Electronic Supplementary Information For

Multifunctional magnetic-fluorescent eccentric-(concentric-Fe_3O_4@SiO_2)@polyacrylic acid core-shell nanocomposites for cell imaging and pH-responsive drug delivery

Lu Li,[a] Cong Liu,[a] Lingyu Zhang,[a] Tingting Wang,[b] Hong Yu,*[c] Chungang Wang,*[a] and Zhongmin Su,[a]

[a] Faculty of Chemistry, Northeast Normal University Changchun 130024, (P. R. China)

E-mail: wangcg925@nenu.edu.cn; yuhong622@yahoo.cn

[b] School of Chemistry & Environmental Engineering, Changchun University of Science and Technology, Changchun, 130022, P. R. China

[c] Jilin Province Institute of Cancer Research , Changchun 130041, P. R. China.

EXPERIMENTAL SECTION

Materials. Superparamagnetic iron oxide NPs (Fe_3O_4 NPs) protected by oleylamine (OMA) and oleic acid (OA) were obtained as a gift from Ocean Nano Tech. 3-Aminopropyltrimethoxysilane (APTMS, 95%), tetraethyl orthosilicate (TEOS, ≥98%), cetyltrimethylammonium bromide (CTAB, ≥99%), fluorescein isothiocyanate (FITC), doxorubicin hydrochloride (DOX) were purchased from Sigma (USA). Anhydrous ethanol, isopropyl alcohol, ytterbium (III) chloride hexahydrate ($YbCl_3·6H_2O$), erbium (III) chloride hexahydrate ($ErCl_3·6H_2O$), gadolinium(III) chloride hexahydrate ($GdCl_3·6H_2O$), oleic acid (OA), ammonium fluoride (NH_4F), and aqueous ammonia solution were purchased from Sinopharm Chemical Reagent Beijing Co., Ltd and used without further purification. Polyacrylic acid (PAA, $M_w \approx 1800$) was obtained from Sigma-Aldrich. Deionized water was used in all experiments.

Characterization. FTIR spectra were obtained on a Magna 560 FTIR spectrometer (Nicolet, USA). The magnetic measurement was carried out by using a superconducting quantum interference device magnetometer (SQUIDMPMS XL-7) with fields up to 1.0 T. Particle size distribution was measured on a Mastersizer 2000 laser particle size analyzer. Transmission electron microscopy (TEM) was performed on a TECNAI G2 F20 transmission electron microscope under 200 kV accelerating voltage. Transmission electron microscopy (TEM) was performed with a JEOL-100CX electron microscope under 80 kV accelerating voltage. Fluorescence spectra were performed with Eclipse fluorescence spectrophotometer (Varian, USA) with the excitation wavelength of 458 nm. Confocal laser scanning microscopy (CLSM) was operated on Olympus Fluoview FV1000. Fluorescence spectra were performed with Eclipse fluorescence spectrophotometer (Varian, USA). UV–Vis absorption spectroscopy was obtained on U-3010 spectrophotometer (Hitachi, Japan). Particle size distribution was measured on a Mastersizer 2000 laser particle size analyzer.

Synthesis of CTAB modification of Fe_3O_4 NPs. Monodisperse CTAB modified Fe_3O_4 NPs were prepared according to the previous report.[1] Typically, 400 μL of oleic acid modification of Fe_3O_4 NPs (~ 25 nm in diameter) were treated with 8 mL, 0.2 mol mL^{-1} of CTAB aqueous solution by magnetic stirring for 30 min. Subsequently, the mixture solution was heated to 60 ^oC and continuously stirred for 20 min, and then cool to room temperature. Finally, the resultant CTAB capped Fe_3O_4 NPs were kept in the oven at 32 ^oC for further experiments.

Synthesis of Fe_3O_4@fmSiO_2 core-shell NPs. To incorporate FITC into silica matrices, we first covalently linked 4 mg of FITC to 44 μL of APTMS in 1 mL of ethanol overnight under dark conditions.[2] The as-prepared CTAB-stabilized Fe_3O_4 NPs and 20 mL pure water were mixed followed by the addition of 20 μL of the FITC-APTMS solution. Then, the pH value of the mixture was adjusted to ~9 with 0.1 M NaOH solution. After that, 50 μL of 20% TEOS in ethanol was injected twelve times at a 30 min interval and subsequently stirred for 24 h at room temperature. The obtained FITC-labeled Fe_3O_4 NPs coated with mesoporous silica shells (Fe_3O_4@fmSiO_2 core-shell NPs) were centrifuged and rinsed with ethanol repeatedly to remove the excess precursors and CTAB molecules.

