Electronic Supporting Information

for

Visual Detection of Cobalt (II) Ion in Vitro and Tissue with a New Type of Leaf-Like Molecular Microcrystal

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Experimental Details

Reagents and materials. All reagents were of analytical grade and used without further purification. PVP, with a molecular weight of 55000, was purchased from Sigma. *p*-Phenylenediamine (*p*-PDA) monomer was gotten from Sinopharm Group Chemical Regent Co, Ltd. (Shanghai, China). 0.1 g/mL PVP working solution and 0.3 g/mL *p*-PDA working solution were prepared respectively by directly dissolving in doubly distilled water under vigorous magnetic stirring, and homogeneous solution at room temperature was available as a consequence.

0.1 M stock solution of $AgNO_3$ was prepared by dissolving solid $AgNO_3$ in doubly distilled water and the working solution was obtained by diluting the stock solution to 0.01 M with water. Doubly deionized water (18.2 MΩ) was used throughout the experiment and all glasswares were cleaned using concentrated nitric acid and subsequently rinsed with a copious amount of doubly deionized water.

Apparatus. Scanning electron microscopy (SEM) was applied to investigate the size and morphology of the newly prepared leaf-like P*p*PD microcrystals, and was carried out with a Hitachi (Tokyo, Japan) S-4800 scanning electron microscope at an accelerating voltage of 20 kV. FTIR spectra were measured on a Perkin Elmer Spectrum GX Fourier Transform Infrared (FTIR) Spectrometer (MA.USA) from 4000 to 500 cm^{-1} at room temperature. X-ray photoelectron spectroscopy (XPS) analysis

was carried out on an ESCRLRB250X (America) cispectrometer with a standard Al K source (1486.6 eV) and X-ray powder diffraction (XRD) was measured on XD-3 system (Beijing Purkinje General Instrument Co. Ltd.) with Cu Kα (1.5405 Å) radiation source under the operating voltage and current of 40 kV and 50 mA, respectively. UV-vis spectra were recorded on a Hitachi UV-3010 spectrophotometer at room temperature.

Preparation of P*p*PD microcrystals. The preparation of P*p*PD microcrystals was very easy to do by using $AgNO_3$ as an oxidant and poly (-*N*-vinylpyrrolidone) (PVP) as a surfactant at room temperature. First, 1 mL of 0.1 g/mL PVP solution and 6 mL of 0.3 g/mL *p*-PDA solution were mixed with 2 mL doubly deionized water in a 50 mL conical flask, and the 1108 μL of 10mM $AgNO_3$ was injected under shaking table running at room temperature for 3 hours. Then, the products were centrifuged and washed 3 times with double-distilled water and absolute ethanol. After that, the P*p*PD microcrystals was suspended in distilled water, and transferred for the characterizations of SEM, FTIR or for the use of visual detection of Co^{2+} and imaging in tissue.

Experimental procedure. To a 1.5 mL tube, solutions were added in the sequence of above-prepared 50 μL 0.35g/L P*p*PD microcrystals solution and 50 μL pH 6.12 Michaelis buffer. The mixture was vortexed and then an appropriate volume of water was added to make the initial volume of 500 μL. At last, sample solution of Co^{2+} was added and the mixture was got mixed thoroughly. After keeping still for 5 min, the mixture was transferred to UV measurements.

Histopathology imaging and analysis. Fish tissues including foregut and anus were fixed separately by immersion in 10% buffered formalin for 24 h, washed twice in phosphate buffered saline and transferred to 75% ethanol until processing. After dehydration and embedding in paraffin, tissues were step-sectioned to obtain longitudinal sections. Sections were pretreated by adding $CoCl_2$ solution and pure double-distilled water respectively for 12 h, and then incubated with P*p*PD mircocrystals for 2 h. At last, the fish tissue was washed several times with

double-distilled water. Histopathological observation of tissues was carried out with an Olympus BX-51 microscope (Tokyo, Japan), which was equipped with a highly numerical dark field condenser (U-DCW) and an Olympus DP72 digital camera (Tokyo, Japan). The analyst was blinded to sample identification and no less than triplex samples from each tissue were analyzed.

Figures

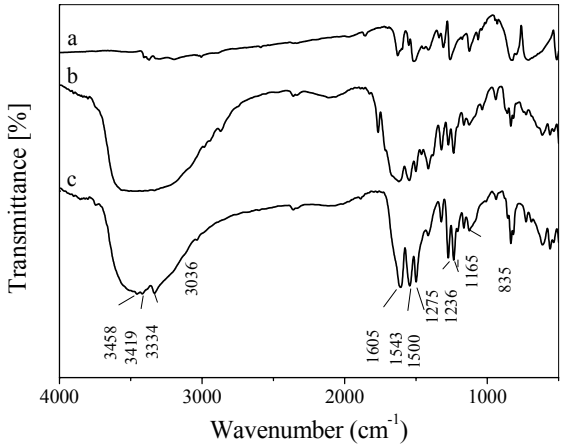

Fig. S1 (a) FTIR spectra of pure *p*-PDA. (b) The FTIR spectra after the P*p*PD microparticles interacted with Co^{2+}. c_{PpPD}, 0.035 g/L; c_{co2+}, 0.05 mM. (c) FTIR spectra of the P*p*PD microparticles. c_{PVP}, 0.01 g/ mL; $c_{p\text{-}PDA}$, 0.03 g/mL; c_{AgNO3}, 1.1 mM.

