Electronic Supplementary Material (ESI) for Analytical Methods.

Supplementary Information for

Inner filter effect-based red-shift and fluorescence dual-sensor platforms with sulfur quantum dots for detection and bioimaging of alkaline phosphatase

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1. Additional information on materials

1.1. Chemicals and apparatus

A Talos F200X transmission electron microscope was used to capture transmission electron microscopy (TEM) images of SQDs. The patterned X-ray diffraction (XRD) of SQDs was obtained on an X-ray diffractometer (D8 ADVANCE). Fourier transform infrared (FTIR) spectra using KBr pellets were collected on a Nicolet iS50 FT-IR spectrometer (Thermo Fisher, USA). The fluorescence lifetime of the SQDs was measured with a FLS1000/FS5 fluorescence spectrophotometer (Edinburgh Instruments, England). A fluorescence spectrophotometer (Shimadzu, Japan, F-6000) was used to record emission spectra. The UV-Vis spectra of SQDs were characterized using a UV-1800 scanning spectrophotometer (Shimadzu, Japan). A confocal laser scanning microscope (Olympus Fluoviewer. 1000, Japan) was used to obtain the fluorescence images.

Sublimed sulfur powder, p-Nitrophenyl phosphate disodium salt hexahydrate (p-NPP), p-Nitrophenol (p-NP), alkaline phosphatase (ALP), albumin from bovine serum (BSA) and glucose oxidase (Gox) were obtained from Aladdin (Shanghai, China); trypsin from Macklin Biochemical Co., Ltd. (Shanghai, China); sodium hydroxide, peroxidase (HRP), hydrochloric acid, hydrogen peroxide, and polyethylene glycol 400 (PEG-400) of analytical grade from Sinopharm Chemical Reagent (Shanghai, China); Histidine (His), Arginine (Arg), Glycine (Gly), Lysine (Lys), Proline (Pro), Cystine (Cys), Serine (Ser) and Methionine (Met) were obtained from Regal (Guangzhou, China); Dulbecco's Modified Eagle's medium (DMEM/High glucose) from HyClone (Utah, USA); fetal bovine serum

(FBS) from Gibco (New York, USA). Tris-HCl buffer solutions were used to control the acidity of testing solutions. All the chemical regents were analytically pure and used without further purification. Ultrapure water (18 MΩ cm^{-1}) was used throughout the experiments.

1.2. Synthesis of SQDs

SQDs were synthesized through a slightly modified H_2O_2 assisted top-down method.[1] Briefly, sublimed sulfur powder (1.40 g), PEG-400 (3.0 mL), and sodium hydroxide (4.00 g) were dissolved in ultrapure water (50.0 mL), followed by transferring the mixture into a 250 mL three-necked round bottom flask and stirring at 70 °C for 72 h. After reaction, the solution was cooled naturally to room temperature, followed by adding H_2O_2 (6.75%) below 40 °C and storage in a refrigerator overnight. After removing the insoluble substance by centrifugation at 10000 rpm for 10 min, the pale-yellow supernatant was obtained, followed by further dialysis for 6 h with a 500-Da MWCO dialysis membrane and storing the purified SQDs aqueous solution at 4 ℃ for future analysis.

1.3. Cell culture and cytotoxicity assay of SQDs

MCF-7 cells (breast cancer cells) were cultured in DMEM with 10% FBS and 1% antibiotics at 37 ℃ in a humidified CO_2 incubator. MCF-7 cells were seeded into 96-well plates and cultured until 80-90% confluence, followed by incubation for 24 h with SQDs in the concentration range of (0, 0.25, 0.50, 0.75, 1.0, 1.25, 1.5, and 2.0 mg mL^{-1}), and evaluating the cell viability by the standard (methyl thiazolyl tetrazolium) MTT assay.[2]

1.4. Detection of ALP activity

SQDs solution (200 μL) was placed into a 1.5 mL scale tube containing Tris-HCl buffer solution (20 mM, pH 10.5) and different concentrations of ALP, followed by adding to the tube a certain amount of standard solution of p-NPP (500 μM) (or sample solution), diluting the mixture with ultrapure water to 1.0 mL, shaking the solution thoroughly and incubation for 20 min at 37 ℃. Finally, the fluorescence of each solution was recorded under the excitation of 355 nm. Meanwhile, a control sample was prepared without adding p-NPP standard solution or sample solution.

