Electronic Supplementary Information (ESI) for:

Enhanced Cancer Cell Killing of a Pt(IV) prodrug Promoted by Outer-Sphere Coordination with Polyethyleneimines

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EXPERIMENTAL SECTION

Materials. The complexes *c,c,t*-$[Pt(NH_3)_2Cl_2(O_2CCH_2CH_2CO_2H)_2]$ (**1**),[1] $[Pt(NH_3)_2(glyct)(O_2CCH_2CH_2CO_2H)_2]$ (**2**)[2] and *c,c,t*-$[Pt(NH_3)_2Cl_2(OH)_2]$ **(3)**[1] were synthesised as previously described. Potassium tetrachloroplatinate(II) (99%) was purchased from Precious Metals Online. Polyethyleneimine (branched, 1.8 kDa, 99%; 36% NH_2, 29% NH and 35% N determined by NMR studies) was purchased from Alfa Aesar. Hydrogen tetrachloroaurate(III) trihydrate (99.99%), succinic anhydride (99%), potassium iodide (≥99.5%), potassium chloride (≥99.0%), silver nitrate (≥99.0%), hydrogen peroxide solution 30% (w/w) in H_2O and glycolic acid (99%) and L-ascorbic acid (>99.5%) were purchased from Sigma Aldrich.

Synthesis of PEI-capped AuNPs. To prepare the PEI-capped AuNPs, PEI dissolved in water was added to an aqueous solution of $HAuCl_4$ in a 2:1 molar ratio and the mixture was stirred at 90 °C. This preparation is slightly modified from recent literature preparations, which used high molecular weight PEI.[3] The formation of AuNPs was monitored by UV-vis, for which UV-vis absorption spectra of the reaction mixture at different time points were recorded on a Cary 5000 UV–Vis-NIR spectrophotometer in 1 cm quartz cuvettes at room temperature. In this study we used PEI-capped AuNPs formed after 2 h. Once the reaction mixture was cooled to room temperature, the PEI-

capped AuNPs were purified by passing through a centrifugal filter unit (MWCO = 50000; Amicon Ultra-4).

Synthesis of FITC-PEI.[4] An aqueous solution of PEI 1.8 kDa (25 mg, 13.9 µmol, 500 µL) was added to FITC (5 mg, 12.8 µmol) dissolved in 5 mL H_2O:DMSO (1:1). The mixture was stirred overnight in the darkness and later dialyzed against H_2O for 2 days (MWCO 2000 kDa) to remove any unbound FITC until point no fluorescence could be detected in the dialysate. The resulting FITC-labeled PEI was then lyophilized.

FITC-PEI-AuNPs were prepared as above by using the FITC-labeled PEI.

Experimental procedures.

Sample preparation. All samples were freshly prepared simply by mixing an aqueous solution of the protonated Pt(IV) complexes at the appropriate concentrations with an aqueous solution of PEI or PEI-capped AuNP at the concentrations given in the figure captions. For the NMR studies the solvent was D_2O and the pH was adjusted by adding small amounts of NaOH over the pH range 4-8, for the electrochemistry we used 10 mM PBS pH 7.4 to prepared the solutions and for the cell cytotoxicity studies we used ultrapure water. In the case of studies with cells prior to the incubation with the cancer cells, the samples were left to interact for ca. 30 minutes and then were diluted 1/10 in cell culture medium and directly added to the cells to ensure no loss of reagents. The concentration of Pt(IV) complexes was determined by ICP-MS at the SGIker analytical facility of the University of the Basque Country (UPV/EHU; Leioa, Spain), the concentration of PEI taking the average MW of 1.8 kDa. In the studies with PEI-AuNPs, the gold concentration refers to Au(0) determined from the UV-Vis spectrum of the nanoparticle stock solution once purified to remove any excess of the reagents. This Au(0) concentration can be directly correlated to the absorbance value at 400 nm.[5]

NMR studies. ^{1}H NMR spectra were recorded on a Bruker Avance 500 NMR spectrometer. Data processing was carried out using Mnova software.^{1}H NMR were obtained in D_2O and the chemical shifts were expressed relative to the water peak at 4.8 ppm.

Electrochemical studies. All electrochemical studies were performed with an Autolab PGSTAT 128N (Metrohm Autolab B.V., The Netherlands) using disposable screen-printed electrodes, which integrate carbon working (4 mm diameter) and counter electrodes and an Ag/AgCl reference electrode onto a ceramic substrate (DRP-110, DropSens, Spain). The platinum(IV) complex (**1**) was dissolved in 10 mM PBS pH 7.4 in the absence and presence of PEI. The solutions were degassed immediately prior to use by bubbling argon through them for at least 30 min.

