Supporting Information

A Versatile Fluorescence Turn-On Assay for Highly Sensitive Detection of Tyrosine Phosphatase Activity

Chenghui Liu,*[ab] Fangfang Wang,[b] Yucong Wang[b] and Zhengping Li *[ab]

[a] *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*

[b] *College of Chemistry and Environmental Science, Hebei University, Baoding 071002, Hebei Province, P. R. China*

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1. Experimental Section

Chemicals and materials. Protein tyrosine phosphatase 1B (PTP1B) was purchased from Sino-Biological (Beijing, China), and was dissolved in HEPES buffer (10 mM, pH 7.5) containing 0.05% bovine serum albumin and 1 mM dithiothreitol (DTT). PTP1B-specific phosphopeptide substrate (DADEpYLIPQQG) was custom synthesized by GL Biochem. (Shanghai, China). All other reagents were commercially purchased and used without further purification.

Detection procedure of phosphate ion (Pi). In a total volume of 200 μL of HEPES reaction buffer (10 mM, pH 7.5), varying concentrations of Pi were firstly incubated with 500 nM of Ce^{3+} at room temperature for 1 h. Subsequently, after addition of calcein (final concentration of 500 nM), the fluorescence signal of the mixture was immediately measured on a Fluorolog 3-211 fluorescence spectrophotometer (Horiba Jobin-Yivon, France) at room temperature.

Standard experimental procedures for the detection of PTP1B activity. Typically, in a 100 μL of PTP1B reaction buffer (10 mM HEPES, pH 7.5), 2 μM of PTP1B-specific phosphopeptide substrate (DADEpYLIPQQG) was incubated with varying concentrations of PTP1B at 30 ºC for 1.5 h in the presence of 1 μM of Ce^{3+}. Afterward, 100 μL of calcein (1 μM) was added to the above reaction system, which was then directly subjected to the measurement of fluorescence signal.

2. Optimization of Experimental Conditions and Results for the Detection of PO_4^{3-} (Pi)

Optimization of Ce^{3+} concentration. Efficient fluorescence quenching of calcein by Ce^{3+} is the basis of this proposed sensing platform, so the optimal concentration of Ce^{3+} is first investigated. It is observed from Fig. S-1 that calcein exhibits strong fluorescence emission around ~512 nm in the absence of Ce^{3+}. However, the fluorescence signals decrease gradually with the increase of Ce^{3+}. When Ce^{3+} is mixed with calcein in a 1:1 stoichiometry, the fluorescence will be quenched almost completely (more than 95%). Furthermore, it can be seen from Fig. S-1b that the fluorescence of calcein can be completely quenched immediately after mixing with Ce^{3+}, indicating that Ce^{3+} ions coordinate with calcein with rather high kinetics, which enables rapid fluorescence detection of Pi as well as PTP1B activity.

