Electronic Supplementary Information

for

Small-Sized Graphene Oxide Supramolecular Assembly for Targeted Delivery of Camptothecin

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Experimental Section

General Method. All chemical reagents were commercially available unless noted otherwise. β-CD-appended GO[1] and adamantyl amine derivative[2] were synthesized according to the reported literatures with slight modification. In the case of β-CD-appended GO, the original size of GO was controlled at around 500 nm for the sake of biological experiments. NMR data were recorded on 400 MHz spectrometer. Mass spectra were performed on an ESI mode MS. Absorption spectra were recorded on a UV/Vis spectrometer. Steady-state emission spectra were recorded in a conventional quartz cell (10 × 10 × 45 mm) at 25 °C on a fluorescence spectrometer. A sample solution for DLS measurement was added into a clean scintillation vial at the concentrations of 0.1 mg/mL at 25 ^{o}C. The thermogravimetric analysis (TGA) was recorded from room temperature to 800 °C with a heating rate of 10 °C min^{-1}. XPS spectra were recorded employing a monochromatic X-ray source (hν = 1486.6 eV). AFM experiments were performed in tapping mode in air at room temperature.

Synthesis of water-soluble hyaluronic amides bearing adamantyl groups (HA-ADA). This compound was synthesized through the amidation reaction using a previously reported method.[3] The acid form of HA (0.5 g, 1.32 mmol) was dissolved in 50 mL DMSO at 60 °C. After the polymer was completely dissolved, the solution was cooled to room temperature. Then, triethylamine (0.92 mL, 6.6 mmol) was added and the reaction solution was stirred for another 10 min. At this moment, ethyl chloroformate (0.38 mL, 4.0 mmol) was added and the mixture was continued to stir at room temperature for 1 h. After the corresponding adamantyl amine (146.6 mg, 0.66 mmol) was added, the reaction mixture was allowed to stir at room temperature for 24 h. Afterwards, the solution was diluted with 50 mL

water and dialyzed (M_w cut off = 8–14 kDa) against 0.1 M of NaCl for 24 h, and then dialyzed against deionized water for 7 days. After dialysis, the sample was freeze-dried as white powder. Using the single-point method from the integrated peak area of adamantyl moiety and HA backbone in NMR spectra, the degree of substitution (DS) was determined as 13%, indicating that adamantane was introduced every 7.7 repeating sugar units on average. ^{1}H NMR (400 MHz, D_2O, ppm): δ 4.24–4.63 (m, 2H), 3.05–3.99 (m, 10.6H), 1.90 (s, 3.3H), 1.57–1.75 (m, 1.6H).

CPT loading on GO-CD/HA-ADA conjugate. The solution of CPT (3.5 mg, 10 mM) in 1 mL DMSO was dropwise added to 10 mL aqueous solution of GO-CD/HA-ADA complex and the mixture was stirred overnight at room temperature in dark. After centrifugation at 5000 rpm for 5 min, the supernatant was filtered through a 0.45 μm filter to remove the excess CPT. Then, the resulting filtrate was dialyzed against deionized water and the homogeneous CPT@GO-CD/HA-ADA solution was stored at 4 °C prior to further characterization. In our case, the percentage loading of CPT onto GO-CD/HA-ADA was estimated by the following equation:

$$\text{Drug loading efficiency (\%)} = 100 \times \frac{m_{\text{CPT in CPT@GO-CD/HA-ADA}}}{m_{\text{total GO-CD/HA-ADA}}}$$

In the drug loading ratio test, the absorption coefficients of GO-CD and GO-CD/HA-ADA at 800 nm were obtained as 7.5 L·g^{-1}·cm^{-1} and 3.7 L·g^{-1}·cm^{-1}, respectively, from Lambert-Beer's law at a certain concentration. The molar extinction coefficient of CPT at 366 nm was 19900 L·mol^{-1}·cm^{-1} using the previously reported result.[4]

According to Lambert-Beer's law: $A = \varepsilon \times b \times c$, where A, ε, b, and c are absorption, absorption coefficients, light length, and sample concentration, respectively, in UV/Vis

spectroscopic experiments, the drug loading efficiency in our case can be calculated as follows:

$c_{\text{GO-CD/HA-ADA}} = A/\varepsilon \cdot b = 0.236/[(3.7\ \text{L}\cdot\text{g}^{-1}\cdot\text{cm}^{-1}) \times 1\ \text{cm}] = 0.064\ \text{g/L}$

$c_{\text{CPT}} = A/\varepsilon \cdot b = 0.12/[(19900\ \text{L}\cdot\text{mol}^{-1}\cdot\text{cm}^{-1}) \times 1\ \text{cm}] = 6.03\times10^{-6}\ \text{mol/L} = 0.0021\ \text{g/L}$

