Supporting Information

Three dimensional graphene oxide supramolecular hydrogel for infrared light-responsive cascade release of two anticancer drugs

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Experimental section

Materials

Graphene oxide (GO, thickness: 0.55-1.2 nm, purity > 99 wt%, size: 2-5 μm) was purchased from Chengdu Organic Chemicals Co., Ltd. Chinese Academy of Sciences. Monomethoxy polyethylene glycol with a molecular weight of 1900 was purchased from Alfa Aesar (Tianjin, China). Camptothecin (CPT) was purchased from Sichuan Jiangyuan Natural Products Co. (China). 5-Fluorouracil (5-FU) was kindly given by Lanzhou University. α-cyclodextrin (α-CD, purity ≥ 98.0%) were purchased from Aladdin Chemical Co. Ltd. (Shanghai, China). N,N′-diisopropylcarbodiimide (DIPC) were purchased from Shanghai GL Biochem Ltd. (China). Dichloromethane (analytical reagent) was used after drying through CaH_2. Other reagents were analytical pure and used without further purification. Ultrapure water was obtained by a water purification system, which was purchased from Shanghai Laikie Instrument Co., Ltd. (China).

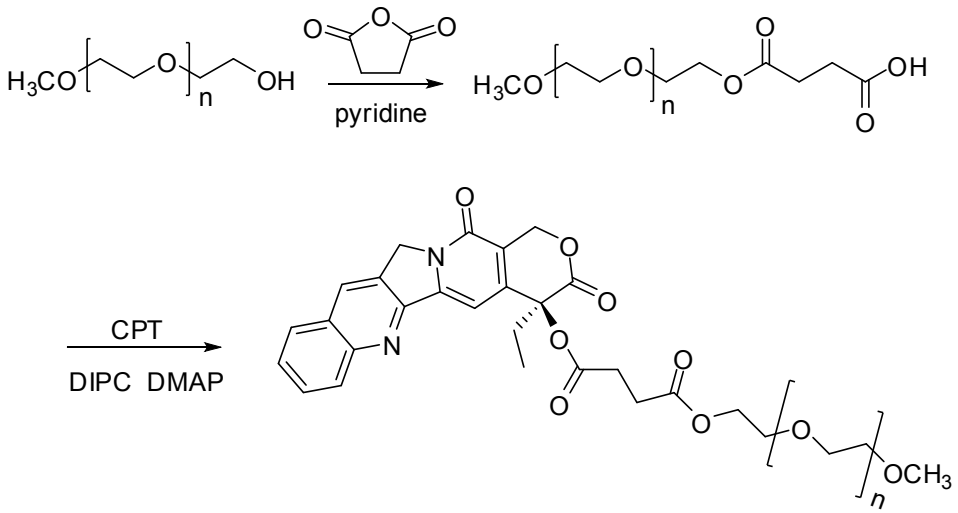
Characterization

UV−vis spectra were performed by a Perkin-Elmer Lambda 35 spectrophotometer with a scanning range of 200 to 800 nm. Fourier transform infrared spectroscopy (FT-IR) spectra were recorded on a Nexus 870 FT-IR spectrophotometer with a scanning range of 4000 to 400 cm^{-1}. ^1H NMR spectra were obtained on a Bruker AVANCE III-400 spectrometers using $CDCl_3$ as the solvent. Fluorescence spectra of CPT-PEG and GO-CPT-PEG in aqueous solution were determined by Perkin-Elmer LS-55 fluorescence spectrophotometer with a slit width of 10.0 and 2.5 nm for excitation and emission. For

fluorescence emission spectra, excitation wavelength was set as 340 nm. The composition of GO-CPT-PEG was analyzed by Thermogravimetric analyses (TGA) on a STA 449C thermal analysis system with a nitrogen atmosphere at a heating rate of 10 °C/min from room temperature to 800 °C. The XRD patterns of the freeze-dried samples were recorded in reflection mode on a PHILP X'Pert PRO diffractometer using Cu Kα (λ = 1.542 Å) irradiation (40 kV, 40 mA) in the range of 2θ=5-80°. The rheological behavior of the hydrogels was investigated by a HAAKE RS6000 rotational rheometer using a 35 mm parallel-plate geometry at 20 °C. The gap distance between the two plates was fixed at 1 mm. Oscillating stress was fixed at 1 Pa for all dynamic tests. The morphologies of GO-CPT-PEG hybrids and hybrid hydrogels were investigated and analyzed on a JSM-5600LV electron microscope after the samples were freeze-dried and coated with gold vapor. Atomic force microscope (AFM) was measured by Nanoscope IIIa, Vecco Instruments, USA.

Synthesis of CPT-PEG prodrug

The CPT-PEG prodrug were prepared according to the procedure reported previously and its structure confirmed by direct comparison with our previously reported spectroscopic data.[1]



Scheme S1. Synthesis route for the CPT-PEG prodrug: (a) CH_2Cl_2, pyridine, N_2, reflux for 3 days; (b) DIPC, DMAP, and CH_2Cl_2, stirred at room temperature for 16 h.

