Supporting Information for

Glutathione-triggered Biodegradable Poly(disulfide)s: Ring-opening Copolymerization and Potent Antibacterial Activity

Jia Guo [a,b], Siqi Zhang [a,b], Yaqi Tao [a,b], Baoer Fan [a,b], Wen Tang [a,b] *

Affiliations:

[a] South China Advanced Institute for Soft Matter Science and Technology, School of Emergent Soft Matter, South China University of Technology, Guangzhou 510640, China.

[b] Guangdong Provincial Key Laboratory of Functional and Intelligent Hybrid Materials and Devices, South China University of Technology, Guangzhou 510640, China.

Corresponding author

Wen Tang (Email address: tangw@scut.edu.cn)

This PDF file includes:

Figures S1 to S30

Tables S1 to S8

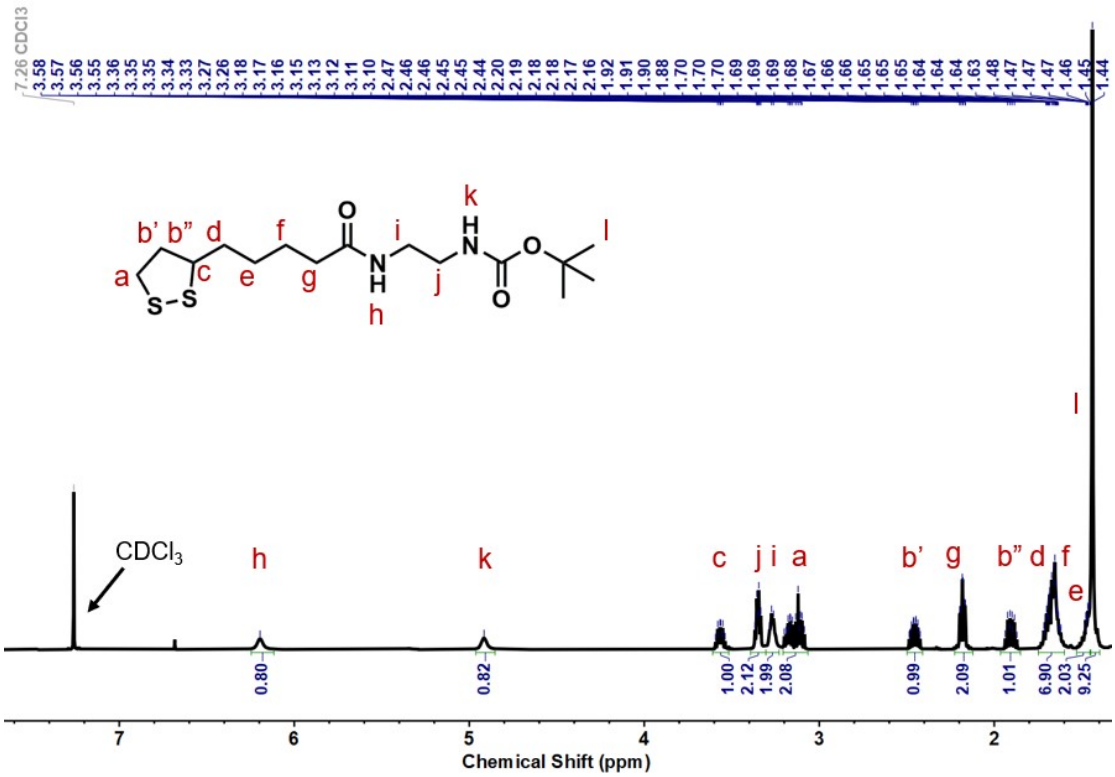


Figure S1. ^{1}H NMR spectroscopy of **M1** in $CDCl_3$.

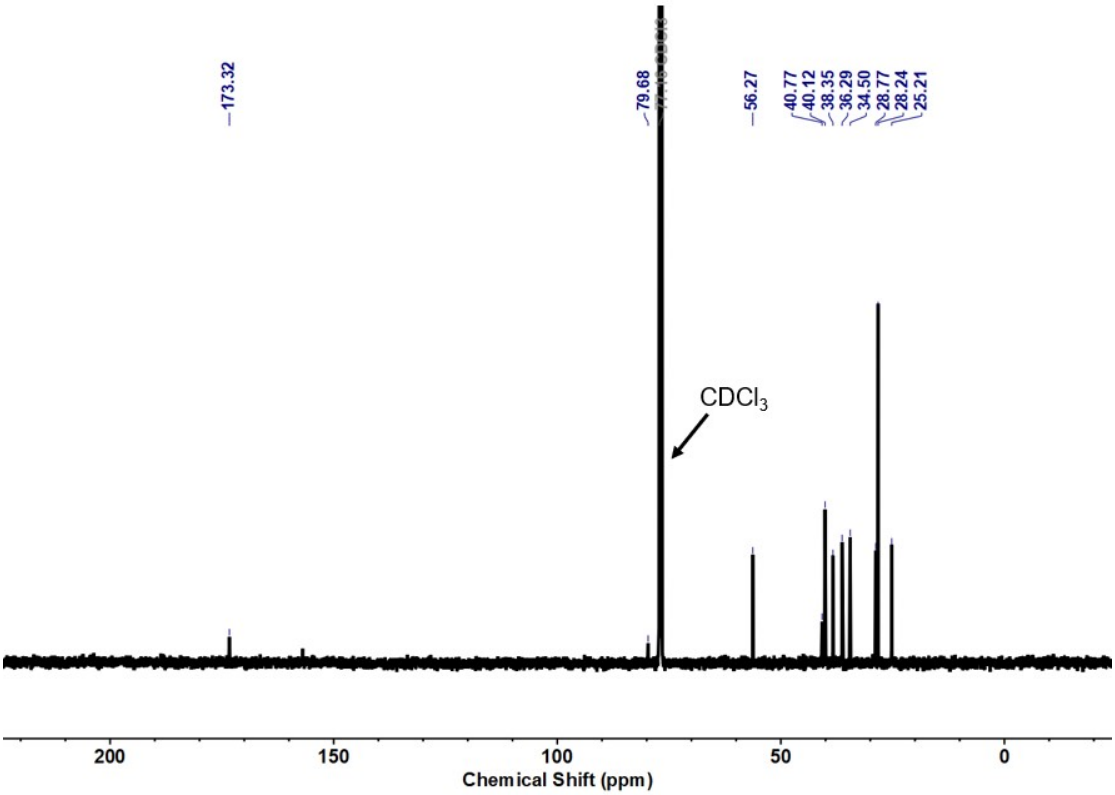


Figure S2. ^{13}C NMR spectroscopy of **M1** in $CDCl_3$.

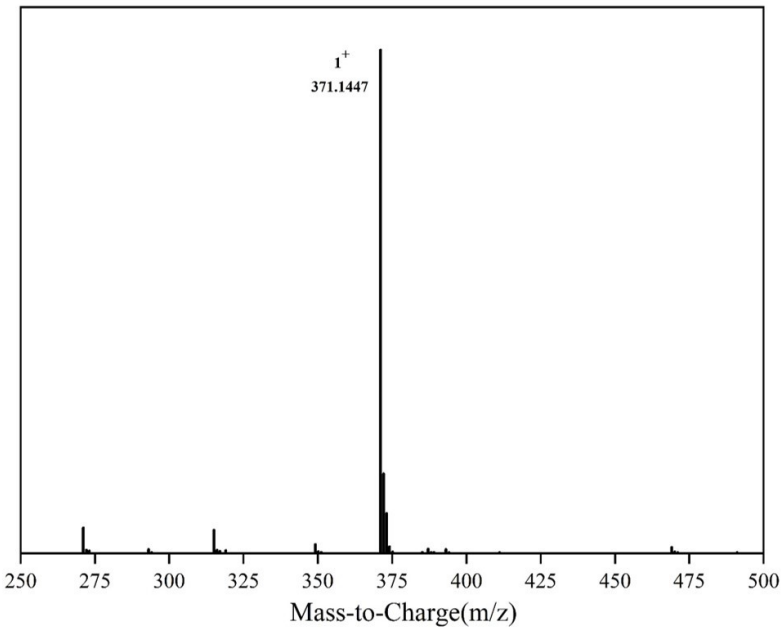


Figure S3. ESI-MS of **M1**. The m/z of **M1** is 371.14 ($[M+Na]^+$).

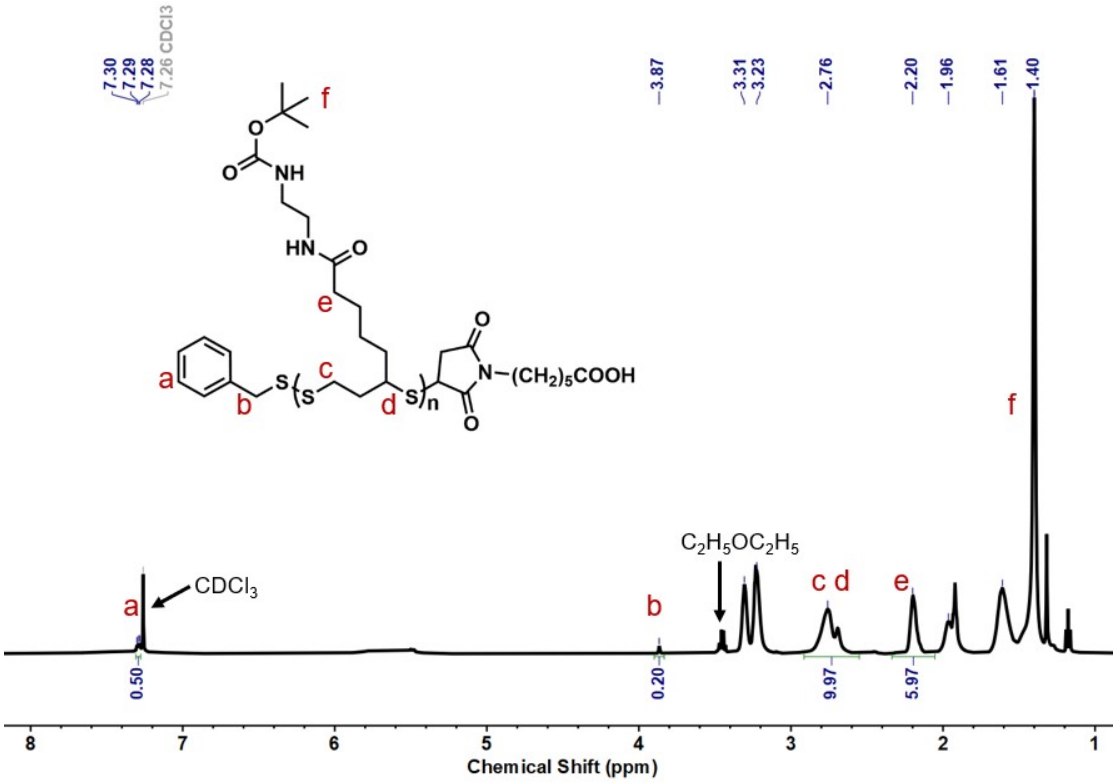


Figure S4. ^{1}H NMR spectroscopy of **P1-35** in $CDCl_3$.

