Electronic Supplementary Information

A Versatile Luminescent Resonance Energy Transfer (LRET)-Based Ratiometric Upconversion Nanoprobe for Intracellular miRNAs Biosensing

Liang Gong,[1,2ζ] Simin Liu,[1ζ] Ya Song, [1] Shaowen Xie,[1] Ziyu Guo,[1] Jianxiong Xu[1]* and Lijian Xu[1]*

[1] Hunan Key Laboratory of Biomedical Nanomaterials and Devices, College of Life Sciences and Chemistry, Hunan University of Technology, Zhuzhou 412007, P. R. China

[2] State Key Laboratory of Chemo/Bio-Sensing and Chemometrics, College of Chemistry and Chemical Engineering, Hunan University, Changsha 410082, P. R. China

Experimental section

Chemicals and Materials. Oleic acid (OA, 90% technical grade) and 1-octadecene (ODE, 90% technical grade) were purchased from Sigma-Aldrich. Tetraethyl orthosilicate (TEOS, 99%) and aminopropyltriethoxysilane (APTES) were purchased from Inno chem Co., Ltd. Ethyl acetate (AR), methanol (AR), ethanol (AR), and cyclohexane (AR) were supplied by Hunan Huihong Reagent Co., Ltd. Sodium oleate (>97.0%) was obtained from TCI. $YbCl_3 \cdot 6H_2O$ (99.9%), $YCl_3 \cdot 6H_2O$ (99.9%), $ErCl_3 \cdot 6H_2O$ (99.9%), hexadecy trimethyl ammonium bromide (CTAB, 99%), ammonium hydroxide ($NH_3 \cdot H_2O$, GR), *n*-hexane (AR, 97%), ammonium fluoride (NH_4F, AR), sodium hydroxide (NaOH, AR) and other chemicals of analytical grade were purchased from Shanghai Aladdin Chemistry Co., Ltd. (China). Tris-HCl buffer (pH 7.4) contained 10 mM tris-HCl, 1mM EDTA, 100 mM NaCl, and 2 mM $MgCl_2$. All chemical reagents were used without any purification.

DNA and RNA oligonucleotides employed in this paper were purchased from Sangon Biological Engineering Technology & Co. Ltd. (Shanghai, China), and all sequences were shown in **Table S1**. The $NaYF_4$:Yb,Er@$NaYF_4$ core/shell nanoparticles were synthesized by a two-step thermal decomposition process in accordance with our previous report.[1, 2]

Instruments. Transmission electron microscopy (TEM), Scanning TEM (STEM) and Energy-dispersive X-ray Spectroscopy (EDX) analysis were carried out by using Titan G260-300 transmission electron microscope. X-ray photoelectron spectroscopy

(XPS) measurements are performed using a Thermo Fisher Scientific ESCALAB 250 Xi system with Al-Ka as the radiation source. The powder X-ray diffraction (XRD) measurement was performed on Rigaku Model D/max-2500 diffractometer, with Cu Kα radiation in the 2θ range of 10-80° with a step size of 0.02°. Fourier transform infrared (FTIR) measurements were recorded using Bruker VECTOR 22 spectrometers with wavenumber precision <0.01 cm^{-1} and signal-to-noise 30000:1. BELSORP-mini II sorption analyzer was used to measure N_2 adsorption and data analyzed by the Brunauer-Emmett-Teller (BET) equation for surface areas and by the Barrett-Joyner-Halenda (BJH) model for pore size distribution. Diffuse reflectance UV-vis spectra were obtained on Beijing Puxi TU-1950 spectrometer. The upconversion luminescent intensity was collected through a Hitachi F-4600 luminescence spectrophotometer which was equipped with an external CNI (2W) 980 nm IR Fiber coupled laser system (Changchun New Industries Optoelectronics Tech. Co., Ltd).

