Electronic Supplementary Information

Preparation of Multi coloured Different sized Fluorescent Gold Clusters from Blue to NIR, Structural Analysis of the Blue Emitting Au_7 Cluster and Cell-Imaging by the NIR Gold Cluster

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Materials:

Reduced glutathione and sodium borohydride were purchased from local chemicals SRL, India. Other chemicals including Na_2HPO_4, Tri-sodium citrate and NaH_2PO_4 were purchased from Merck, Germany. Chloroauric acid was purchased from Spectrochem, India. Reference dyes for quantum yield calculation were purchased from Sigma-Aldrich, USA. Water used for this study was ultra pure Milli Q grade. Dulbecco Modified Eagle Medium (DMEM), 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT), Kanamycin sulfate, Trypsin-EDTA solution,Sodium Chloride, Potassium Chloride and fetal bovine serum were purchased from Sigma Aldrich.2-[4-(2-hydroxyethyl) piperazin-1-yl] ethanesulfonic acid (HEPES) was purchased from Himedia. Penicillin-Streptomycin and colourless DMEM were purchased from Invitrogen Sodium bicarbonate, Potassium dihydrogen phosphate and di-Sodium hydrogen phosphate dehydrate were purchased from Merck. All compounds were used without further purification. A549 (adenocarcinomic human alveolar basal epithelial cells) cell line was purchased from NCCS, pune (India) and cultured in Dulbecco Modified Eagle medium (DMEM) containing 10% (v/v) heat-inactivated fetal bovine serum at 37 °C and 5% CO_2 atmosphere in our lab. Cover glass bottom dishes were purchased from SPL.

Cellular uptake studies:

A549 (adenocarcinomic human alveolar basal epithelial cells) cells were cultured in Dulbecco Modified Eagle medium (DMEM) containing 10% (v/v) heat-inactivated fetal bovine serum at 37^0C and 5% CO_2 atmosphere. 5000 cells were seeded onto a cover glass bottom dish for cellular uptake experiment one day prior to incubation with the near infrared (NIR) gold quantum cluster. 200 µL solution of the near infrared (NIR) gold quantum cluster was dissolved in 100 µL colourless, serum-free DMEM medium (concentration of the near infrared (NIR) gold quantum cluster- 2 mg/mL) and 300 µL of final solution of near infrared (NIR) gold quantum cluster was treated into the cells after washing with the 1X phosphate-buffered saline (PBS) and incubated for 4 hours at 37 °C under 5% CO_2 atmosphere. Finally these cells were washed and the cellular internalization of the near infrared (NIR) gold quantum cluster was imaged by Nikon Eclipse Ti-U inverted fluorescence microscope in 561 nm channel along with bright field (Fig. 10a).

Cytotoxicity study:

Inhibition of cancer cells (adenocarcinomic human alveolar basal epithelial cell line (A549 cell line) proliferation was evaluated by the 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) reduction.[1,2] A549 cells were seeded at a density of 10000 cells per well in a 96-well plate for 18-24 hours before the near infrared (NIR) gold quantum cluster treatment. Then these cells were treated with various concentration of the near infrared (NIR) gold quantum cluster (stock concentration was 100 µg/mL in serum-free DMEM medium) in serum-free DMEM medium for 4h in 96-well plate. After that the cells were washed with DMEM medium, containing 10 % (v/v) heat-inactivated fetal bovine serum and incubated for 24h. 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] × 100.

Synthesis of Gold Clusters

Synthesis of all gold clusters, their fluorescence emission and excitation and cluster sizes have been given in a tabular form (Table S1).

Synthesis of Blue emitting Gold quantum cluster (QC1)

10 mg of reduced glutathione was taken in a 50 mL round bottom flask and 4 mL of 50 mM phosphate buffer of pH 7.46 was added to it. 5.5 mg of $HAuCl_4$ (tetra chloro auric acid) in 500 μL Milli Q water was added to that solution. It was then stirred for a few minutes at room temperature. The round bottom flask was then fitted with a bulb condenser and the reaction environment was made inert by using argon gas balloon fitted to the condenser. The total set up was put on an oil bath with vigorous stirring having bath temperature 140 ˚C. 2 mg of sodium cyanoborohydride ($NaBH_3CN$) in 500 µL Milli Q water was then injected / added to the hot reaction mixture slowly. The progress of reaction was monitored using fluorescence and UV-Vis spectroscopy. After 16 hrs of reaction time, the total solution became very light brown in colour. This solution showed blue colour luminescence behaviour under the UV torch of wavelength 365 nm. This QC1 solution was used for all studies without any purification.

Synthesis of Green emitting Gold quantum cluster (QC2)

The reaction condition for the preparation of green emitting gold quantum cluster (QC2) was same as before, however, the amount of gold precursor ($HAuCl_4$) used was 4.5 mg in 500 µL Milli Q water and trisodium citrate was used as the reducing agent. This as-prepared solution showed a green colour fluorescence upon the UV irradiation at wavelength 365 nm. This QC2 solution was used for all studies without any purification.

