

Multi-Stimuli-Responsive magnetic assemblies as Tunable Releasing Carriers

Supporting Information

Experimental Section

Instruments

The ^1H NMR spectra were recorded using a 600 MHz spectrometer (Advance Bruker-600, Switzerland) and Varian INOVA-400 FT-NMR. The transmission electron microscopy (TEM) were measured by Jeol JEM-100CX and Tecnai G2-20. The dynamic light scattering (DLS) was measured by BI-9000AT, BI-200SM, Brookhaven Instruments Co., USA. UV absorbance of samples was measured by TU-1901 double beams UV-vis spectrophotometer (Beijing Purkine General Instrument Co. LTD., China).

Sample Synthesis

Preparation of β -CD-capped magnetic nanoparticles

According to the traditional co-precipitation method¹, we obtained the Fe_3O_4 magnetic nanoparticles. Then the modification of amino group to MNPs was achieved using APTES as a coating agent². 600 mg MNPs was dispersed in 90 mL ethanol under sonication. With vigorous stirring, 2 mL APTES was added to the solution and then 1 mL deionized water. After reacting for 6 h under nitrogen and vigorous stirring, the reacted mixture was placed on a magnet and the precipitates were collected. The modified MNPs (MNPs-NH_2) were re-suspended in ethanol by sonication and recollected by magnetic decantation for three times. Then, the MNPs-NH_2 were dried at 30 °C for 24 h under vacuum.

To cover β -CD onto the surface of MNPs^3 , the as-synthesized MNPs-NH_2 (0.25 g) and 6-Ts- β -CD (0.0325 g) were suspended in DMF (60 mL) and the mixtures were sonicated for 30 min. Then, the suspension was heated to 70 °C and mechanically agitated. All the procedures were carried out under nitrogen protection to avoid possible oxidization. After 7 h, the reaction solution was cooled to room temperature the product formed was separated by magnetic decantation and re-dispersed in DMF by sonication and recollected by magnetic decantation for three

times. The final sample was defined as CD-MNPs.

Synthesis of Fc-terminated PNIPAM/PEG

The synthetic routes are shown in scheme S1, in brief, mPEG (2.0 g, 1.0 mmol), FcA (0.46 g, 2.0 mmol), and DMAP (0.183 mg, 1.5 mmol) were dissolved in 20 mL of anhydrous dichloromethane followed by cooling the solution to 0 °C. Subsequently, DCC (1.03 g, 5 mmol) dissolved in 10 mL anhydrous dichloromethane was added to the solution under nitrogen atmosphere. The reaction was performed at room temperature for 48 h under nitrogen atmosphere. The byproduct dicyclohexylcarbodiurea (DCU) was removed by filtration, and the filtered solution was evaporated to dryness. The solid was dissolved in chloroform, the obtained solution was extracted with a diluted NaOH solution (pH= 10, 10 mL×3) to remove unreactive FcA. Then precipitated the solution into excess cold diethyl ether, the obtained light yellow product was dried in vacuum (1.85 g, yield: 83.6%).

PNIPAM amine terminated (500 mg, 0.2 mmol), FcA (0.092 g, 0.4 mmol) and HOBT (0.041 mg, 0.3 mmol) were dissolved in 50 ml of anhydrous dichloromethane, followed by cooling the solution to 0 °C. Subsequently, DCC (0.206 mg, 1mmol) dissolved in 10 mL anhydrous dichloromethane was added to the solution under nitrogen atmosphere. The solution was stirred for five days. Then reaction mixture was filtered to remove DCU, the filtered solution was concentrated using a rotavapor at 30°C, followed by a silica gel column with chloroform : methanol =5:1 (v/v) as eluent to give the Fc-terminated PNIPAM. (0.27 g, yield: 50%).

Calculation of the number of β -CD on each of magnetic nanoparticles. By assuming the core shape of nanoparticles to be spherical⁴, the average number of CD units around one nanoparticle (N) could be calculated by the equations listed below:

