Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information

Pyrophosphate ion-responsive alginate hydrogel as an effective fluorescent sensing platform for alkaline phosphatase detection

Experimental section

Chemicals

Alginate and cetylpyridinium chloride (CPC) were purchased from Aladdin (Shanghai, China). Pyrophosphate ion (PPi), amino acids and metal salts were obtained from Sinopharm Chemical Reagent Company (Shanghai, China). All of proteins including alkaline phosphatase (ALP), glucose oxidase (GOx), cholesterol oxidase (ChOx), restriction enzyme EcoR I, and human serum albumin (HSA) were purchased from Sangon Biotech (Shanghai, China).

Synthesis of CDs

The CDs were synthesized by an ultrasonic method according to previous report [1]. Briefly, 9 mL of NaOH (2 M) was added to 100 mL of CPC aqueous solution and mixed well. Then, the mixture was treated by ultrasonic for 30 min at room temperature. After that, the reaction was terminated by adjusting pH of the solution to neutral (pH = 7.0) with HCl, and followed by dialysis to remove unreacted reagents. Finally, the purified products were dried at 65 °C and redispersed in ultrapure water for further use. The excitation and emission spectra of the CDs were recorded by using LS55 fluorescence spectrometer (PerkinElmer, UK).

Preparation of Alg/Cu gel and CDs@Alg/Cu

The Alg/Cu gel was prepared by simple mixing 200 μL of alginate aqueous solution (wt%, 1) and 200 μL of $CuCl_2$ aqueous solution. The gelation assay was carried out for 1 h at room temperature to form insoluble hydrogel. After that, the hydrogel was washed with ultrapure water for three time to remove unreacted reagent.

To prepare CDs@Alg/Cu, 200 μL of CDs was firstly mixed with 200 μL of alginate aqueous solution (wt%, 2) for 30 min. Then, 400 μL of $CuCl_2$ aqueous solution was added to trigger the gelation of alginate. After reacting for 1 h, the insoluble products (CDs@Alg/Cu) were collected by centrifugation (300 rpm, 3 min), and followed by washing with ultrapure water for three time to remove unreacted reagent.

PPi-stimulated sol-gel transition of CDs@Alg/Cu

Typically, 500 μL of PPi aqueous solution with different concentration (0 - 3 mM) was added to the test tube containing as-prepared CDs@Alg/Cu and mixed well. The reaction took place at room temperature with gentle shaking at 150 rpm for 2 h. The volume and fluorescent color of the sol solution was recorded by camera. To determine PPi concentration, diluted sol solution was prepared by adding 100 μL of ultrapure water to 100 μL of original sol solution. Then, the fluorescence of the diluted sol solution was measured by using a fluorescence spectrometer. The emission intensity at 513 nm was used for quantitative analysis of PPi. Same procedures were used for the selective experiments over other anions. All error bars represent standard deviations from three repeated experiments (n = 3).

The detection of ALP

The procedures for the detection of ALP as follows: 50 μL of PPi (mM) was firstly added to 450 μL of HEPES buffer (10 mM, pH 8.5) containing ALP final concentrations from 0 to 400 mU/mL and mixed well. Then, the mixture was incubated at 37 ℃ for 30 min. After that, the reaction solution was added to the test tube containing CDs@Alg/Cu for reacting another 2 h at room temperature under gentle shaking (150 rpm). Finally, the sol solution was diluted by adding equivalent ultrapure water, and followed by recoding the fluorescence of the diluted sol solution. With same procedures, selective experiment was carried out by replacing ALP with GOx, ChOx, EcoR I, and HSA. In addition, for detecting ALP level in real sample, 10-dilueted human serum was employed to prepare spiked serum samples with concentration ranging from 0 to 40 mU/mL. The detection procedures of ALP in the spiked serum sample are same as that of sensitivity experiment.

Supplementary Figures and Tables

Figure S1. Effects of Cu^{2+} with different concentrations on the gelation of alginate.

