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### **Supporting Information**

### Effect of hydrophilic groups on the bioactivity of antimicrobial polymers

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### 1. Characterisation of polymers

### 2.1. HEA family



*Figure S1.* <sup>1</sup>*H NMR spectrum of (A) Boc-protected crude HEA-I1535 after polymerisation, (B) Boc-protected purified HEA-I1535, and (C) Boc-deprotected HEA-I1535.* 

Note: The absence of monomer signals at  $\delta$  5.5-6.4 ppm indicates almost complete monomer conversion. (B) The absence of the signal at  $\delta$  6.8 ppm (attributed to urethane group protons) and 1.4 ppm (attributed to tertbutyl group protons) in the <sup>1</sup>H NMR spectra of the polymers confirmed the successful removal of the Bocprotection group.



**Figure S2.** <sup>1</sup>*H* NMR spectrum of purified HEA-I1040 in DMSO- $d_6$  and molecular weight distribution (inset). The polymer was purified by precipitation in hexane/diethyl ether (1:4). Note: The signal at (d)  $\delta$  4.48 ppm is attributed to the CH in adjacent position of the RAFT end-group (1H, CHSC(=S)S).

Calculation for HEA family polymers containing I as a hydrophobic group (using <sup>1</sup>H NMR of Bocprotected polymers).

For example, monomer composition of HEA-I1040 was calculated as follows:

% Cationic monomer = 
$$\frac{\int a}{\int a + \frac{\int b}{2} + \frac{\int c}{6}} = \frac{27.44}{(27.44 + \frac{10.32}{2} + \frac{125.21}{6})} \times 100\%$$
  
= 51.3%

% HEA monomer= $\frac{\frac{\int b/2}{\int a + \frac{\int b}{2} + \frac{\int c}{6}} = \frac{\frac{10.32}{2}}{(27.44 + \frac{10.32}{2} + \frac{125.21}{6})} \times 100\% = 9.7\%$ 

% I monomer = 
$$\frac{\int c/6}{\int a + \frac{\int b}{2} + \frac{\int c}{6}} = \frac{\frac{125.21}{6}}{(27.44 + \frac{10.32}{2} + \frac{125.21}{6})} \times 100\%$$
  
= 39.0%

Degree of polymerisation (DP<sub>n</sub>) = 
$$\frac{\int a + \frac{\int b}{2} + \frac{\int c}{6}}{\int d} = \frac{(23.1 + \frac{17.52}{2} + \frac{76.05}{6})}{1} = 53.5$$

The experimental cationic: hydrophilic: hydrophobic of (51:10:39) and the  $DP_n$  of 53.5 is in good agreement

with the feed ratio of (50:10:40) and the target  $DP_n$  of 40.



**Figure S3.** <sup>1</sup>*H* NMR spectrum of purified HEA-I1535 in DMSO- $d_6$  and molecular weight distribution (inset). The polymer was purified by precipitation in hexane/diethyl ether (1:4). Note: The signal at (d)  $\delta$  4.48 ppm is attributed to the CH in adjacent position of the RAFT end-group (1H, CHSC(=S)S).



**Figure S4.** <sup>1</sup>*H* NMR spectrum of purified HEA-I2030 in DMSO- $d_6$  and molecular weight distribution (inset). The polymer was purified by precipitation in hexane/diethyl ether (1:4). Note: The signal at (d)  $\delta$  4.48 ppm is attributed to the CH in adjacent position of the RAFT end-group (1H, CHSC(=S)S).



*Figure S5.* <sup>1</sup>*H* NMR spectrum of purified HEA-B1040 in DMSO- $d_6$  and molecular weight distribution (inset). The polymer was purified by precipitation in hexane/diethyl ether (1:4).

