Electronic Supplementary Information

Peptide-templated gold nanoparticle nanosensor for simultaneous detection of multiple posttranslational modification enzymes

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EXPERIMENTAL SECTION

Materials. The peptide substrate (peptide 1: FITC-KGRRPED(Ac)K-Biotin; peptide 2: Biotin-K(Cy5)HRHPRY(p)G), and the non-specific peptide (peptide 3: FITC-KGRRPEDK-Biotin; peptide 4: Biotin-K(Cy5)HRHPRYG) were synthesized and purified by Ontores Biotechnologies (Hangzhou, Zhejiang, China). Histone deacetylase 1 (HDAC 1) and its inhibitor trichostatin A (TSA) were obtained from Cayman Chemical Company (Ann Arbor, Michigan, USA). Endoproteinase rLys-C and protease carboxypeptidase Y (CPY) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Protein tyrosine phosphatase 1B (PTP1B) and sodium orthovanadate (Na_3VO_4) were purchased from Sigma (Shanghai, China). The streptavidin-coated

Au nanoparticles were bought from Nanocs, Inc. (New York, USA). All solutions were prepared with ultrapure water (Millipore, Milford, MA).

Preparation of Peptide-Templated AuNPs. The peptide 1 and peptide 2 (molar ratio, 1 : 1.1) were added to 0.1 mL of AuNP solution (5.7×10^{12} particles/mL) and incubated at 30 °C for 2 h to obtain the peptide-templated AuNP nanostructures. The AuNPs were centrifuged at 14 000 rpm for 20 min to remove the unbound peptides, and then resuspended in 250 μL of PBS buffer (10 mM phosphate, 0.1 M NaCl, pH 7.0), followed by storing at 4 °C. The fluorescence measurement was carried out by using a Hitachi F-7000 fluorescence spectrophotometer (Tokyo, Japan).

Detection of HDAC and PTP1B Activity. The 50 μL of reaction solution containing 30 nM peptides-templated AuNPs, 25 mM Tris-HCl (pH 8.0), 137 mM sodium chloride, 2.7 mM potassium chloride, 1 mM magnesium chloride, 1 μg/mL rLys-C, and 5 U/mL CPY were incubated with varying concentrations of HDAC/PTP1B. The mixture was incubated at 30 ºC for 90 min to allow the cleavage reaction of deacetylation/dephosphorylation peptide 1 / peptide 2. After the reaction, the resultant solution was immediately subjected to fluorescence measurement. All fluorescence spectra were measured by using a quartz cuvette on a Hitachi F-7000 fluorescence spectrophotometer (Tokyo, Japan). The excitation wavelength was 488 nm for FITC, and the emission spectra were recorded over the wavelength range from 490 to 650 nm with a slit width of 5 nm for both excitation and emission. The excitation wavelength was 650 nm for Cy5, and the emission spectra were recorded over the wavelength range from 640 to 800 nm with a slit width of 5 nm for both excitation and emission. The fluorescence intensity of FITC at 520 nm and Cy5 at 670 nm was recorded for data analysis.

TIRF-Based Single-Molecule Detection. The reaction products were diluted 1000-fold with the

imaging buffer (0.4% (w/v) D-glucose, 1 mg/mL glucose oxidase, 50 μg/mL BSA, 67 mM glycine-KOH, 0.04 mg/mL catalase, 2.5 mM $MgCl_2$, 1 mg/mL trolox, pH 9.4). The 10 μL of samples was used for TIRF imaging. The FITC was excited by a 488-nm laser (Coherent, USA), and the Cy5 was excited by a 640-nm laser (Coherent, USA). The FITC / Cy5 signals were collected by Andor Ixon DU897 EMCCD.

