Supplementary Information

For

GSH Responsive Biotinylated Poly(vinyl alcohol) Grafted GO as a Nanocarrier for Targeted Delivery of Camptothecin

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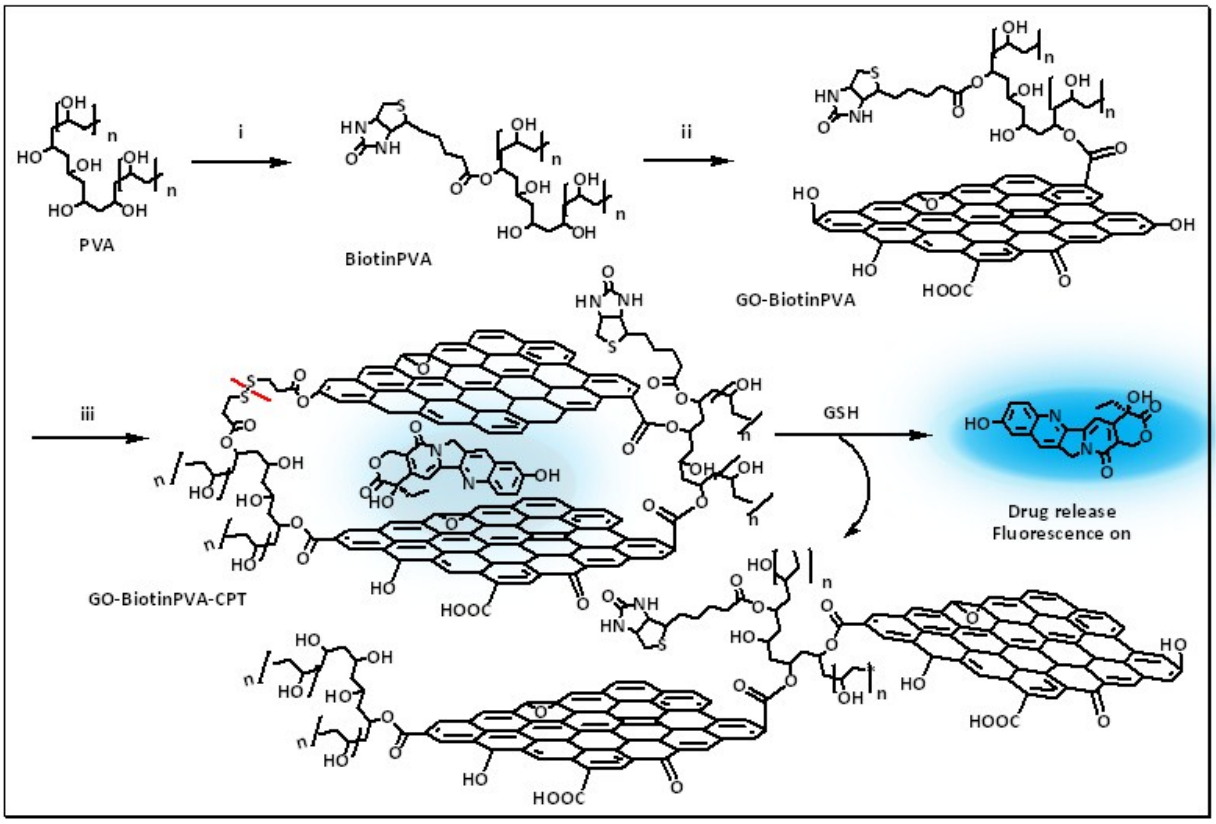
Materials, methods and instrumentations

Graphite with an average size of 100 nm (Aldrich), D-Biotin (Chemexper), EDC.HCl (Himedia, India), Poly (vinyl alcohol) (PVA; MW ~98, 000) (Aldrich), DMSO (Aldrich), camptothecin (CPT) (Chem-Implex Inc.) (GSH (Loba Chem., India), and DCM (Loba Chem. India) were purchased commercially and used without further purification. IR spectra were recorded using Thermo Scientific Nicolet IS10 IR spectrometer. NMR spectra were collected on a 400 MHz spectrometer (Bruker, Germany).UV-Visible spectra were obtained with UV-1800 spectrophotometer. Fluorescence spectra were obtained with Fp-8300 Jasco FL spectrometer. TEM images were obtained using Tecnai F20.AFM images were obtained by XE-70, Park Systems, South Korea.

