Electronic Supplementary Material (ESI) for Chemical Communications

Supramolecular Hydrogel of Kanamycin Selectively Sequesters 16S rRNA

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Supplementary Materials

General. Chemical reagents and solvents were used as received from commercial sources unless otherwise stated. RNA (sequence of 5'-UUGCGUCACACCGGUGAAGUCGC-3') was purchased from Takara Dalian Biochem. ^{1}HNMR spectra were obtained on a 300 MHz Varian XL-300 using DMSO-d_6 as the solvent; Mass spectra were measured on Finnigan TSQ7000 System; circular dichroism spectra were taken on a JASCO J-810 spectropolarimeter; emission spectra were recorded on a Perkin-Elmer LS-55 luminance spectrometer; HPLC analysis were performed on Waters 600E Multi-solvent Delivery System using XTerra MS C_{18} RP column with CH_3CN (0.1% of TFA) and water (0.1% of TFA) as the eluent. Transmission electron micrograph (TEM) was done on JEOL 2010 transmission electron microscope, operating at 200 kV. Rheology test was performed on Rheometrics ARES with a cone and plate (25 mm diameter plate and 0.0999 rad cone angle), the gap opening at the apex of the cone and plate was set to 0.0483 mm. The cryo-dried samples were prepared as following: a copper grid coated with carbon was dipped into the hydrogel and placed into a vial, which was plunged into liquid nitrogen. Then, water was removed from the frozen specimen by a freeze-drier.

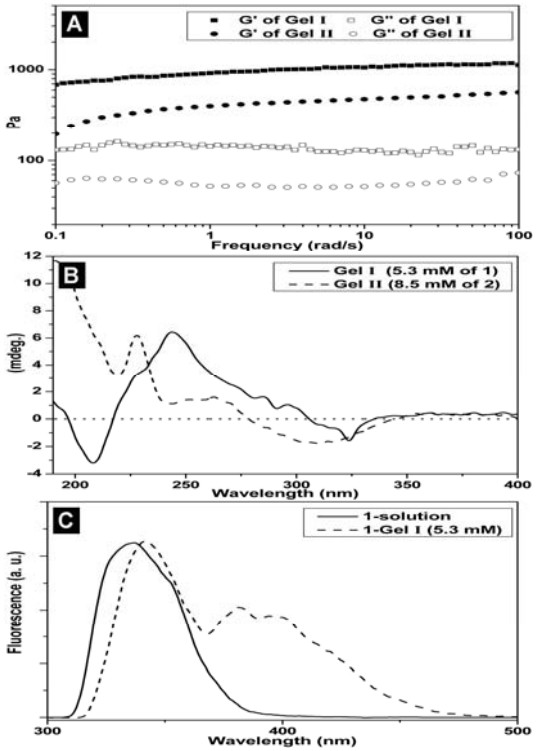


Figure S1. (A) Dynamic frequency sweep of the hydrogels at the strain of 0.3%, T = 25~26 ^{o}C; (B) circular dichroism spectra of the hydrogels; and (C) emission spectrum of **1** in solution and gel phases (ex = 272 nm, Em slit = Ex slit = 5.0 nm).

