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Supplementary Information for

Dual-responsive aggregation-induced emission-active supramolecular nanoparticles for gene delivery and bioimaging

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1. Materials

4-Hydroxy-4'-dimethylaminoazobenzene (>98%, TCI), 6-chloro-hexanol (98%, Alfa Aesar), 1-adamantanecarbonyl chloride (97%, Alfa Aesar), *N,N*-dimethylethylenediamine (DMAE) (99%, Shanghai Wokai Chemical Industry Co. Ltd.), 1,1'-carbonyldiimida (CDI) (98%, Shanghai Wokai Chemical Industry Co. Ltd.), branched polyethyleneimine (PEI) (99%, M_w = 10 kDa, Shanghai Aladdin Reagent Co. Ltd.), triethylamine (TEA) (⩾99%, Shanghai Sinopharm Chemical Reagent Co. Ltd.), potassium carbonate (K_2CO_3) (⩾99%, Shanghai Sinopharm Chemical Reagent Co. Ltd.), sodium sulfate (Na_2SO_4) (⩾99%, Shanghai Sinopharm Chemical Reagent Co. Ltd.), sodium hydroxide (NaOH) (99%, Shanghai Sinopharm Chemical Reagent Co. Ltd.), hydrochloric acid (HCl) (37%, Shanghai Sinopharm Chemical Reagent Co. Ltd.), were used as received without further purification. β-cyclodextrin (β-CD) (Shanghai Sinopharm Chemical Reagent Co. Ltd.) was dried for 48 hours at 60 ^{o}C under vacuum before use.

Dimethyl sulfoxide (DMSO), dimethyl formamide (DMF) and dichloromethane (DCM) from Shanghai Sinopharm Chemical Reagent Co. Ltd., were treated with calcium hydride and distilled before use. Diethyl ether, methanol from Shanghai Sinopharm Chemical Reagent Co. Ltd. and distilled water, were used as received.

2. Instruments and Measurements

Nuclear Magnetic Resonance (NMR)

^1H NMR and ^{13}C NMR spectra were recorded on a Varian Mercury plus 400 NMR spectrometer with deuterated dimethyl sulfoxide (DMSO-d_6) and deuterated chloroform ($CDCl_3$) as solvents at 293 K. The chemical shifts were referenced to residual peaks of deuterated solvents: DMSO-d_6 (2.48 ppm for ^1H NMR, 39.52 ppm for ^{13}C NMR), $CDCl_3$ (7.26 ppm for ^1H NMR, 77.16 ppm for ^{13}C NMR).

Fourier Transform Infrared Spectrometry (FTIR)

FTIR spectra of the obtained compounds were recorded on a Paragon 1000 instrument using the KBr sample holder method. The samples were firstly dried for 30 min to get rid of the residual moisture before measurement.

Electrospray Ionization Mass Spectrometry (ESI-MS)

Mass spectra of the target compounds were recorded on Micromass LCT Premier (ESI) mass spectrometer (Waters Corporation, USA) at ambient temperature with water (DMAE-CD) and chloroform (DMA-Azo-AD) as solvents.

Dynamic Light Scattering (DLS)

DLS measurements were performed on a Malvern Zetasizer NanoS apparatus equipped with a 4.0 mW laser operating at $\lambda = 633$ nm. All samples were measured at a scattering angle of 90° with supramolecular nanoparticle concentration of about 0.5 mg/mL at 25 ^oC. Before measurement, the sample solutions were firstly filtered through some absorbent cotton to eliminate the dust. The sample solutions were then placed in the cell for at least 15 min prior to the measurement to allow for thermal equilibration.

Transmission Electron Microscopy (TEM)

TEM images were acquired from a JEOL JEM-100CX-II instrument at a voltage of 200 kV. The specimens were prepared by directly drop-casting the aqueous solution of supramolecular nanoparticles onto a carbon-coated copper grid, and the excess solution was removed using a piece of filter paper after 5 min. The copper grid was then dried in air at ambient temperature for 48 h.

Atomic Force Microscopy (AFM)

AFM measurements were performed on a Multimode Nanoscope-IIIa Scanning Probe Microscope (Digital Instrument Co., Ltd. U.S.A.) equipped with a MikroMasch silicon cantilever. 40 μL of SNPs/*p*DNA complexes in deionized water containing approximate 0.08 μg of *p*DNA at various N/P ratios (5 and 20) were dropped onto freshly cleaved mica sheets for 5 min, then the excess solution was eliminated with a strip of filter paper. The samples were dried naturally in air at room temperature for 24 h. The samples were visualized using the tapping mode (TM) at room temperature.

UV-Vis Absorption Spectrometry

The UV-Vis absorption spectra of the sample solutions was recorded on a Thermo Electron-EV300 UV-Vis spectrophotometer at room temperature. The slit-width was set as 1 nm, and scan speed was set as 480 nm/min. The wavelength of the spectra ranges from 350 nm to 700 nm.

Steady-State Fluorescence Emission Spectrometry

The fluorescence emission spectra were collected on a PTI- QM/TM/IM steady-state & time-resolved fluorescence spectrofluorometer (USA/CAN Photon Technology International Int.) at ambient temperature. The excitation wavelength of the sample solutions was set at 350 nm. The slit-width was set as 2 nm, and the scan speed was set as

480 nm/min. With the addition of HCl aqueous solution or NaOH aqueous solution, the resulting sample solutions should be maintained in the fluorescence cuvette for at least 10 min prior to the measurement to allow for thermal equilibration and chemical equilibration.

Zeta Potential Measurement

The zeta (ζ) potentials of the sample solutions with pH range from 3 to 12 were determined using a Malvern Zetasizer NanoS at 25 ^{o}C. The cuvettes were filled with the aqueous solution of supramolecular nanoparticles, and the measurement was performed in the ζ-model for a minimum of 10 cycles and a maximum of 100 cycles.

Agarose Gel Electrophoresis

The SNPs/*p*DNA complexes with different N/P ratios were prepared by adding different volumes of SNPs solutions to *p*DNA solutions in PBS buffer containing 0.4 μg *p*DNA, followed by vortexing for 6 s and incubated for 30 min at room temperature. After mixing 5 μL of 0.5 × loading buffer with polyplex solutions, the resulting polyplex solution was loaded on 1% agarose gel containing 0.5 μg/mL ethidium bromide. Gel electrophoresis was carried out in 0.5 × Tris-Borate-EDTA (TBE) buffer at 100 V for 1 h in a Sub-Cell system (Bio-Rad Laboratories, CA). DNA bands were visualized by a UV lamp using a Gel Doc system (Synoptics Ltd., UK).

Cell Cultures

COS-7 cells and HeLa cells were cultured in DMEM (4.5 g/L glucose) supplemented with 10% fetal bovine serum (FBS), penicillin (100 units/mL), and streptomycin (100 μg/mL) at 37 °C under a 5% CO_2 humidified atmosphere. Confluent cells were subcultured every 3 days using standard procedure.

