Electronic Supplementary Information for

Synergistic Regulation on Longitudinal and Transverse Relaxivity of Extremely-Small Iron Oxide Nanoparticles (ESIONPs) Using pH-Responsive Nanoassemblies

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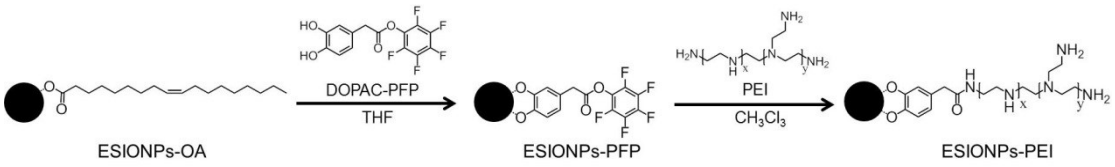

Figure S1. Synthetic route of ESIONPs-PEI.

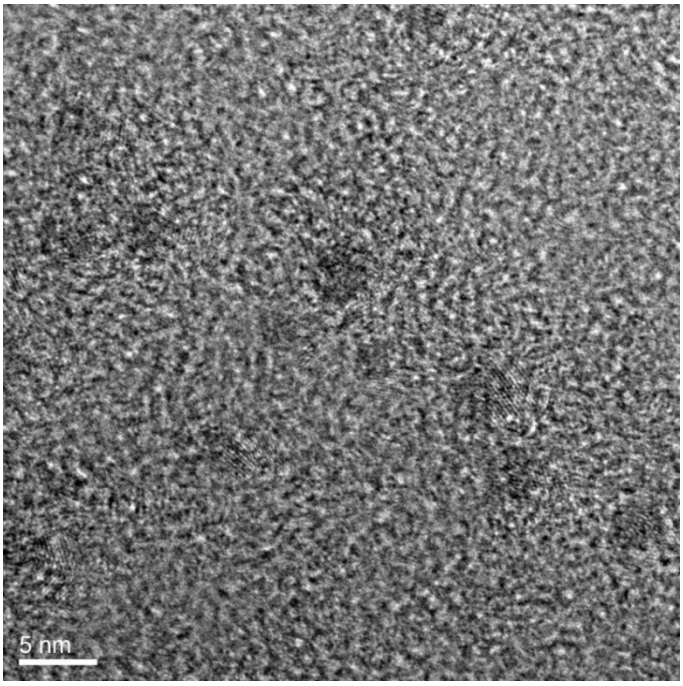

Figure S2. TEM morphology of ESIONPs-OA prepared through the thermal decomposition method.

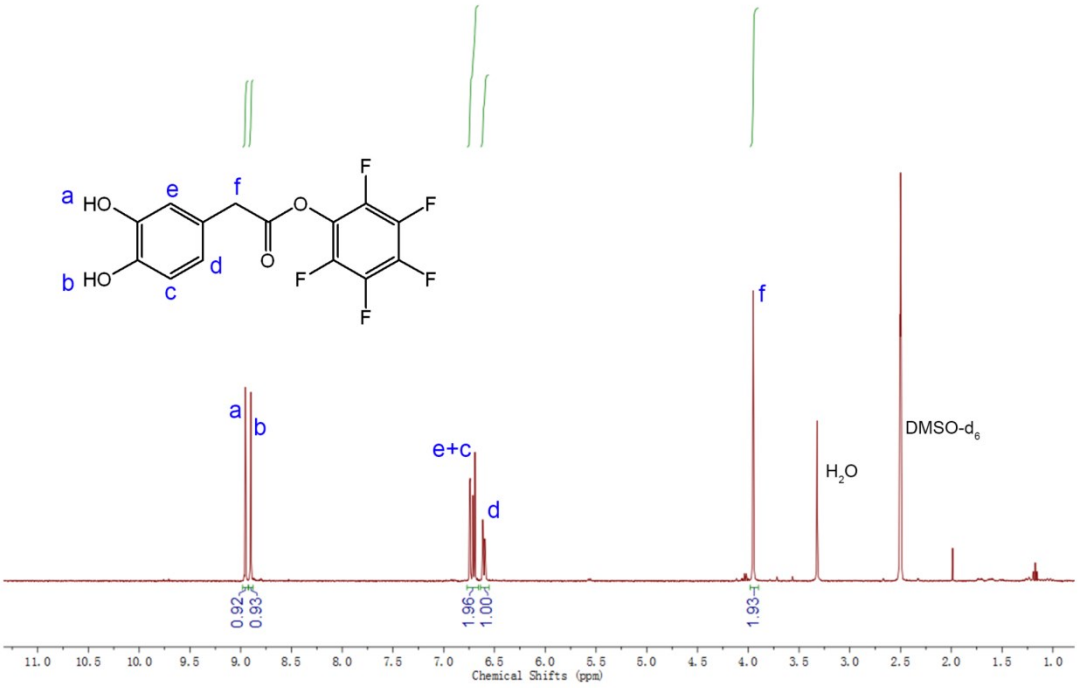

Figure S3. ^{1}H NMR spectrum of DOPAC-PFP (recorded in DMSO-d_6).

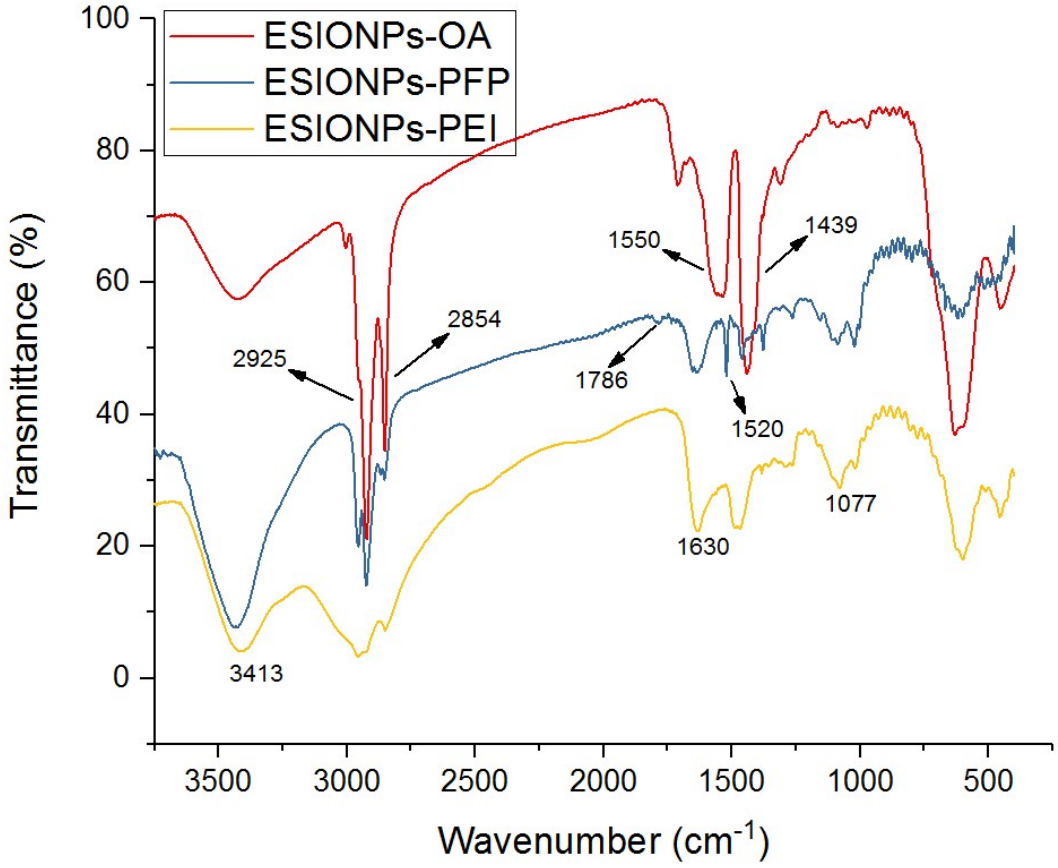

Figure S4. FT-IR spectra of ESIONPs-OA, ESIONPs-PFP and ESIONPs-PEI. For ESIONPs-OA, the bands at 2925 cm^{-1} and 2854 cm^{-1} are attributed to the C-H variations of methyl and methylene group in the oleic acid molecule, and the bands at 1550 cm^{-1} and 1439 cm^{-1} are due to the variations of surface complex carbonyl ($-COO^-$). In ESIONPs-PFP, the band at 1786 cm^{-1} is the characteristic of C=O bond in the active pentafluorophenyl ester, and the band at 1520 cm^{-1} is assigned to the C-F bond in the pentafluorophenyl group. For ESIONPs-PEI, the bands at 3413 cm-1 and 1640 cm-1 are attributed to the variations of N-H bond in the PEI molecule, and the band at 1077 cm^{-1} is due to the vibration of C-N bond in the PEI molecule.

