**Electronic Supplementary Information for**

# Morphology-Controlled Dual Clickable Nanoparticles via Ultrasonic-Assisted Click Polymerization

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## Experimental section

### *Instrumentation and chemical.*

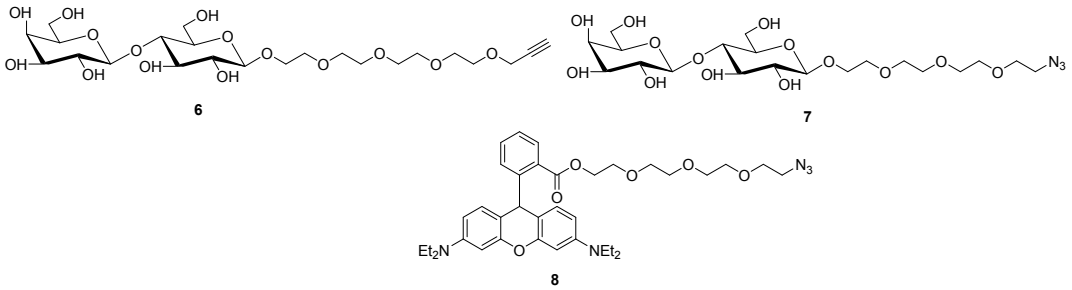
All chemicals used in this work from commercial sources were used as received. Acetonitrile was dried by $CaH_2$ and chloroform was distilled before use. Other solvents were used as received. Column chromatography was performed using silica gel with a grain size of 40-63 μm (Qingdao Haiyang Co., Ltd). 3,5-dibromopyridine was purchased from Beijing Datianfengtuo Chemistry Co., Ltd. 1,3-diethynylbenzene (**2**), tetraglycol, trimethylol propane, and pentaerythritol were obtained from Aladdin.

$^1H$ and $^{13}C$ NMR spectra were recorded on a Bruker Advance 500 instrument in $CDCl_3$, using the residual signals from $CHCl_3$ ($^1H$: $\delta$ 7.25 ppm; $^{13}C$: $\delta$ 77.0 ppm) as internal standard. HRMS (High Resolution Mass Spectrometer) analysis was performed on an Agilent 1290-6540 UHPLC Q-Tof-HRMS. Infrared spectra was measured using KBr pellets and recorded on a FTIR-instrument (BRUKER TENSOR 27, Germany). HPLC tests were performed by LC-20AT (Shimazu, Japan). X-ray photoelectron spectroscopy (XPS) data was obtained by Thermo Scientific K-Alpha K-Alpha. TGA thermograms were recorded on an auto-simultaneous measurement of thermogravimetry and differential thermal analysis (Shimazu DTG-60A, Japan) The Dynamic Light Scattering (DLS) data were obtained by Delsa Nano C analyzer (Beckman Coulter, Inc.). Field emission scanning electron microscopy (FESEM) images were taken on a Hitachi-S4800

instrument operated at 10 KV. A Laser Scanning Confocal Microscope (LSCM, Carl Zeiss LSM 510 UVMETA, Germany) was used to capture images of fluorescent glyconanospheres. Ultrasonic irradiation was produced by SB-5200DT ultrasonic cleaner (Ningbo Scientz Biotechnology Co., Ltd). The centrifuge was performed with H1650-W table-top micro capacity high-speed centrifuge (Hu Nan Xiang Yi Instruments Co., Ltd).

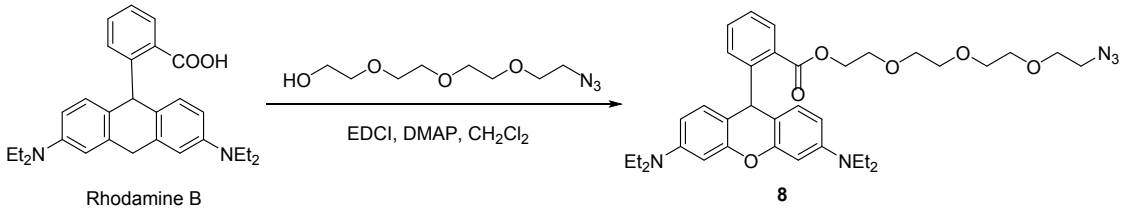
***Synthesis of compounds 1, 3-8.***

The monomer **1**[1], comonomers (**3**,[2] and **4**-**5**[3]), as well as alkyne- and azide-tagged lactoses (**6**[4], **7**[5]) were synthesized according to the published procedures, respectively. Their NMR spectra data are in agreement with those published.




