Supporting Information

Polyethylenimine Coated Graphene-Oxide Nanoparticles for Targeting Mitochondria in Cancer Cells

Abhik Mallick, [a,†] *Aditi Nandi,* [a,†] *and Sudipta Basu* [b,*]

a. Department of Chemistry, Indian Institute of Science Education and Research (IISER)-Pune, Dr. Homi Bhabha Road, Pashan, Pune, Maharashtra, 411008, India

b. Discipline of Chemistry, Indian Institute of Technology (IIT)-Gandhinagar, Palaj, Gandhinagar, Gujarat, 382355, India

† These authors contributed equally to this work.

* Email: sudipta.basu@iitgn.ac.in

1. Material:

All commercially obtained compounds were used without further purification. Graphene oxide (4mg/ml), polyethyleneimine, JC 1 dye, TMRM, Calcein AM, sodium dodecyl sulfate (SDS), and silicon wafer for FE-SEM were bought from Sigma-Aldrich. Cisplatin and topotecan were bought from Selleck Chemicals. H_2DCFDA was purchased from MitoBiogenesis™. DMEM media and 3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were purchased from HiMedia. 96 well plates, 6 well plates, 15 mL and 50 mL graduated sterile centrifuge tubes and tissue culture flasks with filter cap sterile were purchased from Tarsons Product Pvt. Ltd. HeLa cells were obtained from National Centre for Cell Science (NCCS), Pune. The drug loading and cell viability assay were plotted using GraphPad Prism software. The laser scanning confocal microscopy was performed by Zeiss LSM 710 machine. Each sample was done in triplicate.

2. Methods:

2.1 Synthesis of PEI-GTC-NPs:

Graphene oxide (4 mg/mL, 250 μL) was dispersed in distilled water (1 mL). Aqueous solution of topotecan (5 mg) in distilled water (1 mL) was prepared and added to the dispersed graphene oxide solution. The reaction was stirred in dark at room temperature overnight. To remove unreacted topotecan, the reaction mixture was dialysed against distilled water through dialysis membrane (MWCO= 1000 Dalton) for 24 hr. Further aquated cisplatin (5 mg/ml) was added to graphene oxide-topotecan conjugate (GT) and stirred at RT overnight. After completion of reaction, the mixture was again dialysed against distilled water through dialysis membrane (MWCO= 1000 Dalton) for 7 hr to remove excess of aquated cisplatin. Finally the graphene oxide-topotecan-cisplatin (GTC) obtained was coated polyethyleneimine (1mg/mL) by sonicating for 24 hr. The excess polyethyleneimine (PEI) was removed by centrifugation to obtain PEI-GTC-NPs.

2.2 Dynamic light scattering (DLS) and field-emission scanning electron microscopy (FESEM)

The surface charge (zeta potential) of GO, GT, GTC-NP and PEI-GTC-NP was measured by dynamic light scattering (DLS) method using Zetasizer Nano2590 (Malvern, UK). Size and shape was visualized by FESEM over silicon wafer. The silicon wafer was gold coated (30-40 nm thickness) using Quorum, Q150T- E5. The FESEM measurements were done using Carl Zeiss, Ultra plus, scanning electron microscope at an operating voltage of 4.0 KV. Sample preparation for zeta potential and FESEM was performed using the method described in reference 1.

2.3 Fluorescence spectroscopy:

Steady state fluorescence spectroscopy was recorded using a Flouromax-4 (HORIBA scientific, USA). The samples were suspended in water and the emission spectra for topotecan was checked at λ_{max} = 525 nm. The fluorescence spectra of free topotecan in the same concentration was also checked at emission wavelength λ_{max} = 525 nm.

2.4 Resonance Raman Spectroscopy:

Resonance Raman spectra were collected using a Lab RAM HR 800 (Horiba scientific) using laser excitation wavelength of 532 nm excitation with a 50X objective at room temperature. 532 nm was chosen as the excitation to guarantee a good signal/noise ratio. Prior to analysis the baseline of the spectrum was extracted using the software NGSLabSpec.

2.5 Determination of drug loading:

Loading of topotecan and cisplatin in PEI-GTC-NPs was measured by UV-Vis spectroscopy using concentration versus absorbance calibration graph. Sample preparation and calibration graph determination for drug loading was done by using the method described in references 2 and 3.

2.6 Confocal laser scanning microscopy:

2.6.1 Mitochondrial localization:

1.5 X 10^4 HeLa cells were treated with PEI-GTC-NPs at 6 h, 12 h and 24 h time points. The mitochondria were stained by MitoTracker Deep Red. The visualization of the cells were performed under confocal microscopy.[2]

2.6.2 JC1, TMRM, Calcein AM and ROS generation assays:

1.5 X 10^4 HeLa cells were treated with PEI-GTC-NPs for 24 h followed by staining the cells with JC1 or TMRM or Calcein AM or H_2DCFDA dye. The cells were imaged by confocal laser scanning microscopy.[1]

2.7 Cell viability assay:

5 x 10^3 HeLa cells were seeded per well in 96-well microlitre plate and incubated overnight in a 5% CO2 incubator at 37°C for attachment. Cells were then treated with PEI-GTC-NPs and GO, topotecan and cisplatin combination in different concentrations (0.11, 0.23, 0.46, 0.92, 1.87, 3.75, 7.5, 15, 30 μM) for 48 h. The cell viability was measured using the MTT assay. The percent cell viability was calculated considering the untreated cells as 100 percent viability and the effectiveness of GTC and GTCP nanoparticles was compared with the free drug combinations.[3]