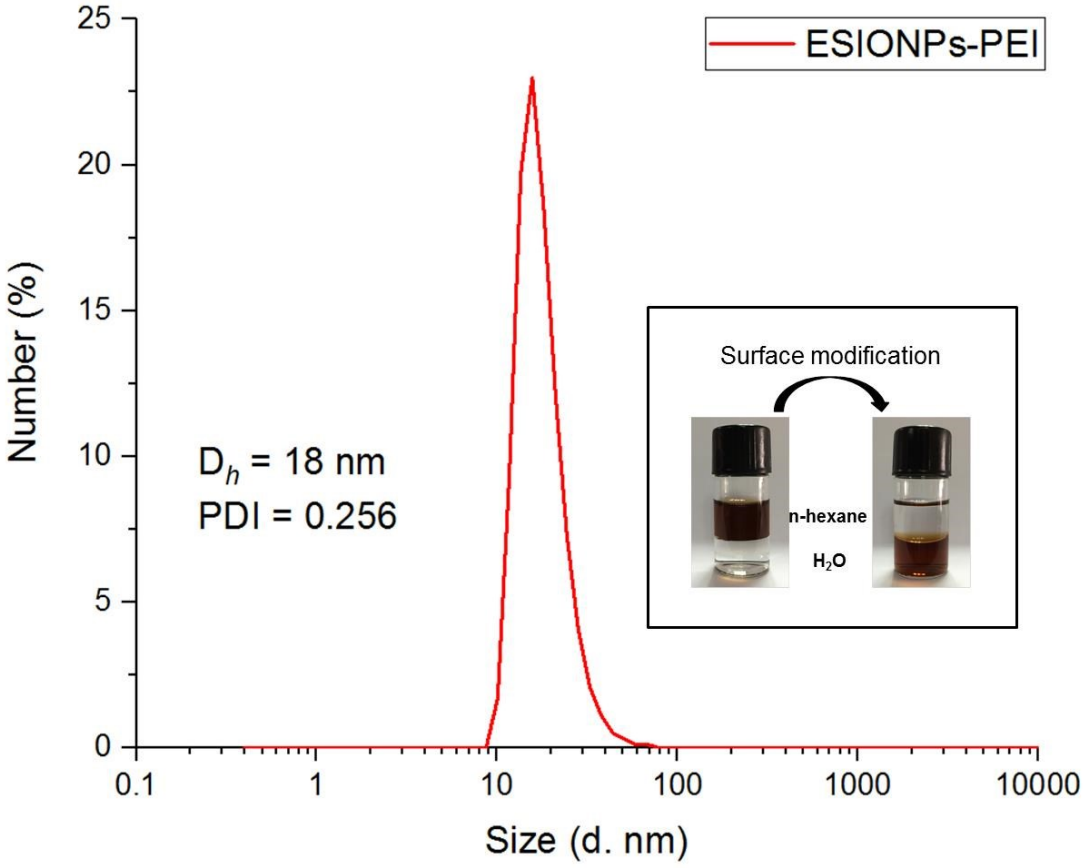

Figure S5. Hydrodynamic size of ESIONPs-PEI. The insert photograph shows the successful transfer of ESIONPs from n-hexane to water phase after PEI modification.

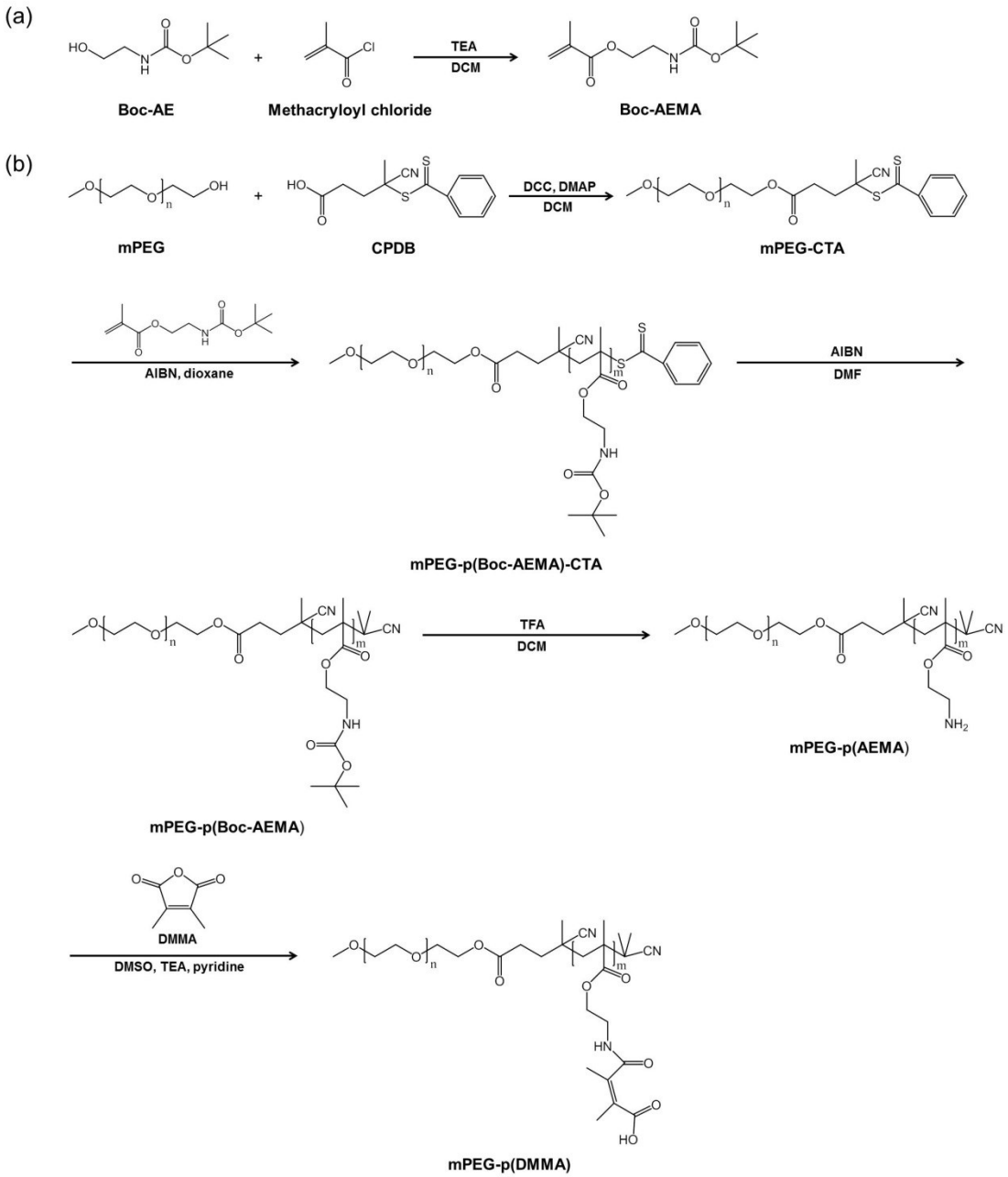

Figure S6. Synthetic routes for (a) Boc-AEMA and (b) mPEG-p(DMMA).

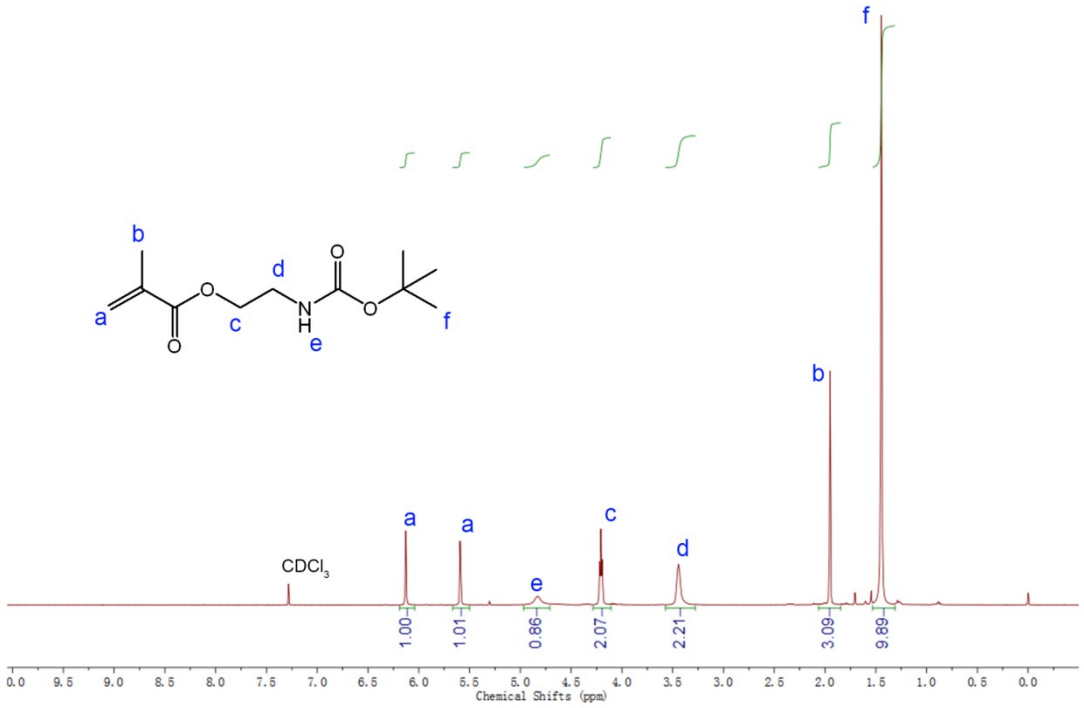

Figure S7. ^{1}H NMR spectrum of Boc-AEMA (recorded in $CDCl_3$).

