

# Electronic Supplementary Information

## Interactive Luminescent Gold Nanocluster Embedded ds DNA and Cisplatin as Model Nanoparticle for Cancer Theranostics

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- 1. Chemicals:** HAuCl<sub>4</sub> (Au, 17 wt % in dilute HCl; 99.99%), mercapto propionic acid (MPA), cis-diamineplatinum (II) dichloride (cisplatin) were obtained from Sigma-Aldrich and used as received. Milli-Q grade water (>18 MΩ cm<sup>-1</sup>, Millipore) was used in all experiments.

2. **Characterization of nano composite:** UV–visible spectra were measured by PerkinElmer Lambda 25 spectrophotometer. Fluorescence spectra were measured by using a spectrophotometer (Fluorolog –3, Horiba Jobin Edison, NY, USA).
3. **Transmission electron microscopy (TEM):** Sample was diluted to optimum level and then 7  $\mu\text{L}$  of it was drop-cast over a TEM grid, allowed to dry for overnight and was then analyzed by a JEM 2100; Jeol machine.
4. **Field Emission Scanning Electron Microscopy (FESEM):** Treated cellular samples were trypsinized and were resuspended in 1 ml phosphate buffer saline(PBS). Cells were fixed with chilled 70 % ethanol from which 7  $\mu\text{L}$  was drop-cast on a clean and autoclaved cover slip, which was allowed to air-dry. Samples were coated by gold before being analyzed by FESEM (Sigma, Zeiss).
5. **Atomic force microscopy (AFM):** For both DNA Au NCs and composite nanoparticles (composite NPs) sample was diluted to optimum level and then 7  $\mu\text{L}$  of it was drop-cast over a cover slip, which was allowed to dry for overnight. Then the sample was analyzed with AFM 5500 series, Agilent.
6. **Synthesis of cisplatin loaded composite NPs:** Different ratios of the cisplatin and DNA were incubated with as- synthesized DNA-Au NCs in the dark for 1 h followed by centrifugation at 10,000 rpm for 10 min. The supernatant was discarded to remove the free cisplatin and redispersed in PBS followed by recording of emission at 580 nm. The binding of cisplatin with DNA-AuNCs resulted in loss in emission intensity of the clusters, which was probed by fluorescence spectroscopy. Then a ratio of cisplatin/base pair 0.55 was chosen for further experiments, which provided sufficient luminescence for cellular bioimaging.
7. **Loading efficiency:** Cisplatin was added with a fixed concentration of the DNA-Au NCs composite followed by the centrifugation at 10,000 rpm, for 10 min at 4 °C. The supernatant was collected and measured the concentration of free cisplatin was

determined by probing the absorbance by UV-Vis spectrometer at 210 nm. The loading efficiency of the composite NPs was calculated with following formula.

$$\text{Loading efficiency (\%)} = \frac{\text{Total Cisplatin} - \text{Cisplatin in supernatant}}{\text{Total Cisplatin}} \times 100 \%$$

Loading efficiency was checked for two ratios of [cisplatin] / [base pair] viz. 0.55 and 2.19. The amounts of cisplatin used in both calculations were 8  $\mu\text{g}$  and 12  $\mu\text{g}$ , respectively. The loading efficiency of the composite NPs was  $86 \pm 4 \%$  and  $46 \pm 2.3 \%$  for the two respective ratios and is in compliance with the fluorescence loss assay. Plasmid DNA content used was 32  $\mu\text{g}$ .

- 8. Confocal, Z stacking and Epi-fluorescence Microscopy:** HeLa cells were grown on a cover slip (which was already placed inside a cell culture plate) for 12 h in DMEM medium in the presence of 5% CO<sub>2</sub> at 37 °C. After that the cells were subjected to the composite NPs treatment (with concentration half of their IC<sub>50</sub> and was added directly in the medium) and were incubated for 3 h in DMEM medium in the presence of 5% CO<sub>2</sub> at 37 °C. The medium was then discarded and the plate was washed with PBS twice and the cells while in PBS were fixed with 0.1 % formaldehyde and then analyzed by microscope (LSM 880, Zeiss) The excitation wavelength for cellular imaging used for the confocal studies was set at 355 nm.
- 9. Release study:** Cisplatin concentration is measured by taking its absorbance at 210 nm by UV-Vis spectroscopy. For release studies the respective samples after the premediated period of time in buffers (both pH 7 and pH 4.5) were centrifuged at 10000 rpm and the supernatant was collected and checked for the amount of cisplatin released into the medium at that particular time interval. The percentage of cisplatin released with the respect to concentration of cisplatin released at 0h.

**10. Cell viability assay by MTT:** For both HeLa and HEK 293 cells  $5 \times 10^3$  cells/well were seeded in 96-well microplate and were allowed to grow for 12 h in DMEM medium in the presence of 5% CO<sub>2</sub> at 37 °C. After that the cells were subjected to various concentrations of the DNA-Au- NC, cisplatin and DNA-Au-NC-cisplatin (composite NPs) for 48 h. Then 7.0 μL of MTT [3- (4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] was added to each well of the microplate and kept for 2 h in 5% CO<sub>2</sub> at 37 °C for the formation of formazan. After 2 h of incubation, the medium was removed and 60 μL of DMSO was added to each well for the development of purple color due to the formation of formazan, the absorbance of which at 550 nm was taken as a measure of cell viability.

