Electronic Supplementary Material (ESI) for Nanoscale


# Protein-Templated Gold Nanoclusters: Size Dependent Inversion of Fluorescence Emission in Presence of Molecular Oxygen

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**Supporting Information**

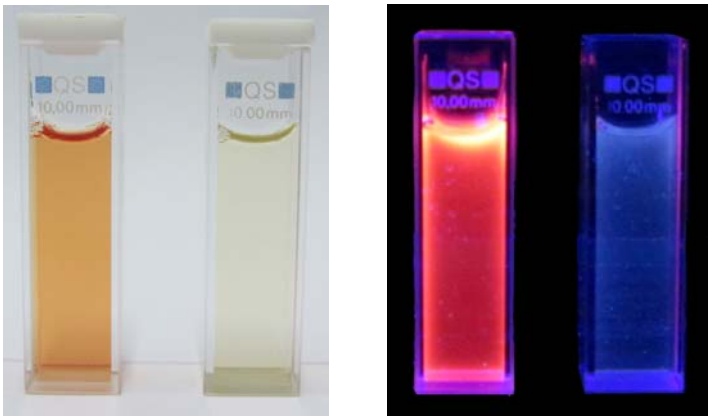

**Figure S1.** BSA directed synthesized Au NCs in aqueous solution under ambient (left panel) and UV light (right panel).

**Determination of number of Au atoms in the blue emitting Au NC using spherical jellium model**

Spherical jellium model applied to nanoclusters can be mathematically represented as:

$$\Delta E_{emission} = \frac{E_{Fermi}}{N^{1/3}} \qquad \text{..... (1)}$$

where, $\Delta E_{emission}$ is the energy of emission from the fluorescing nanocluster, $E_{Fermi}$ is Fermi energy, and N represents the number of metal atoms in the nanocluster.

Equation 1 can be rewritten as,

$h\nu = \frac{E_{Fermi}}{N^{1/3}}$, where h is Planck's constant and ν is the frequency of emission.

Or, $h\frac{c}{\lambda} = \frac{h\frac{c}{\lambda_{Fermi}}}{N^{1/3}}$, where c is the speed of light, λ and $\lambda_{Fermi}$ (= 0.7 nm) are wavelengths of the emitted light from the fluorophore and Fermi wavelength, respectively.

On simplification, one gets,

$N = \sqrt[3]{\frac{\lambda}{0.7}}$. Replacing λ with 410 nm, i.e., emission wavelength of the blue emitting Au NC (considering it to be sphere), we obtain,

$$N = \sqrt[3]{\frac{410}{0.7}} = \sqrt[3]{586} \approx 8.$$

Thus, we can conclude that the blue emitting Au NC emitting at 410 nm contains 8 Au atoms.

**Absorption spectral changes of DAB in presence of singlet oxygen**

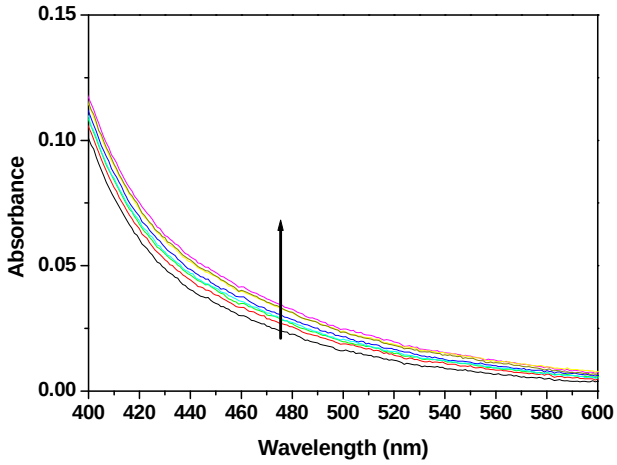


**Figure S2.** Absorption spectrum of diaminobenzidine (DAB) changes on purging with oxygen into a solution of blue emitting Au NC. Oxygen has been purged from 0 to 25 minutes and the enhancement in absorbance at a particular wavelength is monitored.

