Electronic Supplementary Information

Robust Detection of Tyrosine Phosphatase Activity by Coupling Chymotrypsin-Assisted Selective Peptide Cleavage and Graphene Oxide-Based Fluorescent Platform

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Supporting Information

1. Materials and reagents

Protein tyrosine phosphatase 1B (PTP1B) was purchased from Sino Biological Inc. (Beijing, China). Bovine α-chymotrypsin (CMT) and PTP1B inhibitor RK-682 were obtained from Sigma-Aldrich (St. Louis, MO, USA). Fluorescein-attached PTP1B-specific peptide substrate (FITC-DADEpYLRRRRR) was custom synthesized by GL Biochem Ltd. (Shanghai, China). Graphene oxide (GO) was prepared by a modified Hummer's method[1] as described in our previous work.[2] All other reagents were purchased from commercial vendors and used without further purification.

2. Standard experimental procedures for PTP1B and inhibition assay

Typically, in a 100 μL of reaction buffer (10 mM HEPES, pH 7.5), FITC-DADEpYLRRRRR (2 μM) was incubated with varying concentrations of PTP1B and 8 nM of CMT at 30ºC for 60 min to allow the process of dephosphorylation and cleavage reactions. Afterward, this reaction solution was directly mixed with 100 μL of GO (final concentration is 0.2 mg/mL), which was then immediately subject to the measurement of fluorescence spectra (λ_{ex}=480 nm) on a Fluorolog 3-211 fluorescence spectrophotometer (Horiba Jobin-Yivon, France).

For PTP1B inhibition assay, the experiments were carried out in the same procedures as those for PTP1B assay stated above, except for the pre-incubation of a fixed PTP1B concentration with varying concentrations of RK-682 (0-100 μM) or orthovanadate (0-500 nM) in the reaction mixture.

3. Optimization of GO concentration

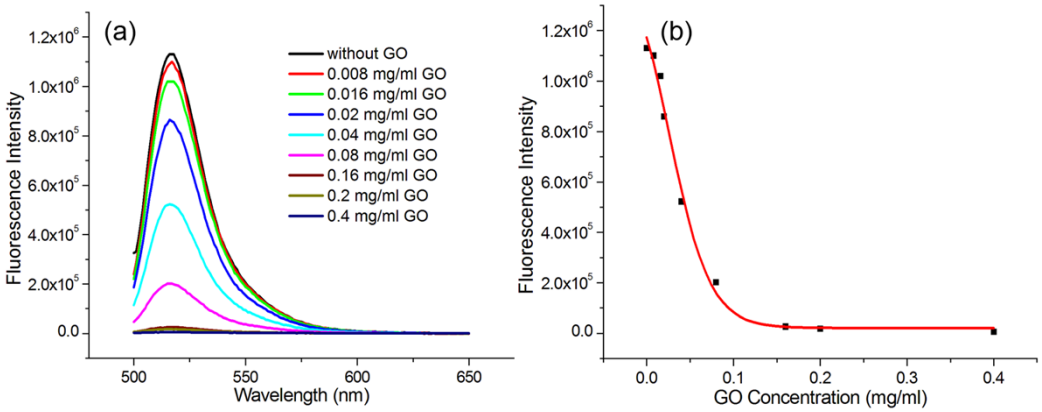

Fig. S1 (a) Fluorescence spectra of the FITC-phosphopeptide (2 μM) after addition of an equal volume of GO with different concentrations; (b) plot of fluorescence intensity at 516 nm with GO

concentrations.

Whether the rationally designed FITC-phosphopeptide could be effectively quenched by GO was crucial for the proposed assay, which was firstly explored in this work. When the concentration of FITC-phosphopeptide was fixed, the quenching efficiency could be strongly influenced by GO concentration. As shown in Fig. S1, with the increasing concentration of GO, the fluorescence intensity of FITC-phosphopeptide decreased obviously and was almost completely quenched when GO reached 0.2 mg/mL. Therefore, 0.2 mg/mL GO was selected for further application in this work to ensure high quenching efficiency. Furthermore, it should be noted that the peptide adsorbed on GO with high kinetics so that the adsorption completed immediately after adding 0.2 mg/mL of GO (with high quenching efficiency of more than 98%).

