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Supporting Information

Sensitive and Accurate Detection of ALP Activity using Fluorescence on-off-on Switch and Mass Barcode Signal Amplification

Chang Shu*, Duo Li, Tengfei Li, Shunli Ji, Li Ding*

[1] Key Laboratory of Drug Quality Control and Pharmacovigilance (China Pharmaceutical University), Ministry of Education, Nanjing 210009, China.

[2] Department of Pharmaceutical Analysis, School of Pharmacy, China Pharmaceutical University, 24 Tongjiaxiang, Nanjing, 210009, P. R. China.

*To whom correspondence should be addressed.

Tel.: +86-025-83271485

E-mail address: shuchang@cpu.edu.cn (Chang Shu);

dinglidlcpu@sina.com (Li Ding)

S.1 Chemicals

The N-Fmoc protected amino acids were obtained from GL Biochem Ltd. (Shanghai, china); Selenium powder (Se), sodium hydrogen boride ($NaBH_4$), zincsulfate ($ZnSO_4•7H_2O$), L-Glutathione (GSH), 1-Hydroxybenzotriazole (HOBt), N,N-diisopropylethylamine(DIPEA), O-benzotriazole-N,N,N',N'-tetramethyl-uroniumhexafluoro-phosphate (TBTU), Tris (2-carboxyethyl) phosphine (TCEP), trifluoroacetic acid (TFA), N,1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide (EDC), N-hydroxysuccinimide (NHS), Fmoc N-hydroxysuccinimide ester (Fmoc-OSu), cystamine dihydrochloride, mercapto-ethylamine, succinic anhydride and mifepristone were purchased from Aladdin Reagent Corporation (Shanghai, China); Cystamine dihydrochloride and Alkali phosphatase (ALP, 10 U/mg) were purchased from J&K Scientific chemical Ltd. (Beijing, China); Other chemicals were of analytical grade and directly used without additional purification. The ultrapure water was prepared by a Milli-Q water purification system (Millipore Corp., MA, America) with an electrical resistance of 18.2MΩ•cm and used in all aqueous solutions.

S.2 Instrumentation

UV-vis absorption and fluorescence spectra were measured by a fluorescence microplate reader (SpectraMax M^{2e}, Molecular devices, America) using a transparent 96-well microplate and a black 96-well microplate (Corning Inc.,NY, U.S.A.), respectively. High resolution transmission electron microscopy (HRTEM) was performed on JEM-2100 (JEOL, Japan). Fourier transform-infrared spectrometer (FT-IR) spectra were recorded on 8400s spectrophotometer (Shimadzu, Japan). The X-ray powder diffraction (XRD) analysis was carried out on a 6000 X-ray diffractometer (Bruker, Germany). The proton spectra nuclear magnetic resonance (^{1}H-NMR) spectra were recorded on a (Bruker 300 MHz, Germany). The Zeta potential was measured by a Mastersizer 2000 Laser Particle Size Analyzer (Malvern, British). LC-MS/MS spectra were recorded on an Agilent 1260 Series HPLC system equipped with an 6410B tandem mass spectrometer (Agilent, America), containing a Turbo-V® ionspray source operated in the positive ESI mode.

S.3 Preparation of mass barcode

Fmoc-OSu (7.4 g, 22.2 mmol) dissolved in 100mL 1,4-dioxane, and dropwise added into mercapto-ethylamine (1.2g, 10.7 mmol) in 100mL 1,4-dioxane. The reaction solution was stirred for overnight at room temperature. The solution was concentrated to a small volume and purified by water and ethyl acetate. Anhydrous Na_2SO_4 was added into the collecting organic layer for 30min. The solution was concentrated to a small volume and purified by using column chromatography (petroleum ether : ethyl acetate = 1:1). The white solid powder of mass barcode was obtained with yield of 82.4%. ESI-MS: $C_{17}H_{17}NO_2S$, calc. MW = 299.1, obsvd. $[M+Na]^+$ = 322.1. ^{1}H-NMR (300 MHz, MeOH-d6).

