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Supplemental Information

High-Throughput Preparation of Antibacterial Polymers from Natural Product Derivatives via the Hantzsch Reaction

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Supporting Data

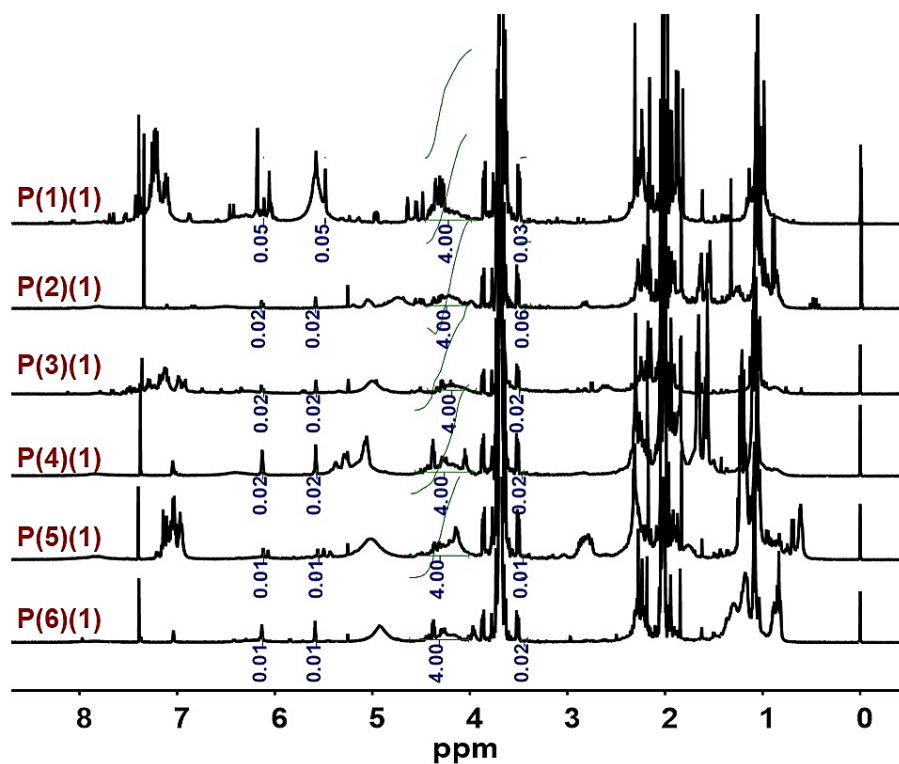


Figure S1. ^1H NMR spectra (CDCl_3 , 400M) of crude **P(X)(1)** after HTP polymerization for conversion calculation. Related to Figure 1.

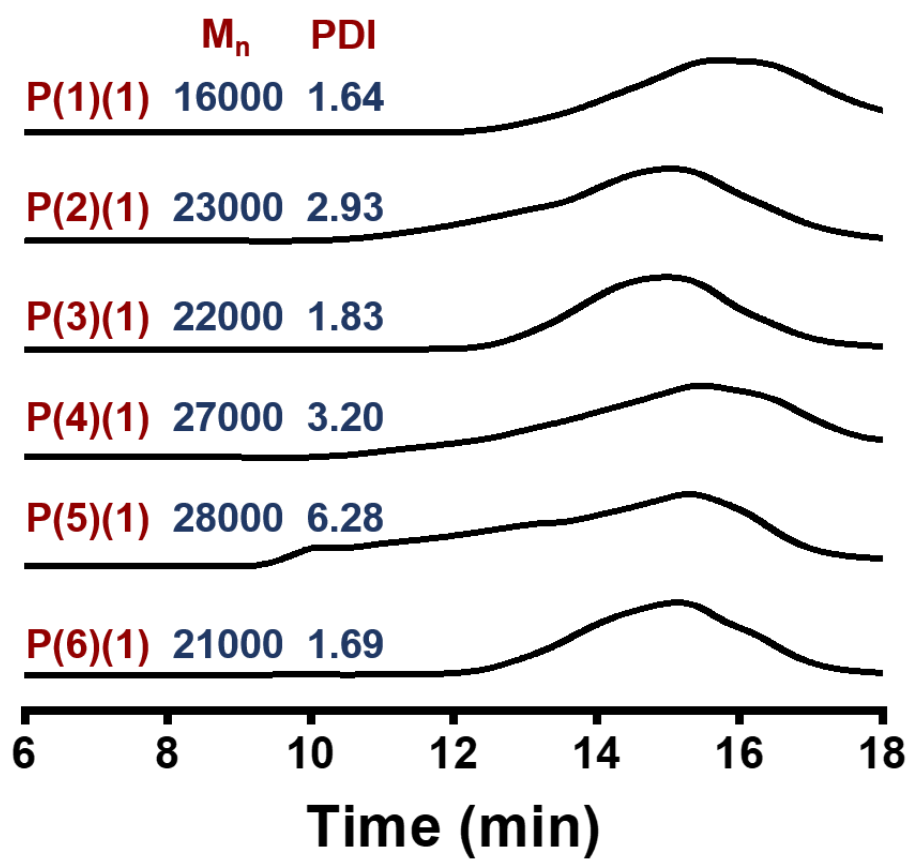


Figure S2. GPC traces of P(X)(1). Related to Figure 1.

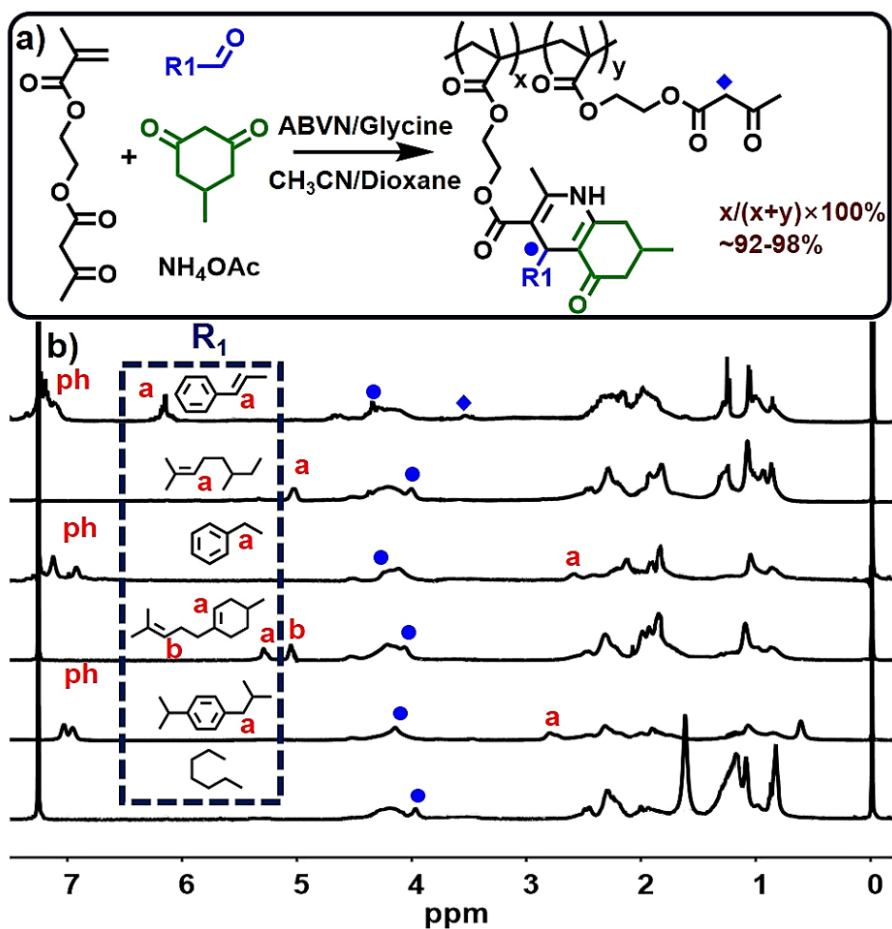


Figure S4. a) Reaction conditions: acetonitrile/dioxane (1/1, v/v) as the solvent, glycine (10% to aldehydes) as the Hantzsch reaction catalyst, ABVN (2% to AEMA) as the initiator for FRP, 75°C, and 12 h. **A(X): B(3):** AEMA: NH_4OAc = 1:1:1:1.5. b) 1H NMR spectra ($CDCl_3$, 400M) of $P(X)(3)$. Related to Figure 1.

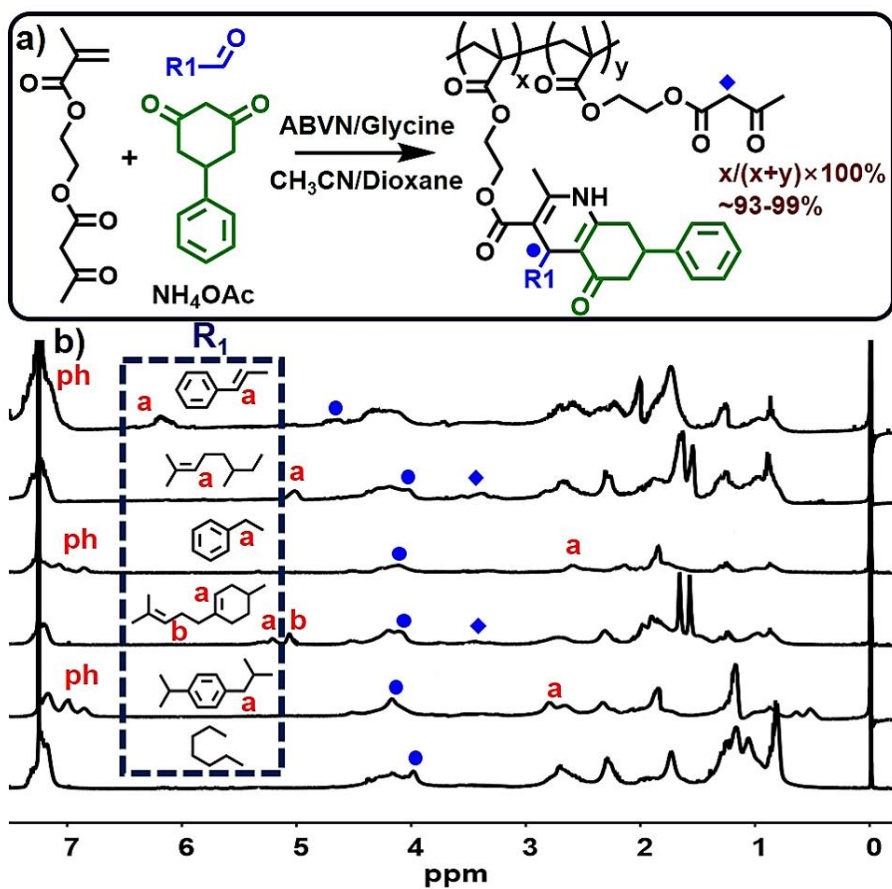


Figure S5. a) Reaction conditions: acetonitrile/dioxane (1/1, v/v) as the solvent, glycine (10% to aldehyde) as the Hantzsch reaction catalyst, ABVN (2% to AEMA) as the initiator for FRP, 75°C, and 12 h. **A(X): B(4): AEMA: NH₄OAc = 1:1:1:1.5.** b) ¹H NMR spectra (CDCl₃, 400M) of **P(X)(4)**. Related to Figure 1.

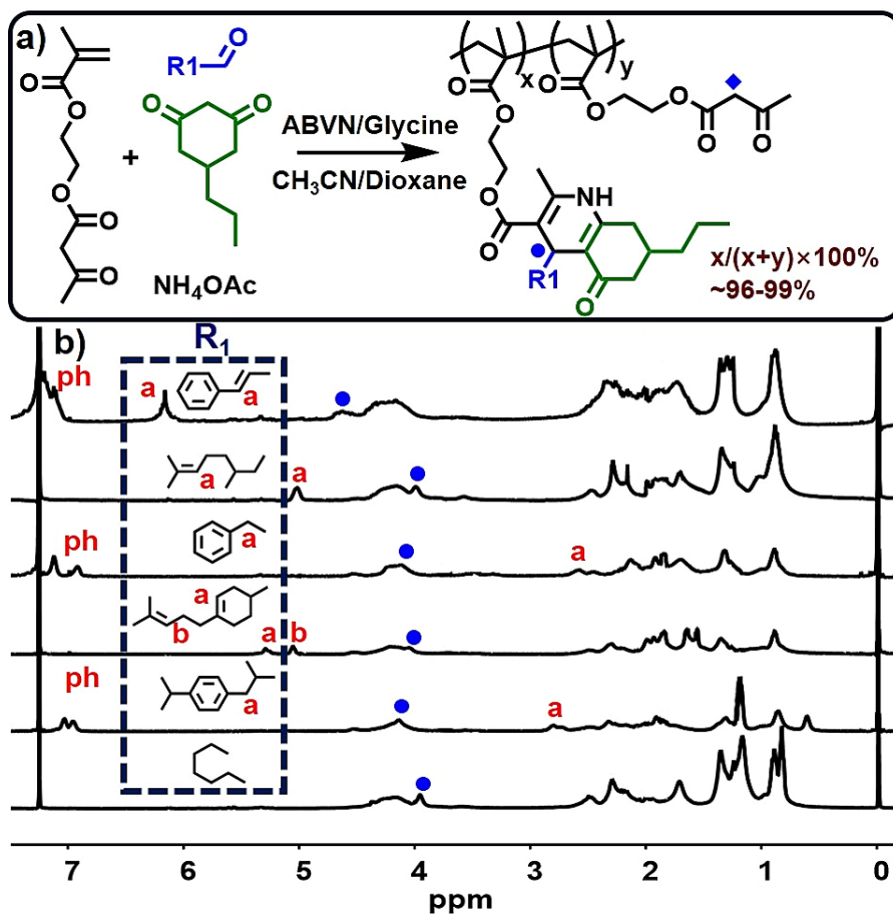


Figure S6. a) Reaction conditions: acetonitrile/dioxane (1/1, v/v) as the solvent, glycine (10% to aldehyde) as the Hantzsch reaction catalyst, ABVN (2% to AEMA) as the initiator for FRP, 75°C, and 12 h. **A(X): B(5): AEMA: NH₄OAc = 1:1:1:1.5.** b) ¹H NMR spectra (CDCl₃, 400M) of **P(X)(5)**. Related to Figure 1.