Cell Culture and Preparation of Cell Extracts. Helen Lane (HeLa) cells were cultured in Dulbecco's modified Eagles medium (DMEM) with 10% fetal bovine serum (FBS) and 50 U/mL penicillin plus 50 μg/mL streptomycin in a 100% humidified chamber containing 5% CO_2 at 37 °C. The nuclear extracts were prepared using a nuclear extract kit (ActiveMotif, Carlsbad, CA, USA) according to the manufacturer's protocol. The obtained supernatant was collected for enzyme activity assay immediately.

Optimization of Experimental Conditions. We optimized the experimental conditions including the AuNP-to-peptide 1 ratio and the AuNP-to-peptide 2 ratio to obtain high quenching efficiency of AuNPs. We optimized the AuNP-to-peptide 1 ratio with the concentration of AuNPs being fixed at 0.005 nM. As shown in Fig. S1A, the F/F_0 value enhances with the increase of AuNP-to-peptide 1 ratio from 1:1 to 1:50 and reaches the highest value at the ratio of 1:30, where F and F_0 are the fluorescence intensity in the presence and absence of HDAC, respectively. We further investigated the AuNP-to-peptide 2 ratio with the concentration of AuNPs being fixed at 0.005 nM. As shown in Fig. S1B, the F/F_0 value reaches the highest value at AuNP-to-peptide 2 ratio of 1:30, where F and F_0 are the fluorescence intensity in the presence and absence of PTP1B, respectively. Therefore, the AuNP-to-peptide ratio of 1:30 is used in the subsequent studies.

In order to obtain a high hydrolysis performance of rLys-C and CPY, the concentration of

rLys-C and CPY should be optimized. As shown in Fig. S1C, 500 nM AuNPs-peptides were cleaved by different concentrations of rLys-C and then mixed with 5 U of CPY. The F/F_0 value enhances gradually with the increasing concentration of rLys-C until it reaches the maximum value at concentration of 1 μg/mL, where F and F_0 are the fluorescence intensity in the presence and absence of HDAC, respectively. Thus, the optimal concentration of rLys-C is 1 μg/mL. We also investigated the influence of CPY concentration upon the assay performance. As shown in Fig. S1D, 500 nM AuNPs-peptides were cleaved by different concentrations of CPY and then mixed with 1 μg/mL rLys-C. The F/F_0 value enhances with the increasing concentration of CPY from 0.6 to 5 U/mL. Thus, 5 U/mL of CPY is used in subsequent research.

