Electronic Supplementary Information

Exploring Tunable Excitation of QDs to Maximize the Overlap with Absorber for Inner Filter Effect-based phosphorescent Sensing of Alkaline Phosphatase

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Outline

1. Experimental Section

Materials and chemicals

ALP was purchased from Shanghai Yuanye Biotechnology Co., Ltd. Glucose oxidase (GOD), bovine serum albumin (BSA), pepsin, papain, lysozyme, cytochrome C and glutathione were purchased from Sigma-Aldrich (Shanghai, China). Ultra-pure water (resistance >18.2 MΩ.cm) purified by a purification system (PCUJ-10, Chengdu Pure Technology Co., Ltd., Chengdu, China), was used for all experiments. *p*-Nitrophenylphosphate (PNPP), amino acids, N-acetyl-cysteine (N-L-Cys), Mercaptosuccinic acid (MPA), $ZnAc_2 \cdot 7H_2O$, $MnAc_2 \cdot 4H_2O$, $Na_2S \cdot 9H_2O$, $CdCl_2 \cdot 2H_2O$, $NaBH_4$, Na_2TeO_3, $C_6H_5Na_3O_7 \cdot 2H_2O$, NaOH, $CuCl_2 \cdot 2H_2O$, Citric Acid, ethylenediamine, 3-mercaptopropionic acid (MPA) (Aladin) and Tris (Sinopharm Chemical Reagent Co., Ltd) were used in this work.

Apparatus

Fluorescence and phosphorescence spectra of the prepared QDs were collected with an F-7000 spectrofluorometer (Hitachi, Japan) equipped with a plotter unit and a quartz cell (1 cm × 1 cm). Fluorescence and phosphorescence lifetime were measured on a Fluorolog-3 spectrofluorometer (Horiba Jobin Yvon) with DeltaDiode (Horiba Scientific, for fluorescence) and SpectraLED (Horiba Scientific, for phosphorescence) as the excitation source and a picosecond photon detection module (PPD-850, Horiba Scientific) as the detector respectively. Absorption spectra were recorded using a UV-1750 UV/Vis spectrophotometer (Shimadzu, Japan). TEM and HRTEM images of QDs were obtained using a Tecnai G2 F20 S-TWIN transmission electron microscope at an

accelerating voltage of 200 kV (FEI Co., USA). XRD patterns were recorded by an EMPYREAN (Panalytical Inc., Netherlands) with a Cuκa radiation; XPS was carried out with an AXIS Ultra DLD 800 X (Kratos, UK).

Synthetic methods of several QDs

BSA-coated Mn-ZnS QDs.[1] In brief, 40 μL BSA (10 mg mL^{-1}), 40 μL $MnAc_2$ (1 mM), 38 μL $ZnAc_2$ (20 mM) and 50 μL Tris-HCl solution (pH = 7.0, 0.1 M) were added to 298 μL ultrapure water for five minutes in room temperature. Then, 34 μL Na_2S (20 mM) was quickly injected into the precursor solution above followed gently vortexing in room temperature. Phosphorescent BSA-capped Mn-ZnS QDs were thus obtained.

L-Cys-capped Mn-ZnS QDs.[1] In brief, 60 μL L-Cys (2 mM), 40 μL $MnAc_2$ (1 mM), 38 μL $ZnAc_2$ (20 mM) and 50 μL Tris-HCl solution (pH = 8.0, 0.1 M) were added to 298 μL ultrapure water for five minutes in room temperature. Then, 34 μL Na_2S (20 mM) was quickly injected into the precursor solution above followed gently vortexing in room temperature. Phosphorescent L-Cys-capped Mn-ZnS QDs were thus obtained.

L-Cys-capped Cu-doped CdS QDs.[2] In a typical synthesis, aqueous solutions of $CdCl_2$ (10 mL, 0.02 M), $CuCl_2$ (50 μL, 0.02 M) and 25 mL deionized water were mixed in a 100 mL three-neck flask. Then, L-Cys (10 mL 0.1 M) was added in the system. The pH of the mixed solution was adjusted to 8.0 with 1.0 M NaOH. After that, 20 mL 0.004 M fresh Na_2S solution dropwise with stirring. Finally, the mixed solution was refluxed at 100℃ with stirring for 45 min in a N_2 atmosphere.

N-L-Cys-capped O/R-CdTe QDs.[3] Briefly, 0.5 mmol of $CdCl_2$, 0.2 g sodium citrate dehydrate and 1 mmol N-acetyl-cysteine were dissolved in 25 mL water. The pH was adjusted about 11 by dropping 1 mol/L NaOH. Then 0.125 mmol Na_2TeO_3 and 50 mg $NaBH_4$ were added quickly, and at last, the solution was refluxed at 100 °C for 0.5 h (O-CdTe QDs) and 1 h (R-CdTe QDs).

MSA-capped G-CdTe QDs.[4] Briefly, 0.5 mmol of $CdCl_2$, and 1 mmol MSA were dissolved in 25 mL citric acid buffer (15 mM) with pH 7. Then 0.125 mmol Na_2TeO_3 and 50 mg $NaBH_4$ were added quickly, and at last, the solution was refluxed at 100 °C for 0.5 h (R-CdTe QDs).

B-CDs.[5] In brief, citric acid (1.0507 g) and ethylenediamine (335 μL) were dissolved in DI-water (10 mL). Then the solution was transferred to a poly (tetrafluoroethylene) (Teflon)-lined autoclave (20 mL) and heated at 180ºC for 8 h. After the reaction, the reactors were cooled to room temperature naturally. The product, which was brown-black and transparent, was subjected to dialysis in order to obtained the B-CDs.

G-CDs.[6] Briefly, 500 μL of ethanediamine and 440 mg catechol were mixed into 8 mL water and then transferred into a 20 mL Teflon-lined autoclave and heated at 180 °C for 12 h. The resultant dark brown mixture centrifuged to remove the large dots at 12 000 rpm for 10 min, and then the supernatant was collected to dialysis in order to obtained the G-CDs.

Procedures for Screening of IFE Pairs and Subsequent IFE sensing

Different concentration of the prepared QDs were mixed with 100 μM PNPP to gain the similar quenching of QDs. The excitation is set at 312 nm. The optimized incubation conditions were set as follows: buffer, 0.01 M Tris-HCl (pH, 8.0); incubation time, 1 h; and temperature, 40 ^{o}C.

Procedures for ALP Detection Based on the PNPP-Mn-ZnS QDs IFE Pair

BSA-Mn-ZnS QDs (50 μL) and PNPP (2 mM, 50 μL) were mixed with 1 mL buffer solution (0.01 M Tris-HCl, pH, 8.0) for quenching the phosphorescence of QDs (λ_{ex} = 312 nm). Then, different concentrations of ALP from different origins (Escherichia coli, human serum, and bovine) were added to catalyze the conversion from PNPP to PNP at 40 ^{o}C and restore the quenched phosphorescence of QDs. The optimized incubation time is 1 h for ALP from Escherichia coli and 3 h for ALP from human serum and bovine.

