

Supplementary information

Synergistic catalysis by gold nanoparticles and metal ions for enhanced chemiluminescence

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Experimental:

Chemicals and instruments: Gold (III) chloridetrihydrate ($\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$), tri-sodium citrate, luminol, urea-hydrogen peroxide ($\text{U-H}_2\text{O}_2$), sodium chloride and metal salts were procured from Sigma Aldrich Chemicals India, Bangalore. The water used in all the experiments was purified in three stage Millipore-MilliQ Plus purification system. Luminol and urea-hydrogen peroxide were prepared in NaOH solution to maintain the desired alkaline pH for CL reaction. Spectral studies were carried out using spectrophotometer UV-1601 (Shimadzu, Japan). CL readings were measured using PMT based Luminoscan TL plus (Thermo-Electron, Finland) luminometer. Transmission electron microscopy (TEM) analysis was done using Jeol 2100 transmission electron microscope (Jeol Ltd., Tokyo, Japan) at 100 kV excitation voltage. Anionic AuNPs were synthesized according to previously reported method.

Synthesis and Characterization of AuNPs: Citrate-capped gold nanoparticles were synthesized using reported protocol.¹ Briefly, a mixture of 5 mL of 2.5 mM $\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$ and 2 mL 1% trisodium citrate was added to 45 mL of boiling Milli-Q water. All the glass ware used for synthesis was cleaned with aqua regia ($\text{HCl}:\text{HNO}_3$ 3:1) solution and dried in hot air oven. The solution was boiled above 100°C under reflux with vigorous stirring for 20 minutes. Appearance of cherry red colour indicated the formation of AuNPs. Size and concentration of the nanoparticles were

calculated using the absorbance data and found to be 22 nm and 0.59 nM respectively² which were further confirmed by Transmission Electron Microscope (TEM) analysis [Fig.S1]. TEM images revealed spherical AuNPs of approximately 20 nm sizes.