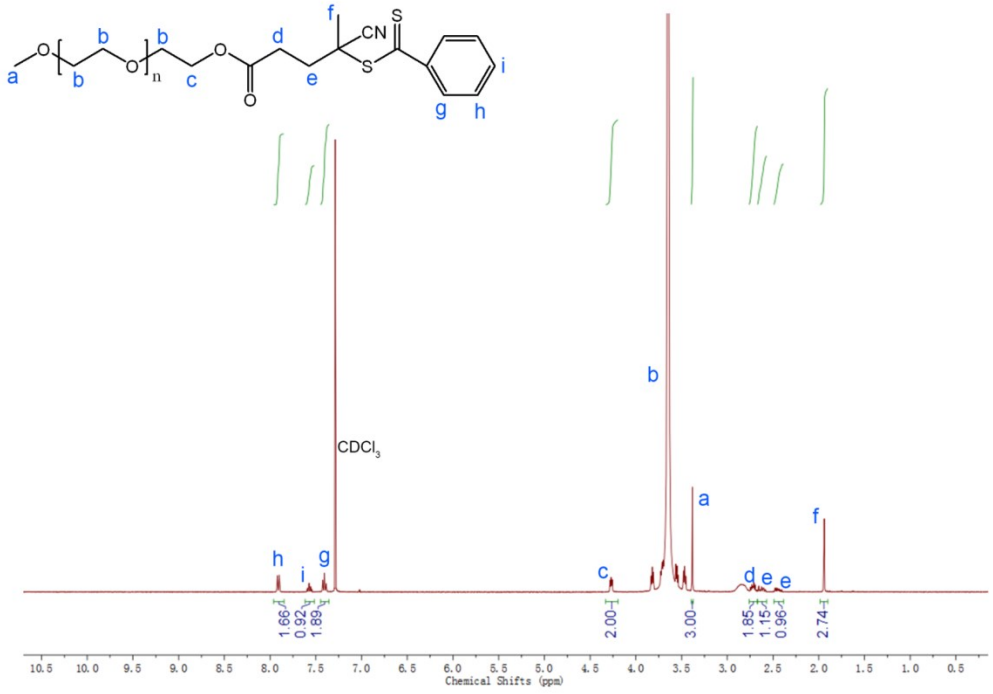

Figure S8. ^{1}H NMR spectrum of mPEG-CTA (recorded in $CDCl_3$).

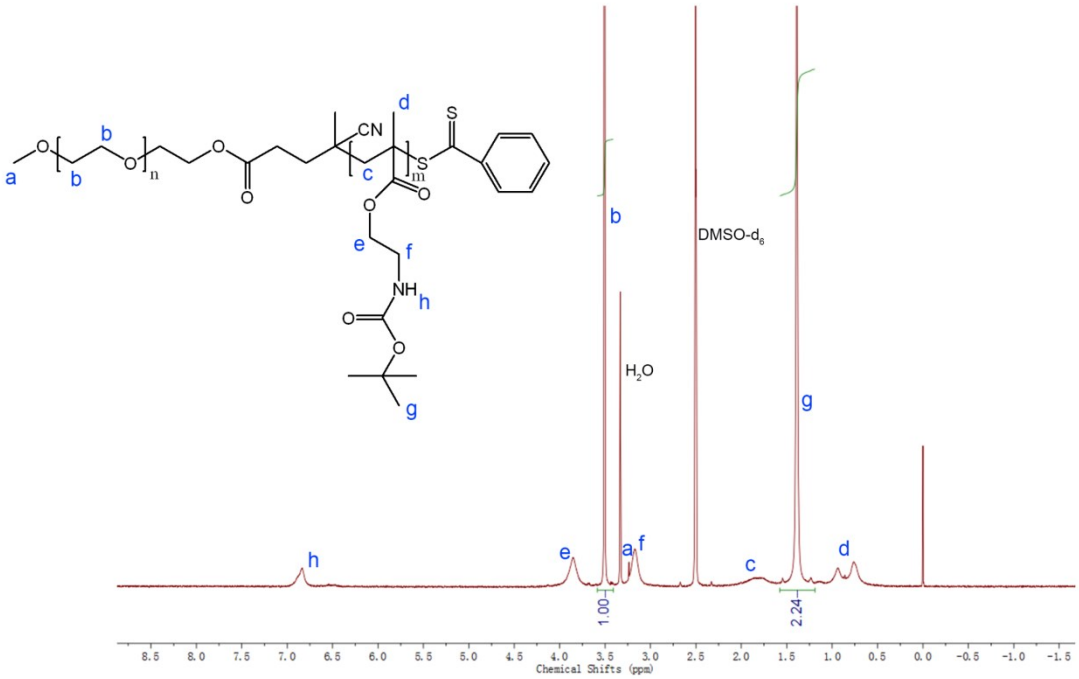

Figure S9. ^{1}H NMR spectrum of mPEG-p(Boc-AEMA)-CTA (recorded in DMSO-d_6).

The polymerization degree (m) of the Boc-AEMA is calculated according to the areas of peak (b) and peak (g), and the calculation formula is shown in Equation 1, which turns out to be 112. Therefore, the molecular weight of mPEG-p(Boc-AEMA)-CTA calculated by ($M_{n,NMR}$) is 30909 g/mol.

$$m = (113*4*A_g)/(9*A_b) \text{ (Equation 1)}$$

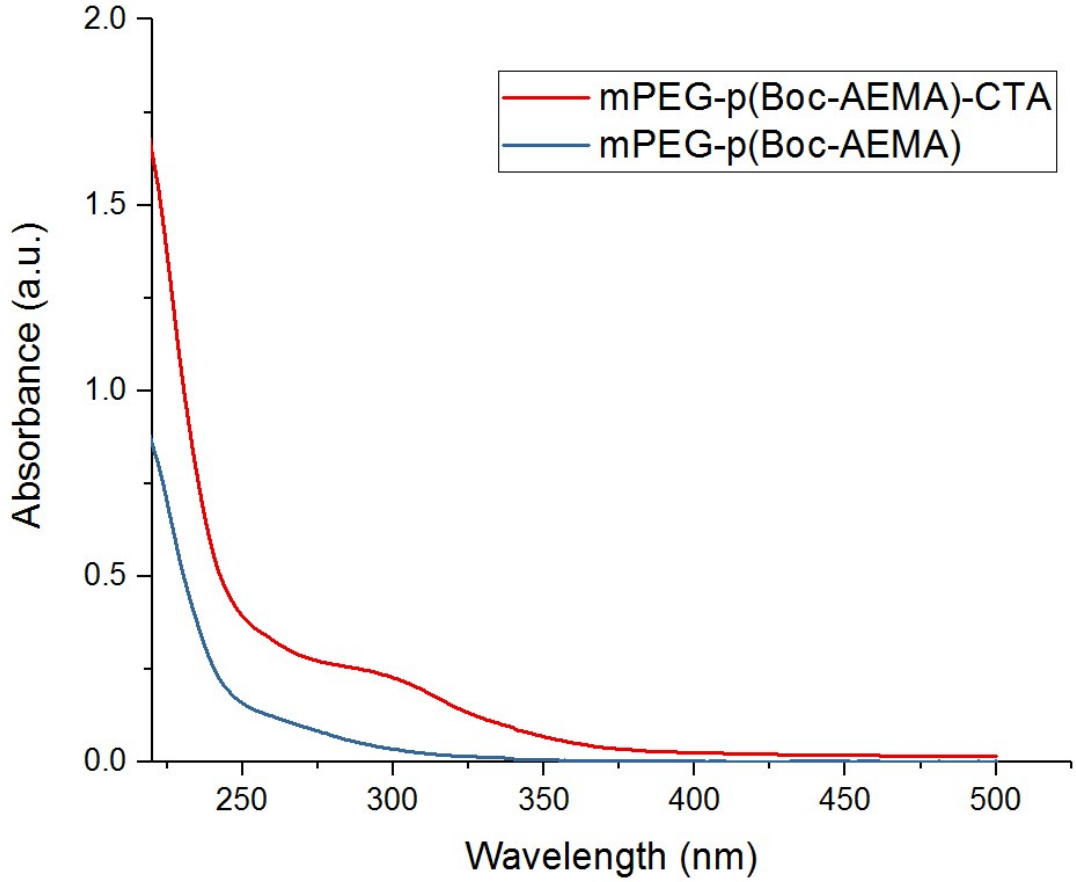

Figure S10. UV-Vis spectra of mPEG-p(Boc-AEMA)-CTA and mPEG-p(Boc-AEMA).