**Scheme S1**. Chemical structures of alkyne- and azide-tagged lactoses (**6** -**7**) and $N_3$-tagged Rhodamine B (**8**).

***$N_3$-tagged Rhodamine B (8)***




To a 25-mL round-bottom flask with a stirrer bar, Rhodamine B (409 mg, 0.85 mmol) and 2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)ethan-1-ol (100 mg, 0.57 mmol) were dissolved in 10 mL $CH_2Cl_2$, followed by the addition of EDC (326 mg, 1.17 mmol) and 4-(dimethylamino)pyridine (DMAP, 5 mg, 0.04 mmol). The reaction mixture was stirred at room temperature overnight. The solvent was removed under reduced pressure. Purification by flash chromatography on silica gel ($CH_2Cl_2$/$CH_3OH$ 30:1 to 10:1 in volume) afforded compound **8** (389.5 mg, 0.60 mmol, 71%) as a thick red oil. $R_f$ = 0.35 ($CH_2Cl_2$/$CH_3OH$, 10:1 in volume). $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 8.34-8.28 (m, 1H, Ar-H), 7.80 (t, $J$ = 7.5 Hz, 1H, Ar-H), 7.72 (t, $J$ = 7.8 Hz, 1H, Ar-H), 7.28 (d, $J$ = 7.5 Hz, 1H, Ar-H), 7.08-7.02 (m, 2H, Ar-H), 6.89 (dd, $J$ = 9.5, 2.4 Hz, 2H, Ar-H), 6.79 (t, $J$ = 5.7 Hz, 2H, Ar-H), 4.16 (t, $J$ = 4.6 Hz, 2H, -$COOCH_2$-), 3.68-3.51 (m, 21H, -$OCH_2CH_2O$-, Ar-CH), 3.36-3.32 (m, 2H, $N_3$-$CH_2$-), 1.28 (t, $J$ = 7.1 Hz, 12H, -$CH_3$) ppm. $^{13}$C NMR (126 MHz, $CDCl_3$) $\delta$ 165.06, 159.03, 157.92, 155.69, 133.83, 133.33, 131.67, 131.49, 130.53, 130.35, 129.86, 114.39, 113.71, 96.43, 70.78, 70.72, 70.62, 70.16, 68.83, 64.83, 50.82, 46.30, 12.82 ppm. ESI HRMS: Calcd for $C_{36}H_{47}N_5O_6$ $[M-H]^-$: 644.3448, found 644.3454.

***Synthesis of DCNPs***

A typical procedure for fabrication of DCNPs is as the following:

To a 10-mL flask, monomer **1** (75 μmol), comonomer (1 equivalent), and 2.4 mL dry solvent were added. The flask was put into an ultrasonic water bath and a certain amount of $Cu(PPh_3)_3Br$ in 100 μL dry chloroform (15 % for DCNSs, 10 % for DCNRs) was injected. The mixture was exposed to ultrasound (40 kHz unless specified) at 13 °C for several hours. The final emulsion was centrifuged at 16500 rpm and the precipitate was washed 3 times with chloroform and dried in vacuum. The specified solvent, time and amount of catalyst were shown in Table S1. For the DCNPs synthesized with 1,3-diethynylbenzene as monomer, the reaction time was 5 h and the teperature was 20 °C.

***The "seed" growth experiment***

The aliquot supernatant of the reaction mixture from **1** + **4** was obtained by centrifugation after general polymerization process was finished, then carefully dried in vacuum. The residue containing unreacted monomer, co-monomer, and catalyst was redissolved into 1 mL solvent. The resulting mixture was kept at room temperature for a certain amount of time.