XPS measurements. XPS experiments were performed in a SPECS Sage HR 100 spectrometer with a non-monochromatic X-ray source (Magnesium Kα line of 1253.6 eV energy and 250 W), placed perpendicular to the analyzer axis and calibrated using the $3d_{5/2}$ line of Ag with a full width at half maximum (FWHM) of 1.1 eV. The selected resolution for the spectra was 15 eV of Pass Energy and 0.15 eV/step for the detailed spectra of the Pt 4f peaks. The deconvolution of the Pt 4f peaks was carried out at 36s of X-ray exposure. The deconvolution allowed to estimate the ratio of the Pt(II) and Pt(IV) states. All measurements were made in an ultra high vacuum (UHV) chamber at a pressure below $5 \cdot 10^{-8}$ mbar. In the fittings Gaussian-Lorentzian functions were used, where the FWHM of all the peaks were constrained while the peak positions and areas were set free.

Cell culture and viability measurements. PC-3 human prostate cancer cell line was obtained from the American Type Tissue Collection (ATCC) and cultured in Ham's F-12K (Kaighn's) medium (Glibco) supplemented with 10% fetal bovine serum (Invitrogen) and 1% penicilin/streptomycin (Invitrogen) at 37°C under 5% CO_2. Cells were passaged at ~70% confluence and a low passage number was maintained using cryopreserved stocks stored in fetal bovine serum supplemented with 10% DMSO (Sigma Aldrich). MDA-MB231 human breast cancer line and PANC-1 human pancreatic carcinoma cell lines were obtained from ATCC and European Collection of Cell Cultures (ECACC) respectively and were cultured in RPMI medium 1640 (Lonza) with 10% fetal bovine serum (Invitrogen), 1% L-glutamine and 1% penicilin/streptomycin (Invitrogen) at 37°C under 5% CO_2. Cells were passaged at ~70% confluence and a low passage number was maintained using cryopreserved stocks stored in FBS supplemented with 10% DMSO (Sigma Aldrich).

The cell viability was measured using the sulforhodamine B assay.[6] For cell viability measurements, PC-3, MDA-MB231 and PANC-1 cells were plated at 2000cell/well density in 96-well plates and allowed to adhere overnight. All the formulations were diluted 1/10 in medium and incubated at 37°C using 200 µL of sample per well in quintuplets. After 72 h, the cells were fixed by the addition of ice cold 25% trichloroacetic acid (TCA) solution prior to staining with the SRB dye solution (0.4% w/v in 1% glacial acetic acid). The plates were washed with 1% glacial acetic acid, air-dried and resuspended in Tris buffer (10 mM, pH 10.5) before reading the absorbance at 550 nm. Curve fitting and interpolation of the IC_{50} values was carried out using GraphPad Prism 4 software. The experiments were repeated three times.

Flow cytometry analysis. PC-3 cells were trypsinized and collected into cytometer tubes (1×10^5cells/tube). The cells were centrifuged (1000 rpm, 5 min, 4 °C) in order to discard the media and the pellet was resuspended in the formulations diluted in cell culture media (200 µL/tube). After 45 minutes of incubation at 4 °C, the cells were centrifuged (1000 rpm, 5 min, 4 °C) and washed once with cold PBS 10 mM. Finally the cell pellet was resuspended in 0.4 mL cold cytometer buffer (1 % bovine serum albumin (BSA) and 0.1 % sodium azide in PBS 10 mM). Cells were kept on ice until its analysis. The cellular uptake of the different formulations was measured using a FACS Canto II (BD Bioscience) and the data was analyzed with FlowJo, LCC software. PC-3 cells were electronically gated based on forward and side scatter parameters and the not-single events leaved out based on the forward area and height scatter parameters. The laser excitation wavelength used for FITC was 488 nm and the emission filter 530/30 nm. Each analysis represented the acquisition of 10000 cells per sample.

Cell imaging. PC-3 cells were seeded in a Ibidi µ-Slide VI 0.4 and allowed to adhere overnight in an incubator (37 °C, 5% CO2) in Ham's F-12K (Kaighn's) medium. The medium was changed and the nuclei were stained with DAPI (5 µg/mL) for 15 minutes, washed with sterile PBS 10 mM and incubated with the FITC containing nanoparticle formulations for 45 minutes. The medium was then removed, cells washed with sterile PBS 10 mM and fresh medium was added. The cells were visualized using a Zeiss Axio Observer wide field fluorescence microscope (Carl, Zeiss, Germany). Brightfield and fluorescence images were collected and processed using AxioVision software.

