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Supporting Information

Fluorescence nanosensor for *in situ* detection of phosphate and alkaline phosphatase in parathyroid dysfunction mice

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EXPERIMENTAL SECTION

Materials and reagents

Ultrapure water (18.2MΩ*cm) was obtained from the Water Pro water purification system (Labconco Corp., Kansas City, MO). Zirconium tetrachloride was purchased from Behringer Technology Co., Ltd (Beijing China). 2-Aminoterephthalic acid was purchased from Shanghai Civic Chemical Technology Co., Ltd (Shanghai, China). Alkaline phosphatase (ALP) was purchased from Sigma-Aldrich (St. Louis, U.S.A.). DNA enzyme, esterase, and adenosine triphosphate (ATP) were purchased from Macklin (Shanghai, China). BSA protein, pyrrole, 3-(4,5-dimethylthiazolyl-2-)-2,5-diphenyltetrazolium bromide (MTT), 4-formylbenzoic acid, L-cysteine Acid and glutathione were purchased from Sigma-Aldrich (Shanghai, China). Other inorganic compounds: $MgSO_4$, NaCl, $CaCO_3$, $Al(OH)_3$, Na_3PO_4, Na_2HPO_4, NaH_2PO_4, Na_2SO_4, Na_2CO_3, $NaHCO_3$, $NaHSO_3$ were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China).

Instruments

TEM and SEM images were performed by Hztachi HT7700 and Zeiss Supra 55 electron microscope, respectively. The UV-Vis absorption spectrum was obtained from a TU-1900 dual-beam UV-vis spectrophotometer. Particle size distribution and stability were obtained from a Malvern instruments Nano-ZS90 Zeta Potentiometer

and DLS Dynamic Light Scatterometer. Fluorescence spectra were obtained from Edingburgh FLS-980 fluorescence spectrometer at room temperature. All pH measurements were made using a Mettler Toledo digital pH meter. The Caliper IVIS Lumina Series III small animal *in vivo* imager was used for the *in vivo* experiments.

Synthesis and characterization of Nano-MOFs Probe

Synthesis of MOFs

Zirconium tetrachloride (0.164 g, 0.7 mmol) and 2-aminoterephthalic acid (0.127 g, 0.7 mmol) were dissolved in a mixture which consists of 20 ml DMF, 3 ml acetic acid and 0.2 ml deionized water at room temperature, Sonicating for 5 min to dissolve sufficiently. Then 36 mg of 5, 10, 15, 20-tetrakis(4-carboxyphenyl)porphyrin was added, and the solution turned from yellow to green. After 5 min of ultrasonication, transfer mixture to a 20 ml Teflon-lined stainless steel autoclave, constant temperature reaction in an oven at 120 °C for 24 hours, then cool to room temperature, and centrifuge at 8000 rpm for 15 min to collect the reddish brown product and wash with DMF three times. The washed product was dispersed in 50 ml of DMF and immersed for 12 hours, and then thoroughly dispersed in a 50 ml ethanol solution for 24 hours. The final product was washed with ethanol and dried in a vacuum oven overnight.

Synthesis of Nano-MOFs Probe

10 mg of MOFs was dissolved in 50 ml of 1.0 mmol/L p-nitrophenyl phosphate (PNPP) solution at room temperature and stirred at 37 °C for 24 hours. Then the

mixture was dialyzed against water, and the precipitate was collected by centrifugation and dried in a vacuum oven.

The experiments with the mice

We selected 15 (5 per group) female Kunming mice for 6-8 weeks(32±2g), and modeled by continuous administration. The weight of the mice in the two experimental groups gradually decreased within 7 days. After modeling, the three groups of mice collected blood by tail-cutting, centrifuged to take the serum, and measured the calcium and phosphorus content in the serum by inductively coupled plasma spectrometer (ICP-OES). The experimental results show that compared with the control group, hyperparathyroidism mice have increased levels of blood phosphorus and calcium due to increased secretion of PTH; mice with hypoparathyroidism have increased hormone levels due to PTH injection, The function of the gland itself is weakened, so blood phosphorus levels are reduced and blood calcium levels are increased. This indicates that we have successfully established two sets of mouse models of parathyroid dysfunction. Experimental mice were purchased from the Animal Center of Shandong University.

All animal care and experimental protocols involved in this article comply with the animal management regulations of the Ministry of Health of the People's Republic of China and have been approved by the Animal Care Committee of Shandong Normal University.

