Supplementary Material (ESI) for Chemical Communications
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Supplementary Information for:

Highly Fluorescent Core-shell Hybrid Nanoparticles from Unimolecular Star Conjugated Polymer for Biological Tool

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Section-1 Materials

Hyperbranched conjugated polymer with aldehyde terminal group (HCP-CHO) was synthesized according to the method described in our previous paper.[1] Sodium borohydride ($NaBH_4$, C.P. grade, Shanghai Chemical Reagent Co.), *N*,*N*-dimethylaminopyridine (98%, Alfa Aesar), 1,1,4,7,7-pentamethyldiethylenetriamine (PMDETA) (98%, Alfa Aesar), 2-bromoisobutyryl bromide (98%, Aldrich) tetraethoxysilane (TEOS, 99%, Alfa Aesar) and 3-aminopropyltrimethoxysilane (APTMS, 99%, Alfa Aesar) were used as received. 2-Dimethylamino ethylmethacrylate (DMAEMA, 99%, Acros) was passed through a column of basic alumina to remove the stabilizing agents. Copper(I) bromide (CuBr, C.P. grade, Shanghai Chemical Reagent Co.) was stirred over 24 h in acetic acid, filtered, washed with ethanol, and then dried *in vacuo*. Tetrahydrofuran (THF) was refluxed over sodium wires and benzophenone until anhydride and then distilled to use immediately. Chloroform ($CHCl_3$) and triethylamine were refluxed with calcium hydride and distilled before use. Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), and phosphate buffered solution (PBS) were purchased from PAA Laboratories GmbH. The other chemical reagents were purchased from domestic suppliers and used as received. Dialysis tube (MWCO, 3.5 kDa) was from Shanghai Lvniao Technology Corp. Clear polystyrene tissue culture treated 6-well, 24-well and 96-well plates were obtained from Corning Costar.

Section-2 Instruments and measurements

Nuclear magnetic resonance (NMR)

NMR analyses were recorded on a Varian Mercury Plus 400 MHz spectrometer with deuterated chloroform ($CDCl_3$), deuterated dichloromethane (CD_2Cl_2) as solvents at 20 °C. Tetramethylsilane (TMS) was used as the internal reference.

Fourier transform infrared spectroscopy (FTIR)

FTIR spectra were recorded on a Paragon 1000 instrument by KBr sample holder

method.

Gel permeation chromatograph (GPC)

The number-average molecular weight (Mn) and the polydispersity (Mw/Mn) were determined by gel permeation chromatography/multi-angle laser light scattering (GPC-MALLS). The gel permeation chromatography system consisted of a Waters degasser, a Waters 515 HPLC pump, a 717 automatic sample injector, a Wyatt Optilab DSP differential refractometer detector, and a Wyatt miniDAWN multi-angle laser light scattering detector. Three chromatographic columns (styragel HR3, HR4, and HR5) were used in series. THF was used as the mobile phase at a flow rate of 1 mL/min at 30 °C. The refractive index increment dn/dc was determined with Wyatt Optilab DSP differential refractometer at 690 nm. Data analysis was performed with Astra software (Wyatt Technology).

Thermal gravimetric analysis (TGA)

TGA measurements were performed on a Perkin-Elmer TGA-7 thermogravimetric analyzer to investigate the thermal stability of all samples in nitrogen atmosphere from ambient temperature to 700 °C at 20 °C/min.

Dynamic light scattering (DLS)

DLS measurements were performed with a Malvern Zetasizer Nano ZS90 apparatus (Malvern Instruments Ltd) equipped with a 4.0 mW He-Ne laser operating at $\lambda = 633$ nm. All samples were measured at room temperature and a scattering angle of 90^{o}.

Transmission electron microscopy (TEM)

TEM studies were performed with a Tecnai G2 Spirit Biotwin instrument at a voltage of 200 kV. Samples were prepared by drop-casting micelle solutions onto carbon-coated copper grids, and then air-drying at room temperature before measurement.

Ultraviolet-visible spectrometry (UV/Vis)

UV/Vis measurements were performed on the Thermo Evolution 300 UV/Vis spectrometer in the range of 200-800 nm.

Photoluminescence spectrometry (PL)

Fluorescent spectra were recorded on QC-4-CW spectrometer, made by Photon Technology International, Int. USA/CAN. The excitation wavelength of all samples was 365 nm.

Time-resolved fluorescence measurement

The time-resolved fluorescence spectra were recorded on a PTI-QM/TM/IM steady-state & time-resolved fluorescence spectrofluorometer (USA/CAN Photon technology international Int.) by using the time-correlated single-photon counting (TCSPC) technique. Utilizing the picosecond pulses from a doubled frequency, the time-resolved fluorescence spectra of sample solutions with excitation of 365 nm were detected at an emission wavelength of 440 nm for lifetime measurements with an emission polarizer and depolarizer.

Fluorescent photobleaching

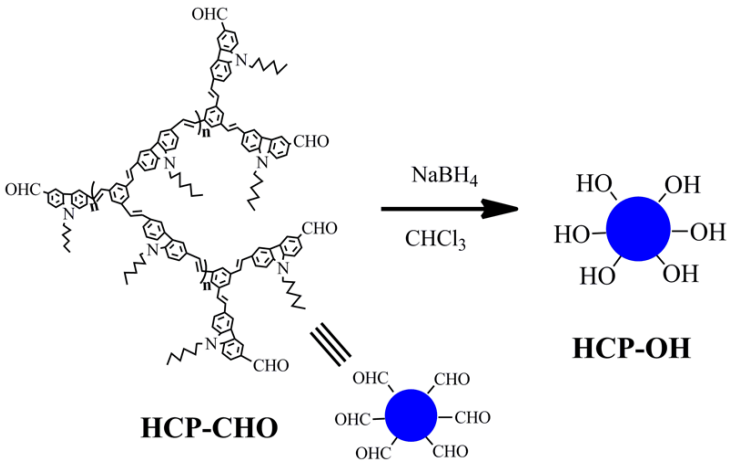
The fluorescent photostability of HCP@SiO_2 was measured continuously under a ZF-1 UV lamp with 30W power. With different exposed time, the fluorescent spectra were recorded on QC-4-CW spectrometer.