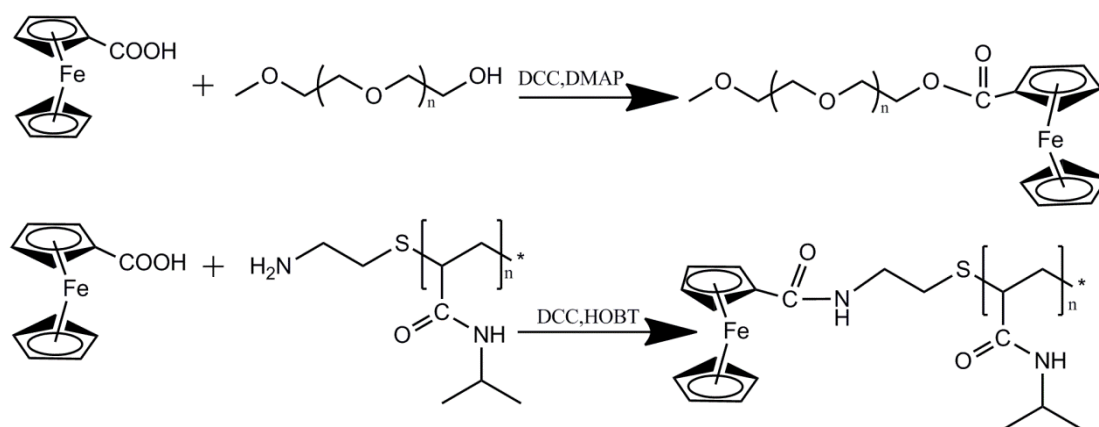
$$M_{MNPs} = \frac{W_{MNPs}}{D_{MNPs} \left(\frac{4}{3} \right) \pi r^3 N_A}$$

$$M_{CD} = \frac{W_{CD}}{MW_{CD}}$$

$$N = \frac{M_{CD}}{M_{MNPs}} = \frac{W_{CD}D_{MNPs}(4/3)\pi r^3 N_A}{W_{MNPs}MW_{CD}}$$

where M_{MNPs} and M_{CD} are the molar number of gold nanoparticles (MNPs) and β -CD, respectively. W_{MNPs} , W_{CD} , and D_{MNPs} are the gold content (obtained from TGA data, Figure S2), cyclodextrin content (obtained from TGA data, Figure S2), Fe_3O_4 density, respectively. r is the radius of nanoparticles (obtained from TEM results). N_A is the Avogadro content (6.02×10^{23}). MW_{CD} is the molecular weight of 6-Ts- β -CD ($M=1288$). N is the number of β -CD on each of nanoparticles.

Synthesis routes of ferrocene-terminated PNIPAM/PEG.