Reaction Scheme: Synthesis of GO-biotinPVA-CPT



Reagents and Reaction conditions: i) D-biotin, EDC.HCl, DMAP, DMSO, RT, 24 h; ii) GO, EDC.HCl, DMAP, DMSO, RT, 24 h; iii) camptothecin (CPT), 3,3′-dithiodipropionic acid, EDC.HCl, DMAP, DMSO, RT, 24h.

Synthesis of GO

GO was synthesized using a modified Hummer's method from natural graphite powder. 3 g of graphite and 1.5 g of $NaNO_3$ were placed into a flask. Then, 250 mL of H_2SO_4 was added with stirring in an ice-water bath, and 9 g of $KMnO_4$was slowly added for about 1h. Stirring was continued for 2h in the ice-water bath. After the mixture was stirred vigorously at room temperature for 2 days, 100 mL of 5 wt% H_2SO_4 aqueous solutions was added for about 1h with stirring and the temperature was kept at 98 °C. The resultant mixture was further stirred at 98 °C for 2h. The temperature was reduced to 60 °C, and 3.0 mL of H_2O_2 (30.0 wt% aqueous solution) was added, and the mixture was stirred at room temperature for 2h. The oxidation product was purified by rinsing with a 10% HCl solution, repeatedly washing with copious amounts of de-ionized water, and filtering through a 0.2 μm Nylon membrane to afford 1 g of GO.

Synthesis of biotinPVA

500 mg of PVA was dissolved in 15 mL DMSO and stirred for 30 min to obtain a homogeneous solution. Then, D-Biotin (200 mg, 0.82 mmol), EDC.HCl (236 mg, 1.24 mmol) and DMAP (75 mg, 1.23 mmol) were added successively into the flask and the resulting mixture was stirred at RT for 2 days. After the reaction was terminated, the reaction mixture was diluted with ethyl acetate, centrifuged and dialyzed to afford biotinPVA as white color solid. This compound was confirmed by H-NMR and FT-IR. One biotin unit present per ~20 mer unit polymer as per proton integration.

Synthesis GO-biotinPVA

200 mg purified GO and BiotinPVA (500 mg) was dissolved in 25 mL of DMSO and stirred for 30 min to obtain a homogeneous solution. The catalysts, EDC.HCl (100.0 mg, 0.52 mmol) and DMAP (20.0 mg, 0.16 mmol) were gradually added into the flask and stirred at RT for 2 days. After the reaction was terminated, the suspension was filtered through a 0.2 μm PTFE microporous membrane, and the obtained solid was washed thoroughly with DMSO and Ethyl acetate in order to eliminate the unreacted PVA and dried in vacuum afforded GO-biotinPVA as black solid. We confirmed by IR.

Synthesis of GO-biotinPVA-CPT

200 mg of GO-biotinPVA (500 mg) was dissolved in 25 mL of DMSO and stirred for 30 min to obtain a homogeneous solution. 3,3-Dithipropionic acid (200.0 mg, 0.952 mmol), EDC.HCl (275.0 mg, 1.5 mmol) and DMAP (20.0 mg, 0.16 mmol) were gradually added into the flask and

then CPT (200 mg, 0.574 mmol) was added to the reaction mixture and stirring continued for 1 day. Excess CPT precipitated as solid was removed by centrifugation. The supernatant was filtered through a 0.8 μm filter to fully remove any solid. The solution was then dialyzed (molecular weight cut-off (MWCO) = 3 kDa) against distilled water for 2 days to remove the small amount of solubilized free CPT and DMSO. The produced compound GO-biotinPVA-CPT was preserved in darkness at 4°C.

Estimation of CPT loading on GO-biotinPVA

We used standard protocol to calculate the drug loading to the GO as described in previous literature.[1] In brief, as per Lambert beer law , absorbance = εcl; where ε =molar extinction coefficient; C = concentration; l= path length. Here l =1; ε =19.9 mM^{-1}; increment of absorbance at 370 nm = 0.1285. Applying the Lambert beer law the concentration of the solution (c) = 0.1285/19.9 : 0.00648 mM. This means in 1000 mL contains 2.257 mg of CPT. We used 15.0 mg/L solution of nanoparticles for this study. Finally it comes 14.99 % = ~ 15%.

