Supporting Information

Single functionalized graphene oxide reconstitutes kinesin mediated intracellular cargo transport, delivers multiple cytoskeleton proteins and therapeutic molecule into the cell

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Experimental Section

Materials:

Chemicals: Graphite powder (<60 μm) was purchased from Loba Chemie. Sodium bicarbonate, di-Sodium hydrogen phosphate dihydrate, Potassium dihydrogen phosphate, Potassium hydroxide, Sulphuric acid, Hydrochloric acid, Sodium nitrate, Magnesium chloride hexahydrate, Hydrogen peroxide (30%), $NiCl_2$ Hexahydrate and N, N´-dimethyl formamide (DMF) were purchased from Merck. Trifluoroacetic acid (TFA) was purchased from Acros Organic. Dimethyl sulphoxide and Uranyl acetate were purchased from Spectrochem. Potassium permanganate, Methanol and Sodium hydroxide were purchased from Fisher Scientific. Triton-X-100 was purchased from SRL. Diamino-polyethylene glycol with MW 3000 Da (NH_2-PEG_{3000}-NH_2) was purchased from Rapp Polymer. N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC), *β*-Mercaptoethanol, 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) and Dulbecco's Modified Eagle's Medium **(**DMEM**)** medium, 4, 6-diamidino-2-phenylindole (DAPI), Kanamycin sulfate, Trypsin-EDTA solution,Sodium Chloride, Potassium Chloride, Guanosine 5′-triphosphate sodium salt hydrate(GTP), PIPES, Paclitaxel, Ethylene glyol-bi(2-aminoethylether)-N, N, N′, N′-tetraacetic acid (EGTA), *β*-casein and formaldehyde were purchased from Sigma Aldrich. Diisopropyl carbodiimide (DIC) was purchased from Fluka. 2-[4-(2-hydroxyethyl) piperazin-1-yl] ethanesulfonic acid (HEPES) and Adenosine 5′-triphosphate disodium salt hydrate (ATP) were purchased from Himedia. Penicillin-Streptomycin, Neutravidin, Alexa Fluor 568 carboxylic acid succinimidyl ester and fetal bovine serum were purchased from Invitrogen. O^{t}Bu protected Tris-(nitrilo Tris-acetic acid) [(O^{t}Bu)Tris-NTA], Cy5-tubulin, biotin-tubulin, Catalase and Glucose oxidase, plasmids of V_HH-His_6, mCherry-XCTK2-His_6, Mal3-EGFP and XMAP215-His_7 were received as kind gift from Dr. Thomas Surrey's laboratory in EMBL, Heidelberg Germany. Anti-alpha Tubulin antibody, Goat Anti-Rabbit IgG H&L (Cy3.5 ®) preadsorbed and Streptavidin protein (hexa His

tag) were purchased from Abcam. All compounds were used without further purification. Cover glass bottom dishes were purchased from SPL. Mica sheet (25 mmX25 mm, grade V-1 Muscovite) was purchased from Spi supplies, USA. Copper Grid-300 mesh was purchased from ProSciTech. UV and FT-IR spectra were recorded on UV-1800 ENG 240V Shimadzu Model and JASCO FT-IR 4200 model respectively.

Protein biochemistry: Tubulin was isolated from goat brain and labelled with Alexa Fluor 568 carboxylic acid succinimidyl ester to obtain the Alexa-568-labelled. The purification of tubulin from goat brain, Alexa-568 labelling of tubulin and the polymerization of microtubules were performed as described in the literature.[1] Deca-histidine tagged EGFP (EGFP-His_{10}) and Kinesin612-His_{10} proteins were expressed in E-coli and purified through Ni-NTA column in our laboratory.

Cell culture: A549 (adenocarcinomic human alveolar basal epithelial cell line) cell line and MCF-7 (human breast cancer cell line) cell line were purchased from NCCS, pune (India) and cultured in Dulbecco Modified Eagle medium (DMEM) containing 10% (v/v) heat-inactivated fetal bovine serum at 37 ^{o}C and 5% CO_2 atmosphere in our lab.

Methods:

Detailed method of preparation of TGO: Graphene oxide was prepared from graphite powder (<60 µm) by a modified Hummers method. Graphene oxide in Milli-Q water (concentration ~4 mg/mL) was ultra-sonicated for an hour followed by additional 3 hour after treatment with 3M NaOH solution. Next, the solution was neutralised (pH 7.0) by adding HCl and filtered through Whatmann 40 paper. The residue GO-COOH was washed several times with Milli-Q water. 100 mg of H_2N-PEG_{3000}-NH_2 was added to 10 mL GO-COOH solution (concentration ~1 mg/mL) and ultra-sonicated for 5 min. Then N-(3-Dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDC) was added into solution mixture to reach 5 mM and sonicated for 30 min. Enough EDC was added to reach 20 mM concentration and the solution was stirred at room temperature for overnight. Finally, 50 µL of *β*-Mercaptoethanol was added to terminate the reaction and the solution was lyophilised to get dry PEGylated GO. Next, dry DMF was added to 100 mg of PEGylated GO and sonicated for 15-30 minutes under N_2 atmosphere. 100 mg O^{t}Bu protected Tris-NTA was added to it, followed by addition of 30 µL DIC as a coupling reagent and stirred under N_2 atmosphere for overnight. Reaction mixture was centrifuged at 12000 rpm for 15 min and the residue was washed with DMF 3-4 times by resuspension and centrifugation process. Then DMF was evaporated and residue was treated with TFA for 6 hour under N_2 at room temperature for deprotection of tertiary butyl group of Tris-NTA. Excess TFA was removed and milli-Q water was added to the residue

followed by sonication for 10 min. Mixture was centrifuged at 12000 rpm for 10 min and the residue was washed with Milli-Q water for 3-4 times. Finally TGO was stored in Milli-Q water at 4 ^{o}C.

UV Spectroscopy: GO and TGO were characterized by UV spectroscopy. The characteristic absorption peaks at 234 and 300 nm were obtained in case of the UV spectrum of GO. However a similar spectral analysis of TGO shows the reduction in intensity of the peak at 234 nm and a significant peak at 300 nm. A new peak at 262 nm was also found in case of TGO, which is attributed to Tris-NTA functionality to the GO surface.

FT-IR Spectroscopy: Both the sample of TGO and GO were lyophilized and FT-IR spectra were recorded by JASCO FT-IR 4200 model. In the FT-IR spectrum of GO, absorbance peaks at 804 cm^{-1} and 970 cm^{-1} are due to the epoxy group present in GO surface. The absorbance peak at 1050 cm^{-1} in GO is distinguished as C-OH stretching frequency which is shifted to 1100 cm^{-1} in case of TGO. The peak at 1723 cm^{-1} corresponds to carbonyl stretching of -COOH group in GO, which remains in the same position in case of TGO due to the presence of -COOH group in Tris-NTA. A new characteristic peak at 1686 cm^{-1} in TGO spectrum corresponds to amide carbonyl stretching from the newly formed amide functional groups, which confirms the covalent functionalization. Absorbance peaks in the region 3000 cm^{-1} to 3700 cm^{-1} in case of all the spectra are due to O-H stretching (both free and H bonded) and amide N-H bond (secondary) stretching vibration. The absorbance peaks at 1403 cm^{-1} and 1454 cm^{-1} in case of GO and TGO respectively are due to aromatic -C=C- present in the GO surface.

