Electronic Supplementary Material (ESI) for Materials Chemistry Frontiers.

Supporting Information

Antibacterial Supramolecular Polymers Constructed via Self-Sorting: Promoting Antibacterial Performance and Controllable Degradation

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Content

1. Materials and instruments

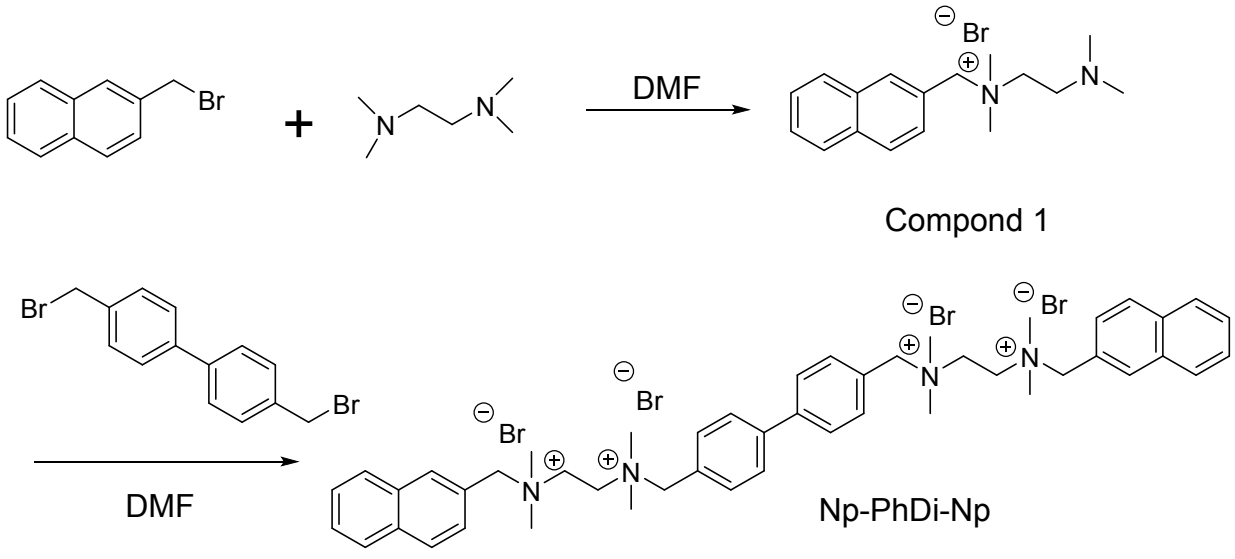
All the chemical materials we used were from commercial suppliers without further purification. JOEL JNM-ECA400 spectrometer (400 MHz) was used to get the ^{1}H NMR spectra. Dynamic light scattering (DLS) was performed on the Malvern ZetaSizerNanoZS90. Isothermal titration calorimetry (ITC) experiments were carried out with a Microcal VP-ITC apparatus in PBS buffer solution at 298.15 K. ESI-MS was carried out on a LTQ LC/MS apparatus. Analytical ultracentrifugation (AUC) experiments were carried out with a Beckman ProteomeLab XL-I apparatus. In the characterization of our supramolecular polymers, the rotator speed was chosen to be 60000 rpm. Sedfit (version 14.7 g) was utilized to fit the sedimentation data of AUC, and then calculate sed coefficients and molecular weights. Density determinations were obtained by an Anton Paar DMA 5000 apparatus to obtain the partial specific volume. The ampicillin-resistant Escherichia coli (*E. coli,* Top 10) was purchased from Beijing Bio-Med Technology Development Co., Ltd.

2. Preparation of bacterial solution

A single colony of Amp^{r} *E. coli* (Top 10) on a solid Luria Broth (LB) agar plate was transferred to 10 mL of liquid LB containing 50 μg / mL ampicillin and was grown at 37 ° C for 6 h. *E.coli* were harvested by centrifuging (7100 rpm for 2 min) and were washed with phosphate buffer saline (PBS, 10 mM, pH = 7.4) by twice. The supernatant was discarded and the remaining *E. coli* were resuspended in PBS, and diluted to an optical density of 1.0 at 600 nm (OD 600 = 1.0).