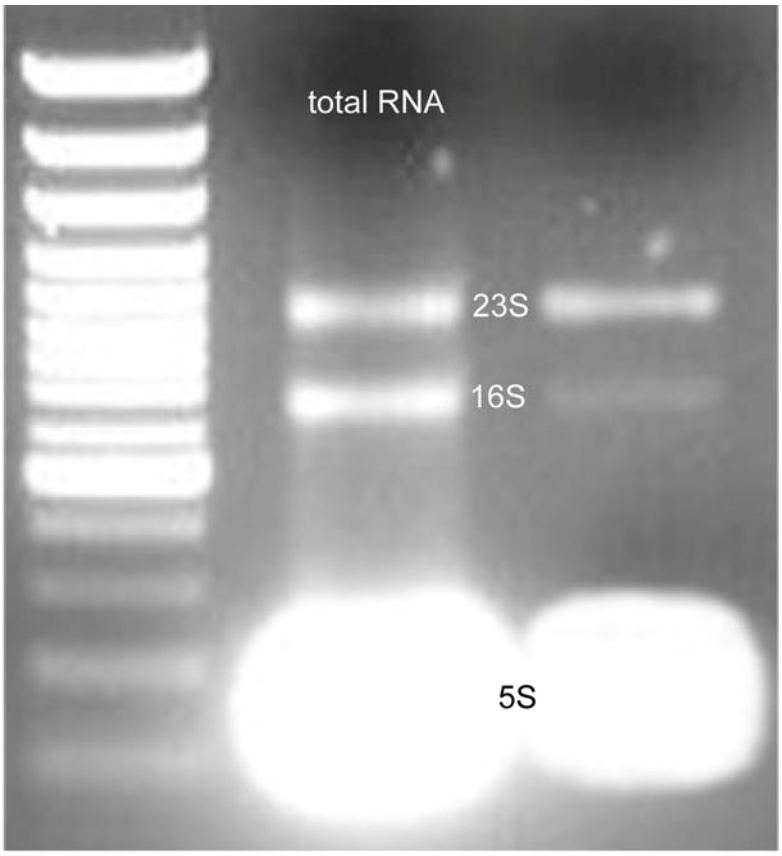


Figure S2. Total RNAs extracted from *E. Coli* before and after uptake (4 hours) by hydrogel of **1.**

Synthesis and characterization

Synthesis of Nap-FF-kanamycin A ***(1):*** After 240 mg (0.5 mmol) of Nap-FF and 58 mg of N-hydroxysuccinimide (NHS) (0.5 mmol) being dissolved in 20 mL of $CHCl_3$, 113 mg of DCC (0.55 mmol) was added. The resulting reaction mixture was stirred at room temperature for 2 hours. The solvent was removed by rotary evaporation, and the resulting white solid was washed with 20 mL of hexane and 20 mL x2 of ethanol, successively. The white solid was collected as activated NHS ester of Nap-FF and used without further purification.

437 mg (0.75 mmol) of kanamycin sulfate and 168 mg (2 mmol) of $NaHCO_3$ were dissolved in 20 mL of H_2O. The activated NHS ester of Nap-FF from previous described procedure was dissolved in 50 mL of acetone and added to the aqueous solution. If necessary, additional water or acetone was used to make the reaction mixture into a clear solution. After being stirred at room temperature for 24 hours, the solvent was removed by purging with N_2. The resulting solid was washed with 10 mL of water, 10 ml x 2 of acetone, successively. The crude product as white powder was dissolved in DMSO and purified by HPLC.

^{1}H NMR of Nap-FF-kanamycin A (**1**) (300MHz, DMSO-d_6) δ (ppm): 8.32-8.44 (4H), 8.18-8.32 (4H), 8.05-8.18 (1H), 7.98-8.07 (1H), 7.88-7.96 (2H), 7.71-7.76 (1H), 7.55-7.68 (2H), 7.22-7.50 (11H), 7.16-7.19 (1H), 6.72-6.85 (1H), 6.00-6.01 (1H), 5.60-5.70 (1H), 5.23-5.40 (2H), 5.12-5.18 (1H), 4.65-4.85 (2H), 3.40-4.00 (28H), 2.84-3.45 (6H), 2.45-2.60 (1H), and 1.80-1.95 (1H). MS: calc. M^+ = 947.0, obsvd. $(M+1)^+$ = 947.5.

Synthesis of Boc-FFFF-kanamycin A ***(2):*** After 354 mg (0.5 mmol) of Boc-FFFF and 58 mg of N-hydroxysuccinimide (NHS) (0.5 mmol) being dissolved in 30 mL of $CHCl_3$, 113 mg of DCC (0.55 mmol) was added. The resulting reaction mixture was stirred at room temperature for 3 hours. The solvent was removed and the resulting white solid was washed with 20 mL of hexane. The crude product was used without further purification.

437 mg (0.75 mmol) of kanamycin sulfate and 168 mg (2 mmol) of $NaHCO_3$ were dissolved in 30 mL of H_2O. The activated NHS ester of Boc-FFFF was dissolved in 40 mL of acetone and added to the aqueous solution. If necessary, additional water or acetone was used to make the reaction mixture into a clear one. After being stirred at room temperature for 24 hours, the solvent was removed by N_2. The resulting solid was dissolved in DMSO and purified by HPLC.