Cell Viability

For MTT assay, COS-7 cells were seeded into 96-well plates at a seeding density of 5000 cells/well in 200 μL medium. After 24 h incubation, the culture medium was removed and replaced with 200 μL of medium containing 50 μL of PEI solutions or SNPs solutions with a series of concentrations (0.001, 0.002, 0.005, 0.01, 0.02, 0.05, 0.1, 0.2 and 0.5 mg/mL). The cells were grown for 48 h. Then, 20 μL of 5 mg/mL MTT assays stock solution in PBS buffer was added to each well. After the cells were incubated for 4 h, the medium containing unreacted dye was carefully removed. The obtained blue formazan crystals were dissolved in 200 μL of DMSO, and the absorbance was measured in a Perkin-Elmer 1420 Multi-label counter at a wavelength of 490 nm.

In Vitro Transfection Assay

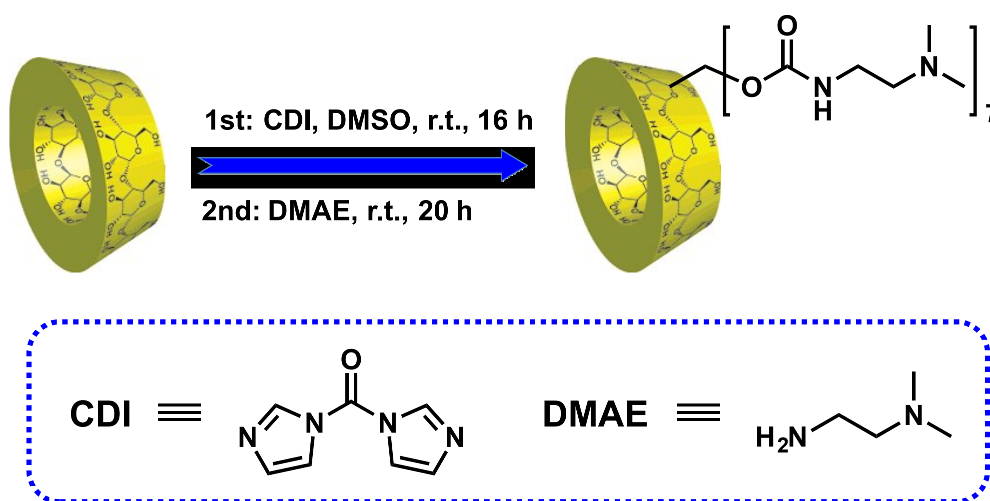
For luciferase expression studies, COS-7 cells and HeLa cells were seeded at a density of 10^4 cells per well in 96-well plates and incubated for 16 to 24 h until 60-70% confluent at 37 °C and 5% CO_2. Immediately prior to transfection, the medium was removed. Then, these cells were washed and replaced with fresh and prewarmed DMEM without or with 10% FBS. Polyplexes were added to each well, and these cells were incubated at 37 °C for 4 h. The medium was then replaced with fresh DMEM with 10% FBS, and then incubated for an additional 48 h. The luciferase assay was carried out according to manufacturer's protocol (Promega, Madison, WI). Relative light units (RLUs) were measured with GloMaxTM 96 microplate luminometer (Promega). The obtained RLUs were normalized with respect to protein concentration in the cell extract determined using the BCA protein assay kit (Beyotime, China).

Confocal Laser Scanning Microscopy Studies (CLSM)

For the CLSM studies, COS-7 cells were seeded in six-well plates at 1×10^5 cells per well in 1 mL of complete DMEM and cultured for 24 h, followed by removing culture medium and adding 1 mL 1 mL SNPs solutions in DMEM medium at a final sample concentration of 10 μM. The cells were incubated at 37 °C for predetermined intervals. Subsequently, the cells were washed with PBS buffer and fixed with 4% paraformaldehyde for 30 min at ambient temperature, and the slides were rinsed with PBS buffer for three times. Finally, the slides were mounted and observed with a LSM510 META.

3. Synthesis Details

3.1 Synthesis of cationic β-CD-(DMAE)$_7$ host



Scheme S1. Synthesis route of β-CD-(DMAE)$_7$.

According to the **Scheme S1**,[1] the synthesis procedure of β-CD-(DMAE)$_7$ was as follows. Dry β-cyclodextrin (2.0 g, 1.75 mmol) was completely dissolved in 20 mL of anhydrous DMSO under stirring, and the above solution was subsequently added dropwise to a solution of CDI (2.2 g, 13.6 mmol) in 20 mL of anhydrous DMSO under vigorous stirring for *ca.* 2 h. The resultant reaction mixture was stirred for 16 h under a

dry nitrogen atmosphere at room temperature. And then DMAE (2.18 g, 24.70 mmol) was slowly added via a syringe to continued reacting for another 20 h with stirring. The resulting reaction solution was concentrated to 15 mL under reduced pressure, and precipitated in 300 mL of diethyl ether and then filtrated off. The precipitate was redissolved in 15 mL of methanol and then reprecipitated in 300 mL of diethyl ether. This reprecipitation and filtration steps were repeated four times before the final product was isolated, collected and dried at 60 ^{o}C in vacuum to yield a white powder (2.44 g, 72%).

^{1}H-NMR (DMSO-d_6, 400 MHz) (**Fig. S1**): δ_{H} (ppm) = 2.13 (br, 42H), 2.28 (br, 14H), 3.05 (br, 14H), 3.40 (br, 7H), 3.61 (m, 14H), 3.76 (br, 7H), 3.98 (br, 7H), 4.23 (br, 7H), 4.85 (bd, J = 21.72 Hz, 7H), 5.6-6.0 (m, 14H), 6.94 (br, 7H). **^{13}C-NMR** (DMSO-d_6, 100 MHz) (**Fig. S2**): δ_{C} (ppm) = 38.94, 45.70, 58.95, 63.86, 69.88, 72.96, 73.57, 82.56, 102.61, 156.90. **FTIR** (KBr) (**Fig. S3**): v (cm^{-1}) = 584, 775, 854, 1031, 1155, 1259, 1463, 1546, 1708, 2944, 3349. **ESI-MS** (*m/z*): calculated for $C_{77}H_{140}N_{14}O_{42}Na_2^{2+}$ $[M+2Na]^{2+}$ 989.4522; found 989.46.

