Electronic Supplementary Material (ESI) for Journal of Materials Chemistry


# Supplementary Information

## Gold nanoshell coated $NaYF_4$ nanoparticles for simultaneously enhanced upconversion fluorescence and darkfield imaging

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### Optimization of PLH and gold amount

The optimum amount of PLH was found out by studying the variation of zeta potential due to the adsorption of positively charged PLH onto the negatively charged silica surface which is shown in following figure.

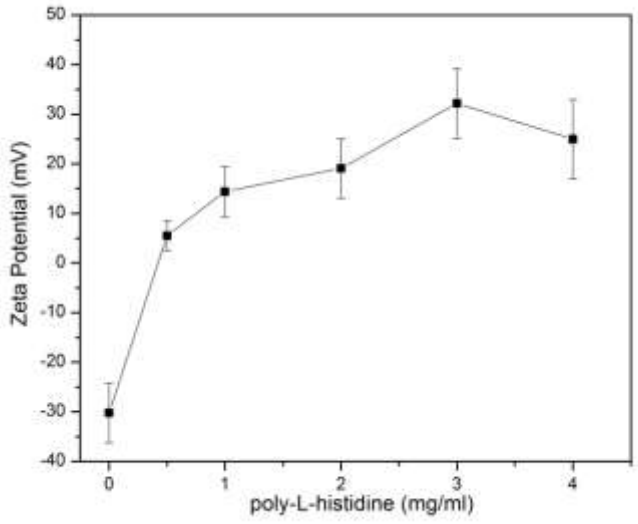
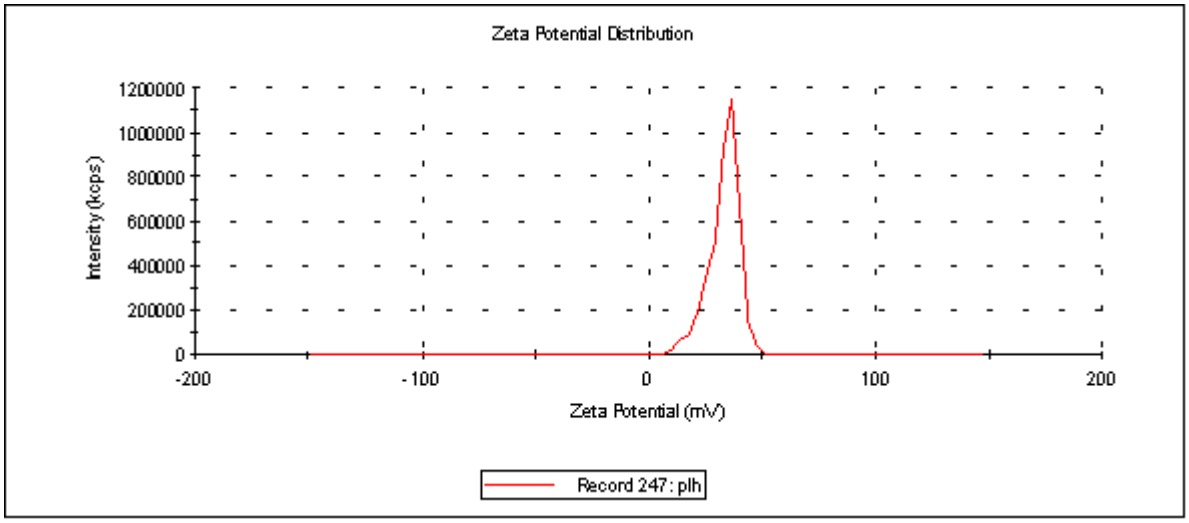



**Figure S1.** Variation of zeta potential after incubation of silica coated $NaYF_4$:Yb,Er UCNs with differing amount of poly-L-histidine

As can be easily seen in the Figure S1, the surface charge becomes increasingly positive upto PLH concentration of 3 mg/ml. No further increase in charge is observed beyond this point which indicates that surface gets saturated at this point. This concentration of PLH was used for the synthesis of gold nanoshell encapsulated UCNs. During the gold-shell encapsulation, the surface charge varies sharply, UCN@$SiO_2$ (-30.1 mV) → UCN@$SiO_2$-PLH (+32.2 mV) → UCN@$SiO_2$-PLH@gold (-39.5 mV).