Preparation of CPT-PEG prodrug modified GO hybrids (GO-CPT-PEG)

100 mg GO and 300 mg CPT-PEG prodrug were dispersed into 50 mL aqueous solution. The hybrid solution was ultrasonicated for 24 h and further incubated at room temperature for 72 h without disturbing. The supernatant was dialyzed against double-distilled water for 3 days to remove free CPT-PEG from the solution. The GO and CPT-PEG content of the GO-CPT-PEG hybrids was determined by TG analysis (Figure S1, Supporting Information).

Preparation of GO-CPT-PEG/α-CD hydrogel

The supramolecular hybrid hydrogel was prepared based on the host-guest interaction between PEG chains on the surface of GO-CPT-PEG and α-CD in aqueous solution. In brief, various amount of α-CD (50, 80, 110 and140 mg) was added into 1 mL of GO-CPT-PEG hybrids solution (15 mg mL^{-1}), the blended solution was ultrasonicated for 5 min and further incubated at room temperature for 72 h without disturbing. For the

formation of 5-FU loaded hydrogel, α-CD (100 mg) and different amount of 5-FU (dose ratio of 5-FU to CPT-PEG ranged from 0:1 to 4:1) was added to 1.0 mL of GO-CPT-PEG buffer solution (PBS, pH 7.4) simultaneously, the solution was mixed thoroughly by sonication for 5 min followed by incubation at room temperature for 72 h before measurements. Finally, the loading efficiency of CPT-PEG at different ratios was calculated to be 1.22%, 1.21%, 1.20%, 1.18%, while the values for 5-FU was 1.20%, 1.88%, 2.62% and 3.93%, respectively.

Thermo-triggered hydrogel gel-sol transition behavior

GO-CPT-PEG/α-CD hydrogel (0.5 mL, containing 7.5 mg GO-CPT-PEG and 50 mg α-CD) formed in the bottom vials were sealed by caps, and then were immersed in a water bath heated to a temperature of 30, 40, 50, and 60 °C, respectively. 30 min later, the vials were taken out for observation and monitored visually according to whether the hydrogels flowed when inverting the vials for 1.0 min.

Self-heating properties of the hydrogel upon NIR irradiation

CPT-PEG/α-CD hydrogel (0.5 mL, containing 10 mg CPT-PEG and 50 mg α-CD) and GO-CPT-PEG/α-CD hydrogel (0.5 mL, containing 7.5 mg GO-CPT-PEG and 50 mg α-CD) formed in the bottom vials were sealed with caps and placed upside-down, and then were irradiated by a NIR laser (MDL-N-808 nm, New Industries Corp., Changchun, China) at a power density of 4.5 W cm^{-2} for 20 min. The time-elapsed temperatures and thermographs of the hydrogel were recorded by using an infrared thermal camera (FLIR t425, USA).

NIR-triggered hydrogel gel-sol transition behavior

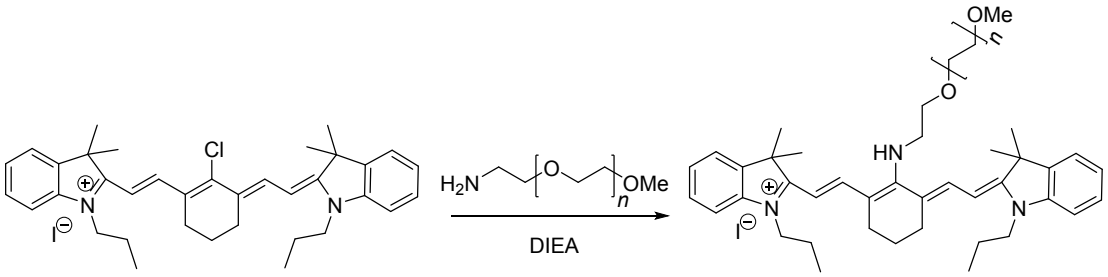
CPT-PEG/α-CD hydrogel (0.5 mL, containing 10 mg CPT-PEG and 50 mg α-CD) and GO-CPT-PEG/α-CD hydrogel (0.5 mL, containing 7.5 mg GO-CPT-PEG and 50 mg α-CD) formed in the bottom of vials were irradiated by NIR laser at a power density of 4.5 W cm^{-2} for 20 min, and the vials were sloped to a certain angle to observe the gel-sol transition behavior of the hydrogels. To determine the degradation percentage of hydrogels, the as-generated sol solution after NIR irradiation was removed by pipette, and the remaining hydrogel was weighed. The degradation percentage of the hydrogel was evaluated by the formula, degradation percentage = (1- remaining mass of hydrogel/ original mass of hydrogel) × 100%.

***In vitro* release kinetics studies**

The 5-FU loaded GO-CPT-PEG/α-CD hydrogels were prepared in a 1.5 mL cuvette. The cuvette was placed upside-down in a test tube with 30.0 mL of phosphate buffered saline (PBS, pH 7.4) and incubated in a 37 °C water bath. The PBS was changed at determined intervals of time. The concentrations of 5-FU and CPT-PEG prodrug released from hydrogels were determined using an Agilent 1260 high performance liquid chromatographic system. Chromatographic separation was performed on an Agilent ZORBAX SB-C18 column (4.6 × 150 mm, 5 μm) at 30 °C with acetonitrile and 0.1% phosphoric acid aqueous solutions (75:25, v/v) as a mobile phase at a flow rate of 1.0 mL min-1. A wavelength of 372 nm was used to detect CPT-PEG and 265 nm to detect 5-FU.