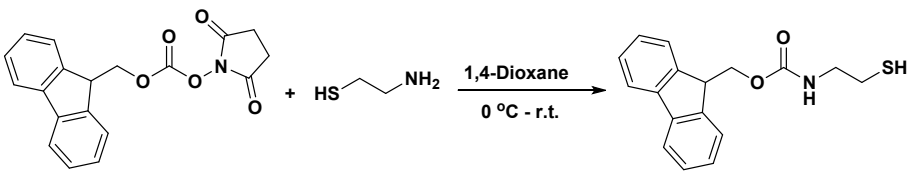


Fig.S1 Synthetic route of mass barcode

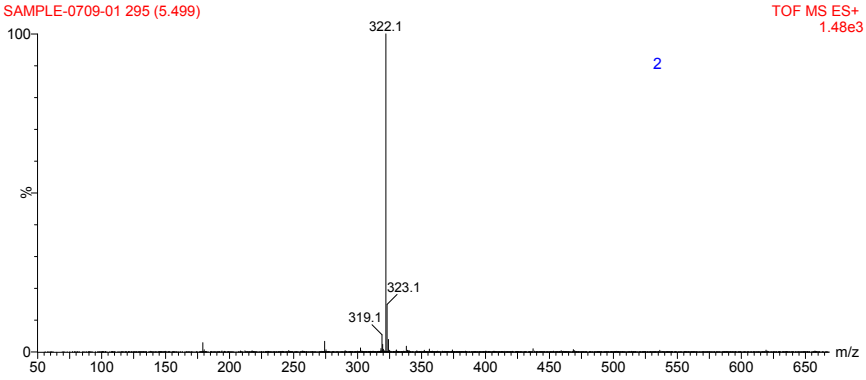


Fig.S2 The MS spectrum of mass barcode

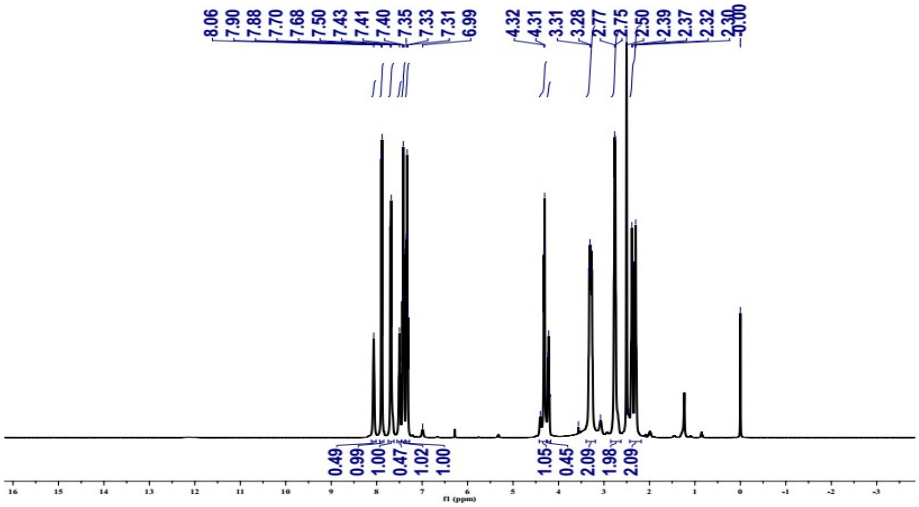


Fig.S3 The ^{1}H-NMR spectrum of mass barcode

S.4 Preparation of Fmoc-succinated cystamine synthesis (Fmoc-SS)

