Folate-assisted targeted photocytotoxicity of red light-activable iron(III) complex co-functionalized gold nanoconjugates (Fe@FA-NPs) against HeLa and triple negative MDA-MB-231 cancer cells

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Supporting Information

Methods and Materials

[$Fe(NO_3)_3.9H_2O$], 2,4-ditertiarybutayl phenol, L-glycine, sodium acetate, formaldehyde (38%), 1,10 phenanthroline-5 amine, folic acid, N-Boc-ethylenediamine, trifluoracetic acid (TFA) (±)-1,2-dithiolane-3-pentanoic acid, DCC, HOBT, tetrachloroauric(III) acid, tri-sodium citrate, Sephadex-g25, MTT, sodium chloride, DPBF, DAPI and MTG were purchased from Sigma-Aldrich (USA). 5,5′,6,6′-Tetrachloro-1,1′,3,3′-tetraethylbenzimidazolo-carbocyanine iodide (JC-1) was obtained from Molecular Probes, USA. L-15 Media (Lonza), DMEM media (MP Biomedicals), Penicillin (Gibco), Streptomycin (Gibco), Fetal Bovine Serum (Gibco), Trypsin EDTA (Sigma-Aldrich), 6 well plates (Tarsons), Nunclon Sphera 3D Ultra-Low Attachment Microplate 96-well Clear Round Bottom (Thermo-Fisher Scientific), MTT Reagent (Invitrogen), Cell culture grade DMSO (MP Biomedicals). Other chemicals and solvents were purchased from TCI Chemicals, Alfa-Aesar, HI-MEDIA, Finnar Chemicals, and used without any further purification. The solvents used were purified according to reported protocols prior using them[1].

UV-visible spectra of the compounds and UV-visible spectroscopic experiments were performed using a Perkin-Elmer UV-visible spectrophotometer. The IR spectra were recorded using a Perkin-Elmer UATR Two FT-IR spectrophotometer. The fluorescence spectra and fluorescence experiments were performed using a Hitachi F-7000 fluorescence spectrophotometer. The conductance measurements were performed using a Eutech Instrument CON 510 (India) conductivity metre. The 1H and 13C NMR spectra were obtained using a Bruker Avance 400 (400 MHz) NMR spectrophotometer with DMSO-d_6 or $CDCl_3$ as the solvent and tetramethylsilane (TMS) as the internal standard. The Q-TOF-ESI mass spectra were recorded using Bruker Esquire 3000. The elemental analysis was performed using a Perkin-Elmer 2400 Series II elemental analyser The DLS and zeta potential were obtained from a Zetasizer Nano ZS90DLS zeta potential analyser High-resolution transmission electron microscopy (HR-TEM) was performed in conjunction with energy dispersive spectrometry. For MTT, DCFDA and Annexin-V/PI assays, one set of **Fe@FA-AuNPs** treated cells were photoirradiated using a Waldmann PDT 1200 L (λ = 600−720 nm, light dose 30 J cm-2). The cellular uptake of compounds was studied by fluorescence-activated cell sorting (FACS)

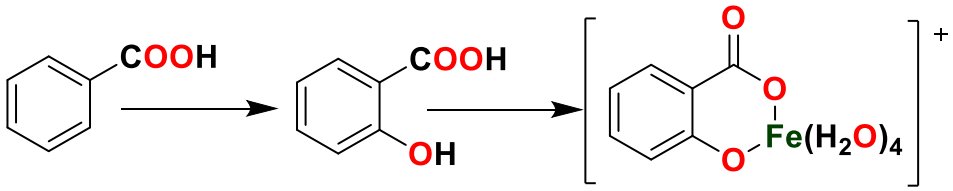
analysis using BD FACSVerse Flow Cytometer. DCFDA and Annexin-V/PI assays were also performed on the same instrument. To study intracellular localization of **Fe@FA-AuNPs**, confocal laser scanning microscopy (CLSM) was done using a Zeiss LSM 880 with an Airyscan microscope containing an oil immersion lens having an amplification of 63×.

Folic acid loading studies

A known concentration of Folic acid lipoic acid conjugate (3.36 mM) was taken in the cuvette and the absorbance spectra was measured. Then gradually known amount of Fe@AuNPs solution (1 mg mL^{-1} stock) was added in the sample and the reference cuvette and the decrease in the absorbance spectra of folic acid lipoic acid conjugate was recorded. After the addition of 500 µg of Fe@AuNPs we found the decrease in the absorbance spectra of folic acid lipoic acid conjugate to be corresponding to 0.815 µM. As, absorbance is directly proportional to the concentration. Hence from the results we conclude that for per mg of the **Fe@FA-AuNPs** nanocomposite, 1.63 µM folic acid is loaded.

Hydroxyl radical generation study[2]

We evaluated the generation of hydroxyl radicals by trapping them by formation of salicylic acid from benzoic acid (0.2 mM) by UV-Visible spectrophotometry. Further the formation of hydroxyl radicals by the photo-Fenton degradation of **Fe@FA-AuNPs** was confirmed by the addition of methanolic solution of $Fe(NO_3)_3.9H_2O$. The spectral traces of Fe(III)-salicylate (λ_{max} = 520 nm) was found to be increasing in presence of **Fe@FA-AuNPs** (1 mg mL^{-1}) upon red light (600 – 800 nm, 30 W) exposure up to 60 min. Gradual increase in A_{520} of Fe(III)-salicylate indicated the generation of OH radical.



Singlet Oxygen ($^1O_2$) generation study[3]

Singlet oxygen generation study measure by DPBF experiment. At first, we take the absorbance value of DPBF with Rose Bengal then DPBF in the presence of the hybrid **Fe@FA-AuNPs** (100 µg mL^{-1}), without photo-exposure were considered as the negative control. The extent of decrease in absorbance (A_{417}) of DPBF, in this case, gives the measure of extent of generation of $^1O_2$. For better graphical representation, we have plotted the extent of decrease of absorbance of DPBF (A_0-A/A_0) against visible light exposure time (t/min), where A was the absorbance of

DPBF at particular time while A_0 was the absorbance of DPBF at t=0 min. We have observed a linear decrease of absorbance of DPBF with exposure time indicating photo-induced generation of $^1O_2$ from $^3O_2$ via type II photo-process. Singlet oxygen quantum yield (ϕ_N) was determined further to correlate photo-cytotoxicity of the complex with the generation of singlet oxygen on photoirradiation. The singlet oxygen quantum yield was determined to be in the range 0.2-0.6 in DMSO which was significant in inducing photocytotoxicity. The $^1O_2$ quantum yield was calculated using the equation below.

$$\phi_N = \phi_{RB} \, X \, \frac{K_N}{K_{RB}} \, X \, \frac{A_{RB}}{A_N}$$

Where ϕ_N is the quantum yield of **Fe@FA-AuNPs**, K is the gradient of the decay rate constants of 1-AuNPs and Rose Bengal, A is the light absorbed which is calculated from the integration of the absorption bands in the wavelength range from 400 to 700 nm.

Partition Coefficient Measurements[4]

Deionized water was stirred with octanol for 24 h and then centrifuged for 5 min to obtain octanol saturated water and water-saturated octanol. The nanohybrid **Fe@FA-AuNPs** was dissolved in the octanol-saturated water, to a typical concentration of 0.03 to 3 mg mL^{-1} and then mixed with water saturated octanol in volumetric ratios of 1:1, 1:2, and 2:1 in duplicate. The mixtures were vortexed for 0.5 h and then centrifuged for 5 min. The layers were separated carefully using a fine gauge needle and then analysed for complex and hybrid content by UV-Visible spectroscopy. The partition coefficient was taken as a ratio of the concentration of complex and hybrid in the octanol layer to that in the aqueous layer ($P = c_{oct}/c_{water}$). The reported error is the standard deviation of the six measurements obtained from this protocol.

HSA binding[5]

A protein binding study was performed by tryptophan fluorescence quenching experiments using human serum albumin stock solution (HSA, 3 X 10^{-5} M, based on its molecular mass of 66000 Da) in 10 mM Tris-HCl buffer (pH = 7.2) to keep the physiological conditions. Fluorescence emission spectra were recorded in the wavelength range 330–500 nm by an excitation wavelength of 295 nm. With increasing the complex concentration, the quenching of the emission intensity of tryptophan residues of HSA at ~342 nm was monitored using the **Fe@FA-AuNPs** (0.1 mg mL^{-1}) as a quencher. Fluorescence measurements were carried out

using a HITACHI F-7000 fluorescence spectrophotometer by keeping the concentration of HSA constant (2 mL, 3 X 10^{-5} M) while varying the quencher concentration at 298 K. A linear fit of the data was done using the Stern–Volmer equation:

$$\frac{I_0}{I} = +kq\tau_0[Q] = 1 + K_{sv}[Q]$$

Cell culture conditions

The Henrietta Lacks cell line (HeLa), human alveolar basal epithelial (A549) cell lines, MDA-MB 231 TNBC human breast cancer cells, human peripheral lung epithelial (HPL1D) cells were purchased from the American Type Culture Collection (ATCC). The cells were in L-15 Media, later the cells were maintained in DMEM media supplemented with penicillin (100 units mL^{-1}), streptomycin (100 mg mL^{-1}), and 10% Fetal Bovine Serum (FBS) and incubated at 37°C in a humidified, 5% CO_2 atmosphere. The cells were passaged using 0.25% Trypsin-EDTA solution. All experiments were performed using passage numbers between 38 – 42.

Cytotoxicity assay (MTT assay)[6-7]

The cytotoxicity of **Fe@FA-AuNPs** hybrid was investigated by MTT assays. Folate positive Henrietta Lacks cell line (HeLa), folate positive triple-negative/basal-B mammary carcinoma (triple negative MDA-MB-231), folate negative adenocarcinomic human alveolar basal epithelial cell line (A549) was cultured in DMEM media supplemented with 10% FBS. The cells were maintained at 37 °C in a humidified atmosphere with 5% CO_2. Approximately, 5000 cells were plated in each well of four 96-well tissue culture plates. The cells were treated with 1.56 μg mL^{-1} to 200 μg mL^{-1} concentrations in 1% DMSO/Dulbecco's modified Eagle's medium (DMEM) of **Fe@FA-AuNPs** hybrid in two sets of 96-well tissue culture plates. One set of the cells were exposed to red light (40-minute exposure, λ = 600-720 nm, light dose = 30 J cm^{-2}) in DPBS (Dulbecco's phosphate-buffered saline) using a Waldmann PDT 1200 L whereas the other set was kept in the dark for the same time period. After exposure to light, DPBS was removed and replaced with fresh medium and the cells were kept at 37 °C for a further period of 19 h. The DPBS buffer containing 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, 20 μL of 5 mg mL^{-1}) was then added into each well and incubated for 4 h. The medium was then discarded, DMSO (200 μL) was added to dissolve the purple formazan crystals, and the absorbance was recorded at 570 nm for each well using a TECAN microplate reader and graphs were plotted by using GraphPad Prism 7 software. Data

were obtained by using three independent sets of experiments done in triplicate for each concentration.