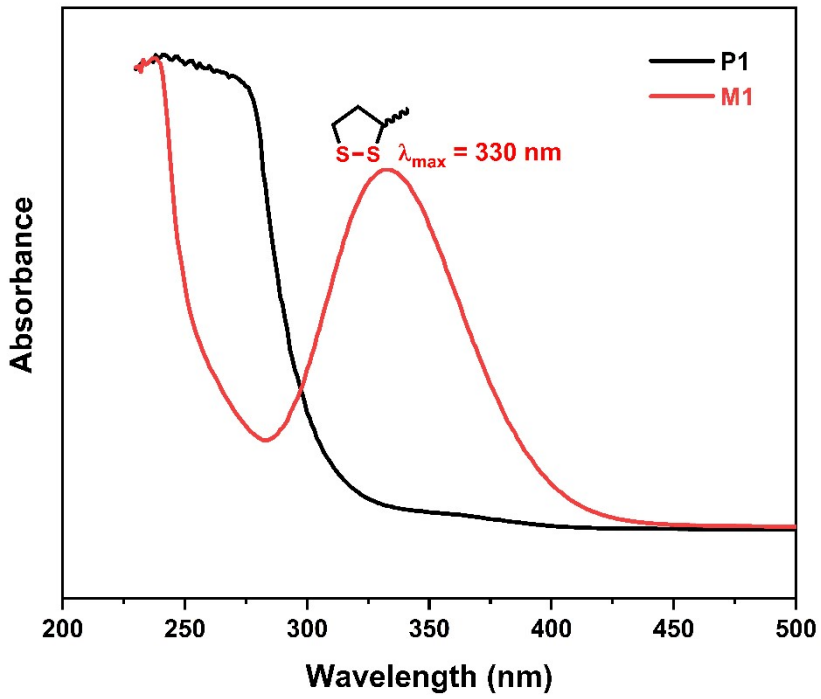


Figure S5. UV−vis absorption spectra of **P1** (10 mg/mL) and **M1** (10 mg/mL).

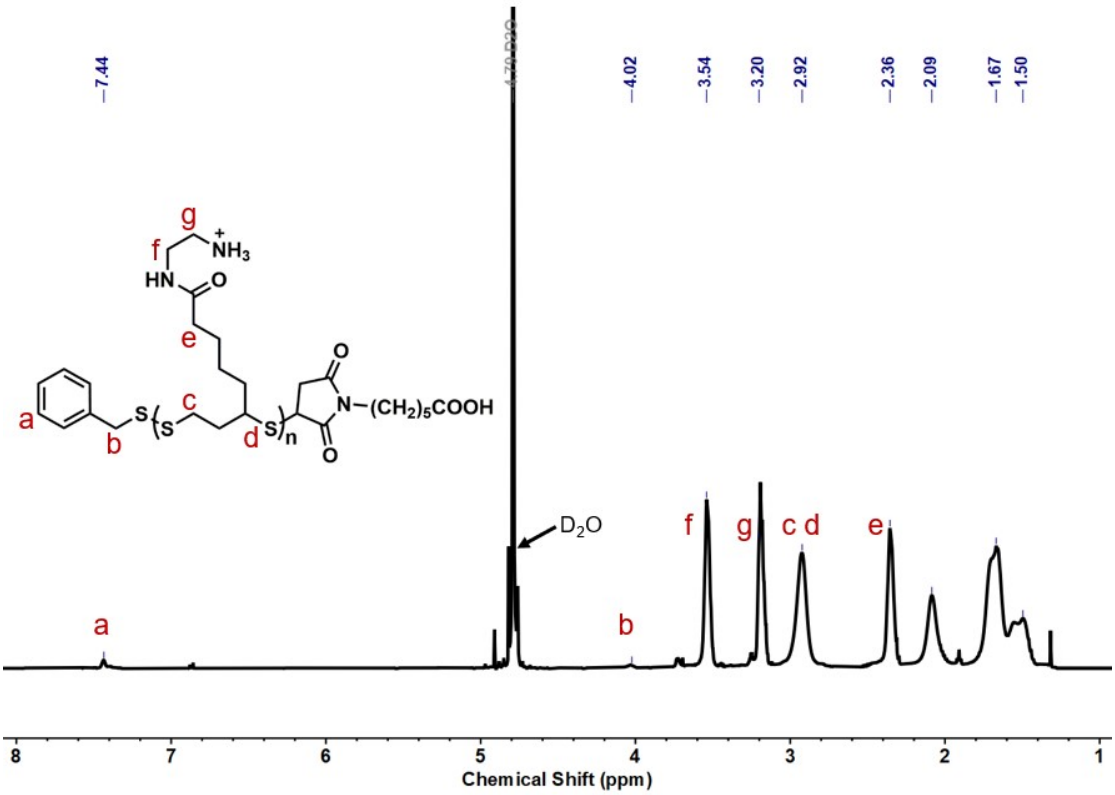


Figure S6. ^{1}H NMR spectroscopy of **P2-35** in D_2O.

Molecular weight (MW) control with different polymerization conditions.

The influence of $[M1]_0$ on the MW of P1.

Summary of polymerization conditions: $[M1]_0$:$[I]_0$:[B]:[Q] = 100:1:2:10, 2 h, 0 ℃.

The M1 (0.35 g, 280 μL, 1.0 mmol, 100 equiv) was dissolved in different amounts of anhydrous THF (370 μL, 270 μL, 200 μL, 145 μL) to obtain different initial concentration of M1 ($[M]_0$ = 1.50 M, 1.75 M, 2.00 M, 2.25 M). As the procedure of the **General synthesis of P1**, after quenched by the 6-maleimidohexanoic acid, these series of samples with different $[M1]_0$ were added dropwise to ice-cold ethyl ether to induce precipitation three times. The precipitation was dried under high vacuum 4-5 h.

Table S1. Polymerization results of M1 with different monomer concentration ($[M1]_0$). Summary of polymerization conditions: $[M1]_0$:$[I]_0$:[B]:[Q] = 100:1:2:10, 2 h, 0 ℃.

$[M1]_0$ (mol/L)	Mn (kDa)[a]	$Đ$[b]	Conversion (%)
1.5	11	1.22	43
1.75	13	1.21	52
2.00	15	1.20	46
2.25	16	1.31	37

[a, b] Determined by GPC analysis (RI)

The influence of monomer-to-initiator ratios on the MW of P1.

Summary of polymerization conditions: [I]:[B]:[Q] = 1:2:10, $[M1]_0$ = 1.75 mol/L, 2 h, 0 ℃.

The M1 (0.5 mmol, 1.0 mmol, 1.5 mmol, 2.0 mmol) was dissolved in different amounts of anhydrous THF (125 μL, 270 μL, 410 μL, 555 μL) to obtain the same concentration of M1 ($[M1]_0$ = 1.75 M). Then, the initiator solution was prepared with BnSH (3 μL, 0.025 mmol, 1 equiv) and Base (15 μL, 0.05 mmol, 2 equiv) in 50 μL anhydrous THF ($[M1]_0$:$[I]_0$:[B]:[Q] = 50:1:2:10, 100:1:2:10, 150:1:2:10, 200:1:2:10). The next procedure to realize ring-opening polymerization (ROP) was similar to the methods for the **General synthesis of P1**. These series of samples with different monomer-to-initiator ratios were added dropwise to ice-cold ethyl ether to induce precipitation for three times, finally dried under high vacuum 4-5 h.

Table S2. Polymerization results of M1 with different monomer-to-initiator ratios ($[M1]_0$:$[I]_0$). Summary of polymerization conditions: [I]:[B]:[Q] = 1:2:10, $[M1]_0$ = 1.75

mol/L, 2 h, 0 °C.

$[M1]_0:[I]_0$	Mn (kDa)[a]	$Đ$[b]	Conversion (%)
50:1	9	1.18	47
100:1	13	1.21	52
150:1	19	1.27	59
200:1	18	1.24	38

[a, b] Determined by GPC analysis (RI).

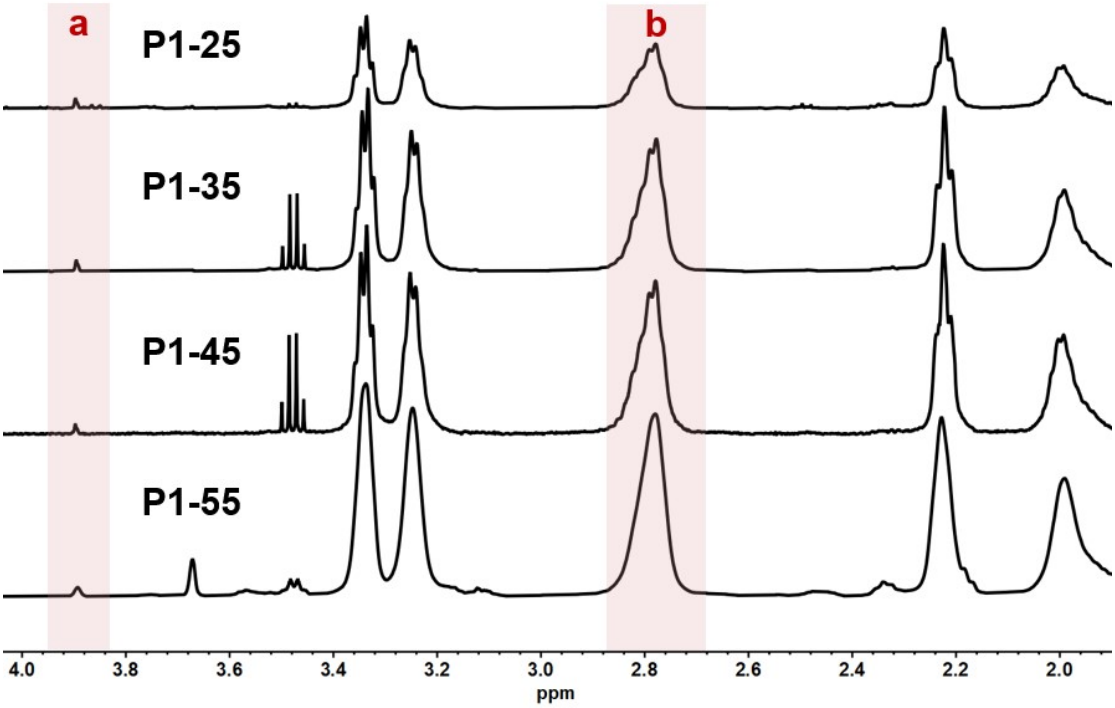


Figure S7. ^{1}H NMR spectroscopy of series P1 with different DP (DP = 25, 35, 45, and 55) after purification by recycling preparative GPC in $CDCl_3$.

