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Electronic Supplementary Information for：

Construction of Biodegradable Core Cross-Linked Nanoparticles from Near Infrared Dyes Encoded Polyprodrug Amphiphiles and Synergistic Anticancer Investigation

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Experimental Section

Instruments

The ^{1}H nuclear magnetic resonance (NMR) spectra were recorded using a Bruker 600 MHz spectrometer operated in the Fourier Transform mode. Chemical shifts are reported in delta (δ) units and expressed in parts per million (ppm) downfield from tetramethylsilane using the residual proton solvent as an internal standard. Molecular weight (M_n) and molecular weight distribution (M_w/M_n) were determined by gel permeation chromatography (GPC) equipped with a G1310B Iso. pump, a G1316A PL gel column, and a G1362A differential refractive index detector. The eluent was DMF with 1 g L^{-1} LiBr at a flow rate of 1.0 mL min^{-1}. FT-IR spectra were recorded on Perkin-Elmer Spectrum BX FT-IR system using KBr pellets at 25 ^{o}C. UV-vis spectra were performed on UNIC 4802 UV/vis double beam spectrophotometers, quartz cells with 1.0 mm lengths were used in UV-vis measurements. Fluorescence spectra were recorded using a RF-5301/PC (Shimadzu) spectrofluorometer. The temperature of the water-jacketed cell holder was controlled by a programmable circulation bath. The slit widths were set at 5.0 nm for both excitation and emission. Transmission electron microscopy (TEM) observations were conducted on a JEM-2100F electron microscope operating at an acceleration voltage of 100 kV. The samples for TEM observation were prepared by casting the corresponding solutions of polymers onto copper mesh grids and drying in air at room temperature. Dynamic light scattering (DLS) measurements were carried on a Nano-ZS90 Zetasizer of Malvern (UK) instrument, all data were averaged over three times measurements.

Materials

(*S*)-(+)-camptothecin (CPT), *tert*-butyldimethylsilyl chloride (TBSCl), imidazole, 4-dimethyaminopyridine (DMAP), ammonium chloride (NH_4Cl), 2-(dodecylthlo carbonothloylthlo)-2-methyl-proplonlc acid (DTCTMPA), oligo (ethylene glycol) methyl ether methacrylate (OEGMA), and triphosgene were purchased and used as received unless otherwise specified. Tetrahydrofuran (THF) was further dried over sodium benzophenone ketyl, distilled onto $LiHAl_4$ under nitrogen, and distilled under high vacuum just before use. Dichloromethane (DCM) and triethylamine was distilled

over CaH_2. Reduction-responsive CPT monomer (CPTM) was synthesized according to previously reported literature.[1] All chemicals were purchased from Aladdin, Aldrich, Alfa Aesar and Across, and were used as received. Water was deionized with a Milli-Q SP reagent water system (Millipore) to a specific resistivity of 18.0 MΩ cm.

Methods

Synthesis of 2-((2-hydroxyethyl)disulfaneyl)ethyl methacrylate (HEMA). HEMA was synthesized by an esterification between 2,2'-dithiodiethanol and methacryloyl chloride. Typically, 2,2'-dithiodiethanol (5.0 g, 32 mmol), triethylamine (4.93 g, 48 mmol), and dry THF (120 mL) were charged into a 250 mL round-bottom flask. After cooling to 0 ^{0}C in an ice-water bath, methacryloyl chloride (3.34 g, 32 mmol) in 65 mL of dry THF was added dropwise over a period of 1 h under vigorous magnetic stirring. After the addition was completed, the reaction mixture was allowed to stir at room temperature overnight. After filtration and evaporating all the solvents, the residues were diluted with ethyl acetate and washed twice with water and brine, respectively. The organic layer was collected and dried over anhydrous Na_2SO_4. The crude product was finally purified by silica gel column chromatograph using ethyl acetate/petroleum ether (1/2 v/v) as the eluent, affording HEMA as a yellowish liquid (4.5 g, yielding: 54 %). The ^{1}H NMR spectrum was shown in Figure S1a.