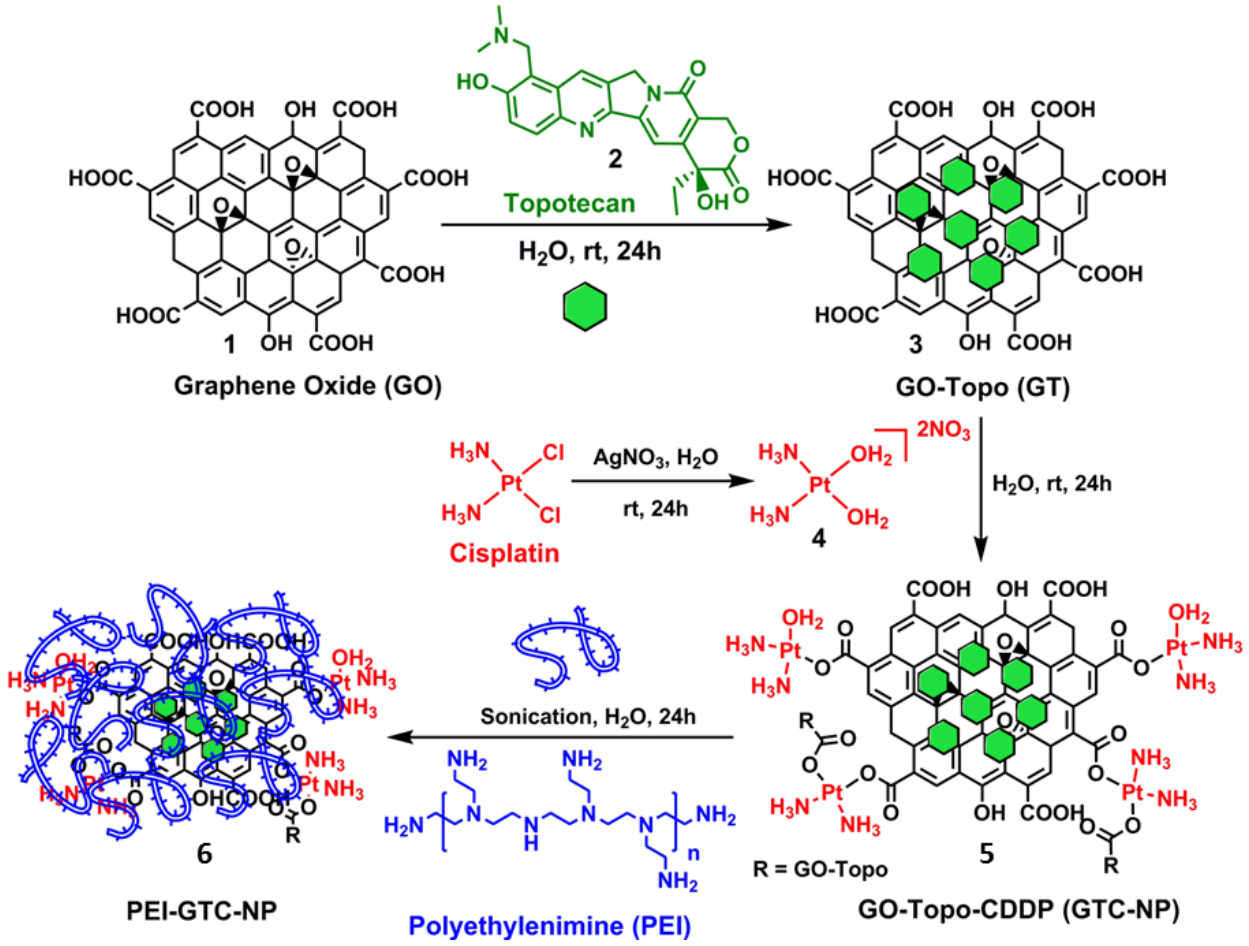


Figure S1: Synthetic scheme of PEI-GTC-NPs from graphene oxide.

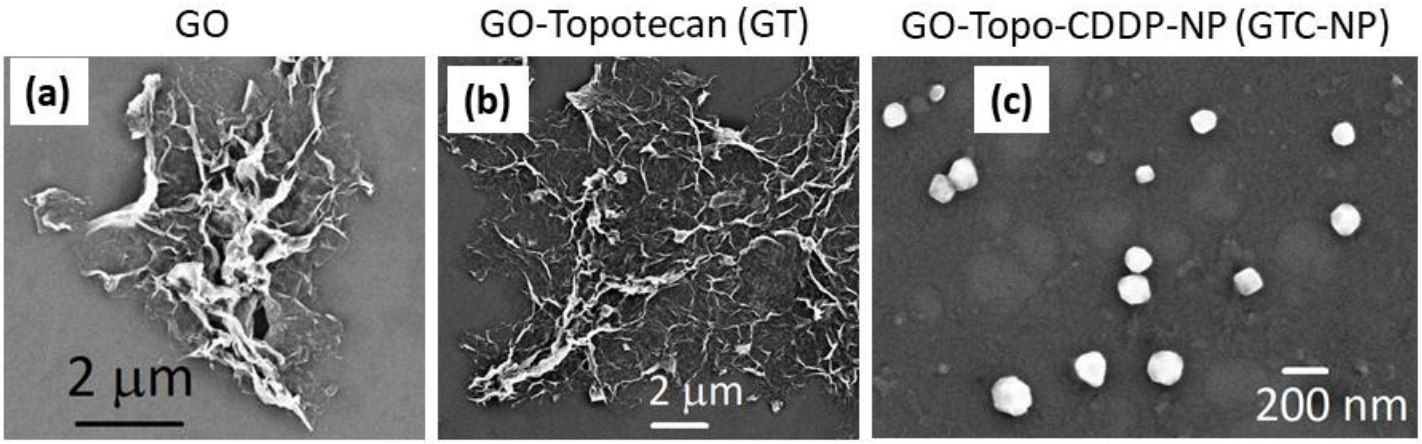


Figure S2: FESEM images of (a) GO, (b) GO-Topotecan conjugate (GT) and (c) GTC-NPs.

