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# **Supplementary Material**

## Fluorometric and colorimetric analysis of alkaline phosphatase

### activity based on nucleotide coordinated copper ion as mimicking

#### polyphenol oxidase

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**Figure S1** Comparison of catalytic activities of nanozymes synthesized in four kinds of buffer solutions. All the buffers are 10 mM, pH=8.5.



**Figure S2** Comparison of catalytic activities of nanozymes in four buffers in the chromogenic reaction. All the buffers are 30 mM (pH=6.7).



Figure S3 The fluorescence spectra of nucleotides. The concentrations of ATP, ADP and AMP are 10 mM in 60 mM MES buffer (pH = 6.7). Ex = 220 nm.



Figure S4 The zeta potential of prepared nanozymes.



**Figure S5** Steady-state kinetic assay of ATP-Cu (A and B) and laccase (C and D). The concentration of 2,4-DP was 0.0625, 0.125, 0.25, 0.5, 1 mM. The error bars represent the standard deviation of three measurements.

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Catalysts	V <sub>max</sub> (mM/min)	$K_{m}(mM)$
ATP-Cu	0.0022	0.207
Laccase	0.0003	0.891

Table S1 The kinetic parameters of ATP-Cu and laccase



**Figure S6** The catalytic activity of the supernatant and precipitation of the nanozymes cycled for 7 times, respectively.



Figure S7 Control experiment comparing single Cu<sup>2+</sup> and ATP-Cu for PPO-like activity.

Table 52 comparison of unrefert methods for the determination of ALA.					
System	Linear range	Detection	Ref.		
	(U/L)	limit (U/L)			
Near infrared Ag <sub>2</sub> S quantum dots and calcein	2-100	1.28	S1		
Facile colorimetric assay	60-100	5.4	S2		
Gold nanoparticles-based colorimetric assay	100-600	10	S3		
High-Resolution Colorimetric Assay	5-100	3.3	S4		
Real-time Ratiometric Fluorescent Assay	25-200	10	S5		
This work	1-30	0.3	-		

Table S2 Comparison of different methods for the determination of ALP.



**Figure S8** The selectivity of the GTP-based method for ALP assay. Absorbance intensity at 510 nm for reaction solutions containing BSA (10  $\mu$ M), GSH (3 mM), L-Glutamate (100  $\mu$ g/mL), L-aspartic acid (100  $\mu$ g/mL), Glycine (100  $\mu$ g/mL), L-alanine (100  $\mu$ g/mL), Cl<sup>-</sup> (3 mM), Br<sup>-</sup> (3 mM), and OH<sup>-</sup> (3 mM) respectively in the presence of 30 U/L of ALP.

#### **Supplementary References**

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