Real Serum Samples Detection

Human serum samples were collected from Sichuan Provincial People's Hospital. 50 μL the prepared Mn-ZnS QDs, 50 μL PNPP (2 mM), and 100 μL Tris-HCl (pH, 8.0, 0.1 M) and 5 μL human serum were added to 1 mL water at 40 ^{o}C 3 h. Then, the quantitation of ALP activity of serum samples was carried out according to the above procedure.

2. Characterization of several QDs

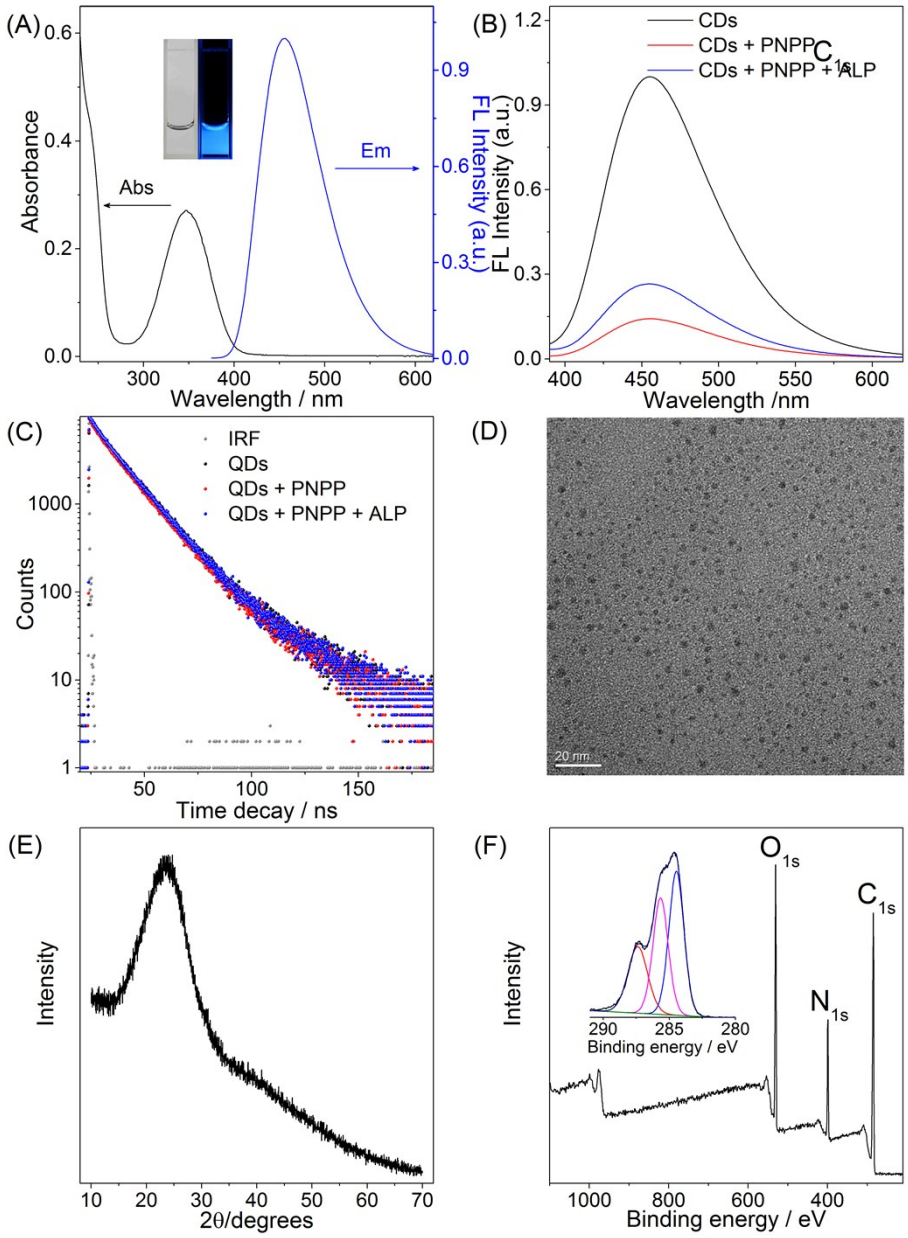

Fig. S1 Characterization of the B-CDs: (A) UV-Vis absorption spectra and fluorescence emission spectra (the inset are the photographs of B-CDs in ambient light (left) and under irradiation by UV light (right)); (B) fluorescence intensity and (C) fluorescencent decay curve of B-CDs in the absence and presence of PNPP (100 μM), and in the presence of PNPP (100 μM) + ALP (10 U/L). (D) TEM image; (E) XRD pattern and (F) XPS of CDs. (excitation source: DeltaDiode 320 nm, time range: 400 ns, the data is fitted with the second order exponential decay).

