Supplementary Information

The Function of Peptide-Mimetic Anionic Groups and Salt Bridges in the Antimicrobial Activity and Conformation of Cationic Amphiphilic Copolymers

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[a] RB and LF and GR: Equal contribution

1. Monomer and Polymer characterization
    a. NMR spectra
    b. GPC traces
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3. Solvent accessible surface area of M1

1. Monomer and Polymer characterization

1-1a 1H NMR and ^{13}C NMR spectra of monomers and assignment of peaks

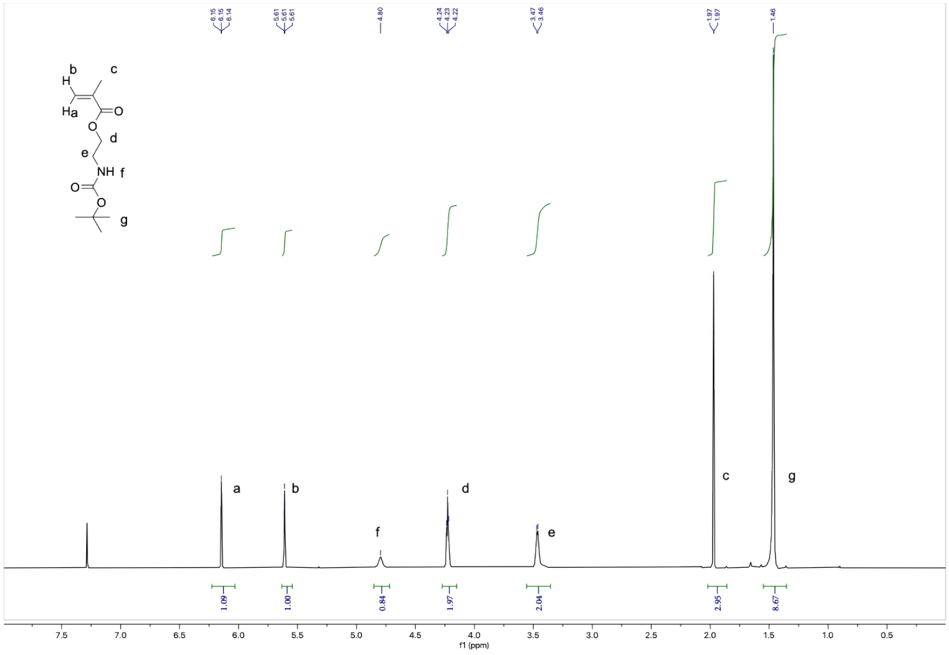


Figure S 1. ^{1}H NMR spectrum of Boc-aminoethyl methacrylate monomer in $CDCl_3$