1.5. Monitor

MCF-7 cells were seeded into confocal dishes at the density of 1×10^5 cells per dish and cultured for 24 h, followed by removing the culture medium from dishes and three washes with cold PBS. Next, the dishes were separated into 5 groups: a control with the culture medium and the remaining 4 groups for incubation with SQDs (1.0 mg mL^{-1}) for 2 h, followed by using cold PBS to wash off the extracellular SQDs and adding different concentrations of p-NPP (0, 1.0, 1.5 and 2.5 mM) to each of the SQDs-treated dishes for 2 h. After three washes with cold PBS, all the dishes were fixed by 4% paraformaldehyde at room temperature for 30 min, followed by using a confocal laser scanning microscope (CLSM) to obtain the fluorescence images at the excitation wavelength of 345 nm.

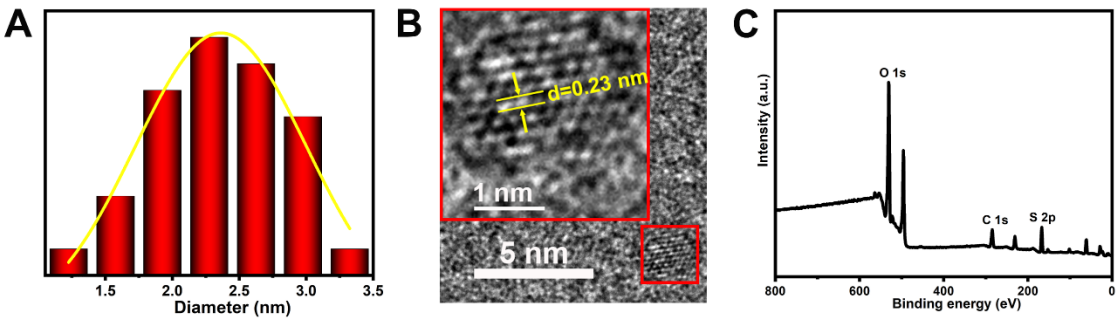

Figure. S1. (A) Size distribution, (B) HRTEM image, and (C) XPS spectra of SQDs.

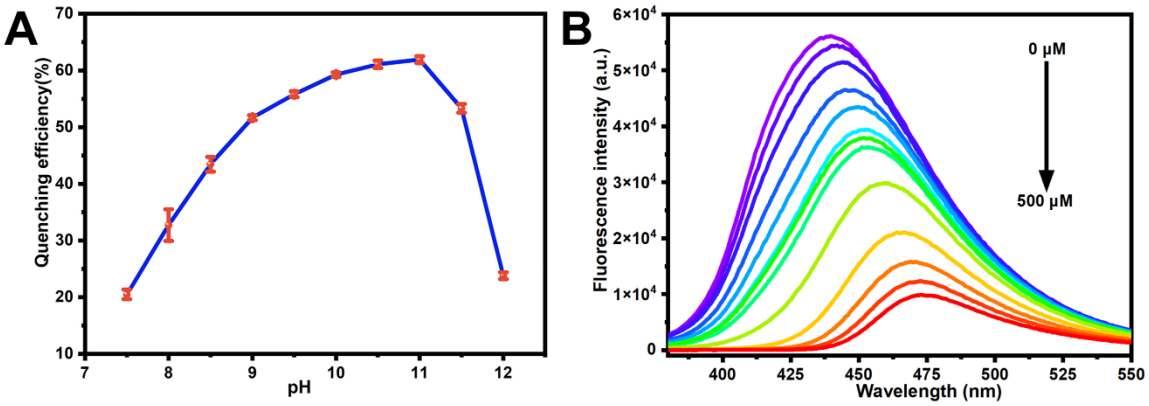

Figure. S2. (A) Quenching efficiency of SQDs supplemented with p-NPP and ALP at different pH values and (B) fluorescence spectra of SQDs supplemented with different concentrations of p-NP.