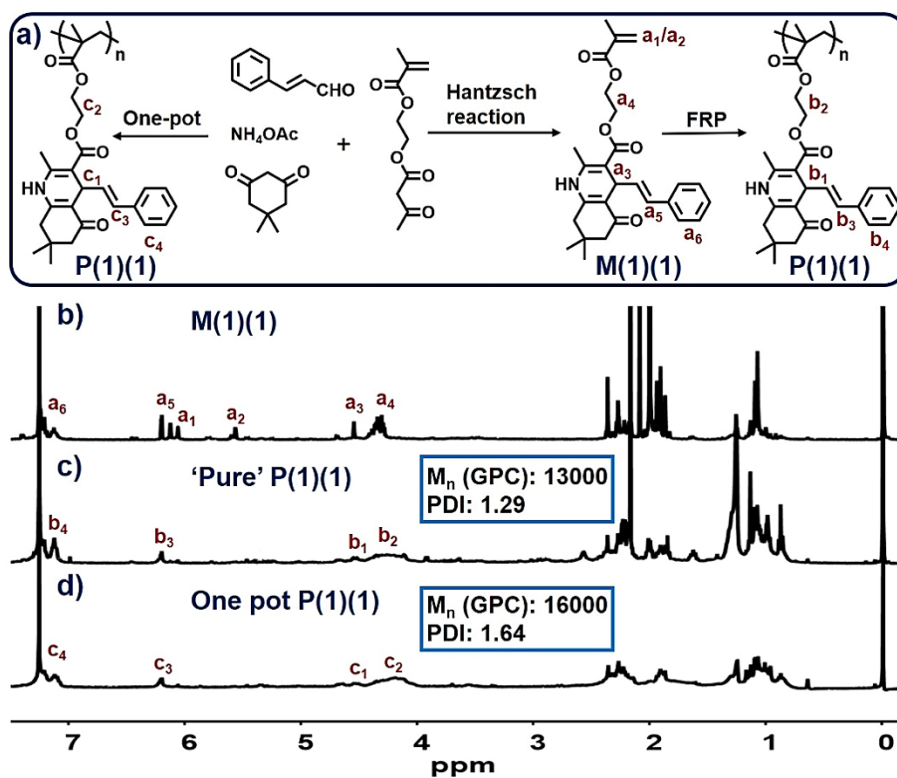


Figure S7. a) Preparations of **P(1)(1)** from the traditional monomer–polymer method and one-pot strategy. ^1H NMR spectra (CDCl_3 , 400M) of b) **M(1)(1)**, c) “pure” **P(1)(1)**, and d) **P(1)(1)** via the one-pot Hantzsch–FRP method. Related to Figure 1.

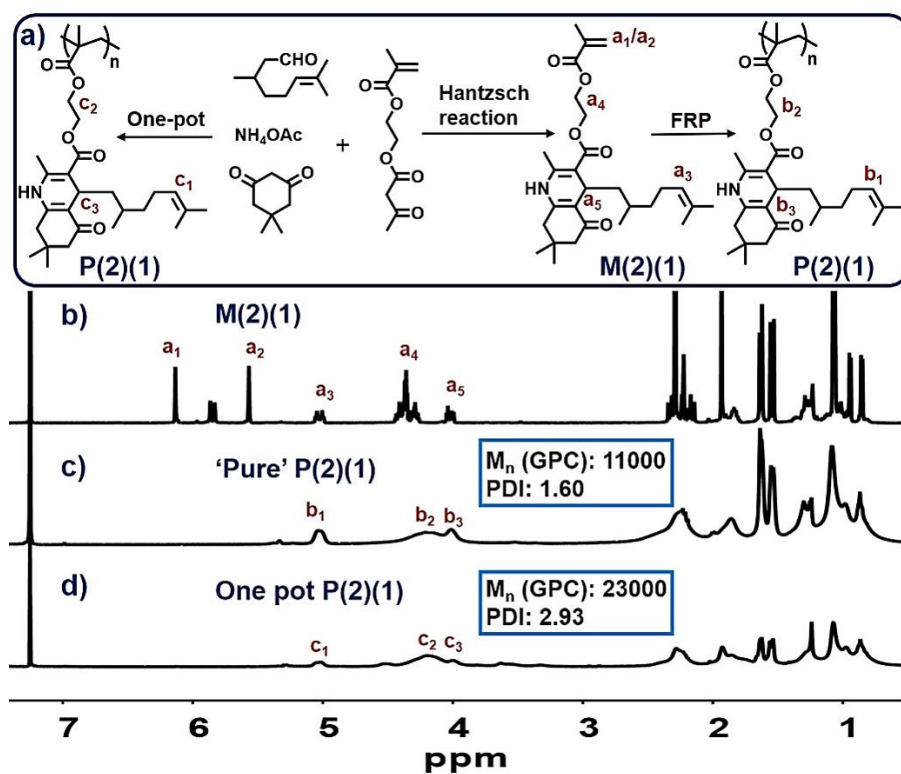


Figure S8. a) Preparations of **P(2)(1)** from the traditional monomer–polymer method and one-pot strategy. ¹H NMR spectra (CDCl₃, 400M) of b) **M(2)(1)**, c) “pure” **P(2)(1)**, and d) **P(2)(1)** *via* the one-pot Hantzsch–FRP method. Related to Figure 1.

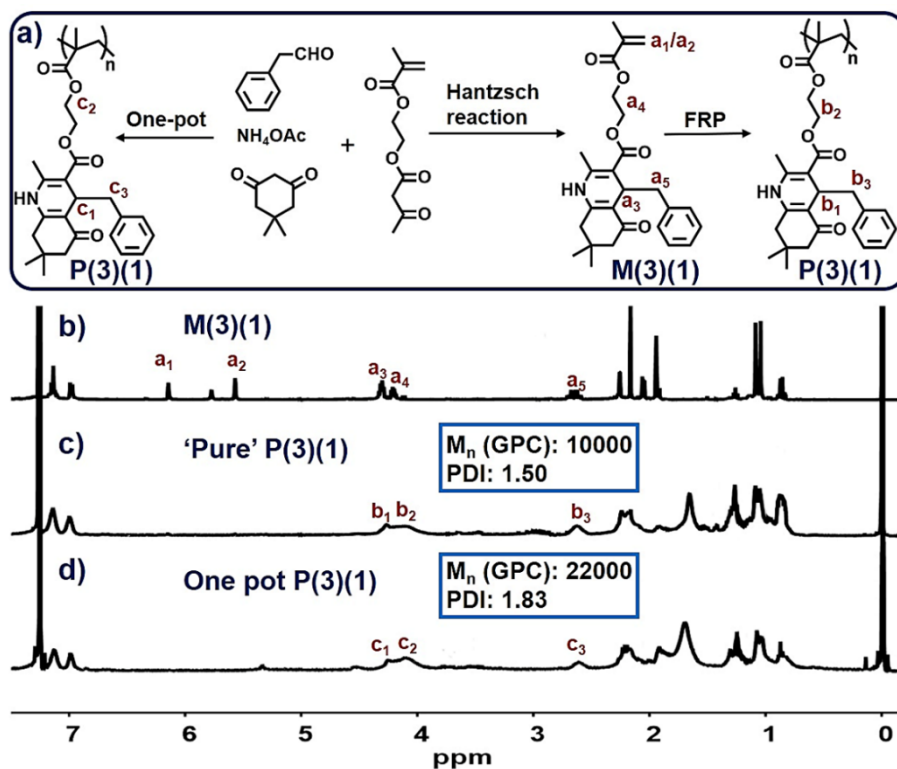


Figure S9. a) Preparations of **P(3)(1)** from the traditional monomer–polymer method and one-pot strategy. ¹H NMR spectra (CDCl₃, 400M) of b) **M(3)(1)**, c) “pure” **P(3)(1)**, and d) **P(3)(1)** *via* the one-pot Hantzsch–FRP method. Related to Figure 1.

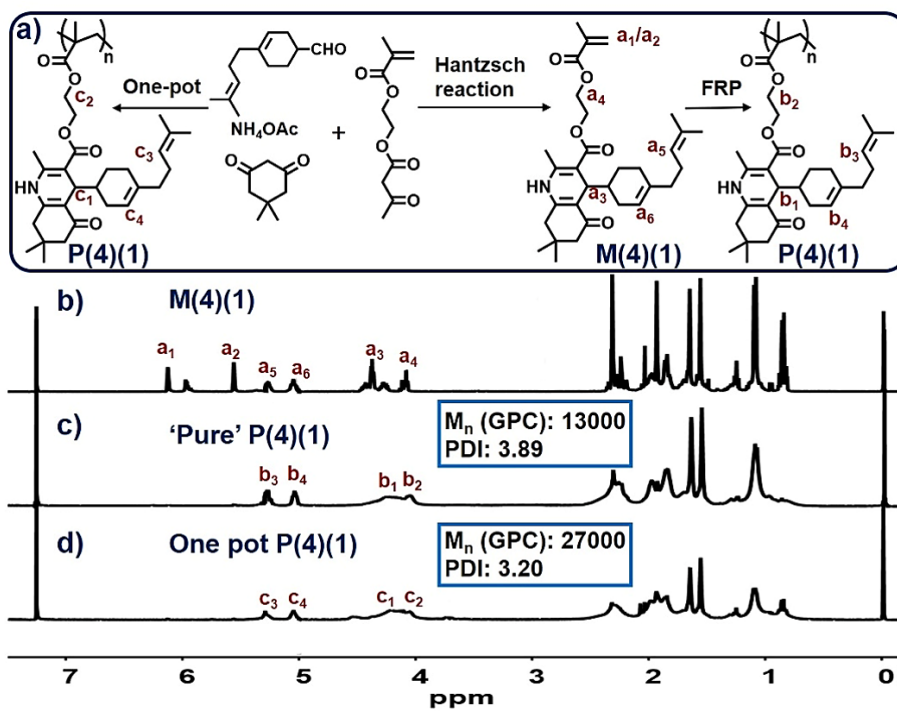


Figure S10. a) Preparations of P(4)(1) from the traditional monomer–polymer method and one-pot strategy. ¹H NMR spectra (CDCl₃, 400M) of b) M(4)(1), c) “pure” P(4)(1), and d) P(4)(1) via the one-pot Hantzsch–FRP method. Related to Figure 1.

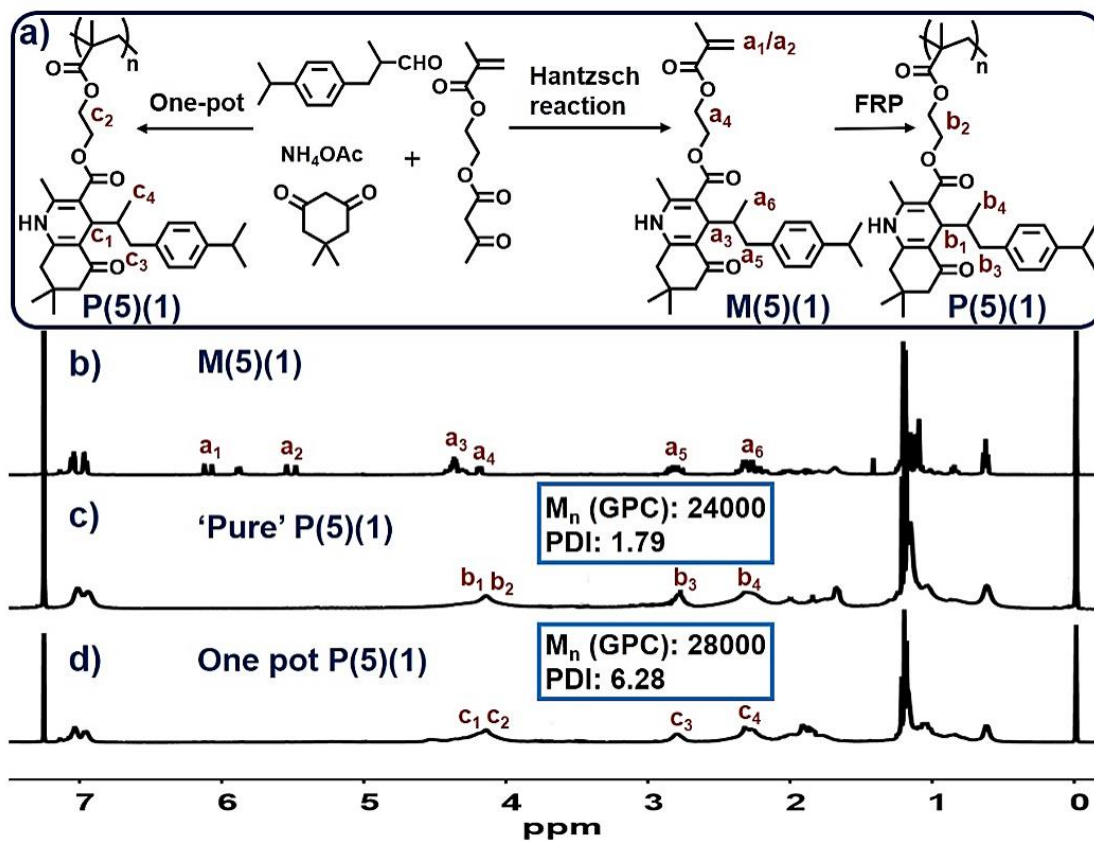


Figure S11. a) Preparations of **P(5)(1)** from the traditional monomer–polymer method and one-pot strategy. ^1H NMR spectra (CDCl_3 , 400M) of b) **M(5)(1)**, c) “pure” **P(5)(1)**, and d) **P(5)(1)** via the one-pot Hantzsch–FRP method. Related to Figure 1.

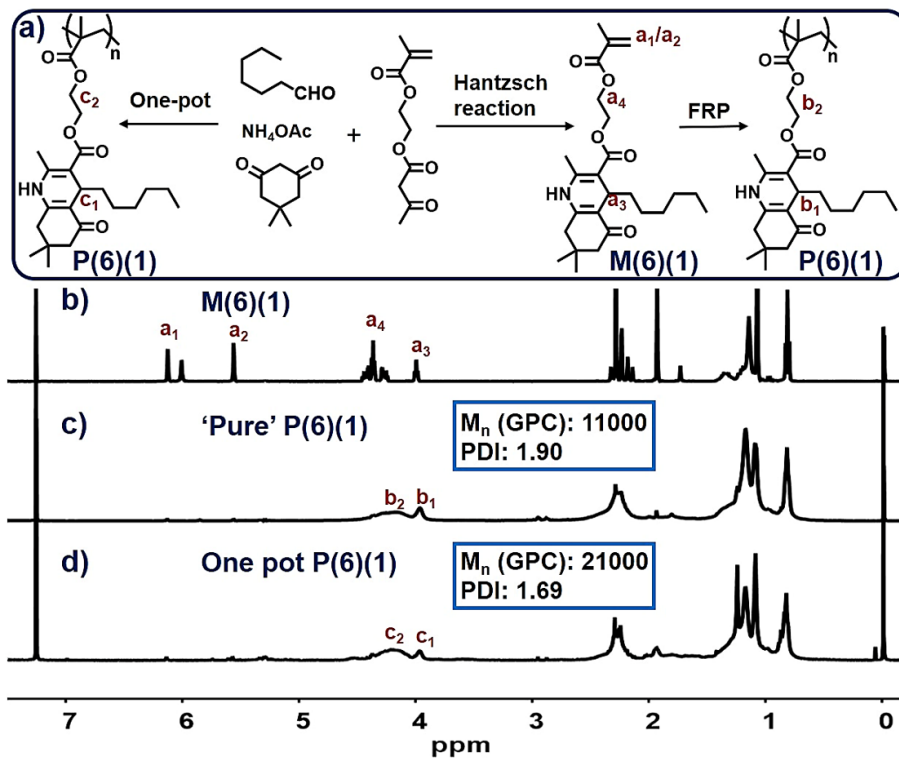


Figure S12. a) Preparations of **P(6)(1)** from the traditional monomer–polymer method and the one-pot strategy. ¹H NMR spectra of (CDCl₃, 400M) of b) **M(6)(1)**, c) “pure” **P(6)(1)**, and d) **P(6)(1)** *via* a one-pot Hantzsch–FRP system. Related to Figure 1.