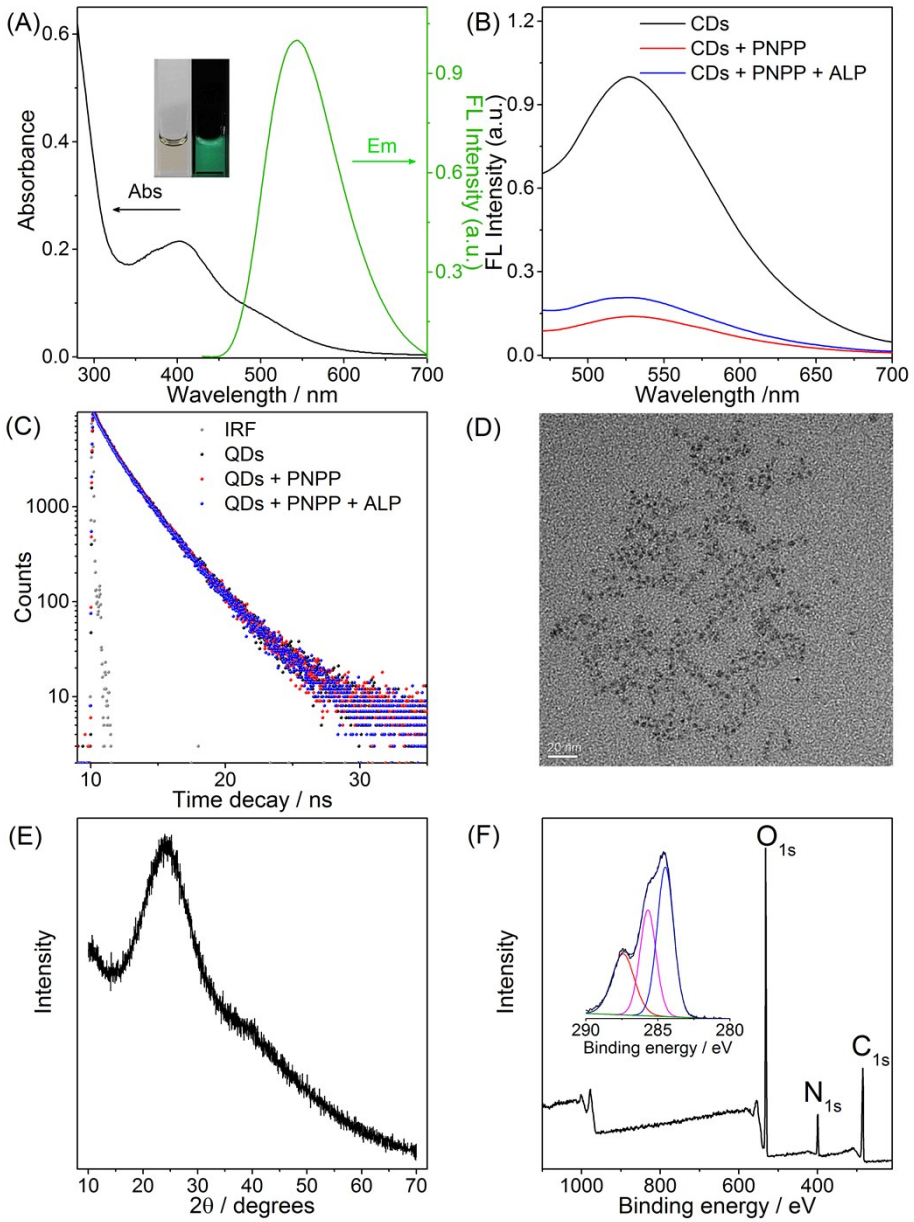

Fig. S2 Characterization of the G-CDs: (A) UV-Vis absorption spectra and fluorescence emission spectra (the inset are the photographs of G-CDs in ambient light (left) and under irradiation by UV light (right)); (B) fluorescence intensity and (C) fluorescencent decay curve of G-CDs in the absence and presence of PNPP (100 μM), and in the presence of PNPP (100 μM) + ALP (10 U/L). (D) TEM image; (E) XRD pattern and (F) XPS of CDs. (excitation source: DeltaDiode 320 nm, time range: 200 ns, the data is fitted with the second order exponential decay).

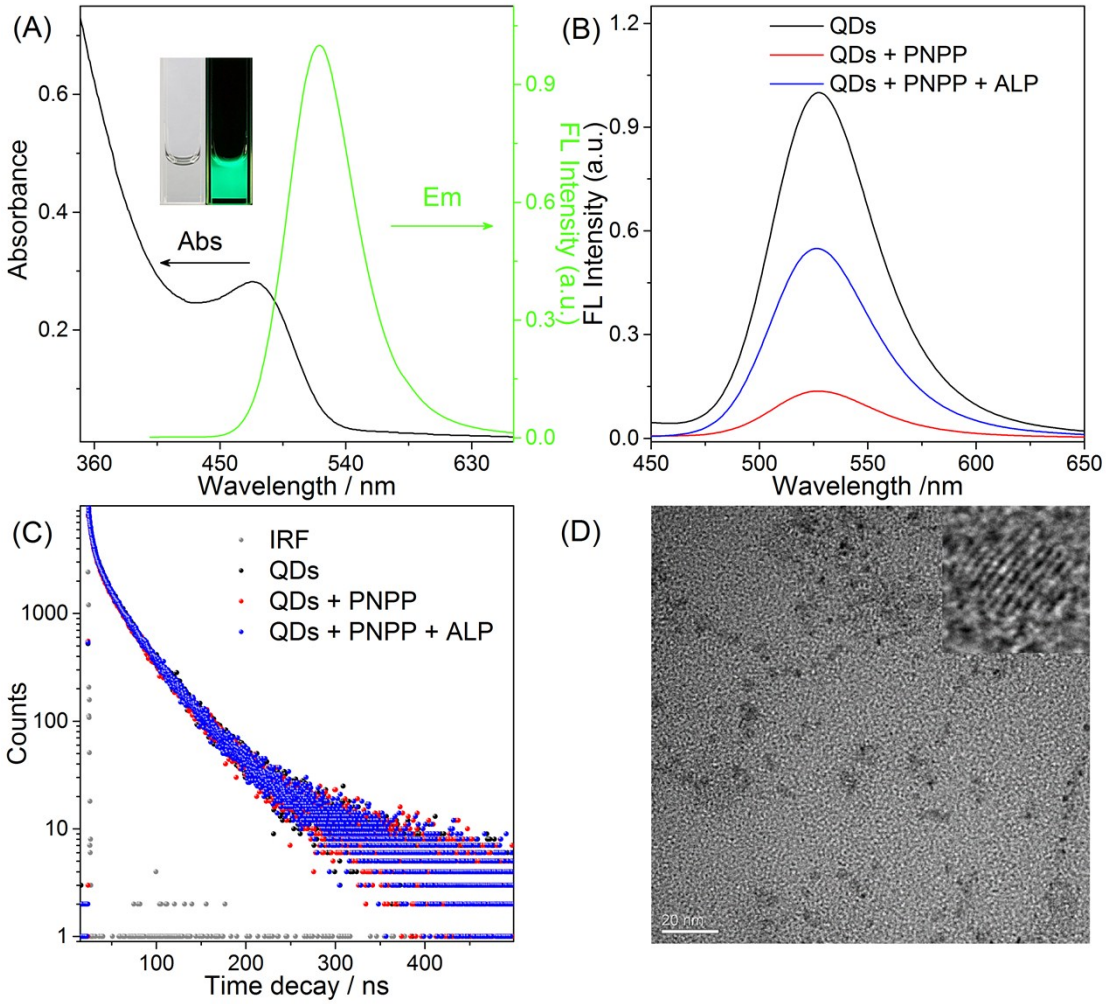

Fig. S3 Characterization of the G-CdTe QDs: (A) UV-Vis absorption spectra and fluorescence emission spectra (the inset are the photographs of G-CdTe QDs in ambient light (left) and under irradiation by UV light (right)); (B) fluorescence intensity and (C) fluorescencent decay curve of G-CdTe QDs in the absence and presence of PNPP (100 μM), and in the presence of PNPP (100 μM) + ALP (10 U/L). (D) TEM image of QDs with a high-magnification TEM image shown in the inset. (excitation source: DeltaDiode 320 nm, time range: 800 ns, the data is fitted with the second order exponential decay).