Absorption and fluorescence studies

All fluorescence and UV-Visible spectra were obtained with Fp-8300Jasco FL spectrometer with a 1cm standard quartz cell and UV-1800 spectrophotometer, respectively. Stock solutions (1mM) of various analytes (H_2O_2, NO_2^-, Fe^{2+}, Zn^{2+}, NO_3^-, GSH, ·OH, and Cu^{2+}) were prepared in deionized water. The stock solution of GO-biotinPVA-CPT was prepared in PBS buffer (pH = 7.4) with 10% DMSO. Excitation was carried out at 370 nm with excitation and emission slit width 2.5 nm each. The Fluorescence experiments (solution test) of GO-biotinPVA-CPT (300 μg/mL) recorded in the presence of increasing concentrations of GSH (0–15mM) in PBS buffer (pH = 7.4).

Microscopic studies (TEM & AFM)

The GO, GO-biotinPVA and GO-biotinPVA-CPT dispersions were characterized by transmission electron microscopy (Tecnai F20) fitted with a CCD camera at an acceleration voltage of 200kV. The samples for TEM measurements were prepared by dissolving it in the distilled water and casted a small drop of dilute GO, GO-biotinPVA and GO-biotinPVA-CPT solution on a carbon-coated copper grid, allowed to dry in air, at room temperature. Atomic force microscopy (AFM) was conducted in the noncontact mode at a resonance frequency of ~250 kHz

of the tip. A drop of dilute sample solution (GO, GO-biotinPVA and GO-biotinPVA-CPT) was casted on a clean silicon wafer surface, and the morphology of the vacuum-dried sample was studied using an AFM instrument (XE-70, Park Systems, Korea).

Cell Culture

HeLa cells and 3T3-L1 mouse fibroblast cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10 % fetal bovine serum (v/v), 1 % penicillin, 1 % streptomycin and 0.1 % Amphotericin B.

Cell Viability Assay

The cell viability was measured using MTT (3-(4, 5-dimethyl-2-thiazolyl)-2, 5-diphenyl-2H-tetrazolium bromide) assay. HeLa cells and 3T3-L1 cells were seeded at a density of 5000 cells/well in a 96 well microtiter plate and incubated overnight. The cells treated with and without CPT and GO-biotinPVA-CPT at a range of concentrations varying from 0-160 nM. MTT was added at a concentration of 5 mg/mL into each well and incubated for 3 hours at 37 °C. DMSO was used for MTT solubilisation. The absorbance was read at 590 nm with a reference filter of 620 nm using Synergy HT Multi-Mode Microplate Reader (BioTek). MTT assays were performed after 48h of treatment. The same protocol was followed for checking the viability of HeLa cells pre-treated with 100.0 μM biotin for 2h and then treated with GO-biotinPVA-CPT.

Effect of CPT and GO-biotinPVA-CPT on morphology of HeLa cells and 3T3L1 cells

HeLa and 3T3L1 cells were seeded at a density of 0.5x 10^6 cells/ml and were cultured in DMEM medium supplemented with 10% FBS and incubated overnight. The cells were treated in the absence (control cells), or in the presence of CPT and CPT in GO-biotinPVA-CPT at 372 μg/L for 24 h at 37°C. Morphological changes of treated cells were observed by inverted microscopy and compared with control cells.