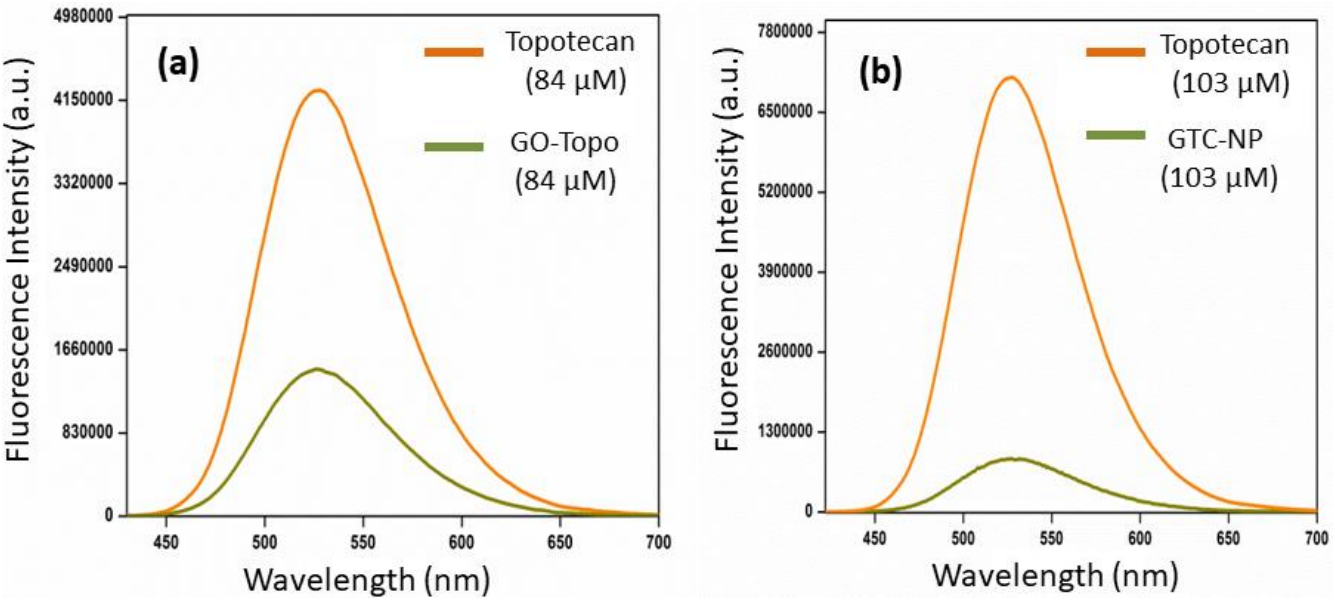


Figure S3: Fluorescence emission spectra of (a) GO-topotecan conjugate (GT) and (b) GTC-NP showing the fluorescence quenching of topotecan after stacking with GO surface.