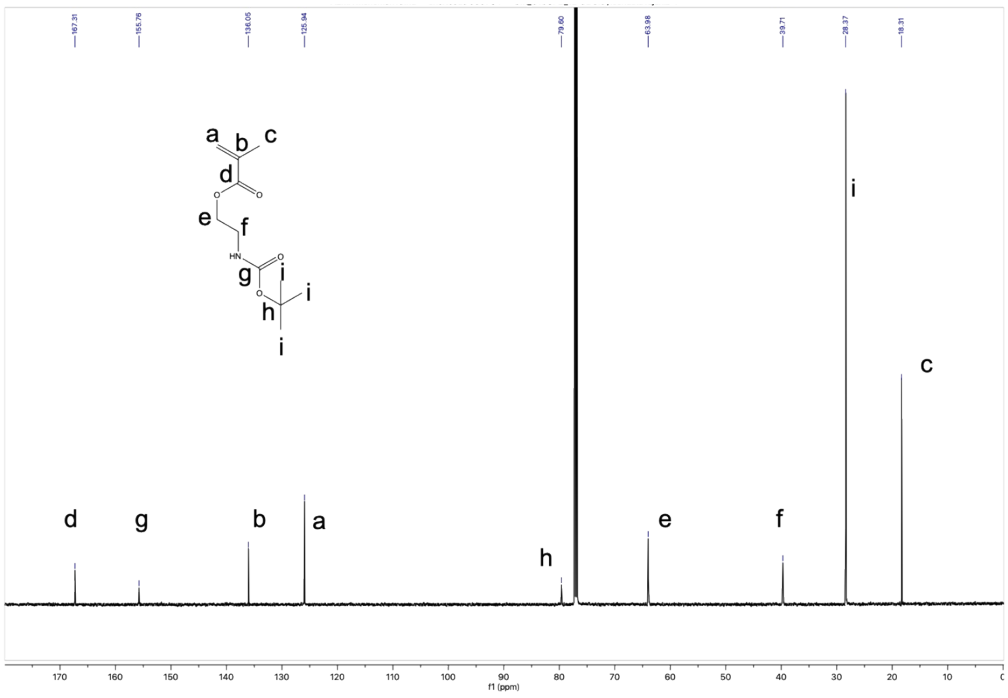


Figure S 2. ^{13}C NMR spectrum of Boc-aminoethyl methacrylate monomer in $CDCl_3$