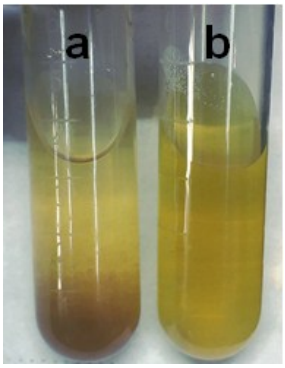

Fig. S1 Visual comparison of the dispersion of (a) MOFs and (b) nanosensor; (a) and (b) are same quality of MOFs and nanosensor after standing for 3 days at room temperature.

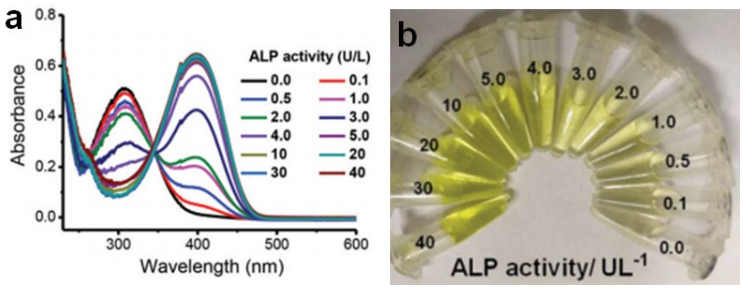

Fig. S2 (a) Ultraviolet absorption spectra of nanosensor at different concentrations of phosphate and ALP. (b) Changes of nanosensor under different concentrations of ALP.

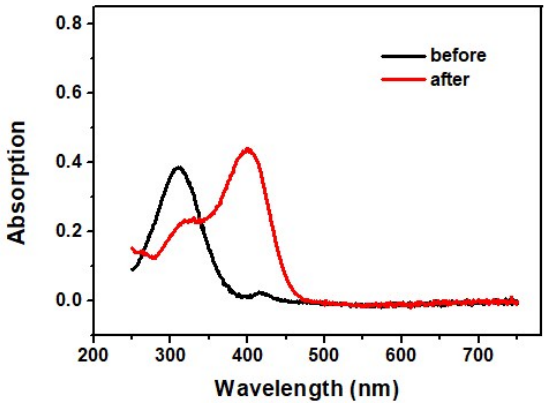

Fig. S3 UV-Vis absorption diagram before and after reaction of nanosensor with ALP. In the control group (black line) , (100 μL, 0.1 mg/L) of nanosensor solution was diluted into 1.4 ml of 10 mmo/L Tris-HCl buffer. The nanosensor solution in the experimental group (red line) , (100 μL, 0.1 mg/L) was diluted to 1.4 ml of Tris-HCl buffer (10 mmo/L) containing (0.2 U/mL) ALP. Each group of experiments was measured in parallel three times.

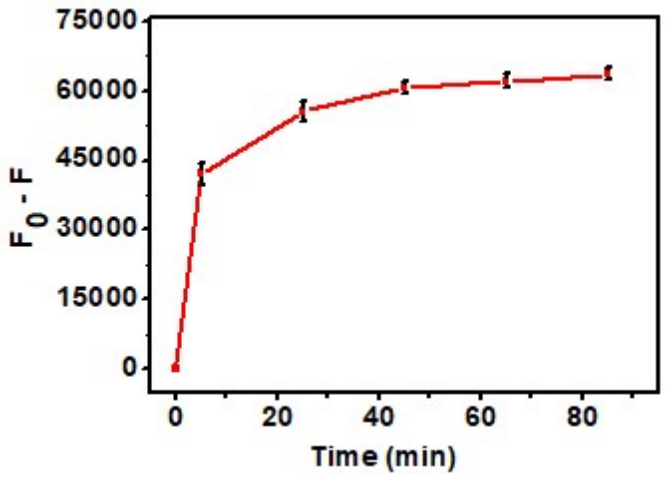

Fig. S4 Response of nanosensor to ALP at different response times. Dilute nanosensor (100 μL, 0.1 mg/L) solution to 1.4 ml of Tris-HCl buffer containing ALP (0.2 U/mL), the final concentration of the nanosensor was 6.67 μg/ml. The fluorescence intensity at 650 nm of the nanosensor solution at 405 nm excitation was recorded every 10 minutes, until the fluorescence intensity longer changed significantly.