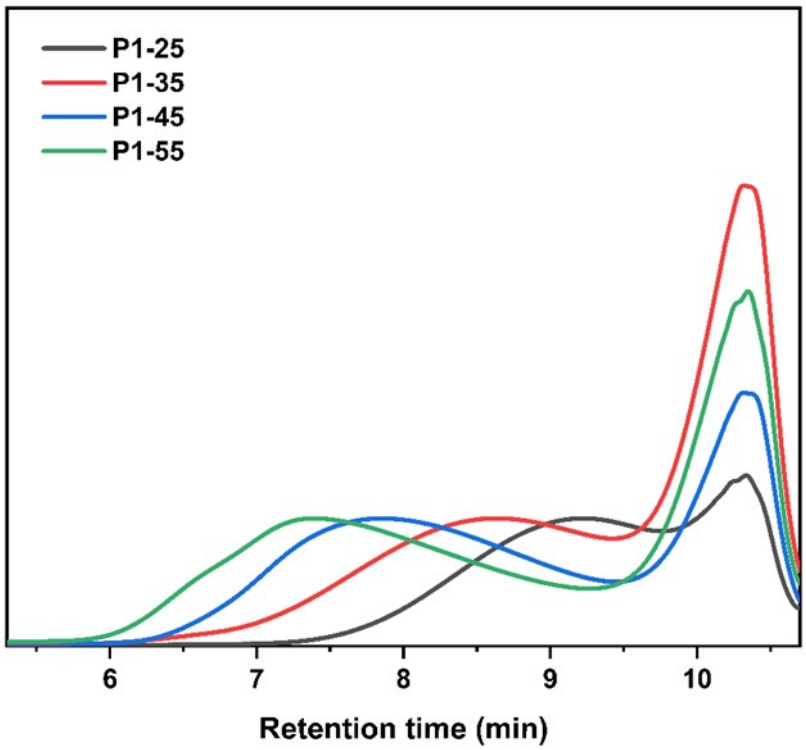


Figure S8. GPC characterization of P1 with different DP (DP = 25, 35, 45, and 55). Inevitable cyclic oligomers are present in the ring-opening polymerization (retention = 9.5-10.5 min) due to the effect of pendant group of M1.

Table S3. Polymerization conditions and results of M1 to provide a series of P1 with different DP. Summary of polymerization conditions: [I]:[B]:[Q] = 1:2:10, 2 h, 0 ℃.

Polymer	$[M1]_0$:$[I]_0$	$[M1]_0$ (mol/L)	Mn (kDa)[a]	$Đ$[b]	DP[c]
P1-25	25:1	1.75	9	1.22	25
P1-35	100:1	1.5	12	1.31	35
P1-45	100:1	2.0	15	1.32	45
P1-55	150:1	1.75	19	1.28	55

[a, b, c] Determined by GPC analysis (RI).

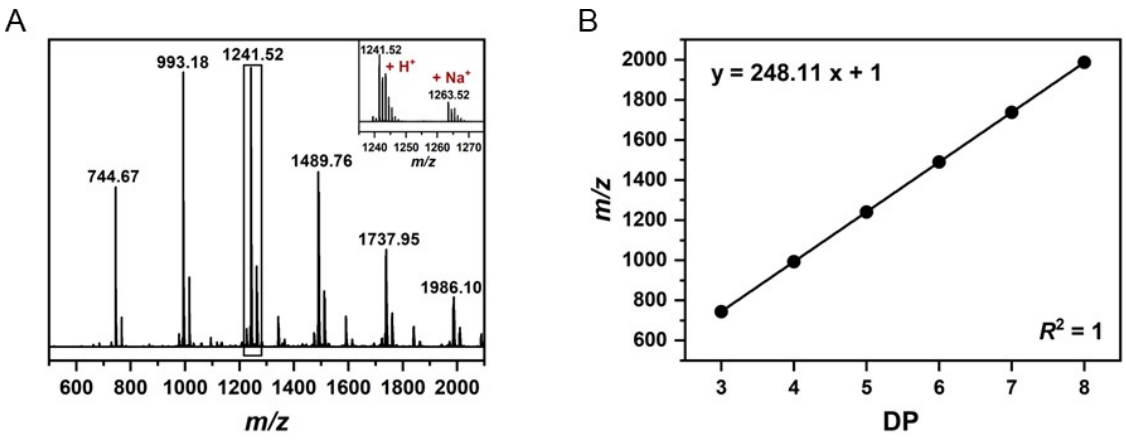


Figure S9. (A) MALDI-TOF mass spectra (MALDI-MS) analysis of the oligomer after deprotection the NH-Boc groups. (B) Linear fitting of the MALDI-MS. The results shown that the mass of the oligomer is an integer multiple of that of the repeating unit, which confirmed the cyclic structure of oligomer.

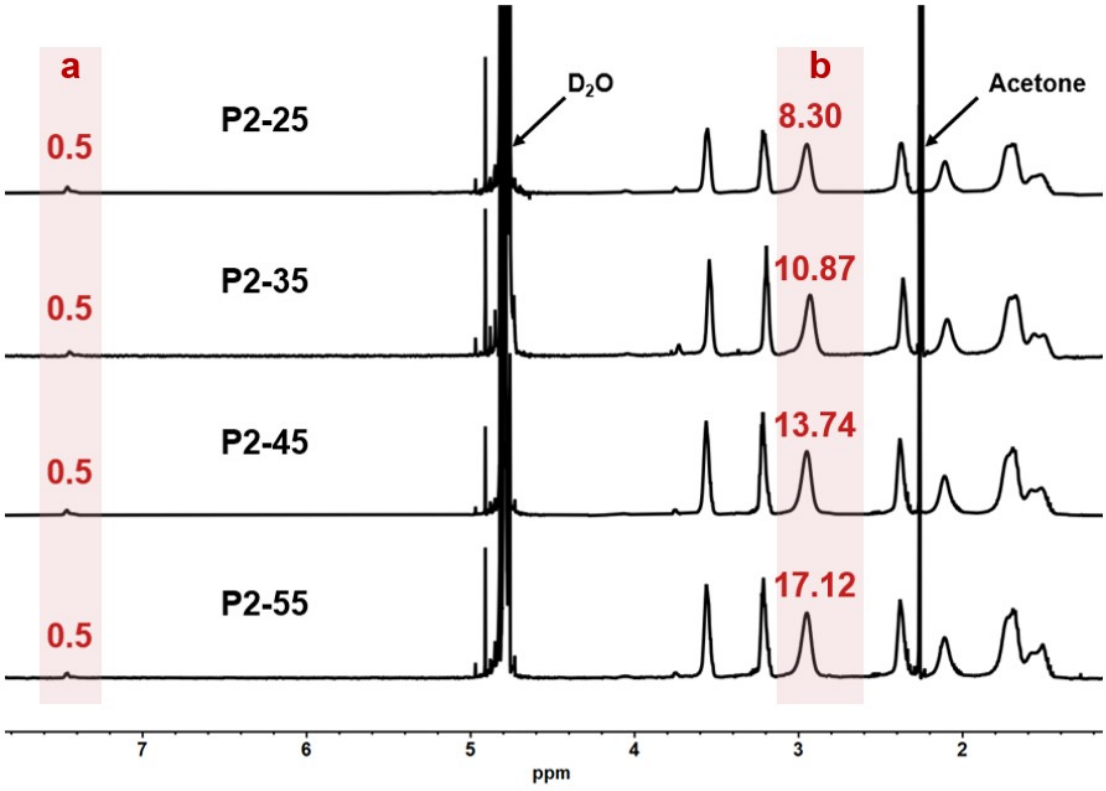


Figure S10. ^{1}H NMR spectroscopy of series P2 with different DP (DP = 25, 35, 45, and 55) in D_2O.

DP = $(I_b/3)/(I_a/5)$, i.e., DP (P2-25) = (8.30/3)/(0.5/5) = 28.

Table S4. Polymerization results of M1 to provide a series of P2 with different DP. The polymerization conditions was the same as those in **Table S3**.

Polymer	Mn (kDa)[a]	$Đ$[b]	DP[c]	DP[d]
P2-25	9	1.22	25	28
P2-35	12	1.31	35	36
P2-45	15	1.32	45	46
P2-55	19	1.28	55	57

[a, b, c] Determined by GPC analysis (RI) before deprotection;

[d] Determined by ^{1}H NMR spectroscopy (Figure S10).

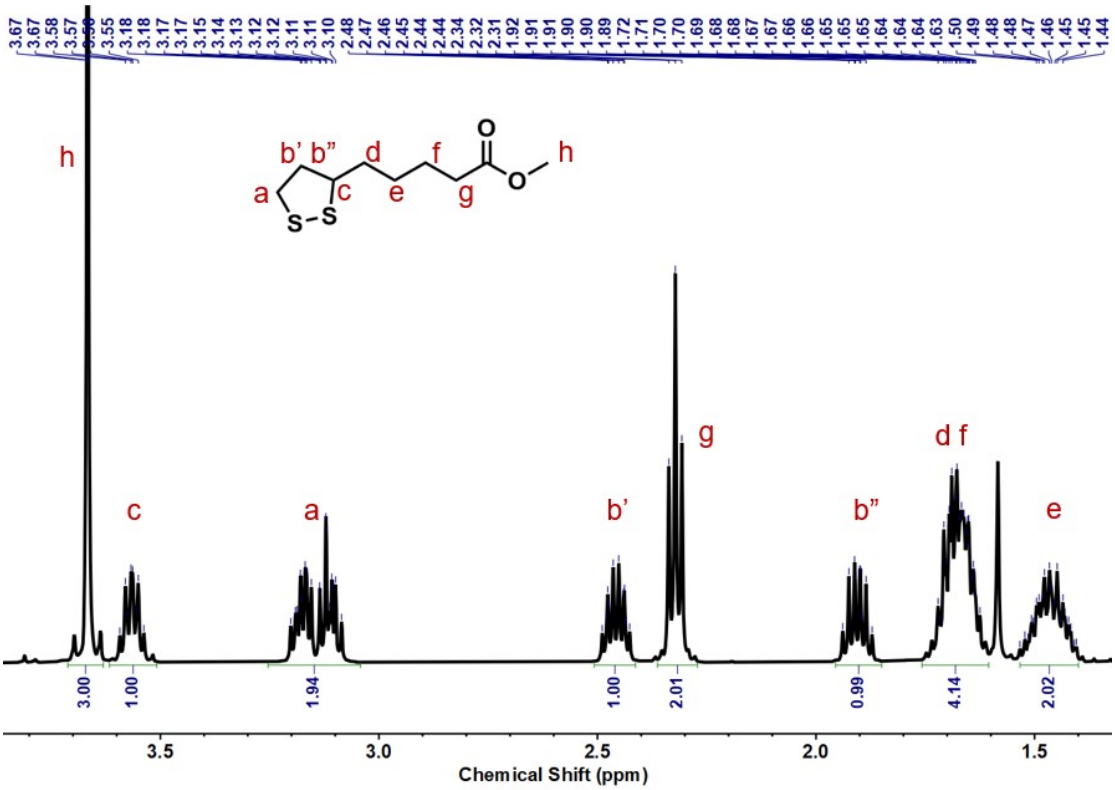


Figure S11. ^{1}H NMR spectroscopy of M2 in $CDCl_3$.

