Electronic Supplementary Material (ESI) for Photochemical & Photobiological Sciences.

S.1. Materials

S.1.1 Chemicals

Tetrachloroauric acid trihydrate ($HAuCl_4.3H_2O$), trisodium citrate, silver nitrate ($AgNO_3$), D-luciferin, adenosine 5′-triphosphate magnesium salt (ATP), dodecanal, Bovine serum albumin, lysozyme, Firefly luciferase from *Photinus pyralis* and EDTA were obtained from Sigma-Aldrich, St. Louis, USA. Acetic acid, ethylene glycol, tris(hydroxymethyl) aminomethane, ascorbic acid, flavin mononucleotide (FMN) and magnesium sulphate were from Sisco Research Laboratories, Mumbai, India. Sodium chloride (NaCl), glycerol, ammonium chloride, dipotassium hydrogen phosphate, potassium dihydrogen phosphate, cetyltrimethylammonium bromide (CTAB), ferrous sulphate and hydrochloric acid (HCl) were the products of Ranbaxy Fine Chemicals Limited, New Delhi, India. Sodium alginate, Luria Bertani (LB) broth, yeast extract, and tryptone were procured from Himedia Laboratories, Mumbai, India. Calcium carbonate, sodium chloride, calcium chloride, magnesium sulphate, ammonium chloride, potassium dihydrogen phosphate, ethanol, ferrous sulphate and potassium chloride were purchased from Qualigens Fine Chemicals, Mumbai, India. Sodium dithionite was procured from Loba Chemie, Mumbai, India. All the reagents used in the experimentation were of analytical grade and extra pure. The water used in all the experiments was purified in three stage Millipore- Milli Q plus purification system. Glasswares used in the present work were properly washed with aqua regia, rinsed many times with distilled water and dried.

S.1.2 Instruments

Spectral analysis of Au-Ag colloids was done in the range from 300 to 800 nm using spectrophotometer UV-1601 (Shimadzu, Japan). Particle size characterization of

synthesized nanoparticles (NPs) was carried out using Transmission Electron Microscopy (Jeol 2100, USA). Luminescence emission from luciferase enzyme activity and whole cells was performed using luminometer (Luminoskan TL Plus, generation II, type 1254 built by Thermo Labsystems, Finland). Centrifugation was done using REMI centrifuge (REMI Instruments, India), Incubator shaker (Ecotron, Infors HT, France) was used for growing bacterial cells.

S.2. Experimental section

S. 2.1 Synthesis and characterization of gold silver alloys

Spherical gold nanoparticles were synthesized according to the method described by Xia et al [18], modified to our requirements. In brief, 5mL of $HAuCl_4$ aqueous solution (0.1% wt.) and a known amount of $AgNO_3$ solution (0.1% wt.) added to a given amount of citrate aqueous solution (1% wt.).The volume was made up to 10 mL and allowed to incubate for 10 minutes. The reaction mixture was then added to 40 mL of boiling water under reflux with constant stirring. Immediately the reaction mixture turns to black and then orange to ruby red colour within 3 minutes. The reaction was allowed to proceed for 30 minutes to form uniform spherical nanoparticles. The cooled NPs were filtered through 1µm filter to remove any aggregates and stored at 4˚ C till further use.

Different concentrations of $AgNO_3$ ($5.9x10^{-6}$ to $5.9x10^{-5}$M) was added to the reaction mixture to study the effect of silver nitrate. Trisodium citrate concentration was kept constant for the study ($6.8x10^{-3}$M). The colloids formed were visibly different in color i.e. from ruby red to dirty green. Absorption spectra for all the samples were taken (Figure S2).

The UV–vis absorbance spectra of Au-Ag colloid were acquired at room temperature. TEM studies were carried at 100 kV accelerating voltage. Samples were prepared by depositing a drop of diluted colloidal nanoparticle solution on carbon grid and dried in vacuum (Figure S3).

S.2.2 Effect of tri-sodium citrate concentration nanoparticle size

Keeping silver nitrate concentration constant at 0.1% wt, we varied citrate concentration (10-100mM) while synthesizing Au-Ag colloids. The resulting colloids were characterized by UV-Vis spectrophotometry and TEM (Figure S4).

S.2.3 Studies on reaction time for nanoparticle growth

After the addition of reaction mixture containing 5 mL of (0.1% wt) $HAuCl_4$ aqueous solution, 40 µL $AgNO_3$ (0.1% wt) and 2 mL Trisodium citrate (1% wt) to 45 mL of boiling water, the samples were collected periodically and spectral readings of the samples were taken (Figure S1).