ζ-Potential measurement

The ζ-Potential values of HCP-*star*-PDMAEMA, HCP@SiO_2 and HCP@SiO_2-NH_2 in PBS buffer were performed with a Malvern Zetasizer Nano ZS90 apparatus (Malvern Instruments Ltd) at 25 °C.

Section-3 Synthetic procedures

Synthesis of hyperbranched conjugated polymer with hydroxyl terminal group (HCP-OH)



Scheme S1. Synthesis of hyperbranched conjugated polymer with hydroxyl terminal group

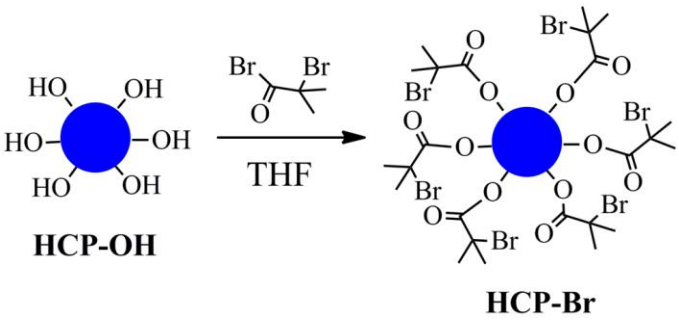
The HCP-CHO (1 g, 0.1 mmol) was fully dissolved in chloroform (50 mL) at 0 °C, and then the solution was stirred with addition of several drops of methanol. The sodium borohydride powder (113.5 mg, 3 mmol) was added slowly into the solution. After standing for 4 h at 0 °C, the temperature of reaction was heated to room temperature for another 20 h. The solution was extracted three times with distilled water, and dried with anhydrous magnesium sulfate. The resultant concentrated under reduced pressure and added slowly into methanol at room temperature to precipitation. Reprecipitation was performed three times, and a bright yellow polymer was obtained after drying under vacuum at 40 °C. Yield: 86%.

^{1}H NMR ($CDCl_3$, 400 MHz, ppm) δ: 0.42-0.98 (-CH_3), 0.98-1.45 (-C$\underline{H_2}$C$\underline{H_2}$$CH_3$), 1.45-2.45 (-$NCH_2C\underline{H_2}CH_2$-), 3.60-4.65 (-NC$\underline{H_2}CH_2$-), 4.50-4.90 (Ar-OH), 5.78-8.85 (Ar -CH).

^{13}C NMR ($CDCl_3$, 400 MHz, ppm) δ: 14.25, 22.75, 27.10, 29.12, 29.96, 31.71, 43.42, 109.07, 118.93, 121.38, 123.19, 123.49, 125.00, 126.05, 126.94, 128.98, 131.36, 134.60, 138.34, 140.85.

IR (cm^{-1}): 3390, 3014, 2952, 2924, 2853, 1623, 1602, 1582, 1487, 1384, 1349, 1240, 1193, 1152, 1153, 1093, 1047, 958, 878, 800 and 691.

Synthesis of macroinitiator HCP-Br



Scheme S2. Synthesis of hyperbranched conjugated polymer with bromine terminal group

The ATRP initiator was synthesized by reaction of terminal hydroxyl groups of the HCP-OH precursors with 25% molar excess of 2-bromoisobutyrylbromides. Typically, HCP-OH (0.8 g, 0.81 mmoL) was dissolved in 60 mL dried THF. After degassing for three cycles by pulling a vacuum and back-filling with nitrogen gas, *N*,*N*-dimethylaminopyridine (160 mg, 1.31 mmoL) and triethylamine (0.5 mL, 3.6 mmol) were added. At 0 °C, 2-bromo-isobutyryl bromide (350 mg, 1.52 mmol) of THF (30 mL) solution was added dropwise while stirring. Then, it was reacted for 72 h. The resulting solution was filtered and then THF was removed under reduced pressure. The crude products were dissolved in $CHCl_3$, and washed twice with water, 0.5 mol/L hydrochloric acid, and 0.5 M $NaHCO_3$. Then, the concentrated sample was precipitated in cold methanol. The resultant product was dried in vacuum for 24 h to give the yellow solid. Yield: 80%.

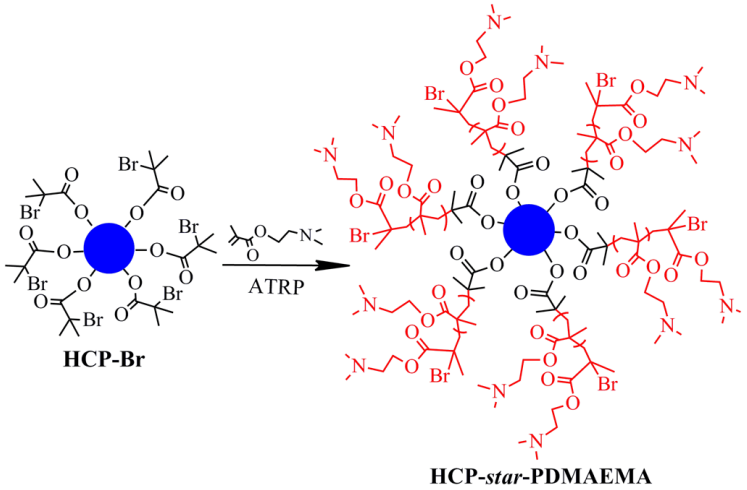
^{1}H NMR ($CDCl_3$, 400 MHz, ppm) δ: 0.42-0.98 (-CH_3), 0.98-1.45 (-C$\underline{H_2}$C$\underline{H_2}$$CH_3$), 1.45-2.54, 1.96 (-$NCH_2C\underline{H_2}CH_2$-, -$C(CH_3)_2Br$), 3.60-4.65 (-NC$\underline{H_2}CH_2$-), 4.50-4.90 (Ar-OH), 5.78-8.85 (Ar-CH).