Scheme S1. The synthetic routes of ferrocene-terminated PNIPAM and PEG

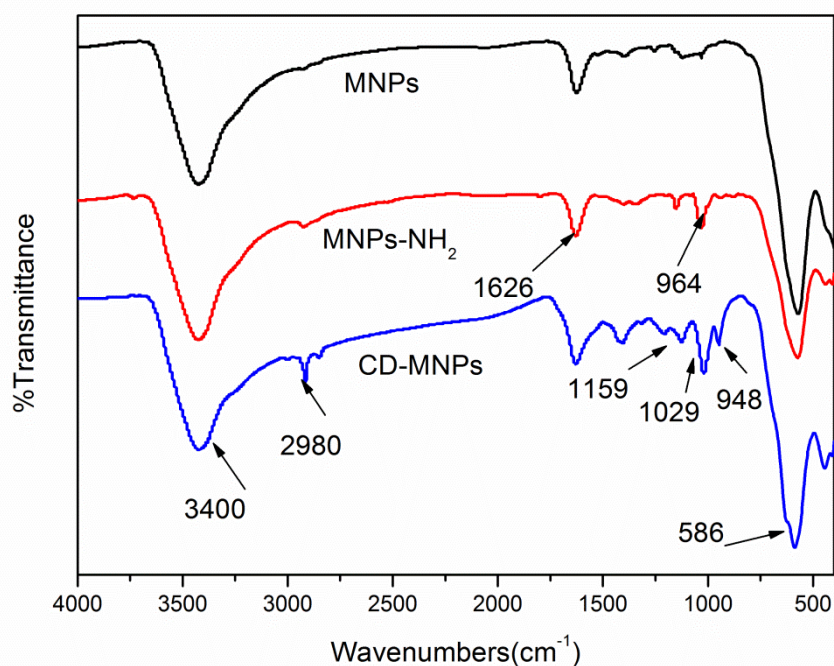


Fig. S1 FTIR spectra of Fe_3O_4 MNPs and $MNPs-NH_2$, CD-MNPs

Fig. S1 shows the FT-IR spectra of MNP, MNP-NH₂ and CD-MNPs. For MNPs, the characteristic absorption peak at around 586 cm⁻¹, corresponding to the Fe-O bonds in tetrahedral sites of bare magnetic nanoparticles. The broad band at 3300~3450 cm⁻¹ is assigned to the -OH stretching vibration arising from surface hydroxyl groups on the nanoparticles. Compared with MNPs, MNP-NH₂ presents absorption peaks at 2924 and 2854 cm⁻¹ assigned to stretching vibration of C-H bond of the propyl amine group². In addition, the absorption peaks around 964 and 1110 cm⁻¹ are attributed to the Si-O-H and Si-O-Si groups, confirming the modification of APTES to MNPs. And in the spectrum of CD-MNPs, the three characteristic peaks at 948 cm⁻¹, 1029 cm⁻¹ and 1159 cm⁻¹ can be observed due to immobilized β-CD on the surface of MNPs³. These results indicated that each step of modification was successfully done.

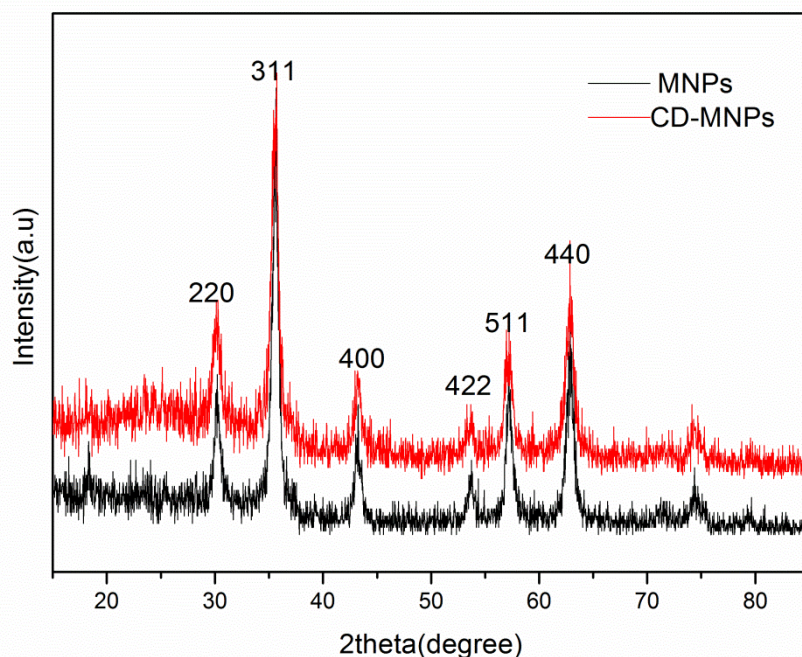


Fig. S2 XRD patterns of MNPs and CD-MNPs.

Fig. S2 presents the XRD diffraction patterns of the prepared MNPs and CD-MNPs. The XRD patterns of the particles show six characteristic peaks ($2\theta = 30.1, 35.5, 43.1, 53.4, 57.0, 63.1$), these are related to their corresponding indices (220), (311), (400), (422), (511) and (440), respectively^{2,4}. It is implied

that the resultant nanoparticles are pure Fe_3O_4 with reveal a cubic iron oxide phase a spinel structure and the modifying process did not change any phase change of Fe_3O_4 .

