# Support Information

# Organic Dye-Modified Upconversion Nanoparticle as A Multi-Channel Probe to Detect $Cu^{2+}$ in Living Cells

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**Chemicals and Materials**

Column chromatography was conducted over silica gel (mesh 200–300). OA, 1-octadecane (ODE 90%), and $NH_4F$ were purchased from Sigma-Aldrich. $YCl_3 \cdot 6H_2O$ (99.9%), $YbCl_3 \cdot 6H_2O$ (99.9%), $ErCl_3 \cdot 6H_2O$ (99.9%), and $TmCl_3 \cdot 6H_2O$(99.9%) were purchased from Alfa Aesar. Absolute ethanol, methanol, cyclohexane, dimethyl sulfoxide and methylene chloride were of analytical grade. All the chemicals are used without further purification.

**Instrumentation**

$^1$H-NMR was measured on a BrukerAV-400 spectrometer with chemical shifts reported in ppm (in $CDCl_3$, $CD_3OD$ or DMSO-d6; TMS as internal standard). Electrospray ionization mass spectrum (ESI-MS) was carried out on a Micromass LCTTM system. X-ray diffraction was performed on a Shimadzu XRD-6000 diffractometer at a scanning rate of 1 °/min with the 2θ range from 10 to 90° (Cu Kα radiation, λ = 1.54056 Å). HR-TEM was carried out on a JEOL JEM-2100F transmission electron microscope with an accelerating voltage of 200 kV. UV–Vis spectrum was recorded by a Shimadzu UV-2501 spectrometer. The UCL spectrum was obtained by a DM150i monochromator equipped with a R928 photon counting photomultiplier tube (PMT), in conjunction with a 980nm diode laser.

**Synthesis of OA-UCNPs**

OA-UCNP was synthesized through a modified procedure according to a previous report.[1-4] $YCl_3 \cdot 6H_2O$ (235.7 mg, 0.777 mmol), $YbCl_3 \cdot 6H_2O$ (77.5 mg, 0.20 mmol), $ErCl_3 \cdot 6H_2O$ (6.9 mg, 0.018 mmol) and $TmCl_3 \cdot 6H_2O$ (1.9 mg, 0.005 mmol) were dissolved in 10 ml methanol under sonication. After removing methanol, 7 ml oleic acid and 15 ml 1-octadecene were added. The mixture was heated up to 160°C for 30 minutes and a homogeneous solution was formed. After cooling to room temperature, 10 ml methanol solution containing NaOH (100 mg, 2.5 mmol) and $NH_4F$ (148 mg, 4 mmol) were added. In an Argon environment, the resulting colloidal mixture was slowly heated up to 140°C for 10 minutes to remove methanol, and then increased to 305°C, and maintained this temperature for 1.5 hours. After cooling the solution naturally, the nanoparticles were obtained by adding ethanol, followed by centrifugation, and washed with ethanol for three times.

**Synthesis of RB-FC-UCNPs**

RB-FC-UCNPs were synthesized according to a modified procedure according to a previous report.[3] Igepal CO-520 (0.2 ml) was dispersed into cyclohexane (8.0 ml). Then, cyclohexane solution containing dispersed OA-UCNPs (4.0 ml, 10.0 mg $ml^{-1}$) was added into Igepal CO-520

solution. After stirring the mixture vigorously until the solution becomes transparent, ammonium hydroxide (29.4% (wt/wt), 80 µl) was added into the mixture, and stirred the mixture vigorously again to make a transparent solution. Followed by adding TEOS (40 µl) to the mixture, and stirred the mixture gently for 48 h at room temperature. Acetone (20 ml) was added into the resulting nanocomposites, and then the precipitates were separated through centrifugation (7500 rpm, 15 min, 25 °C). Silica-coated UCNPs were obtained after washing the collected nanocomposites with ethanol for three times. Silica-coated UCNPs and RB-FC were dissolved in DMSO, stirred, and heated under 70 °C for 24 h. The final solution was RB-FC-UCNPs.

