Electronic Supplementary Information (ESI) for Nanoscale
This journal is © The Royal Society of Chemistry 2017

Versatile Upconversion Surface Evaluation Platform for Bio-nano Surface Selection for Nervous System

Libing Fu, [a,b,c] *Marco Morsch,* [b] *Bingyang Shi,** [a,b,c] *Guoying Wang,* [b] *Albert Lee,* [b] *Rowan Radford,* [b] *Yiqing Lu,* [a,c,d] *Dayong Jin,* [e] *and Roger Chung* [b]

[a] International Joint Center for Biomedical Innovation, School of Life Sciences, Henan University, Jin Ming Avenue, Kaifeng, Henan, 457004, China
[b] Department of Biomedical Sciences, Faculty of Medicine & Health Sciences, Macquarie University, Sydney, NSW 2109, Australia
[c] ARC Centre for Nanoscale Biophotonics, Macquarie University, Sydney, NSW 2109, Australia
[d] Department of Physics and Astronomy, Faculty of Sciences & Engineering, Macquarie University, Sydney, NSW 2109, Australia
[e] Institute for Biomedical Materials and Devices(IBMD), Faculty of Science, University of Technology Sydney, Sydney, NSW2007, Australia
E-mail: bingyang.shi@mq.edu.au

Methods:

Synthesis of the Original $NaYF_4$:20%Yb,4%Tm upconversion nanocrystals (UCNPs). The original UCNPs were synthesized following an oxygen-free protocol described in the literature.[1] In a typical procedure, 3.5 mL of methanol solution (0.76mmol YCl_3, 0.2mmol $YbCl_3$ and 0.04mmol Tm) was magnetically mixed with OA (6.5 mL) and ODE (15 mL) in a 100 mL three-neck round-bottom flask. The mixture was degassed under Ar flow and heated to 150 ˚C for 30 min to form a clear solution, then cooled to room temperature. 5 mL of methanol solution containing NH_4F (4 mmol) and NaOH (2.5 mmol) was added into the solution of YCl_3 in ODE and stirred for 30 min at room temperature. The mixture solution was slowly heated up to 110 ˚C and kept at 110 ˚C for 30 min to remove methanol and water completely. Then the mixture solution was quickly heated to reaction temperature 310˚C and aged for 1.5 h. After the solution cooled down to room temperature, ethanol was added to precipitate the nanocrystals. After the product was washed with cyclohexane, ethanol and methanol for 3 times, the final $NaYF_4$ nanocrystals were re-dispersed in 10mL cyclohexane at 4°C for further use.

Synthesis of OA-free UCNPs. OA ligand on the UCNPs surface was removed according to literatures[2-4] with modifications. 1ml of cyclohexane containing oleate-capped UCNPs (20 mg/ml) was added into 4ml absolute ethanol and performed with sonicating for 10min. The solution turned to be cloudy, because the UNCPs were participated. Then the solution was centrifuge for 10min at 10000 rpm to separate UCNPs. The as prepared UCNPs was dispersed in aqueous solution (5 mL) and the pH was adjusted to 2 by adding 0.5 m HCl solution. The reaction was performed with sonication for 10min. During this reaction the carboxylate groups of the oleate ligand were protonated (to yield oleic acid). The UCNPs in the solution were recuperated and treated with 5ml HCl solution (pH 4) by sonicating for 20 min. Afterwards, the particles were recuperated and washed with ethanol for three times. Finally, the particles were dispersed in water.

NSC-34 Cell Culture. Mouse NSC-34 hybrid cell line (NSC-34; kindly provided by Dr. Vinod, University of Macquarie) were cultured in Dulbecco's modified Eagle's medium (DMEM), including 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin solution (P/S).[5] Cells were maintained under standard conditions (humidified, 5% CO_2, 37°C). Cells were subcultured every 3-4 days. The in vitro experiments were designed at different concentrations of UCNPs and different incubation time. After the incubation the cells were washed with phosphate buffered saline (PBS) buffer for three times. Control experiments were performed with growth medium without nanoparticles.