3D spheroid MTT Assay[8-9]

Briefly, 100 µl complete medium containing 1.5×10^3 cells was seeded in each well in an ultra-low attachment 96-well plate (Nunclon Sphera 3D Ultra-Low Attachment Microplate 96-well Clear Round Bottom). Plates were centrifuged at 300 × g for 3 min and the plate was incubated at 37°C for 72 hrs in a CO_2 incubator. After the formation of the spheroid, treatment was performed by adding 100 µl of fresh complete medium with FA-AuNPs at concentrations 5, 25, 50, 75, 100, and 125 µg mL^{-1} and incubated for 24 hours. The old media was discarded and 100 µl fresh media containing MTT at the concentration of 5 mg mL^{-1} was added and the plate was incubated for 24 hours. The next day media was discarded and 100 µl of DMSO was added to solubilize formazan crystals and incubated for 1 hrs. Absorbance was measured with a Multimode Microplate Reader. Results were expressed as mean value ± S.E.M. percent optical density (OD) vs. vehicle-treated control cells.

Cell imaging study[10]

For imaging study, the Henrietta Lacks (HeLa) cells were plated in treated 35 mm Ibidi Corning dishes at a seeding density of 0.60×10^5 cells per dish and allowed to grow to confluence (48 h). The dishes were aspirated and cells treated with hybrid (**Fe@FA-AuNPs**) nanoparticles, 40 µg mL^{-1}, suspended in mixture PBS and media (without FBS). The cells were further incubated from time to time for 1h, 2h and 3h at 37 °C in a humidified atmosphere of 5% CO_2 in air. Finally, the cells were washed twice with PBS buffer and placed in PBS with Trypan blue for imaging studies.

DCFDA assay[11]

Cellular reactive oxygen species (ROS) was detected by 7'-dichlorofluorescein diacetate (DCFDA) assay. Cellular ROS oxidizes cell permeable DCFDA generating a fluorescent DCF having emission maxima at 528 nm. The percentage population of cells generating ROS was determined by Flow Cytometry analysis. HeLa cells were incubated with the compounds at their IC_{50} value 28 µg mL^{-1} for 4 h and then irradiated with red light for 40 min (λ = 600-720 nm, light dose = 30 J cm^{-2}) using Waldmann PDT 1200 L. The cells were harvested by trypsinization and a single cell suspension was prepared. The cells were subsequently treated with 1 µM DCFDA (solution prepared with DMSO) in dark for 20 min at room temperature.

The distribution of DCFDA stained A549 cells was obtained by flow cytometry in the FL-1 channel.

Cellular Uptake Studies[11]

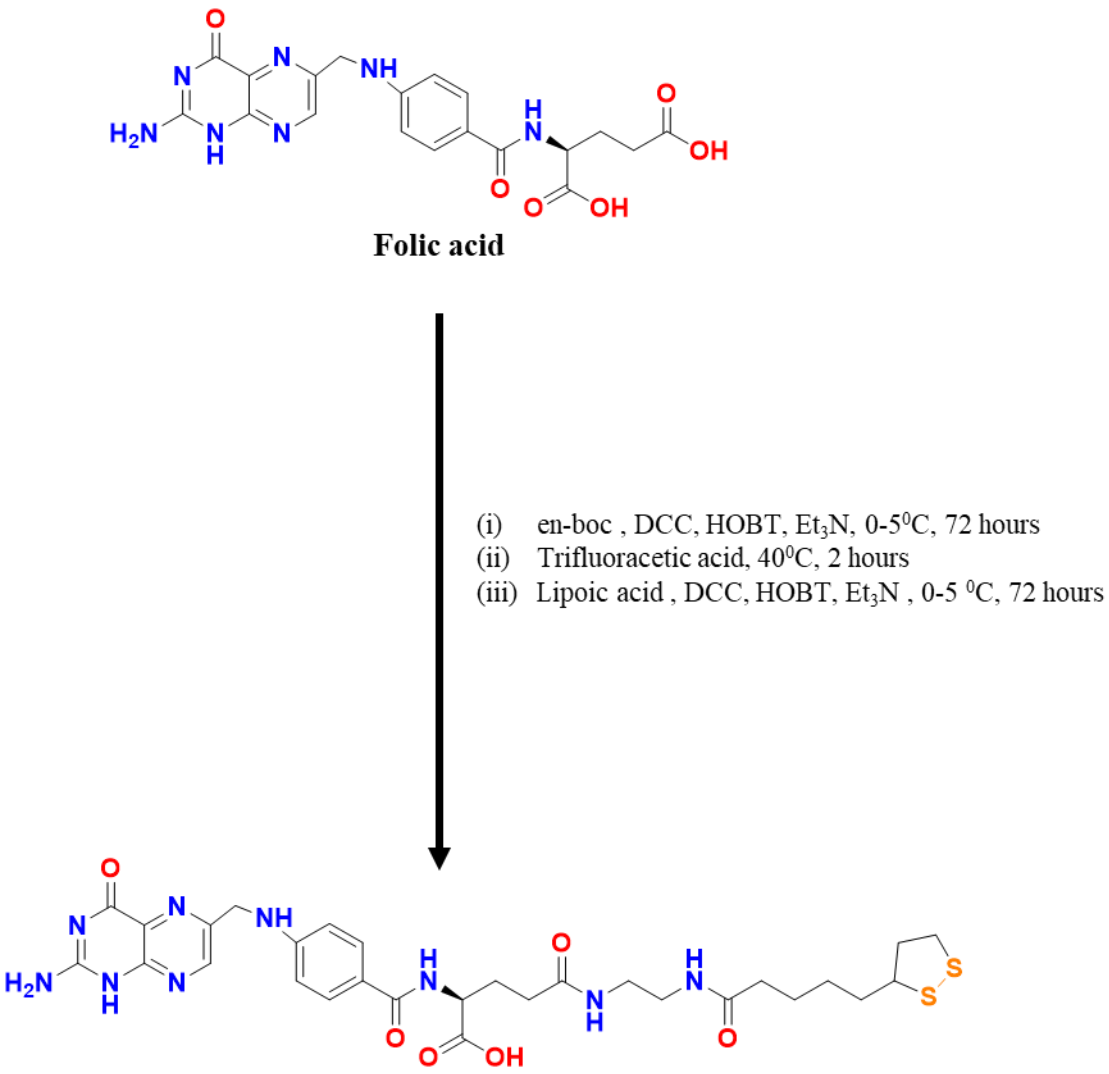
HeLa cells, A549 cells and human peripheral lung epithelial (HPL1D) cells (about 5 x 10^4) were plated on glass cover slips in 12 well tissue culture plates and incubated at 37 °C and 5% CO_2 atmosphere for 24 h. Then the cells were incubated with nanohybrid (40 μg mL^{-1}) at 37 °C for 4 h. The cells were then washed once with chilled phosphate buffer saline and analyzed by Flow Cytometry on an FL2 channel.

Annexin-V/FITC Assay[11]

About 4 x 10^5 HeLa cells were plated in 96 well plates and grown for 24 h and cells were treated with the **Fe@FA-AuNPs** hybrid (40 μg mL^{-1}) for 4 h. One of the plates was exposed to photo-irradiation (600-720 nm, 30 Jcm-2, 40 min) in DPBS followed by addition of fresh media. The cells were further incubated for 1 h, trypsinized and washed in DPBS twice. The cells were then re-suspended in 400 μL of 1X binding buffer and 1 μL of annexin V-FITC and 2 μL of PI were added to each cell suspension. These tubes were then incubated at room temperature for 20 min in dark and the fluorescence of the cells was measured immediately with a flow cytometer. Cells that are early in the apoptotic process were stained with the annexin V-FITC alone while live cells showed no staining by either PI or annexin V-FITC. Late apoptotic cells were stained by both PI and annexin V-FITC while the dead cells were only stained by PI.

Scratch wound healing assay

The MDA-MB-231 cells were seeded in a 6-well plate at a concentration of 5 × 10^5 cells/well and allowed to form a 90% confluent monolayer. Cells were then incubated in serum-free media for 24 hours. Using a sterile pipette tip (200 μl), a straight scratch was made simulating a wound in each of the wells and washed with serum-free medium (SFM) to remove floating cells and photographed (time 0). The cells were treated with FA-AuNPs at concentrations of 39.91 μg mL^{-1} (in light) and 200 μg mL^{-1} (in dark) for 24 hours. Cells were then photographed again after 24 hours at 3 randomly selected sites per well.



Scheme S1: Synthetic route for preparation of FA-en-LA

Table S1: Size, Zeta potential and the powder XRD data of the nanoconjugates.

Sl. No	Average Size (nm) (TEM)	Avarage hydrated Size (nm) (DLS)	Avarage Zeta-potential (mV)	Powder XRD
AuNPs	15 ± 3	41 ± 15	-24 ± 6	38.22°, 44.22°, 64.72°, and 77.69° [(111), (200), (220), and (311) planes]
Fe@AuNPs*	22 ± 4	142 ± 14	-9.36 ± 5	21.09°, 38.22°, 44.22°, 64.72°, and 77.69° [(100), (111), (200), (220), and (311) planes]
Fe@FA-AuNPs	25 ± 3	159 ± 18	-11 ± 5	22.11°, 38.22°, 44.22°, 64.72°, and 77.69° [(100), (111), (200), (220), and (311) planes]

*The characteristic data of **Fe@AuNPs** is consistent with our previous report "M. Pal, V. Ramu, D. Musib, A. Kunwar, A. Biswas and M. Roy, Inorganic Chemistry, 2021, 60, 6283–6297"

