Supporting Information

Quick and simple estimation of bacteria using a fluorescent Paracetamol Dimer-Au nanoparticle composite

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MATERALS AND METHODS

Fluorescence study of composite in presence of different contaminants

The HeLa cells (cervical cancer cell line) were grown in the Dulbecco's modified Eagle's medium supplemented with L-glutamine (4 mM), penicillin (50 units mL^{-1}), streptomycin (50 mg mL^{-1}), and 10% (v/v) fetal bovine serum in 5% CO_2 humidified incubator at 37 ^{0}C. To collect the cells, the medium was removed, washed with PBS, trypsinized, and pelleted down by centrifugation. The cell fixation was done in glutaraldehyde/paraformaldehyde solution. The fixed cells were dehydrated in graded ethanol solutions and finally resuspended in absolute ethanol after counting them by Haemocytometer.

The plant cells obtained from callus culture of Mung bean (*Vigna radiata*) was a kind gift from Dr. Lingraj Sahoo. The sand used was obtained from banks of Brahmaputra. The fluorescence study of composite in presence of sand was done by correlating the number of bacteria with weight of sand. The wet weight of single *E.coli* bacteria is ~1×10^{-15} kg. Thus, 1.0 µg/ml of sand corresponds to10^6 CFU/mL.

RESULTS

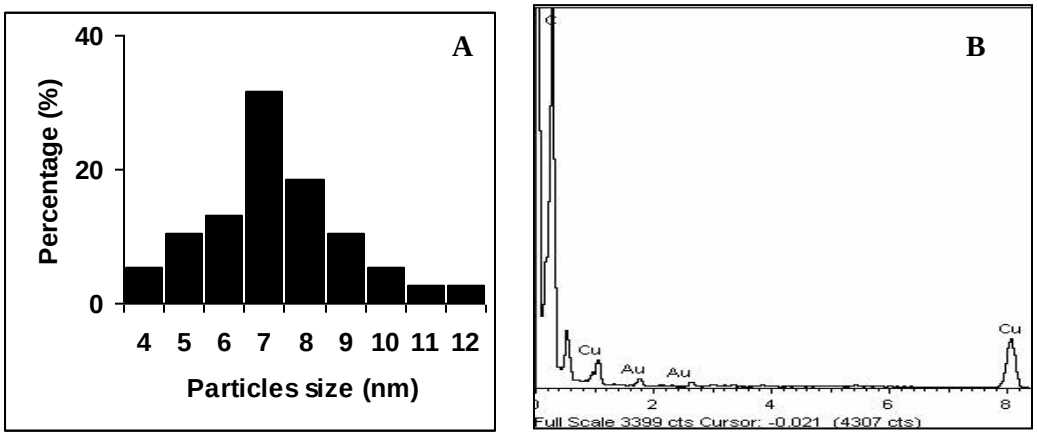

Figure S1: (A) Particle size distribution of Au NPs. The average particle size was 7 ± 0.5 nm. (B) EDX spectrum of single NP.

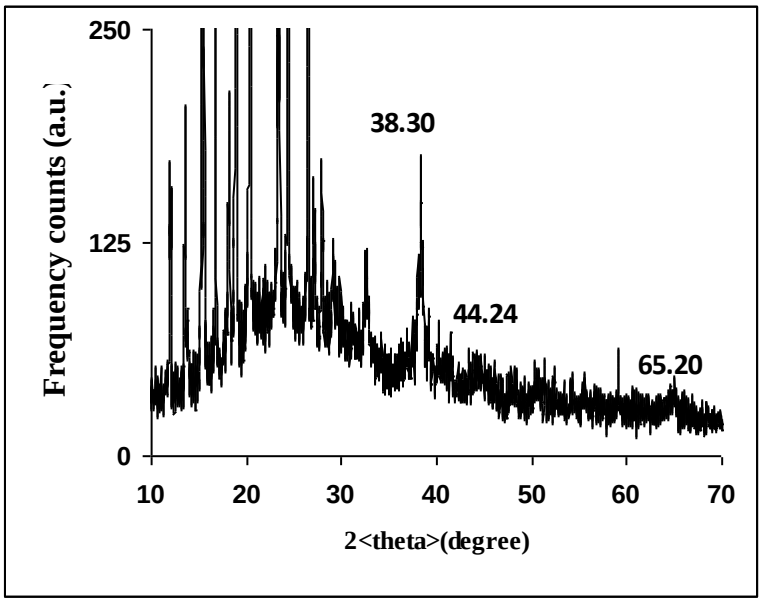

Figure S2: Powder X-ray diffraction pattern of the composite.

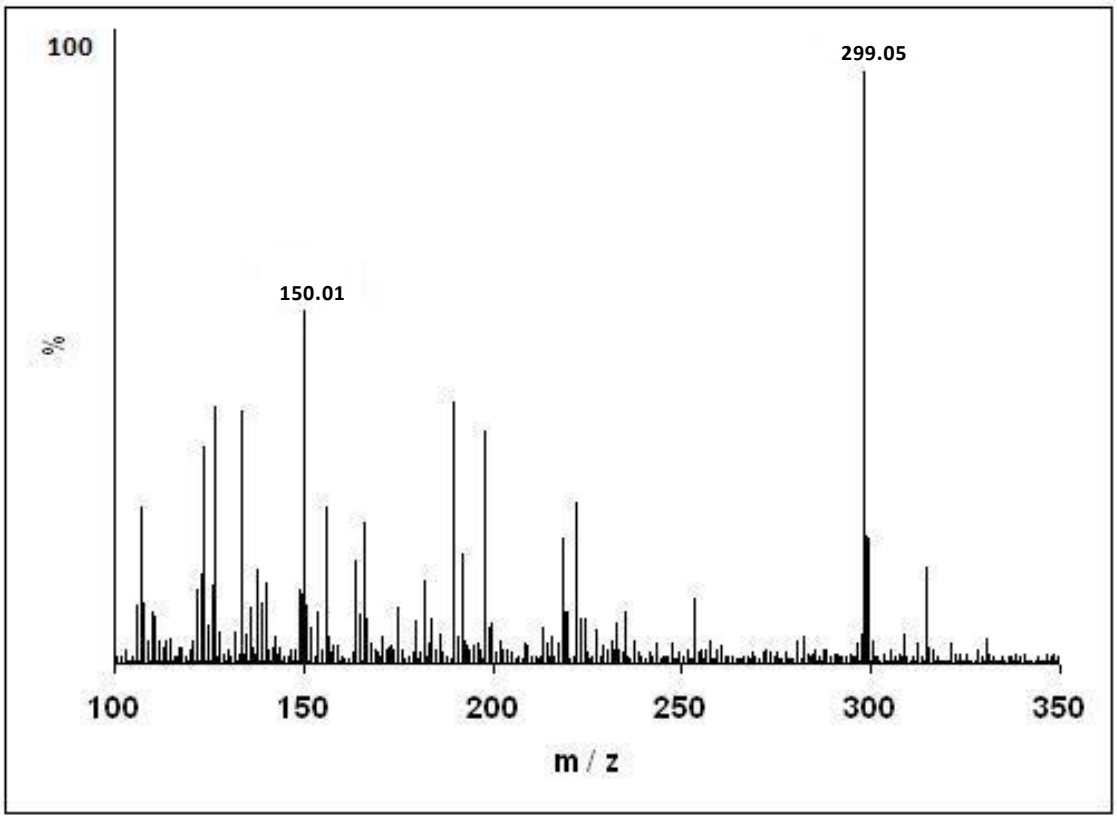

Figure S3: ESI (-) Mass Spectra of the composite.

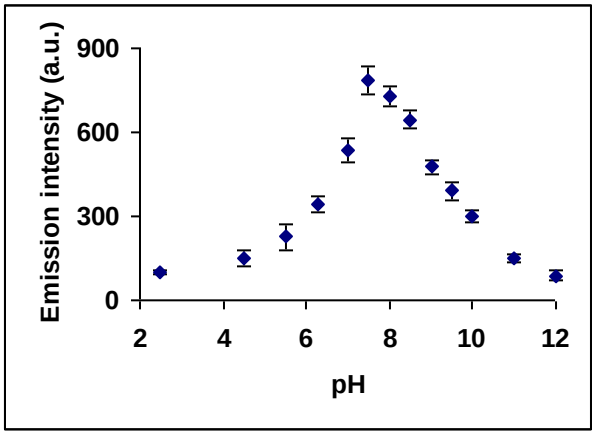

Figure S4: Effects of pH on the emission intensity of the composite at 435 nm when excited at 320 nm. The optimal pH of maximum fluorescence was 7.5. The data represents mean ± S.D. of three experimental results.

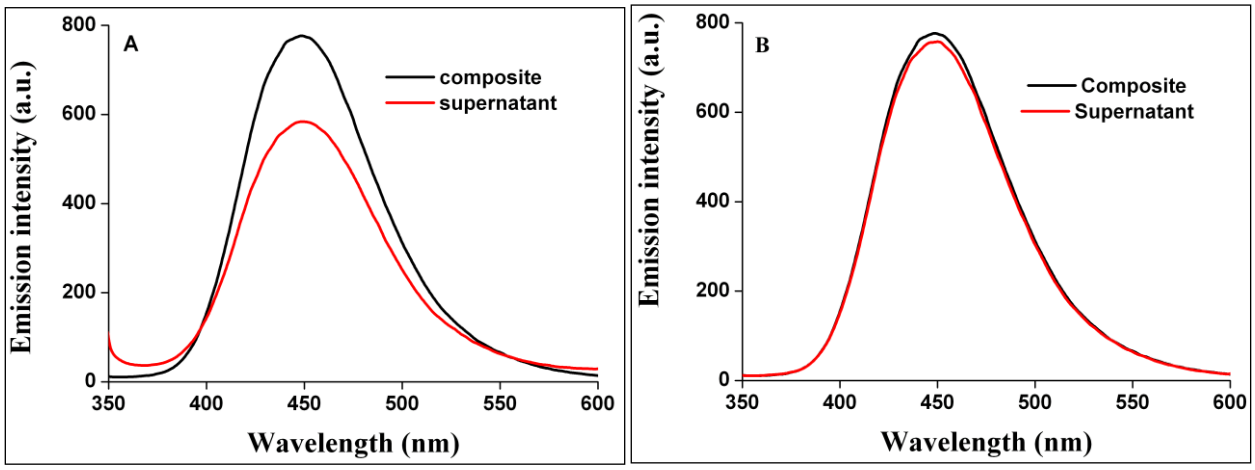

Figure S5: (A) Emission of the composite (in the buffer medium) and supernatant (B) emission of the composite incubated with buffer (without bacteria) under the same condition. The composite was incubated with bacterial cells for 15 min at 37 ^{0}C followed by centrifugation. The fluorescence spectrum of the supernatant was then recorded. The supernatant in A refers to thus obtained supernatant.

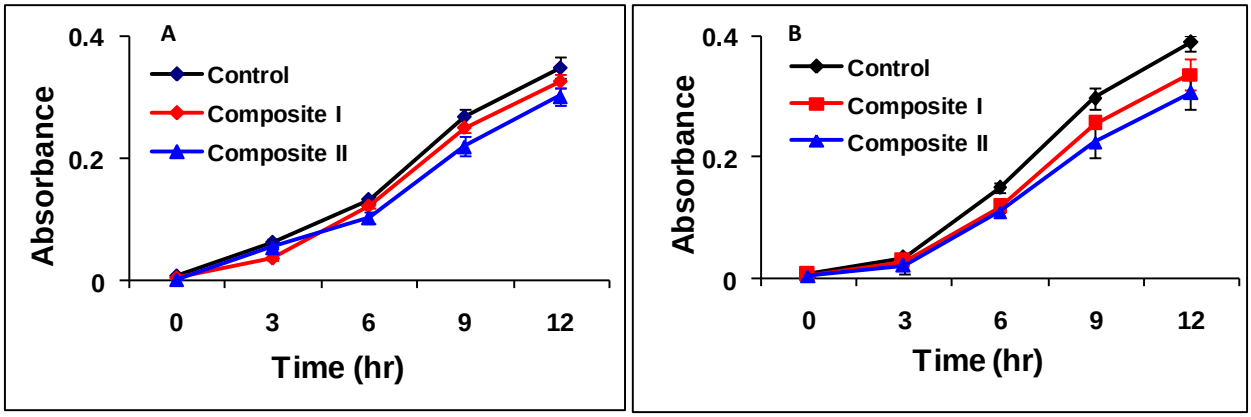

Figure S6: Growth curve of (A) *Enterococcus faecalis* MTCC439 (Gram-positive) and (B) *Escherichia coli* MTCC433 (Gram-negative) bacteria in presence of composite, where control is untreated bacteria, composite I is working concentration of the composite (0.49 mM pHA and

5.0 µM Au) and composite II is three times higher concentration of the composite. The data represents mean ± S.D. of three experimental results.

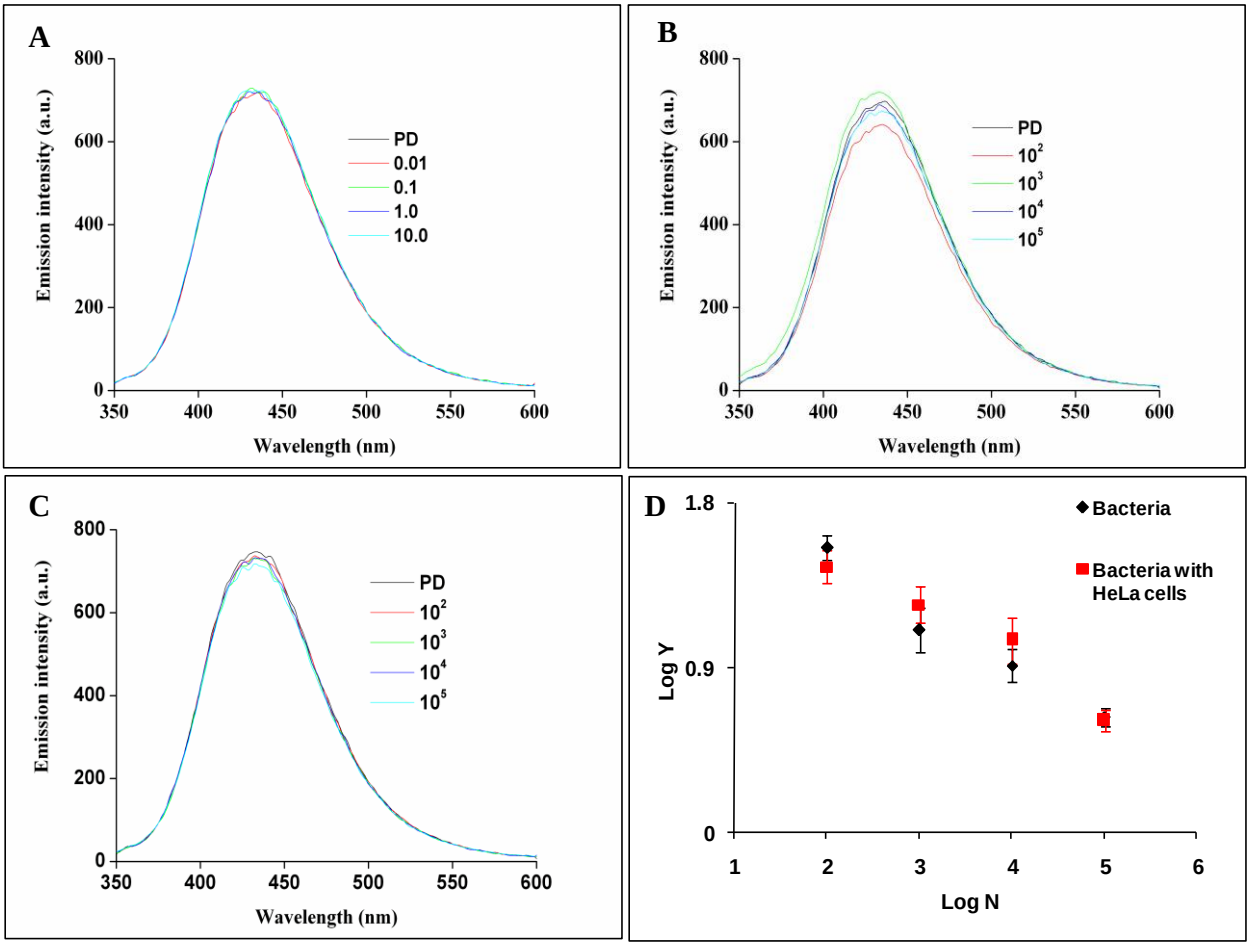

Figure S7: Decrease in fluorescence intensity of PD-Au NPs composite at 435 nm in presence of different amount of (A) sand (µg/mL), (B) plant cells (cells/ml) and (C) mammalian cells (cells/ml) and **(**D**)** Decrease in fluorescence intensity of PD-Au NPs composite as a function of bacterial cell number, where Y= (I_f^{Co} / I_f^{Co} - I_f^{C}) and N is the number of bacteria (CFU/ mL) for bacteria (*E.coli* MTCC 433) and mixture (bacteria with HeLa cells). The data represents mean ± S.D. of three experimental results.

Chi square test

Chi square test was performed to establish the statistical significance of our method. According to this, if the expected frequency is E and observed frequency is O then

Chi square ($\chi 2$) = $\sum_{i=1}^{i=n} (Oi - Ei)^2 / \mathrm{E}_i$

For Chi square test, 24 different bacterial samples (in the range of 10^2 to 5×10^3 CFU/ mL) were randomly chosen and the number of bacteria corresponding to each concentration was determined from both the fluorescence quenching experiment and plate count method. The obtained number of bacteria was divided into three sets viz. 10^2-10^3, 10^3- 2×10^3 and 2×10^3-5×10^3. The expected frequency (N_E) corresponded to the frequency (number of times) of appearance of each set in the fluorescence quenching experiment and observed frequency (N_O) corresponded to frequency of each set appearing in the plate count method as shown in Table S1.

Table S1. Chi square test of GFP-expressing recombinant *E. coli.*

Serial No. (n)	Number of bacteria (CFU/ mL)	Expected frequency (N_E) from fluorescence quenching experiment	Observed frequency (N_O) from plate count
1	10^2-10^3	7	6
2	10^3- 2×10^3	8	10
3	2×10^3-5×10^3	9	8

Chi square (χ^2) value = 0.75

Degrees of freedom (df) = n-1

= 2

From the chi square probability distribution table [50] the chi square value corresponding to probability (p) ≤ 0.05 and the degrees of freedom 2 is 5.99. However, the obtained value in our

case was 0.75 which is less than table value. This indicates that there is ≥ 95% probability that the observed results are similar to expected values. Thus the observed and expected values are not significantly different indicating authenticity of our method.