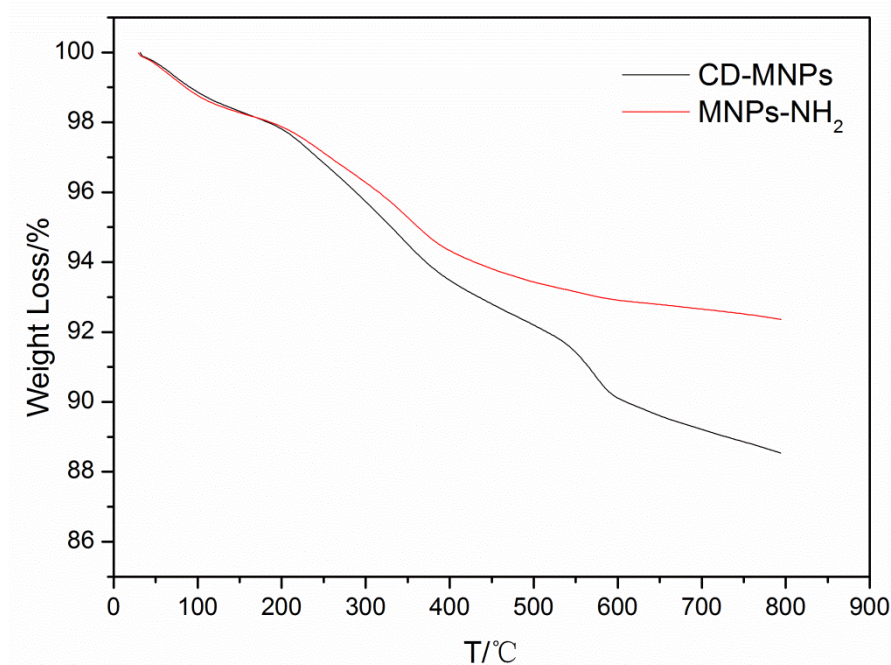


Fig. S3 TGA spectrums of MNPs-NH₂ and CD-MNPs

To estimate the amount of β -CD covered on MNPs, thermo gravimetric analyses of MNPs-NH₂ and CD-MNPs were investigated. As illustrated in Fig.S3, for the TGA of MNPs-NH₂, the weight loss (5.1 wt%) in the range of 200-650°C is contributed from the thermal decomposition of APTES⁴. Compared with that of the MNPs-NH₂, the weight loss of CD-MNPs was 11.0 wt% in the range of 200-650°C. The increase of the weight loss is ascribed to the β -CD covered on the MNPs. Thus, The TGA curves also confirmed the successful grafting of β -CD molecules onto the magnetic surface.

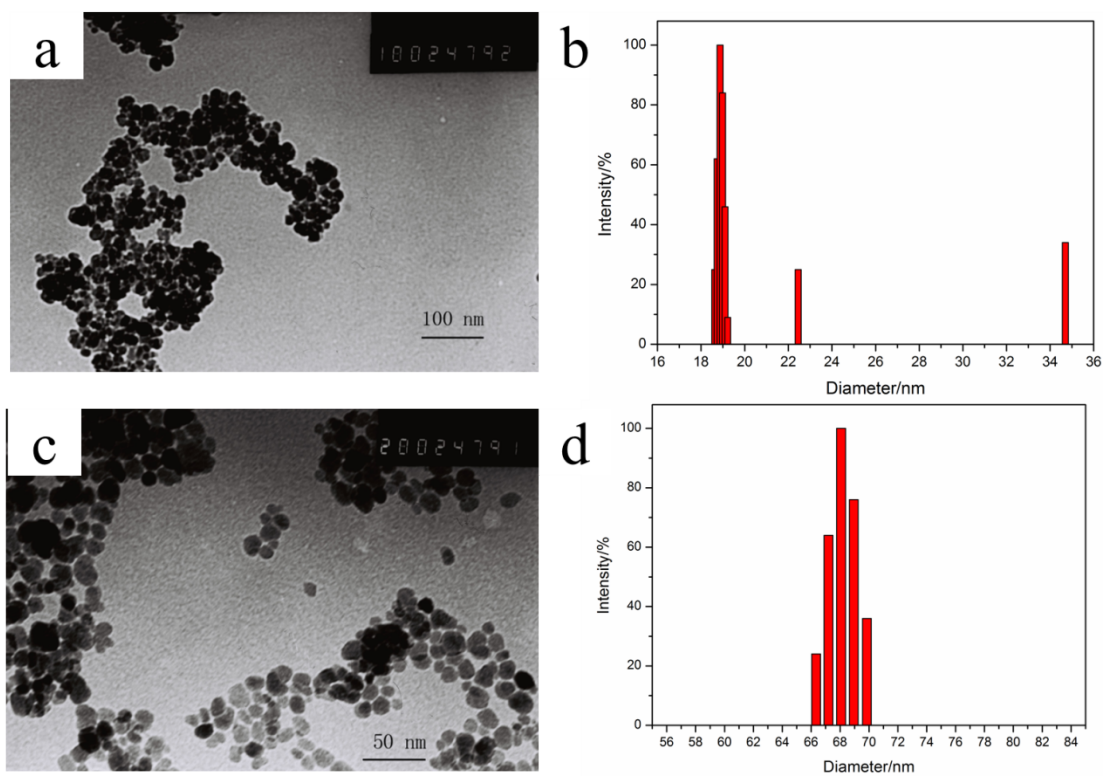


Fig. S4 TEM images of (a) MNPs, (c) CD -MNPs and dynamic light scattering (DLS) of (b) MNPs, (d) CD-MNPs.