**11. Caspase 3 assay:** HeLa cells were seeded at  $1 \times 10^5$  cells/well in a six-well plate and were allowed to grow for 12 h in DMEM medium in the presence of 5% CO<sub>2</sub> at 37 °C. Then the cells were treated with the DNA-Au-NC, cisplatin, and DNA-Au-NC-cisplatin (composite NPs) at their IC<sub>50</sub> values for 48 h. Then cells were trypsinized and were further fixed with 0.1% formaldehyde in PBS for 15 min at room temperature. After recollecting cells by centrifuging them at 650 rcf for 5 min and at 4 °C they were re-suspended in PBS with 0.5 % Tween 20 (membrane permeabilization solution) and centrifuged immediately at 650 rcf for 7 min. Then the cells were washed twice with PBS and were re-suspended in PBS. Finally cells were treated with PE conjugated Rabbit Anti-Active-Caspase-3 (Catalog no. 550821, BD Biosciences) for 30 min in the dark at 37 °C and then the samples were analyzed by flow cytometer using Cell Quest pro in FL 2 H (red emission ) fluorescence channel for 15000 cells for each sample.

**12. Cell cycle analysis:** HeLa cells were seeded at  $1 \times 10^5$  cells/well and were allowed to grow for 12 h in DMEM medium in the presence of 5% CO<sub>2</sub> at 37 °C. Then the cells were treated with the DNA-Au-NC, cisplatin, and DNA-Au NC- cisplatin (composite NPs) at their IC<sub>50</sub> values for 48 h. Then cells were trypsinized and were recollecting by

centrifuging them at 650 rcf for 5 min at 4 °C. Cells were then fixed with 1 mL 70 % chilled ethanol by adding it drop by drop while vortexing it and then were kept at -20 °C for 1 h. Cells were then centrifuged at 650 rcf for 5 min at 4 °C; supernatant was discarded and the pellet was resuspended in 1 mL PBS. Cells were then subjected to RNase (100 µg/mL) treatment and were incubated at 55 °C for 1 h. Then propidium iodide (40 µg/ mL) was added with 0.01% Triton X-100 into the cells and samples were analyzed by flow cytometer using Cell Quest pro in FL 2 A (red emission) fluorescence channel for 15000 cells for each sample.

**13. Reactive oxygen species (ROS) generation measurement:** HeLa cells were seeded at  $1 \times 10^5$  cells/well and were allowed to grow for 12 h in DMEM medium in the presence of 5% CO<sub>2</sub> at 37 °C. Then the cells were treated with the DNA-Au-NC, cisplatin, and DNA-Au NC- cisplatin (composite NPs) at their IC<sub>50</sub> values for 3 h. Then cells were trypsinized and were recollected by centrifuging them at 650 rcf for 5 min at 4 °C. The cells were then re-suspended in 1 mL fresh DMEM with 5.0 µL of 1.0 mM of DCFH-DA being added to it and incubated for 10 min at 37 °C. After incubation, the samples were analyzed for the fluorescence of DCF by flow cytometer using Cell Quest pro in FL1 H (green emission) fluorescence channel for 15 000 cells for each sample.

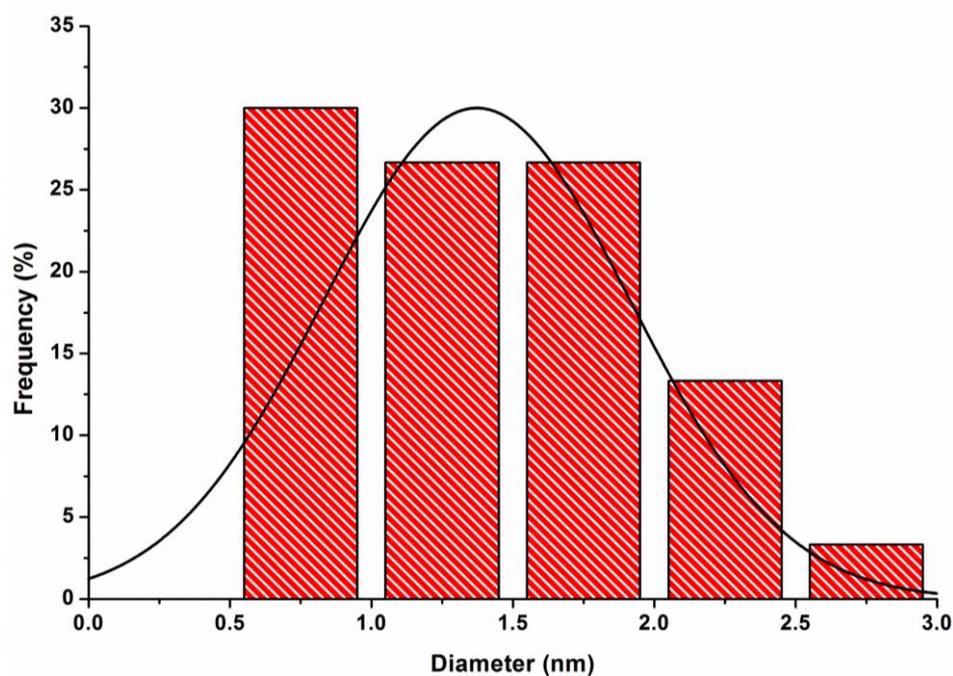
**14. Quantum yield measurement:** Quantum yield (QY) of DNA-Au-NC was measured by established protocol and taking quinine sulfated in 0.10 M H<sub>2</sub>SO<sub>4</sub> solution as the standard. Equation used for calculation is

$$Q_{\text{DNA NC}} = Q_{\text{Ref}} \frac{m_{\text{DNA NC}}}{m_{\text{Ref}}} \frac{n_{\text{DNA NC}}^2}{n_{\text{Ref}}^2}$$

