Electronic Supplementary Information

The as-prepared Gold Cluster-Based Fluorescent Sensor for the Selective Detection of As^{III} Ions in Aqueous Solution

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Experimental section:

General Methods and Materials.

L-cysteine and triphenylmethanol were purchased from sigma chemicals. HOBt (1-hydroxybenzotriazole) and DCC (dicyclohexylcarbodiimide), TFA (trifluoroacetic acid), Formic acid, Sodium Borohydride were purchased from SRL (Local Chemicals). Sodium acetate, absolute ethanol etc. were purchased from Merck.

Synthesis of dipeptides.

The dipeptide was synthesized by conventional solution-phase methods using racemization free fragment condensation strategy. [1] The Boc group was used for N-terminal protection, the C-terminus was protected as a methyl ester and the free thiol group was protected by tri-phenyl methanol. Couplings were mediated by dicyclohexylcarbodiimide/1-hydroxybenzotriazole (DCC/HOBt). Methyl ester deprotection was performed via the saponification method and the Boc group was deprotected by 98% formic acid. 300 MHz ^{1}H NMR and mass spectrometry characterized all the intermediates. The final compounds were fully characterized by 300 MHz ^{1}H NMR spectroscopy, ^{13}C NMR spectroscopy, ESI-Mass Spectromety and FT-IR spectroscopy**.**

1. Preparation of S-Tri-Phenylmethyl-L-Cysteine.

(10.90g, 90 mM) of L-Cysteine was dissolved in trifluoroacetic acid (165 ml). Trityl alcohol (23.4 g, 90 mM) was added to it and the reaction mixture was kept for 30 minute at room temperature. [2] Trifluoroacetic acid was removed under reduced pressure and di-methyl ether (200 ml) was added to the reddish-brown gummy compound. Then to this ethereal solution 30% aqueous solution of NaOAc was added to bring the pH near

neutrality and at this point white precipitate was obtained. It was filtered and washed with cold double distilled water (200 ml), EtOH (70 ml) and dried in vaccu. The white solid was taken in di-ethyl ether (200 ml) and filtered through a buckner funnel and dried in vaccu. Yield- 29.7g (91·1 %), mp: 182 °c.

2. Boc-S-Tri-Phenylmethyl-L-Cysteine.

A solution of s-triphenylmethyl-L-cysteine (10.89 g, 30mM) in a mixture of dioxane (60ml), water (30ml), and 1 (N) NaOH (30 ml) was stirred and cooled in an ice–water bath. Ditertbutylpyrocarbonate (7.2 g, 33mM) was added and stirring was continued at room temperature for 6 h. The solution was then concentrated in a vacuum to about 20-30 ml, cooled in an ice–water bath, covered with a layer of ethyl acetate (about 70 ml), and acidified with a dilute solution of $KHSO_4$ to pH 2–3 (Congo-red). The aqueous phase was extracted with ethyl acetate and this operation was done at least three times. The ethyl acetate extracts were pooled, washed with water and dried over anhydrous Na_2SO_4 and evaporated in a vacuum. A white material was obtained. Yield: 12.84 g (27.75 mM, 92.5 %), mp : 195 °c

3. Boc-S-Tri-Phenylmethyl-L-Cysteine (1)- S-Tri-Phenylmethyl-L-Cysteine (2)-OMe.

9.26 g (20 mM) of Boc-S-triphenylmethanol-L-cysteine (1)-OH was dissolved in 10 ml of DMF in an ice–water bath. H_2N- S-triphenylmethanol-L-cysteine (2)-OMe was isolated from 15.08 g (40 mM) of the corresponding methyl ester hydrochloride by neutralization, subsequent extraction with ethyl acetate and ethyl acetate extract was concentrated to 10 ml. It was then added to the reaction mixture, followed immediately by 4.12 g (20 mM) of dicyclohexylcarbodiimide (DCC) and 2.7 g (20 mM) of HOBt. The

reaction mixture was allowed to come to room temperature and stirred for 3 days. The residue was taken up in ethyl acetate (40 ml) and dicyclohexylurea (DCU) was filtered off. The organic layer was washed with 1 (N) HCl (3 × 30 ml), brine (1 × 30 ml), 1 (M) sodium carbonate (3 × 30 ml) and brine (2 × 30 ml); dried over anhydrous sodium sulphate; and evaporated in a vacuum. A yellowish white material was obtained. This compound was purified by column chromatography using silica (100–200 mesh) gel as stationary phase and 1:1 ethyl acetate/toluene as an eluent. The purified final compounds were fully characterized by ^{1}H NMR spectroscopy and mass spectrometry.

Yield: 13.89 g (16.9 mm, 84%). ^{1}H NMR (300 MHz, chloroform-*d*, 25°c, TMS): δ 7.24-7.08 (m, 30H, aromatic, CH); 6.49 (br, 1H, NH); 4.69 (br, 1H, NH); 4.40-4.36 (m, 1H, $C^{\alpha}H$); 4.07-4.03 (m, 1H, $C^{\alpha}H$); 3.57 (s, 3H, OCH_3); 2.63-2.54 (m, 2H, $C^{\beta}H$'s); 2.48-2.43 (m, 2H, $C^{\beta}H$'s); 1.33 (s, 9H, $(OCH_3)_3$); anal. Calcd for $C_{50}H_{50}N_2O_5S_2$ (822.3161): C, 72.96; H, 6.12; N, 3.46. Found: C, 72.11; H, 6.03; N, 3.21. ESI-MS: found m/z $(M+Na)^+$ = 844.7012; M_{cald} = 822.3161, mp : 211 °c

4. Boc-S-Tri-Phenylmethyl-L-Cysteine (1)- S-Tri-Phenylmethyl-L-Cysteine (2)-COOH.

To 13.89 g (16.9 mM) of 3 were added 50 ml of MeOH and 20 ml of 2 (N) NaOH was added and the progress of saponification was monitored by thin-layer chromatography (TLC). The reaction mixture was stirred. After 10 h, methanol was removed under vacuum and the residue was taken in 50 ml of water and washed with diethyl ether (2 × 50 ml). Then the pH of the aqueous layer was adjusted to 2 using 1 (M) HCl and it was extracted with ethyl acetate (3 × 50 ml). The extracts were pooled, dried over anhydrous

sodium sulphate and evaporated under vacuum to yield 11.31 g (14 mM) of (4). The purified final compounds were fully characterized by ^{1}H NMR spectroscopy and mass spectrometry.

