Supporting information

Rapid colorimetric sensing of Gadolinium by EGCG-derived AgNPs: the development of nanohybrid bioimaging probe

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Experimental

Materials

Silver Nitrate, (-)-Epigallocatechin gallate (EGCG) (95%), ferric chloride, zinc nitrate, nickel chloride, cobalt chloride, mercuric chloride, lead nitrate and cadmium chloride gadolinium acetate, ytterbium chloride, europium chloride, Neodymium chloride were purchased from Sigma Chemical Co.

Methods

Preparation of Epigallocatechin gallate-silver nanoparticles

EGCG solution was prepared by dissolving required amount of EGCG in deionized water (1ml) which was pre- adjusted with pH 5, 7 and 9 respectively, and then it was added dropwise in 50 ml of 1mM $AgNO_3$ solution with continuous stirring at 27^0C for 10 min in all pre-made EGCG solution.

Characterization of nanoparticles

UV-visible spectroscopy

The preliminary characterizations of AgNPs were carried out using Ultraviolet-visible (UV-vis) spectrophotometer. The bioreduction of silver ions were monitored by periodic sampling of aliquots (0.2 mL) of the reaction mixture and recording the UV-vis spectral reading of the solutions. UV-vis spectral data were taken as a function of time of reaction on Shimadzu UV 1800 -visible Spectrophotometer from 200 to 700nm.

HRTEM analysis

A drop of sample was loaded and dried on a carbon coated copper grid and kept in vacuum desiccators before loading onto specimen holder to analyze the AgNPs. The micrograph images were recorded on Tecnai G2, F30 (HR-TEM) instrument operating at a voltage of 300 kV.

FTIR

The FTIR measurements of AgNPs were performed using Lab India (Alpha FTIR). The lyophilized sample was grinded with KBr pellet and the resulting was placed into the sample holder for FTIR measurement.

Detection of heavy metals and REEs

Stock solutions of ferric chloride, zinc nitrate, nickel chloride, cobalt chloride, mercuric chloride, lead nitrate and cadmium chloride gadolinium acetate, ytterbium chloride, europium chloride, Neodymium chloride were prepared in water. For detection of metal, corresponding aqueous metal salt (90µM) was mixed with AgNPs solution and volume was adjusted to 500µl. UV-vis spectral data were taken on Shimadzu UV 1800 - visible Spectrophotometer ranging from 200 to 700nm.

Limit of detection study

Aqueous solution of gadolinium acetate (1mM) was prepared as stock. Gadolinium acetate (1-10µM) solution was mixed with AgNPs solution and volume was adjusted to 1 ml. Absorption spectral reading of the resulting solution was taken at 410 nm using 96 well ELISA plate reader and all measurements were conducted in triplicate. The relative absorption intensity calculation was described as $\Delta A = A_0 - A$, where A_0 and A represent the absorbance of AgNPs in the absence and presence of gadolinium, respectively. The limit of detection for Gd^{3+} was determined using the linear regression relationship ($y = 0.030x + 0.312$, $R^2 = 0.994$), between the absorbance intensity changes and the concentration of Gd^{3+} ion over the range from 1µM to 10µM.

Fluorescence spectroscopy

Fluorescence spectra were taken by using Cary Eclipse Fluorescence Spectrophotometer (Agilent Technologies).

Fluorescence microscopy

HCT 116 human colon cancer cells were cultured in Dulbecco's modified Eagle Medium (DMEM, gibco, USA), supplemented with 10% fetal bovine serum, 1% penicillin/ streptomycin and 2% L- Glutamine (Himedia Chemical Co.). Adherent cells HCT 116 were seeded for overnight on glass coverslips in six well plates. Then the cells were treated with Gd-AgNPs solution at a concentration of 200 nM of Gd^{3+} ions. After 4h of incubation, the cell were intensively washed with PBS to remove residual metal ions and fixed with 4% paraformaldehyde solution in PBS for 20 min. The fluorescence images of the cells were obtained by using fluorescence microscope (Olympus BX61).