^{13}C NMR ($CDCl_3$, 400 MHz, ppm) δ: 14.25, 22.75, 27.10, 29.12, 29.96, 31.71, 43.42, 109.07, 118.93, 121.38, 123.19, 123.49, 125.00, 126.05, 126.94, 128.98, 131.36, 134.60, 138.34, 140.85, 171.89.

IR (cm^{-1}): 3434, 2963, 2925, 2853, 1732, 1624, 1584, 1488, 1467, 1385, 1261, 1097,

1024, 960, 872, 802 and 689.

Synthesis of star conjugated copolymer (HCP-*star*-PDMAEMA)



Scheme S3. Synthesis of HCP-*star*-PDMAEMA

The star copolymers of HCP-*star*-PDMAEMA were synthesized through an ATRP path by using HCP-Br as macroinitiator, CuBr as catalyst, and PMDETA as the ligand. The general procedure was as follows. The macroinitiator (225 mg, ~0.02 mmol) and CuBr (2.88 mg, 0.02 mmol) were placed in an oven-dried reaction tube, and degassed for three cycles. Then, dried THF (20 mL) was added via a syringe, and the system was degassed for another three cycles after the macroinitiator was dissolved completely. PMDETA (10.36 mg, 0.06 mmol) was subsequently added by syringe. When the solution turned to light blue, DMAEMA monomer was added by syringe. The reaction was carried out at 60 °C for 12 h. The sample was further diluted with THF, passed through a column of neutral alumina to remove copper salts, and then precipitated in cold *n*-hexane for three times. The yellow product was dried in vacuum at 40 °C.

^{1}H NMR ($CDCl_3$, 400 MHz, ppm) δ: 0.63-0.96 (-CH_3), 0.96-1.49 (-C$\underline{H_2}$C$\underline{H_2}$$CH_3$, -($CH_2CC\underline{H_3}$)$_n$-), 1.45-2.06 (-NC$H_2C\underline{H_2}$$CH_2$-, -(C$H_2CCH_3$)$_n$-, -CCO($CH_3$)-), 2.10-2.40 (-$CH_2$N(C$H_3$)$_2$), 2.44-2.67 (-C$H_2$N($CH_3$)$_2$) 3.80-4.65 (-NC$\underline{H_2}$$CH_2$-, -OC$H_2$$CH_2$N($CH_3$)$_2$), 4.50-4.90 (Ar-OH), 5.78-8.85 (Ar-CH).

^{13}C NMR ($CDCl_3$, 400 MHz, ppm) δ: 14.27, 16.84, 18.76, 22.76, 27.17, 29.66, 29.92, 31.51, 31.79, 34.44, 44.90, 45.25, 45.99, 52.70, 54.37, 57.26, 63.22, 109.01, 121.48, 123.48, 125.73, 128.87, 135.90, 138.68, 140.73, 144.16, 176.79, 177.60, 177.95, 178.26.

IR (cm^{-1}): 3434, 2927, 2853, 2822, 2770, 1730, 1603, 1457, 1385, 1265, 1148, 1042, 1017, 964, 854, 802, 779, 748 and 524.

Preparation of core-shell hybrid nanoparticles from HCP-*star*-PDMAEMA

The star conjugated copolymer (HCP-*star*-PDMAEMA, 20 mg) was dissolved in the THF (1000 mL) with the concentration of 0.02 mg/mL. After stirring thoroughly for 2 days, 50 mL aqueous solution with pH=5 was added dropwise into the THF solution and stirred continuously for 2 day to make sure the protonation of PDMAEMA. 500 μL TEOS was drop slowly into the solution during the stirring. After stirring for 2 days, the solution was at rest for 2 day to keep silica reaction completely. The as-synthesized solution was concentrated, washed three times to remove the unreacted species and redispersed in ethanol. The HCP@SiO_2 aqueous solution was prepared by dialysis against distilled water for 3 days. The final concentration of solution was adjusted by adding some distilled water.

Preparation of hybrid nanoparticles HCP@SiO_2 with amine terminal group

The surfaces of HCP@SiO_2 were functionalized with amine groups by treatment with APTMS. 100 μL of APTMS was introduced to 20 mL HCP@SiO_2 ethanol solution (1.5 mg/mL), and the solution was stirred for 24 h. The as-synthesized nanoparticles were concentrated and washed three times to remove the unreacted species and redispersed in ethanol. The HCP@SiO_2 with amine group aqueous solution was obtained by dialysis against distilled water for 3 days. The final concentration of solution was adjusted by adding some distilled water.

Section-4 Cell experiment

Cell cultures

HeLa cells and NIH-3T3 cells were cultured in DMEM (Dulbecco's modified Eagle's medium) supplied with 10% FBS (fetal bovine serum), and antibiotics (50 units/mL penicillin and 50 units/mL streptomycin) at 37 °C under a humidified atmosphere containing 5% CO_2

In vitro cytotoxicity measurements of HCP@SiO_2

The relative cytotoxicity of HCP@SiO_2 against NIH/3T3 cells was estimated by MTT viability assay. In the MTT assay, NIH/3T3 cells were seeded into 96-well plates with a density of 1.0×10^4 cells per well in 200 μL of medium. After 24 h of incubation, the culture medium was removed and replaced with 200 μL of a medium containing serial dilutions of hybrid nanoparticles. The cells were grown for another 48 h. Then, 20 μL of 2.5 mg/mL MTT assays stock solution in PBS was added to each well. After incubating the cells for 4 h, the medium containing unreacted dye was removed carefully. The obtained blue formazan crystals were dissolved in 200 μL per well DMSO and the absorbance was measured in a BioTek Elx800 at a wavelength of 490 nm.

Cellular uptake of HCP@SiO_2 and HCP@SiO_2-NH_2 by HeLa cells

The cellular uptake experiments were performed on flow cytometry. For flow cytometry, HeLa cells were seeded in six-well plates at 6×10^5 cells per well in 1 mL complete DMEM and cultured for 24 h. Then the HCP@SiO_2 aqueous solution with hydroxyl and amine terminal group dissolved in DMEM culture medium with a concentration of 250 μg/mL was added to different wells and the cells were incubated at 37 °C for 15, 30, 120, and 240 min. Thereafter, samples were prepared for flow cytometry analysis by removing the cell growth media, rinsing with PBS, and treating with trypsin. Data for 1.0×10^4 gated events were collected and analysis was performed

by means of a BD LSRFortessa flow cytometer.