Synthesis of Reduction-Responsive CPT Monomer (CPTM). Typically, camptothecin (CPT; 1.80 g, 5.16 mmol) and DMAP (1.568 g, 12.8 mmol) were suspended in dry DCM (15 mL) under argon atmosphere. Triphosgene (635 mg, 2.14 mmol) was added and the mixture was stirred for 2 hours at room temperature. HEMA (950 mg, 4.28 mmol, in 4 mL dry THF) was added dropwise *via* a constant pressure funnel. The reaction mixture was allowed to stir overnight. After filtration and evaporating all the solvents, the residues were diluted with diethyl acetate and washed once with water, and twice with brine, respectively. The organic layer was dried over anhydrous Na_2SO_4, filtered, and concentrated on a rotary evaporator. The crude product was purified by column chromatography using ethyl acetate as eluent to give CPTM as a pale solid powder (1.31 g, yield: 61%). The ^{1}H NMR spectrum was shown in Figure S1b.

Synthesis of alkynyl-IR780. Typically, IR-780 iodide (400 mg, 0.6 mmol) and propargylamine (100 mg, 1.8 mmol) were dissolved in anhydrous DMF (3 mL). The solution was heated at 80 ^{o}C for 10 hours. The solvent was then removed under vacuum and the residue was purified by silica gel column chromatography with CH_2Cl_2/MeOH (40:1, v/v), yielding *alkynyl*-IR780 as a deep blue solid (130 mg, 32%). The ^{1}H NMR spectrum was shown in Figure S2.

Synthesis of P(CPTM-co-GMA) Copolymers. Typically, chain transfer agent DTCTMPA (12.23 mg, 0.033 mmol), CPTM (800 mg, 1.34 mmol), GMA (71.5 mg, 0.5 mmol), and AIBN (0.82 mg, 0.005 mmol) were charged into a glass ampoule containing 3 mL 1,4-dioxane and DMSO mixed solvents (1:1, v/v). The ampoule was then degassed *via* three freeze-pump-thaw cycles and flame-sealed under vacuum. It was then immersed into an oil bath thermostated at 70 ^{o}C to start the polymerization. After 12 h, the ampoule was quenched into liquid nitrogen to terminate the copolymerization. The mixture was precipitated into an excess of cold ethyl ether to generate pale residues, the residues were then dissolved in DCM and precipitated into ethyl ether again, and the above dissolution-precipitation cycle was repeated for three times. The final product was dried in a vacuum oven overnight at room temperature, yielding a pale solid powder (660 mg, yield: 73.3 %). The ^{1}H NMR spectrum was shown in Figure S3a.

Synthesis of Amphiphilic P(CPTM-co-GMA)-b-POEGMA Copolymers. Typically, P(CPTM-*co*-GMA) (400 mg, 0.028 mmol), OEGMA (271 mg, 0.6 mmol), and AIBN (3.0 mg, 0.003 mmol) were charged into a glass ampoule containing 3 mL mixed solvent of DMSO and $CHCl_3$ (4:1, v/v). The ampoule was then degassed *via* three freeze-pump-thaw cycles and flame-sealed under vacuum. It was then immersed into an oil bath thermostated at 70 ^{o}C to start the polymerization. After 12 h, the ampoule was quenched into liquid nitrogen to terminate the polymerization. The mixture was precipitated into an excess of cold ethyl ether to generate viscous residues. The residues were dissolved in DCM and precipitated into ethyl ether again, and the above dissolution-precipitation cycle was repeated for three times. The final product was dried in a vacuum oven overnight at room temperature, yielding a yellow solid

powder (450 mg, yield: 56.3%). The ^{1}H NMR spectrum was shown in Figure S3b.

Synthesis of Amphiphilic P[CPTM-co-GMA(-N_3/-OH)]-b-POEGMA Copolymers. The P(CPTM-*co*-GMA)-*b*-POEGMA block copolymer was prepared through the epoxide ring-opening reaction of PGMA segments. Typically, P(CPTM-*co*-GMA)-*b*-POEGMA (0.40 g), NaN_3 (0.15 g), NH_4Cl (0.11 g) and DMF (5 mL) were charged into a flask and stirred at 50 °C. After 24 h, the mixture was precipitated into an excess of cold diethyl ether and filtered. The residues were dissolved in DCM and precipitated into an excess of ethyl ether. The above dissolution-precipitation cycle was repeated for three times. The final P[CPTM-*co*-GMA(-N_3/-OH)]-*b*-POEGMA was dried in a vacuum oven overnight at room temperature to afford a yellow solid (0.24 g, yield: 59.3%). The ^{1}H NMR spectrum was shown in Figure S3c.