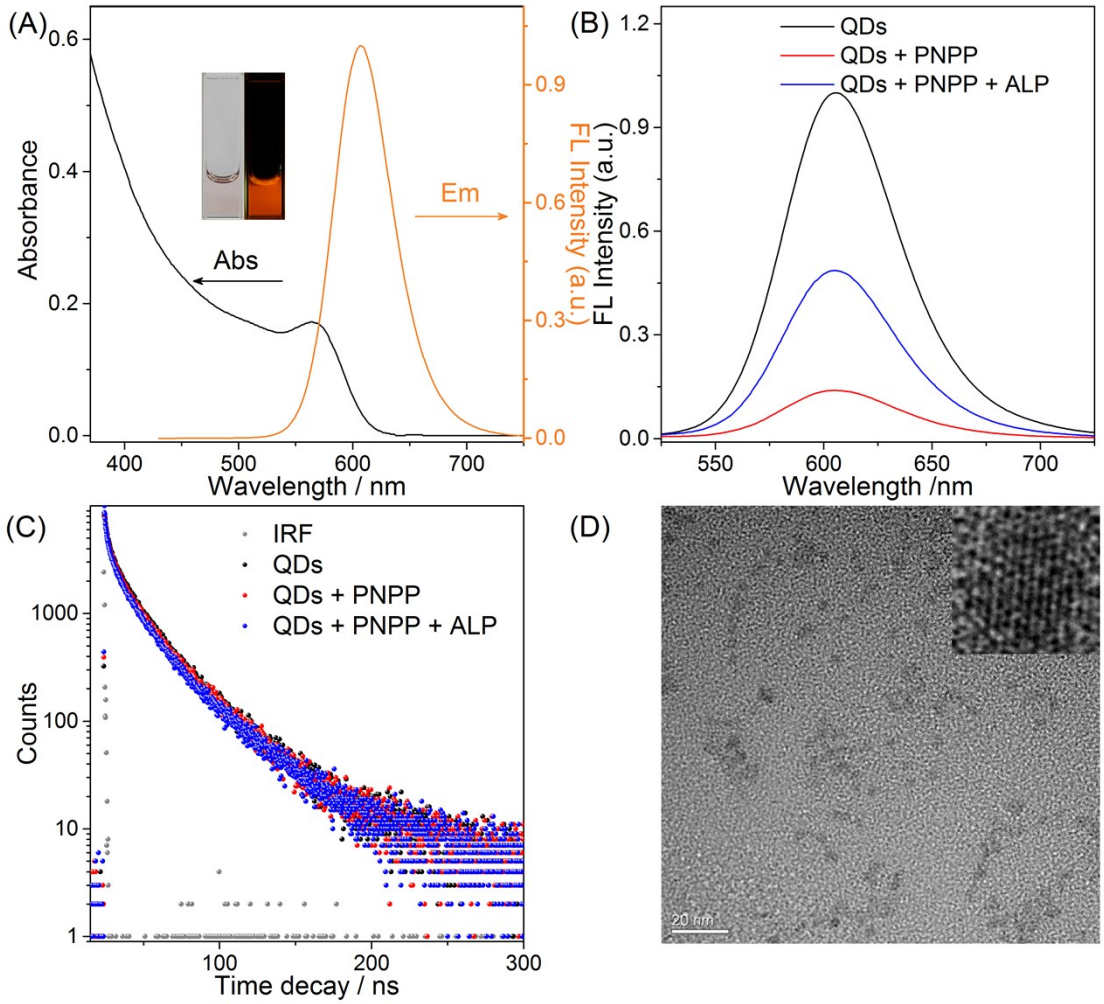

Fig. S4 Characterization of the O-CdTe QDs: (A) UV-Vis absorption spectra and fluorescence emission spectra (the inset are the photographs of O-CdTe QDs in ambient light (left) and under irradiation by UV light (right)); (B) fluorescence intensity and (C) fluorescencent decay curve of O-CdTe QDs in the absence and presence of PNPP (100 μM), and in the presence of PNPP (100 μM) + ALP (10 U/L). (D) TEM image of QDs with a high-magnification TEM image shown in the inset. (excitation source: DeltaDiode 320 nm, time range: 800 ns, the data is fitted with the second order exponential decay.)