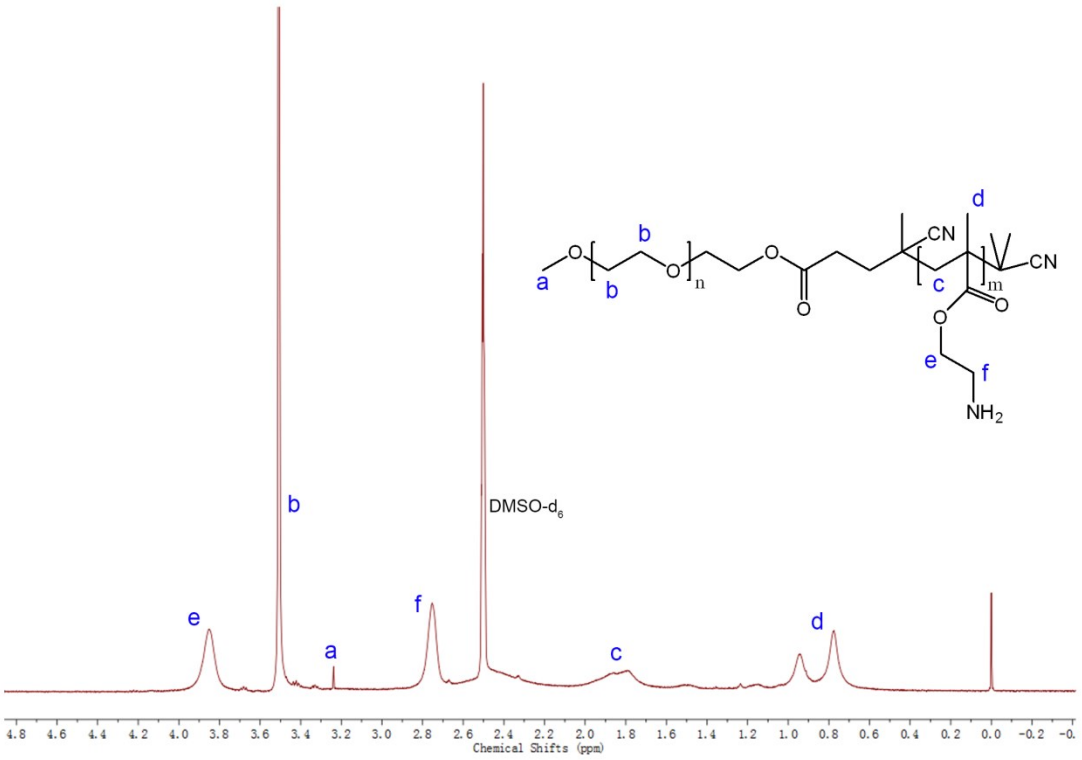

Figure S11. ^{1}H NMR spectrum of mPEG-p(AEMA) (recorded in DMSO-d_6).

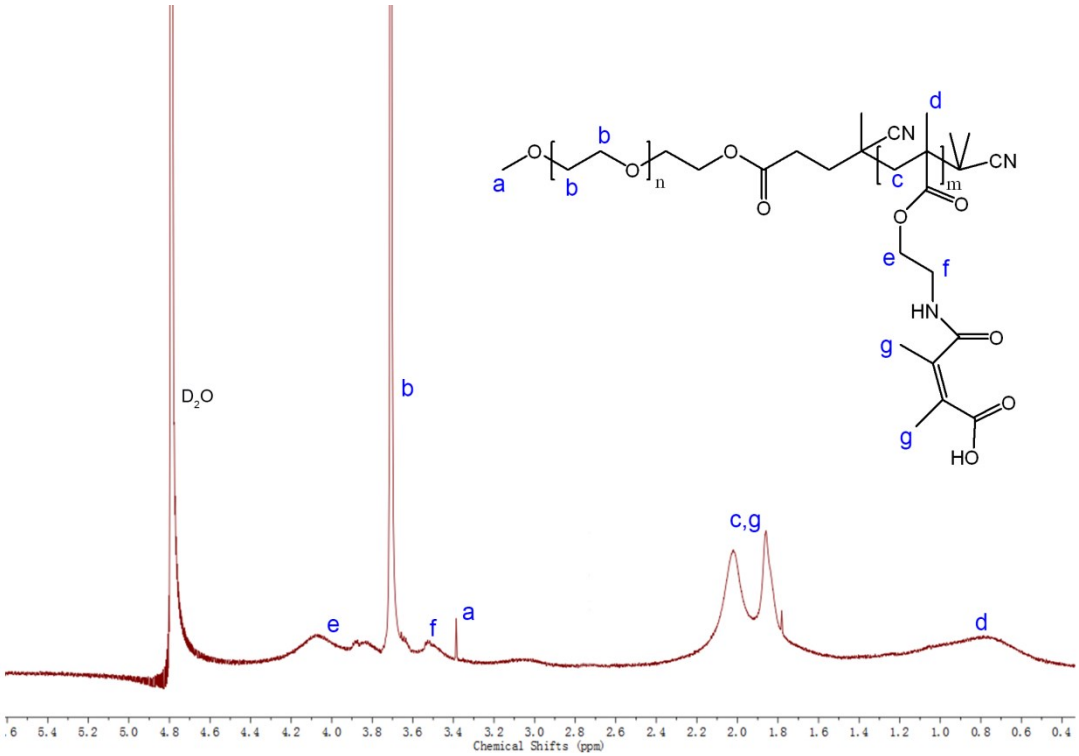

Figure S12. ^{1}H NMR spectrum of mPEG-p(DMMA) (recorded in D_2O).

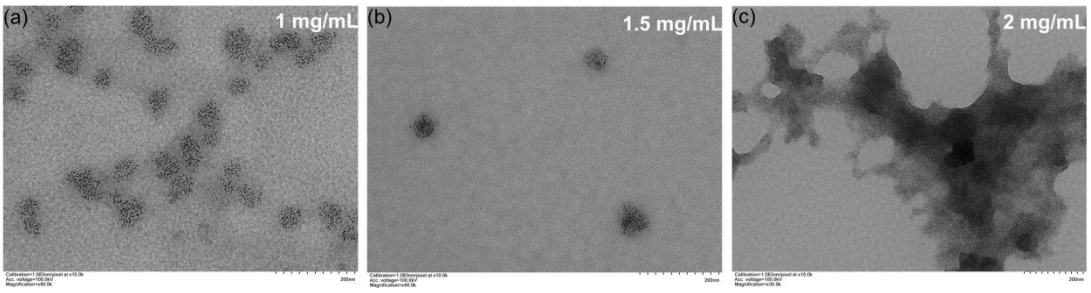

Figure S13. TEM images of ESIONPs micelle prepared with various concentrations of ESIONPs-PEI (1 mg/mL, 1.5 mg/mL and 2 mg/mL) together with 1 mg/mL mPEG-p(DMMA). (a) When the concentration ratio is low (ESIONPs-PEI = 1 mg/mL, mPEG-p(DMMA) = 1 mg/mL), irregular nanoclusters with the loosen structure form. (b) At the situation that the ratio is moderate (ESIONPs-PEI = 1.5 mg/mL, mPEG-p(DMMA) = 1 mg/mL), uniform spherical nanoclusters are achieved and this process is very efficient. (c) When the ratio goes to a high level (ESIONPs-PEI = 2 mg/mL, mPEG-p(DMMA) = 1 mg/mL), the aggregation of nanoclusters appears.

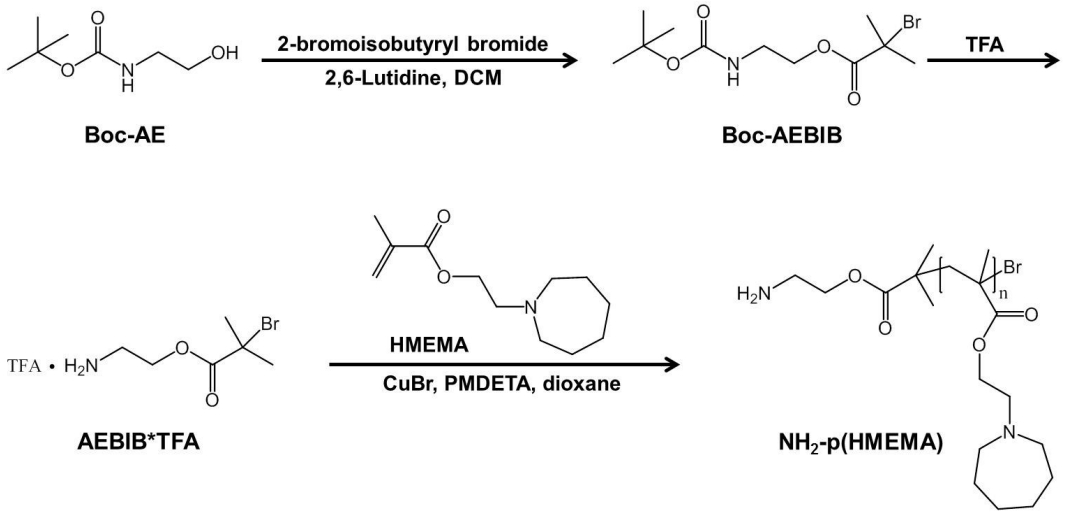

Figure S14. Synthetic route for NH_2-p(HMEMA).