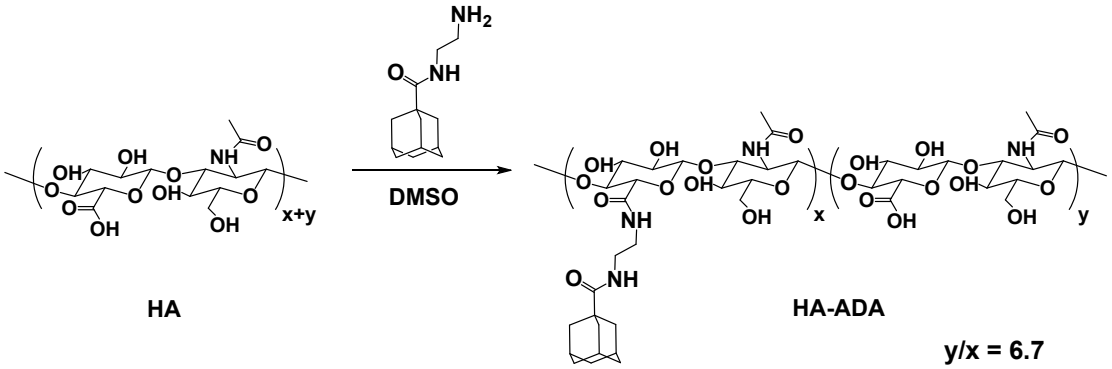
Therefore, the drug loading efficiency (%) = 0.0021/0.064 = 3.3%.

CPT releasing *in vitro*. To further examine CPT releasing from CPT@GO-CD/HA-ADA, 5 mL prepared CPT@GO-CD/HA-ADA solution was placed in an inner dialysis bag (M_w cut off = 8–14 kDa) and then dialyzed against 100 mL PBS buffer (pH 7.2 and I = 0.01 M) in an outer beaker at 37 °C. At each selected time interval (10 min, 20 min, 30 min, 1 h, 2 h, 3 h, 5 h, 10 h, 15 h, and 24 h), 3 mL dialyzate was taken out from the beaker and an equal volume of fresh PBS buffer was replaced to it. The released drug was evaluated by analyzing the emission intensity of CPT at 451 nm. Please note that the releasing curve of CPT alone cannot be obtained under the same experimental condition, because of its rather lower solubility in water.

Cytotoxicity Experiments. MDA-MB-231 human breast cancer cells and NIH3T3 mouse embryonic fibroblasts were cultured in Dulbecco's modified Eagle's medium (DMEM), which was supplemented with 10% fetal bovine serum (FBS), in 96-well plates (4×10^4 cells mL^{-1}, 0.1 mL per well) for 24 h. Then, the cells were incubated with CPT, GO-CD/HA-ADA, CPT@GO-CD/HA-ADA, CPT@GO-CD/HA-ADA in the presence of excess HA, and CPT@GO-CD ([CPT] = 1.0 μM, [GO-CD/HA-ADA] = 10.5 μg/mL, and [HA] = 0.6 mg/mL). After incubation for 24 h and 48 h, the relative cellular viability was measured using MTT assay. All data were shown as the mean ± standard deviation. Statistical data was analyzed by

using Student's t-test. Statistically significant differences were considered when the P value was < 0.05.

Preparation of GO-CD/HA-ADA conjugate. HA-ADA (10 mg) was added to an aqueous solution of GO-CD (2 mg/mL, 5 mL) and the mixture was ultrasonicated for 30 min. Then, the resulting solution was diluted to 50 mL and the homogeneous GOCD-HAADA solution was stored at 4 °C after filtering through a 0.45 μm filter.



Scheme S1. Synthetic route of HA-ADA.

