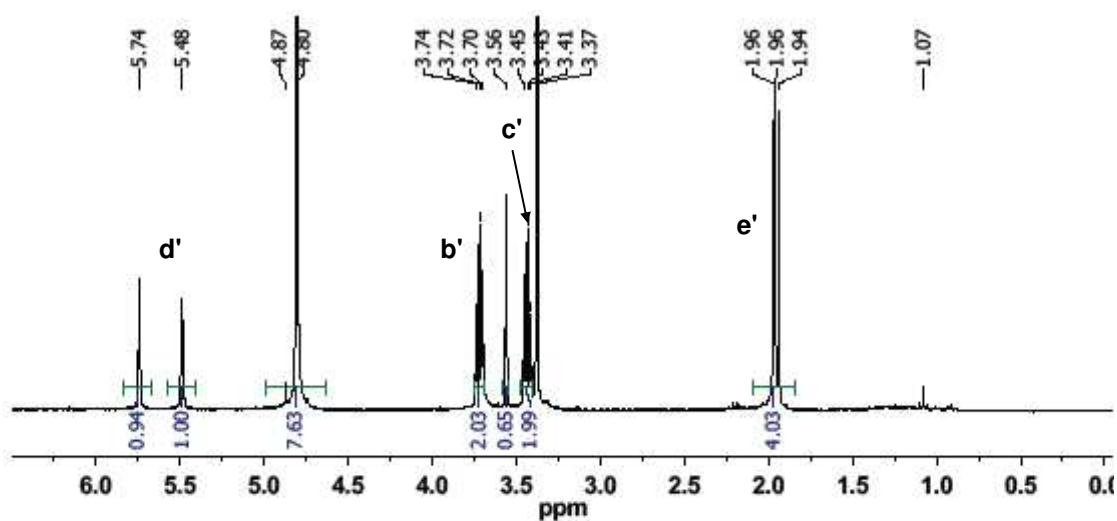**Figure S13**. ^{1}H NMR of HEMAm (300 MHz, methanol-d$_4$)

x

Hemolysis induced by Triton X-100 in different buffers pH 6-8.

Triton X-100 is a non-ionic surfactant used as a positive control in our experiments. HC_{50} ~ 120 uM in all buffers pH 6-8. The accepted value of Triton X-100 CMC (reported by Sigma) is shown as the dotted line.
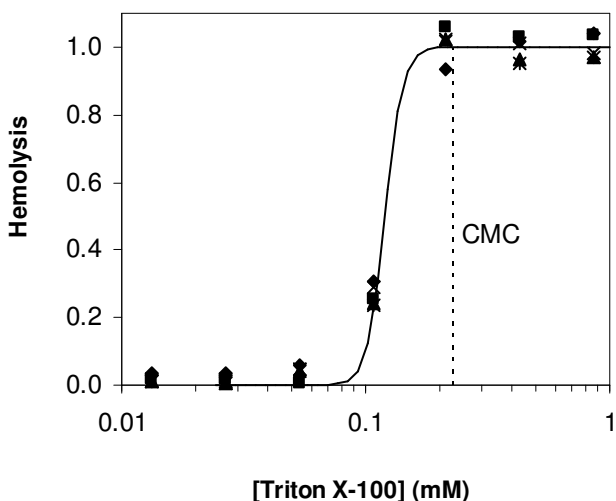

Figure S14. Hemolysis data for Triton X in buffer of pH 6 (triangles), 7 (squares), and 8 (circles).

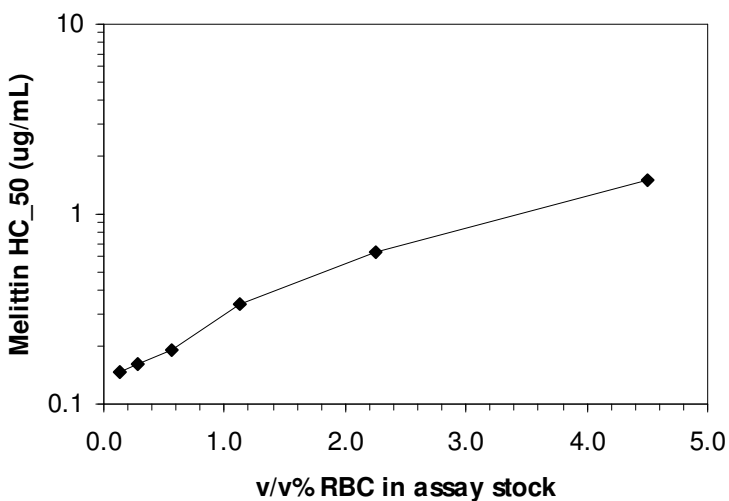*Hemolysis depends on the concentration of red blood cells in assay stock*

Figure S15. HC_{50} value of melittin in different assay conditions. The HC_{50} is strongly dependent on the concentration of red blood cells used in the assay. For this reason, it is important to compare hemolytic activity of compounds tested in identical assay conditions. In this report, we used melittin as an internal standard for comparison, with the concentration of red blood cells fixed at 3% v/v.

E. coli ATCC 25922 viability in phosphate buffered saline at room temp.

Bacteria grown to mid-logarithmic phase, diluted in buffer of pH 6, 7, or 8. The suspensions were then incubated at room temp for 2 hours (non-growing condition). About every 20 min, an aliquot of the bacteria suspension was taken and diluted 10^3 fold, then spread on an agar plate. Plates were incubated at 37 ^{o}C overnight and the number of colonies counted to determine the cfu/mL in the original suspension.

In our MBC experiments, polymers were shown to cause at least a 3 log reduction in the number of cfu/mL after 90 min incubation at room temperature.
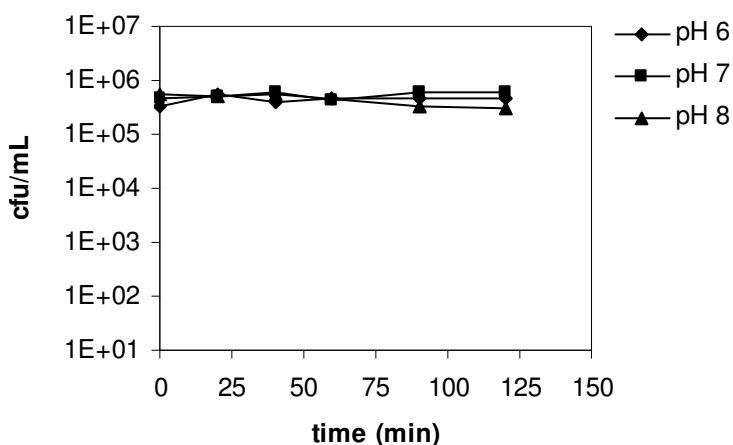

Figure S16. Viability of *E. coli* as a function of time and pH in the non-growing assay condition.