**Cytotoxicity Assay**

To verify the cytotoxic effect of RB-FC-UCNPs, an MTT assay was performed by treating A549 cells with 24 h incubation. Cells were passed and plated to a 70% confluence in 96-well plates, and cultured in growth medium at 37°C and 5% $CO_2$ for 24h. Different doses of RB-FC-UCNPs (100, 200, 500, 1000 µg $ml^{-1}$) were added into the A549 cells under the same condition, followed by incubated with 5 mg $ml^{-1}$ MTT reagent for 4h and the absorbance of each well was measured by a microplate reader (SPECTRA SLT; Labinstruments, Salzburg, Austria). Each treatment was done in six wells, and the experiments were repeated twice. Cytotoxicity was calculated relative to the absorbance of the control for each treatment. The reported percent of cell survival values were relative to untreated control cells.

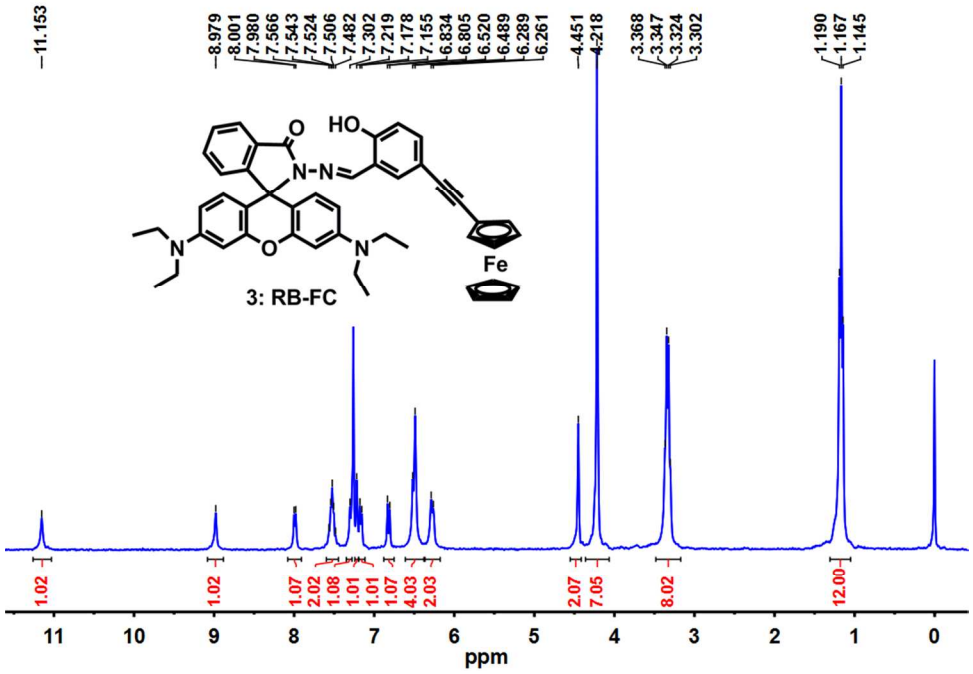



**Figure S1**.[1]H-NMR spectrum of RB-FC.

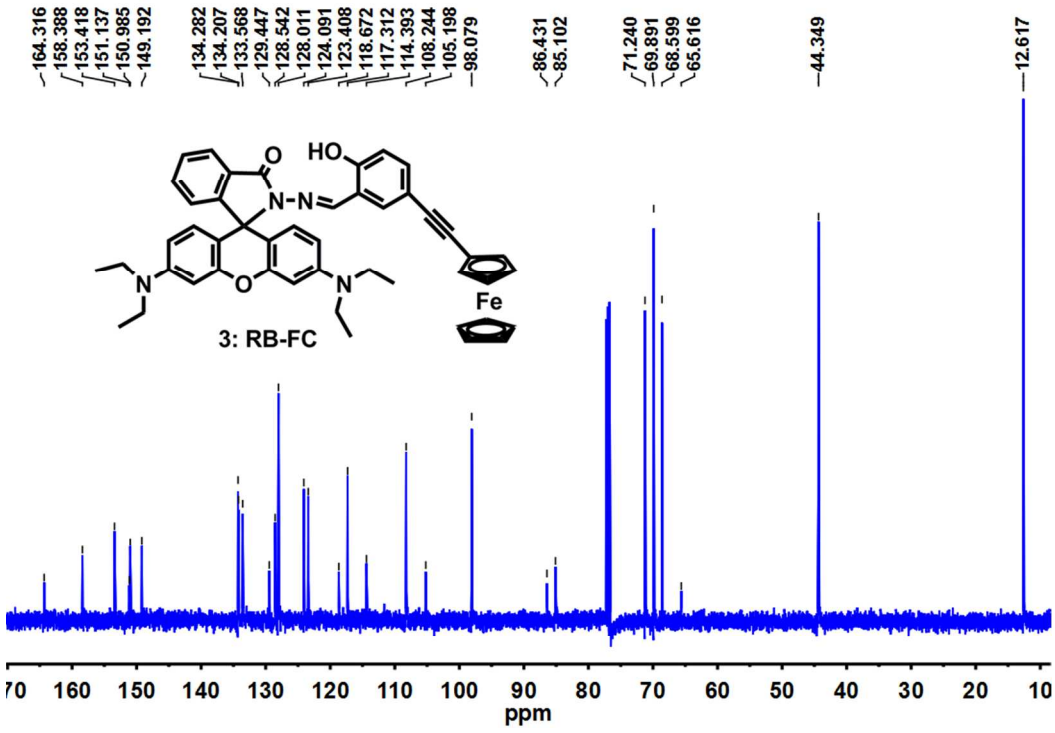



**Figure S2**.[13]C-NMR spectrum of RB-FC.

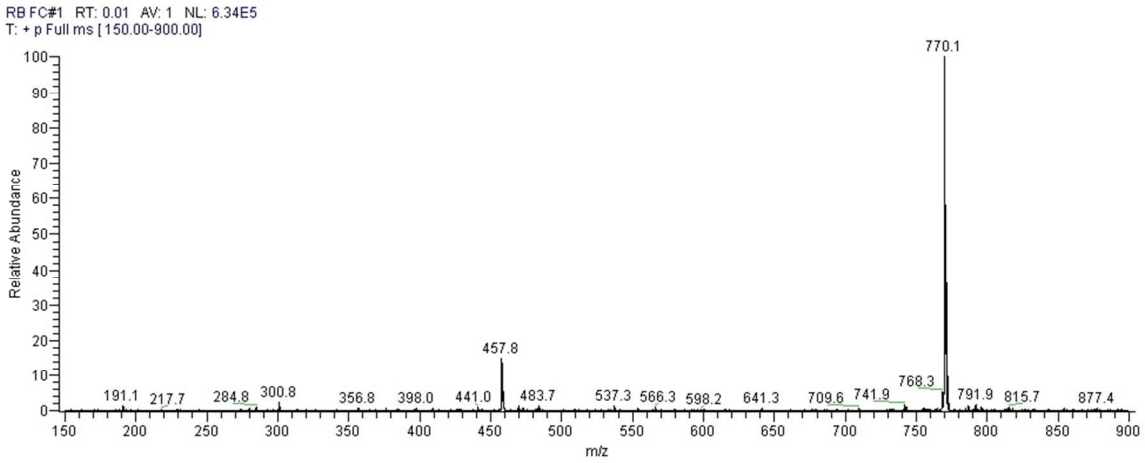



