Supporting Information

Experimental

Chemicals and Materials. All of the chemicals used were of analytical grade and were used without further purification. Glutaraldehyde (50%), tris (hydroxymethyl)aminomethane (Tris, 98%), avidin (99%) were purchased from Sigma-Aldrich. Other reagents were obtained from Beijing DingGuo Biotechnology co. Ltd. Adenosine 5`-triphosphate (ATP), adenosine 5`-diphosphate (ADP), guanosine 5`-triphosphate (GTP), cytosine 5`-triphosphate (CTP) were all subscribed from Shanghai Sangon Biotechnology Co. Ltd. Deionized water was purified through Milli-Q water purification system and the resistivity was 18.2 MΩ·cm. The oligonucleotides were synthesized by Shanghai Sangon Biotechnology Co. Ltd. The sequences of several single stranded DNA involved are listed in Table S1. There are two segments within the sequence of UCNPs_DNA: ATP binding domain (underlined letters), and nonsense domain (italicized letters). The 12-nt TAMRA_DNA is complementary to the last five nucleotides of this nonsense region as well as the seven adjacent nucleotides on the aptamer element. There were only two single-bases difference between UCNPs_DNA and Mutant_DNA, at the 6th-base position (G to C) and 14th-base position (T to A) from the 3`-end as indicated by the framed letters.

Instrumentation and characterization. Size and morphology of UCNPs were determined using a Hitachi H-8100 IV transmission electron microscopy (TEM) at 200 kV. Ultraviolet-visible (UV-vis) absorption of TAMRA and NBT were measured at room temperature using a UV-3101 spectrophotometer, respectively. Luminescence

spectra of resonance energy transfer and TAMRA were detected with a Hitachi F-4500 fluorescence spectrofluorimeter. An additional 980 nm laser diode and the Xenon in the spectrophotometer were used as excitation source, respectively.

Synthesis of water-soluble $NaYF_4$: Yb^{3+}, Er^{3+} UCNPs. The oleyl amine (OM) capped $NaYF_4$: 20% Yb^{3+}, 2% Er^{3+} UCNPs were synthesized according to the published methods.[1] A typical synthesis is as follows: the mixture of CF_3COONa (2 mmol), $(CF_3COO)_3Y$ (0.78 mmol), $(CF_3COO)_3Yb$ (0.2 mmol), and $(CF_3COO)_3Er$ (0.02 mmol) was dissolved in OM (10 mL), the mixture was heated to 120 °C to remove water and oxygen, with vigorous magnetic stirring under argon flow for 1h. Then the mixture was heated to 330 °C in the presence of argon for protection from oxidation. After 2 h, heating was stopped. The transparent yellowish reaction mixture was allowed to cool to 80 °C before ethanol (20 mL) was added. The particle purification were accomplished through centrifugation, and then stored in chloroform before further treatment.

The ligand exchange process was carried out according to our previous work, with some modifications.[2] 200 mg of Aminoethanephosphonic acid (AEP) was dispersed in 10 ml mixture of water and ethanol (volume ratio is 3:2), The hydrophobic UCNPs solution (~20mg, purified and dispersed in 5 ml of chloroform) were mixed with the AEP solution and stirred vigorously over 48 h at 30 °C, The UCNPs transferred from the bottom chloroform layer to the top H_2O/CH_3CH_2OH layer, the H_2O/CH_3CH_2OH layer was collected and centrifuged twice and the obtained nanoparticles were redispersed in deionized water for analysis. No obvious precipitation and aggregation was found after storing the resulted aqueous dispersions of nanocrystals for more than two months under ambient conditions.

Determination of ROI production. To evaluate the photoactivation role of UCNPs compared with QDs, the detection of ROI experiment was performed by an indirect method using a chemical probe. In a typical assay, three kinds of different luminescent nanoparticels were dispersed in 1 ml of 100 μM NBT solution (1:1 water/DMSO mixture), respectively. The final concentrations of sample were 20 nM CdSe/ZnS QDs, 20 nM CdTe QDs, and 0.1mg/ml $NaYF_4$:Yb^{3+}, Er^{3+} UCNPs. The photo-irradiation experiments were performed under ambient condition and irradiation by an argon-488nm laser or a 980nm laser diode for 1 h, and then the intensities of NBT absorption in the 450-700 nm range were obtained.

Agarose gel electrophoresis. Strand breakage of aptamer was examined using agarose gel electrophoresis. Equal volumes of aptamer (1 μM) with QD (5μM) or UCNPs (8 mg/ml) were photoactivated under 400 nm (10 $\mu W/cm^2$) or 980 nm (300 mW output power) light illumination for 45 min, respectively. A parallel set of controls was also used, in which the void aptamer were either exposed to 400 nm or 980 nm light irradiation or not. The samples were mixed with the gel loading buffer and loaded into wells in 1.5 % agarose gel, which was immersed in the TAE buffer in a horizontal tank. Aptamer bands was run at 75 V for 5 min and visualized by ethidium bromide staining under ultraviolet light.