## Calculation for HEA polymers containing B as hydrophobic monomer (using <sup>1</sup>H NMR of Boc-protected polymers)

For example, monomer composition of HEA-B1040 was calculated as follows:

% Cationic monomer = 
$$\frac{\int a}{\int a + \frac{\int b}{2} + \frac{\int c}{5}} = \frac{50}{(50 + \frac{20.16}{2} + \frac{186.64}{5})} \times 100\%$$
  
= 51.3%

$$\frac{\int b/2}{\int a + \frac{\int b}{2} + \frac{\int c}{5}} = \frac{\frac{39.39}{2}}{(50 + \frac{20.16}{2} + \frac{186.64}{5})} \times 100\%$$
= 10.3%

$$\% \text{ B monomer} = \frac{\frac{\int c/5}{\int a + \frac{\int b}{2} + \frac{\int c}{5}} = \frac{\frac{186.64}{5}}{(50 + \frac{20.16}{2} + \frac{186.64}{5})} \times 100\%$$

The experimental cationic: hydrophilic: hydrophobic of (51:10:38) is in good agreement with the feed ratio of (50:10:40)



*Figure S6.* <sup>1</sup>*H NMR spectrum of purified HEA-B1535 in DMSO-d*<sub>6</sub> and molecular weight distribution (inset). *The polymer was purified by precipitation in hexane/diethyl ether (1:4).* 



**Figure S7.** <sup>1</sup>*H* NMR spectrum of purified AM-I2030 in DMSO- $d_6$  and molecular weight distribution (inset). The polymer was purified by precipitation in hexane/diethyl ether (1:4). Note: The signal at (d)  $\delta$  4.48 ppm is attributed to the CH in adjacent position of the RAFT end-group (1H, CHSC(=S)S).

### Calculation for AM-family polymers using <sup>1</sup>H NMR of Boc-protected polymers.

For example, monomer composition of AM-I2030 was calculated as follows:

% Cationic monomer = 
$$\frac{\int a}{\int a + \frac{\int b}{8} + \frac{\int c}{6}} = \frac{50}{(50 + \frac{133.13}{8} + \frac{175.85}{6})} \times 100\%$$
 = 52.1%

% AM monomer = 
$$\frac{\frac{\int b/8}{\int a + \frac{\int b}{8} + \frac{\int c}{6}} = \frac{\frac{133.13}{8}}{(50 + \frac{133.13}{8} + \frac{175.85}{6})} \times 100\% = 17.3\%$$

% I-monomer = 
$$\frac{\int c/6}{\int a + \frac{\int b}{8} + \frac{\int c}{6}} = \frac{\frac{125.21}{6}}{(50 + \frac{133.13}{8} + \frac{175.85}{6})} \times 100\%$$
  
= 30.5%

Degree of polymerisation (DP<sub>n</sub>) = 
$$\frac{\int a + \frac{\int b}{8} + \frac{\int c}{6}}{\int d} = \frac{(50 + \frac{133.13}{8} + \frac{175.85}{6})}{2.2} = 43.6$$

The experimental cationic: hydrophilic: hydrophobic of (52:17:31) and the DP<sub>n</sub> of 43.6 is in good agreement with the feed ratio of (50:20:30) and the target DP<sub>n</sub> of 40.



**Figure S8.** <sup>1</sup>*H* NMR spectrum of purified PEG-AA-I1040 in DMSO-d<sub>6</sub> and molecular weight distribution (inset). The polymer was purified by precipitation in hexane/diethyl ether (1:1). Note: The signal at (d)  $\delta H$  4.48 is attributed to CH in adjacent position of RAFT end-group (1H, CHSC(=S)S).

### Calculation for PEG-AA family polymers using <sup>1</sup>H NMR of Boc-protected polymers.

For example, monomer composition of PEG-AA-I1040 was calculated as follows:

% Cationic monomer = 
$$\frac{\int a}{\int a + \frac{\int b}{2} + \frac{\int c}{6}} = \frac{50}{(50 + \frac{22.82}{2} + \frac{233.61}{6})} \times 100\%$$
 = 49.8%

% PEG-AA monomer = 
$$\frac{\frac{\int b/2}{\int a + \frac{\int b}{2} + \frac{\int c}{6}} = \frac{\frac{22.82}{2}}{(50 + \frac{22.82}{2} + \frac{233.61}{6})} \times 100\% = 11.4\%$$

$$\% \text{ I monomer} = \frac{\frac{\int c/6}{\int a + \frac{\int b}{2} + \frac{\int c}{6}}}{(50 + \frac{22.82}{2} + \frac{233.61}{6})} \times 100\%$$

Degree of polymerization (DP<sub>n</sub>) = 
$$\frac{\int a + \frac{\int b}{2} + \frac{\int c}{6}}{\int d} = \frac{(50 + \frac{22.82}{2} + \frac{233.61}{6})}{2.6} = 38.6$$

The experimental cationic: hydrophilic: hydrophobic of (50:11:39) and the  $DP_n$  of 39 is in good agreement with the feed ratio of (50:10:40) and the target  $DP_n$  of 40.