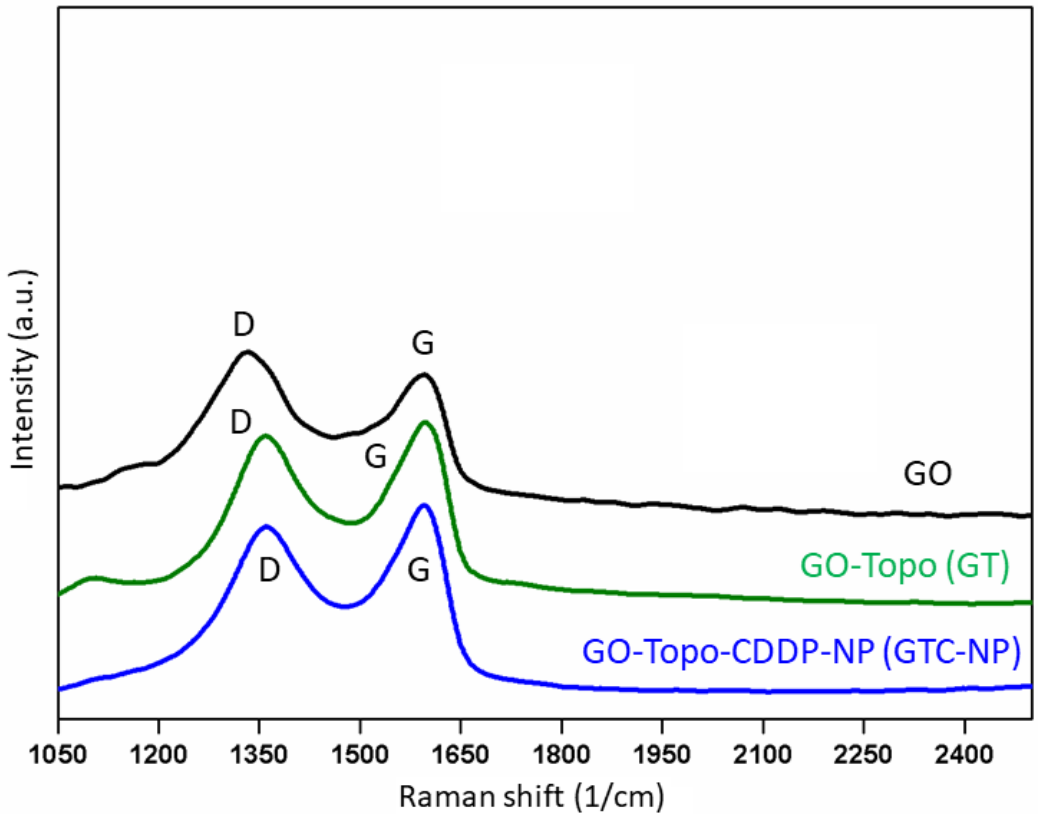


Figure S4: Resonance Raman spectra of GO, GT and GTC-NP showing the characteristic D and G bands to confirm the presence of GO.

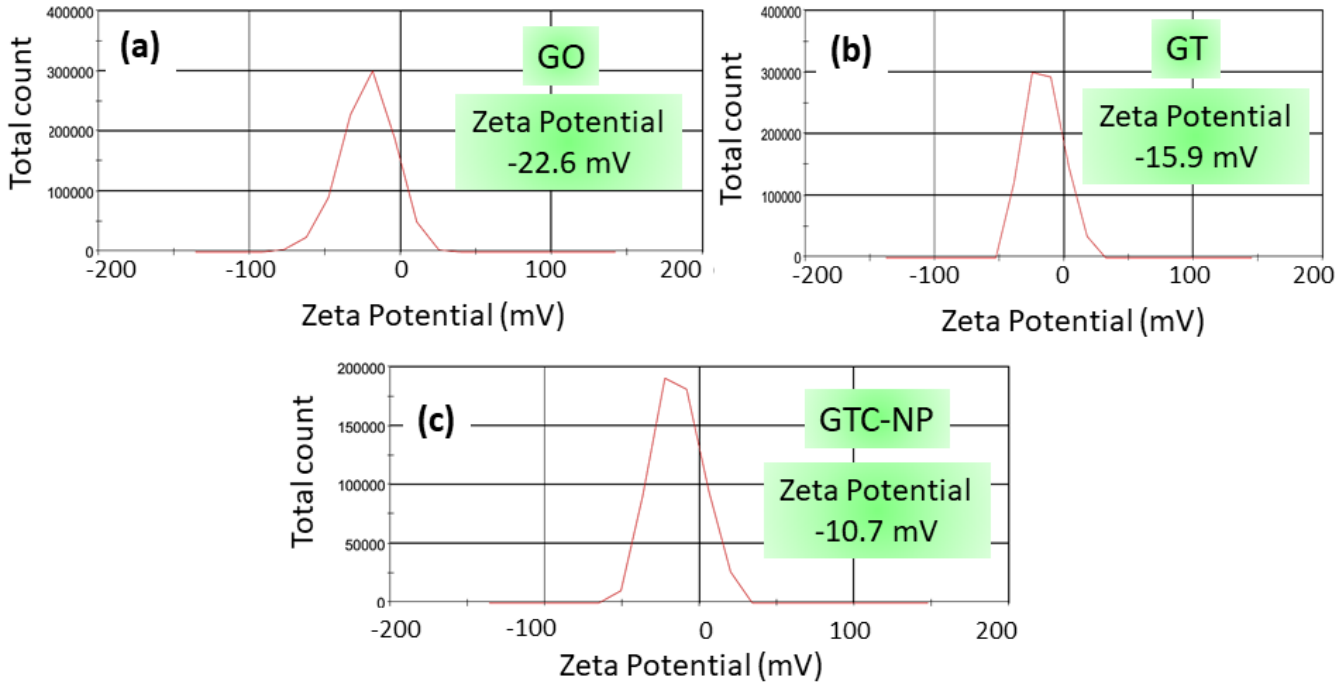


Figure S5: Zeta potential of (a) GO, (b) GT and (c) GTC-NP to show their surface charge.