Synthesis of Orange Red emitting Gold quantum cluster (QC3)

QC3 was prepared by using the following procedure:

(i) At first, 5.5 mg of the gold precursor ($HAuCl_4$) was taken keeping the other conditions same as synthesis of QC2. However, after the progress of the reaction for 1 hour at 140 ˚C, 1 mL of the reaction mixture was taken out and kept at room temperature leaving the rest in stirring reflux condition at 140 ˚C.

(ii) After 30 mins the reaction mixture that was kept at room temperature was added to the reaction vessel and the total reaction was stirred for furthur 1 hour.

This process (taking out aliquot from the reaction mixture and its addition after standing at room temperature) was repeated 5 times to obtain the best result. Under the UV torch of wavelength 365 nm, a orange red fluorescence colour was observed. This QC3 solution was used for all studies without any purification.

Synthesis of Red emitting Gold quantum cluster (QC4)

A striking difference of the synthesis of this cluster from the rest is that other clusters were prepared in hot conditions and this synthesis was successfully carried under ice-cold condition. 5 mg of reduced glutathione was taken in a 5 mL glass vial. 1.8 mL of 50 mM sodium acetate buffer of pH 7.46 was added to it. 1 mg of $HAuCl_4$ was added to that solution and it was stirred for a few minutes at ice cold condition. 2 mg of $NaBH_4$ was dissolved in 1 mL of cold Milli Q water and 200 μL of cold sodium borohydride solution was added to the ice-cold solution mixture slowly. The progress of the reaction was monitored by fluorescence and UV-Vis spectroscopic studies. After 30-40 minutes of reaction time, the total solution became reddish in colour. This solution showed a red colour fluorescence under the UV torch of wavelength 365 nm. This QC4 solution was used for all studies without any purification.

Synthesis of NIR emitting Gold quantum cluster (QC5)

10 mg of reduced glutathione was taken in a 50 mL round bottom flask. 4 mL of 50 mM phosphate buffer of pH 7.46 was added to it. 8 mg of $HAuCl_4$ in 500 μL Milli Q water was added to that solution and it was stirred for a few minutes at room temperature. The round bottom flask was then fitted with a bulb condenser and the reaction environment was made inert by using argon gas balloon fitted to the condenser. The total set up was put in to an oil bath with vigorous stirring having bath temperature 110 ˚C. 2 mg of sodium borohydride ($NaBH_4$) in 500 μL Milli Q water was then injected / added to the hot reaction mixture slowly. The progress of reaction was monitored using fluorescence spectroscopic and UV-Vis studies. After 16 hrs of reaction time, the total solution becomes very light brown in colour. This solution shows red colour fluorescence behaviour under UV torch of wavelength 365 nm. This QC5 solution was used for all studies (except cell-imaging) without any purification.

This NIR cluster was used for the cell-imaging studies. For better reproducibility determination of the exact concentration of the NIR cluster in the solution was necessary. Though only Au22 clusters are present (as known from the MALDI-TOF spectra), there can be some bulk gold present in the sample and these bulk gold do not participate in fluorescence. Methanol was added to the aqueous dispersion of NIR clusters in such a way that the water : methanol ratio becomes 1:1 in the cluster solution and this was done for removing bulk gold. Then this solution was centrifuged for 20 minutes at a speed of 13,000 rpm and some black particles were precipitated out. The precipitate does not give any fluorescence after re-dissolving, while fluorescence peak of the supernatant liquid is similar to the cluster solution before purification. Thus, bulk gold particles were removed and this purified NIR cluster solution was used for the cell imaging study.

Instrumentation

UV-Vis spectroscopic analysis

We used a Cary Varian 50 scan UV-Vis optical spectrometer equipped with 'Cary Win' UV software to elucidate the optical properties of gold quantum clusters.

Fluorescence spectroscopy

Fluorescence studies of gold quantum clusters in a sealed cuvette were carried out in a Perkin Elmer LS55 Fluorescence Spectrometer instrument. All the experiments were carried out with the excitation slit width 5 nm and emission slit width 5 nm.

Time-Correlated Single Photon Counting (TCSPC) study

TCSPC measurements were performed by means of Horiba Jobin Yvon IBH having MCP PMT Hamamatsu R3809 detector instrument and all data were fitted using Data Station v2.3. We have used NANO-LED source for excitation of samples at 340 nm and LASER source for excitation of samples at 440 nm.

MALDI-TOF MS study

The MALDI-TOF MS analyses were done using Bruker Daltonics flexAnalysis mass spectrometer.

Raman spectroscopic study

A Kr^+ laser (Sabre Innova, model SBRC-DBW-K) from Coherent and a spectrograph with 1.5 mm slit width (model Trivista 555) fitted with an electronically cooled Pixis CCD from Princeton Instruments were used to collect the Raman data. The 676.4 nm red laser was used for the Raman experiments.