^{1}H NMR (300 MHz, dmso-d_6, 25°c, TMS): δ 12.82 (b, 1H, -CO_2H); 8.01 (d, j = 9Hz, 1H, NH); 7.30-7.14 (m, 30H, aromatic, CH); 6.94 (d, 1H, j = 9Hz, NH); 4.49 (br, 1H, C^{α}H); 4.06-4.02 (m, 1H, C^{α}H), 3.47-3.32 (m, 1H, C^{β}H's); 2.42-2.29 (m, 1H, C^{β}H's); 1.35 (s, 9H, O(CH_3)$_3$). Anal. Calcd for $C_{49}H_{48}N_2O_5S_2$ (808.3005): C, 72.74; H, 5.98; N, 3.46. Found: C, 72.45; H, 5.78; N, 3.35. ESI-MS: found m/z $(M+Na)^+$ =831.8125; M $_{cald}$ = 808.3005, $(M+K)^+$= 847.8, mp : 205 °c

5. H_3N^+-S-Tri-Phenylmethyl-L-Cysteine (1)- S-Tri-Phenylmethyl-L-Cysteine (2)-COO^-.

To 11.31 g (14 mM) of Boc-S-triphenylmethyl-L-cysteine (1)- S-triphenylmethyl-L-cysteine (2)-OH was dissolved in 6 ml of formic acid for the removal of Boc group. It was monitored by TLC. After 8 h, formic acid was removed under vacuum. The residue was taken in water (20 ml) and washed with diethyl ether (2 × 30 ml). Then the pH of the aqueous layer was adjusted to 7 using aqueous ammonia solution and it was extracted with ethyl acetate (3 × 50 ml). The extracts were pooled, dried over anhydrous sodium sulphate and evaporated under vacuum to yield 7.08 g (10 mM) of 5. The purified final compounds were fully characterized by ^{1}H NMR spectroscopy and mass spectrometry.

^{1}H NMR (300 MHz, dmso-d_6, 25°c, TMS): δ 7.36 (d, j = 6 Hz, 1H, NH); 7.29-77.19 (m, 30H, CH, aromatic); 6.58 (br, 1H, NH_2, secondary amine); 4.77 (br, 1H, C^{α}H); 4.49-4.43 (m, 1H, C^{α}H); 2.68-2.62 (m, 2H, C^{β}H's); 2.58-2.54 (m, 2H, C^{β}H's); anal. Calcd. For $C_{44}H_{40}N_2O_3S_2$ (708.248): C, 72.54; H, 5.69; N, 3.95. Found: C, 72.41; H, 5.54; N, 3.73.

ESI-MS: Found m/z $(M+Na)^+$ =731.4636; $(2M+Na)^+$ = 1417.142; M_{cald} =708.248, mp : 208 °c

6. H_3N^+-L-Cysteinyl-L-cysteine-COO^-.

S-triphenylmethyl-L-cysteine (1)- S-triphenylmethyl-L-cysteine (2) (1.41 g, 2.0 mM) was dissolved in dry dichloromethane (30 mL) and then triethylsilane (4.77 mg, 4 mM) was added to the solution and stirred for 5 min. Trifluoroacetic acid (TFA) (10 mL) was added to the reaction system and the reaction mixture was stirred for 1 hour. The solution was then concentrated under *vacuum* and diethyl ether was added. The precipitate formed which was then dried and dissolved in minimum volume of methanol and re-precipitated by adding diethyl ether, this process was repeated three times and the material was dried in high vacuum pump. The purified final compounds were fully characterized by ^{1}H NMR spectroscopy, mass spectrometry, FT-IR, ^{13}C NMR and DEPT 135.

Yield: 0.34 g (1.5 mm, 75%); ^{1}H NMR (50 MHz, DMSO-d_6, 25°c, TMS): δ 8.30 (d., 3H, NH_3^+, j =3.42); 6.57 (br, 1H, NH); 4.27 (br, 1H, $C^{\alpha}H$); 4.05-3.98 (m, 1H, $C^{\alpha}H$); 2.97-2.87 (m, 2H, $C^{\beta}H$'s), 2.68 (br, 2H, $C^{\beta}H$'s); 1.52-1.44 (m, 1H each, 2 SH).

^{13}C NMR (75 MHz, DMSO-d_6, 25 °c): δ = 170.73 (C of COO^-), 170.66 (C of CONH), 52.62 (cysteine c^{α}), 51.95 (cysteine c^{α}), 34.38 (cysteine c^{β}), 33.13 (C^{α} cysteine).

DEPT-135 (DMSO-d_6, 25 °c): δ = 52.67, 52.00, 34.42 and 33.17; (disappear) 170.73 and 170.66 and (positive) 52.67 and 52.00; 3(negative) 34.42 and 33.17.

Anal. Calcd. For $C_6H_{12}N_2O_3S$ (224.0289): C, 32.13; H 5.39; N 12.49: found C 32.01; H 5.30; N 12.33. ESI-MS: m/z $(M+H)^+$ =225.13; $(M+Na)^+$= 247.10, $M_{cal.}$= 224.03, $(2M+Na)^+$= 471.215.

FT-IR. Amide A- 3373.61, amide I- 1666.55, amide II- 1595.18 cm^{-1}, -SH- 2592.41 cm^{-1}.

Calculation of Quantum Yield of Gold Clusters.

Actual quantum yields are generally measured relative to an optically dilute standard fluorophore solution that exhibits a well-known quantum yield (Φ_s). The quantum yields of unknown fluorophore (Φu) were determined by using the parker-rees method.[3]

$\Phi_u = (A_s F_u n_u^2 / A_u F_s n_s^2) \Phi_s$

In this expression, au denotes the absorbance of unknown sample at the excitation wavelength, F_u represents the total, integrated fluorescence intensity for the unknown sample when excited at the same excitation wavelength, F_s is the integrated fluorescence intensity of the reference sample when excited at the same excitation wavelength. The refractive indices of the solvents in which the unknown and the standard samples are prepared, are given by N_u and N_s respectively. Here, in our study we have chosen coumarin c-480 in water by sonication as a standard and its quantum yield (Φ_s) is known to be 0.66 in water. The quantum yield of the gold clusters was calculated as 0.412.

Instrumentation.

FT-IR studies.

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

Raman studies.

The confocal micro-Raman studies have been carried out in backscattering geometry using DILOR-XY instrument equipped with a liquid nitrogen-cooled charge coupled device (CCD) detector.

NMR Spectroscopy.

All NMR studies were carried out on a Bruker dpx 300MHz spectrometer at 300 k. Peptide concentrations were in the range 1–10 mg in $CDCl_3$ and $(CD_3)_2SO$.