Cell imaging of HeLa with HCP@SiO_2 and HCP@SiO_2-NH_2

HeLa cells (1.0×10^4 cells per well) were seeded on coverslips in a 24-well tissue culture plate. After 24 h of culture, the HCP@SiO_2 aqueous solution with hydroxyl or amine terminal group dissolved in DMEM culture medium with a concentration of 250 μg/mL was added to different wells, respectively, and the cells were incubated at 37 °C for 30 min, 2 h, 4 h and 24 h. After being washed with PBS, the cells were fixed with 4% formaldehyde for 30 min at room temperature, and the slides were rinsed with PBS three times. Finally, the slides were mounted and observed with a Leica DMI6000B inverted fluorescence microscope. The excitation wavelength of all samples was 364 nm.

Section-5 ^{1}H NMR spectra of HCP precursors and HCP-*star*-PDMAEMA

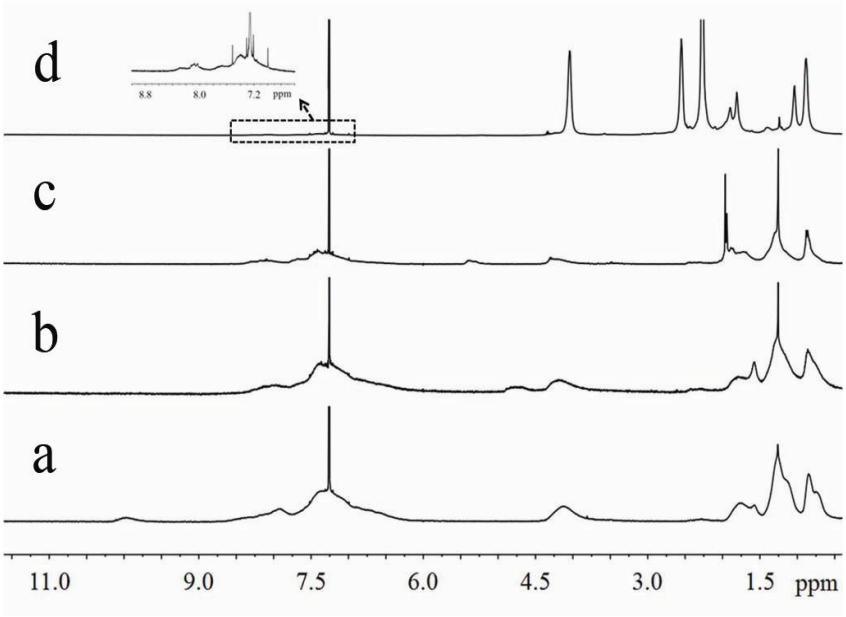


Figure S1. ^{1}H NMR spectra of: (a) HCP-CHO, (b) HCP-OH, (c) HCP-Br, (d) HCP-*star*-PDMAEMA. (400 MHz, in $CDCl_3$, 298 K, the solvent is chloroform)

In Figures S1a, the proton signals at δ=10 ppm are assigned to –C*H*O, while the peak at δ=5.00 ppm assigned to -CH_2Br does not exist. This indicates that the terminal group

of HCP is only aldehyde group. After reduction with sodium borohydride, the proton signal at δ=10 ppm disappears in Figure S1b, which indicates that the terminal group of HCP becomes the hydroxyl group completely. With esterification of 2-bromoisobutyryl bromide, a new signal at δ=1.96 ppm attributed to the methyl group of 2-bromoisobutyryl bromide appears in Figure S1c, which indicates the macroinitiator HCP-Br is obtained successfully. The HCP-Br is used as the macroinitiator to initiate the ATRP copolymerization of DMAEMA monomer to get the star conjugated copolymers. As shown in Figure S1d, peaks at δ=2.28, 2.57 and 4.05 ppm belong to protons of methyl group and methylene group in PDMAEMA.

Section-6 FTIR spectra of HCP precursors and HCP-*star*-PDMAEMA

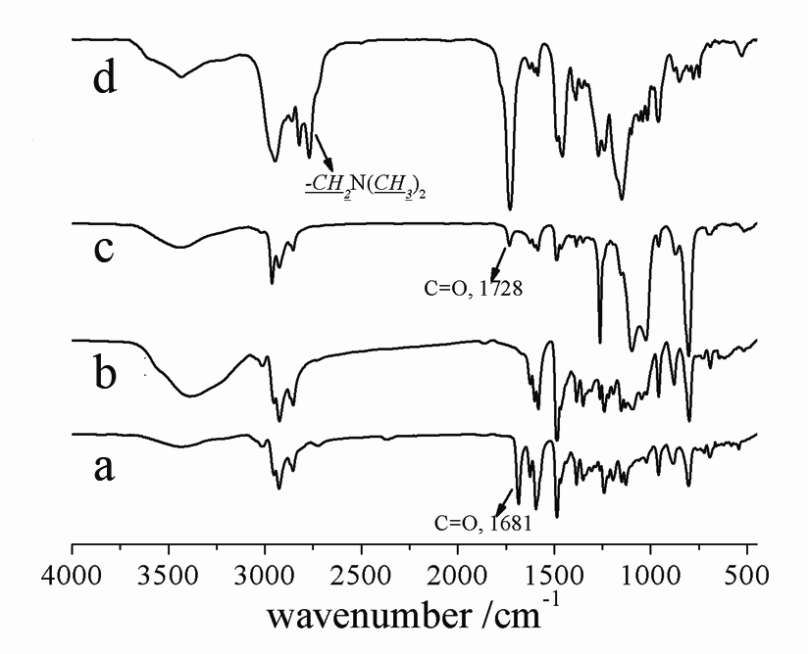


Figure S2. The FTIR spectra of: (a) HCP-CHO, (b) HCP-OH, (c) HCP-Br, (d) HCP-*star*-PDMAEMA.