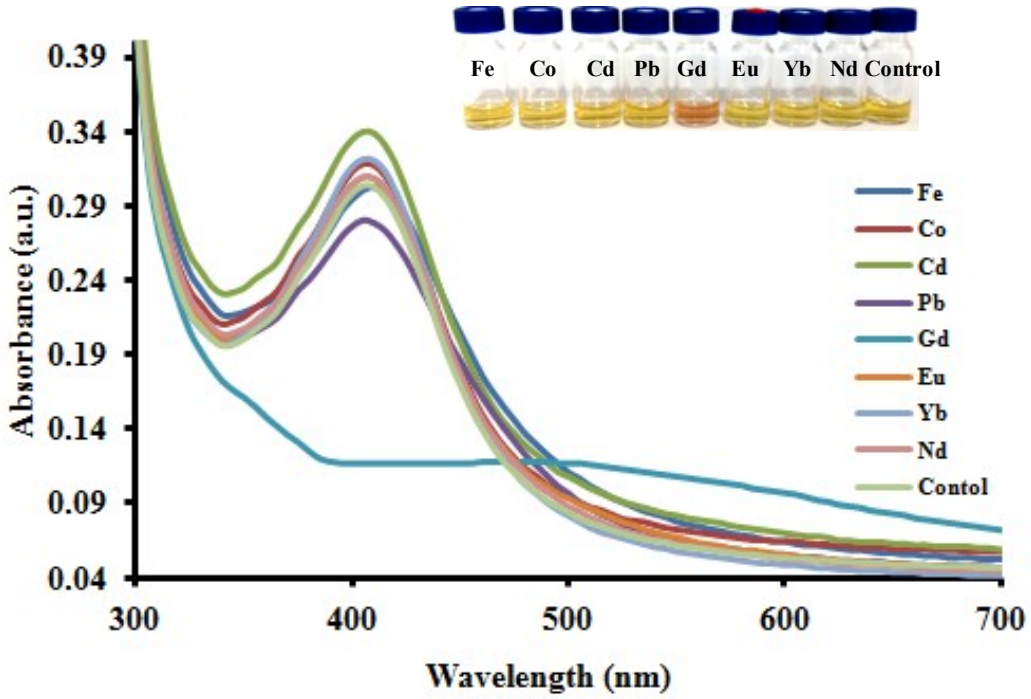


Fig S1. UV-vis spectra of AgNPs upon the addition of different metal ions (concentration=10 µM)

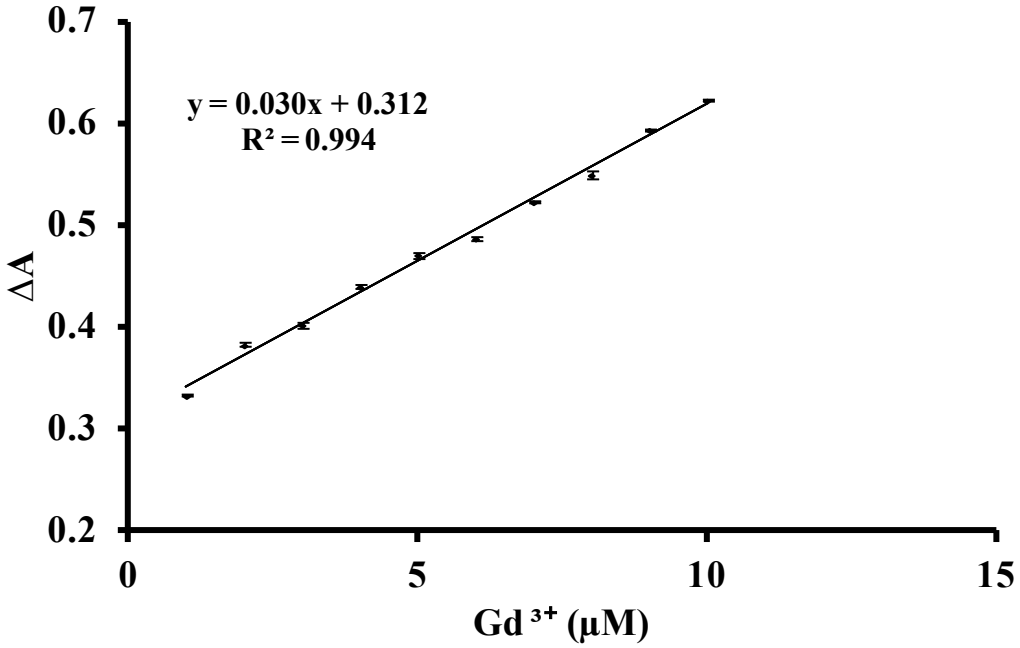


Fig S2. Plot of absorbance intensity at 410 nm versus Gd^{3+} ions (1–10 μM) concentration