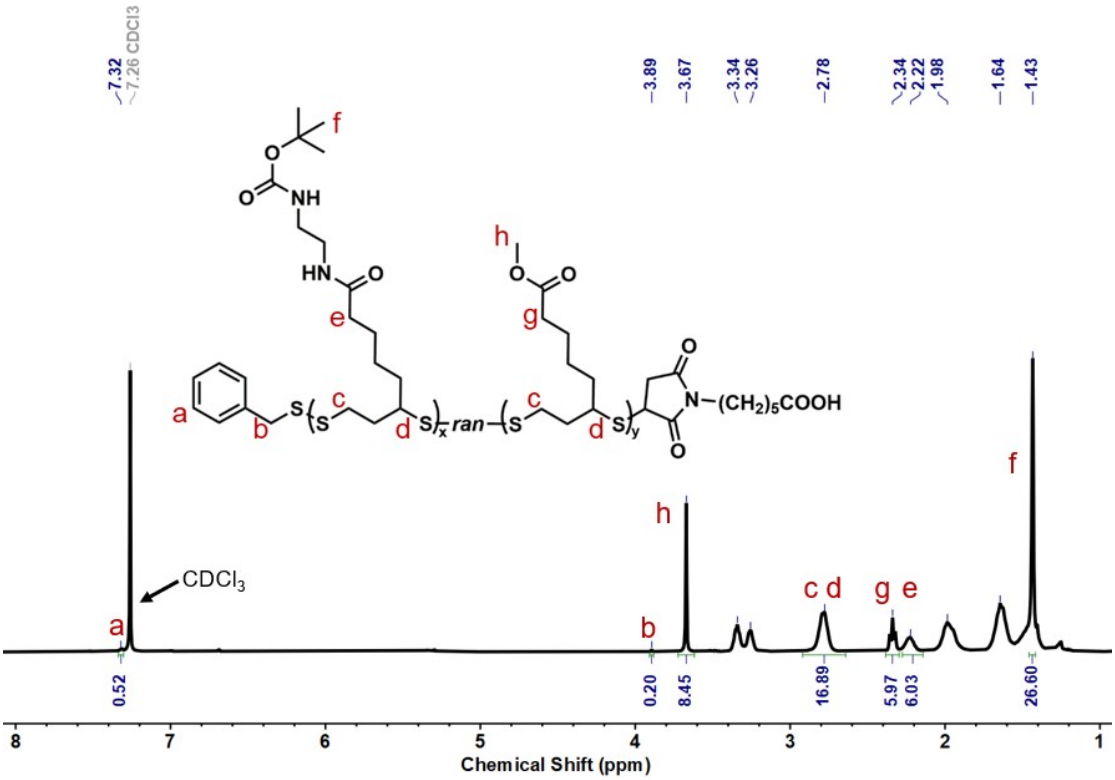


Figure S12. ^{1}H NMR spectroscopy of **P3-50%** in $CDCl_3$.

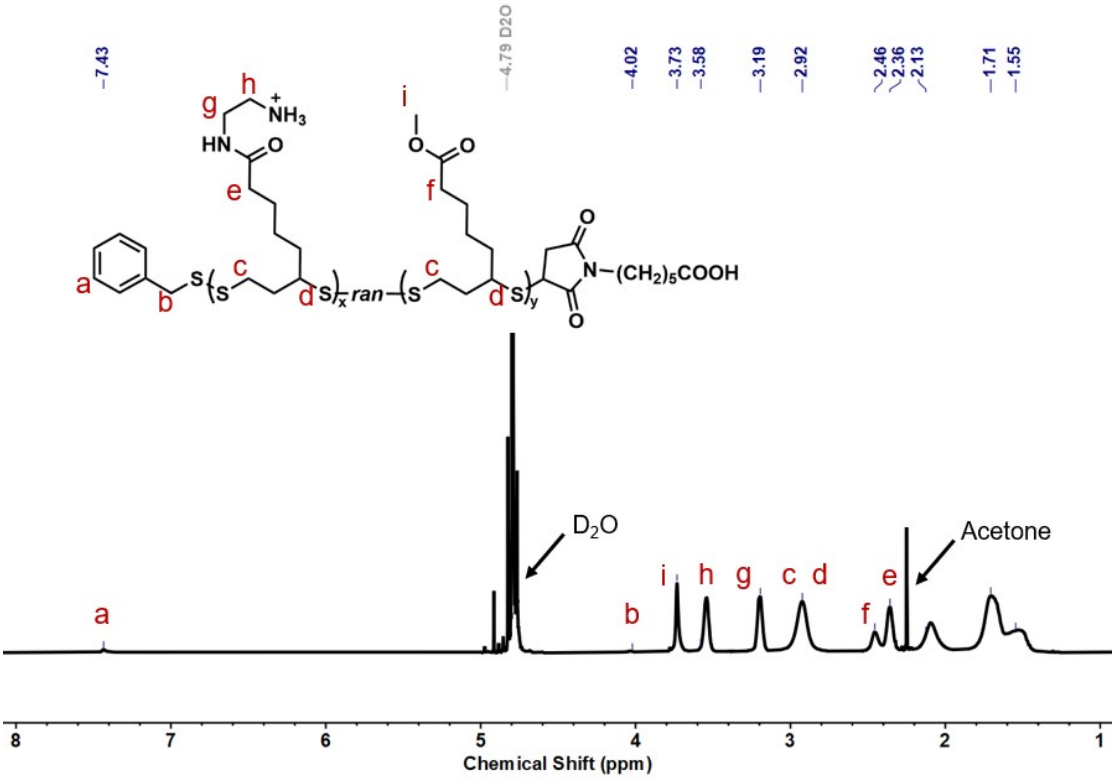


Figure S13. ^{1}H NMR spectroscopy of **P4-50%** in D_2O.

Cationic-Hydrophobic Balance Control with different polymerization conditions.

The cationic-hydrophobic balance control of the final antibacterial poly(disulfide)s 4 (P4) depends on the feeding ratio of M1 to M2 during the copolymerization. And the copolymerization with different feeding ratios of M1 to M2 was achieved by procedures similar to the **General synthesis of P3**. Briefly, to a thick-walled pressure bottle was added M1 and M2 (the ratios range from 1:3, 1:1, 2:1 to 3:1, 2.0 M, 100 equiv) in anhydrous THF, evacuated, and backfilled with Ar. The initiator solution was prepared with BnSH (0.02 M, 1 equiv) and Base (0.04 M, 2 equiv) in anhydrous THF, then, injected into the monomer solution. The reaction mixture was stirred for 2 h at 0 °C. The polymerization reaction was quenched by the 6-maleimidohexanoic acid (0.2 M, 10 equiv) solution. The mixture was also added dropwise to ice-cold ethyl ether three times to induce precipitation. Finally, these series of samples were dried under high vacuum to obtain pure P3 with different fraction of hydrophobic repeating units as white powder.

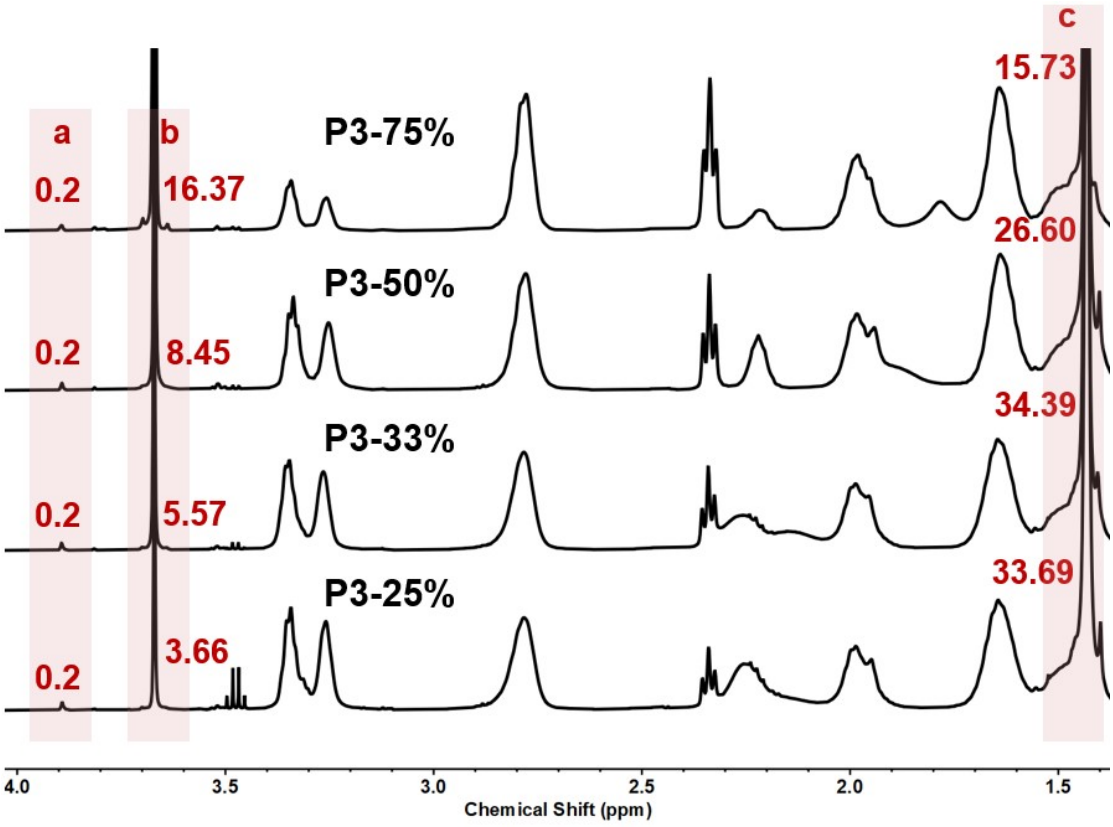


Figure S14. ^{1}H NMR spectroscopy of series P3 with different fraction of hydrophobic repeating units (75%, 50%, 33%, and 25%) after purification by recycling preparative GPC in $CDCl_3$.

