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Supporting Information

A novel ratiometric MALDI-MS quantitation strategy for alkaline phosphatase activity with homogenous reaction and tunable dynamic range

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Experimental

Materials and Reagents. The coded phosphopeptide GRETKES(p)PDDGHT-OH (CPP, Mw 1508.40) was obtained from APeptides Co., Ltd (Shanghai, China). Ethylenediaminetetraacetic acid (EDTA), sodium orthovanadate (Na_3VO_4), $MgCl_2$ and $ZnCl_2$ were purchased from Shanghai Reagent Co. (Shanghai, China). Alkaline phosphatase (ALP), bovine serum albumin (BSA), glucose oxidase (GOD), cytochrome C (Cyt C), hemoglobin (Hb) , tris(hydroxymethyl)aminomethane (Tris), myoglobin (Mb), α-cyano-4-hydroxycinnamic acid (CHCA) and trifluoroacetic acid (TFA, ≥90%) were obtained from Sigma-Aldrich (USA). Acetonitrile (ACN) was obtained from Merck (Darmstadt, Germany). All these reagents were used as received without further purification. The clinical serum samples were from the People's Hospital of Liaocheng. Deionized water was prepared with a Milli-Q water purification system (Millipore, Milford, MA).

ALP Assay. ALP was firstly dissolved in Tris-HCl buffer (TBS, pH 9.0: 10 mM Tris-HCl, 5 mM $MgCl_2$, 0.1 mM $ZnCl_2$) as the storage solution (1.0×10^4 U/L). The stock solution was sub-packed and kept at -20 °C prior to use. For identifying the feasibility of the proposed strategy, 1×10^2 U/L (or 5×10^3 U/L) of ALP in 10 mM Tris-HCl buffer (pH 9.0, 5 mM $MgCl_2$，0.1 mM $ZnCl_2$) was added to equal volume of the freshly prepared CPP (0.1 mM, 10 μL) and incubated for 30 min at 37 °C, then the above-mentioned solution was heated up to 90 °C for 5 min in order to deactivate the ALP. The reacted product could be directly analyzed by MALDI-TOF MS.

For monitoring the reaction process, 1×10^2 U/L ALP was mixed with equal volume of the freshly prepared 0.1 mM CPP and incubated for different times (10, 20, 30, 40, 50 and 60 min) at 37 °C, after heated for 5 min at 90 °C, MALDI-TOF MS analysis was carried out.

To perform the quantitative detection, ALP at different final activities (5×10^{-2} to 5×10^3 U/L) in TBS was incubated with 0.1 mM CPP at 37 °C for 30 min, and then the mixture was heated for 5 min at 90 °C. Afterward, the mass spectra was recorded by MALDI-TOF MS. The dynamic range can be tuned by simply changing the concentration of substrate CPP (e.g. 0.1 mM CPP for 5×10^{-2} to 5×10^3 U/L ALP or 1 μM for 5×10^{-4} to 5×10^1 U/L ALP, respectively).

To testify the selectivity of this strategy, control experiments were carried using 1×10^2 U/L ALP, EDTA-treated ALP, heat-treated ALP, or 1 mg/mL interference protein (BSA, Cyt C, GOD, Hb, Mb) prepared in the TBS. The EDTA-treated ALP was obtained by mix 0.1 μL of 100 μM EDTA with 10 μL of 1×10^2 U/L ALP. To obtain heat-treated ALP, ALP was incubated at 100 °C for 10 min.

ALP Inhibition Assay. To evaluate the effect of ALP inhibitor, different concentrations of EDTA or Na_3VO_4 were mixed with 1×10^2 U/L ALP, and 10 μL of the mixtures were added to the 10 μL of 0.1 mM CPP solution at 37 °C for 30 min. The following detection process was the same as the procedure of ALP detection.

Analysis of ALP in Real Serum Samples. The serum samples were obtained by coagulating the human blood samples and extracting the supernate. Then, each sample was divided into two parts. One was tested by the clinical mature method (colorimetric assay), while the other was assayed by the proposed strategy. Specifically, 10 μL of diluted 10% human serum was incubated with 10 μL of 0.1 mM CPP at 37 °C for 30 min. Then, the quantitation of ALP was actualized in accordance with the ALP activity assay procedure described in section of ALP Assay.

MS Analysis. After 1 μL of the reaction solution was deposited on the MALDI plate and dried, 1 μL of 10 mg/mL CHCA in 60% ACN (v/v) containing 0.1% TFA was introduced as matrix. The MALDI-TOF MS experiments were performed on a 5800 Plus MALDI-TOF/TOF Mass Spectrometer (AB SCIEX). All spectra were taken from signal-averaging of 800 laser shots with the laser intensity kept at a proper constant. The MS data were acquired in positive reflective mode. MS data analysis was performed with a Data ExplorerTM Software from AB SCIEX. The presence of ALP could be rapidly identified from one mass spectrum through matching a pair of peaks with an m/z shift of 80.0.