S.2.4 Effect of pH on the stability of the nanoparticles

Au-Ag alloys were synthesized in varying pH conditions to probe the influence of hydrogen ion concentration on nanoparticles growth. The molar ratio of the reaction mixture was kept constant for all the reactions. Only the pH of the reaction mixtures was adjusted to 3.0, 5.0, 7.0 and 9.0 prior to the experiment. The experimental conditions remained the same as mentioned in previous sections. The spectral variation was recorded (Figure S5).

S.2.5 Bioluminescence using luciferase enzymes

For luciferase enzyme extraction from *P. leiognathi*, 10 mL cell suspension was pelleted and further incubated for 1 hour at 25 ºC in 1 mL of (100 µg/mL) lysozyme (4,000 U) in 100 mM of phosphate buffer, pH 7.4 and 10 mM EDTA. Thereafter, the cell suspension was frozen at -20 ºC for 1 hour and thawed at 25 ºC. This cycle was repeated two times. Thereafter, the cell homogenate was centrifuged at 4 ºC at 12,000 rpm for 5 min. The supernatant containing crude enzyme was collected in a separate vial and kept in 100 mL aliquots at -20 ºC till further use. The assay buffer for carrying out luminescence assay was prepared using 100 mM of phosphate buffer, pH 7.4, 0.2 % BSA, 0.5 mM dithiothreitol and 5 mM EDTA.

Commercial firefly luciferase enzyme from *P. pyralis* (10-40 x 10^4 LU) was obtained and used for the luminescence assay. The assay buffer used in the firefly luciferase assay included 100 mM tris acetate buffer, pH 7.8, 0.1 % BSA, 30.0 mM $MgSO_4$, 2.0 mM EDTA and 0.5 mM DTT.

Supplementary figures

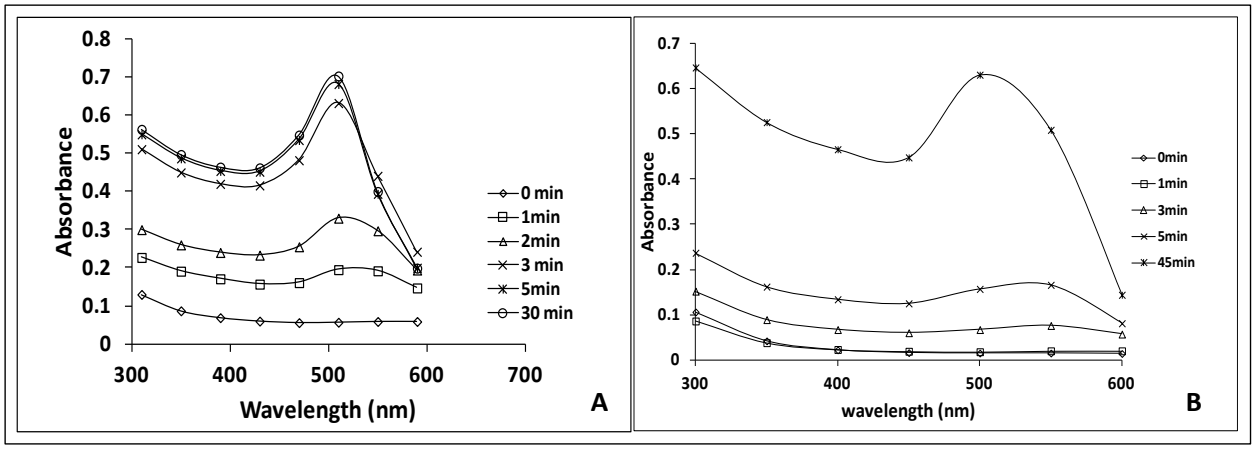

Figure S1: Effect of reaction time on nanoparticle growth. There is little increase in SPR peak of the Au-Ag alloy after 5 minutes (1A) showing almost complete growth and formation in that time in comparison time taken in Turkevich method was 45 minutes (1B).

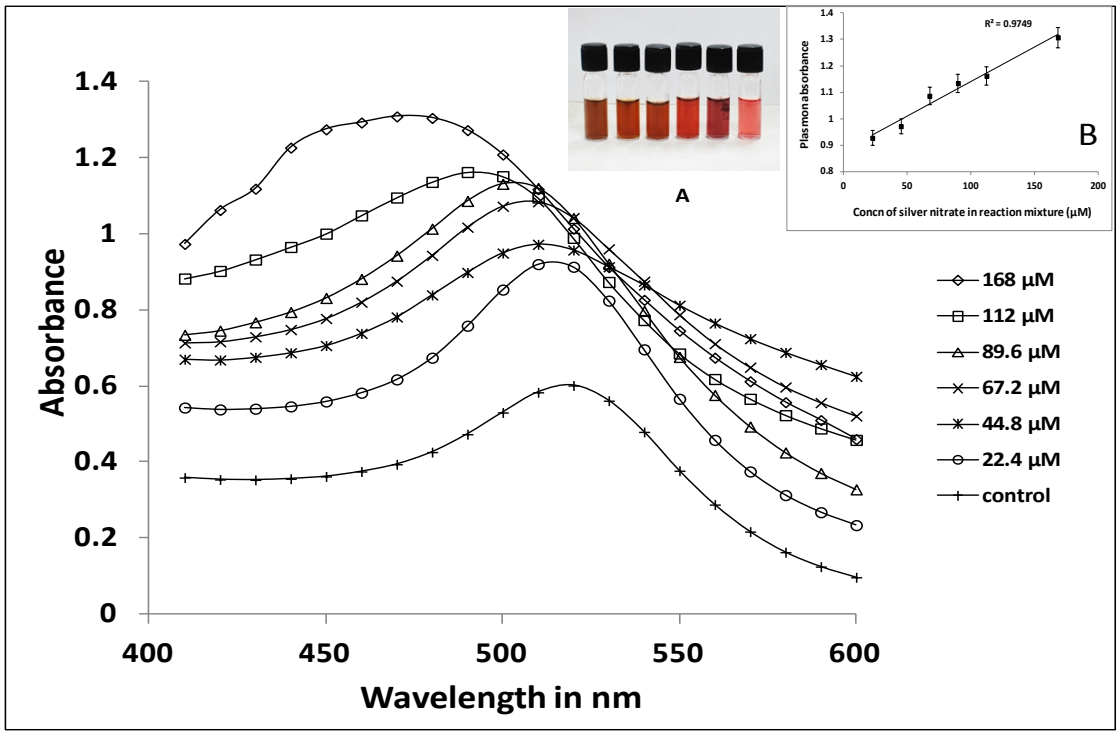

Figure S2: Effect of different silver nitrate concentrations on the formation and characteristics of the synthesized alloy nanoparticles. The SPR wavelength shows blue shift with increase in silver nitrate concentration. Inset figure B shows the variation in the plasmon absorbance with silver nitrate concentration. Inset figure A shows the visual colour differences in the prepared colloids substantiating their absorption profiles.

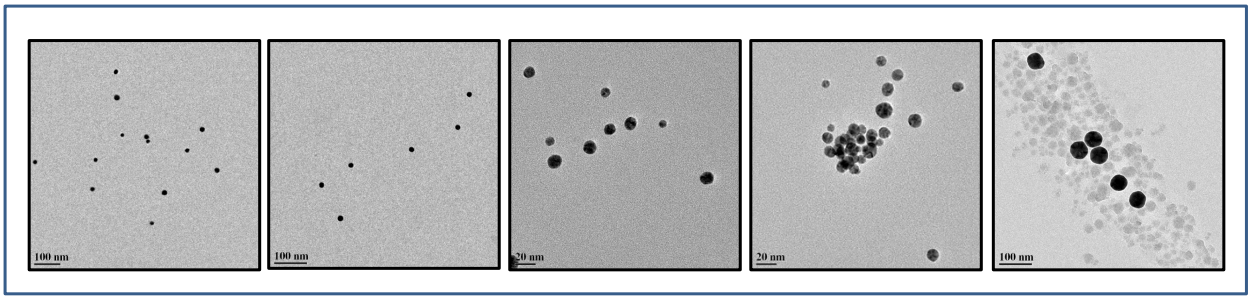

Figure S3: The variation in size of the nanoparticles (as seen in TEM) with changing tri-sodium citrate concentration. Sizes are ca. 10 nm, 15 nm, 20 nm, 25 nm and 50 nm. Increase in citrate concentration resulted in decreasing nanoparticle size.