Synthesis of $NaYF_4$:Yb,Er@$NaYF_4$@NH_2-mSiO_2

In a typical procedure, 10 mL cyclohexane containing 0.05 g $NaYF_4$:Yb,Er@$NaYF_4$ was introduced to 20 mL CTAB solution (containing CTAB 0.5 g) and sonicated for 2 h at 150 W. Then, the mixed solution was heated at 70 °C under vigorous stirring to remove cyclohexane until a transparent solution was formed. A mixture of 60 mL water, 4 mL ethyl acetate and 0.6 mL of ammonia was added to the surfactant solution with stirring for 2 h. Subsequently, 0.2 mL of TEOS was added dropwise to the mixed solution and kept stirring overnight at room temperature. Then,

solid crude product was collected by centrifugation (12000 rpm, 10 min), washed with ethanol several times.

To remove the surfactant template (CTAB), 0.1 g the as-synthesized products were refluxed for 72 h in 10 mL methanol solution containing 100 μL concentrated HCl. The resulting materials (denoted as $NaYF_4$:Yb,Er@$NaYF_4$@$mSiO_2$) were then centrifuged (12000 rpm, 5 min), washed with ethanol. To obtain amine-functionalized $NaYF_4$:Yb,Er@$NaYF_4$@$mSiO_2$, 0.1 g $NaYF_4$:Yb,Er@$NaYF_4$@$mSiO_2$ was dispersed in 10 mL ethanol and injected dropwise with 100 μL APTES, then kept stirring for 6 h. After that, the products were centrifuged (12000 rpm, 5 min), and washed thoroughly water. The product was denoted as $NaYF_4$:Yb,Er@$NaYF_4$@NH_2-$mSiO_2$.

Synthesis of $NaYF_4$:Yb,Er@$NaYF_4$@NH_2-$mSiO_2$/rhodamine B Nanocomposite

In order to obtain $NaYF_4$:Yb,Er@$NaYF_4$@NH_2-$mSiO_2$/rhodamine B nanocomposite, 1 mL 1.0 mg/mL of $NaYF_4$:Yb,Er@$NaYF_4$@NH_2-$mSiO_2$ aqueous solution was mixed with different concentrations of rhodamine B range from 0.03 mM to 0.3 mM, respectively. Then the mixture solution was stirred at room temperature for 6 h in a dark area. After adsorption equilibrium, the $NaYF_4$:Yb,Er@$NaYF_4$@NH_2-$mSiO_2$/rhodamine B nanocomposites were separated by centrifugation (10000 rpm, 5 min). The adsorption amount of rhodamine B was calculated by comparing the UV-vis absorbance of the initial rhodamine B solution and the supernatant after adsorption equilibrium. The obtained $NaYF_4$:Yb,Er@$NaYF_4$@NH_2-$mSiO_2$/rhodamine B nanocomposites was then re-dispersed in tris-buffer with concentration of 5.0 mg mL^-

[1]. A small portion of the aqueous dispersion was further diluted to 1.0 mg mL^{-1} and subjected to luminescent measured to evaluate the quenching efficiency. The nanocomposite with completely quenching of the upconversion luminescence at 540 nm was selected for next experiment.

Preparation of the LBRU Nanoprobe

To construct optimal LBRU nanoprobe, C-DNA (10 μL, 100 μM) with different lengths were respectively added to 200 μL 1.0 mg mL^{-1} of the above optimized nanocomposite compounded of $NaYF_4$:Yb,Er@$NaYF_4$@NH_2-$mSiO_2$ nanoparticles incubated with rhodamine B (0.3 mM). Then kept the solution stirring for 2 h so that the C-DNA could cap the channels on the surface of nanocomposites to construct LBRU nanoprobe. After the surface-adsorbed dye washed with tris-HCl buffer and removed by centrifugation (10000 rpm, 5 min), the LBRU nanoprobes were obtained and resuspended in tris-HCl buffer. Next, stimulus responses of these LBRU nanoprobes wrapped by different length of C-DNA to miRNA-21 were measured. Chose optimal C-DNA according to the highest SBR value and re-dispersed in tris-buffer with concentration of 5.0 mg mL^{-1}, stored it at 4 ℃.