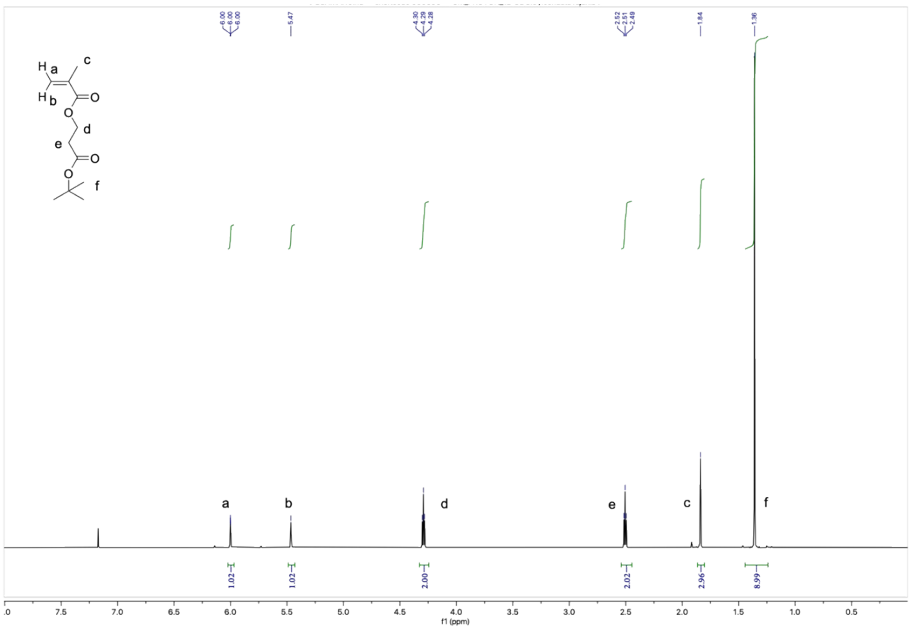


Figure S 3. ^{1}H NMR of ^{t} butyl propanoic acid methacrylate monomer

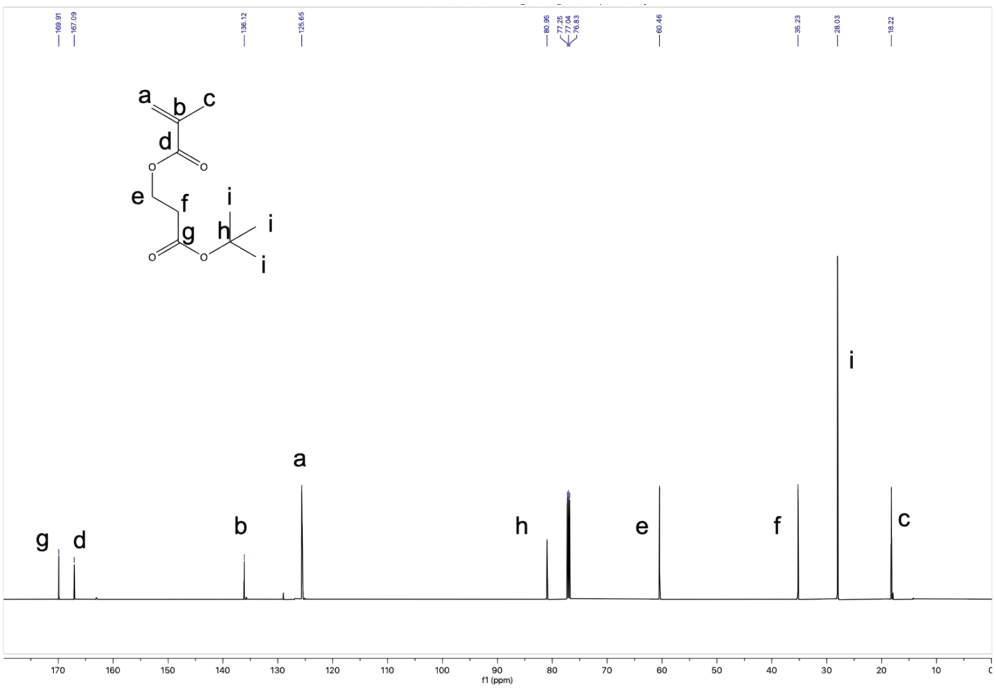
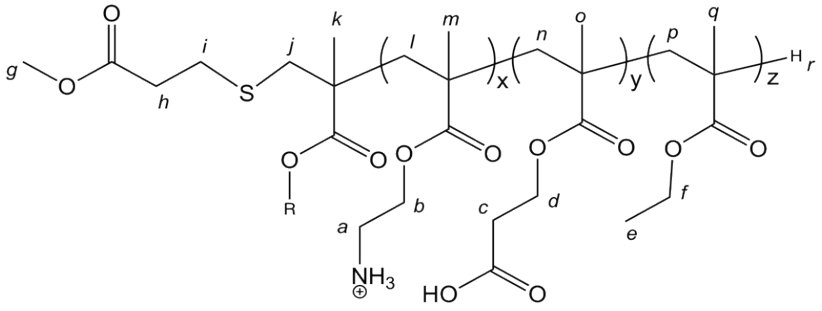


Figure S 4. ^{13}C NMR of ^{t} butyl propanoic acid methacrylate monomer

1-1a. ^{1}H NMR spectrum of $AE_{39}PA_{17}E_{44}$ in CD_3OD as solvent and assignment of peaks