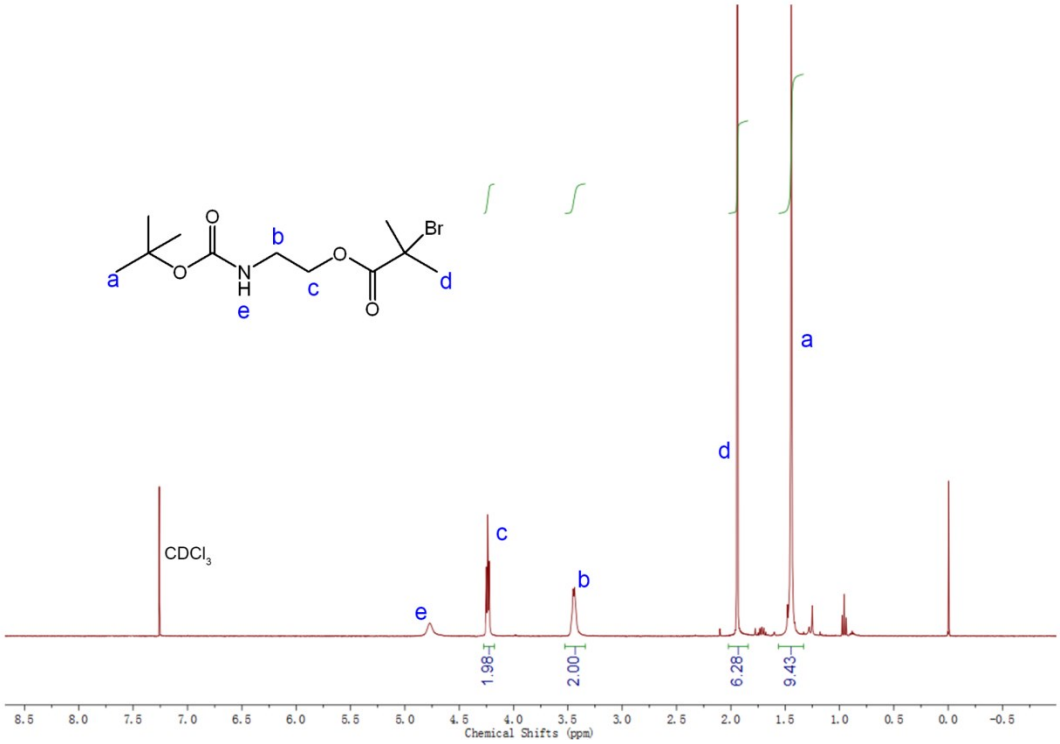

Figure S15. ^{1}H NMR spectrum of Boc-AEBIB (recorded in $CDCl_3$).

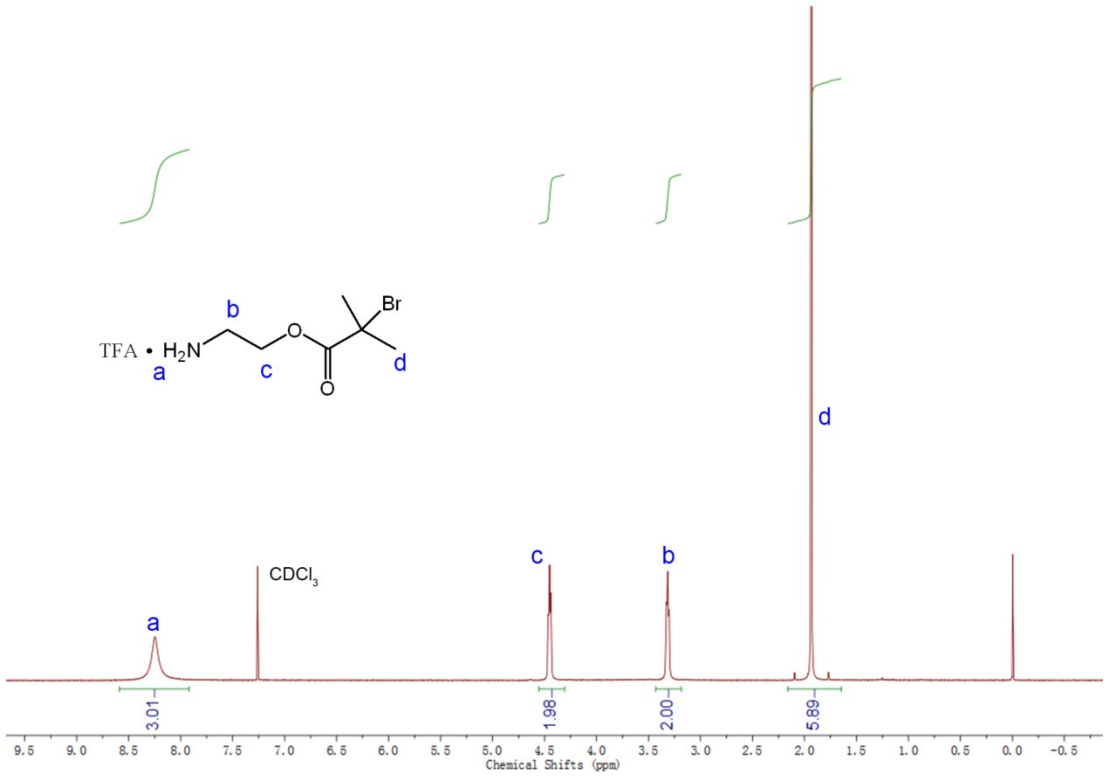

Figure S16. ^{1}H NMR spectrum of AEBIB*TFA (recorded in $CDCl_3$).

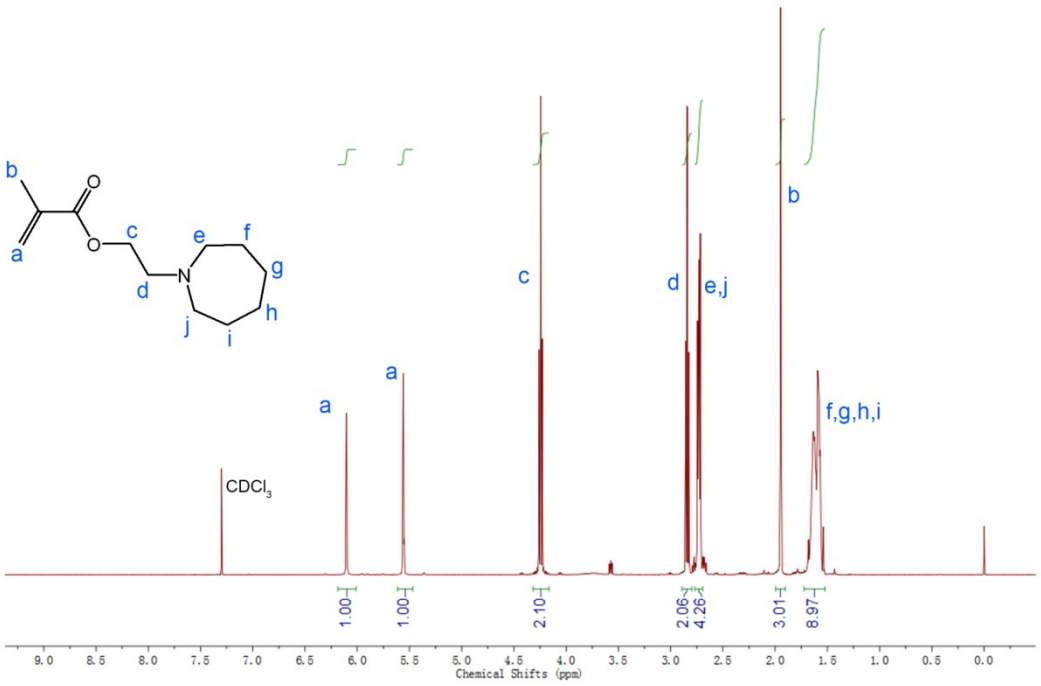

Figure S17. ^{1}H NMR spectrum of HMEMA (recorded in $CDCl_3$).

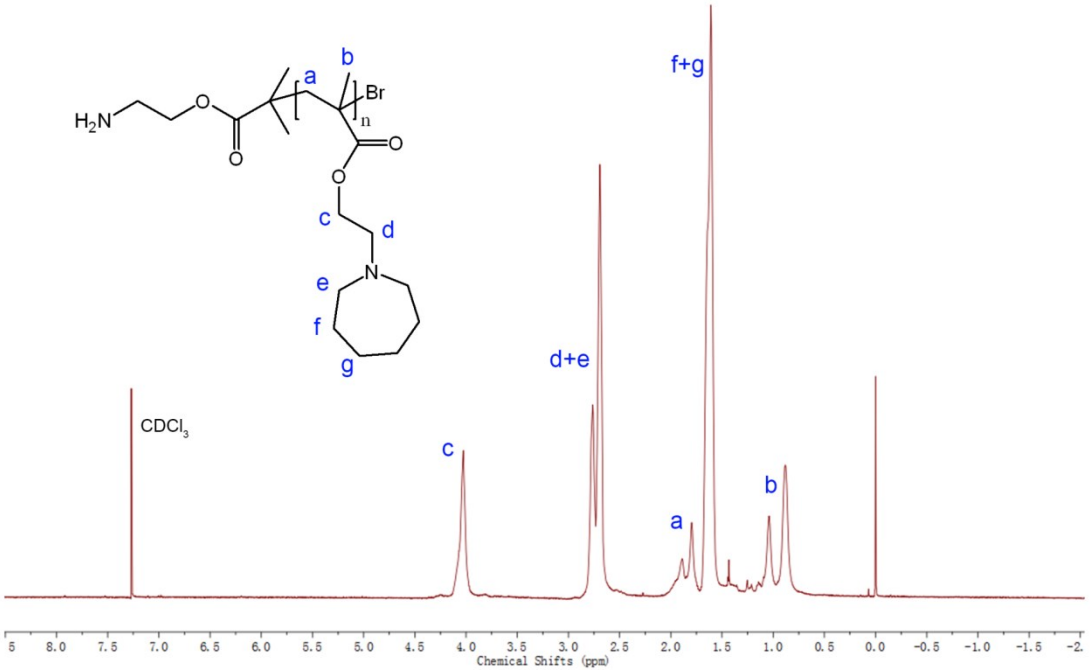

Figure S18. ^{1}H NMR spectrum of NH_2-p(HMEMA) (recorded in $CDCl_3$).

