

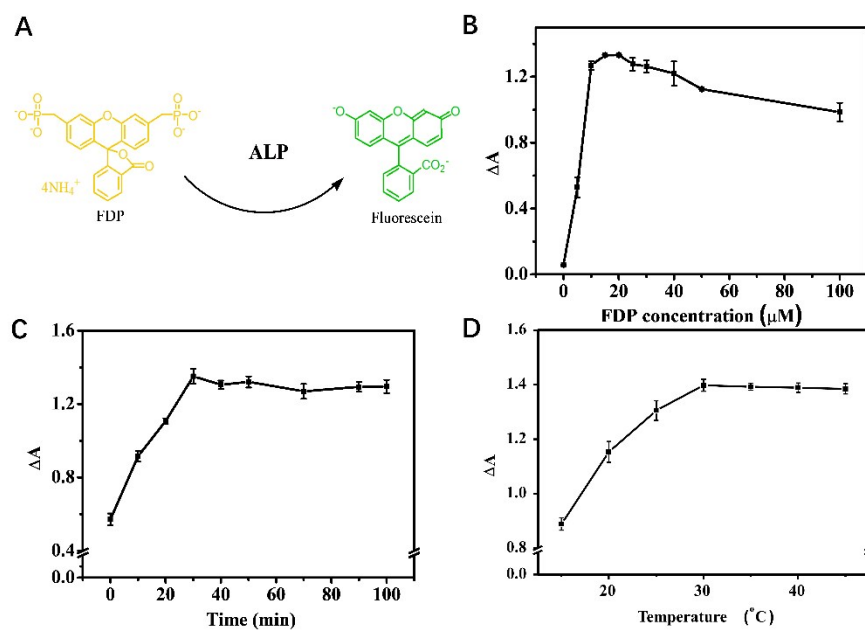
## *Supplementary materials*

### **Colorimetric Sensing Alkaline Phosphatase and $\alpha$ -Fetoprotein based on the Photoinduced Oxidase Activity of Fluorescein**

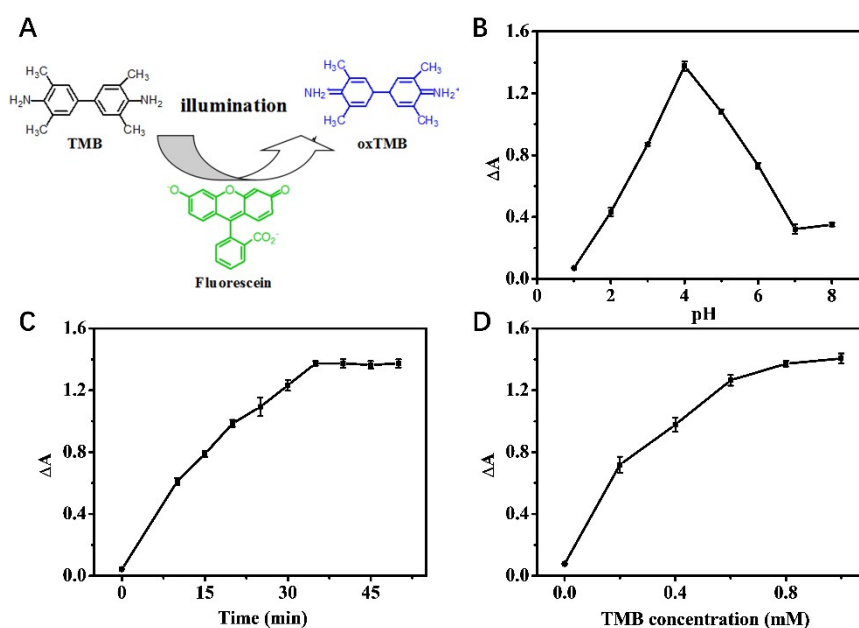
**Xuemei Hu, Chaoqun Sun, Ying Shi, Yijuan Long, Huzhi Zheng\***

Key Laboratory of Luminescent and Real-Time Analytical Chemistry (Southwest University), Ministry of Education, College of Chemistry and Chemical Engineering, Southwest University, Beibei, Chongqing 400715, China