I_a: The integration of the peak at 3.89 ppm; I_b: The integration of the peak at 3.67 ppm;

I_c: The integration of the peak at 1.43 ppm;

N(M1) and N(M2): The amount of M1 and M2 in P3;

N(M1):N(M2) = $[(I_c/9)/(I_a/2)]:[(I_b/3)/(I_a/2)]$, i.e., N(M1):N(M2) (P3-25%) = (3.74/0.1):(1.22/0.1) = 3.07:1.

Table S5. Polymerization results of M1 and M2 with different fraction of hydrophobic repeating units (75%, 50%, 33%, and 25%). Summary of polymerization conditions: $[M1+M2]_0:[I]_0:[B]:[Q] = 100:1:2:10$, $[M1+M2]_0 = 2.0$ mol/L, 2 h, 0 °C.

$[M1]_0:[M2]_0$	Mn (kDa)[a]	$Đ$[b]	Conversion (%)	N(M1):N(M2)[c]	Hydrophobic Fraction (%)
25:75	18	1.39	35	0.32:1	75
50:50	19	1.29	41	1.05:1	50
66:34	17	1.30	55	2.06:1	33
75:25	17	1.29	53	3.07:1	25

[a, b] Determined by GPC analysis (RI); [c] Determined by ^{1}H NMR spectroscopy.

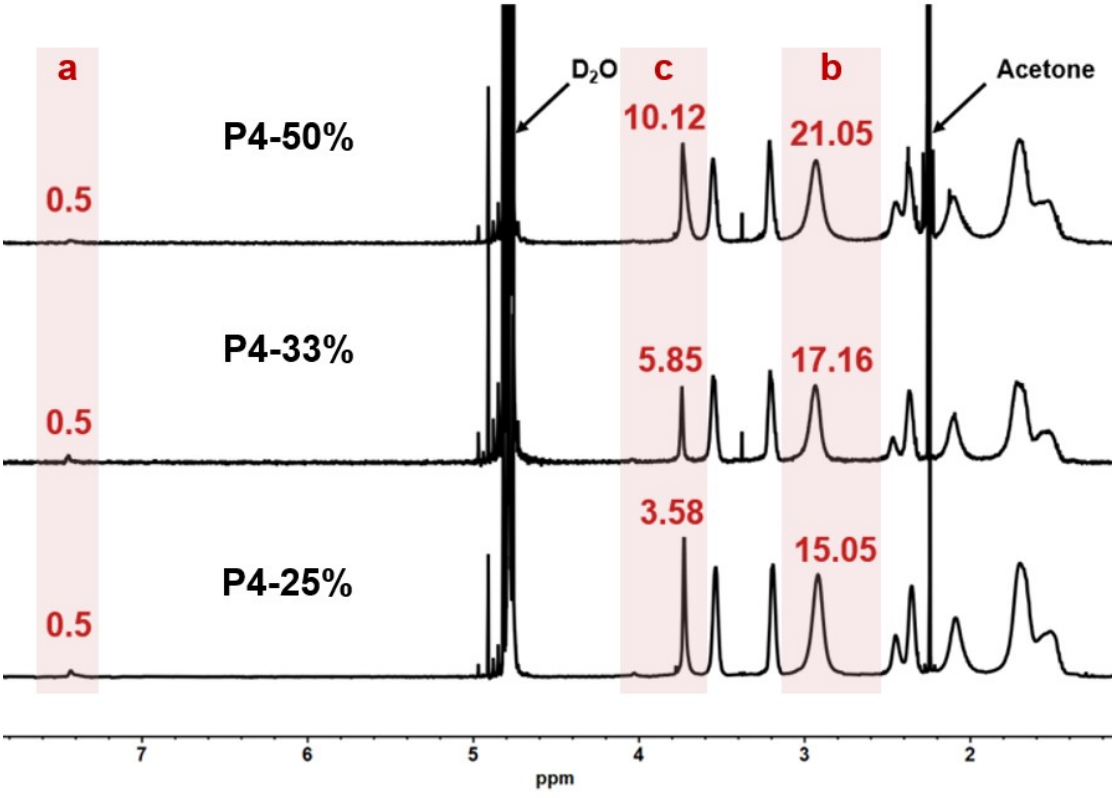


Figure S15. ^{1}H NMR spectroscopy of series P4 with different fraction of hydrophobic repeating units (50%, 33%, and 25%) in D_2O.

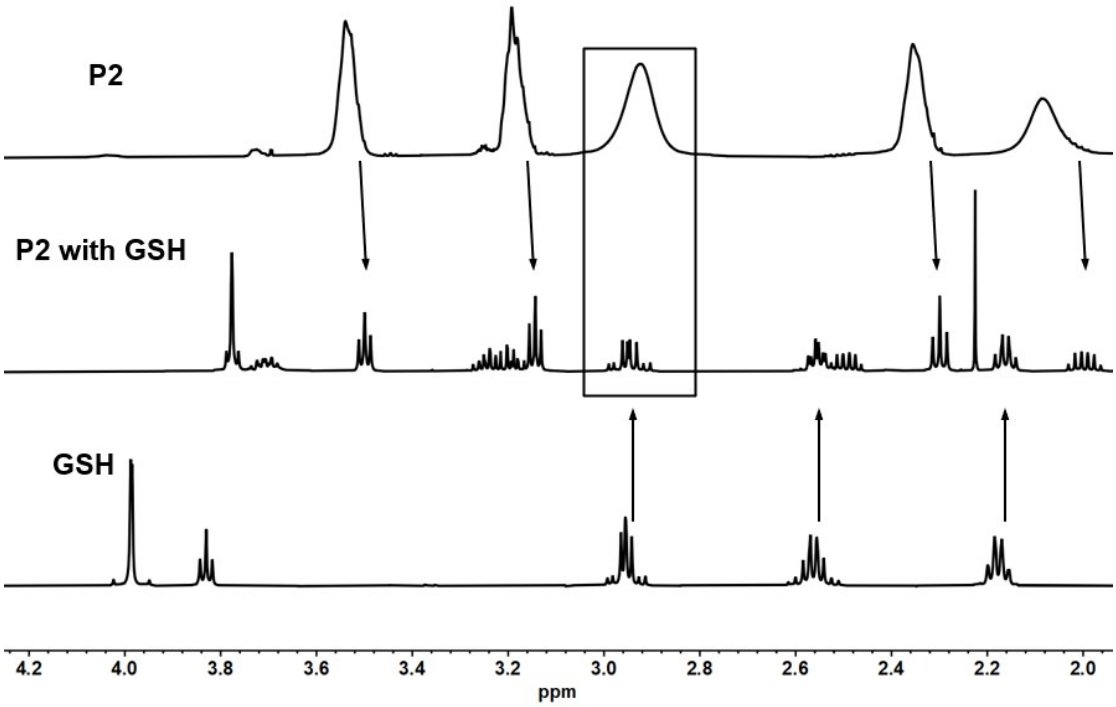


Figure S16. ^{1}H NMR spectroscopy of the P2-55 before and after treated with GSH in D_2O in pH = 7.0 for 5 min at 37 ℃.

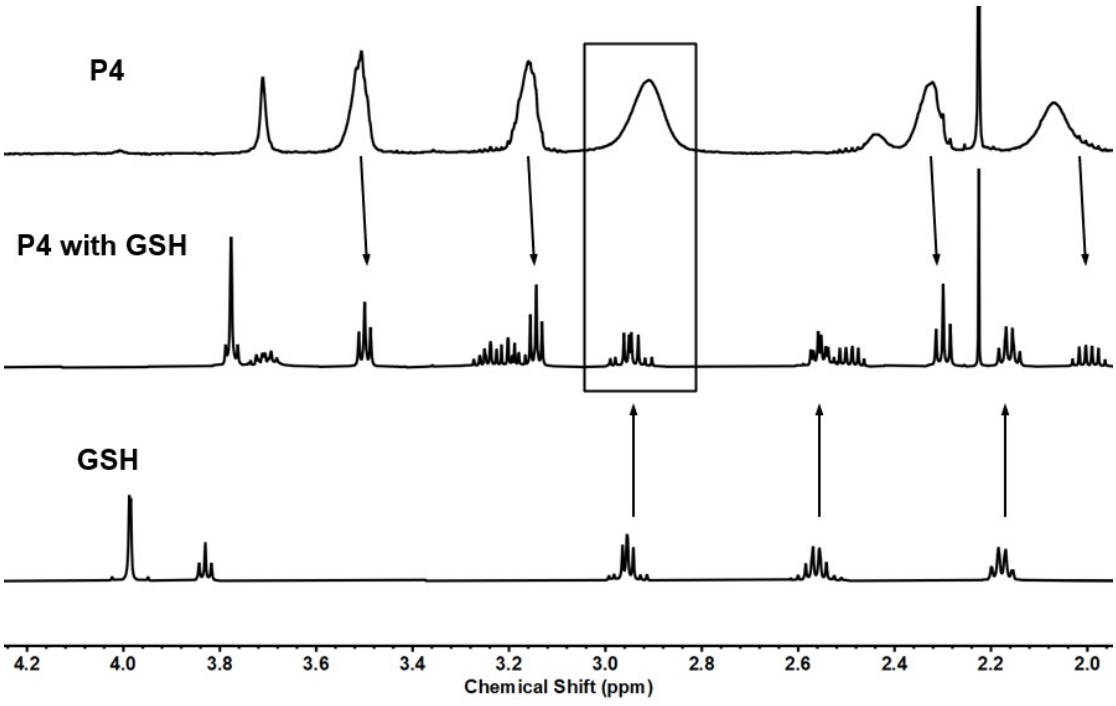


Figure S17. ^{1}H NMR spectroscopy of the P4-25% before and after treated with GSH in D_2O in pH = 7.0 for 5 min at 37 ℃.