3. Synthesis of molecules

3.1 Synthesis of Np-PhDi-Np



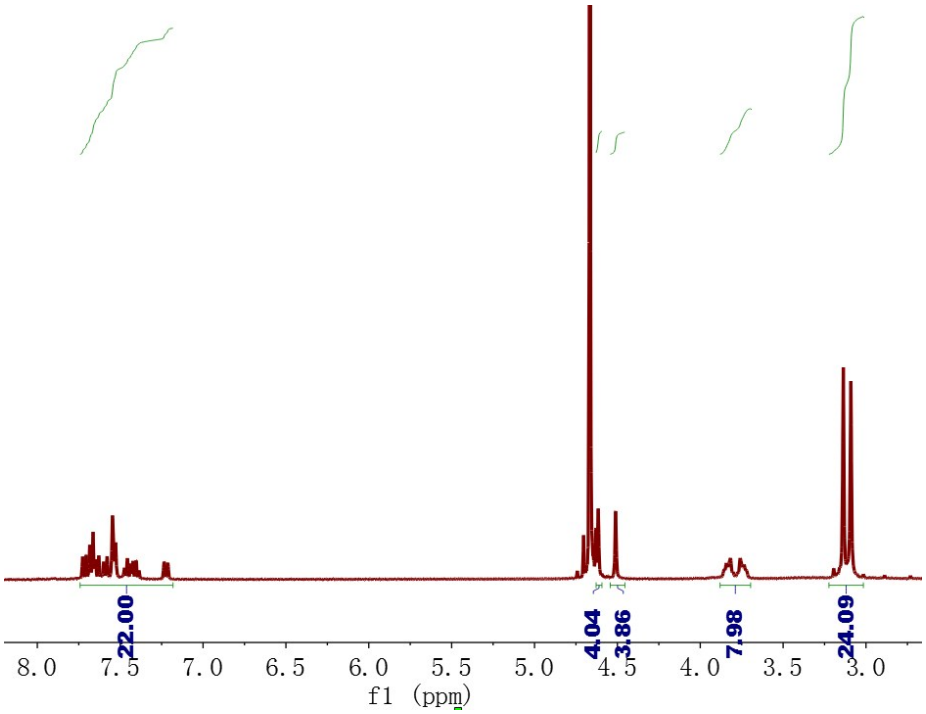
Scheme S1. Synthetic route of Np-PhDi-Np.

0.54 g 2-(Bromomethyl)naphthalene and 10 mL N, N, N', N'-Tetramethylethylenediamine were mixed in a flask with 10 mL DMF. The mixture was heated at 80 °C in a nitrogen environment overnight (around 12 h), altering the color to light yellow. The light yellow solution was added dropwise into 150 mL of diethyl ether and filtered. The filter residue was washed by diethyl ether for three times. The white crystal-like precipitate was compound 1, with a yield of 88% (0.72 g).

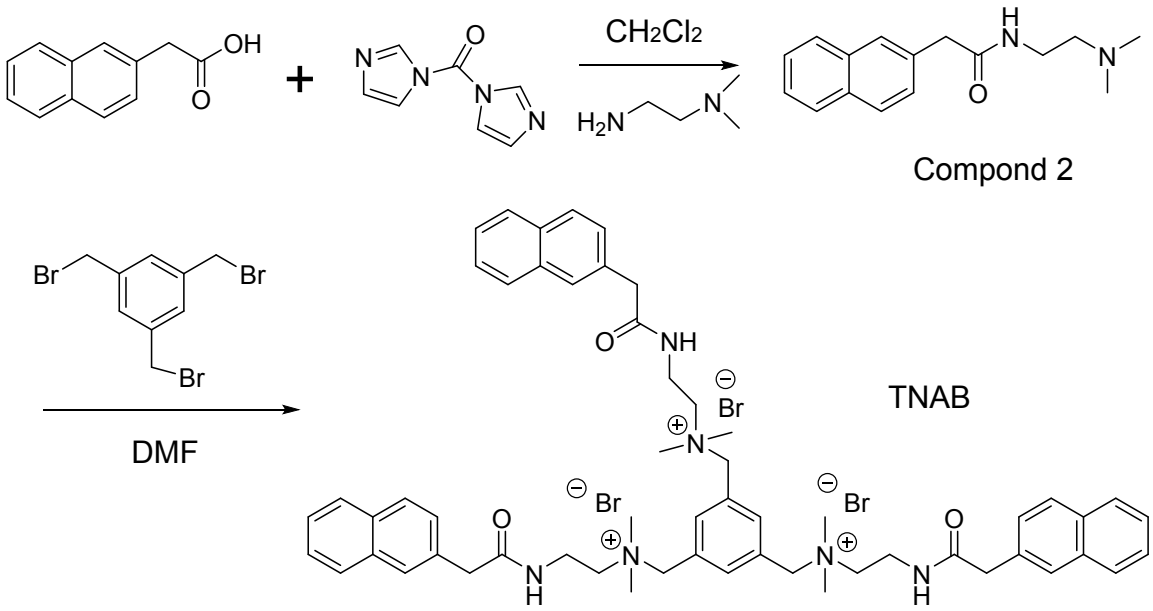
0.12 g 4,4'-Bis(bromomethyl)biphenyl and 1.1 g compound 1 were mixed with 20 mL DMF. The mixture was poured into a flask and then heated up to 90 °C for 10 h in a nitrogen environment. The white solid precipitate (Np-PhDi-Np) was filtered directly and washed by acetonitrile and diethyl ether for three times. The yield was 95% (0.34 g).