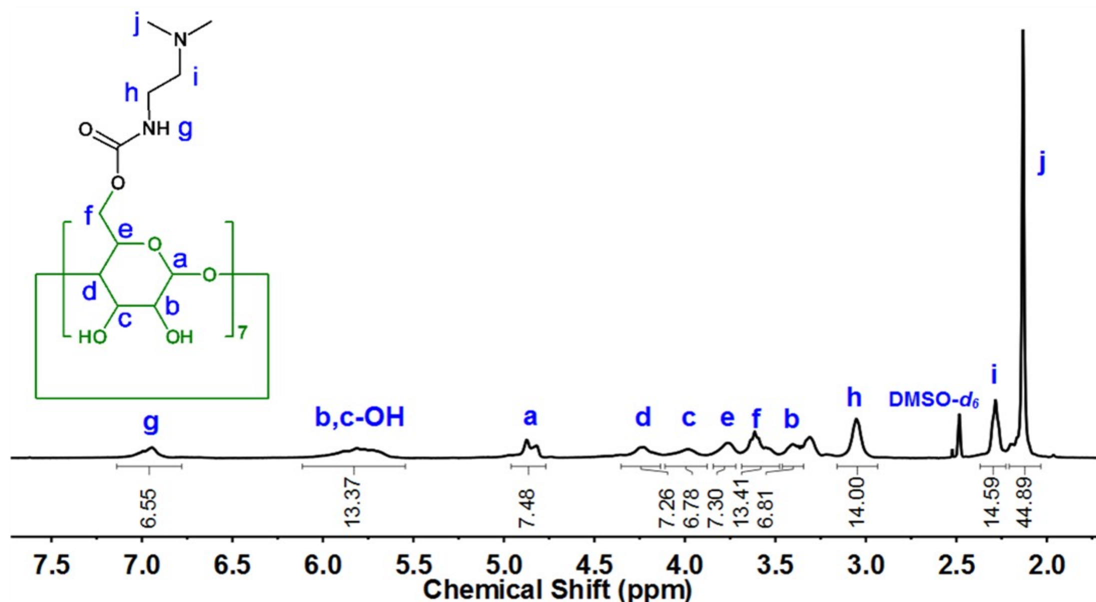


Fig. S1. ^{1}H-NMR spectrum of β-CD-(DMAE)$_7$ in DMSO-d_6.

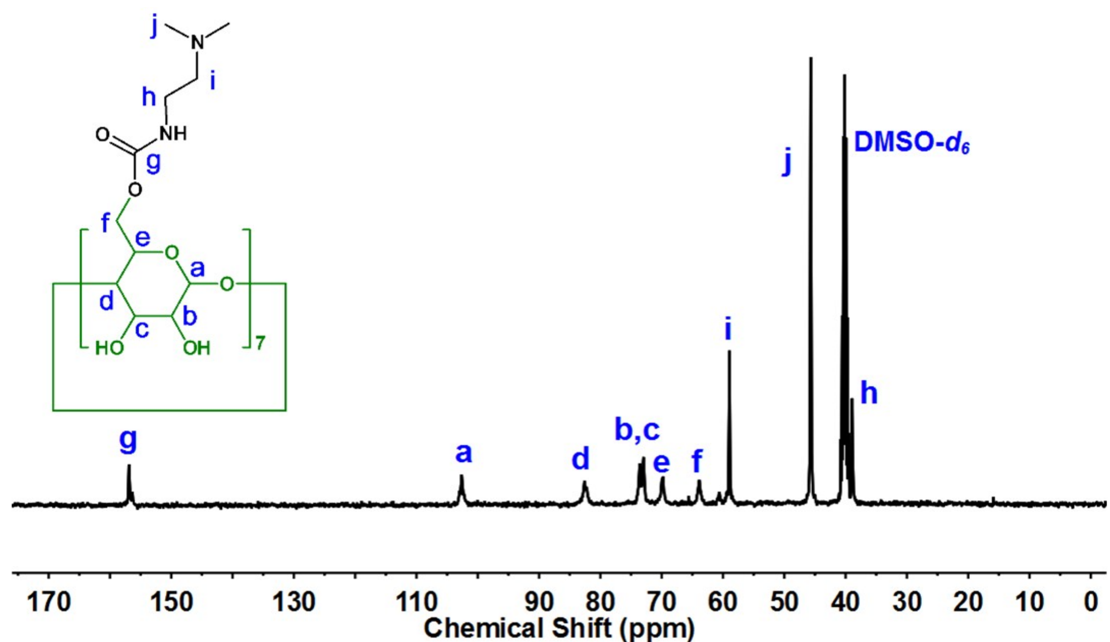


Fig. S2. ^{13}C-NMR spectrum of β-CD-$(DMAE)_7$ in DMSO-d_6.

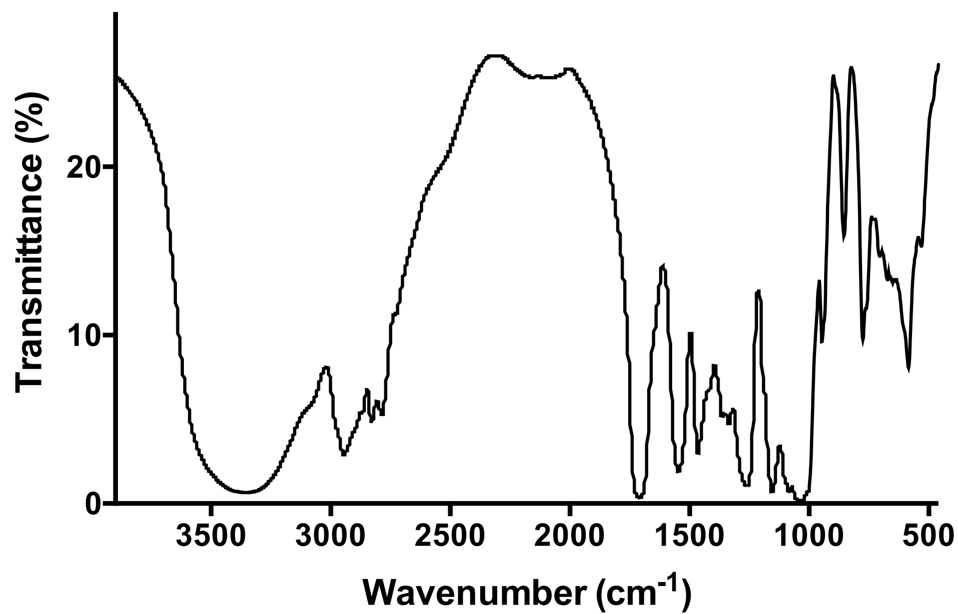
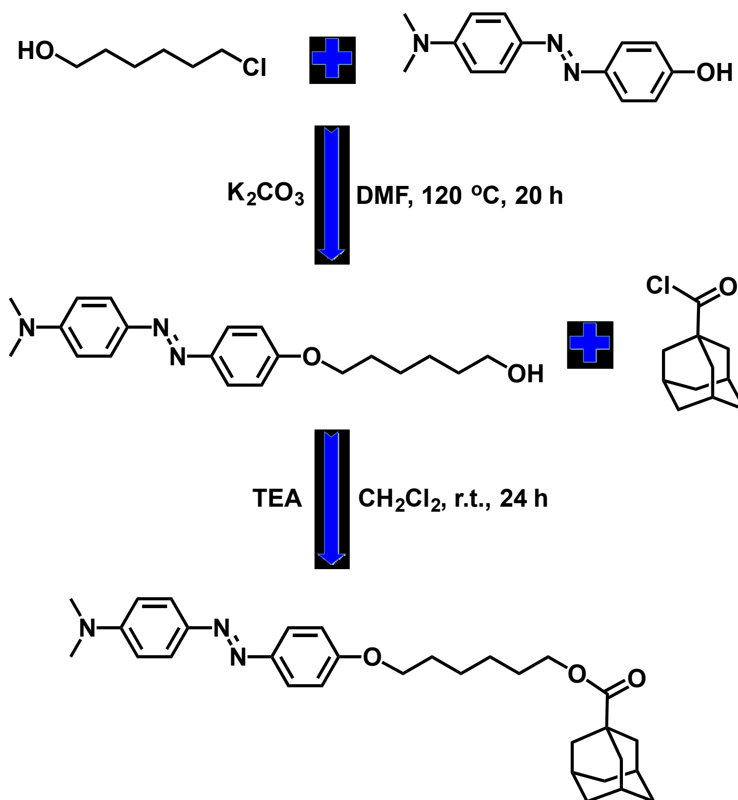


Fig. S3. The FTIR spectrum of β-CD-$(DMAE)_7$.