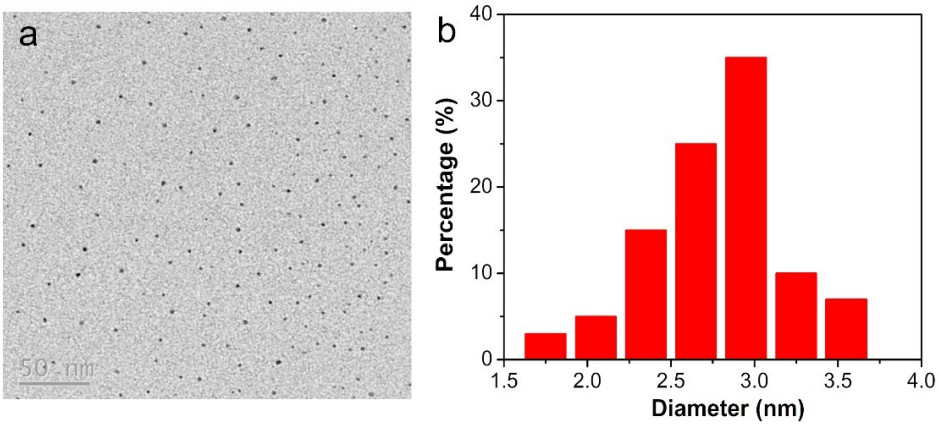

Figure S2. TEM image (a) and size distribution (b) of CDs.

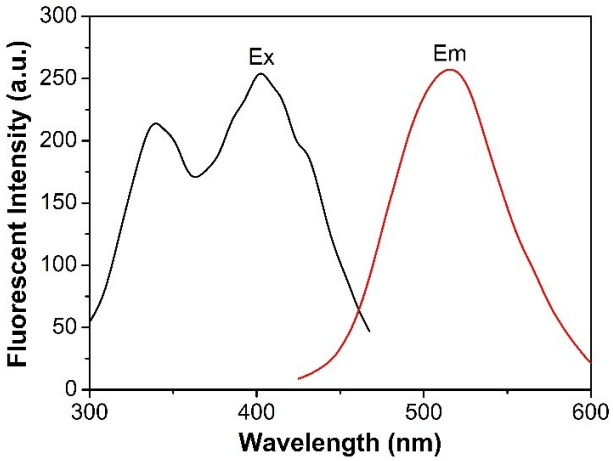

Figure S3. Excitation (black line) and emission (red line) spectra of CDs.

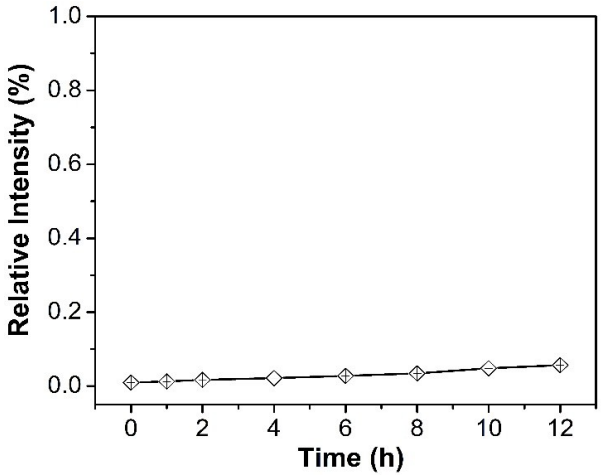

Figure S4. Relative fluorescent intensity of the supernatant of CDs@Cu/Alg after storing for different time at room temperature.