Cell viability assays. For the viability assays, 1.0×10^4 NSC-34 cells were seeded into a 96-well plate (200μL medium/well) and incubated overnight at 37 °C with 5% CO_2. The media was replaced with increasing UCNPs concentrations (0, 1, 10, 20, 50 and 100mg/ml). The plates were incubated for 24 h. a) A colorimetric MTT assay with minor modifications was conducted by adding 10uL of the 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium-bromide (MTT, 5.0 mg/mL in PBS) to each well. Cells were further incubated at 37 °C for 4 h to allow precipitation of insoluble purple formazan crystals. Then, the supernatant was carefully removed, and 150uL dimethyl sulfoxide (DMSO) was added to the wells and left for 2h in the dark at room temperature. Finally, the absorbance was measured using the Biotek Microplate Reader (Biotek, U.S.A.) at a wavelength of 570 nm. The control well was assumed have 100% viability. Therefore, the viable cells number was calculated based on the absorbance of the control cultures. b) LDH assay followed the LDH Assay Kit (601170): 1.0×10^4 of cells

at a density of 5 × 10^5 cells/ml (DMEM containing 10% FBS) were seeded in each well of 96-well plates and grown for 24 h before NP incubation. The cells were washed with PBS three times and dosed with different concentrations of NPs (0, 1, 10, 20, 50 and 100mg/ml) in DMEM medium containing 10% FBS. After 24 h incubation, 20ul of Triton X-100(10%) solution was added to three wells and 20ul of Assay Buffer was added to another three wells before incubation at room temperature for one hour. Transfer 100ul of cell supernatant to a new 96-well assay plate. 100ul of Reaction Solution was added to each well. After that, the plate was incubated with gentle shaking on an orbital shaker (150rpm) for 30 minutes at 37 degrees. Finally, the absorbance of the plate was measured at 490nm with Biotek Microplate Reader (Biotek, U.S.A.). The LDH activity of the samples was obtained by measuring the decreasing rate of NADH absorbance over time.