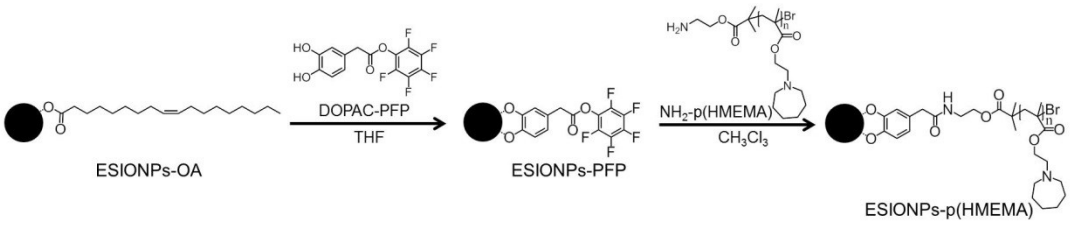

Figure S19. Synthetic route for ESIONPs-p(HMEMA).

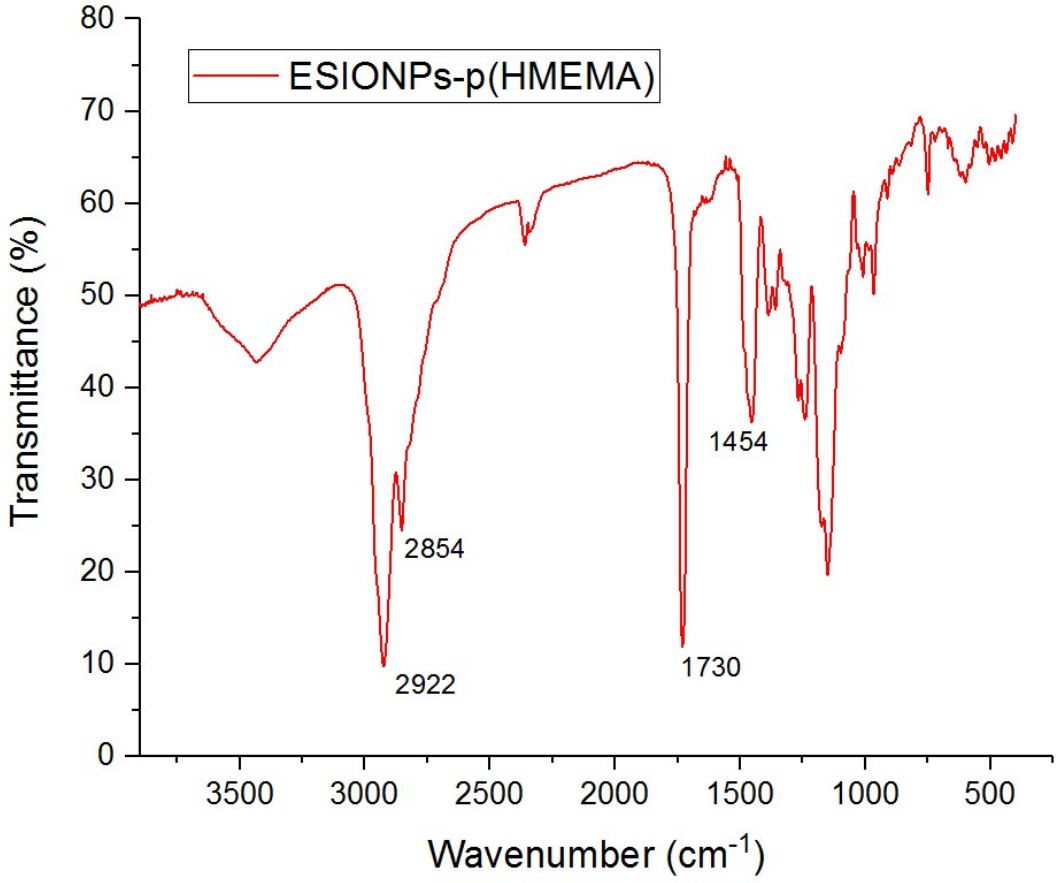

Figure S20. FT-IR spectrum of ESIONPs-p(HMEMA). The bands at 2922 cm^{-1}, 2854 cm^{-1} and 1454 cm^{-1} are assigned to the variations of C-H in the methylene group. The band at 1730 cm^{-1} is ascribed to C=O bond.

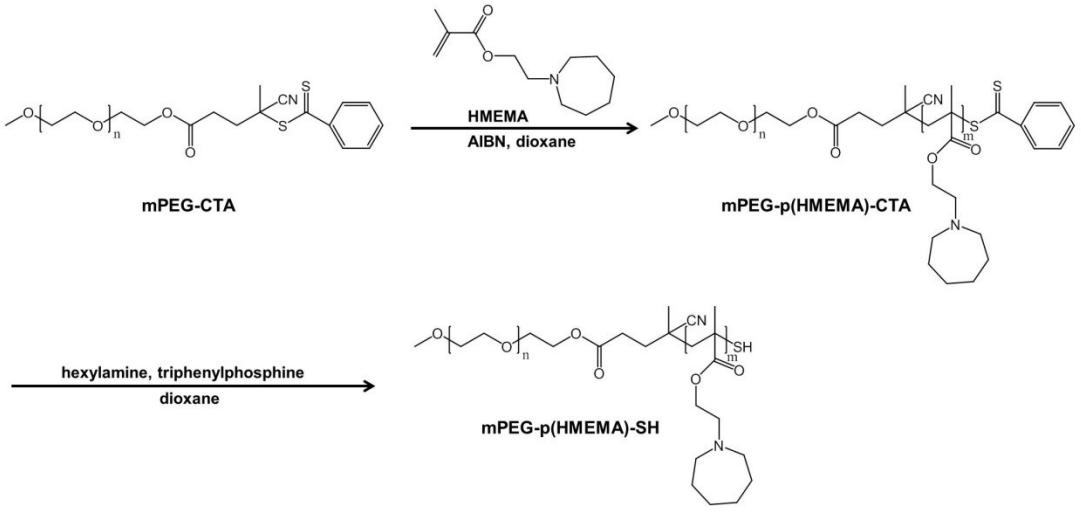

Figure S21. Synthetic route for mPEG-p(HMEMA)-SH.

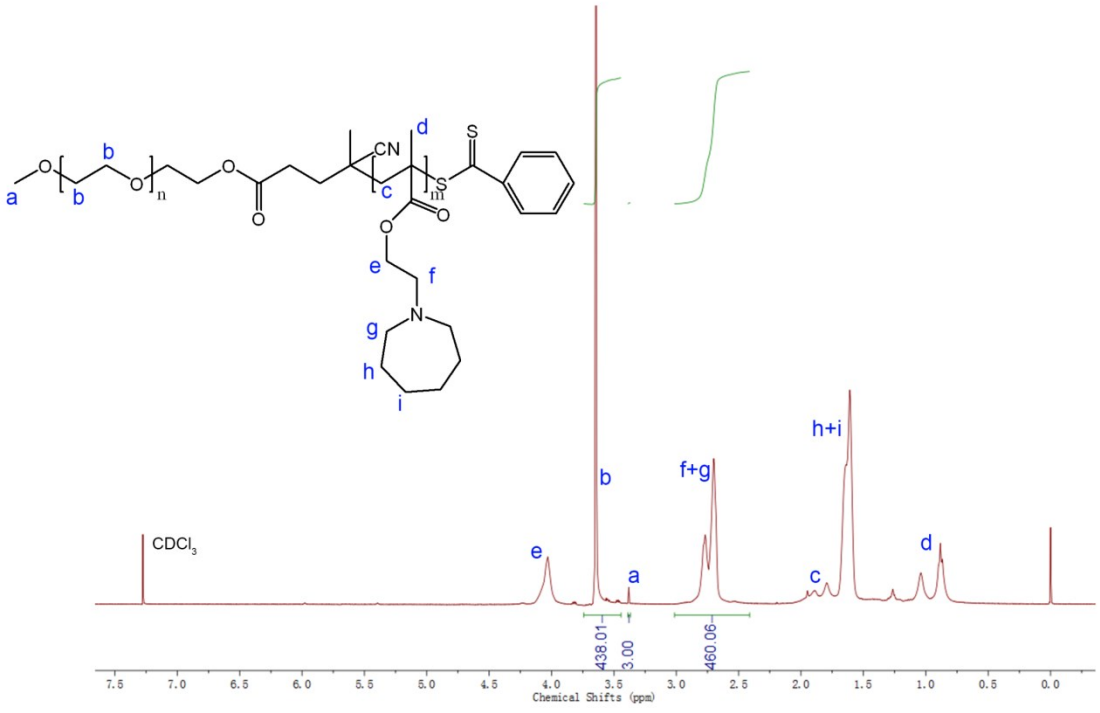

Figure S22. ^{1}H NMR spectrum of mPEG-p(HMEMA)-CTA (recorded in $CDCl_3$).