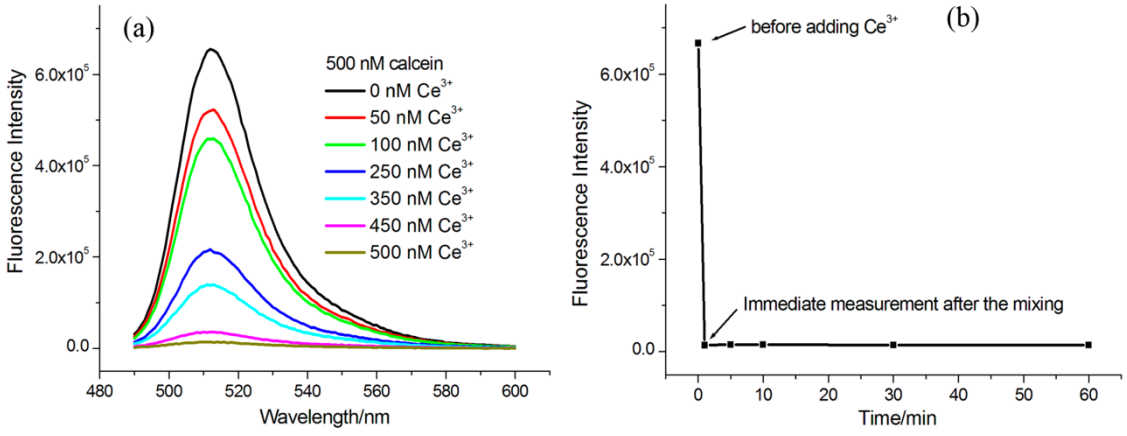


Fig. S-1. (a) Fluorescence spectra of calcein (500 nM) in the presence of varying concentrations of Ce^{3+} ion; (b) fluorescence quenching of calcein (500 nM) by Ce^{3+} (500 nM) as a function of time.

Optimization of the reaction time between Ce^{3+} and Pi. For the detection of Pi, the assay was performed by firstly incubating Ce^{3+} with Pi for a certain time to form $CePO_4$. Then fluorescence spectra were recorded immediately after blending the mixture with calcein. So the reaction time between Ce^{3+} and Pi is optimized. As shown in Fig. S-2, the fluorescence signal increases sharply at

the beginning with increasing incubation time of Ce^{3+} and Pi. However, when the reaction time is longer than 30 min, no obvious fluorescence increase will be observed, indicating the efficient formation of $CePO_4$. In contrast, the fluorescence signal of the blank control (without Pi) keeps almost a constant. Therefore, to ensure high reaction efficiency, 60 min incubation time for Ce^{3+} and Pi is selected in this work for Pi analysis.

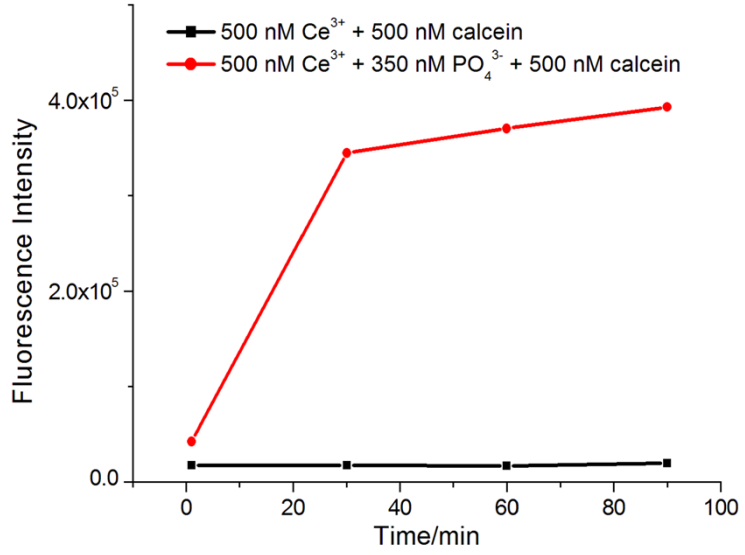


Fig. S-2. Optimization of reaction time between Ce^{3+} and Pi. Red line: 350 nM of Pi was incubated with 500 nM of Ce^{3+} for different times in HEPES buffer (10 mM, pH 7.5), and then mixed with calcein (500 nM) for fluorescence intensity measurement at 512 nm; Black line, blank control without the addition of Pi.

Analytical performance for the detection of Pi. According to the standard protocols stated in the experimental section, the analytical performance of the proposed approach for the quantitative determination of Pi is investigated. Fig. S-3a shows the fluorescence responses for different Pi concentrations. It is observed that the fluorescence signals increase gradually as the concentrations of Pi vary from 5 nM to 500 nM. One can also see from Fig. S-3a that when 500 nM of Pi is

introduced, the fluorescence is almost fully recovered to the initial intensity of the unquenched calcein with a striking 43-fold fluorescence enhancement, indicating that the high binding efficiency between PO_4^{3-} and Ce^{3+} effectively prevent calcein from quenching. The dependence of the fluorescence intensities recorded at 512 nm on Pi concentrations is plotted in Fig. S-3b. It can be seen that as low as 5 nM Pi can be discriminated from the blank control, and the fluorescence intensities are linearly proportional to the Pi concentrations in the ranges of 5 nM to 200 nM, and 200 nM to 400 nM, respectively. Correspondingly, the linear regression equations are $I_F = 525\ C_{Pi} + 12358$ (R=0.9993), and $I_F = 1888\ C_{Pi} - 268164$ (R=0.9985), respectively.