Atomic Force Microscopy (AFM): Both the sample of GO and TGO were imaged with an atomic force microscope (Pico plus 5500 ILM AFM, Agilent Technologies USA) operating under Acoustic AC mode (AAC), with the aid of cantilever (Micro fabricated silicon cantilevers of 225 µm in length with a nominal spring force constant of 21-98 N/m and resonant frequency was 150-300 kHz from Nano Sensors, USA) and a piezo scanner (maximum range of 9 µm). The images were captured in air at room temperature with a scan speed of 0.5 lines/sec. Images were processed using Pico view 1.1 software and data analysis was done through Pico Image Advanced version software (Agilent Technologies, USA). 10µL of the samples were deposited onto freshly cleaved mica sheet (25 mmX25 mm, grade V-1 Muscovite, spi supplies, USA) after sonication and dried in air. Significant changes in the surface morphology and surface height were observed from the AFM images of TGO in comparison with GO. The average height, measured from multiple images were 1.2 ± 0.36 nm for GO, 3.58 ± 1.44 nm for TGO. There was ~ 2.38 nm enhancement in the surface height in case of TGO compared to GO due to functionalization.

Transmission Electron Microscopy (TEM): A 10 µL suspensions of GO and TGO in water were placed after sonication on a carbon coated copper grid. Excess fluid was removed after 1 min and the grid was

stained with 2% uranyl acetate in water. Excess staining solution was removed from the grid after two minutes and the grids were vacuum dried. Samples were viewed using a TECNAI G2 SPIRIT BIOTWIN CZECH REPUBLIC 120 KV electron microscope operating at 80 kV. TEM images of the TGO and GO clearly indicate that there is a change in the surface morphology after functionalization.

High Resolution Transmission Electron Microscopy (HR-TEM): Samples were prepared as previously described method and viewed using a JEOL JEM-2011 electron microscope operating at 200 kV. HR-TEM images of the TGO and GO revealed highly resolved surface morphology, which also clearly indicates the change in surface morphologies after functionalization. In case of GO, well organised pattern like structure was found where as this patterned structure disappeared in case of TGO surface.

Fluorescence Microscopic Imaging after loading multiple proteins on TGO: For fluorescence microscopic imaging, TGO (concentration- 3 mg/mL) was equilibrated with 10 mM $NiCl_2$ solution using a mechanical shaker for 15 min followed by centrifugation at 10000 rpm for 10 min. Residue was washed with Milli-Q water for 3 times with intermittent sonication and centrifugation. Next, TGO was equilibrated with BRB80 at 4 ^{o}C followed by incubation with *β*-casein (concentration-1mg/mL) by gentle shaking for 15 min at 4 ^{o}C. Excess *β*-casein was removed by centrifugation at 10000 rpm for 10 min at 4 ^{o}C followed by washing with BRB80 through sonication and centrifugation method at 4 ^{o}C. TGO was incubated with the mixture of 1 µM V_HH-His_6, 100 nMmCherry XCTK2 (His tagged) and 160 nMXMAP215-$His_7$by gentle shaking for 15 min at 4 ^{o}C. Washing was done following previously described method for the removal of excess proteins. Next, protein loaded TGO was incubated with the mixture of 18.5 µM Cy5-tubulinand 250 nMMal3-EGFP in BRB80 by gentle shaking on mechanical shaker for 15 min at 4 ^{o}C. Excess proteins were removed by centrifugation at 10000 rpm and washed with BRB80 by sonication/resuspension method at 4 ^{o}C (2 times). Finally, 20 µL of multiple proteins bound TGO suspension in BRB80 was loaded on ethanol cleaned microscopic glass slide and on top of that solution another 22 mm/22 mm coverslip was placed for making a sandwich. Sandwich was sealed with VALAC and imaged using 488, 561 and 638 nm laser through Nikon Eclipse Ti-U inverted fluorescence microscope.

Control Experiment: Control experiment was carried out with PEGylated GO. For that It was treated with Ni^{+2}, *β*-casein and all the proteins following previously described method (similar method as described in Fluorescence Microscopic Imaging for TGO). Finally, imaging was done as previously described method and PEGylated GO sheets was observed in bright field, 488, 561 and 638 nm laser light separately. No fluorescent signal was observed at 488, 561 and 638 nm laser light, which clearly states that protein binds to the TGO only through functionalization.

Particle size measurement: The average size of TGO after 15 min probe sonication and multiple cytoskeleton proteins loaded TGO nanoparticles (proteins were loaded to TGO as previouly described) were measured in DMEM by Malvern particle size analyser (Model no. ZEN 3690 ZETASIZER NANO ZS 90). Average particle size of TGO was found to be 213 nm with PDI-0.384 and that of multiple cytoskeleton proteins loaded TGO was 434.6 with PDI-0.589.

Preparation of the sample of multiple cytoskeleton proteins loaded TGO nanoparticles for TEM study: Proteins were loaded to TGO following the previously described method. After loading proteins on TGO, 10 μL suspensions of multiple cytoskeleton proteins loaded TGO in BRB80 was placed on a carbon coated copper grid. After 5 minutes excess buffer was removed and the grid was washed with milliQ water for 2 times. The grid was stained with 2% uranyl acetate in water. Excess staining solution was removed from the grid after two minutes and the grids were vacuum dried. Samples were viewed using a TECHNAI POLARA 200 KV electron microscope operating at 200 kV.

Tranport of EGFP-His_{10} loaded TGO nanoparticle through microtubule network:

Preparation of biotin and Alexa Fluor568 labeled Taxl stabilized microtubules: Mixture A: 0.5 μL Alexa568 Tubulin (15 mg/mL, 65% labeling ratio) + 0.5 μLbiotinylated Tubulin + 4 μL Tubulin 27 mg/mL + 4.6 μL BRB80 (80 mM PIPES, 1 mM EGTA, 1 mM $MgCl_2$, pH was adjusted to 6.8 using KOH solution) + 0.4 μL GTP-Mg (25 mM).

Mixture B: 180 μL of BRB 80 and 0.36 μLTaxol (10 mM).

Mixture **A** and **B** was warmed at 37 ºC for 20 minutes and then 90 μL mixture B was added into the mixture **A** and mixed carefully. Then the mixture was centrifuged for 8 min at 12000 rpm at 37 ºC. Then the supernatant was carefully taken out and the colored pellet was resuspended into the 20 μL mixture **B** solution and kept at room temperature covering with aluminum foil.