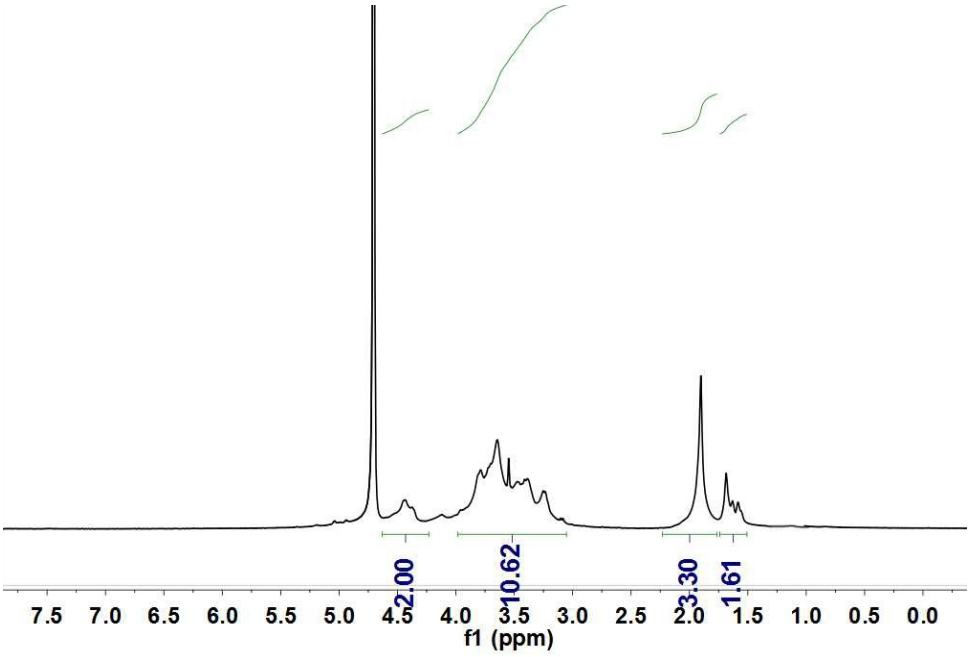


Fig. S1. ^{1}H NMR spectrum of HA-ADA (D_2O, 400 MHz, 25 °C). The degree of substitution was determined as 13% by the integral ratio of ethyl protons in adamantane (δ 1.57–1.75, 12H)

relative to the protons in HA backbone (δ 4.24–4.63, 2H).

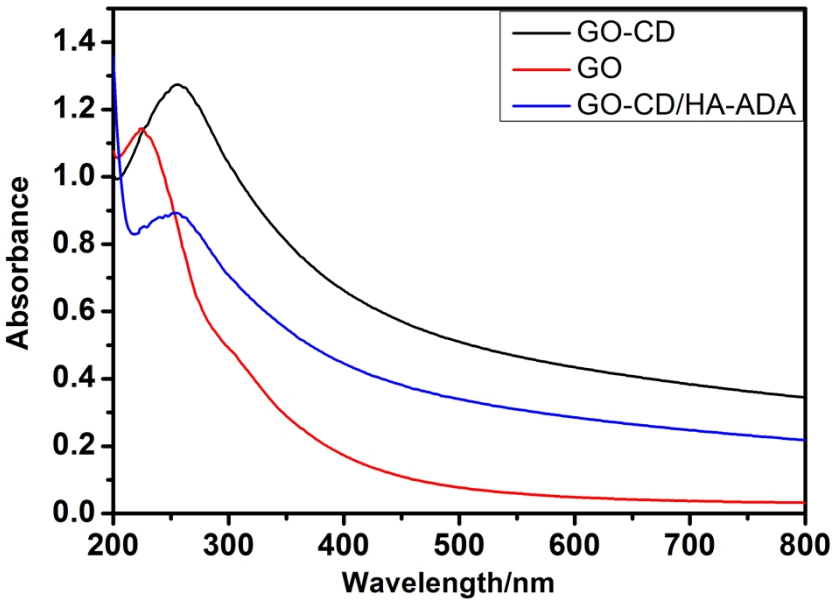


Fig. S2. UV/Vis spectra of (a) GO, (b) GO-CD, and (c) GO-CD/HA-ADA, respectively.

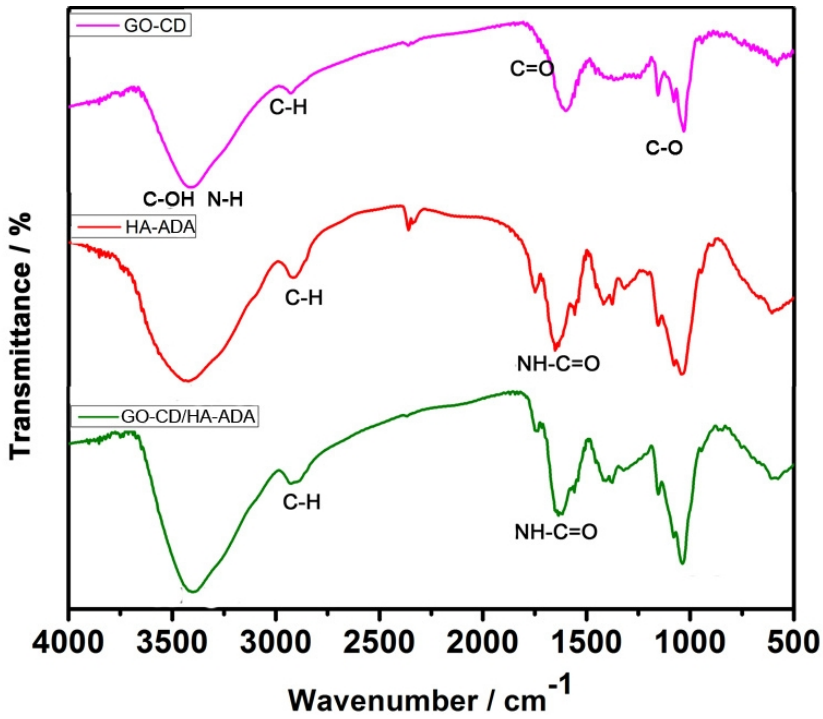


Fig. S3. FTIR spectra of GO-CD, HA-ADA, and GO-CD/HA-ADA complex, respectively.