References

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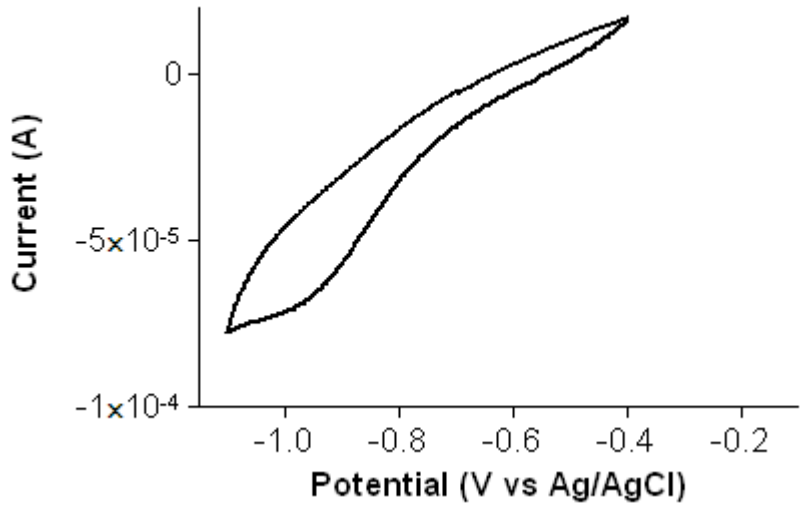

Fig. S1. Cyclic voltammogram of (**1**) in 10 mM PBS pH 7.4 (scan rate = 100 mV/s).

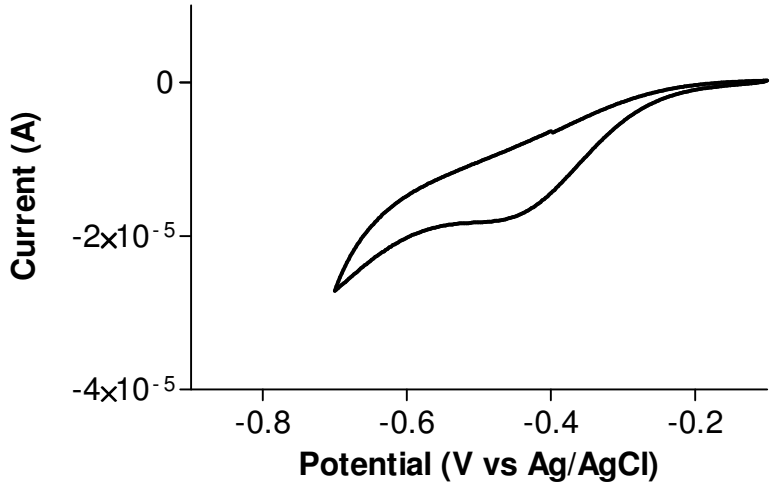

Fig. S2. Cyclic voltammograms of (**1**) in the presence of PEI in 10 mM PBS pH 7.4 (scan rate = 100 mV/s).