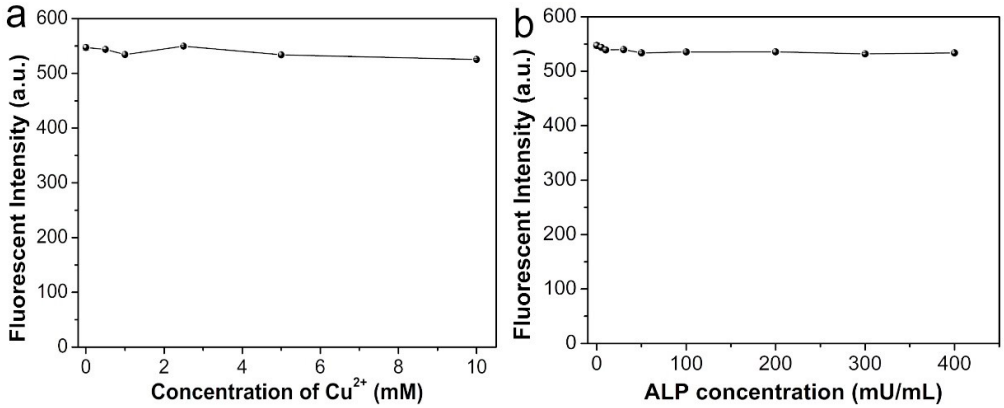

Figure S5. Effects of different concentrations of Cu^{2+} (a) and PPi (b) on the fluorescence of free CDs.

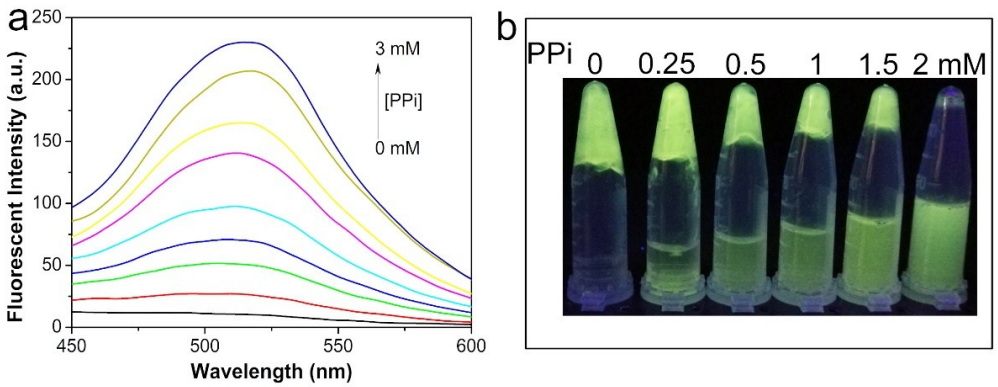

Figure S6. (a) Emission spectra of the sol solutions of CDs@Cu/Alg after adding PPi with different concentrations. (b) Visual dissolution CDs@Cu/Alg in the presence of different concentrations of PPi.

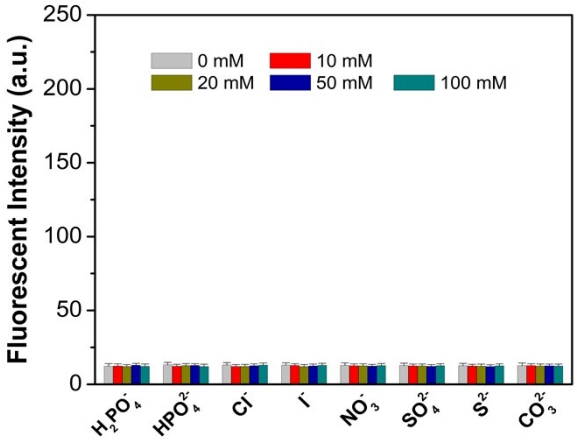

Figure S7. Fluorescent responses of CDs@Cu/Alg to various anions with different concentrations.