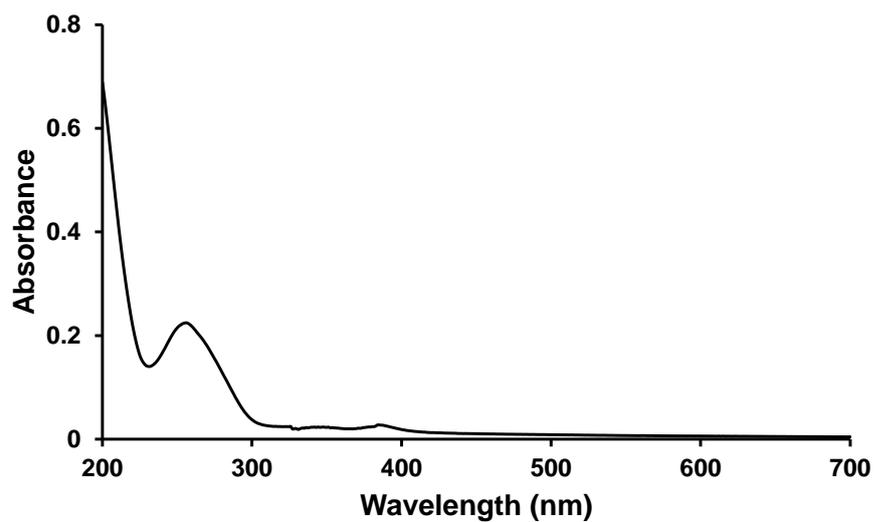
Here Ref stands for standard, Q is quantum yield, m stands for the slope of the plot of integrated fluorescence intensity vs. absorbance and n is for the refractive index (solvent

in both cases is water). We used the same solution for UV-vis and fluorescence measurements.

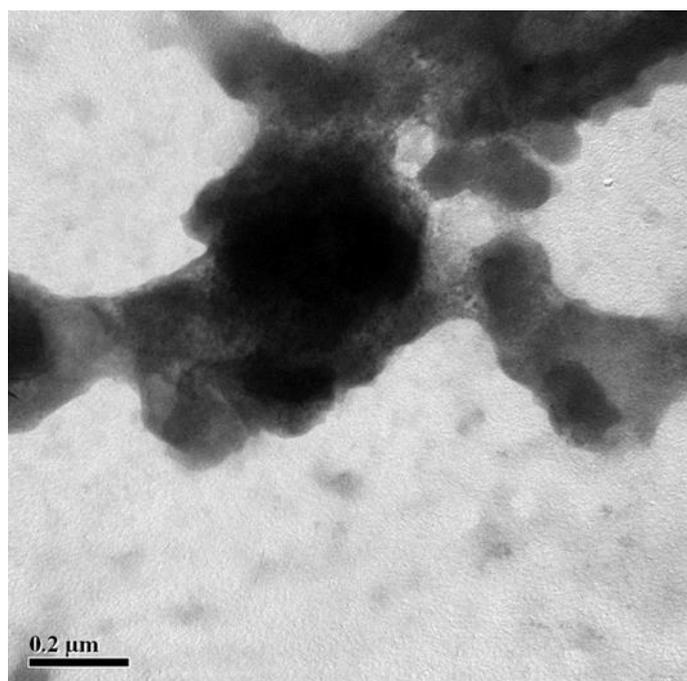
**15. Stastical analysis:** Statistical studies on MTT data and cell cycle data have been performed with one-way ANOVA test.



**Fig.S1.** Size distribution of the synthesized gold nanoclusters (DNA Au NCs) embedded in DNA. The average size of the particle was calculated to be  $1.38 \pm 0.54$  nm in the TEM analysis.



**Fig. S2.** UV-vis absorption spectrum of the synthesized DNA Au NCs marking the absence of characterstic SPR peak of gold. The peak at 260 can be attributed to the absorption by DNA.



**Fig. S3.** TEM image of the as-synthesized gold nanoclusters (DNA Au NCs).

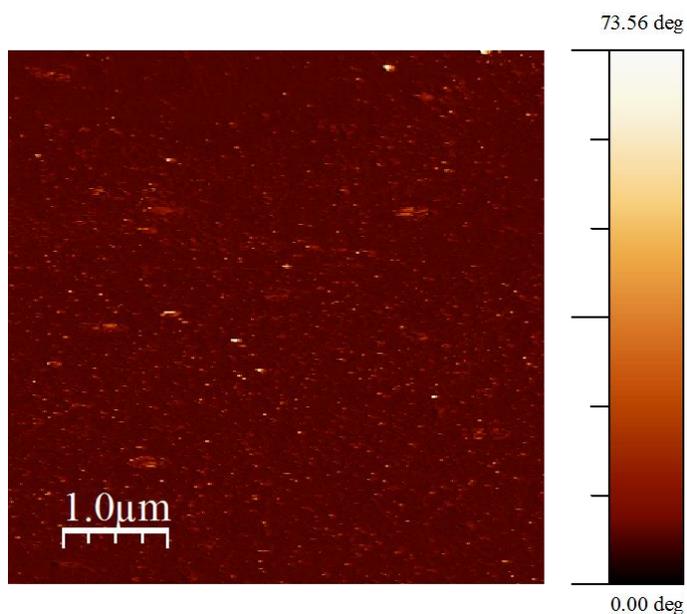


Fig. S4. AFM image of the synthesized gold nanoclusters (DNA Au NCs).