**Surface charge on PLH in native state:** When 6 g of poly-L-histidine hydrochloride is dissolved in 2 ml of deionized water, the pH of the resulting solution was 6.8 and zeta potential was found to be +30.4 mV. The zeta potential plot is given below:



**Characterization of crystalline phases by electron diffraction:**

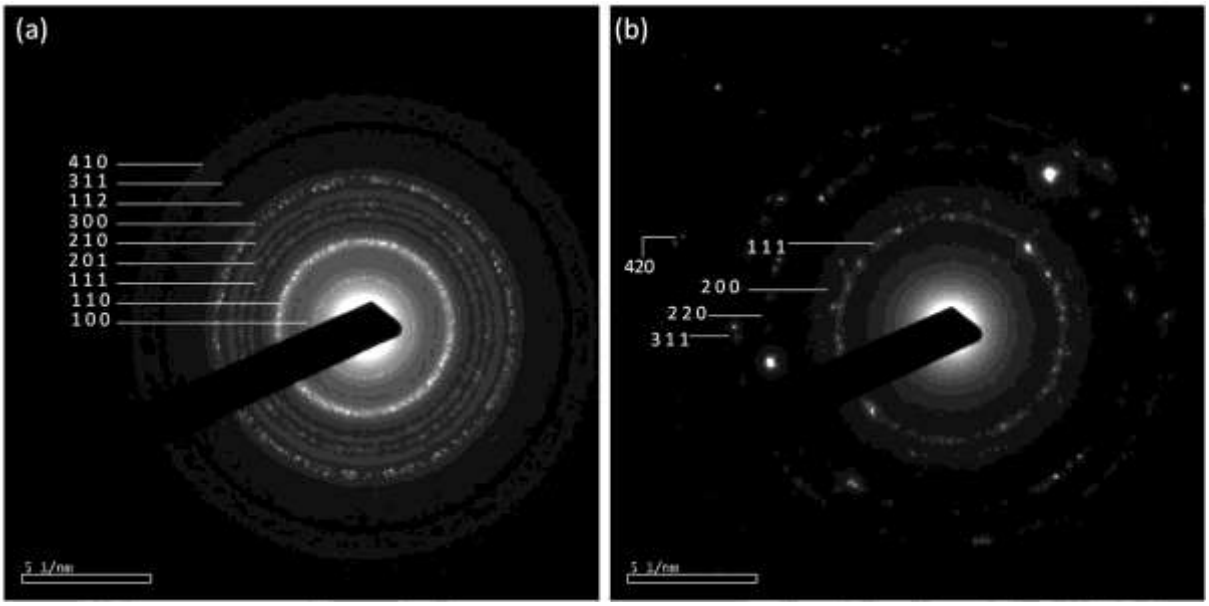



**Figure S2.** The selected area electron diffraction (SAED) pattern of (a) $NaYF_4$:Yb,Er nanocrystals and (b) $NaYF_4$:Yb,Er@$SiO_2$@Au nanocrystals [All the rings corresponding to different interplanar spacings (h k l) have been labeled]

The SPR peak position of the gold nanoshell was tuned by changing the shell thickness but keeping the size of silica coated particles the same. This was achieved by varying the amount of $HAuCl_4$ and hydroxylamine solution. With change in the amount of gold ions (1% aq. solution ), the SPR peak moved in the following manner:

1. 400 µl of $Au^{3+}$ , 800 µl of hydoxylamine, SPR: 580 nm
2. 300 µl of $Au^{3+}$, 600 µl of hydroxylamine, SPR: 650 nm
3. 200 µl of $Au^{3+}$, 400 µl of hydroxylamine, SPR:830 nm
4. 100 µl of $Au^{3+}$, 200 µl of hydroxylamine, SPR: 900 nm

Attempts were also made to make the shell thinner so as to get an SPR band of 980 nm. But, further reduction in the amount of gold ions didnot yield any favourable results. Below 100 µl

of gold ions (1% solution), the particles are not uniformly coated. As shown in Fig S2, some of the particles get thickly coated while the rest do not get the gold-coat at all.