Mass Spectrometry.

Mass spectra were recorded on a Hewlett Packard series 1100 msd mass spectrometer by positive mode electro spray ionisation.

MALDI-TOF MS analysis.

MALDI-TOF MS analyses have been performed by using Applied Biosystems MALDI TOF/TOF Analyzer.

To probe the size of the gold clusters Au_xL_y, matrix-assisted laser-desorption ionization (MALDI) spectrometric analysis was carried out using this colorless solution of gold clusters in methanol-water solvent and α-cyano-4-hydroxycinnamic acid (CHCA) as a matrix in a negative mode.

Transmission Electron Microscopic Study.

The morphologies of the reported compounds were investigated using transmission electron microscope (TEM). The transmission electron microscopic studies of all the gold clusters solution were done on carbon-coated copper grids (200mesh) by slow evaporation and allowed to dry in vacuum at 27°c for two days. Images were taken at an accelerating voltage of 200 kV. Tem was done by a jem-2010 electron microscope.

UV/Vis Spectroscopy.

UV/Vis absorption spectra were recorded on a Hewlett-Packard (model 8453) UV/Vis spectrophotometer (Varian carry 50.bio).

Fluorescence Study.

The fluorescence spectra were obtained using a Perkin-Elmer Spectrofluorimeter and excitation and emission wavelengths of 300 and 410 nm respectively.

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 DataStation v2.3.

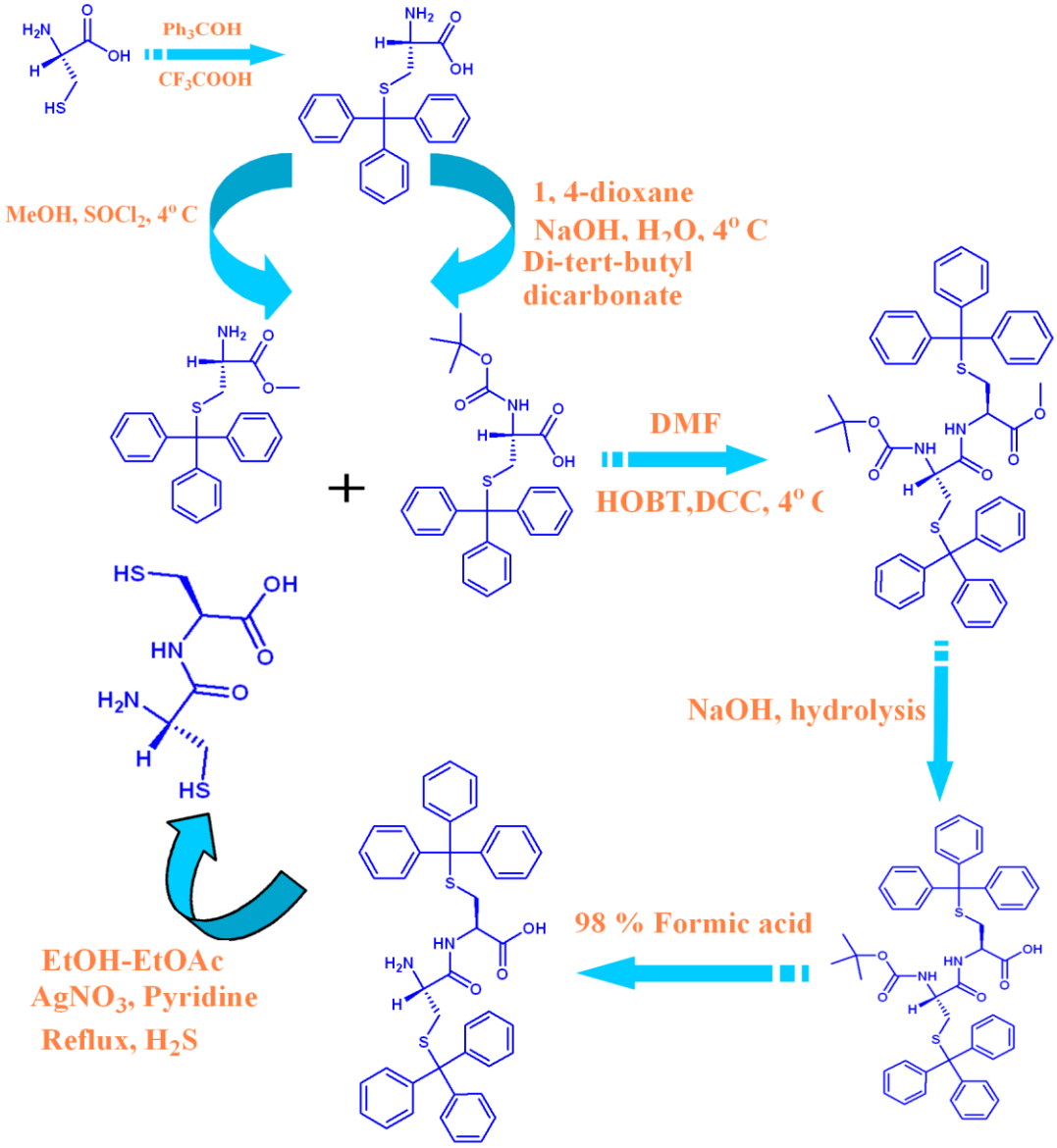
X-ray Diffraction Study.

For X-ray diffraction study of gold clusters, the clusters solution was drop casted on a glass slide, dried and then dried under vacuum for 2 days. Samples are done on a Seifert x-ray diffractometer (C 3000).

Conductivity Mesurements (I-V).

A thin film of gold clusters was prepared on glass substrate using spin coater for electrical conductivity measurements. The film was well dried in vacuum for 3 days. The current-voltage (I-V) analysis were carried out using Ag electrodes in coplanar configuration using a Keithley model 6517A Electrometer at 27° C. The thickness of the film was analyzed using a surface profilometer (STYLUS, Model No. DEKTAK 6M), and it was found to be uniform in thickness.

Schematic representation of peptide synthesis.



Scheme S1. Schemetic representation of the reaction procedure for the synthesis of the dipeptides L-cysteinyl-L-cysteine.