Preparation of avidin-functionalized UCNPs. The avidin was directly immobilized onto the amino-functionalized nanoparticles with well-established glutaraldehyde method as shown below.[3] 2 mg nanoparticles was dispersed into phosphate buffer saline (PBS, 0.01M, pH 7.4) containing 5% glutaraldehyde and then stirred for about

12 h at room temperature. After centrifugation and washed with water, the nanoparticles were re-dispersed in PBS buffer and 1 mg avidin was added. The linkage reaction was allowed to proceed at 4°C for 48 h with shaking. Finally, the avidin-functionalized nanoparticles were centrifuged, washed and suspended into a buffered solution (pH 8.2, containing 20mM Tris-HCl, 5 mM KCl, 1 mM $MgCl_2$).

Preparation of aptamer-functionalized UCNPs. To activate aptamer hybridization duplex, a mixture of TAMRA_DNA (50 μM) and UCNPs_DNA (25μM) in a signaling buffer (pH 8.2, containing 20 mM Tris-HCl, 5 mM KCl, 1 mM $MgCl_2$) was stirred at 70 °C for 3 min to facilitate the thermally equilibrated hybridization reaction, and slowly cooling to room temperature to allow the maximal duplex strands formation. The solution was subsequently diluted into a serious of designed concentrations and titrated into the aforementioned avidin-functionalized UCNPs solution (0.5 mg/mL). After incubated for about 1 hour, the reaction system was taken out for luminescence spectrum measurement under continuous-wave excitation at 980 nm.

ATP detection assay. A signaling mixture made of avidin-functionalized UCNPs (0.5 mg/mL), UCNPs_DNA (700 nM) and TAMRA_DNA (1400 nM) is prepared. The detecting experiment was performed by keeping the concentration of DNA-functionalized UCNPs constant while varying the concentration of Target ATP. Different concentrations of ATP were added into signaling mixture solution to incubate for 1 hour at 37 °C and then luminescence spectra were recorded. The

nonspecific control experiments were conducted in a manner identical to the assays described above with a Mutant_DNA, ADP, CTP, and GTP.

Table S1. DNA sequences used in this aptamer-UCNPs bioconjugates study

Oligonucleotide	Sequence
UCNPs_DNA	5`-/biotin/*TTCACTG*ACCTGGGGGAGTATTGCGGAGGAAGGT-3`
Mutant_DNA	5`-/biotin/*TTCACTG*ACCTGGGGGAGTAATGCGGAGCAAGGT-3`
TAMRA_DNA	5`-CCCAGGTCAGTG/TAMRA/-3`

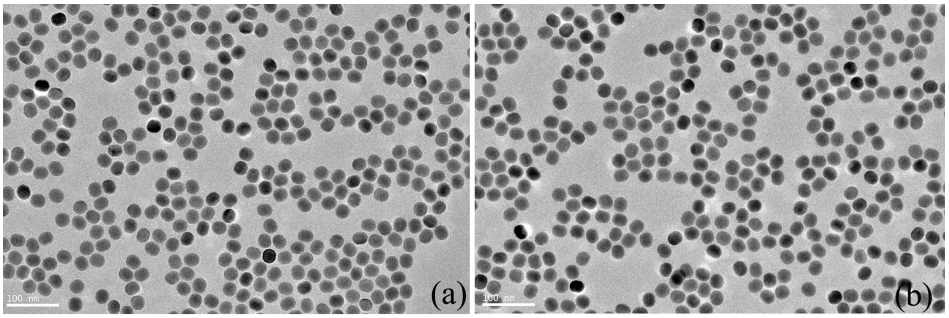

Fig. S1. TEM images of $NaYF_4$:Yb^{3+}, Er^{3+} UCNPs before (a) and after (b) ligand exchange. Compared with the original hydrophobic nanoparticles, the size and shape of the nanoparticles after ligand exchange do not show apparent changes.