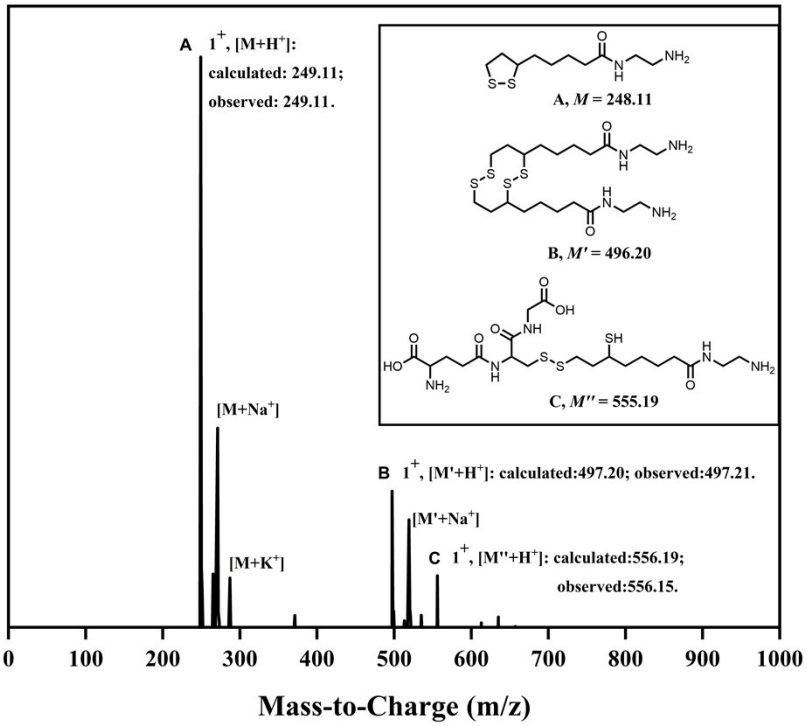


Figure S18. ESI-MS of degraded P2 treated with 10 mM GSH in pH = 7.0 for 5 min at 37°C.

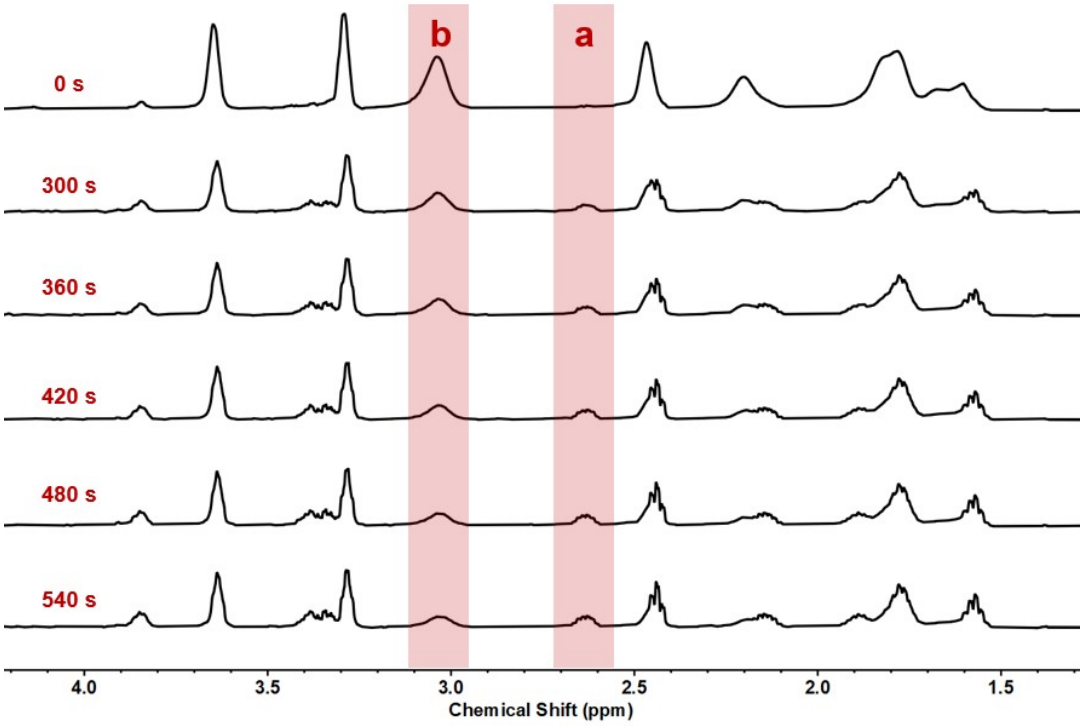


Figure S19. ^{1}H NMR spectroscopy of P2-45 ([Repeating Unit, RU]$_0$ = 0.045 M) during depolymerization triggered by 0.2 mM GSH in pH = 7.0 at 37°C. The [RU] is calculated by the equation: $[RU] = [RU]_0 \times (I_b/3)/(I_a+I_b/3)$. After mixed with GSH solution, the ^{1}H NMR spectra was recorded first time at 300 s, and test every 60 s until the polymer was

half degraded.

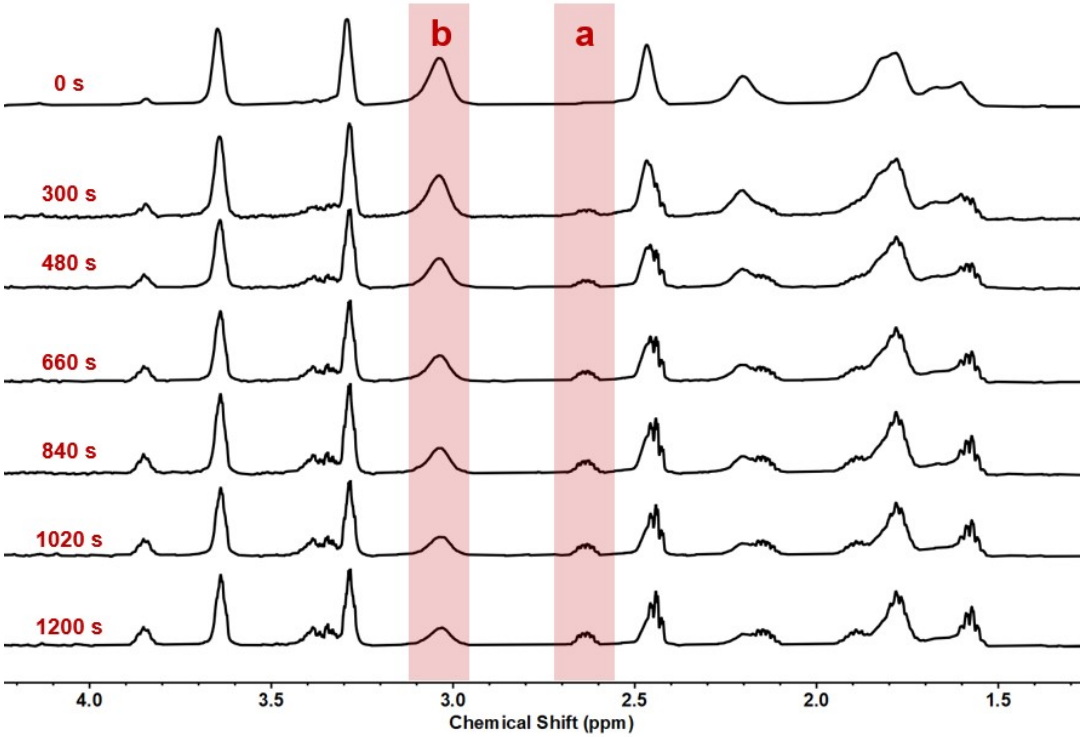


Figure S20. ^{1}H NMR spectroscopy of P2-45 ($[RU]_0$ = 0.045 M) during depolymerization triggered by 0.1 mM GSH in pH = 7.0 at 37℃. After mixed with GSH solution, the ^{1}H NMR spectra was recorded first time at 300 s, and test every 180 s until the polymer was half degraded.

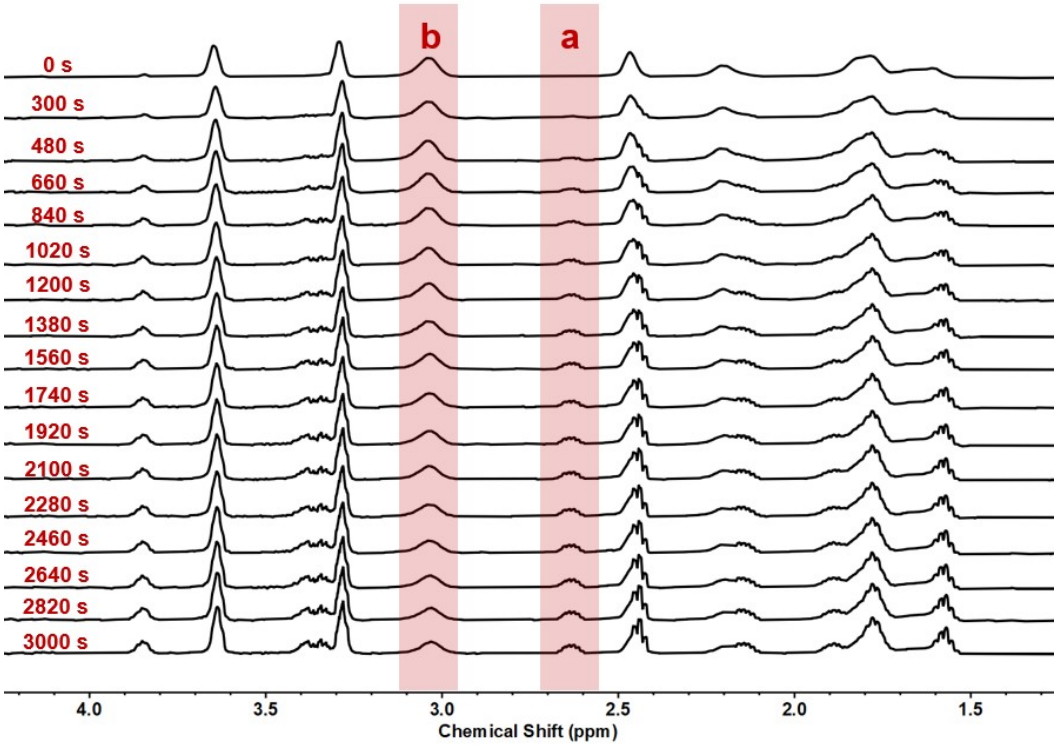


Figure S21. ^{1}H NMR spectroscopy of P2-45 ($[RU]_0$ = 0.045 M) during

depolymerization triggered by 0.05 mM GSH in pH = 7.0 at 37℃. After mixed with GSH solution, the ^{1}H NMR spectra was recorded first time at 300 s, and test every 180 s until the polymer was half degraded.