3.2 Synthesis of DMA-Azo-AD guest



Scheme S2. Synthesis route of DMA-Azo-AD.

The adamantane-modified dimethylamino-azobenzene (DMA-Azo-AD) guest was synthesized according to the following route.

1st step (DMA-Azo-hexanol):[2] 4-Hydroxy-4'-dimethylaminoazobenzene (4.82 g, 20 mmol) and 6-chloro-1-hexanol (3.0 g, 22 mmol) were dissolved in 50 mL of DMF, and then K_2CO_3 (3.04 g, 22 mmol) was added to the above solution. The resulting solution was refluxed at 120 ^{o}C for 20 h. After being concentrated to 15 mL, the reaction mixture was precipitated in 200 mL of methanol/water (1/3, *v/v*) for twice. Finally, the product was collected and dried in vacuum oven at 40 ^{o}C for 24 h, yielding 6.25 g (18.3 mmol, 91.5%) as a yellow solid.

^{1}H-NMR ($CDCl_3$, 400 MHz) (**Fig. S4**): δ_H (ppm) = 7.94 (m, 4H), 6.97 (m, 2H), 6.76 (d, J = 8.94 Hz, 2H), 4.02 (t, J = 6.48, 12.98 Hz, 2H), 3.65 (t, J = 6.50, 13.05 Hz, 2H),

3.08 (s, 6H), 1.30-1.90 (m, 8 H). **^{13}C-NMR** ($CDCl_3$, 100 MHz) (**Fig. S5**): δ_C (ppm) = 25.79, 26.11, 29.45, 32.89, 40.67, 63.05, 68.34, 111.98, 114.85, 124.04, 124.83, 143.87, 147.34, 152.18, 160.66. **FTIR** (KBr) (**Fig. S6**): ν (cm^{-1}) = 552, 843, 1020, 1150, 1250, 1360, 1520, 1600, 2940, 2860, 3370.

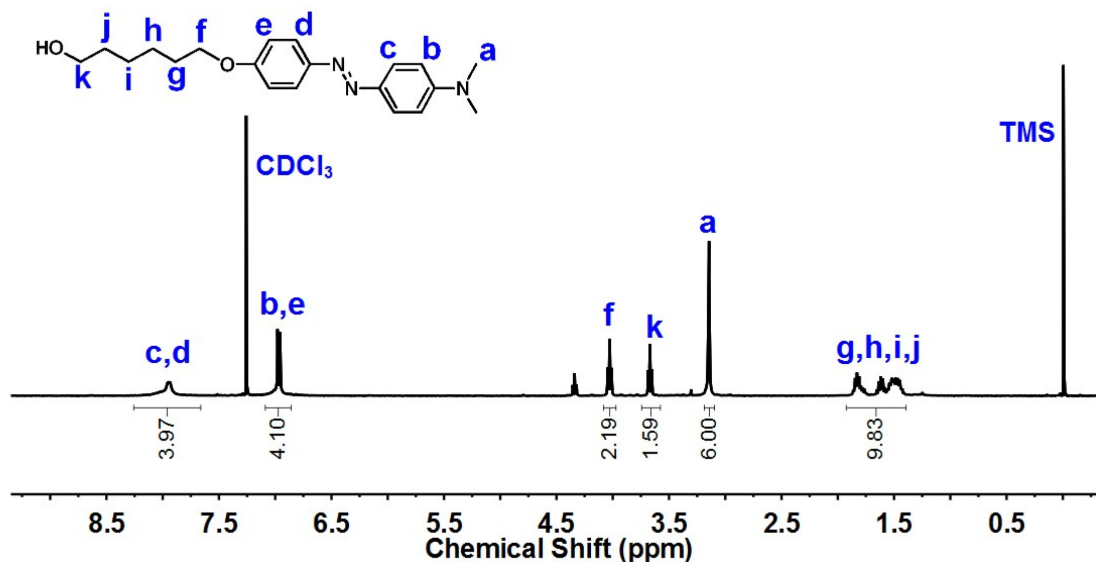


Fig. S4. ^{1}H-NMR spectrum of DMA-Azo-hexanol in $CDCl_3$.

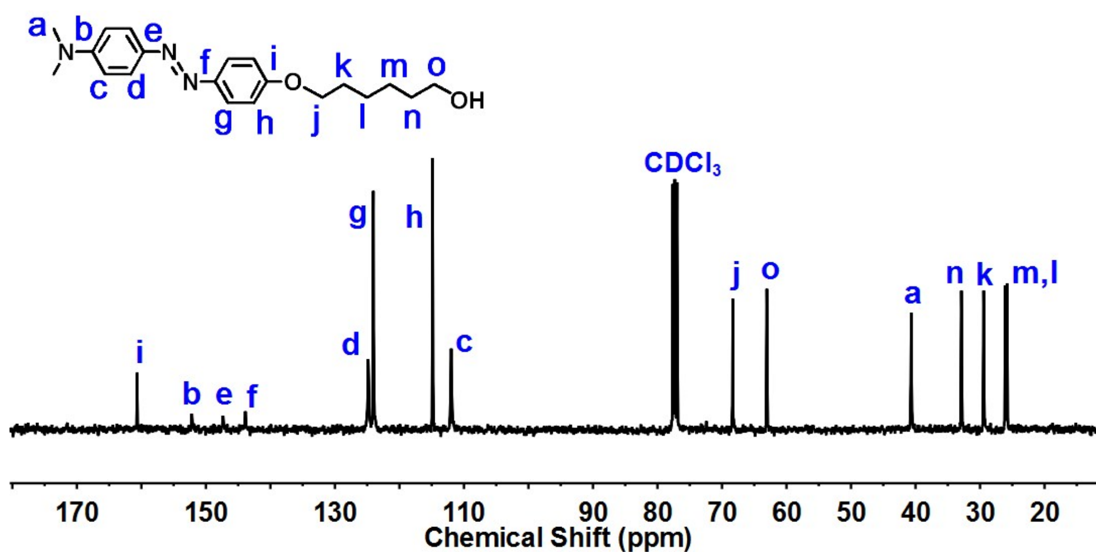


Fig. S5. ^{13}C-NMR spectrum of DMA-Azo-hexanol in $CDCl_3$.

