

Supporting Information

Photo-controlled host–guest interaction as a new strategy to improve the preparation of “breathing” hollow polymer nanospheres as controlled drug delivery

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1. Synthesis and characterization of AZO-PIEMA-*b*-PNIPAM

1.1. Synthesis of aminoazobenzol-2-bromo-2-methylpropionyl (AZO-Br)

AZO-Br was synthesized according to the method reported in the literatures.^[1] A solution of 2-Bromo-2-methylpropionyl bromide (2.42 g, 11.0 mmol) in 5.0 mL of dry methylene chloride cooled to 0°C under argon was added a solution of aminoazobenzol (1.97 g, 10 mmol) and triethylamine (1.01 g, 10 mmol) in 10.0 mL of dry methylene chloride. The reaction mixture was stirred overnight and filtrated. The filtrate was washed five times with water, dried over MgSO₄, and concentrated. A purified product was easily obtained by passing through a silica gel column (3.74 g, Yield: 91%). ¹H NMR (DMSO-*d*₆): δ 10.16 (Ar-NH-CO), 7.98-7.87 (6H, azobenzene), 7.62-7.53 (3H, azobenzene), 2.05 (6 H, (CH₃)₂-C).

1.2. Synthesis of aminoazobenzol-ended poly(2-hydroxyethyl methacrylate)(AZO-PHEMA-Br)

AZO-PHEMA-Br was synthesized by the atom transfer radical polymerization (ATRP) of HEMA monomer using AZO-Br as the initiator. In a typical example, HEMA monomer (3.38 g, 15.0 mmol), PMDETA (6.93 mg, 0.04 mmol), AZO-Br (0.206 g, 0.6 mmol), and acetone/isopropano mixed solutions (6 mL, 1/1, v/v) were charged into a reaction flask. The flask was capped with a rubber plug and purged with pure nitrogen for 30 min. CuBr (0.0864 g, 0.6 mmol) was then introduced under protection of N₂ flow to start the polymerization at 30 °C under a nitrogen atmosphere. After 5h, the reaction mixture was then exposed to air and diluting with methanol. The copper catalyst was removed by passing the solution through alumina. The polymer was obtained from the resulting reaction mixture by addition to cold diethyl ether to precipitate the solids which were filtered and dried under high vacuum at room temperature for 12 h. The isolated polymer was reprecipitated from methanol into cold diethyl ether three times and dried under vacuum at 25 °C for 24 h, a pale yellow powder was obtained (1.43 g, yield: 40.8%) ($M_{n,SEC-MALLS} = 2290$, $M_w/M_n = 1.17$). The number average DP of AZO-PHEMA was calculated to be 23 by ¹H NMR analysis in DMSO-*d*₆.

1.3. Synthesis of aminoazobenzol-ended poly(2-hydroxyethyl methacrylate)-block-poly(N-isopropylacrylamide) (AZO-PHEMA-*b*-PNIPAM)

AZO-PHEMA-*b*-PNIPAM was synthesized by the ATRP of NIPAM monomer using AZO-PHEMA-Br as the initiator. Typically, AZO-HEMA-Br (0.821 g, 0.02 mmol), NIPAM (48 mg, 0.6 mmol) and Me₆TREN (138 mg, 0.6 mmol) were introduced into a Schlenk flask in acetone/isopropano (2 mL, 1/1, v/v). After degassed by one freeze-thaw cycle, CuBr (2.88 mg, 0.02 mmol) was added rapidly. The Schlenk flask was immersed into a thermostated oil bath at 50 °C for 24 h. The reaction mixture was passed through a neutral alumina column in order to remove copper salt, and precipitated in excess of diethyl ether and dried under vacuum at 25 °C for 24 h. A pale yellow powder was obtained (0.53 g, yield: 64.0%). $M_{w,SEC-MALLS}=7850$, $M_w/M_n=1.19$). The final DP of NIPAM was determined to be 44 by SEC-MALLS analysis.