4. Effect of chymotrypsin (CMT) concentration on the PTP1B assay

The phosphate-dependent differential CMT cleavage rate provided a possibility that an appropriate amount of CMT could be chosen such that the dephosphorylated peptide was cleaved, yet most of the phosphorylated peptide remained intact within the time frame of the assay. So the CMT concentration was another key issue for this proposed assay. In this work, to evaluate the cleavage ability of CMT towards phosphorylated and dephosphorylated peptides, a series of FITC-phosphopeptides were treated with a high concentration of PTP1B (fixed at 0.2 μg/mL, to ensure high dephosphorylation efficiency of the peptides) and different concentrations of CMT (0 nM, 0.8 nM, 1.6 nM, 3.2 nM, 8 nM, 16 nM, 32 nM, 80 nM, 160 nM). Meanwhile, FITC-phosphopeptides treated with only such varying concentrations of CMT but without PTP1B were used as the blank control.

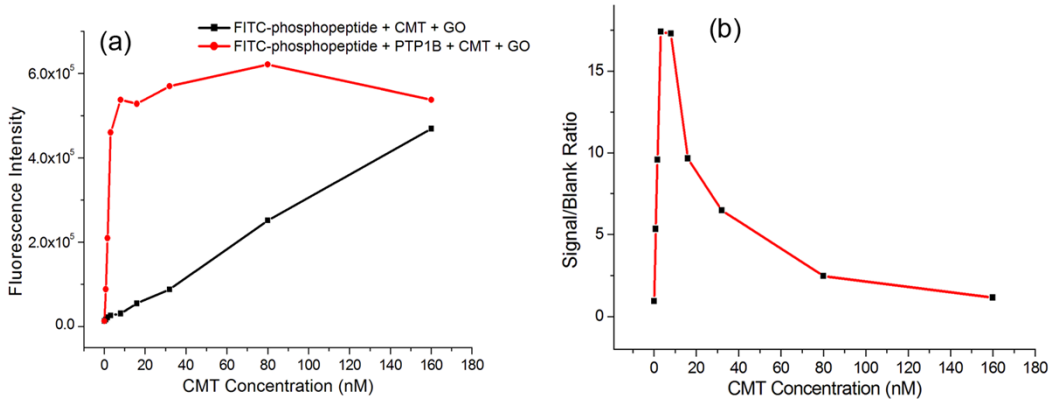

Fig. S2 (a) Effect of CMT concentration on the PTP1B assay. Black line: blank control refers to the FITC-phosphopeptides treated with only CMT but without PTP1B; Red line: FITC-

phosphopeptides treated with both 0.2 μg/mL PTP1B and CMT; (b) plot for the ratios of the signals produced by PTP1B/CMT to the blank produced only by CMT. Other conditions are the same as described in the standard assay procedures.

As can be seen from Fig. S2a that in the presence of PTP1B, the fluorescence intensity (peak value at ~516 nm) increased sharply with the increasing CMT concentration from 0 to 8 nM. However, no obvious fluorescence increase was observed when further elevating the CMT from 8 nM to 160 nM. As a contrast, for the control samples treated with only CMT but without PTP1B, the fluorescence signals always increased gradually with the increasing of CMT concentration, indicating that the more CMT involved, the more possibility the phosphorylated peptides be cleaved. According to the results shown in Fig. S2b, when 4 nM or 8 nM CMT was used, the ratio of the signal produced by both PTP1B/CMT to the blank produced only by CMT reached its highest. Therefore, we selected 8 nM as the optimal concentration of CMT for further analytical applications in this study.