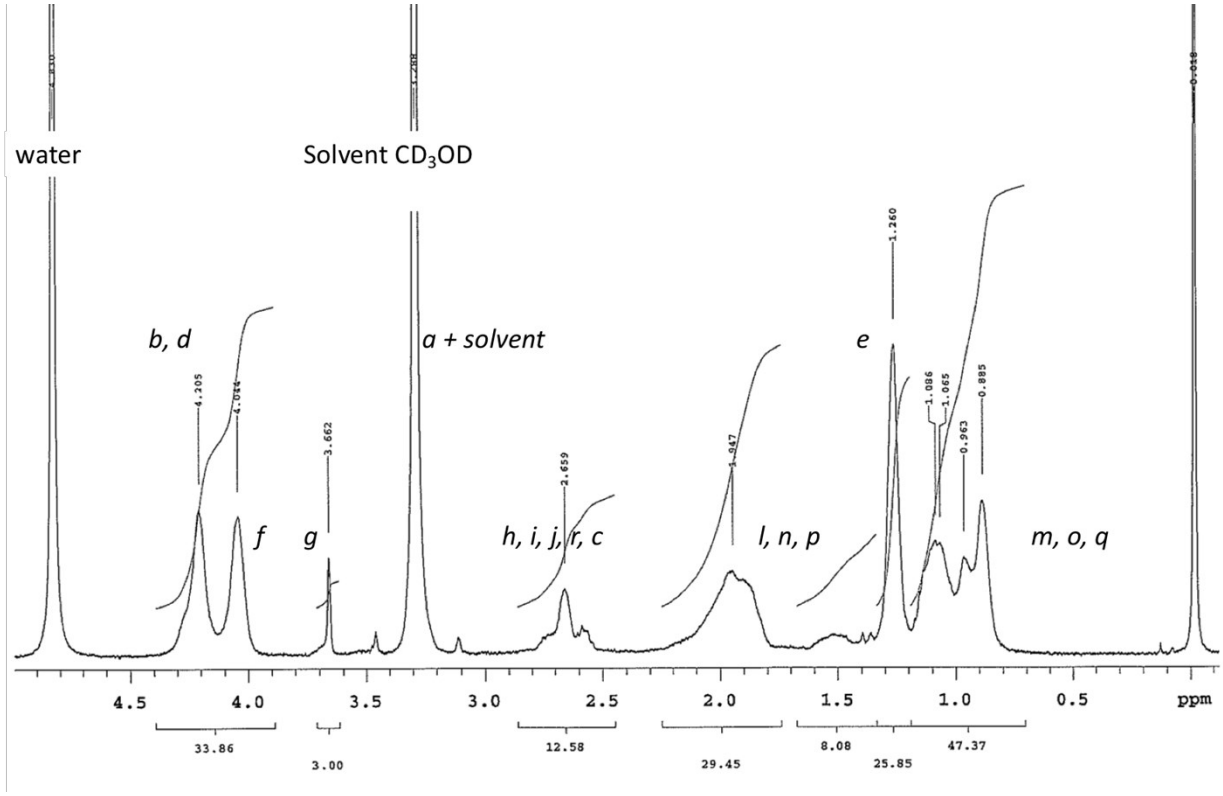


Figure S 5. ^{1}H NMR of $AE_{39}PA_{17}E_{44}$

The DP and mol. % of monomers were determined as following:

DP = 33.86/2 (*b, d, f*) =16.93

PAMA = [12.58 (*c*) – 7 (*h, i, j, r*)] / 33.86 (*b, d, f*) = 16.5%

EMA = *f* / *(b, d, f)* = 26.5 mm / (26.5 mm + 33.5 mm) * = 44 % (* = heights of integration curve)