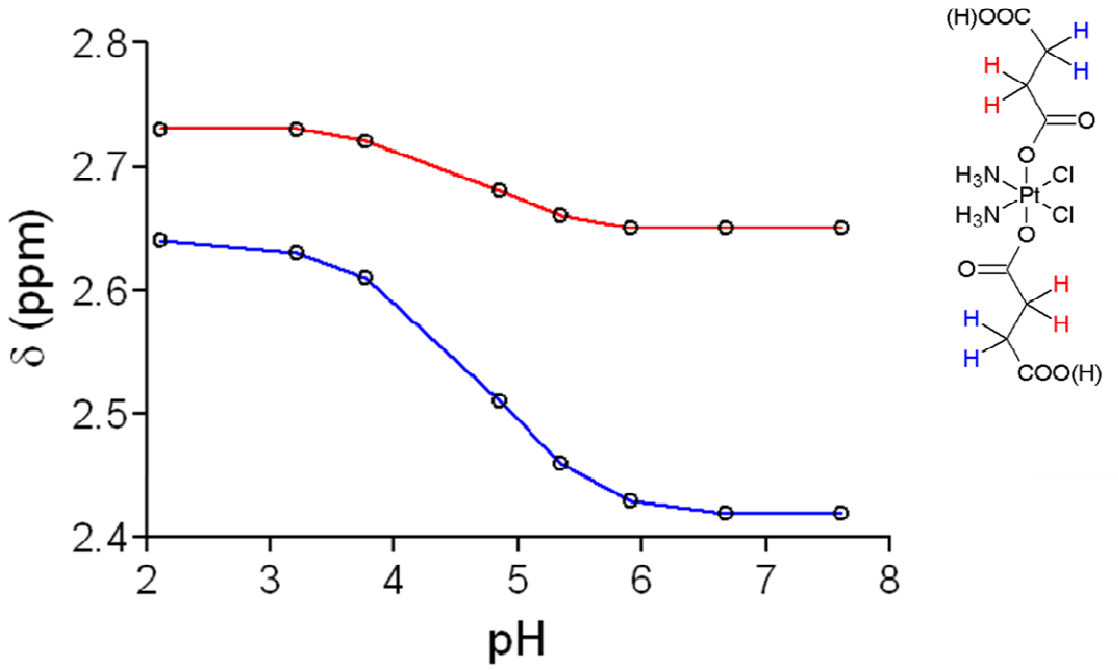

Fig. S3. pH dependent variation in the chemical shift of the succinic protons of (**1**).

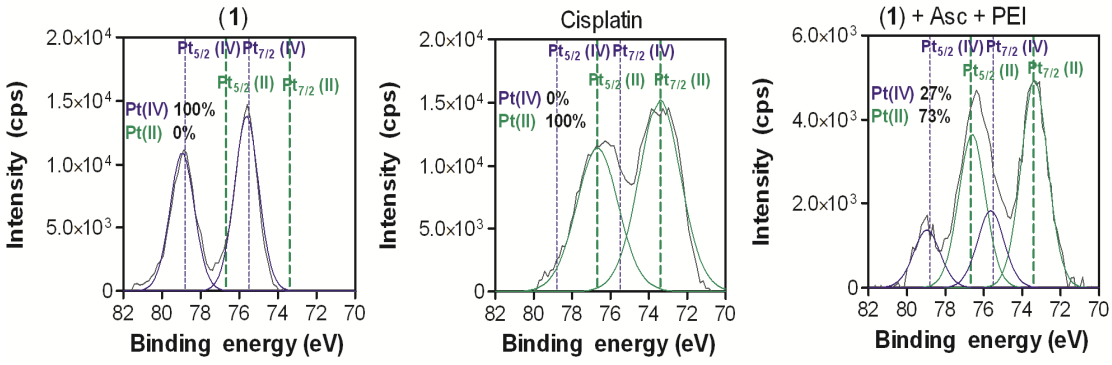

Fig. S4 XPS spectra showing the Pt 4f region of (**1**), cisplatin and PEI-(**1**) outer sphere complexes in the presence of ascorbic acid 24 h after mixing in water at pH 7.

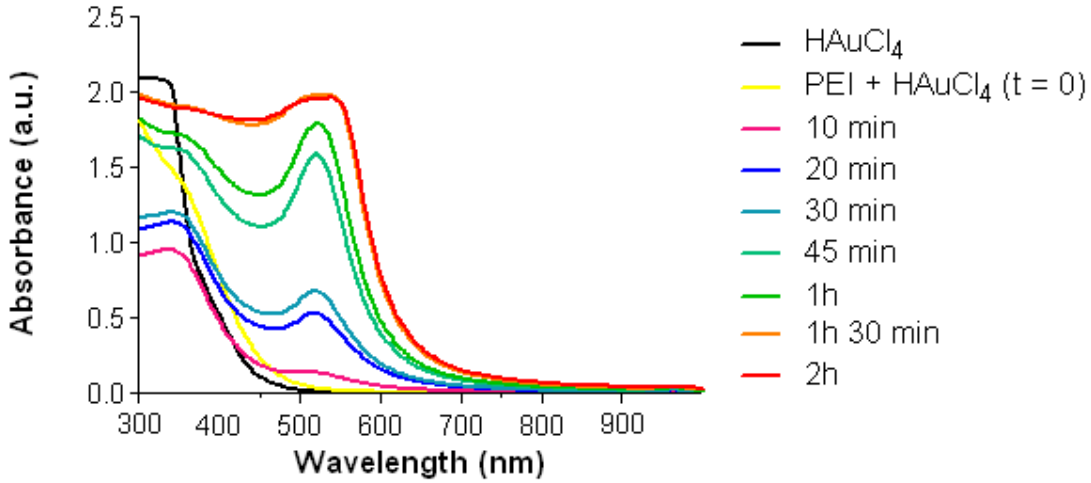

Fig. S5. UV-vis spectra of the solutions of $HAuCl_4$ and 1.8 kDa PEI at the different stages of the synthesis of the PEI-capped AuNPs in water.