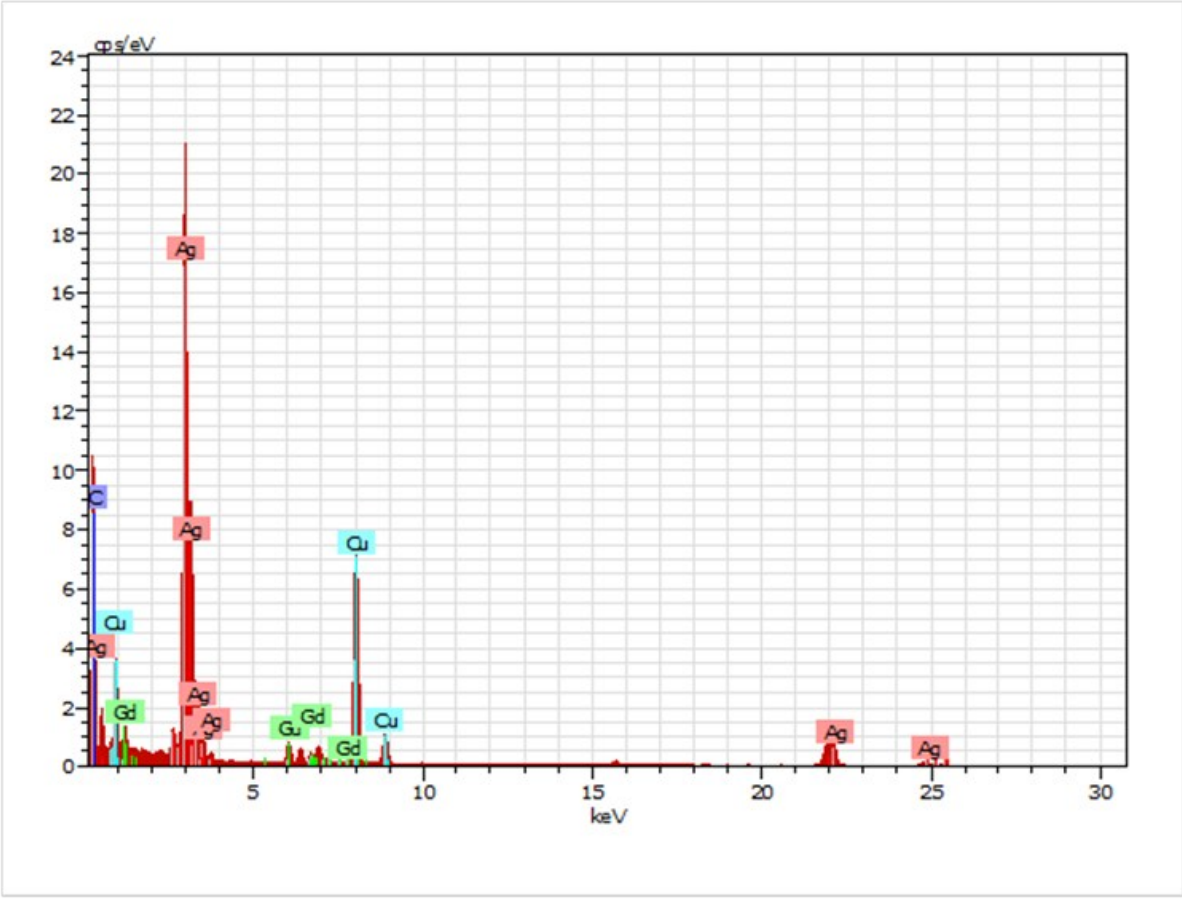


Fig S3. EDX profile of Gd containing Ag nanocrystals

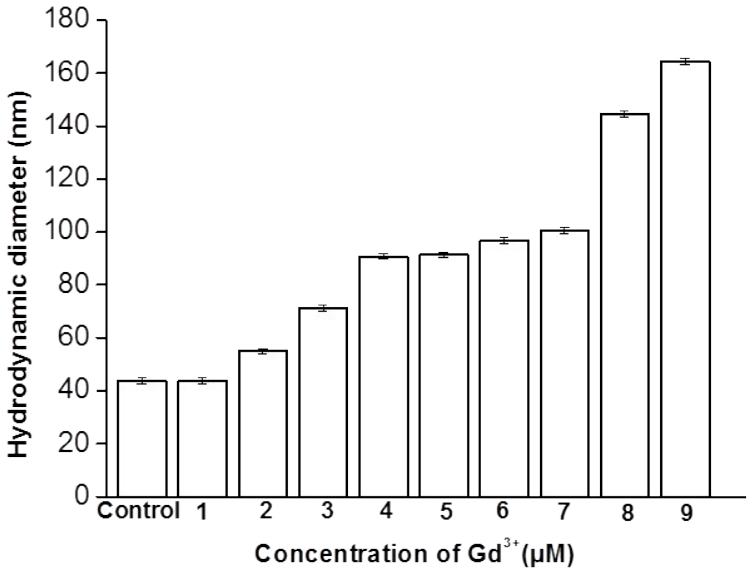


Fig S4. DLS analysis of AgNPs upon the addition of increased concentration of Gd^{3+} ions