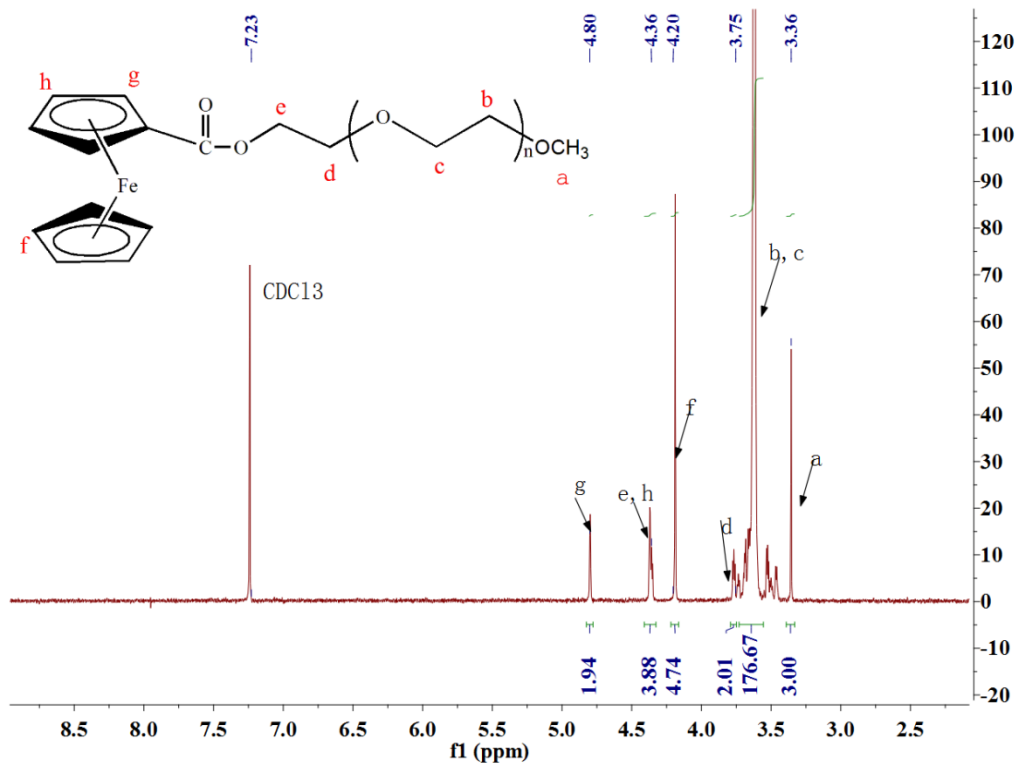


Fig. S5 ^1H -NMR spectra recorded in CDCl_3 for ferrocene-terminated PEG.