The Fmoc-SS was prepared according to our previously report with minor modification. In brief, cystamine dihydrochloride (5 g, 22.2 mmol) and DIPEA (7.7 mL, 44.4mmol) were added in 25ml methanol with ice-water bath for 30 min. Succinic anhydride (2.22 g, 22.2 mmol) was dissolved in 50mL 1,4-dioxane, and dropwise added into above reaction solution. After stirred at room temperature for 15 min, the mixture solution was removed the solution through reducing

pressure distillation. The obtained solution was purified with saturated Na_2CO_3 aqueous solution and diethyl ether. Na_2CO_3 (3.8 g，36 mmol) was added into the collecting water layer and stirred with ice-water bath until completely dissolved. Fmoc-OSu (7.4 g, 22.2 mmol) dissolved in 25mL 1,4-dioxane was dropwise added into above reaction solution. The mixture solution was stirred for overnight at room temperature. The obtained solution was filtered, and the filtrate was added diethyl ether. Collecting water layer, the pH value was adjust to 1~2 with HCl (1M), and the extraction with DCM twice. Anhydrous Na_2SO_4 was added into the collecting organic layer for 30min. The solution was concentrated to a small volume and purified by using column chromatography (petroleum ether : ethyl acetate : acetic acid = 1:1:0.1). The white solid powder of Fmoc-SS was obtained with yield of 82.5%. ESI-MS: $C_{23}H_{26}N_2O_5S_2$, calc. MW = 474.1, obsvd. $[M+H]^+ = 475.1$, $[M+Na]^+ = 497.2$, $[M+K]^+ = 513.2$. ^{1}H-NMR (300 MHz, MeOH-d6).