Synthesis of *ecc*-(*con*-Fe_3O_4@fmSiO_2)@PAA core-double shell nanoparticles. In a 100 mL of flask, 5 mg of Fe_3O_4@fmSiO_2 core-shell nanoparticles were firstly dispersed in 10 mL deionized water by ultrasonication to form a suspension. Then, 50 μL of PAA aqueous solution (0.2 g mL^{-1}) and 75 μL of $NH_3 \cdot H_2O$ (2 mol L^{-1}) added into the suspension, ultrasonically dispersed for 30 min. After that, 90 mL of isopropyl alcohol were dripped into the flask under magnetic stirring, to obtain the *ecc*-(*con*-Fe_3O_4@fmSiO_2)@PAA core-double shell nanoparticles.

Synthesis of $NaYF_4$: Yb/Er/Gd@SiO_2 core-shell NRs. Uniform and monodisperse OA capped NaYF4:Yb/Er/Gd NRs were fabricated using the previously reported method.[3] Typically, 3 mL of the oleic acid stabilized $NaYF_4$:Yb/Er/Gd NRs in cyclohexane was mixed with 12 mL CTAB (0.2 M) under vigorous magnetic stirring for 30 min. And the mixture was heated to 80 ºC for 30 min to volatilize cyclohexane. Then, 20 mL water was added and sonicated for 30 min. Sodium hydroxide solution (NaOH 0.1 M) was added to adjust pH to 8.0~9.0. Finally, 300 μL of 20% TEOS in ethanol was dropped into the solution and added 50 μL every 30 min interval. The mixture was stirred for 48 h, and then $NaYF_4$: Yb/Er/Gd@SiO_2 NRs with a mesoporous silica shell were obtained. The obtained $NaYF_4$: Yb/Er/Gd@SiO_2 NRs were then centrifuged and washed with ethanol at least five times to remove the unreacted species as well as CTAB molecules.

Synthesis of *ecc*-(*con*-$NaYF_4$:Yb/Er/Gd@SiO_2)@PAA core-double shell NCs. In a 250 mL of flask, 5 mg of $NaYF_4$:Yb/Er/Gd@SiO_2 core-shell NRs were firstly dispersed in 10 mL deionized water by ultrasonication to form a suspension. Then, 60 μL of PAA aqueous solution (0.2 g mL^{-1}) and 90 μL of $NH_3 \cdot H_2O$ (2 mol mL^{-1}) were added into the suspension, dispersed ultrasonically for 30 min. After that, 80 mL of isopropyl alcohol were dripped into the flask under magnetic stirring to obtain the *ecc*-(*con*-$NaYF_4$:Yb/Er/Gd@SiO_2)@PAA core-double shell NCs.

Loading DOX into *ecc*-(*con*-Fe_3O_4@fmSiO_2)@PAA core-double shell nanoparticles. UV–Vis spectroscopy was used to determine the amount of DOX loaded into the *ecc*-(*con*-Fe_3O_4@fmSiO_2)@PAA core-double shell nanoparticles. In a typical procedure, the drug-loaded *ecc*-(*con*-Fe_3O_4@fmSiO_2)@PAA core-double shell nanoparticles were prepared by mixing DOX aqueous solution (10 mg mL^{-1}, 20 μL) with *ecc*-(*con*-Fe_3O_4@fmSiO_2)@PAA core-double shell nanoparticles mixed solution (0.08 mg mL^{-1}, 1.0 mL) of 100 μL H_2O and 900 μL isopropyl alcohol for 24 h and then magnetic separation by an external magnet. To evaluate the DOX-loading efficiency (LE), the contents of original DOX and residual DOX in supernatant were determined by UV–Vis measurements at 480 nm and compared to the standard curve created previously. The DOX-loading efficiency was calculated by eqn (1):

$$LE\ (\%) = [m_{(\text{total DOX})} - m_{(\text{DOX in supernatant})}]/m_{(\text{total DOX})} \times 100\% \quad (1)$$