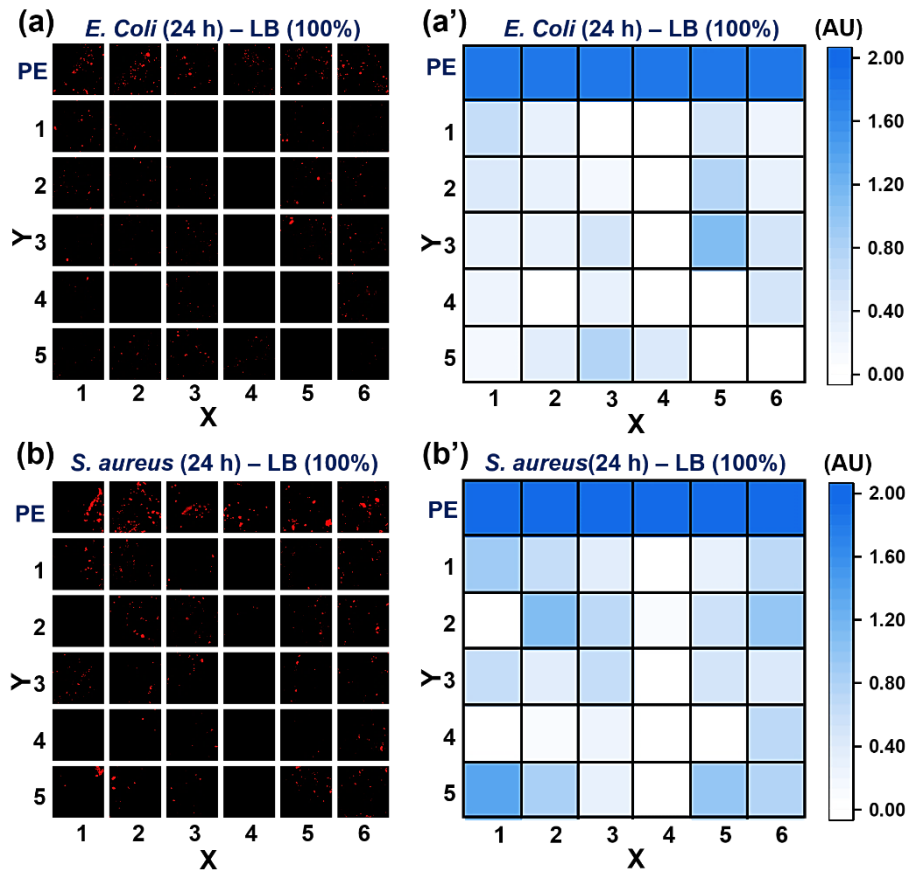


Figure S13. a) LSCM images and a') intensity map of *E. coli* on sample disks, 24 h, 100% LB medium. b) LSCM images and b') intensity map of *S. aureus* on sample disks, 24 h, 100% LB medium. Related to Figure 2.

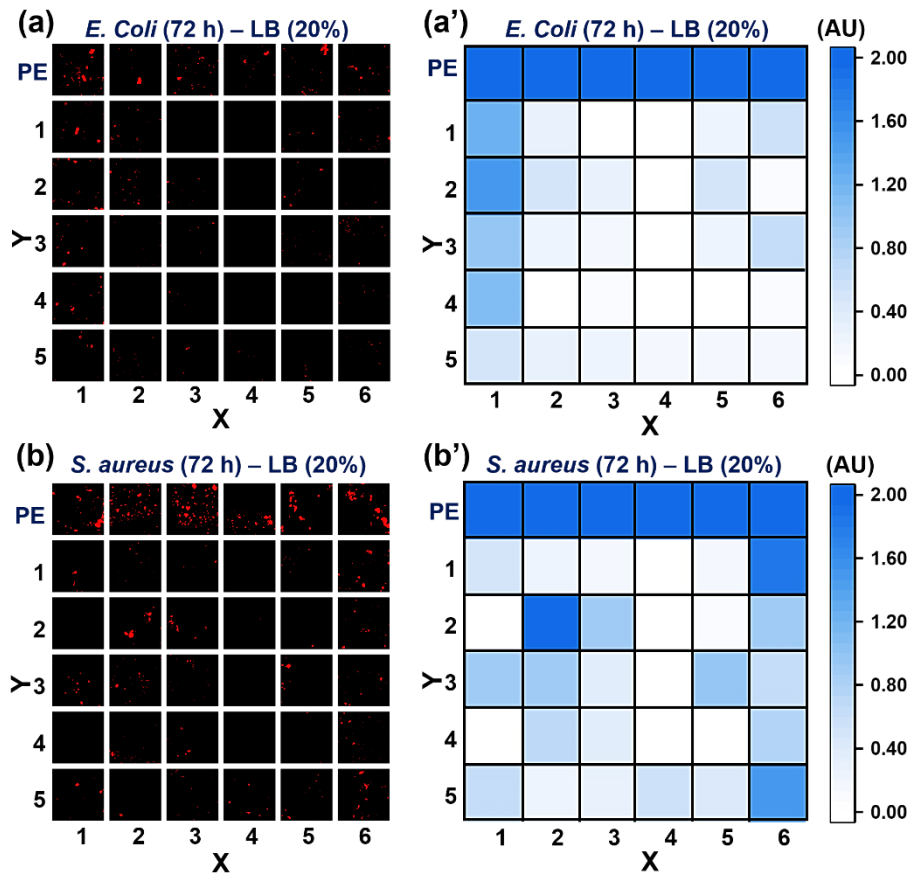


Figure S14. a) LSCM images and a') intensity map of *E. coli* on sample disks, 72 h, 20% LB medium. b) LSCM images and b') intensity map of *S. aureus* on sample disks, 72 h, 20% LB medium. Related to Figure 2.

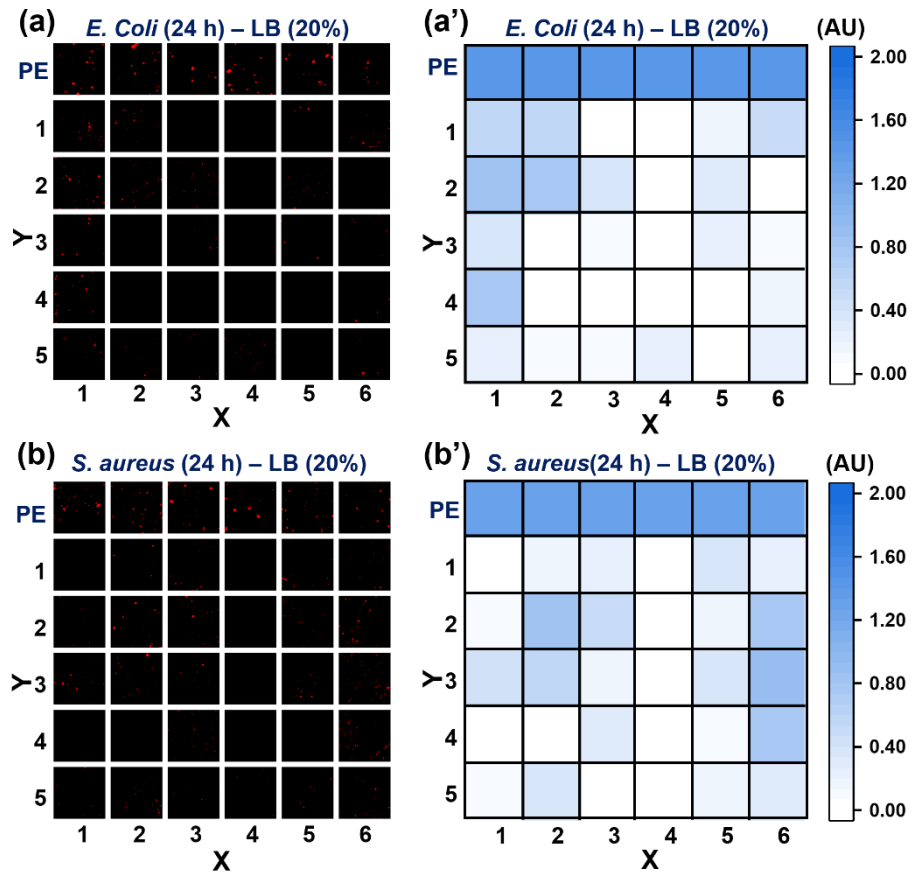


Figure S15. a) LSCM images and a') intensity map of *E. coli* on sample disks, 24 h, 20% LB medium. b) LSCM images and b') intensity map of *S. aureus* on sample disks, 24 h, 20% LB medium. Related to Figure 2.

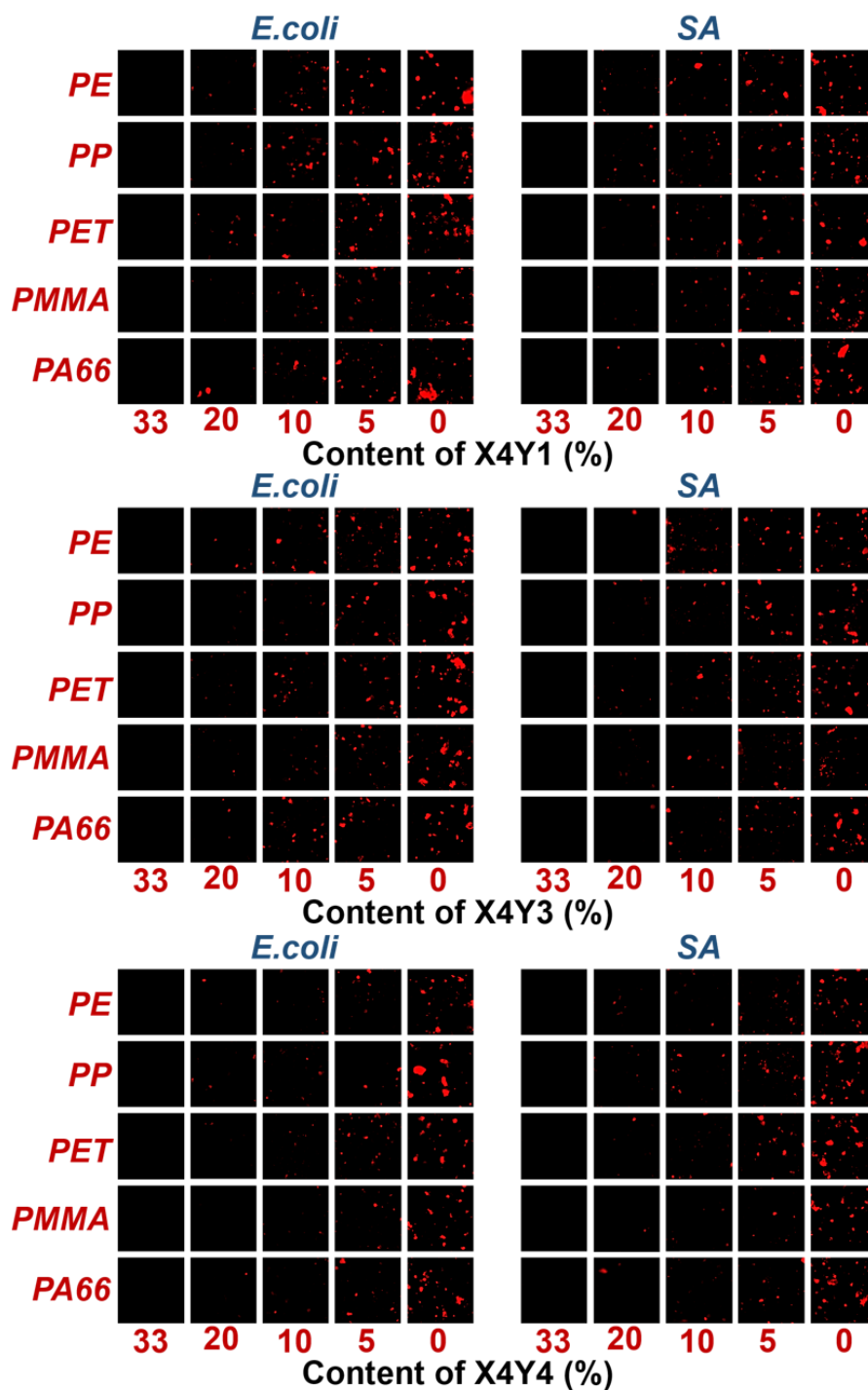


Figure S16. LSCM images of bacteria on sample disks after 72-h incubation in 100% LB medium. Three replicate samples were tested, commodity polymers served as the controls. a) Commodity polymers + **P(4)(1)**. b) Commodity polymers + **P(4)(3)**. c) Commodity polymers + **P(4)(4)**. Related to Figure 3.

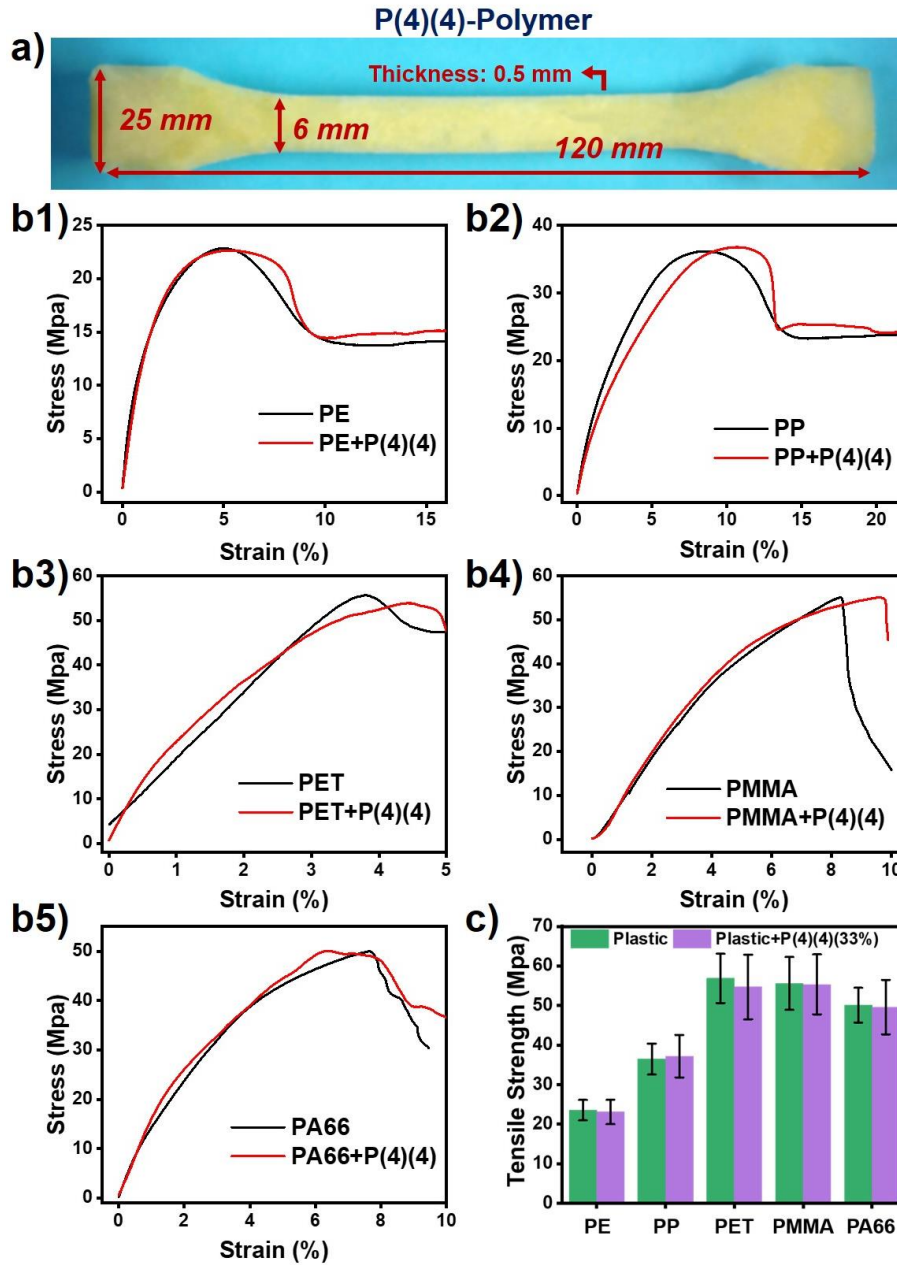


Figure S17. a) Typical samples of P(4)(4)-polymers. b1–5) Stress–strain curves of commodity polymers (black) and P(4)(4)-polymer blends (red). c) Tensile strength of commodity polymers and P(4)(4)-polymer blends. Data are represented as mean \pm SD, n = 6. Related to Figure 4.