^{1}H NMR (400 MHz, D_2O): 7.20-7.75 (m, 22H), 4.60 (s, 4H), 4.51 (s, 4H), 3.65-3.90 (m, 8H), 3.14 (s, 12H), 3.09 (s, 12H).

ESI-MS: m/z (Np-PhDi-Np - 2Np) = 206.18 (Calculated for (Np-PhDi-Np - 2Np): m/z = 206.18)



3.2 Synthesis of TNAB



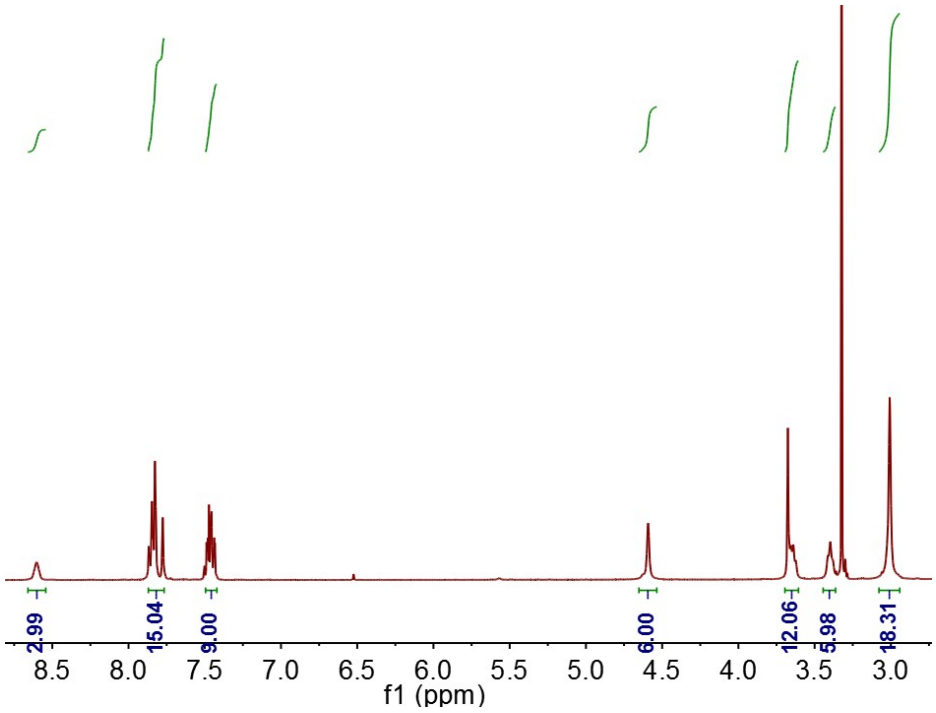
Scheme S2. Synthetic route of TNAB.

1.0 g 2-Naphthylacetic acid and 1.5 g 1, 1'-Carbonyldiimidazole were mixed in a flask with 25 mL dry CH_2Cl_2. The mixture was stirred overnight (around 12 h) at room temperature in a nitrogen environment. Then the 2-Dimethylaminoethylamine (3.2 g) was added into the flask, stirring for another 12 h in a nitrogen environment. After washing by 50 mL of 1 N $NaHCO_3$ and 25 mL of 1 N hydrochloric acid, the product was extracted by 75 mL of CH_2Cl_2, and then dried over Na_2SO_4. After evaporation of the solvent, the compound was purified by column chromatography on silica gel using dichloromethane / methanol (9:1) as eluent. The yield of compound 2 was 70% (0.96 g).

0.85 g compound 2 and 0.10 g 1,3,5-Tris(bromomethyl)benzene were mixed with 20 mL DMF. The mixture was poured into a flask, heating up to 90 °C for 10 h in a nitrogen environment. Then the solution was added dropwise into 150 mL of diethyl ether and filtered. The filter residue was washed by diethyl ether for three times. The yield of TNAB was 62% (0.20 g).

^{1}H NMR (400 MHz, DMSO-d6): 8.60 (br, 3H), 7.70-7.85 (m, 15H), 7.35-7.50 (m, 9H), 4.55 (s, 6H), 3.50-3.70 (m, 12H), 3.32-3.40 (m, 6H), 2.97 (s, 18H).

ESI-MS: m/z = 295.18 (Calculated: m/z = 295.18)



3.3 Synthesis of Np

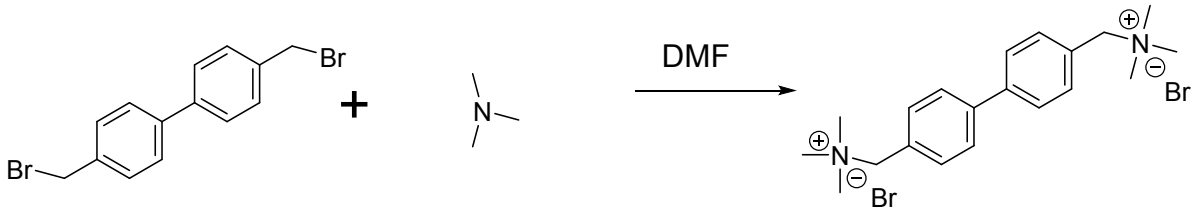
Scheme S3. Synthetic route of Np.