Synthesis of Amphiphilic P[CPTM-co-GMA(-N_3/-OH)]-b-POEGMA Copolymers. This amphiphilic copolymer was synthesized *via* copper(I) catalyzed azide-alkyne cycloaddition click reaction. Typically, P[CPTM-*co*-GMA(-N_3/-OH)]-*b*-POEGMA (40 mg), PMDETA (2.3 mg, 0.014 mmol), *alkynyl*-IR780 (23 mg, 0.04 mmol), and DMF (1.0 mL) were charged into a glass ampoule equipped with a magnetic stirring bar. The mixture was degassed by three freeze-pump-thaw cycles, and then CuBr (2.0 mg, 0.014 mmol) was introduced under nitrogen atmosphere. The mixture could stir for 24 h at 35 ^{o}C, and then azide functionalized Merrifield resin was added. After another 2 hours, the ampoule was exposed to air and diluted with DCM. The solution was then passed through neutral alumina column using DCM as the eluent to remove copper catalysts. After removing most of the solvents on a rotary evaporator, the residuals were then precipitated into an excess of diethyl ether. The above dissolution-precipitation cycles were repeated for three times. The final product was dried in a vacuum oven overnight at room temperature to afford a solid (20 mg, yield: 47.3%). The ^{1}H NMR spectrum was shown in Figure S2.

Fabrication of Core-Crosslinked Nanoparticles. The procedure employed for the fabrication of core-crosslinked nanoparticles from HCCP and P[CPTM-*co*-GMA (-IR 780/-OH)]-*b*-POEGMA copolymers was according to our previously reported reference.[2-3] Typically, into a 50 mL dried flask, P[CPTM-*co*-GMA (-IR 780/-OH)]-

b-POEGMA (20 mg, 1.45 μmoL; [-OH]: 34.8 μmol), HCCP (6.0 mg, 17.4 μmol), and dried DMF (3.0 mL) were added under a nitrogen atmosphere. Then, TEA (0.5 mL) was added as catalyst and acid-binding agent. The mixture was allowed to react at 25 ^{o}C for 24 hours. After the reaction was completed, the resultant complex, core cross-linked nanoparticles were obtained with a brown color by precipitation with cold diethyl ether.

Preparation of Anti-Cancer Drug (Curcumin) Loaded HCNPs. Hydrophobic curcumin was loaded into the hydrophobic region of HCNPs during the co-solvent self-assembly process. Typically, the DMF dispersion of HCNPs (1.0 g/L, 1 mL) and curcumin (0.2 g/L) were prepared in advance and then mixed together. Under vigorous stirring, 9.0 mL DI water was added *via* a syringe pump at a flow rate of 0.5 mL/min. After the addition was completed, the mixture was left stirring for another 4 h. DMF was then removed by dialysis (MWCO 3.5 kDa) against pure water for 24 h. Fresh water was replaced approximately every 6 h. The obtained dispersion could stand at room temperature for more than one week, suggesting the formation of stable curcumin@HCNPs complex. Free curcumin removed by passing through a 0.22 μm Millipore filter. The final dispersion was diluted with phosphate buffer solution (PBS; pH 7.4) for further use.

Photothermal Effect of HCNPs. For photothermal efficiency measurements, 2.0 mL of HCNPs dispersion (0.5 g/L; pH 7.4) was charged into a cuvette, the temperature after being subjected to light (660 nm, 1.0 Wcm^{-2}) irradiation for 10 min was directly measured by thermometer probe. For comparison, pure PBS buffer was also tested.

In Vitro Cargo Release Profile. The cargo release from HCNPs was measured by the dialysis method. Briefly, the HCNPs dispersion (1.0 g/L; 10.0 mL) was placed in a dialysis tube (MWCO 3.5 kDa) and then immersed into 500 mL of water with Tween 20 (1.0% total volume) under gentle stirring at 37 ^{o}C. Then, the system was treated by DTT (5.0 mM) and acid (pH 5.5) in turn or simultaneously according to the need. At different time intervals, 20 mL external water solution was removed and replaced with equal volume of fresh water. The separated solution was lyophilized and then dissolved in DMSO, the cargo concentration was quantified by measuring the

absorbance against a standard calibration curve.