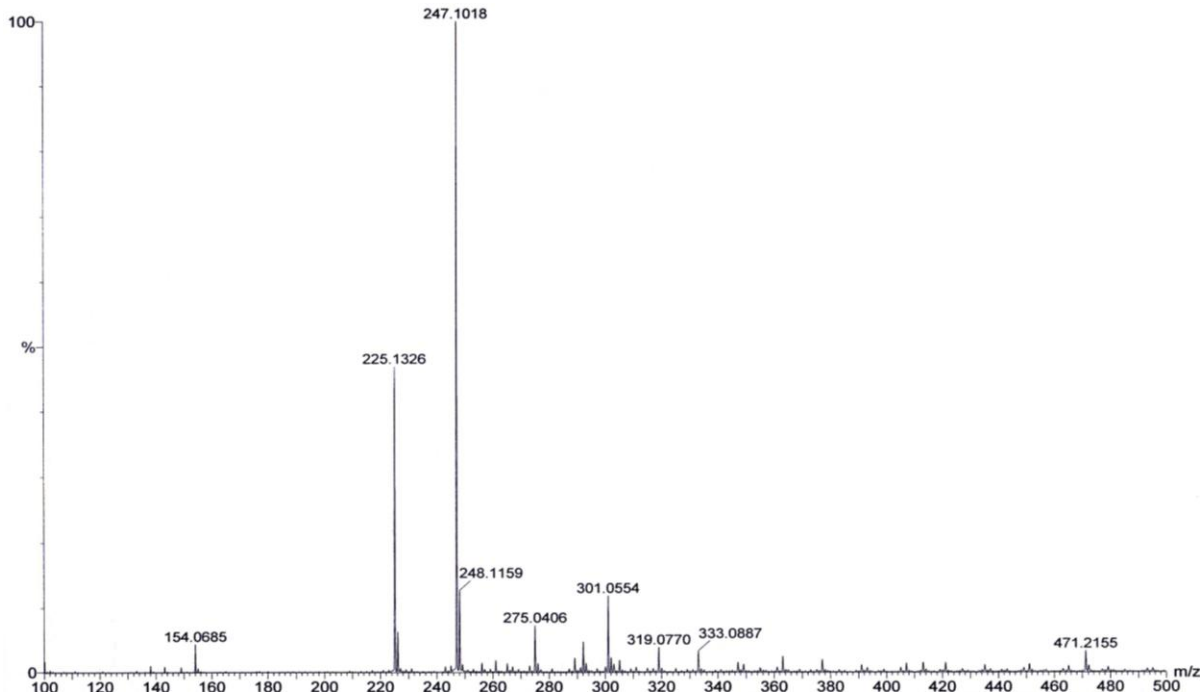


Figure S1 ESI-Mass spectum of L-cysteinyl-L-cysteine.

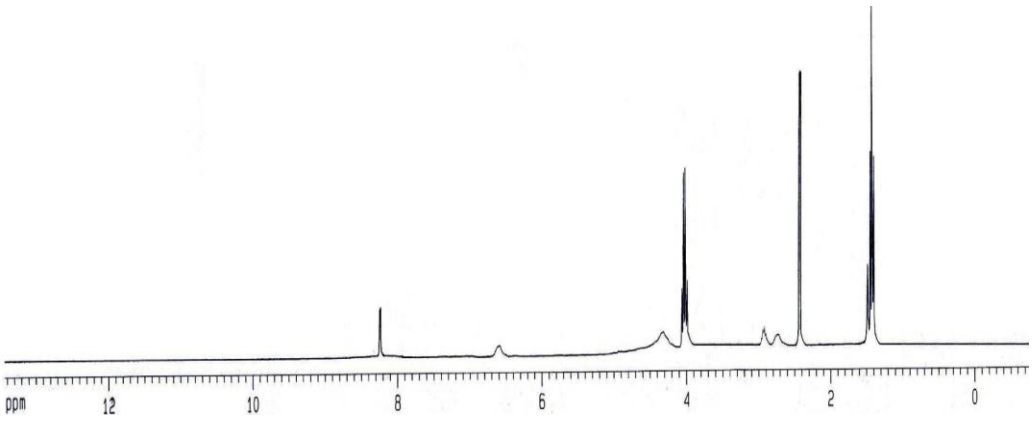


Figure S2 ^{1}H NMR spectum of L-Cysteinyl-L-Cysteine.

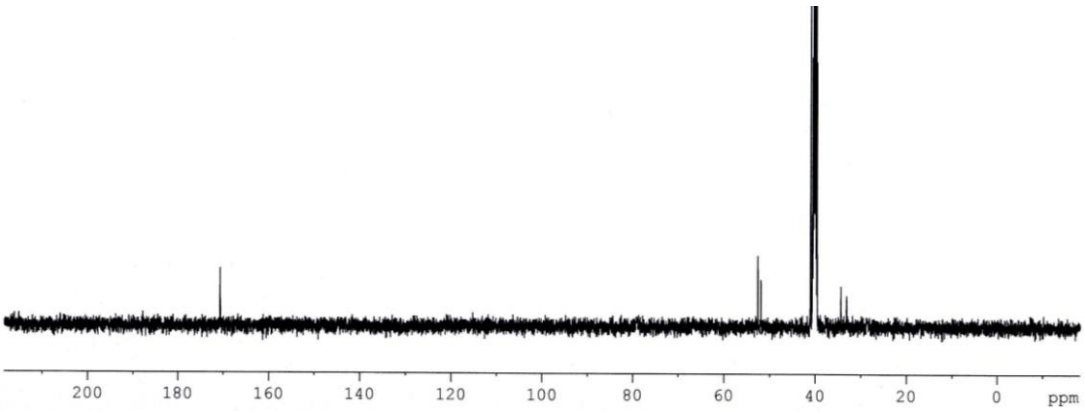


Figure S3 ^{13}C NMR spectrum of L-Cysteinyl-L-Cysteine.