Transport through microtubule network: Ni^{+2} and β-casein were loaded onto TGO nanoparticles (concentration-3 mg/mL) following previously described method. Then TGO was incubated with 100 nM EGFP-His_{10} in BRB80 by gentle shaking on mechanical shaker for 15 min at 4 ^oC. Removal of excess dye and washing was performed following previously described method. EGFP-His_{10} bound TGO was suspended in motility buffer containing BRB80, 50 nM kinesin612-$His_{10,}$ 18 μM taxol, oxygen scavenger (50 mM glucose, 1 mg mL^{-1} glucose oxidase, and 0.5 mg mL^{-1} catalase), 4 mM ATP and 5 mM $MgCl_2$ and kept in ice. Next, a flow chamber was constructed with biotinylated glass following our previously described method.[2] Now transport assay was performed following sequential steps: (i) flow chamber was washed with BRB80 and incubated with β-casein solution for 10 min on ice and excess β-casein was

removed by washing with BRB80 in ice. (ii) it was equillibrated with 100 nMneutravidin solution for 10 min followed by washing with BRB80 in ice. (iii) Then it was equillibrated with 20 μL of motility buffer (BRB80 containing 2.5 mM ATP, 20 μM taxol and 1.5 mM β-Mercaptoethanol) at room temperature. and (iv) it was equillibrated with taxol stabilized Alexa 568 and biotin labeled microtubules in motility buffer (BRB80, 2 mM GTP, 5 mM $MgCl_2$, 20 μMtaxol and an oxygen-scavenger system (50 mM glucose, 1 mg mL^{-1} glucose oxidase, 0.5 $mgmL^{-1}$ catalase)for 10 min. Finally, 20 μL solution of Kinesin-Tris-NTA-EGFP in motility buffer was flowed inside the flow chamber, containing immobilized biotin and Alexa568-labeled microtubules at room temperature followed by immediate capturing of time lapse images in streaming mode under IX-81 total internal reflection fluorescence (TIRF) microscope (Olympus) with a 60x/1.45 NA TIRF M objective (Olympus) and an Andor iXon3 897 camera at 37 °C with simultaneous 488 and 561 nm dual channel laser illumination.

Cellular uptake studies: Cellular uptake of the multiple proteins loaded TGO was studied in A549 and MCF-7 cell lines. Both the cells were seeded differently at a density of 5000 cells per cover glass bottom dish in 6 cover glass bottom dish (3 for each cell line) one day prior to incubation with proteins loaded TGO. Proteins binding to TGO was carried out as previously described procedure. Then both the cells were treated with 200 μL of protein loaded TGO solution in serum free DMEM medium (final concentration of protein loaded TGO is 10 μg/mL) for each cover glass bottom dish for 4 hour. After that the cells were washed with phosphate buffer saline and both the A549 and MCF-7 cells were viewed with an NIKON inverted microscope (Model Ti-U).

Synthesis of antimitotic biotinylated dodecapeptide: Biotinylateddodecapeptide (Biotin-FRRKAFLHWYTG) was synthesized by solid phase peptide synthesis method using Rink Amide AM resin. Crude peptide was purified by reverse phase HPLC and characterized by MALDI Mass Spectroscopy.

Immobilization of biotinylated dodecapeptide to TGO: For immobilization of biotinylated dodecapeptide to the TGO, First TGO was equilibrated with $NiCl_2$ solution and β-casein following previously described procedure. Then TGO solution in BRB80 was incubated with Streptavidin protein (His tag) in BRB80 having concentration 10 μg/mL by gentle shaking for 15 min at 4 ^oC. Excess streptavidin protein (hexa His tag) was removed by centrifugation at 10000 rpm for 10 min at 4 $^\circ C$ followed by washing with BRB80 through sonication and centrifugation method at 4 ^oC. Then streptavidin protein (hexa His tag) loaded TGO was incubated with biotinylated dodecapeptide solution by gentle shaking for 15 min at 4 ^oC. Excess biotinylated dodecapeptide was removed by centrifugation followed by washing with BRB80. Finally dodecapeptide-TGO solution was stored for cellular study.

Cytotoxicity study: Cytotoxicity of TGO towards the A549 and MCF-7 cancer cell line as well as the same of biotinylated dodecapeptide and TGO-streptavidin-bio-dodecapeptide towards MCF-7 cell line were evaluated by the 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) reduction. Both the cells were seeded differently at a density of 10000 cells per well in a 96-well plate one day prior to the traetment. Then A549 cells were treated with TGO and MCF-7 cells were treated with TGO, biotinylated dodecapeptide and dodecapeptide-TGO (maximum final concentrations of all the compounds were 50 μg/mL) in fetal bovine serum free DMEM medium for 4 h in different 96-well plate. After that the medium was changed with fresh DMEM containing FBS. Following the termination of experiment, cells were washed and promptly assayed for viability using MTT. Results were expressed as percent viability = [A550 (treated cells)-background/A550 (untreated cells)-background] x 100.

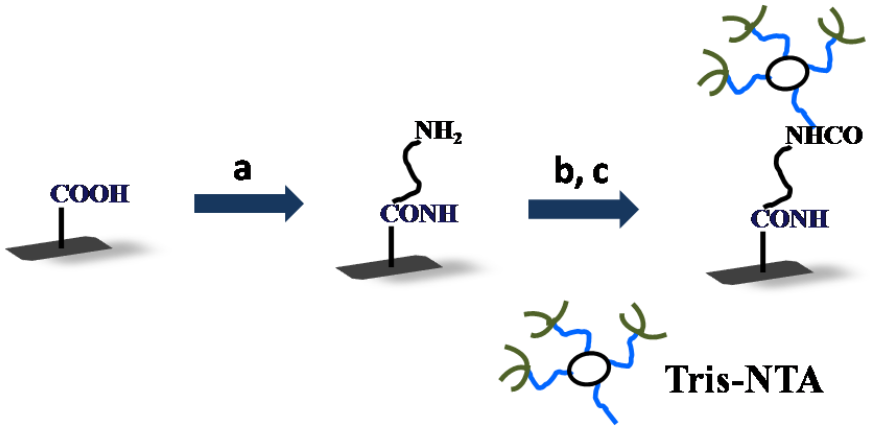
Study the effect of the microtubule network of MCF-7 cell line after TGO, biotinylateddodecapeptideanddodecapeptide-TGO treatment: Change in the microtubule network of human breast cancer cell line (MCF-7 cell line) after TGO, biotinylateddodecpeptide and dodecapeptide-TGO treatment was studied by following method. The cells were seeded at a density of 5000 cells per cover glass bottom dish in four cover glass bottom dish for 18-24 hours before the compounds' treatment. After washing, the cells were treated with 50 μg/mL concentration of each compound in serum free DMEM medium for 4h keeping one dish as a control. After that the medium was changed with fresh DMEM containing FBS and kept for overnight. Then the cells were washed with 1X Phosphate Buffer Saline (PBS), fixed with 4% paraformaldehyde solution and permealized by 0.1% Triton-X-100. Then the fix cells were treated with primary antibody (Anti-alpha Tubulin (1:300)) for 1 hour and it was followed by the secondary antibody (Goat Anti-Rabbit IgG H&L(1:500)) treatment for 1 hour. Finally the cells were treated with 4, 6-diamidino-2-phenylindole (DAPI) at a concentration 3 μM for 30 minutes. Following the termination of the experiment, the cells were washed with 1X PBS and observed in the inverted microscope (Model Nikon Eclipse Ti-U).

Image Analysis and Data Calculation: Image analysis and data calculation were performed using Image J software.