**Figure S3**. Mass spectrum of RB-FC.

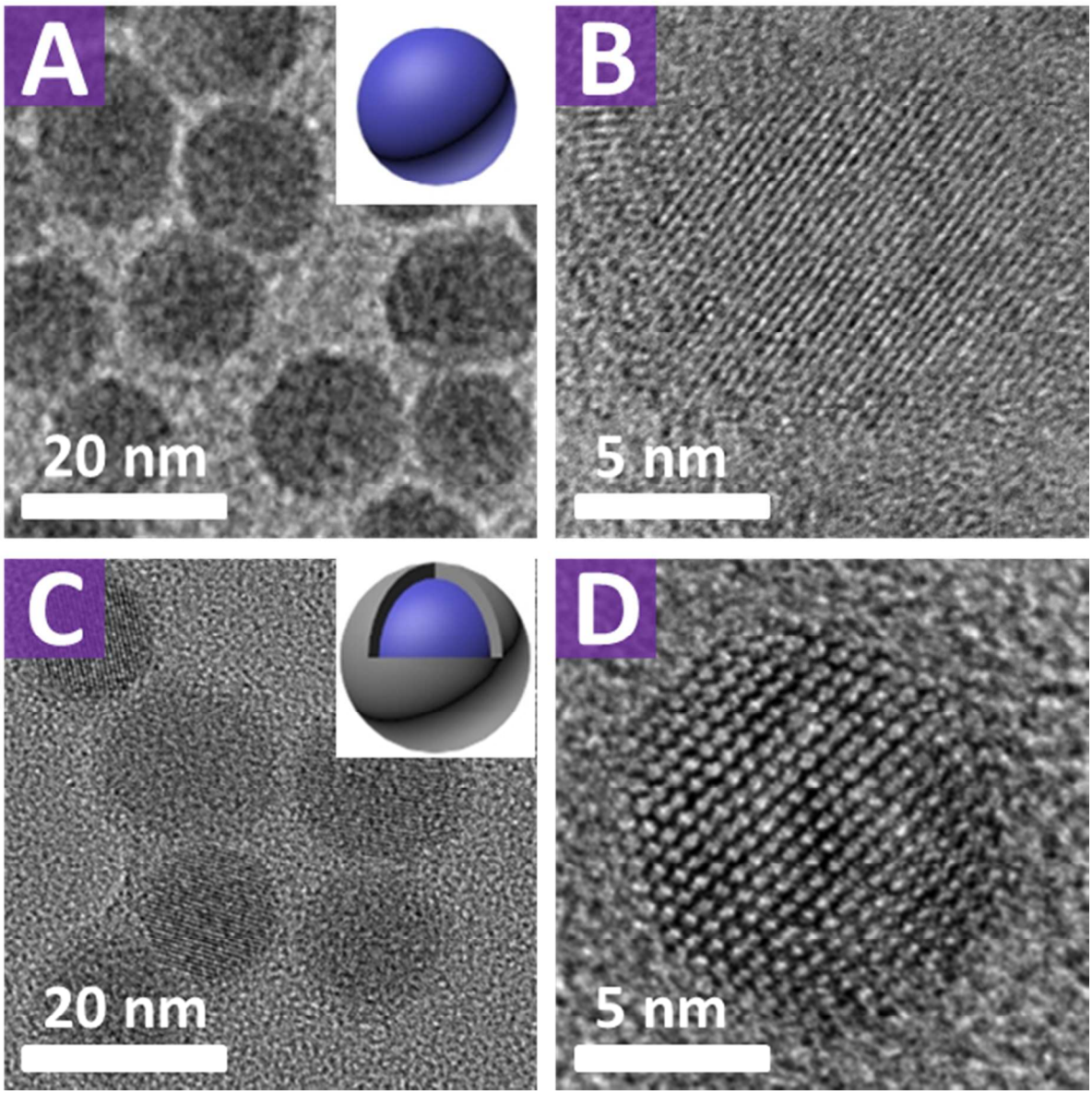



**Figure S4**. TEM (A) and HR-TEM (B) images of OA-UCNPs. TEM (C) and HR-TEM (D) images of UCNP@$SiO_2$.

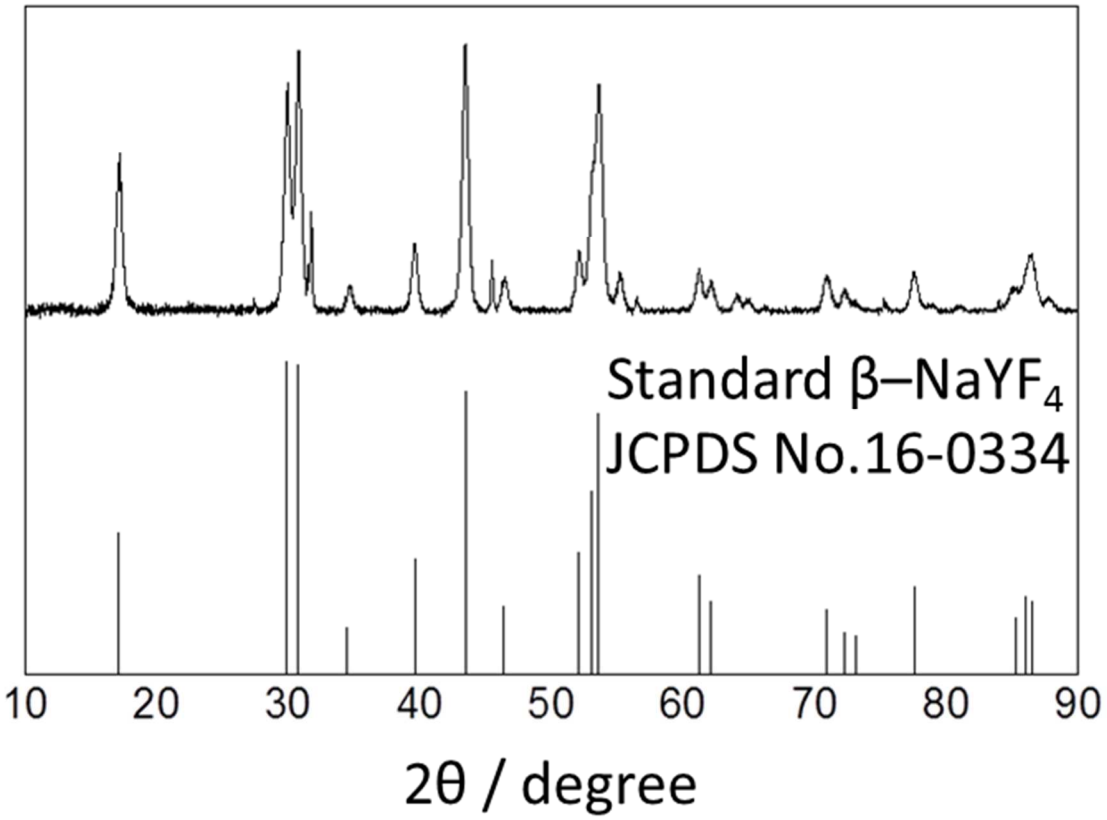
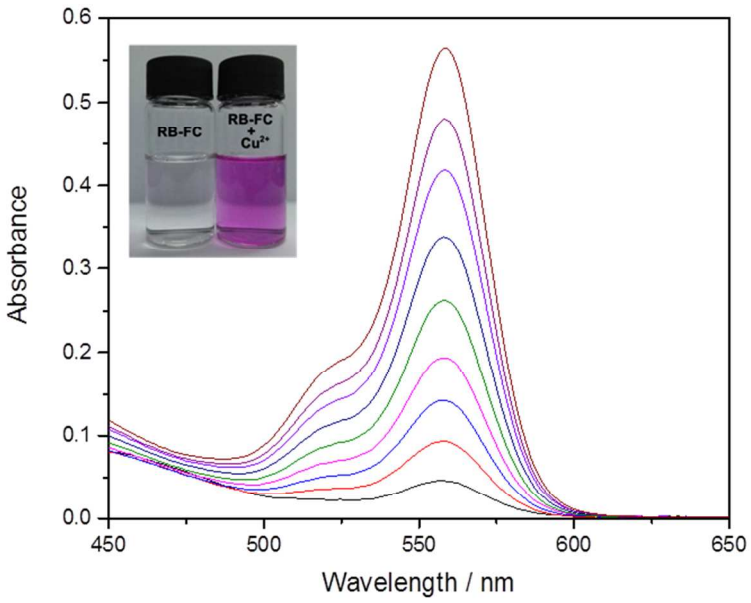
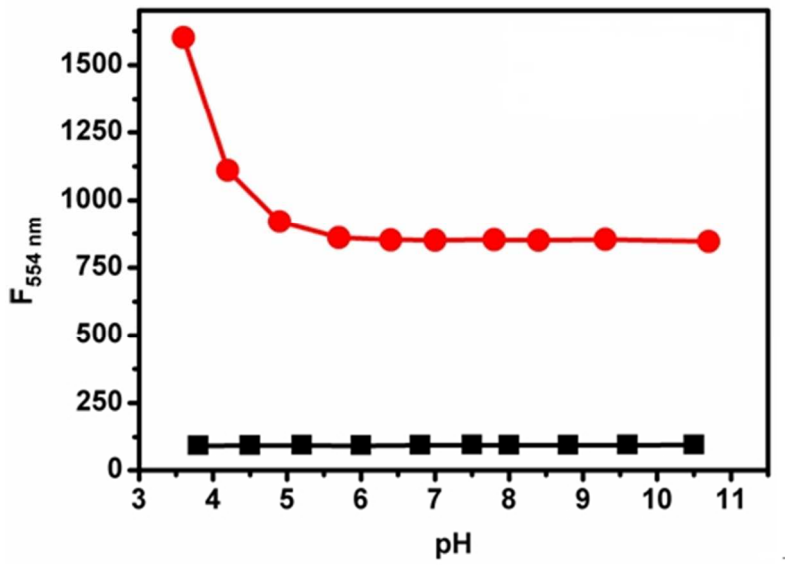