TEM study

TEM study of the NIR clusters were carried out in a JEOL 2100 KeV Ultra High Resolution Field Emisson Gun (UHR FEG) TEM with voltage 200 KeV.

FTIR study

The FT-IR spectra were taken by using Shimadzu (Japan) model FT-IR spectrophotometer. In the solid state FT-IR studies, powdered were mixed with KBr for preparing thin films.

X-ray Photoelectron Spectroscopic (XPS) Study

XPS analysis of dried NIR quantum cluster was carried out by using an X–ray photoelectron spectroscopic (XPS, Omicron, model: 1712–62–11) method. Measurement was done by using an Al-Kα radiation source under 15 kV voltages and 5 mA current.

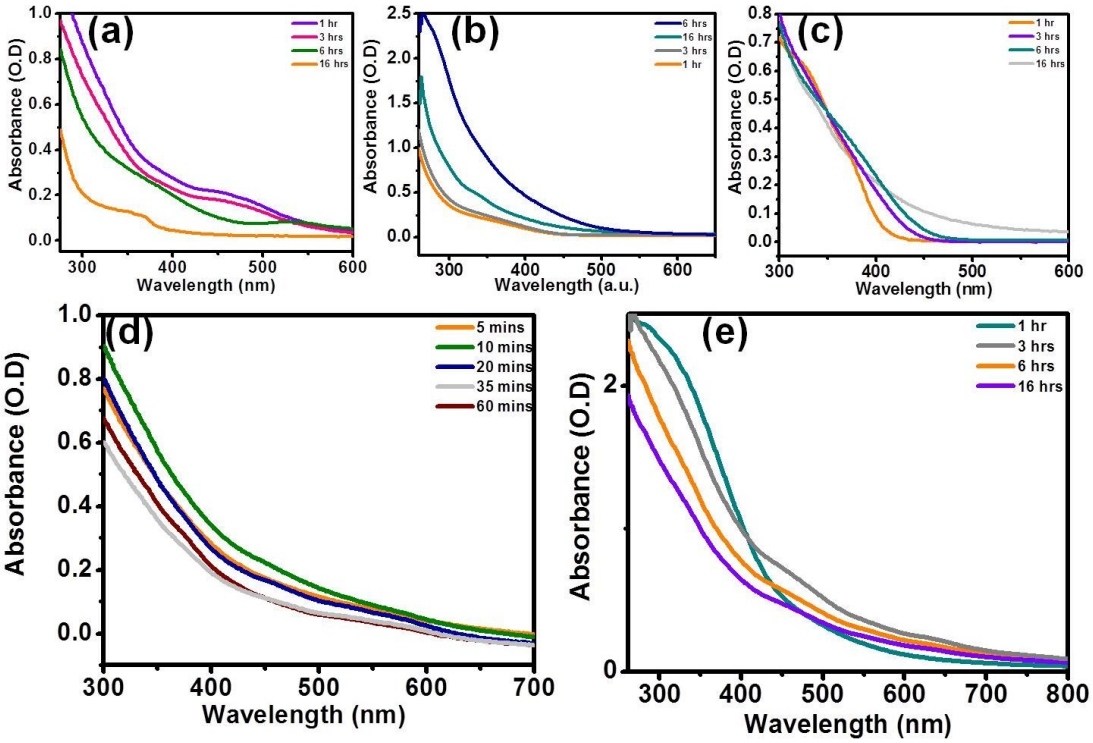

Fig. S1 UV-Vis formation kinetics of gold quantum clusters.