References:

1. A. Hyman, D. Drechsel, D. Kellogg, S. Salser, K. Sawin, P. Steffen, L. Wordeman and T. Mitchison, *Methods in Enzymology*, 1991, **196**, 478.

2. A. Biswas, A. Saha, B. Jana, P. Kurkute, G. Mondal and S. Ghosh, *ChemBioChem*, 2013**, 14,** 689.



Scheme S1. Synthetic scheme for TGO. (a) diaminopoly(ethylene glycol) (H_2N-PEG_{3000}-NH_2 N-(3-Dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDC), *β*-Mercaptoethanol. (b) O-tert butyl protected Tris-[nitrilotris (acetic acid)] [(OtBu)Tris-NTA] and Diisopropylcarbodiimide (DIC), dry N, N′-dimethyl formamide (DMF). (c) Trifluoroacetic acid (TFA).

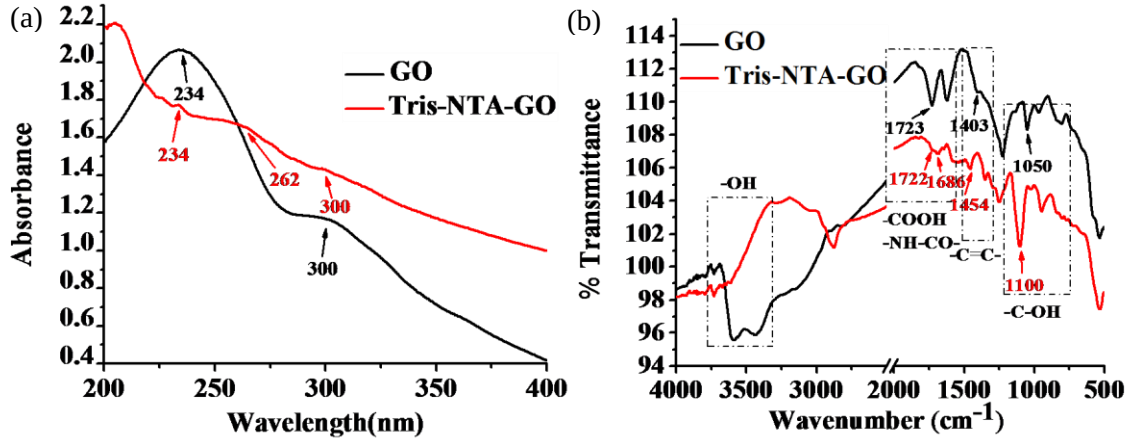


Figure S2. (a) UV spectra of GO and TGO; (b) FT-IR spectra of GO and TGO.

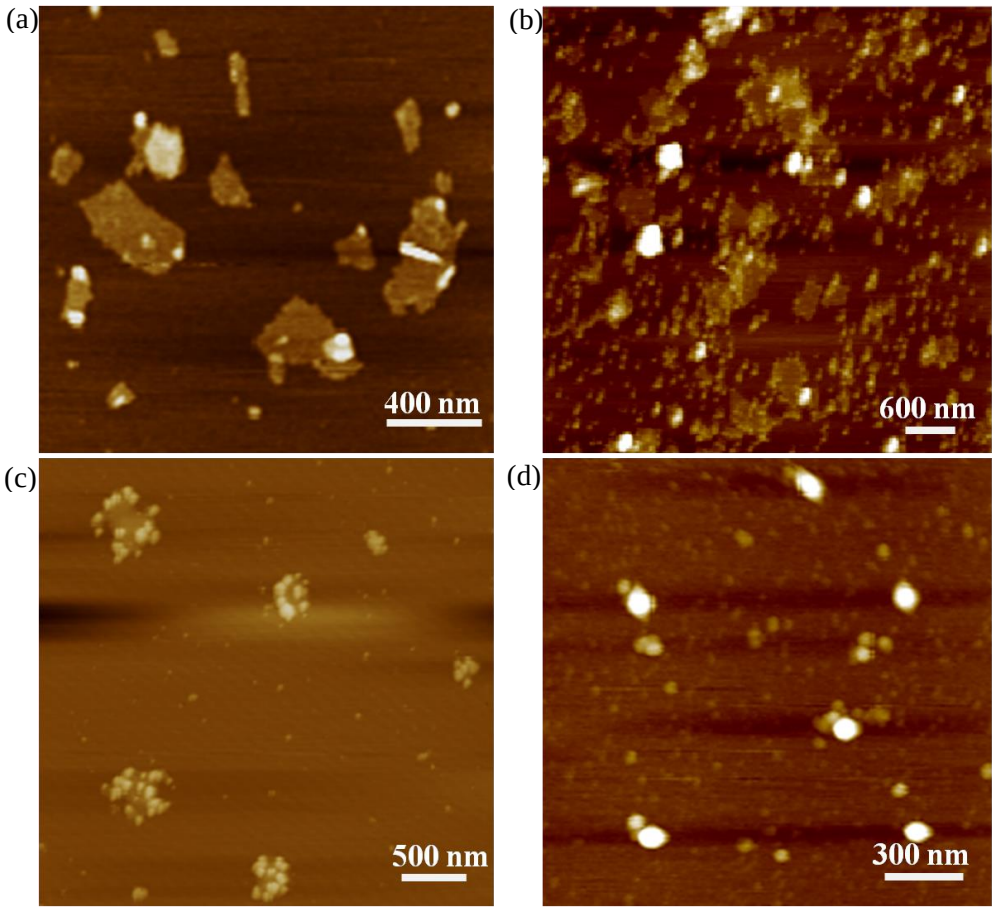


Figure S3. AFM images of GO sheets of (a) larger and (b) smaller sized particles. AFM images of TGO nanoparticles having (c) larger and (d) smaller size.

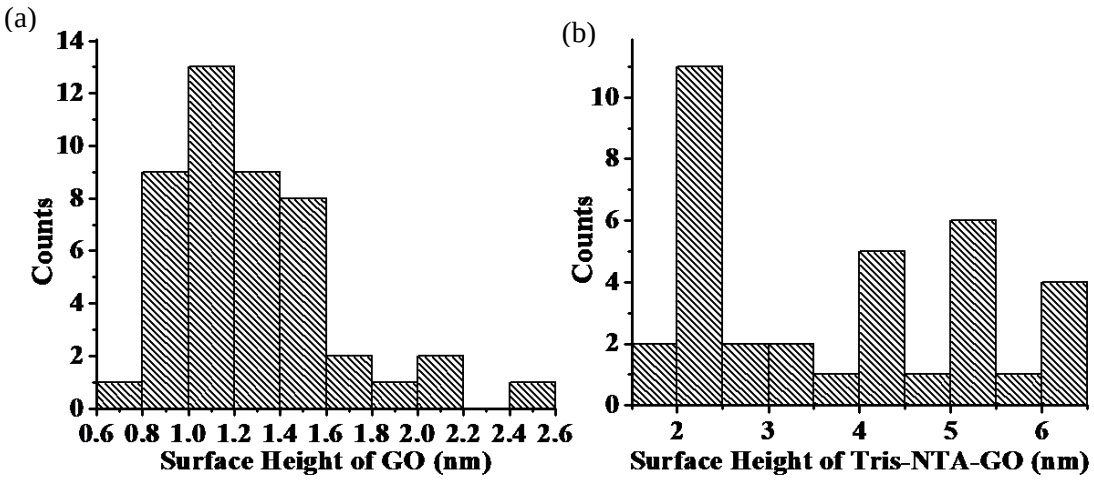


Figure S4. Histogram of surface height. (a) GO and (b) TGO nanoparticles.