**Changes in fluorescence due to adsorption of molecular oxygen on L-cysteine directed blue emitting Au NCs**

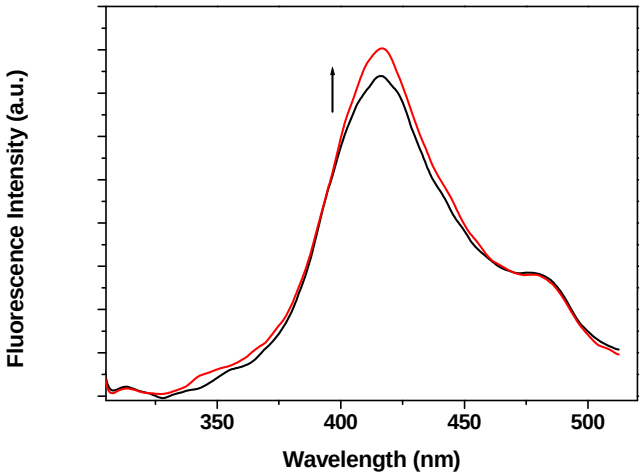


***Figure S3.*** Enhancement in emission intensity of L-cycteine directed blue emitting Au NC in presence of molecular oxygen. Oxygen has been purged for 30 minutes. The system has been excited at 280 nm.

**Cell culture and cytotoxicity assays**

MCF7 (human breast cancer) cell-line and HeLa cells were used independently for this study. The cells were maintained in high-glucose Dulbecco's modified Eagle's medium (DMEM). Media contained fetal bovine serum (10%), l-glutamine (2.9 $mgmL^{-1}$), streptomycin (1 $mgmL^{-1}$), and penicillin (1000 $unitsmL^{-1}$). All cells were cultured at 37°C in water-saturated air supplemented with 5% $CO_2$.

Cells were plated in 96-well microtiter plates at initial densities of 1400 and 2800 cells per well and kept for 24 h allowing the cells to attach to the plate. The cytotoxicity of nanoparticles was determined by the MTT assay. For cytotoxicity measurements, 24 h after seeding, fresh medium containing blue emitting and red emitting nanoclusters (12 µl in 200 µl) was added to different wells and the cells were incubated for another 24 h. Phosphate-buffered saline (PBS) containing MTT (5 $mgmL^{-1}$) was dispensed into each well and the plates were incubated for 2.5 h. This assay measures the conversion of the yellowish water-soluble tetrazolium salt to a water-insoluble purple formazan product within viable breathing cells as a proxy of cell number and viability. The water-insoluble formazan was dissolved in dimethyl sulfoxide (DMSO). Absorption of the samples was measured with an ELISA plate reader at 515 nm. The amount of formazan produced is directly proportional to the number of living cells in the well. There were six replica of each experimental set.

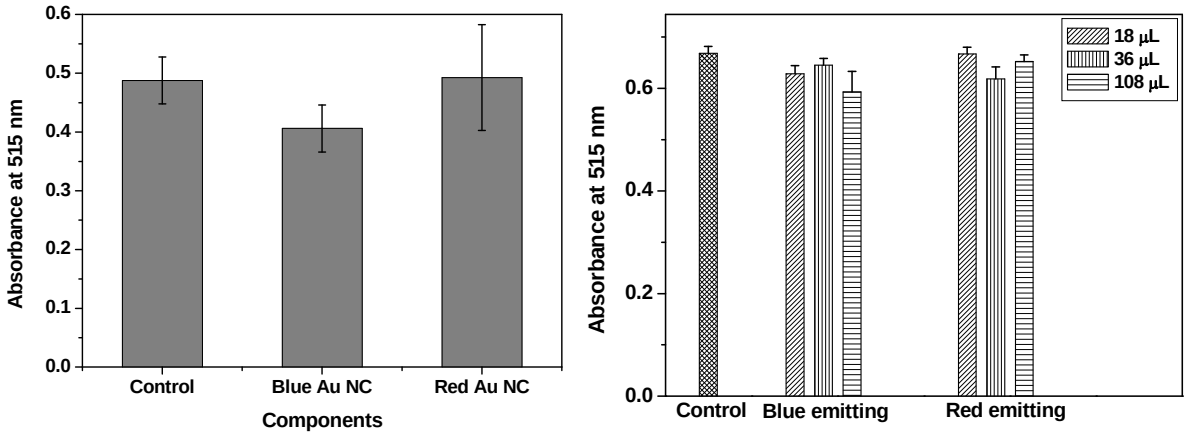


**Figure S4.** Cytotoxicity effect of blue and red emitting Au NCs on (left panel) MCF7 (human breast cancer) cell-line and (right panel) HeLa cells after 24 hours of incubation. The inset in the lower panel signifies the different volumes of Au NC stocks added to 900 µL of water.

Figure S4 shows that blue and red emitting Au NCs does not have significant cytotoxicity. We have performed the test with different concentrations of the nanoclusters and incubated for 24 hours. The data shows that there is no appreciable cell death. This corroborates with the previous reports on the cytotoxicity of Au NCs.[1-4] Moreover, compared to quantum dots, Au NCs are much superior probes to be used as biological labels.[5,7]

References

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