# Supporting information

## High-Efficiency in Vitro and in Vivo Detection of $Zn^{2+}$ by Dye-Assembled Upconversion Nanoparticles

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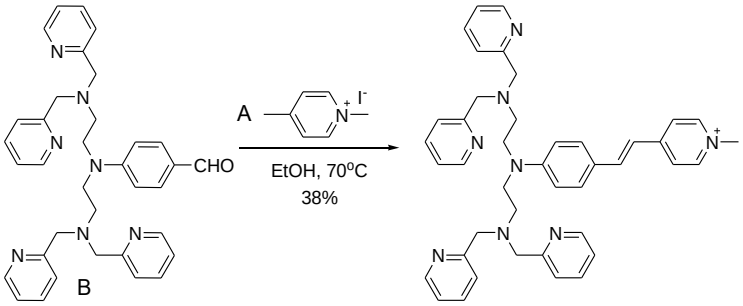
**Scheme S1. Synthetic route of ligand compound 1.**




**Synthesis of compound 1**

Compound **1** was synthesized by the reported paper,[1] typically, to a solution of B (57.2 mg, 0.1 mmol) in EtOH (4 mL), A (35.5 mg, 0.15 mmol) and pyrimidine (10 μL) were added. The reaction mixture was allowed to stir in the microwave oven (80 W) at 70 $^{o}$C for 1 hr. Removal of the organic solvent gave the crude product, which was further purified by reverse phase semi-prep HPLC (Gilson RP-HPLC with a C18 column (100 mm × 21.2 mm, Axia column from Phenomenex Inc.) using water and acetonitrile as eluents to give the pure product compound **1** as red oil (30.0 mg, Yield: 38%).

**Compound 1** $^{1}$H NMR (500 MHz, DMSO-$d_6$) δ 2.55 (4H, t, $J$ = 7.1 Hz), 3.79 (8H, s), 4.16 (3H, s), 6.41 (2H, d, $J$ = 8.9 Hz), 7.10 (1H, d, $J$ = 15 Hz), 7.22-7.25 (4H, m), 7.35 (2H, d, $J$ = 8.8 Hz), 7.47-7.48 (4H, m), 7.70-7.73 (4H, m), 7.80 (1H, d, $J$ = 15 Hz), 8.01 (2H, d, $J$ = 6.8 Hz), 8.47 (4H, d, $J$ = 4.2 Hz), 8.63 (2H, d, $J$ = 6.7 Hz) $^{13}$C NMR (125 MHz, DMSO-$d_6$) δ 46.30, 48.44, 50.02, 60.02, 111.21, 116.64, 121.89, 122.00, 122.18, 122.68, 130.15, 136.48, 141.74, 144.17, 148.72, 149.48, 153.35, 158.98. HRMS-ESI: calculated for $[M]^+$ ($C_{42}H_{45}N_8$) 661.3762, found 661.3755

IUPAC name of compound **1**:

(E)-4-(4-(bis(2-(bis(pyridin-2-ylmethyl)amino)ethyl)amino)styryl)-1-methylpyridin-1-ium.

**Reaction mechanism between compound 1 and $Zn^{2+}$**

Based on the multiple pyridyl $Zn^{2+}$ chelating agent structure and previously reported $Zn^{2+}$ fluorescent sensors, we conclude that intracellular charge transfer (ICT)

plays a major role in the emission shift. ICT usually occurs by company of adiabatic photoreactions that lead to two conformations of one molecule, with difference in the dihedral angle between amino group and phenyl ring.[2, 3] Therefore ICT leads to a different sub-excited level (charge transfer state) that will induce wavelength shift (**Figure S1**).[4] In our sensing system, compound **1** with absorption peak at 475 nm was chosen as energy acceptor because of their wide spectral band overlap with blue UCL (475 nm) of UCNPs. In the presence of $Zn^{2+}$, the coordination between $Zn^{2+}$ and compound **1** leads to a significant blue-shift from 475 to 360 nm of compound **1**, (**Figure S9**) thereby resulting in energy mismatch between compound **1** and UCNPs. Other metal ions did not show this blue-shift, demonstrating that there is no ICT.