In the FTIR spectra, Figure S2a exhibits a strong absorption band at 1681 cm^{-1} associated with the stretching of aldehyde group, while it disappears in Figure S2b. It suggests that the aldehyde group is reduced completely by sodium borohydride. After esterification with 2-bromoisobutyryl bromide, Figure S2c shows a strong C=O

stretching band of ester bond of HCP-Br at 1728 cm^{-1}, indicating the graft of 2-bromoisobutyryl bromide onto HCP-OH. When DMAEMA monomer is polymerized from macroinitiator HCP-Br by ATRP, strong absorption bands of methyl group and methylene group in PDMAEMA at 2768 and 2820 cm^{-1} appear in Figure S2d.

Section-7 Characterization of the degree of branching (DB) of HCP core

To simplify the calculation, we neglect the *cis-trans* isomer of double bond in this system. The calculation details of hyperbranched conjugate polymer are shown in Ref. 1. Base on the integrated area of peaks in the ^1H NMR spectrum, the DB is about 0.70.

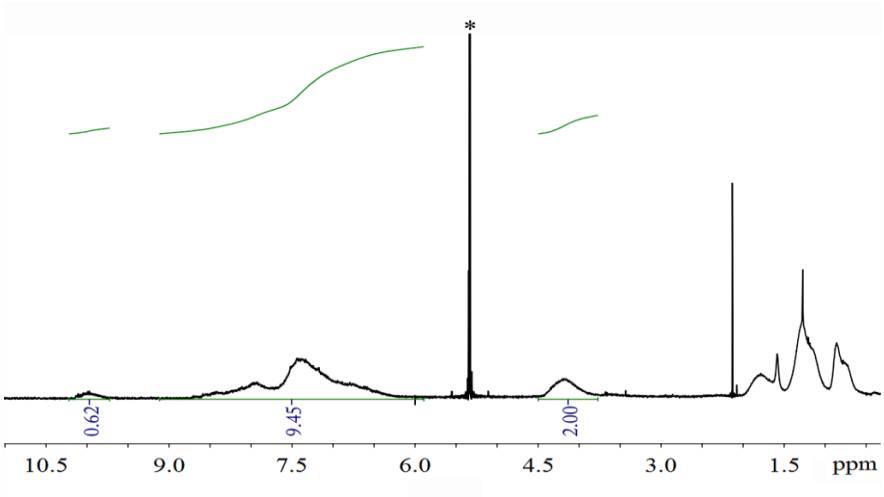


Figure S3. ^1H NMR spectrum of hyperbranched conjugated polymer. (400 MHz, in CD_2Cl_2, 298 K, the solvent peaks are marked with asterisks.)

Section-8 Molecular weight of HCP precursors and HCP-*star*-PDMAEMA

Table S1. Characterization of HCP precursors and HCP-*star*-PDMAEMA

Sample	M_n [a)] ($\times 10^4$ g mol^{-1})	M_w ($\times 10^4$ g mol^{-1})	PDI [a)]
HCP	0.51	0.75	1.45
HCP-*star*-PDMAEMA	2.82	3.13	1.11

a) Determinated by GPC-MALLS.

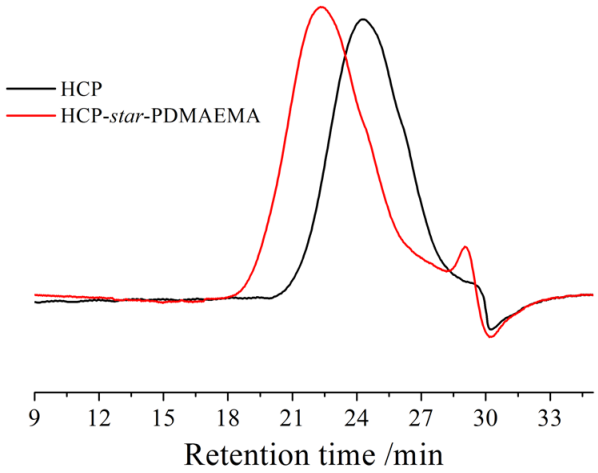


Figure S4. GPC traces of HCP (black line) and HCP-*star*-PDMAEMA (red line).

Section-9 Thermogravimetric analysis (TGA) of HCP precursors, HCP-*star*-PDMAEMA and HCP@SiO_2

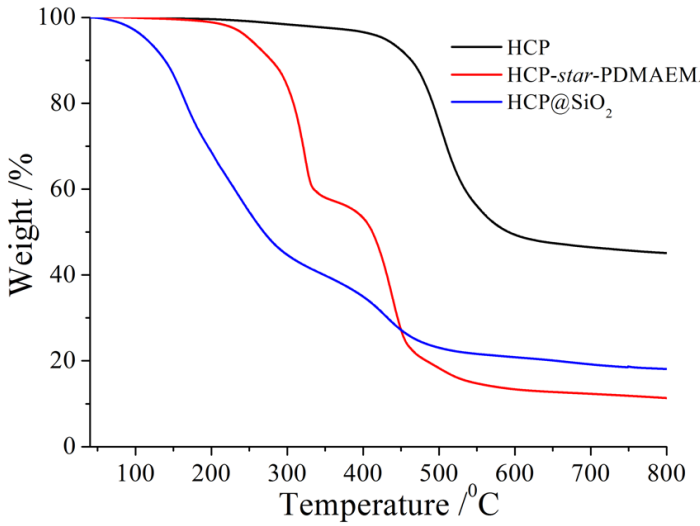


Figure S5. The TGA curves of HCP HCP-*star*-PDMAEMA and HCP@SiO_2.