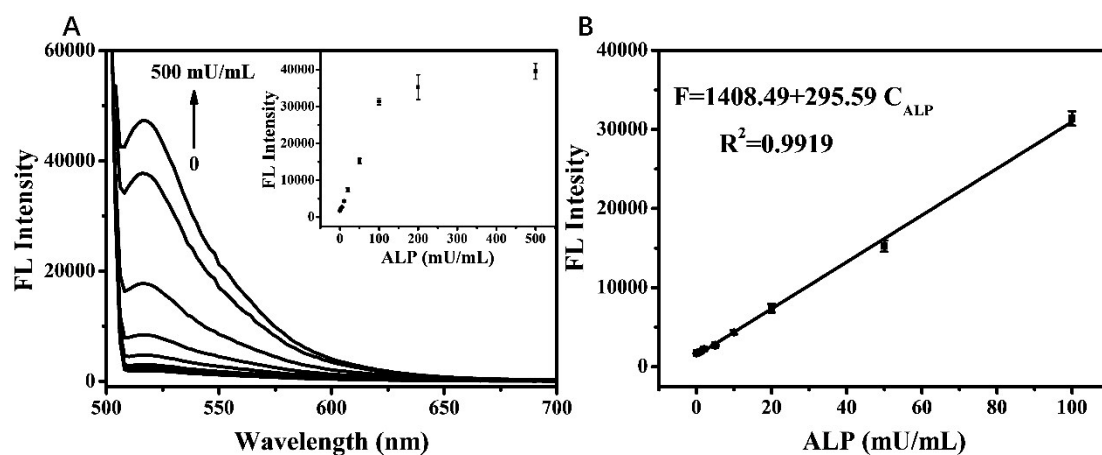
Corresponding author: Huzhi Zheng, E-mail: [zhenghz@swu.edu.cn](mailto:zhenghz@swu.edu.cn).



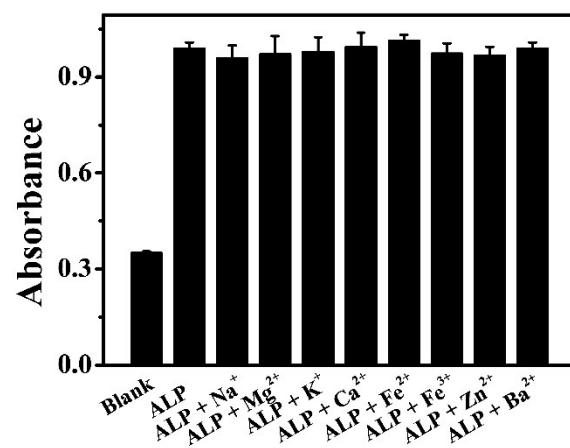
**Fig. S1** Condition optimization of hydrolysis process. (A) Hydrolysis diagram of FDP. (B) Optimization of FDP concentrations. hydrolysis time, 40 min; hydrolysis temperature, 35°C. (C) hydrolysis time. FDP, 20  $\mu$ M; hydrolysis temperature, 35°C. (D) hydrolysis temperature. FDP, 20  $\mu$ M; hydrolysis time, 40 min.  $\Delta A = A - A_0$ , where A and  $A_0$  represents the absorbance at 652 nm in the presence and absence of 50 mU/mL ALP, respectively. TMB, 0.80 mM; illumination time, 40 min; hydrolysis buffer, pH 8.0 Tris-HCl; chromogenic buffer, pH 4.0 HAc-NaAc. The error bars represent the standard deviation of three measurements.



**Fig. S2** Condition optimization in the process of catalytic chromogenic reaction. (A) Photoinduced TMB color change diagram; (B) optimization of pH; TMB, 0.80 mM; illumination time, 40 min. (C) optimization of illumination time; TMB, 0.80 mM; chromogenic buffer, pH 4.0 HAc-NaAc; (D) optimization of TMB concentrations. illumination time, 40 min; chromogenic buffer, pH 4.0 HAc-NaAc.  $\Delta A = A - A_0$ , where A and  $A_0$  represents the absorbance at 652 nm in the presence and absence of 50 mU/mL ALP, respectively. FDP, 20  $\mu$ M; hydrolysis time, 40 min; hydrolysis temperature, 35°C; hydrolysis buffer, pH 8.0 Tris-HCl. The error bars represent the standard deviation of three measurements.