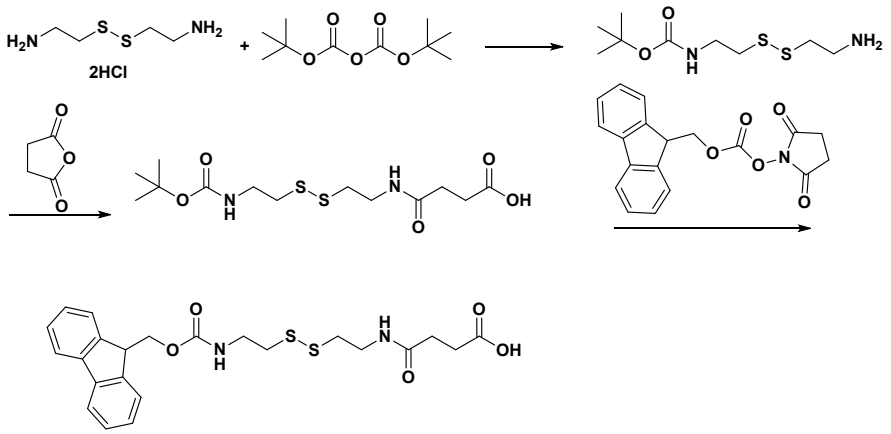


Fig.S4 Synthetic route of Fmoc-SS

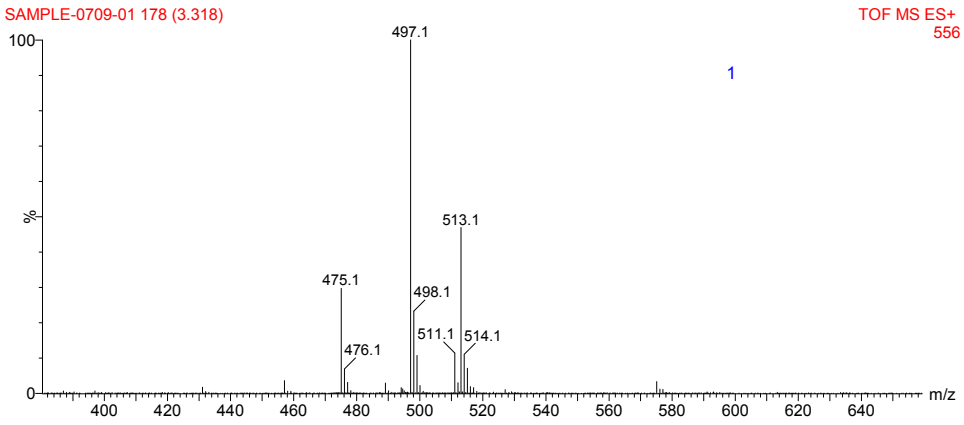


Fig. S5 The MS spectrum of Fmoc-SS

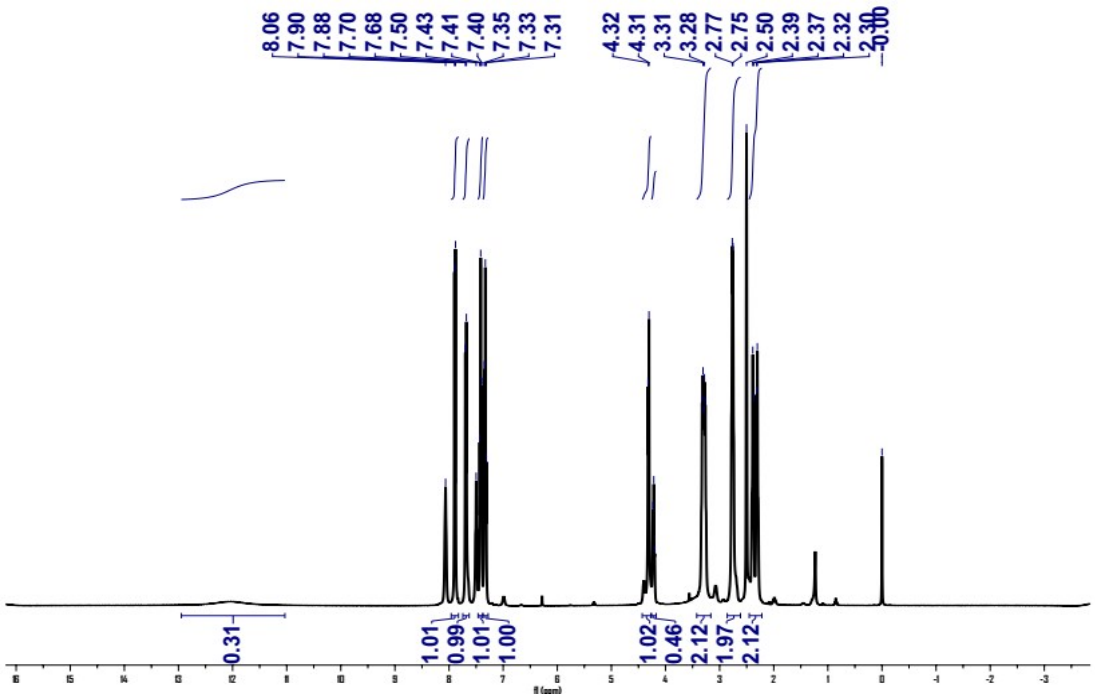


Fig.S6 The ^{1}H NMR spectrum of Fmoc-SS

S.5 Preparation of Gly-Gly-Phe-Phe-Tyr(OPO_3H_2) peptide (GGFFYp)

The Gly-Gly-Phe-Phe-Tyr(OPO_3H_2) peptide was prepared by standard solid-phase peptide synthesis (SPPS), which used 2-chlorotrityl chloride resin (1.0~1.2 mmol/g) and Fmoc-amino acids. The white solid powder of peptide was obtained with yield of 98.2%. ESI-MS: $C_{31}H_{36}N_5O_{10}P$, calc. MW = 669.2, obsvd. $[M+H]^+$ = 670.2. ^{1}H-NMR (300 MHz, DMSO-d6).

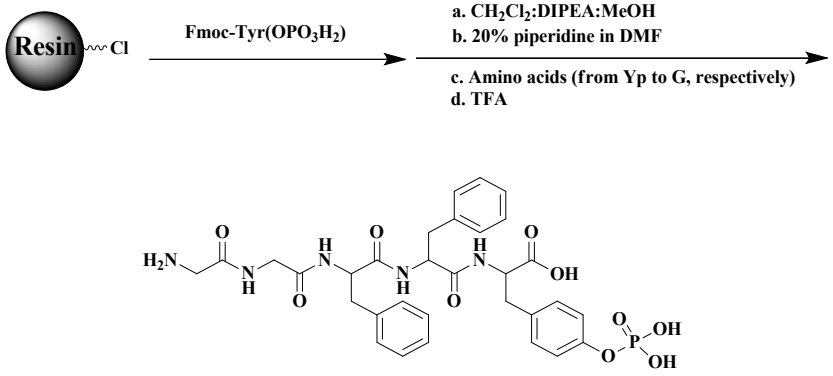


Fig.S7 Synthetic route of GGFFYp peptide.

