# *Supporting Information*

# Fluorescent and Colorimetric Dual-Readout Assay for Inorganic Pyrophosphatase with $Cu^{2+}$-Triggered Oxidation of o-Phenylenediamine

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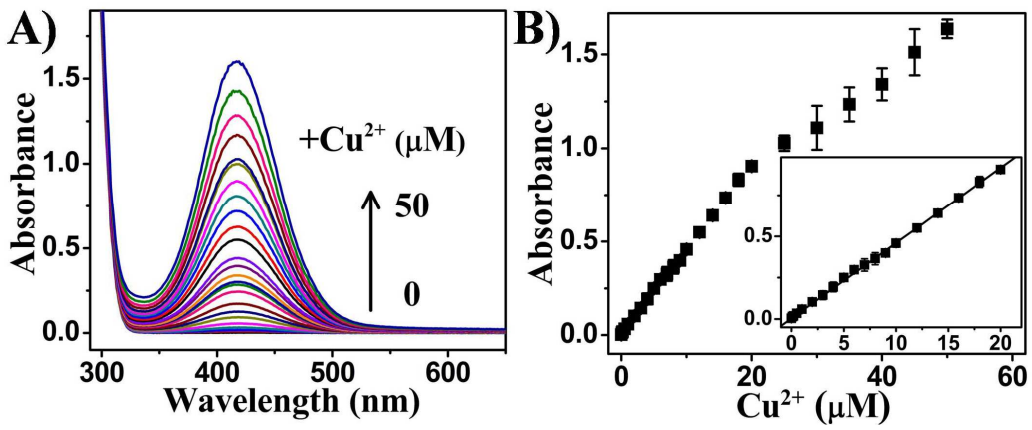


**Figure S1.** (A) Absorption spectra of the OPD (1 mM) solution in Tris-HCl buffer (10 mM, pH 7.4) in addition of varied concentrations of $Cu^{2+}$ (0–50 μM). (B) The absorbance values at 417 nm for the analytical solution versus the $Cu^{2+}$ concentrations. Inset: linear curve.

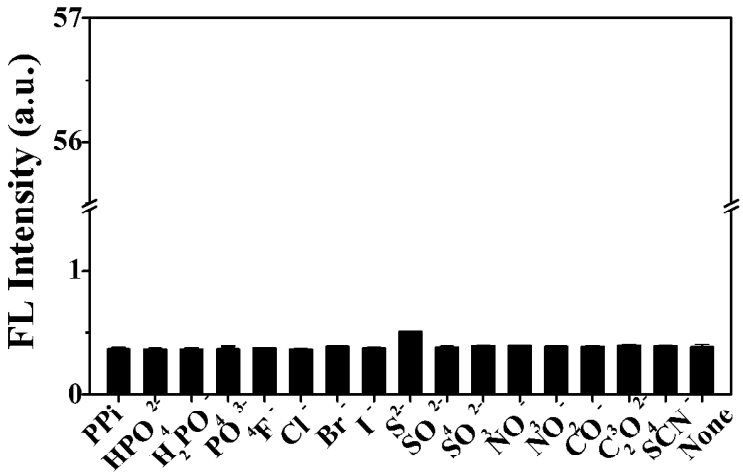


**Figure S2.** The fluorescence intensities at 560 nm of the OPD (1 mM) solution in Tris-HCl buffer (10 mM, pH 7.4) by adding different inorganic anions (10 μM).

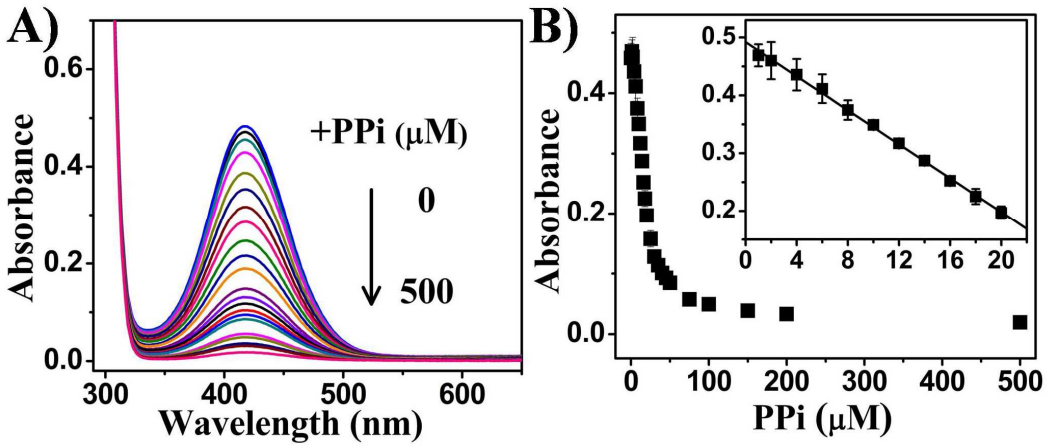


**Figure S3.** (A) Absorption spectra of the analytical solution prepared by adding the mixture of $Cu^{2+}$ and varied concentrations of PPi (0–500 μM) into Tris-HCl buffer (10 mM, pH 7.4) containing OPD (1 mM). (B) The absorbance values at 417 nm for the analytical solution versus the PPi concentrations. Inset: linear curve.