1.1 g 2-(Bromomethyl)naphthalene and 10 mL Trimethylamine (ca. 25% in methanol, ca. 3.2 mol/L) were mixed in a flask with 15 mL DMF. The mixture was heated at 80 °C overnight (around 12 h), altering the color to light yellow. The light yellow solution was added dropwise into 150 mL of diethyl ether and filtered. The filter residue was washed by diethyl ether for three times. The white crystal-like precipitate was Np, with a yield of 74% (1.0 g).

^{1}H NMR (400 MHz, D_2O): 8.00-8.25 (m, 4H), 7.60-7.75 (m, 3H), 4.68 (s, 2H), 3.17 (s, 9H).

ESI-MS: m/z = 200.14 (Calculated: m/z = 200.14)

3.4 Synthesis of PhDi



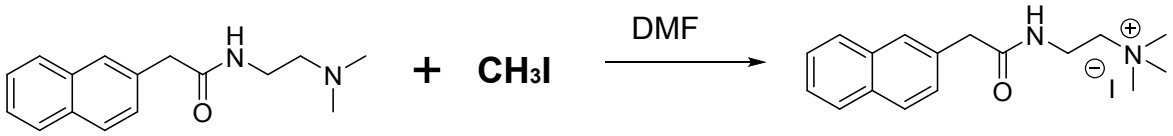
Scheme S3. Synthetic route of PhDi.

1.3 g 4,4'-Bis(bromomethyl)biphenyl and 10 mL Trimethylamine (ca. 25% in methanol, ca. 3.2 mol/L) were mixed in a flask with 15 mL DMF. The mixture was heated at 80 °C overnight (around 12 h). The mixture was added dropwise into 150 mL of diethyl ether and filtered. The filter residue was washed by diethyl ether for three times. The white powder-like precipitate was PhDi, with a yield of 76% (1.3 g).

^{1}H NMR (400 MHz, D_2O): 7.88 (d, *J* = 8.0 Hz, 4H), 7.71 (d, *J* = 8.4 Hz, 4H), 4.58 (s, 4H), 3.16 (s, 18H).

ESI-MS: m/z = 149.12 (Calculated: m/z =149.12)

3.5 Synthesis of NpAM



Scheme S4. Synthetic route of NpAM.

0.21 g 4,4'-Bis(bromomethyl)biphenyl and 1.0 mL Iodomethane were mixed in a flask with 10 mL DMF. The mixture was heated at 70 °C in a nitrogen environment overnight (around 12 h). The mixture was added dropwise into 100 mL of diethyl ether and filtered. The filter residue was washed by diethyl ether for three times. The white powder-like precipitate was NpAM, with a yield of 75% (0.24 g).

^{1}H NMR (400 MHz, D_2O): 7.80-8.00 (m, 4H), 7.40-7.65 (m, 3H), 3.82 (s, 2H), 3.65-3.75 (m, 2H), 3.47 (t, *J* = 6.4 Hz, 2H), 3.10 (s, 9H).

ESI-MS: m/z = 271.18 (Calculated: m/z = 271.18)

4. Experimental procedures

4.1 Antibacterial experiments

4.1.1 Antibacterial experiments in PBS solution

0.02 OD *E. coli* were respectively incubated with 50 μM TNAB, supramolecular polymer backbone and antibacterial supramolecular polymer in PBS at 37 °C for 23 min. And then all of the *E. coli* suspensions were serially diluted 1×10^4 fold with PBS. A 100 μL portion of the dilution with bacteria was spread on the solid LB (supplemented with 50 μg / mL ampicillin) agar plate, and the colonies formed after 18 h incubation at 37°C were counted. The inhibition ratio was determined by dividing the number of colony-forming unit (CFU). The inhibition ratio was calculated according to the following equation:

$$CFU\ Ratio = \frac{C}{C_0} \times 100\%$$

C is the CFU of the experimental group treated with different drugs, and C_0 is the CFU of the control group without any treatments.

4.1.1 Antibacterial experiments in LB nutrient solution

Bacteria (*E. coli*) were seeded in 96-well plates at a density of 5×10^5 cfu/well in the experiments with LB nutrient solution. The bacteria were incubated with different concentrations of antibacterial supramolecular polymer and TNAB at 37 ℃ for 24 h. After shaking the plate for 2 min, the absorbance at 600 nm of each well was read by a microplate reader. The antibacterial inhibition ratio (%) was calculated according to the following equation:

$$\mathrm{I}\ (\%) = \{[(\mathrm{A\text{-}B}) - (\mathrm{C\text{-}B})]/\ (\mathrm{A\text{-}B})\} \times 100\%$$

where A is the absorbance of the bacteria control (without adding antibacterial agents, other operations were identical to the experiment group), B is the absorbance of the culture medium control (only added equivalent culture medium used in experiment group, other operations were identical to the experiment group) and C is the absorbance of the experiment group.