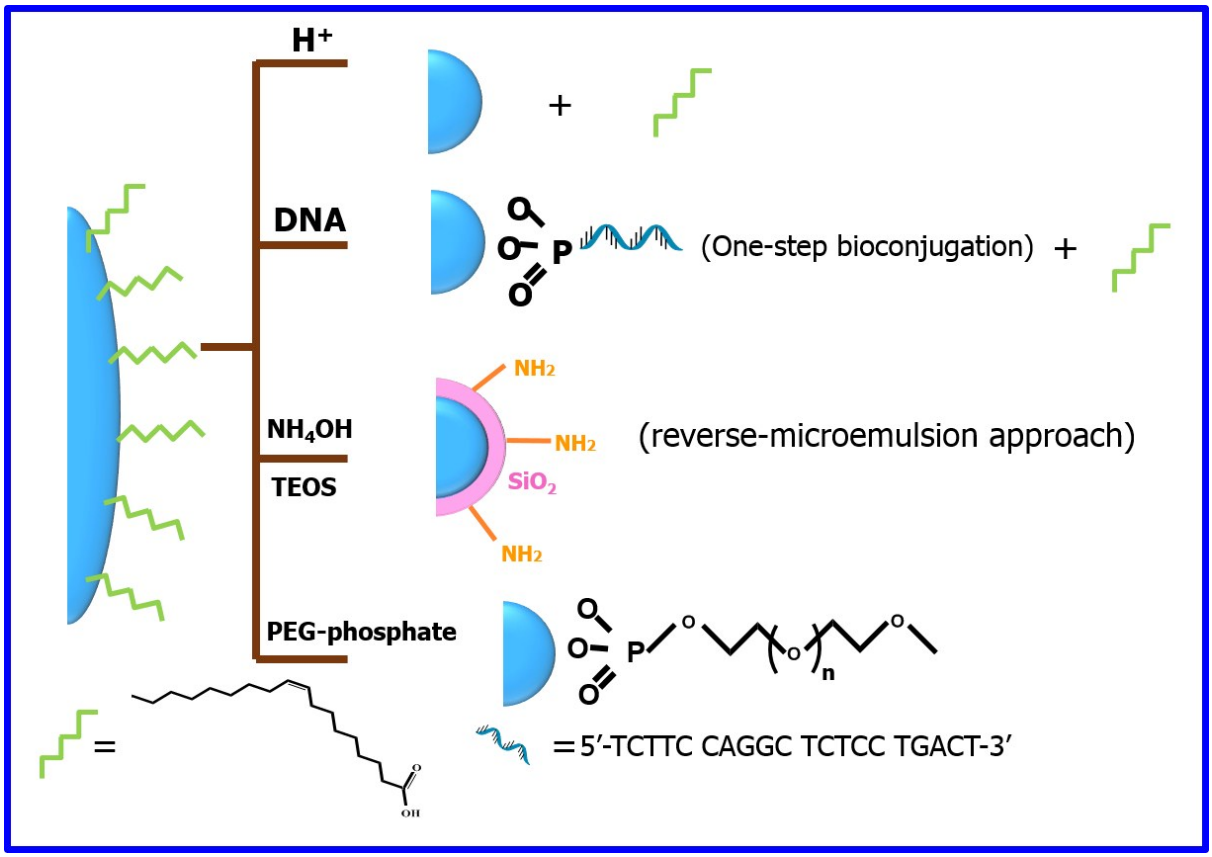

Fig. S1 Schematic illustration showing the fabrication of the surface-modified UCNPs: OA-free (employing acid to remove OA on the surface), DNA-modified (using one-step bioconjugation approach), Silica-coated (utilizing reverse-microemulsion method) and PEG-COOH functionalized (ligand exchange approach) UCNPs.

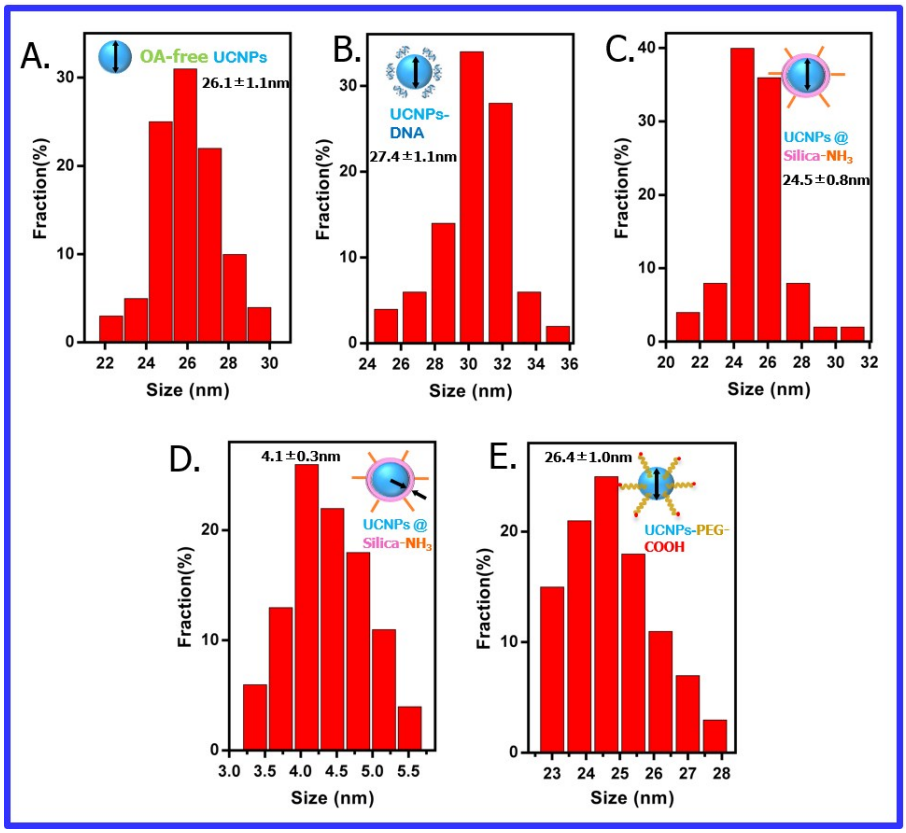

Fig S2. The size distribution characterization of the UCNPs core. A) OA-free, B) DNA-modified, C) silica-coated, D) silica layer of (C) and E) PEG-capped UCNPs. The size of upconversion core of these types of modified UCNPs are similar (about 26nm~28nm).