Release profile of DOX from DOX-loaded *ecc*-(*con*-Fe_3O_4@fmSiO_2)@PAA core-double shell nanoparticles. In vitro DOX release from DOX-loaded *ecc*-(*con*-Fe_3O_4@fmSiO_2)@PAA core-double shell nanoparticles was evaluated using a semipermeable dialysis bag diffusion technique right after the preparation of DOX loaded. Two portions of the asprepared DOX-loaded *ecc*-(*con*-Fe_3O_4@fmSiO_2)@PAA core-double shell nanoparticles at equal amount were redispersed in 0.5 mL of PBS (pH 7.4) and 0.5 mL of acetate buffer (pH 5.1), respectively. Both of the release media were transferred into pretreated semipermeable dialysis bags and then immersed into 3 mL of deionized water at 37 ^{o}C with gentle shaking, respectively. At selected time intervals, the amount of released DOX moved out of semipermeable dialysis bag into water was measured by fluorescence spectrophotometer with emission at 591 nm and excitation at 479 nm.

Cell culture. PC3M cells were grown as a monolayer in a humidified incubator at 37 ^{o}C in a 95 % air/5 % CO_2 in DMEM supplemented with 10 % fetal bovine serum.

Cell uptake. 1 × 10^5 PC3M cells were seeded onto glass cover slips in a 24-well plate in DMEM medium containing 10% fetal bovine serum for 24 h at 37 °C in a humidified atmosphere with 5 % CO_2 to allow the cells to attach. Then, 1 μg mL^{-1} of DOX-loaded *ecc*-(*con*-Fe_3O_4@fmSiO_2)@PAA core-double shell nanoparticles were added to the PC3M cells. After incubation for 6 h, the cell monolayer on the coverslip was washed with PBS for several times to remove the remaining particles and dead cells. Finally, the observations were performed using a CLSM.

In vitro release and cytotoxicity of DOX-loaded *ecc*-(*con*-Fe_3O_4@fmSiO_2)@PAA core-double shell nanoparticles against PC3M cells. In vitro release and cytotoxicity of DOX-loaded *ecc*-(*con*-Fe_3O_4@fmSiO_2)@PAA core-double shell nanoparticles against PC3M cancer cells. In vitro release of DOX from DOX-loaded *ecc*-(*con*-Fe_3O_4@fmSiO_2)@PAA core-double shell nanoparticles was evaluated using a semipermeable dialysis bag diffusion technique right after the DOX loading. The asprepared DOX-loaded *ecc*-(*con*-Fe_3O_4@fmSiO_2)@PAA core-double shell nanoparticles were redispersed in 0.5 mL PBS (pH 7.4) and 0.5 mL acetate buffer (pH 5.1), respectively. Both of the release mediums were placed into pretreated semipermeable dialysis bags and then immersed into 2 mL deionized water at 37 °C with gentle shaking, respectively. At certain time intervals, DOX concentration moved out of semipremeable dialysis dag into water was measured by fluorescence spectrophotometer. The amount of DOX released was determined by fluorescence emission at 591 nm with excitation at 479 nm.

The in vitro cytotoxicity of empty *ecc*-(*con*-Fe_3O_4@fmSiO_2)@PAA core-double shell nanoparticles and DOX-loaded *ecc*-(*con*-Fe_3O_4@fmSiO_2)@PAA core-double shell nanoparticles were evaluated by standard 3-(4,5-dimethylthialzol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assays and PC3M cancer cells were used. Cells were seeded in a 96-well plate at a density of 2.5 × 10^4 (100 μL) per well and incubated at 37 °C in a humidified atmosphere with 5% CO_2 in DMEM medium containing 10% fetal bovine serum for 24 h to attach. Then serial concentrations of empty *ecc*-(*con*-Fe_3O_4@fmSiO_2)@PAA core-double shell nanoparticles, DOX-loaded *ecc*-(*con*-Fe_3O_4@fmSiO_2)@PAA core-double shell nanoparticles and free DOX in serum-free medium with 100 μL were added, respectively. One row of a 96-well plate was used as a control with 100 μL culture medium only. After incubation 24 h, each well was washed three times with PBS, then 20 μL 5 mg mL^{-1} MTT solution was added to each well and the mixture was incubated for another 4 h. The amount of dark-blue formazan crystals generated by the live cells was proportional to the number of live cells. The medium was then replaced with DMSO (150 mL) and the absorbance was monitored with a microplate reader at a wavelength of 490 nm. Cell viability was determined by eqn (2):

$$\text{Cell viability (\%)} = Abs_{(\text{test cells})}/Abs_{(\text{reference cells})} \times 100\% \quad (2)$$