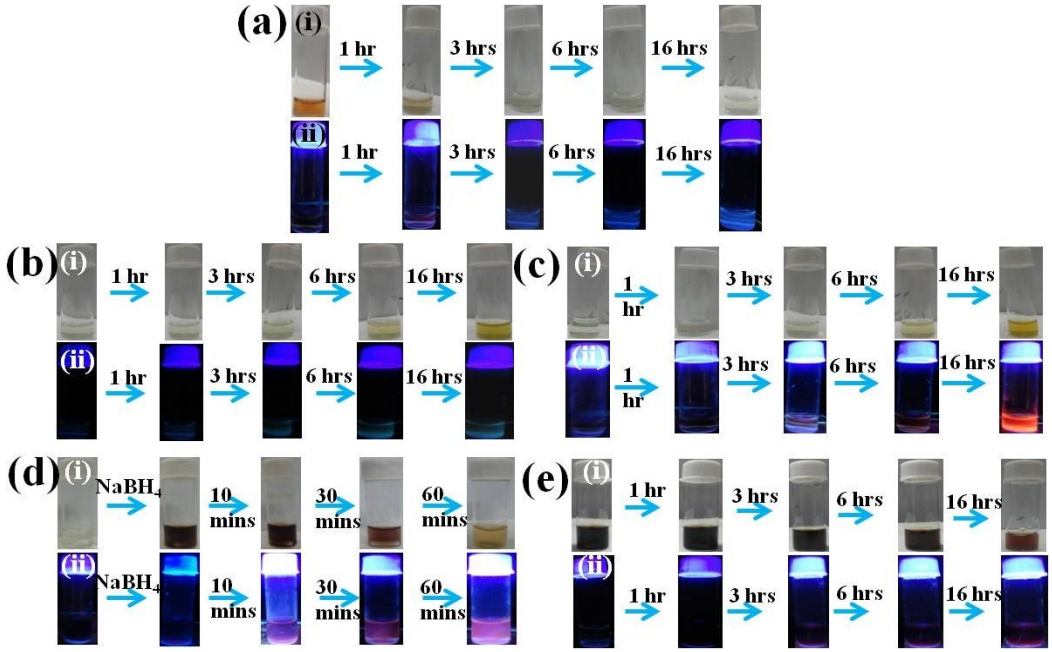

Fig. S2 Colour changes during the formation of gold quantum clusters for (a) blue, (b) green, (c) orange red, (d) red and (e) NIR. In each image (i) represent the colour changes under visible light and (ii) represent under UV light irradiation.

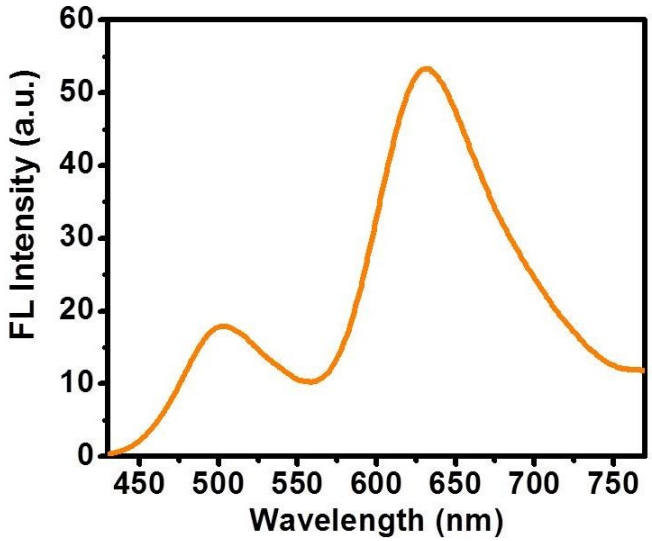

Fig. S3 Presence of a minor peak at lower region for QC3 at 503 nm.