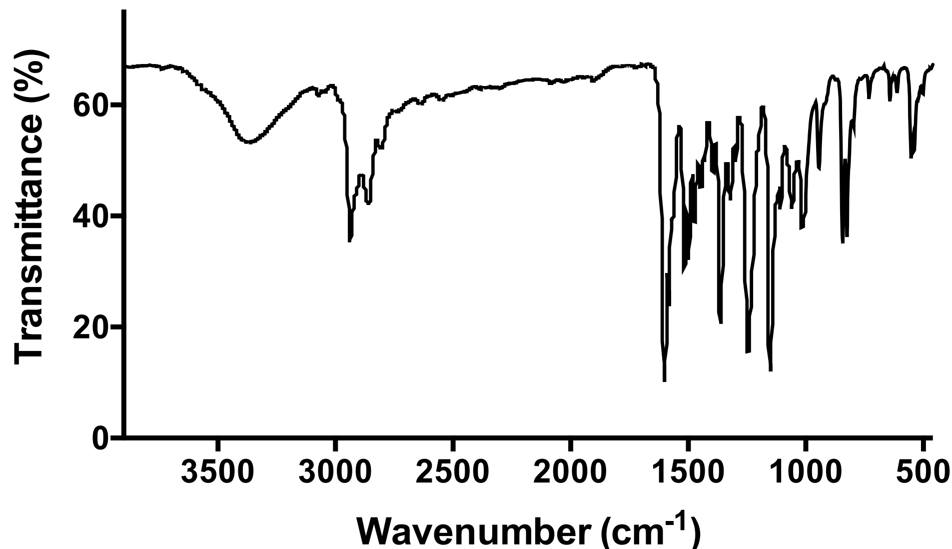


Fig. S6. The FTIR spectrum of DMA-Azo-hexanol.

2nd step (DMA-Azo-AD): The solution of 1-adamantanecarbonyl chloride (1.2 g, 6 mmol, 1.2 equiv.) in 10 mL of CH_2Cl_2 was slowly added dropwise to a solution of 4-dimethylamino-4'-(6-hydroxy hexyloxy) azobenzene (1.7 g, 5 mmol, 1.0 equiv.) and triethylamine (0.5 g, 5 mmol, 1.0 equiv.) in 20 mL of CH_2Cl_2 at ca. 0 °C for about 1 h. Subsequently, the reaction mixture was left to react for another 24 h at room temperature under stirring. The resulting solution was washed in series with 1 M HCl aqueous solution, deionized water, 1 M NaOH aqueous solution, and deionized water for three times. After being dried with Na_2SO_4, the organic phase was concentrated to 5 mL, and then precipitated into 60 mL of methanol/water (1/3, *v/v*) for twice. Finally, the product was collected and dried in vacuum oven at 40 °C for 24 h, yielding 2.14 g (4.25 mmol, 85%) as a yellow solid.

^{1}H-NMR ($CDCl_3$, 400 MHz) (**Fig. S7**): δ_H (ppm) = 7.89 (m, 4H), 6.96 (m, 2H), 6.83 (m, 2H), 4.04 (dt, 4H), 3.09 (s, 6H), 1.30-2.20 (m, 23H). **^{13}C-NMR** ($CDCl_3$, 100 MHz) (**Fig. S8**): δ_C (ppm) = 25.96, 28.19, 28.81, 29.36, 36.65, 38.85, 40.60, 64.23, 68.26, 111.86, 114.81, 124.04, 124.73, 143.90, 147.48, 152.19, 160.60, 178.04. **FTIR** (KBr) (**Fig. S9**): ν (cm^{-1}) = 540, 819, 1149, 1240, 1363, 1518, 1600, 1724, 2854, 2904, 3423.

UV-vis (**Fig. S10**): λ = 408 nm. **ESI-MS** (*m*/*z*): calculated for $C_{31}H_{42}N_3O_3^+$ $[M+H]^+$ 504.3148; found 504.31.

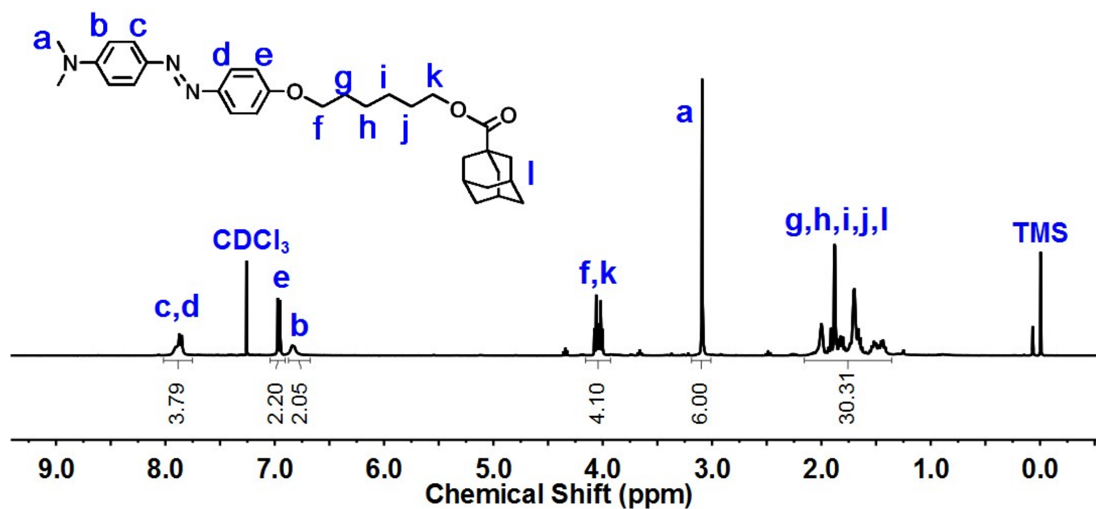


Fig. S7. ^{1}H-NMR spectrum of DMA-Azo-AD in $CDCl_3$.