**Table S1** Properties of fluorescent $Zn^{2+}$ probes

| Probes | $\lambda_{ex}$ nm | $\lambda_{em}$ nm | Φ free | Application | Solution |
|---|---|---|---|---|---|
| ZP1 [5] | 505 | 525 | 0.13 | HeLa cells | HEPES (pH=7.5, 50 mM, 100 mM KCl) |
| ZS5 [6] | 497 | 522 | 0.36 | HeLa cells | PIPES (pH=7, 50 mM, 100 mM KCl). |
| ZP3 [7] | 502 | 521 | 0.15 | Live Hippocampal Neurons | PIPES (pH=7, 50 mM, 100 mM KCl). |
| WZS [8] | 449 | ~ 550 | 0.03 | HeLa cells | Tris-HCl (pH=7.0, 0.1 M) Ethanol:water=1:9, v/v, |
| NBD-TPEA [9] | 469 | 550 | 0.003 | HeLa cells, zebrafish | HEPES (pH=7.4, 50 mM, 100 mM $KNO_3$,), DMSO:water=1:9 |
| CTMPA [10] | 510<br>670 | 730<br>590 | 0.033 | C2C12 cells, zebrafish | HEPES (pH=7.4, 10 mM, 10% $CH_3CN$) |
| Eu·L [11] | 280 | 616 | --- | --- | Buffered aqueous |
| Eu-7 [12] | 320 | 614 | --- | HeLa cells | HEPES (pH=7.4, 100 mM) |
| DTH-$Fe_3O_4$@$SiO_2$ NPs [13] | 390 | ~470 | --- | --- | Ethanol |
| Dye-2@sensor-1 core–shell silica NPs [14] | 320 | ~482 | --- | HeLa cells | Tris-HCl (pH=7.10, 0.01 M) |
| PEG-b-P($MEO_2$MA-*co*-OEGMA-*co*-ZQMA) [15] | 364 | 482 | --- | HeLa cells | Phosphate buffer (pH=7.4) |
| Mesoporous silica [16] | 324 | 483 | --- | --- | HEPES (pH = 7.0, 0.05 M) Ethanol:water = 3:7 |
| Gold [17] | Colorimetric λ=600 | --- | --- | --- | Borate buffer (pH=7.4,50 mM) |
| ZincBY-1 [18] | 533 | 560 | 0.052 ±0.002 | Mammalian egg | HEPES (pH=7.2, 50 mM, 100 mM $KNO_3$) |