*Figure S9.* <sup>1</sup>*H* NMR spectrum of purified PEG-AA-I1535 in DMSO- $d_6$  and molecular weight distribution (inset). The polymer was purified by precipitation in hexane/diethyl ether (1:1).



*Figure S10.* <sup>1</sup>*H* NMR spectrum of purified PEG-AA-I2030 in DMSO- $d_6$  and molecular weight distribution (inset). The polymer was purified by precipitation in hexane/diethyl ether (1:1). Note: The signal at (d)  $\delta$  4.48

ppm is attributed to the CH in adjacent position of the RAFT end-group (1H, CHSC(=S)S).



**Figure S11.** <sup>1</sup>*H* NMR spectrum of purified PEG-A-I1040 in DMSO-d<sub>6</sub> and molecular weight distribution (inset). The polymer was purified by precipitation in hexane/diethyl ether (1:1). Note: The signal at (d)  $\delta$  4.48 ppm is attributed to the CH in adjacent position of the RAFT end-group (1H, CHSC(=S)S).

Calculation for PEG-A family polymers containing I as a hydrophobic group (using <sup>1</sup>H NMR of Bocprotected polymers).

For example, monomer composition of PEG-A-I1040 was calculated as follows:

% Cationic monomer = 
$$\frac{\int a}{\int a + \frac{\int b}{3} + \frac{\int c}{6}} = \frac{\frac{50}{(50 + \frac{30.55}{3} + \frac{235.18}{6})} \times 100\%} = 50.3\%$$

% PEG-A monomer = 
$$\frac{\frac{\int b/3}{\int a + \frac{\int b}{3} + \frac{\int c}{6}} = \frac{\frac{30.55}{3}}{(50 + \frac{30.55}{3} + \frac{235.18}{6})} \times 100\% = 10.2\%$$

% I monomer = 
$$\frac{\int c/6}{\int a + \frac{\int b}{3} + \frac{\int c}{6}} = \frac{\frac{235.18}{6}}{(50 + \frac{30.55}{3} + \frac{235.18}{6})} \times 100\%$$
  
= 39.4%

Degree of polymerisation (DP<sub>n</sub>) = 
$$\frac{\int a + \frac{\int b}{3} + \frac{\int c}{6}}{\int d} = \frac{(50 + \frac{30.55}{3} + \frac{235.18}{6})}{2.6} = 38.2$$

The experimental cationic: hydrophilic: hydrophobic of (50:10:39) and the  $DP_n$  of 38 is in good agreement with the feed ratio of (50:10:40) and the target  $DP_n$  of 40.



*Figure S12.* <sup>1</sup>*H* NMR spectrum of purified PEG-A-I1535 in DMSO-d<sub>6</sub> and molecular weight distribution (inset). The polymer was purified by precipitation in hexane/diethyl ether (1:1). Note: The signal at (d)  $\delta$  4.48 ppm is attributed to the CH in adjacent position of the RAFT end-group (1H, CHSC(=S)S).



**Figure S13.** <sup>1</sup>*H* NMR spectrum of PEG-A-I2030 in DMSO- $d_6$  and molecular weight distribution (inset). Note: The signal at (d)  $\delta$  4.48 ppm is attributed to the CH in adjacent position of the RAFT end-group (1H, CHSC(=S)S).



**Figure S14.** <sup>1</sup>*H* NMR spectrum of PEG-A-I3020 in DMSO- $d_6$  and molecular weight distribution (inset). Note: The signal at (d)  $\delta$  4.48 ppm is attributed to the CH in adjacent position of the RAFT end-group (1H, CHSC(=S)S).



*Figure S15.* <sup>1</sup>*H* NMR spectrum of purified PEG-A-B1040 in DMSO- $d_6$  and molecular weight distribution (inset). The polymer was purified by precipitation in hexane/diethyl ether (1:1).



*Figure S16.* <sup>1</sup>*H* NMR spectrum of purified PEG-A-B1535 in DMSO- $d_6$  and molecular weight distribution (inset). The polymer was purified by precipitation in hexane/diethyl ether (1:1).