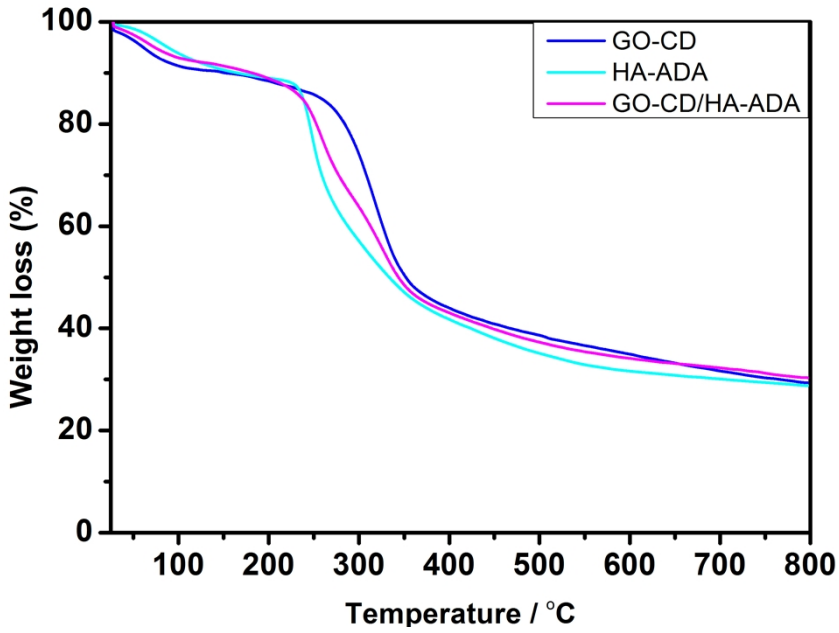


Fig. S4. TGA curves of GO-CD, HA-ADA, and GO-CD/HA-ADA complex, respectively.

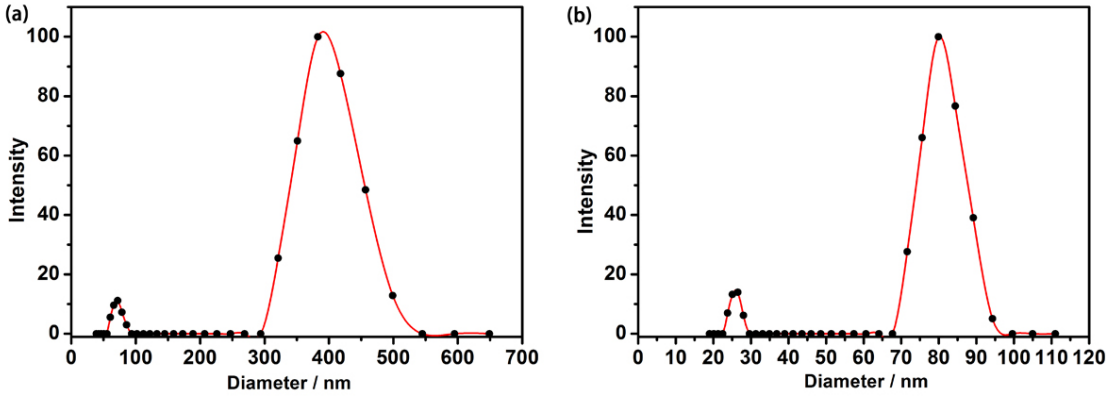


Fig. S5. Diameter distributions of (a) GO-CD and (b) GO-CD/HA-ADA (0.1 mg/mL).