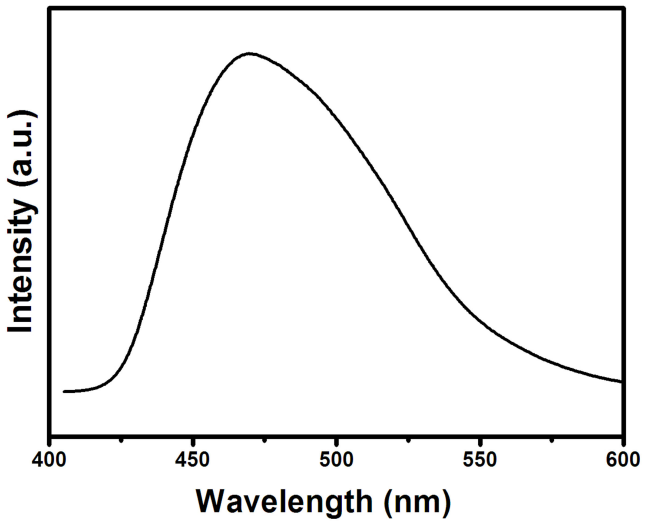

Fig. S2. Fluorescent emission spectra of fluorescamine upon irradiation at 390nm

The existence of amino group on the surface of nanoparticles was proved by fluorometric method using non-fluorescent fluorescamine reagent for rapid amino assay. The reaction of primary amines with fluorescamine can result in fluorophore products, and the excess fluorescamine can be hydrolyzed into non-fluorescent products very fast. By measuring the emission band centered at 470nm (λex = 390 nm), the presence of amino guroup can be validated. Fig. S2 illustrates the emission spectrum of phase transferred nanoparticles excited by 390nm, a strong peak centered at 470 was clearly observed, confirming the existence of amino groups

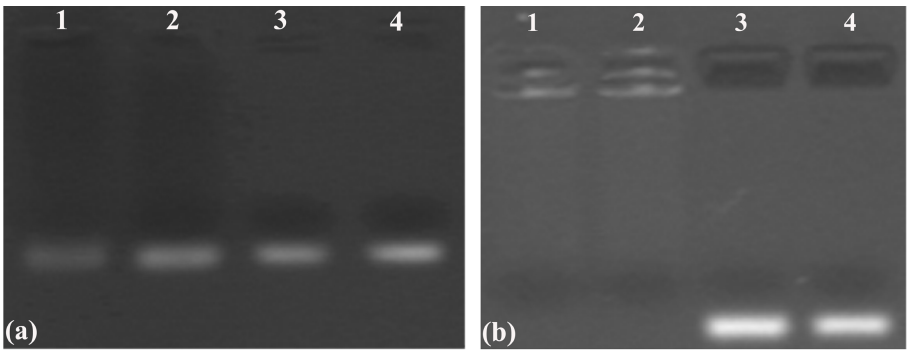

Fig. S3. (a) The agarose gel images of aptamer with and without QD before and after photoactivation. Lane 1: aptamer with QDs photoactivated using UV light of 400 nm for 45 min, lane 2: aptamer with QDs incubated in the dark for 45 min, lane 3: aptamer photoactivated using UV light of 400 nm for 45 min, lane 4: aptamer incubated in the dark for 45 min; (b) the agarose gel images of aptamer with and without UCNPs before and after photoactivation. Lane 1: aptamer with UCNPs photoactivated at 980 nm for 45 min, lane 2: aptamer with UCNPs incubated in the dark for 45 min, lane 3: aptamer photoactivated at 980 nm for 45 min, lane 4: aptamer incubated in the dark 45 min.

Fig. S3a shows the agarose gel images of aptamer with and without QD before and after photoactivation, as compared with that of void aptmer. It is apparent that the intensity of the aptamer with QDs was considerably decreased after 45 min of photoactivation using UV light of 400 nm (lane 1). The gel images of aptamer kept in the dark (lane 4) and under direct irradiation of 400 nm without QDs (lane 3) were essentially not affected. This indicates that the photosensitized breakage and damage of aptamer was solely attributed to the photosensitized reactions between aptamer and

QDs. It is noted that the intensity of aptamer in lane 2 was high compared to lane 4, which was ascribed to the superposition in intensity from QDs under 400 nm light irradiation. For comparing, the agarose gel images of aptamer are given in Fig. S3b as well with and without UCNPs before and after photoactivation. It can be seen that there was no migrating band using agarose gel electrophoresis in lane 1 and lane 2, which may relate with the fact that the size of UCNPs was too large to pass the channels of 1.5% agarose gel. However, the intensity of the aptamer stayed in the hole of both lanes is similar, indicating that the photoactivated UCNPs were not effective in inducing aptamer damage. There was also no significant difference between lane 3 and lane 4, indicating that the subsequent irradiation of 980nm laser light alone had innoxious effect on aptamers. All these results suggest that UCNPs, possessing excellent optical properties and innoxious photosensitized breakage and damage of aptamer molecules, are good candidates for donors in LRET-based aptamer biosensor.

Notes and references

1 Q. B. Zhang, K. Song, J. W. Zhao, X. G. Kong, Y. J. Sun, X. M. Liu, Y. L. Zhang, Q. H. Zeng and H. Zhang, *J. Colloid. Interface. Sci.,* 2009, **336,** 171.

2 H. X. Mai, Y. W. Zhang, R. Si, Z. G. Yan, L. D. Sun, L. P. You and C. H. Yan, *J. Am. Chem. Soc.,* 2006, **128,** 6426.

3 M. V. Kiselev, A. K. Gladilin, N. S. Melik-Nubarov, P. G. Sveshnikov, P. Miethe and A. V. Levashov, *Anal. Biochem.,* 1999, **269,** 393.