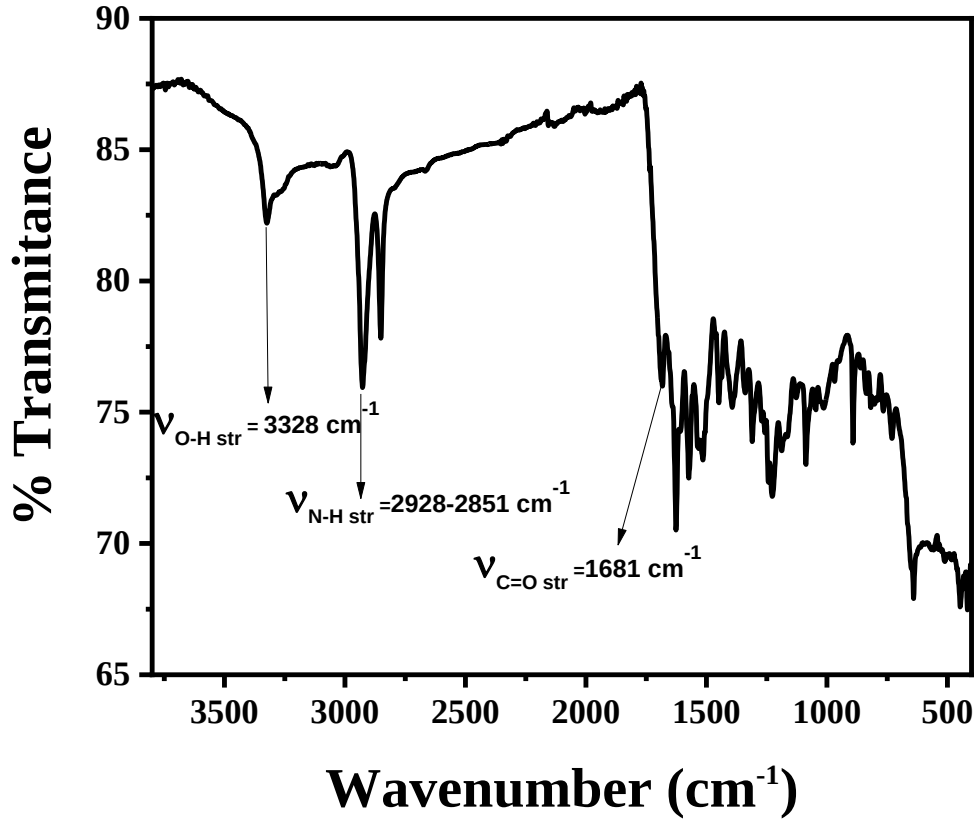


Figure S1: FT-IR Spectra of FA-en-LA recorded in KBr phase using Perkin-Elmer UATR TWO FT-IR Spectrometer.

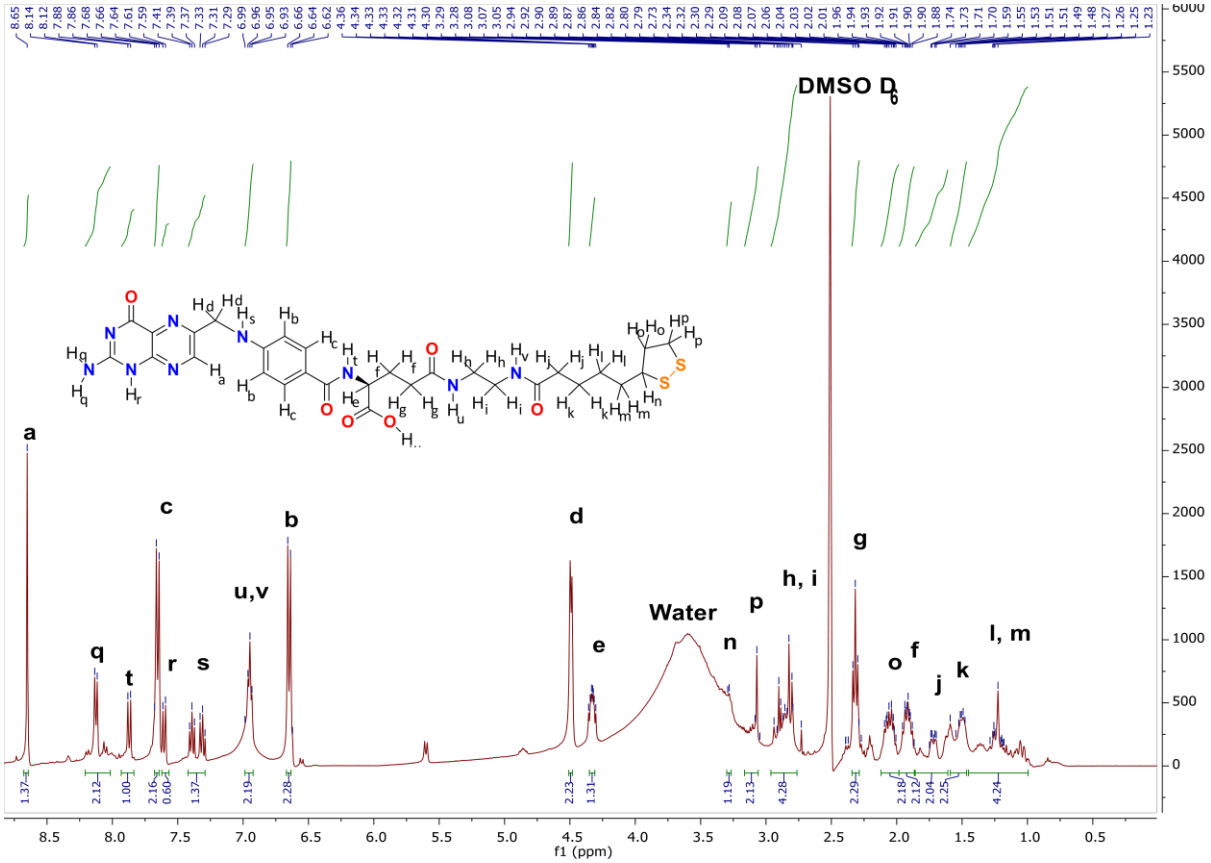


Figure S2: ^{1}H NMR of FA-en-LA recorded in DMSO-d_6 using Bruker Avance 400 (400 MHz) spectrometer (DMSO-d_6 = 2.5 ppm).

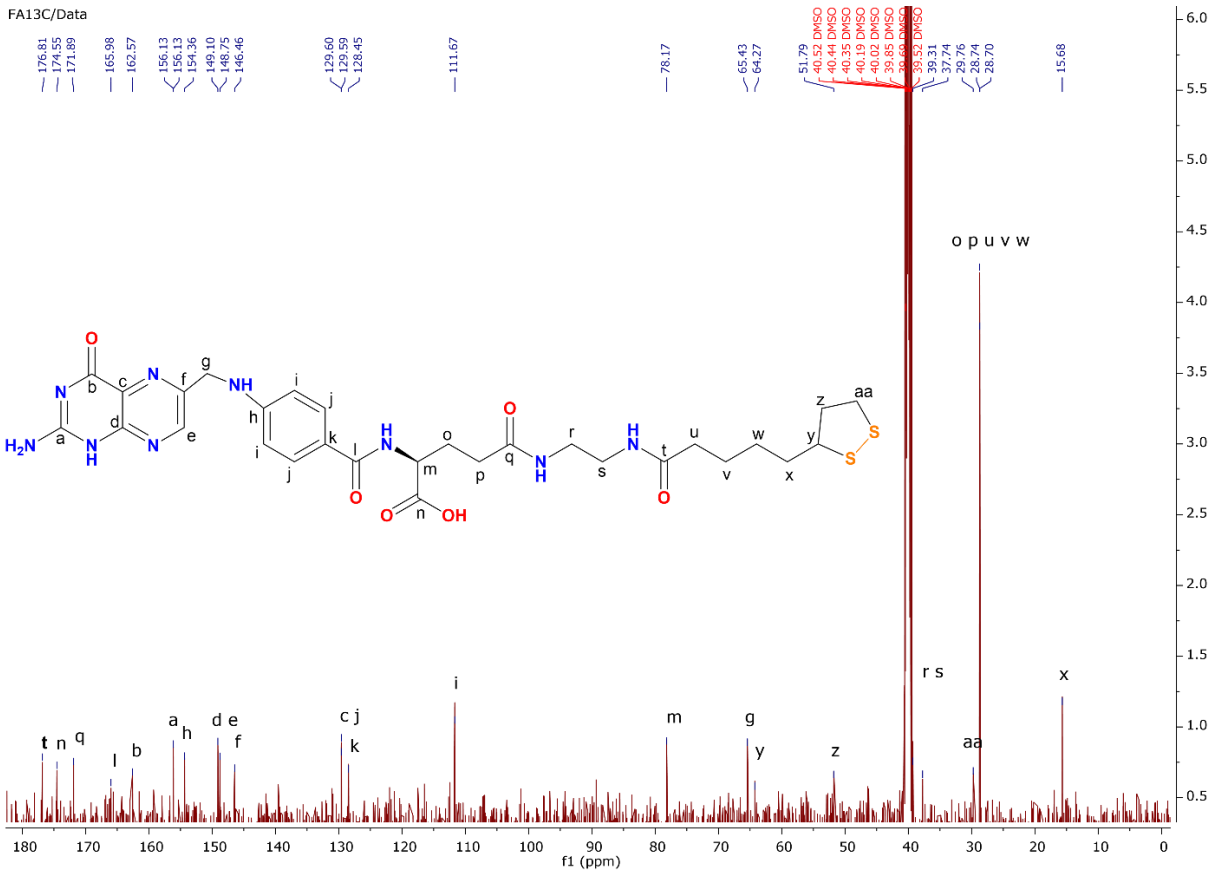


Figure S3: ^{13}C NMR of FA-en-LA recorded in DMSO-d_6 using Bruker Avance 400 (100 MHz) spectrometer.