***Synthesis of glyconanoparicles***

The glyconanoparicles were prepared as the following: 1) To a suspension of DCNSs (10.0 mg) in $CH_3OH/H_2O$ (6 mL, 5:1 in volume), alkyne-functionalized lactose (**6**, 10.0 mg), $CuSO_4•5H_2O$ (7.5 mg) and sodium ascorbate (12 mg) were added. The mixture was stirred at room temperature for 24 h. The yellow emulsion was centrifuged at 16500 rpm. The precipitate was washed three times with $H_2O$ and one time with methanol. 2) Azide-functionalized lactose (**7**, 10 mg) was used to replace **6**, the process was repeated with the precipitate obtained in Step 1). Finally, the reaction mixture was dialyzed using a dialysis membrane (8 000-14 000 MWCO) against PBS buffer to remove residual small molecules to obtain GNPs buffer emulsion. GNPs were characterized by FESEM and FTIR.

***Preparation of Rhodamine-labelled glyconanoparicles (FGNPs)***

FGNPs were prepared in two steps: 1) To a suspension of DCNPs (10.0 mg) in chloroform (6 mL), azide-tagged Rhodamine B (**8**, 10.0 mg) and $Cu(PPh_3)_3Br$ (6.8 mg) were added. The mixture was stirred at room temperature for 24 h. The dark red emulsion was centrifuged at 16 500 rpm. The precipitate was washed 3 times with chloroform. 2) The precipitate obtained in Step 1 was dispersed in $CH_3OH/H_2O$ (6 mL, 5:1 in volume). Following the process described in GNPs preparation, alkyne-tagged lactose (**6**, 10 mg) was conjugated to the surface of the nanospheres. The final emulsion was dialyzed using a dialysis membrane (8 000-14 000 MWCO) against PBS buffer to remove residual small molecules. FGNPs were characterized by FESEM, LSCM, and FTIR (Fig. 4 and Fig. S10).

***Cell culture and incubation of Hela cells with glyconanoparicles***

The cell culture was performed according to the published literature.[6] HeLa cells were grown in DMEM medium supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin. Prior to incubation, the cells were seeded in the 6-well plate with a cell density of $1\times10^5$ cells/well in 1.9 mL of growth medium before the plates were incubated in a humidified, 5 % $CO_2$ atmosphere at 37 °C for 24 h, then 100 μL of glyconanoparicles suspensiom

was added. The concentration of glyconanoparicles in the incubation solution was 200 μg/mL, and incubation duration of the time dependent incubation experiments were 12 h, 24 h, and 48 h, respectively. After incubation, the cells were washed with PBS and stained with propidium iodide (PI) for flow cytometry analysis.

**Table S1.** Summary of optimal conditions for morphology control of DCNPs.[a]

| Entry | Monomer + Comonomer | Solvent[b] | Shape[c] | $D_H$[d] (nm) | PDI[d] | Aspect ratio[e] |
|---|---|---|---|---|---|---|
| 1 | **1** + **4** | $CHCl_3$ | Nanorod | - | - | 7.1 |
| 2 | **1** + **3** | $CHCl_3$ | Nanorod | - | - | 8.1 |
| 3 | **1** + **4** | $CH_3CN/CHCl_3$ | Nanoshpere | 674 ± 72 | 0.392 | 1 |
| 4 | **1** + **5** | $CH_3CN/CHCl_3$ | Nanoshpere | 590 ± 57 | 0.423 | 1 |

[a] Reaction conditions: monomer/comonomer = 1:1 equivalent. $Cu(PPh_3)_3Br$ for nanospheres was 15 mol %, and for nanorods was 10 mol %. Reaction time was 2.5 h for Entry 1, 2 h for Entry 2, and 3 h for Entry 3 and 4. Temperature was 13 °C. [b] $CH_3CN/CHCl_3$ = 96:4 in volume. [c] FESEM images: Fig. 1a and 1b for Entry 1 and 3. Fig. 2 for Entry 2 and 4. [d] Average values of three measurements by DLS (Fig. S9). [e] Calculated with Adobe Photoshop.

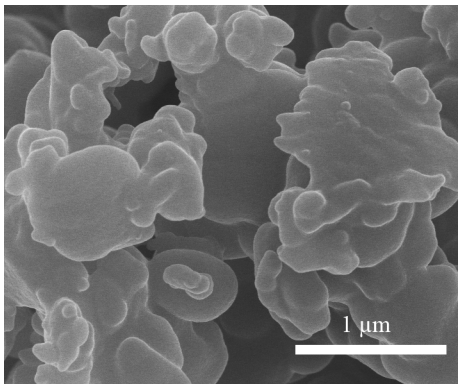



**Fig. S1**. FESEM image of polymeric particles synthesized by click polymerization between **1** and **4** (1:1 equivalent) in $CH_3CN/CHCl_3$ (96:4 in volume) catalyzed by $Cu(PPh_3)_3Br$ (15 mol %). The reaction mixture was stirred by a magnetic stir bar at room temperature for 2 h.