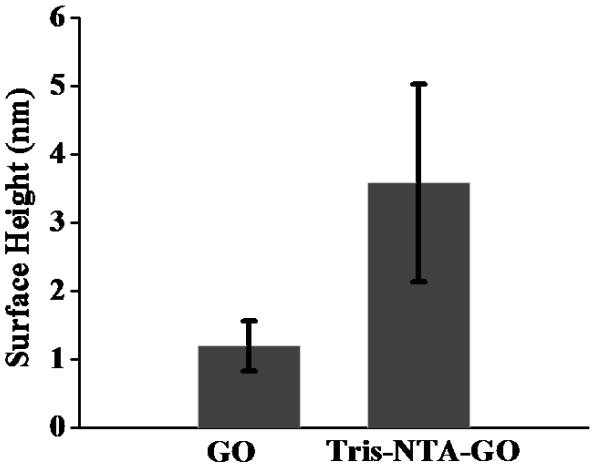


Figure S5. Graph represents the enhancement of surface height of GO after functionalisation with Tris-NTA.

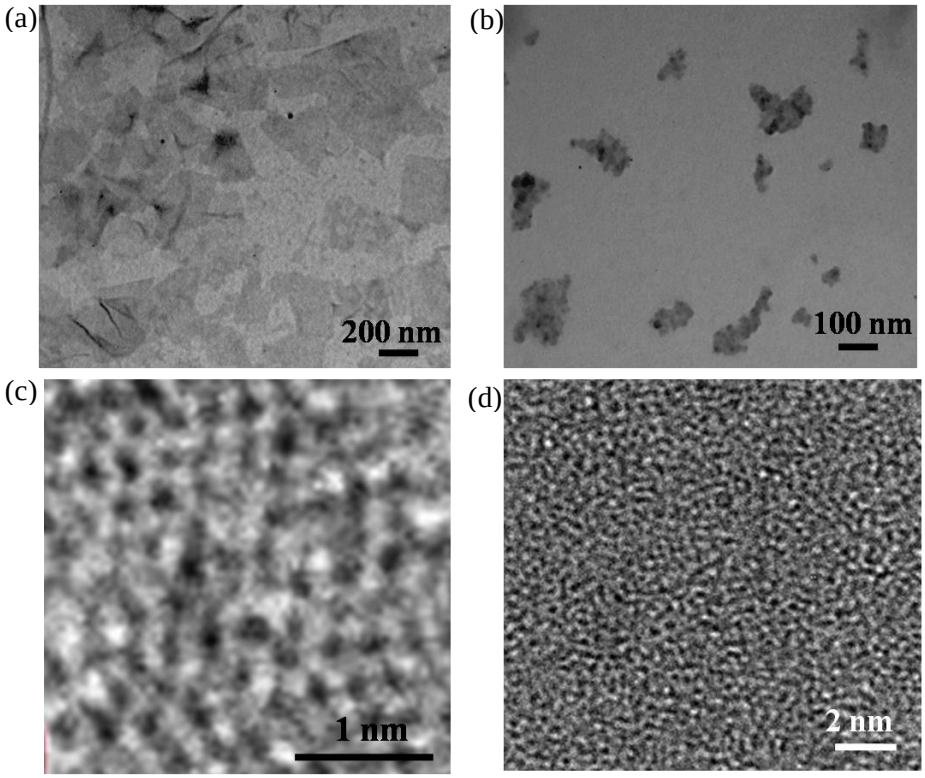


Figure S6. TEM images of (a) GO sheet and (b) TGO nanoparticles. HR-TEM images of (c) GO sheet and (d) TGO nanoparticles.

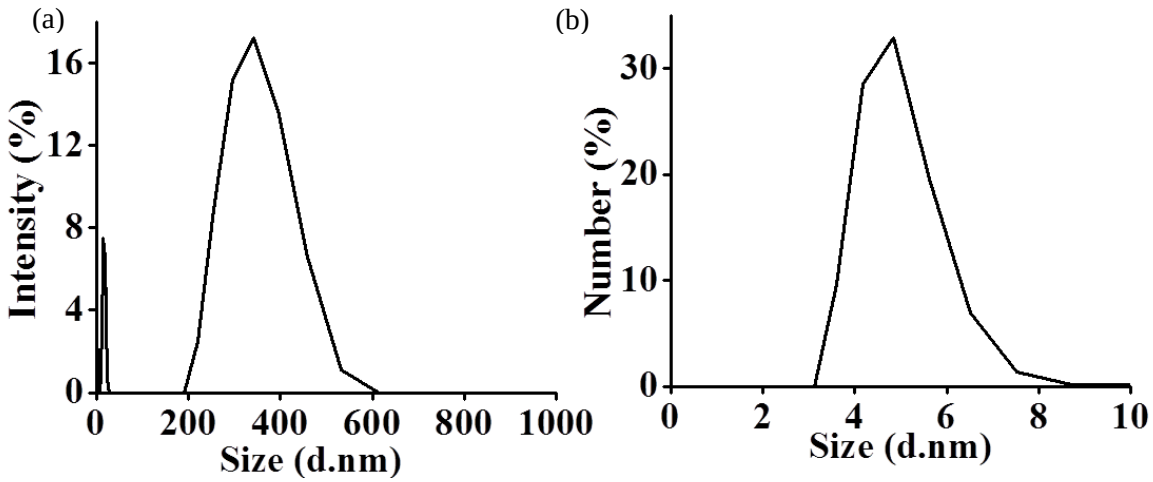


Figure S7. Dynamic light scattering micrograph of TGO nanoparticles, which clearly indicates that the average particle size of TGO nanoparticles is 213 nm. (a) % Intensity graph; (b) % number graph.