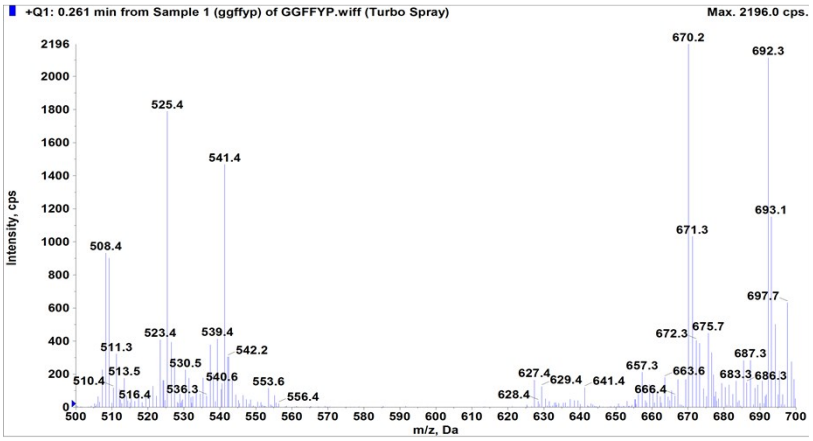


Fig.S8 The MS spectrum of GGFFYp peptide

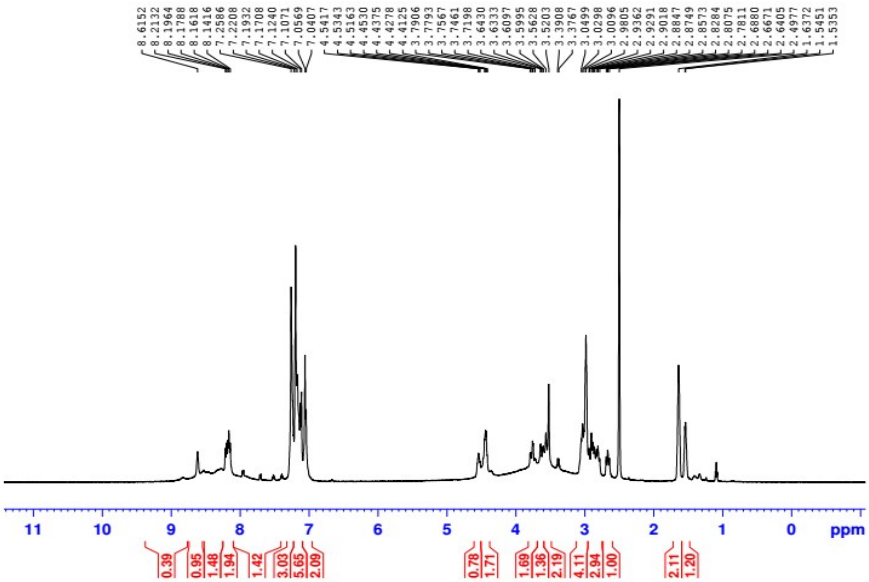


Fig.S9 The ^{1}H NMR spectrum of GGFFYp peptide

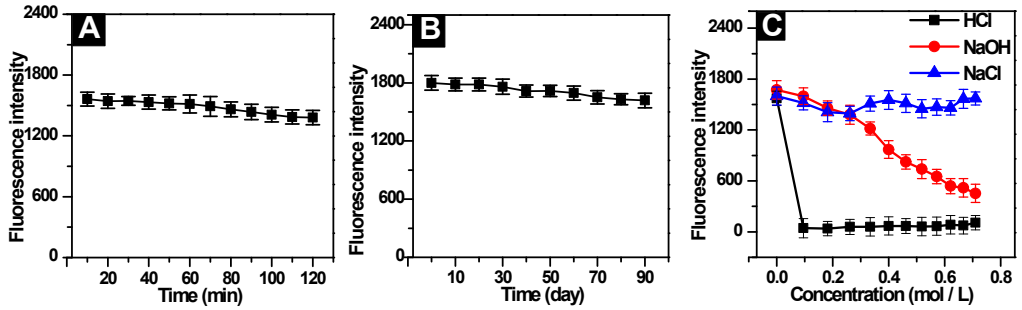


Fig. S10 The Fluorescence stability of