AEMA = 100 mol. % – 44 mol. % (EMA) – 16.5 mol. % = 38.5 mol. %

1-2. Polymer GPC traces

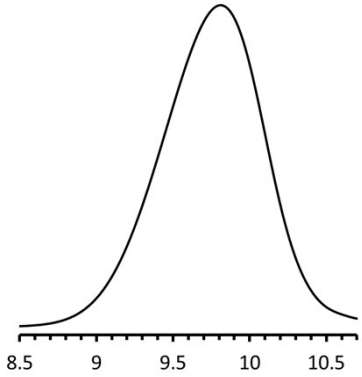


Figure S 6*:* SEC trace of $AE_{00}PA_{55}E_{45}$ using RI detector

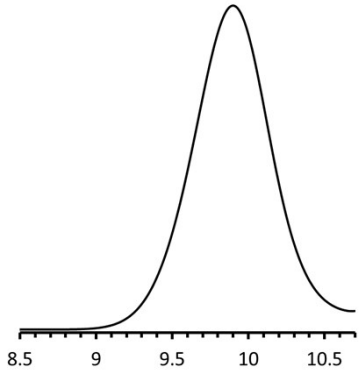


Figure S 7*:* SEC trace of $AE_{7}PA_{47}E_{46}$ using RI detector

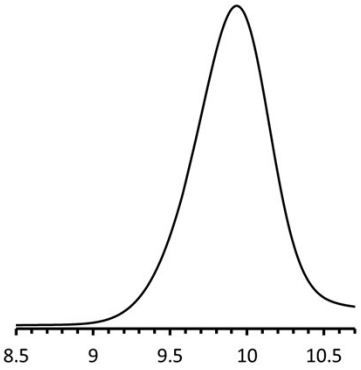


Figure S 8*:* SEC trace of $AE_{21}PA_{42}E_{37}$ using RI detector

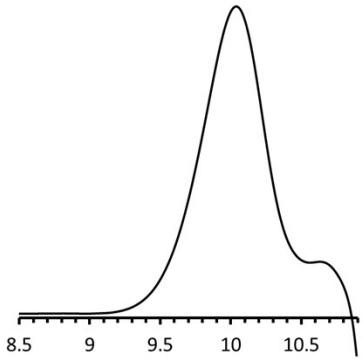


Figure S 9*:* SEC trace of $AE_{29}PA_{27}E_{44}$ using RI detector

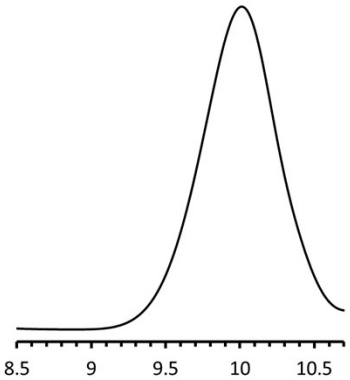


Figure S 10*:* SEC trace of $AE_{37}PA_{24}E_{39}$ using RI detector

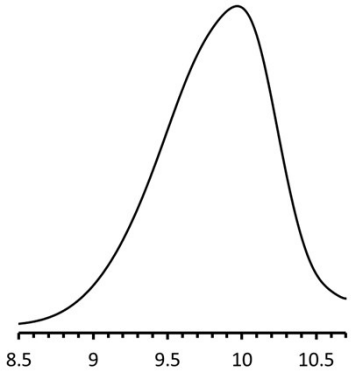


Figure S 11*:* SEC trace of $AE_{39}PA_{22}E_{41}$ using RI detector

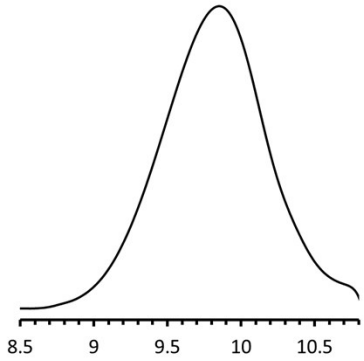


Figure S 12*:* SEC trace of $AE_{39}PA_{17}E_{44}$ using RI detector

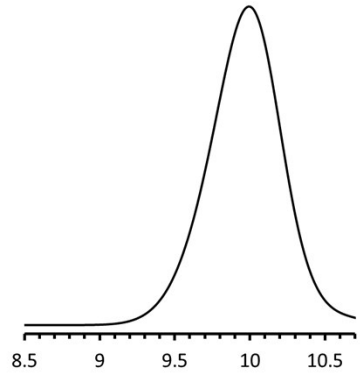


Figure S 13*:* SEC trace of $AE_{46}PA_{12}E_{42}$ using RI detector

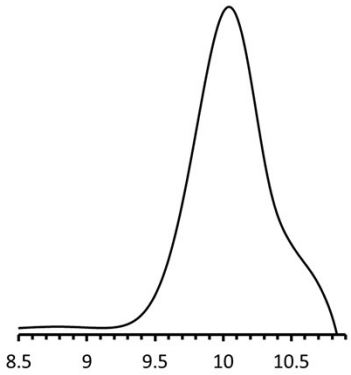


Figure S 14*:* SEC trace of $AE_{59}PA_{00}E_{41}$ using RI detector

1-3 Hemolysis Curves

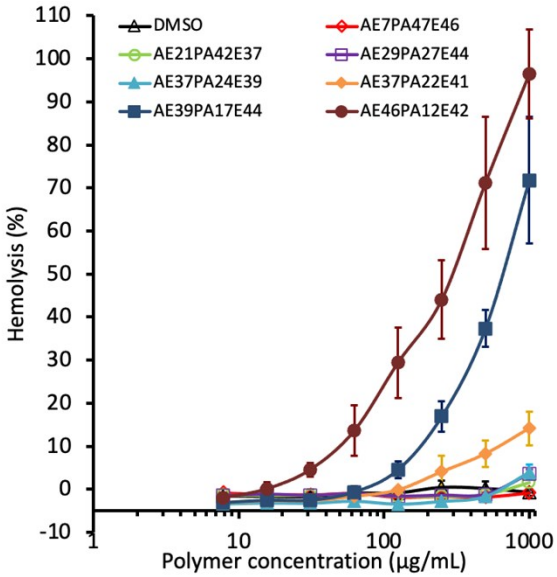


Figure S 15. Representative hemolysis curves. Each data point and error represent the average and standard deviation of triplicates