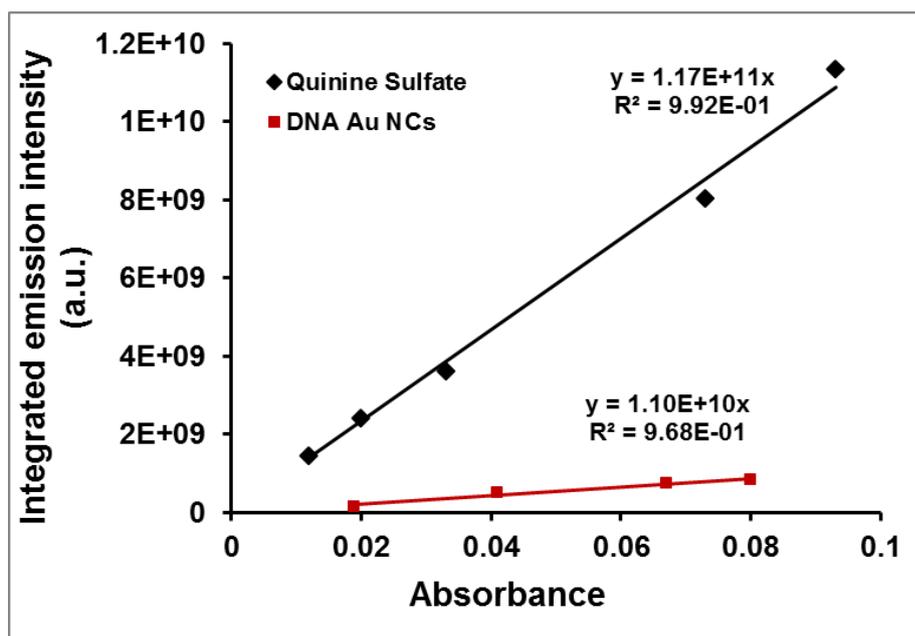
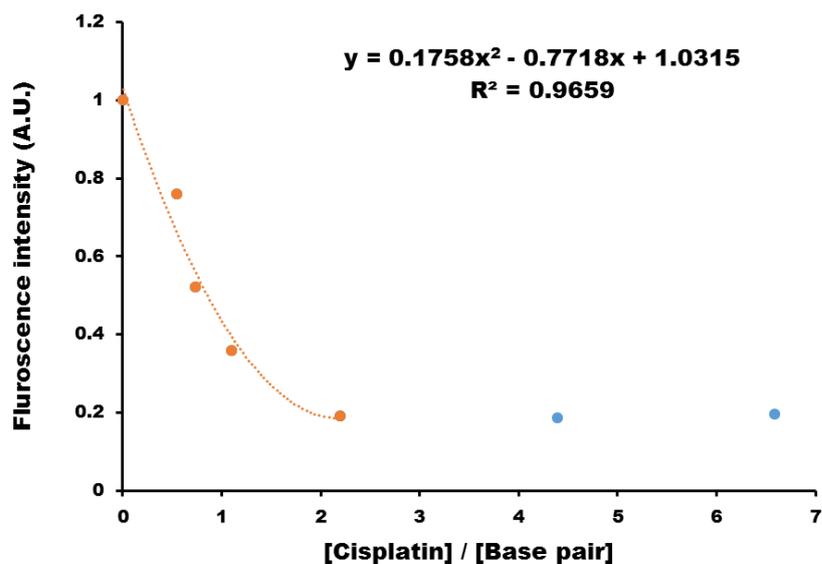
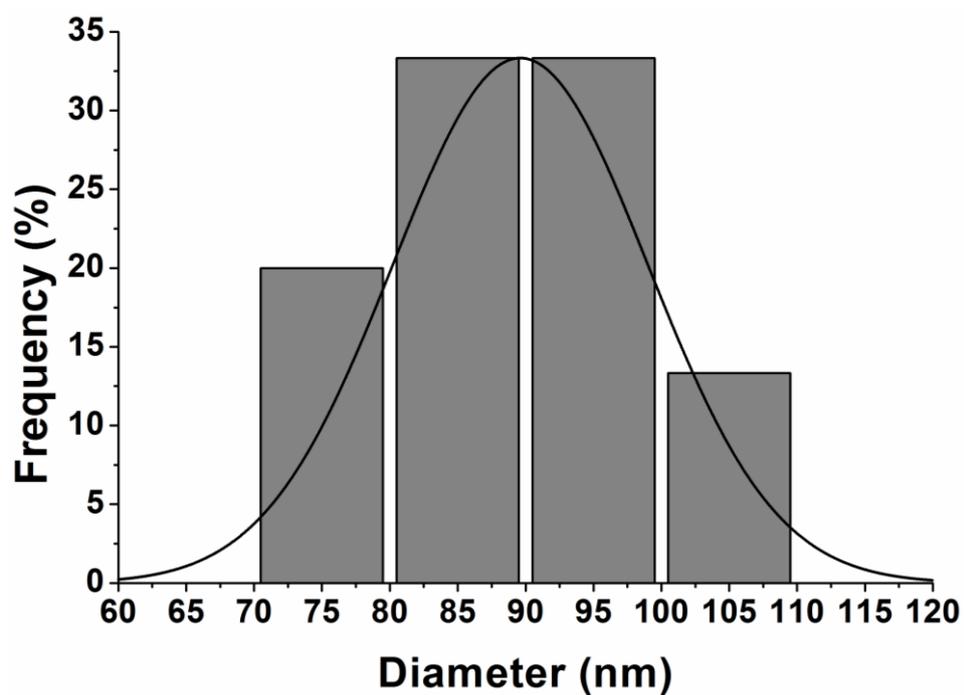