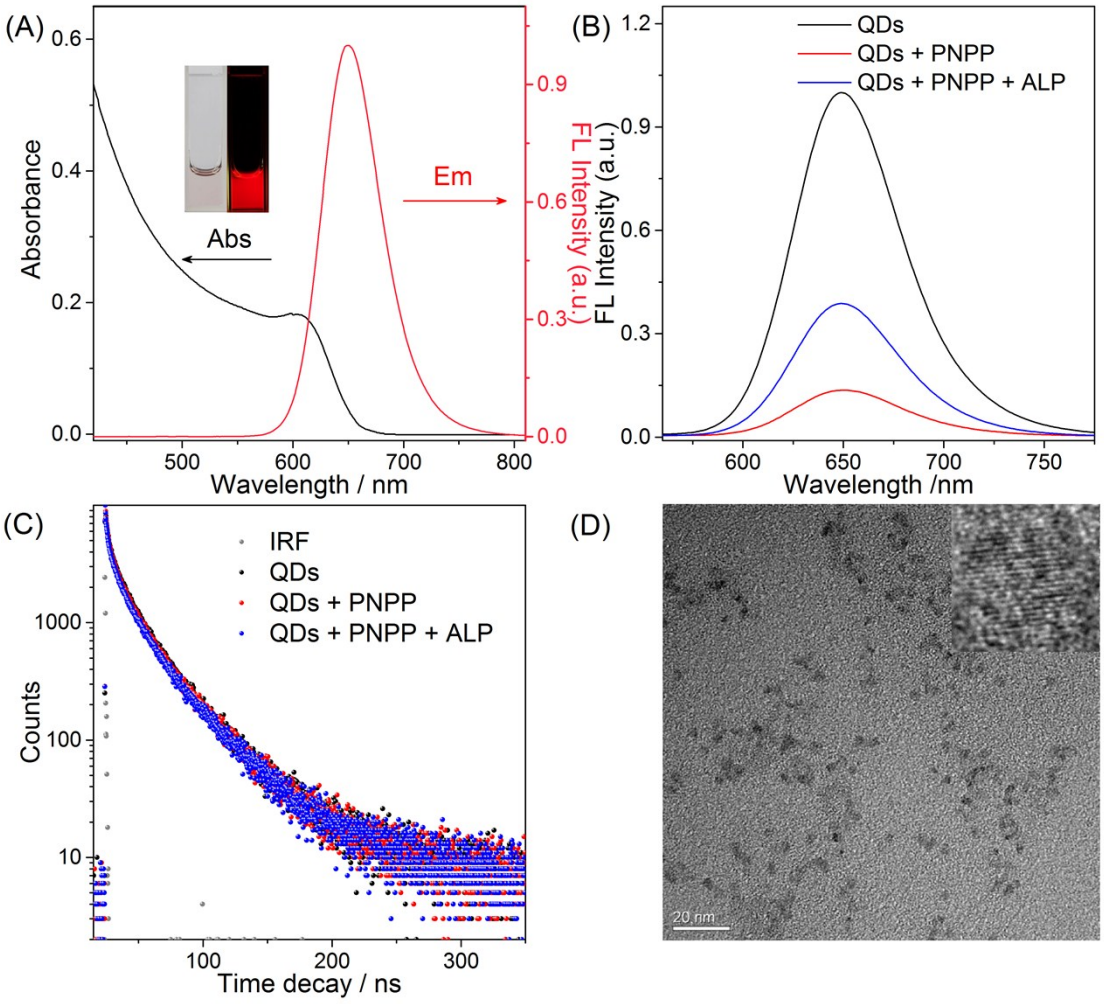

Fig. S5 Characterization of the R-CdTe QDs: (A) UV-Vis absorption spectra and fluorescence emission spectra (the inset are the photographs of R-CdTe QDs in ambient light (left) and under irradiation by UV light (right)); (B) fluorescence intensity and (C) fluorescencent decay curve of R-CdTe QDs in the absence and presence of PNPP (100 μM), and in the presence of PNPP (100 μM) + ALP (10 U/L). (D) TEM image of QDs with a high-magnification TEM image shown in the inset. (excitation source: DeltaDiode 320 nm, time range: 800 ns, the data is fitted with the second order exponential decay).

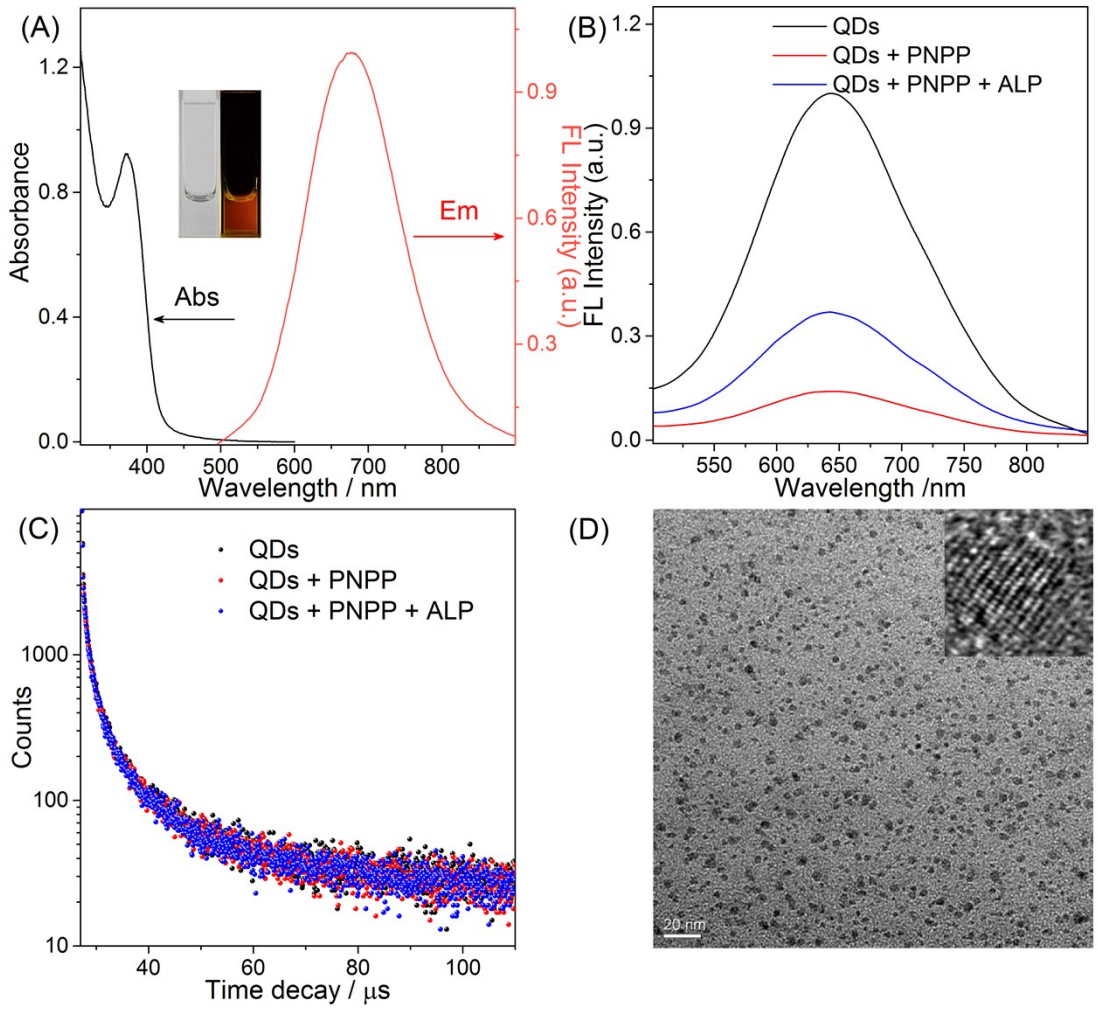

Fig. S6 Characterization of the Cu-CdS QDs: (A) UV-Vis absorption spectra and fluorescence emission spectra (the inset are the photographs of R-CdTe QDs in ambient light (left) and under irradiation by UV light (right)); (B) fluorescence intensity and (C) fluorescencent decay curve of R-CdTe QDs in the absence and presence of PNPP (100 μM), and in the presence of PNPP (100 μM) + ALP (10 U/L). (D) TEM image of QDs with a high-magnification TEM image shown in the inset. (excitation source: SpectraLED 355 nm, time range: 340 μs, the data is fitted with the third order exponential decay).

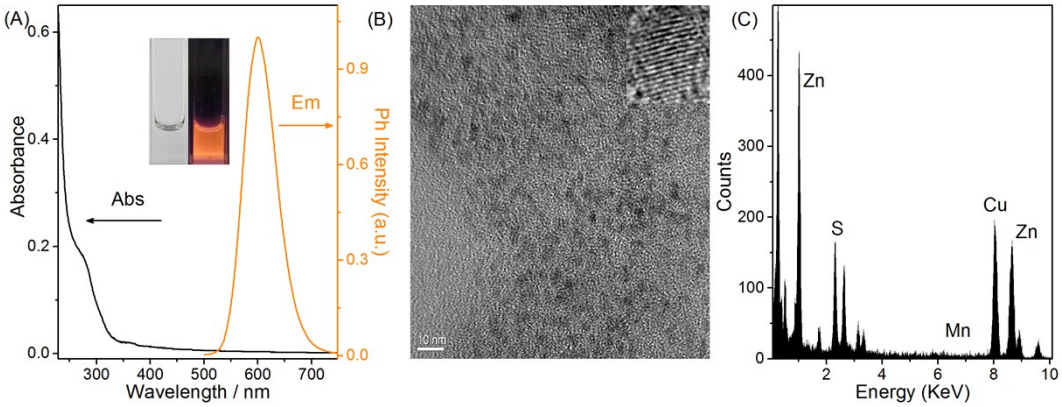

Fig. S7 Characterization of the Mn-ZnS QDs: (A) UV-Vis absorption spectra and fluorescence emission spectra (the inset are the photographs of Mn-ZnS QDs in ambient light (left) and under irradiation by UV light (right)); (B) TEM image of the Mn-ZnS QDs, with a high-magnification TEM image shown in the inset; and (C) the EDX of the Mn-ZnS QDs.