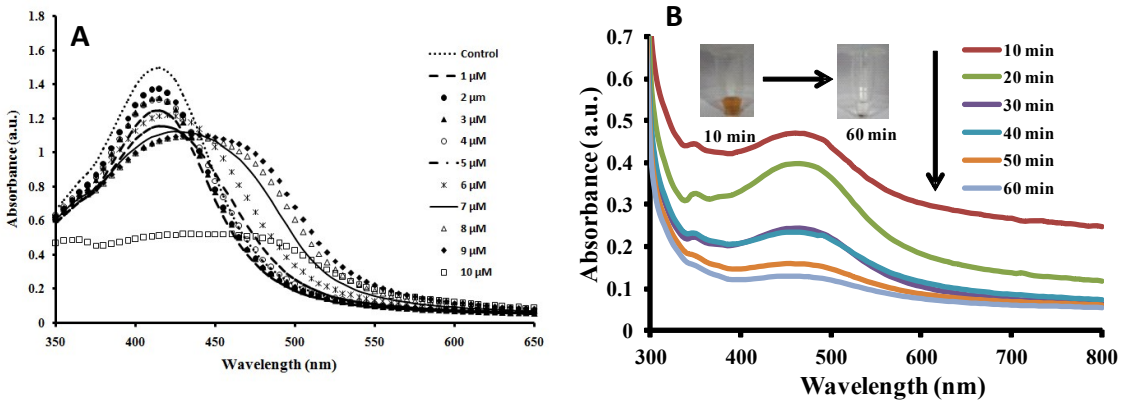


Fig S5. (A) UV-vis spectra of AgNPs upon the addition of different concentrations of Gd^{3+} ions, (B) Kinetic study of AgNPs- Gd^{3+} ions (10 μM) mixture.