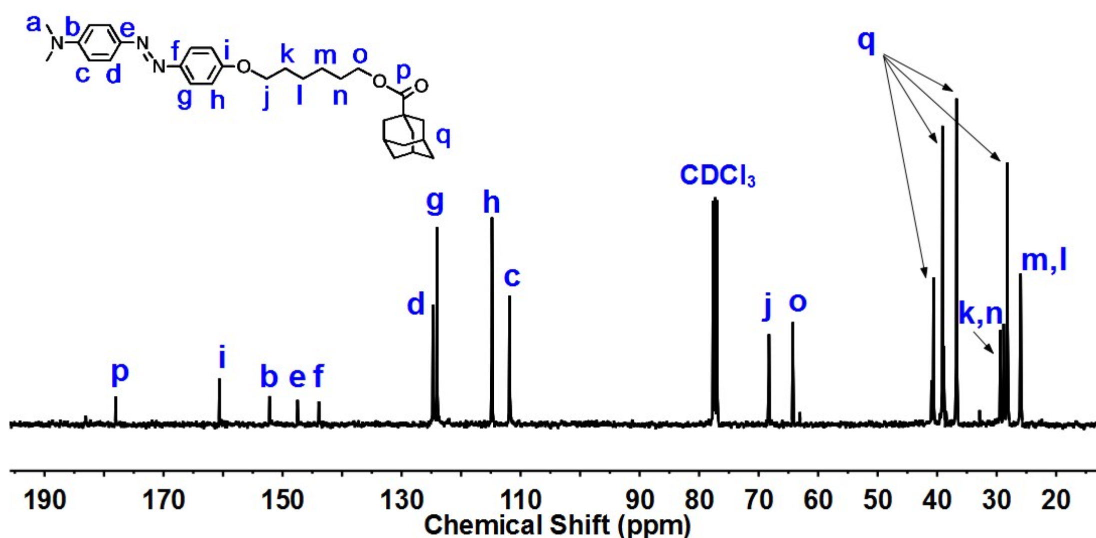


Fig. S8. ^{13}C-NMR spectrum of DMA-Azo-AD in $CDCl_3$.

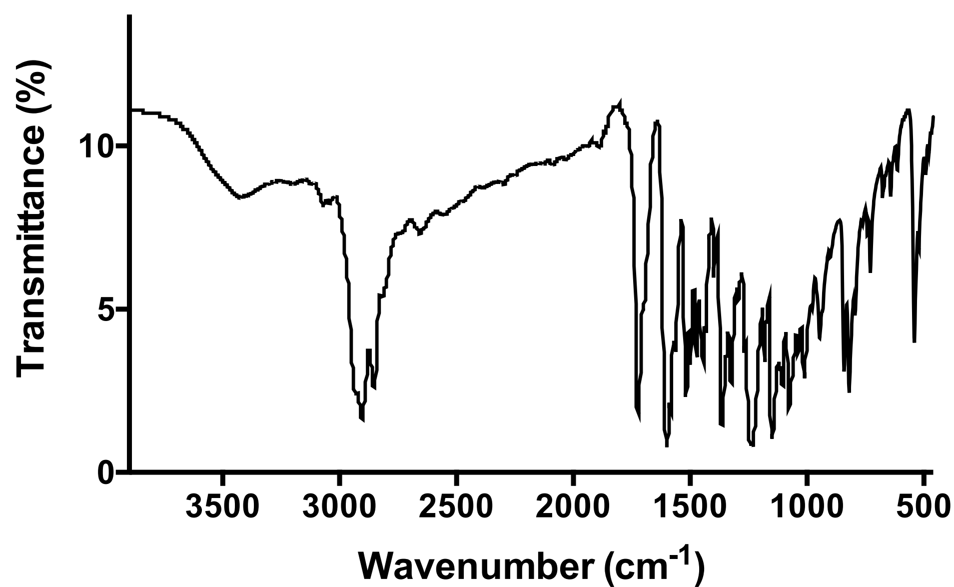


Fig. S9. The FTIR spectrum of DMA-Azo-AD.

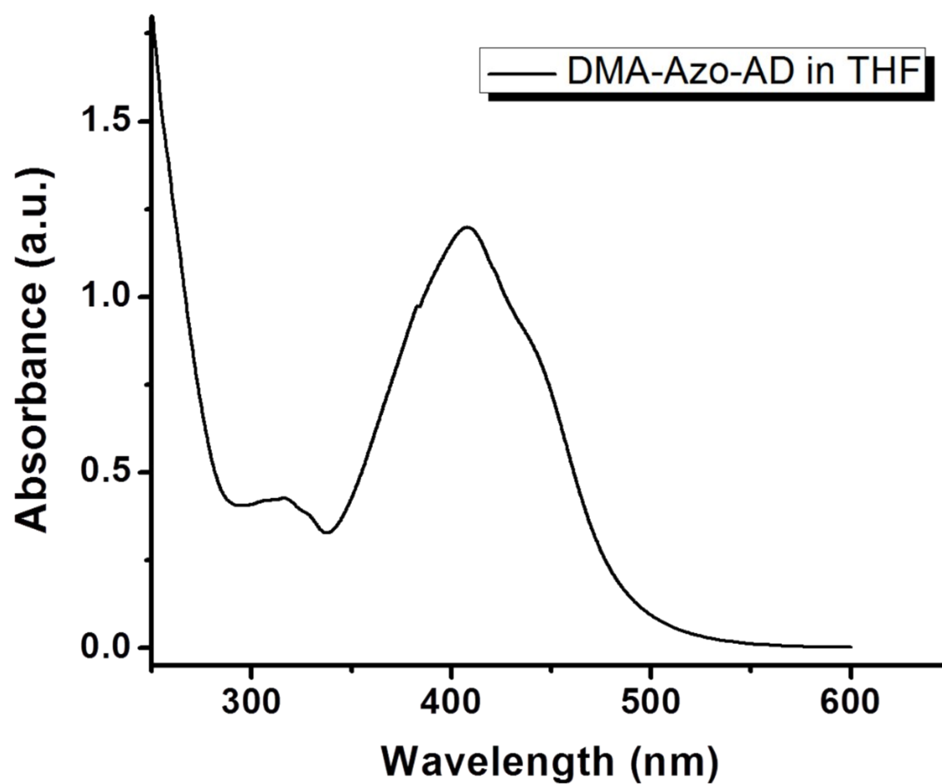


Fig. S10. The UV-Vis spectrum of the THF solution of DMA-Azo-AD.

4. Preparation of Supramolecular Nanoparticles

Equimolar β-CD-$(DMAE)_7$ and DMA-Azo-AD were dissolved in deionized water and THF, respectively. The THF solution of DMA-Azo-AD was added dropwise into the aqueous solution of β-CD-$(DMAE)_7$ over 30 min. Subsequently, the resulting self-assembly solution was evaporated under vacuum to remove the THF, followed by dilution with deionized water to obtain 0.5 mg/mL sample solution for further experiment.