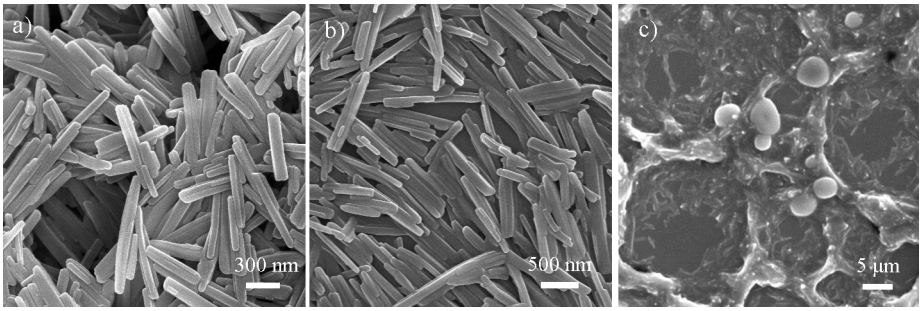



**Fig. S2** FESEM images of polymers obtained by **1** + **4** under ultrasonic for 2.5 h in the mixtures of $CHCl_3$/DCM (v/v). (a) 90%; (b) 80%; (c) 70%.

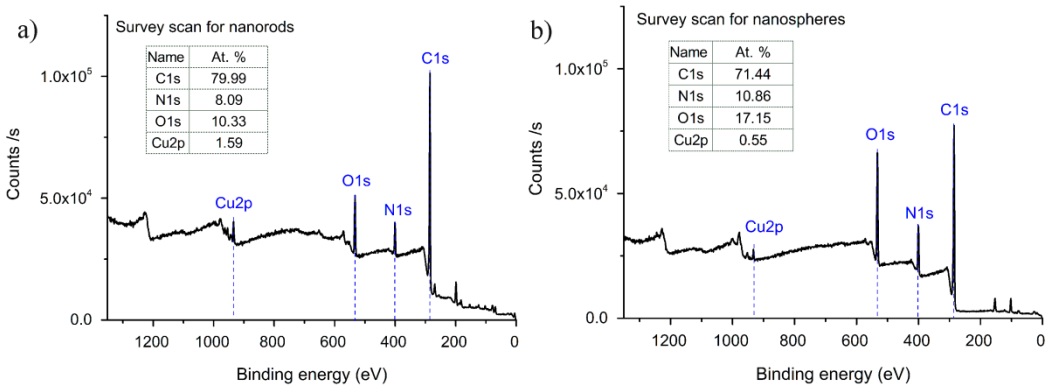



**Fig. S3** XPS data of DCNRs (a) and DCNSs (b) synthesized from **1** + **4** (Entry 1 and 3 in Table S1).

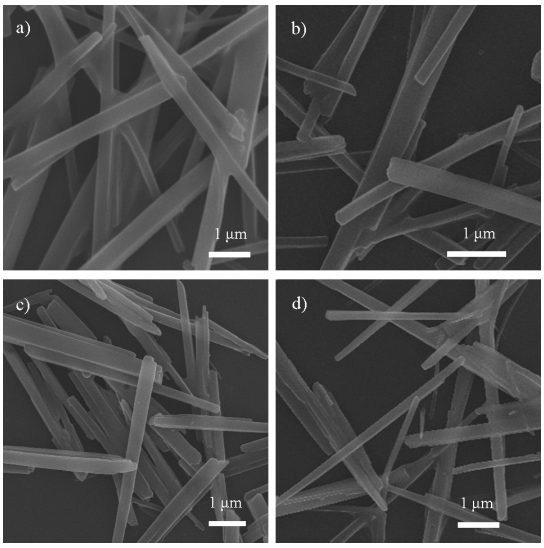



**Fig. S4** FESEM images of rodlike polymers synthesized from **1** + **4** by incubating the "seeds" of nanorods in different solvents for 3 days. a) $CHCl_3$; b) DMF; c) THF; d) DCM.