**Figure S5**. XRD of UCNP ($NaYF_4$: 20%Yb, 1.8%Er, 0.5%Tm) and standard pattern of β–$NaYF_4$ (JCPDS No.16-0334).




(a)




(b)

**Figure S6**. (a) Absorption spectrum of 26 μM RB-FC with the addition of $Cu^{2+}$ from 4-20 μM in DMSO. The inset is the color change from light green to red. (b) Fluorescence intensity at 554 nm in the absence (black) and presence (red) of 3 equiv. $Cu^{2+}$ in EtOH/$H_2O$ (1:1, v/v) at different pH. The excitation wavelength was 500 nm

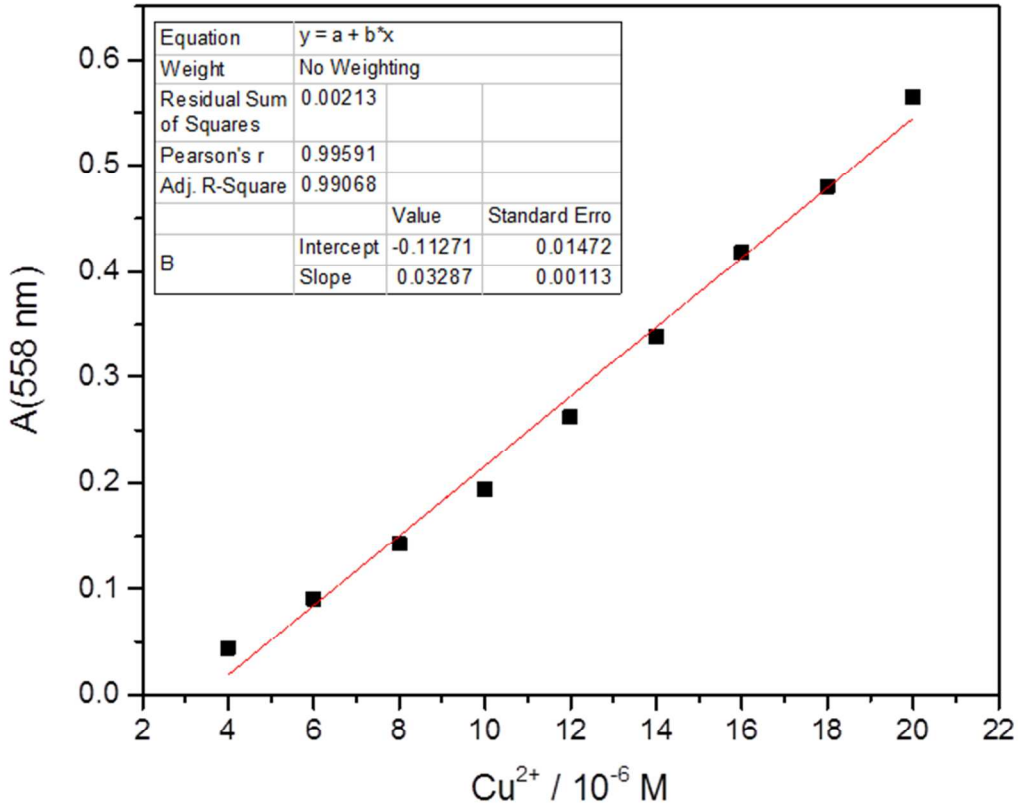



**Figure S7**. Linear relationship between absorption at 558 nm and $Cu^{2+}$ concentration.

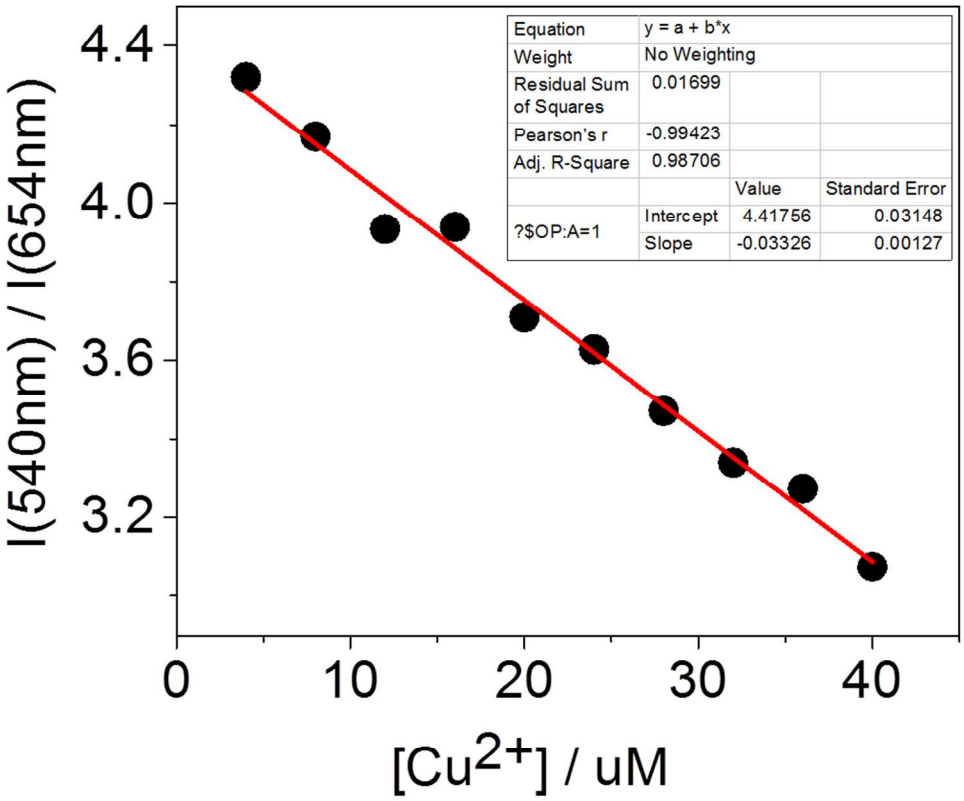