Cell Culture and in Vitro Cytotoxicity Assessment. HeLa cells (5×10^3 cells/well) in Dulbecco's modified Eagle's medium (DMEM) complete medium were plated into a 96-well plate and incubated overnight. Then, the cells were exposed to HCNPs with different concentrations at 37 °C for up to 30 h in DMEM complete medium. Then, cells were rinsed with PBS buffer and DMEM complete medium. Cytotoxicity was assessed by adding 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl tetrazolium bromide (MTT) for another 4h. Cells incubated with PBS were served as positive control.

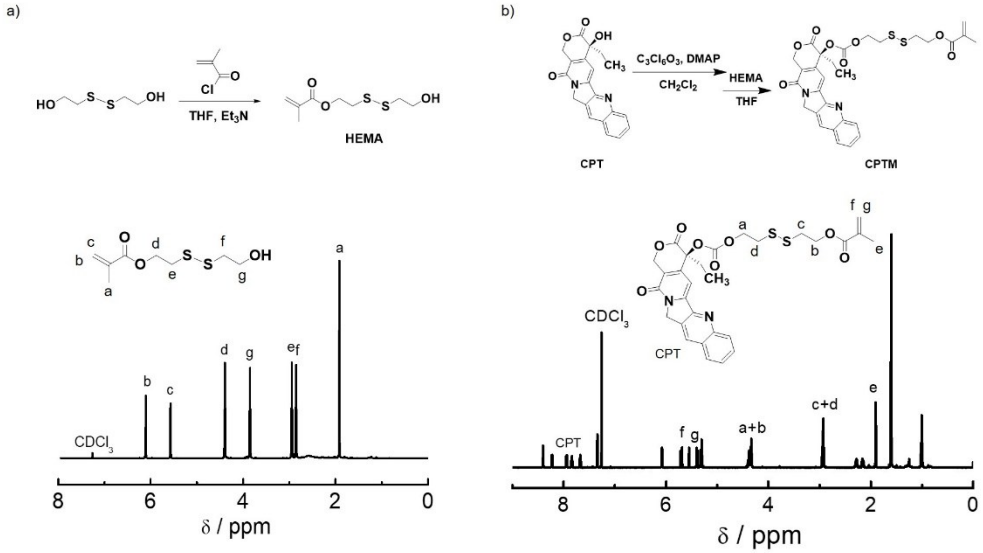

Figure S1. ^{1}H NMR spectra obtained for (a) HEMA and (b) CPTM using $CDCl_3$ as solvent at 25 ^{o}C.

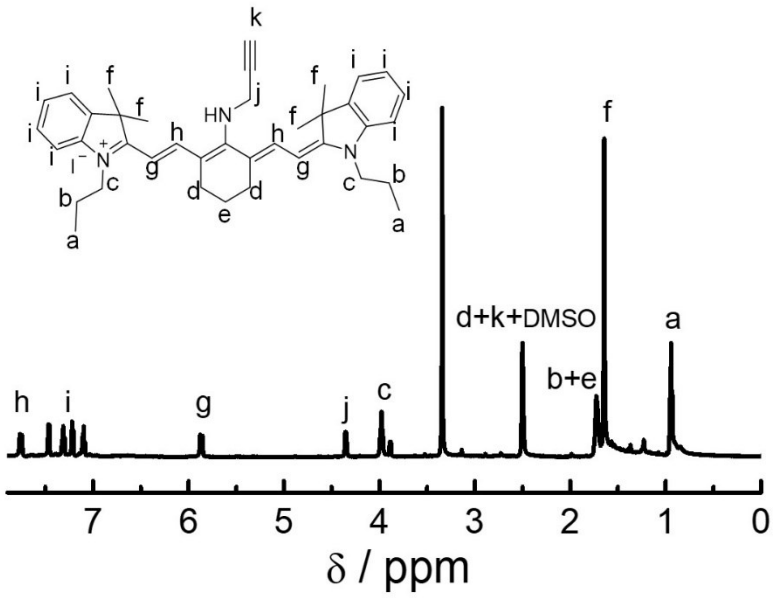

Figure S2. ^{1}H NMR spectrum obtained for *alkyne*-terminated IR780 using DMSO-d_6 as solvent at 25 ^{o}C.