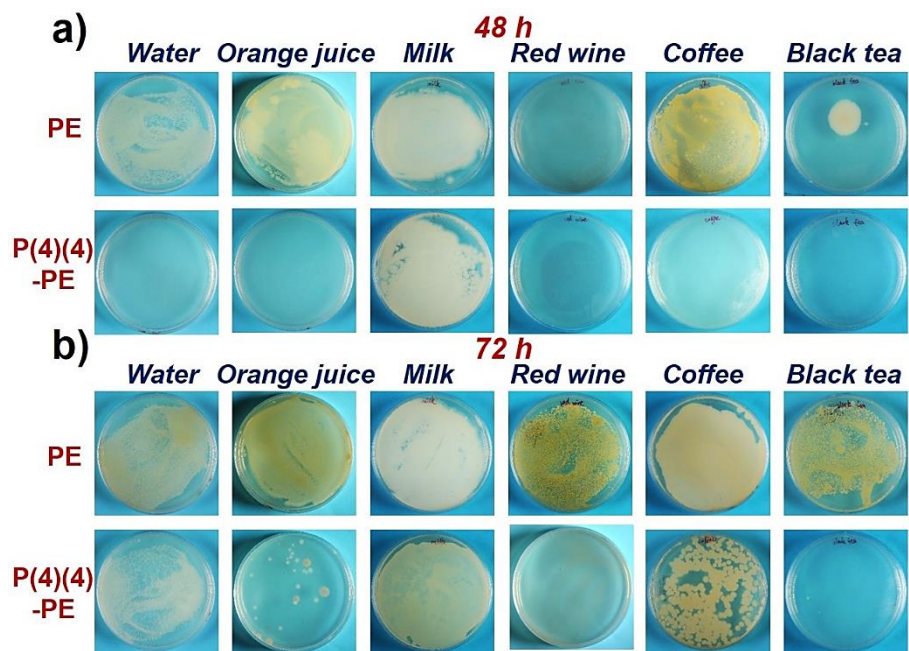


Figure S18. Images of the plate-streaking experiment: 100 μ L sample on LB agar (diameter: 90 mm, thickness: 6 mm, LB: 1.5 g/L), a) 48 h culture, and b) 72 h culture.

Related to Figure 4.

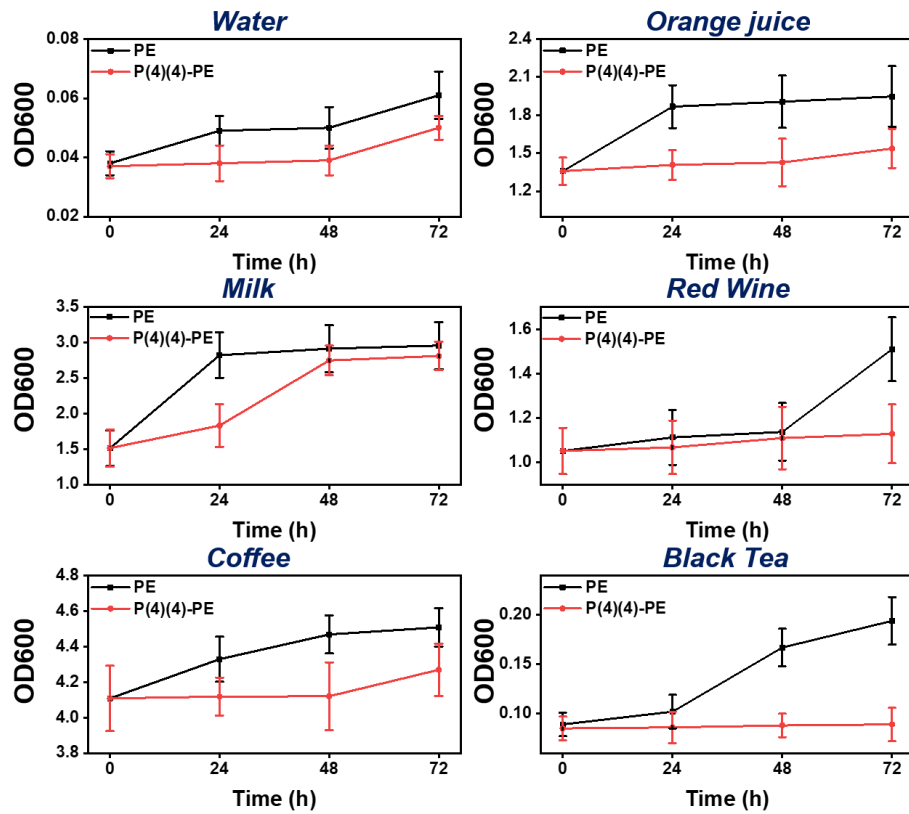


Figure S19. OD600 values versus time in the presence of different commercially available beverages. Data are represented as mean \pm SD, $n = 5$. Related to Figure 4.

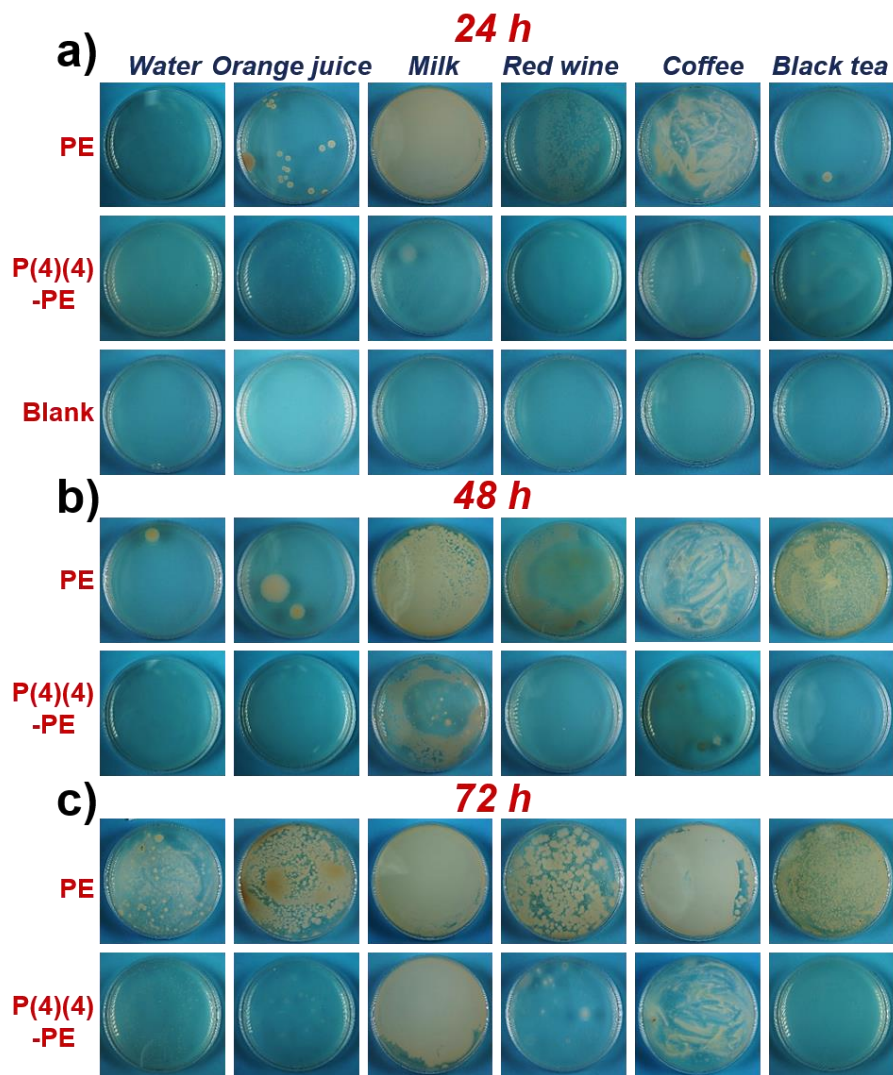


Figure S20. Images of the antibacterial capability test of bowls via a standard method.

S. aureus was used as a model bacterium, 100 μ L sample on LB agar (diameter: 90 mm, thickness: 6 mm, LB: 1.5 g/L). a) 24 h culture, b) 48 h culture and c) 72 h culture.

Related to Figure 4.

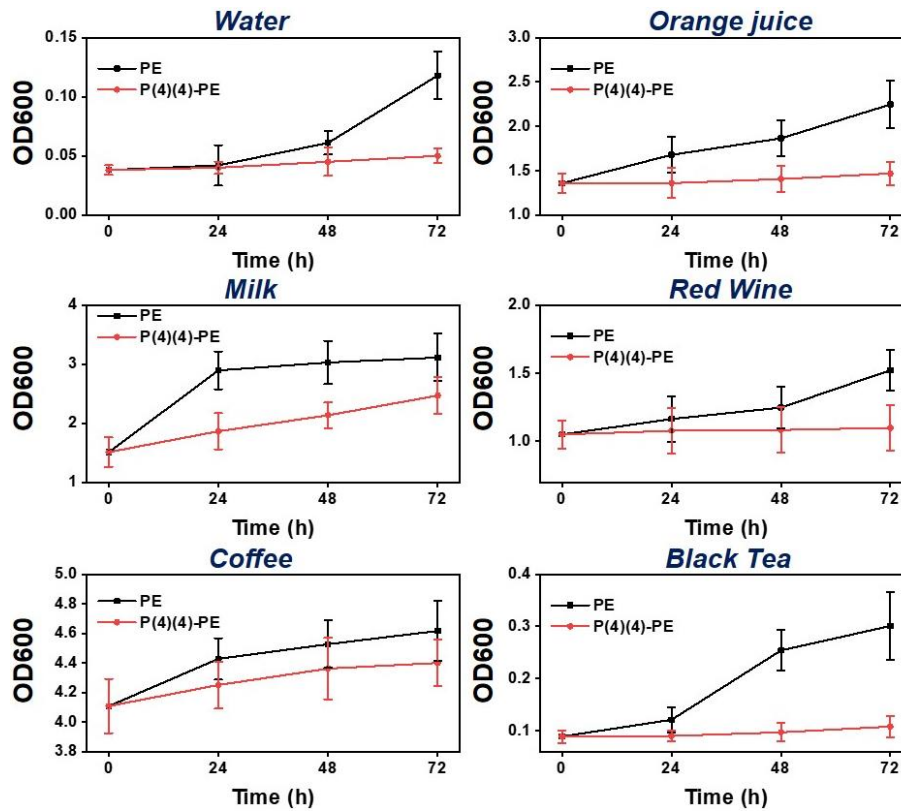


Figure S21. OD600 values versus time in the presence of different commercially available beverages in the standard antibacterial capability test of bowls, *S. aureus* was used as a model bacterium. Data are represented as mean \pm SD, n = 5. Related to Figure 4.

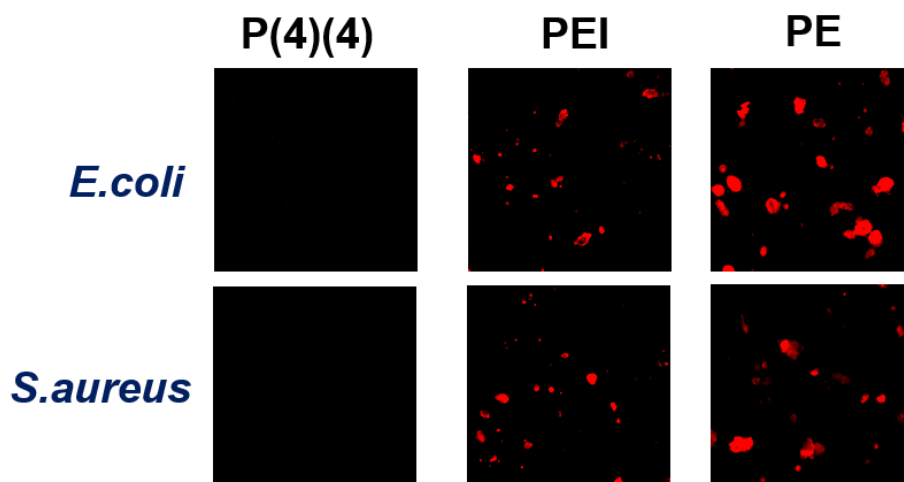


Figure S22. LSCM images of *E. coli* and *S. aureus* on sample disks after 72-h incubation. PE served as the control. Each image is $200 \times 200 \mu\text{m}$. Related to Figure 5.

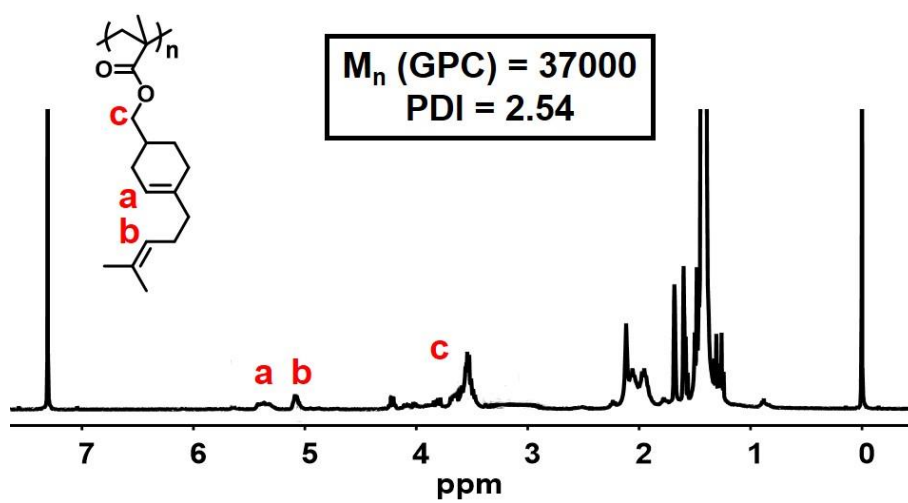


Figure S23. ^1H NMR spectrum (CDCl_3 , 400M) of **P1**. Related to Figure 6.