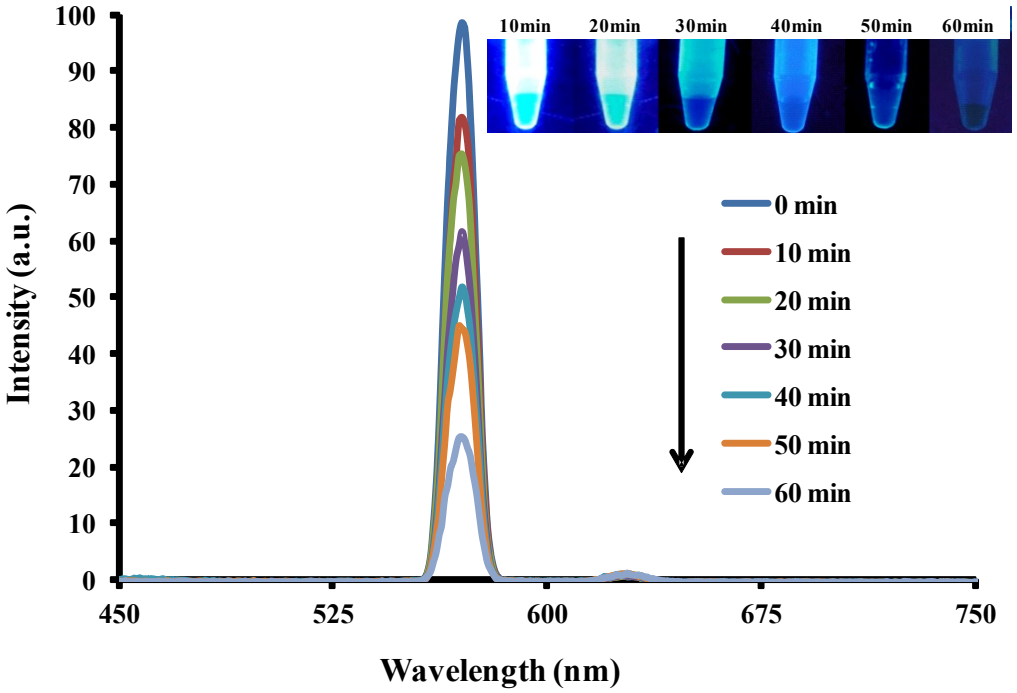


Fig S6. Time dependent fluorescence spectra of AgNPs- Gd^{3+} ions (10 µM) mixture

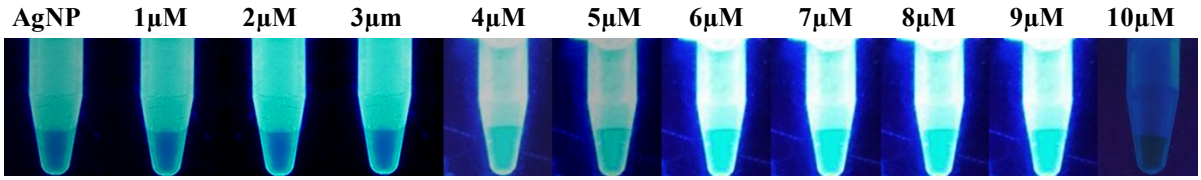
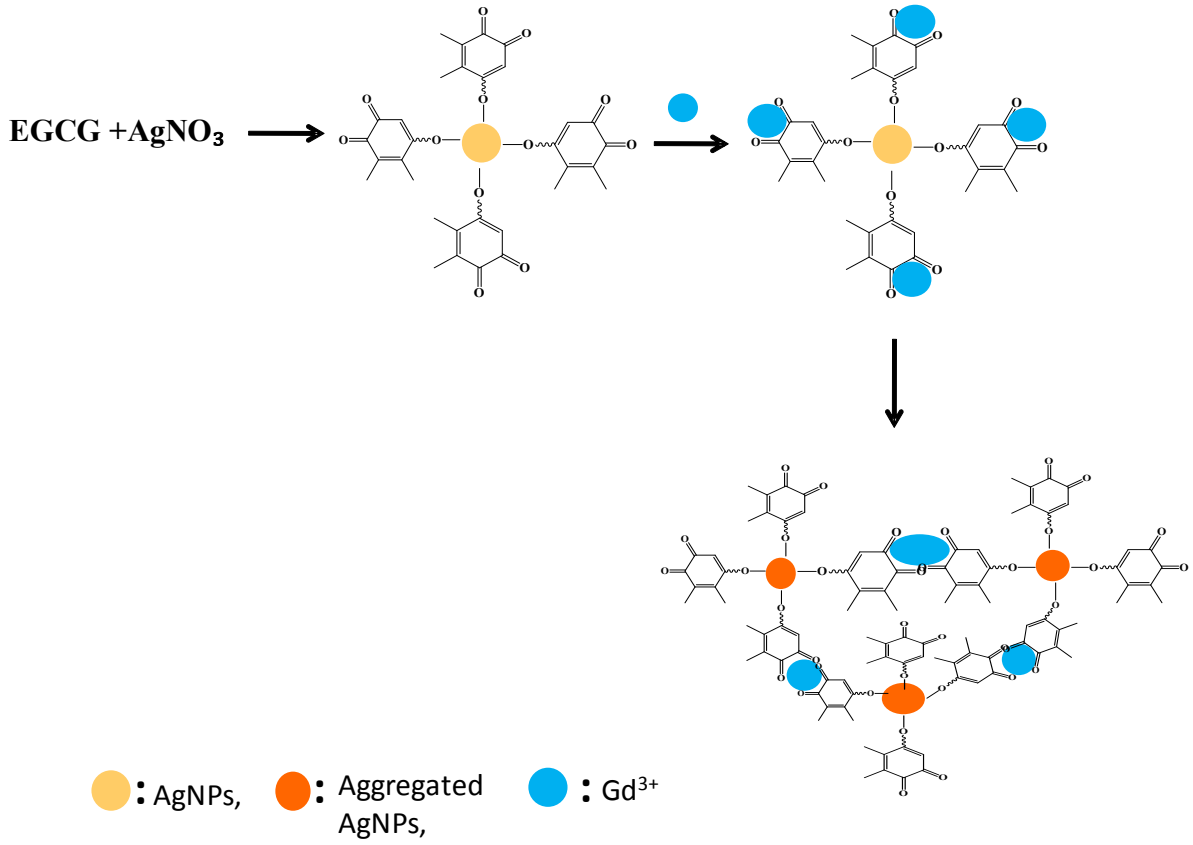