5. Electrophoresis results of the FITC-attached phosphopeptide treated with different enzyme components

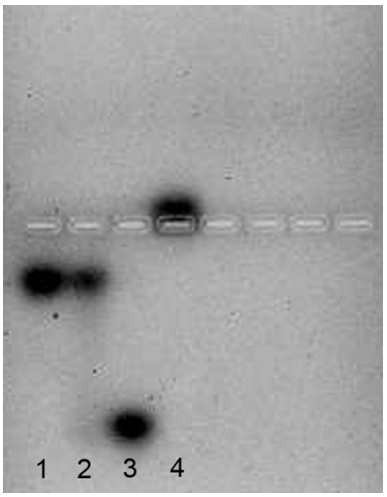

Fig. S3 Electrophoresis results of the FITC-phosphopeptide treated with different enzyme components. Lane 1: pure FITC-phosphopeptides (20 μM); Lane 2: FITC-phosphopeptides (20 μM) treated with 80 nM CMT only; Lane 3: FITC-phosphopeptides (20 μM) treated with 80 nM CMT and 0.01 μg/mL PTP1B; Lane 4: FITC-phosphopeptides (20 μM) treated with 0.01 μg/mL PTP1B only.

When the FITC-phosphopeptides were treated by PTP1B or the combination of PTP1B/CMT, the products might have different lengths as well as net charges due to the dephosphorylation and

CMT cleavage reaction. Therefore, gel electrophoresis may be used to verify the validity of our proposed assay. In this study, the PTB1B and/or CMT reactions were carried out in the similar procedures as those for PTP1B assay stated above (without adding GO), but the reaction components such as PTP1B, CMT as well as FITC-phosphopeptides were all 10-fold concentrated to make the electrophoresis band (signal originated from the fluorescence of FITC) more clear. 3% agarose gel electrophoresis (110 V, 1h) was performed for the analysis of peptide products, and the results were provided in Fig. S3.

Lane 1 was the band of pure FITC-phosphopeptides. Probably due to their phosphate groups, the peptides showed a short migration towards the positive electrode. Meanwhile, when the FITC-phosphopeptides was reacted with PTP1B, the dephosphorylation of substrate peptides made them positively charged (pI of ~10.61) in the electrophoresis buffer (pH 8.0), so the dephosphorylated FITC-peptide tended to migrate to the negative electrode (Lane 4). If the FITC-phosphopeptides were incubated with the combination of PTP1B/CMT, the cleaved peptide fragments bearing the fluorescein probes were short FITC-DADEY (pI of ~3.49) with negative charge in the electrophoresis buffer, which moved obviously to the positive electrode, as shown in lane 3. The result shown in lane 3 can also demonstrate that the high efficiency of PTP1B-assisted CMT cleavage reaction, because the FITC-phosphopeptides treated with CMT but without PTP1B only showed a sharply decreased peptide cleavage rate (Lane 2). The electrophoresis results were well consistent with the fluorescence results shown in Fig. 2. It should be pointed out that to make the peptide bands more clear in the electrophoresis study, the concentrations of CMT and the FITC-phosphopeptide were both 10-fold concentrated than those in the standard PTP1B assay system, so the undesirable cleavage of phosphopeptides (Lane 2) would be further reduced under lower CMT concentration according to the standard PTP1B protocol.

6. Detection of PTP1B activity by the malachite green-based colorimetric assay

In a 100 μL of reaction system, 50 μM of phosphopeptide substrate was incubated with varying concentrations of PTP1B at 30ºC for 60 min to perform the dephosphorylation reaction. Then the reaction solution was directly subject to the malachite green-based colorimetric assay. This colorimetric assay is based on the formation of a complex between malachite green molybdate and free phosphate ion released from the PTP1B-catalyzed dephosphorylation reaction. By recording the absorbance of the formed complex at 620~650 nm, the amount of phosphate ions

which are correlated with the PTP1B activity 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,[3,4] and the results were shown in Fig. S4.