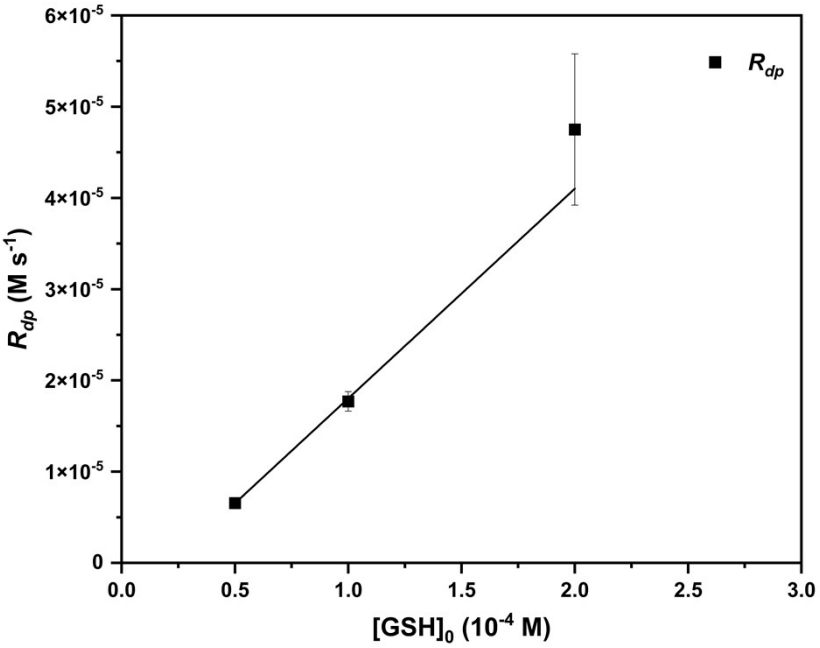
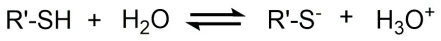
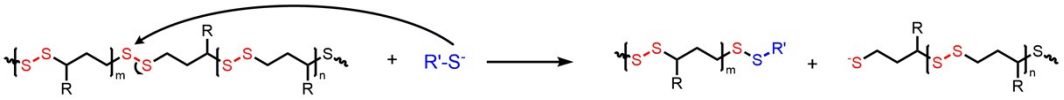


Figure S22. Scatter plot of depolymerization rates (R_{dp}, R_{dp} = -d[RU]/dt) of P2-45 ([RU]$_0$ = 0.045 M) with different concentrations of GSH ([GSH]$_0$ = 0.05 mM, 0.1 mM, 0.2 mM) in pH = 7.0 at 37℃. After linear fitting, the slope is k_{dp}^{app} = 0.23±0.02 s^{-1}, which means: R_{dp} = k_{dp}^{app}[GSH]^1. Accordingly, we proposed the degradation mechanism includes the bellowing three steps:

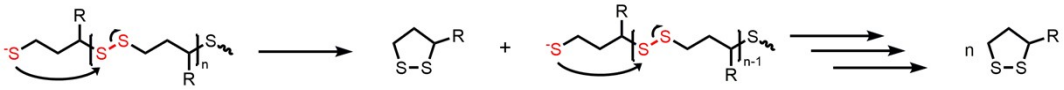
(1) GSH (R'-SH) ionizes S$^-$ in water, K$_a$ = $10^{-9.2}$:



(2) Thiolate attack disulfide bonds in the polymer backbone:



(3) Ring-closing depolymerization, k$_{rc}$ = 0.95 s^{-1} (calculated by Matile *et al.*[48]):



When the [R'-S$^-$] ionized from GSH in solution is much smaller than the [RU], R_{dp} = k_{dp}^{app}[GSH]^1, and k_{dp}^{app} = 0.23±0.02 s^{-1}, the step (2) is the rate-determining step of the degradation of poly(disulfide)s by GSH.

Table S6. Summary of MIC values measured multiple times for different P2.

Bacteria	Number of times	Degraded P2	P2-25	P2-35	P2-45	P2-55
MIC aginst *S. aureus*	1	400	100	100	200	100
	2	400	100	100	200	100
	3	400	100	100	200	100
	Average	400	100	100	200	100
MIC aginst *E. coli*	1	800	100	100	200	200
	2	800	200	200	400	200
	3	800	200	200	400	200
	Average	800	167±47	167±47	333±94	200

Table S7. Summary of MIC values measured multiple times for different P4.

Bacteria	Number of times	P4-50%	P4-33%	P4-25%
MIC aginst *S. aureus*	1	400	400	100
	2	400	200	100
	3	400	400	100
	Average	400	333±94	100
MIC aginst *E. coli*	1	400	400	100
	2	400	200	100
	3	400	200	100
	Average	400	267±94	100

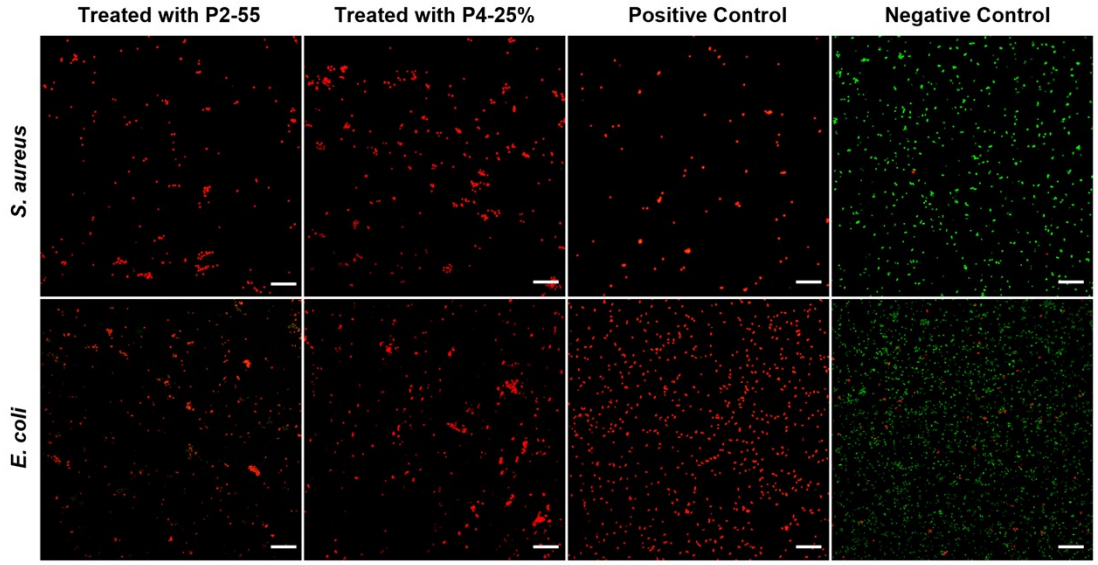


Figure S23. Fluorescence confocal images of *S. aureus* and *E. coli* treated with P2-55 or P4-25% (2×MIC) for 4 h and then subjected to live/dead staining (merged). Isopropyl alcohol (70%) treated bacteria were used as positive controls and untreated bacteria

were used as negative controls. SYTO 9 stain (green fluorescence) generally labels bacteria with intact membranes (live), while propidium iodide (PI, red fluorescence) penetrates only bacteria with damaged membranes (dead). The scale bar is 20 μm.

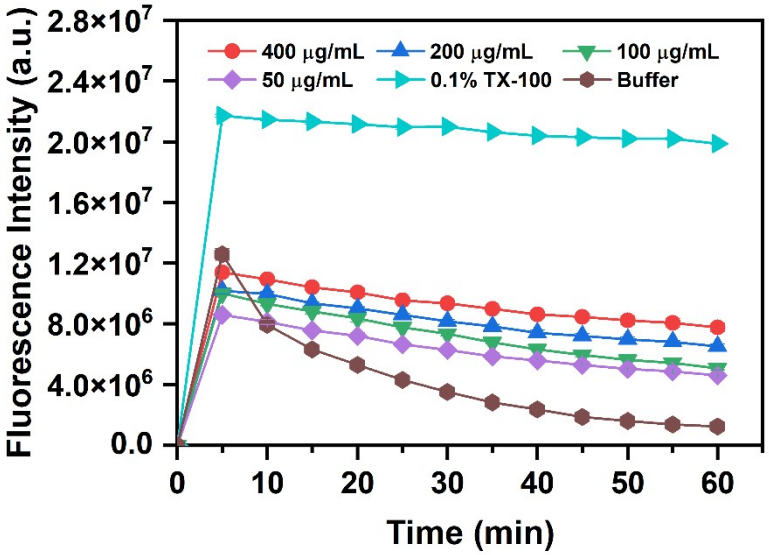


Figure S24. The P2-55 caused cytoplasmic membrane depolarization in *E. coli* quantified with the fluorescence of DISC3(5).

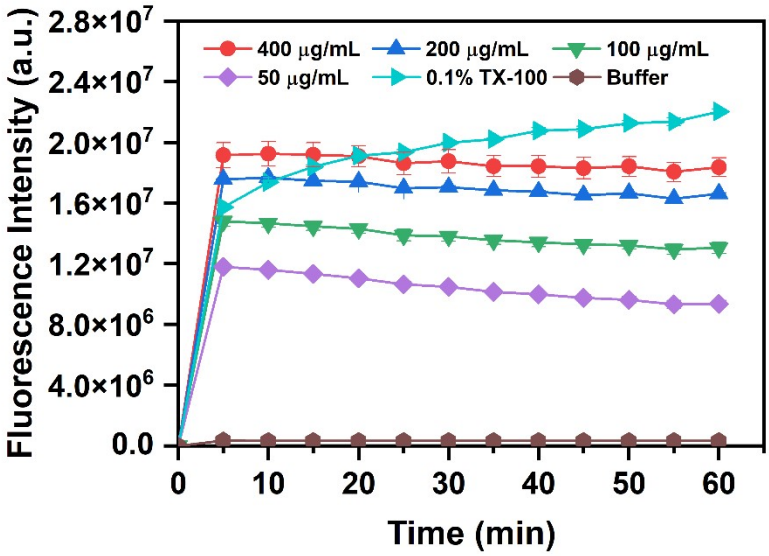


Figure S25. The P4-25% caused cytoplasmic membrane depolarization in *S. aureus* quantified with the fluorescence of DISC3(5).