QDs-SS-Yp solutions under light illumination (A) and room temperature (B). Fluorescence intensity (C) of QDs-SS-Yp with different concentration of HCl, NaOH and NaCl solution.

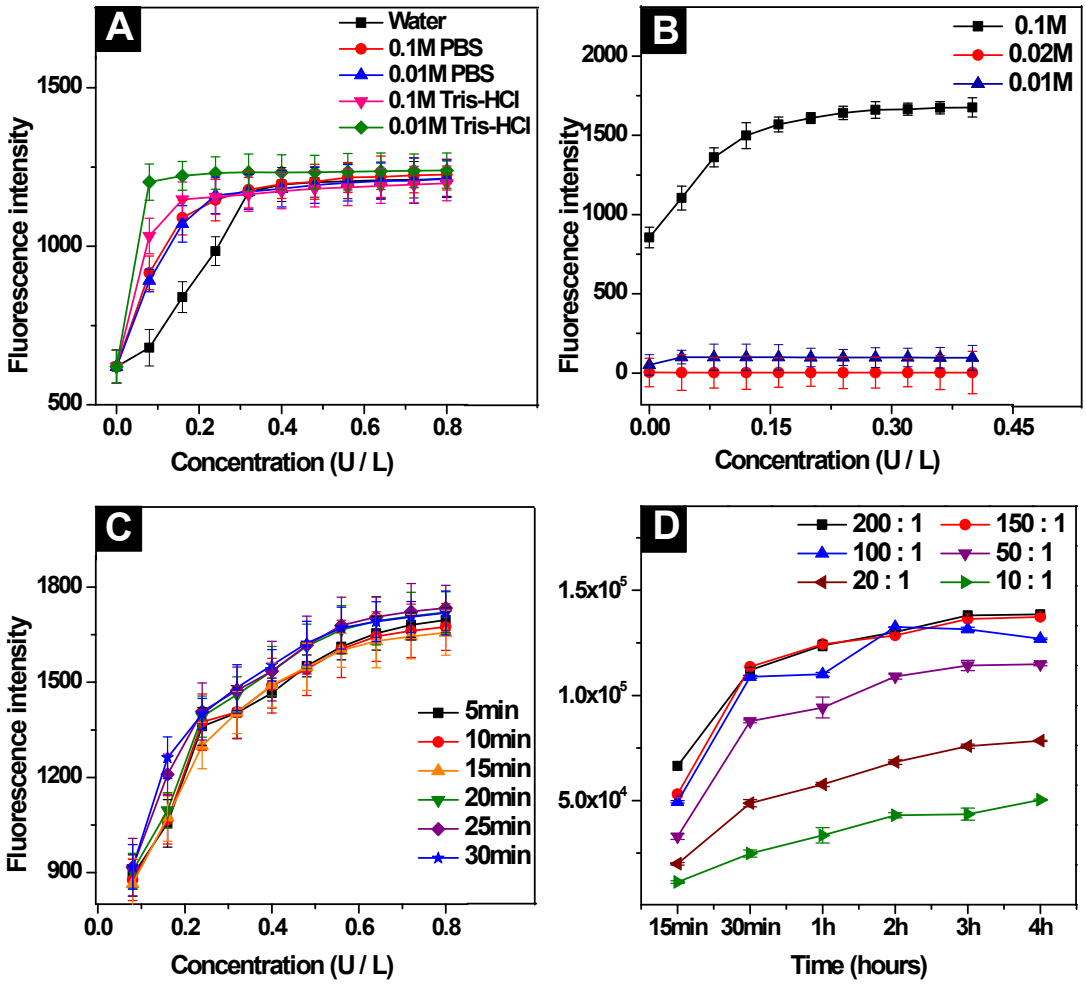
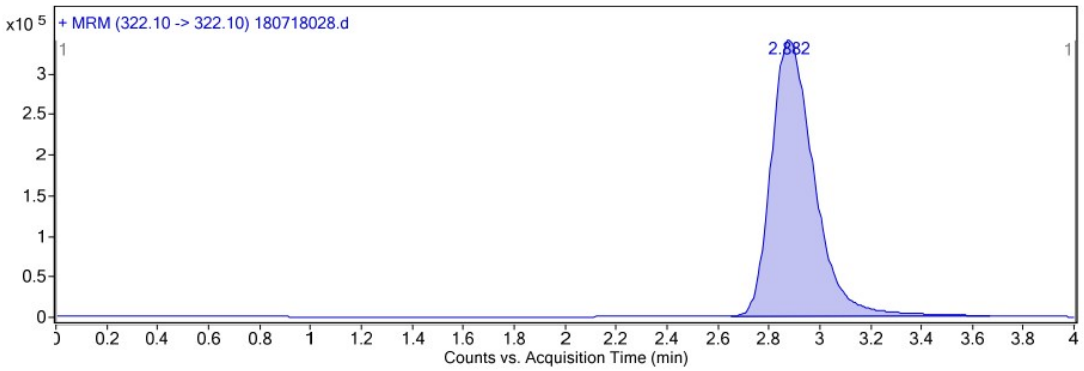