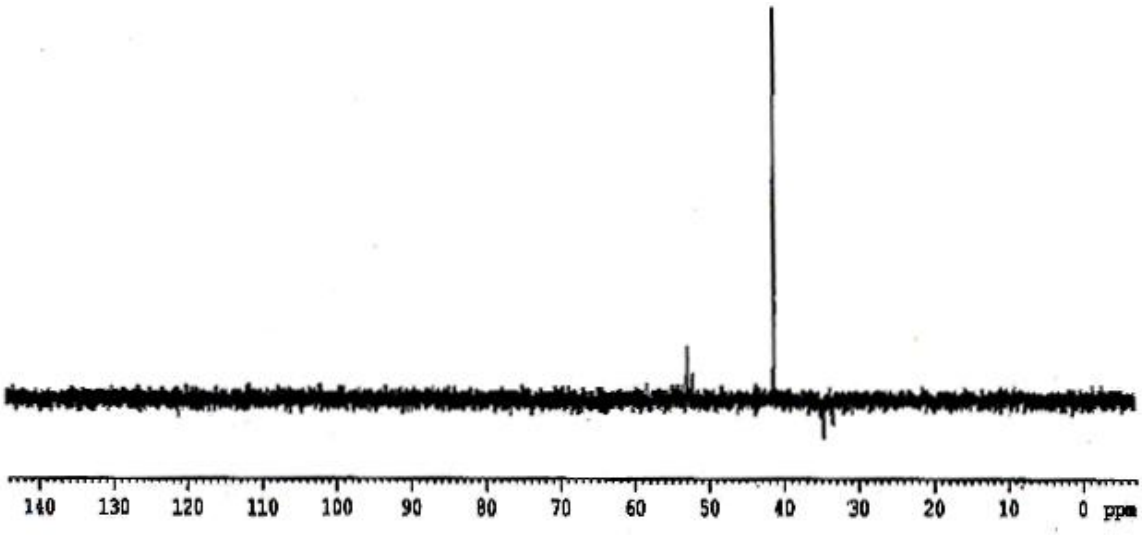


Figure S4 DEPT-135 spectrum of L-Cysteinyl-L-Cysteine.

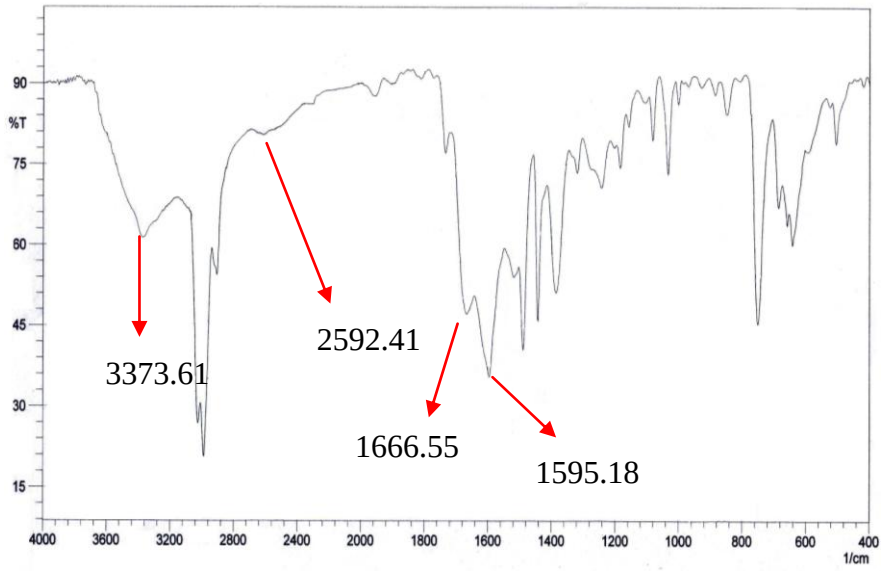


Figure S5 FT-IR spectrum of L-Cysteinyl-L-Cysteine.

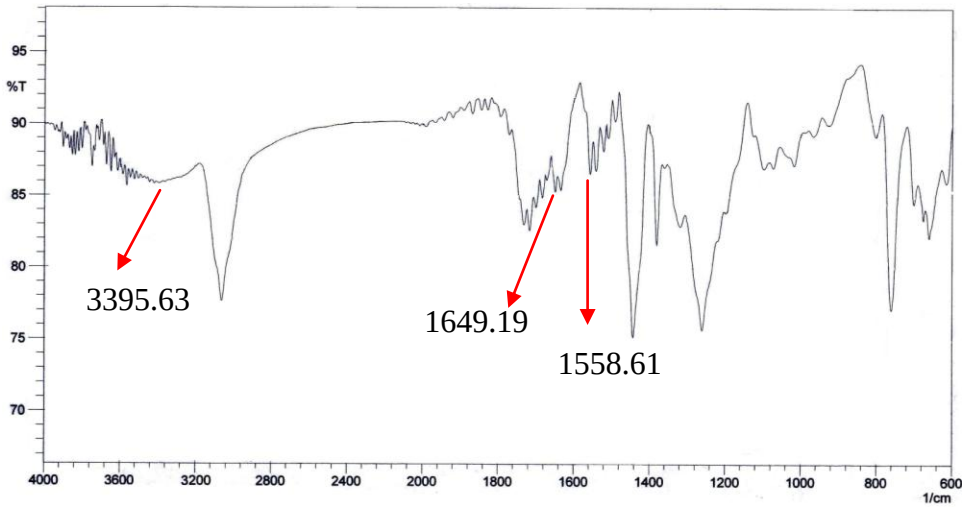


Figure S6 FT-IR spectrum of gold clusters stabilised by L-Cysteinyl-L-Cysteine.

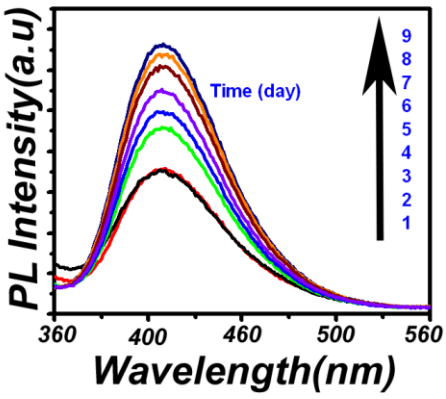


Figure S7 Time dependent PL spectra of the dicysteine capped gold nanoparticles solution, PL excitation at 300 nm.

