Supporting Information

Cucurbit[8]uril-based supramolecular polymer nanocapsules as an

effective siRNA delivery platform for gene therapy

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Materials

Tris(2-aminoethyl)amine, bromoacetyl bromide and 4,4'-bipyridine were purchased from Aladdin Reagent Co., Ltd., (Shanghai, China) and used directly. Cucurbit[8]uril (CB[8]) was purchased from Sigma Aldrich. DEPC-treated water was bought from Sangon Biotech. Survivin siRNA was supplied from Gene Pharma (Shanghai, China), which contained the antisense sequence of 5'-UAUCACUCUAUUCUGUCUCTT-3'. The antisense sequence of scrambled siRNA was 5'-UCUACGUCUUAUCCAAUGCTT-3'. RNase A solution, RPMI 1640 medium, fetal bovine serum, 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), Hoechst 33342 staining solution, Annexin V-FITC/PI Apoptosis Detection Kit and trypsin were purchased from Dalian Meilun Biotechnology Co., Ltd. Antibodies, cell lysis buffer for Western and IP and other solutions were obtained from Beyotime Institute of Biotechnology (Shanghai, China) and Beijing Biosynthesis Biotechnology Co., Ltd. Lipofectamine 2000 was obtained from InvitrogenTM. All other chemical reagents we used were analytical reagent grade without any further purification. All the solvents were purchased from Beijing chemical plant.

Methods and instruments

Dynamic light scattering and Zeta potential (Malvern Instrument Zetasizer Nano ZS90 instrument) was performed at 25 °C. SEM images were captured on scanning electron microscopy JEOL JSM 6700F. The sample was prepared by dripping a drop of the aqueous solution onto a silicon wafer and freeze-dried. TEM images were captured on a transmission electron microscope JEM-2100F instrument with 200 kV accelerating voltage. A drop of the aqueous solution was dripped on a 300-mesh, carboncoated copper grid and freeze-dried before the measurement. AFM images were captured on Bruker Dimension Fast Scan Atomic Force Microscopy in tapping mode. The sample was prepared by dripping a drop of the aqueous solution onto a silicon wafer and removed after a few minutes.

Experimental Section



Scheme S1 Synthesis route of the triviologen-based guest molecule.

1. Synthesis of N,N',N"-(nitrilotris(ethane-2,1-diyl))tris(2-bromoacetamide) (TBr)

N,N',N''-(nitrilotris(ethane-2,1-diyl))tris(2-bromoacetamide) was synthesized according to the previous procedures.¹⁻⁵ Tris(2-aminoethyl)amine (1.50 mL, 0.010 mol) and dry triethylamine (6.95 mL, 0.050 mol) were added and mixed in dry dichloromethane. Then bromoacetyl bromide (4.34 mL, 0.050 mol) was added dropwisely into the solution at 0 °C and the mixture was stirred at room temperature for 6 h. After the reaction, the mixture was poured into plenty of cool water to remove the impurities. Next collect the organic layer and condense them under vacuum. The solid was purified by column chromatography and the final product N,N',N''-(nitrilotris(ethane-2,1-diyl))tris(2-bromoacetamide) was obtained as a black solid (4.60 g). The ¹H-NMR and ESI-MS spectrum were shown in Figure S1 and Figure S2. CDCl₃: δ = 7.26 ppm.



Figure S1 ¹H-NMR (500 MHz, chloroform-d, 25 °C, TMS) spectrum of TBr. δ (ppm) = 7.05 (s, 3H), 3.95 (s, 6H), 3.38 (t, *J* = 5.5 Hz, 6H), 2.65(t, *J* = 6.13 Hz, 6H).



Figure S2 ESI-TOF mass spectrum of TBr. ESI-MS spectrometric analysis was performed at the Thermo Finnigan LCQ AD System. ESI-MS: m/z 506.9 ([M+H]⁺).

2. Synthesis of 1,1",1""-(((nitrilotris(ethane-2,1-diyl))tris(azanediyl))tris(2-oxoethane-2,1-diyl))tris(([4,4'-bipyridin]-1-ium))(TBP)

N,*N'*,*N''*-(nitrilotris(ethane-2,1-diyl))tris(2-bromoacetamide) (2.545 g, 0.005 mol) was dissolved in dry *N*,*N*-dimethylformamide (DMF). Then 4,4'-Bipyridine (7.03 g, 0.045 mol) was added in the solution later. The reaction mixture was refluxed at 120 °C for 72 h. The mixture was concentrated under vacuum to obtain the crude product. The crude product was purified by column chromatography and the final product obtained as a black solid (2.95 g).¹⁻⁵ The ¹H-NMR, ESI-MS spectrum and ¹³C-NMR were shown in Figure S3, Figure S4 and Figure S5.



Figure S3 ¹H-NMR (500 MHz, deuterium oxide-d, 25 °C, TMS) spectrum of TBP. δ (ppm) = 8.82 (d, *J* = 6.3 Hz, 6H), 8.55 (d, *J* = 7.94 Hz, 6H), 8.26 (d, *J* = 7.09 Hz, 6H), 7.68 (d, *J* = 6.27 Hz, 6H), 5.41 (s, 6H), 3.43 (t, *J* = 7.7 Hz, 6H), 2.82 (t, *J* = 6.13 Hz, 6H).



Figure S4 ESI-TOF mass spectrum of TBP. ESI-MS spectrometric analysis was performed at the Thermo Finnigan LCQ AD System. ESI-MS: m/z 245.8 (z = 3).



Figure S5 ¹³C-NMR (500 MHz, deuterium oxide-d, 25 °C, TMS) spectrum of TBP. δ (ppm) = 165.74, 154.20, 150.04, 146.29, 141.65, 125.44, 122.25, 61.46, 52.03, 37.22.

3. UV spectrum of the TBP



UV-vis absorption spectrum was recorded on a Shimadzu 3100 UV-vis spectrophotometer. λ max=264 nm.

Figure S6 UV spectrum of TBP in H_2O .

4. IR spectrum of the TBP

IR spectrum was recorded on Bruker VERTEX 80 V FT-IR spectrometer. IR (cm⁻¹): 814, 1220, 1410, 1545, 1641, 1674, 3038, 3225, 3443.



Figure S7 IR spectrum of TBP.

5. Job's plot experiments to determine the molar ratio between host molecule and guest molecule.



Figure S8 Job's plot obtained by recording the absorbance at 264 nm for the solution of TBP and CB[8] in water at 25 °C (TBP + CB[8]= 1.0^{-4} M), confirming the 2:3 stoichiometry of their complex.

6. Zeta potential of the nanocapsules.



Figure S9 Apparent zeta potential (+30.6 mV) of the supramolecular nanocapsules measured by DLS.



Figure S10 Hydrodynamic sizes of the supramolecular nanocapsules in PBS measured by DLS. (A), (B), (C), (D), (E), (F), (G) and (H) represent the nanocapsules which stay for 0, 1, 2, 4, 6, 8, 10 and 12 h.



8. The stability of the complexes with siRNA in PBS at 37 °C.



Figure S11 Zeta potential of the complexes with siRNA in PBS at 37 °C when the N/P is 5. (A), (B), (C), (D), (E), (F), (G) and (H) represent the complexes which stay for 0, 1, 2, 4, 6, 8, 10 and 12 h.

8. Agarose gel electrophoresis experiments.



Figure S12 Electrophoretic retardation analysis of the complexes with siRNA (N/P: 5) in PBS at 37 °C for different time (0, 1, 2, 4, 6, 8, 10 and 12 h).

References

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