To research the release kinetics of hydrogel upon NIR irradation, the 5-FU loaded CPT-PEG/α-CD (0.5 mL) and GO-CPT-PEG/α-CD hydrogels (4 samples, 0.5 mL) were prepared in bottom of a cuvette and then 1.8 mL PBS was added into the cuvette. The cuvettes were placed upside-down and irradiated by NIR laser for 40 min. The laser power density of 1.0, 2.5, 4.5 and 5.5 W cm^{-2} were selected for GO-CPT-PEG/α-CD hydrogel samples, while the power density for CPT-PEG/α-CD hydrogel sample was 4.5 W cm^{-2}, respectively. While the NIR irradiation, an aliquot of the PBS solution (0.3 mL) was collected at a time point of 0, 5, 10, 20, 30, and 40 min, respectively, and then was analyzed by using HPLC to determine the concentration of the released 5-FU and CPT-PEG prodrug. For each collection of the PBS solution, the same amount of PBS was fed back to the cuvettes to keep the constant solution volume.

Synthesis of CyN-PEG



Scheme S2. Synthesis route of CyN-PEG

IR-780 iodide (130 mg, 0.2 mmol), PEG-NH_2 (600 mg, 0.3 mmol), and N,N-diisopropylethylamine (DIPEA, 52 μL, 0.4 mmoL) were dissolved in acetonitrile (ACN) (3 mL). The mixture was stirred at 80 ^oC for 4 h. The solution was cooled to room temperature. The residues were purified by silica gel column chromatography (CH_2Cl_2/MeOH, 300:1 to 15:1, v/v) to afford the CyN-PEG as blue solid (yield 51%).

^{1}H NMR ($CDCl_3$, 400 MHz, δ): 7.76 (d, *J* = 13.0 Hz, 2H), 7.30–7.28 (m, 4H), 7.10–7.06 (m, 2H), 6.90 (d, *J* = 8.0 Hz, 2H), 5.68 (d, *J* = 13.0 Hz, 2H), 3.55–3.84 (m), 3.39 (s, 3H), 2.47-2.51 (t, *J* = 8.0 Hz, 4H), 2.03 (m, 6H), 1.83–1.86 (m, 4H), 1.70–1.71 (m, 12H), 1.05 (t, *J* = 8.0 Hz, 6H).

***In vivo* NIR-triggered drug release from GO-CPT-PEG/α-CD hydrogel**

Chinese KunMing mice with average weight of 18-20 g were purchased from Lanzhou University. All the operation process was conformed to the principles of laboratory animal operation regulation and was approved by the Experimental Animal Use and Care Committee, Lanzhou University. The H22 tumor xenograft model was established by injection of H22 ascites sarcoma cells (~106 cells) suspended in 100 μL PBS into the right hind limbs of mice via a percutaneous approach. The mice with an average tumor volume of 500 mm3 were ready for experiment. GO-CPT-PEG/α-CD hydrogel laden with CyN-PEG (50 mm^3) was injected into the tumors of two groups of mice. The IVIS images (Kodak In-vivo Imaging System FX Pro coupled with a 625 excitation filter and a 700 nm emission filter, USA) of the mice were recorded after injection, and then one group of mice were irradiated by NIR laser at a power density of 4.8 W cm^{-2} for 20 min. The final IVIS images of the two groups of mice were captured at 20 min to determine the gel-sol transition of the hydrogel and the drug release.

***In vitro* anticancer activities**

The freeze-dried powder sample of CPT-PEG/α-CD hydrogel and 5-FU loaded GO-CPT-PEG/α-CD hydrogels with different dose ratio of 5-FU and CPT-PEG (from 0:1

to 4:1) were put into 5 mL of RPMI 1640 cell culture medium in a 50 mL centrifuge tube, and then placed in a shaker incubator (37 °C, 60 rpm) for 2 days. After that, the media were filtered with 0.22 μm sterile filter into a sterile container and stored in a refrigerator at 4 °C before use.

A549 cells were seeded in a 96 well culture plate at a density of 8000 cells per well and cultured in RPMI 1640 medium supplemented with 10 % fetal bovine serum at 37 °C in a humidified environment of 5 % CO_2 for 1 day. Thereafter, the cells were incubated with CPT, CPT-PEG prodrug and the extracted leached medium from the hydrogel at varying concentrations for 72 h. Then, the RPMI 1640 medium was aspirated and replaced with 100 μL fresh medium. 24 h later, 15 μL of 5 mg/mL MTT solution was added to each well and incubated for further 4 h. The medium solution was then replaced with 150 μL DMSO to dissolve the MTT-formazan that was generated by live cells, and the plate was shaken for 30 min to produce a homogeneous coloured solution. Absorbance was read at 570 nm on a microplate reader. The relative cell viability (%) was expressed as a percentage of that of the control culture. The release experiments were carried out six times. The results presented are the average data.