The polymerization degree (m) of the HMEMA is calculated according to the areas of peak (a) and peak (f+g), and the calculation formula is shown in Equation 2, which turns out to be 115. Therefore, the molecular weight of mPEG-p(HMEMA)-CTA

calculated by ($M_{n,NMR}$) is 29526 g/mol.

$$m = (3*A_{f+g})/(4*A_a) \text{ (Equation 2)}$$

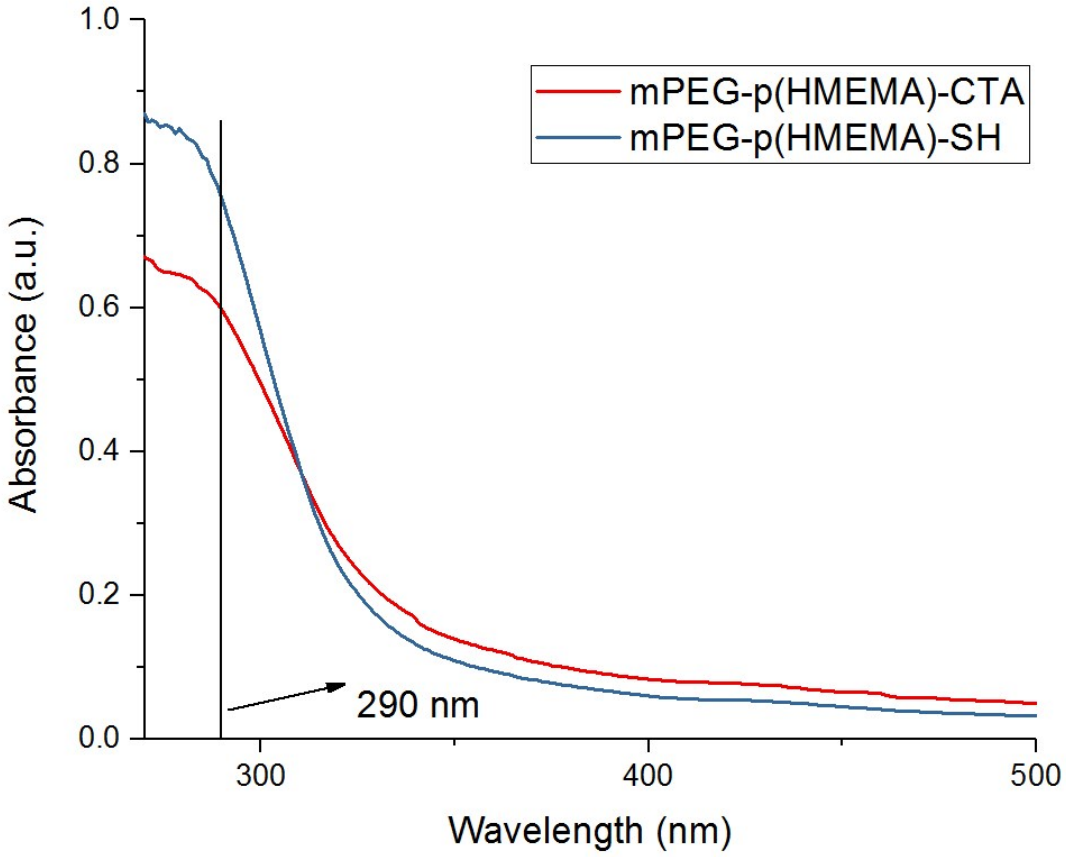

Figure S23. UV-Vis spectra of mPEG-p(HMEMA)-CTA and mPEG-p(HMEMA)-SH.

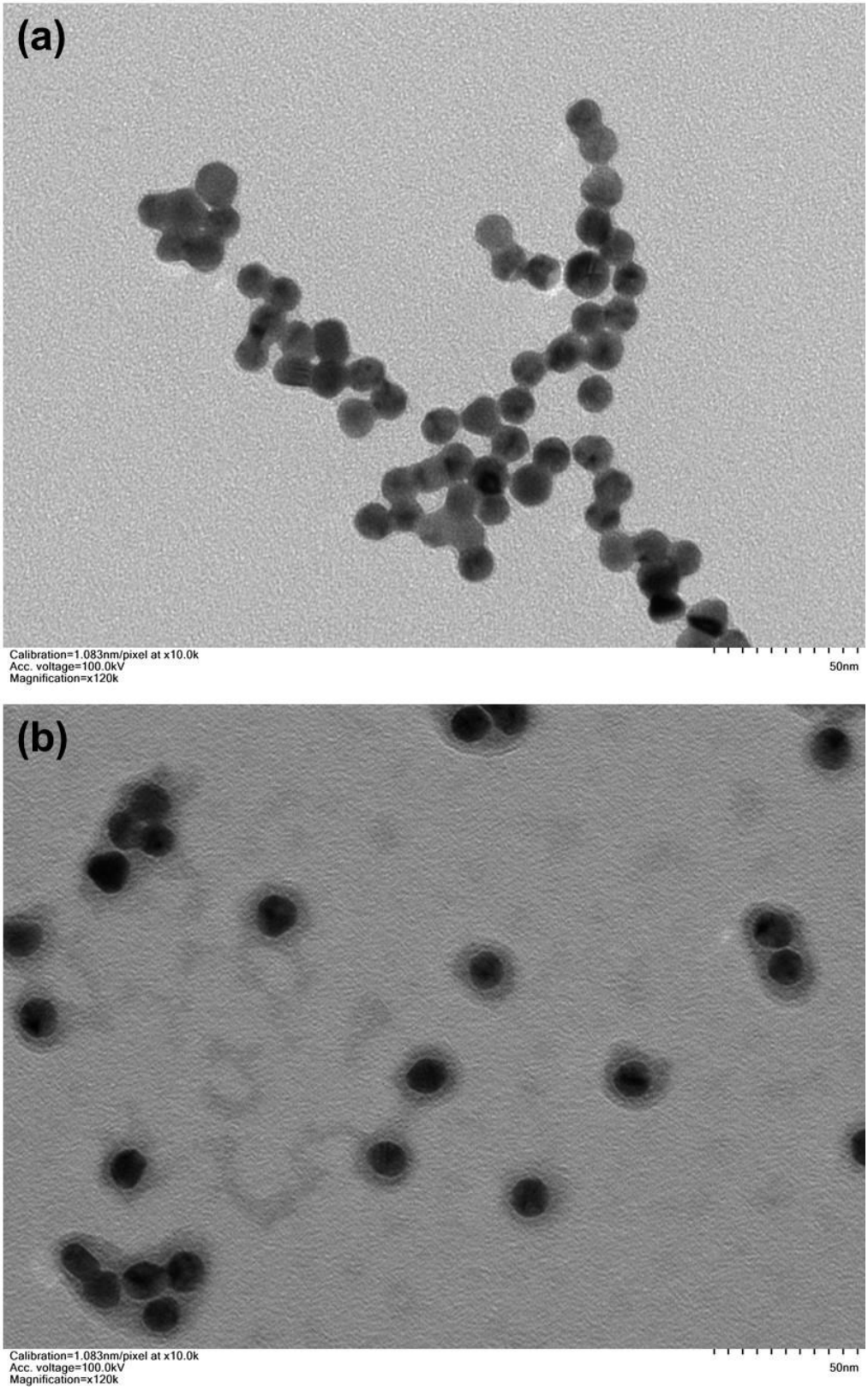

Figure S24. TEM morphology of (a) AuNPs-CA and (b) AuNPs-p(HMEMA)-mPEG (stained with the phosphotungstic acid).

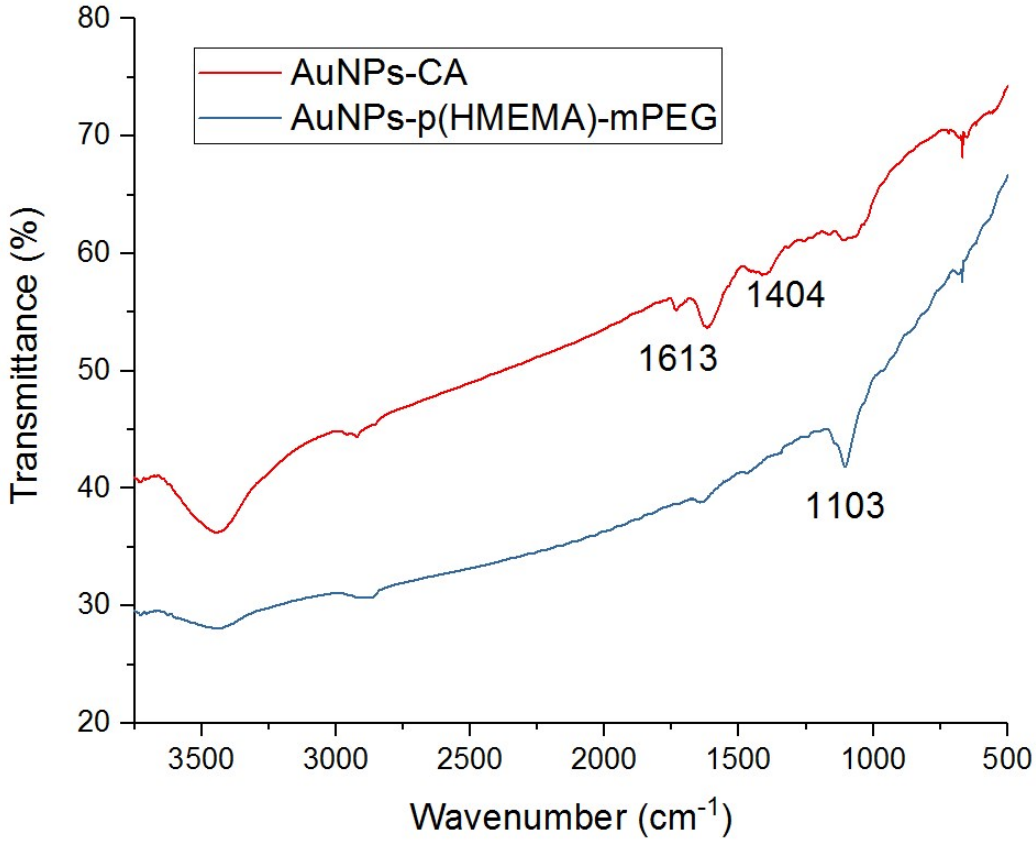

Figure S25. FT-IR spectra of AuNPs-CA and AuNPs-p(HMEMA)-mPEG. For AuNPs-CA, the bands at 1613 cm^{-1} and 1404 cm^{-1} are assigned to the vibration of C=O and C-O band in the citric acid molecule, respectively. For AuNPs-p(HMEMA)-mPEG, the band at 1103 cm^{-1} is the characteristic of C-O-C bond in the mPEG block.