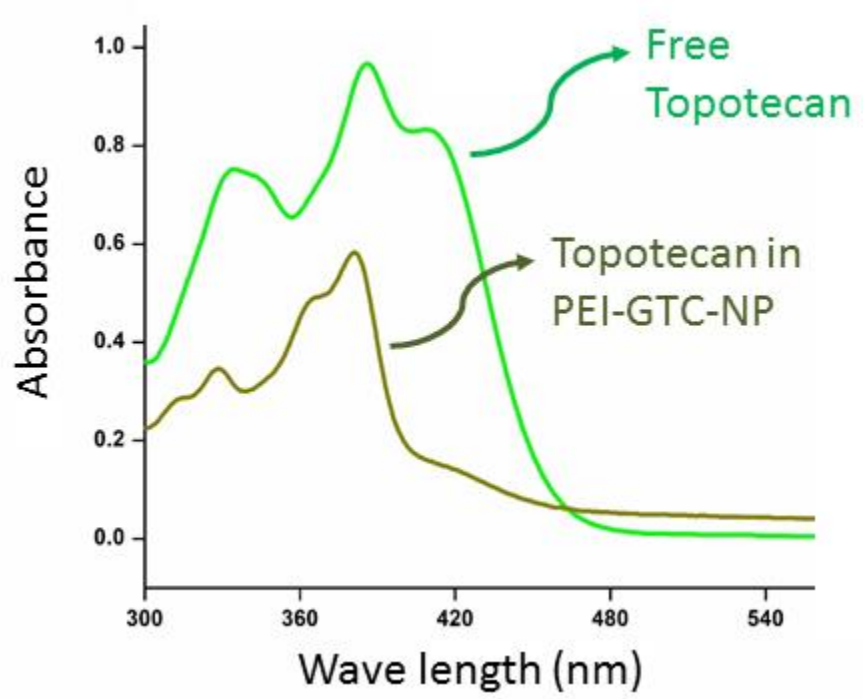
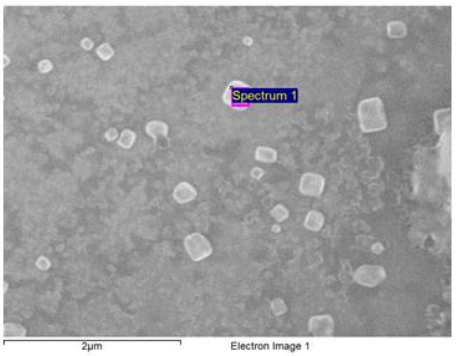


Figure S6: UV-Vis spectra of free topotecan and PEI-GTC-NPs to show the presence of topotecan in the nanoparticle.



Element	Weight%	Atomic%
C	44.05	53.83
N	22.54	23.62
O	23.79	21.83
Pt	9.61	0.72

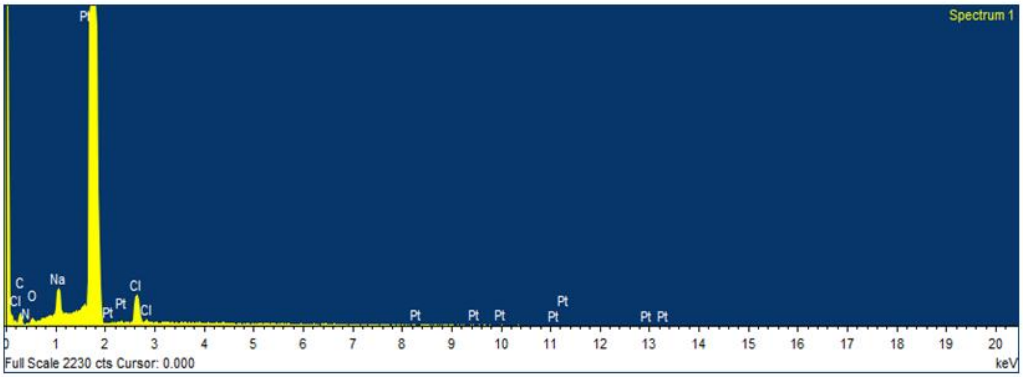


Figure S7: EDX of PEI-GTC-NPs from FESEM showing the presence of cisplatin.

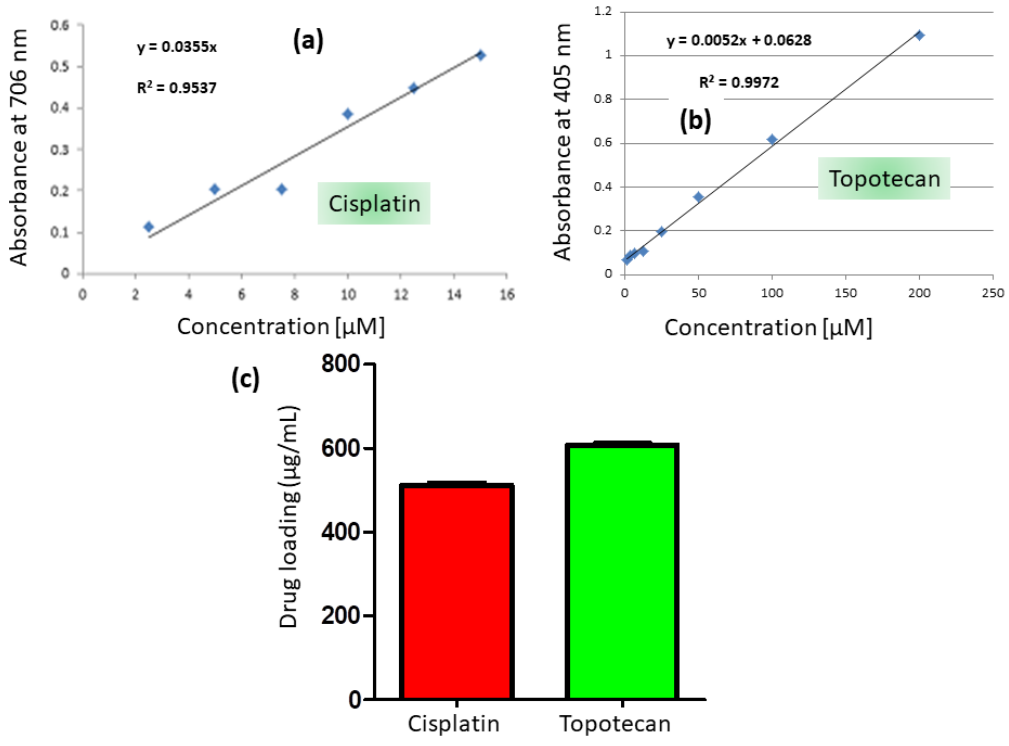


Figure S8: (a, b) Absorbance versus concentration calibration graph of cisplatin and topotecan at characteristic λmax = 706 nm and 405 nm respectively from UV-Vis spectra. (c) Loading of cisplatin and topotecan in PEI-GTC-NPs.