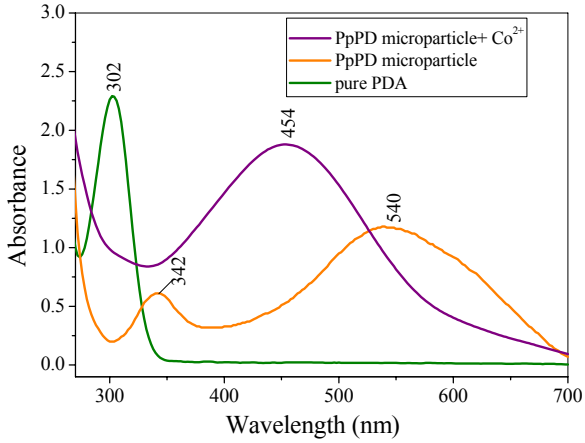

Fig. S2 The UV-vis spectra of pure *p*-PDA, the P*p*PD microparticle synthesized and the P*p*PD microparticle interacted with Co^{2+}, respectively. Curve 1 (green): Pure *p*-PDA; Curve 2 (purple): P*p*PD microparticles after interacted with Co^{2+}. c_{PpPD}, 0.035

g/L; c_{co}^{2+}, 0.05 mM. Curve 3 (yellow): P*p*PD microparticles. c_{PVP}, 0.01 g/ mL; $c_{p\text{-}PDA}$, 0.03 g / mL, c_{AgNO3}, 1.10 mM.

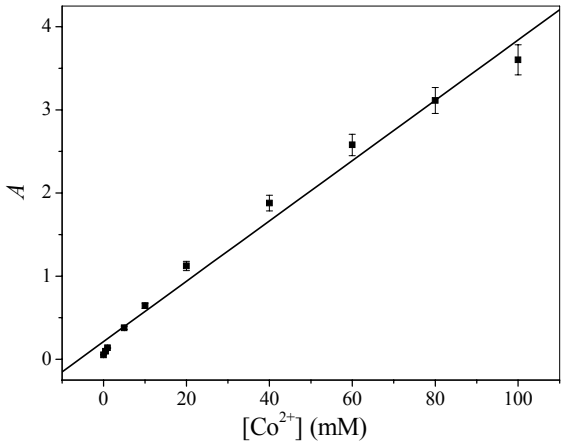

Fig. S3 Linear response of the absorbance of the P*p*PD microcrystals with the addition of Co^{2+} (0.5-100 μM). The linear regression equation is $A = 0.193 + 0.036\ c$ with the corresponding correlation coefficient (r) of 0.9930. c_{PpPD} , 0.035 g/L; pH 6.12; λ, 454 nm.

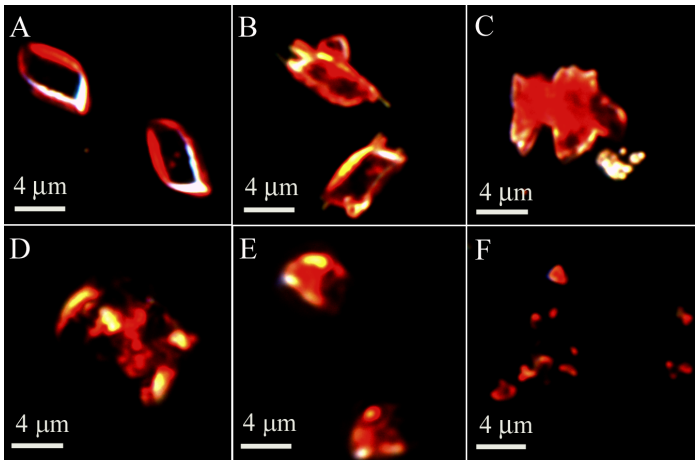

Fig. S4 Etching effect of Co^{2+} on the PpPD microcrystals as displayed by dark-field light scattering images. c_{Co2+}, (A) 0.0 (B) 1.0 (C) 10.0 (D) 40.0 (E) 80.0 (F) 100.0 μM; c_{PpPD}, 0.035 g/L. pH 6.12.

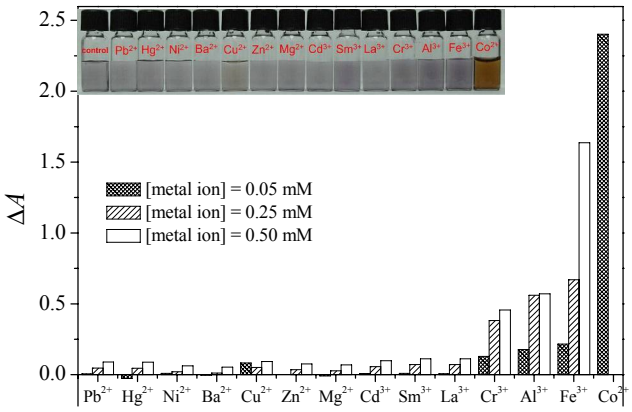

Fig. S5 The absorbance change upon the addition of different metal ions. $c_{P\textit{p}PD}$, 0.035 g/L; pH 6.12; λ, 454 nm. (The inset picture shows the color change of the P*p*PD microcrystals in presence of different metal ions with 0.05 mM concentration.)

Table

Table S1. FTIR peak Assignments in P*p*PD microcrystals

Wave number, cm^{-1}	**Assignments**
3458, 3419, 3334	N–H stretching vibrations of the –NH– group,
3036	Aromatic C–H stretching vibration
1605	C=N stretching vibrations in the phenazine ring
1543	C=C stretching vibrations in the phenazine ring
1500	Stretching of the benzene ring
1275	C–N stretching in the benzenoid
1236	C–N stretching in the quinoid imine units (–C—N–)
1165, 1006	Aromatic C–H in plane bending mode
835	C–H out-of-plane bending vibrations of benzene nuclei in the phenazine skeleton