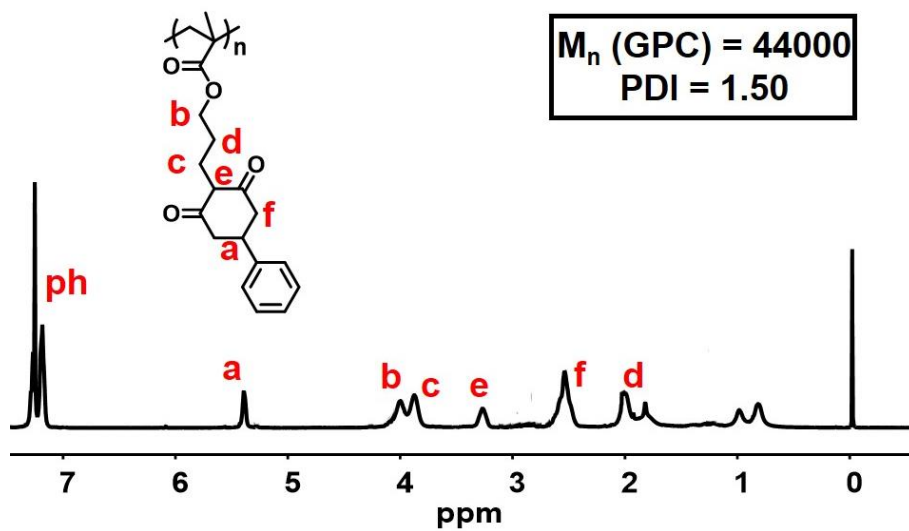


Figure S24. ^1H NMR spectrum (CDCl_3 , 400M) of **P2**. Related to Figure 6.

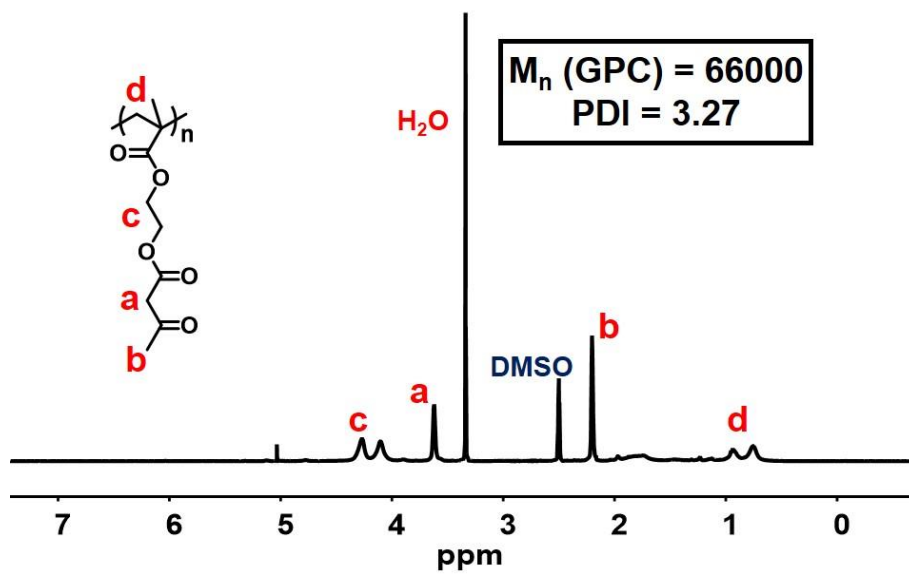


Figure S25. ^1H NMR spectrum ($\text{DMSO-}d_6$, 400M) of P(AEMA). Related to Figure 6.

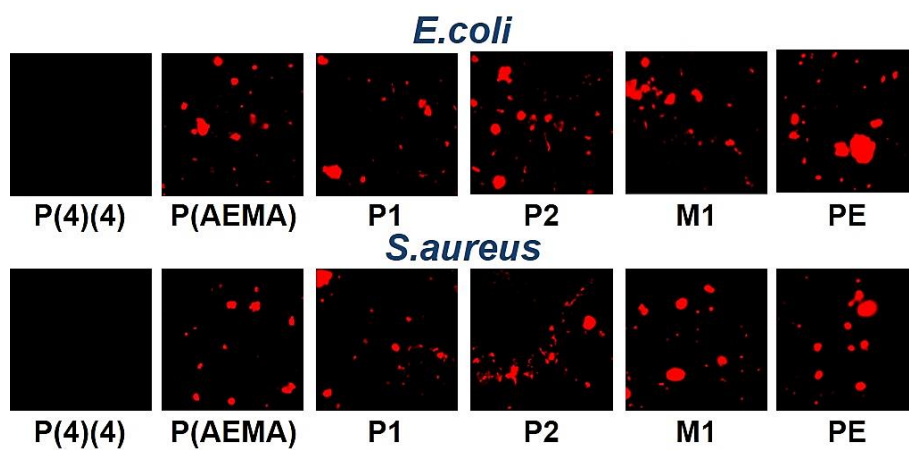


Figure S26. LSCM images of *E. coli* and *S. aureus* on sample disks after 72 h incubation. PE served as the control; each image is $200 \times 200 \mu\text{m}$. Related to Figure 6.

Table S1. The Hantzsch-type polymers (P(X)(Y)). Related to Figure 1.

Polymer	Conv.(vinyl) ^a	Conv.(Hantzsch) ^a	M _n ^b	PDI ^b	T _g ^c (°C)	T _d ^d (°C)
P(1)(1)	95%	97%	16000	1.64	115.4	207
P(2)(1)	98%	94%	23000	2.93	88.2	252
P(3)(1)	98%	98%	22000	1.83	102.6	232
P(4)(1)	98%	98%	27000	3.20	106.8	221
P(5)(1)	99%	99%	28000	6.28	111.3	227
P(6)(1)	99%	98%	21000	1.69	121.3	308
P(1)(2)	97%	95%	16000	1.39	90.6	228
P(2)(2)	97%	92%	19000	1.85	88.3	263
P(3)(2)	97%	93%	23000	1.96	98.7	251
P(4)(2)	98%	94%	28000	1.35	102.9	216
P(5)(2)	98%	95%	22000	3.42	105.7	226
P(6)(2)	99%	98%	19000	1.48	85.2	283
P(1)(3)	97%	93%	20000	1.48	117.2	208
P(2)(3)	96%	92%	24000	1.77	93.6	256
P(3)(3)	98%	93%	23000	2.14	105.6	236
P(4)(3)	99%	94%	30000	1.37	100.0	224
P(5)(3)	99%	95%	20000	3.35	108.9	267
P(6)(3)	98%	98%	22000	1.48	103.5	282
P(1)(4)	98%	93%	24000	1.26	112.6	237
P(2)(4)	98%	98%	25000	1.71	73.9	292
P(3)(4)	98%	98%	26000	1.94	97.9	219
P(4)(4)	97%	98%	23000	1.83	97.1	208
P(5)(4)	99%	99%	20000	2.30	103.6	268
P(6)(4)	99%	99%	26000	1.64	101.1	283
P(1)(5)	95%	97%	23000	1.47	56.6	246
P(2)(5)	96%	97%	21000	1.79	84.2	250
P(3)(5)	97%	96%	24000	2.38	112.7	238
P(4)(5)	98%	99%	19000	2.07	109.6	256
P(5)(5)	98%	98%	25000	3.33	99.8	259
P(6)(5)	99%	99%	21000	1.55	96.4	307

a. Calculated by ¹H NMR (CDCl₃, 400 MHz).

b. Measured by GPC using DMF as the eluent (1 mL/min).

c. Measured by DSC at a scanning rate of 10°C/min.

d. Temperature at weight-loss ratio of 5%, measured by TGA with a heating rate of 20°C/min.

Table S2. Concentrations of polymers and M1 on PE surface^a. Related to Figure 2 and Figure 6.

		Atom N% (Expected) ^b	Atom N% (Observed) ^a	Concentration on PE surface %	Deviation (%) ^c
A1	B1	1.03	1.12	35.88	7.65
	B2	1.10	1.32	39.61	18.84
	B3	1.06	0.87	27.08	-18.75
	B4	0.93	1.06	37.61	12.84
	B5	1.00	1.05	34.65	3.96
A2	B1	0.99	1.05	35.00	5.01
	B2	1.05	1.27	39.91	19.74
	B3	1.02	0.99	32.03	-3.90
	B4	0.89	1.06	39.30	17.91
	B5	0.96	1.00	34.38	3.15
A3	B1	1.06	1.14	35.49	6.48
	B2	1.13	1.21	35.34	6.03
	B3	1.09	1.24	37.54	12.63
	B4	0.95	1.05	36.47	9.42
	B5	1.03	1.01	32.36	-2.91
A4	B1	0.91	0.83	30.10	-9.69
	B2	0.96	1.07	36.78	10.35
	B3	0.94	1.10	38.62	15.87
	B4	0.83	0.84	33.40	0.21
	B5	0.89	1.07	39.67	19.02
A5	B1	0.91	0.84	30.46	-8.61
	B2	0.97	1.04	35.38	6.15
	B3	0.94	0.97	34.05	2.16
	B4	0.83	0.85	33.80	1.41
	B5	0.89	0.95	35.22	5.67
A6	B1	1.08	1.04	31.78	-4.65
	B2	1.16	1.25	35.56	6.69
	B3	1.12	1.13	33.29	-0.12
	B4	0.97	1.10	37.42	12.27
	B5	1.05	1.07	33.63	0.90
M1		0.92	0.82	29.51	-11.46

a. Measured by X-ray photoelectron spectroscopy (XPS).

b. Calculated according to the bulk concentration (33%).

c. $(\text{Concentration on PE surface} - 33)/33 \times 100\%$.

Table S3. The fluorescence intensity of *E. coli* and *S. aureus* on the sample disks^a. Related to Figure 2.

		<i>E.coli</i>	A.E.(%) ^b (<i>E. coli</i>)	<i>S.aureus</i>	A.E.(%) ^b (<i>S. aureus</i>)
	PE	3.828±0.653	0	4.386±0.583	0
A1	B1	1.528±0.207	60.06	1.034±0.150	76.42
	B2	2.338±0.344	38.92	0.460±0.157	89.51
	B3	2.207±0.281	42.35	2.116±0.271	51.76
	B4	1.042±0.142	72.78	0.004±0.001	99.91
	B5	0.805±0.103	78.97	1.962±0.240	55.27
A2	B1	0.790±0.111	79.36	1.703±0.257	61.17
	B2	1.397±0.158	63.51	3.806±0.519	13.22
	B3	0.511±0.086	86.65	1.721±0.251	60.78
	B4	0.244±0.036	93.63	0.816±0.114	80.37
	B5	1.211±0.172	68.36	1.827±0.238	58.34
A3	B1	0.248±0.039	93.52	1.896±0.250	56.77
	B2	1.076±0.120	71.89	2.283±0.307	47.95
	B3	1.032±0.130	73.04	2.112±0.271	51.85
	B4	0.532±0.071	86.10	2.968±0.392	32.33
	B5	2.276±0.316	40.54	1.421±0.186	67.60
A4	B1	0.004±0.001	99.90	0.004±0.001	99.91
	B2	0.244±0.036	93.63	0.816±0.114	80.37
	B3	0.097±0.019	97.47	0.237±0.032	95.60
	B4	0.002±0.000	99.95	0.004±0.001	99.91
	B5	1.164±0.162	69.59	0.053±0.010	98.79
A5	B1	0.786±0.119	79.47	1.311±0.178	70.11
	B2	1.818±0.237	52.51	1.601±0.227	63.50
	B3	1.456±0.207	61.96	2.407±0.312	45.12
	B4	0.142±0.024	96.29	0.427±0.041	90.26
	B5	0.130±0.013	96.60	2.396±0.307	45.37
A6	B1	1.157±0.158	69.78	1.182±0.157	73.12
	B2	0.664±0.097	82.65	2.968±0.407	32.33
	B3	0.708±0.109	81.50	2.431±0.327	44.57
	B4	0.711±0.103	81.43	2.270±0.307	48.24
	B5	1.728±0.206	54.86	2.622±0.339	40.22

a. 37°C, 72 h culture.

b. The antibacterial efficiency (A.E.) of polymers, PE served as the control.

Table S4. The fluorescence intensity of the bacterial on sample disks^a. Related to Figure 3.

		% ^b	<i>E.coli</i>	A.E.(%) ^c (<i>E. coli</i>)	<i>S.aureus</i>	A.E.(%) ^c (<i>S. aureus</i>)
P(4)(1)	PE	33	0.004±0.001	99.90	0.004±0.001	99.91
		20	0.304±0.029	92.06	0.693±0.101	84.20
		10	2.323±0.347	39.32	0.650±0.089	85.18
		5	2.130±0.309	44.36	1.418±0.517	67.67
		0	3.828±0.653	0	4.386±0.583	0
	PP	33	0.010±0.001	99.75	0.003±0.001	99.90
		20	0.340±0.059	91.52	0.406±0.051	86.97
		10	1.417±0.199	64.65	1.256±0.189	59.69
		5	1.555±0.187	61.20	2.080±0.331	33.25
		0	4.008±0.527	0.00	3.116±0.402	0
	PET	33	0.008±0.001	99.77	0.007±0.001	99.78
		20	0.599±0.087	82.73	0.232±0.039	92.70
		10	0.780±0.116	77.52	0.662±0.102	79.16
		5	2.080±0.287	40.04	1.962±0.379	38.22
		0	3.469±0.716	0	3.176±0.394	0
	PMMA	33	0.011±0.002	99.72	0.009±0.001	99.72
		20	0.056±0.011	98.59	0.090±0.010	97.18
		10	0.571±0.071	85.66	0.424±0.057	86.71
		5	1.481±0.249	62.82	1.681±0.210	47.30
		0	3.983±0.497	0	3.190±0.422	0
PA-66	33	0.009±0.001	99.78	0.010±0.002	99.75	
	20	0.840±0.146	79.43	0.159±0.029	96.03	
	10	1.061±0.267	74.02	0.746±0.175	81.37	
	5	1.654±0.257	59.50	1.598±0.264	60.09	
	0	4.084±0.622	0	4.004±0.517	0	
P(4)(3)	PE	33	0.002±0.001	99.95	0.004±0.001	99.91
		20	0.050±0.008	98.69	0.254±0.048	94.21
		10	0.330±0.069	91.38	0.616±0.089	85.96
		5	1.601±0.248	58.18	2.010±0.421	54.17
		0	3.828±0.653	0	4.386±0.583	0
	PP	33	0.007±0.001	99.83	0.003±0.001	99.90
		20	0.316±0.041	92.12	0.267±0.037	91.43
		10	1.589±0.308	60.35	1.425±0.245	54.27
		5	1.973±0.307	50.77	1.737±0.207	44.26
		0	4.008±0.527	0	3.116±0.402	0
	PET	33	0.015±0.003	99.57	0.007±0.002	99.78
		20	0.221±0.037	93.63	0.242±0.037	92.38
		10	1.112±0.200	67.94	1.110±0.145	65.05
		5	1.549±0.194	55.35	1.447±0.206	54.44
		0	3.469±0.716	0	3.176±0.394	0
	PMMA	33	0.007±0.002	99.82	0.004±0.001	99.87
		20	0.126±0.027	96.84	0.254±0.038	92.04
		10	0.362±0.042	90.91	0.426±0.057	86.65
		5	1.438±0.037	63.90	1.419±0.029	55.52
		0	3.983±0.497	0	3.190±0.422	0
PA-66	33	0.018±0.004	99.56	0.014±0.003	99.65	
	20	0.183±0.028	95.52	0.155±0.028	96.13	
	10	1.732±0.207	57.59	0.767±0.107	80.84	
	5	1.708±0.216	58.18	1.115±0.270	72.15	
	0	4.084±0.622	0	4.004±0.517	0	
P(4)(4)	PE	33	0.004±0.001	99.90	0.013±0.005	99.70
		20	0.340±0.049	91.12	0.211±0.049	95.19
		10	0.491±0.097	87.17	0.695±0.109	84.15
		5	0.553±0.105	85.55	1.291±0.208	70.57
		0	3.828±0.653	0	4.386±0.583	0

PP	33	0.018±0.004	99.55	0.028±0.009	99.10
	20	0.157±0.022	96.08	0.216±0.047	93.07
	10	0.244±0.039	93.91	0.308±0.044	90.12
	5	0.829±0.100	79.32	1.122±0.112	63.99
	0	4.008±0.527	0	3.116±0.402	0
PET	33	0.019±0.002	99.45	0.005±0.002	99.84
	20	0.083±0.014	97.61	0.233±0.057	92.66
	10	0.303±0.049	91.27	0.671±0.094	78.87
	5	1.481±0.027	57.31	1.462±0.028	53.97
	0	3.469±0.716	0	3.176±0.394	0
PMMA	33	0.007±0.001	99.82	0.012±0.003	99.62
	20	0.004±0.001	99.90	0.077±0.022	97.59
	10	0.193±0.038	95.15	0.194±0.029	93.92
	5	0.694±0.103	82.58	0.390±0.062	87.77
	0	3.983±0.497	0	3.190±0.422	0
PA-66	33	0.009±0.001	99.78	0.007±0.001	99.83
	20	0.095±0.017	97.67	0.299±0.042	92.53
	10	0.313±0.041	92.34	0.542±0.071	86.46
	5	1.250±0.203	69.39	1.055±0.151	73.65
	0	4.084±0.622	0	4.004±0.517	0

- 37°C, 72 h culture.
- The ratio of selected polymers (**P(4)(1,3,4)**) added in several typical commodity polymers.
- The antibacterial efficiency (A.E.) of polymers. Commodity polymers served as the controls.