4.2 SEM measurements

In order to study the mechanism of TNAB and antibacterial supramolecular polymer in killing bacteria, SEM was applied. After the treatment described in antibacterial experiments, *E. coli* was immediately fixed with glutaraldehyde (0.5%) in PBS at room temperature for 30 min. The bacteria were centrifuged (10000 g for 5 min) and the supernatant was removed, and then the pellets were suspended in sterile water. 2-3 μL of *E. coli* suspension was dropped onto clean silicon slices followed by drying naturally in the air. Once the specimens became dry, 0.1% glutaraldehyde was added to fix it for 1 h and then 0.5% glutaraldehyde for another 2 h. Next, the specimens were washed twice with

sterile water and then were dehydrated by adding ethanol in a graded series (70% for 6 min, 90% for 6 min, and 100% for 6 min), and dried after that. Finally, the specimens were platinum-coated before being put into the experiment of SEM.

4.3 NMR spectra of Np-PhDi-Np with different ratios of CB[8]

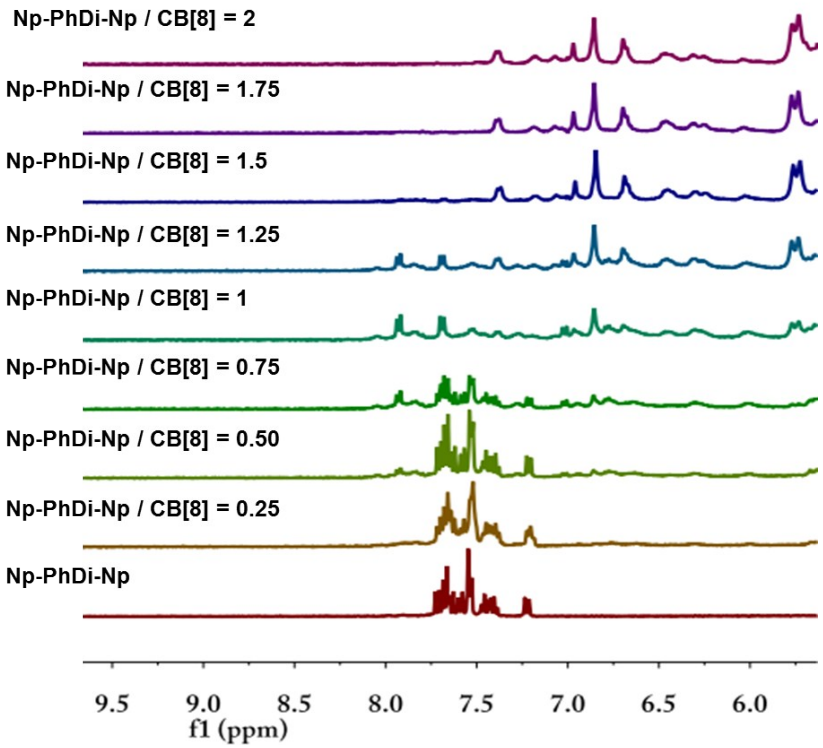


Figure S1 The change of ^{1}H NMR spectra by addition of CB[8] to Np-PhDi-Np.

From ^{1}H NMR spectra we found that when the molar ratio of CB[8] to Np-PhDi-Np increased from 0 to 1.0, the peaks of naphthalene moiety shifted up-field, while the peaks of phenylbenzene moiety shifted down-field. These shifting peaks indicated that the CB[8] combined with naphthalene moieties of Np-PhDi-Np primarily, acting as the driving force for supramolecular polymerization. When the molar ratio of CB[8] to Np-PhDi-Np increased from 1.0 to 2.0, the peaks of phenylbenzene moiety shifted up-field, which indicated that the CB[8] combined with the phenylbenzene moiety of Np-PhDi-Np subsequently.