Section-10 Dimension of HCP-*star*-PDMAEMA and as-synthesized HCP@SiO_2

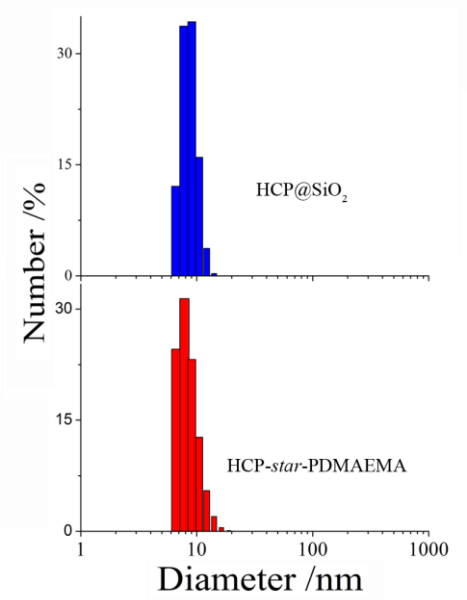


Figure S6. Number-averaged size distribution of HCP-*star*-PDMAEMA and as-synthesized HCP@SiO_2 at 0.02 mg mL^{-1} concentration solution in THF

Section-11 Electronic dispersive X-ray spectroscopy of as-synthesized HCP@SiO_2

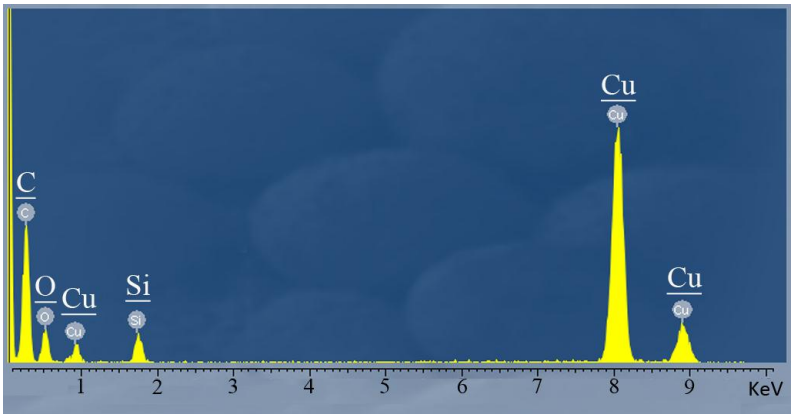


Figure S7. Electronic dispersive X-ray spectroscopy (EDS) analysis of as-synthesized HCP@SiO_2

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Section-12 The optical properties of HCP and HCP-*star*-PDMAEMA in THF solution

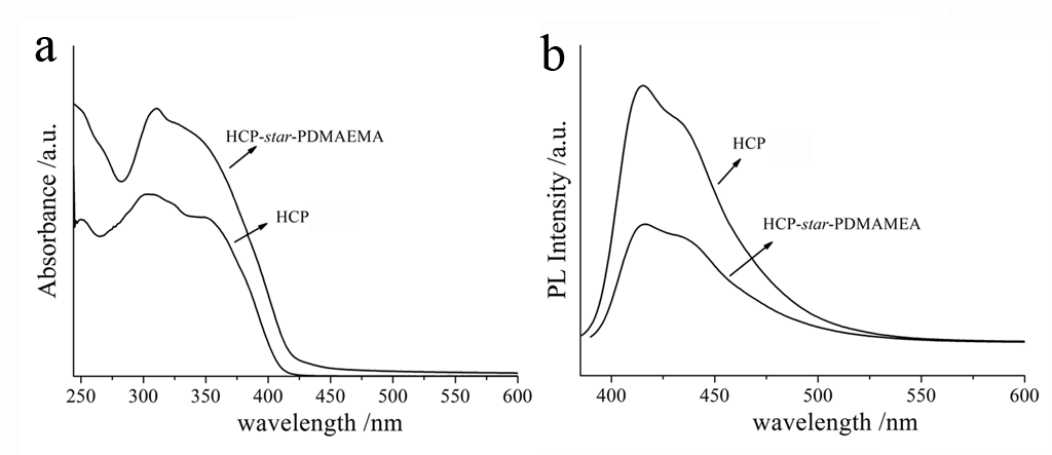

Figure S8. The UV-Vis spectra (a) and PL spectra (b) at 1 mg mL^{-1} concentration of HCP and HCP-*star*-PDMAEMA in THF solution

Section-13 The time-resolved fluorescence of HCP-*star*-PDMAEMA and HCP@SiO_2 in different solvent

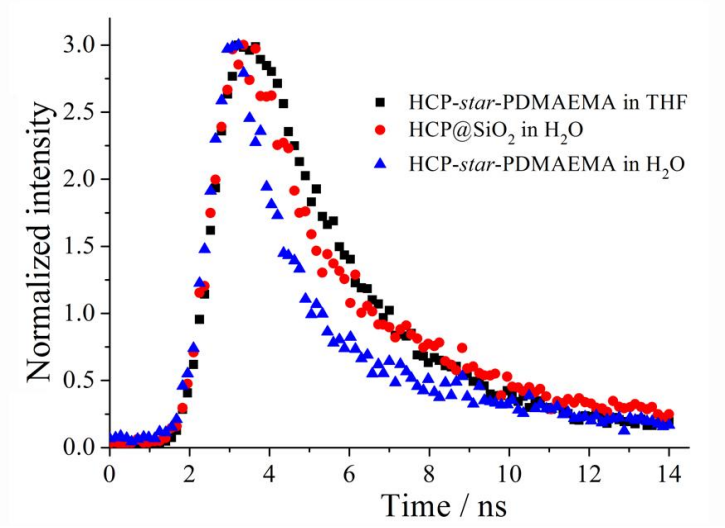

Figure S9. The time-resolved fluorescence of HCP-*star*-PDMAEMA in THF solvent (black line), in water solvent (blue line) and HCP@SiO_2 water solution (red line) at 1 mg mL^{-1} concentration

In Figure S9, The emission lifetime of HCP-star-PDMAEMA in the good solvent THF is 2.70±0.05 ns, which is higher than 2.07±0.11 ns of polymer in poor solvent water. The decreased lifetime of HCP-star-PDMAEMA is caused by the polymer aggregation. However, owing to protection of silica shell, the emission lifetime of HCP@SiO2 is increased to 2.40±0.08 ns.