Table S5. Interaction between polymers and polysaccharides^a. Related to Figure 5.

	lipopolysaccharide	peptidoglycan
P(4)(4)	0.10 × 10 ⁻³	0.14 × 10 ⁻³
PE	0.20 × 10 ⁻³	0.26 × 10 ⁻³

- Measured by an Octet assay by using a BLI Automated Biosensor.

Transparent Methods

EXPERIMENTAL SECTION

1. Materials

All chemicals and solvents were purchased from commercial sources and used without further purification. 2-(Acetoacetoxy)ethyl methacrylate (AEMA, Aladdin, 95%), cinnamic aldehyde (MREDA, 99%), citronellal (MERYER, 99%), hyacinthin (J&K, 97.5%), myrac aldehyde (Energy, 97%), cyclamen aldehyde (MAKLIN, 92%), phellandral (MAKLIN, 97%), 5,5-dimethyl-1,3-cyclohexanedione (MAKLIN, 99%), 1,3-cyclohexanedione (Shaoyuan, 97%), 5-methyl-1,3-cyclohexanedione (NineDing, 98%), 5-phenyl-1,3-cyclohexanedione (Energy, 97%), 5-n-propyl-1,3-cyclohexanedione (Energy, 97%), ammonium acetate (MAKLIN, 98%), glycine (Ouhe, 98%), 2,2'-azobisisoheptonitrile (ABVN, Energy, 98%), polyethylene (PE, 500 mesh, ZhongLian Plastic), polypropylene (PP, 500 mesh, ZhongLian Plastic), polyethylene terephthalate (PET, 500 mesh, ZhongLian Plastic), poly(methyl methacrylate) (PMMA, 500 mesh, ZhongLian Plastic), polyamide 66 (PA66, 500 mesh, ZhongLian Plastic), poly(ethylene imine) (PEI, $M_w \sim 10000$, 50 wt/% H_2O), 3-bromo-1-propanol (Ark-Pharm, 97%), methacryloylchloride (HEOWNS, 99%), sodium borohydride ($NaBH_4$, LanGe, 98%). Drinking natural mineral water (Nongfu Spring), orange juice (NFC), drinking pure milk (Jizhi), red wine (ChangYu), coffee (Nestle), black tea (Oriental leaves).

Escherichia coli (*E. coli*), BL21 (competent cell of *E. coli*, Merck Millipore), *Staphylococcus aureus* strain (*S. aureus*, CMCC), pCMV-N-mCherry (Beyotime), Luria-Bertani (LB) broth (Gibco), LB agar (Gibco), tryptic soy broth (TSB, Gibco), tryptic soy agar (TSA, Gibco), kanamycin sulfate (inalco), isopropyl β -D-thiogalactoside (IPTG, inalco), phosphatic buffer solution (PBS, Gibco) were used as purchased.

2. Instruments

Gel permeation chromatography (GPC) analyses of polymers were performed using *N, N*-dimethyl formamide (DMF) containing 50 mM LiBr as the eluent. The GPC system is a Shimadzu LC-20AD pump system consisting of an auto injector, a MZ-Gel SDplus 10.0 μm guard column (50×8.0 mm, 10^2 Å) followed by two PLgel 5 μm MIXED-D columns (300×7.5 mm), and a Shimadzu RID-10A refractive index detector. The system was calibrated with narrow molecular weight distribution polystyrene standards ranging from 200 to 10^6 g mol⁻¹.

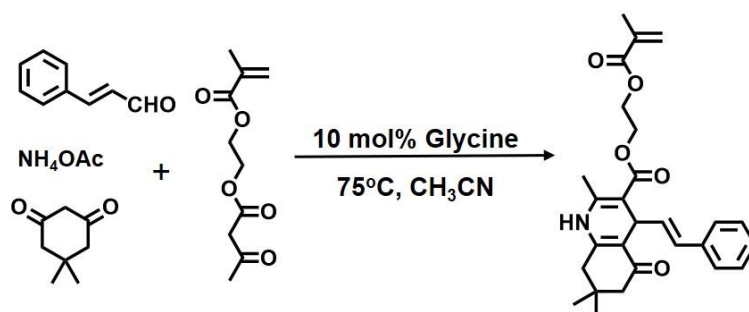
NMR spectra were obtained using a JEOL JNM-ECA400 spectrometer for all samples. The ESI-MS data were collected using a Micro TOF-QII Bruker. The FT-IR spectra were recorded in a transmission mode on a Perkin-Elmer Spectrum 100 spectrometer (Waltham, MA, USA).

Differential scanning calorimetry (DSC) was performed using a TA instrument Q2000 operated at a scanning rate of 10°C/min. Thermal gravimetric analysis (TGA) was conducted on a TA instrument Q50 with a heating rate of 20°C/min. The fluorescent

images of bacteria were recorded by a Laser scanning Confocal Microscopy (Zeiss LSM 780). Bio-Rad Electroporator was used for the electrotransformation of *E. coli* and *S. aureus*. The tensile shear strength was measured using an MTS SYSTEMS (CHINA) Co. Ltd. SANS CMT6503 electromechanically universal testing machine. Injection molding was performed by a by micro injection molding machine (WZS10D, Shanghai Xinshuo precision instrument co., LTD). X-ray photoelectron spectroscopy (XPS) data were obtained by an ESCALAB 250 Xi electron spectrometer from VG Scientific using 300 W Al K α radiation with a pass energy of 100.0 eV; binding energy of all the elements was calibrated relative to the carbon impurity with a C1s at 284.8 eV. Biosensor experiment was performed by BLI (BioLayer Interferometry) on Octet RED96 (Pall ForteBIO LLC, Fremont, USA).

3. Methods

3.1 2-(Methacryloyloxy)ethyl (E)-2,7,7-trimethyl-5-oxo-4-styryl-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (M(1)(1))



The monomers M(X)(1) were prepared via the Hantzsch reaction by different combinations of aldehydes (A(X)) and 5,5-dimethyl-1,3-cyclohexanedione (B(1)). Typically, A(1) (cinnamaldehyde, 660 mg, 5.0 mmol), AEMA (1.07 g, 5.0 mmol), B(1)

(700 mg, 5.0 mmol), and ammonium acetate (578 mg, 7.5 mmol) were put in a 15-mL centrifuge tube. Then, glycine (38 mg, 0.5 mmol) and acetonitrile (6.0 mL) were added. The mixture was kept in an oil bath (75°C) for 4 h. After removing volatiles under vacuum, the crude was purified by a column chromatography (petroleum ether/ethyl acetate: 5/1) to get monomer M(1)(1) as a yellow powder (1.65 g, yield: 73.6%).

All other monomers were prepared through the same procedure.

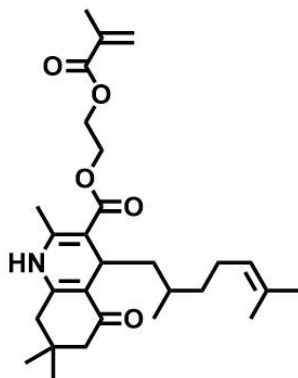
¹H NMR (400 MHz, CDCl₃, δ/ppm): 7.06-7.29 (m, 5H, ph), 6.20 (s, 1H, CHCH=CH), 6.19 (s, 1H, CHCH=CH), 6.12 (s, 1H, CH₂=C), 6.06 (s, 1H, NH), 5.57 (s, 1H, CH₂=C), 4.54 (s, 1H, CCHC), 4.25-4.44 (m, 4H, COOCH₂CH₂), 2.36 (s, 2H, CH₂C=O), 2.09 (s, 3H, NHCCH₃), 2.00 (s, 3H, CH₃C=CH₂), 1.90 (s, 2H, NHCCH₂), 1.08 (s, 6H, CH₃CCH₃).

¹³C NMR (100 MHz, CDCl₃, δ/ppm): 196.15, 167.70, 167.29, 149.84, 147.27, 145.23, 135.96, 128.02, 127.98, 127.97, 127.96, 127.95, 126.96, 126.95, 126.19, 111.79, 105.18, 62.80, 61.51, 50.93, 40.58, 36.70, 32.70, 29.59, 27.13, 19.29, 18.38.

IR (v/cm⁻¹): 3287, 2959, 1716, 1692, 1603, 1488, 1377, 1279, 1214, 1149, 1113, 1082, 1045, 941, 884, 814, 783, 695, 658, 566.

ESI-MS: observed (expected): 450.2286 (450.2280) [M + H⁺].

3.2 2-(Methacryloyloxy)ethyl 4-(2,6-dimethylhept-5-en-1-yl)-2,7,7-trimethyl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (M(2)(1))



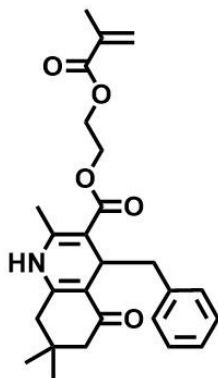
^1H NMR (400 MHz, CDCl_3 , δ/ppm): 6.12 (s, 1H, $\text{CH}_2=\text{C}$), 5.83 (s, 1H, NH), 5.57 (s, 1H, $\text{CH}_2=\text{C}$), 5.05 (m, 1H, $\text{CH}=\text{C}(\text{CH}_3)_2$), 4.25-4.44 (m, 4H, $\text{COOCH}_2\text{CH}_2$), 4.05 (s, 1H, CCHC), 2.36 (s, 2H, $\text{CH}_2\text{C}=\text{O}$), 2.09 (s, 3H, NHCCH_3), 2.00 (s, 3H, $\text{CH}_3\text{C}=\text{CH}_2$), 1.90 (s, 2H, NHCCH_2), 1.60 (s, 3H, $\text{CH}=\text{CCH}_3$), 1.55 (s, 3H, $\text{CH}=\text{CCH}_3$), 1.25 (m, 7H, $\text{CH}_2\text{CHCH}_3\text{CH}_2\text{CH}_2$), 1.08 (s, 6H, $\text{CH}_2\text{C}(\text{CH}_3)_2$), 0.86 (d, 3H, CHCH_3 , $J = 4.2$ Hz).

^{13}C NMR (100 MHz, CDCl_3 , δ/ppm): 196.28, 167.70, 167.27, 148.98, 145.00, 136.03, 130.73, 128.93, 126.21, 112.53, 105.74, 62.85, 61.53, 50.98, 44.86, 41.34, 38.32, 37.49, 32.42, 29.85, 27.70, 27.43, 25.81, 25.62, 20.24, 19.51, 18.40, 17.68.

IR (v/cm^{-1}): 3675, 3285, 2971, 2901, 1719, 1694, 1637, 1604, 1480, 1451, 1405, 1393, 1319, 1241, 1214, 1164, 1075, 1056, 938, 891, 813, 776, 661.

ESI-MS: observed (expected): 472.3061 (472.3063) $[\text{M} + \text{H}^+]$.

3.3 2-(Methacryloyloxy)ethyl 4-benzyl-2,7,7-trimethyl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (M(3)(1))



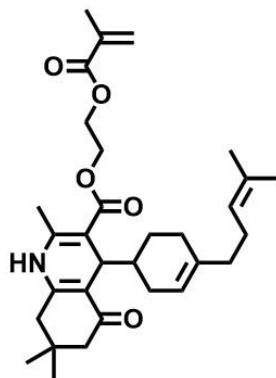
^1H NMR (400 MHz, CDCl_3 , δ/ppm): 6.95-7.25 (m, 5H, ph), 6.12 (s, 1H, $\text{CH}_2=\text{C}$), 5.76 (s, 1H, NH), 5.57 (s, 1H, $\text{CH}_2=\text{C}$), 4.31 (s, 1H, CCHC), 4.18-4.30 (m, 4H, $\text{COOCH}_2\text{CH}_2$), 2.62 (m, 2H, phCH_2), 2.36 (s, 2H, $\text{CH}_2\text{C}=\text{O}$), 2.09 (s, 3H, NHCCH_3), 2.00 (s, 3H, $\text{CH}_3\text{C}=\text{CH}_2$), 1.90 (s, 2H, NHCCH_2), 1.08 (s, 6H, CH_3CCH_3).

^{13}C NMR (100 MHz, CDCl_3 , δ/ppm): 196.08, 167.99, 167.21, 149.74, 145.27, 137.36, 135.94, 131.24, 126.10, 124.50, 120.43, 110.02, 103.37, 62.74, 61.74, 50.94, 41.99, 41.31, 37.53, 34.11, 32.39, 29.85, 27.63, 26.48, 19.45, 17.67.

IR (v/cm^{-1}): 3675, 3286, 3062, 2959, 1719, 1693, 1603, 1486, 1452, 1383, 1320, 1296, 1279, 1220, 1165, 1106, 1079, 1048, 1004, 941, 814, 753, 699, 595.

ESI-MS: observed (expected): 438.2287 (438.2280) $[\text{M} + \text{H}^+]$.