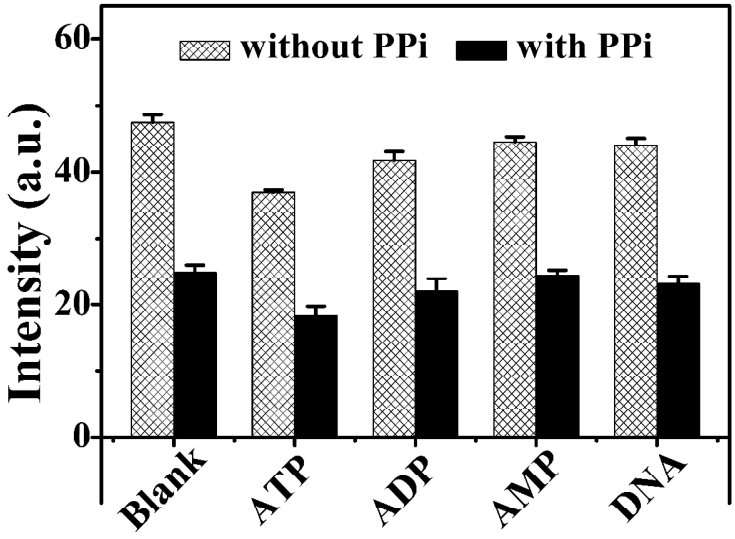


**Figure S4.** The fluorescence intensities of the OPD–$Cu^{2+}$ system in the presence of the control biomolecules (50 μM of ATP, ADP, AMP and 50 μg/mL of fish sperm DNA) without and with the coexistence of PPi ions (20 μM).

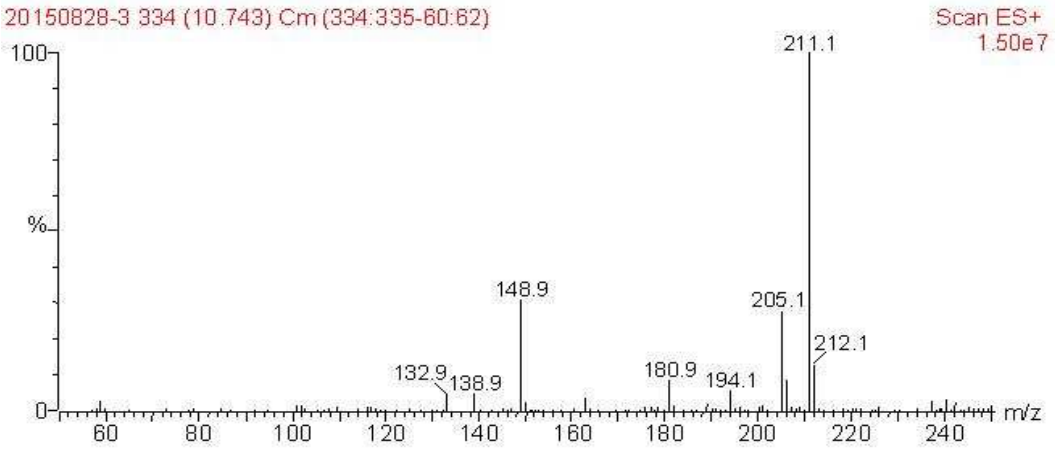


**Figure S5.** The ESI-MS spectrometry of the final as-purified product of the incubated mixture solution of $Cu^{2+}$ and OPD (OPDox).