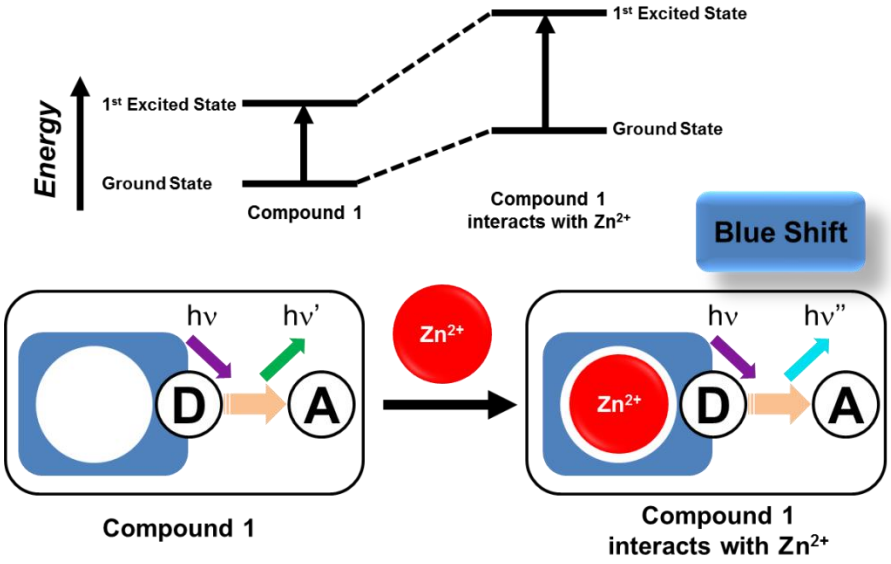



**Figure S1**. A simple scheme of ICT process.

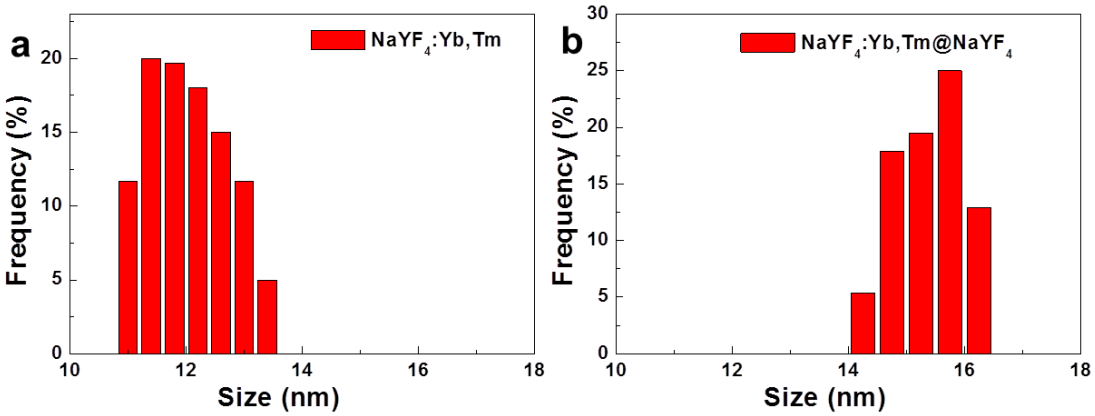



**Figure S2.** Size distribution of $NaYF_4$:Yb/Tm and $NaYF_4$:Yb/Tm@$NaYF_4$

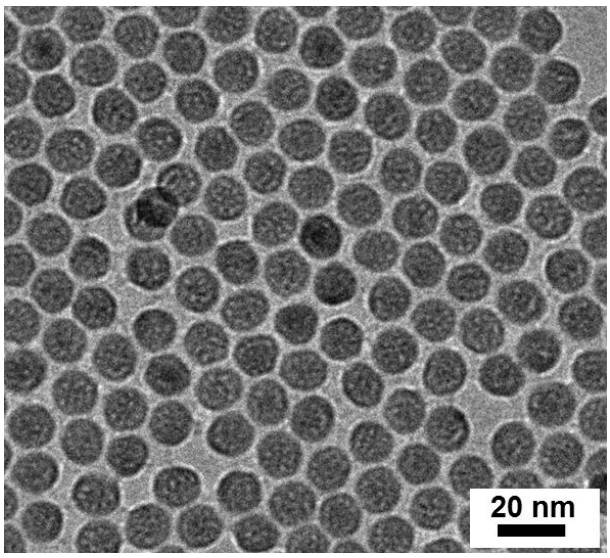



**Figure S3.** TEM images of OA-$NaYF_4$:Yb/Tm.

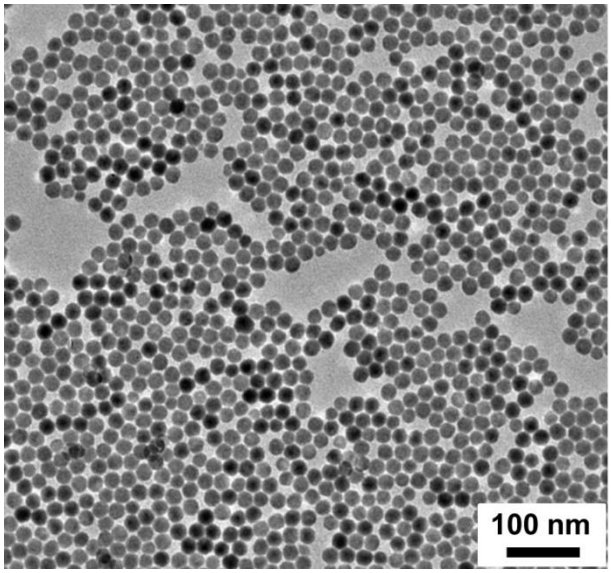



**Figure S4.** TEM images of bare $NaYF_4$:Yb/Tm@$NaYF_4$.

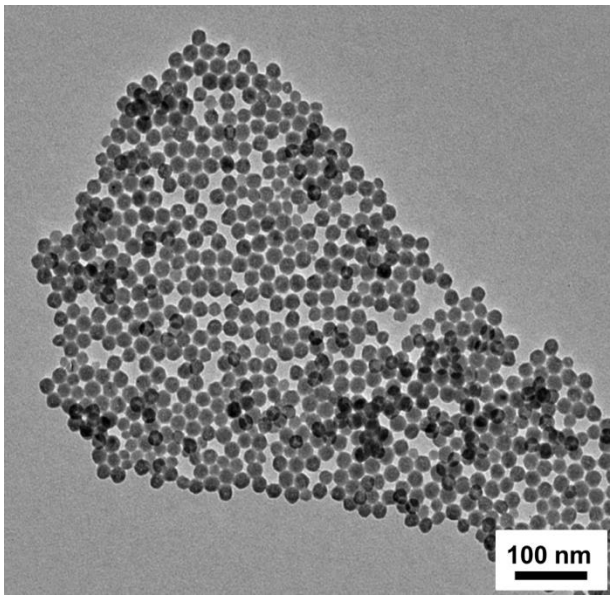