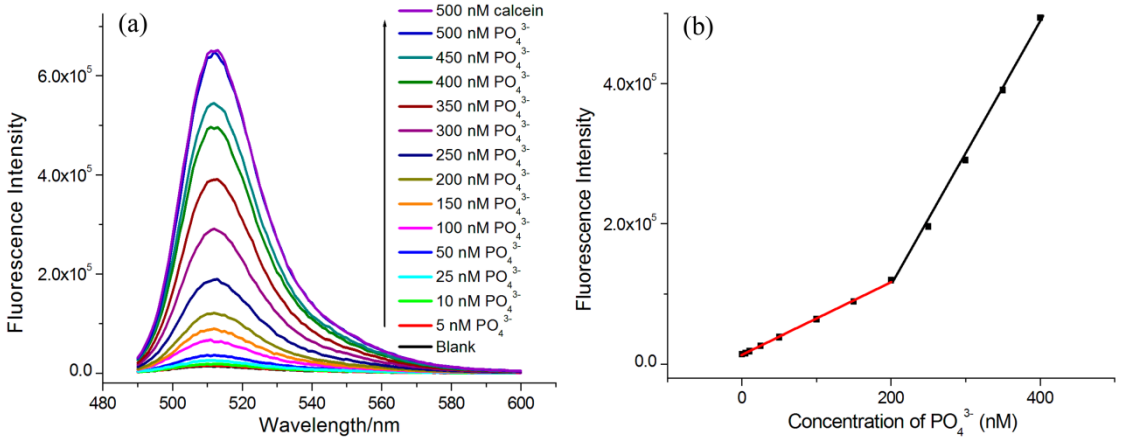


Fig. S-3. (a) Fluorescence spectra of the proposed calcein/Ce^{3+}-based sensing system in the presence of varying concentrations of Pi. Pi concentration (from bottom to the top): 0, 5 nM, 10 nM, 25 nM, 50 nM, 100 nM, 150 nM, 200 nM, 250 nM, 300 nM, 350 nM, 400 nM, 450 nM and 500 nM, respectively; (b) plot between the fluorescence intensities (I_F) at 512 nm and Pi concentrations. Experimental conditions: calcein, 500 nM; Ce^{3+}, 500 nM.

Detection sensitivity is one of the most important aspects to assess a new bioassay. Up to now, most of the commercially available kits for Pi are based on the malachite green-based colorimetric

assay. The sensitivity of the proposed method is further compared with that of malachite green-based assay. This colorimetric protocol is based on the formation of a complex between malachite green molybdate and free Pi. By recording the absorbance of the formed complex at 630 nm, the amount of Pi can be quantitatively determined. In this study, the preparation of the malachite green working reagents and the detection procedures were all according to a standard protocol reported previously.[1,2] Varying concentrations of Pi was mixed with malachite green working reagent to a final volume of 200 μL. After incubating for 10 minutes, absorbance spectra were recorded on a TU-1901 UV-Vis spectrophotometer (Pgeneral, Beijing).

Fig. S-4 displays the Pi detection results by using such colorimetric method, from which the detection limit of Pi is estimated to be ~0.5 μM. Therefore, it can be seen that the sensitivity of the proposed calcein/Ce^{3+}-based assay is about two orders of magnitude higher than the most widely used malachite green-based colorimetric method.