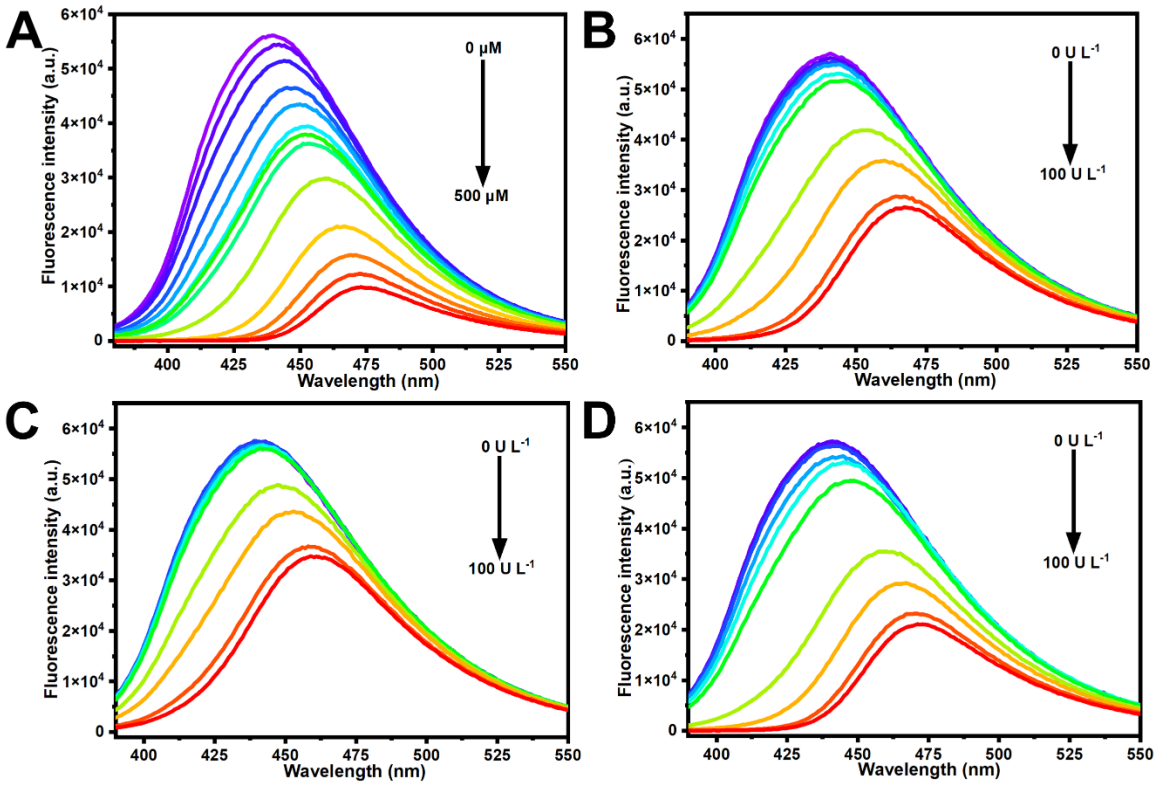

Figure. S3. Fluorescence spectra of SQDs supplemented with p-NPP in the concentration range of 0.25-100 U L^{-1} ALP in (A) Na_2HPO_4- $C_6H_5Na_3O_7$ buffer, (B) (Britton-Robinson) BR buffer, (C) phosphate buffer, and (D) H_3BO_3 buffer.