Gel electrophoresis experiment

The agarose gel electrophoresis was performed as following: 5 μL DNA marker (5 μM) as indicator, 5 μL $NaYF_4$:Yb,Er@$NaYF_4$@$mSiO_2$ (1.0 mg/mL), 5 μL $NaYF_4$:Yb,Er@$NaYF_4$@$mSiO_2$/rhodamine B nanocomposites (1.0 mg/mL), 5 μL LBRU nanoprobe (1.0 mg/mL), 5 μL (LBRU nanoprobe + miRNA-21) (1.0 mg/mL), 5

μL miRNA-21(5 μM), and 5 μL C-DNA (5 μM) was added 1.5 μL loading buffer, respectively. Samples were injected into 3.5% agarose gel containing ethidium bromide. After carried out at 120 V in TAE buffer for 30 min, the electrophoresis resulting was obtained under UV irradiation.

Preparation of cell extracts.

The L02 cells (1.0×10^6/mL cells) were first centrifuged for 3 min at 800 rpm to remove the supernatant, and then re-dispersed cells precipitate in 1 mL PBS buffer to subject a sonication treatment (30 min with 3s on and 3s off) in an ice-water bath using a probe-type sonicator (150 W). After that, the disrupted cell suspension was centrifuged (10000 rpm) for 15 min to collect the supernatant. Finally, the resulting cell extracts was stored at 4 °C for further use.

Detection of miRNA-21 in Buffer Solution

100 μL the LBRU nanoprobe with concentration of 50 μg/mL was incubated with a series of concentrations of miRNA-21 from 10 nM to 10 μM. After incubated at 37 ℃ for 2 h, luminescent spectra of all of the mixture solutions were recorded by F-4600 fluorescence spectrophotometer under the excitation of 980 nm laser. To investigate specificity of the nanoprobe to miRNA-21, some interfering agents (SMmiRNA-21, miRNA-141, miRNA-143 and random DNA at concentration of 100 nM and other compounds at concentration of 100 μM including GSH, ATP, BSA, Cys, NaCl, KCl, $CaCl_2$, $MgSO_4$, and $Zn(NO_3)_2$) in place of miRNA-21 were test with same conditions.

The detection of miRNA 141 in buffer solution is similar with detecting miRNA-21. In brief, 100 μL the nanoprobe wrapped by C-141 with concentration of 50 μg/mL was incubated with different concentration of miRNA 141 (0, 80 nM, 10 μM) respectively. After incubated at 37 ℃ for 2 h, luminescent spectra of all of the mixture solutions were recorded by F-4600 fluorescence spectrophotometer under the excitation of 980 nm laser.

Detection of miRNA-21 in cell extracts.

Different concentrations of miRNA-21solution were added respectively into the cell lysates of L02 cells to achieve a series of samples with miRNA-21 concentrations from range from 20 nM to 100 nM. Then, just like detecting miRNA-21 in buffer solution, 10 μL LBRU nanoprobes (50 μg/mL) were incubated with 90 μL the above prepared solution for 2 h. After that, the upconversion luminescent measurement was measured.

Cytotoxicity of the LBRU nanoprobe

Hela cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) cell medium containing 10% fetal bovine serum and 1% penicillin/streptomycin in a humidified incubator at 37°C under 5% CO_2 atmosphere. Cell viability was measured by MTT assay. Briefly, Hela cells were cultured on a 96-well plate at a density of 10,000 cells per well for 24 h, then the medium was replaced with 100 μL of fresh medium containing different concentrations of LBRU nanoprobe (from 0 μg/mL to 400 μg/mL). After incubation for overnight, the medium was replaced by fresh medium

(100 μL) containing MTT (0.8 mg/mL) and incubated for 4 h at 37°C with 5% CO_2. Subsequently, the medium in each well was replaced with DMSO and incubated for another 10 min, and absorbance of the solution was measured to assess the viability of cells using a Bio-Rad 680 microplate reader. The cell viability was expressed as: $(OD_{test}/OD_{control}) \times 100\%$, where OD represented the absorbance at 570 nm of cells.

Upconversion Fluorescent Microscopy Imaging

To investigate the intracellular tracing efficiency of the LBRU nanoprobe, Hela cells and MCF-7 cells were plated on a 35 mm confocal dishes with 10 mm bottom well and cultured for 20 h before the experiments. After washed for three times with PBS buffer, cells were incubated with culture medium containing 200 μg/mL nanoprobe for 3 h, and then co-stained with 5 μL Hoechst 33342 for 10 min at 37 °C in 5% CO_2. After washed three times with PBS, cells were subjected to confocal upconversion-luminescence microscopy analysis with Olympus FV1000 confocal scanning system employing Spectra-Physics Mai Tai HP pulsed laser, the luminescent emission spectrum was collected in the range of 510-560 and 630-675 nm.