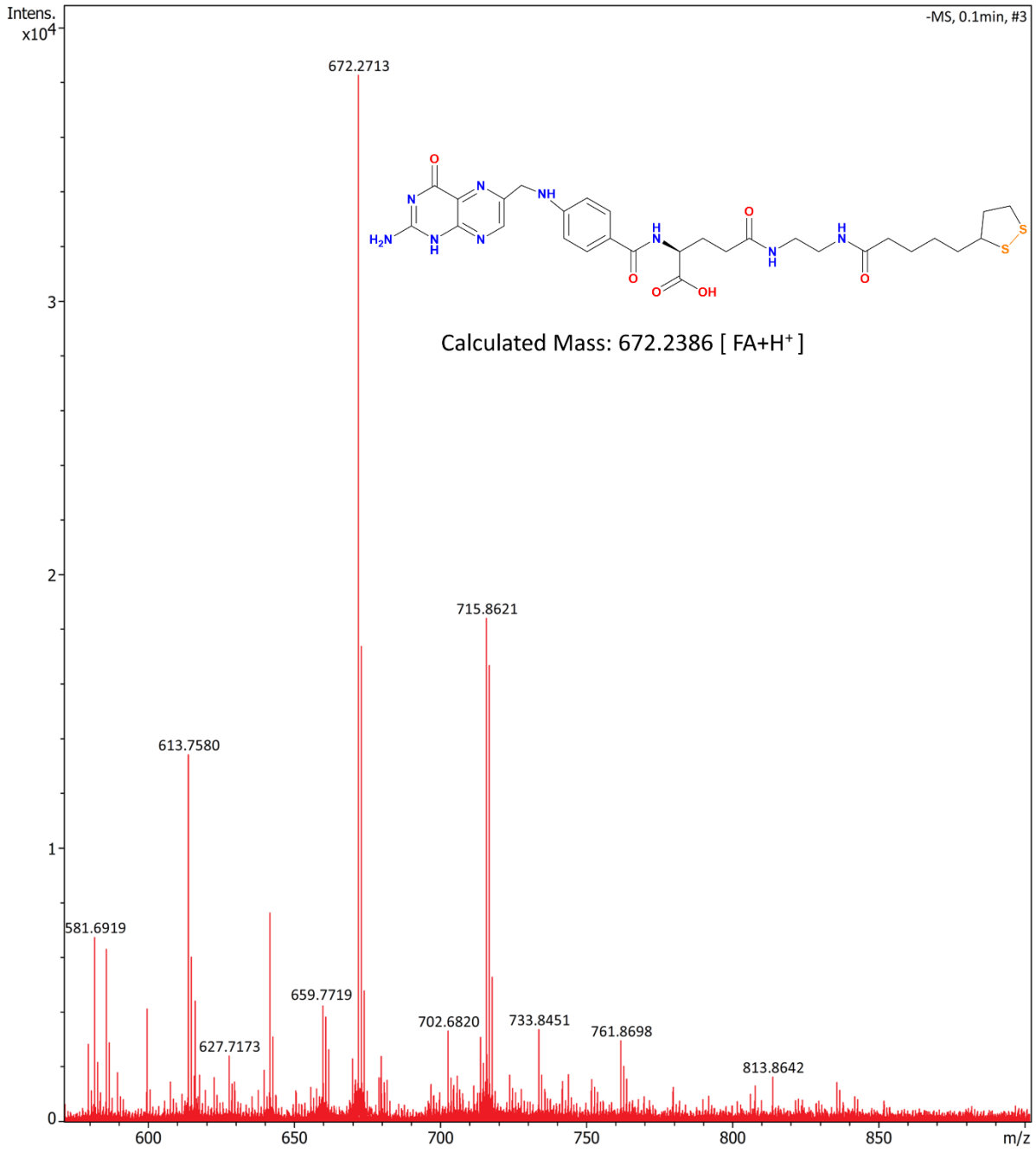


Figure S4: Q-TOF ESI Mass spectra of the FA-en-LA recorded in DMF using Bruker Esquire 3000 Plus Spectro-photometer (Bruker-Franzen Analytic GmbH, Bremen, Germany). The peak at m/z 672.2713 corresponds to the species $[FA\text{-}en\text{-}LA@H]^+$.

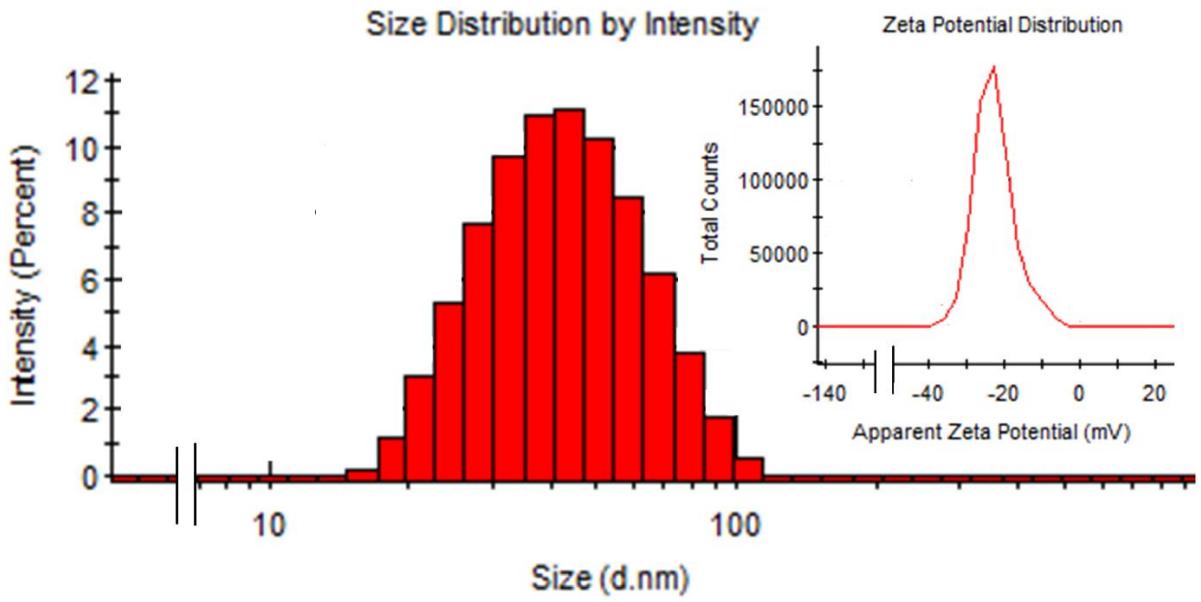


Figure S5: Distribution of the size of the Gold-nanoparticles (AuNPs) determined by using DLS spectrometer in 5% DMSO-H_2O at pH 7.2 in 298 K. (41 nm). Inset: Zeta potential graph of AuNPs determined from DLS measurements 5% DMSO-H_2O at pH 7.2 in 298 K. (-24.3 mV).

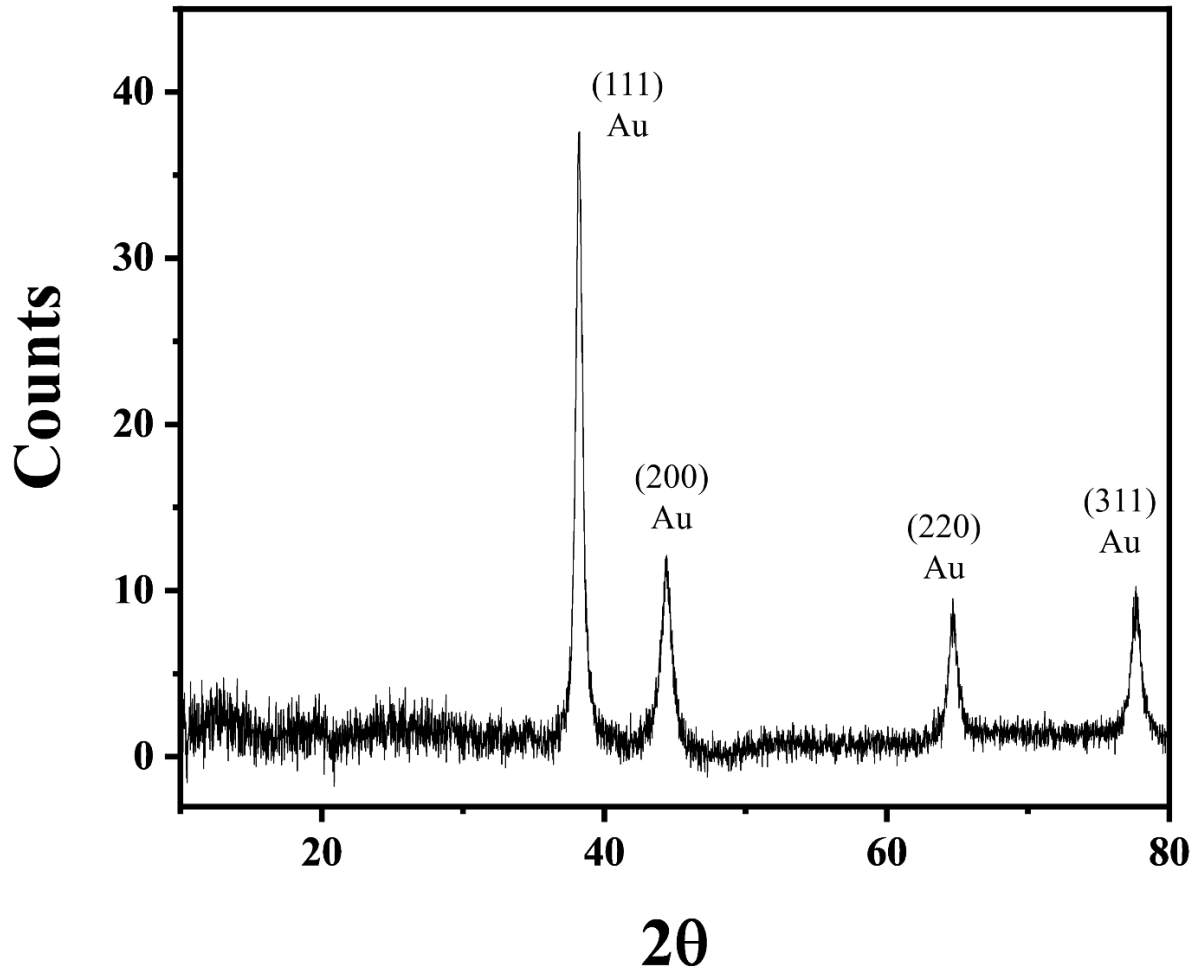


Figure S6: Powder XRD of AuNPs.

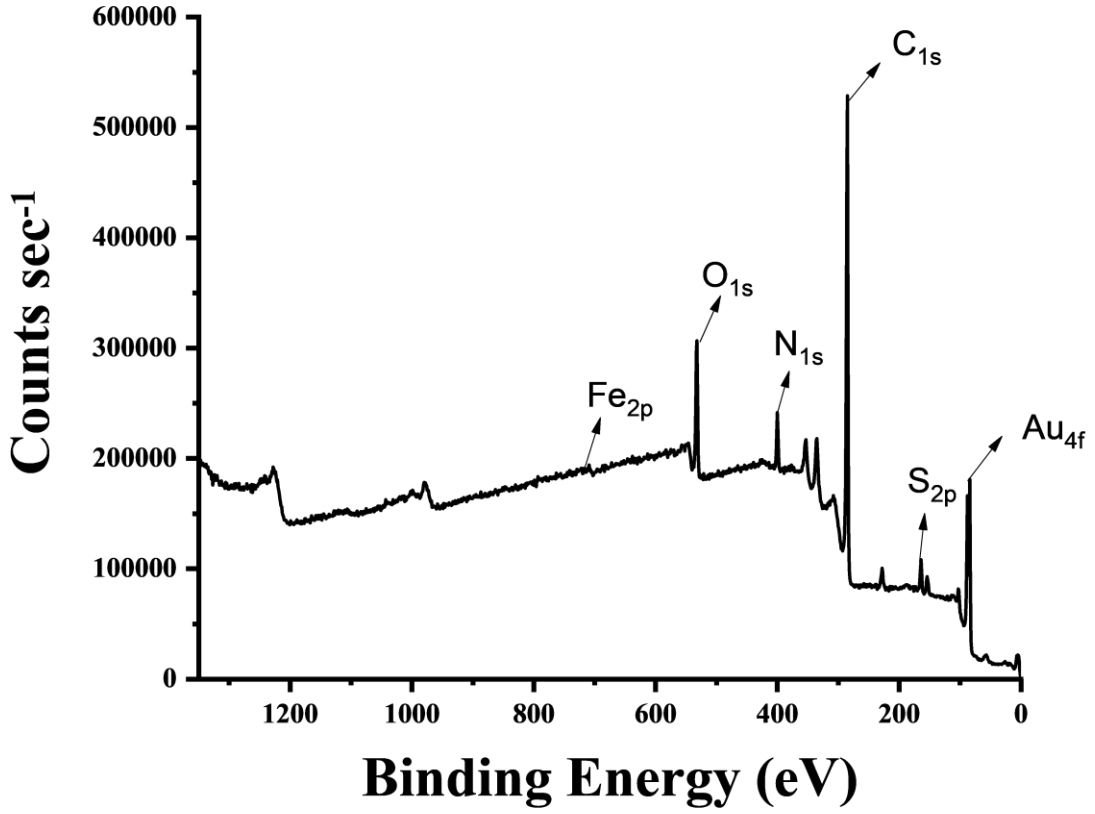


Figure S7: XPS survey scan of **Fe@FA-AuNPs**, indicating the presence of Fe, O, N, C, S and Au atoms.