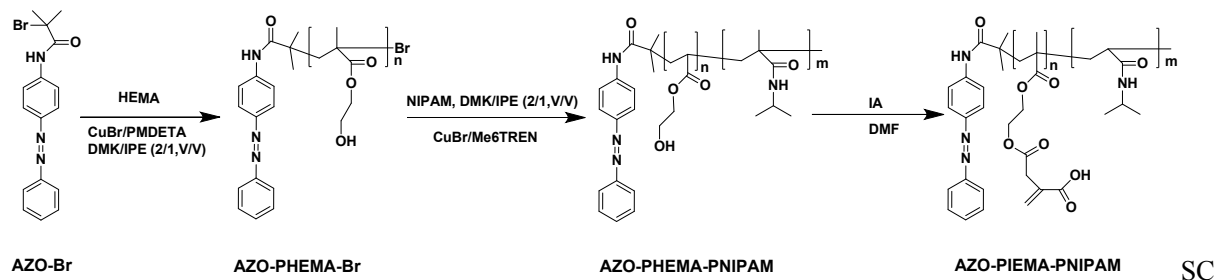
1.4. Synthesis of AZO-PIEMA-*b*-PNIPAM

AZO-PIEMA-*b*-PNIPAM was synthesized by the esterification of the PHEMA moiety in AZO-PHEMA-*b*-PNIPAM. The hydroxy groups of the HEMA residues of the block copolymer were reacted using itaconic anhydride (IA) with different molar ratio of 2:1:1, ($n_{IA}:n_{hydroxy\ group\ in\ AZO-PHEMA-b-PNIPAM}:n_{TEA}$), respectively in DMSO at 25°C. The reaction mixture was precipitated into excess diethyl ether to remove the small molecule impurities. The final AZO-PIEMA-*b*-PNIPAM copolymers were dried under vacuum overnight to obtain a white product in 78% yield.

1.5. Characterization of AZO-PIEMA-*b*-PNIPAM

ATRP polymerization has frequently been used to prepare block copolymers due to the good control over polymerization of various monomers.^[2] In the current work, the diblock copolymer AZO-PIEMA-*b*-PNIPAM was synthesized via successive ATRP polymerization of HEMA and NIPAM, and subsequent esterification between Itaconic anhydride (IA) and hydroxyl groups (-OH) in PHEMA units. The general approach employed for the preparation

of AZO-PIEMA-*b*-PNIPAM was shown in Scheme S1.



Scheme S1 Synthesis routes of AZO-PIEMA-*b*-PNIPAM.

As shown in Figure S1, ¹H NMR analysis revealed that after amidation reaction, the appearance of characteristic signal of NH ($\delta=10.16$ ppm) and CH₃ ($\delta=2.05$ ppm), suggesting the quantitative end group transformation.