1-4 Titration curves

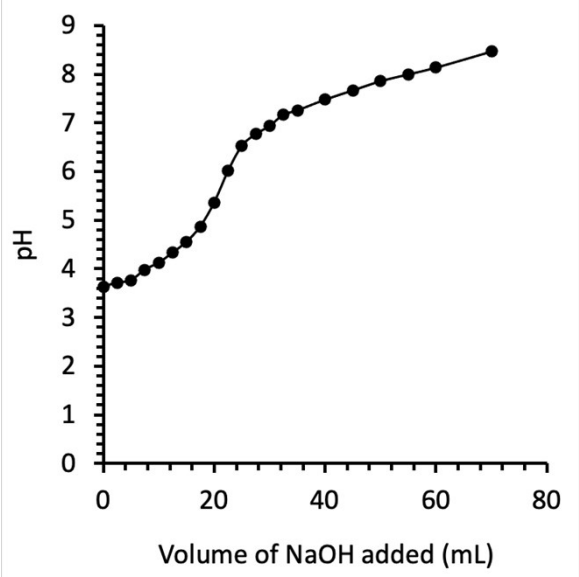


Figure S 16. Representative potentiometric titration curve of $AE_{39}PA_{17}E_{44}$ copolymer. The titration curve was measured at the polymer concentration of 0.5mg/mL due to its low availability.

Table S 1. Characterization of protected and deprotected copolymers with EMA 40 mol. %

	Feed composition (mole%)			Protected copolymers [a]						Deprotected copolymers [a]						
Polymer	AEMA	PAMA	EMA	AEMA	PAMA	EMA	DP	Conv.	Yield (%)	AEMA	PAMA	EMA	DP	M_n[a] w/TFA	M_n w/o TFA	Yield (%)
$AE_0PA_{55}E_{45}$	0	60	40	0.0	55.6	45.4	18.8	-	-	0	54.8	45.2	19.6	2770	2770	16
AE_7 $PA_{47}E_{46}$	10	50	40	7.6	50.9	41.5	15.7	88.5	35	6.7	46.6	46.7	15.3	2330	2190	16
AE_{21} $PA_{42}E_{37}$	20	40	40	25.5	38.9	35.6	15.6	91.6	-	21.4	41.6	37	14.8	2490	2130	28
AE_{29} $PA_{27}E_{44}$	30	30	40	30.5	27.2	42.3	15.1	-	58	29.4	26.8	43.8	17.1	2930	2360	50
AE_{37} $PA_{24}E_{39}$	40	20	40	35.2	22.7	42.1	15.2	-	-	36.6	23.2	40.2	15.3	2780	2140	50
AE_{37} $PA_{22}E_{41}$	35	25	40	38.0	22.5	39.5	14.8	97.4	72	37.3	21.7	41.0	15.4	2740	2100	60
AE_{39} $PA_{17}E_{44}$	40	20	40	43.3	15.9	40.8	15.9	-	60	39.4	16.5	44.1	16.9	3040	2290	55
AE_{46} $PA_{12}E_{42}$	45	15	40	45.2	12.8	42.0	16.5	95.2	65	46.3	12.1	41.6	18.6	3440	2480	58
AE_{59} PA_0E_{41}	60	0	40	61.1	0.0	38.9	14.8	-	88	59.3	0.0	40.7	14.4	2860	1900	81