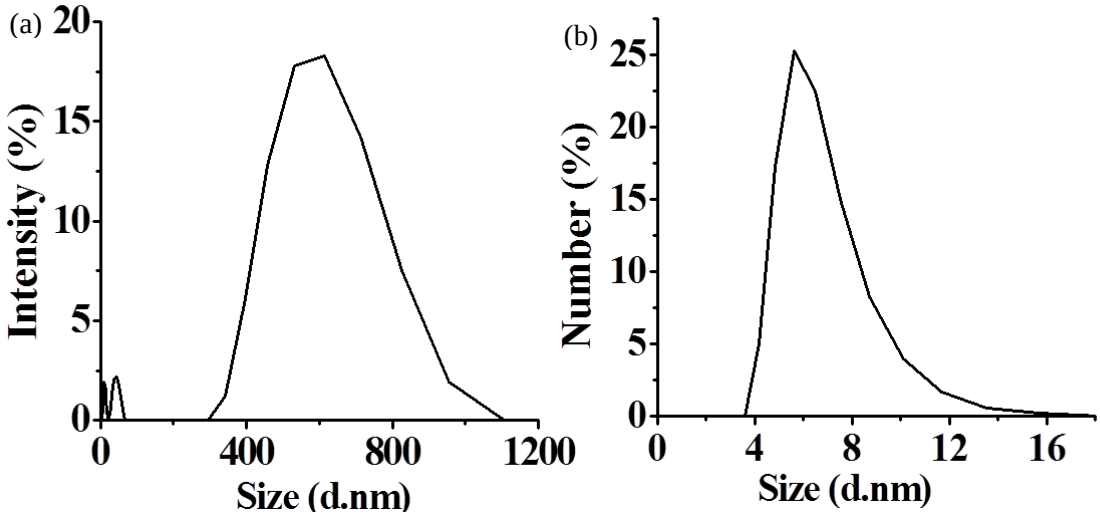


Figure S8. Dynamic light scattering micrograph of multiple cytoskeleton proteins loaded TGO nanoparticles, which indicates that the average particle size of TGO nanoparticles after proteins loading is 434.6 nm. (a) % Intensity graph; (b) % number graph.

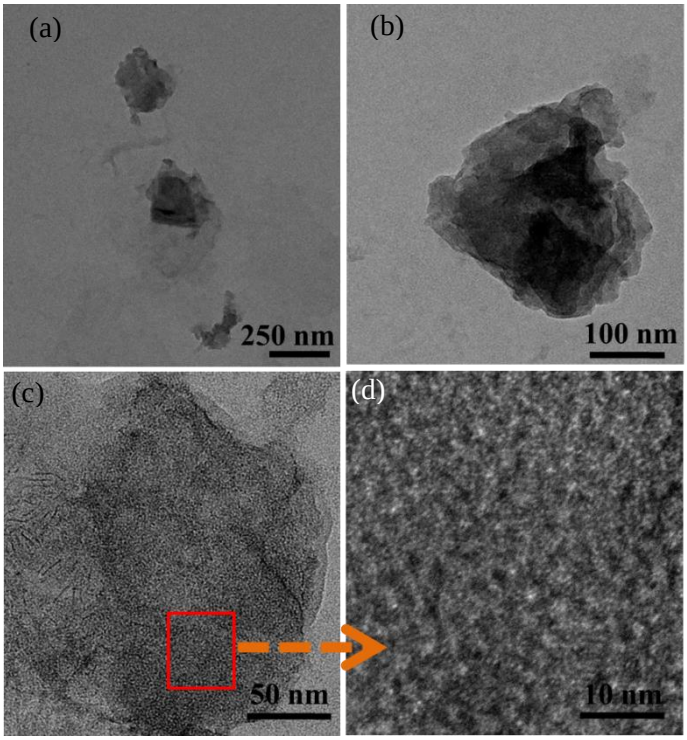


Figure S9. TEM images of multiple cytoskeleton proteins loaded TGOin (a,b) lower and (c,d) higher magnification.

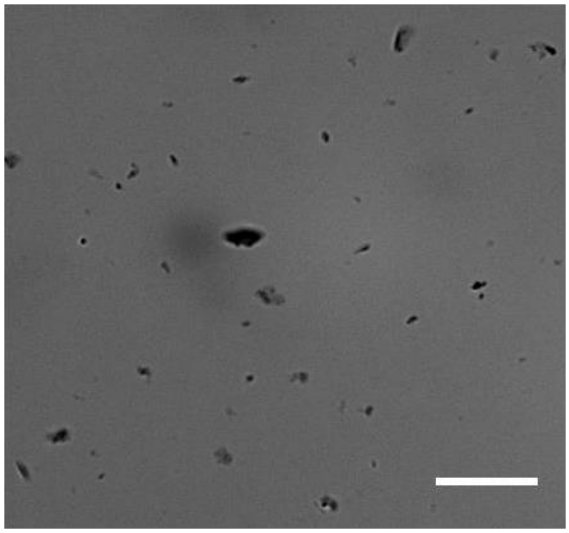


Figure S10. Microscopic image of multiple cytoskeleton proteins loaded TGO in DIC mode. Scale bar corresponds to 10 µm.

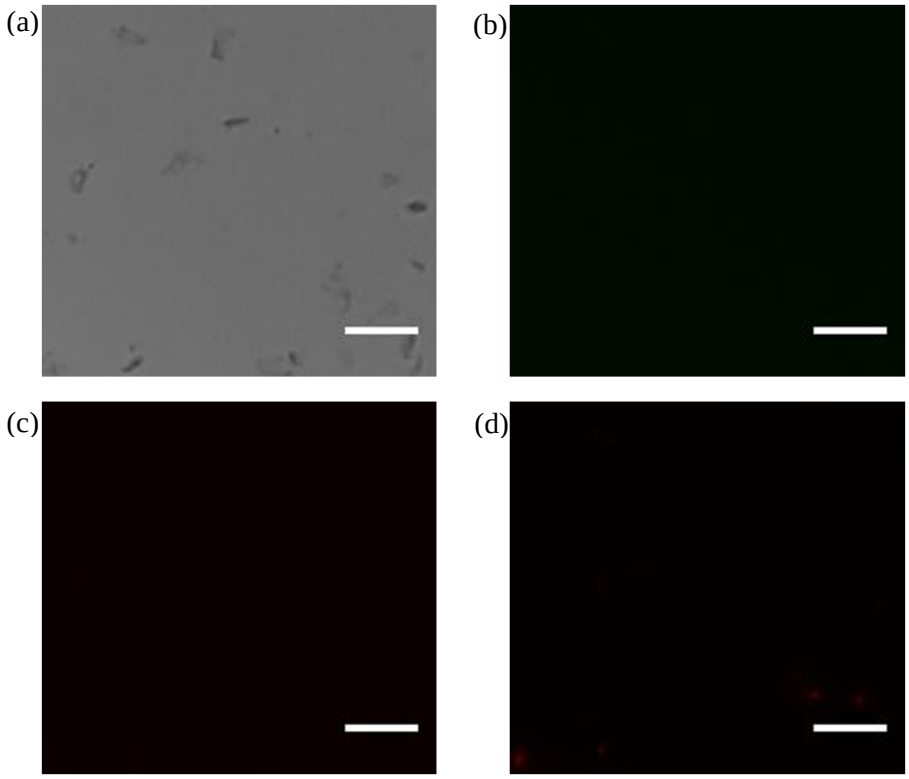


Figure S11. Control experiment: Fluorescence microscopic images of multiple proteins loaded PEGylated-GO (a) DIC mode; (b) 488, (c) 561 and (d) 638 nm channel. Scale bar corresponds to 10 µm.