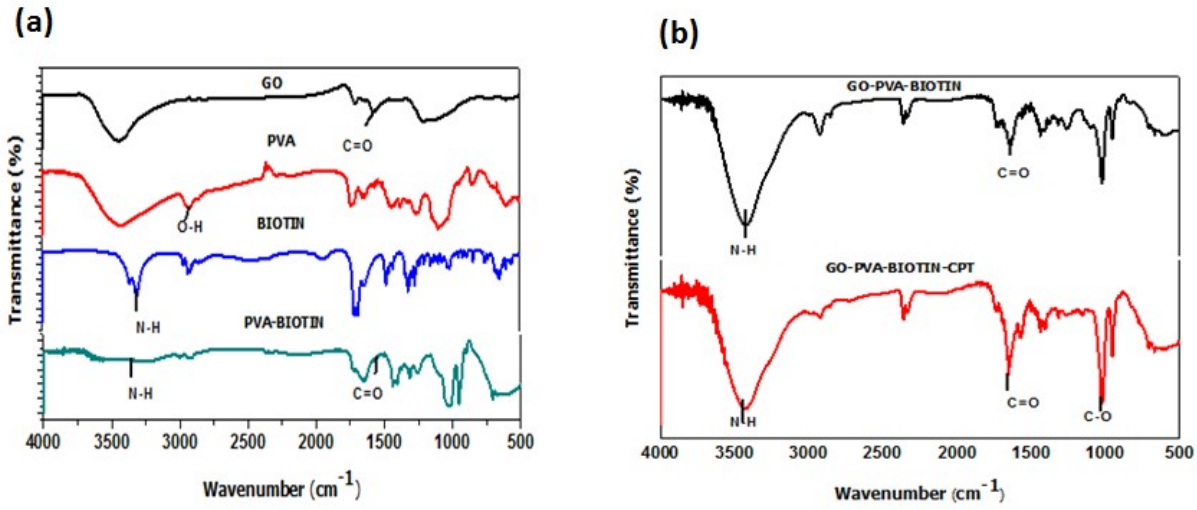


Fig. S1 FTIR spectra for (a) biotin, PVA, GO, biotinPVA; (b) GO-biotinPVA, GO-biotinPVA-CPT.

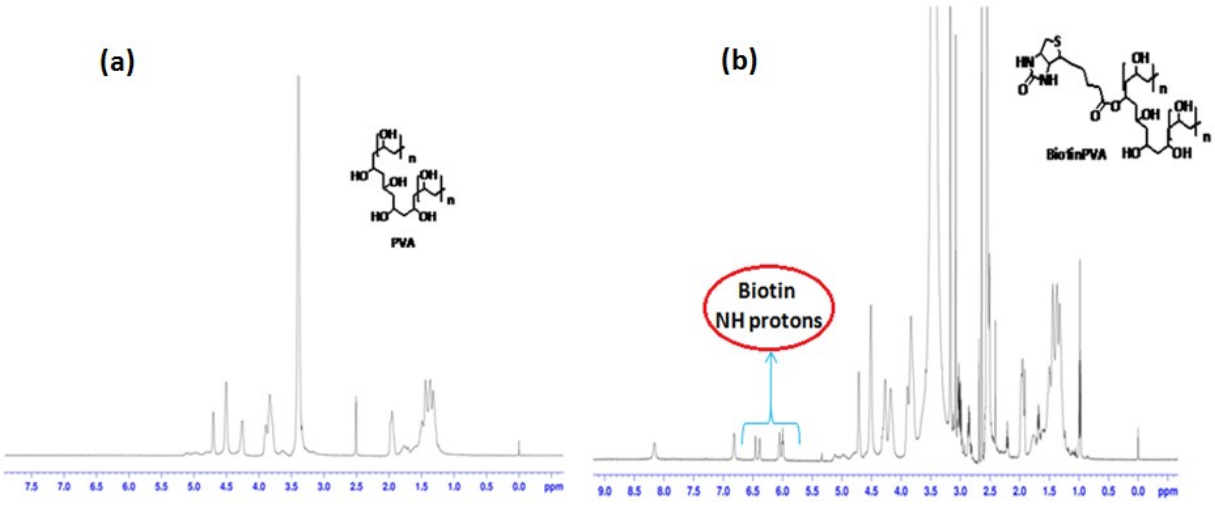


Fig. S2 ^{1}H-NMR spectra of (a) PVA; (b) biotinPVA in DMSO-d_6.