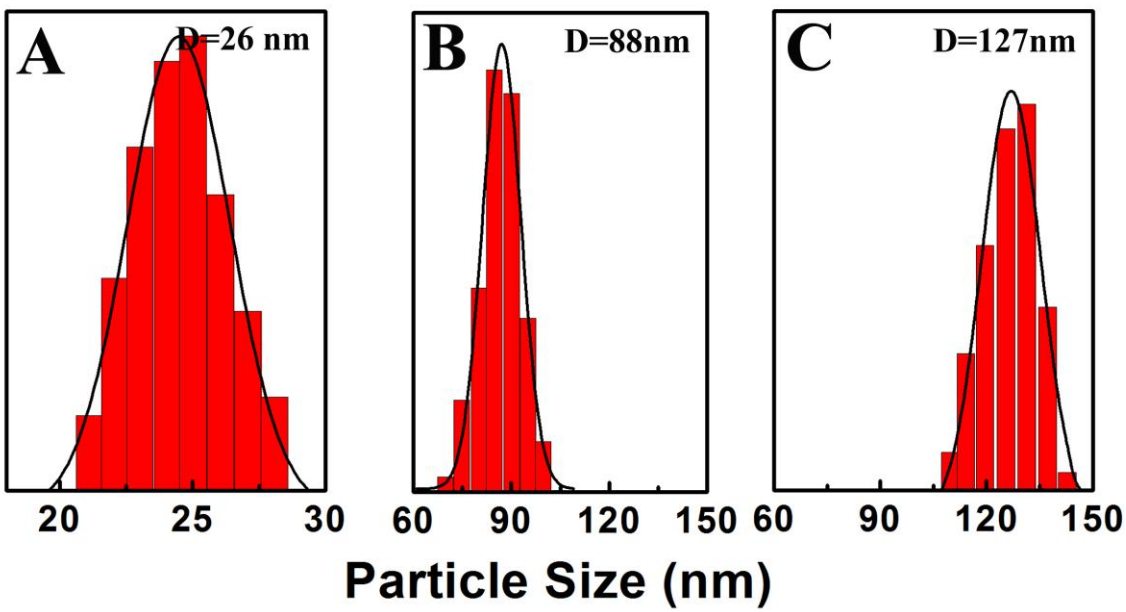

Fig. S1 Size distribution of particle diameter of CTAB modified Fe_3O_4 NPs (A), *con*-Fe_3O_4@fmSiO_2 core-shell NPs (B) and *ecc*-(*con*-Fe_3O_4@fmSiO_2)@PAA core-double shell NCs(C).