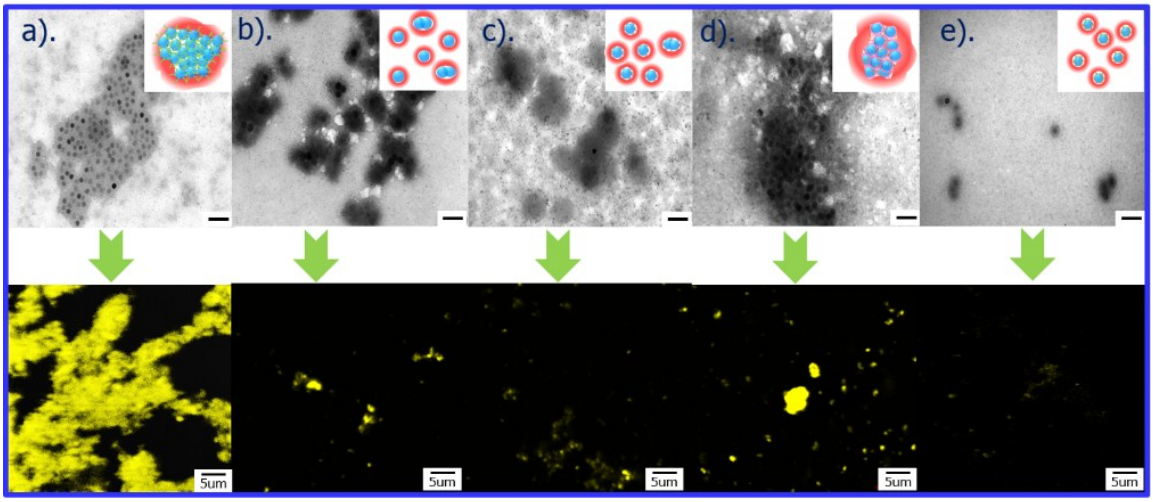

Fig. S3 TEM images of the UCNPs (the upper row): unmodified (a), DNA-modified (b), OA-free (c), Silica-coated (d) and PEG-COOH functionalized UCNPs dispersed in cell culture media for 4h at 37 °C. The corresponding UCNPs (the bottom row) imaged with confocal microscope under 980nm laser excitation.

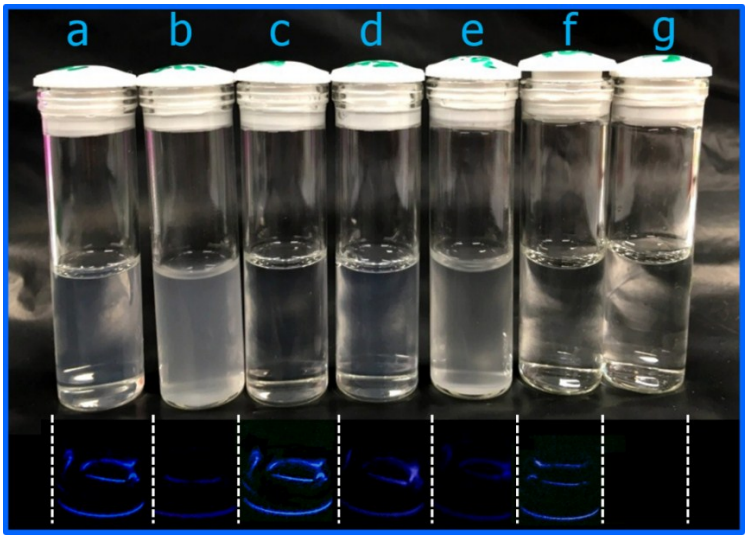

Fig. S4 The photograph of the upcoversion nanoparticles: a) OA-capped UCNPs in 1wt% cyclohexane solution (as the positive control for comparison); b) OA-capped UCNPs, c) OA-free UCNPs, d) UCNPs-DNA, e)UCNPs@Silica-NH_2, f) UCNPs-PEG-COOH (b-f were dispersed in 1wt% water) and MiliQ water (as the blank control). The bottom row showed the upconversion fluorescence (blue) of corresponding UCNPs under 980nm NIR excitation. Note the NIR emission of $NaYF_4$:20%Yb,4%Tm at 800nm is invisible to both the human eye and the camera.