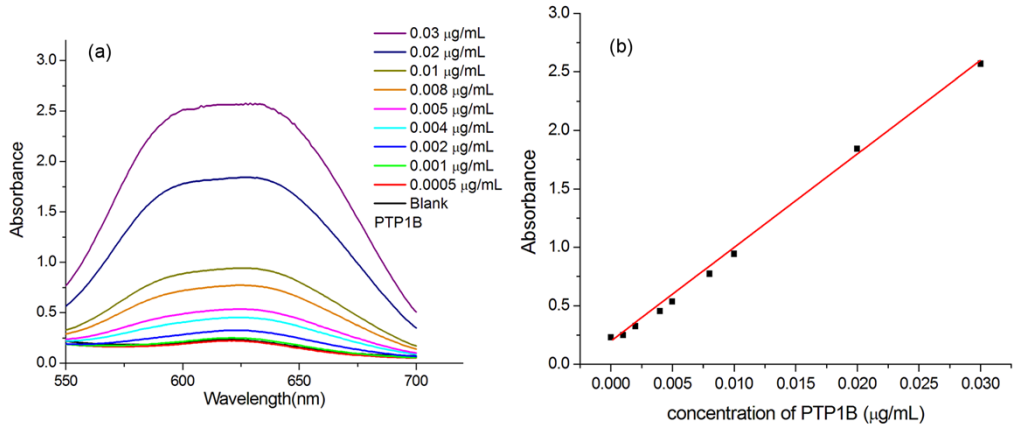

Fig. S4 (a) Absorbance spectra of the malachite green-based colorimetric assay system in the presence of different concentrations of PTP1B. PTP1B concentrations from bottom to top according to the peak at 630 nm: 0 (blank), 0.0005, 0.001, 0.002, 0.004, 0.005, 0.008, 0.01, 0.02, and 0.03 μg/mL; (b) the relationship between the absorbance at 630 nm and PTP1B concentrations.

7. Orthovanadate-based PTP1B inhibition assay

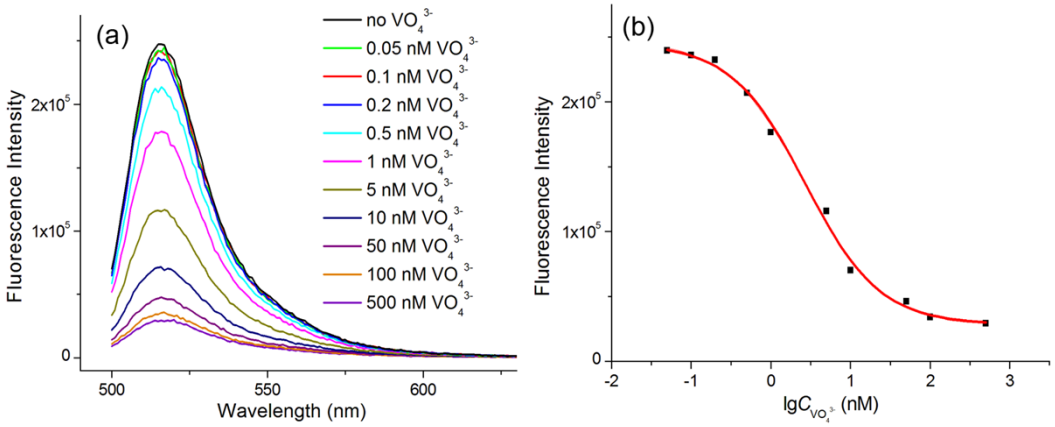

Fig. S5 (a) Fluorescence spectra of the proposed assay system in the presence of different concentrations of orthovanadate (0 ~ 500 nM) by fixing PTP1B concentration at 0.0005 μg/mL; (b) the relationship between the fluorescence intensity at 516 nm and orthovanadate concentrations on the logarithm scale. The corresponding IC_{50} value is calculated to be ~2.8 nM.

References:

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