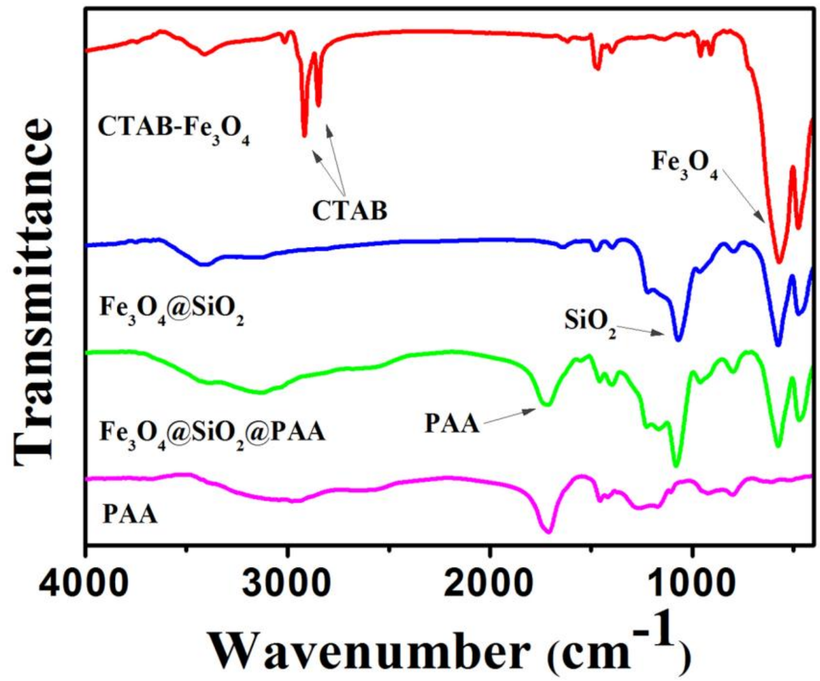

Fig. S2 FT-IR spectroscopic analysis of the CTAB-Fe_3O_4 (red line), Fe_3O_4@fmSiO_2 NPs (blue line), *ecc*-(*con*-Fe_3O_4@fmSiO_2)@PAA core-double shell NCs (green line), and PAA (pink line).

The spectra have the characteristic peaks of both Fe_3O_4 and SiO_2. The peaks in the 400-800 cm^{-1} region are due to the multiple lattice absorptions of partially ordered Fe_3O_4 and the peaks at 1070 cm^{-1} are attributed to the vibration bands of Si-O-Si. At the same time these C-H peaks derived from CTAB all disappeared, confirms the all CTAB be washed off. After coated with PAA, new adsorption peaks appeared at 1714 cm^{-1}, which could be assigned to the C=O stretching vibration in the carboxyl group, which confirms the presence of a PAA coating in the Fe_3O_4@fmSiO_2.

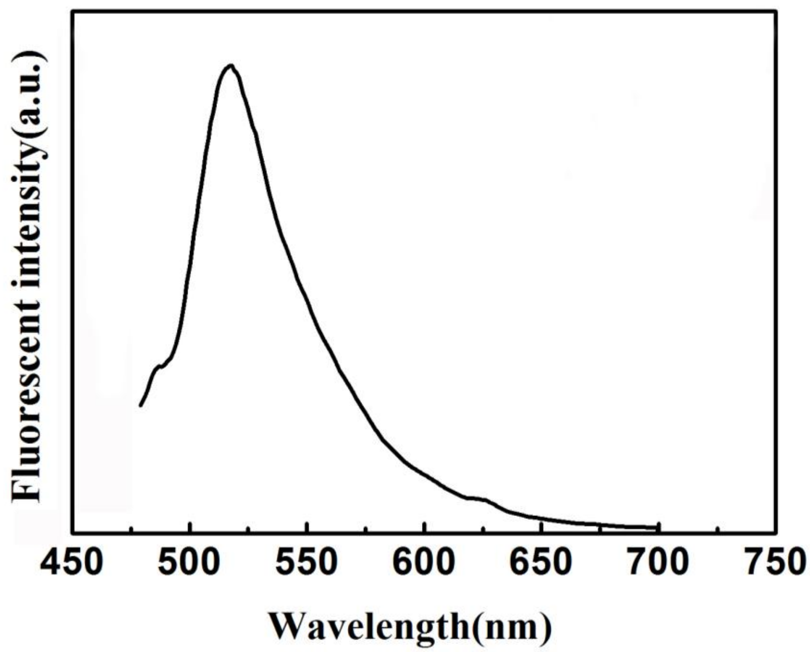

Fig. S3 Fluorescence spectrum of *ecc*-(*con*-Fe_3O_4@fmSiO_2)@PAA core-double shell NCs with the excitation wavelength of 458 nm.