**Figure S8**. Linear relationship between UCL intensity ratio of 540 nm to 654 nm and $Cu^{2+}$ concentration.

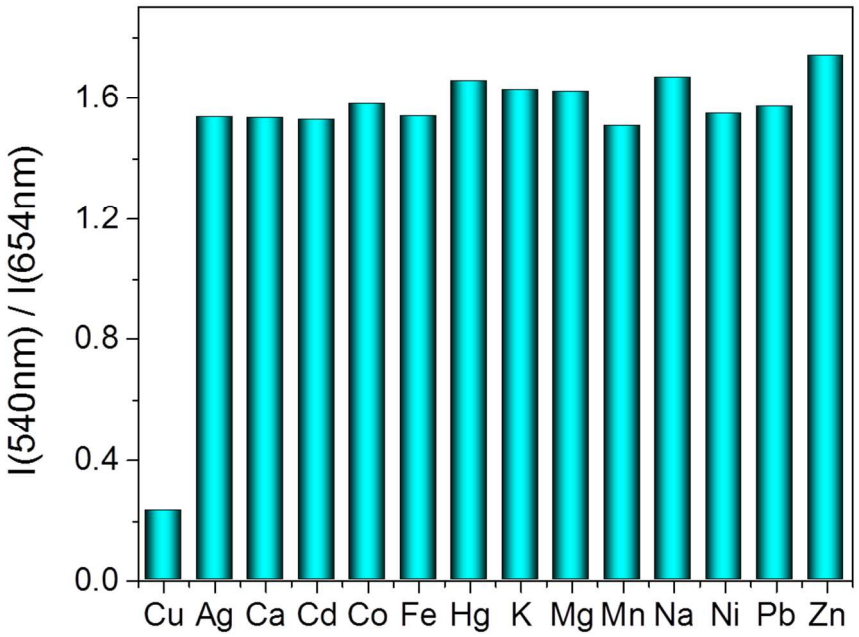



**Figure S9**. Selectivity test of RB-FC-UCNPs to $Cu^{2+}$ over other metal ions.

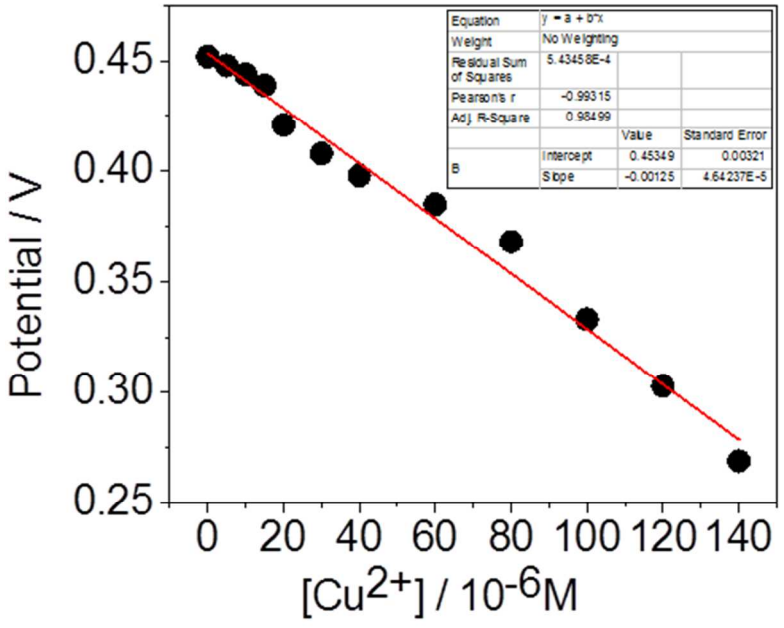



**Figure S10**. Linear relationship between reduction peak value and $Cu^{2+}$ concentration.

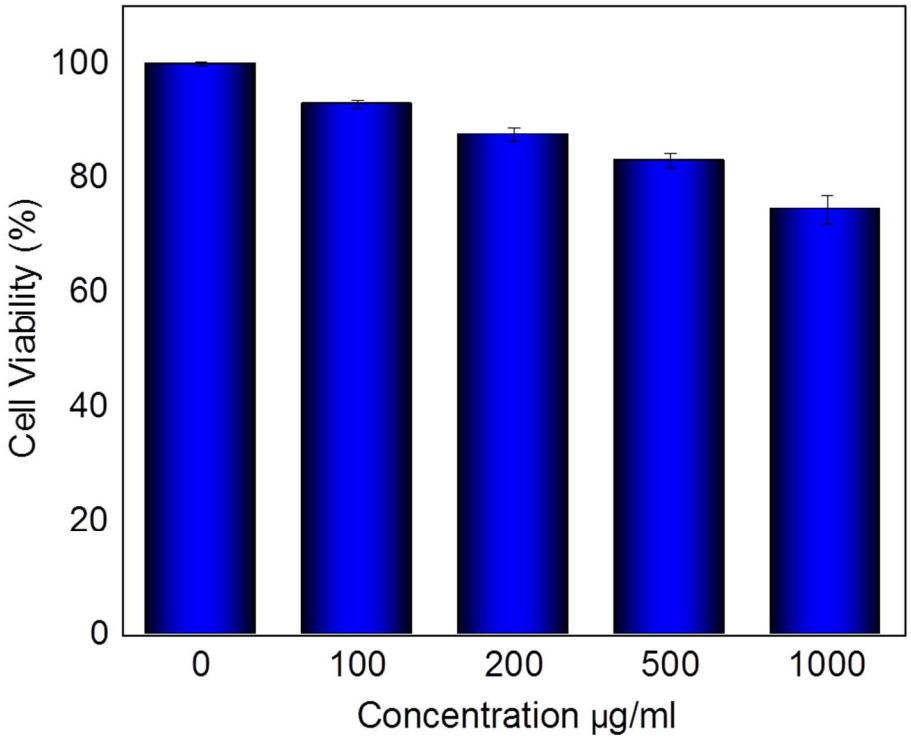



**Figure S11**. Cell viability was quantified by MTT assay (A549 cells, 24h).

References:

(1) Li, Z.; Zhang, Y.; Jiang, S. Multicolor Core/Shell-Structured Upconversion Fluorescent Nanoparticles. *Adv. Mater.* **2008,***20* (24), 4765-4769.
(2) Wang, X.; Zhuang, J.; Peng, Q.; Li, Y. A general strategy for nanocrystal synthesis. *Nature* **2005,***437* (7055), 121-124.
(3) Liu, Q.; Feng, W.; Yang, T.; Yi, T.; Li, F. Upconversion luminescence imaging of cells and small animals. *Nat. Protoc.* **2013,***8* (10), 2033-2044.
(4) Qian, H.-S.; Zhang, Y. Synthesis of Hexagonal-Phase Core−Shell NaYF4 Nanocrystals with Tunable Upconversion Fluorescence. *Langmuir* **2008,***24* (21), 12123-12125.