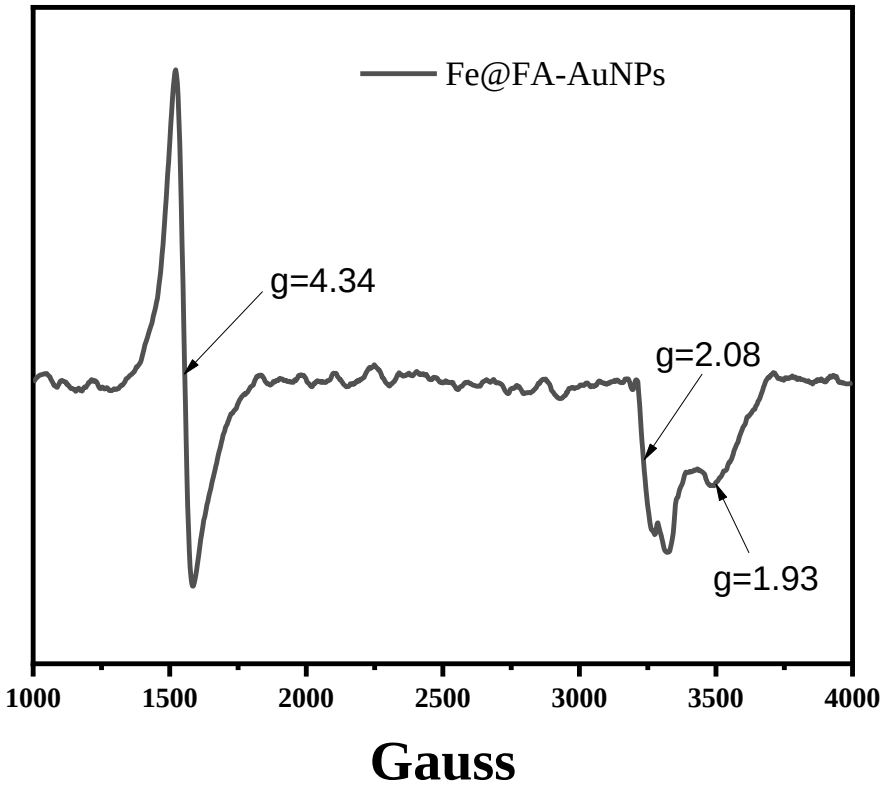


Figure S8: EPR spectra of nanohybrid **Fe@FA-AuNPs** in 5% DMSO-H_2O solution recorded at 77K.

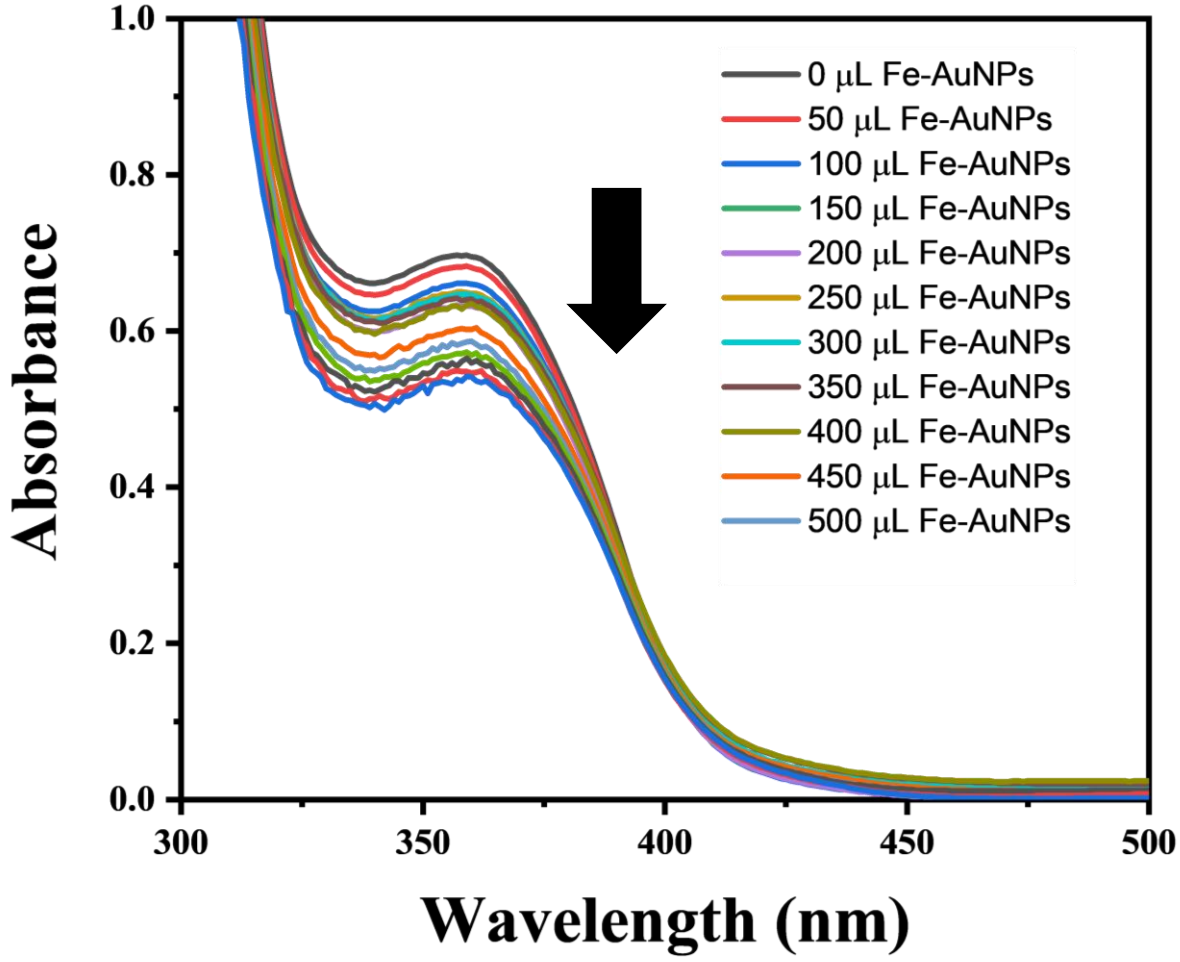


Figure S9: Folic acid loading study in Fe@AuNPs using mM folic acid lipoic acid conjugate and gradual addition of stock (1 mg mL^{-1}) solution of Fe-AuNPs in 5% DMSO-H_2O at pH 7.2 in 298 K.

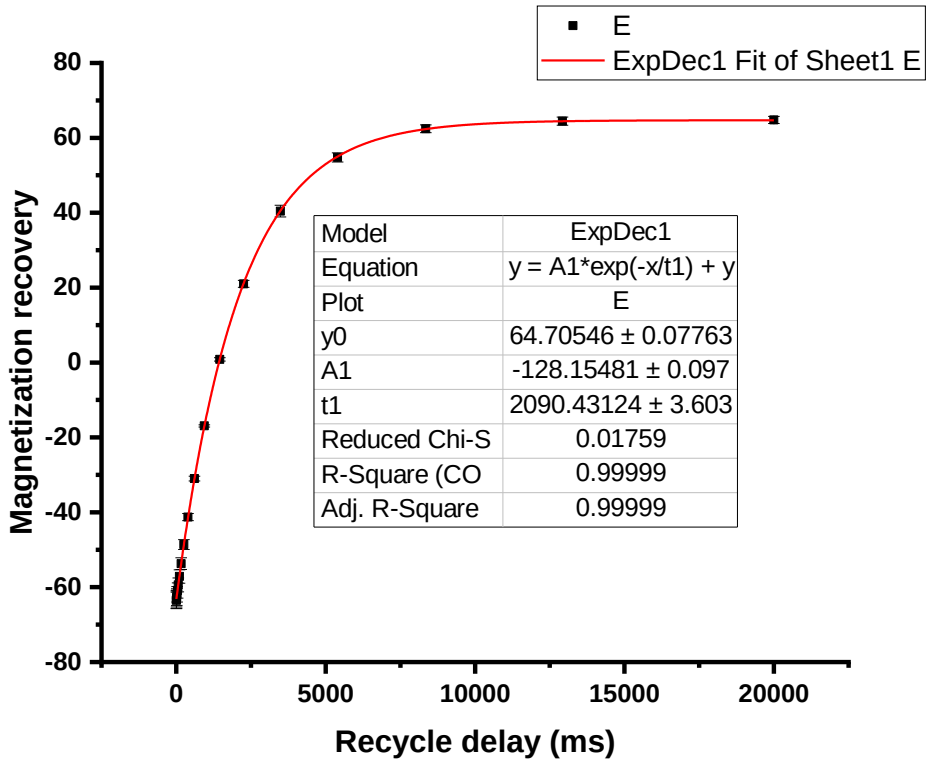


Figure S10: T1 relaxation time measurement of **Fe@FA-AuNPs** (1 mg mL^{-1}) in 5% DMSO-H_2O at pH 7.2 in 298 K.