Fig S7. Fluorescence images of varying concentrations of Gd^{3+} containing AgNPs under UV light



Scheme 2. Possible mechanism of detection of Gd^{3+} ions

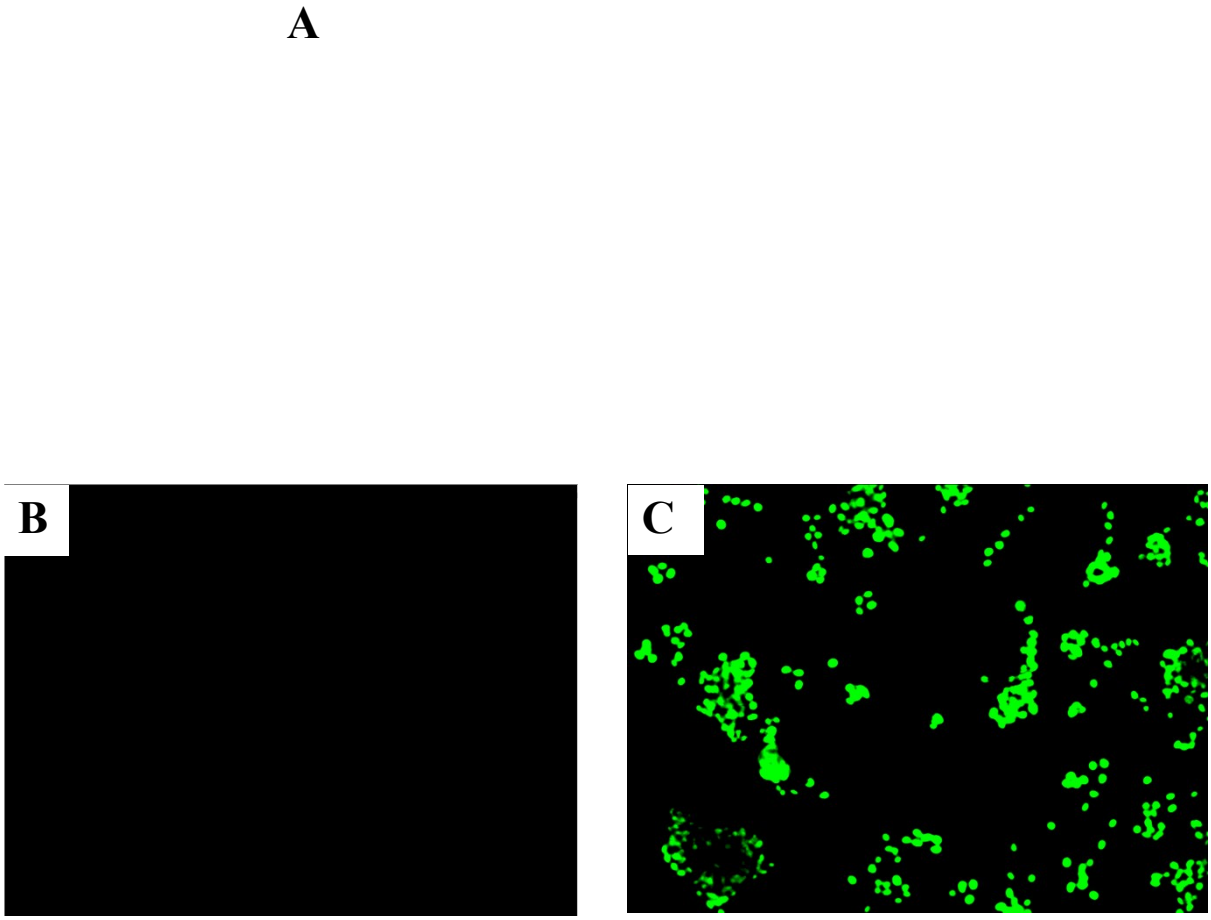


Fig S8. Fluorescence emission spectra of control Gd^{3+} ions at different excitation wavelengths (A), Fluorescence microscopy image of control cells with Gd^{3+} ions (B) and Gd^{3+} -AgNPs nanoprobe treated cells (C)

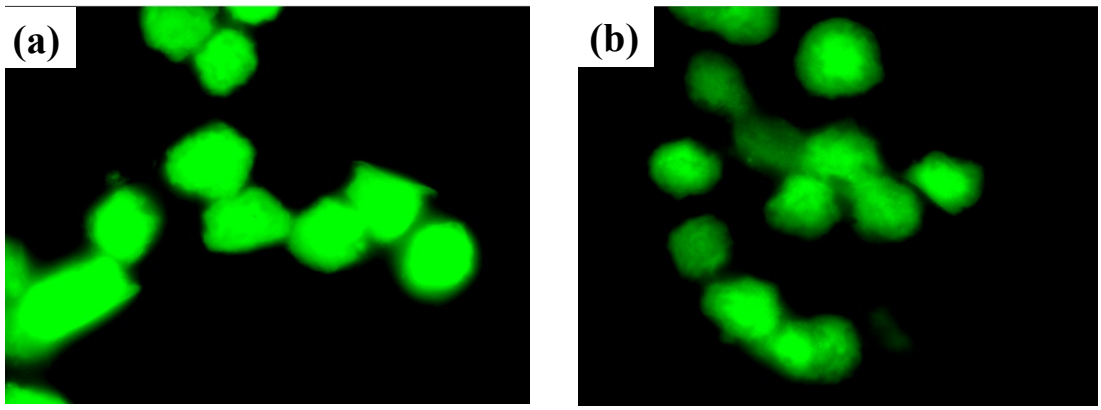


Fig S9. Bioimaging **(100X)** of HCT 116 cell using Gd-AgNPs without serum (a) and with serum (b)