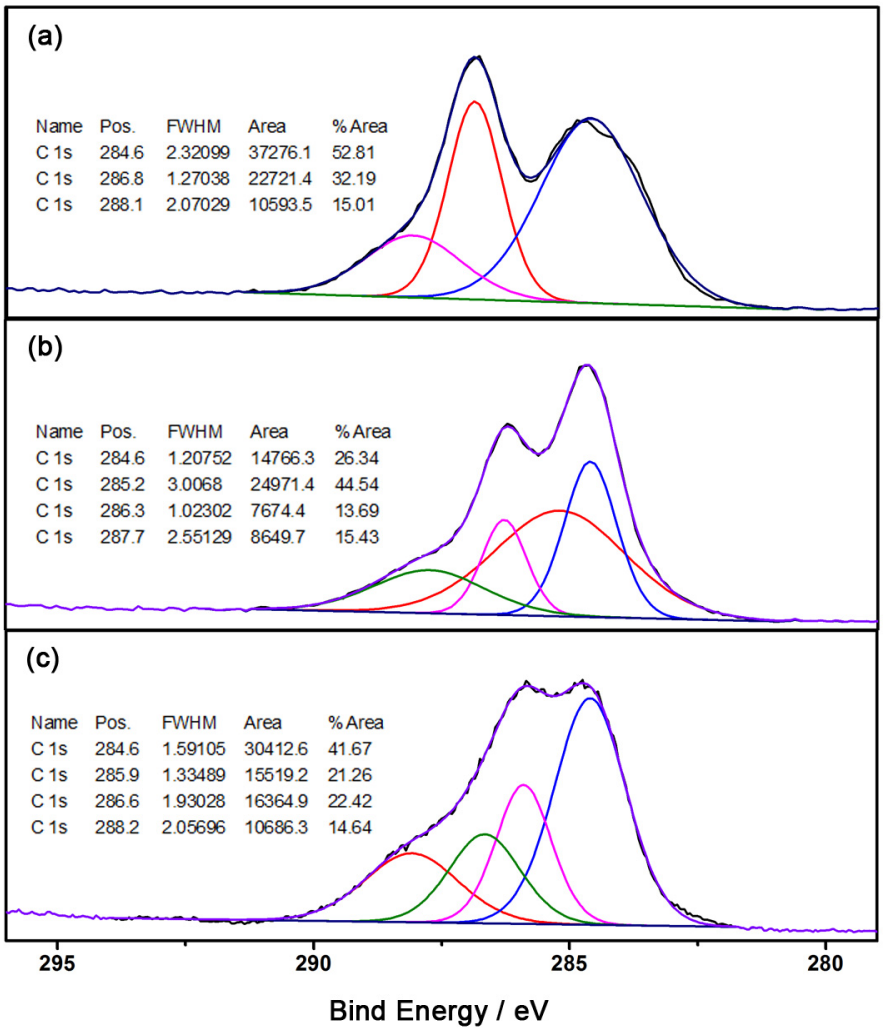


Fig. S6. Selected XPS C1s spectra of (a) GO, (b) GO-CD, and (c) GO-CD/HA-ADA complex. The C1s spectrum of GO was composed of three components (284.6, 286.8, and 288.1 eV). The peak at 284.6 eV was contributed to non-oxygenated graphite-like carbon, whereas the ones at 286.8 and 288.1 eV were assigned to C–O and C=O, respectively. After grafting with CD, the intensity of C–O at 286.3 eV was declined, directly proving that the epoxy groups were successfully removed in the amine-epoxy reaction. Moreover, the peaks at 285.2 eV in GO-CD and 285.9 eV in GO-CD/HA-ADA were originated from C–N groups in CD and HA units.

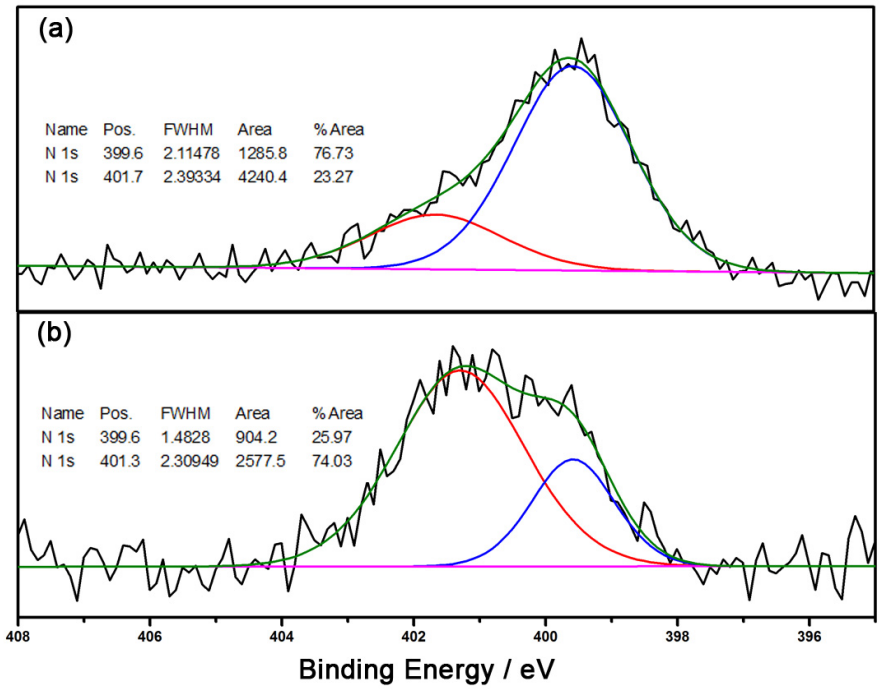


Fig. S7. Selected XPS N1s spectra of (a) GO-CD and (b) GO-CD/HA-ADA complex. The peaks at 399.6 and 401.7 eV in N1s spectrum of GO-CD could be assigned to C–N and $–NH_3^+$ (CO–NH), respectively. In comparison to GO-CD, XPS survey of GO-CD/HA-ADA gave a significant amount of N1s at 401.3 eV, which is ascribe to the amide groups on HA-ADA polymer.