Supplementary Data

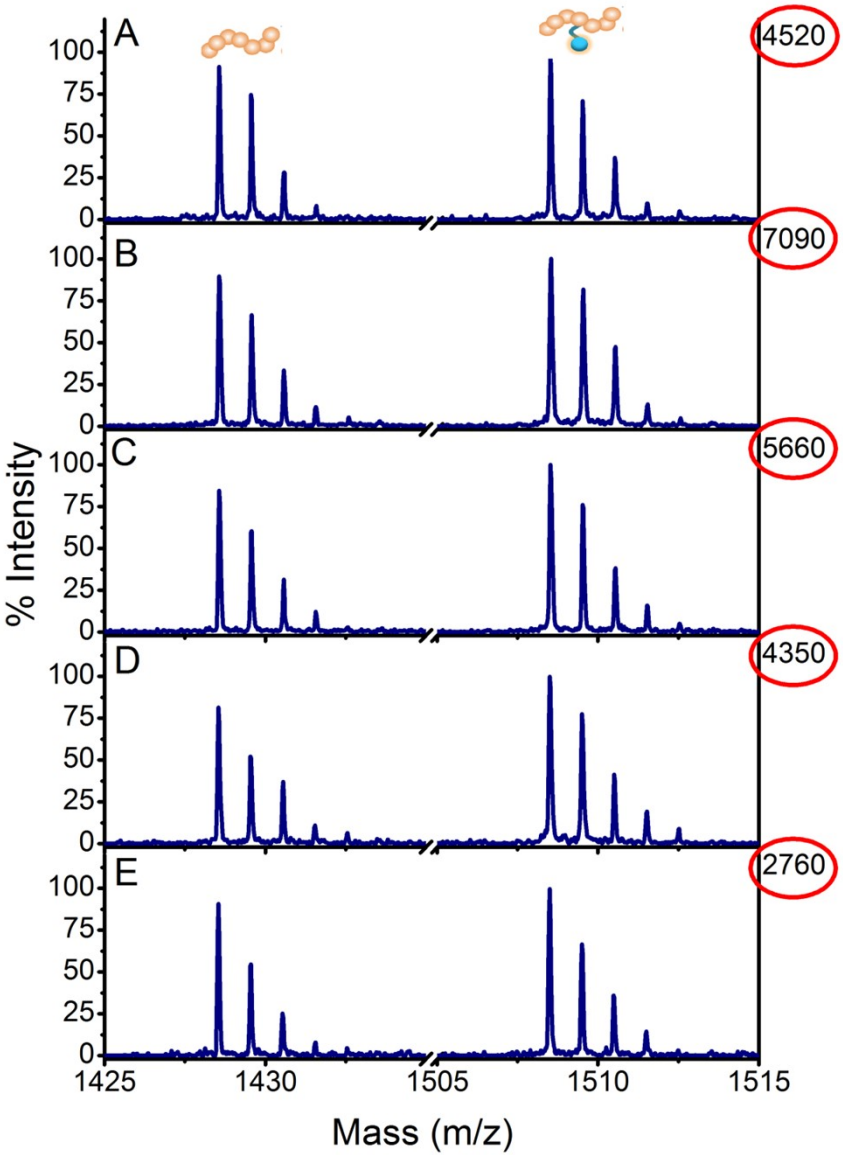

Fig. S1 Spot-to-spot reproducibility for analysis of 0.1 mM CPP incubate with 5 U/L ALP. protonated DPP, protonated CPP.

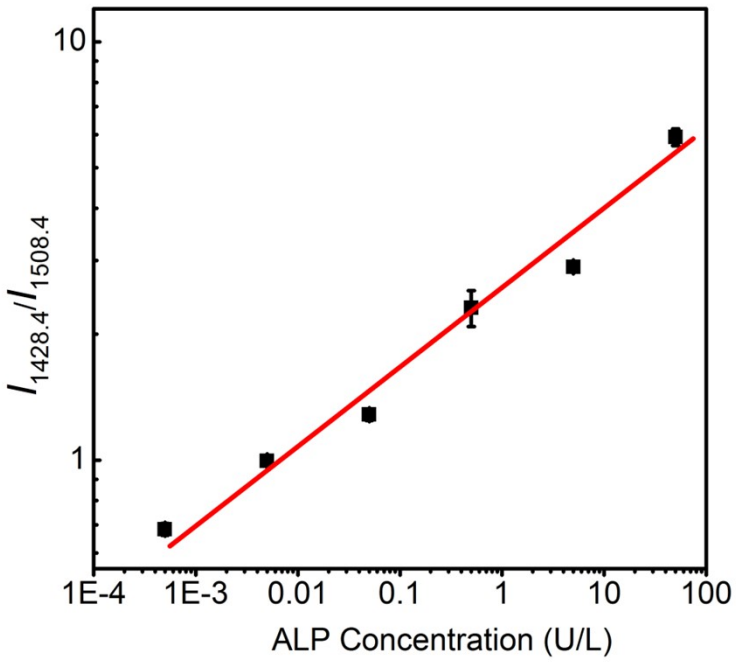

Fig. S2 Ratiometric MALDI-MS quantitation calibration curve of ALP using 1 μM CPP as substrate.

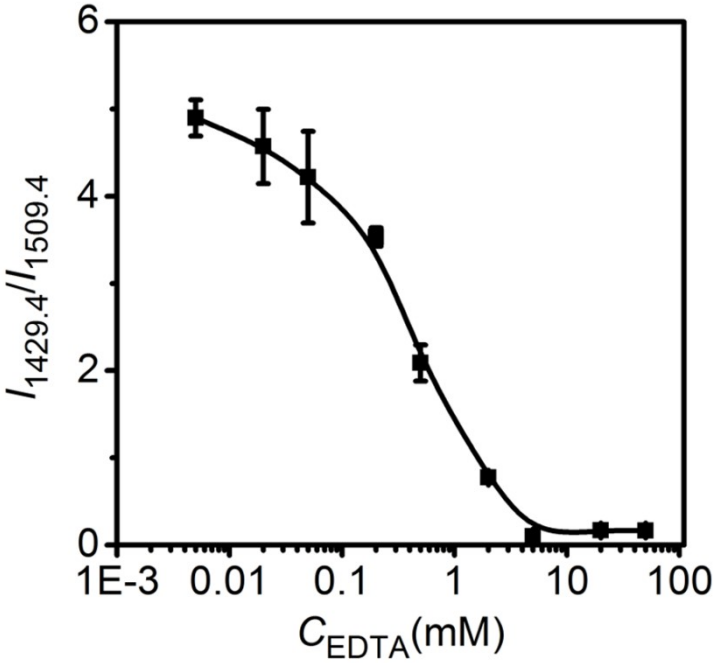

Fig. S3 Dependence of the peak intensity ratio of DPP to CPP of ALP-responsive system on the concentration of EDTA.

Table S1. The linear equation, regression coefficient (R) dynamic range, and detection limit for different concentration of CPP.

CPP concentration	linear equation	regression coefficient	Dynamic range (U/L)	Detection limit (U/L)
0.1 mM	$\log (I_{1429.4}/I_{1509.4}) = -0.4072+0.6855 \log C_{ALP}$	0.9982	$5\times10^{-2} - 5\times10^{3}$	1×10^{-2}
1 μM	$\log (I_{1429.4}/I_{1509.4}) = 0.3762+0.1669 \log C_{ALP}$	0.9968	$5\times10^{-4} - 5\times10^{1}$	3×10^{-4}

Table S2. Comparison of the proposed method with other reported methods for ALP detection.

Analytical method	Assay time[a]	Real sample analysis	Detection limit (U/L)	Linear range (U/L)	Refs.
Fluorescence	~ 3 h	human embryonic kidney 293 cells and HeLa cells	0.02	0.05 – 1	(1)
Fluorescence	60 min	human serum and 1 cancer cell	3.2×10^{-1}	5×10^{-1}–1×10^{3}	(2)
Fluorescence	~ 2 h	human serum	3.5×10^{-2}	6×10^{-2}–6×10^{2}	(3)
Fluorescence	~ 2 h	HeLa cells and HT-29 cells	2×10^{-4}	1×10^{-3}–1×10^{2}	(4)
Colorimetry	~ 2 h	1% human serum	0.84	20−200	(5)
Electrochemiluminescence	~ 3 h	1% human serum	7×10^{-4}	2×10^{-3}–5×10^{1}	(6)
Photoelectrochemistry	over 2 days	human serum	0.18	1−500	(7)
Photoelectrochemistry	~1 days	human serum	0.33	0.5−40	(8)
Surface-enhanced Raman scattering	~ 10 h	N/A[b]	1	1−50	(9)
colorimetric	~ 2 h	human serum	0.94	0−220	(10)
Electrochemical	~ 3 h	2% human serum	0.5	0.5−20	(11)
RM-MALDI-MS	~ 2 h	10% human serum	3×10^{-4}	5×10^{-4} -5×10^{3}	This work

[a] Assay time includes the total time of preparation, reaction and detection.

[b] "N/A" represents "not applicable."

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