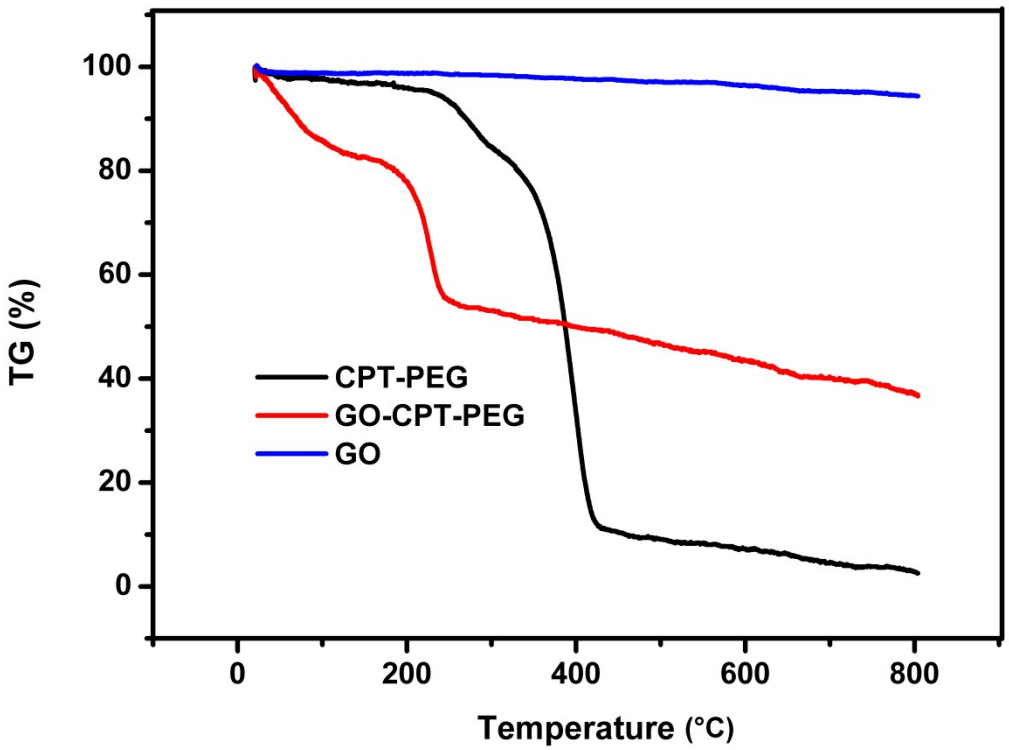


Figure S1. TGA (in N_2) curves of GO, CPT-PEG, and GO-CPT-PEG.

The TGA data indicated GO-CPT-PEG had a 63.4% weight loss at 800 °C in N_2, while the values for GO and CPT-PEG were 5.7% and 97.5%, respectively. So the ratio of modified CPT-PEG was estimated according to the following reported equation.[2]

$$0.057x + 0.975y = 0.634 \quad (1)$$

$$x + y = 1 \quad (2)$$

x and y are weight percentage of GO and CPT-PEG.

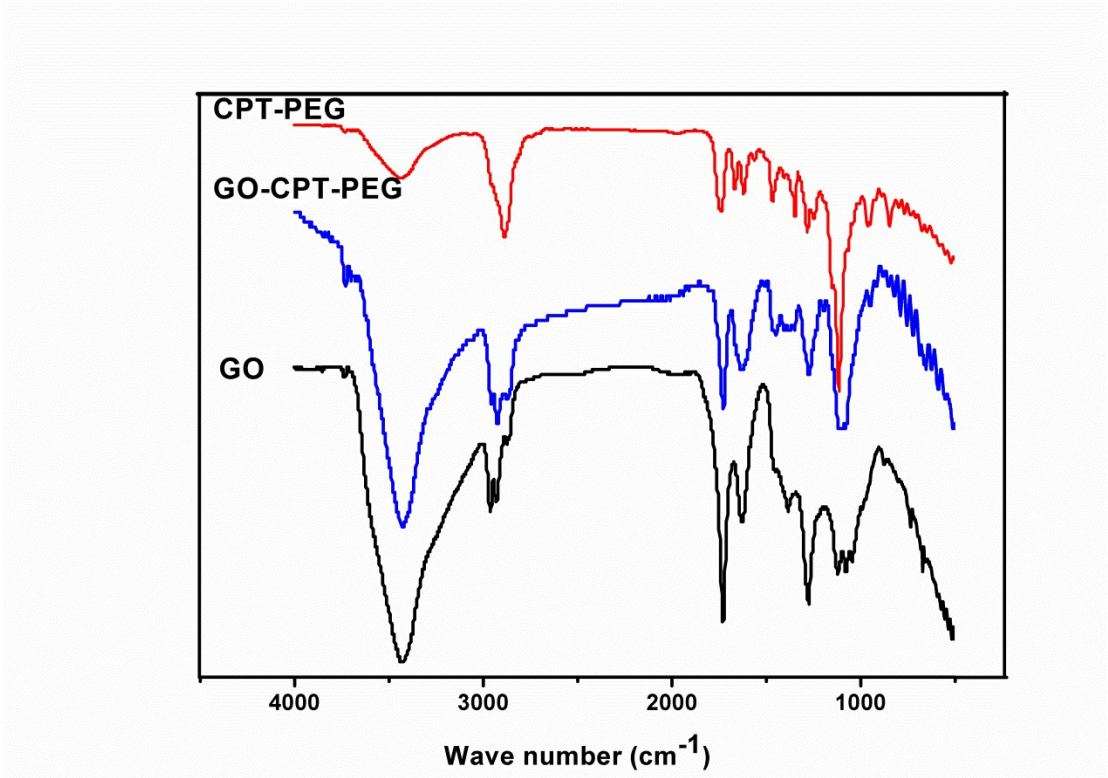


Figure S2. FT-IR spectra of GO, CPT-PEG and GO-CPT-PEG.

