# Electronic Supplementary Information

## Highly Sensitive Detection of Protein Kinase Activity Using Upconversion Luminescent Nanoparticles

Wenxia Yang,[a] Yucong Wang,[a] Lijuan Chang,[a] Chenghui Liu,*[a,b] Jie Bai,[a] Zhengping Li[a,b]

[a]*Key Laboratory of Medicinal Chemistry and Molecular Diagnosis, Ministry of Education; College of Chemistry and Environmental Science, Hebei University, Baoding 071002, Hebei Province, P. R. China*

[b]*Key Laboratory of Analytical Chemistry for Life Science of Shaanxi Province, School of Chemistry and Chemical Engineering, Shaanxi Normal University, Xi'an 710062, Shaanxi Province, P. R. China.*

**List of Contents:**

**1.** Materials and reagents

**2.** Preparation of avidin-UCNPs bioconjugates

**3.** Synthesis procedures for ZrMBs

**4.** Standard procedures for detection of PKA activity

**5.** Detection of PKA activity by using FITC-peptides

**1. Materials and reagents**

cAMP-dependent protein kinase A (PKA, catalytic subunit) was purchased from New England Biolabs and H-89 was obtained from EMD Bioscience. PKA specific substrate peptide (Biotin-LRRASLG) was supplied by GL Biochem Ltd. (shanghai, China). Avidin was purchased from Calbiochem. 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) and N-hydroxysulfosuccinimide sodium salt (sulfo-NHS) were obtained from Sigma-Aldrich and Alfa Aesar, respectively. The culture and drug stimulation of Hela cells as well as the preparation of cell lysates were performed according to our previous report.[1] All of the reagents used in this work were of analytical grade and used as purchased without further purification.

**2. Preparation of avidin-UCNPs bioconjugates**

Poly(acrylic acid) (PAA)-functionalized β-$NaYF_4$:Yb,Er UCNPs with an average diameter of ~30 nm (Fig. S-1) were prepared according to our previous reported approach.[2]

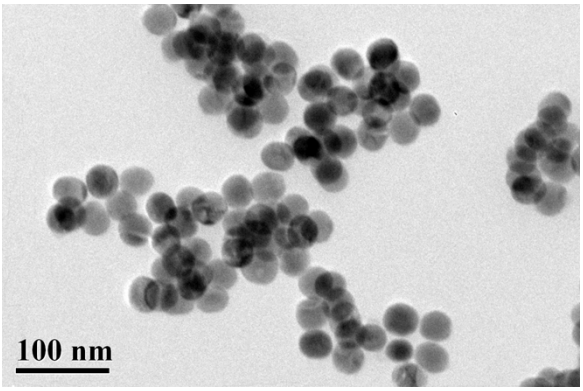


**Fig. S-1.** Typical TEM image of the β-$NaYF_4$:Yb,Er UCNPs

Standard EDC/sulfo-NHS-assisted procedures [3] were employed to crosslink carboxylic acid groups on UCNPs with amine-containing avidin. Typically, EDC (2 mM) and sulfo-NHS (5 mM) were added to 1 mL of MES buffer (10 mM, pH 5.5) containing 2 mg PAA-functionalized UCNPs to activate the carboxylic acid groups. The mixture was incubated at 30 °C for 2 h with shaking. After centrifugation and washed with HEPSE buffer (10 mM, pH 7.5), the precipitate was added to 1 mL of HEPES buffer containing 2 mg avidin. The linkage reaction was allowed to proceed at 30 °C for 10 h. Afterwards, 20 mg BSA was added to block any unreacted active sites on the

UCNPs. Finally, avidin-UCNPs bioconjugates were centrifuged, washed several times and dispersed in 0.5 mL Tris-HCl buffer (50 mM, pH 7.5) for further applications.

**3. Synthesis procedures for ZrMBs**

*Synthesis of magnetic $Fe_3O_4$ nanoparticles.* Magnetic $Fe_3O_4$ nanoparticles were prepared by a modified co-precipitation method.[4,5] Briefly, 2.36 g of $FeCl_3$•$6H_2O$ and 0.86 g of $FeCl_2$•$4H_2O$ (molar ratio of 2:1) were dissolved in 40 mL of deionized water with vigorous stirring under a nitrogen atmosphere. When the solution was heated to 90 °C, 5 mL of ammonium hydroxide was added quickly under rapid stirring, and the reaction was kept at 90 °C for 1 h. After cooled down to room temperature, the as-prepared black magnetic nanoparticles were separated magnetically, washed with deionized water and ethanol thoroughly. The products were then re-dispersed in 150 mL of 0.05 M citrate sodium (pH 5.4, coating reagent) solution for 3 h under rapid mechanical stirring. Subsequently, acetone was added to precipitate the citrate-capped $Fe_3O_4$ nanoparticles. The products were finally collected magnetically and dried at 60°C under vacuum.