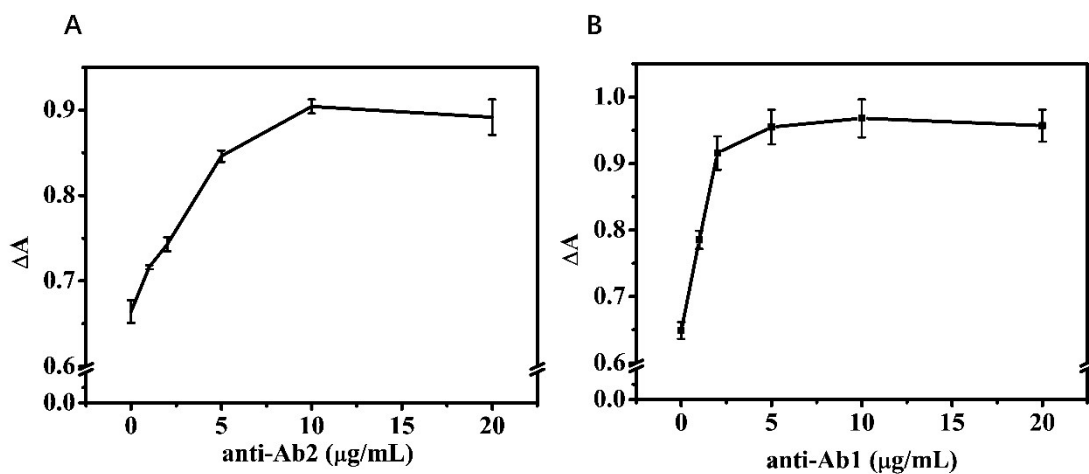
**Fig. S3** Conventional fluorescence method to measure different concentrations of ALP (0, 1, 2, 5, 10, 20, 50, 100, 200 mU/mL); Experimental conditions for the enzymatic reaction: FDP, 20  $\mu$ M; hydrolysis time, 40 min; hydrolysis temperature, 35°C; hydrolysis buffer, pH 8.0 Tris-HCl; Ex: 480 nm, Em: 530 nm. The error bars represent the standard deviation of three measurements



**Fig. S4** Effects of coexisting inorganic salt ions on the sensing of ALP. Experimental conditions for the reaction: FDP, 20  $\mu$ M; ALP, 20 mU/mL; other inorganic salt ions (Na<sup>+</sup>, Mg<sup>2+</sup>, K<sup>+</sup>, Ca<sup>2+</sup>, Fe<sup>2+</sup>, Fe<sup>3+</sup>, Zn<sup>2+</sup> and Ba<sup>2+</sup>, 10  $\mu$ M). The error bars represent the standard deviation of three measurements.

**Table S1** Comparison of the current work other reported methods for the detection of ALP.

Method	Sensing system	Detection range(mU/mL)	LOD (mU/mL)	Ref.
Chemiluminescence	CSPD substrate	0.01-10	0.01	[1]
Fluorometry	CdS QDs	0-50	0.5	[2]
Fluorometry	TPDA-PPI	0-200	0.09	[3]
Colorimetry	AuNPs/ATP	100-600	10	[4]
Colorimetry	AuNPs/ATP/Ca <sup>2+</sup>	5-100	0.1	[5]
	AuNPs/ATP/Pb <sup>2+</sup>	0.2-20	0.1	
Colorimetry	Redox active nanoceria	0.04-2	0.04	[6]
Colorimetry	Calcein-Ce <sup>3+</sup>	0.1-0.4 0.4-1.2	0.023	[7]
Colorimetry	FDP-ALP-TMB	0.2-80	0.18	This work



**Fig. S5** Optimizing of AFP anti-Ab2 and anti-Ab1 concentrations for ELISA. (A) Optimization of anti-Ab2 concentrations. anti-Ab1, 5  $\mu\text{g/mL}$ ; AFP, 20  $\text{ng/mL}$ . (B) Optimization of anti-Ab1 concentrations. anti-Ab2, 10  $\mu\text{g/mL}$ ; Other conditions are the optimized conditions for the reaction.  $\Delta A = A - A_0$ , where A and  $A_0$  represents the absorbance at 652 nm in the presence and absence of AFP, respectively. The error bars represent the standard deviation of three measurement.