Fig.S11 (A) Fluorescence intensity of QDs-SS-Yp containing DNS solution in the presence of various concentrations of ALP from 0 to 0.8U/L in different buffer solutions. (B) Fluorescence intensity of QDs-SS-Yp containing DNS solution in different concentrations of Tris-HCl buffer solutions with various concentrations of ALP from 0 to 0.8U/L. (C) Time-dependent fluorescence intensity of QDs-SS-Yp containing DNS solution in the presence of various concentrations of ALP from 0 to 0.8U/L. (D) The response intensity of the mass with concentrations of ALP mixture gradually increases with increasing reaction time and concentration of TCEP.

(A)



(B)

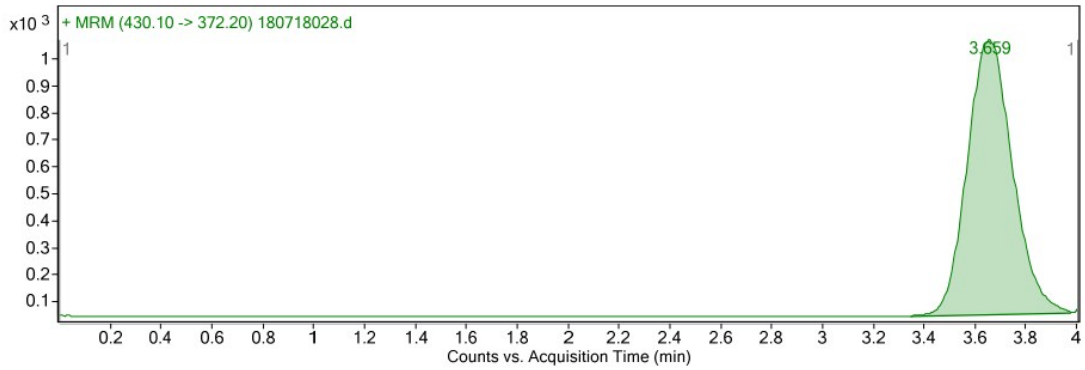


Fig.S12 LC-MS/MS chromatograms of mass barcode (A) and inner standard (B)