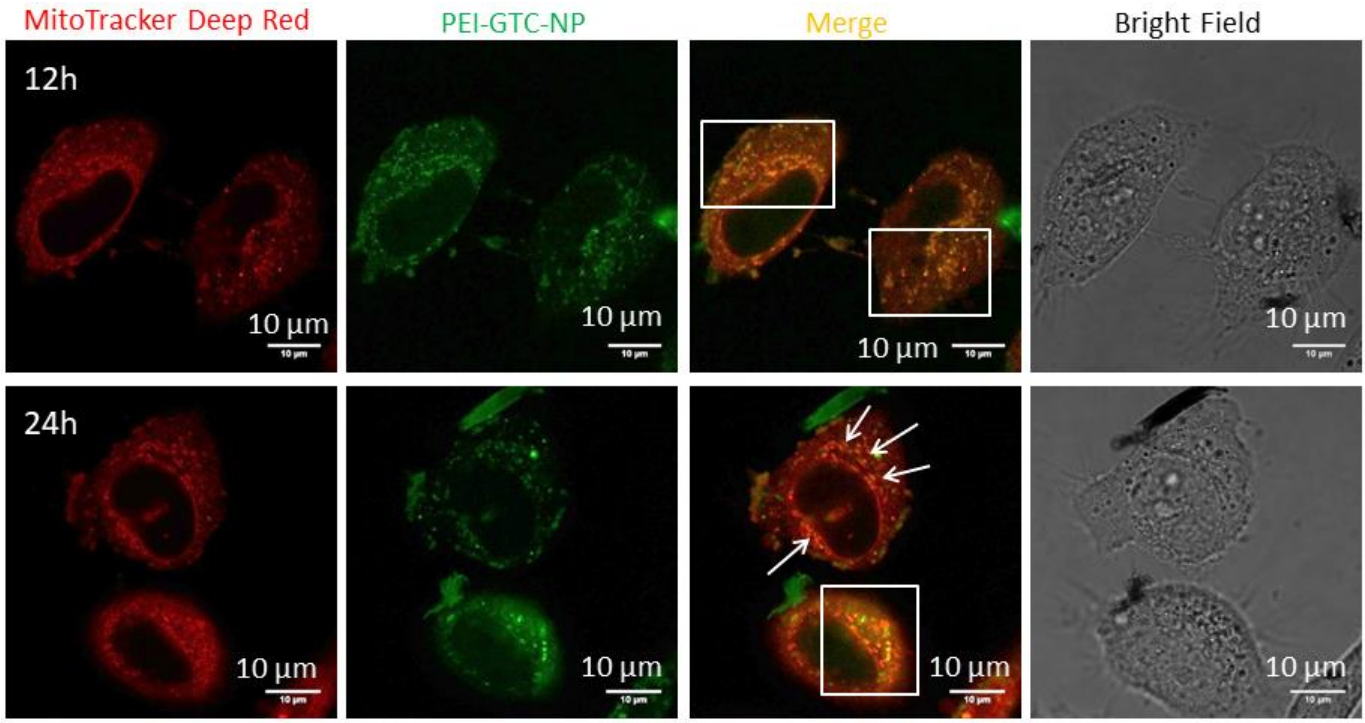


Figure S9: Confocal laser scanning microscopy (CLSM) of HeLa cells treated with PEI-GTC-NPs for 12 h and 24 h. Mitochondria were stained with MitoTracker Deep Red dye. Yellow regions in merged images are showing the localization of PEI-GTC-NPs in mitochondria. Scale bar = 10 µm.