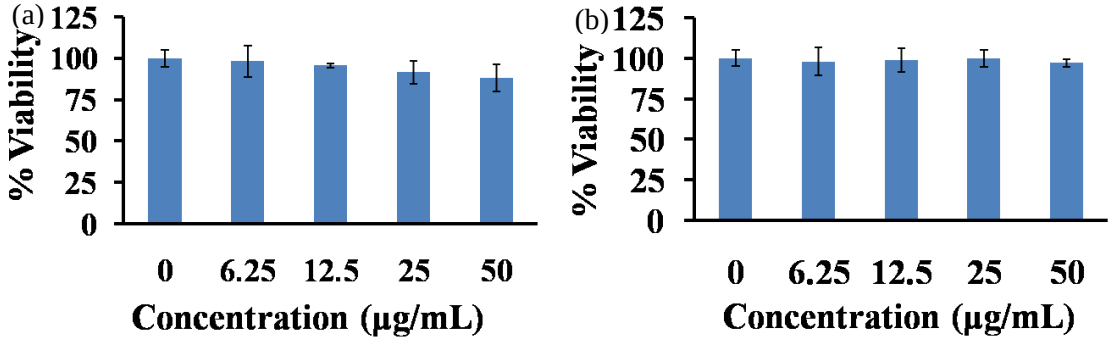


Figure S12. Survival of (a) human lung cancer cell line (A549 cell line) and (b) human breast cancer cell line (MCF-7 cell line) was assessed by MTT assay for 24 hour after TGO treatment, which indicates non-cytotoxic nature of TGO nanoparticles.

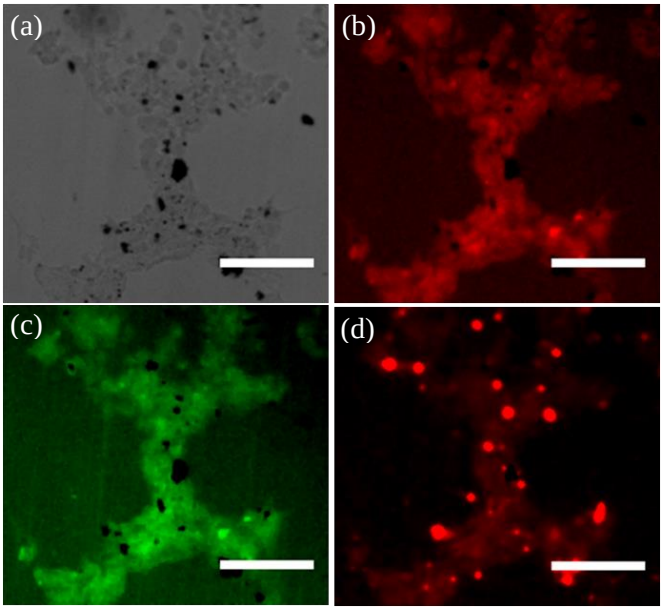


Figure S13. Cellular uptake of multiple cytoskeleton proteins loaded TGO in MCF-7 cell line. (a) DIC image of MCF-7 cell line after cytoskeleton proteins loaded TGO Images at (b) 561 nm channel, (c) 488 nm channel and (d) 638 nm channel indicate uptake of multiple cytoskeleton proteins loaded TGO by MCF-7 cells. Scale bar corresponds to 100 µm.

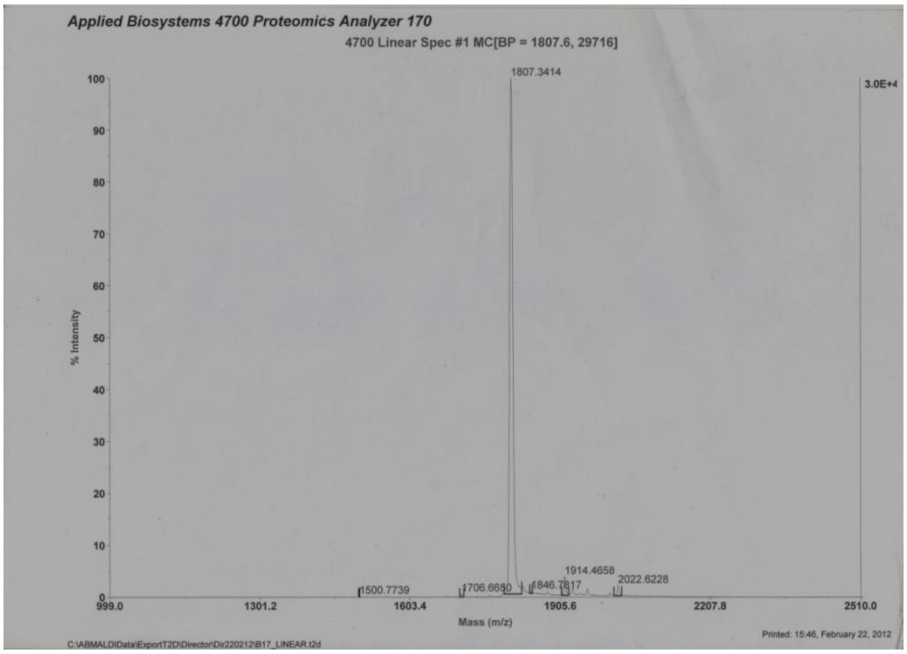


Figure S14. MALDI mass spectrum of biotinylated dodecapeptide.

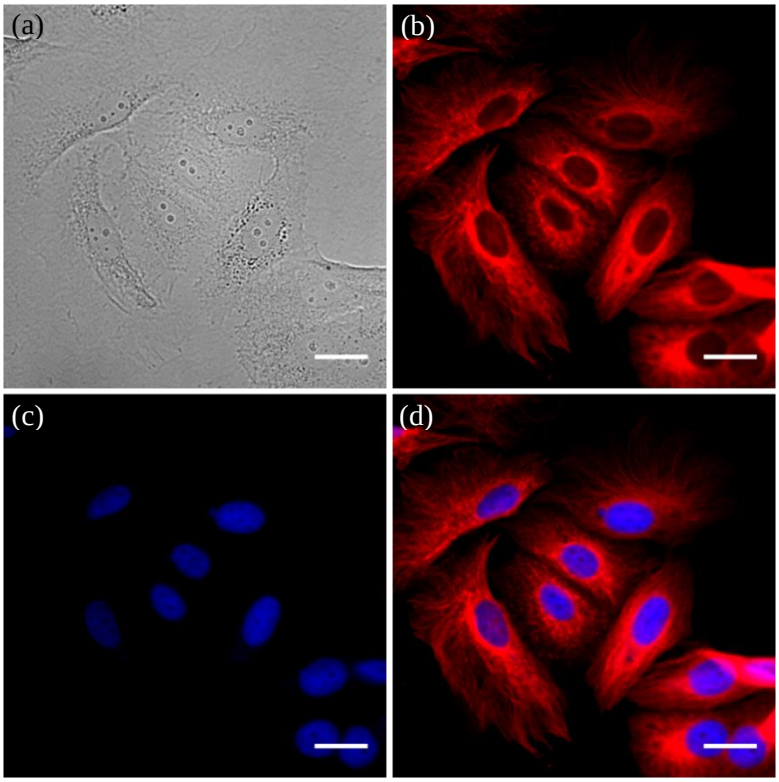


Figure S15. Microtubules network of MCF-7 cell lines.Fluorescence microscopic images of MCF-7 cells in (a) DIC mode, (b) 561, (c) 405 nm channel after antibody and DAPI treatment and (d) merged image. Scale bar corresponds to 30 µm.