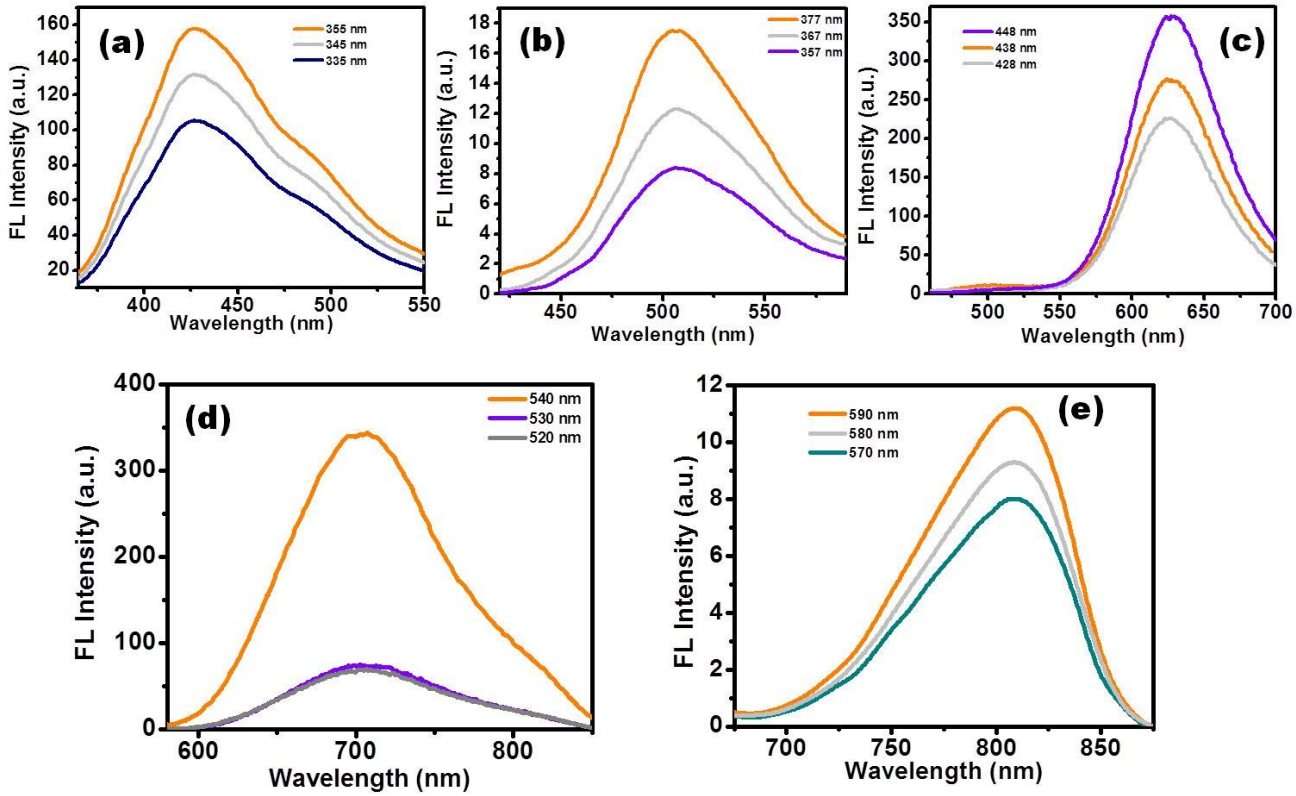

Fig. S4 Fluorescence emission profile of the gold quantum clusters (a) QC1, (b) QC2, (c) QC3, (d) QC4 and (e) QC5 at different fluorescence excitation wavelengths.

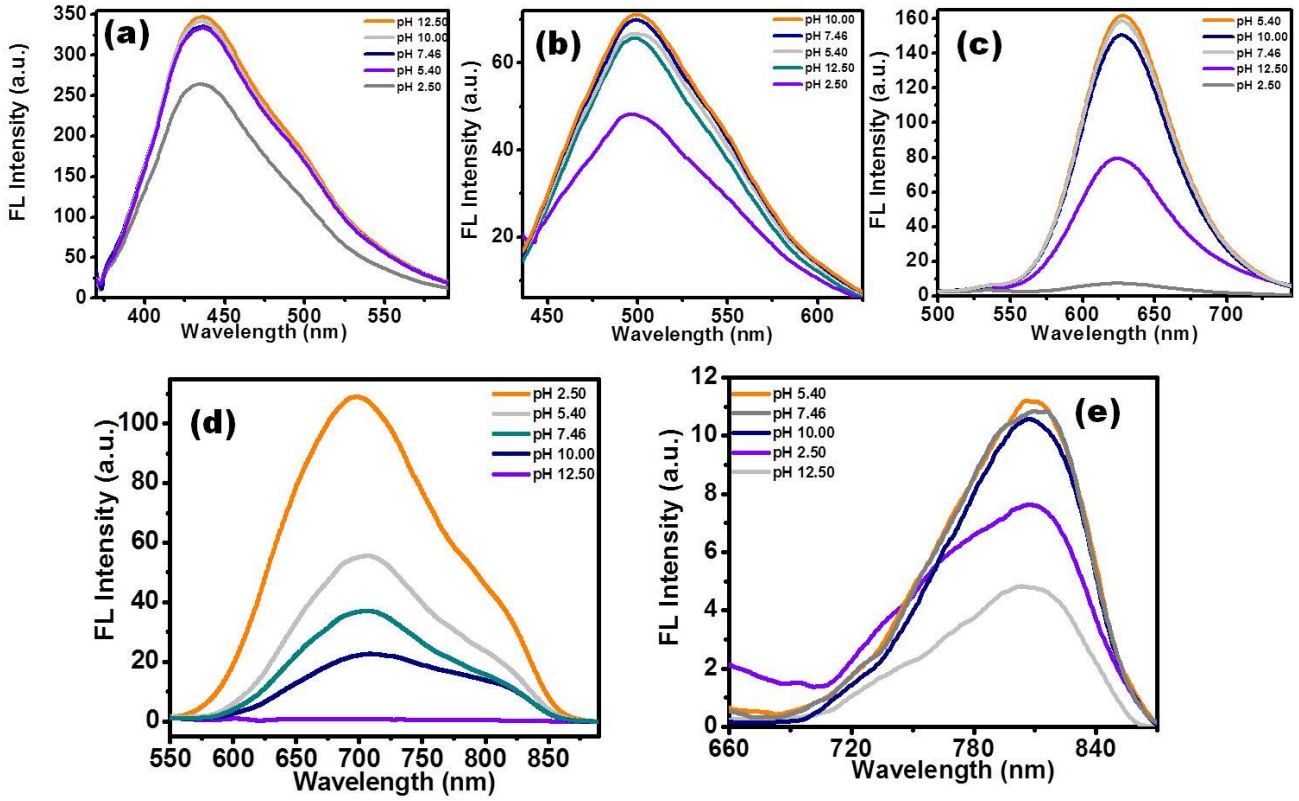

Fig. S5 Fluorescence emission profile of the gold quantum clusters (a) QC1, (b) QC2, (c) QC3, (d) QC4 and (e) QC5 at different pHs.

