Supporting Information

Ratiometric Sensing of Alkaline Phosphatase Based on the

Catalytical Activity from Mn-Fe Layered Double Hydroxides

Nanosheets

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EXPERIMENTAL SECTION

Preparation of MnO₂ nanosheeets. The MnO₂ nanosheets are prepared according to the previous work.¹ Typically, 1 mL of 10 mM KMnO₄ is introduced to 10 mL of 0.01 M MES buffer and the mixture keeps sonication for 30 min by ultrasonic cell crusher. The MnO₂ nanosheets are obtained after centrifugation under 10000 rpm for 10 min and wash with distilled water for many times. The product are dispersed in distilled water and the final concentration of MnO₂ nanosheets is 0.862 mM according to the results from inductively coupled plasma-mass spectrometry.

Synthesis method of CoOOH nanosheets. Referring to the synthetic method reported in the literature.² In brief, 1.5 mL of 1.0 M NaOH and 50 mL of 10 mM $CoCl_2$ are uniformly mixed under ultrasonic conditions for 5 min, then centrifuge at 4000 rpm for 20 min and the obtained precipitate is dispersed into 50 mL of distilled water. Finally, 2.5 mL of 0.9 M NaClO solution is added to the above solution under ultrasonic treatment for 20 min, and then centrifuge at 12000 rpm for 10 min with distilled water washed.

Synthesis method of spherical Fe_3O_4 . Synthesis of Fe_3O_4 based on literature reports.³ First, 50 mL of 1 M FeCl₃ aqueous solution and 10 mL of 2 M FeCl₂ solution in hydrochloric acid are mixed and oxygen is removed by N₂ for 10 min. Then, the above mixed solution is added to 500 mL of 0.7 M the aqueous ammonia solution while vigorously stirring for 30 minutes at room temperature (the solution is deoxidized and protected by N₂), Fe₃O₄ obtained by the reaction is centrifuged and washed three times with distilled water and then redispersed in water and stored at

room temperature for future use.

Synthesis method of FeOOH nanosheets. Synthesis of FeOOH nanosheets based on literature reports.⁴ Use CuO as template. First, 0.5 g of CuCl is dissolved in 70 mL water and stirs for 30 min, then ammonia water (2.5 mL, 25 %) is introduced, and the mixture is heated at 150 °C for 20 h, then the resulting mixture is dried at 60 °C overnight to obtain CuO template. After dissolving 80 mg of CuO in 150 mL water and sonicating for 30 min and then introducing N₂ for 30 min, 80 mg of FeCl₂•4 H₂O is added and the mixture is sealed in a beaker at room temperature for 24 h. The suspension gradually turns brownish yellow. Finally, aqueous ammonia (15 %) is introduced the mixture and the CuO template is removed by centrifugation three times with secondary water and dried to obtain FeOOH nanosheets.



Figure S1. XRD spectrum of Mn-Fe LDH.



Figure S2. Schematic illustration of the reaction between OPD and AA.



Figure S3. (A) Fluorescence spectra in the presence of 5 mM OPD ,10 μ M AA and Mn²⁺, Fe³⁺, Fe²⁺. (B) Fluorescence intensity at 425 nm in the presence of 5 mM OPD ,10 μ M AA and Mn²⁺, Fe³⁺, Fe²⁺.



Figure S4. The pH-dependent fluorescence spectra in the presence of 5 mM OPD, 10 μ LMn -Fe LDH in the absence of AA.



Figure S5. (A) The fluorescence responses using different amount of Mn-Fe LDH in the presence of 5 mM OPD ,10 μ M AA and (B) UV-vis absorption of spectrum of the Mn-Fe LDH (red line) and the fluorescence emission spectrum of OPD + AA + Mn-Fe LDH (black line).



Figure S6. (A) The fluorescence responses of the sensing system against the AA concentration: The AA concentration are 0.5, 1, 4, 8, 10, 15, 20, 25 Mm. (B) The linear relationship of $\Delta F_{425 \text{ nm}}/\Delta F_{565 \text{ nm}}$ against AA concentration.



Figure S7. ALP determination based on the present strategy using real samples with standard additional method, real sample (black line), successive addition of 2 mU ALP (pink line), 4 mU ALP (green line), 6 mU ALP (blue line), 8 mU ALP (red line).

Added ALP	Measured ALP	Recovery
(mU)	(mU)	(%)
2	1.86	93.2
6	5.82	97.0
8	7.99	99.9

Table S1. Recovery Test Results of ALP in human plasma samples.

REFERENCES

- 1 R. Deng, X. Xie, M. Vendrell, Y. T. Chang and X. Liu, J. Am. Chem. Soc., 2011, 133, 20168-20171.
- 2 S. Liu, L. Han, N. Li, Y. Fan, Y. Yang, N. Li and H. Luo, *Sensor. Actuat. B-Chem.*, 2019, **283**, 515-523.
- 3 Y. Song, Y. Cao, J. Wang, Y.-N. Zhou, F. Fang, Y. Li, S. Gao, Q. Gu, L. Hu and D. Sun, *ACS Appl. Mater. Interfaces*, 2016, **8**, 21334-21342.
- 4 H. Wei and E. Wang, Anal. Chem., 2008, 80, 2250-2254.