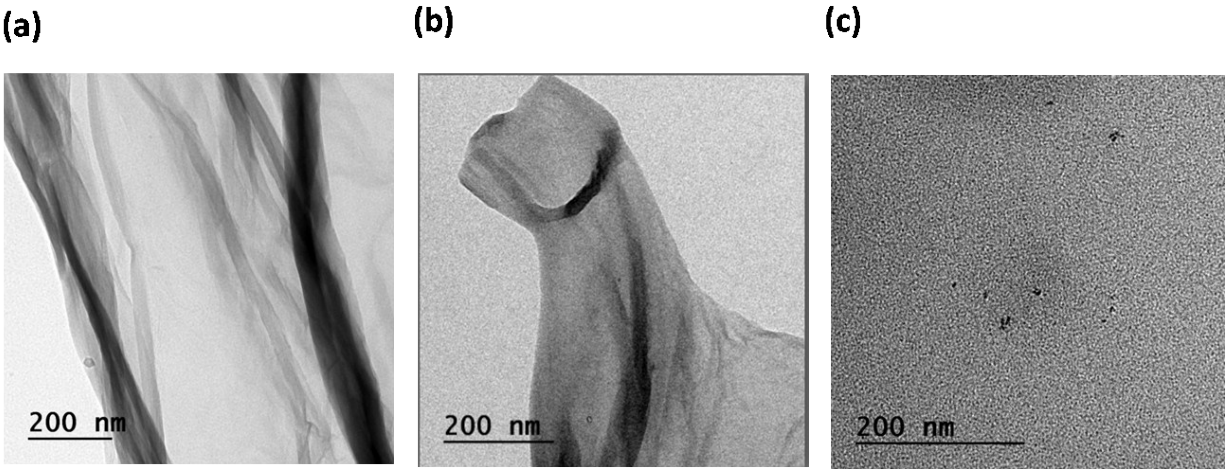


Fig. S3 TEM images of (a) GO; (b) GO-biotinPVA; (c) GO-biotinPVA-CPT.

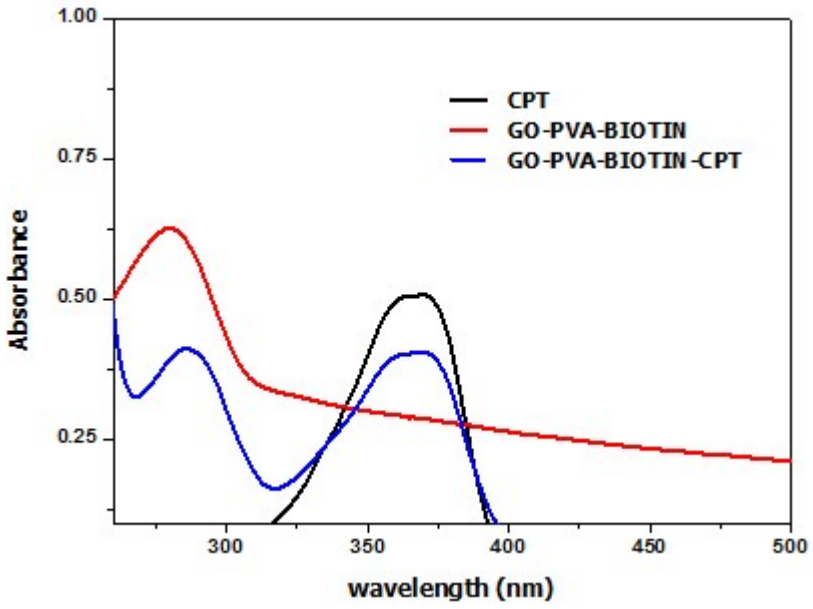


Fig. S4 UV absorbance spectra of GO-biotinPVA (15.0 mg/L) after GO-biotinPVA- CPT (15.0 mg/L) and CPT (10.μM) in to the GO –biotinPVA in PBS buffer solution (pH 7.4) at 37 °C.