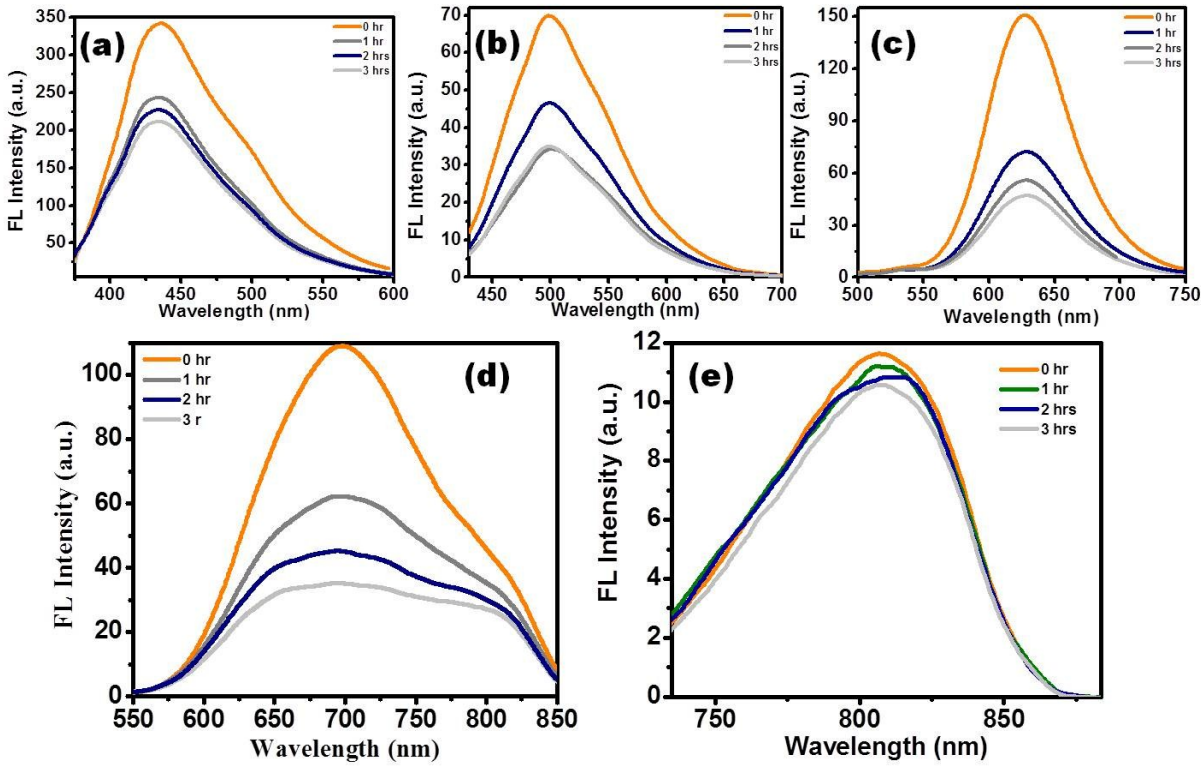

Fig. S6 Fluorescence emission profile of the gold quantum clusters (a) QC1, (b) QC2, (c) QC3, (d) QC4 and (e) QC5 under UV light irradiation.

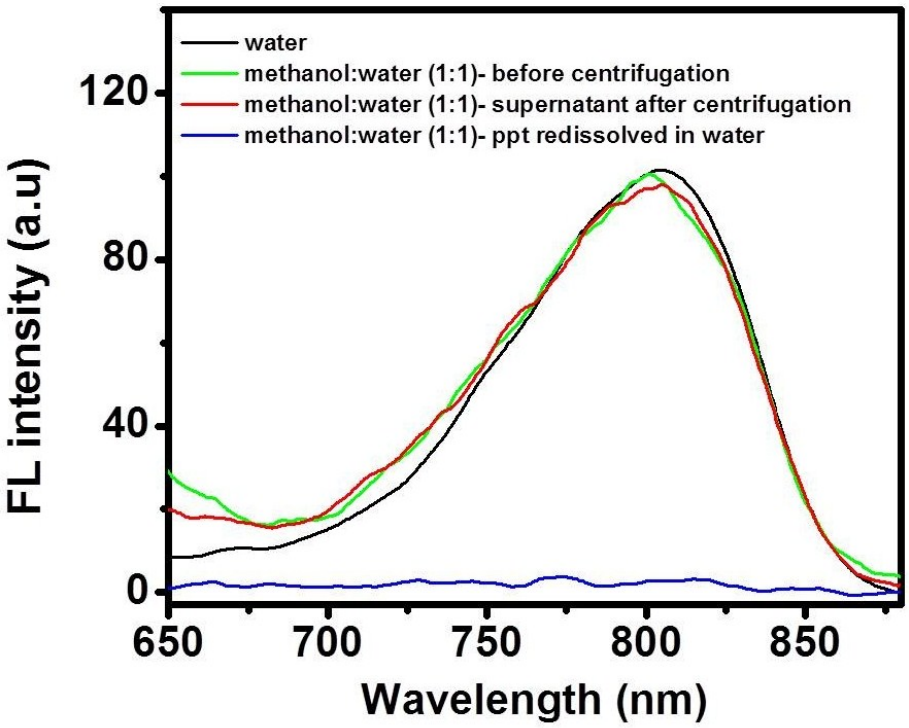

Fig. S7 FL emission of NIR clusters before and after purification.