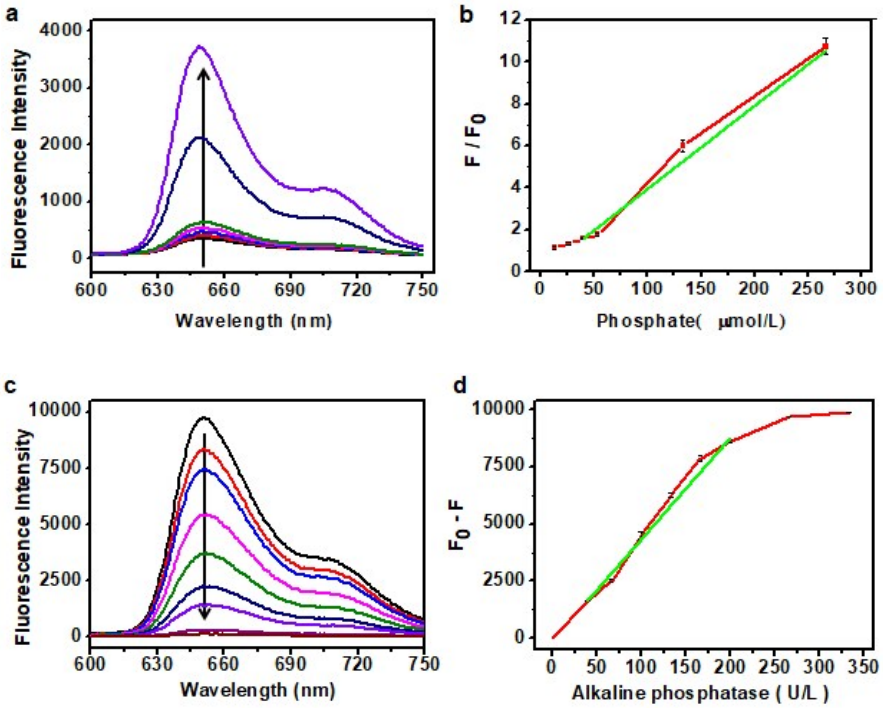

Fig. S5 (a) Fluorescence response of nanosensor to phosphate with gradient concentration. The phosphate gradient concentrations were: 20 μmol/L, 40 μmol/L, 60 μmol/L, 80 μmol/L, 200 μmol/L, and 400 μmol/L, respectively. nanosensor solution (100 μL, 0.1 mg/L) and the corresponding concentrations were added to 10 mmol/L HEPES buffer to maintain the total volume of the blank group and the experimental group at 1.5 ml. (b) Linear working curve of phosphate ion detected by nanosensor. The linear correlation coefficient was 0.979. (c) Response of nanosensor to gradient concentration ALP. The gradient concentrations of ALP were: 40 U/L, 66.7 U/L, 100 U/L, 133.3 U/L, 166.7 U/L, 200 U/L, 266.7 U/L, and 333.3 U/L. The nanosensor solution (100 μL, 0.1 mg/L) and the corresponding concentrations to be tested were added to 10 mmol/L Tris-HCl buffer, the blank group and the experimental group were 1.5 ml in total. (d) Linear working curve of ALP detected by nanosensor. The linear correlation coefficient is 0.998. The excitation wavelength was 405 nm, and the slit width was 5 nm.

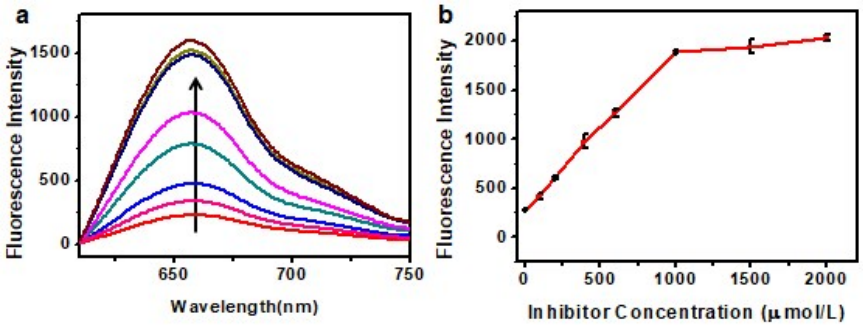

Fig. S6 (a) Fluorescence response of nanosensor to ALP inhibitor concentrations. The gradient concentrations of the inhibitors were: 0 μmol/L, 100 μmol/L, 200 μmol/L, 400 μmol/L, 600 μmol/L, 1000 μmol/L, 1500 μmol/L, 2000 μmol/L. Add 100 μL (0.1 mg/L) of nanosensor. solution, 200 U/L of ALP and the respective inhibitor concentrations in 10 mmol/L Tris-HCl buffer to keep experimental groups consistent with blank. The total volume was 1.5 ml. The excitation wavelength was 405 nm, and the emission wavelength was 650nm, the slit width was 5 nm. (b)Linear relationship between fluorescence and inhibitor concentration of nanosensor.