5. Supplemented Figures

5.1. Determination of critical aggregation concentration (CAC) by fluorescence method

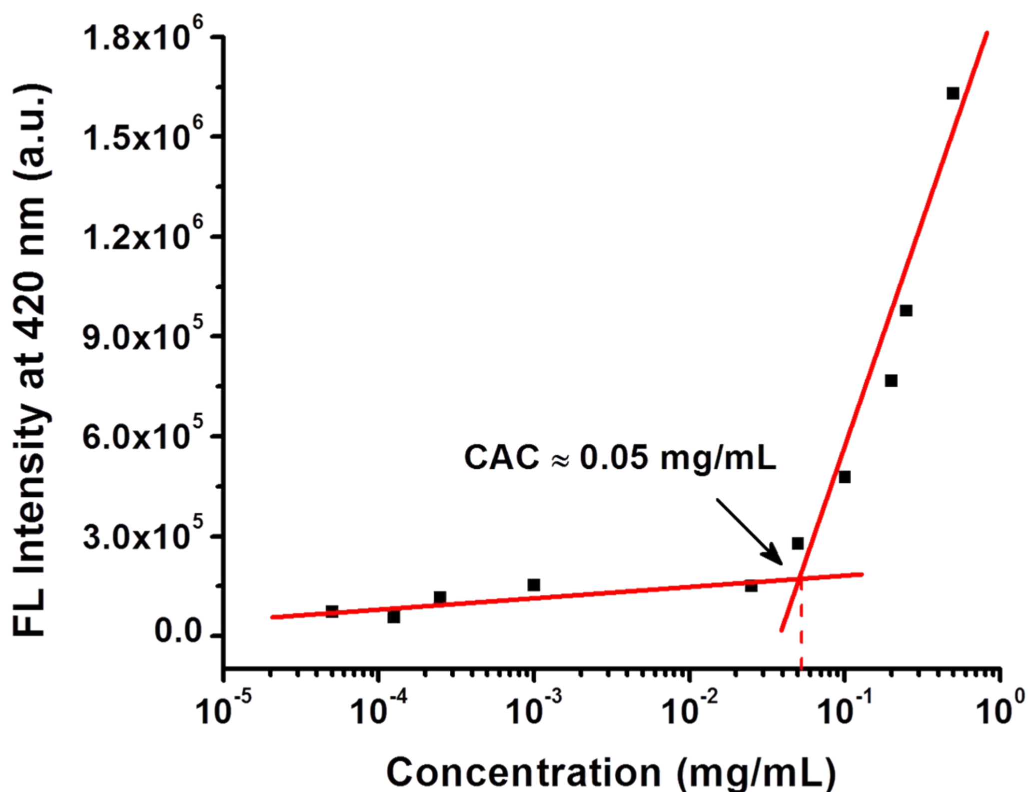


Fig. S11. The fluorescent intensity at 420 nm as a function of concentration of the aqueous solution of supramolecular aggregates.

5.2. Intensity-averaged size distribution of supramolecular nanoparticles

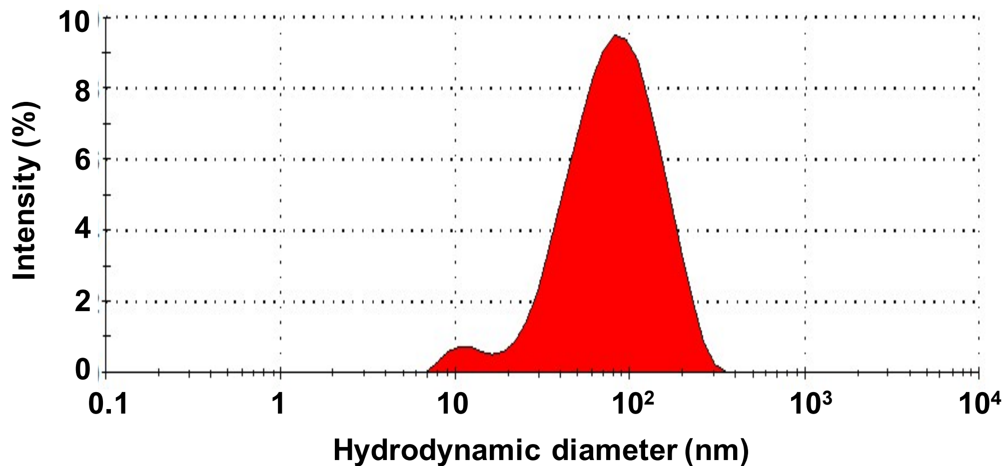


Fig. S12. DLS profile of the aqueous solution of supramolecular nanoparticles at pH of 7.

5.3. UV light-triggered size variation of supramolecular nanoparticles

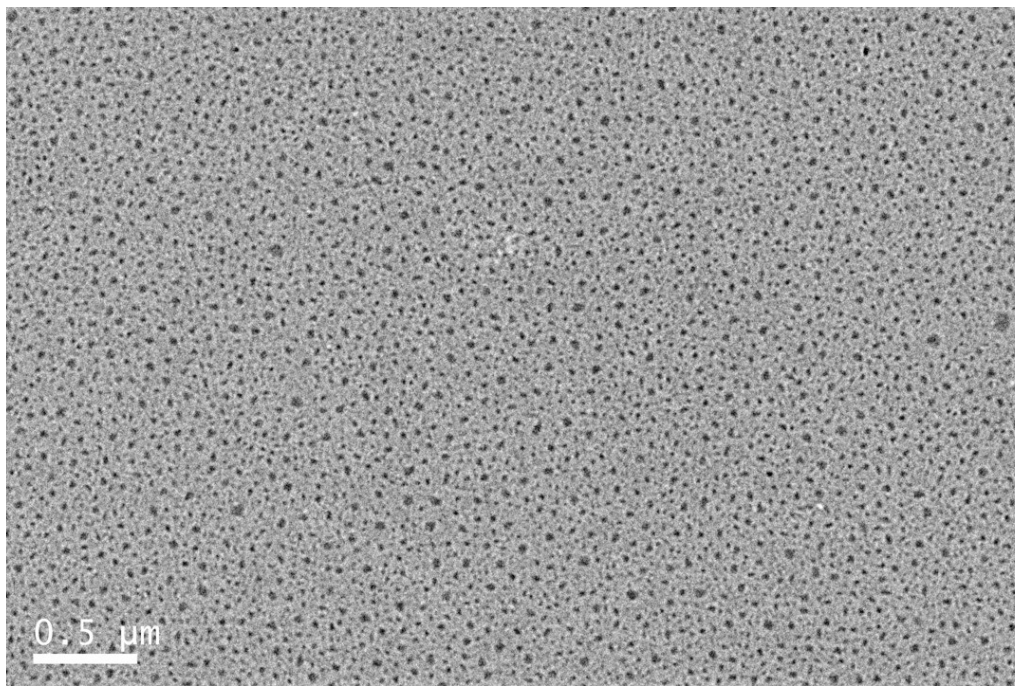
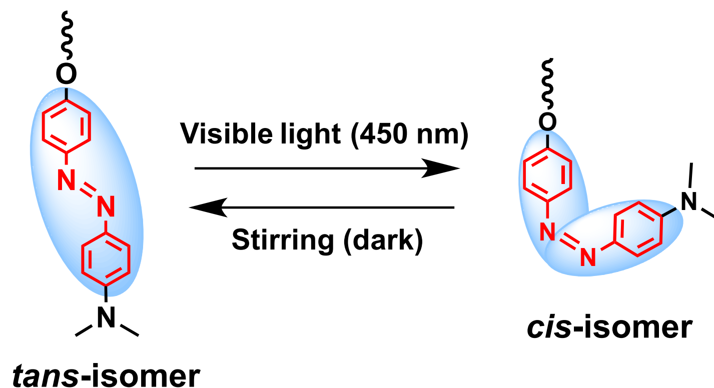


Fig. S13. TEM image of supramolecular nanoparticles after UV irradiation for 30 min.