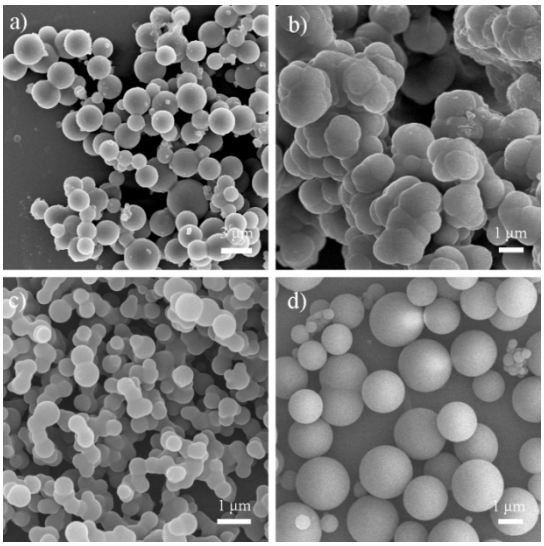



**Fig. S5** FESEM images of spherical polymers synthesized from **1** + **4** obtained by incubated the "seed" of nanospheres in different solvents for 2 days. a) $CHCl_3/CH_3CN$; b) DMF; c) THF; d) DCM.

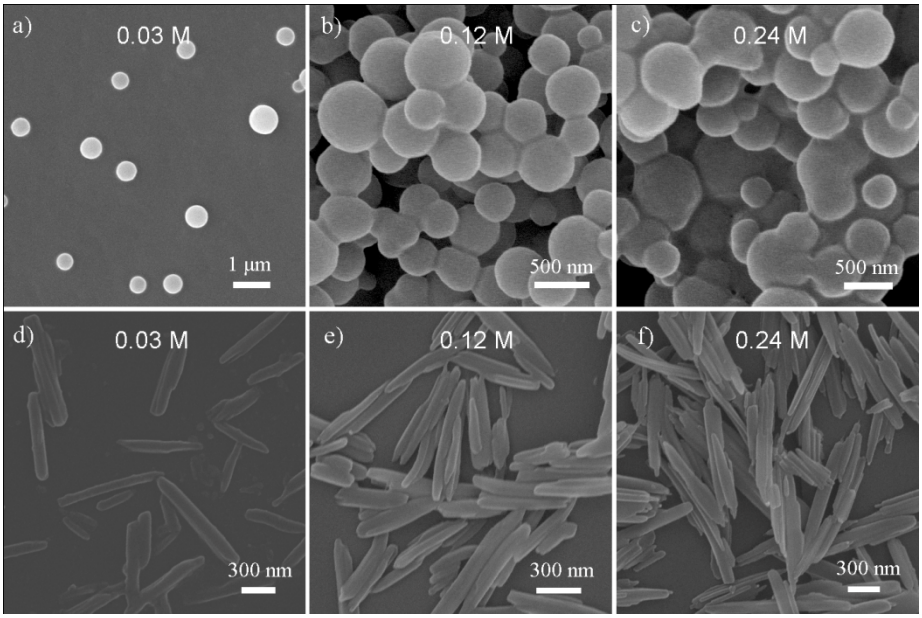



**Fig. S6** The effect of monomer concentration on the morphology of polymer particles from **1** + **4** under ultrasonication by FESEM. (a) - (c) DCNSs: $CH_3CN/CHCl_3$ (96:4 v/v), $Cu(PPh_3)_3Br$ 15 %; (d) - (f) DCNRs: $CHCl_3$, $Cu(PPh_3)_3Br$ 10 %. The reaction time was 3 h for (a), 2.5 h for (b) - (d), 2 h for (e) and (f).