*Preparation of magnetic silica microbeads (MBs).* Magnetic microbeads (MBs) were prepared by assembling citrate-capped $Fe_3O_4$ nanoparticles on $SiO_2$ microspheres. Typically, 1.3 g of $SiO_2$ microspheres (purchased from BaseLine Chromtech, 5~6 μm in diameter) were dispersed in 20 mL deionized water under ultrasonication. Then 3 mL of polyethyleneimine solution (PEI, 50 mg/mL) was added into the $SiO_2$ microspheres. After vortexing for 10 min, the $SiO_2$ microspheres were isolated via centrifugation (6000 rpm for 5 min), washed four times by deionized water and then dispersed in 20 mL water. As such, PEI was deposited on the $SiO_2$ microspheres to facilitate subsequent adsorption of $Fe_3O_4$ magnetic nanoparticle. Afterwards, a layer of $Fe_3O_4$ nanoparticles were assembled on the silica microspheres by adding 10 ml of aqueous citrate-capped $Fe_3O_4$ nanoparticles (5 mg/mL) to the PEI-coated $SiO_2$ microspheres, allowing 15 min for $Fe_3O_4$ adsorption and purification by three repeated magnetic isolation/water wash/re-dispersion cycles. In this study, MBs were fabricated by three cycles of PEI/$Fe_3O_4$ adsorption according to the assembly steps mentioned above, and the final products were dried at 60°C under vacuum.

Subsequently, 0.1 g of such MBs was dispersed in 4 mL of absolute alcohol, and followed by the addition of 0.8 mL of deionized water, 25 μL of TEOS and 20 μL of $NH_3•H_2O$. The mixture was incubated at room temperature for 3 h to coat the MBs with a thin layer of $SiO_2$. After purification, the MBs were further re-suspended in 4 mL of ethanol and 0.8 mL of water, and then 25 μL of 3-aminopropyltriethoxysilane and 20 μL of $NH_3•H_2O$ were added. The mixture was allowed to stand at ambient temperature for another 3 h under stirring to implement the amination of the MBs. Finally, the $NH_2$-MBs were washed four times with ethanol and collected magnetically.

*Preparation and characterization of ZrMBs.* The synthesis of ZrMBs was performed according to literature methods with some modifications.[6,7] 0.1 g of $NH_2$-MBs were dispersed in 7.5 mL of anhydrous toluene, and then 0.25 mL of pyridine and 0.25 mL of $POCl_3$ were added to the mixture. The reaction was allowed to proceed at ambient temperature for 18 h under shaking to produce the phosphonate-modified MBs. After washed with toluene and water respectively, the as-prepared phosphonate-modified MBs were suspended in 2 mL of deionized water. Afterwards, 20 mg of the phosphonate-modified MBs were stirred in 9.6 mL of water with 0.6446 g $ZrOCl_2•8H_2O$ overnight for the loading of $Zr^{4+}$. Finally, the as-synthesized ZrMBs were washed with water for three times to remove the excess $Zr^{4+}$ and re-dispersed in 2 mL water containing 30% acetonitrile (ACN).

The ZrMBs was characterized by SEM, and a typical SEM image was shown in Fig. S-2. It can be seen that the ZrMBs have an average diameter of ~6 μm. The representative energy-dispersive X-ray analysis (EDX) result of ZrMBs (Fig. S-3) reveals the existence of Zr, Fe and Si elements, and the EDX results of several randomly selected microspheres show similar atomic composition ratios of Si/Fe/Zr (Si/Fe/Zr=23.92/2.23/0.84 (w%)), confirming the successful formation of ZrMBs.

