Supporting information:

Inhibition of dsDNA-templated copper nanoparticles by pyrophosphate as a label-free fluorescent strategy for alkaline phosphatase assay

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Experimental Section

Reagents and Apparatus. The DNA oligonucleiotides used in this work were synthesized by Shanghai Sangon Biological Engineering Technology & Services Co., Ltd. (China). The sequences of the DNA oligonucleotides were as follows:

P1: 5′-TACTCATACGCTCATACGTTCATCACGACTACACA-3′

P2: 5′-TGTGTAGTCGTGATGAACGTATGAGCGTATGAGTA-3′

Alkaline phosphatase from bovine intestinal mucosa, thrombin from human plasma, glucose oxidase from *aspergillus niger* (GOx), lysozyme from chicken egg white, avidin from egg white and bovine serum albumin (BSA) were purchased from Sigma-Aldrich (Shanghai, China). Other chemicals used in this work were of analytical grade and directly used without additional purification. The solutions were prepared using ultrapure water which was obtained through a Millipore Milli-Q water purification system (Billerica, MA) and had an electric resistance >18.2 MΩ.

The fluorescence measurements were carried out on an FL-7000 spectrometer (Hitachi, Japan). The fluorescence emission spectra of CuNPs were recorded from 510 nm to 645 nm at room temperature (~20℃) with a 335 nm excitation wavelength.

Preparation of dsDNA template. In order to prepare dsDNA templates, 1 μM of ssDNA probes (P1, P2) were mixed together in 10 mM Tris-HCl (pH 7.4, 100 mM NaCl) containing 2 mM freshly prepared ascorbate. The mixture was denatured at 95℃ for 10 min, and then cooled slowly to room temperature.

Evaluation of pyrophosphate inhibition on CuNPs formation. The inhibition of PPi on CuNPs formation was firstly evaluated. Briefly, pyrophosphate of different

concentrations were mixed with Cu^{2+} in 50 μL 10 mM Tris-HCl buffer (pH 7.4, 100 mM NaCl) for 10 min at 37℃. Then, 50 μL as-prepared ascorbate-contained dsDNA were added into the mixture and the fluorescence intensity was recorded at a fixed reduction time of 4 min.

Alkaline phosphatase assay. ALP of different final concentrations reacted with PPi (final concentration of 250 μM) at 37℃ for 60 min in 25 μL 10 mM Tris-HCl (pH 7.4, 100 mM NaCl). Cu^{2+} (final concentration of 200 μM) was then added to give a final volume of 50 μL and incubated for 10 min. After that, 50 μL as-prepared ascorbate-contained dsDNA templates were added into the mixture and the fluorescence intensity was recorded at a fixed reduction time of 4 min.

For ALP detection in complex samples, ALP solutions of different final concentrations were mixed with PPi (final concentration of 250 μM) at 37℃ for 60 min in 25 μL 10 mM Tris-HCl (pH 7.4, 100 mM NaCl) containing 1% diluted human serum. The followed detection procedure was the same as shown in the aforementioned experiment for ALP detection in clean Tris-HCl buffer.

Inhibition effect of phosphate on ALP activity. To study the inhibition of Pi on ALP activity, ALP (final concentrations of 2.5 nM) was added into 25 μL 10 mM Tris-HCl (pH 7.4, 100 mM NaCl) containing PPi (final concentration of 250 μM) and Pi of different final concentrations, and then incubated at 37℃ for 60 min. The followed detection procedure was the same as shown in the aforementioned experiment for ALP detection.