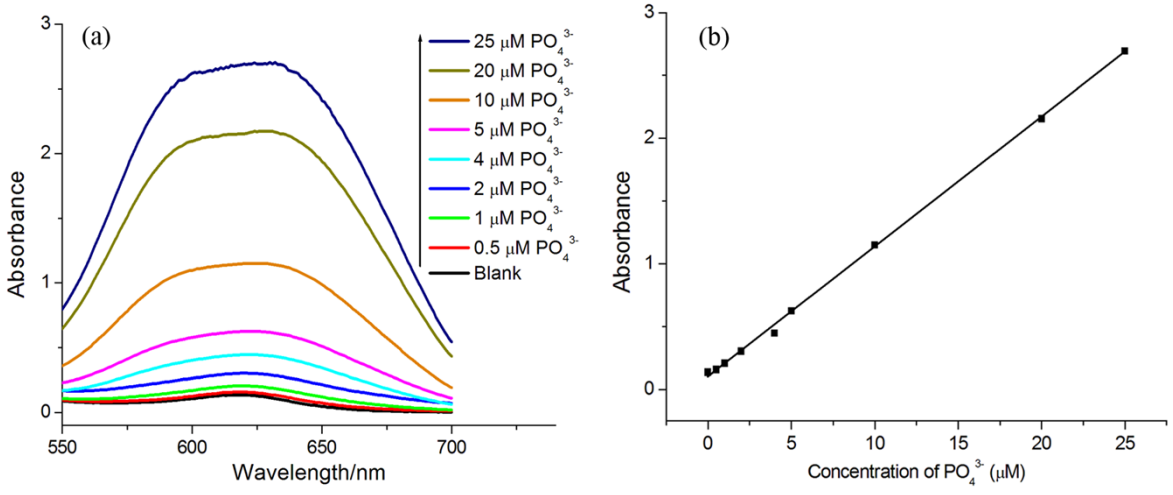
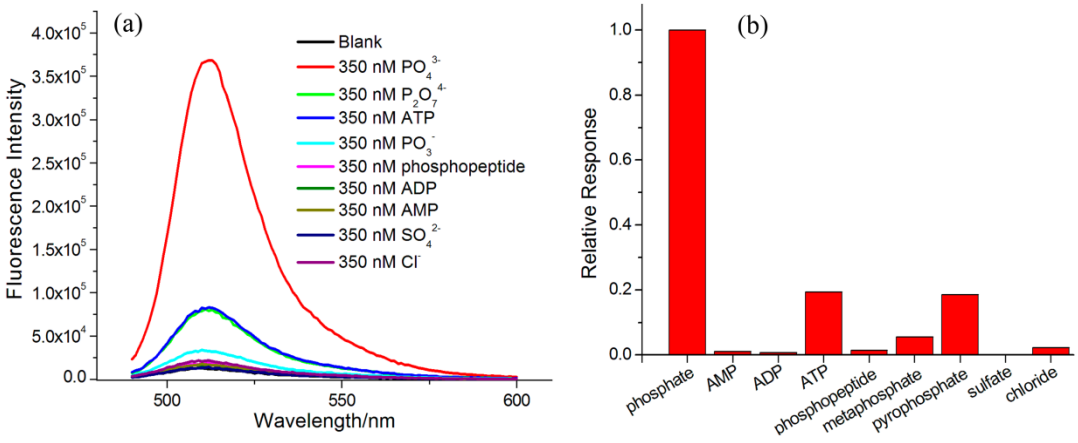


Fig. S-4. Detection of Pi by malachite green-based colorimetric method. (a) absorbance spectra of the assay system in the presence of different concentrations of Pi; (b) the relationship between the

absorbance at 630 nm and Pi concentrations.

Specificity evaluation for Pi analysis. Furthermore, to evaluate its specificity, the proposed assay is also challenged by $P_2O_7^{4-}$, AMP, ADP, ATP, PO_3^-, phosphopeptide (DADEpYLIPQQG), SO_4^{2-}, and Cl^- under the same conditions as in the case of Pi detection. As shown in Fig. S-5a, only Pi can arouse remarkable fluorescence enhancement and the responses of AMP, ADP, PO_3^-, phosphopeptide (DADEpYLIPQQG), SO_4^{2-} and Cl^- are all rather low. Although the interferences of ATP or $P_2O_7^{4-}$ seem relatively higher than other compounds, the responses are both below 20% of the Pi-induced signal (Fig. S-5b), indicating that the selectivity of the proposed method is satisfactory for detection of Pi. In addition, as shown in Fig. S-5c, the co-existence of some common cations such as Fe^{2+}, Fe^{3+}, K^+, Mg^{2+} and Ca^{2+} in the calcein/Ce^{3+}-based system also do not interfere with the detection of Pi. More importantly, the results shown in Fig. S-5 demonstrate that the interference of phosphopeptides is negligible for Pi detection, indicating that the organic phosphate groups on the phosphopeptides are unable to complex with Ce^{3+}. Therefore, this proposed sensing platform shows high discrimination ability towards free Pi and phosphopeptides, which provides a sound basis for the fabrication of a phosphatase assay.