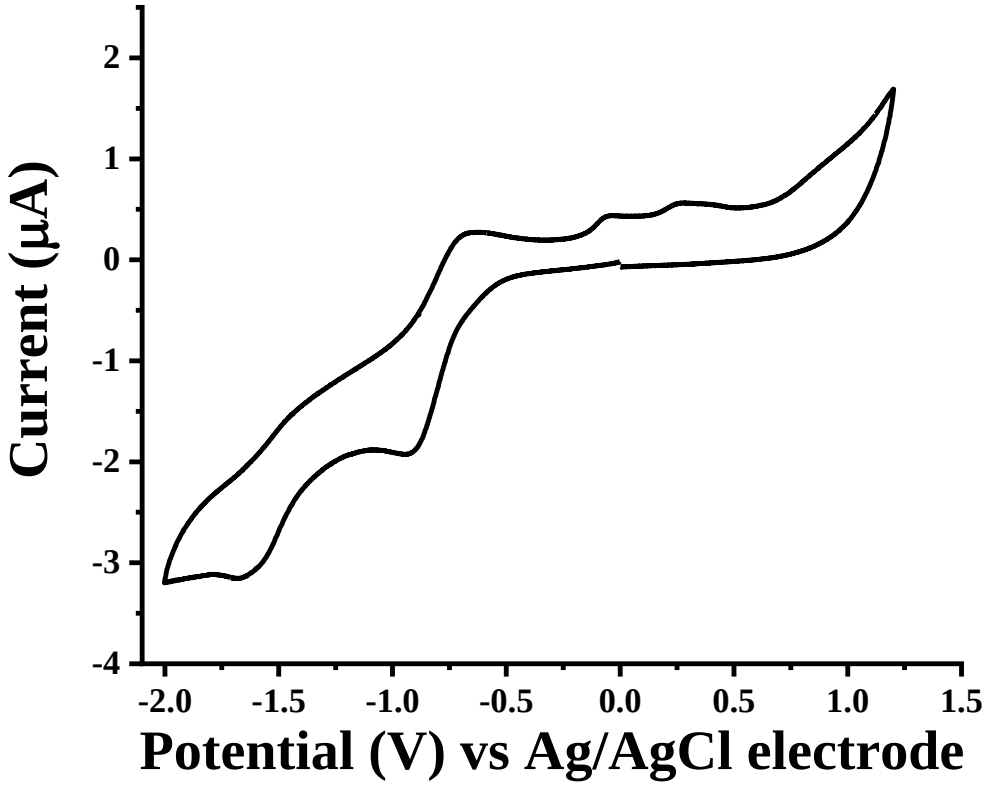


Figure S11: Cyclic Voltammogram of **Fe@FA-AuNPs**, using 1mg mL^{-1} solution of **Fe@FA-AuNPs** in 5% DMSO-H_2O in at pH 7.2 in 298 K using Glassy Carbon electrode as working electrode, Ag/AgCl electrode as reference electrode and Pt electrode as counter electrode and TBAP (Tetrabutaylammonium perchlorate) 0.1 M as supporting electrolyte, at scan rate 50 mV/s which shows 2 electron reduction at -0.46 V and -1.01 V.

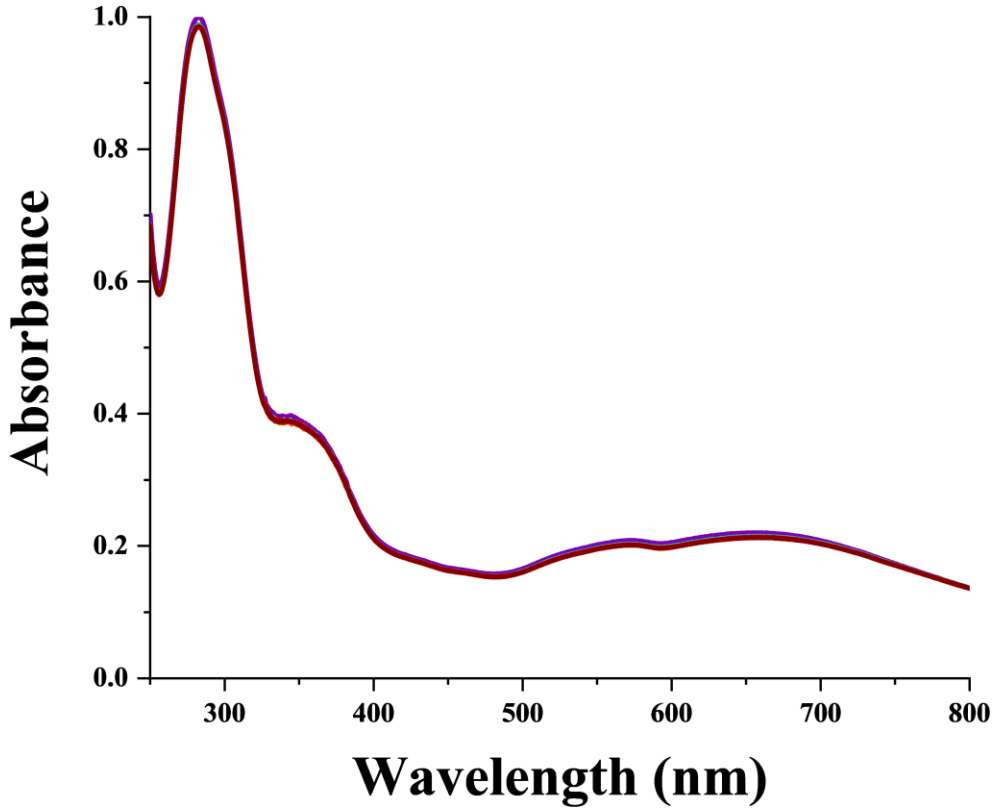


Figure S12: Stability of **Fe@FA-AuNPs** in 5% DMSO-H_2O in at pH 7.2 in 298 K solution medium at dark up to 48 hours.

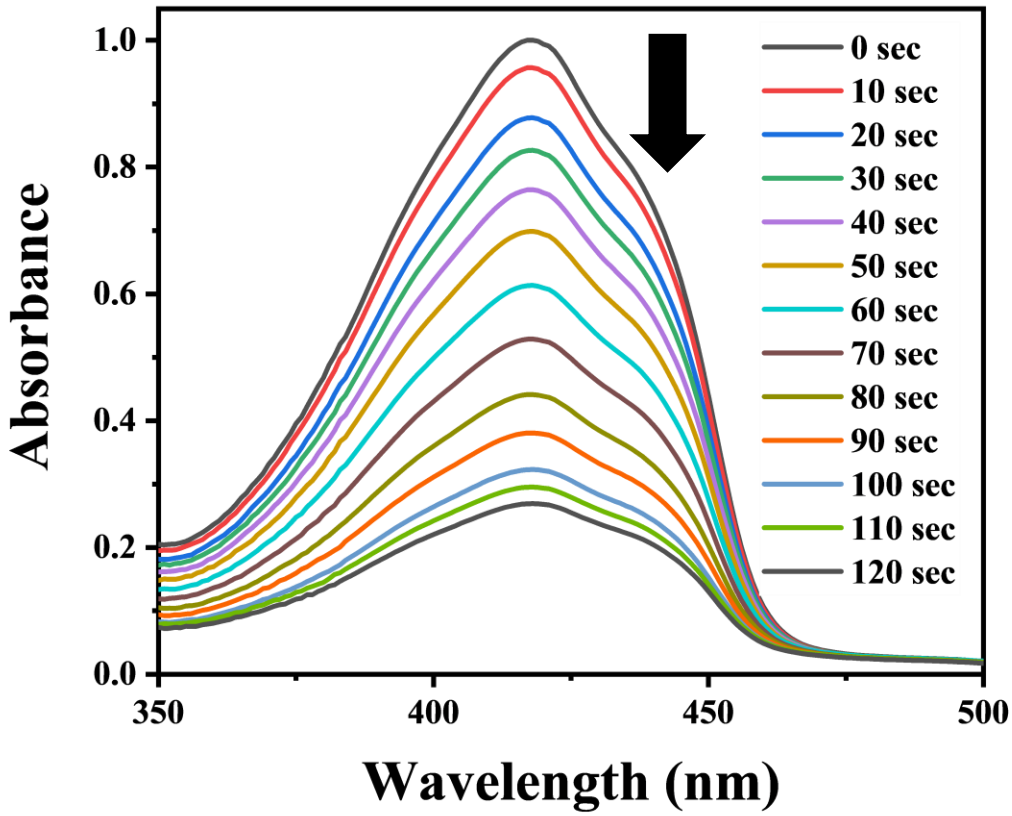


Figure S13: Spectroscopic studies on the generation of singlet oxygen by **Fe@FA-AuNPs** (100 μg mL^{-1}) upon photo-activation in Red light (30 Watt, 620-700 nm) in DMSO at 298 K.

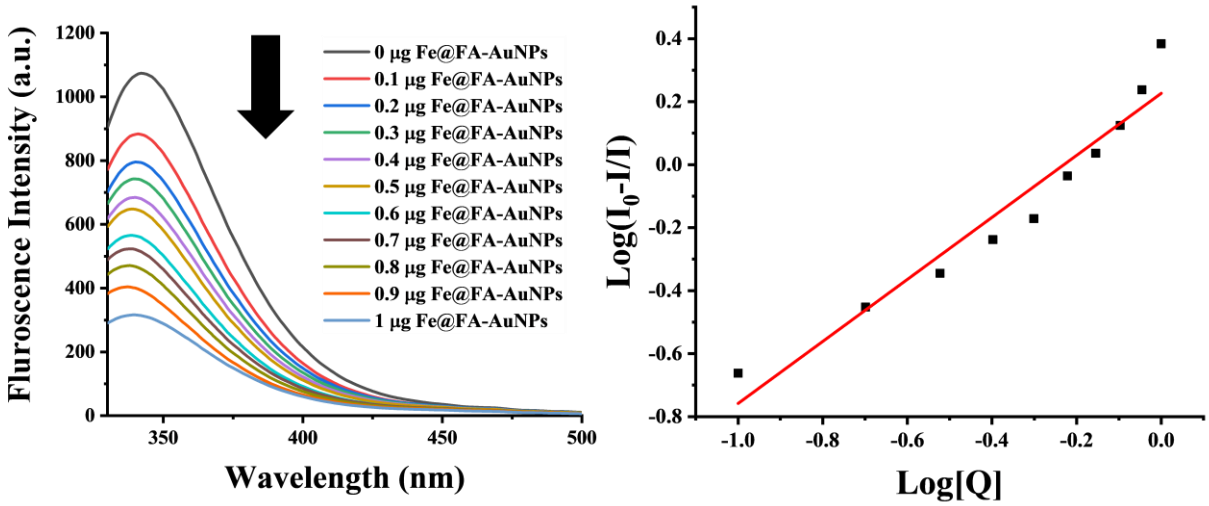


Figure S14: HSA binding studies of **Fe@FA-AuNPs** (10 mL 1mg mL^{-1} stock solution) with 3 X 10^{-5} M Human serum albumin, Scatchard plot of **Fe@FA-AuNPs** in Tris-HCl-buffer (5 mM, pH 7.2) at 298 K.