ADDITIONAL FIGURES

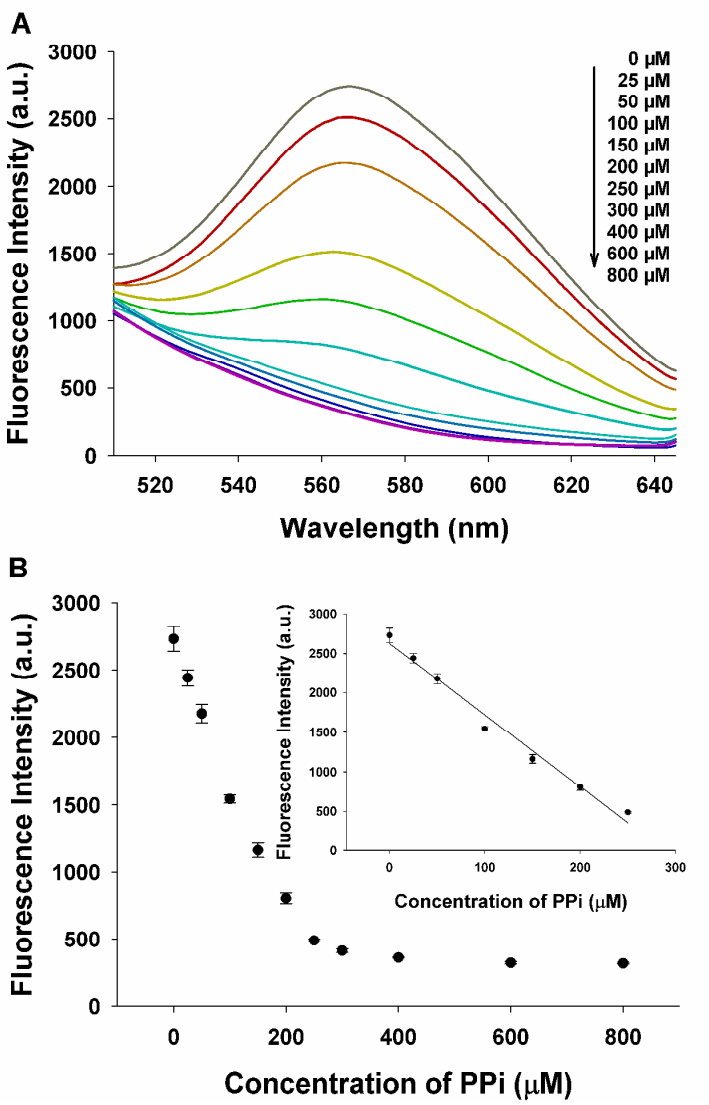

Figure S1. (A) Typical fluorescence spectral responses of obtained CuNPs under various PPi concentrations at a fixed reduction time of 4 min. (B) Plot of peak intensity of obtained CuNPs with respect to PPi concentrations. Inset: the linearity of peak intensity with respect to PPi concentrations. Error bars were estimated from three replicate measurements.

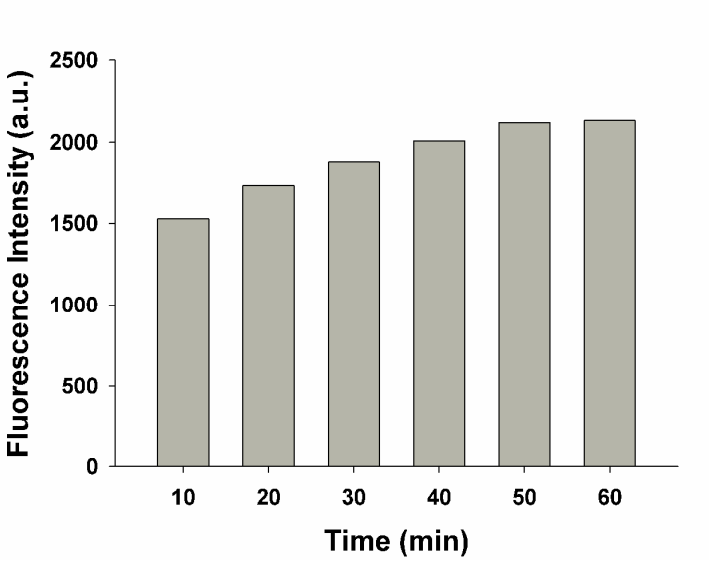

Figure S2. The effect of hydrolysis time on the assay performance. The concentration of ALP was 2.5 nM.

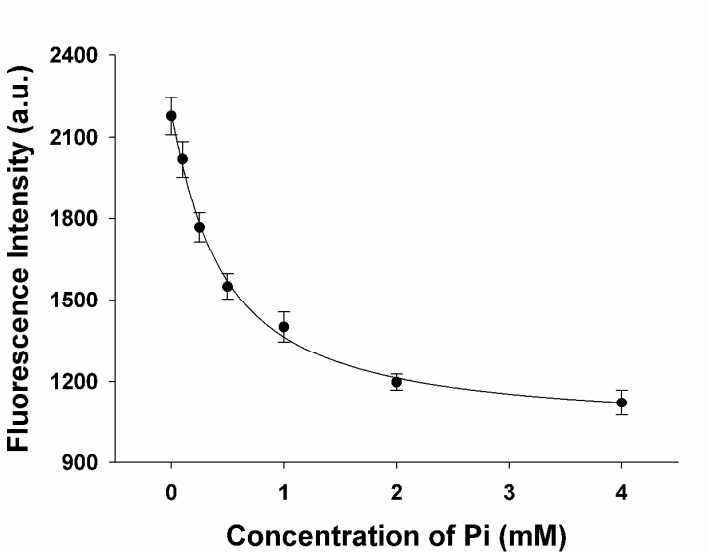

Figure S3. The inhibition effect of Pi on ALP activity.

