Supporting Information

Continuous and Sensitive Acid Phosphatase Assay Based on A Conjugated Polyelectrolyte

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Experimental

Materials. 4-nitrophenyl phosphate disodium salt (pNPP) and 4-nitrophenol (pNP) were obtained from Aladdin (Shanghai, China). Acid phosphatase (ACP) from potato was obtained as lyophilized powder (3.0-10.0 units/mg solid) from Sigma (USA). Prostatic acid phosphatase (PAP) was purchased from R&D Systems (USA). The broad-spectrum phosphatase inhibitor, sodium vanadate (Na_3VO_4) was purchased from Bio Basic Inc. (Toronto, Canada). All chemicals were used as received, unless otherwise noted. Stock solutions of pNPP and pNP were prepared in DMSO just before use and their concentrations were adjusted to 1 mM. The water used in all experiments was prepared on a SG water purification system and displayed a resistivity of ≥ 18.2 MΩ-cm^{-1}. PPE4+ was synthesized[1] and provided by Prof. Kirk S. Schanze in University of Florida. All test solutions of PPE4+ were diluted to 10 μM in Hepes buffer (pH 5.2) from its concentrated stock solution.

Instrumentation. Steady state fluorescence quenching experiments were carried out on a SPEX FL3 spectrometer with 380 nm as the excitation wavelength and 400-650 nm as the emission scanning range. Fluorescence turn-on assays were measured on a 96-well plate using Thermo-Fisher Varioskan Flash Multimode Reader with 380 nm as the excitation wavelength and 500 nm as the emission wavelength.

Fluorescence turn-on effect with the presence of ACP. 20 μM pNPP was added to the 2 mL solution of 10 μM PPE4+ in 10 mM Hepes buffer (pH 5.2) to make a pre-quenched test solution. Then 20 nM ACP was added into the solution. The resulting solution was incubated at 25 ℃ for a certain period of time (from 0 to 180 min), and its fluorescence spectrum was measured on the SPEX FL3 spectrometer.

Calculation of limit of detection (LOD) of ACP assay. PPE4+/pNPP assay was used for a continuous real-time ACP activity study and the ACP-catalyzed hydrolysis of pNPP as a function of time upon incubation with different ACP concentrations (0 -

20 nM) was investigated. The LOD of ACP in this assay was obtained using the following equation:[2]

$$LOD = 3 \times \frac{S_0}{S} \quad \text{(Eq. S1)}$$

where S_0 is the standard deviation of the background and S is the sensitivity.

Calculation of concentrations of the substrate pNPP and the product pNP as function of time. In order to quantitatively analyze the enzymes' kinetics, concentrations of un-hydrolyzed substrate pNPP at different times were derived from fluorescence intensity data based on Eq. S2.[21]

$$[S]_t = [S]_0 \frac{I_0 / I_t - 1}{I_0 / I_q - 1} \quad \text{(Eq. S2)}$$

where $[S]_t$ is the substrate concentration at time t; $[S]_0$ is the initial substrate concentration; I_0 is the fluorescence intensity of the polymer with the enzyme in a set of control experiments without the substrate; I_q is the fluorescence intensity quenched by the substrate before the reaction of enzyme; and I_t is the fluorescence intensity at time t after reaction.

The derived product pNP concentrations at different time were calculated as in Eq. S3:

$$[pNP]_t = [S]_0 - [S]_t \quad \text{(Eq. S3)}$$

where $[S]_0$ is the initial substrate concentration and $[S]_t$ is the substrate concentration at anytime t, which was derived from Eq. S2.

Calculation of kinetic parameters. Kinetic parameters of ACP were determined with assays at different initial substrate concentrations in the range from 0 to 30 μM. The original fluorescence intensity change *vs* time is shown as in Figure S1.

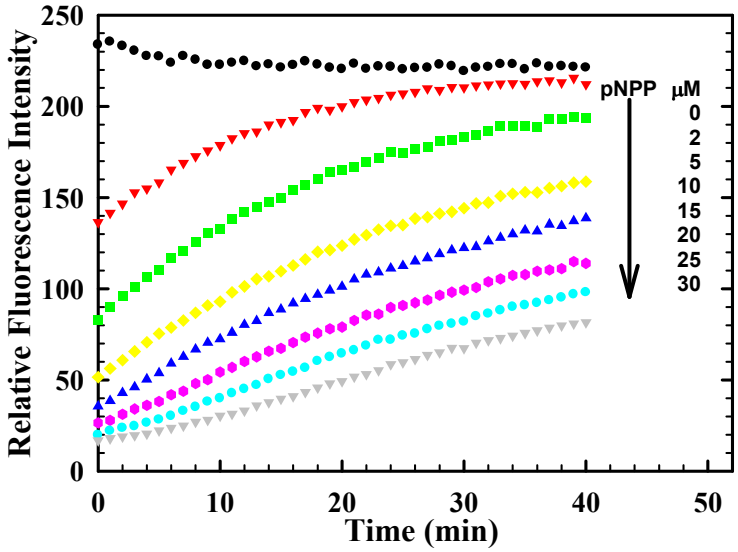

Figure S1. Real-time fluorescence turn-on effect of ACP assays at different initial pNPP concentrations (0, 2, 5, 10, 15, 20, 25 and 30 μM). The assays were carried out using 20 nM ACP and 10 μM PPE4+ in 10 mM Hepes buffer (pH 5.2) at 25 °C.

Lineweaver–Burk plot was obtained using double reciprocal data of initial rate vs. substrate concentration as in Eq. S4:

$$\frac{1}{V} = \frac{K_m}{V_{max}[S]_0} + \frac{1}{V_{max}} \qquad \text{(Eq. S4)}$$

where V is the initial rate and calculated from the slopes of the plots in Figure 4 using the beginning 5 points; $[S]_0$ is the initial substrate concentration; K_m is the Michaelis constant; V_{max} is the maximal velocity.

***In vitro* inhibitor screening.** In the inhibitor screening assays, varying concentrations of the inhibitor Na_3VO_4 (0, 1, 10, 100, 500, 1000, 5000, and 10000 nM) were pre-incubated with 20 nM ACP for 15 min before adding into the 200 μL pre-quenched assay solution. The initial rate of ACP-catalyzed hydrolysis reaction in the presence of different amounts of inhibitor (V) were calculated from the initial five points of fluorescence intensity change with time, and V_0 was the catalysis rate in

the absence of inhibitor. IC_{50} value was obtained by sigmoidal fit of the data using SigmaPlot 10.0.

References

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(2) An, L.; Liu, L.; Wang, S. *Biomacromolecules* **2009**, *10*, 454.