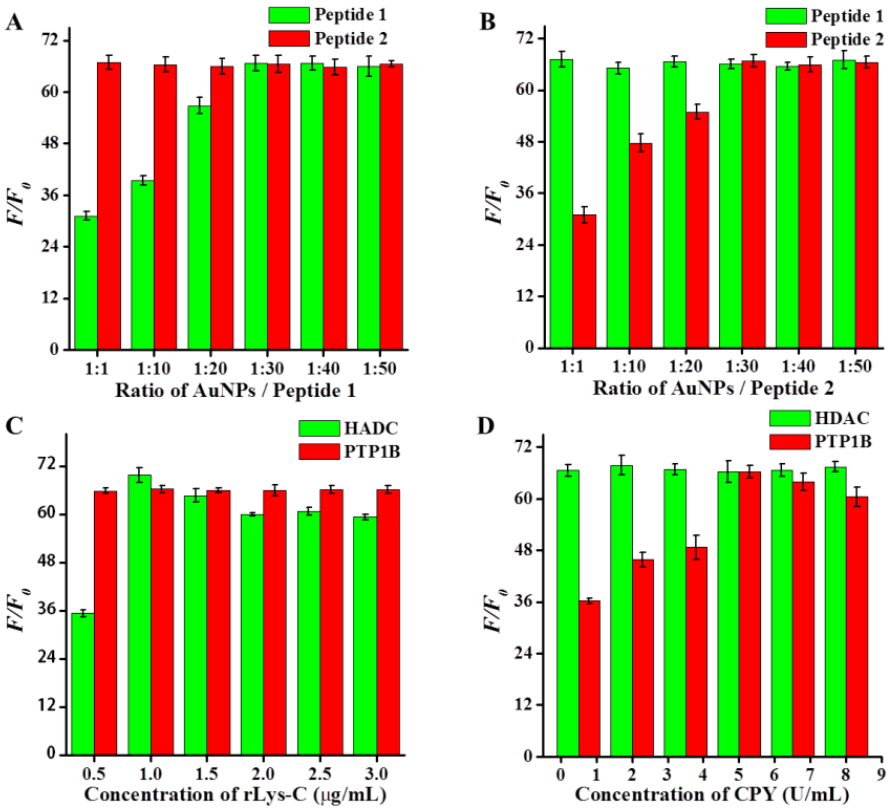

Fig. S1. (A) Variance of the F/F_0 value with the AuNP-to-peptide 1 ratio with the concentration of AuNPs being fixed at 0.005 nM. F and F_0 are the fluorescence signals in the presence and absence

of HDAC, respectively. The HDAC concentration is 1 μM. (B) Variance of the F/F_0 value with the AuNP-to-peptide 1 ratio with the AuNP concentration being fixed at 0.005 nM. F and F_0 are the fluorescence signals in the presence and absence of PTP1B, respectively. The PTP1B concentration is 3 nM. (C) Variance of F/F_0 value with the concentration of rLys-C. F and F_0 are the fluorescence signals in the presence and absence of HDAC, respectively. The HDAC concentration is 1 μM. (D) Variance of F/F_0 value with the concentration of CPY. F and F_0 are the fluorescence signals in the presence and absence of PTP1B, respectively. The PTP1B concentration is 3 nM. Error bars show the standard deviation of three experiments.

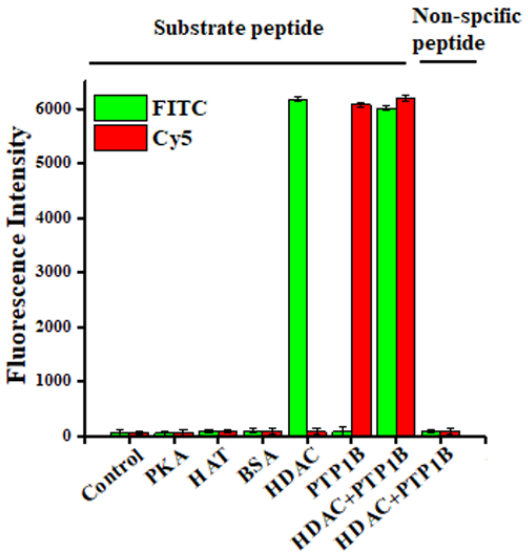

Fig. S2 Measurement of FITC fluorescence intensity (green color) and Cy5 fluorescence intensity (red color) in response to 500 nM PKA, 500 nM HAT, 10 μg/mL BSA, 1 μM HDAC, 3 nM PTP1B, and the co-existence of 1 μM HDAC and 3 nM PTP1B, respectively. The sample without any enzyme is used as the control. Error bars show the standard deviation of three experiments.

To evaluate the selectivity of the proposed nanosensor, we used bovine serum albumin (BSA),

protein kinase (PKA), and histone acetyltransferase (HAT) as the nonspecific proteins. HATs can catalyze the acetylation by transferring the acetyl moiety from acetyl-coenzyme A to the ε-amino group of specific lysine residues.[1] PKA can catalyze the phosphorylation of protein substrates at serine/threonine residues.[2] In theory, none of these proteins can generate the deacetylated/dephosphorylated peptide for the digestion by rLys-C and CPY. As shown in Fig. S2, neither FITC nor Cy5 fluorescence signal is observed in the presence of PKA, HAT, BSA, and the control group without any proteins. In contrast, a high FITC fluorescence signal is observed only in the presence of HDAC, and a high Cy5 fluorescence signal is detected only in the presence of PTP1B, and the simultaneous detection of FITC and Cy5 fluorescence signals can be achieved only when both HDAC and PTP1B are present. We further used the non-specific peptide to investigate the substrate specificity (Fig. S2). Neither FITC nor Cy5 fluorescence signal can be detected even in the presence of HDAC and PTP1B (Fig. S2). These results clearly demonstrate the high specificity of the proposed nanosensor towards HDAC and PTP1B.