**Figure S5.** TEM images of PAA-$NaYF_4$:Yb/Tm@$NaYF_4$.

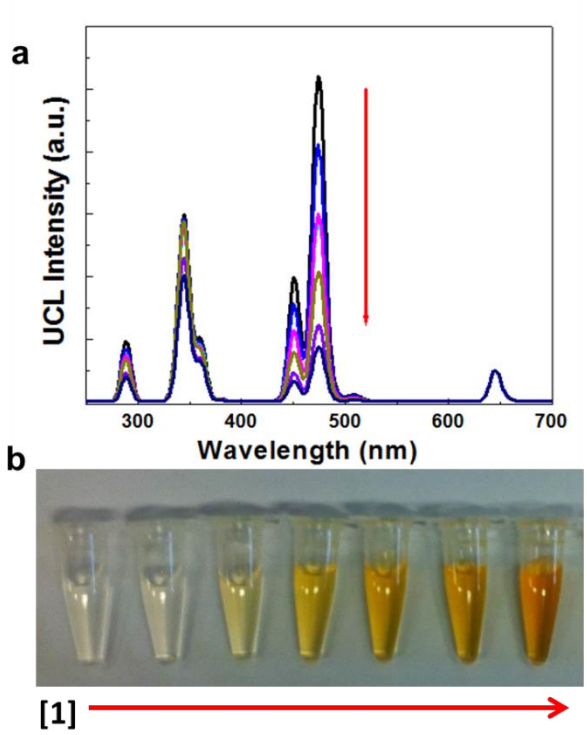



**Figure S6.** Normalized upconversion emission spectra (a) of UCNPs modified with compound **1** at different concentrations (0-0.1 mM). The concentrations of UCNPs are fixed at 500 μg/mL. (b) Corresponding photographs of the samples shown in (a), showing a tunable solution color change as a function of compound **1** concentration.