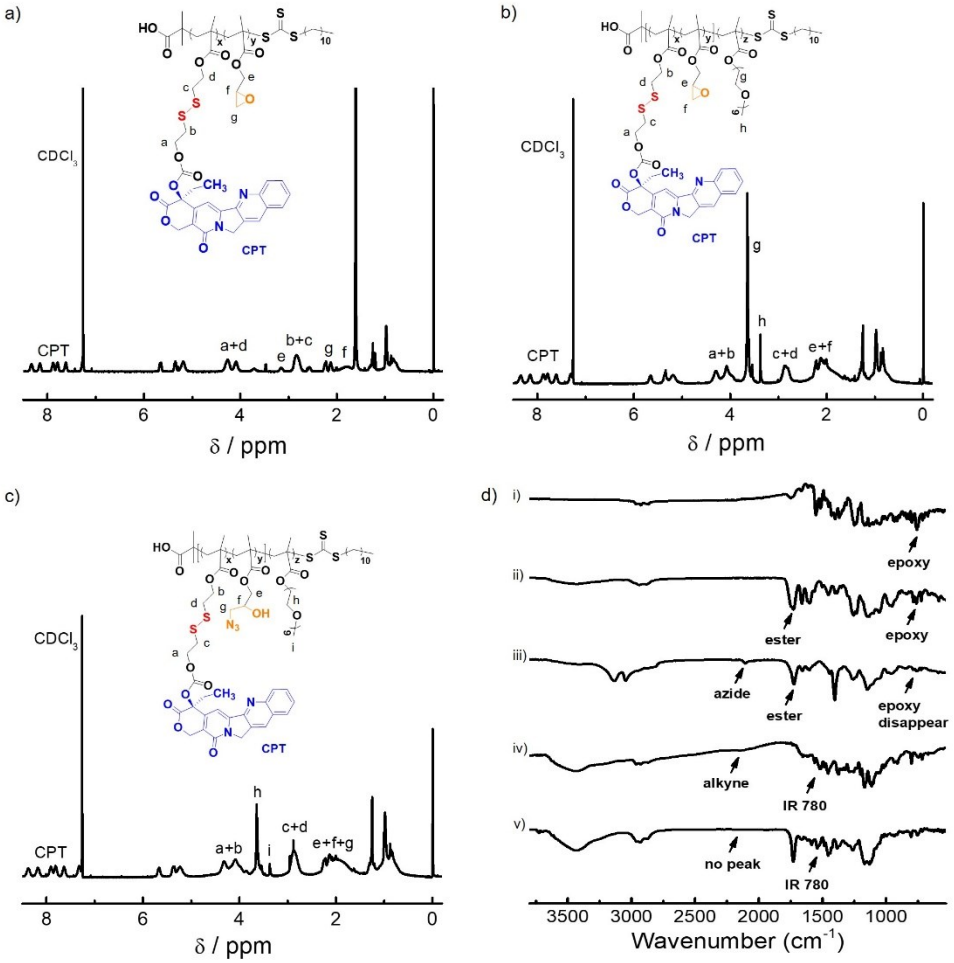

Figure S3. ^{1}H NMR spectra obtained for (a) PCG, (b) PCGP, and (c) PCAHP using $CDCl_3$ as solvents at 25 ^{o}C. (d) FT-IR spectra obtained for i) PCG, ii) PCGP, iii) PCAHP, iv) *alkyne*-terminated IR780, and v) PCIHP at 25 ^{o}C using KBr pellets.

Table S1. Summary of the PDI values of PCIHP NPs obtained from DLS measurement.

Samples	PDI
before cross-linking, in water	0.19
after cross-linking, in water	0.16
after cross-linking, in THF	0.17

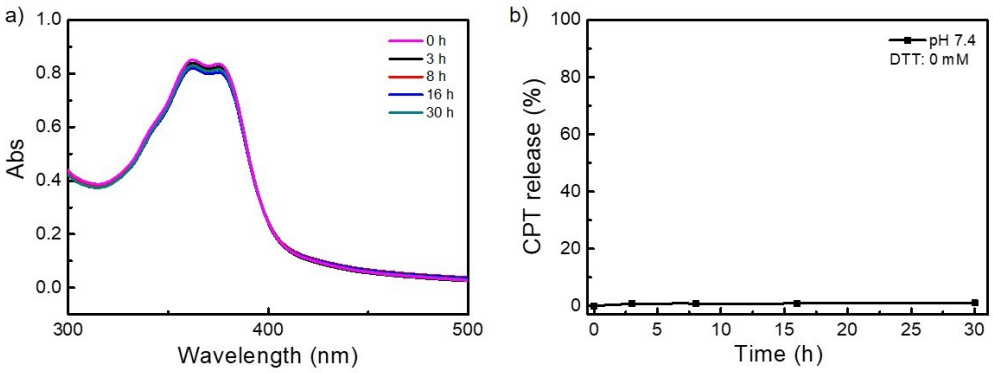

Figure S4. (a) UV-vis spectra recorded for the aqueous dispersion of HCNPs at different times in the absence of DTT and pH 7.4. (b) CPT release profile of HCNPs in the absence of DTT and pH 7.4.