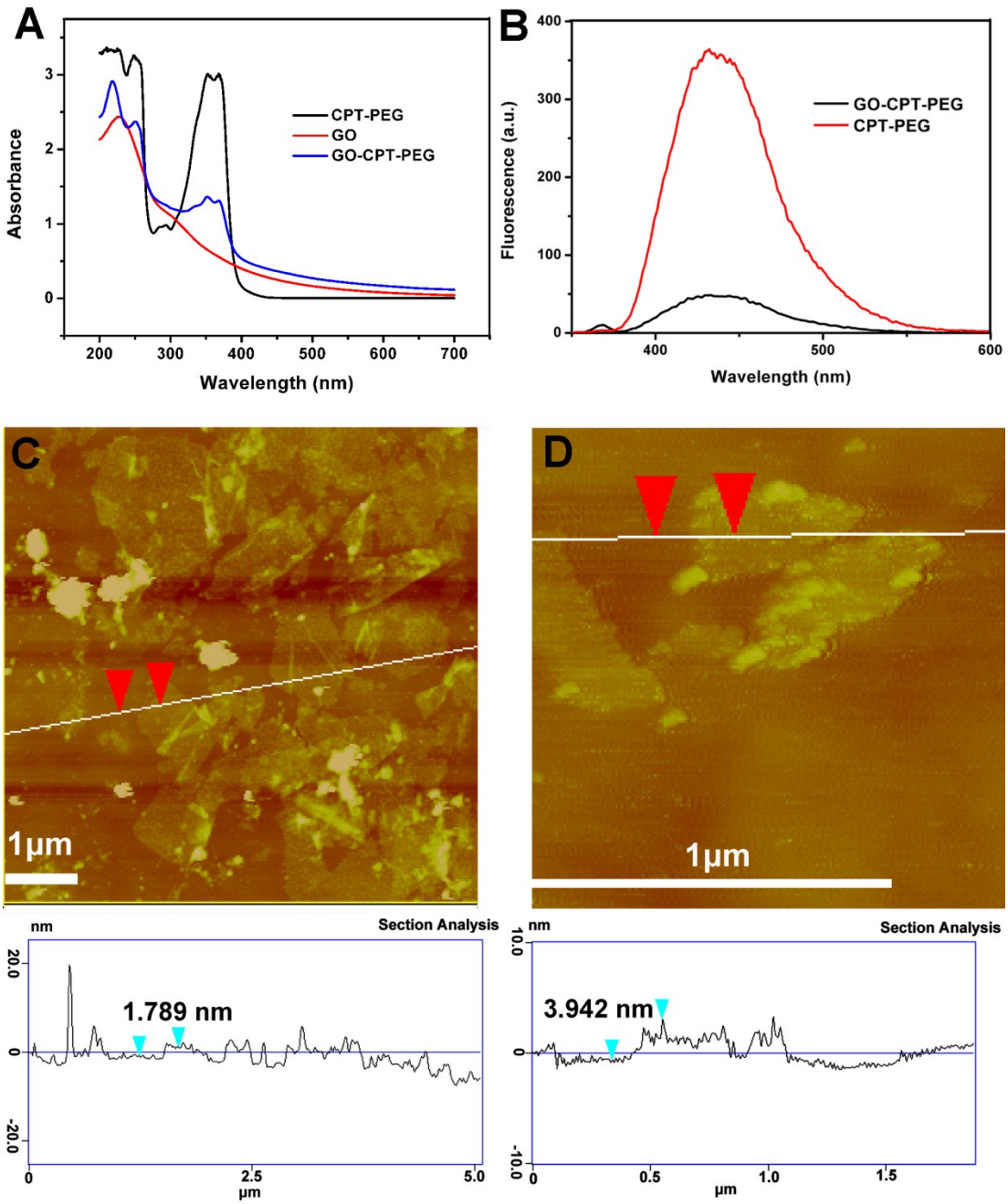


Figure S3. (A) UV-vis absorption spectra of CPT-PEG, GO and GO-CPT-PEG hybrids. (B) Fluorescence spectra of CPT-PEG and GO-CPT-PEG solution (λex: 340 nm). AFM images of (C) GO and (D) GO-CPT-PEG hybrids.

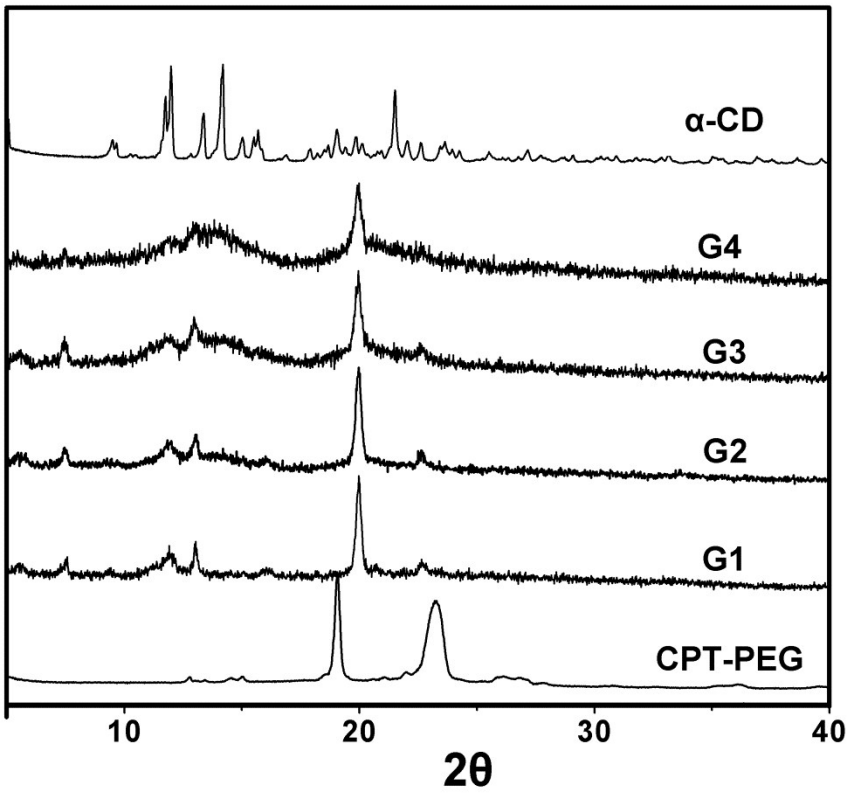


Figure S4. X-ray diffraction patterns for freeze-dried α-CD, CPT-PEG, GO-CPT-PEG/α-CD hydrogel (G1, G2, G3 and G4).

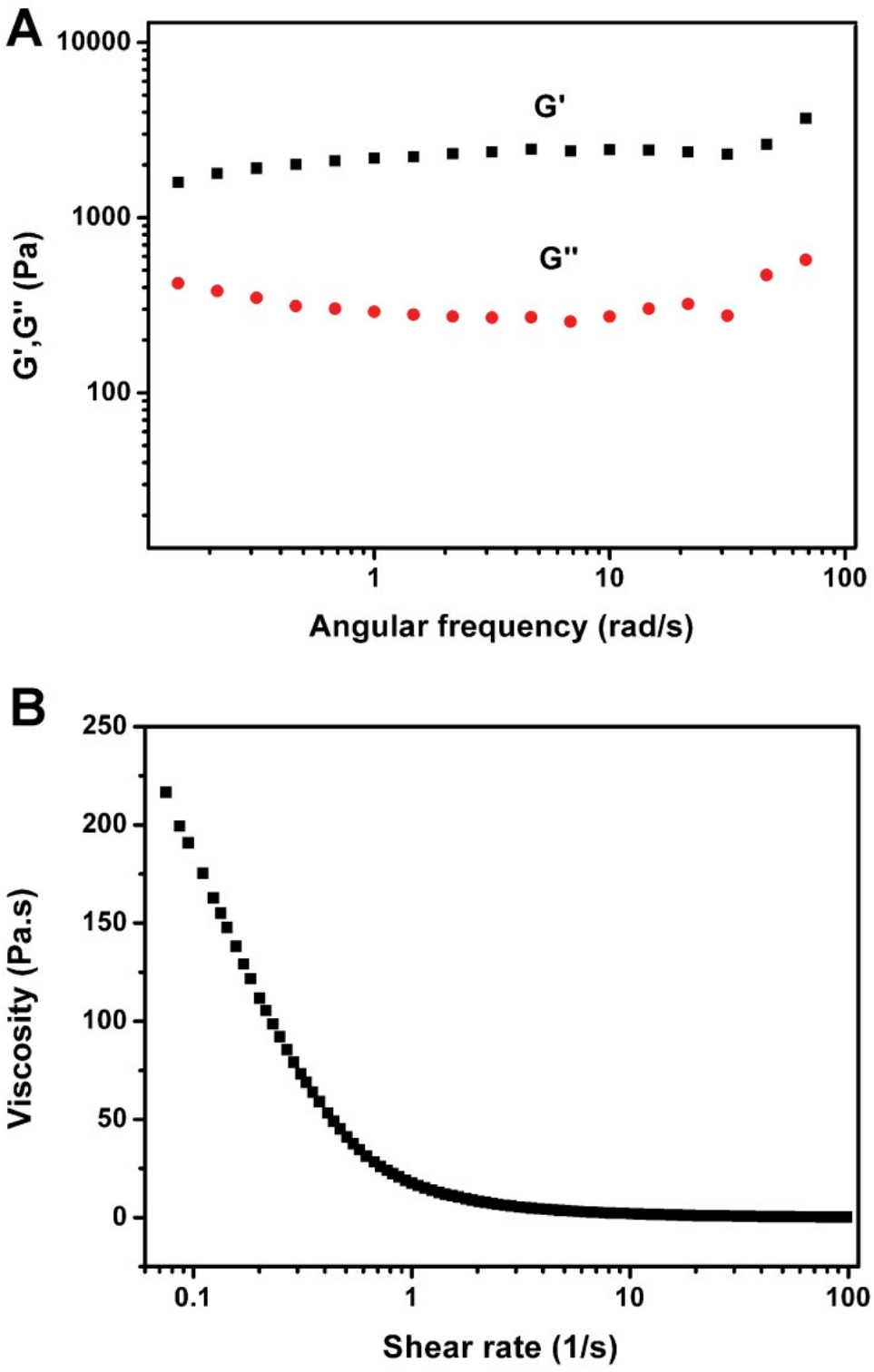


Figure S5. (A) Dynamic and (B) steady rheological behaviors of the GO-CPT-PEG/α-CD hydrogel (**G1**).