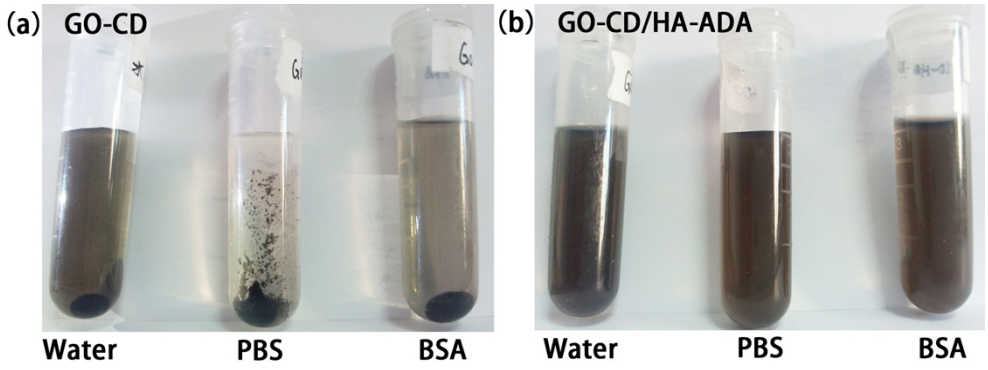


Fig. S8. Photos of (a) GO-CD and (b) GO-CD/HA-ADA (0.2 mg/mL) in water, PBS (0.1 M, pH 7.2), and BSA (38 mg/mL) solutions, respectively, after centrifugation at 10000 rpm for 10 min.

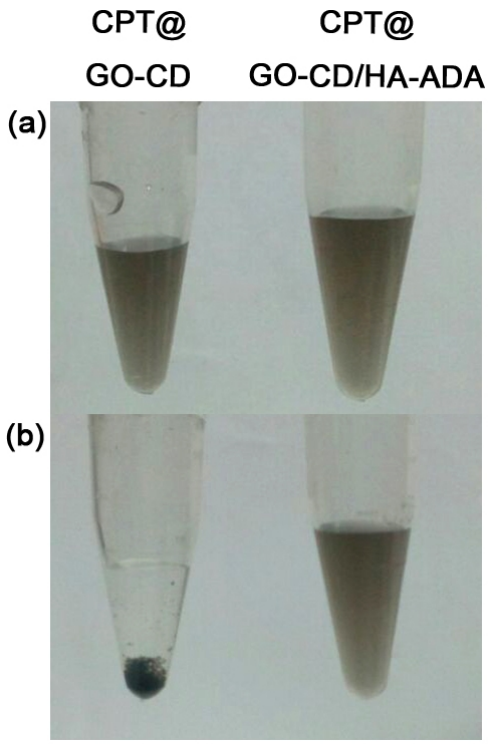


Fig. S9. Photos of CPT@GO-CD and CPT@GO-CD/HA-ADA (a) before and (b) after standing for 10 h in PBS (0.1 M, pH 7.2).

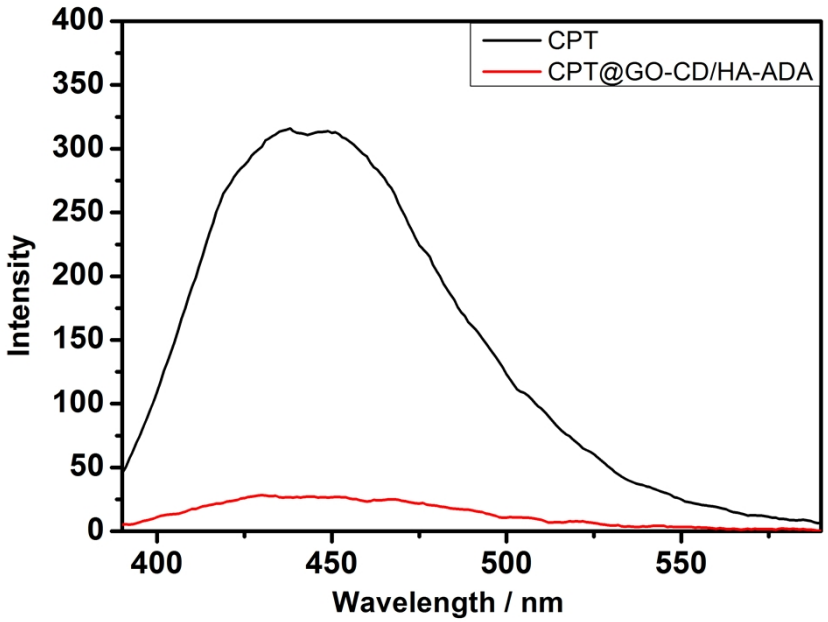


Fig. S10. Emission spectral changes of CPT and CPT@GO-CD/HA-ADA in water, respectively ([CPT] = 4.0 μM). The fluorescent spectrum of free CPT was obtained using 1%

DMSO as co-solvent.