3. Screening of IFE Pairs Based on Tunable Excitation of QDs

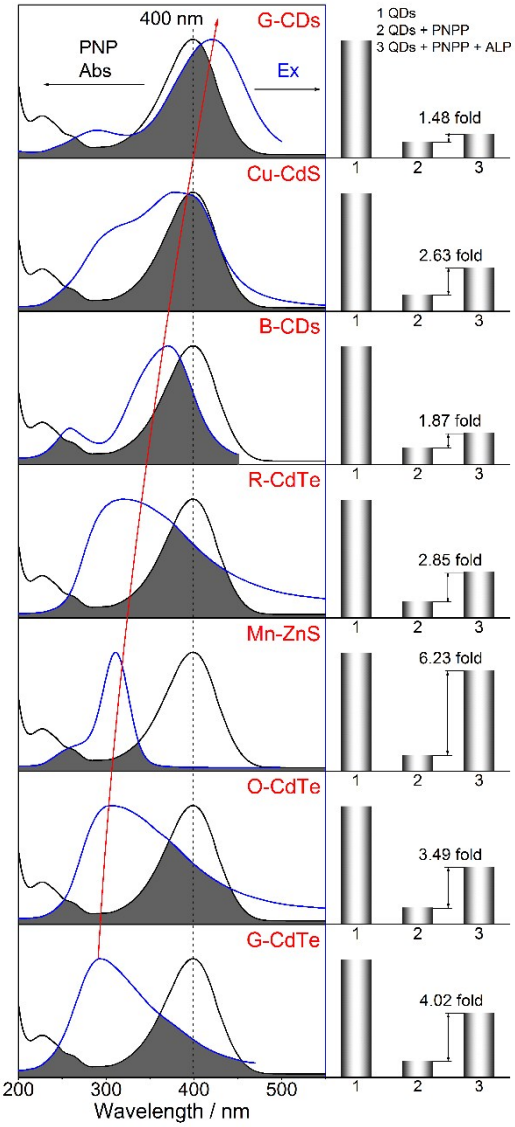

Fig. S8 Screening of the IFE pair based on tunable excitation of various QDs: the left part shows the overlap between the absorption of PNP (black lines) and the excitation of various QDs (blue lines), and the exact overlapped area was shadowed; the right panel shows the corresponding normalized fluorescence intensity of QDs (1), quenched fluorescence intensity in the presence of PNP (2), and the restored fluorescence intensity due to ALP enzymatic reaction (3).

4. Definition of areas ($A_{overlap}$ and $A_{excitation}$) of spectral overlap

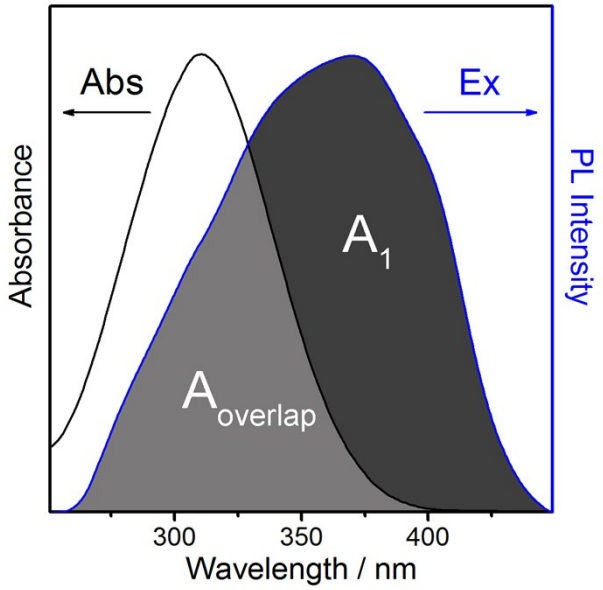

Fig. S9 The spectra overlap of excitation and absorbance. The $A_{overlap}$ stands the part of overlap (gray shadow), the A_1 stands the remaining part of excitation (blank shadow), $A_{overlap}$ plus A_1 is the $A_{excitation}$. Therefore, the $A_{overlap}/A_{excitation}$ is the overlap ratio of total part of the excitation.

5. Optimization of experimental conditions

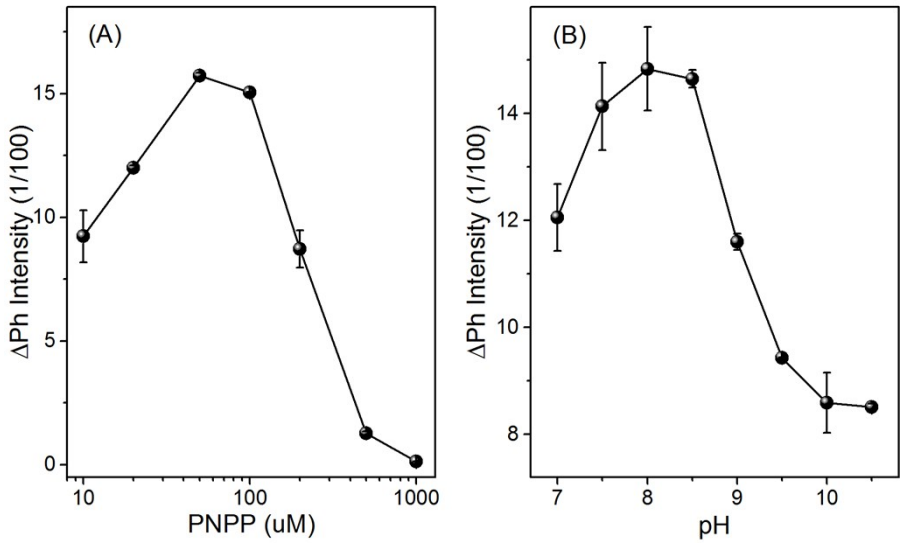

Fig. S10 Optimization of the (A) concentration of PNPP and (B) pH in the conditions of incubation with ALP and Mn-ZnS QDs. The buffer solution is 0.01 M Tris-HCl.

