Experimental Supplementary Information

Fluorescence Detection of Alkaline Phosphatase Activity with β-Cyclodextrin-Modified Quantum Dots

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Experimental section

Preparation of GSH capped QDs:
The CdTe colloidal solutions were prepared using the reaction between Cd\(^{2+}\) and NaHTe solution following the method described previously in the presence of glutathione (GSH) as the stabilizing agent \(^{[1]}\). The molar ratio of Cd\(^{2+}\): Te\(^{2-}\): GSH was 1: 0.5: 2.4. The pH value of the solution was 10. No fluorescence was observed with the crude solution. Under reflux, the fluorescence of the solution appeared and could be tuned in color by prolonging the refluxing time. The reaction was terminated when the fluorescence color became orange and the fluorescence intensity had reached maximum. The excess of glutathione was removed by two successive precipitation steps, and the purified particles were dissolved in HEPES buffer, 0.01 M, pH 7.4.

Preparation of p-Aminophenylboronic acid capped QDs(APBA/QDs):
To the 1 nmol of the GSH-capped QDs in HEPES buffer, Sulfo-NHS (3 \(\times\)10\(^{-7}\) mol) and EDC.HCl (3 \(\times\)10\(^{-7}\) mol) were added, the mixture was shaken for 15 min. The QDs were purified by precipitation, dissolved in 10 mM HEPES buffer (pH=7.4) to which was added 100 μl of a p-aminophenylboronic acid (APBA) stock solution (1 mg/ml), and the resulting solution was shaken for 1.5 hours. Finally, the excess of APBA was removed by two successive precipitation steps, and the purified particles were dissolved in 200 μL of phosphate buffer, 0.1 M, pH 10.4.

Preparation of β-CD-capped QDs\(^{[2]}\):
To 1 nmol of APBA/QDs was added 1 μmol of β-cyclodextrin (β-CD), dissolved in 100 μL phosphate buffer, and the mixture was shaken for 12 hours. The excess of β-cyclodextrin was removed by repeated centrifugation (12 000 rpm, 10 min) with DMSO/CH\(_3\)CN (v/v=1:1). Then the modified quantum dots were dissolved in triple-distilled water, and precipitated again with DMSO/CH\(_3\)CN (v/v=1:1). The resulting QDs were dried overnight under vacuum for further experiments.

ALP activity assay
A typical procedure was carried out as follows: First, β-CD/QDs and p-nitrophenyl-phosphate (60 μM) in 2 mL Tris-HCl buffer solution (0.1 M, pH 9.5, containing 1 mM ZnCl\(_2\), 1 mM MgCl\(_2\)) was placed in the cuvette and allowed to
thermally equilibrate at 25 °C. And then different concentrations of ALP were added into the cuvettes, respectively. The enzyme assays were carried out in Tris-HCl buffer at 25 °C. For the real-time assays, the fluorescence spectra were recorded at 1 min intervals over 30 min with excitation at 400 nm. The plots of the fluorescence intensity at 580 nm versus the ALP incubating time were obtained.

**Instruments**

UV/vis spectra were carried out with a UV/vis Shimadzu UV-2505 spectrometer. Fluorescence measurements were carried out on a LS-55 Luminescence Spectrometer (PerkinElmer).

![Fig. S1](image1.png)

**Fig. S1** The FT-IR spectra of β-CD, GSH/QDs and β-CD/QDs

![Fig. S2](image2.png)

**Fig. S2** (a) UV/vis absorption spectra and (b) Fluorescence emission spectra of GSH/QDs and β-CD/QDs in Tris buffer.
**Fig. S3** Fluorescence emission spectra of (a) GSH/QDs (b) APBA/QDs (c) β-CD/QDs with 60 μM NP, respectively. (d) The luminescence quenching at 580nm of the β-CD/QDs and APBA/QDs upon the addition of different concentration of NP.

**Fig. S4** Fluorescence emission spectra of β-CD/QDs with 60 μM p-nitro-benzaldehyde, phenol, NPP and NP, respectively.
Fig. S5 Fluorescence emission spectra of β-CD/QDs with ALP (800 mU/mL) only and inhibitor (25 μM) only, respectively.

References for supporting information: