Supporting Information

In situ Synthesis of Luminescent Au Nanoclusters on Bacterial Template for Rapid Detection, Quantification and Distinction of Kanamycin Resistant Bacteria

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Chemicals and growth Media

Gold chloride (17 wt. % solution of $HAuCl_4$ in dilute HCl; 99.99%) and 3-Mercaptopropionic acid of high purity were obtained from Sigma-Aldrich Chemicals, U.S.A. Nutrient broth (NB), Brain–Heart Infusion (BHI), growth media were procured from HiMedia, Mumbai, India. Water used in all the experiments was high purity Milli-Q grade water (>18 MΩ cm^{-1}, Millipore).

Bacterial Strains

Four bacterial strains were chosen as template for cluster synthesis and detection, which included two Gram-positive strains: *Bacillus cereus* MTCC 1305, *Enterococcus faecalis* MTCC 439 and two Gram-negative strains: *Escherichia coli* MTCC 433, *Pseudomonas aeruginosa* MTCC 2488 strains. *Enterococcus faecalis* MTCC 439 was grown in BHI at 37° C at 220 rpm for 12 h and all the other strains were grown in NB medium keeping the conditions intact.

Synthesis of Au NCs

The serial dilution was conducted on overnight grown bacteria after centrifugation in de-ionized water. On that 90µL (10 mM) of $HAuCl_4$ along with 30 µL (0.11M) of MPA was added and thoroughly mixed keeping the pH constant at 7.The samples were heated at 50 °C (less than pasteurization temperature) for 2 min followed by cooling. Bright luminescence was observed under UV trans illuminator (Excitation 305 nm).The luminescence peak was obtained at 580 nm on excitation at 320 nm on Au NCs synthesized bacteria, which was not there in case of control bacteria.

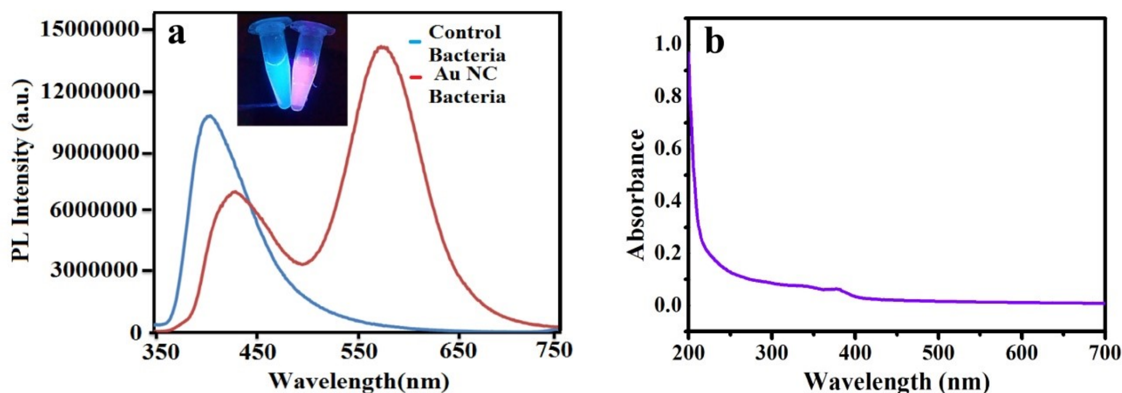

Figure S1. (a) Luminescence spectra of control bacterium and Au NCs synthesized on bacterium along with the inset image (b) UV-Vis spectra of Au NCs synthesized on bacterium.

Characterization

Luminescence Measurements

All luminescence data were obtained from Flurolog-3, Horiba JovinYvon, Edison, NY, USA. For this purpose, first the overnight grown bacterial culture was harvested by centrifuging at 10,000 rpm for 1 min. The cell pellet was redisposed in de- ionised water. The absorbance value of each bacterial strain is noted at 595 nm in UV- Vis spectrophotometer and is kept constant for all the strains. To carry out detection of bacteria, serial dilutions from 10^6 - 10^2 were made and synthesis was carried out in these, keeping all the reaction conditions intact. Further, before synthesis 100 µl from each set was spread on agar plate to obtain colonies and kept for overnight incubation at 37 °C. The number of colonies obtained is related with luminescence of each set of bacteria as luminescence obtained is directly proportional to bacterial number. The luminescence spectra obtained directs the Au NCs formation on the bacterial surface, which was not the characteristic peak of control bacteria. Luminescence spectra with different bacterial concentration of two Gram positive (*Bacillus cereus* MTCC 1305, *Enterococcus faecalis* MTCC 439) and two Gram negative strains (*Escherichia coli*

MTCC433, *Pseudomonas aeruginosa* MTCC 2488) are shown. To find out the slope between different bacteria the logarithm of number of bacteria Log (N) CFU/mL with normalized emission intensity was plotted which exhibited a linear relation for both Gram positive and Gram negative (where I_f = Final emission intensity I_i = Initial emission Intensity).

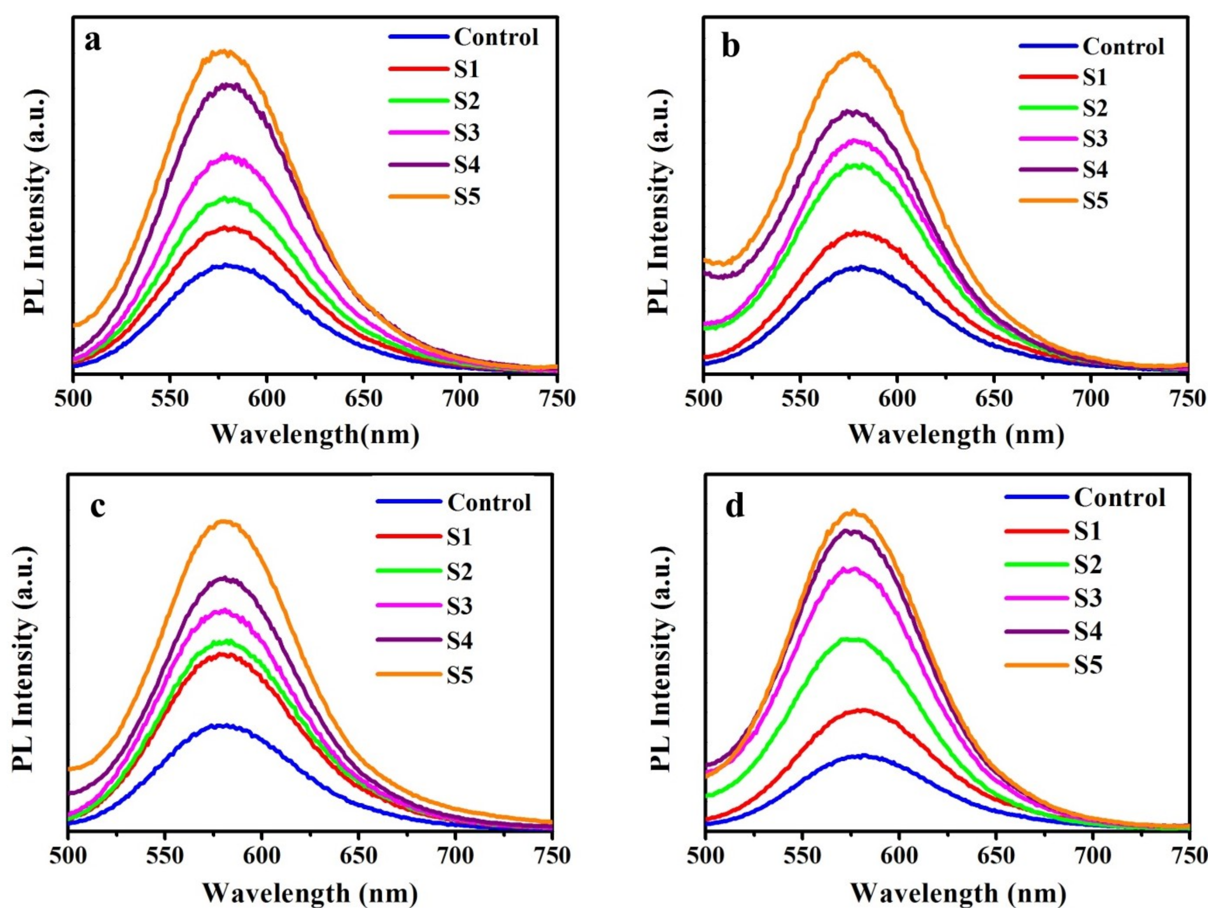

Figure S2. Increase in luminescence intensity of Au NCs synthesized on bacteria with increase in number of bacterial cells when excited at 320 nm. (a) *Bacillus cereus* MTCC 1305 (b) *Enterococcus faecalis* MTCC 439 (c) *Escherichia coli* MTCC 433 and (d) *Pseudomonas aeruginosa* MTCC 2488.

X- Ray Photoelectron Spectroscopy (XPS)

To find out the electronic state of Au NCs, X- Ray Photoelectron Spectroscopy was carried out in PHI 5000 Versa Probe II scanning XPS microprobe. For this, synthesized Au NCs on bacterial surface was taken and pellet was collected by centrifuging at 10,000 rpm for 1 min. The pellet was dried by lyophilisation and measurements were taken.

Matrix-Assisted Laser Desorption Ionization Time of Flight Mass Spectroscopy (MALDI-TOF-MS) Analysis

MALDI –TOF analysis was carried out in Applied Bio systems 4800 Plus MALDI TOF/TOF Analyzer where sinapinic acid was used as a matrix for the samples. The matrix was prepared by dissolving sinapinic acid (10 mg) in a mixture of 1 mL of 50 % acetonitrile (ACN) and 0.05% of trifluroacetic acid (TFA) and were mixed in the ratio of 1:2 (volume ratio) with Au NCs synthesized bacteria carefully and then spotted.

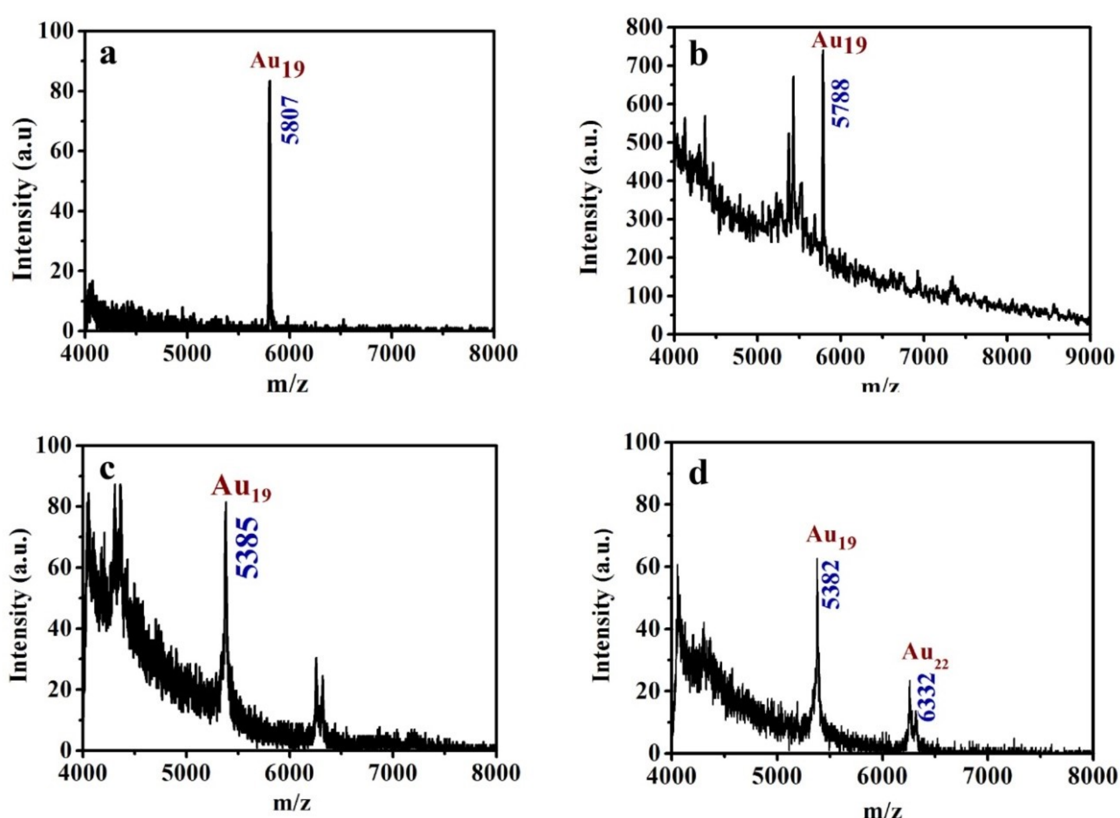

Figure S3. MALDI-TOF spectrum of Au NC synthesized on bacteria showing peaks at 5807, 5788 ,5385 , 5382, 6332 , which corresponds to 19 and 22 atoms of gold.

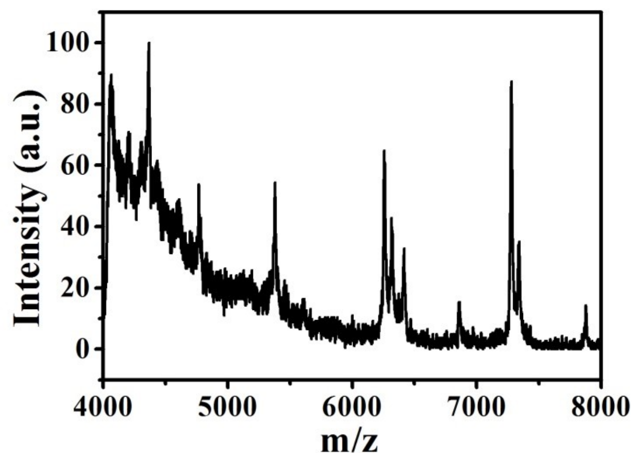

Figure S4. MALDI -TOF MS spectra of control bacteria.

Confocal Laser Scanning Microscopy and Deconvulation microscopy Analysis

For CLSM studies, first the overnight grown bacteria (*Escherichia coli* MTCC 433) were taken centrifuged (10,000 rpm for 1 min) and serially diluted in Milli-Q water followed by Au NCs synthesis. After synthesis it was centrifuged again to remove the unreacted materials and pellet was fixed with 4% formaldehyde. From this, 60 µL of samples were taken and drop cast on a clean glass slide and was covered with a coverslip. The prepared samples were observed under CLSM (Zeiss microscope LSM 880) with λ_{ex} 405 nm. For Delta Vision Deconvolution Microscope (GE Healthcare) imaging was carried out in agar pads which were prepared with 0.6% agar in LB medium, where 10 µL of Au NCs synthesized bacteria were drop casted for imaging.

Figure S5. Bright field image of the bacterium mentioned in Figure 1c of the manuscript (i.e., treated with precursors for the synthesis of Au NCs).

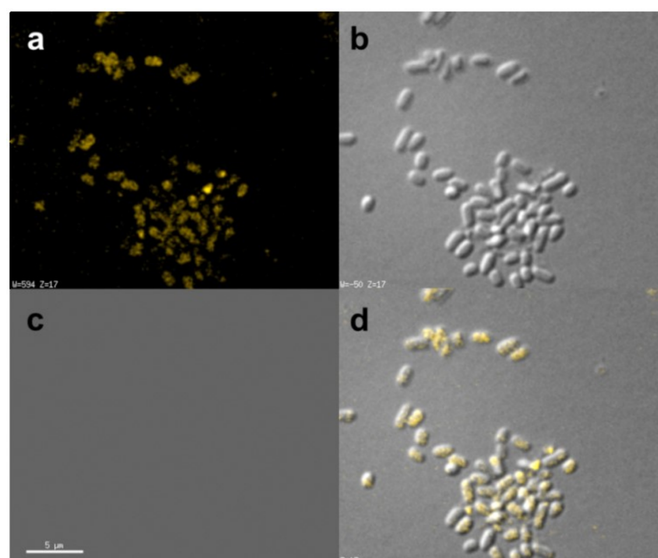

Figure S6. Deconvulation microscopy images of bacteria after synthesis of Au NCs. (a) Luminescent image of bacterial cell after Au NC synthesis (b) Bright field image of the same bacteria (c) Background of the image (d) Merged image of a and b.

Transmission Electron Microscopy (TEM)

To analyse the size of Au NCs, high resolution TEM (JEM-2100, JEOL, Japan) was used, which operates at an accelerating voltage of 200 kV. For TEM analysis, the sample as mentioned above was prepared, centrifuged and washed with water to remove the residual media, gold and MPA and analysed by adding 8.0 µL of the sample on carbon-coated copper grid . After complete drying the samples were taken for measurements.

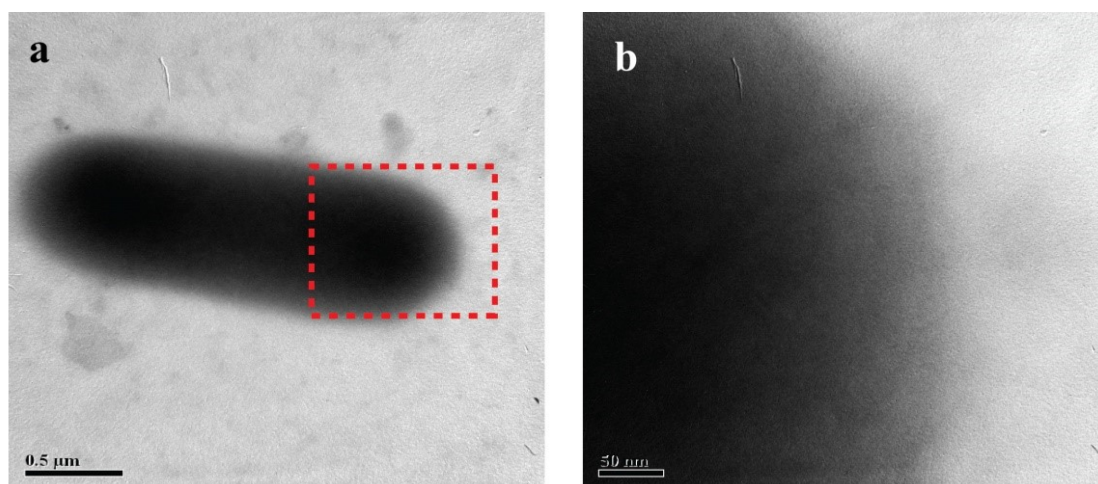

Figure S7. (a) TEM image of control Gram negative bacterium (*Escherichia coli* MTCC 433), the portion needs to be magnified is highlighted in red and **(b)** Magnified image of the same sample.

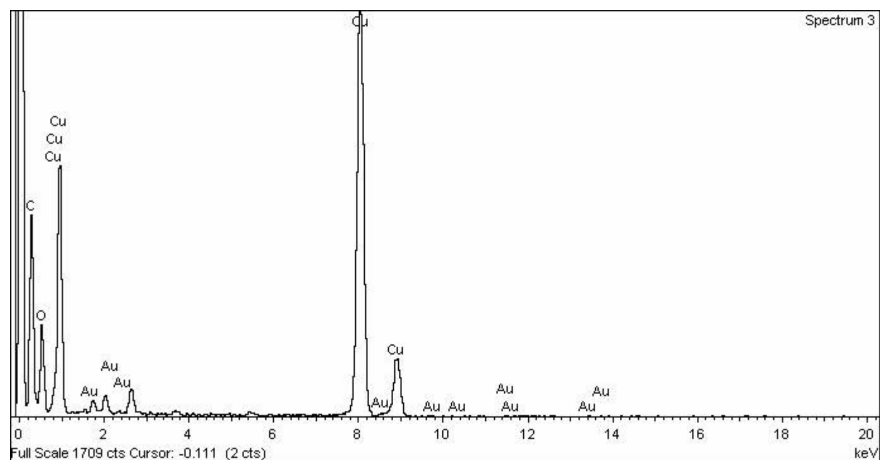

Figure S8. EDX of Au NCs synthesized bacterium.

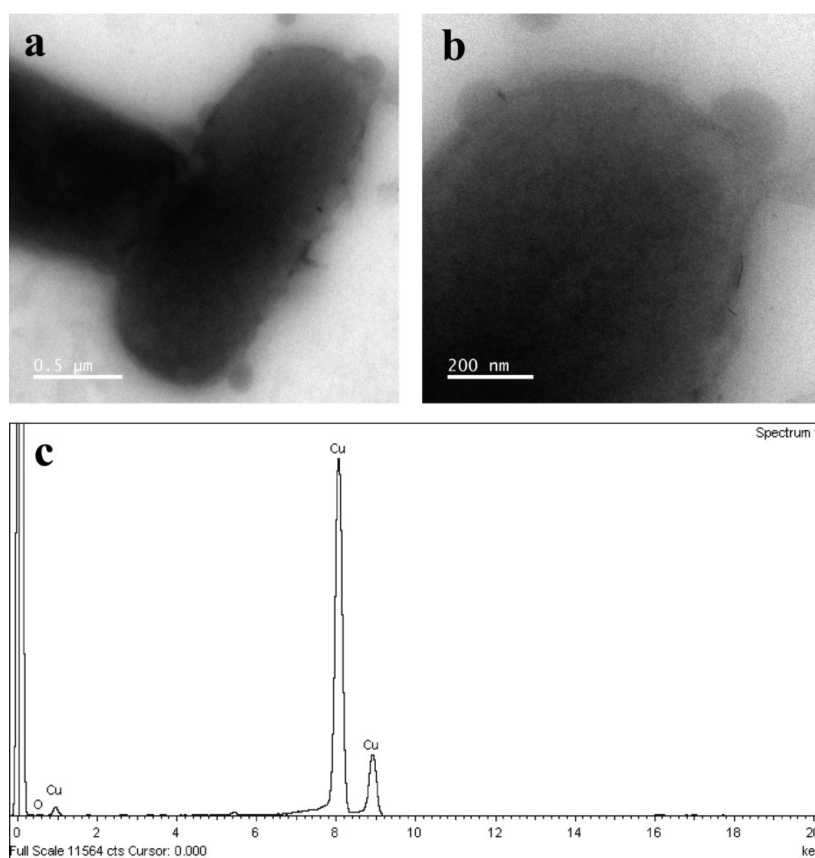

Figure S9. (a) TEM image of control bacterium (i.e., bacterium without Au NCs). (b) Magnified image of the same bacterium. (c) Energy dispersive X-ray spectrum (EDX) of the same bacterium.

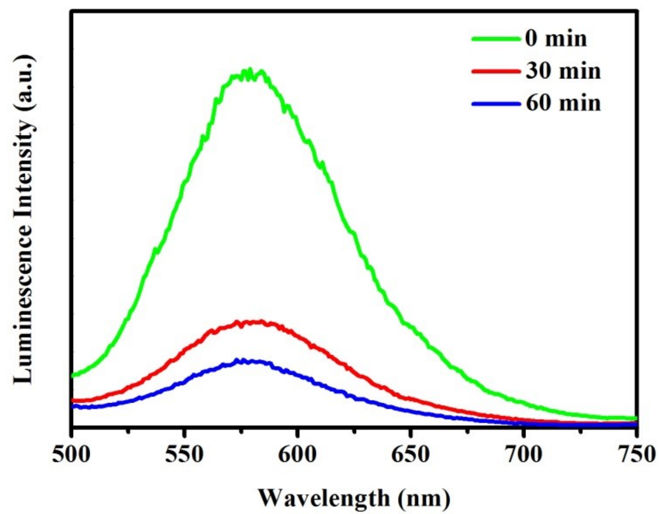

Figure S10. Luminescence of control (without bacteria) with precursors of Au NCs ($HAuCl_4$ and MPA) at different time points.

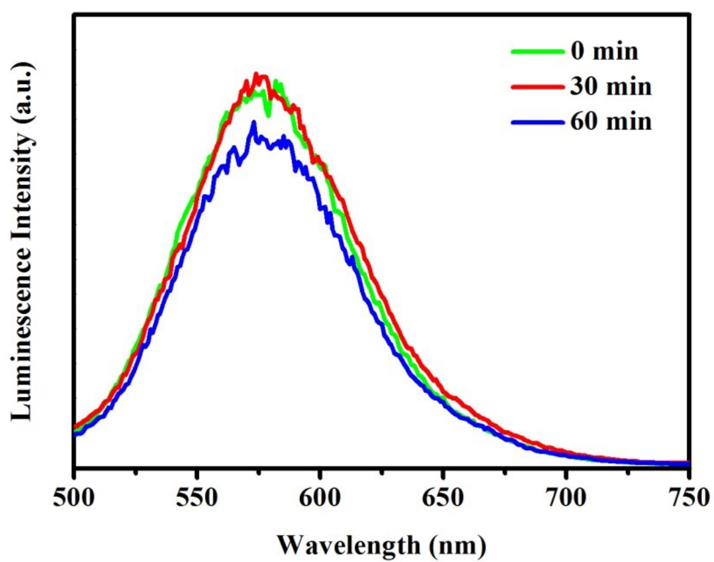

Figure S11. Luminescence of Au NCs synthesized on bacteria at different time points.

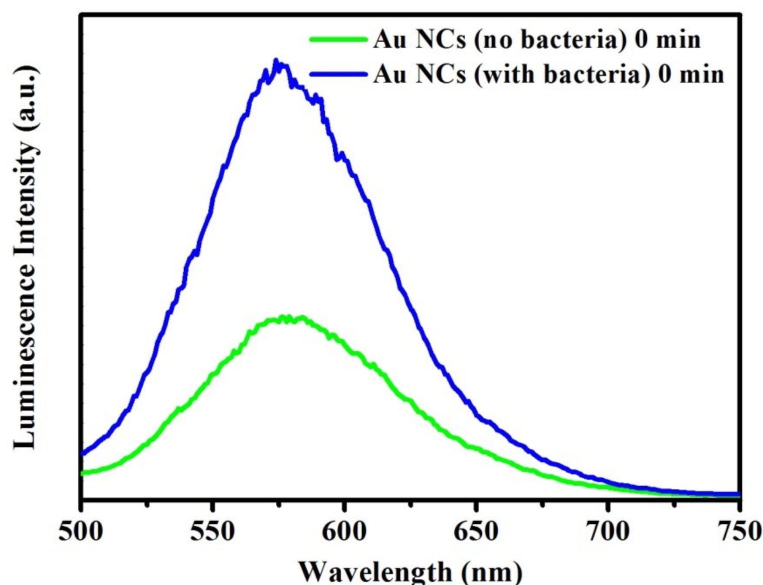

Figure S12. Luminescence of control Au NCs (without bacteria) and Au NCs synthesized on bacteria at 0 min with same concentration of precursors ($HAuCl_4$ and MPA).

Quantum Yield

The photoluminescence quantum yield of flurophore Au NCs synthesized on bacteria was determined relative to the reference of quantum yield of known compound quinine sulphate. If both the sample and a reference sample are excited at same wavelength keeping same slits and bandwidths, then it can be calculated by using the formula:

$$QY = \frac{QY_r\, m}{m_r} \frac{n}{n_r}$$

Where, m denotes the slope of the plot of integrated fluorescence intensity vs. absorbance, n is the refractive index. The reference fluorophore i.e. quinine sulphate solution is represented by suffix r .For the experiment the same solutions were used to record both UV-Vis spectra (Perkin Elmer LS 45) and fluorescence emission .The absorbance value was kept less than 0.01 to minimize re-absorption effects. The refractive index of solvent (water) is 1.33 and quantum yield of the standard (QY_r) is 0.54.

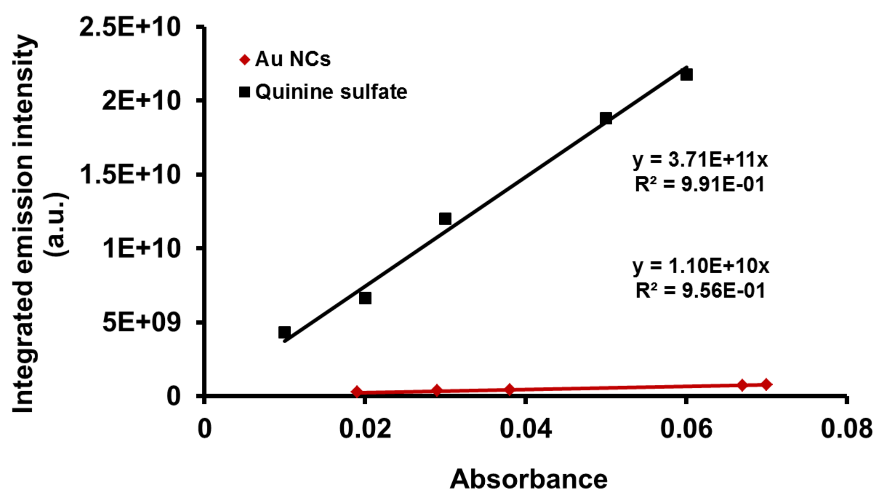

Figure S13. Quantum yield of Au NCs synthesized on bacteria.

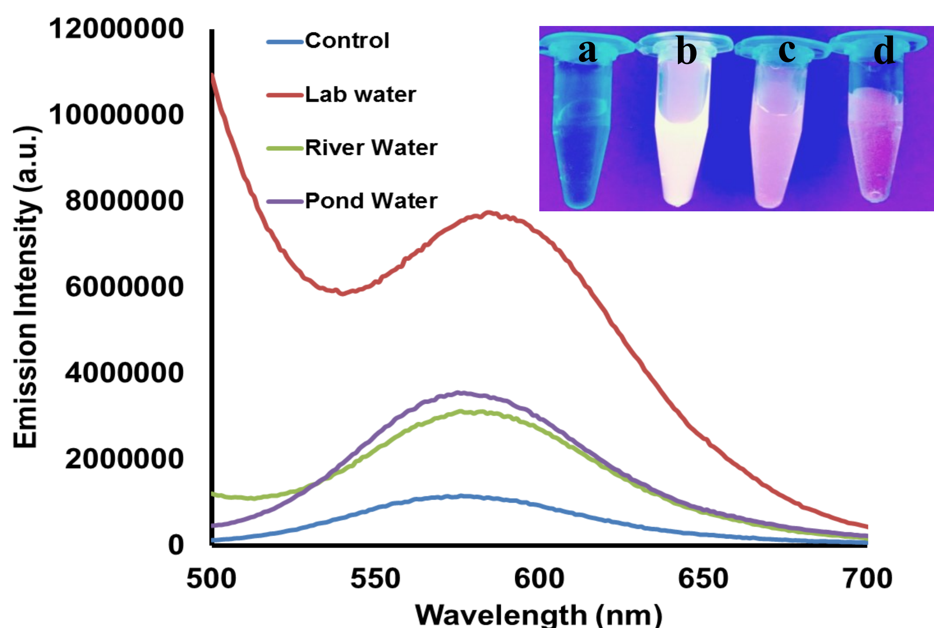

Figure S14. Luminescence spectra of bacteria in four different water samples i.e. control (Milli-Q water), lab waste water, river water, pond water.

Cell Viability MTT Assay

To study the cell viability of Au NCs synthesized bacteria MTT assay was carried out after 24 h of treatment on HEK-293 cells. Briefly, 1×10^4 cells were seeded in each well of 96 well plates and were incubated overnight for attachment. After this treatments were given at varied concentrations for 24 h, in triplicates. Thereafter, MTT was added in each well and the

formed formazon was dissolved in DMSO. The absorbance of formazan, was then measured at 550 nm with background reference measured at 655 nm in Multiplate Reader (Tecan). The cell viability was calculated as:

$$\% \text{ of Cell Viability} = \frac{(A_{550} - A_{655})_{sample}}{(A_{550} - A_{655})_{control}} \times 100$$

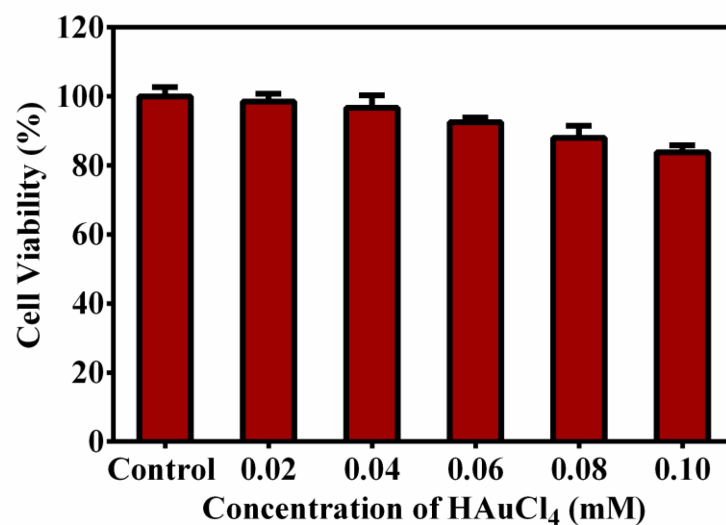

Figure S15. MTT assay of Au NCs synthesized on bacteria (at various concentrations) on HEK-293 cells performed following incubation for 24 h.

Table S1. Surface roughness and indentation of statistics of control bacteria and bacteria after Au NCs synthesis quantified using Gwiddion software analysis.

Statistical Quantities of Control

File: D:\NEW\Controlled 433_00006.gwy
Data channel: Topography
Selected area: 512 × 512 at (0, 0) px
2.500 × 2.500 at (0.000, 0.000) µm
Mask in use: No
Minimum: 0.000 nm
Maximum: 97.177 nm
Average value: 37.644 nm
Median: 21.206 nm
Ra (Sa): 24.024 nm
Rms (Sq): 26.386 nm
Rms (grain-wise): 26.386 nm
Skew: 0.6956
Kurtosis: -1.223
Surface area: 6.589594 µm^2
Projected area: 6.250000 µm^2
Variation: 1.5874 µm^2
Entropy: -16.806
Entropy deficit: 0.77403
Inclination θ: 0.14 deg
Inclination φ: 80.17 deg

Statistical Quantities of treated

File:
D:\NEW\bac_00003_1_2d.tif
Data channel: Detail 4
Selected area: 309 × 309 at (0, 0) px
3.018 × 3.018 at (0.000, 0.000) µm
Mask in use: No
Minimum: 0.00 nm
Maximum: 208.00 nm
Average value: 113.82 nm
Median: 113.89 nm
Ra (Sa): 34.56 nm
Rms (Sq): 41.44 nm
Rms (grain-wise): 41.44 nm
Skew: -0.06817
Kurtosis: -0.7388
Surface area: 9.410890 µm^2
Projected area: 9.105778 µm^2
Variation: 2.0591 µm^2
Entropy: -15.619
Entropy deficit: 0.038534
Inclination θ: 0.44 deg
Inclination φ: -91.88 deg