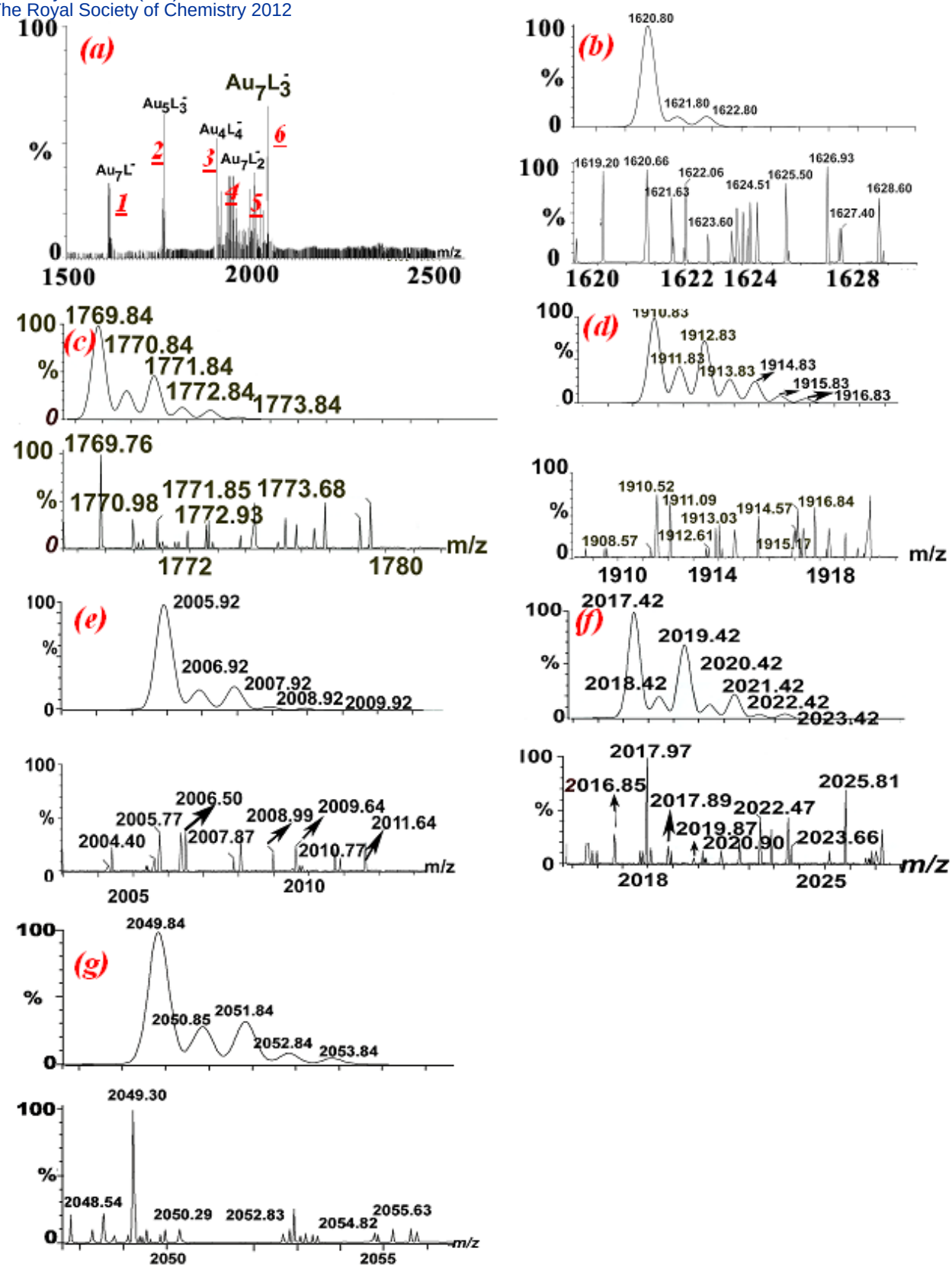


Figure S8 ESI-MS spectra of gold clusters (negative ion mode). (a) Shows the ESI-MS spectrum from m/z 1500 to 2500. (b) Experimental and simulated curve for peak 1. (c)

Experimental and simulated curve for peak 2. (d) Experimental and simulated curve for peak 3. (e) Experimental and simulated curve for peak 4. (f) Experimental and simulated curve for peak 5. (g) Experimental and simulated curve for paek6. In each spectrum from (b) to (g) the upper curve is simulated curve and the lower one is experimental.

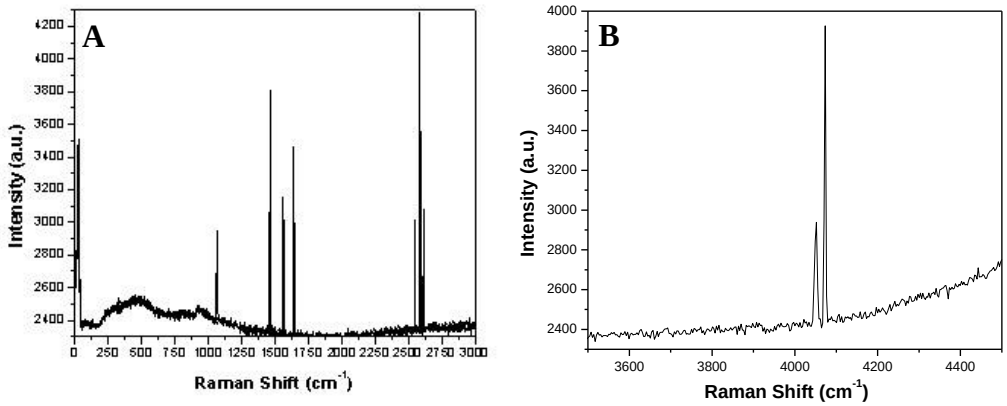


Figure S9 Raman spectra (a) for the dipeptide and (b) for the Au clusters.

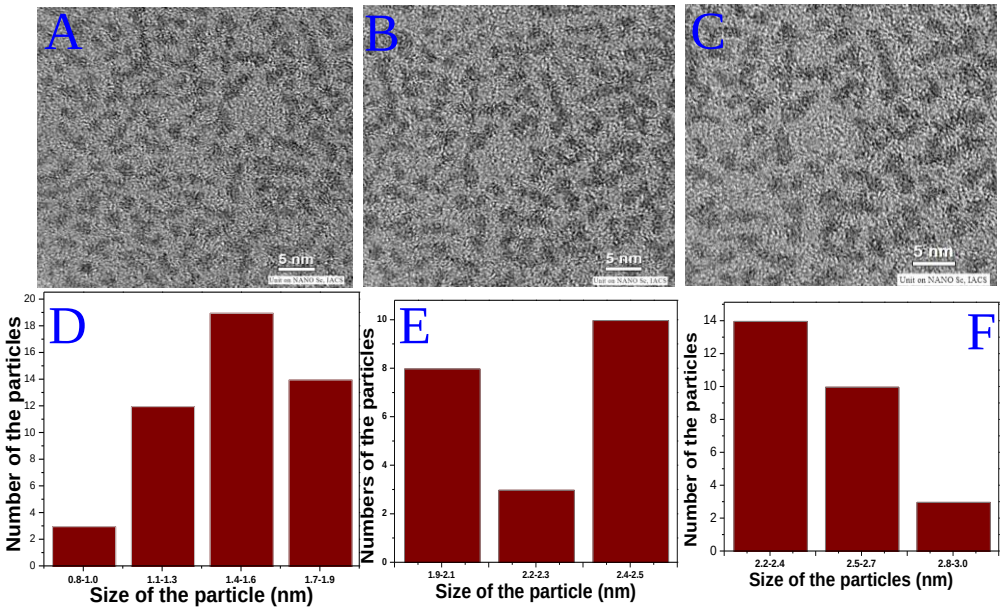


Figure S10 (A), (B) and (C) are the Transmission Electron Microscopic (TEM) images of gold clusters after 5, 10 and 15 min. of exposure under the electron beam respectively

and (D), (E) and (F) are the particles size distribution diagram of these gold clusters after 5, 10 and 15 min. of exposure under the electron beam respectively.