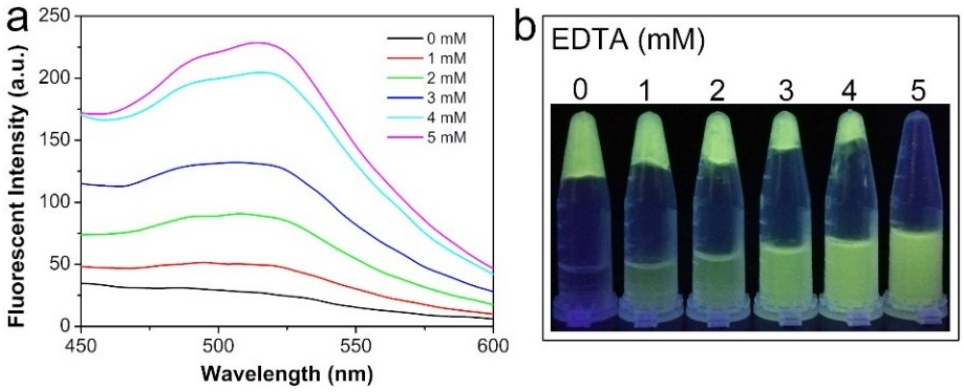

Figure S8. Emission spectra (a) and photography (b) of the sol solutions of CDs@Cu/Alg after adding EDTA with different concentrations.

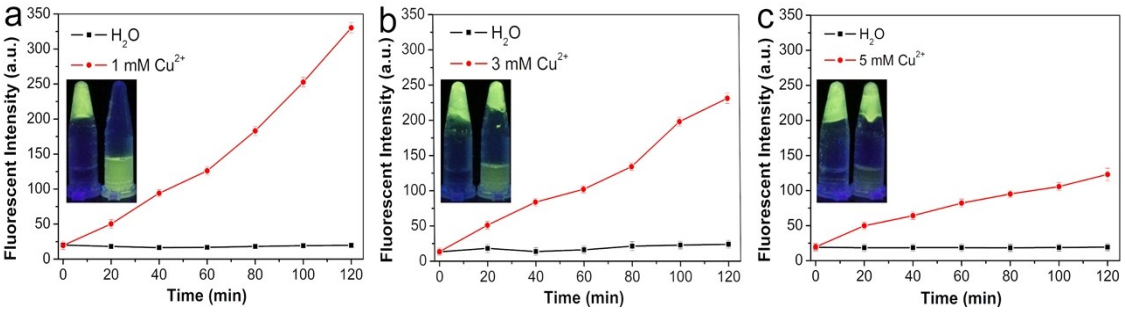

Figure S9. Dissolution rates of CDs@Cu/Alg formed with different concentrations of Cu^{2+} (1, 3, 5 mM) in the presence of 2 mM PPi.

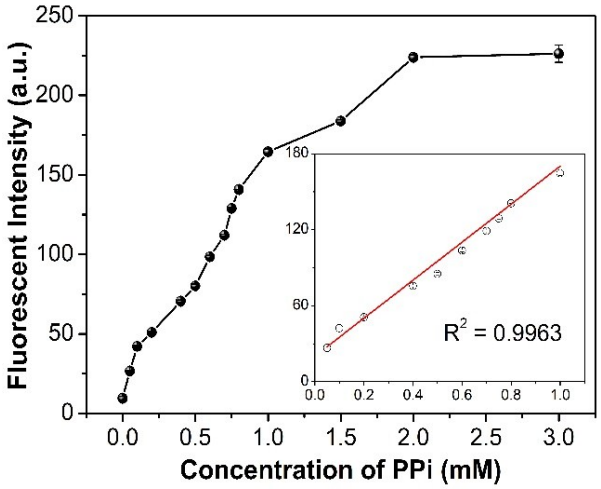

Figure S10. Fluorescent intensity at 513 nm of the sol solution of CDs@Cu/Alg after adding different concentrations of PPi. Inset is the linear curve of fluorescent intensity at 513 nm versus PPi concentrations.