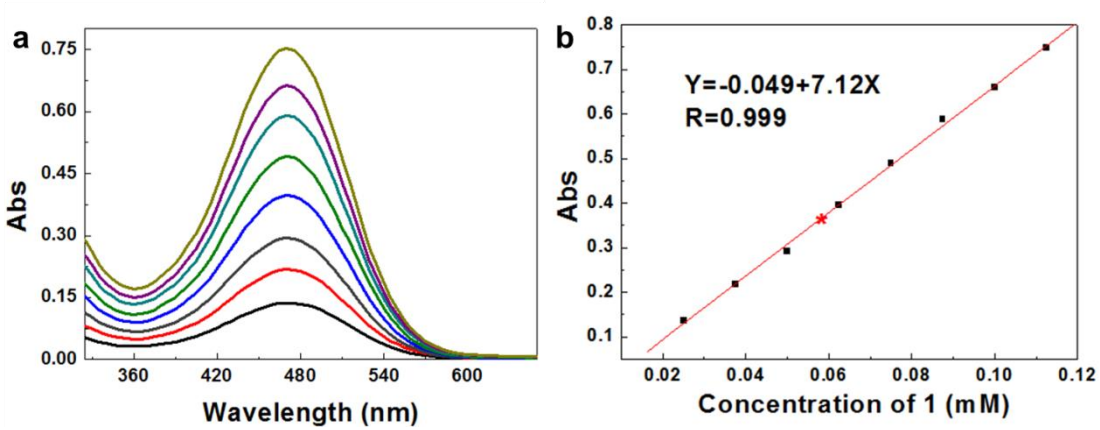



**Figure S7.** (a) The absorption spectra of free compound **1** with different concentrations (0.05-0.113 mM). (b) The absorbance at 475 nm as a function of compound **1** concentration. The amount of compound **1** loaded on the UCNPs surface was determined as 0.058 mM (0.5 mg/mL UCNPs).

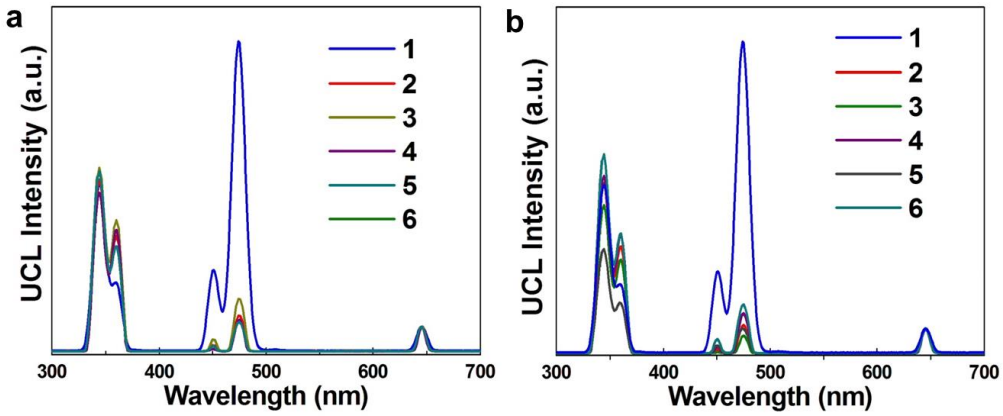



**Figure S8** (a) UCL spectra of 1, PAA-UCNPs dispersed in water, and **1**-PAA-UCNPs dispersed in different media: 2, water, 3, PBS (0.1 M, pH=7.0) 4, HEPESS (1 M, pH =7.0), 5, DMEM medium (containing 10% FBS) or 6, cell extracts. (b) UCL spectra of as-prepared nanoprobes dispersed in different media for 24 hr.

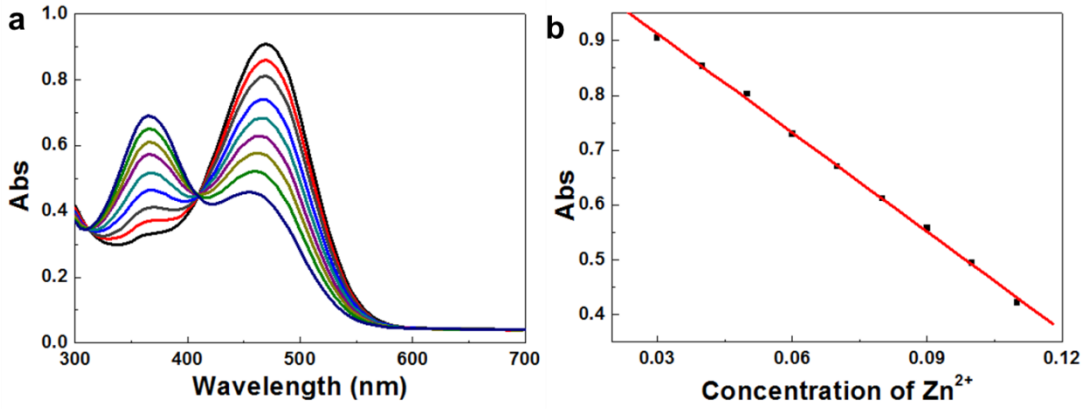



**Figure S9.** (a) Changed in the absorption spectra of compound **1** (0.9 mM) in the aqueous solution upon gradual addition of $Zn^{2+}$ ions (from 0 to 115 μM). (b) Plot of absorption intensity at 475 nm as a function of $Zn^{2+}$ concentration.