Similarly, to study the capability of LBRU nanoprobe for monitoring intracellular miRNA-21 levels in MCF-7 cells and L02 cells, cells were incubated with culture medium containing 200 μg/mL LBRU nanoprobe for 3 h at 37 °C in 5% CO_2. After washed three times with PBS, cells were subjected to confocal upconversion-luminescence microscopy analysis with the same instruments and conditions as above.

Table S1. Sequences of employed nucleic acid.

Name of nucleic acid	Sequences of nucleic acid (5' →3')
C-21	TCAACATCAGTCTGATAAGCTA
C-30	TCAACATCAGTCTGATAAGCTACCAAATCA
C-35	$(CTT)_4$CTCAACATCAGTCTGATAAGCTA
C-40	$(CTT)_6$TCAACATCAGTCTGATAAGCTA
C-50	$(CTT)_9$CTCAACATCAGTCTGATAAGCTA
C-141	CCATCTTTACCAGACAGTGTTA
miRNA-21	UAGCUUAUCAGACUGAUGUUGA
SMmiRNA-21	UAGCUUAUCAGACUGAUGAUGA
miRNA-141	UAACACUGUCUGGUAAAGAUGG
miRNA-143	UGAGAUGAAGCACUGUAGCUCA
random DNA	CTATCAAGCTATCAATCTATCTGTC

The surface elemental evolution for synthesis of $NaYF_4$:Yb,Er@$NaYF_4$@$mSiO_2$ was demonstrated by XPS. As shown in **Figure S1**, both $NaYF_4$:Yb,Er (core) nanoparticles and $NaYF_4$:Yb,Er@$NaYF_4$ (core/shell) nanoparticles showed had similar XPS peaks, including four strong peaks of Y 3d, Y 3p, F 1s, and Na 1s at 161.0, 303.5, 688.7, and 1072.8 eV, as well as two additional peaks at 286.0 and 532.5 eV corresponding to OA molecules on the surface of the two nanoparticles. Besides, a weak peaks at 186.8 eV for Yb 4d were also observed in $NaYF_4$:Yb,Er matrix. However, after coated by $NaYF_4$ on the surface of $NaYF_4$:Yb,Er, the weak peaks disappeared as shown, which attributed to the formation of $NaYF_4$:Yb,Er@$NaYF_4$ core-shell nanoparticles. Upon coating of SiO_2 on the surface of $NaYF_4$:Yb,Er@$NaYF_4$ core-shell nanoparticles, $NaYF_4$:Yb,Er@$NaYF_4$@$mSiO_2$ presented distinct peaks at 532.0 eV and 103.2 eV corresponding to O 1s and Si 2p of SiO_2, and the intensity of the other peaks, such as 161.0 eV (Y 3d), 303.5 eV (Y 3p), 688.7 eV (F 1s), and 1072.8 eV (Na 1s) decreased significantly.

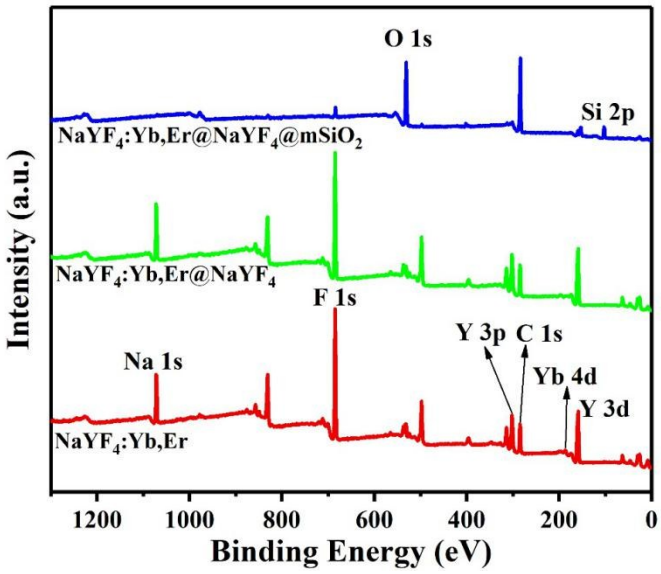

Figure S1. XPS spectra of $NaYF_4$:Yb,Er (core), $NaYF_4$:Yb,Er@$NaYF_4$ (core/shell),

and $NaYF_4$:Yb,Er@$NaYF_4$@$mSiO_2$ (sandwich-structured).