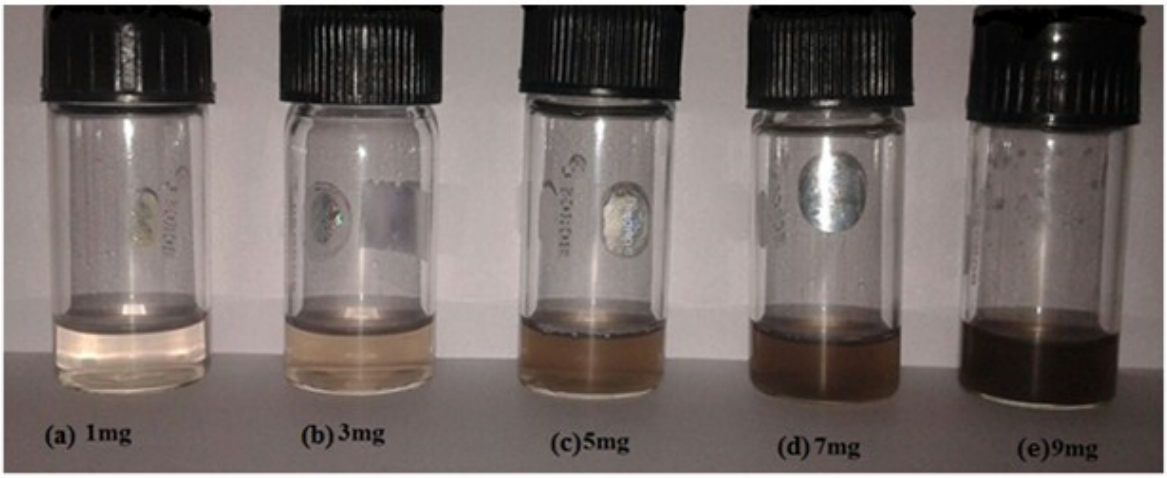


Fig. S5 Photographs of GO-biotinPVA-CPT at different concentrations 0 mg, 3.0 mg, 5.0 mg, 7.0 mg, and 9.0 mg in 3 mL PBS buffer (pH 7.4) solution.

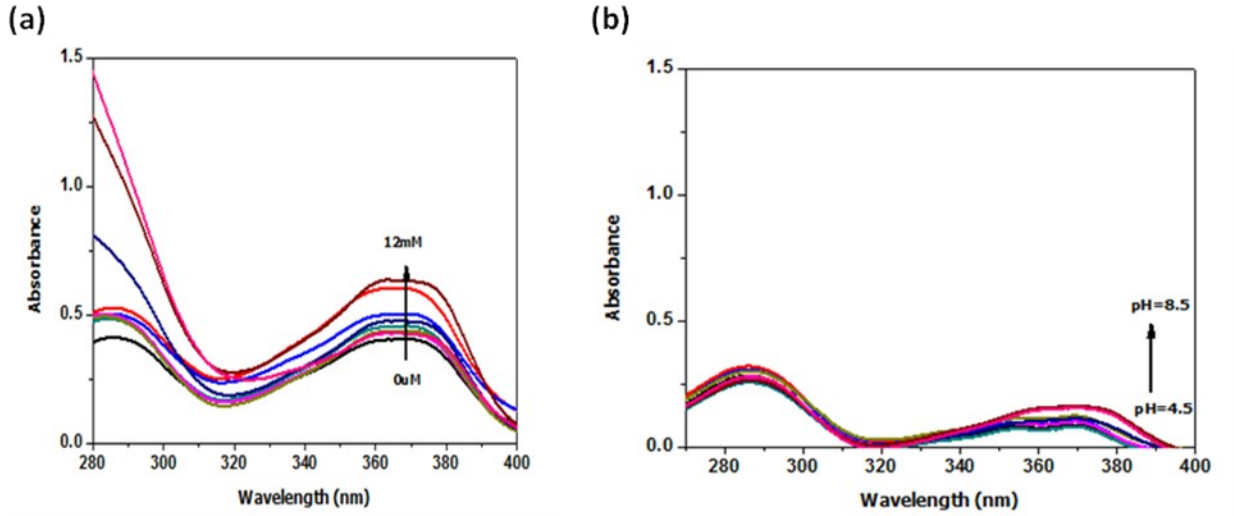


Fig. S6 UV absorbance spectra of (a) GO-biotinPVA-CPT,(300 µg/mL) in the presence of different concentrations of GSH (0.0 µM – 12.0 mM) in PBS buffer solution (pH=7.4; 10% DMSO) at 37 °C; (b) GO-biotinPVA-CPT,(300.0µg/ mL) for different pH (4.5-8.5) in PBS buffer solution at 37 °C.