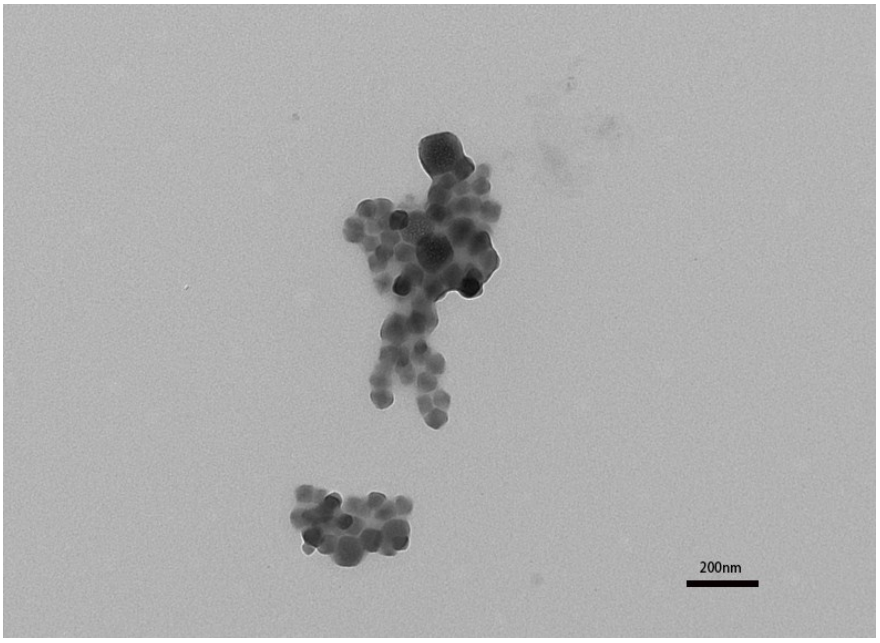

Fig. S7 More visual field of aggregated MOFs particles (Fig. 1(c)). Significant aggregation of MOFs particles, almost no separate MOFs particles.

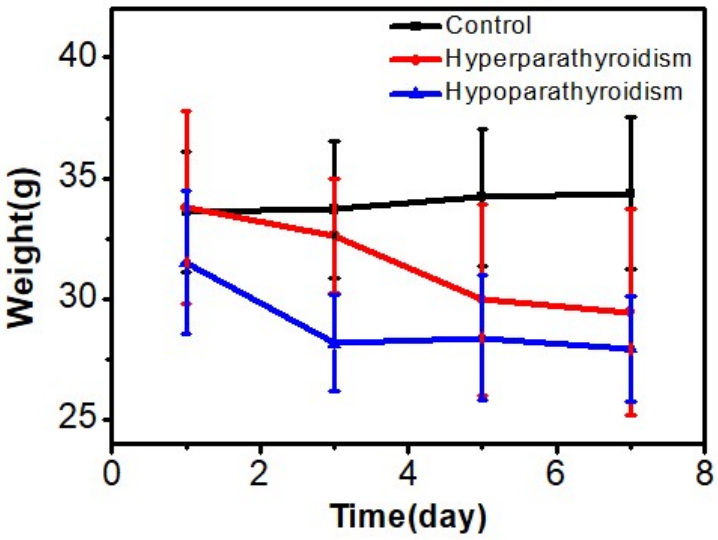

Fig. S8 Changes in mouse weight over time during modeling. 15 mice divided into three groups female Kunming mice for 6-8 weeks (32±2g), and modeled by continuous administration. Weigh the mice every 2 days, each mouse was weighed five times in succession to take the average, and the weight of the five mice in each group was averaged to obtain the weight change trend. The black line is the control group, the red line is the hyperparathyroidism group, and the blue line is the hypoparathyroidism group.