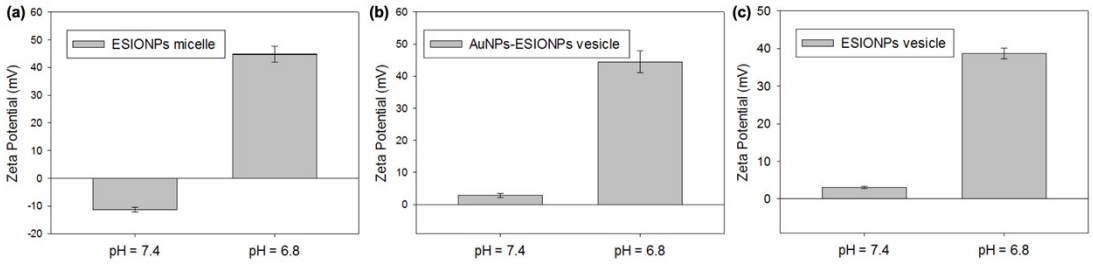

Figure S26. Zeta potentials of (a) ESIONPs micelle, (b) AuNPs-ESIONPs vesicle and (c) ESIONPs vesicle at pH = 7.4 and pH = 6.8.

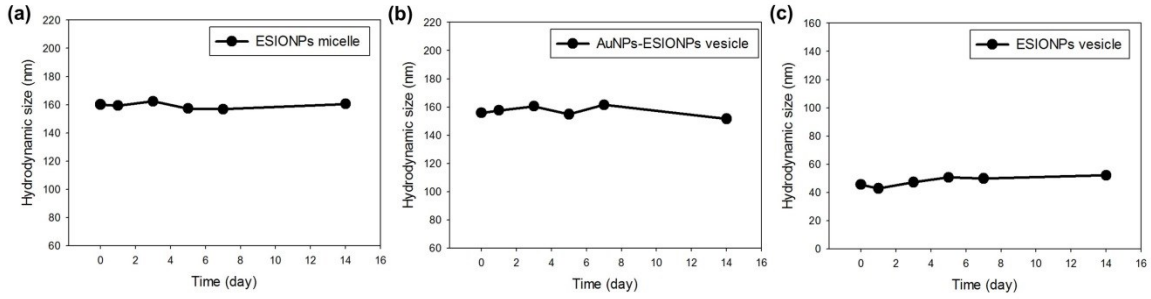

Figure S27. Variation of hydrodynamic size for (a) ESIONPs micelle, (b) AuNPs-ESIONPs vesicle and (c) ESIONPs vesicle in water in 14 days.

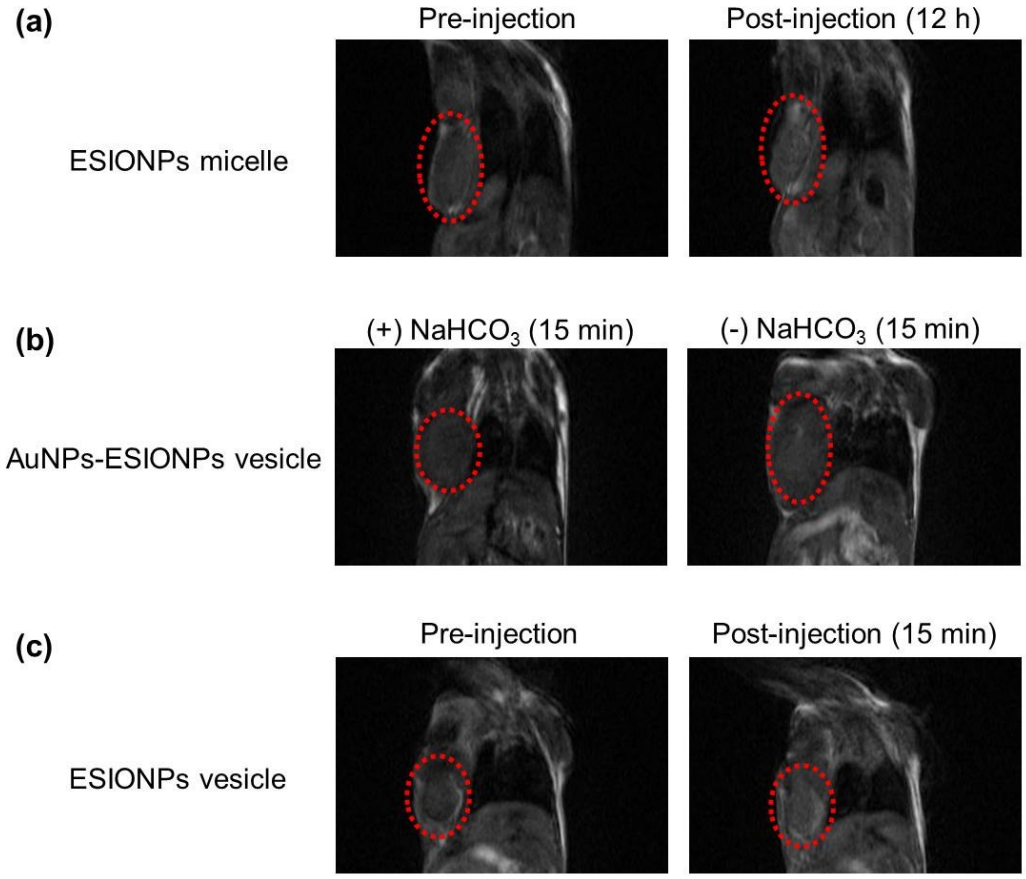

Figure S28. T_1-weighted MRI images of tumor-bearing mice (red dashed circles indicate tumor location). (a) Pre-injection and post-injection (12 h) of ESIONPs micelle through intratumoral injection with an iron dose of 0.0002 mmol. Obvious T_1 contrasting enhancement at the tumor location is observed after the injection. (b) With

and without neutralizing weakly acid microenvironment of tumors using $NaHCO_3$ before intratumoral injection of AuNPs-ESIONPs vesicle with an iron dose of 0.0002 mmol. The images were obtained at the time point of 15 min after the injection. Compared to the neutralized group, the amplification of T_1 contrasting enhancement is visible at the tumor location based on the pH-responsiveness in the unneutralized group. (c) Pre-injection and post-injection (15 min) of ESIONPs vesicle through intratumoral injection with an iron dose of 0.0002 mmol. T_1 contrasting enhancement is significant at the tumor location after the injection. (T_1 MRI images of tumor-bearing mice were acquired on a 1.5 T scanner using spin-echo sequence. The parameters for T_1 imaging were set as follows: TR/TE = 100 ms/14.12 ms, matrix = 512 × 256, FOV = 80 × 45 mm^2, slice thickness = 0.3 mm, 128 slices and no gap between slices.)

Table S1. Information of yield, quantity, quality and reproducibility for the organic molecules used in the present study.

sample	yield	quantity	quality	reproducibility
DOPAC-PFP	68%	2.5 g	good (^{1}H NMR)	good
Boc-AEMA	24%	2 g	good (^{1}H NMR)	good
HMEMA	80%	10 g	good (^{1}H NMR)	good
mPEG-CTA	66%	4.5 g	good (^{1}H NMR)	good
mPEG-p(Boc-AEMA)-CTA	71%	1.4 g	good (^{1}H NMR)	good
mPEG-p(Boc-AEMA)	80%	0.7 g	good (UV-Vis)	good
mPEG-(AEMA)	89%	350 mg	good (^{1}H NMR)	good
mPEG-(DMMA)	65%	110 mg	good (^{1}H NMR)	good
Boc-AEBIB	53%	8.2 g	good (^{1}H NMR)	good
AEBIB*TFA	65%	4 g	good (^{1}H NMR)	good
NH_2-p(HMEMA)	22%	390 mg	good (^{1}H NMR)	good
mPEG-p(HMEMA)-CTA	44%	2.1 g	good (^{1}H NMR)	good
mPEG-p(HMEMA)-SH	90%	450 mg	good (UV-Vis)	good