4.4 Thermodynamic information of host-guest interaction between model molecules and CB[8]

4.4.1 Thermodynamic information of host-guest interaction between Np and CB[8]

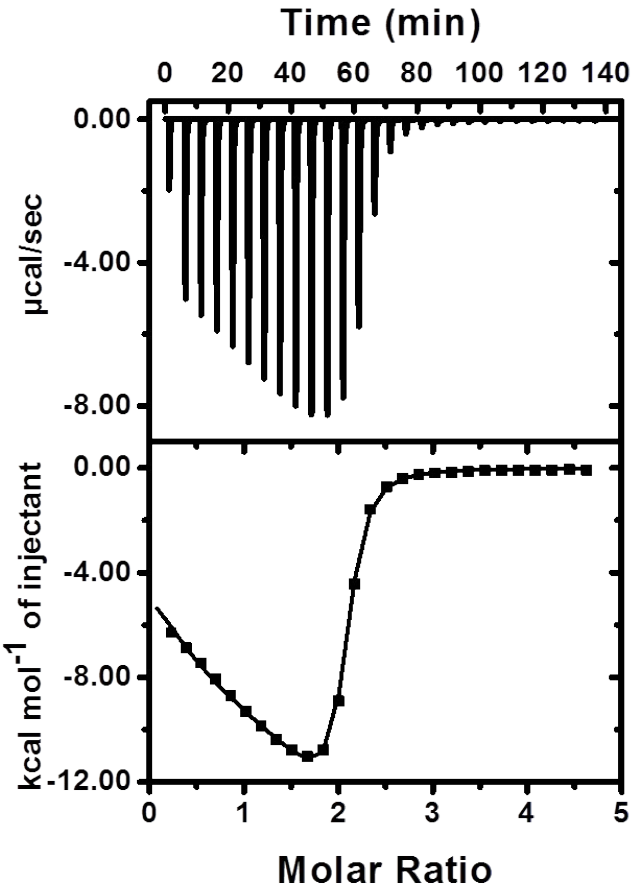


Figure S2 Thermodynamic information of host-guest interaction was collected through ITC. Simulated with two-sites binding model, the two-step binding constants of Np to CB[8] were calculated as $2.4 \times 10^6\ M^{-1}$ and $9.5 \times 10^5\ M^{-1}$, respectively. ITC data measured as Np titrated into CB[8]. The concentrations were fixed as c(Np) = 2.0 mM, c(CB[8]) = 0.1 mM.

4.4.2 Thermodynamic information of host-guest interaction between PhDi and CB[8]

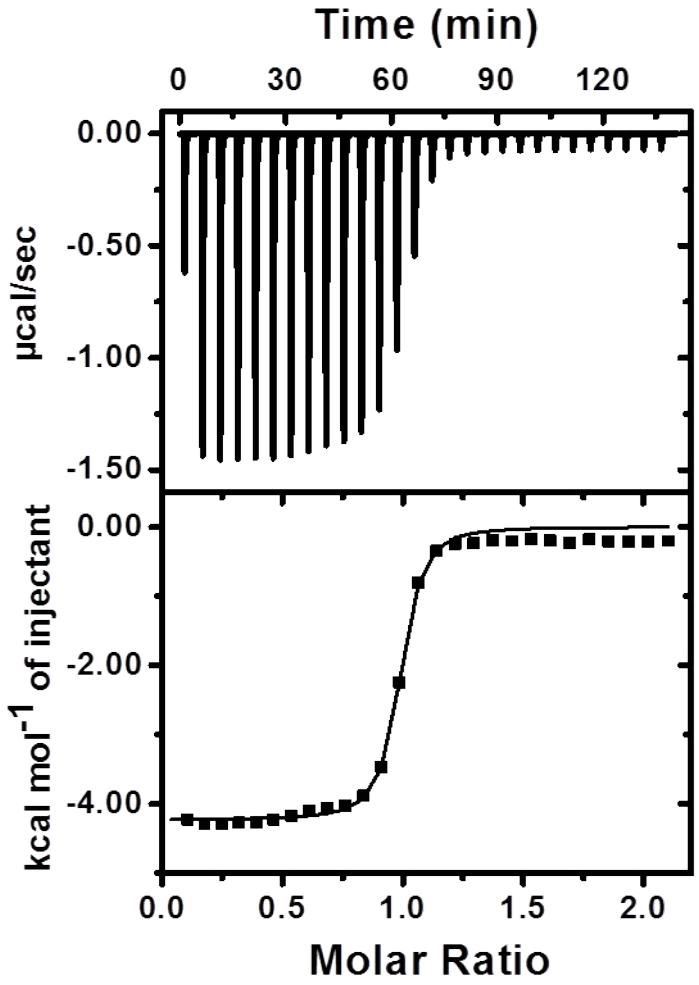


Figure S3 Thermodynamic information of host-guest interaction was collected through ITC. Simulated with one site binding model, the binding constant of PhDi to CB[8] was calculated as 5.1×10^6 M^{-1}. ITC data measured as PhDi titrated into CB[8]. The concentrations were fixed as c(PhDi) = 1.0 mM, c(CB[8]) = 0.1 mM.