# Calculation for PEG-family polymers using <sup>1</sup>H NMR of Boc-protected polymers containing B as a hydrophobic group (using <sup>1</sup>H NMR of Boc-protected polymers).

For example, monomer composition of PEG-A-B1535 was calculated as follows:

% Cationic monomer =  $\frac{\int a}{\int a + \frac{\int b}{3} + \frac{\int c}{5}} = \frac{50}{(50 + \frac{46.5}{3} + \frac{161.8}{5})} \times 100\%$  = 51.1%

% PEG-A monomer = 
$$\frac{\frac{\int b/3}{\int a + \frac{\int b}{3} + \frac{\int c}{5}} = \frac{\frac{46.5}{3}}{(50 + \frac{46.5}{3} + \frac{161.8}{5})} \times 100\% = 15.8\%$$

% B monomer = 
$$\frac{\int c/5}{\int a + \frac{\int b}{3} + \frac{\int c}{5}} = \frac{\frac{161.8}{5}}{(50 + \frac{46.5}{3} + \frac{161.8}{5})} \times 100\%$$
  
= 33.1%

The experimental cationic: hydrophilic: hydrophobic of (51:16:33) is in good agreement with the feed ratio of (50:15:35)

#### 2. Kinetics studies of copolymerisation of representative polymers



*Figure S17.* <sup>1</sup>*H* NMR spectra of the monomers and their mixture used in the kinetic study of PEG-AA-I2030. Insert shows <sup>1</sup>*H* NMR spectra of acrylamide bonds for pure Boc-AEAm, I, and PEG-AA monomers and their mixture before polymerisation.



**Figure S18.** <sup>1</sup>H NMR spectra of polymerisation mixture at different time points. Note: The monomer conversions for each monomer were calculated based on the gradual decreases of the different signals. The signals were normalised by the signal at (d)  $\delta$  0.87 ppm which is attributed to (-CH<sub>3</sub>) of hydrophobic side chain (I) as internal standard.

We conducted kinetic studies and monitored the monomer conversions using <sup>1</sup>H NMR according to the reported protocol<sup>1-3</sup> to determine if the three monomers were preferentially incorporated in the polymer. Owing to the distinct chemical shifts for the double bonds, it was possible to monitor the overall and individual monomer conversions in copolymerization. **Figure S17-S18** illustrated the detection of individual monomer peaks and their quantification for calculating their monomer conversion of PEG-AA-I2030 copolymerization. More details regarding a mathematical calculation of individual monomer conversions can be found in the previous studies of our group.<sup>1-3</sup>



*Figure S19. Total and individual monomer conversions versus time for the preparation of (A) HEA-I2030, (B) AM-I2030, (C) PEG-A-I2030 and (D) PEG-AA-I2030 copolymers.* 

*Table S1*. Detail recipe to synthesize representative polymer of each family.

Copolymers	BTPA (mg)	DMSO (V/µL)	Monomer stock solution (V/µL)		ZnTPP (V/µL)
HEA-I2030	3.45	51.57	Boc-AEAm	170.54	
			HEA	36.11	39.31
			Ι	70.49	
AM-I2030	3.35	52.72	Boc-AEAm	165.54	
			A.M	42.92	38.16
			Ι	68.42	
PEG-AA-I2030	2.30	64.74	Boc-AEAm	113.38	
			PEG-AA	114.67	26.13
			Ι	46.86	
PEG-A-I2030	2.43	63.25	Boc-AEAm	119.84	
			PEG-A	105.79	27.62
			Ι	49.53	
PEG-A-B1535	2.53	62.09	Boc-AEAm	124.90	
			PEG	82.69	28.79
			В	64.91	

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2. Judzewitsch, P. R.; Nguyen, T.-K.; Shanmugam, S.; Wong, E. H. H.; Boyer, C., Towards Sequence-Controlled Antimicrobial Polymers: Effect of Polymer Block Order on Antimicrobial Activity. *Angewandte Chemie International Edition* **2018**, *57* (17), 4559-4564.

3. Judzewitsch, P. R.; Zhao, L.; Wong, E. H. H.; Boyer, C., High-Throughput Synthesis of Antimicrobial Copolymers and Rapid Evaluation of Their Bioactivity. *Macromolecules* **2019**, *52* (11), 3975-3986.