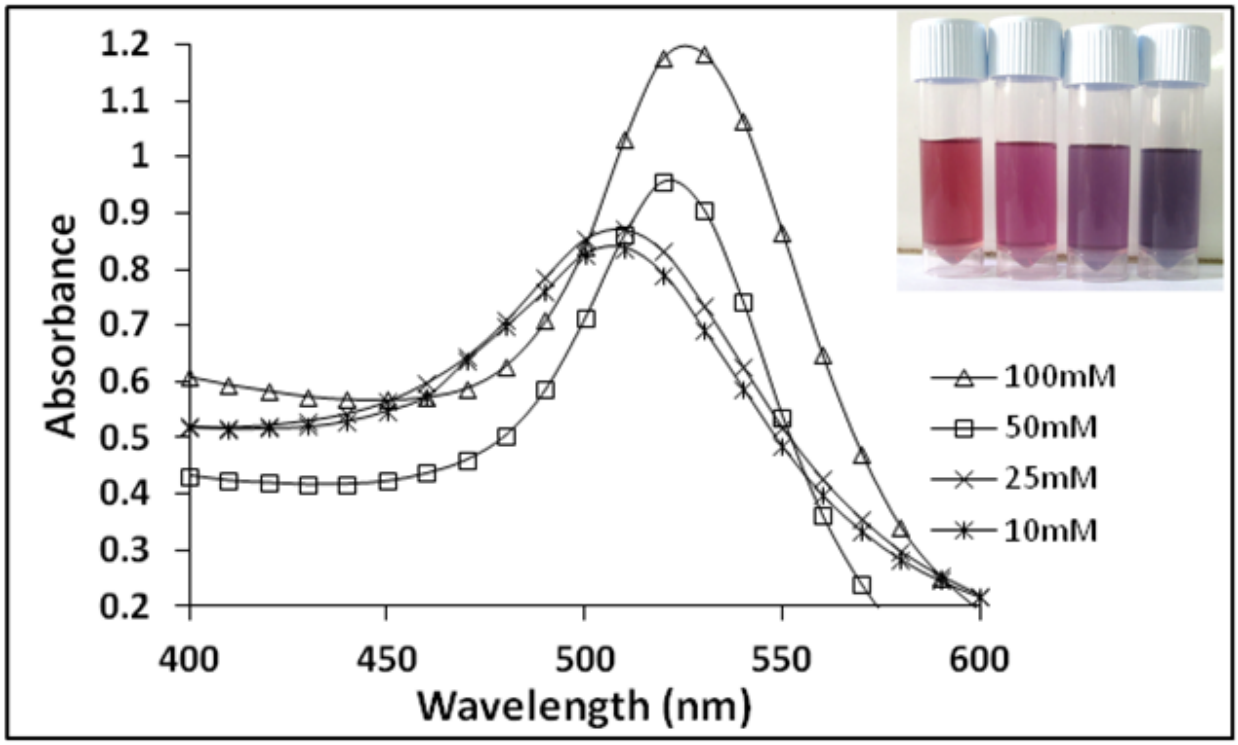

Figure S4. Variation in the plasmon characteristics of the colloids with change in concentration of Trisodium citrate

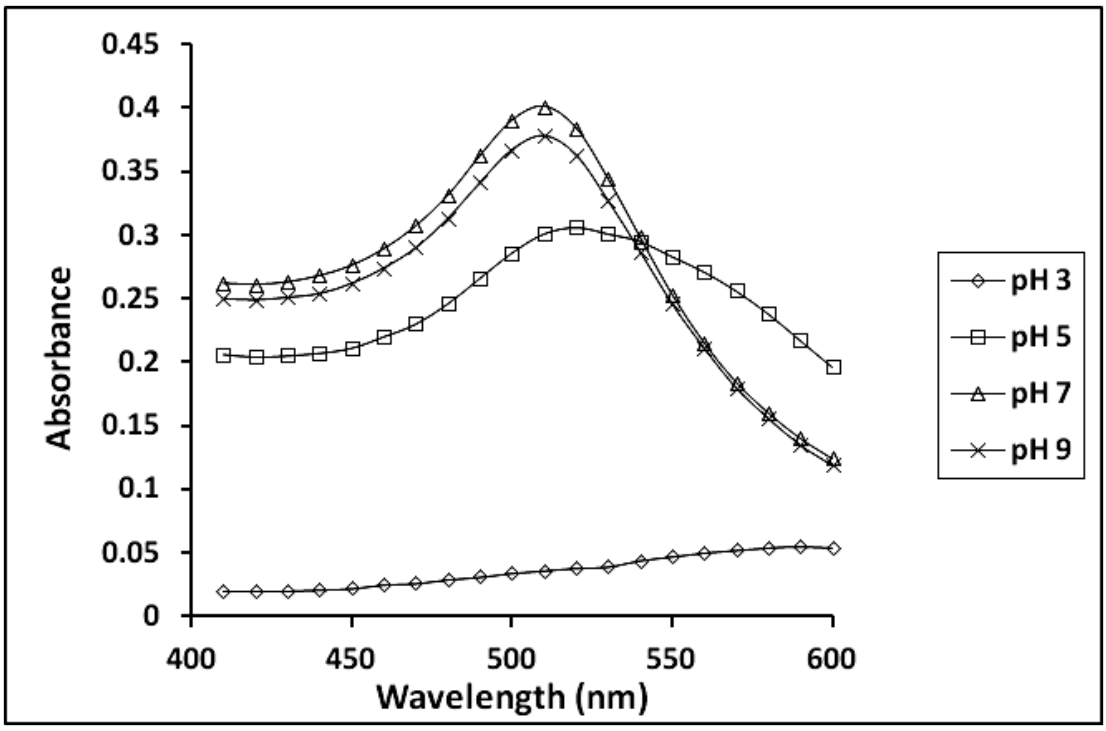

Figure S5: The effect of reaction pH on the stability of the nanoparticles.