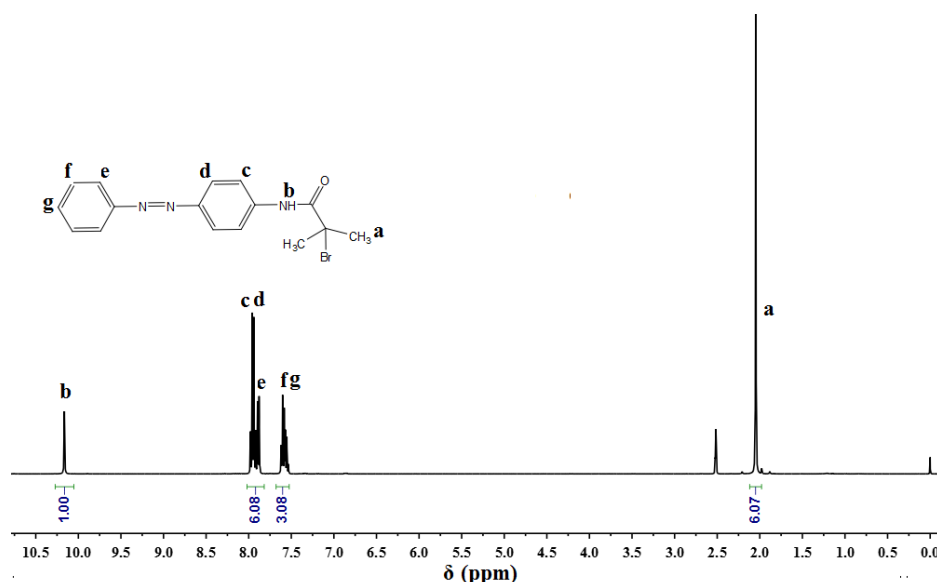


Figure S1 ¹H NMR spectrum of AZO-Br in DMSO-*d*₆

The ATRP reaction of AZO-Br with HEMA afforded macro-ATRP initiator AZO-HEMA-Br. SEC-MALL analysis revealed an M_w of 3180 and an M_w/M_n of 1.17 (Table S1 and Figure S3). The number average degree of polymerization (DP) of HEMA was 23 by ¹H NMR analysis in DMSO-*d*₆. AZO-PHEMA-Br was subsequently used as the macro ATRP agent to control the polymerization of NIPAM. The ¹H NMR spectrum of AZO-PHEMA-PNIPAM was shown in Figure S1B, and all signals characteristic of AZO, PNIPAM and PHEMA moieties can be clearly observed. The final DP of NIPAM was determined to be 44 by SEC-MALLS analysis. SEC-MALLS analysis revealed a M_n of 7650 and an M_w/M_n of 1.19 (Table S1). The AZO-

PHEMA-*b*-PNIPAM diblock copolymer precursors were converted into the final AZO-PIEMA-*b*-PNIPAM by esterification of the hydroxyl groups on the PHEMA blocks with IA with molar ratio of 2:1 ($n_{\text{IA}}:n_{\text{hydroxy group in AZO-PNIPAM-PHEMA}}$).^[3]As shown in Figure S1C, -OH signals of PHEMA were no longer detected at 4.9 by ¹H NMR, indicating very high degrees of esterification. The molecular weight of AZO-PIEMAA-*b*-PNIPAM could be calculated as 9660 which was consistent with the result determined by SEC-MALL measurement (Table S1 and Figure S3).

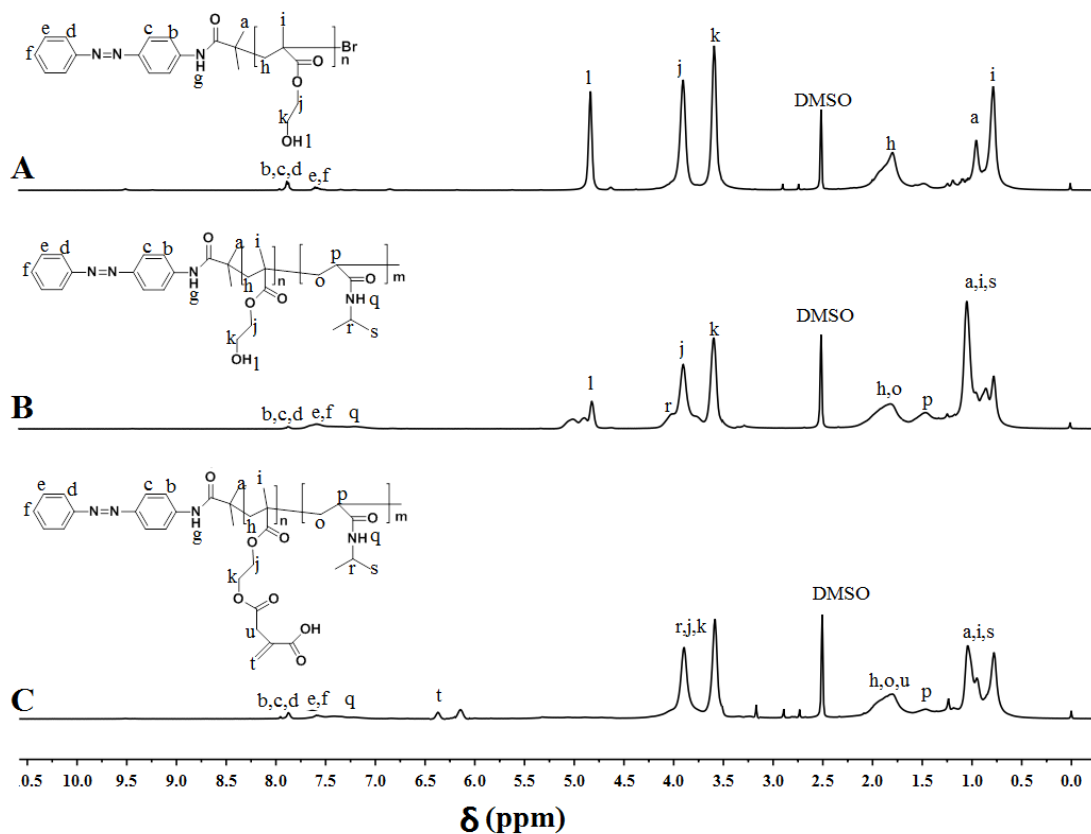


Figure S2 ¹H NMR spectra of AZO-PHEMA (A), AZO-PHEMA-*b*-PNIPAM (B) and AZO-PIEMA-*b*-PNIPAM (C) in DMSO-*d*₆.