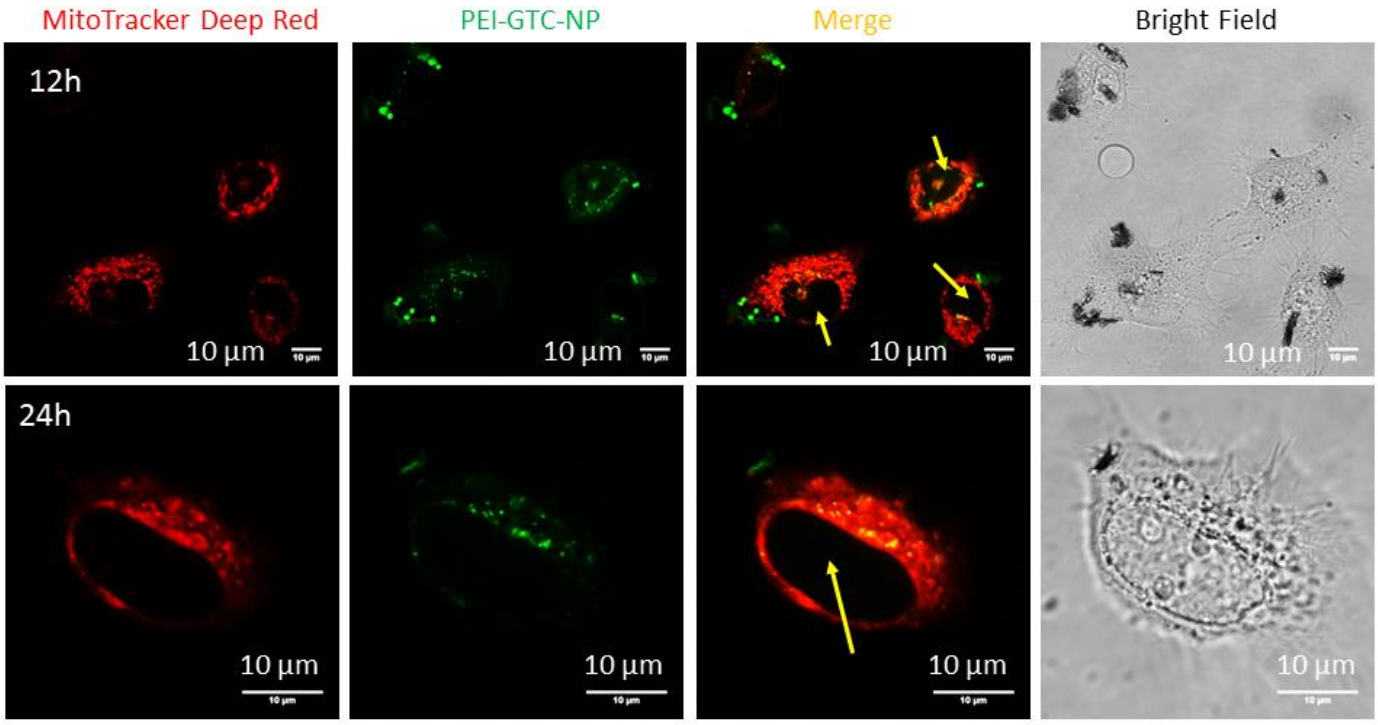


Figure S10: Confocal images of HeLa cells treated with PEI-GTC-NPs for 12 h and 24 h. Mitochondria were stained with MitoTracker Deep Red dye. Scale bar = 10 µm.

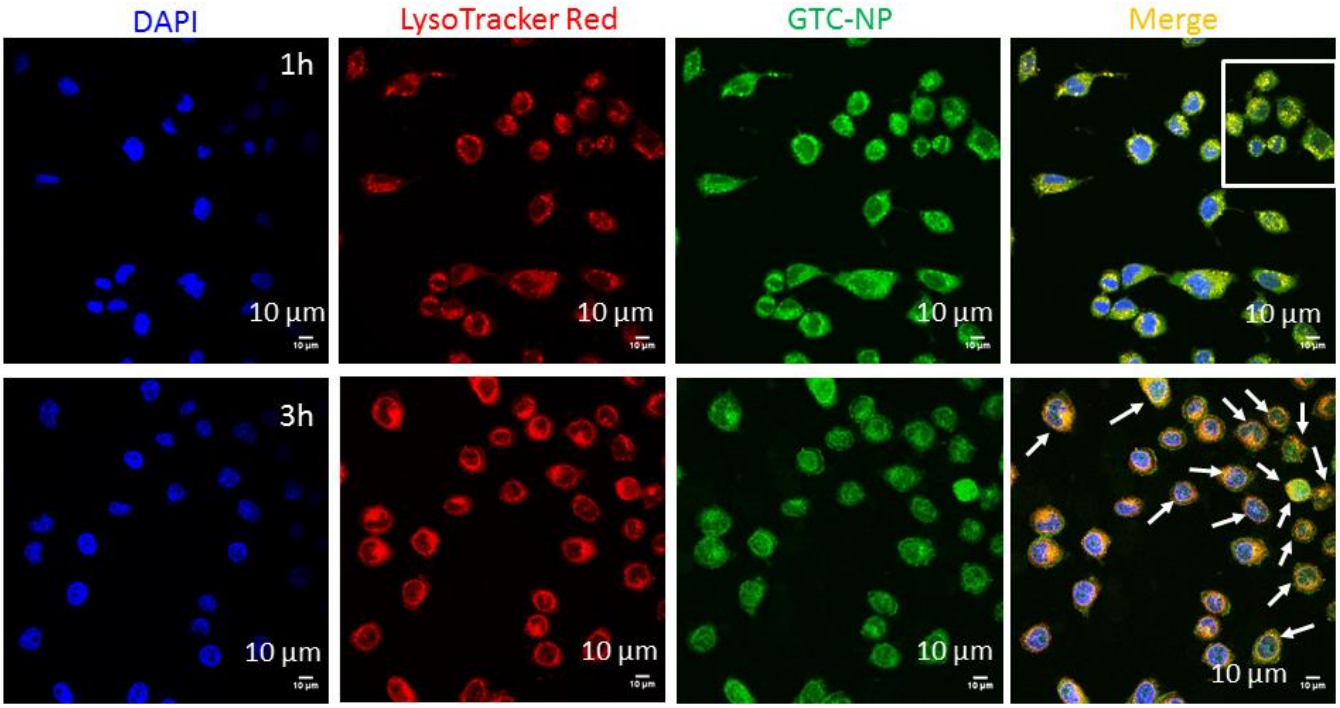


Figure S11: Confocal images of HeLa cells treated with GTC-NPs for 1 h and 3 h. Nucleus and lysosomes were stained with DAPI (blue) and LysoTracker Red (red) dyes. Scale bar = 10 µm.