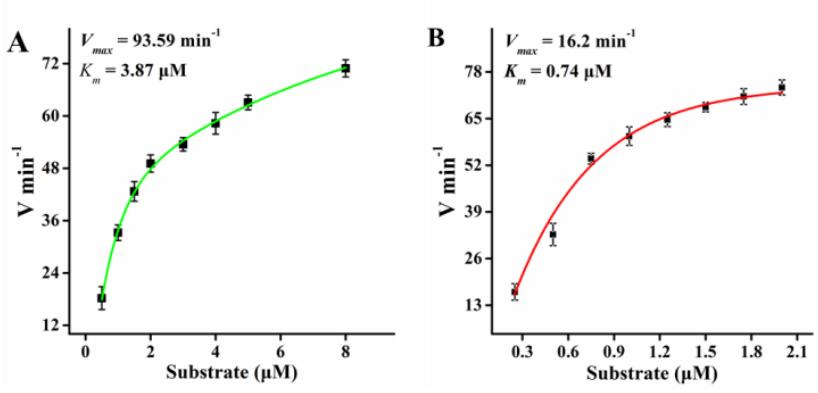

Fig. S3 Variance of initial velocity with the concentration of peptide substrates in response to HDAC (A) and PTP1B (B), respectively. Error bars show the standard deviation of three experiments.

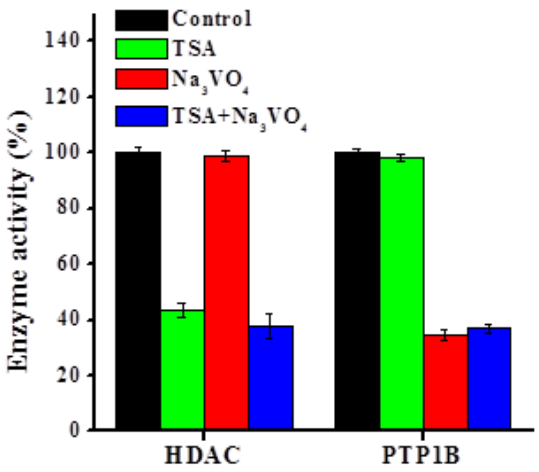

Fig. S4 Simultaneous detection of HDAC and PTP1B activities in crude cell extracts after the treatment with various inhibitors. TSA (2 nM) is used as the HDAC inhibitor, and Na_3VO_4 (5 μM) is used as the PTP1B inhibitor. Error bars show the standard deviation of three experiments.

The proposed nanosensor can be further used to measure the cellular PTM enzymes after treatment with various inhibitors (Fig. S4). The relative activity of PTM enzymes can be calculated according to A (%) = (F_i) / F_0) × 100, where F_i is the fluorescent intensity in the presence of inhibitors, and F_0 is the fluorescent intensity in the control sample without inhibitor. As shown in Fig. S4, the addition of 2 nM TSA alone induces the decrease of HDAC activity, while the addition of 5 μM Na_3VO_4 alone induces the decrease of PTP1B activity. The addition of 2 nM TSA and 5 μM Na_3VO_4 can induce the decrease of both HDAC and PTP1B activities. These results further confirm that the proposed nanosensor can be applied for simultaneous detection of multiple PTM enzymes in real samples.

References

[1] F. Ma, M. Liu, Z. Y. Wang and C. Y. Zhang, *Chem. Commun.*, 2016, **52**, 1218-1221.

[2] P. Cohen, *Nat Cell Biol*, 2002, **4**, E127-130.