3.4 2-(Methacryloyloxy)ethyl 2,7,7-trimethyl-4-(4-(4-methylpent-3-en-1-yl)cyclohex-3-en-1-yl)-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (M(4)(1))



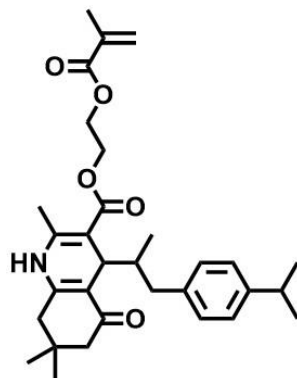
^1H NMR (400 MHz, CDCl_3 , δ/ppm): 6.12 (s, 1H, $\text{CH}_2=\text{C}$), 5.95 (s, 1H, NH), 5.57 (s, 1H, $\text{CH}_2=\text{C}$), 5.29 (m, 1H, $\text{CHCH}_2\text{CH}=\text{C}$), 5.05 (d, 1H, $\text{CH}_2\text{CH}_2\text{CH}=\text{C}$, $J = 6.9$ Hz), 4.25-4.44 (m, 4H, $\text{COOCH}_2\text{CH}_2$), 4.12 (s, 1H, CCHC), 2.36 (s, 2H, $\text{CH}_2\text{C}=\text{O}$), 2.09 (s, 3H, NHCCH_3), 2.00 (s, 3H, $\text{CH}_3\text{C}=\text{CH}_2$), 1.95-2.06 (m, 4H, $\text{CCH}_2\text{CH}_2\text{CH}$), 1.70-2.10 (m, 7H, $\text{CH}_2\text{CHCH}_2\text{CH}_2$), 1.90 (s, 2H, NHCCH_2), 1.81 (s, 3H, CHCCH_3), 1.76 (s, 3H, CHCCH_3), 1.08 (s, 6H, CH_3CCH_3).

^{13}C NMR (100 MHz, CDCl_3 , δ/ppm): 196.08, 167.80, 167.21, 149.70, 145.27, 137.36, 135.97, 131.24, 126.10, 124.50, 120.43, 110.14, 103.37, 62.74, 61.47, 50.97, 41.99, 41.31, 37.53, 34.11, 32.39, 29.85, 29.23, 27.63, 27.34, 26.48, 25.70, 19.48, 18.30, 18.30, 17.67.

IR (v/cm^{-1}): 3675, 3281, 2968, 2901, 1720, 1694, 1606, 1483, 1383, 1280, 1214, 1153, 1105, 1076, 1045, 997, 938, 883, 788, 754, 610, 588, 565.

ESI-MS: observed (expected): 510.3224 (510.3219) $[\text{M} + \text{H}^+]$.

3.5 2-(Methacryloyloxy)ethyl 4-(1-(4-isopropylphenyl)propan-2-yl)-2,7,7-trimethyl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (M(5)(1))



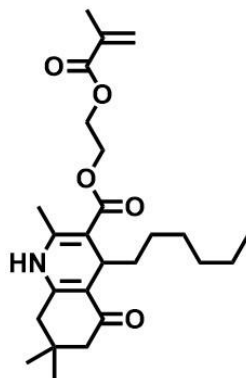
^1H NMR (400 MHz, CDCl_3 , δ/ppm): 6.95-7.09 (m, 4H, ph), 6.12 (s, 1H, $\text{CH}_2=\text{C}$), 5.89 (s, 1H, NH), 5.57 (s, 1H, $\text{CH}_2=\text{C}$), 4.32-4.44 (m, 4H, $\text{COOCH}_2\text{CH}_2$), 4.19 (s, 1H, CCHC), 2.92 (m, 2H, CH_2ph), 2.30 (m, 1H, phCH), 2.36 (s, 2H, $\text{CH}_2\text{C}=\text{O}$), 2.09 (s, 3H, NHCCCH_3), 2.00 (s, 3H, $\text{CH}_3\text{C}=\text{CH}_2$), 1.90 (s, 2H, NHCCCH_2), 1.55-1.65 (m, 1H, CH_2CHCH_3), 1.10 (d, 3H, CH_2CHCH_3 , $J = 6.5$ Hz), 1.08 (s, 6H, CH_3CCH_3), 0.67 (d, 6H, CH_3CHCH_3 , $J = 6.5$ Hz).

^{13}C NMR (100 MHz, CDCl_3 , δ/ppm): 197.13, 167.23, 164.74, 146.93, 139.16, 137.36, 135.94, 135.41, 129.02, 126.51, 126.07, 125.03, 124.04, 110.04, 103.57, 63.15, 62.36, 51.98, 46.55, 40.24, 33.78, 32.99, 32.40, 30.03, 28.36, 27.19, 25.31, 24.16, 18.37, 17.87, 15.22.

IR (v/cm^{-1}): 3285, 2958, 1720, 1697, 1604, 1483, 1382, 1275, 1217, 1150, 1113, 1076, 1048, 998, 938, 886, 856, 812, 783, 654, 590, 566, 554.

ESI-MS: observed (expected): 508.3069 (508.3063) $[\text{M} + \text{H}^+]$.

3.6 2-(Methacryloyloxy)ethyl 4-hexyl-2,7,7-trimethyl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (M(6)(1))



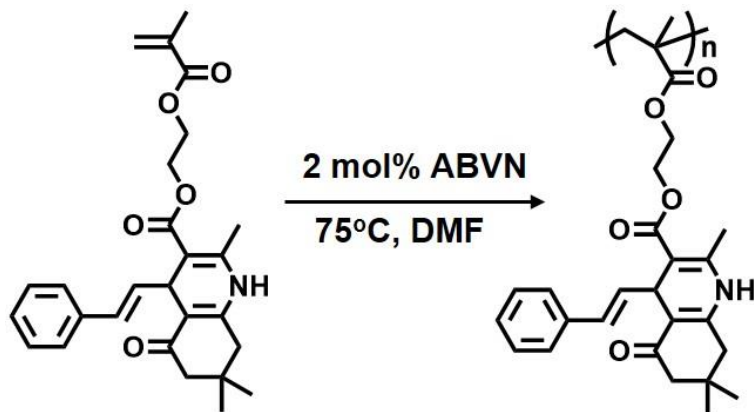
¹H NMR (400 MHz, CDCl₃, δ/ppm): 6.12 (s, 1H, CH₂=C), 6.05 (s, 1H, NH), 5.57 (s, 1H, CH₂=C), , 4.25-4.44 (m, 4H, COOCH₂CH₂), 4.00 (s, 1H, CCHC), 2.36 (s, 2H, CH₂C=O), 2.09 (s, 3H, NHCCH₃), 2.00 (s, 3H, CH₃C=CH₂), 1.90 (s, 2H, NHCCH₂), 1.09-1.50 (m, 10H, CH(CH₂)₅CH₃), 1.08 (s, 6H, CH₃CCH₃), 0.86 (s, 3H, CH₂CH₃).

¹³C NMR (100 MHz, CDCl₃, δ/ppm): 196.14, 167.62, 167.29, 149.90, 145.38, 136.08, 126.15, 111.33, 104.67, 62.81, 61.47, 51.02, 41.08, 36.34, 32.59, 32.01, 29.84, 29.75, 27.09, 25.01, 22.76, 19.43, 18.36, 14.16, 14.15.

IR (v/cm⁻¹): 3281, 2926, 1722, 1693, 1605, 1483, 1386, 1278, 1216, 1163, 1094, 1048, 995, 954, 884, 813, 780, 747, 641, 618, 587, 575, 558.

ESI-MS: observed (expected): 432.2759 (432.2750) [M + H⁺].

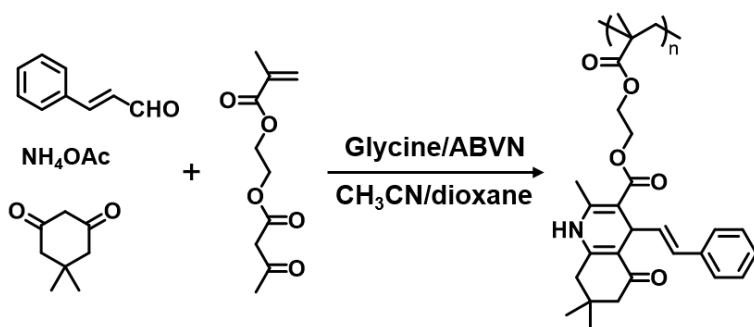
3.7 Polymerization of M(X)(1) to get P(X)(1)



The ‘pure’ polymers P(X)(1) were prepared by radical polymerization of M(X)(1). For example, monomer M(1)(1) (450 mg, 1.0 mmol), 2,2'-azobis-(2,4-dimethylvaleronitrile) (ABVN, 5 mg, 0.02 mmol) were dissolved in dimethyl formamide (DMF, 1.0 mL). The mixture was purged by nitrogen flow for 20 min, then kept in an oil bath (75°C) for 12 h. The polymerization was quenched in an ice–water bath. After precipitation in diethyl ether three times, P(1)(1) was obtained as a yellow powder (400 mg, yield: 88.9%).

All other ‘pure’ polymers were prepared through the same procedure by using different monomers.

3.8 HTP-One pot preparation of polymers (P(X)(Y))



Polymers P(X)(Y) were prepared *via* a one-pot HTP method. For a representative synthesis, A(1) (cinnamaldehyde, 660 mg, 5.0 mmol), AEMA (1.07 g, 5.0 mmol), B(1) (5,5-dimethyl-1,3-cyclohexanedione, 700 mg, 5.0 mmol), and ammonium acetate (578 mg, 7.5 mmol) were charged to a 15-mL centrifuge tube. Then, glycine (38 mg, 0.5 mmol), ABVN (25 mg, 0.1 mmol), and acetonitrile/1,4-dioxane (3.0/3.0 mL) were added. The mixture was purged by bubbling nitrogen for 20 min, then sealed and placed in an oil bath (75°C) for 12 h. The polymerization was quenched in an ice–water bath. A 20–50 μ L aliquot was taken for ^1H NMR and GPC analyses. The polymer was purified by precipitation in water followed by re-precipitation from THF with diethyl ether to obtain a yellow powder (P(1)(1) (1.85 g, yield: 82.3%).

All other polymers were simultaneously prepared using the same procedure with the respective A and B starting materials.

3.9 Red-fluorescent-protein (RFP) transferred bacteria

RFP transferred bacteria were prepared according to previous literatures (Nickoloff, 1995). BL21 is a competent cell line of *E. coli*. BL21 cells were transferred to a chilled electroporation cuvette with a precooled plasmid solution (2 μ L, pCMV-N-mCherry in PBS, 100 μ g/mL, kanamycin resistance). This cuvette was put in the electroporation instrument for electric shock with an exponential decay pulse (2.5 kV, 200- Ω resistance, 25- μ F capacitance, 4.5 ms). Then, LB medium (1 mL) was added to the electroporation cuvette, and the contents were transferred to a 1.5-mL tube for incubation (1.5 h, 180 r/min, 37°C). The mixture was plated on a LB agar plate containing antibiotic

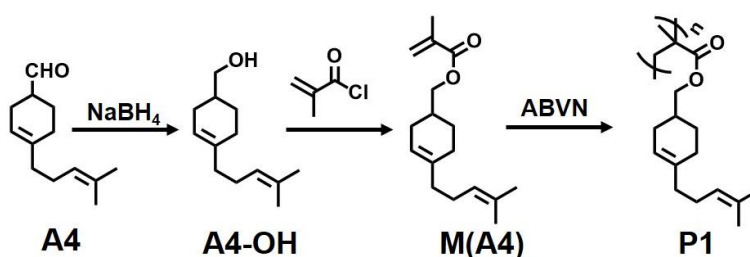
(kanamycin sulfate: 50 $\mu\text{g}/\text{mL}$) and incubated at 37°C for 12 h. One small colony was isolated and incubated in 200 mL of LB medium for 2 h. Then, IPTG (200 mM, 2 mL) was added to induce gene expression (28°C, 4 h) to obtain the RFP-*E. coli*.

RFP-*S. aureus* was similarly prepared.

3.10 Poly(acetoacetoxy ethyl methacrylate) (P(AEMA))

AEMA (2.14 g, 10 mmol) and ABVN (50 mg, 0.2 mmol) were dissolved in DMF (20 mL) and put in a 50 mL polymerization tube. This tube was sealed with a rubber septum and purged by nitrogen flow for 20 min, and kept in an oil bath (75°C) for 12 h. The polymerization was quenched in an ice–water bath, and the polymer (P(AEMA)) was purified by precipitation in diethyl ether three times as a white powder (1.82 g, yield: 85.0%).

3.11 Poly((4-(4-methylpent-3-en-1-yl)cyclohex-3-en-1-yl)methyl methacrylate) (P1)



A4-OH: Myrac aldehyde (A4, 9.62 g, 50 mmol) and sodium borohydride (NaBH₄, 2.27 g, 60 mmol) were dissolved in methanol (50 mL), and put in an ice bath for 4 h. After removing volatiles under vacuum, A4-OH was purified by a column chromatography (petroleum ether/ethyl acetate = 10/1) as a colorless oil (8.59 g, 88.4% yield).

^1H NMR (400 MHz, CDCl_3 , δ/ppm): 5.45 (d, 1H, $\text{CHCH}_2\text{CH}=\text{C}$, $J = 6.8$ Hz), 5.05 (t, 1H, $\text{CH}_2\text{CH}_2\text{CH}=\text{C}$, $J = 6.9$ Hz), 3.58 (m, 2H, CH_2OH), 2.20-2.10 (m, 7H, $\text{CH}_2\text{CHCH}_2\text{CH}_2$), 2.10-2.00 (m, 4H, $\text{CH}_2\text{CH}_2\text{CH}=\text{C}(\text{CH}_3)_2$), 1.85 (s, 3H, $\text{CH}=\text{CCH}_3\text{CH}_3$), 1.82 (s, 3H, $\text{CH}=\text{CCH}_3\text{CH}_3$).

^{13}C NMR (100 MHz, CDCl_3 , δ/ppm): 137.89, 131.47, 124.44, 119.54, 67.91, 37.79, 36.44, 28.30, 27.88, 26.55, 25.79, 25.75, 17.77.

IR (v/cm^{-1}): 3324, 2965, 2913, 1670, 1437, 1376, 1261, 1232, 1139, 1087, 1049, 943, 892, 825, 673, 600, 581, 563.

ESI-MS: observed (expected): 195.1757 (195.1749) $[\text{M} + \text{H}^+]$.

M(A4): A4-OH (1.94 g, 10 mmol) and trimethylamine (2.0 g, 20 mmol) was solved in CH_2Cl_2 (20 mL) followed by adding methacryloyl chloride (1.3 g, 12 mmol) dropwise slowly. The mixture was kept at 20°C for 6 h. M(A4) was purified by a column chromatography (petroleum ether/ethyl acetate = 10/1) as a colorless oil (1.95 g, 74.4% yield).

^1H NMR (400 MHz, CDCl_3 , δ/ppm): 6.12 (s, 1H, $\text{CH}_2=\text{CCH}_3$), 5.67 (s, 1H, $\text{CH}_2=\text{CCH}_3$), 5.45 (d, 1H, $\text{CHCH}_2\text{CH}=\text{C}$, $J = 6.8$ Hz), 5.05 (d, 1H, $\text{CH}_2\text{CH}_2\text{CH}=\text{C}$, $J = 6.9$ Hz), 3.58 (m, 2H, CHCH_2O), 2.10-2.20 (m, 7H, $\text{CH}_2\text{CHCH}_2\text{CH}_2$), 2.00-2.10 (m, 4H, $\text{CH}_2\text{CH}_2\text{CH}=\text{C}$), 2.00 (s, 3H, $\text{CH}_3\text{C}=\text{CH}_2$), 1.85 (s, 3H, $\text{CH}=\text{CCH}_3\text{CH}_3$), 1.82 (s, 3H, $\text{CH}=\text{CCH}_3\text{CH}_3$).