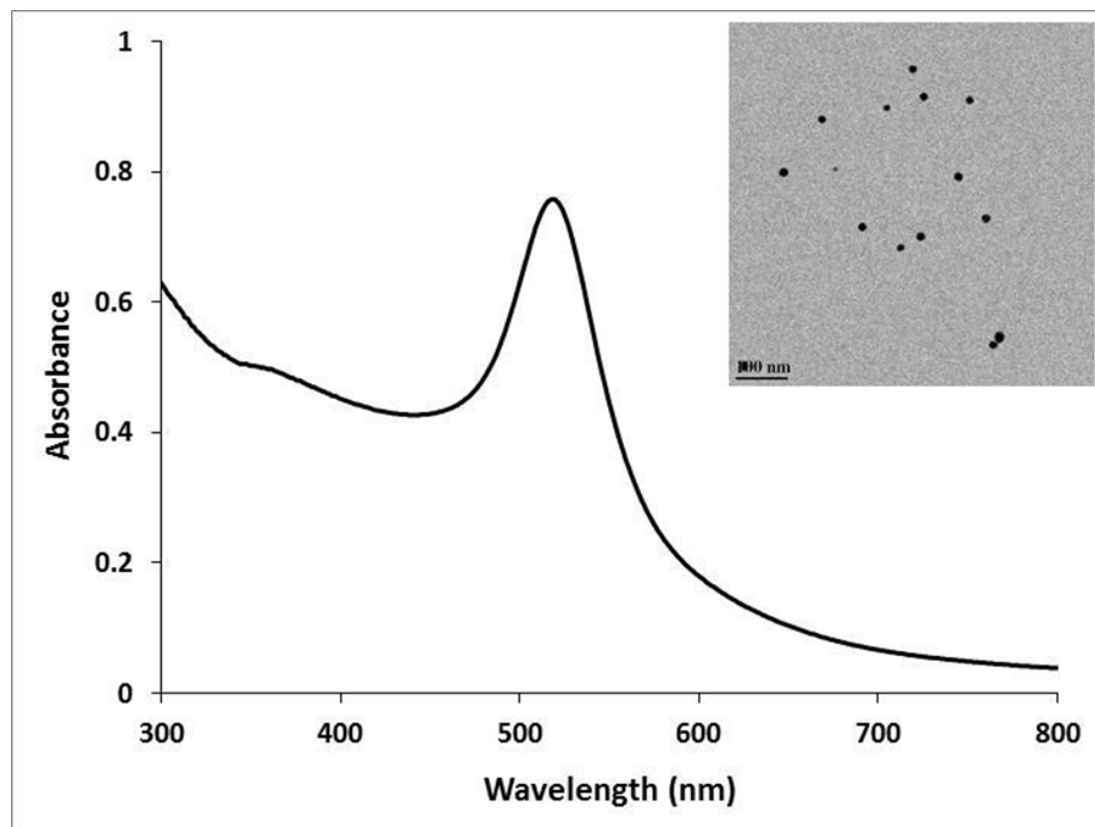


Fig.S1: Characteristic surface plasmon resonance peak of AuNPs at 518nm. Inset: TEM image of AuNPs.

Optimization of parameters for CL enhancement: Reaction parameters of CL like luminol, U- H_2O_2 , AuNPs and pH were optimized to attain uniform and precise results. Gold colloid was diluted to required concentration with Milli-Q water in luminometric cuvettes and CL was measured. To check the effect of metal ions as well as aggregated NPs on CL, we optimized the

concentration of NaCl required for aggregation of AuNPs. The optimal concentration was used for other salts.

Optimization of AuNPs volume for chemiluminescence enhancement: Different volumes of AuNPs (10, 20, 30, 40 and 50 μL) were added to optimized concentrations of alkaline solution of luminol and $\text{U-H}_2\text{O}_2$ (0.15 mM and 0.5 mM respectively) to get a total volume of 150 μL . Since no saturation in CL increase was seen till 50 μL and higher volumes of AuNP are not suited for practical purposes, this amount was chosen for CL studies [Fig.S2]. The final concentration of AuNP for this volume was found to be 0.2 nM, and this was maintained for the CL assay.