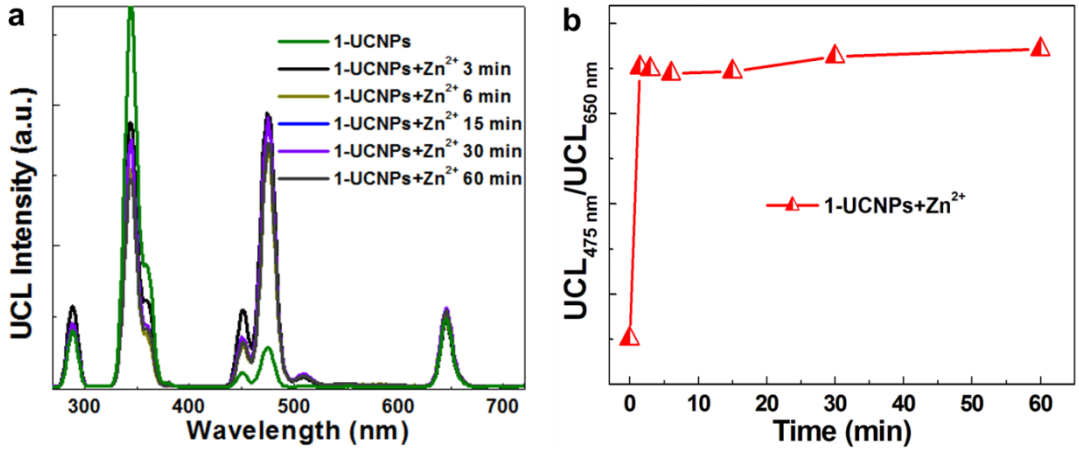



**Figure S10.** (a) Photoluminescence response of **1**-PAA-UCNPs (1 mg/mL) incubated with $Zn^{2+}$ (0.1 mM) at different time. (b) Plot of Photoluminescence intensity at 475 nm as a function of time.

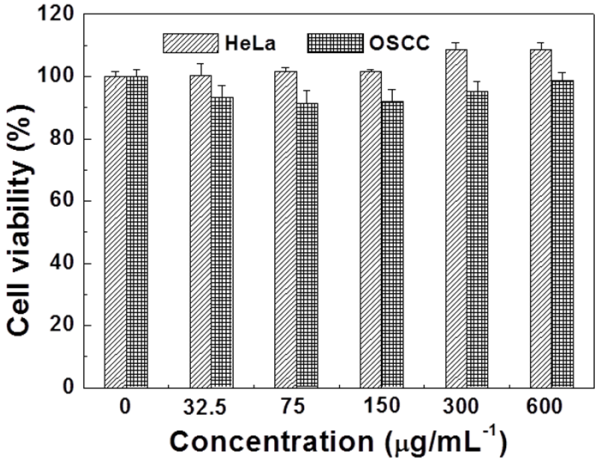



**Figure S11.** *In vitro* cell viability of HeLa and OSCC cells incubated with PAA-UCNPs at different concentrations (0, 32.5, 75, 150, 300 and 600 μg/mL) for 24 hr at 37 ℃.

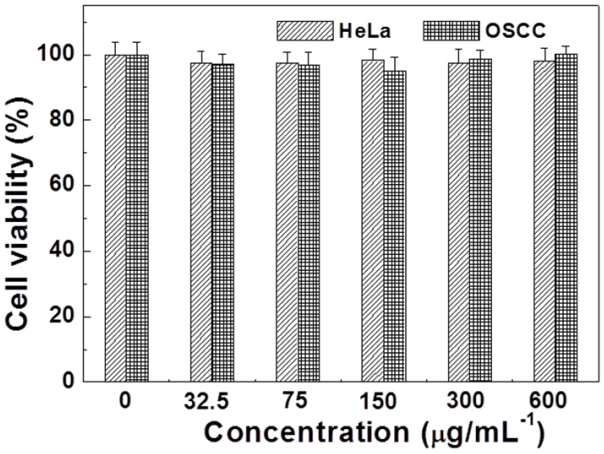



**Figure S12.** *In vitro* cell viability of HeLa and OSCC cells incubated with **1**-PAA-UCNPs at different concentrations (0, 32.5, 75, 150, 300 and 600 μg/mL) for 24 hr at 37 ℃.