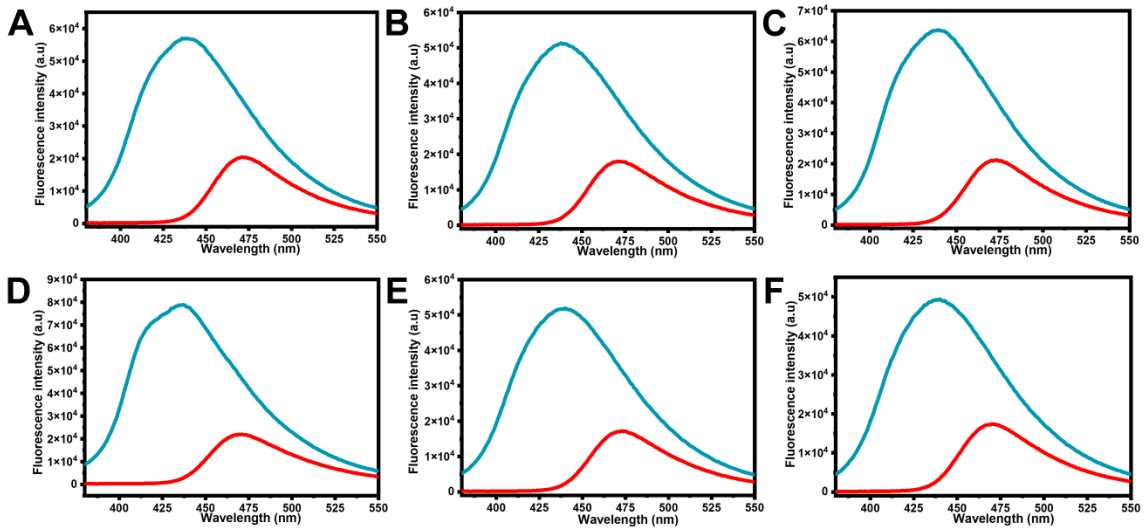

Figure. S4. Fluorescence spectra of SQDs with p-NP (500 μM) in (A) DMF, (B) CH_3OCH_3, (C) DMSO, (D) THF, (E) CH_3CH_2OH, and (F) CH_3CN solvents.

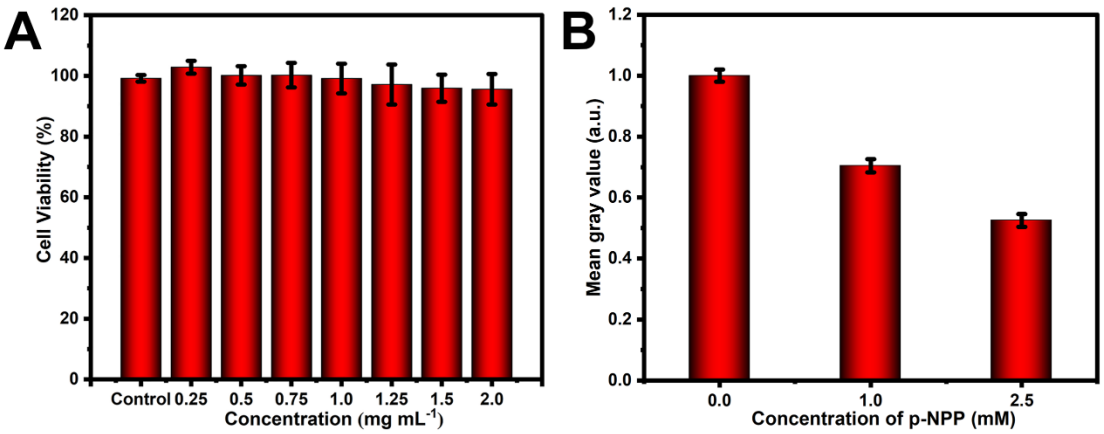

Figure. S5. (A) Cell viability of MCF-7 cells after incubation with different concentrations of SQDs for 24 h, and (B) the fluorescence intensity of real-time monitored cells measured by ImageJ.

Table S1. Comparison of different analytical methods for ALP detection

Probes	LOD (U L^{-1})	Linear range (U L^{-1})	Ref
CdTe/CdS QDs	0.34	2.2-220	[3]
Gold nanoclusters	0.78	0.8-16	[4]
Carbon dots	0.001	0.01-25	[5]
MoS_2 QD_S and AuNCs	0.08	0.5-50	[6]
APN	0.16	0-100	[7]
OPD(S)-AuNPs	0.3	1-20	[8]
β-CD-Mn doped ZnS QDs	0.15	0.5-10	[9]
Polydopamine Nanoparticles	0.34	1-80	[10]
SQDs (RSEW)	0.08	0.25-100	This work
SQDs (fluorescence quenching)	0.10	0.25-100	This work

Table S2. Determination of ALP in 0.2% bovine serum samples by the RSEW method (n = 3, mean ± SD, U L^{-1})

ALP added (U L^{-1})	Measured (U L^{-1})	Recovery (%)
0.50	0.49 ± 0.056	98.0
10.00	9.39 ± 0.031	93.9

Table S3. Determination of ALP in 0.2% bovine serum samples by fluorescence intensity (n = 3, mean ± SD, U L^{-1})

ALP added (U L^{-1})	Measured (U L^{-1})	Recovery (%)
0.50	0.54 ± 0.059	108.0
10.00	10.23 ± 0.013	102.3

Reference

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