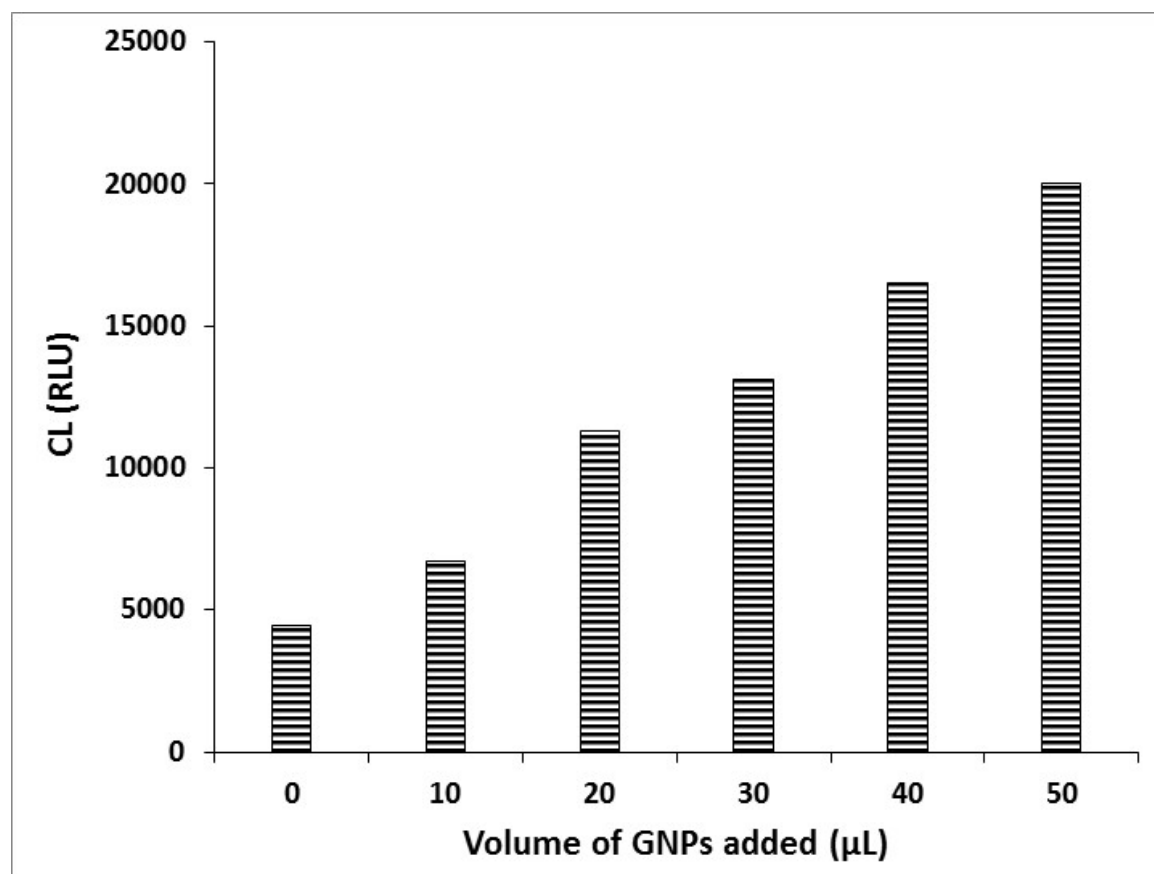


Fig.S2: Optimization of AuNPs volume for CL enhancement.

Optimization of NaCl for aggregation of AuNPs: AuNPs (300 μ L) were mixed with common salt solutions (50 μ L) of different concentrations (ranging from 0.1 M NaCl to 5 M NaCl) in aqueous medium. The mixture was incubated for 15 minutes, after which the UV-Vis spectrum was recorded. NaCl concentration of 0.5 M was enough to aggregate the nanoparticles as seen by the spectrum and the A_{640}/A_{520} (Absorbance at 640 nm and 520 nm respectively) ratio plot against NaCl concentration [Fig S3].