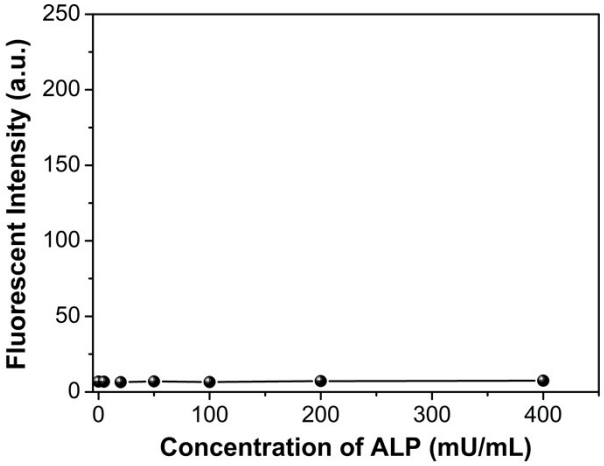

Figure S11. Fluorescent intensity at 513 nm of the sol solution of CDs@Cu/Alg after adding ALP with different concentrations.

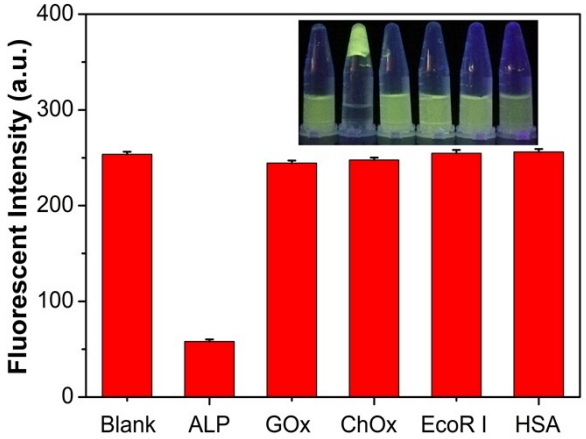

Figure S12. Selectivity of the PPi-responsive CDs@Cu/Alg based sensing system to ALP.

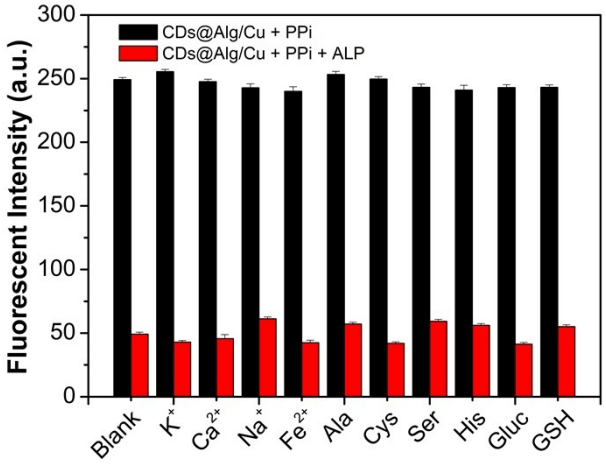

Figure S13. The effects of metal ions and biological species that are commonly found in serum sample on the PPi-stimulated gel dissolution assay and its corresponding ALP hydrolysis assay.

Table S1. Comparison of various fluorescent sensors using PPi as substrate for ALP detection

Sensors	**Linear range (mU/mL)**	**Detection limit (mU/mL)**	**Refs**
Upconversion nanoparticles	62.5 - 875	19	2
Conjugated polyelectrolyte polymer	0 - 450	1.5	3
dsDNA-templated CuNPs	0.3 - 7.5	0.3	4
Carbon quantum dots	16.7 - 782.6	1.1	5
Carbon dots-Cu(II)	2.5 - 40	1	6
CDs@Cu/Alg	1 - 100	0.55	This work

Table S2. Determination of the level of ALP in serum samples

Sample	Founded (mU/mL)	Added (mU/mL)	Detected (mU/mL)	Recovery (%)	RSD (n = 5, %)	pNPP assay
1	2.87	5	7.94	101.20	1.95	8.28
2	2.87	10	12.53	96.60	1.67	12.50
4	2.87	20	24.34	107.35	1.42	24.18

References

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