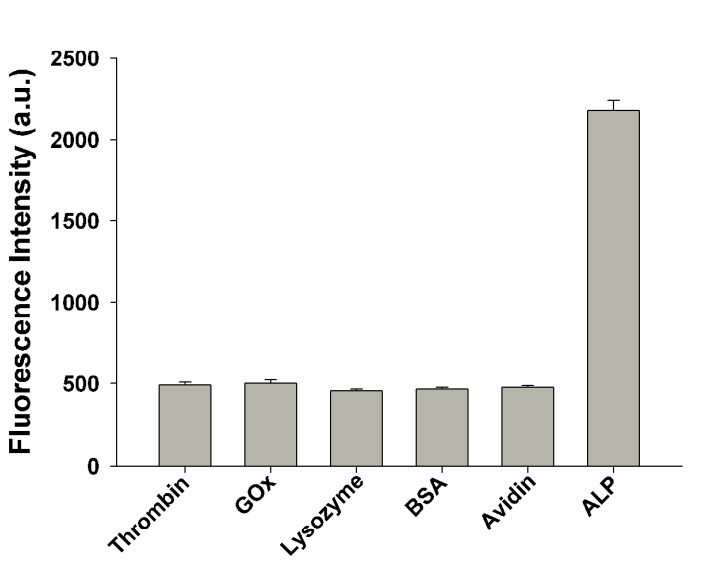

Figure S4. The selectivity of the CuNPs-based method for ALP assay. The concentration was 2.5 nM for ALP and 10 nM for each other interfering proteins. Error bars were estimated from three replicate measurements.

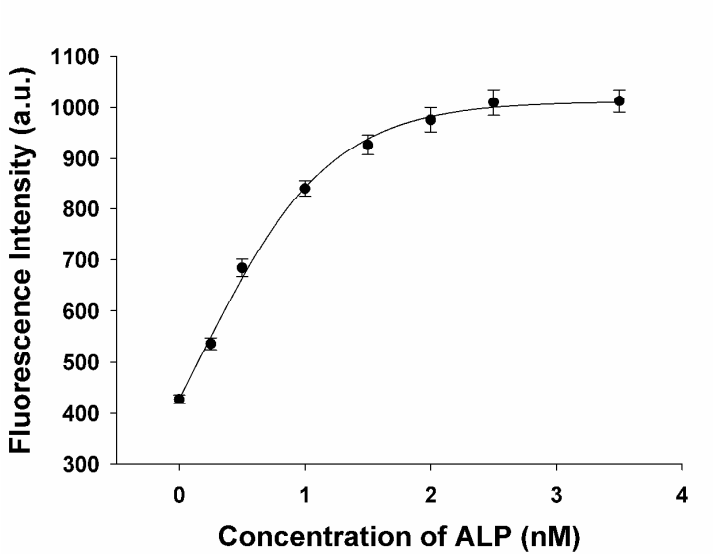

Figure S5. The calibration curve for ALP detection in diluted serum. Error bars were estimated from three replicate measurements.

Table S1 Recovery experiments of ALP in diluted serum samples

Samples	Added ALP (nM)	Detected ALP [a] (nM)	Recovery (%)
1	0.375	0.35 ± 0.01	93.3
2	0.5	0.49 ± 0.04	98.0
3	0.75	0.74 ± 0.05	98.7
4	1	1.03 ± 0.07	103.0
5	1.25	1.22 ± 0.05	97.6
6	1.5	1.47 ± 0.07	98.0

[a] Average of three determinations ± standard deviation

ADDITIONAL DISCUSSION

ALP Activity Calculation. The ability of the proposed method to determine ALP activity is a prerequisite for clinical applications. Thus, we explored the potential ability of this method to determine the ALP activity using the working curves for ALP and PPi (Figure 3, and Figure S1). At low ALP concentration range from 0 to 0.5 nM, the fluorescence intensity exhibits a quasi-linear correlation to ALP concentrations. From Figure 3, 0.1 nM and 0.5 nM ALP have fluorescence response of ~656 (a. u.) and ~1293 (a.u.) respectively, which amount to ~217 μM and ~147 μM PPi as estimated using the linear working curve in Figure S1. This means ~70 μM PPi were converted into Pi when ALP concentration was increased from 0.1 to 0.5 nM, indicating the turnover of PPi is ~1.75×10^5. And the activity of 1 nM ALP is estimated to be ~3 U/L (One unit of ALP activity is defined as the amount of enzyme

hydrolyzing 1 μmol PPi per minute under the above conditions of assay). The ability of our assay to determine ALP activity suggests the significance and utility of this method.