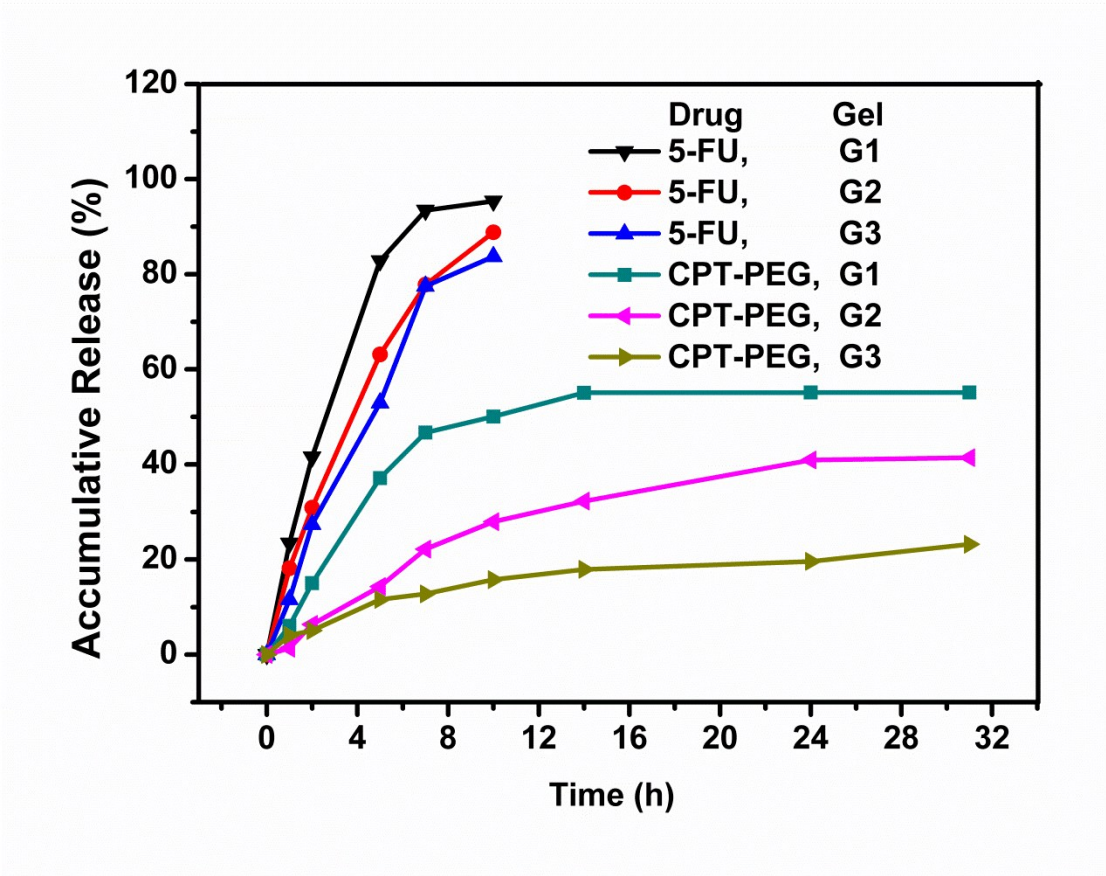


Figure S6. 5-FU and CPT-PEG prodrug release kinetics from G1, G2 and G3 in PBS at pH 7.4 and 37 °C.