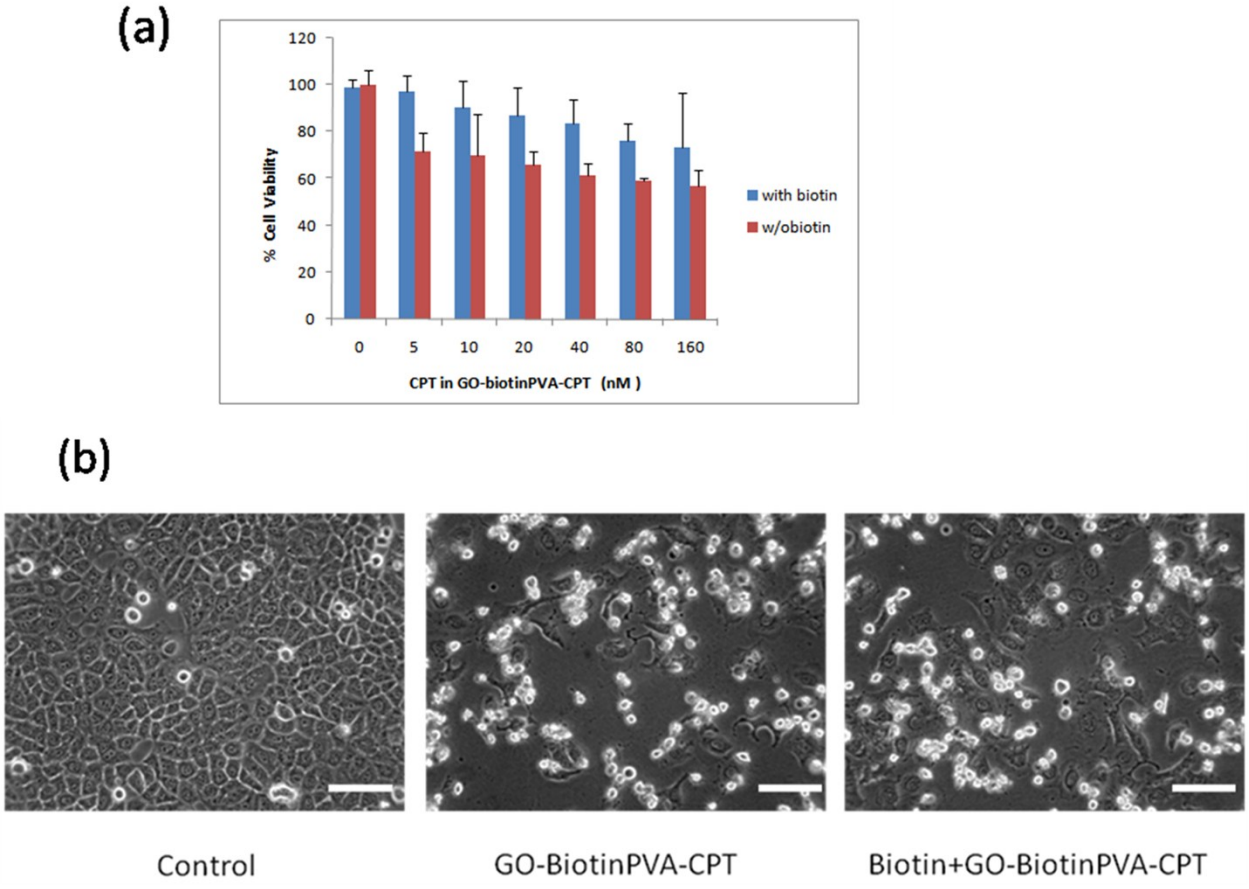


Fig. S7 Mechanism of cellular uptake of GO-biotinPVA-CPT in Hela cells: (a) Viability of the cells pretreated with 100 μM biotin and then further treated with different concentration of CPT in the GO-biotinPVA-CPT was measured by MTT assay after 24h. (b) Inverted microscopic images of control HeLa cells, GO @ GO-biotinPVA-CPT (160 nM) treated and biotin (100 μM) + CPT @ GO-biotinPVA-CPT (160 nM) treated cells (Scale bar 100 μm).

References

1. N. G. Sahoo, H. Bao, Y. Pan, M. Pal, M. Kakran, Henry K. F. Chen, L. Li and L. P. Tan, *Chem. Commun.,* 2011, **47**,5235.