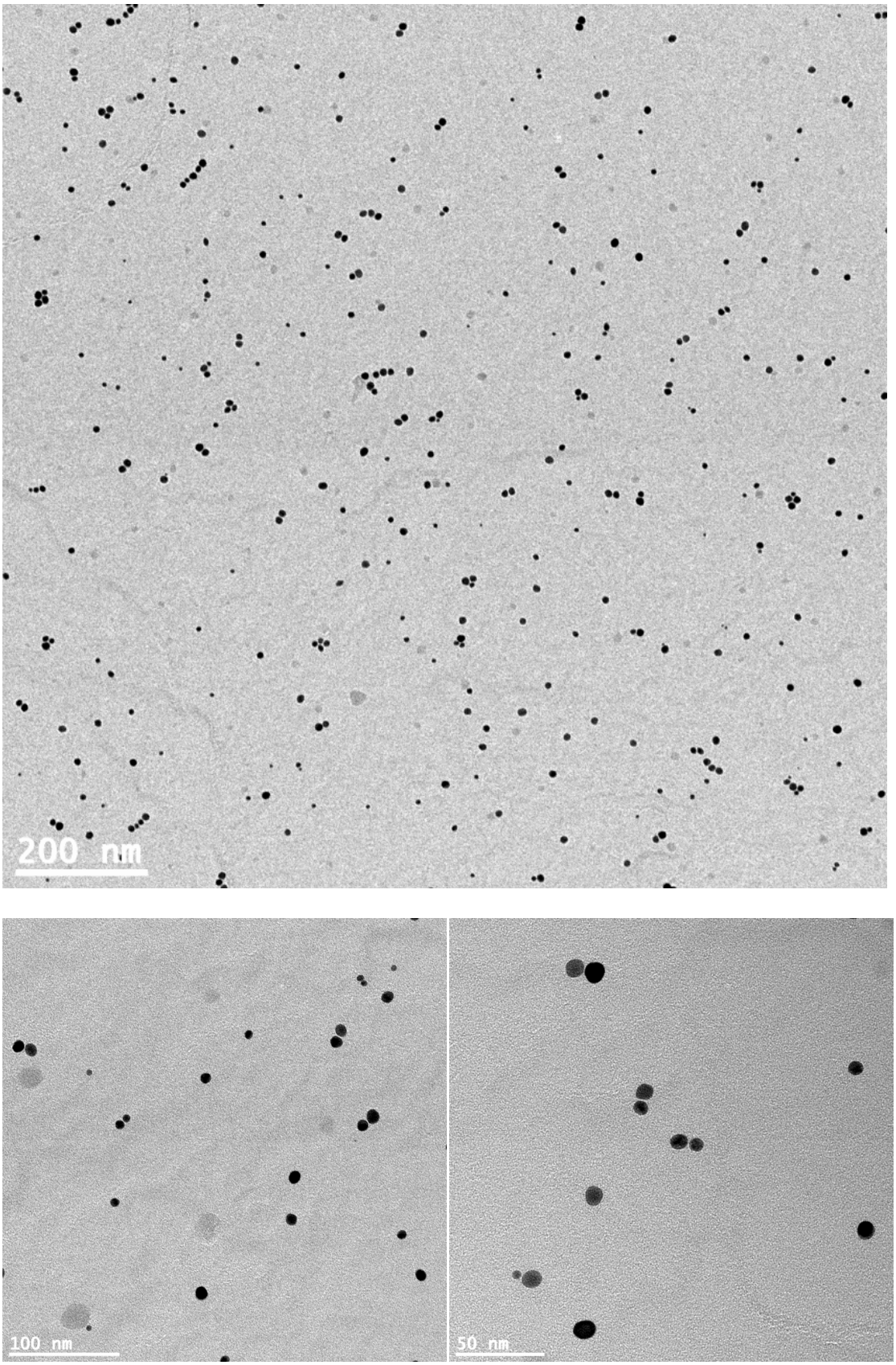

Fig. S6. Representative low and high magnification TEM images of the synthesized PEI-capped AuNPs.