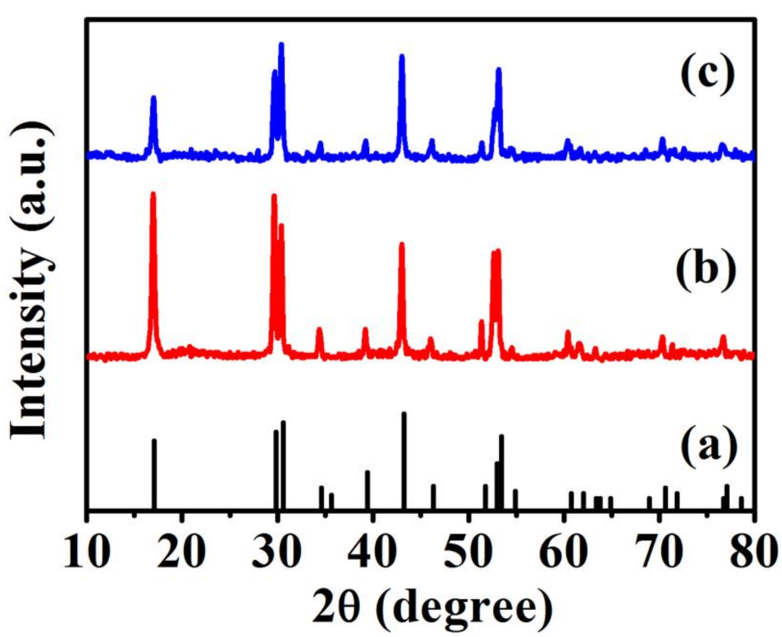

Fig. S4 The standard JCPDS card 16-0334 of (a) $NaYF_4$, wide-angle XRD patterns (b) of $NaYF_4$:Yb/Er/Gd@SiO_2 NRs and (c) *ecc*-(*con*-$NaYF_4$:Yb/Er/Gd@SiO_2)@PAA core-double shell NCs, respectively.

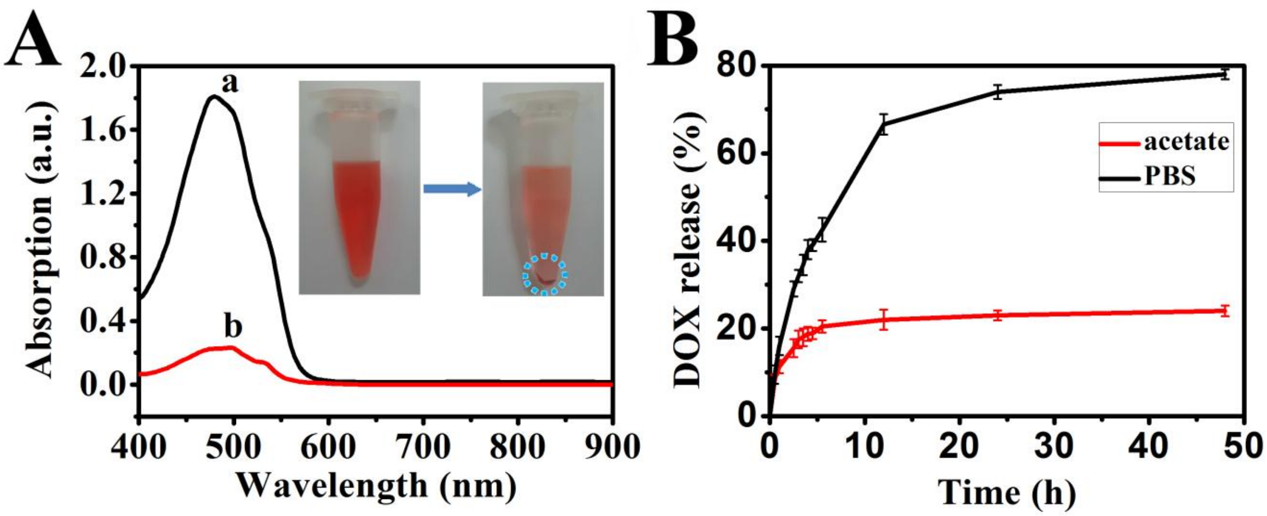

Fig. S5. (A) UV-Vis absorption spectra of DOX solution before (a) and after (b) interaction with *ecc*-(*con*-$NaYF_4$:Yb/Er/Gd@SiO_2)@PAA NCs. Inset: digital pictures of DOX solution before (left) and after (right) interaction with NCs. (B) DOX-release profiles for DOX-loaded NCs measured at pH 5.1 in acetate buffer and at pH 7.4 in PBS buffer, respectively, at 37°C.

1. J. Kim, H. S. Kim, N. Lee, T. Kim, H. Kim, T. Yu, I. C. Song, W. K. Moon and T. Hyeon, *Angew. Chem. Int. Ed.,* 2008, **47**, 8438.
2. J. E. Lee, N. Lee, H. Kim, J. Kim, S. H. Choi, J. H. Kim, T. Kim, I. C. Song, S. P. Park, W. K. Moon and T. Hyeon, *J. Am. Chem. Soc.,* 2009, **132**, 552.
3. F. Wang, Y. Han, C. S. Lim, Y. H. Lu, J. Wang, J. Xu, H. Y. Chen, C. Zhang, M. H. Hong and X. G. Liu, *Nature,* 2010, **463**, 1061.