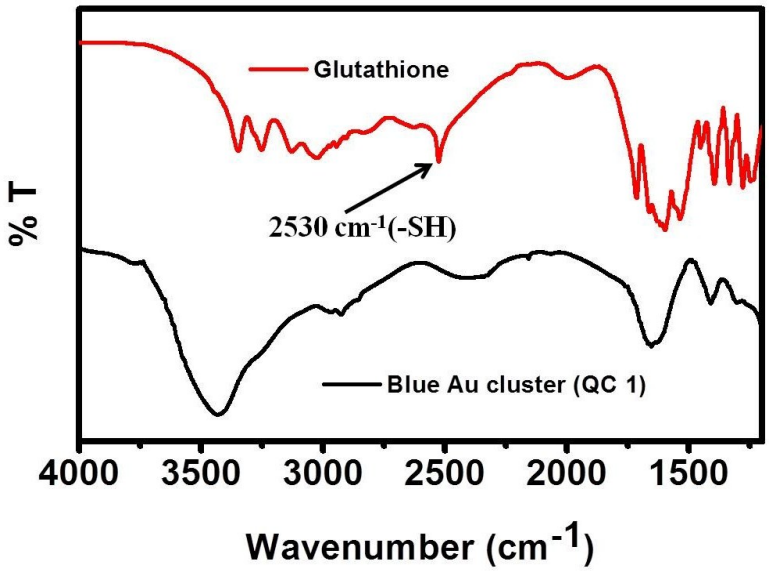

Fig. S8 FTIR study of QC1 and glutathione (reduced).

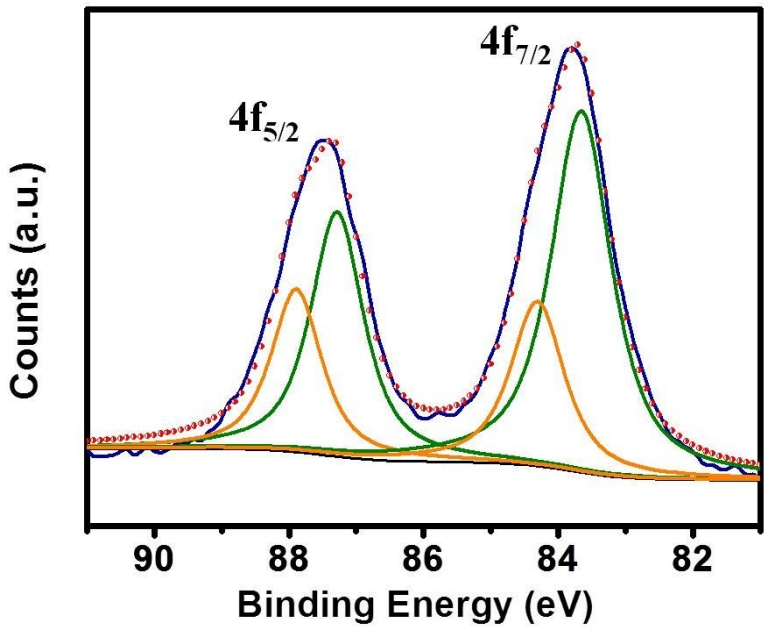

Fig. S9 XPS spectra of the NIR gold cluster. The Au $4f_{7/2}$ binding energy (blue line) could be deconvoluted into two components, which give peaks at 84.28 eV (orange line) and 83.64 eV (green line). These two components could be assigned to Au(I) and Au(0) respectively. The fitted result is shown as the dotted line.