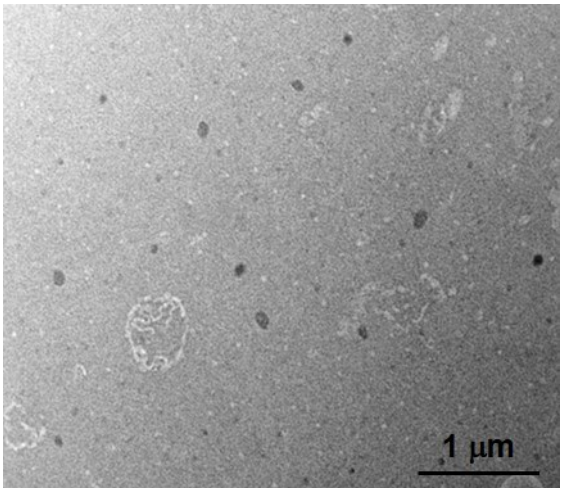

Figure S5. TEM image obtained for HCNPs after incubating with DTT (5mM) at 37 ^{o}C.

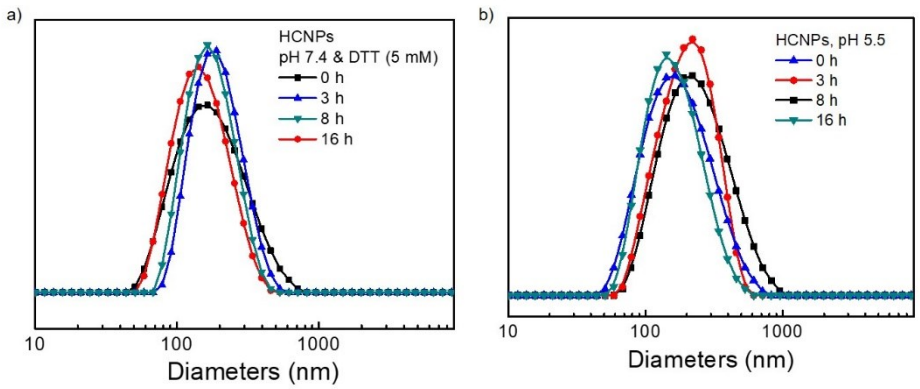

Figure S6. Hydrodynamic diameter distribution obtained for the aqueous dispersion of HCNPs in different conditions at 37 °C: (a) pH 7.4, DTT (5.0 mM), (b) pH 5.5.

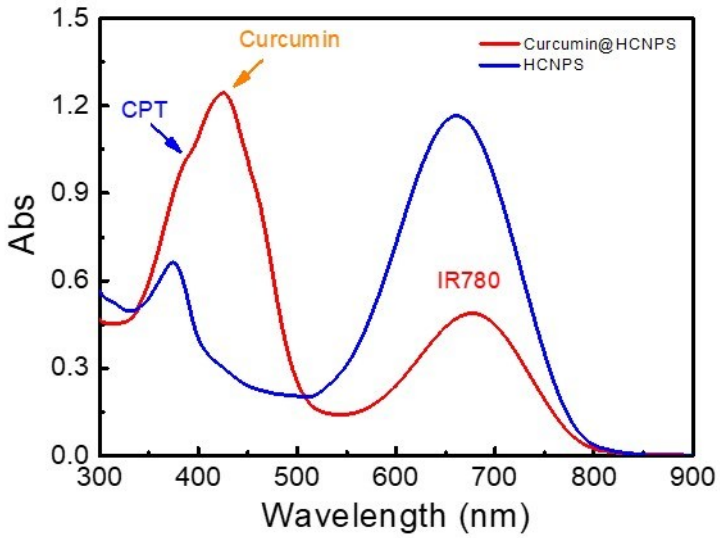

Figure S7. UV-vis spectra recorded for the aqueous dispersions of HCNPs and curcumin@HCNPs at pH 7.4 and 25 ^{o}C.