Section-14 Size and morphologies of HCP@SiO_2 aqueous solution

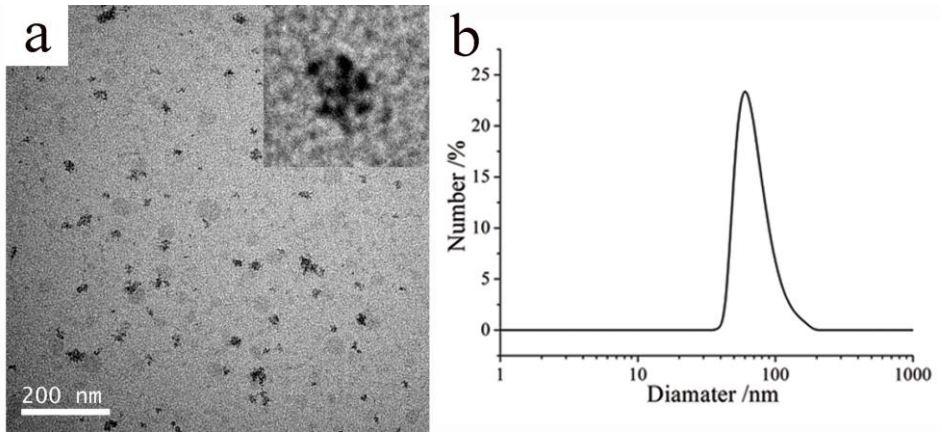

Figure S10. TEM photograph (a) and typical number-weighted DLS plot (b) of HCP@ SiO_2 aggregates at 1 mg mL^{-1} concentration solution in the aqueous medium; the scale bar represents 200 nm for a.

Section-15 The proposed aggregation model of hybrid core-shell HCP@SiO_2

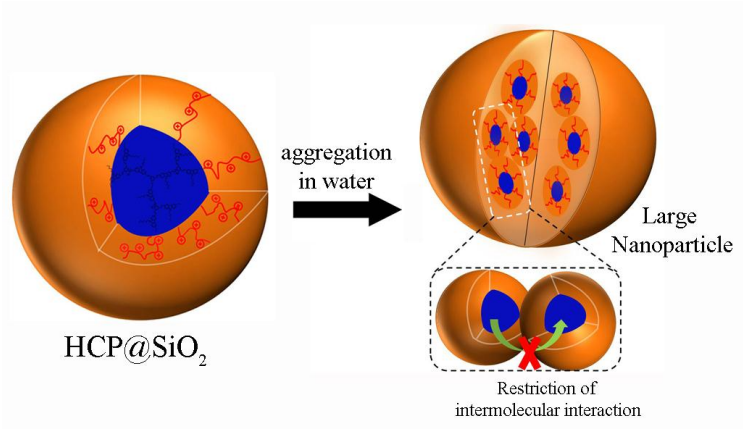

Figure S11. The illustration for aggregation of large nanoparticles from unimolecular

hybrid HCP@SiO_2

Section-16 *In vitro* cytotoxicity of HCP@SiO_2

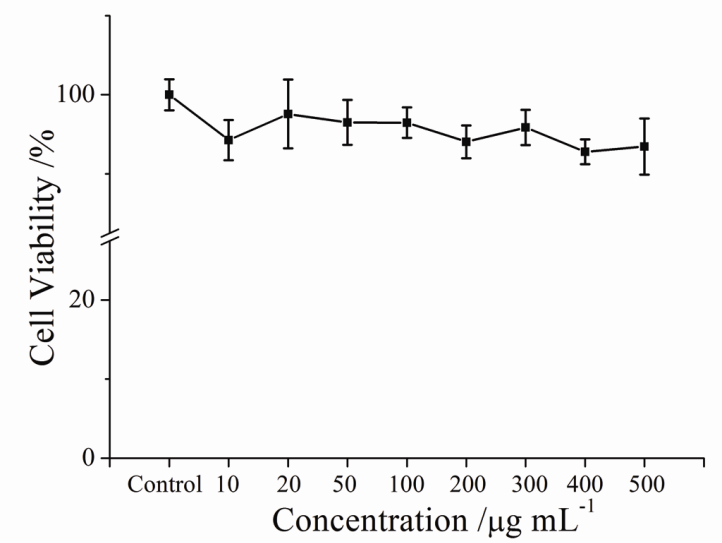

Figure S12. Cell viability of NIH-3T3 cells against HCP@SiO_2 after cultured for 24 h with different concentrations.

The in vitro cytotoxicity of HCP@SiO_2 has been evaluated by MTT assay using NIH-3T3 normal cells. The MTT assay demonstrates that compared to untreated cells, the cell viability after 24 h incubation with HCP@SiO_2 up to 0.5 mg mL^{-1} still remains above 90%, indicating the low cytotoxicity of hybrid nanoparticles from the protection of silica shell.[2]

Section-17 Fluorescent photostability of HCP@SiO_2

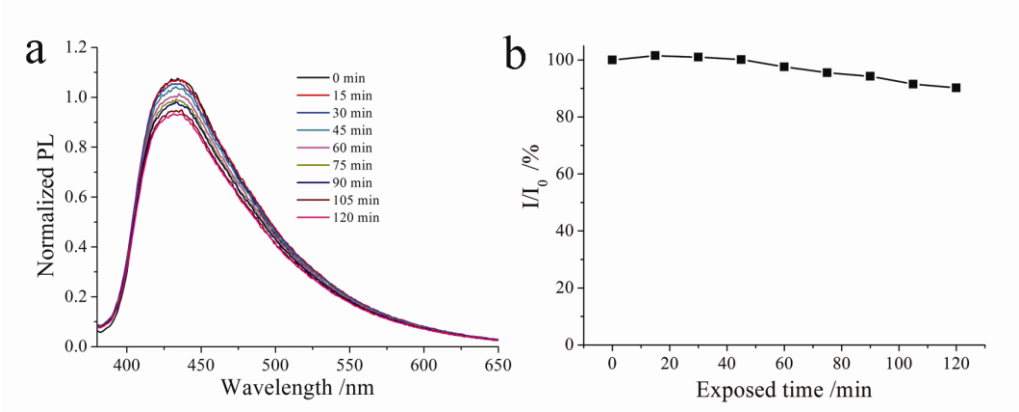

Figure S13. The photobleaching curves of HCP@SiO_2 in water under UV lamp with different time.