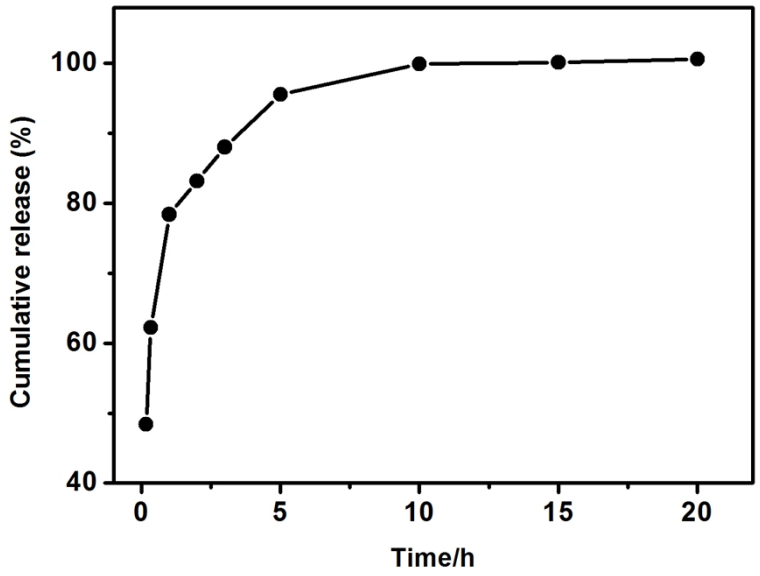


Fig. S11. Releasing profile of CPT from the CPT@GO-CD/HA-ADA conjugate *in vitro* in PBS (pH 5.7, I = 0.01 M) at 37 °C.

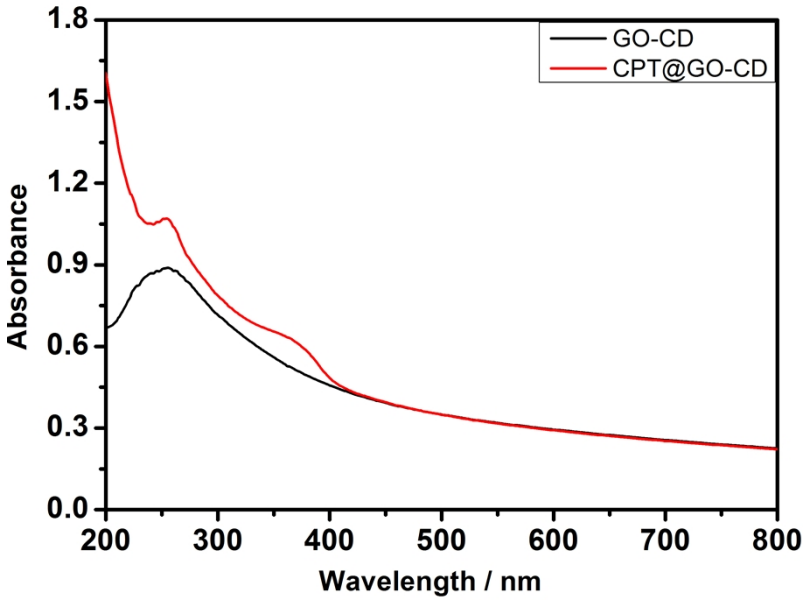


Fig. S12. UV/Vis absorption of GO-CD and CPT@GO-CD, respectively ([GO-CD] = 30 μg/mL).

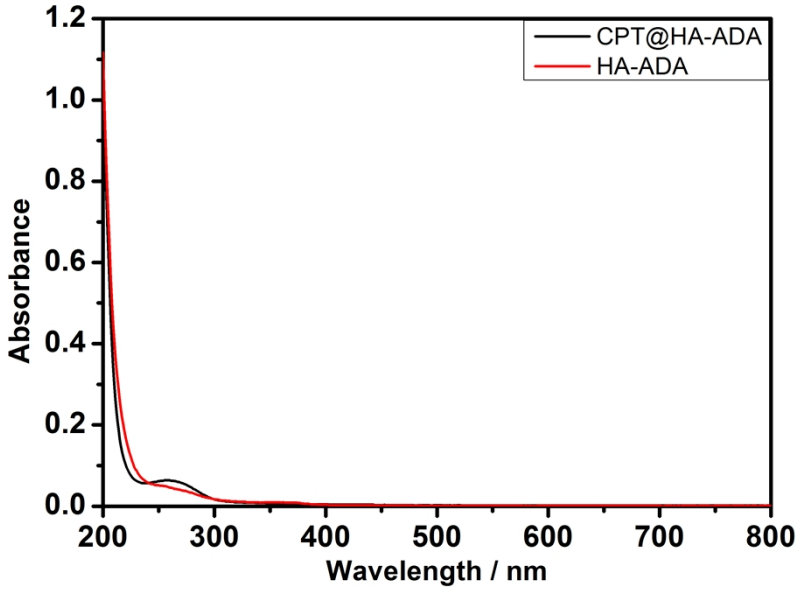


Fig. S13. UV/Vis absorption of HA-ADA and CPT@HA-ADA, respectively ([HA-ADA] = 30 μg/mL).