Table S1 SEC-MALLS data of AZO-PHEMA, AZO-PHEMA-*b*-PNIPAM and AZO-PIEMA-*b*-PNIPAM copolymers

Sample	M_n^a (g/mol)	M_w^b (g/mol)	PDI ^a (M_w/M_n)
AZO-PHEMA	2290	2680	1.17
AZO-PHEMA- <i>b</i> -PNIPAM	6420	7650	1.19
AZO-PIEMA- <i>b</i> -PNIPAM	7990	9670	1.21

^{a,b}The weight-average molecular weight (M_w) and number-average molecular weight (M_n) were determined by SEC-MALLS; ^c Polydispersity index was determined by SEC-MALLS.

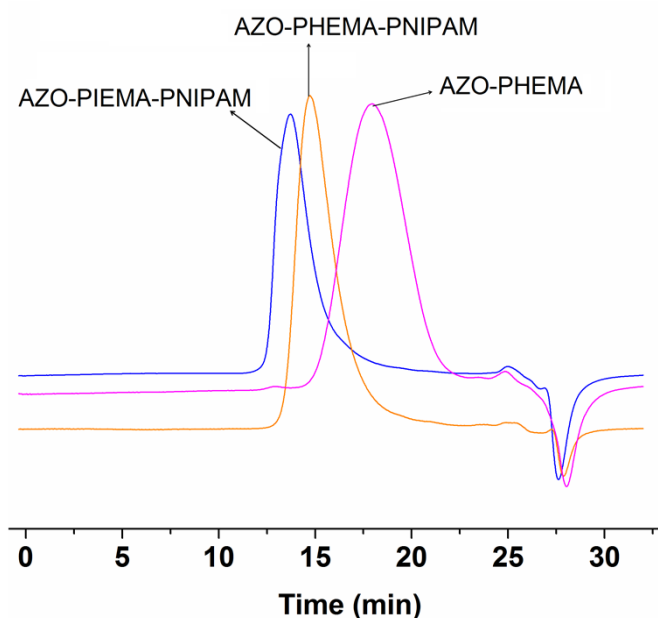


Figure S3 DRI signals of SEC/MALLS chromatograms of AZO-PHEMA, AZO-PHEMA-*b*-PNIPAM and AZO-PIEMA-*b*-PNIPAM (0.5mg mL⁻¹).

2. Synthesis and characterization of β -CD-PDEA

2.1 Synthesis of β -cyclodextrin-based ATRP initiator (β -CD-Br)

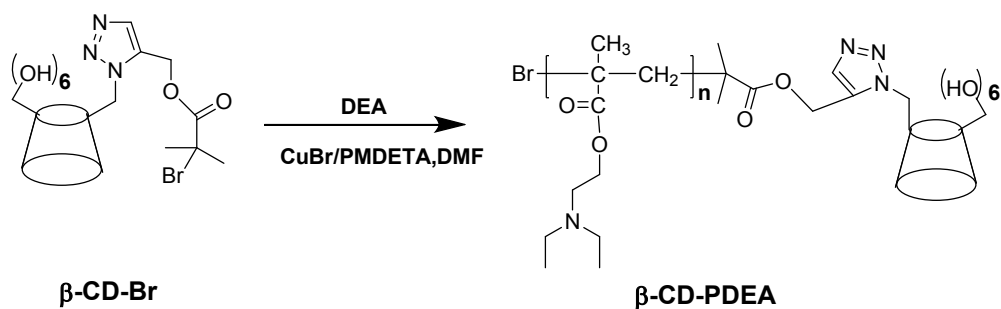
β -CD-Br was prepared via the click reaction of β -CD-N₃ with a slight excess of PBIB, and a typical procedure was as follows. β -CD-N₃ (2.32 g, 2.0 mmol), PBIB (0.612 g, 3.0 mmol), PMDETA (710 μ L, 3 mmol), and anhydrous DMF (10 mL) were introduced into an oven-dried Schlenk tube equipped with a magnetic bar. The tube was capped with a rubber plug and