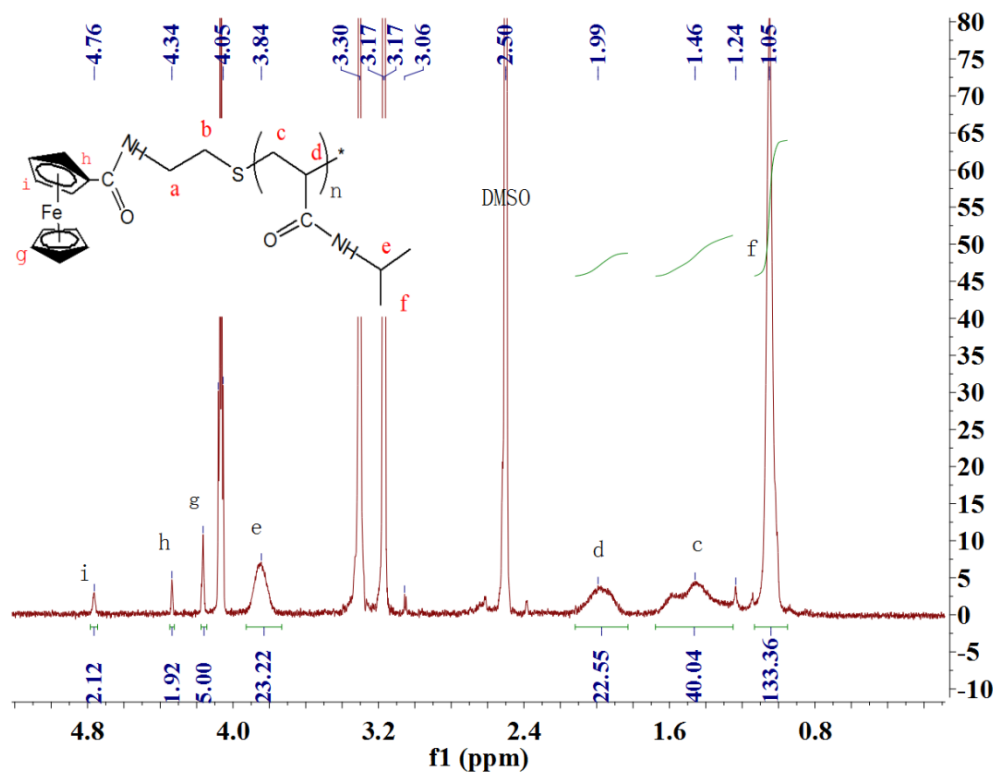


Fig. S6 ¹H -NMR spectra recorded in DMSO for ferrocene-terminated PNIPAM.

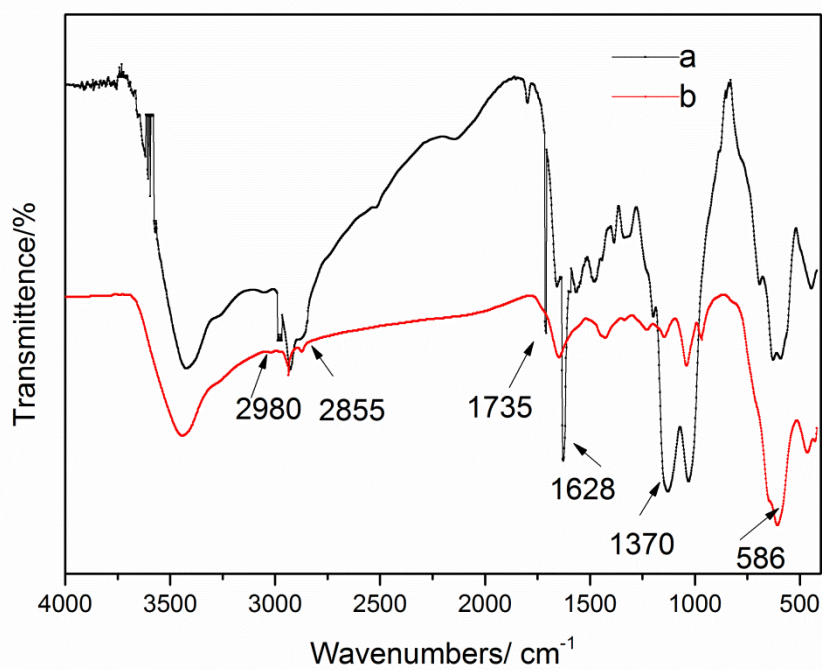


Fig. S7 FTIR spectra of PEG/PNIPAM/CD-MNPs (a) and CD-MNPs (b)

Fig. S7 represented the FT-IR spectra of PEG/PNIPAM/CD-MNPs(a) and CD-MNPs(b). Comparing with Fig. S7b, there appeared many new peaks, the

characteristic absorption peak at around 1736 cm^{-1} , corresponding to the C=O bonds in the PEG-Fc. Absorption peaks 1370 cm^{-1} are assigned to stretching vibration of C-H bond of the isopropyl group on the PNIPAM repetitive units, as shown in Fig. S7a, while the absorption peaks at 2924 and 2854 cm^{-1} assigned to stretching vibration of C-H bond of became stronger. All the results indicated that the polymer PEG and PNIPAM was successfully attached on the surface of CD-MNPs.