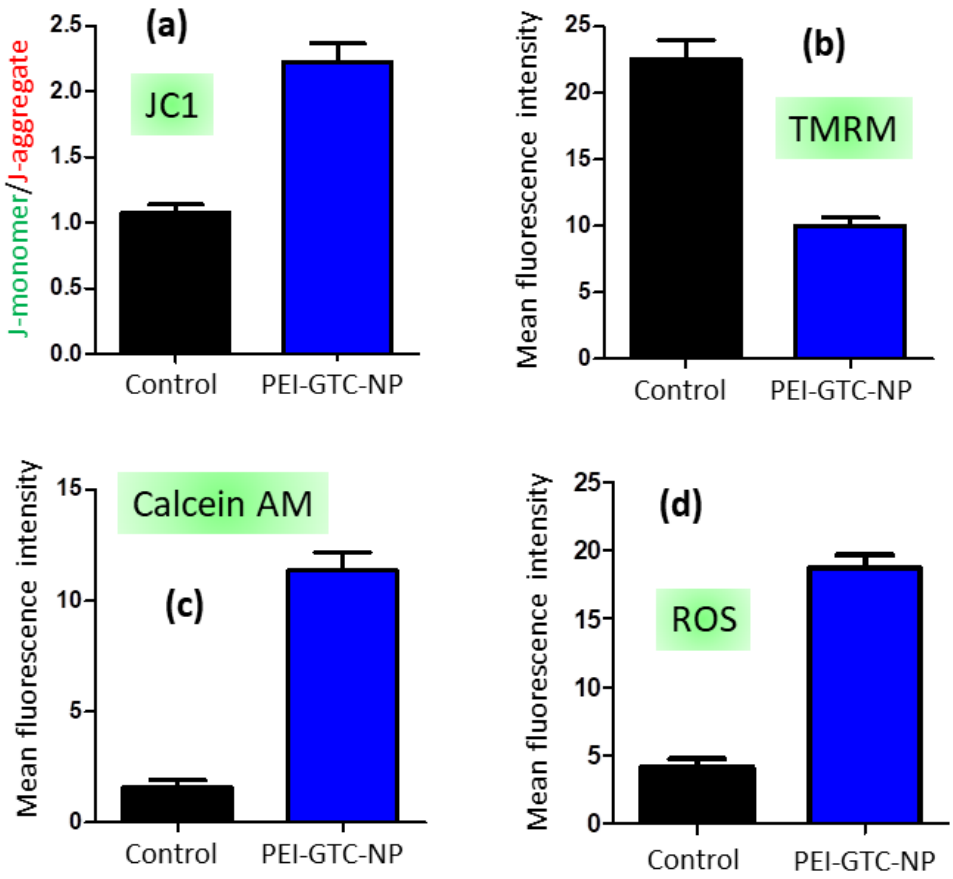


Figure S12: Quantification of (a) JC1, (b) TMRM, (c) Calcein AM and (d) ROS from CLSM images.

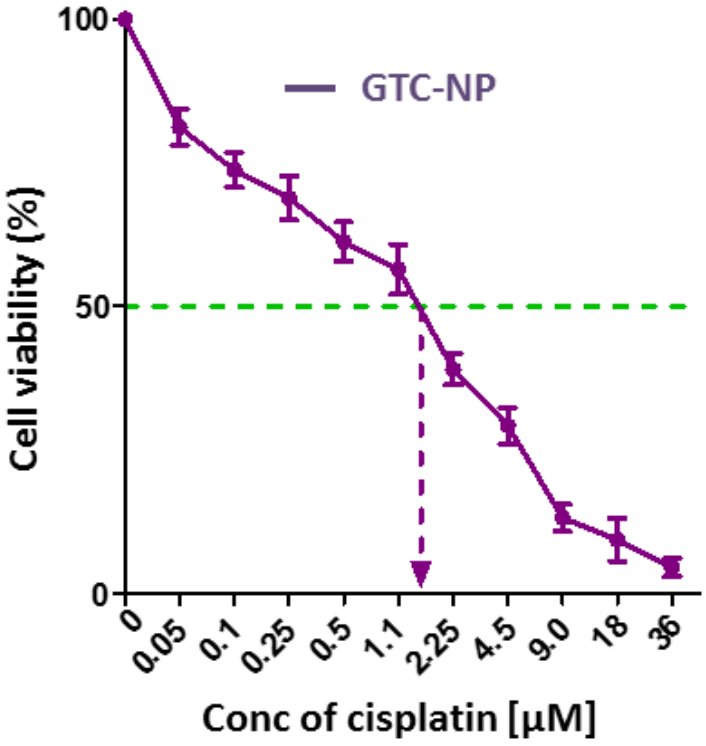


Figure S13: Viability of HeLa cells at 48 h after treatment with GTC-NPs in a concentration dependent manner determined by MTT assay.

References:

1. Mallick, A.; More, P.; Syed, M. M. K.; Basu, S. Nanoparticle-Mediated Mitochondrial Damage Induces Apoptosis in Cancer. *ACS Appl. Mater. Interfaces* **2016**, *8*, 13218−13231.

2. Mallick, A.; Kuman, M. M.; Ghosh, A.; Das, B. B.; Basu, S. Cerberus Nanoparticles: Cotargeting of Mitochondrial DNA and Mitochondrial Topoisomerase I in Breast Cancer Cells. *ACS Appl. Nano Mater.* **2018**, *1*, 2195−2205.

3. Mallick, A.; More, P.; Ghosh, S.; Chippalkatti, R.; Chopade, B.A.; Lahiri, M.; Basu, S. Dual Drug Conjugated Nanoparticle for Simultaneous Targeting of Mitochondria and Nucleus in Cancer Cells. *ACS Appl. Mater. Interfaces* **2015**, *7*, 7584−7598.