a) Values determined by ^{1}H NMR

2. Atomistic MD simulations of model polymer M2

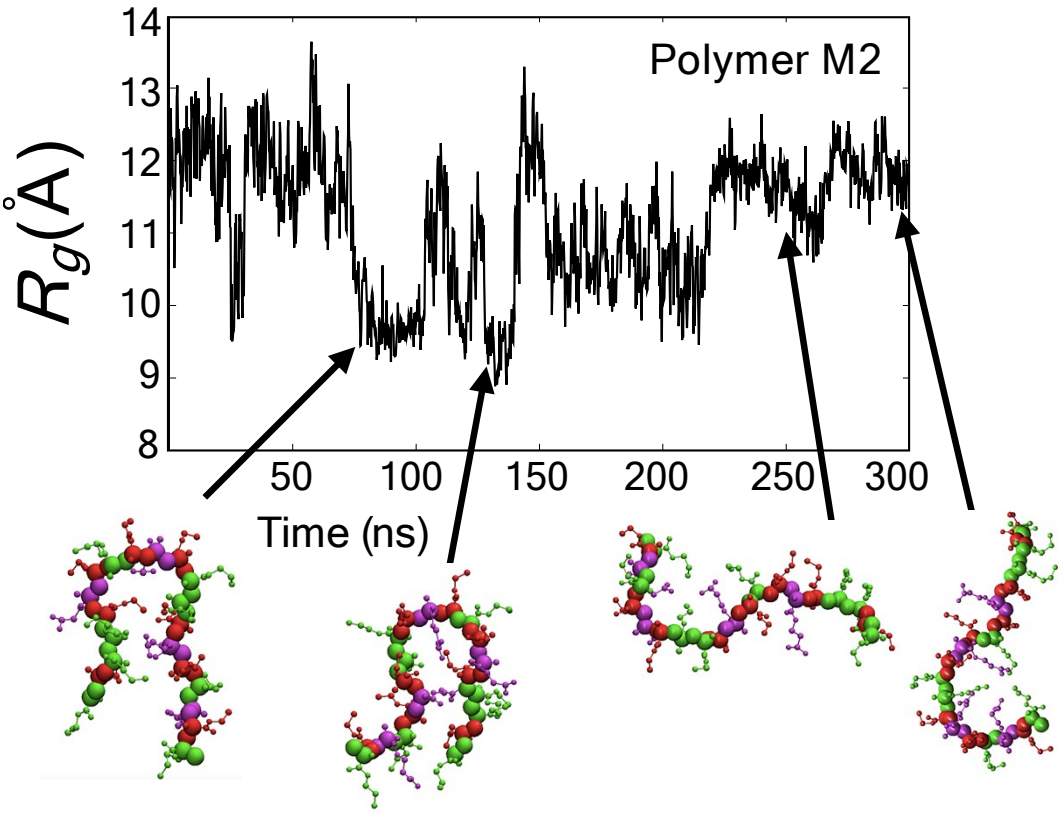


Figure S 17. Radius of gyration (R_g) for the model polymer (M2) as a function of simulation time. Representative snapshots to illustrate the conformations of the polymers are also presented, with arrows indicating the corresponding simulation time and R_g values.