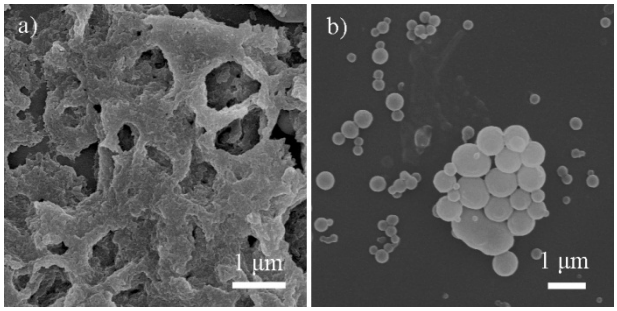



**Fig. S7** FESEM images of the polymers synthesized from **1** + **4** at 80 kHz in different solvents for 3 h at 13 $^oC$. a) $CHCl_3$, $Cu(PPh_3)_3Br$ 10 %; b) $CH_3CN/CHCl_3$ (96:4 v/v), $Cu(PPh_3)_3Br$ 15%.

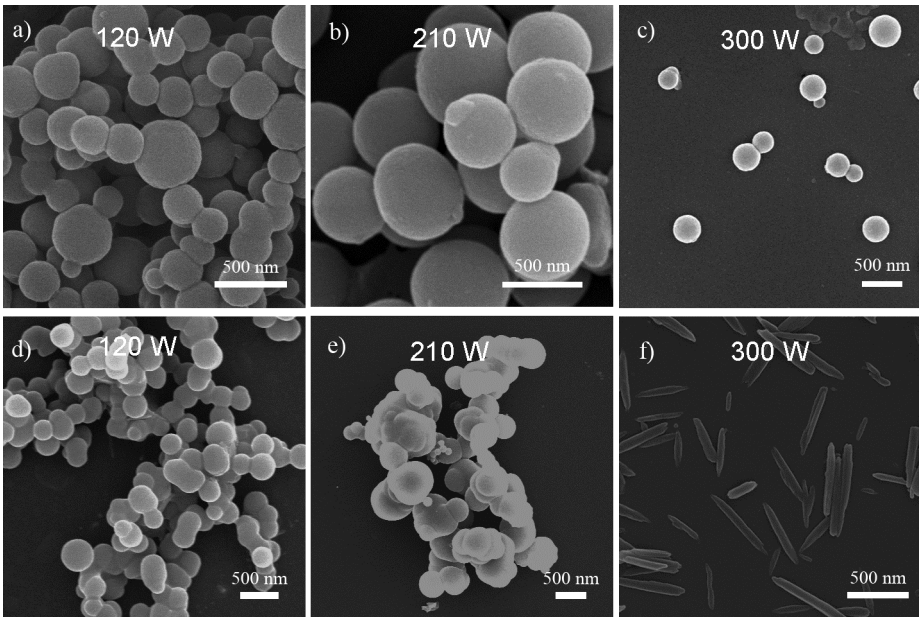



**Fig. S8** FESEM images of nanostructures synthesized from **1** + **4** under ultrasonic (40 kHz) with different powers. (a) - (c) DCNSs: $CH_3CN/CHCl_3$ (96:4, v/v), $Cu(PPh_3)_3Br$ 15 %; (d) - (f) DCNRs: $CHCl_3$, $Cu(PPh_3)_3Br$ 10 %.

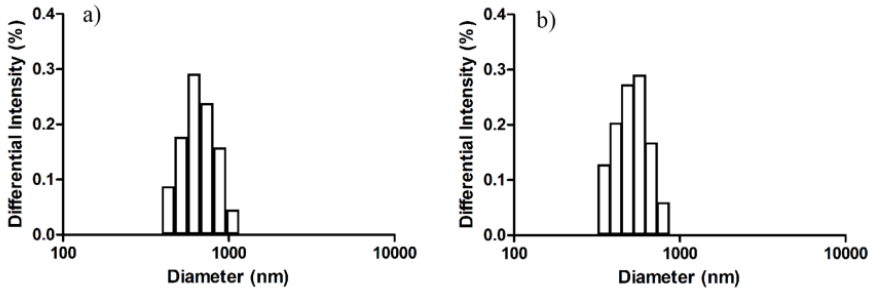



**Fig. S9** DLS analysis of DCNSs, (a) - (b) were corresponding to Entry 3 and 4 in Table S1, respectively.

***Determination of the amount of azide and alkyle groups on the surface of DCNSs***

The amount of azide and alkyle groups on the surface of DCNSs was determined by HPLC according to the following procedure:

To a solution of ethynylbenzene (or benzyl azide) in chlorofom (0.1 mg/mL, 5 mL), DCNSs (15.0 mg) were suspended, followed by the addition of $Cu(PPh_3)_3Br$ (2.0 mg). The mixture was incubated on a Shaker at room temperature for 24 h. Thereafter, the emulsion was filtered with 0.45 μm filter and the concentration of ethynylbenzene (or benzyl azide) in the filtrate was analyzed by HPLC (Shimadzu LC 20AT, 150 × 4.6 mm C18 analytical column with particle size of 5 μm). A control experiment was performed without the catalyst. The analysis was carried out at 25 °C using a mobile phase A (water/acetonitrile 90:10, v /v + 0.1 % TFA) and B (MeCN + 0.1 % TFA) at a flow rate of 1.0 mL/min. The following gradient was applied: A linear increase from solution 30 % to 100 % B in 8 min, then held for 2 min. The detection wavelength was 215 nm for ethynylbenzene and 295 nm for benzyl azide, respectively. The amount of azide groups (or alkyne grous) was calculated according to the following equation:

$$N = \frac{(C_0 - C) \times V}{m}$$

Where C stands for the concentration of ethynylbenzene (or benzyl azide) in the supernatant after the reaction (μmol/mL); $C_0$ stands for the concentration of ethynylbenzene (or benzyl azide) in control experiment (μmol/mL); m stands for the mass of DCNSs (g); V stands for the volume of the reaction solvent (mL); N stands for the amount of functional group (μmol/g polymer).

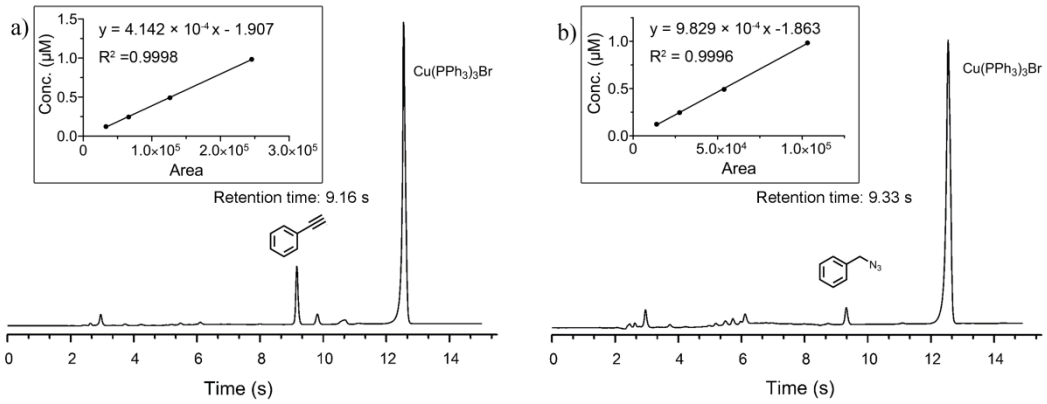



**Fig. S10** The determination of the amount of azide and alkyne groups on the surface of DCNSs synthesized from **1** + **4** by HPLC. (a) HPLC chromatogram for the supernatant from the reaction between DCNSs and ethynylbenzene; Inset: the standard curve of ethynylbenzene. (b) HPLC chromatogram for the supernatant from the reaction between DCNSs and benzyl azide; Inset: the standard curve of benzyl azide.

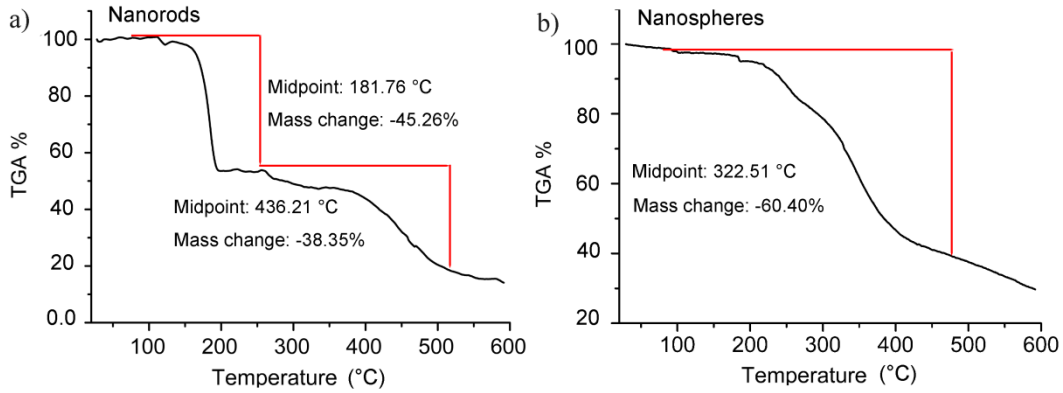



**Fig. S11** TGA thermograms of DCNPs synthesized from **1** + **4,** recorded under nitrogen at a heating rate of 10 °C/min.