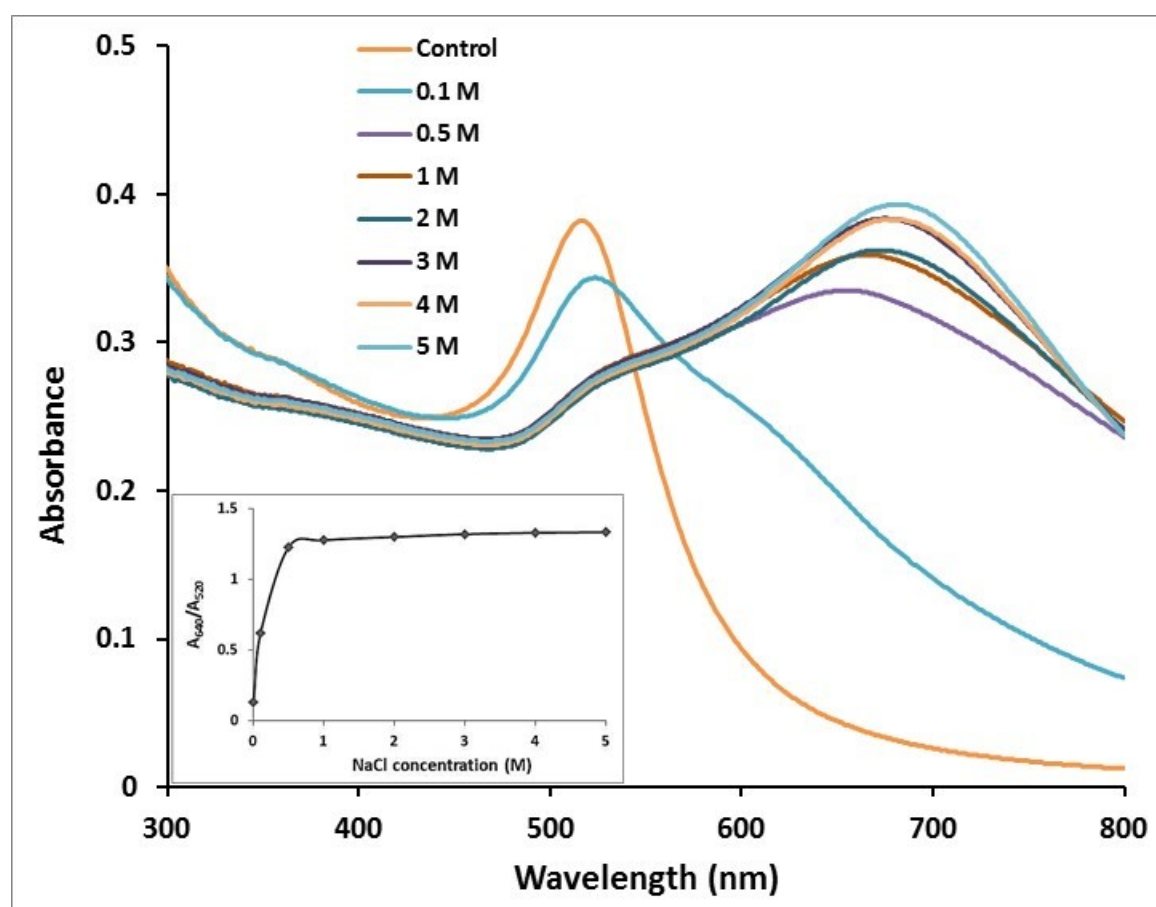


Fig.S3: Aggregation pattern of AuNPs to various concentrations of NaCl. Inset: A_{640}/A_{520} ratio of AuNPs to different concentrations of NaCl.

Maintenance of alkaline pH of the reaction mixtures: The pH of the reaction mixture is maintained at optimal (for CL reaction) levels by addition of alkaline luminol solution. The neutral salts used in the study cannot alter the pH value. However to confirm this fact, we measured the pH of the solutions after adding all the reaction components. The pH data is shown in Table S1. All the reaction mixtures showed pH values within the range 12 to 14, which is optimum and used for luminol-U-H₂O₂ CL. The pH of mixtures containing ammonium salts was adjusted to 11. The chemiluminescence was repeated for all the salt solutions (pH maintained) and the results obtained were nearly the same as the earlier reported data. A minor increase was observed for ammonium salts (data incorporated in Fig 3, main article)

Table S1: pH values after addition of all reaction components.

Anion/Cation	Concentration	pH
Control (Luminol + UH ₂ O ₂)		12
Control (AuNPs + Luminol + UH ₂ O ₂)		12
NaCl	0.5M	12
KCl	0.5M	12
Li ₂ SO ₄	0.5M	12
(NH ₄) ₂ SO ₄	0.5M	9
NH ₄ COOCH ₃	0.5M	11
NH ₄ Cl	0.5M	11
NaOH	0.5M	12
KCl	0.5M	12
H ₂ SO ₄	0.5M	1-2
HCl	0.5M	1-2
CH ₃ COOH	0.5M	5-6
H ₂ SO ₄	1mM	12

HCl	1mM	12
CH ₃ COOH	1mM	12

Effect of different salts on CL enhancement: To check the effect of increasing NaCl concentration on CL, optimized values of AuNPs, salt, luminol and U-H₂O₂ were added in sequence in cuvettes and signals were noted immediately after the addition of U-H₂O₂ (Refer results and discussion). When the system was used to measure the effect of ions, different salts and alkalis (Li₂SO₄, NaCl, NaOH, KCl, KOH, NH₄Cl, (NH₄)₂SO₄, NH₄COOCH₃) were added to the gold colloid in the experimental set, whereas in the control set only salts (without AuNPs) were added (**Refer SI**). To study sole effect of anions, hydrochloric, sulphuric and acetic acid were added to the reaction mixture. Results are shown in Fig S4.