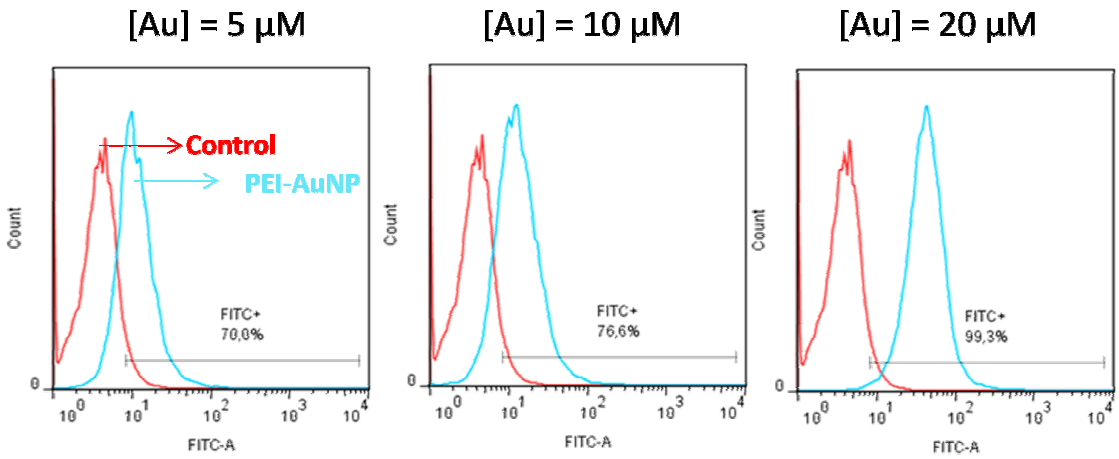

Fig. S7. Flow cytometric histograms of PC-3 cells incubated with FITC-PEI-AuNPs for 45 min at different gold concentrations.

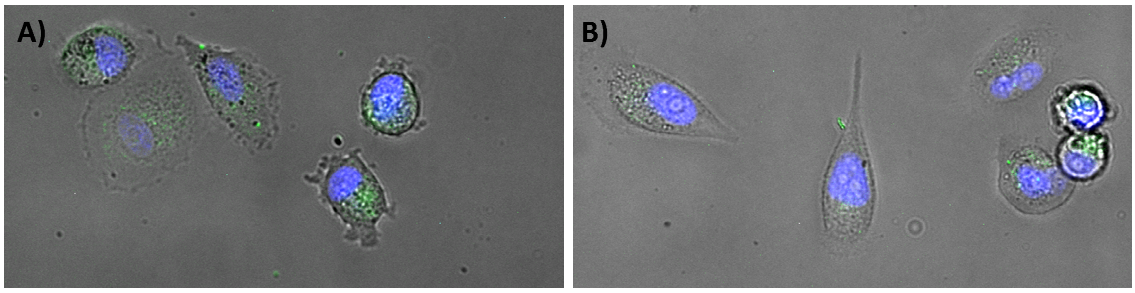

Fig. S8. Overlay of brighfield and fluorescence microscopy imagines taken of live PC-3 cells after 45 min incubation with A) FITC-PEI-AuNPs and B) FITC-PEI-AuNPs/(**1**) complexes (green), followed by extensive washing to remove adherent nanoparticles. The nuclei are stained with DAPI (blue).

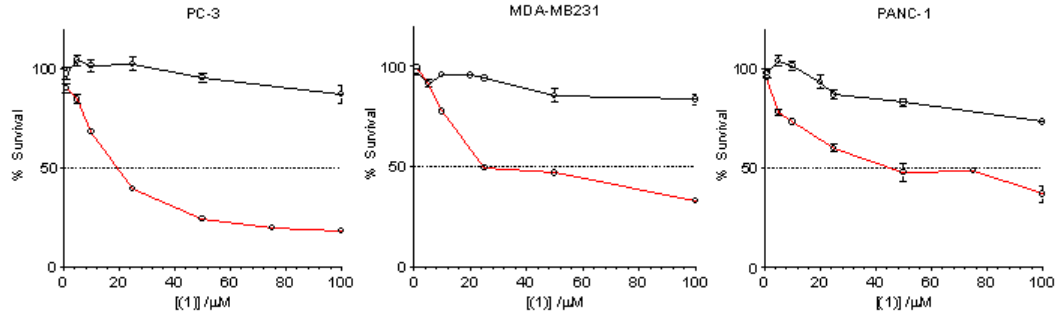

Fig. S9. Cytotoxicity profiles of PEI-(**1**) mixtures (red) and (**1**) (black) in PC-3, MDA-MB231 and PANC-1 cell lines. Results are the means ± SEM from three independent experiments performed in quintuplets.