4.4.3 Thermodynamic information of host-guest interaction between NpAM and supramolecular polymer backbone

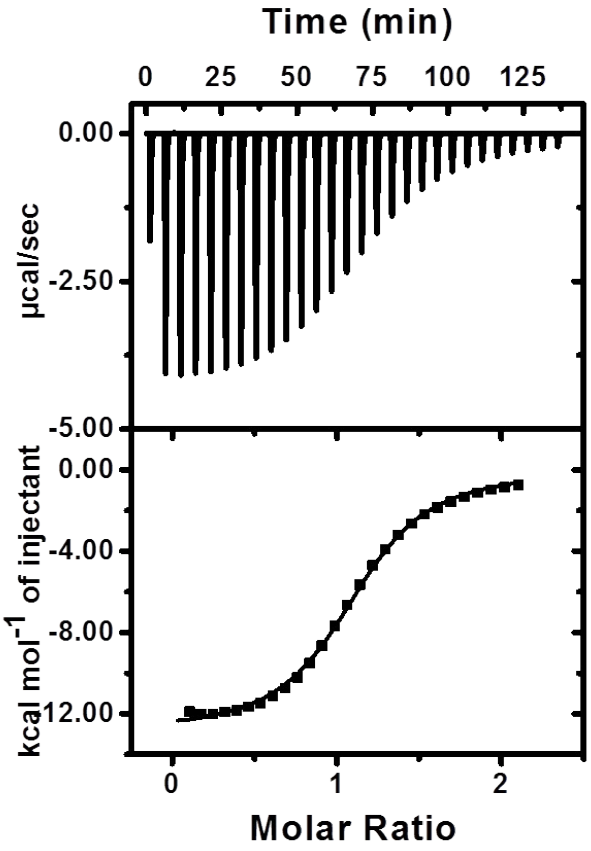


Figure S4 Thermodynamic information of host-guest interaction was collected through ITC. Simulated with one site binding model, the binding constant of NpAM to supramolecular polymer backbone was calculated as $2 \times 10^5\ M^{-1}$. ITC data measured as NpAM titrated into supramolecular polymer backbone. The concentrations were fixed as c(NpAM) = 1.0 mM, c(supramolecular polymer backbone) = 0.1 mM.

4.5 The molecular weight of supramolecular polymers detected by analytical ultracentrifugation

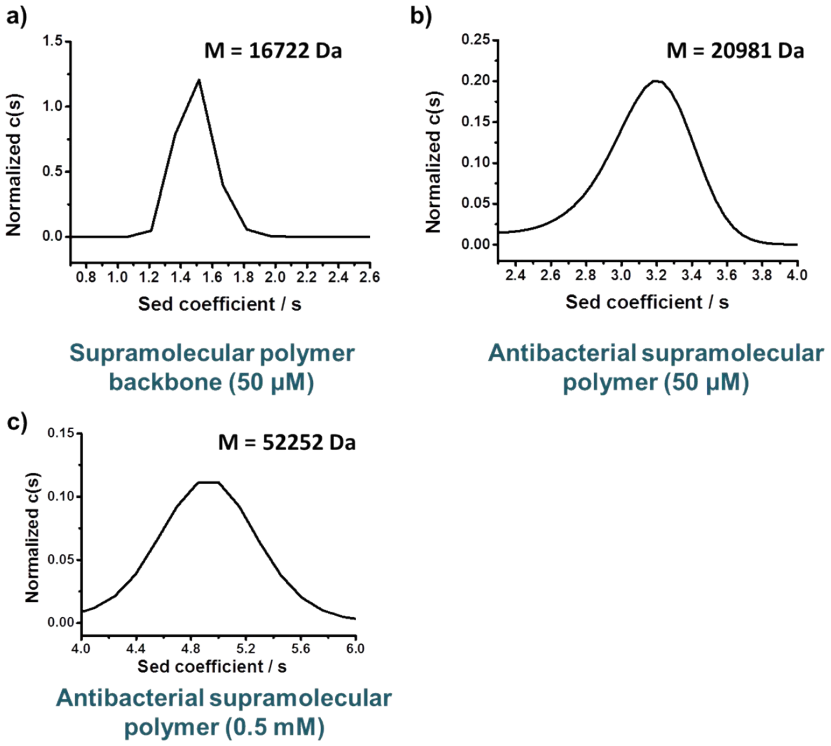


Figure S5 The molecular weight of a) 50 μM supramolecular polymer backbone; b) 50 μM antibacterial supramolecular polymer; c) 0.5 mM antibacterial supramolecular polymer detected by analytical ultracentrifugation.

4.6 The diffusion coefficient of supramolecular polymer backbone detected by DOSY under addition of disassembling agent

Groups	Diffusion coefficient (10^{-10} m^2/s)
0.25 mM Supramolecular polymer backbone + 0.25 mM Disassembling agent	**1.54**
0.25 mM Supramolecular polymer backbone + 0.20 mM Disassembling agent	**1.47**
0.25 mM Supramolecular polymer backbone + 0.15 mM Disassembling agent	**1.42**
0.25 mM Supramolecular polymer backbone + 0.10 mM Disassembling agent	**1.40**
0.25 mM Supramolecular polymer backbone + 0.05 mM Disassembling agent	**1.31**
0.25 mM Supramolecular polymer backbone	**1.10**

Table S1 DOSY measurements for diffusion coefficient of supramolecular polymer backbone with addition of disassembling agents.