purged with pure nitrogen for 30 min. CuBr (432 mg, 3.0 mmol) was then added under the protection of nitrogen and the mixture was then stirred at 50 °C for 24 h. The reaction mixture was then exposed to air and precipitated into an excess of acetone/H₂O (6:1 v/v). Recovery by suction filtration and drying overnight in a vacuum oven yielded a pure white solid (1.98 g, yield: 73.3%). ¹HNMR (DMSO-*d*₆): δ=8.13 (methine proton in 1,2,3-triazole), 5.93–5.59 (2,3-OH), 5.23 (-OCH₂-1,2,3-triazole), 4.98–4.72 (1-H), 4.59–4.39 (6-OH), 4.28 (-O-CH₂-CH₂), 3.84–3.47 (3,5,6-H), 3.46–3.12 (2,4-H), 3.18-2.98 (-CH₂-N), 1.88 (-CH₂-), 1.25 (-CH₂CH₃), 1.12 (C-CH₃)

2.2 Synthesis of β-CD-PDEA

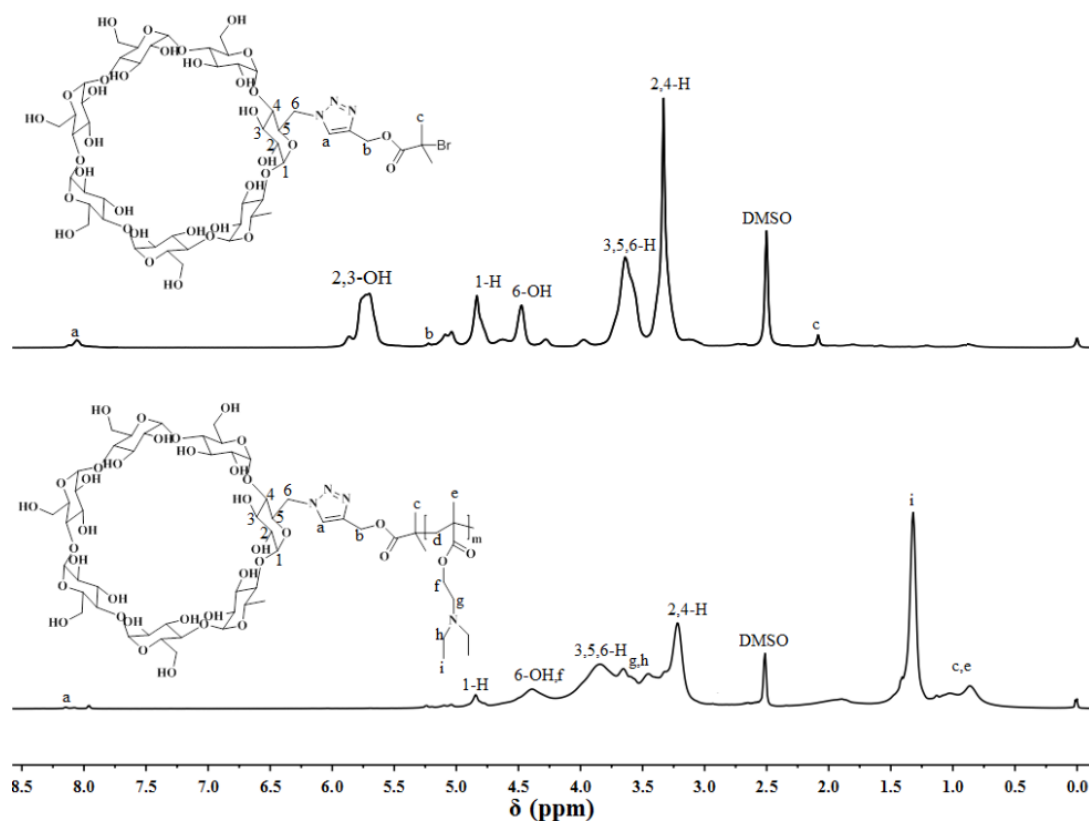
β-CD-PDEA was synthesized by the ATRP reaction of DEA monomer using β-CD-Br as the initiator. In a typical example, DEA monomer (2.78 g, 15 mmol), PMDETA (117 μL, 0.56 mmol), β-CD-Br (0.764 g, 0.56 mmol) and DMF (5 mL) were charged into a reaction flask. The flask was capped with a rubber plug and purged with pure nitrogen for 30 min. CuBr (0.0806 g, 0.56 mmol) was then introduced under protection of N₂ flow to start the polymerization at room temperature under a nitrogen atmosphere. After 4h, the reaction mixture was then exposed to air and precipitated into cold petroleum ether (-20 °C) to remove residual monomer. The crude product was dissolved in 5 mL DMF, enclosed in dialysis membrane (MWCO 2000Da), and then purified by dialyzing in deionized water for 3d to remove the excess β-CD-Br. After removal of the water by freeze drying, a white powder was obtained (1.11 g, yield: 31.1%, M_{w,SEC-MALL}=8740, M_w/M_n=1.26). The degree of polymerization (DP) for DEA was calculated to be 58 by SEC-MALLS analysis.