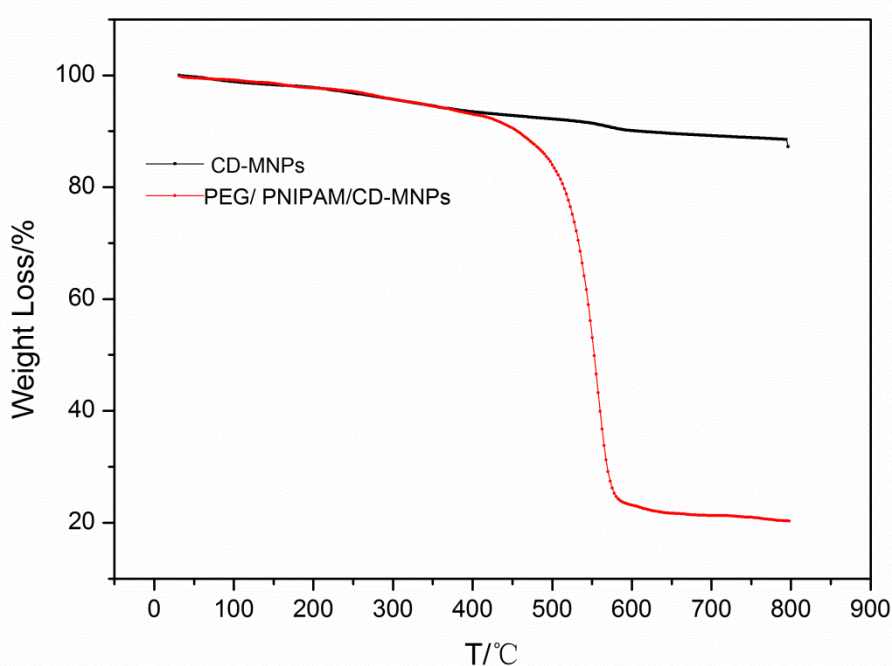


Fig. S8 TGA data of CD-MNPs and PEG/PNIPAM/CD-MNPs

Thermo gravimetric analyses of CD-MNPs and PEG/PNIPAM/CD-MNPs were investigated to estimate the amount of polymers attached on CD-MNPs. As illustrated in Fig.S8, the obvious increase of the weight loss (60%) is attributed to the polymers attached on CD-MNPs. Thus, The TGA curves also confirmed the successful grafting of polymers onto the magnetic surface.

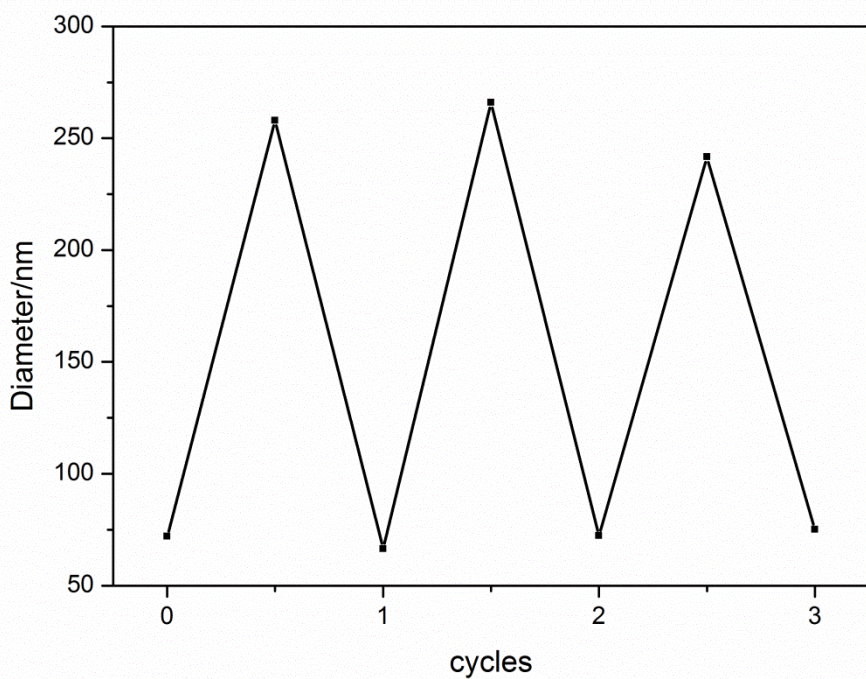


Fig. S9 Three repeated diameter of the PEG/PNIPAM/CD-MNPs in aqueous media after changing the temperature from 25 to 37 °C