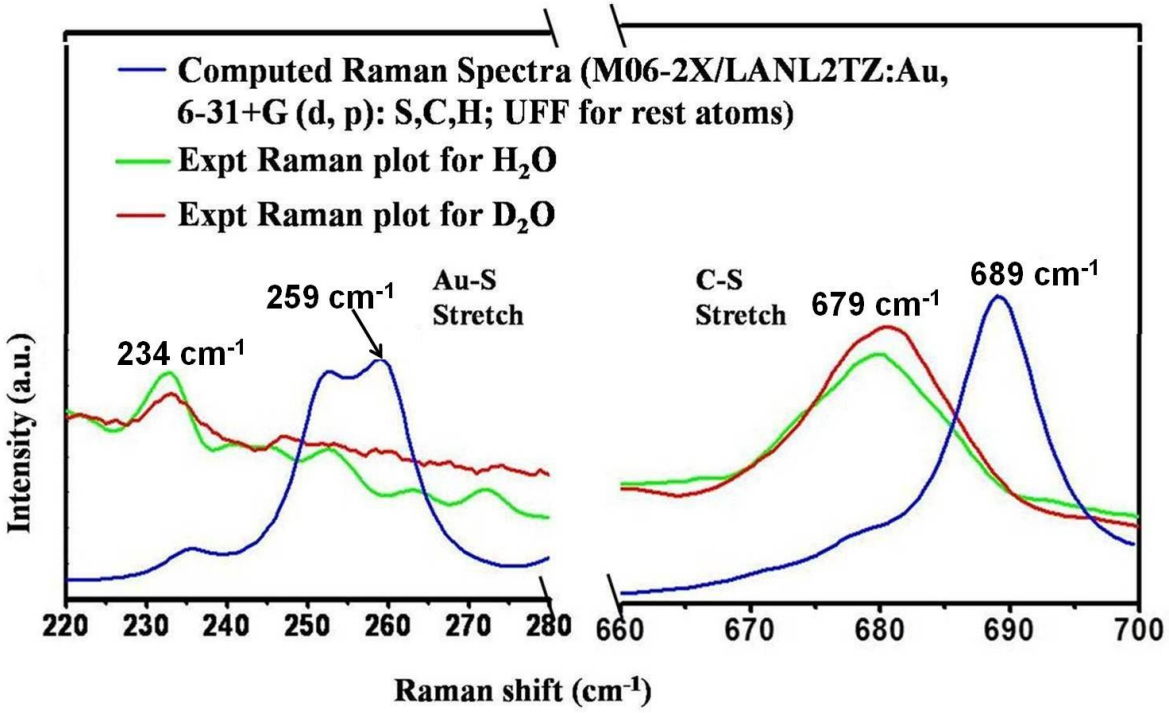

Fig. S10 Observed and the computed Raman spectra for the $Au_7(glutathionate)_2$ cluster. No change on deuterated cluster confirms thiolate –gold instead of thiol-gold interaction.

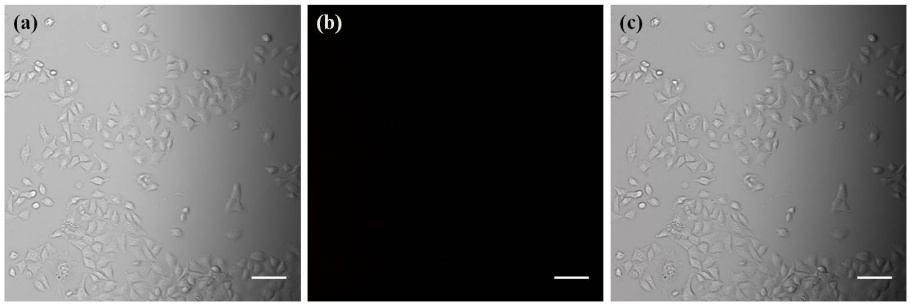

Fig. S11 Autofluorescence of A549 cell line: (a) DIC image of A549 cell line, (b) Fluorescence image of A549 cell line in 561 nm channel and (c) Merged image. Scale bar 20 μm.

Table S1. Reaction details of clusters formation, FL, FLE and clusters sizes.

Reaction Condition	**Fluorescence emission (FL) and fluorescence excitation (FLE) (nm) maxima**	**Band gap (eV)**	**Cluster Size (Au_xL_y)(from MALDI-TOF MS)**
$HAuCl_4$ (mg): 5.5, $NaBH_3CN$ (mg): 2, Temperature (˚C): 140, Capping agent (glutathione) (mg): 10, Medium: phosphate buffer (pH 7.46)	FL: 426 nm, FLE: 355 nm	3.49	x = 7, y = 2
$HAuCl_4$ (mg): 4.5, trisodium citrate (mg): 2, Temperature (˚C): 140, Capping agent (glutathione) (mg): 10, Medium: phosphate buffer (pH 7.46)	FL: 507, FLE: 338 nm and 378	3.28	x = 16, y = 8
$HAuCl_4$ (mg): 5.5, trisodium citrate (mg): 2, Temperature (˚C): 140, Capping agent (glutathione) (mg): 10, Medium: phosphate buffer (pH 7.46)	FL: 628 (major) and 503 (very less intense), FLE: 448	2.76	x = 19, y = 5
$HAuCl_4$ (mg): 1, $NaBH_4$ (mg): 200 µL (2mg/1 mL), Temperature (˚C): 0-4, Capping agent (glutathione) (mg): 5, Medium: sodium acetate buffer (pH 7.46)	FL: 705 , FLE: 540 and 576	2.15	x = 21, y = 8
$HAuCl_4$ (mg): 8, $NaBH_4$ (mg): 2, Temperature (˚C): 110, Capping agent (glutathione) (mg): 10, Medium: phosphate buffer (pH 7.46)	FL: 805, FLE: 590	2.10	x = 22, y = 5

References:

(1) B. Jana, G. Mondal, A. Biswas, I. Chakraborty, S. Ghosh, *RSC Advances* **2013**, 3, 8215-8219.
(2) M. Jiang, O. Huang, X. Zhang, Z. Xie, A. Shen, H. Liu, M. Geng, K. Shen, *Molecules* **2013**, *18,* 701-720.