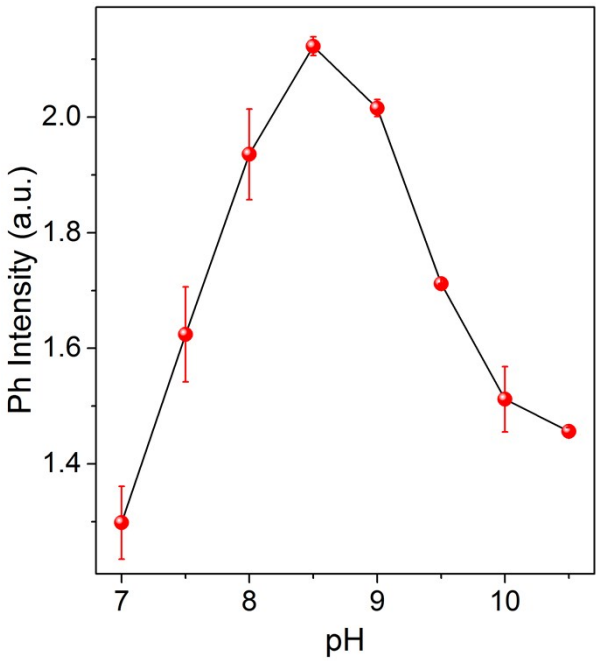

Fig. S11 Phosphorescence intensity of Mn-ZnS QDs at different pH of Tris-HCl buffer.

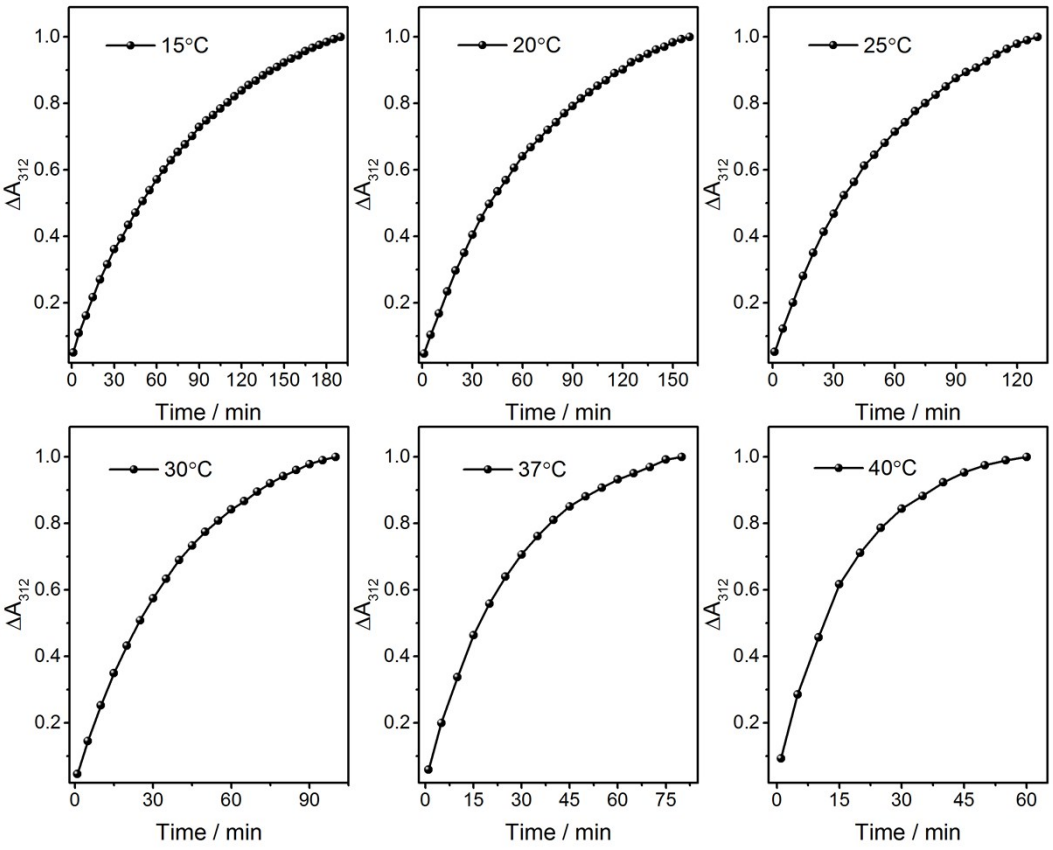

Fig. S12 Optimization of the temperature and time in the conditions of incubation with ALP.

6. Comparison of analytical performance for ALP detection

Table S1 Analytical performance of some assays for ALP detection.

Analytical methods	Principle of sensing	LOD (U/L)	References
Colorimetric assay	cerium oxide-based redox reaction	0.04	7
Fluorescence assay	CDs quenched with Cu^{2+}	1.1	8
Fluorescence assay	CDs quenched with Ce^{3+}	1.4	9
Fluorescence assay	AuNCs quenched with Cu^{2+}	0.1	10
Fluorescence assay	AuNCs-based redox reaction	0.002	11
Fluorescence assay	AuNCs-based IFE	0.002	12
Fluorescence assay	CDs-based IFE	0.001	6
Fluorescence assay	Fluorescence dye based sol−gel transition	60	13
Ratiometric fluorescence assay	Betaine-modified PEI-based Excimer/Monomer Conversion	100	14
Phosphorescence spectroscopy	Mn-ZnS QDs-based IFE	4×10^{-4}	This work

7. Analytical performance of UV-Vis assay for ALP (Escherichia coli) detection

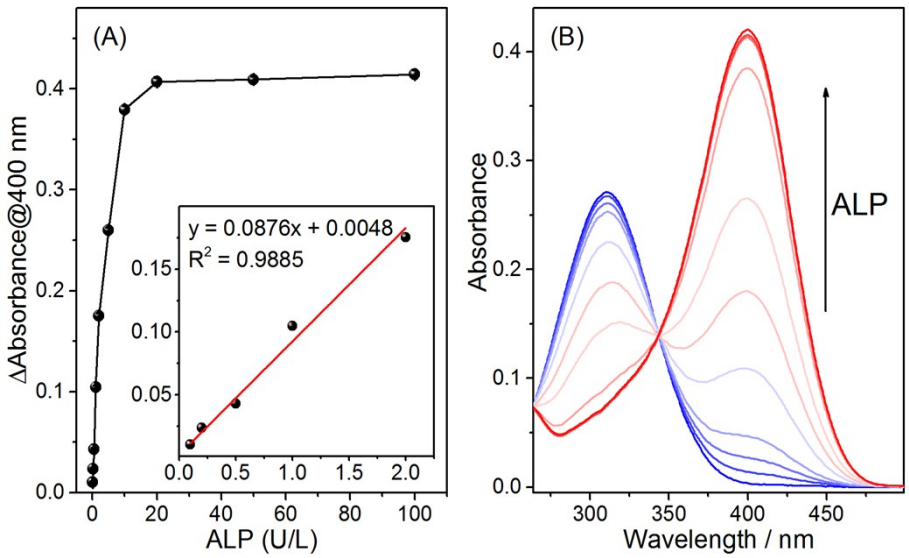

Fig. S13 Analytical performance of the UV-Vis assay for ALP detection: (A) plots of the increased absorbance as a function of ALP concentration; and (B) UV-Vis spectra of PNPP and PNP in the presence of increasing amounts of ALP.

8. Interferences of relevant metal ions and other biomolecules

Table S2 Effect of co-existing substances on the quenched RTP intensity of Mn-doped ZnS QDs by 1 U/L alkaline phosphatase.