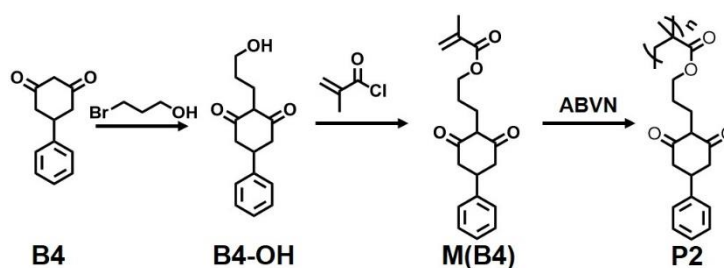
^{13}C NMR (100 MHz, CDCl_3 , δ/ppm): 167.63, 137.82, 136.62, 131.52, 125.32, 124.38, 119.25, 69.01, 37.76, 33.21, 28.45, 27.73, 26.53, 25.87, 25.79, 18.44, 17.77.

IR (ν/cm^{-1}): 3676, 2967, 2914, 1718, 1638, 1452, 1403, 1376, 1320, 1296, 1104, 1066, 1049, 1013, 984, 937, 813, 760, 726, 683, 580.

ESI-MS: observed (expected): 285.1839 (285.1831) $[\text{M} + \text{Na}^+]$.

P1: M(A4) (2.62 g, 10 mmol) and ABVN (50 mg, 0.1 mmol) were dissolved in DMF (20 mL). The mixture was purged by nitrogen flow for 20 min, and kept in an oil bath (75°C) for 12 h. The polymerization was quenched in an ice–water bath, and P1 was precipitated in diethyl ether three times as a white powder (2.08 g, 79.4% yield).

3.12 Poly(3-(2,6-dioxo-4-phenylcyclohexyl)propyl methacrylate) (P2)



B4-OH: 5-Phenyl-1,3-cyclohexanedione (B4, 9.40 g, 50 mmol), potassium iodide (KI, 10 g, 60 mmol), potassium carbonate (K_2CO_3 , 13.8 g, 50 mmol) and 3-bromo-1-propanol (6.9 g, 50 mmol) were dissolved in acetone (50 mL). This solution was refluxed for 12 h. After removing acetone under vacuum, B4-OH was purified by a column chromatography (petroleum ether/ethyl acetate = 5/1) as a colorless oil (8.86 g, 72.1% yield).

^1H NMR (400 MHz, CDCl_3 , δ/ppm): 7.10-7.45 (m, 5H, ph), 5.48 (s, 1H, CH_2OH), 4.06 (m, 2H, CH_2OH), 3.92 (m, 1H, CCHC), 3.41 (t, 1H, CH_2CHCH_2 , $J = 5.2$ Hz), 2.62 (m, 4H, CH_2CHCH_2), 2.60 (m, 2H, CHCH_2CH_2), 2.00 (m, 2H, $\text{CH}_2\text{CH}_2\text{OH}$).

^{13}C NMR (100 MHz, CDCl_3 , δ/ppm): 199.18, 199.18, 142.70, 128.90, 128.90, 127.17, 126.77, 126.77, 67.75, 62.27, 43.91, 43.91, 39.43, 36.71, 31.50.

IR (v/cm^{-1}): 3403, 3029, 2944, 1717, 1598, 1497, 1465, 1453, 1399, 1374, 1352, 1265, 1231, 1209, 1139, 1089, 1061, 1000, 968, 868, 825, 760, 733, 699, 657, 615, 600.

ESI-MS: observed (expected): 247.1339 (247.1334) $[\text{M} + \text{H}^+]$.

M(B4): B4-OH (2.5 g, 10 mmol) and trimethylamine (2.0 g, 20 mmol) were solved in CH_2Cl_2 (25 mL). Methacryloyl chloride (1.3 g, 12 mmol) was added dropwise slowly. The mixture was kept at 25°C for 1 h. After removing the white solid by filtration and the volatiles under vacuum, M(B4) was purified by a column chromatography (petroleum ether/ethyl acetate = 5/1) as a colorless oil (2.67 g, 85.1% yield).

^1H NMR (400 MHz, CDCl_3 , δ/ppm): 7.10-7.45 (m, 5H, ph), 6.12 (s, 1H, $\text{CH}_2=\text{C}$), 5.57 (s, 1H, $\text{CH}_2=\text{C}$), 4.21 (t, 2H, CH_2O , $J = 6.2$ Hz), 4.02 (m, 1H, CCHC), 3.41 (t, 1H, CH_2CHCH_2 , $J = 6.7$ Hz), 2.62 (m, 4H, CH_2CHCH_2), 2.60 (m, 2H, CHCH_2CH_2), 2.00 (m, 2H, $\text{CH}_2\text{CH}_2\text{CH}_2$), 1.98 (s, 3H, $\text{CH}_2=\text{CCH}_3$).

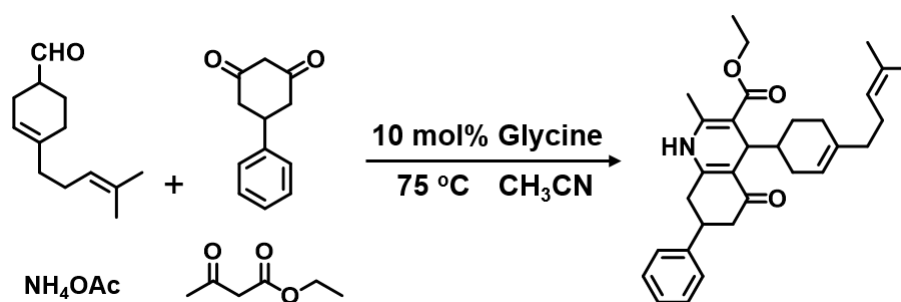
^{13}C NMR (100 MHz, CDCl_3 , δ/ppm): 198.93, 176.91, 167.34, 142.68, 136.19, 128.90, 128.90, 127.16, 126.77, 126.77, 125.91, 102.80, 65.38, 61.08, 43.91, 39.40, 36.60, 28.06, 18.40.

IR (v/cm^{-1}): 3675, 2958, 2901, 1716, 1654, 1602, 1497, 1469, 1454, 1428, 1403, 1374, 1351, 1318, 1296, 1257, 1231, 1207, 1161, 1044, 1007, 975, 940, 910, 869, 816, 760, 733, 699, 656, 613, 588.

ESI-MS: observed (expected): 337.1422 (337.1416) [M + Na⁺].

P2: M(B4) (1.57 g, 10 mmol) and ABVN (50 mg, 0.2 mmol) were dissolved in DMF (20 mL). This solution was purged by nitrogen flow for 20 min, then kept in an oil bath (75°C) for 12 h. The polymerization was quenched in an ice–water bath. P2 was purified by precipitation in diethyl ether three times as a white powder (1.37 g, 87.3% yield).

3.13 Ethyl 2-methyl-4-(4-(4-methylpent-3-en-1-yl)cyclohex-3-en-1-yl)-5-oxo-7-phenyl-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (M1)



Myrac aldehyde (A(4), 1.92 g, 10 mmol), ethyl acetoacetate (1.30 g, 10 mmol), 5-phenyl-1,3-cyclohexanedione (B(4), 1.89 g, 10 mmol), and ammonium acetate (1.16 g, 15 mmol) were put in a 15-mL centrifuge tube. Then, glycine (76 mg, 1 mmol) and acetonitrile (9.0 mL) were added. The mixture was kept in an oil bath (75°C) for 4 h. Acetonitrile was removed under vacuum, and the crude was purified by a column chromatography (petroleum ether/ethyl acetate: 5/1) to get M1 as a yellow oil (3.86 g, yield: 81.6%).

¹H NMR (400 MHz, CDCl₃, δ/ppm): 7.10-7.33 (m, 5H, ph), 6.08 (s, 1H, NH), 5.29 (s, 1H, CHCH₂CH=C), 5.09 (s, 1H, CH₂CH₂CH=C), 4.13 (m, 2H, CH₂O), 4.12 (s, 1H,

CCHC), 3.51 (m, 1H, phCH), 2.36 (m, 2H, CH₂C=O), 2.09 (s, 3H, NHCCH₃), 1.90 (s, 2H, NHCCH₂), 1.95-2.06 (m, 4H, CCH₂CH₂CH), 1.70-2.10 (m, 7H, CH₂CHCH₂CH₂), 1.81 (s, 3H, CHCCH₃), 1.76 (s, 3H, CHCCH₃), 1.17 (m, 3H, CH₃CH₂O).

¹³C NMR (100 MHz, CDCl₃, δ/ppm): 195.62, 168.44, 150.57, 144.00, 142.64, 137.34, 131.33, 128.79, 128.79, 127.05, 126.84, 126.84, 124.61, 120.49, 111.40, 104.00, 59.88, 43.78, 41.97, 41.57, 39.58, 37.64, 34.90, 34.55, 29.32, 27.68, 26.63, 25.80, 19.36, 17.78, 14.49.

IR (v/cm⁻¹): 3288, 2980, 2908, 1738, 1696, 1641, 1605, 1485, 1449, 1372, 1236, 1215, 1157, 1105, 1070, 1045, 937, 915, 846, 786, 762, 699, 634, 607, 560.

ESI-MS: observed (expected): 474.3015 (474.3008) [M + H⁺].

3.14 HTP measurements of the antibacterial ability of polymers

Preliminary screening: As a typically example, P(1)(1) (1.5 g) and PE powder (3.0 g) were mixed and grinded evenly in a mortar. The mixture was manufactured to a square membrane (40 × 40 mm, thickness: 0.5 mm) by the hot-press technology under 150°C. Then, P(1)(1)-PE discs (diameter: 5 mm) were prepared by using a puncher. Other P(X)(Y)-PE samples were similarly prepared.

All P(X)(Y)-PE samples were attached on glass slides (25 mm × 75 mm × 1 mm) to get mini-arrays (10 samples/piece). These mini-arrays were used to test the antibacterial ability of P(X)(Y) with PE as the control. Briefly, these polymer mini-arrays were sterilized by a 75% ethanol aqueous solution and UV light (254 nm, 40 w, 30 min), then put into a Luria-Bertani (LB) broth (200 mL) followed by addition of a

suspension of planktonic *E. coli* or *S. aureus* (10 μ L). The optical density of this suspension at UV~600 nm (OD600) is approximately 1.0. The polymer arrays were incubated with bacteria for 72 h followed by washing twice with water and fixation with paraformaldehyde (4%) prior to observation by laser scanning confocal microscopy (LSCM, excitation wavelength: 543 nm; emission wavelength: 566–719 nm).

Secondary screening: P(X)(Y)-polymer samples were prepared by abovementioned method. These samples were attached on glass slides for antibacterial test as abovementioned. Commodity polymers (PE, PP, PET, PMMA, PA-66) were used as the controls.

Antibacterial capabilities of all other polymers and small molecules (PEI, P(AEMA), P1, P2, and M1) were similarly tested through the polymer-array method.

3.15 Mechanical properties of P(4)(4)-polymer samples

The tensile shear strength of P(4)(4)-polymer samples were tested according to the national standard method (GBT 1040.3-2006). As a typical example, P(4)(4) (5 g) was mixed with PE (10 g) to prepare dumbbell-shaped splines by hot pressing. The dumbbell-shaped splines were in agreement with the national standard (length: 120 mm, breadth: 25 mm, thickness: 0.5 mm; length of narrow part: 25 mm, breadth of narrow part: 6 mm, thickness of narrow part: 0.5 mm). These splines were tested by an MTS SYSTEMS (CHINA) Co. Ltd. SANS CMT6503 electromechanically universal testing machine (tensile speed: 5 mm/min; 25°C).

All other P(4)(4)-polymer splines were prepared and tested through the same procedure. General polymers were used as the controls. The data are presented as mean \pm SD (n = 6).

3.16 Injection molding for plastic bowls

The plastic bowls were produced by injection molding technology. Typically, the uniform mixture of P(4)(4)-PE (33 wt.% of P(4)(4), 3 g) was added to the machine and heated (150 °C, 2 minutes) to melt-down. Then, the molten polymer was injected into a mold for bowl (0.6 Mpa, 1 minute) followed by keeping at 25°C (normal atmosphere, 10 minutes) to get a plastic P(4)(4)-PE bowl.

The PE bowls were prepared through the same procedure.

3.17 Antibacterial capability of bowls

The antibacterial capability of bowls has been characterized according to a standard method (QB/T2591-2003). Briefly, bowls were sterilized by a 75% ethanol aqueous solution, and incubated with a suspension of *S. aureus* in LB medium (5 mL, OD600 = 1.0) for 24 h at 37°C. Then, these bowls were washed with sterilized PBS for 10 times prior to addition of different beverages (drinking natural mineral water (Nongfu Spring), orange juice (NFC), drinking pure milk (Jizhi), red wine (ChangYu), coffee (Nestle), black tea (Oriental leaves)) (5 mL). These bowls containing beverages were covered with glass and incubated at 37°C. Aliquots were taken at different time intervals for analyses.

The experiment to simulate actual application of bowls was similarly performed by keeping bowls on a laboratory bench (face up) for 24 h prior to adding different beverages.

3.18 Plate-streaking experiment

A plate-streaking experiment was performed to test for the presence of viable bacteria. Typically, after 24 h culture, beverage aliquots (100 μ L) were taken and evenly coated on the surface of LB agar-coated petri dishes (diameter: 90 mm, thickness: 6 mm, LB: 1.5 g/L). These petri dishes were incubated at 37°C for 24 h prior to observation. Original beverages were used as the controls.

Beverage samples taken at 48 h and 72 h were similarly tested.

3.19 Interaction between P(4)(4) and polysaccharides

The interaction between P(4)(4) and two polysaccharides on bacteria surface (lipopolysaccharide and peptidoglycan) were tested via an Octet assay (Abdiche et al., 2008) by using a BLI Automated Biosensor. Streptavidin (SA) biosensors and kinetic buffer (PBS, 0.02% Tween) were used. Each polysaccharide (1 μ g/mL) was biotinylated by Sulfo-NHS-Biotin (Pierce Biotechnology, Rockford, IL) at a 1:1 ratio. P(4)(4) was dissolved in DMSO (0.1 mL, 400 μ g/mL) then added in the kinetics buffer (1.9 mL) prior to measurement.

3.20 Statistical Analyses

Results were analyzed with SPSS 25.0 and MedCalc 18.1 and are presented as mean \pm SD as indicated. Comparisons were performed between two groups using a two-tailed Student's t-test or ANOVA test when comparing more than two conditions. For all analyses, $*p < 0.05$ and $**p < 0.01$ were considered statistically significant. Each experiment was carried out at least three times independently. The sample size is pre-estimated to ensure statistical analysis and no sample was optionally excluded from analysis. No blinding was done in the analyses and quantifications.

Reference:

Abdiche, Y., Malashock, D., Pinkerton, A., and Pons, J. (2008). Determining kinetics and affinities of protein interactions using a parallel real-time label-free biosensor, the Octet. *Anal Biochem* 377, 209-217.

Nickoloff, J.A. (1995). *Electroporation protocols for microorganisms* (Totowa, N.J.: Humana Press).