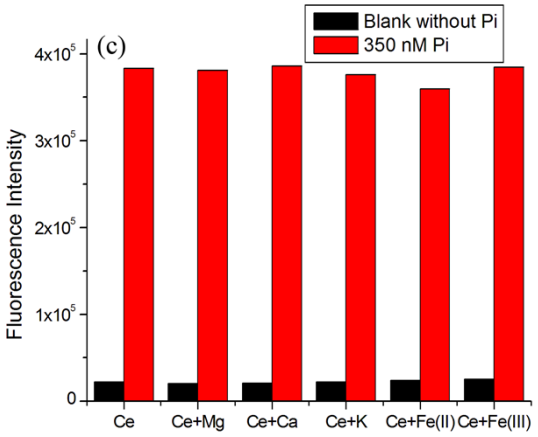


Fig. S-5. Specificity evaluation of the proposed Pi assay. (a) fluorescence spectra of the sensing system in the presence of different targets (with the same concentration of 350 nM); (b) relative responses of the same concentrations of interferents compared to Pi. Experimental conditions: Ce^{3+}, 500 nM; calcein, 500 nM; (c) effect of some common cations on the detection of Pi as they co-exist with Ce^{3+} in the calcein/Ce^{3+}-based sensing system. Experimental conditions: Ce^{3+}, 500 nM; calcein, 500 nM; Pi, 350 nM; all of the cation interferents are controlled at 350 nM.

3. Detection of PTP1B Activity by Using Malachite Green-Based Colorimetric Method

In a 100 μL of reaction buffer, 50 μM of phosphopeptide substrate was incubated with varying concentrations of PTP1B at 30 ºC for 1.5 h. Then the reaction solution was subsequently subjected to the malachite green-based colorimetric assay,[1,2] and the results are shown in Fig. S-6.

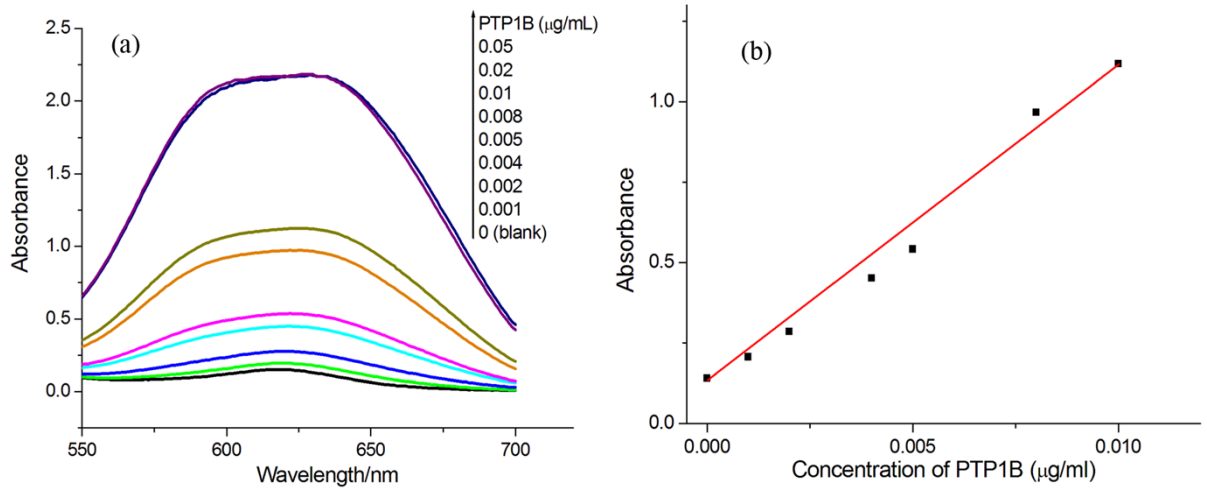


Fig. S-6. (a) Absorbance spectra of the malachite green-based assay system in the presence of varying concentrations of PTP1B; (b) the relationship between the absorbance at 630 nm and PTP1B concentrations.