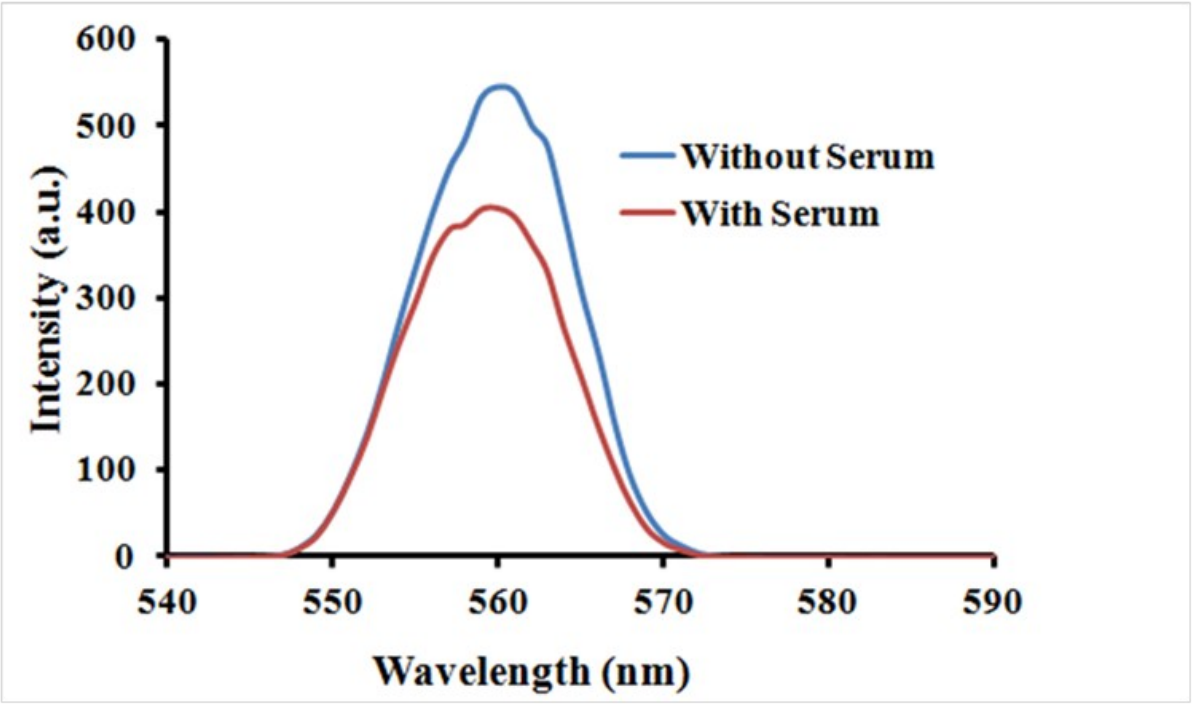


Fig S10. Fluorescence spectra of AgNPs-Gd^{3+} ions in the presence of serum.

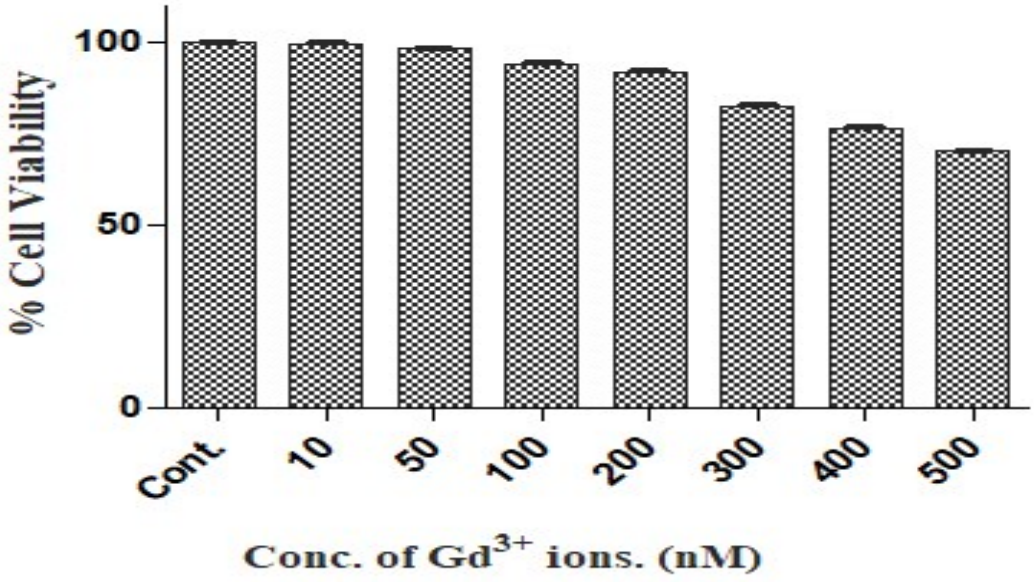


Figure S11. Cytotoxicity assay in HCT116 cells using Gd^{3+} containing AgNPs