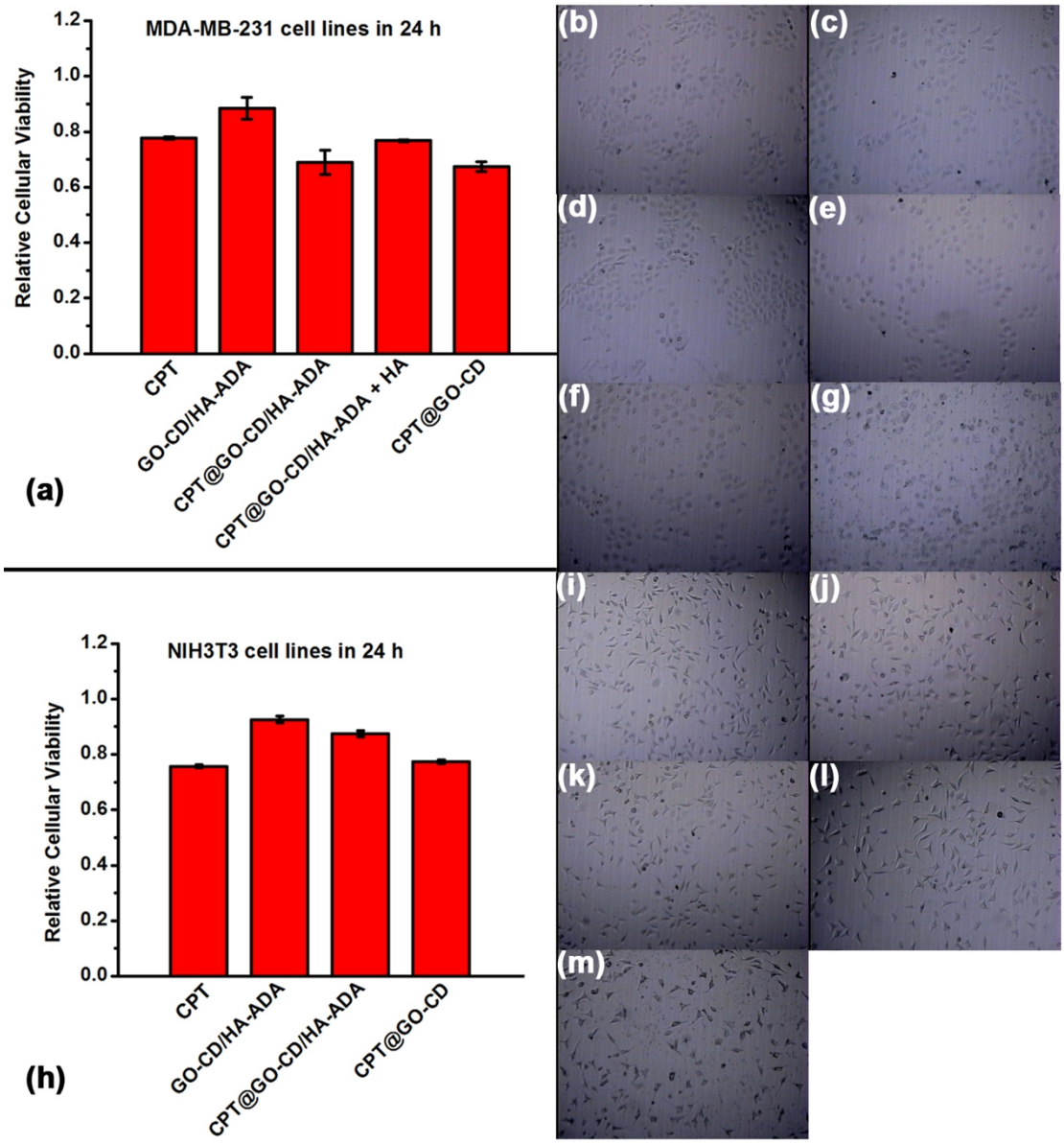


Fig. S14. Relative cellular viability and cell photos of (a-g) MDA-MB-231 and (h-m) NIH3T3 cell lines after the treatment with blank (b and i), CPT (c and j), GO-CD/HA-ADA (d and k), CPT@GO-CD/HA-ADA (e and l), CPT@GO-CD/HA-ADA with an excess of HA (f), and CPT@GO-CD (g and m), respectively, in 24 h incubation ([CPT] = 1.0 μM).

References

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