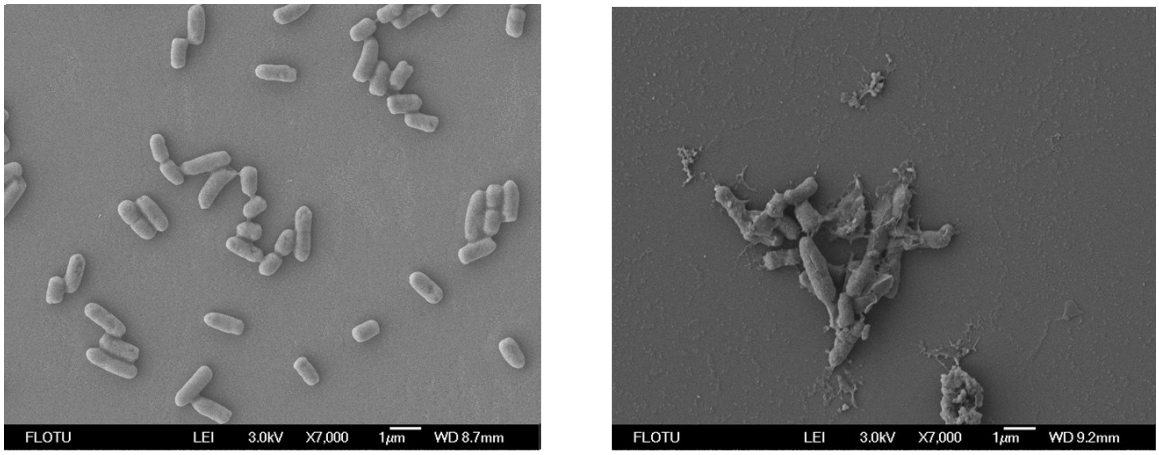
4.7 Zeta potential measurements for surface charge of bacteria

Samples	Zeta potentials (ζ, mV)
E. coli	**-48.0 ± 1.4**
E. coli **+ TNAB**	**-12.9 ± 0.95**
E. coli **+ Supramolecular polymer backbone**	**5.25 ± 0.42**
E. coli **+ Antibacterial supramolecular polymer**	**19.9 ± 2.5**

Table S2 Zeta potential measurements for surface charge of bacteria

0.02 OD *E. coli* were respectively incubated with 50 μM TNAB, supramolecular polymer backbone and antibacterial supramolecular polymer in 1X PBS solution for 30 min at 37 ^{o}C. The bacteria were gotten by centrifuging for two times (first centrifuging condition: 7000 g for 2 min 4 ^{o}C; second centrifuging for ultrapure water washing condition: 1000 g for 5 min, 4 ^{o}C). The precipitated pellets were suspended in ultrapure water and the suspensions were kept on ice for zeta potential measurements. As blank controls, untreated bacteria were also disposed under exactly the same process.

4.8 SEM observation of the bacteria



E. coli

E. coli + Antibacterial supramolecular polymer

Figure S6 Scanning electron microscope (SEM) was used to observe the membrane disruption of bacteria by addition of antibacterial supramolecular polymer (50 μM)

4.9 Antibacterial experiment results in LB nutrient solution

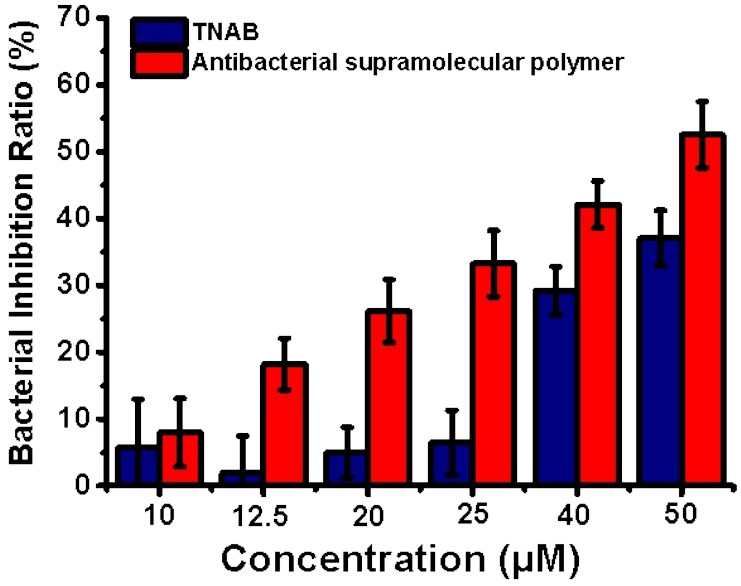


Figure S7 Bacterial inhibition ratio (%) in LB nutrient solution were calculated by addition of different concentration (10 μM, 12.5 μM, 20 μM, 25 μM, 40 μM, 50 μM) of antibacterial supramolecular polymer and TNAB.