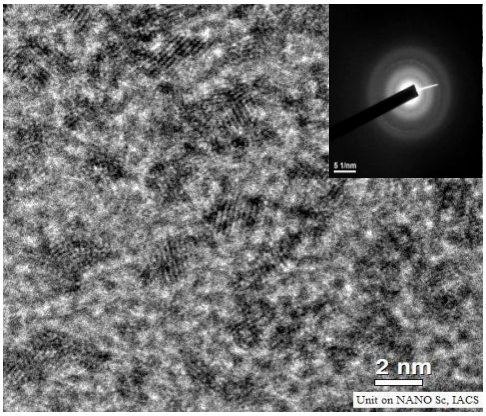


Figure S11 High Resolution-Transmission Electron Microscopic (HR-TEM) imaging of the lattice plane after 20 min. of exposure under the electron beam and inset of the Figure indicates the diffraction pattern of the gold clusters (10:1).

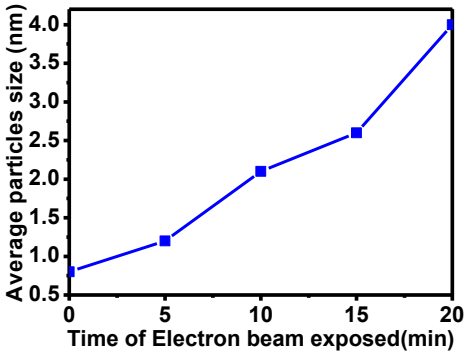


Figure S12 Time dependent average particle size of the gold clusters (10:1) during the exposure under electron beam.

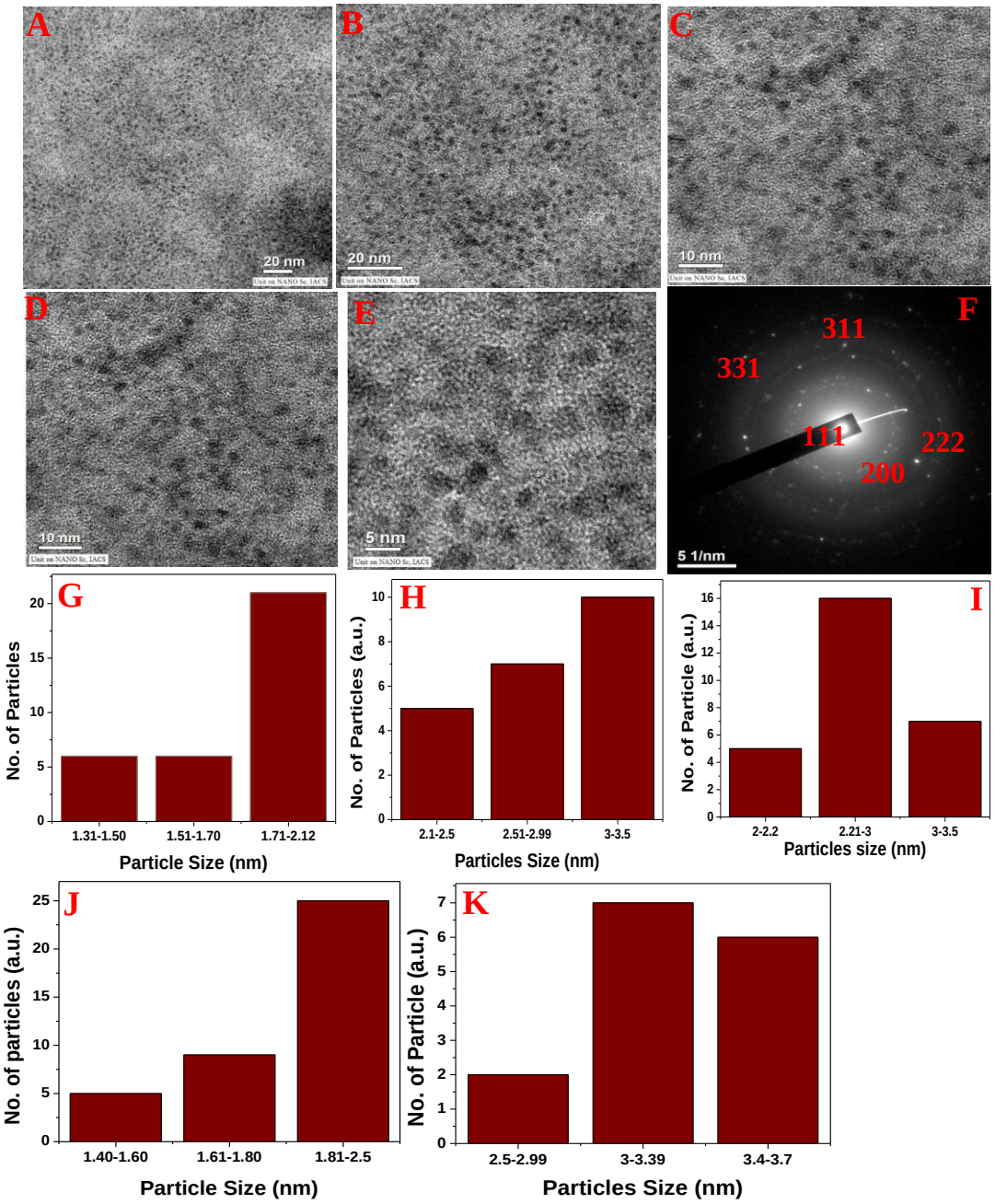


Figure S13 (A), (B), (C), (D) and (E) are the transmission electron microscopy (TEM) images of the gold clusters (5:1) initial, after 5, 10, 15, 20 min. of the exposure under the electron beam respectively and (G), (H), (I), (J) and (K) are the particle size distribution diagram of the gold cluster (5:1) when initial, after 5, 10, 15, 20 min. of the exposure

under the electron beam respectively. Particle size distribution of the gold cluster (5:1) after 20 min. exposure under the electron beam. (F) Represents the electron diffraction pattern of the (5:1) gold clusters.