**Table S2** Comparison of our AFP detection method with other methods.

Method	Sensing system	Detection range(ng/mL )	LOD (ng/mL)	Ref.
Chemiluminescence	Luminol-H <sub>2</sub> O <sub>2</sub> -HRP-PBP	0.1–5.0	0.01	[8]
Immunochemistry	QD-based ICTS	—	1.0	[6]
Fluorescence-anisotropy	Methylene blue-doped SiNPs	1.9–51.9	—	[9]
Fluorometry	Calcein-Ce <sup>3+</sup>	0.2–1.0, 1.0–4.0	0.041	[7]
Colorimetry	silver nanoparticles	1–100	0.23	10
Colorimetry	SA-HRP-Cu-3(PO <sub>4</sub> ) (2) hybrid nanoflowers	0.1-50	0.078	[11]
Colorimetry	FDP-ALP-TMB	0.5-50	0.2	This work



1. Blum J, Li R, Ag, Barry M An optimized method for the chemiluminescent detection of alkaline phosphatase levels during osteodifferentiation by bone morphogenetic protein 2. *Journal of Cellular Biochemistry*, (2015) 80:532-537.
2. Malashikhina N, Garaiibabe G, Pavlov V Unconventional Application of Conventional Enzymatic Substrate: First Fluorogenic Immunoassay Based on Enzymatic Formation of Quantum Dots. *Analytical Chemistry*, (2013) 85:6866-6870.
3. Jian S, Tao H, Chen C, Dan Z, Fan Y, Yang X Fluorescence Immunoassay System via Enzyme-Enabled in Situ Synthesis of Fluorescent Silicon Nanoparticles. *Analytical Chemistry*, (2016) 88:9789-9795.
4. Kim TI, Kim H, Choi Y, Kim Y A fluorescent turn-on probe for the detection of alkaline phosphatase activity in living cells. *Chemical Communications*, (2011) 47:9825-9827.
5. Zhao W, Chiuman W, Lam JC, Brook MA, Li Y Simple and rapid colorimetric enzyme sensing assays using non-crosslinking gold nanoparticle aggregation. *Chemical Communications*, (2007) 36:3729-3731.
6. Yang Q, Gong X, Song T, Yang J, Zhu S, Li Y, Cui Y, Li Y, Zhang B, Chang J Quantum dot-based immunochromatography test strip for rapid, quantitative and sensitive detection of alpha fetoprotein. *Biosensors & Bioelectronics*, (2011) 30:145-150.
7. Chen C, Zhao J, Lu Y, Jian S, Yang X Fluorescence Immunoassay Based on the Phosphate-Triggered Fluorescence Turn-on Detection of Alkaline Phosphatase. *Analytical Chemistry*, (2018) 90:3505-3511.
8. Bi S, Yan Y, Yang X, Zhang S Gold Nanolabels for New Enhanced Chemiluminescence Immunoassay of Alpha-Fetoprotein Based on Magnetic Beads. *Chemistry - A European Journal*, (2010) 15:4704-4709.
9. Deng T, Li JS, Jiang JH, Shen GL, Yu RQ Preparation of Near-IR Fluorescent Nanoparticles for Fluorescence-Anisotropy-Based Immunoagglutination Assay in Whole Blood. *Advanced Functional Materials*, (2010) 16:2147-2155.
10. Ren RR, Cai GN, Yu ZZ, Zeng RY, Tang DP.. Metal-polydopamine framework: an innovative signal-generation tag for colorimetric immunoassay. *Analytical Chemistry*, *acs.analchem*. (2018) 90:11099-11105.
11. Liu Y, Chen J, Du M, Wang X, Ji X, He Z The preparation of dual-functional hybrid nanoflower and its application in the ultrasensitive detection of disease-related biomarker. *Biosensors & Bioelectronics*, (2017) 92:68-73.

## References