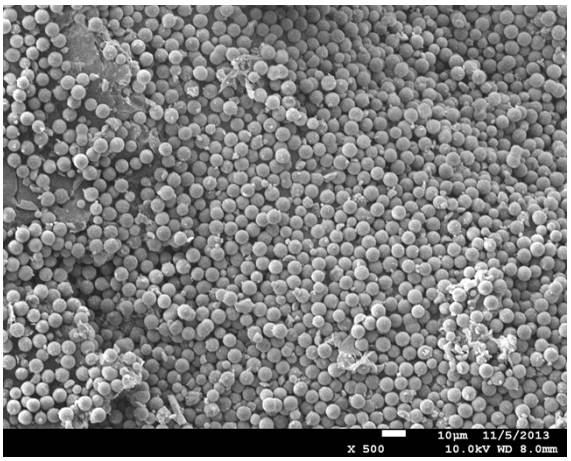


**Fig. S-2.** Typical SEM images of the as-prepared ZrMBs

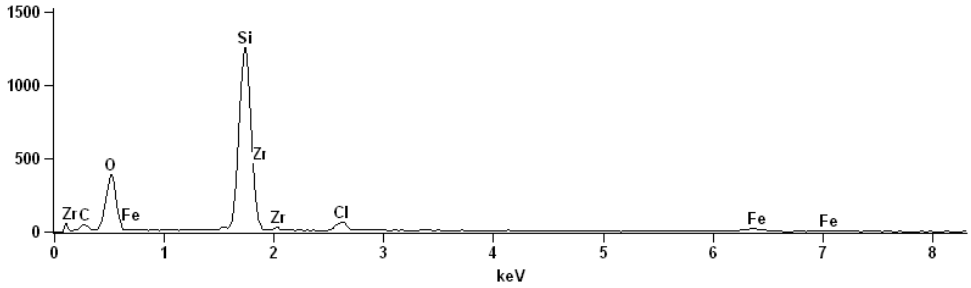


**Fig. S-3.** Representative EDX result of the ZrMBs

**4. Standard procedures for detection of PKA activity**

In a typical 100 μL phosphorylation reaction system, biotin-peptides (1.5 μM) were treated with a certain amount of PKA at 30 °C for 1 h in 50 mM Tris-HCl buffer (pH 7.5) containing 10 mM $MgCl_2$ and 12 μM ATP. After the phosphorylation reaction, the mixture was directly mixed with an equal volume of acetonitrile containing 1.0 mg/mL of ZrMBs and incubated for 0.5 h at room temperature to capture the phosphorylated biotin-peptides. The phosphopeptides-loaded ZrMBs were washed with water and then incubated with avidin-UCNPs (0.2 mg/mL) in Tris-HCl buffer containing 1% BSA. After magnetic separation and washing steps, the amount of UCNPs anchored on ZrMBs was proportional to the PKA activity. The UCNPs-avidin/biotin-phosphopeptides complexes can be dissociated from ZrMBs surface after incubation with 0.5% $NH_3 \cdot H_2O$ solution for 0.5 h. By recording the UC luminescence signal of the dissociated UCNPs solutions, quantitative analysis of PKA activity can be realized. UC photoluminescence spectra

were recorded using a Fluorolog 3-211 fluorescence spectrophotometer (Horiba Jobin-Yivon, France) with an external 980 nm laser diode (Viasho, China) as the excitation source.

For PKA inhibitor assay, the experiments were carried out via similar procedures as those for PKA assay stated above, except for the pre-incubation of a fixed PKA concentration of 0.02 U/μL and varied concentrations of H-89 (0~5 μM) in the reaction mixture.

**5. Detection of PKA activity by using FITC-peptides**

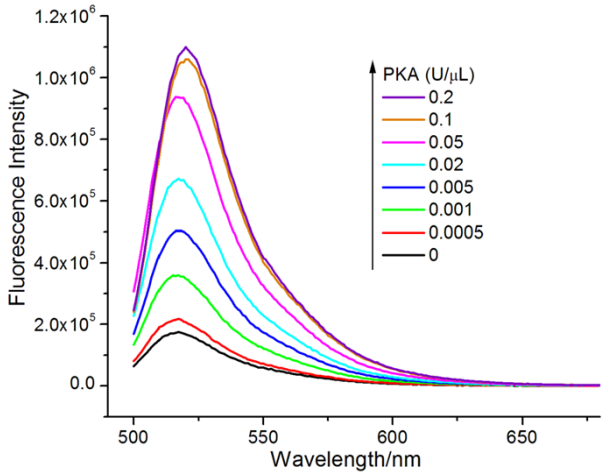


**Fig. S-4.** Fluorescence spectra of the PKA assay system by using FITC-LRRASLG peptides. The concentrations of ZrMBs and peptides are the same as those used in Fig. 2.

**References**


[1] J. Bai, Y. Zhao, Z. Wang, C. Liu, Y. Wang and Z. Li, *Anal. Chem.*, 2013, **85**, 4813.

[2] C. Liu, H. Wang, X. Li and D. Chen, *J. Mater. Chem.*, 2009, **19**, 3546.

[3] G. T. Hermanson, *Bioconjugate Techniques (2nd Edition)*, Academic: San Diego, 2008, pp. 595.

[4] Z. Y. Ma, Y. P. Guan and H. Z. Liu, *J. Magn. Magn. Mater.*, 2006, **301**, 469.

[5] Z. Y. Ma, D. Dosev, M. Nichkova, S. J. Gee, B. D. Hammock and I. M. Kennedy, *J. Mater. Chem.*, 2009, **19**, 4695.

[6] L. Hu, H. Zhou, Y. Li, S. Sun, L. Guo, M. Ye, X. Tian, J. Gu, S. Yang and H. Zou, *Anal. Chem.*, 2009, **81**, 94.

[7] L. Zhao, R. Wu, G. Han, H. Zhou, L. Ren, R. Tian and H. Zou, *J. Am. Soc. Mass Spectrom.*, 2008, **19**, 1176.