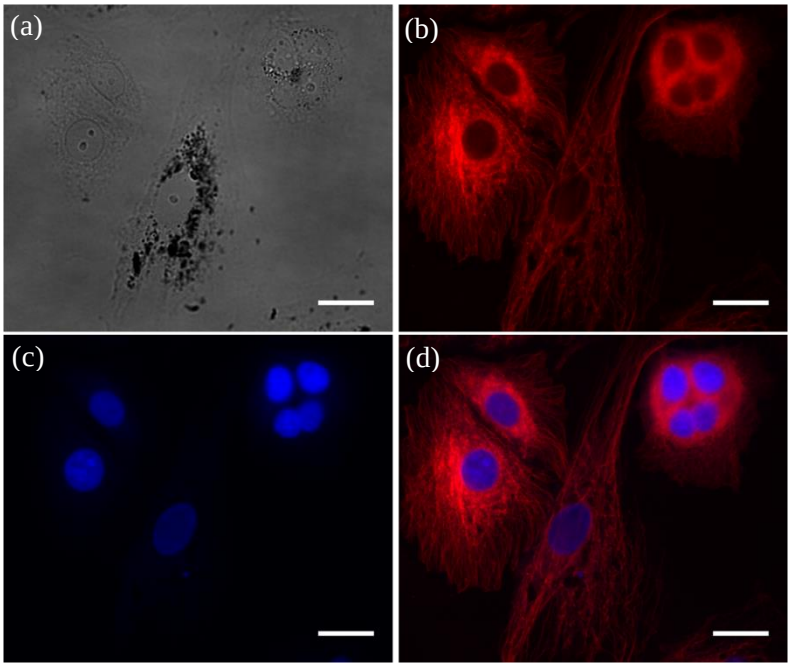


Figure S16. Microtubules network of MCF-7 cell lines after TGO treatment.Fluorescence microscopic images of MCF-7 cells in (a) DIC mode, (b) 561, (c) 405 nm channel after antibody and DAPI treatment and (d) merged image. Scale bar corresponds to 30 µm.

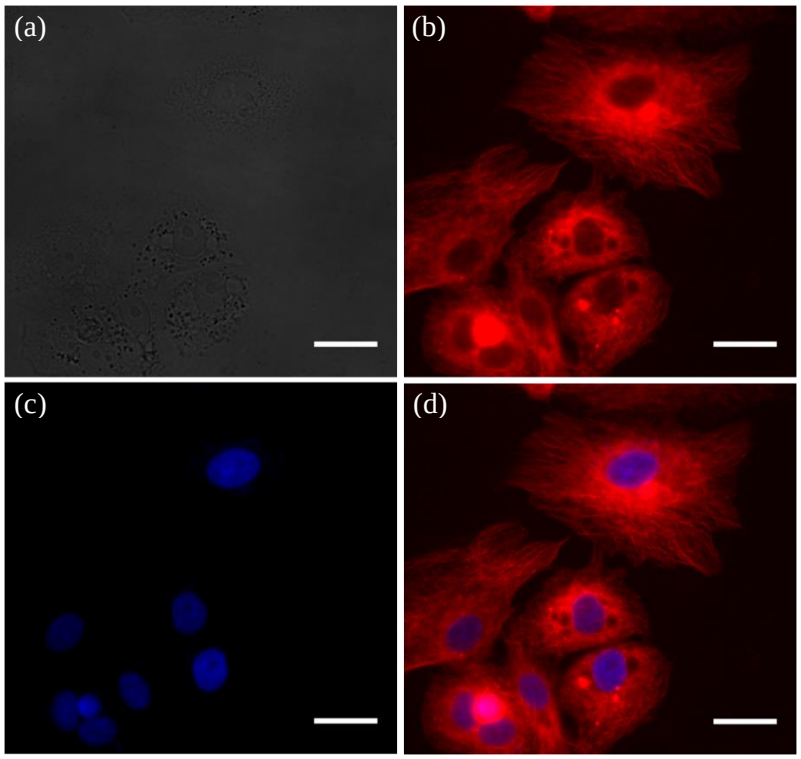


Figure S17. Microtubules network of MCF-7 cell lines after biotinylateddodecapeptidetreatment.Fluorescence microscopic images of MCF-7 cells in (a) DIC mode, (b) 561, (c) 405 nm channel after antibody and DAPI treatment and (d) merged image. Scale bar corresponds to 30 µm.

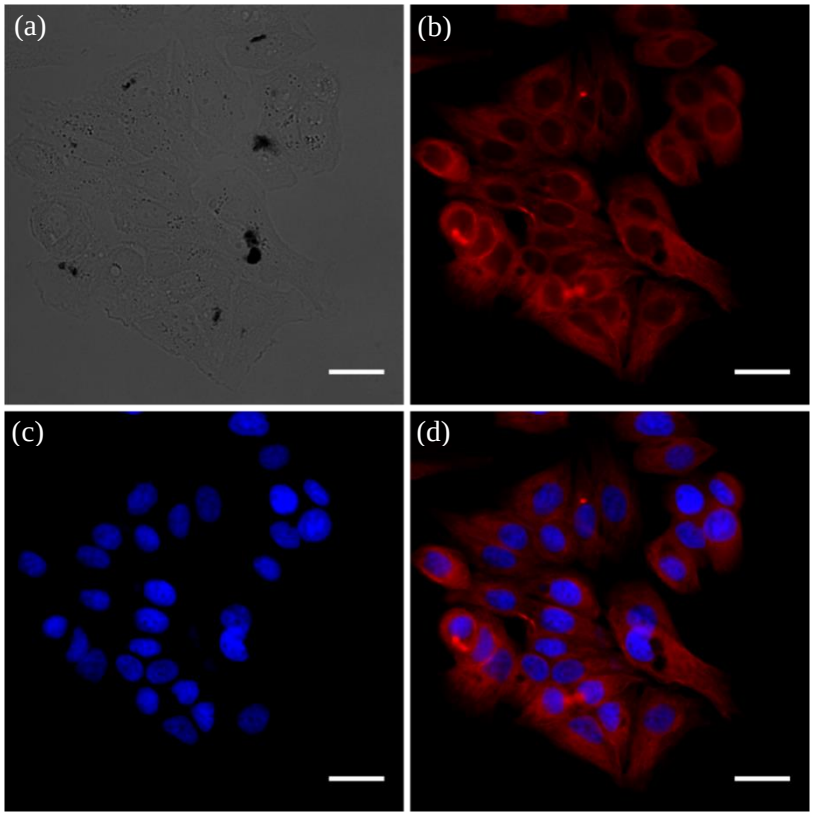


Figure S18. Microtubules network of MCF-7 cell lines after dodecapeptide-TGOtreatment.Fluorescence microscopic images of MCF-7 cells in (a) DIC mode, (b) 561, (c) 405 nm channel after antibody and DAPI treatment and (d) merged image. Scale bar corresponds to 30 µm.

Supplementary Movie 1: Kinesin1-TGO-EGFP nanoparticles are excellently walking along the microtubules as soon as they land onto the microtubules lattice as we observed that the green colored TGO nanoparticles are walking along the red colored immobilized microtubules. Scale bar corresponds to 10 µm.