5.4. Visible light-induced reversible photoisomerization



Scheme S3. The reversible *trans-cis* isomerization triggered by visible light irradiation (450 nm).

5.5. Zeta potentials of supramolecular nanoparticles in PBS buffer with different pHs

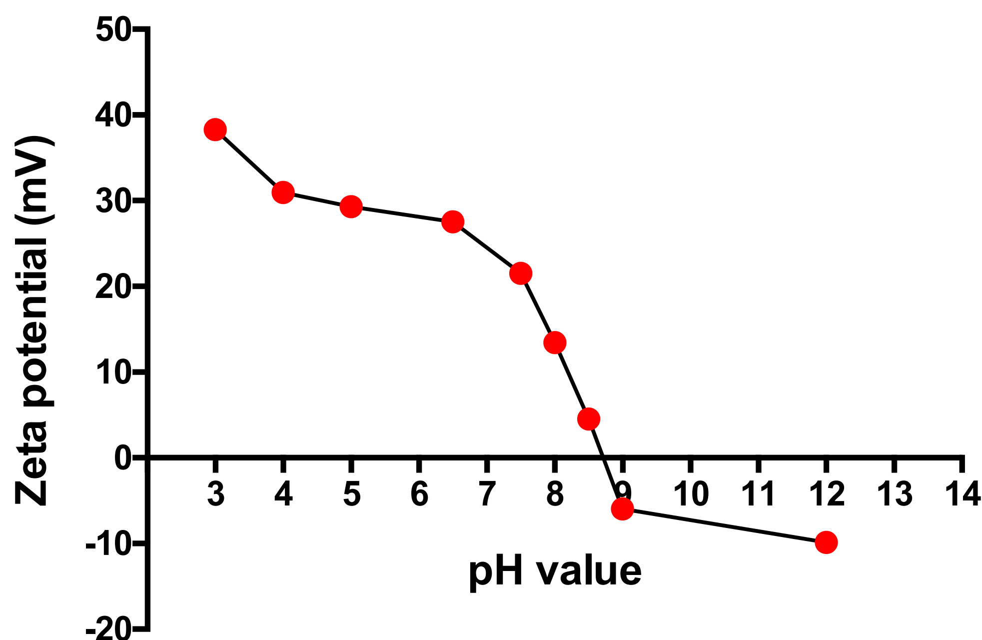


Fig. S14. Zeta potentials of the supramolecular nanoparticles in PBS buffer with pH range from 3 to 12.

5.6. *p*DNA condensation by branched PEI

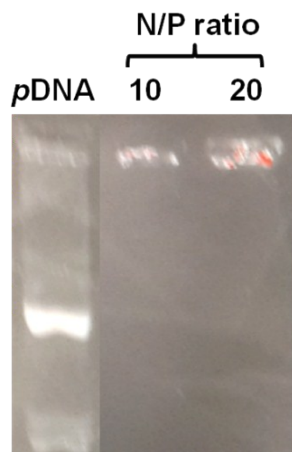


Fig. S15. Agarose gel electrophoresis retardation of *p*DNA by branched PEI (10 kDa) at N/P ratios of 10 and 20.

5.7. Cell viability of supramolecular nanoparticles

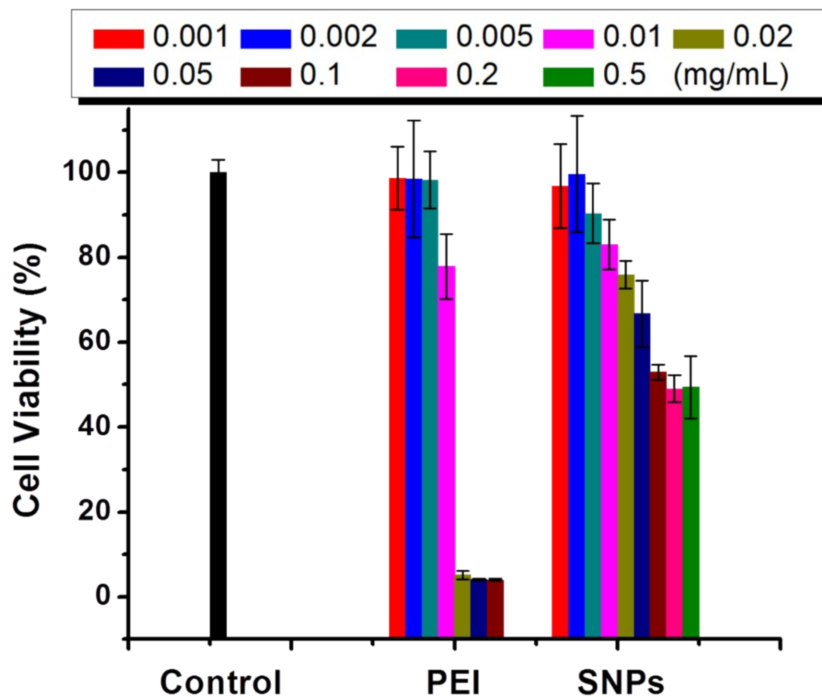


Fig. S16. Cell viability assay of supramolecular nanoparticles and branched PEI (10 kDa) incubated in COS-7 cells for 48 h. Black bars represent the mean values ($n = 5$).

5.8. Imaging of supramolecular nanoparticles in COS-7 cells

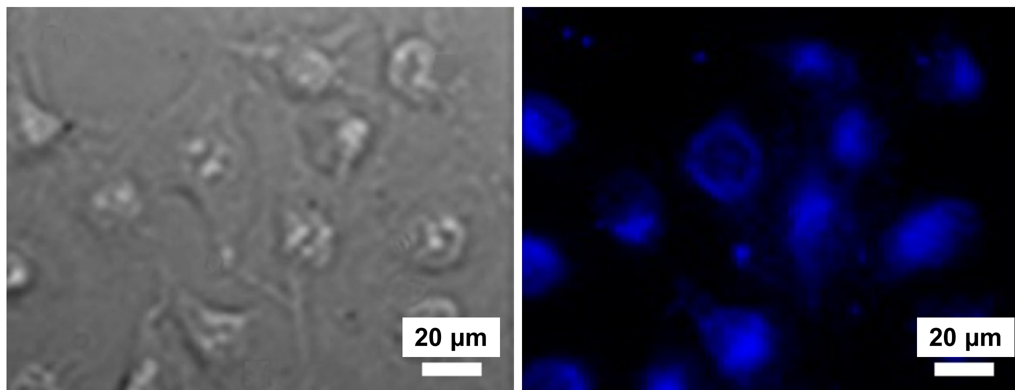


Fig. S17. Confocal laser scanning microscopy (CLSM) images of COS-7 cells that incubated with AIE-active supramolecular fluorescent nanoparticles at 37 °C for 1 h with a final sample concentration of 10 μM.

6. References

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[2] R. Dong, B. Zhu, Y. Zhou, D. Yan and X. Zhu, *Angew. Chem. Int. Ed.*, 2012, **51**, 11633–11637.