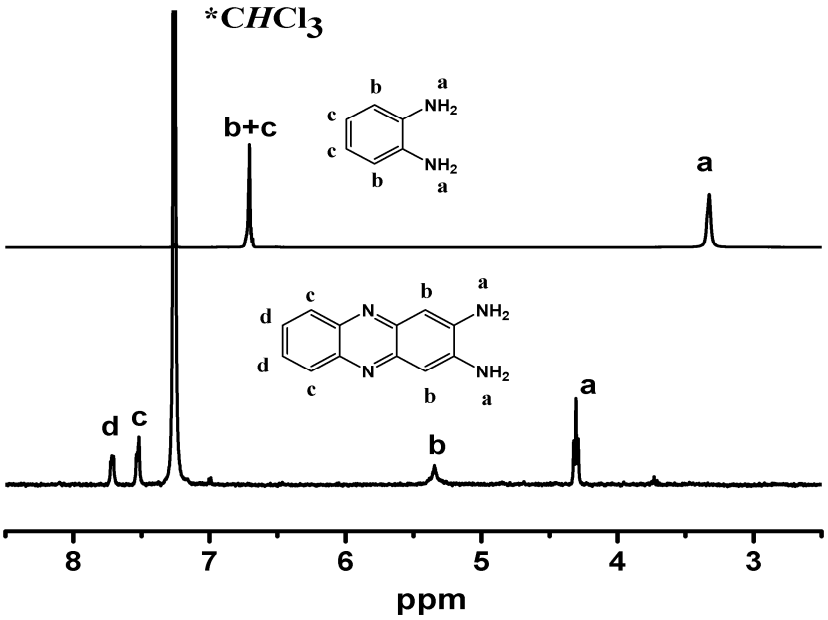


**Figure S6**. The $^{1}H$ NMR spectrometry of OPD and the final as-purified product of the incubated mixture solution of $Cu^{2+}$ and OPD (OPDox).

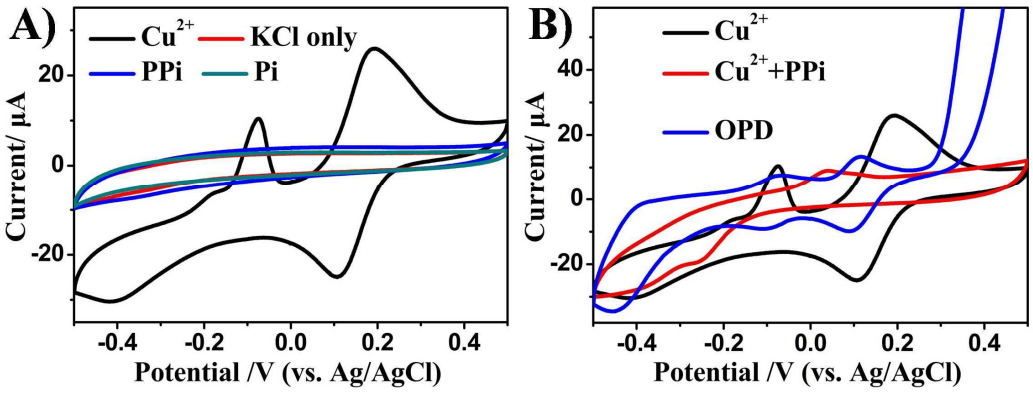


**Figure S7.** (A) Cyclic voltammograms of the $CuSO_4$ (black), blank (red), PPi (blue), and Pi (green), respectively, in KCl solution. (B) Cyclic voltammograms of the $CuSO_4$ (black), $CuSO_4$ in presence of PPi (red), and OPD (blue), respectively, in KCl solution.

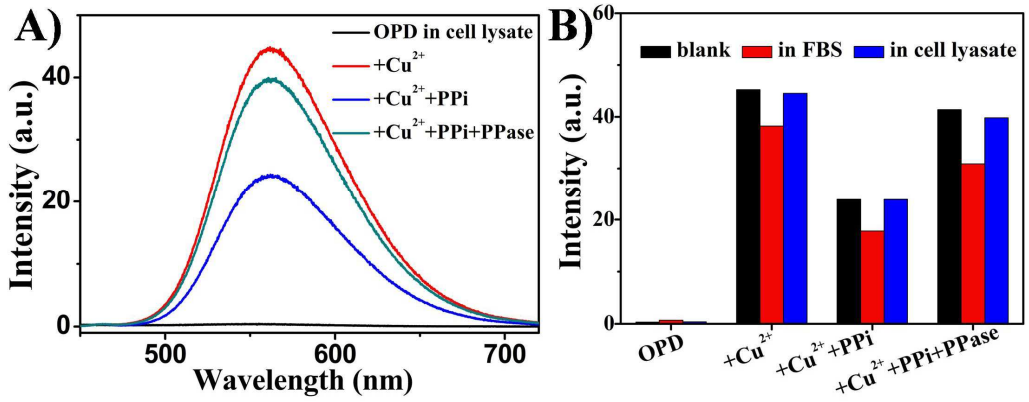


**Figure S8.** (A) Fluorescence spectra of the OPD (black), OPD + $Cu^{2+}$ (red), OPD + $Cu^{2+}$ + PPi (blue), and OPD + $Cu^{2+}$ + PPi + PPase (green) in 2% 3T3L1 cell lysate. (B) The fluorescence intensities of the OPD, OPD + $Cu^{2+}$, OPD + $Cu^{2+}$ + PPi, and OPD + $Cu^{2+}$ + PPi + PPase in the standard assay system (black), the 2% fetal bovine serum (red) and the 2% 3T3L1 cell lysate (blue).