Electronic Supplementary Information

LaF_3 Nanoparticle-Assisted Sensitive Detection of Protein Kinase Activity

Yunlong Fan, Yucong Wang,* Peipei Wang and Zhengping Li

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

List of Contents:

1. Optimization of LaF_3 NPs concentration

LaF_3 concentration was optimized by using commercially synthesized fluorescein-labeled and phosphorylated peptides (FITC-LRRApSLG, p indicates that the serine is phosphorylated), which would be captured on LaF_3 NPs directly. Series dilutions of LaF_3 NPs were respectively incubated with the peptide of a fixed concentration (2 μM). After centrifugation and redispersion, the fluorescence intensities of the LaF_3 NPs bearing the FITC-LRRApSLG peptides were recorded respectively. As can be seen from Fig. S-1, the fluorescence signal increases gradually when the concentration of LaF_3 is lower than 3 mg/mL. However, the fluorescence signals become stable when the concentration of the phosphopeptides is higher than 3 mg/mL. Therefore, it is reasonable to conclude that the 3 mg/mL of LaF_3 NPs is enough for capturing 2 μM phosphorylated peptides. To ensure high binding efficiency, 5 mg/mL of LaF_3 (2.5 mg/mL in the final 200 μL detection system) is used in this work.

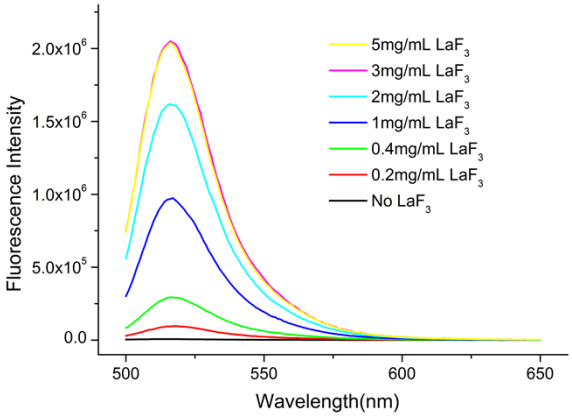

Fig. S-1. Fluorescence spectra of the LaF_3-based system in the presence of different concentrations of LaF_3 NPs

2. Effect of incubation time between the PKA reaction system and LaF_3 NPs

According to the procedures of the LaF_3 NPs-Based PKA assay, we further optimized the incubation time between the PKA reaction system and LaF_3 NPs. As can be seen from Fig. S-2, the fluorescence signal increases gradually with extending the incubation time and reaches its

maximum at 60 min. Meanwhile, the highest S/B ratio is also obtained at 60 min. Therefore, we selected 60 min for further analytical applications in this work.

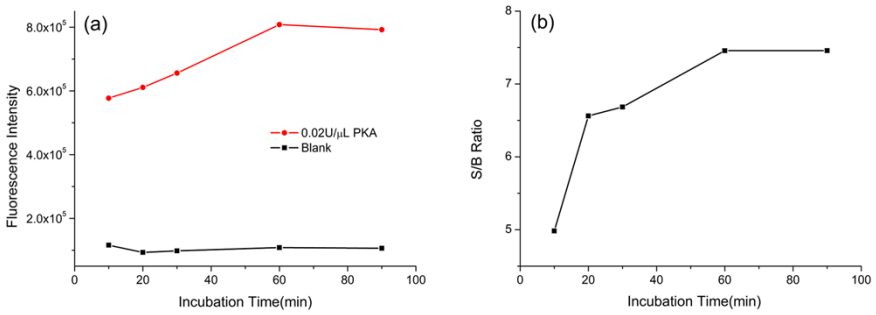

Fig. S-2. Effect of incubation time between the PKA reaction system and LaF_3 NPs

3. Detection of drug-stimulated activation of PKA in cell lysates

MCF-7 breast cancer cells (1×10^6 cells) were cultured in DMEM with 10% fetal bovine serum, 1% insulin-transferrin-selenium-A supplement, penicillin (100 U/mL), streptomycin (100 mg/mL), and amphotericin B (0.25 mg/mL) at 37 ºC in a 5% CO_2 – 95% air incubator. Then the culture medium was replaced by a serum-free medium, and the cells were incubated for 4 h before stimulation. Afterward, 10 μM of forskolin and 20 μM of IBMX in dimethyl sulfoxide (DMSO) were added to the medium to activate intracellular PKA. DMSO (equal volume) instead of forskolin/IBMX solution was added to the medium for unstimulated sample. After 30 min of stimulation, the cells were removed by scraping and lysed in Dulbecco's phosphate-buffered saline (D-PBS). The mixture was sonicated three times for 2 s × 60 times at an interval of 3 s for each time and then centrifuged at 22000 rpm for 60 min at 4 °C. The resulting cell lysate was stored at –20 °C before use. Total protein concentrations in cell lysates were quantified by using the improved Bradford protein assay dye reagent kit (SK3051, Sangon) with BSA as the standard.

In this study, the total protein concentration of cell lysate was diluted to 10 μg/mL for the proposed kinase activity assay. As shown in Fig. S-3, the cell lysate without drug stimulation has a relative low fluorescence signal. Meanwhile, the fluorescence response increases obviously when the MCF-7 breast cancer cell is stimulated with the combination of

forskolin/IBMX. These results indicate that the proposed method is feasible for the detection of drug-triggered activation of PKA in cells. Therefore, the LaF_3-basd method is potentially applicable for PKA analysis in complex biological samples.

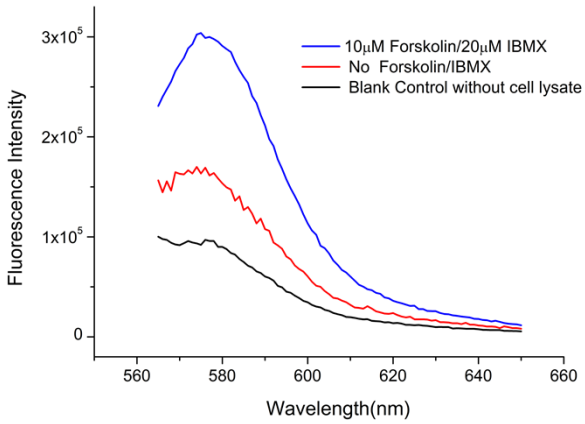

Fig. S-3. Fluorescence spectra of the LaF_3-based system for detection of cell PKA activities. The total protein concentrations of MCF-7 cell lysates are all 10 μg/mL.