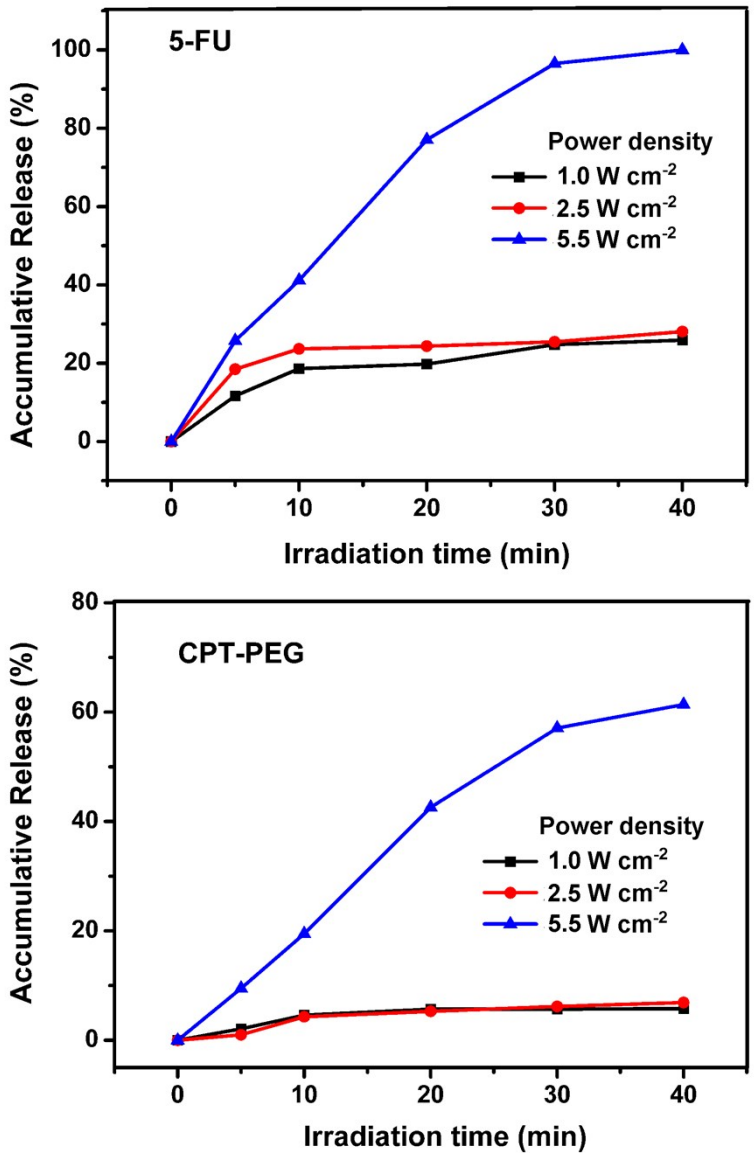


Figure S7. 5-FU and CPT-PEG prodrug release kinetics from GO-CPT-PEG/α-CD hydrogel upon NIR irradiation with different power density (1.0, 2.5 and 5.5 W cm^{-2})**.**

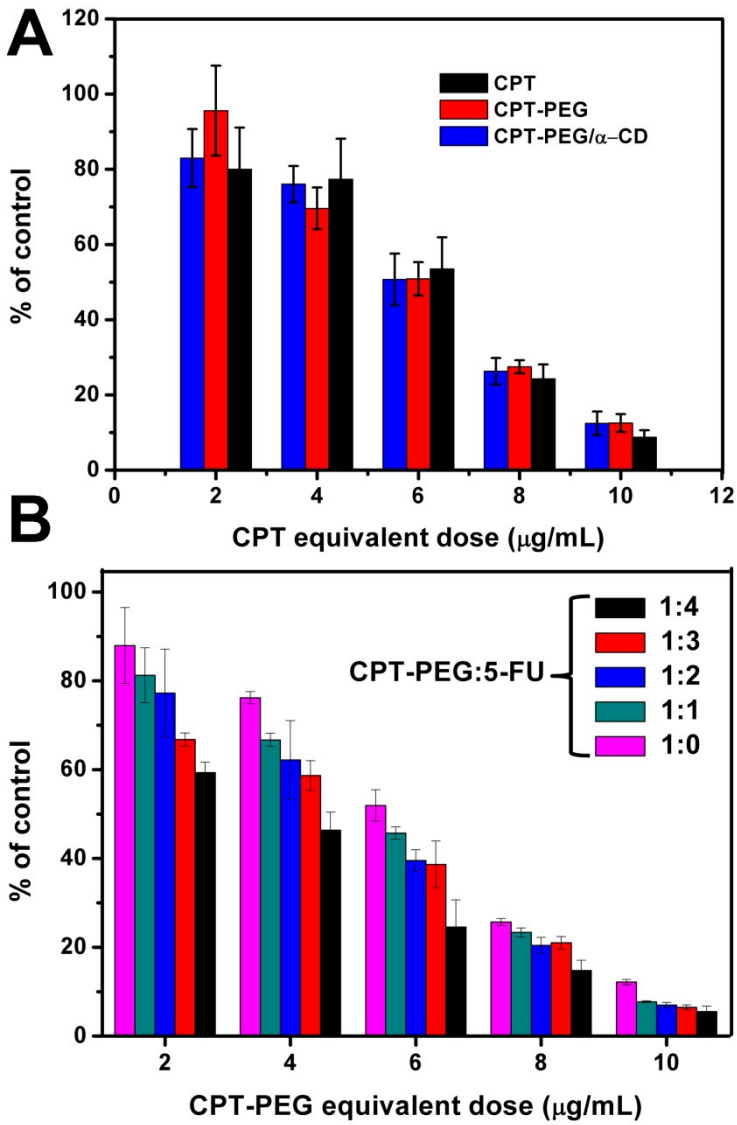


Figure S8. *In vitro* cytotoxicity of free CPT, CPT-PEG prodrug, CPT-PEG/α-CD hydrogel (A) and GO-CPT-PEG/α-CD hydrogel laden with different amount of 5-FU (B) to A549 lung cancer cells determined by MTT assay.

Reference

[1] W. Ha, J. Yu, X. Y. Song, J. Chen, Y. P. Shi, *ACS Appl. Mater. Interfaces* **2014**, *6*, 10623.

[2] Z. Y. Xu, S. Wang, Y. J. Li, M. W. Wang, P. Shi, X. Y. Huang, *ACS Appl. Mater. Interfaces* **2014**, *6*, 17268.