Existing substances	Concentration	Changed RTP intensity (%)
Tryptophan	100 μM	0.4
Isoleucine	100 μM	-1.3
Glycine	100 μM	4.4
Lysine	100 μM	5.6
L-cysteine	20 μM	3.0
Valine	100 μM	-2.7
Proline	100 μM	3.5
Alanine	50 μM	8.2
Leucine	100 μM	6.8
Glutamic acid	100 μM	4.0
Asparagine	100 μM	8.1
Arginine	50 μM	5.2
Methionine	100 μM	5.4
Threonine	100 μM	4.4
Tyrosine	50 μM	5.4
Asparaginic acid	100 μM	2.5
Histidine	100 μM	5.3
Ascorbic acid	1 μM	3.5
GSH	1 μM	7.9
Glucose	1 mM	5.7
Ca^{2+}	100 μM	-3.7
Cu^{2+}	200 nM	-1.7
Fe^{3+}	500 nM	-2.3
K^{+}	1 mM	-2.3
Mg^{2+}	50 μM	1.4

Mn^{2+}	500 nM	-3.0
Na^{+}	1 mM	5.6
Zn^{2+}	2 μM	1.9

9. L-Cys capped Mn-ZnS QDs detect ALP based on IFE assay.

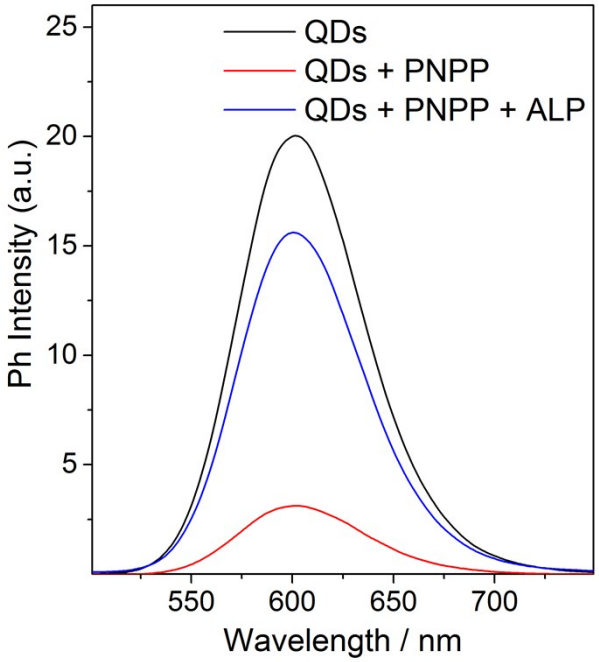

Fig. S14 Phosphorescence intensity of L-Cys-Mn-ZnS QDs in the absence and presence of PNPP (100 μM), and in the presence of PNPP (100 μM) + ALP (10 U/L).

10. Analytical performance of UV-Vis assay for ALP (human) detection

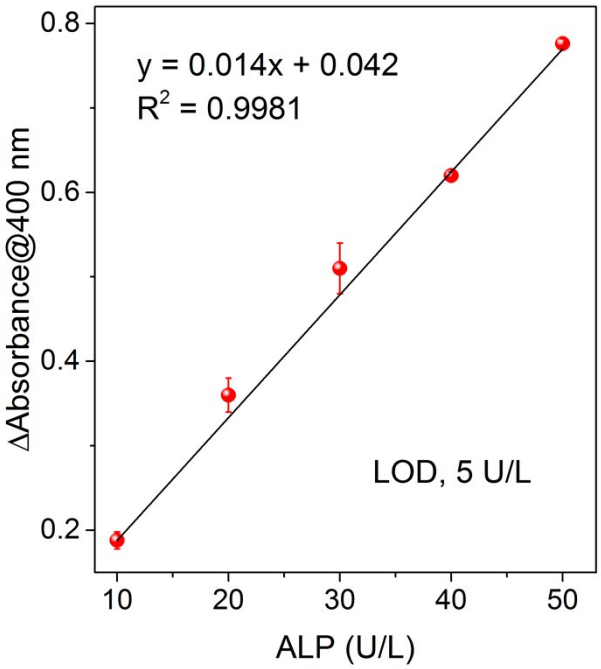

Fig. S15 Analytical performance of the UV-Vis assay for ALP (human) detection with the LOD of 5 U/L.

11. ALP detection in human serum samples

Table S3 Alkaline phosphatase detection in human serum samples by this probe and testing in hospital.

Samples	ALP amount (U/L) by this probe	ALP amount (U/L) test in hospital
Serum 1	78.3 ± 3.5	79
Serum 2	125.9 ± 8.6	130
Serum 3	46.3 ± 3.5	49
Serum 4	97.4 ± 6.9	91
Serum 5	108.1 ± 12.9	105
Serum 6	89.0 ± 9.0	96
Serum 7	354.6 ± 10.0	341
Serum 8	290.9 ± 29.8	292
Serum 9	2591.8 ± 69.3	2599
Serum 10	226.8 ± 6.9	220
Serum 11	90.3 ± 6.9	88

12. Fluorescence, phosphorescence and UV-Vis background of serum samples

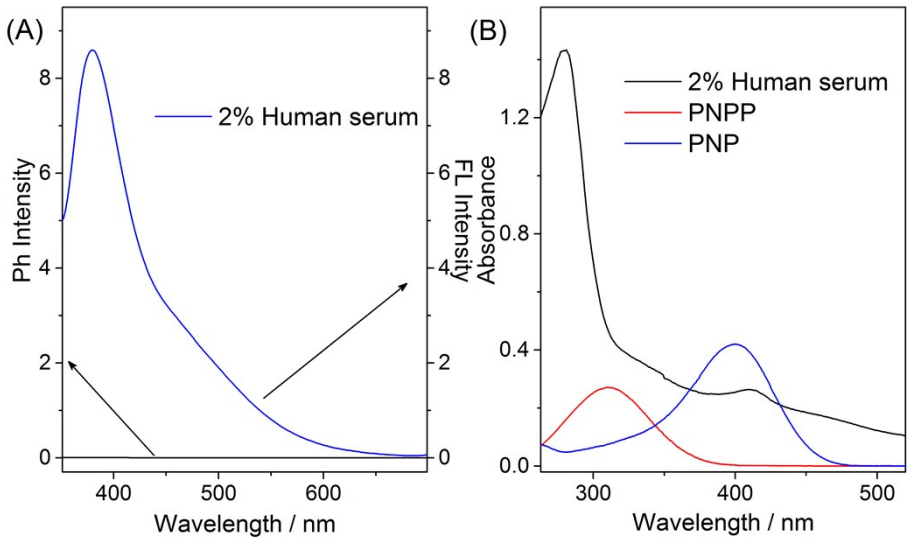

Fig. S16 Fluorescence, phosphorescence and UV-Vis background of serum sample.

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