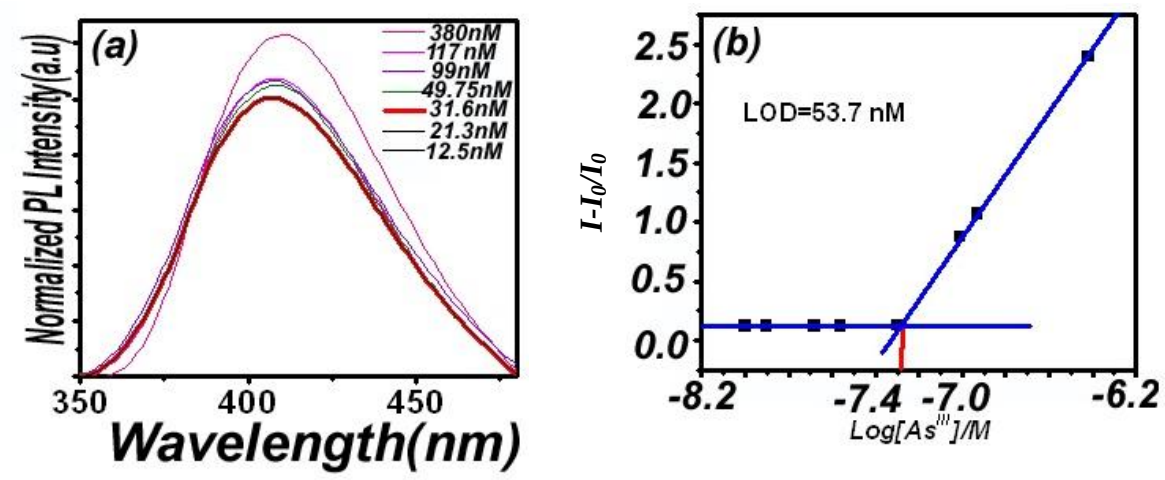


Figure S14 (a) Minimum concentration required for sensing of As^{III} ions by fluorescent gold clusters in water in presence of other interfering metal ions (Ba^{II}, Ca^{II}, Mg^{II}, Mn^{II}, Fe^{II}, Co^{II}, Ni^{II}, Zn^{II}, Sr^{II}, Pb^{II}, Sb^{III}, Bi^{III}, Al^{III}, Cr^{III}, Au^{III}) is 53.7 nM for As^{III} ions. (b) $I-I_0/I_0$ vs Log $[As^{III}]$/M plot where I_0 indicates the initial fluorescence intensity of the gold clusters and I represent the fluorescence intensity after the addition of measured amount of As^{III} ions. LOD represents lower detection limit.

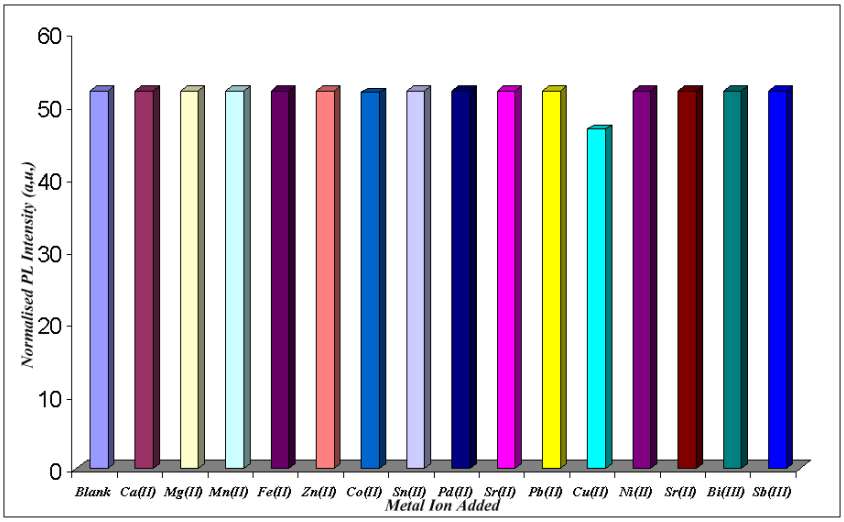


Figure S15 Sensitivity of the di-cysteine capped gold clusters towards the bivalent and trivalent metal ions in water medium.

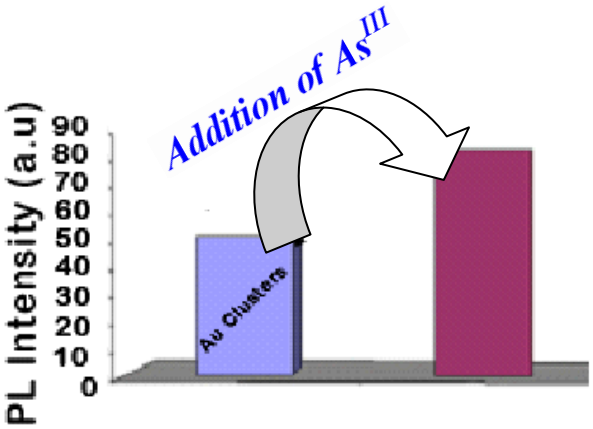


Figure S16 Sensitivity of the di-cysteine capped gold cluster towards As^{III} present in water medium.

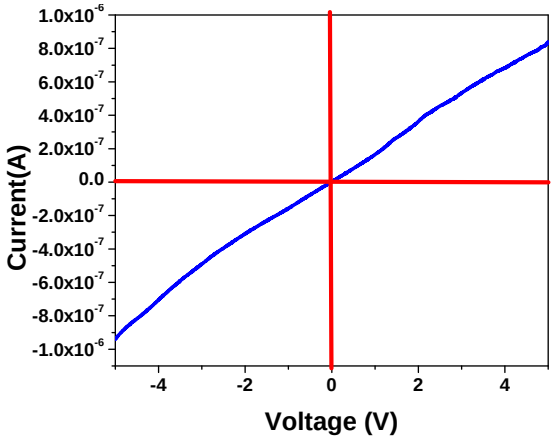


Figure S17. I-V curve of the monodisperse gold clusters with a potential scan rate 5 mv/s.

1. M. Bodanszky and A. Bodanszky, *the practice of peptide synthesis, Springer: Berlin* 1984, 1–282.
2. B. J. Cleynhens, G. M. Bormans, H. P. Vanbilloen, d. V. Vanderghinste, d. M.; Kieffer, T. J. Degroot and A. M. Verbruggen, *Tetrahedron letters,* 2003, **44**, 2597.
3. G. Palui and A. Banerjee, *J. Phys. Chem. B,* 2008, **112**, 10107.