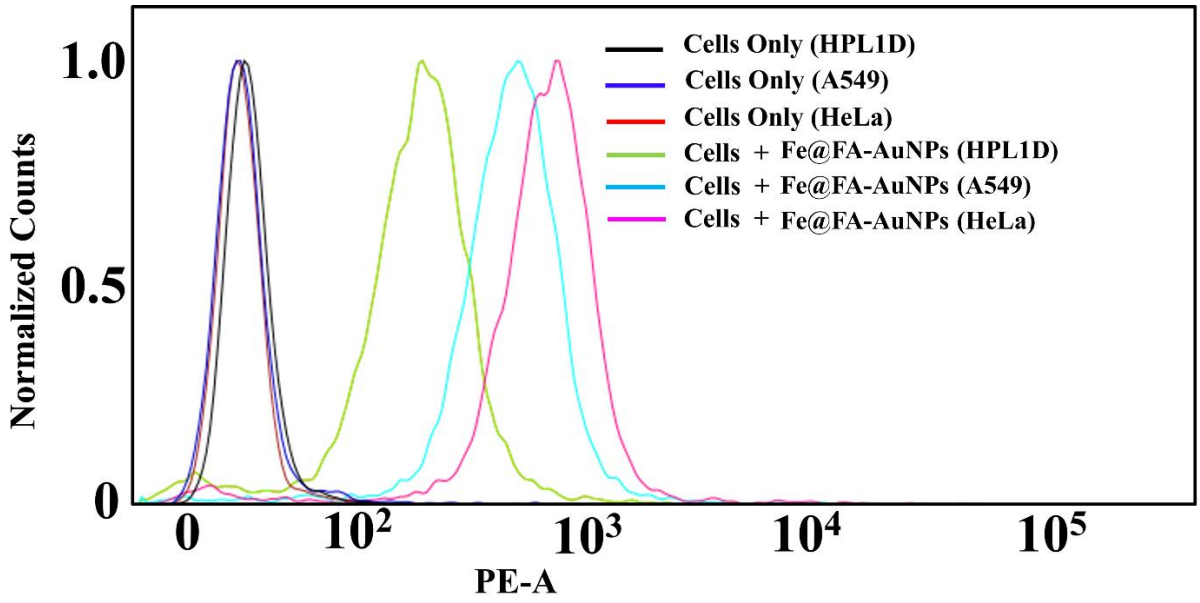


Figure S15: Fluorescence-assisted cell sorting analysis of the cellular uptake of **Fe@FA-AuNPs** (40 µg mL^{-1}) in the HeLa (Henrietta Lacks cell line, folate positive cancer cell line) A549 (adenocarcinomic human alveolar basal epithelial cells, folate negative cancer cell line), HPL1D (human peripheral lung epithelial cells, normal cell line) cell line after 4 h of incubation in the dark.

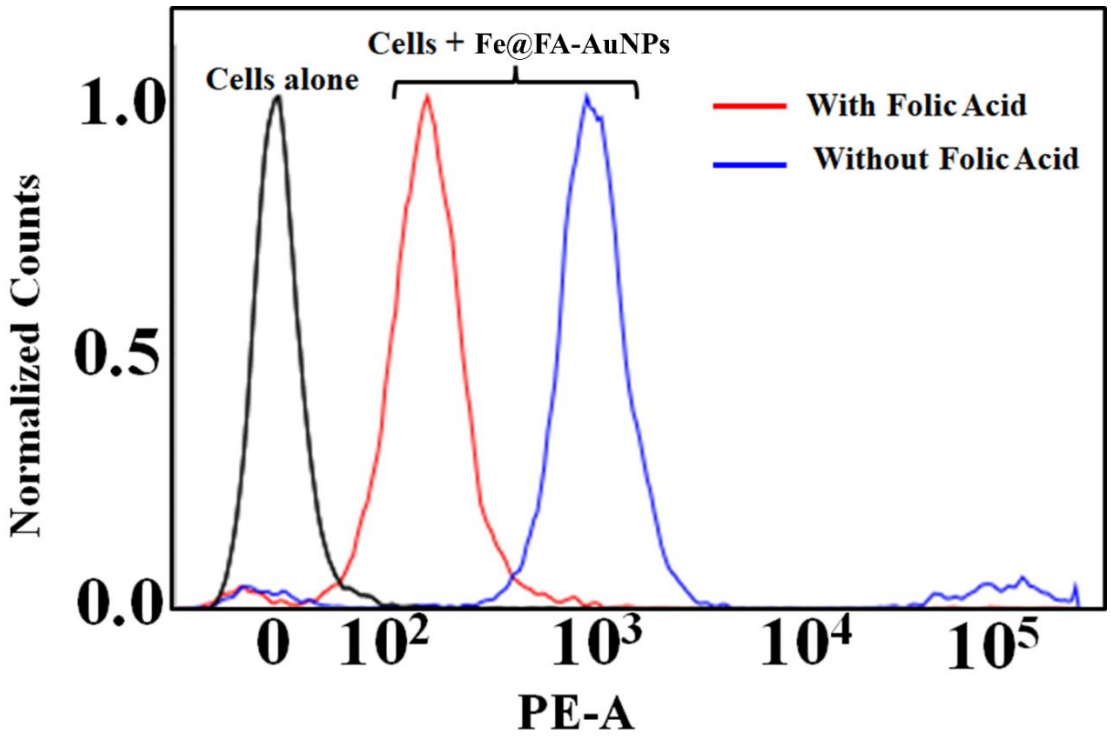


Figure S16: Fluorescence-assisted cell sorting analysis of the cellular uptake of **Fe@FA-AuNPs** (40 μg mL^{-1}) in the HeLa (Henrietta Lacks cell line, folate positive cancer cell line) cell line after 4 h of incubation in the dark, in presence and in absence of external folic acid.

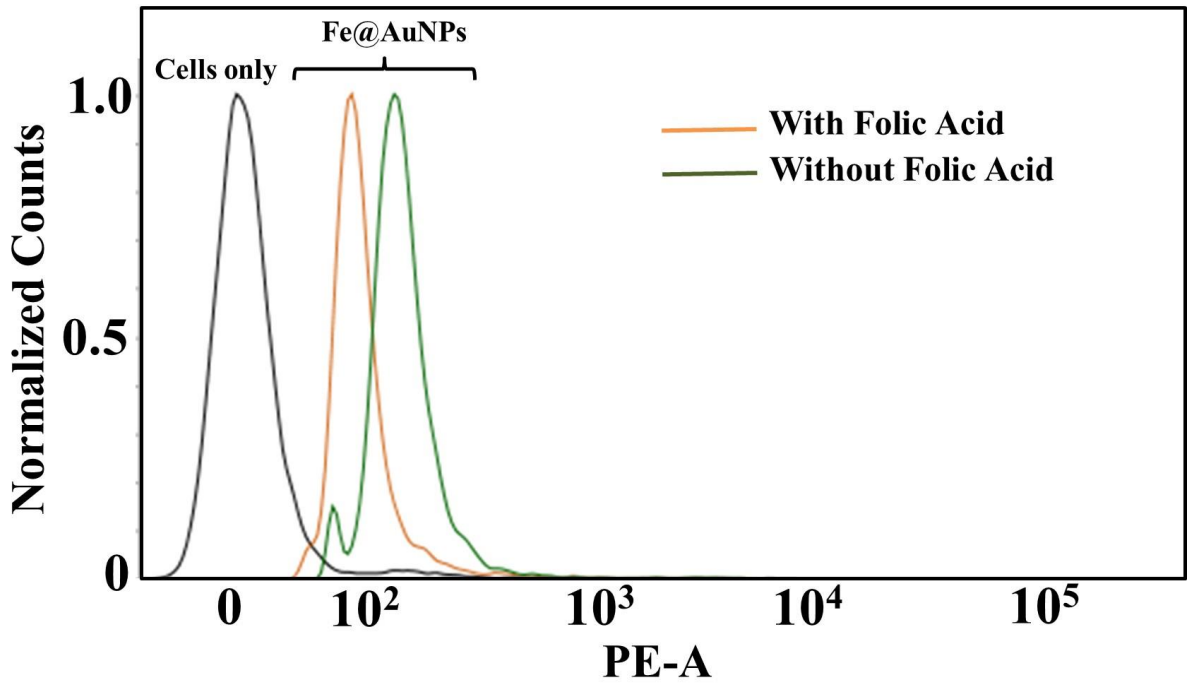


Figure S17: Fluorescence-assisted cell sorting analysis of the cellular uptake of **Fe@AuNPs** (40 μg mL^{-1}) in the HeLa (Henrietta Lacks cell line, folate positive cancer cell line) cell line after 4 h of incubation in the dark, in presence and in absence of external folic acid.

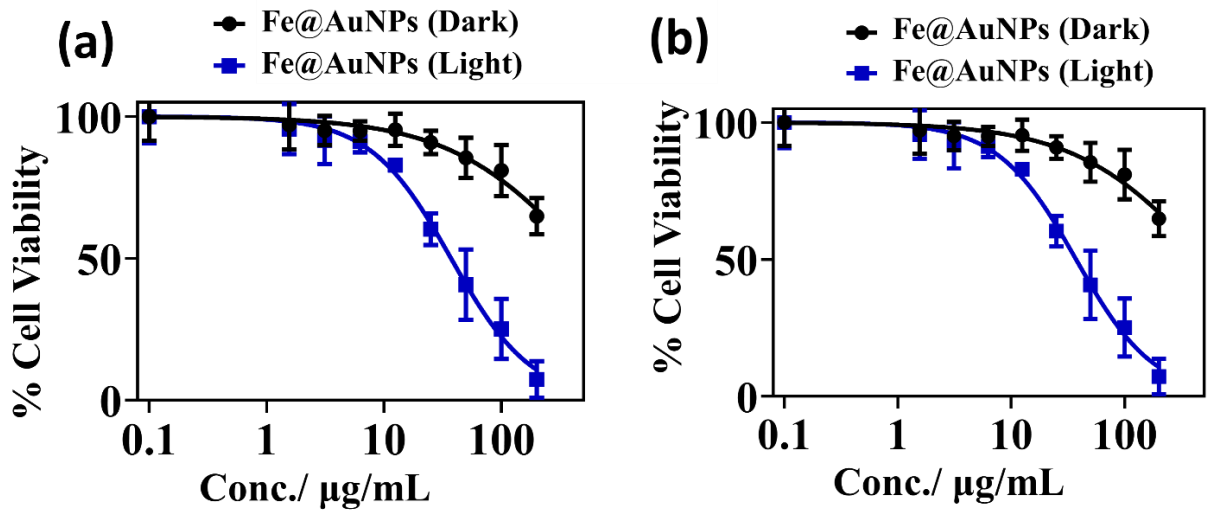


Figure S18: Cell viability (MTT assay) plots showing the cytotoxicity of the Fe@AuNPs in HeLa cells (a), MDA-MB-231 (b) in the dark (black symbols) and in the presence of red light (blue symbols, 600-720 nm, 30 J cm^{-2}).