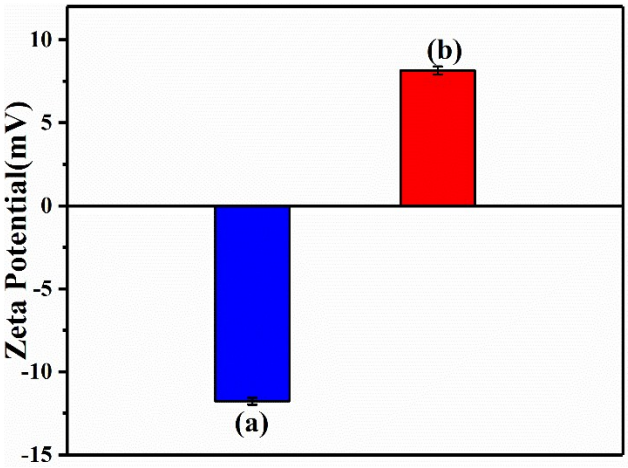

Figure S2. Zeta-potential of $NaYF_4$:Yb,Er@$NaYF_4$ @$mSiO_2$ (a) and $NaYF_4$:Yb,Er@$NaYF_4$@NH_2-$mSiO_2$ (b).

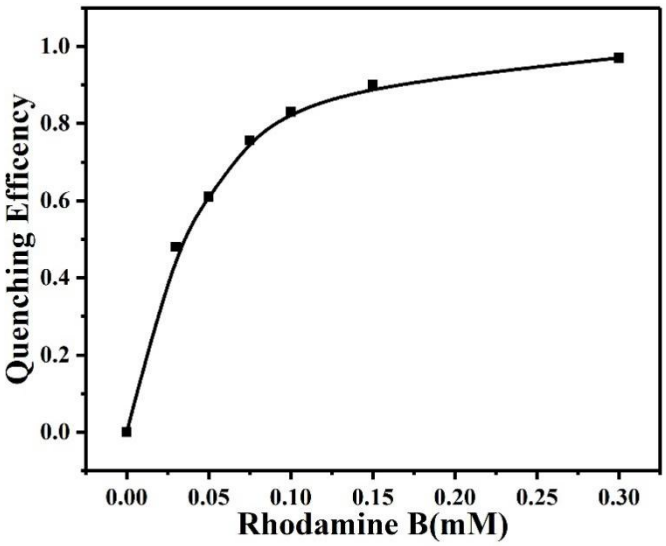

Figure S3. Plot of up-conversion luminescence quenching efficiency *vs.* rhodamine B concentrations.

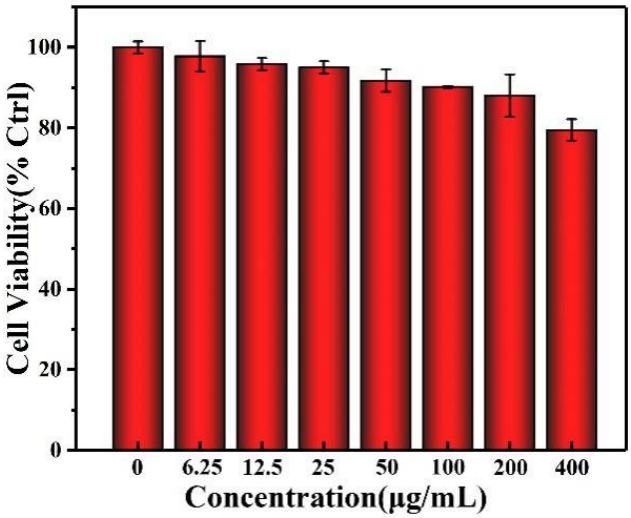

Figure S4. The viability of Hela cells treated with different concentrations of LBRU nanoprobe.