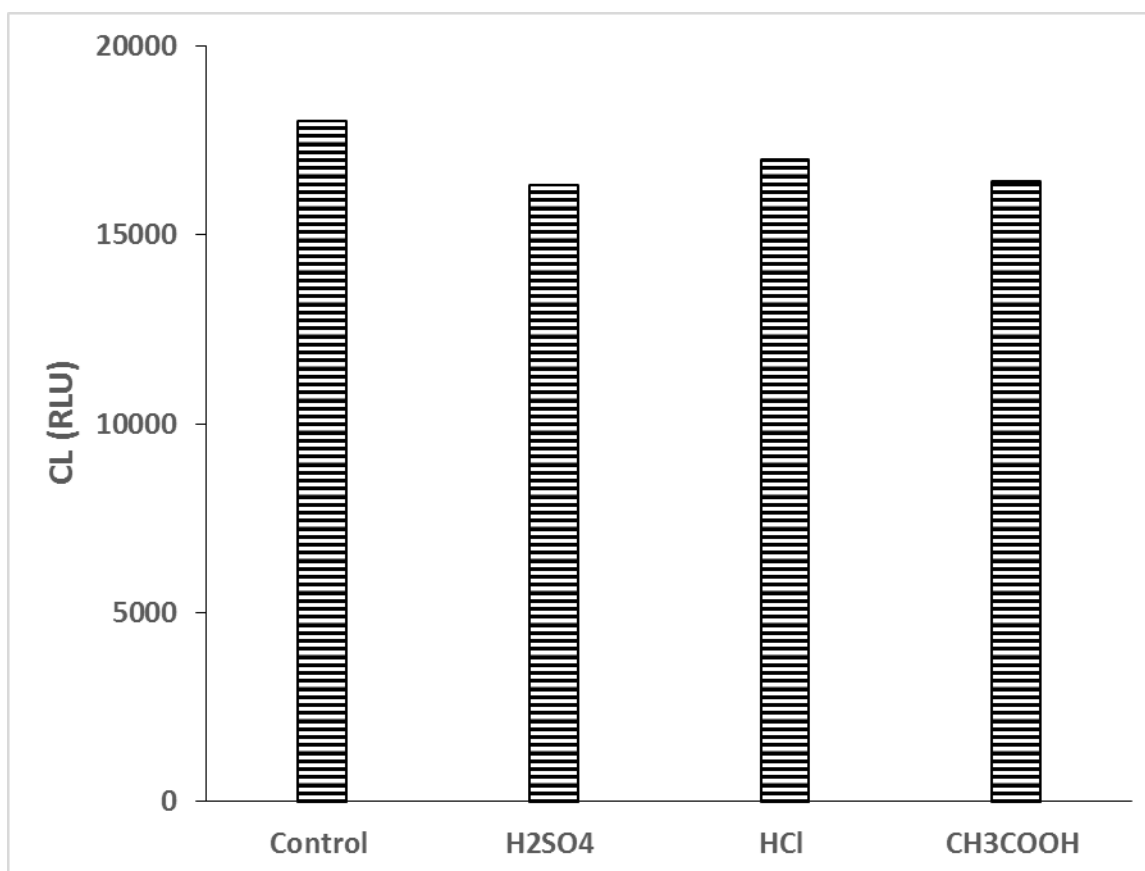


Fig.S4: Effect of anions (1mM) on AuNPs enhanced luminol and U-H₂O₂ system.

Effect of different concentrations of NaCl on AuNPs-enhanced CL: Ratios of AuNPs and NaCl, which were obtained in aggregation assay, were maintained here. From the aggregated AuNP-salt mixture, 100 µL was drawn, and luminol and U-H₂O₂ was added to it. The CL was recorded in a luminometer (Fig 1, main article).

Chemiluminescence assay: For the CL assay, 50 µL of 0.5 M salt was added into 300 µL of AuNPs. The mixture was allowed to stand for 15 minutes for aggregation. From the total volume of 350 µL, three aliquots each of 100 µL were drawn for CL assay. To each AuNP-salt aliquot in luminometric cuvette, luminol (100 µL) and U-H₂O₂ (50 µL) was added so as to maintain final optimized concentration for all the components. In the samples without AuNPs, the volume was made up with water. Control samples did not contain salts. The CL signals from the final solution of volume 250 µL was obtained from the luminometer and recorded (Fig 2 and 3, main article). The initial reading was taken without addition of U-H₂O₂, to observe the baseline, which was almost constant for all samples. A dead time of 5 seconds was kept constant for all measurements.

References:

- (1) J. Turkevich, P. C. Stevenson and J. Hillier. A study of the nucleation and growth processes in the synthesis of colloidal gold. *Discuss. Faraday Soc.* **1951**, 11, 55-75.
- (2) W. Haiss, N. T. K. Thanh, J. Aveyard and D. G. Fernig. Determination of size and concentration of gold nanoparticles from UV-Vis spectra. *Anal. Chem.* **2007**, 79, 4215-4221.