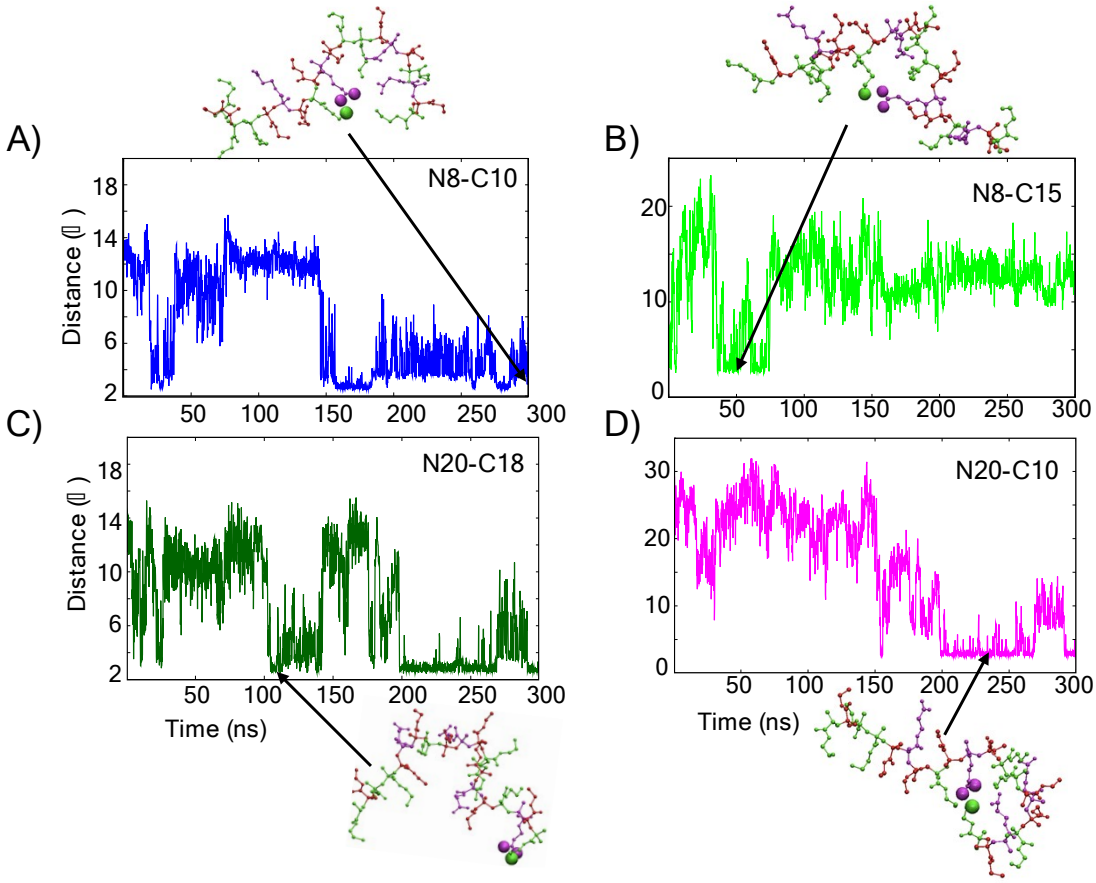


Figure S 18. Time evolution of distance between the nitrogen atom of NH_3^+ in AEMA group and the carboxyl oxygen atoms of COO^-. A representative snapshot of the polymer is shown with arrows pointing to the corresponding distance between nitrogen (shown as green ball) and oxygen atoms (shown as magenta balls) and simulation time.

3. Solvent accessible surface area of M1

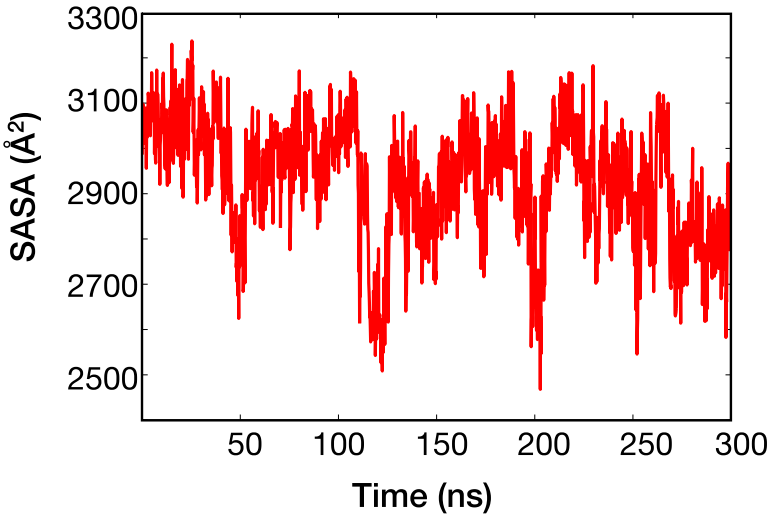


Figure S 19. Time evolution of SASA value of M1.

References

1. Stromstedt, A. A.; Pasupuleti, M.; Schmidtchen, A.; Malmsten, M., Evaluation of Strategies for Improving Proteolytic Resistance of Antimicrobial Peptides by Using Variants of EFK17, an Internal Segment of LL-37. *Antimicrob Agents Ch* 2009, *53* (2), 593-602.