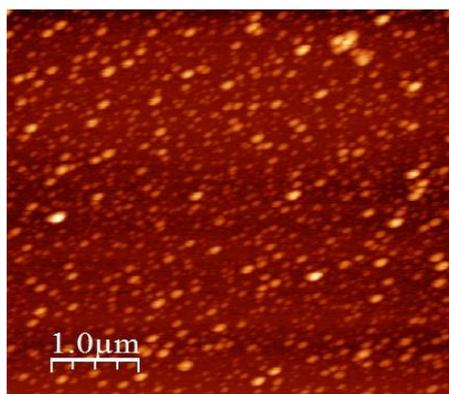
Fig. S5. Integrated fluorescence intensity vs absorbance plot of DNA Au NCs and quinine sulphate. Quinine sulphate was used here as a standard to determine the quantum yield of the synthesized DNA-Au-NC (which was found to be 5.01%).



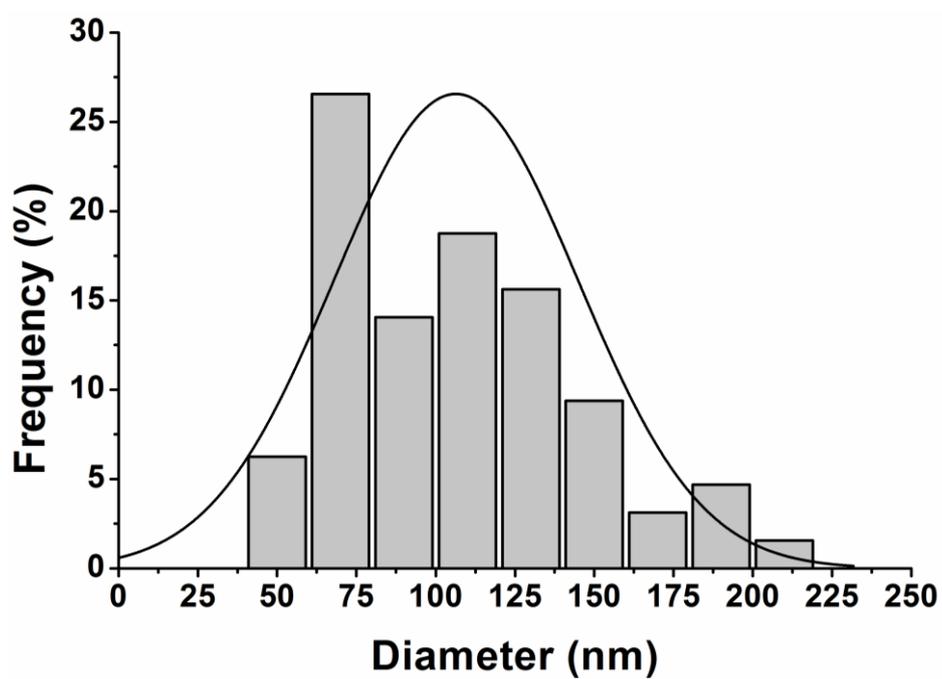
**Fig. S6.** Gradual loss in fluorescence of DNA Au NCs with increasing concentration of cisplatin. The plot was fitted with a second order polynomial relationship between fluorescence intensity and [cisplatin]/ [base pair].



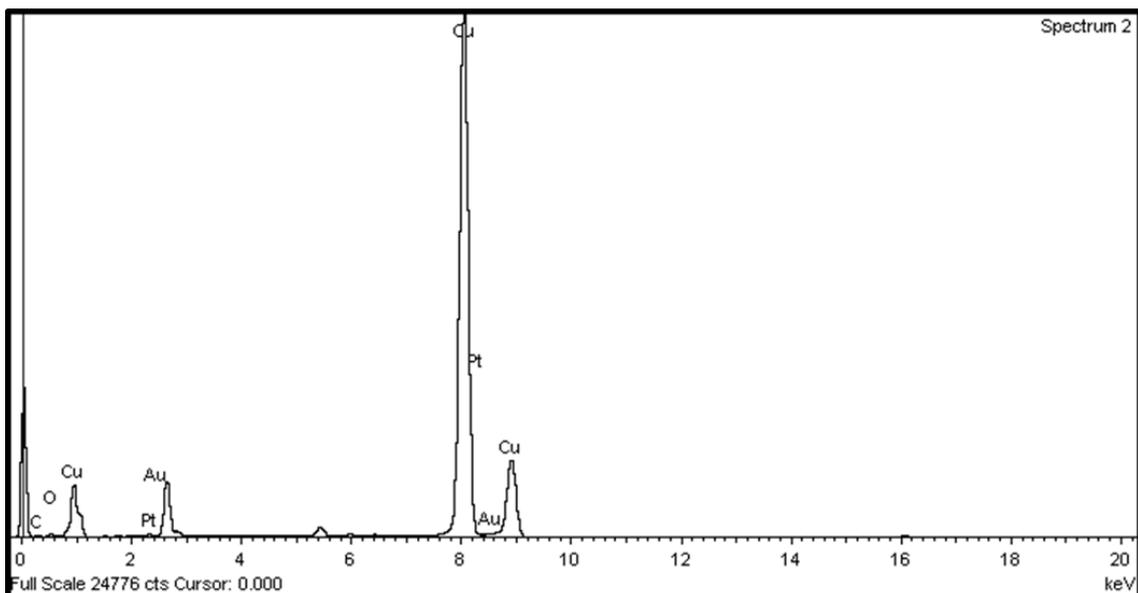
**Fig. S7.** Composite NPs size distribution based on TEM image .