Section-18 ζ-Potential of HCP-*star*-PDMAEMA, HCP@SiO_2 and HCP@SiO_2-NH_2

Table S2 ζ-Potential value of HCP-*star*-PDMAEMA, HCP@SiO_2 and HCP@SiO_2-NH_2

Material	HCP-*star*-PDMAEMA	HCP@SiO_2	HCP@SiO_2-NH_2
ζ vaule	22.47±0.90	-0.95±0.30	16.13±3.26

Section-19 Size and morphology of HCP@SiO_2-NH_2 aqueous solution

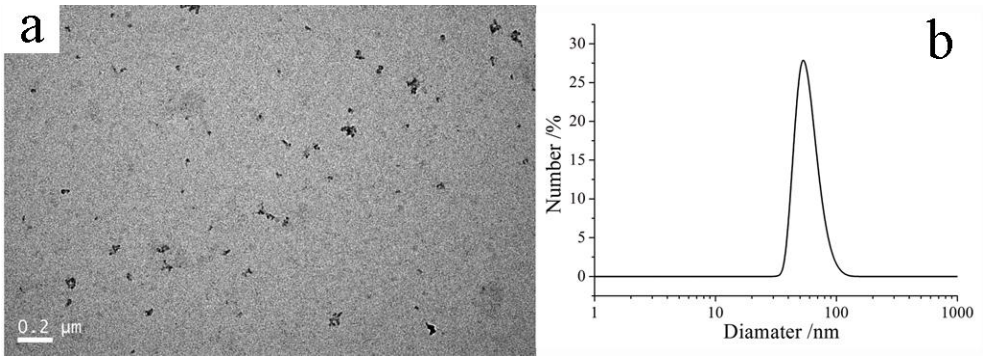

Figure S14. (a) TEM photograph and (b) typical number-weighted DLS plot of HCP@SiO_2-NH_2 aggregates at 1 mg mL^{-1} concentration solution in the aqueous medium.

Section-20 Cellular internalization of HCP@SiO_2 and HCP@SiO_2-NH_2 by flow cytometry measurement

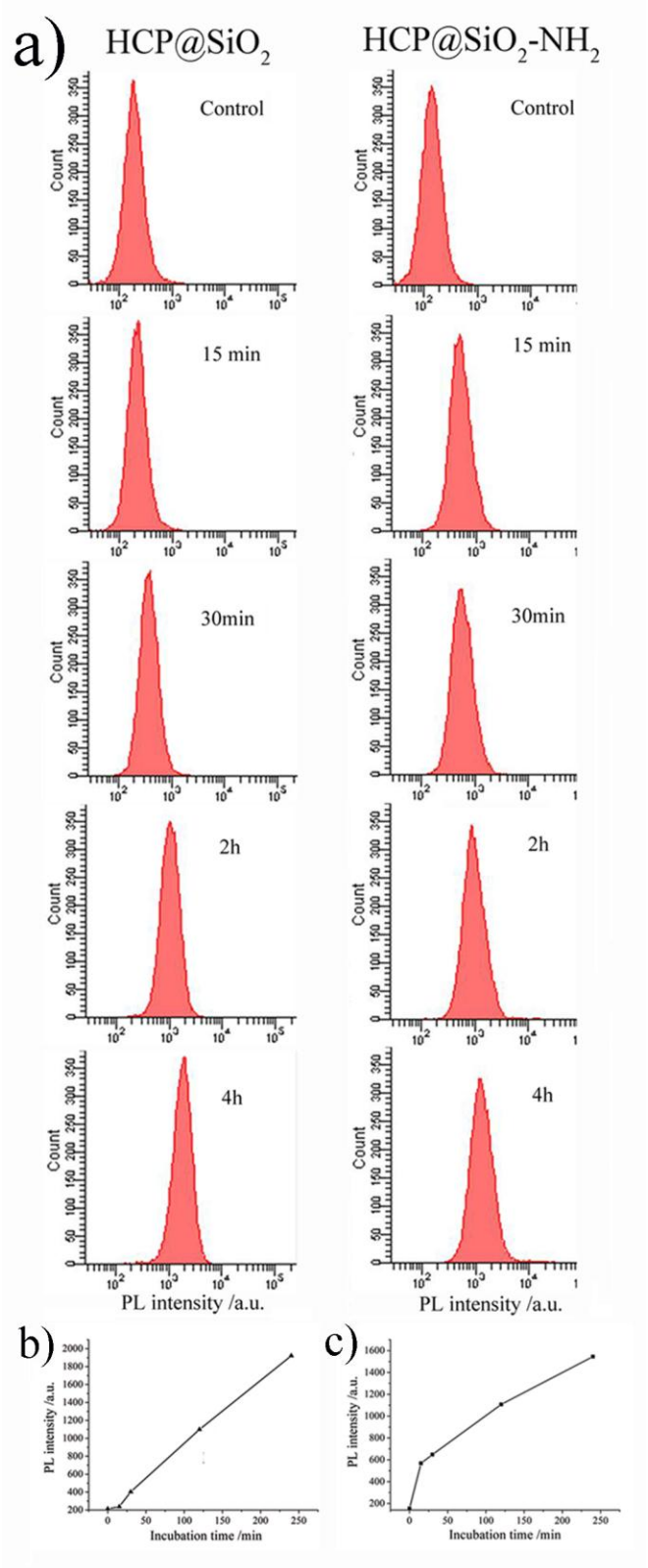

Figure S15. (a) Detailed Flow cytometry histogram profiles of HeLa cells incubated with

HCP@SiO_2 and HCP@SiO_2-NH_2, respectively; Cellular uptake of HCP@SiO_2 (b) and HCP@SiO_2-NH_2 (c) by HeLa cell versus the incubation time by flow cyctometry.

Section-21 Reference

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