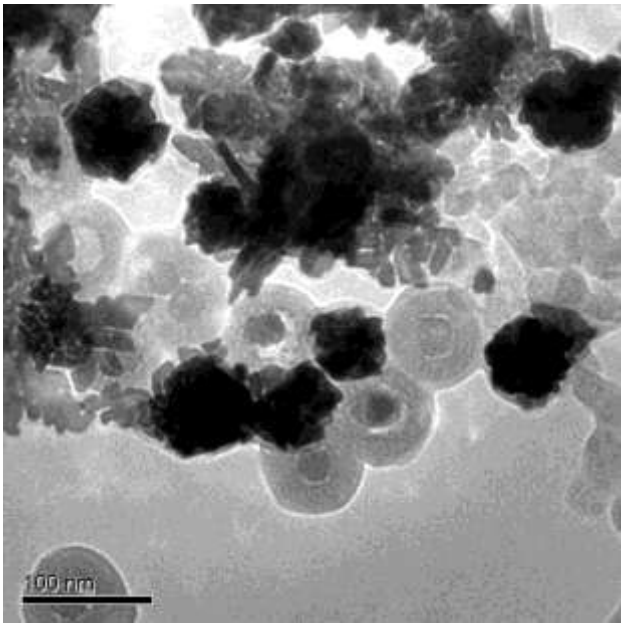



**Figure S3.** TEM image showing non-uniform coating of gold layer on silica coated $NaYF_4$:Yb,Er UCNs on reduction in the amount of gold ions below 100 µl (1% aq. $HAuCl_4$).

**Photostability of the gold-shell encapsulated silica coated $NaYF_4$:Yb,Er UCNs**

To check the photostability, the nanoparticles were continuously irradiated with 980 nm laser at a power of 500 mw and the spectra was recorded periodically at an hourly interval.As shown in Figure S3, no significant change in the luminescence intensity was observed even after 12 hours of irradiation.

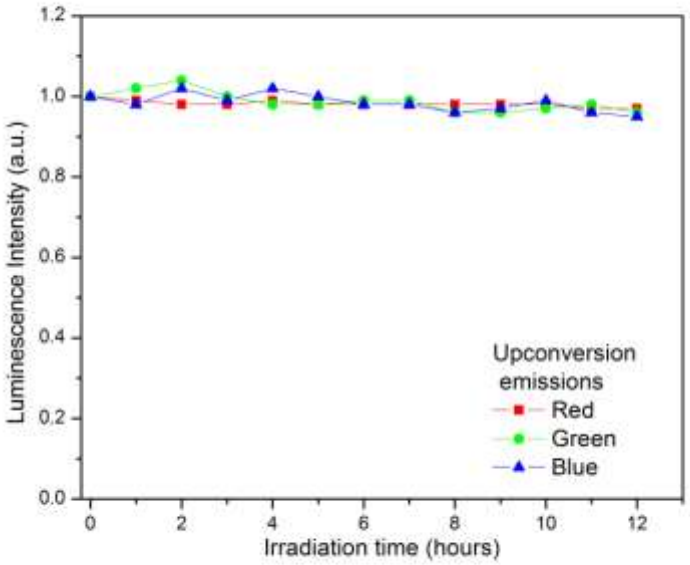



**Figure S4.** Variation of luminescence intensity on irradiation with 980 nm laser (power 500 mw) for 12 hours.

**Chemical Stability of the gold-shell encapsulated silica coated $NaYF_4$:Yb,Er UCNs**

**(a) Effect of pH:**

The nanoparticle-solution were taken in different glass vials and pH was adjusted by using HCl (1N) and NaOH (1N) solution. It can be seen (Figure S4) that the hydrodynamic diameter and the luminescence remain largely unaffected with the drastic variation in pH from acidic to basic range.