4. Detection of PTP1B activity by the calcein/Ce^{3+}-based assay with high concentrations of sensing elements

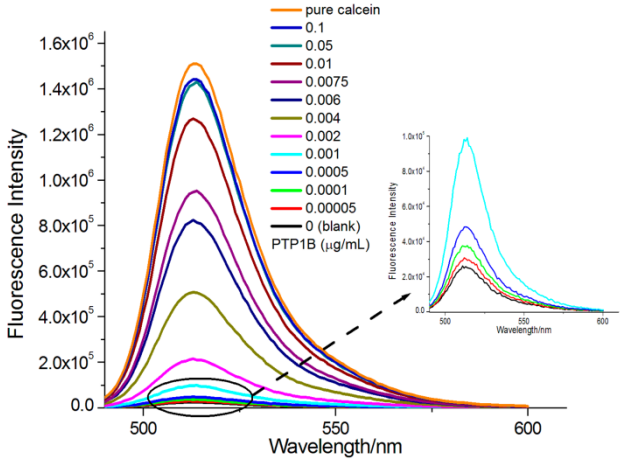


Fig. S-7. Analytical performance of the calcein/Ce^{3+}-based system with high concentrations of sensing elements for detection of PTP1B activity. Experimental conditions: calcein, 10 μM; Ce^{3+}, 10 μM; phosphopeptides substrate, 20 μM.

5. Detection of PTP1B in cell lysate media

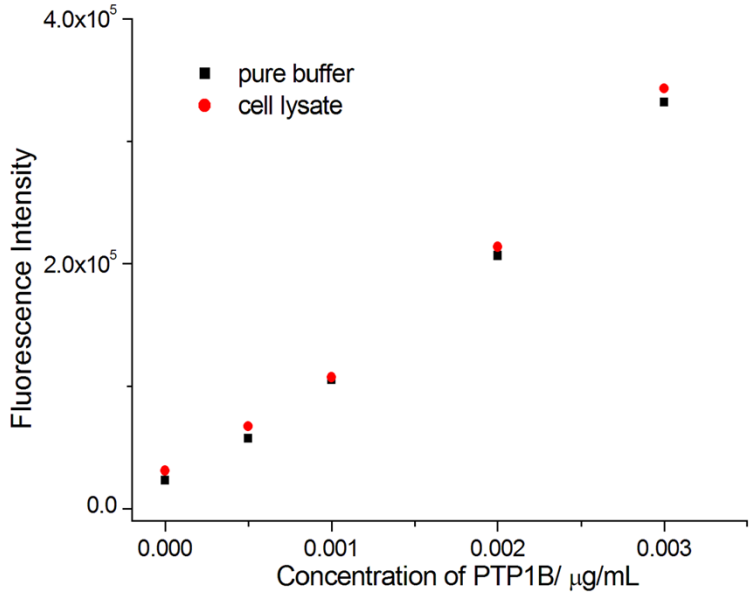


Fig. S-8. Comparison of the proposed PTP1B assay in the media of cell lysates (red, 5 μg/mL total protein concentration for each cell lysate sample) and clean buffer (black). Experimental conditions: calcein, 10 μM; Ce^{3+}, 10 μM; phosphopeptides substrate, 20 μM. The culture of HeLa cells as well as the preparation of cell lysates were performed according to our previous report.[3]

References:

[1] Mercan, F.; Bennett, A. M. *Curr. Protoc. Mol. Biol.* **2010**, *91*, 18.16.1.

[2] Baykov, A. A.; Evtushenko, O. A.; Avaeva, S. M. *Anal. Biochem.* **1988**, *171*, 266.

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