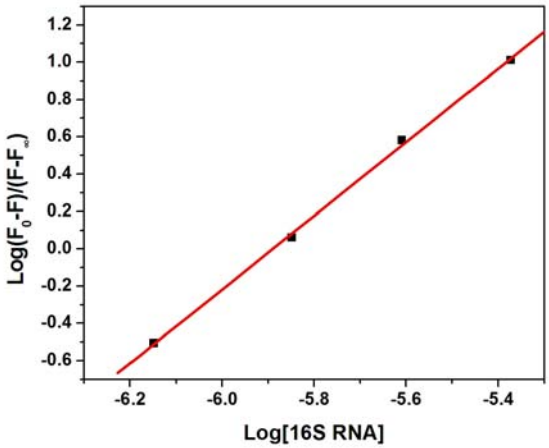
^{1}H NMR of Boc-FFFF-kanamycin A (**2**) (300MHz, D_2O) δ (ppm): 8.05-8.41 (14H), 7.23-7.52 (24H), 7.08-7.16 (2H), 7.00-7.08 (1H), 6.66-6.80 (1H), 5.98-6.06 (1H), 5.58-5.70 (1H), 5.25-5.42 (2H), 5.12-5.18 (1H), 4.60-4.82 (2H), 3.40-3.96 (28H), 2.80-3.40 (12H), 2.45-2.60 (1H), 1.80-1.94 (1H), and 1.35-1.50 (9H). MS: calc. M^+ = 1172.3, obsvd. $(M+1)^+$ = 1173.3.

Figure S3. Optical image of the hydrogel of **1** in water (0.6 wt%, pH = 7.4).

Table S1. Eluting gradient for HPLC purifications of compound **1** and **2**.

Time (minute)	Flow (ml/min.)	H_2O %	CH_3CN %
0	5.0	90	10
25	5.0	20	80
34	5.0	20	80
35	5.0	90	10
40	5.0	90	10

Calculation of binding constant:[1]



Linear Regression for Data1_B: Y = A + B * X

Parameter	Value	Error
A	11.6275	0.25976
B	1.97426	0.04516

R	SD	N	P
0.99948	0.02597	4	5.2284E-4

Protocol for the extraction of total RNAs from E. Coli by TRIzol kit:

1. Pellet 10^8 cells by centrifugation in a 1.5 ml Eppendorf, and remove all supernatant (PBS or culture medium). Resuspend cell pellet by flicking tube.

2. Add appropriate volume of trizol carefully, avoiding spillage, especially with 1ml TRIzol. Pipette mix the suspension until all cell debris dissolves, and no lumps are apparent. Use vortex to finish the job. Incubate mixture at room temperature for 5 minutes to complete homogenisation.

3. Prepare a heavy Phase Lock Gel (PLG; Eppendorf) tube by centrifuging at full speed (~16,000x*g*) for 20 seconds. Add homogenised sample to PLG tube, add appropriate volume of chloroform, close cap securely and shake vigorously by hand for 15 seconds. Then incubate on bench for 2 – 3 minutes.

4. Spin sample at 12,000x*g* · 4ºC for 15 minutes. Carefully remove the upper colourless aqueous layer using a p10 tip for the final drop, and place into a fresh 1.5ml tube.

5. Slowly add an equal volume of 70% ethanol to the aqueous phase and mix the tube gently afterwards, by inversion. Once the ethanol has been mixed in, pipette the appropriate volume of the mixture into the RNA binding column.

6. Centrifuge the column using the appropriate conditions of the kit, e.g. 8000x*g* for 15 seconds, and discard flow through.

7. Column washing and RNA elution by non-DPEC treated RNase-free water.

8. After this maintain the RNA solution at 4ºC by keeping the tube buried in ice.

Procedure for the uptake of RNA from aqueous solution into gel phase:

1. 200 μL solution of total RNAs from E. Coli was added to 1 mL hydrogel of **1** (0.6 wt%, pH = 7.4)
2. 20 μL of upper clear solution was taken to run Agar gel electrophoresis.

Reference:

(1) Chipman, D. M.; Grisaro, V.; Sharon, N. *J. Biol. Chem.* 1967, *242*, 4388.