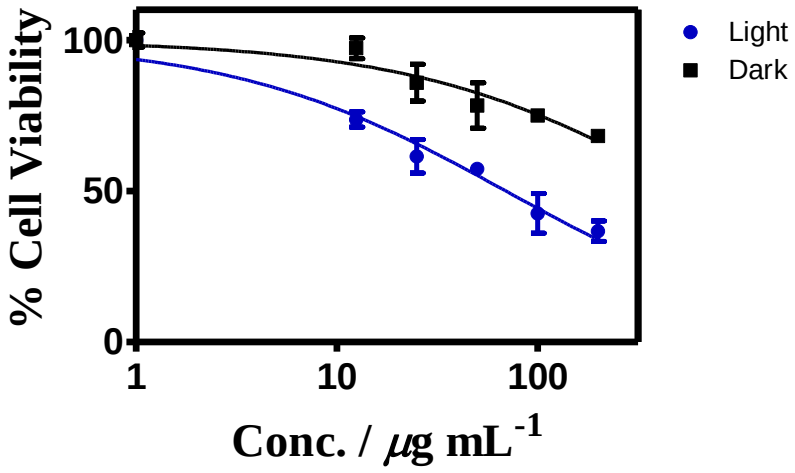


Figure S19: Cell viability (MTT assay) plots showing the cytotoxicity of the Fe@AuNPs in A549 in the dark (black symbols) and in the presence of red light (blue symbols, 600-720 nm, 30 J cm^{-2}).

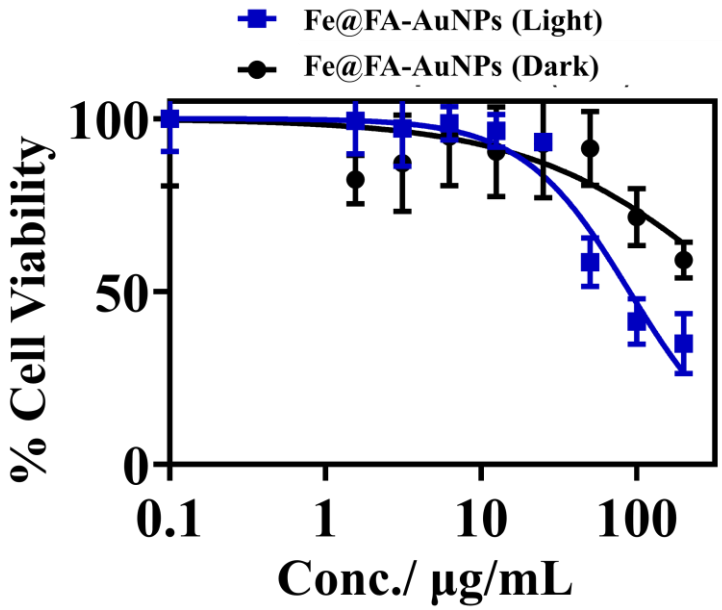


Figure S20: Cell viability (MTT assay) plots showing the cytotoxicity of the Fe@FA-AuNPs in HPL1D in the dark (black symbols) and in the presence of red light (blue symbols, 600-720 nm, 30 J cm^{-2}).

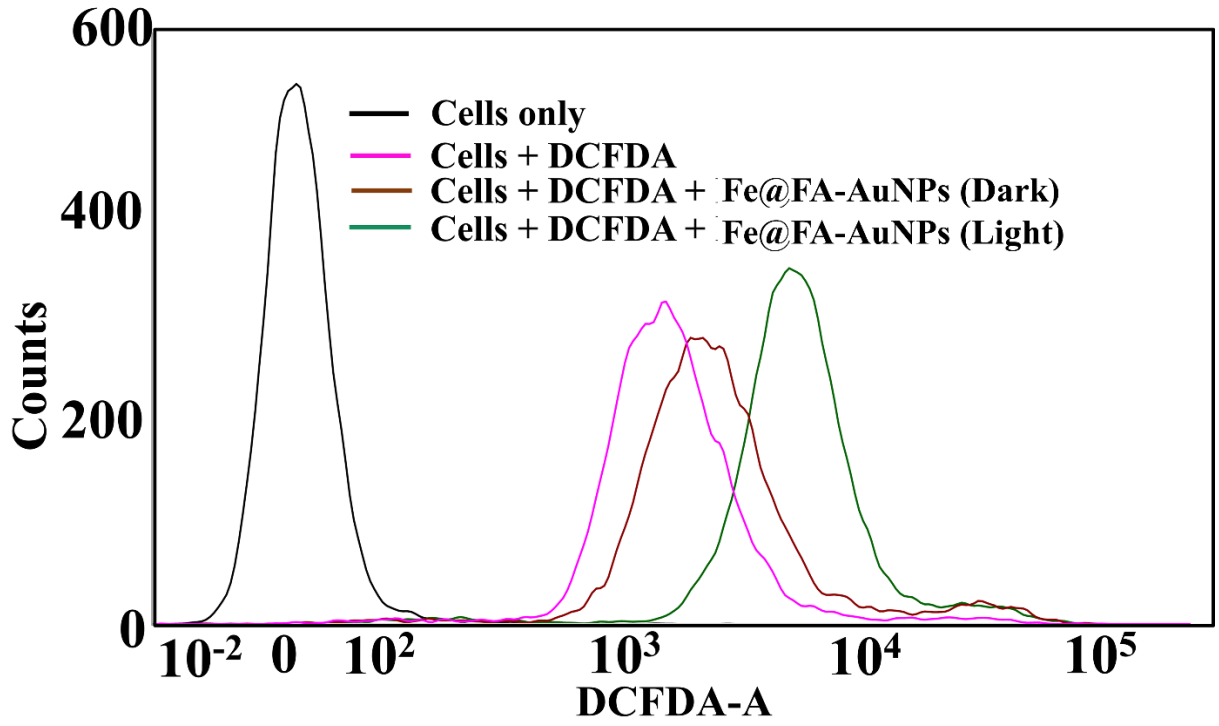


Figure S21: Shift in the band position of the cells alone, cells treated with DCFDA (2',7'-dichlorofluorescin diacetate), cells treated with **Fe@FA-AuNPs** (40 µg mL^{-1}) in Dark, cells treated with **Fe@FA-AuNPs** (40 µg mL^{-1}) in HeLa cells upon light irradiation (λ = 600–720 nm, light dose = 30 J cm^{-2}).

References

1. Pangborn, A.B.; Giardello, M.A.; Grubbs, R.H.; Rosen, R.K.; Timmers, F.J.; Safe and Convenient Procedure for Solvent Purification, *Organometallics* **1995**, 15, 1518-1520.
2. Gazi, S.; Ananthakrishnan, R.; Semi-Quantitative Determination of Hydroxyl Radicals by Benzoic Acid Hydroxylation: An Analytical Methodology for Photo-Fenton Systems, *Curr. Anal. Chem.*, **2012,** (*8*), 143.
3. Musib, D.; Banerjee, S.; Garai, A.; Soraisam, U.; Roy, M.; Synthesis, Theory and In Vitro Photodynamic Activities of New Copper (II)-Histidinito Complexes. *Chemistry Select.* **2018,** 3, 2767-2775.
4. Wilson, J. J.; and Lippard, S. J.; In Vitro Anticancer Activity of cis-Diammineplatinum(II) Complexes with β-Diketonate Leaving Group Ligands, *J. Med. Chem.*, **2012**, 55, 5326-5336.

5. Shao, J.; Bao, W.G.; Tian, H.; Li, B.; Zhao, X. F.; Qiao, X.; Xu, J.Y.; Nuclease activity and protein-binding properties of a novel tetranuclear thiosemicarbazide Pt(II) complex. *Dalton Trans.*, **2014**, 43, 1663.
6. Skehan, P.; Storeng, R.; Scudiero, D.; Monks, A.; McMahon, J.; Vistica, D.; Warren, T.J.; Bokesch, H.; Kenney, S.; Boyd, R. M.; New colorimetric cytotoxicity assay for anticancer-drug screening. *J. Natl. Cancer Inst*., **1990**, 82, 1107-1112.
7. Raza, K.; Gautam, S.; Garai, A.; Mitra, K.; Kondaiah, P.; Chakravarty, A. R.; Monofunctional BODIPY-Appended Imidazoplatin for Cellular Imaging and Mitochondria-Targeted Photocytotoxicity. *Inorg. Chem.* , **2017**, 56, 11019-11029.
8. Bresciani G.; Hofland L. J.; Dogan F.; Giamas G.; Gagliano T.; Zatelli M. C.; Evaluation of Spheroid 3D Culture Methods to Study a Pancreatic Neuroendocrine Neoplasm Cell Line. *Front Endocrinol (Lausanne)*. **2019**, 10, 682.
9. Adcock A. F.; Trivedi G.; Edmondson R.; Spearman C.; Yang L.; Three- Dimensional (3D) Cell Cultures in Cell-based Assays for *in-vitro* Evaluation of Anticancer Drugs. J Anal Bioanal Tech, **2015**, 6, 247.
10. Elmes, R. B. P.; Orange, K. N.; Cloonan, S. M.; Williams, D. C.; Gunnlaugsson, T.; Luminescent Ruthenium(II) Polypyridyl Functionalized Gold Nanoparticles; Their DNA Binding Abilities and Application As Cellular Imaging Agents. *J. Am. Chem. Soc.*, **2011** 133, 15862–15865.
11. Musib, D.; Pal, M.; Raza, M. K.; Roy, M.; Photo-physical, theoretical and photo-cytotoxic evaluation of a new class of lanthanide(iii)–curcumin/diketone complexes for PDT application. *Dalton Trans*., **2020**, 49 10786-10798.
12. Majumder M.; Debnath S.; Gajbhiye R. L.; et al. Ricinus communis L. fruit extract inhibits migration/invasion, induces apoptosis in breast cancer cells and arrests tumor progression in vivo. *Sci Rep*. **2019**; 9, 14493.
13. Justus C. R.; Leffler N.; Ruiz-Echevarria M.; Yang L. V.; In vitro cell migration and invasion assays. *J Vis Exp*. **2014**, 88, 51046.