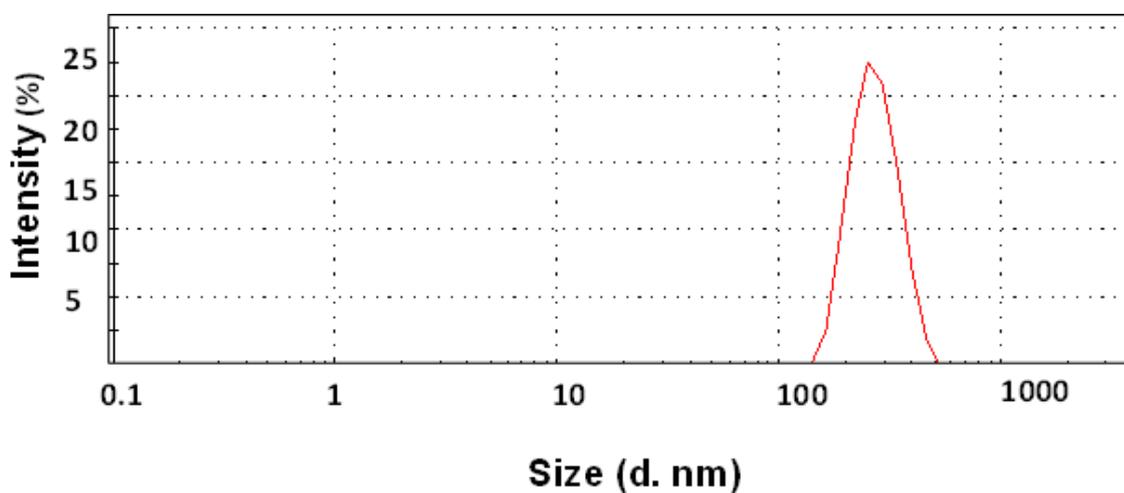
**Fig. S8.** AFM image of the composite NPs.



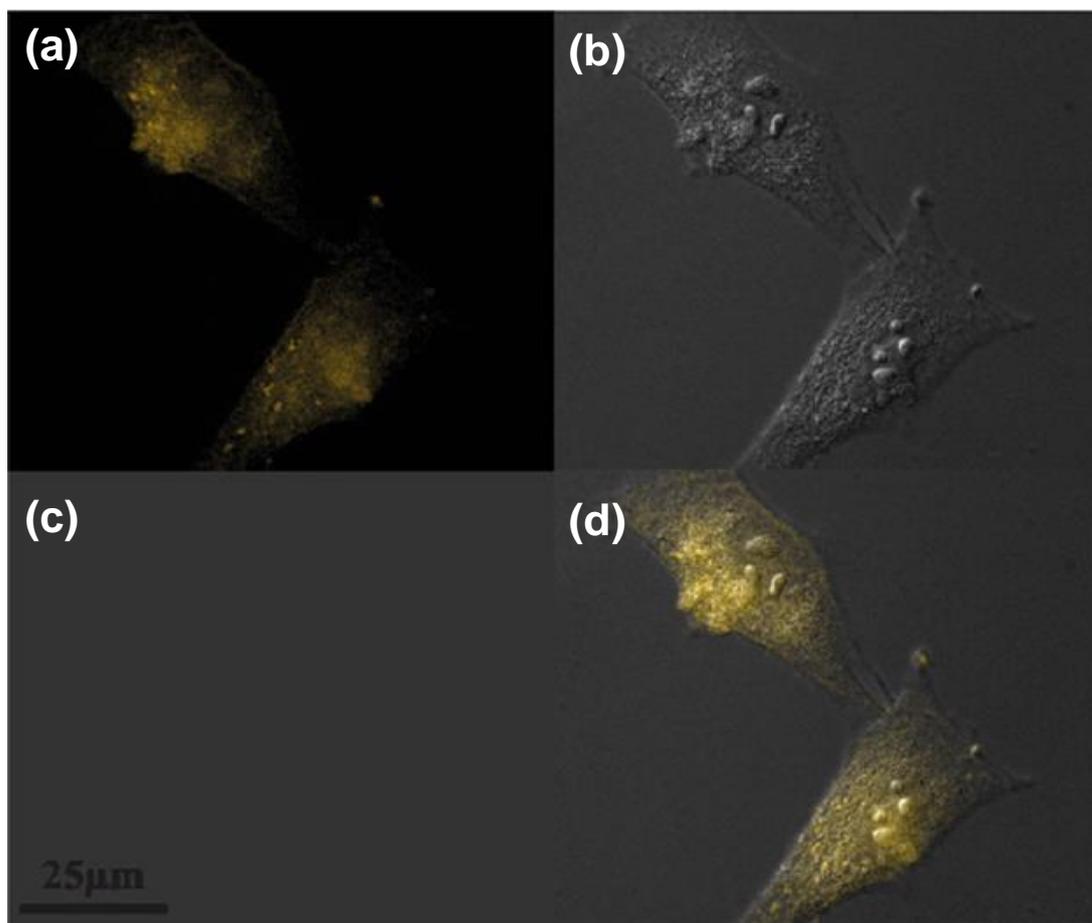
**Fig.S9.** Composite NPs size distribution based on AFM images.