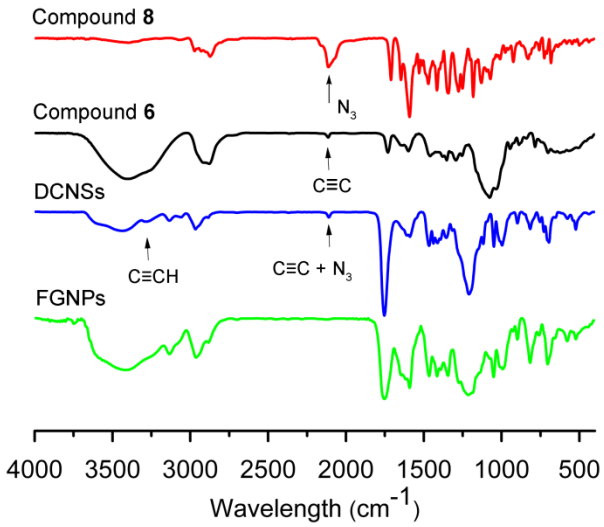



**Fig. S12** IR spectra of **8** (red), **6** (black), DCNSs from **1** + **4** (blue), and fluorescent glyconanopaticles (FGNPs, green).

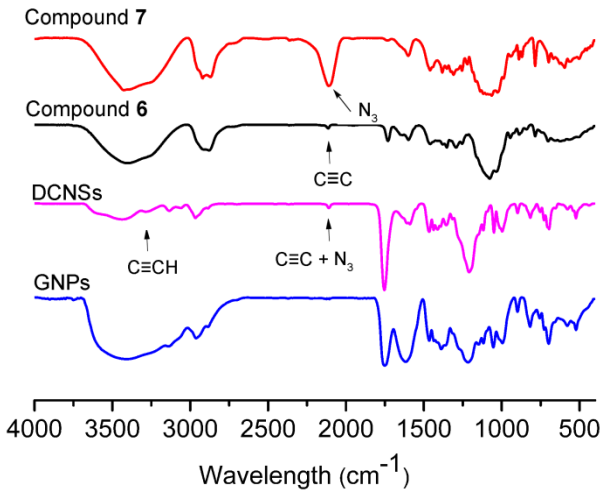



**Fig. S13** IR spectra of **7** (red), **6** (black), DCNSs from **1** + **4** (pink), and glyconanopaticles (GNPs, blue).

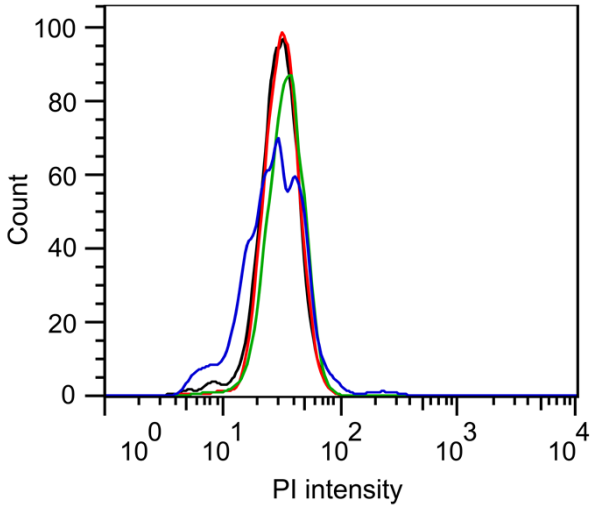



**Fig. S14** Viability of Hela cells. The cells were stained with PI prior to analysis, and the viability was assessed immediately after incubation by flow cytometry: control (black), cells treated with GNPs (200 μg/mL) for 12 h (red), 24 h (green), and 48 h (blue).

## Reference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