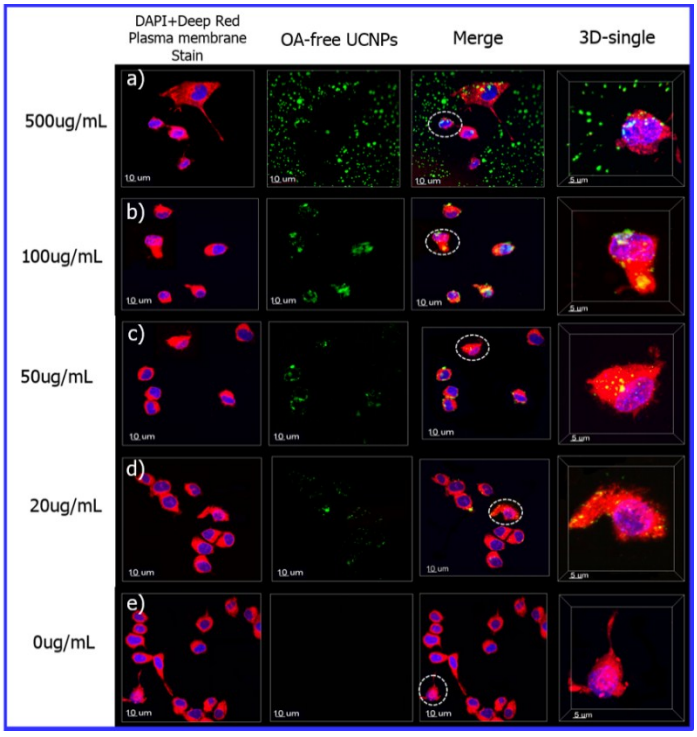

Fig. S5 Confocal microscopy images of NSC-34 cells after 4h incubation at 37°C with OA-free UCNPs at different concentrations (a-e: 500ug/ml, 100ug/ml, 50ug/ml, 20ug/ml and 0ug/ml): Fluorescent images of cell nucleus (blue, stained with Hoechst), cell membrane (red, labeled with deep red plasma membrane stain) and OA-free UCNPs (set to green, upconversion luminescence of UCNPs collected at 800nm), overlays of cell images & OA-free UCNPs and 3D-single cell with OA-free UCNPs. The OA-free UCNPs were utilized as sample for optimization the concentration of UCNPs used for cell uptake experiment.

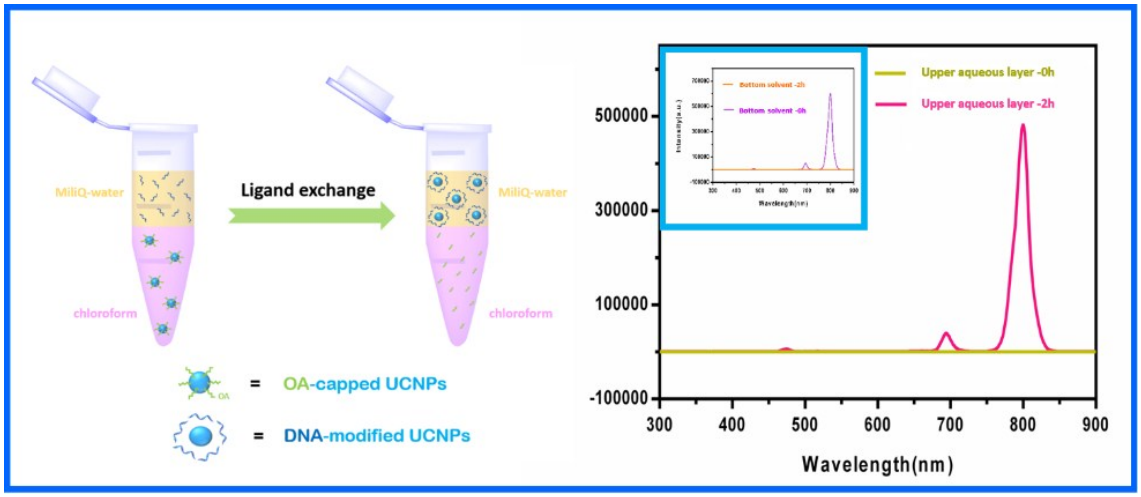

Fig. S6 The change of emission spectrum in the upper aqueous layer after and before 2h-incubation of original UCNPs with ssDNA. The inset figure shows the change of UCNPs-containing organic solvent before and after incubation with ssDNA. The negligible spectrum cure after incubation suggests that nearly all the UCNPs were successfully transferred out from the bottom solvent layer to the upper aqueous layer.

Supplementary References

[1] Z. Q. Li, Y. Zhang and S. Jiang, *Adv. Mater.*, 2008, **20**, 4765-4769.
[2] Z. Yu, Q. Sun, W. Pan, N. Li and B. Tang, *ACS nano*, 2015, **9**, 11064-11074.
[3] L. Zhao, J. Peng, Q. Huang and C. Li, *Adv. Funct. Mater.*, 2014, **24**, 363-371.
[4] N. Bogdan, F. Vetrone, G. A. Ozin and J. A. Capobianco, *Nano letters*, 2011, **11**, 835-840.
[5] N. R. Cashman, H. D. Durham, J. K. Blusztajn, K. Oda, T. Tabira, I. T. Shaw, S. Dahrouge and J. P. Antel, *Dev. Dyn.*, 1992, **194**, 209-221.
[6] D. K. Yi, S. T. Selvan, S. S. Lee, G. C. Papaefthymiou, D. Kundaliya and J. Y. Ying, *J. Am. Chem. Soc.*, 2005, **127**, 4990-4991.