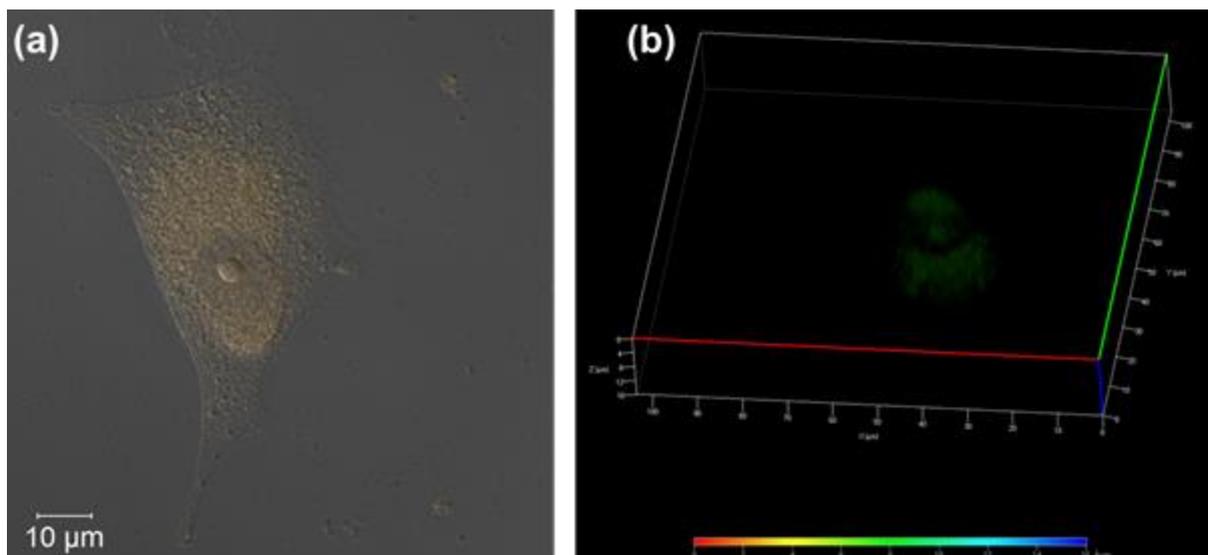
**Fig. S10.**Energy dispersive X-ray (EDX) spectrum of the synthesized composite NPs showed the presence of both gold and platinum and it meant the presence of DNA Au NCs and cisplatin both in the synthesized composite NPs.



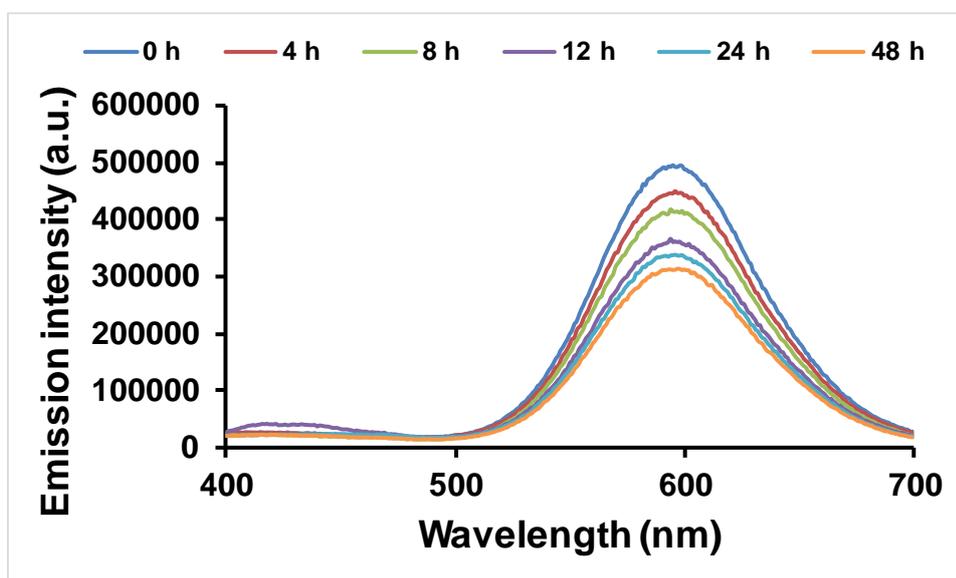
**Fig. S11.**Hydrodynamic size distribution of the composite NPs . The average hydrodynamic diameter of the composite NPs was 280 nm.