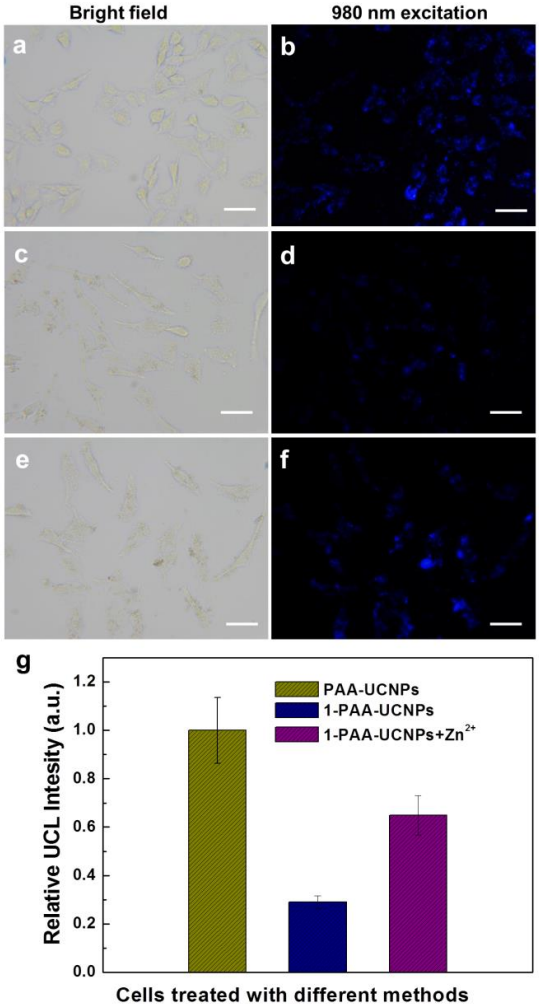



**Figure S13**. (a, b) Bright-field images and upconversion luminescence images of HeLa cells incubated with 500 μg/mL PAA-UCNPs for 2 hr. (c, d) Upconversion luminescence images and bright-field images of HeLa cells incubated with 500 μg/mL **1**-PAA-UCNPs for 2 hr, and then incubated with 0.25 mM $Zn^{2+}$ for 30 min (e, f). (g) Normalized UCL intensity of HeLa cells measured after treatment with different chemicals and upconversion nanoparticles. The scale bars are 10 μm.

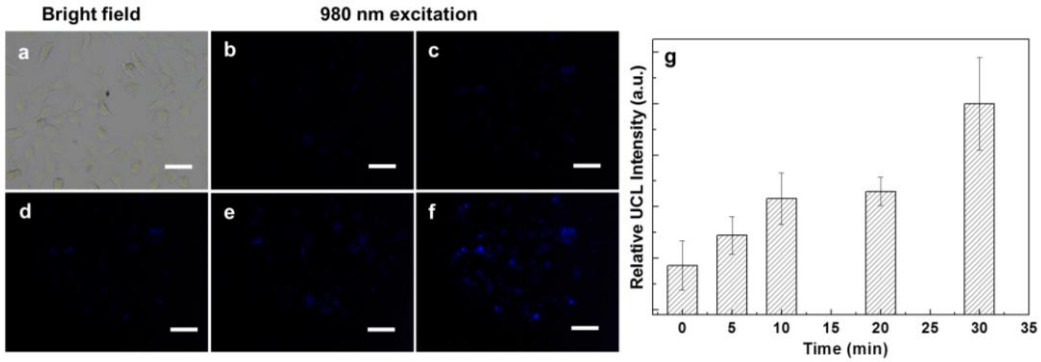



**Figure S14**. (a) Bright-field images and upconversion luminescence images of OSCC cells incubated with 500 μg/mL **1**-PAA-UCNPs for 1 hr. (b, c, d, e, f) Upconversion luminescence images OSCC cells incubated with 500 μg/mL 1-PAA-UCNPs for 1 hr, and then incubated with 0.25 mM $Zn^{2+}$ at different time, 0, 5, 10, 20, 30 min. The scale bars are 10 μm. (g) Normalized UCL intensity of OSCC cells measured after treatment with **1**-PAA-UCNPs and $Zn^{2+}$ at different time.