2.3 Characterization of β-CD-PDEA



Scheme S2 Synthesis route of β -CD-PDEA

As shown in scheme S2, β -CD-PDEA was prepared via ATRP of DEA monomer using β -CD-Br as the initiator and CuBr/PMDETA as catalysts. Firstly, the β -CD-based ATRP initiator, β -CD-Br, was prepared by the click reaction of β -CD- N_3 with PBIB. The subsequent ATRP of DEA using β -CD-Br initiator readily afforded β -CD-PDEA homopolymers. The ^1H NMR spectra of β -CD-Br and β -CD-PDEA were shown in Figure S3. As shown in Figure S3b, all signals characteristic of β -CD and PDEA moieties can be clearly observed. SEC-MALLS (Figure S5) analysis revealed a M_w of 8740 and a M_w/M_n of 1.26. The number average DP of PDEA was determined to be 58 by SEC-MALLS analysis.



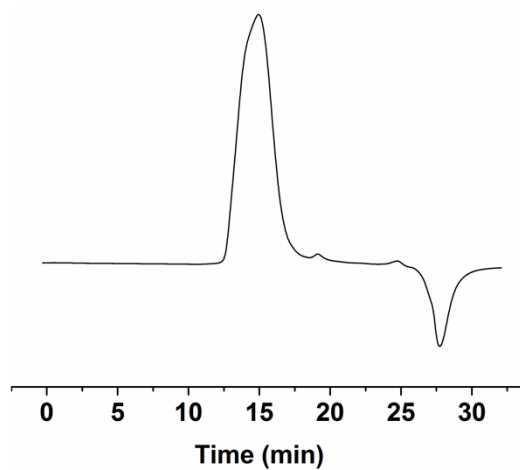


Figure S5 DRI signal of SEC/MALLS chromatograms of β -CD-PDEA (0.5 mg mL^{-1}).