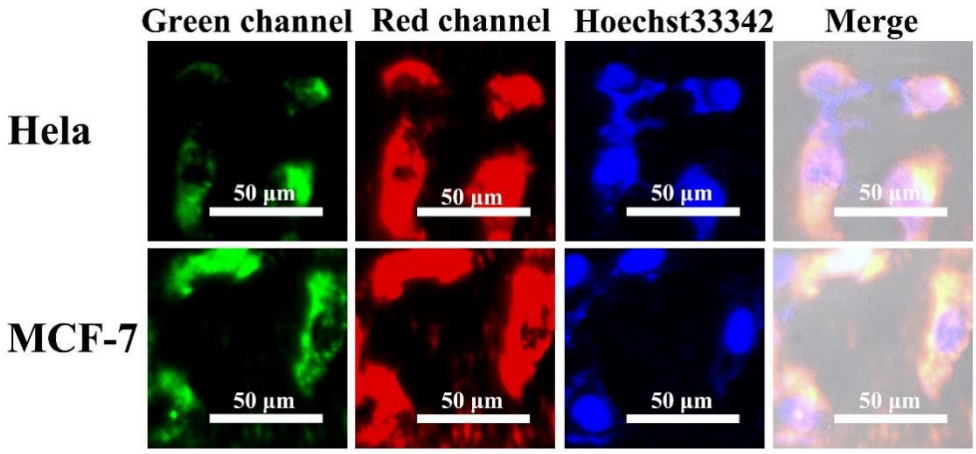

Figure S5. Upconversion luminescence imaging of Hela cells and MCF-7 cells incubated with 200 μg/mL with LBRU Nanoprobe and co-stained with Hoechst 33342. Luminescence signals was collected by green channel at 510-560 nm and red channel at 575-675 nm under excitation at 980 nm. Merge of bright field, green, and red upconversion luminescence images.

Figure S6 displays typical upconversion luminescent spectra of the LBRU nanoprobe which wrapped with C-141 in response to miRNA-141 under different concentrations. Similarly, with the increase concentrations (0.08 to 10 μM) of miRNA-141, the luminescence recovery at 540 nm (I_{540}) increased, while the luminescent intensity at 660 nm (I_{660}) remained unchanged.

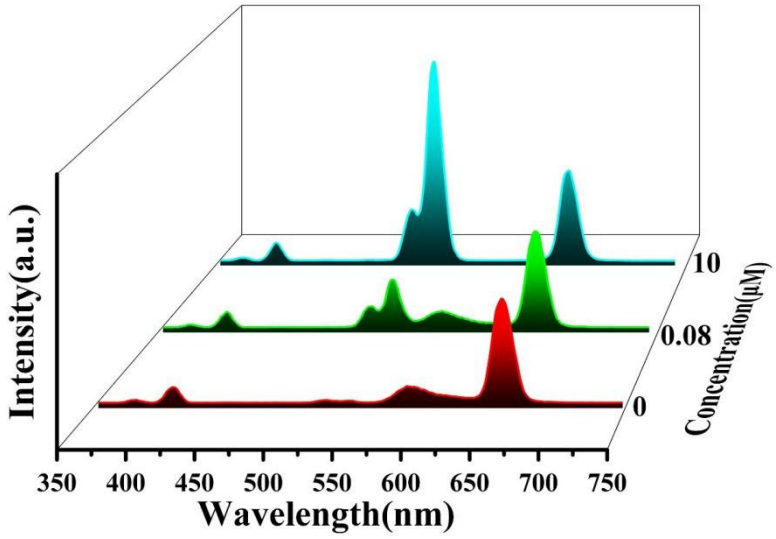

Figure S6. Upconversion luminescent spectra of LBRU nanoprobe in response to different concentrations of miRNA-141.

References

1. G. Gong, Y. Song, H. Tan, S. Xie, C. Zhang, L. Xu, J. Xu and J. Zheng, *Composites Part B: Engineering*, 2019, **179**, 107504.

2. S. Xie, G. Gong, Y. Song, H. Tan, C. Zhang, N. Li, Y. Zhang, L. Xu, J. Xu and J. Zheng, *Dalton Transactions*, 2019, **48**, 6971-6983.