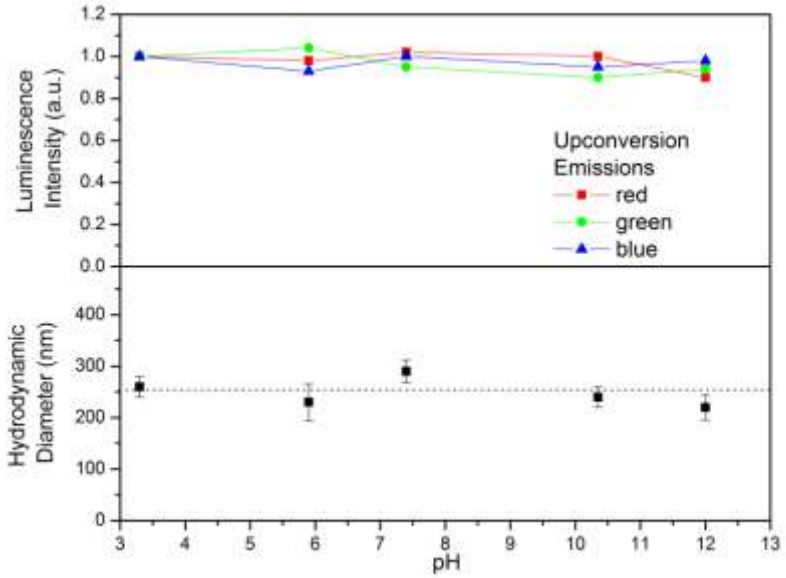
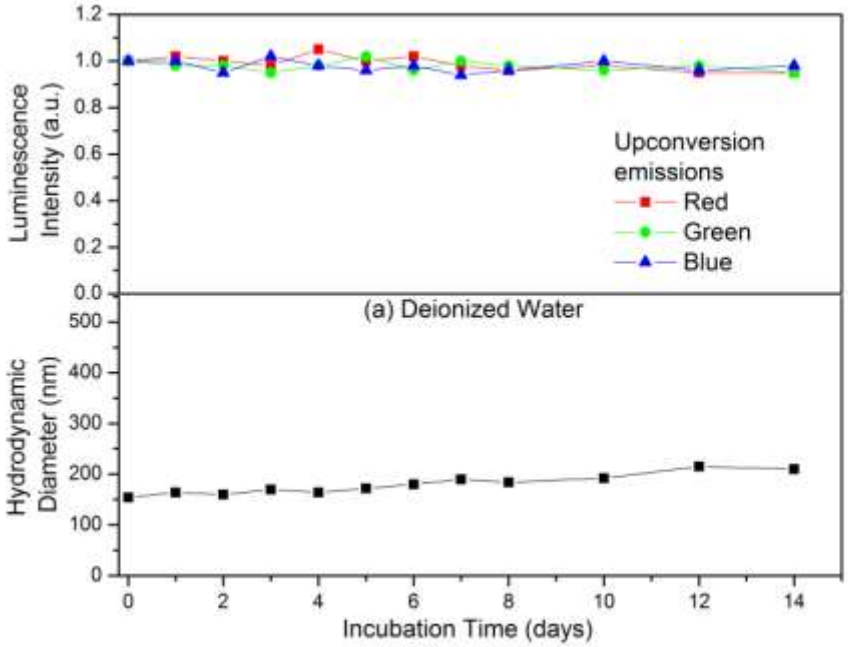
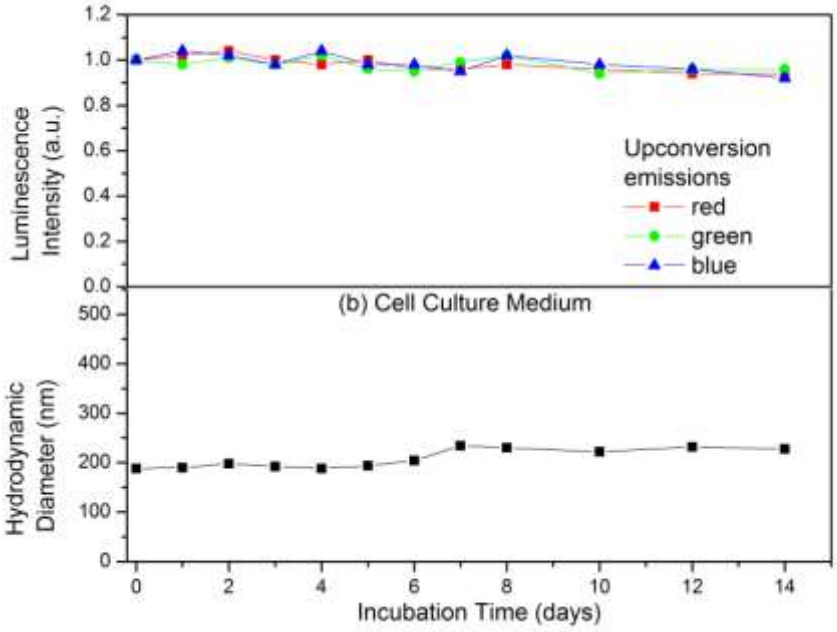



**Figure S5.** Variation of luminescence intensity and hydrodynamic diameter with change in pH of the solution.

**(b) Incubation with different biologically relevant media:**

The nanoparticles were incubated in three biologically relevant media: (a) deionized water, (b) cell culture medium (DMEM, glucose and 10% FBS) and (c) PBS (phosphate buffer). The fluorescence intensity and the hydrodynamic diameter of the nanoparticles were monitored for 14 days on a daily basis. As shown in the figure, the luminescence intensity is quite stable over the entire period in all the cases. However, the hydrodynamic diameter increases significantly in PBS over a period of 8 days after which it gets stabilized. This indicates that particle tend to aggregate in PBS.