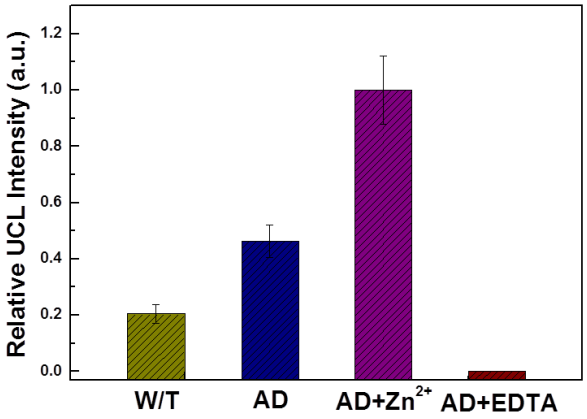



**Figure S15**. Normalized UCL intensity of wild-type tissue (W/T) and AD brain tissue without treating (AD), AD brain tissue pre-incubation with 100 μM $ZnCl_2$ for 1 hr ($AD+Zn^{2+}$), AD brain tissue pre-incubation with 5 mM EDTA for 1 hr (AD+EDTA), then stained with **1**-PAA-UCNPs (500 μg/mL).

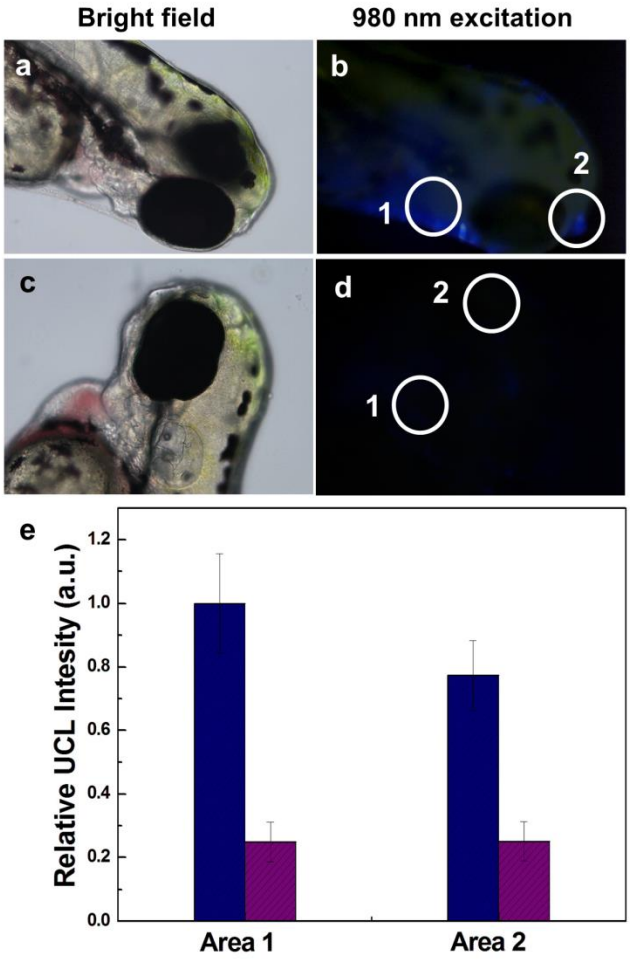



**Figure S16**. Bright-field image (a) and UCL images (b) of 3-day-old zebrafish incubated with 500 μg/mL **1**-PAA-UCNPs for 2 hr. Bright-field (c) and upconversion luminescence images (d) of zebrafish incubated with EDTA 250 μM for 2 hr before **1**-PAA-UCNPs incubate for 2 hr, the total length is ~3 mm. (e) Normalized UCL intensity of Zebra fish measured different area after treatment with different chemicals and upconversion nanoparticles. Zebrafish incubated with 500 μg/mL **1**-PAA-UCNPs (500 μg/mL) for 2 hr. Zebrafish incubated with EDTA 250 μM for 2 hr before **1**-PAA-UCNPs (500 μg/mL) incubate for 2 hr,

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