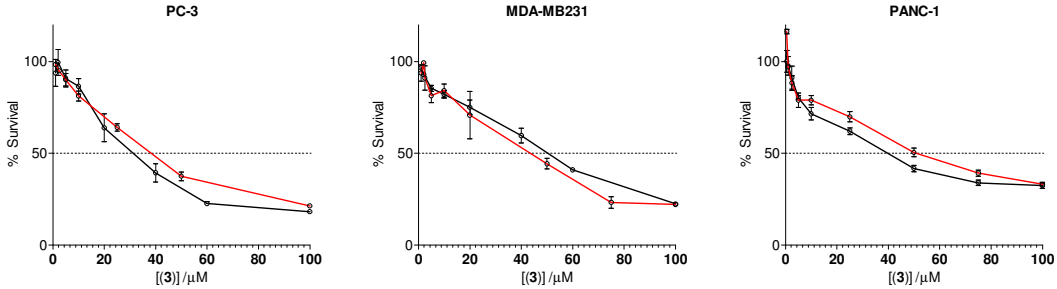

Fig. S10. Cytotoxicity profiles of PEI-(**3**) mixtures (red) and (**3**) (black) in PC-3, MDA-MB231 and PANC-1 cell lines. Results are the means ± SEM from three independent experiments performed in quintuplets.

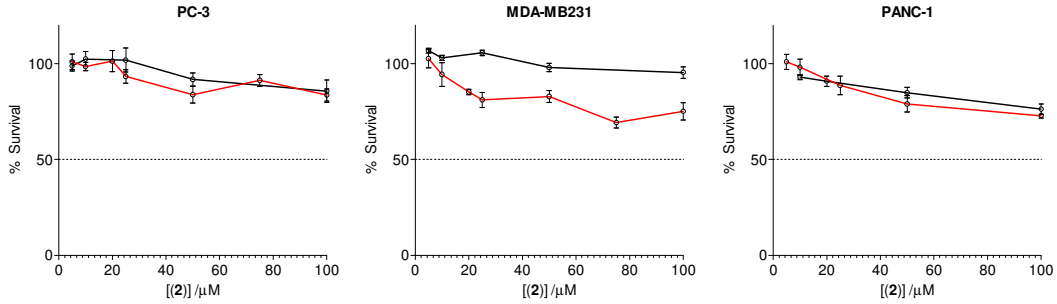

Fig. S11. Cytotoxicity profiles of PEI-(**2**) mixtures (red) and (**2**) (black) in PC-3, MDA-MB231 and PANC-1 cell lines. Results are the means ± SEM from three independent experiments performed in quintuplets.