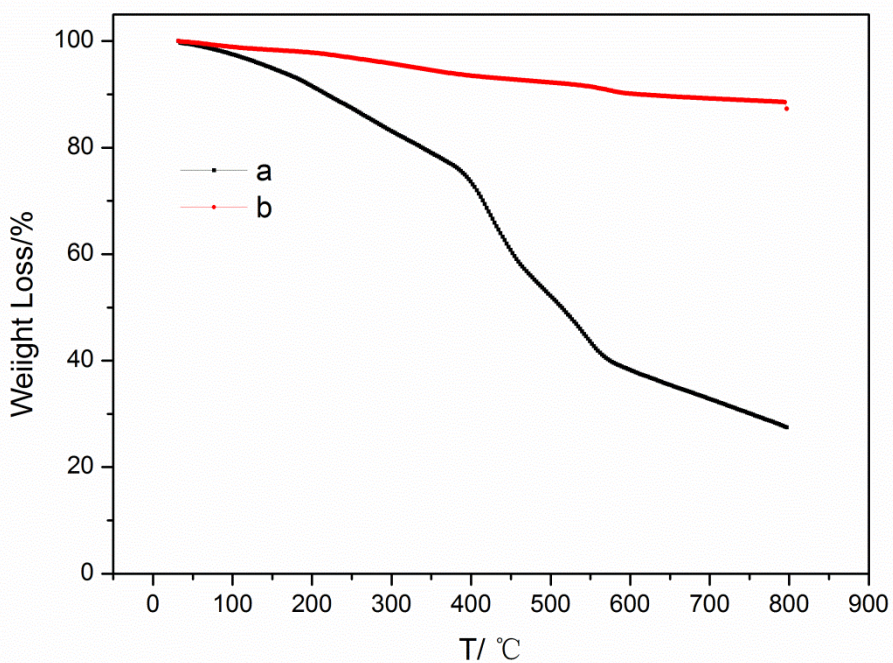


Fig. S10 TGA data of micellar aggregates in the absence of H₂O₂ (a) and in the presence of H₂O₂ (b)

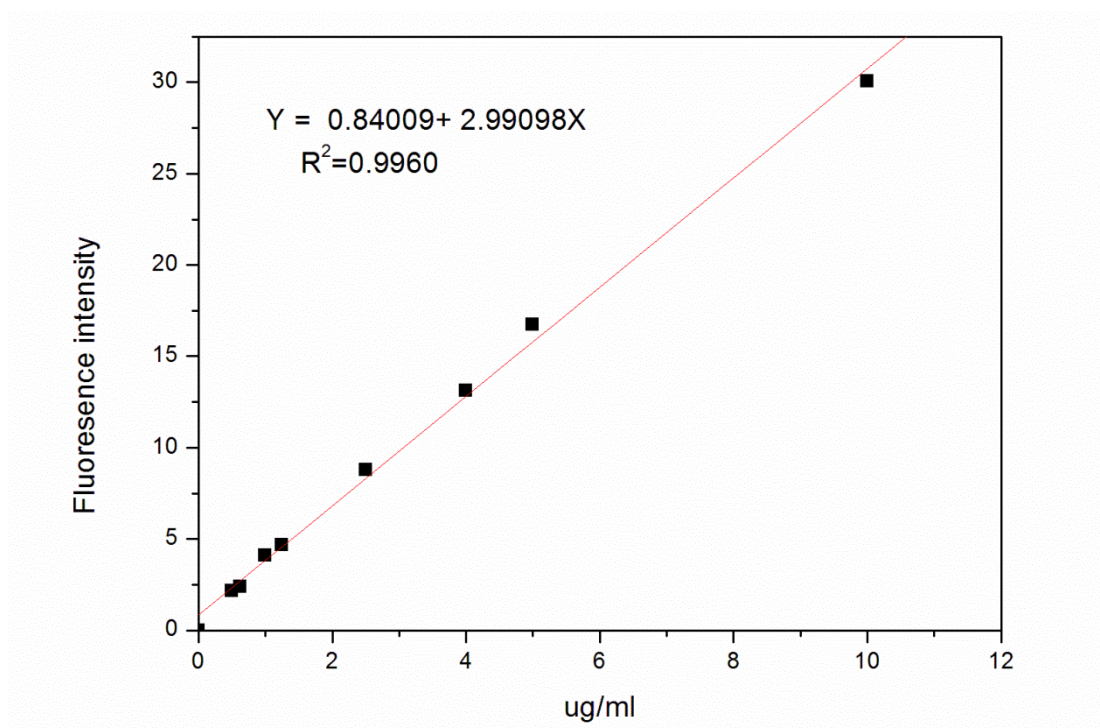


Fig. S11 Standard curve plotted by the ΔA_{485nm} of the standard versus the DOX concentration.

Reference

- 1 R. Massart, *IEEE Trans. Magn.*, 1981, **17**, 1247-1248.
- 2 X. Q. Liu, Z. Y. Ma, Xing J.M., *J. Magn. Magn. Mater.*, 2004, **270**, 1-6.
- 3 A.Z.M. Badruddoza, K. Hidajat, M.S. Uddin, *J. Colloid. Interf. Sci.*, 2010, **346**, 337-346.
- 4 H. Wang, Y. Chen, X. Y. Li and Y. Liu, *Mole. Pharm.*, 2007, **4**, 189-198.