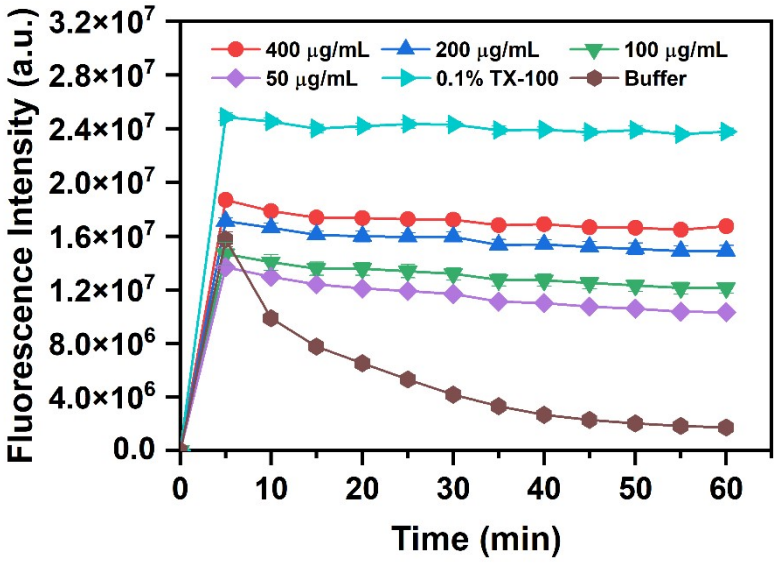


Figure S26. The P4-25% caused cytoplasmic membrane depolarization in *E. coli* quantified with the fluorescence of DISC3(5).

Table S8. The percentage of membrane depolarization of P2-55 and P4-25% against *S. aureus* and *E. coli* with different concentrations.

Polymer	Bacteria	Concentration (μg/mL)	Depolarization (%, Average)
P2-55	*S. aureus*	50 (0.5×MIC)	25
		100 (1×MIC)	28
		200 (2×MIC)	38
		400 (4×MIC)	44
	E. coli	50 (0.25×MIC)	18
		100 (0.5×MIC)	21
		200 (1×MIC)	28
		400 (2×MIC)	35
P4-25%	*S. aureus*	50 (0.5×MIC)	42
		100 (1×MIC)	59
		200 (2×MIC)	75
		400 (4×MIC)	83
	E. coli	50 (0.5×MIC)	39
		100 (1×MIC)	47
		200 (2×MIC)	60
		400 (4×MIC)	68

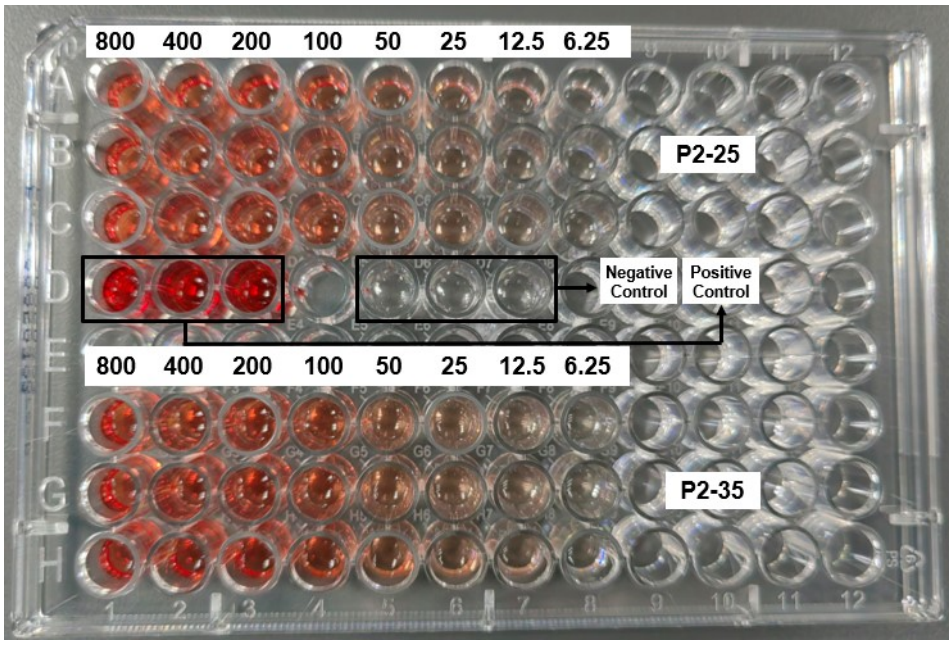


Figure S27. Hemolysis image on a 96-well plate of dilutions of P2-25 and P2-35 (μg mL^{-1}).

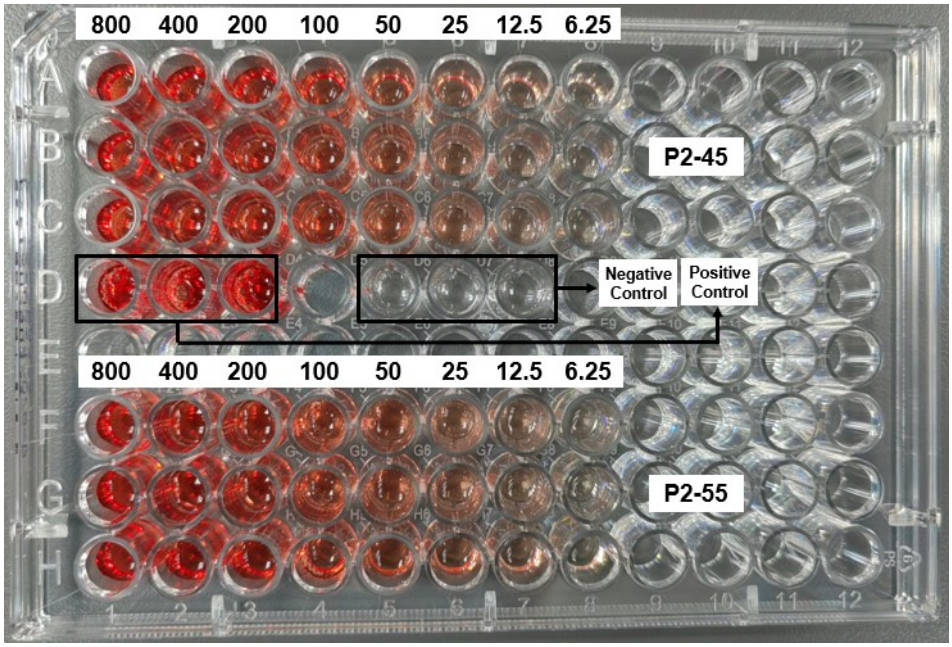


Figure S28. Hemolysis image on a 96-well plate of dilutions of P2-45 and P2-55 (μg mL^{-1}).

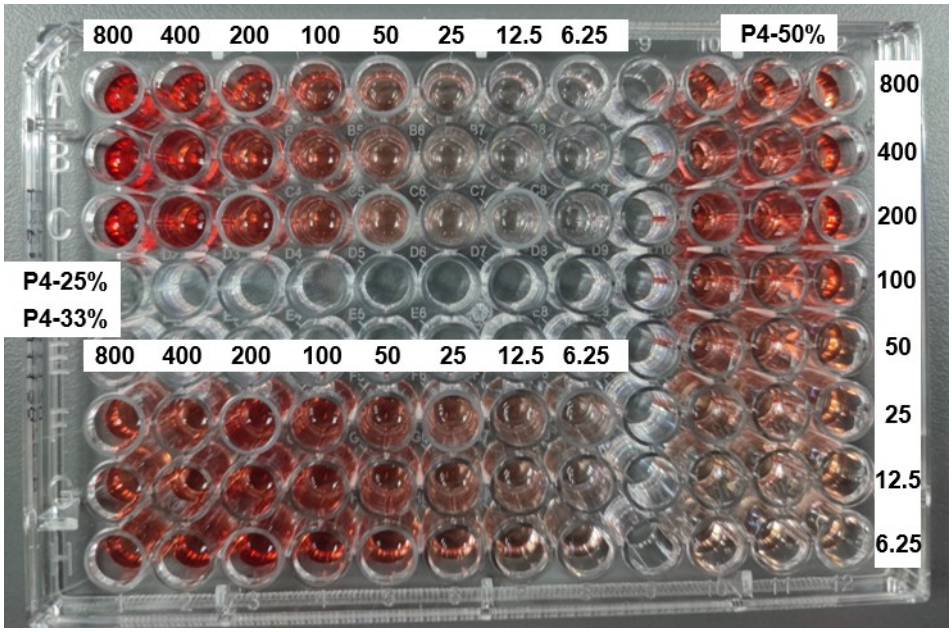


Figure S29. Hemolysis image on a 96-well plate of dilutions of P4-25%, P4-33% and P4-50% (μg mL^{-1}).

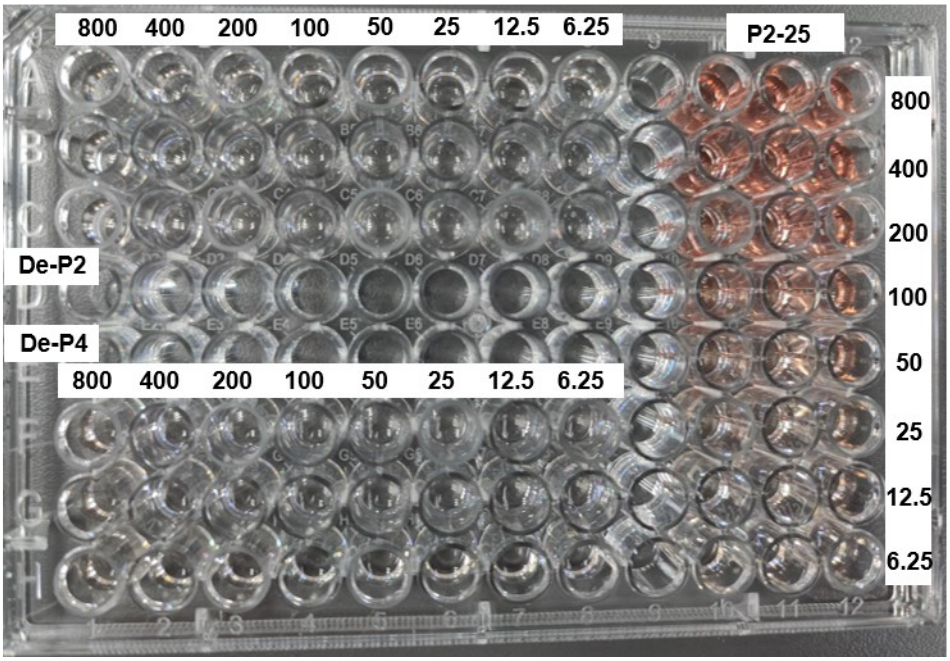


Figure S30. Hemolysis image on a 96-well plate of dilutions of Degraded P2 and Degraded P4 (μg mL^{-1}).