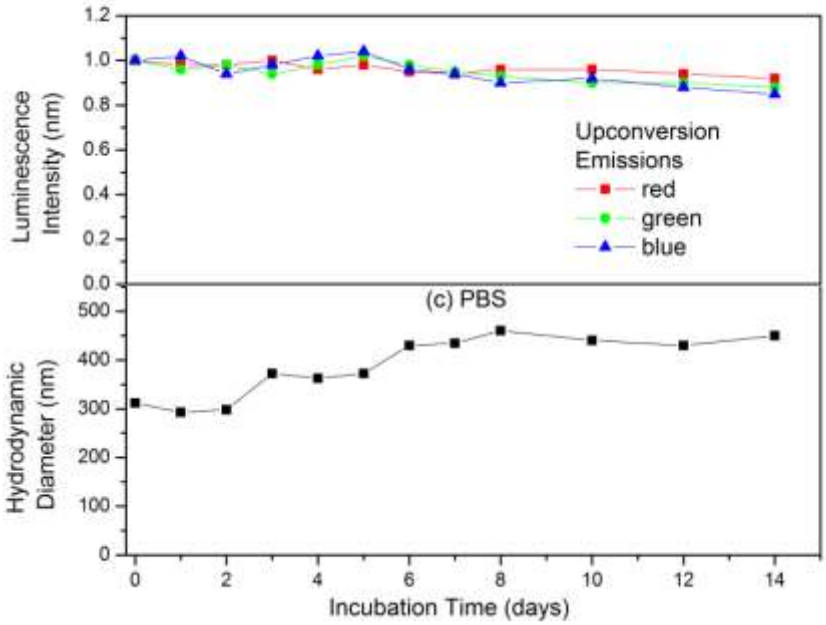



**Figure S6.** Variation of luminescence intensity and hydrodynamic diameter on incubation with different biological media: (a) deionized water, (b) cell culture medium and (c) PBS.

**Cytotoxicity study**

*In vitro* cytotoxicity of the nanoparticles was assessed on B16-F0 cells. The cells were seeded as described above and incubated with the nanoparticles at an extracellular concentration ranging from 0 to 0.1 mg/ml for 48 h at 37 °C in a humidified, 5 % $CO_2$ atmosphere. After which, the number of cells that remained viable at the end of the incubation were assessed by CellTiter 96® $AQ_{ueous}$ One Solution Cell Proliferation Assay (Promega, Madison, WI, USA) as per manufacturer's instructions. Cell viability was then calculated as follows: $[A]_{X\ mg/mL} / [A]_{0\ mg/mL} \times 100$; where $[A]_{X\ mg/mL}$ is the absorbance of the test sample incubated with X mg/mL of the nanoparticles and $[A]_{0\ mg/mL}$ is the absorbance of the control sample incubated with 0 mg/mL of the nanoparticles.

B16-F0 melanoma cells incubated with the nanoparticles at various concentrations normally used for cell labeling were assessed for their cell viability using an MTS assay. After 48 h round of incubation, viability of cells exposed to 0.01 to 0.1 mg/ml of the nanoparticles remained high at a value greater than 95 % (Figure S7), which is not significantly different compared to control cells that has not been exposed to the nanoparticles (assumed to have a viability of 100 %). Interestingly, this value is higher than that previously observed when silica-coated UCNs at a concentration of 0.1 mg/mL was incubated with skeletal myoblasts or bone marrow derived stem cells for 48 h[1], which showed cellular viability dropping to around 68 %.

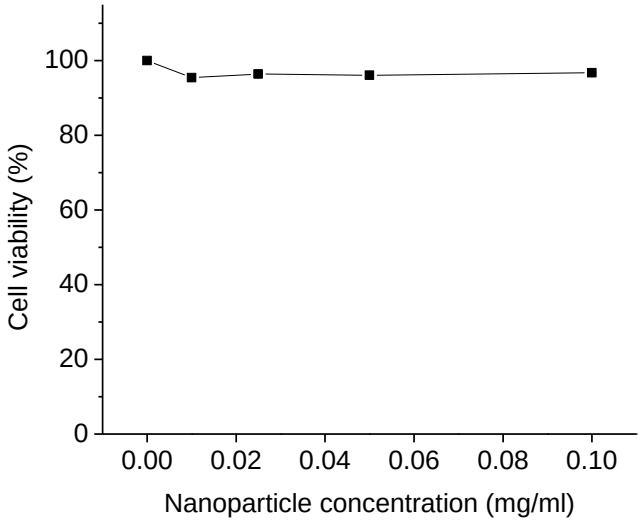



**Figure S7.** Cell viability of B16-F0 cells incubated with gold-shell encapsulated UCNs for 48 hours.

**Reference**

Jalil, R.A. & Zhang, Y. Biocompatibility of silica coated NaYF4 upconversion fluorescent nanocrystals. *Biomaterials* **29**, 4122-4128 (2008).