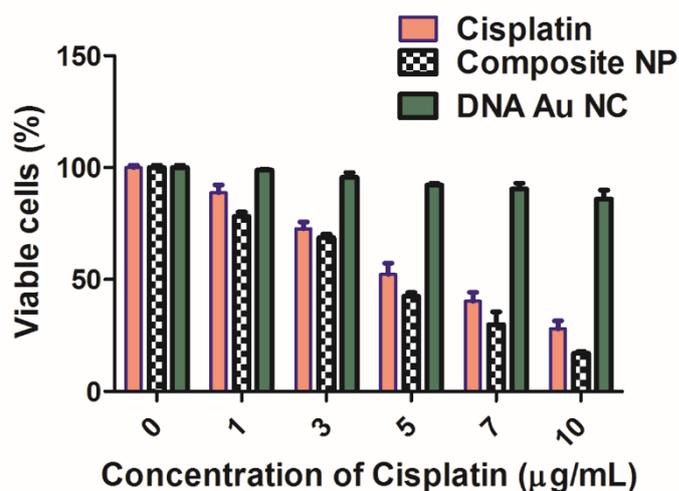
**Fig. S12.** Fluorescence images of HeLa cells treated with the composite NPs. (a) Fluorescence image of the HeLa cells showing characteristic yellow emission of the DNA - Au NCs; (b) bright field image of the same cells; (c) only background image and (d) merged image of the A and B, Scale bar 25  $\mu\text{m}$ .



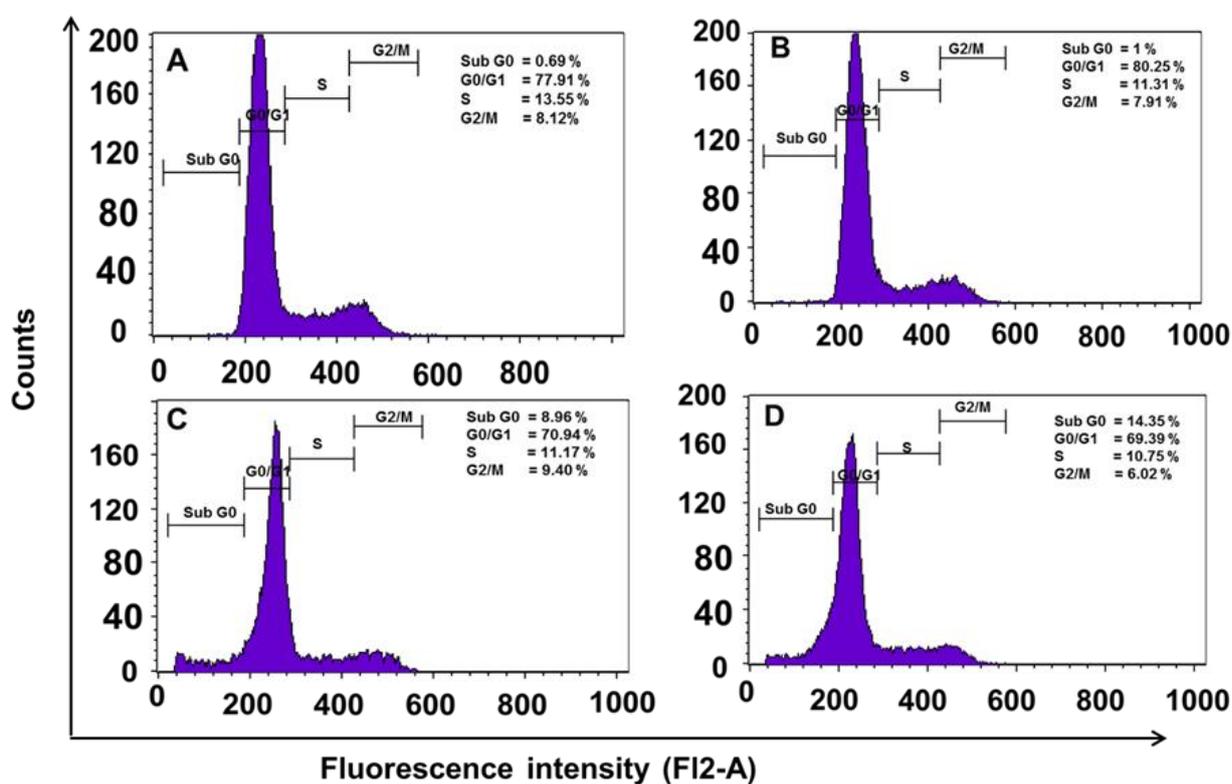
**Fig. S13. Fluorescence Cellular imaging of the HeLa cells with DNA Au NCs.** (a) Merged image of HeLa cells after treatment with DNA Au NCs for 3h, (b) a 16 μm Z-stacking image of the same cell confirming the uptake of the DNA-Au NCs. The image of the control cells (untreated cells) remained the same as with the composite NPs.



**Fig. S14.** Emission of the composite NPs in PBS at 0 h, 4 h, 8 h, 12 h, 24 h and 48 h.



**Fig. S15.** MTT assay results showing a better cytotoxicity of the composite NPs against only drug with HEK 293 cells. All data are represented as the mean  $\pm$  S.D.



**Fig. S16.** Cell cycle analysis with propidium iodide (PI) staining (A-D). The results correspond to (A) control, (B) DNA-Au-NC, (C) cisplatin and (D) composite NPs treated cells. The results confirmed the evolved cytotoxicity of the composite NPs over free cisplatin.