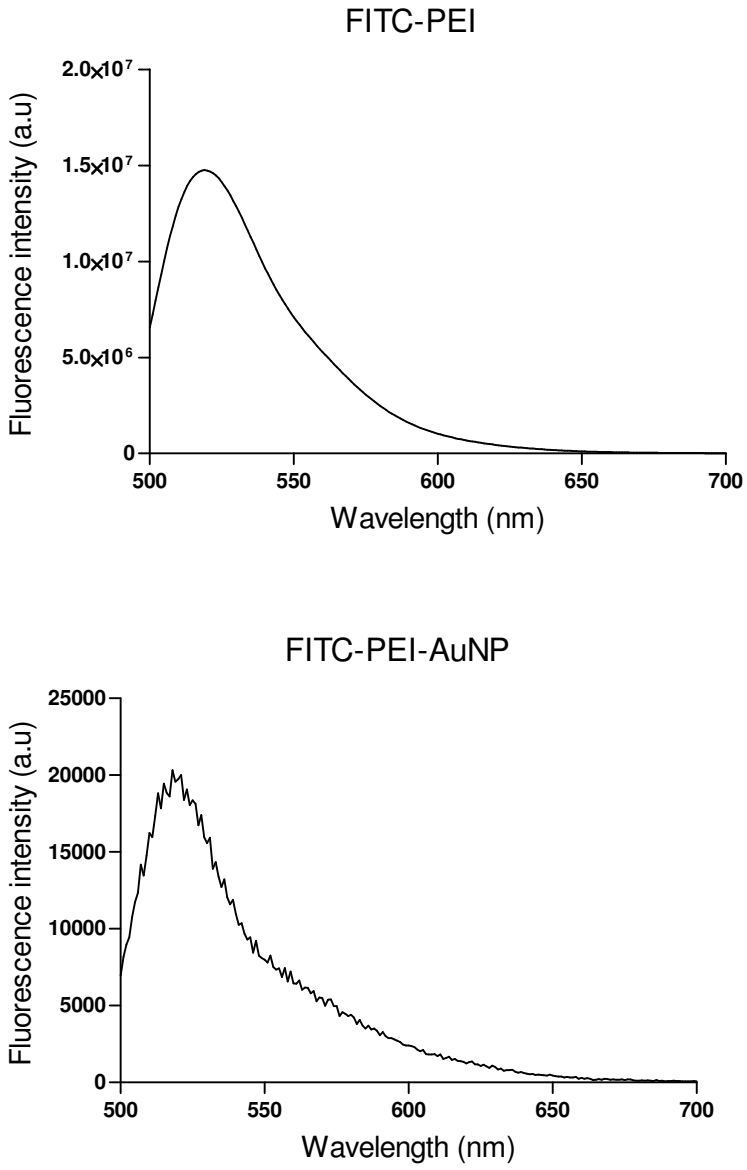

Fig. S12. Fluoresecence spectra of the FITC-PEI and FITC-PEI-AuNPs.

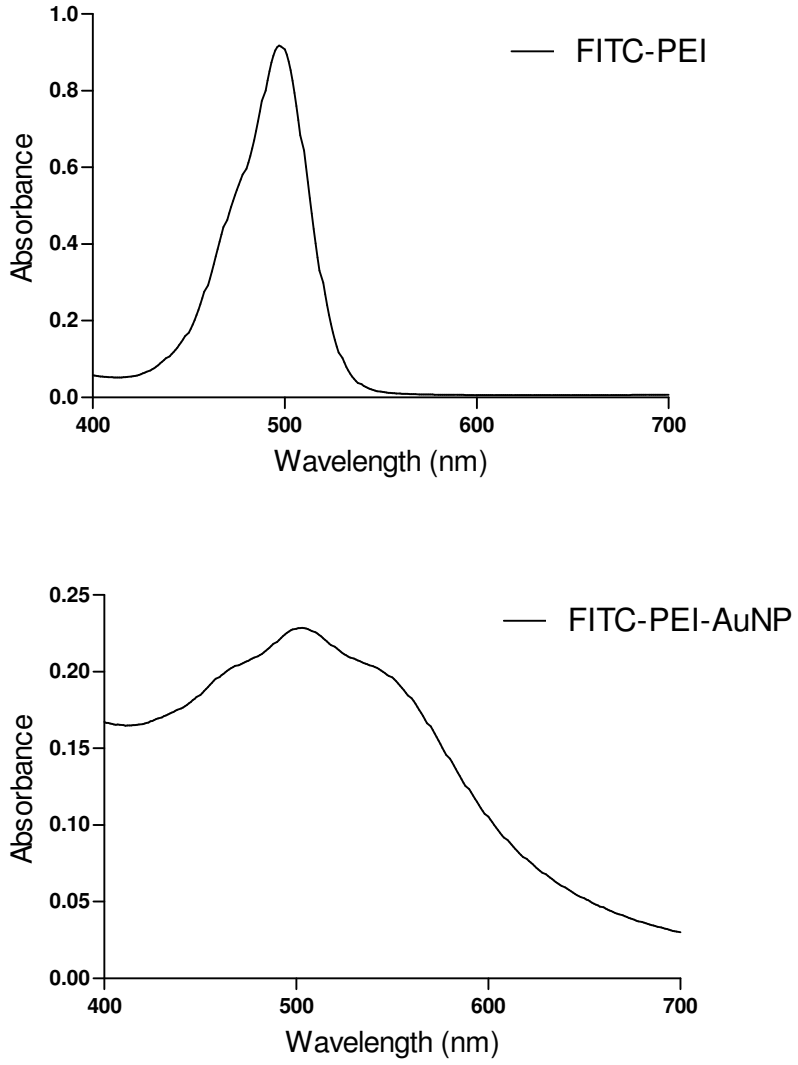

Fig. S13. UV-vis spectra of the FITC-PEI and FITC-PEI-AuNPs.