3. 2D ^1H NMR NOESY spectra of solid and hollow nanospheres

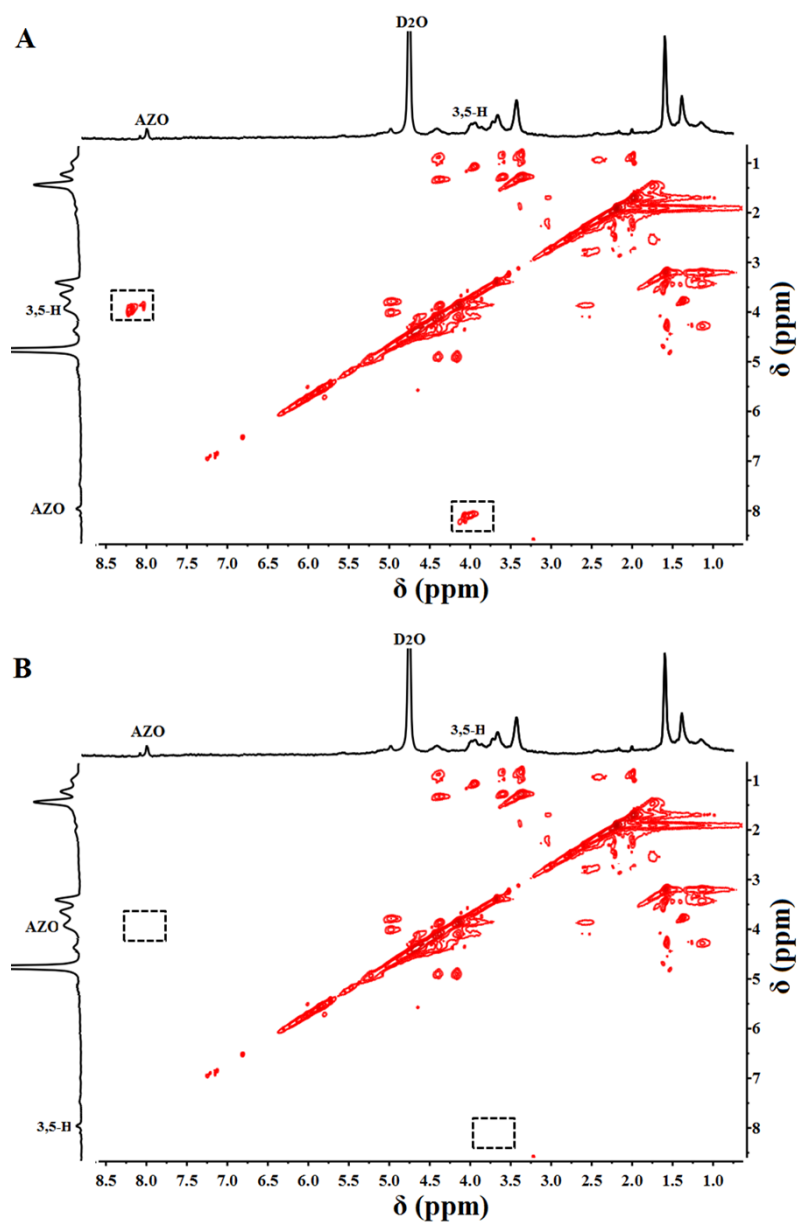


Figure S6 2D ^1H NMR NOESY spectra of shell cross-linked nanospheres in aqueous solution before (A) and after (B) 365 nm UV light irradiation at pH 6.0 and 25 °C.

4. Drug Loading

Although various types of hollow microspheres or microcapsules were reported, very few studies concerning the drug release behavior of the temperature responsive hollow nanospheres were published yet.^[4] To explore the feasibility of using PIEMA-PNIPAM hollow nanospheres as a controlled drug-delivery vehicle, the anticancer drug DOX was loaded into PIEMA-PNIPAM hollow nanospheres, and then its release profile was investigated by dialysis in vitro. UV-vis spectra showed that DOX was been successfully loaded in the PIEMA-PNIPAM hollow nanospheres since its characteristic absorbance appeared at 253 and 485 nm in Figure S7A. TEM characterization provided further proof of drug loading (Figure S7B). Since their interiors are darker than their shells, it can be deduced that the loaded drugs were mainly located in the cavity of the hollow nanospheres.

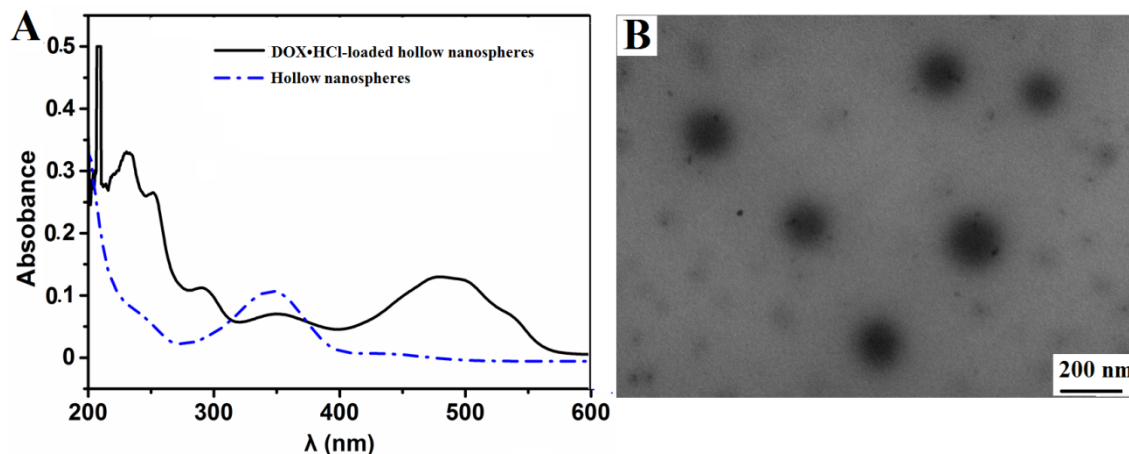


Figure S7 (A) UV-vis absorption spectra of unloaded hollow nanospheres and DOX•HCl-loaded hollow nanospheres at 25 °C; (B)TEM images of DOX•HCl-loaded hollow nanospheres.

5. Reference

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