Electronic supplementary information for

Chemical redox modulated switch-on fluorescence of carbon dots for probing alkaline phosphatase and its application in immunoassay

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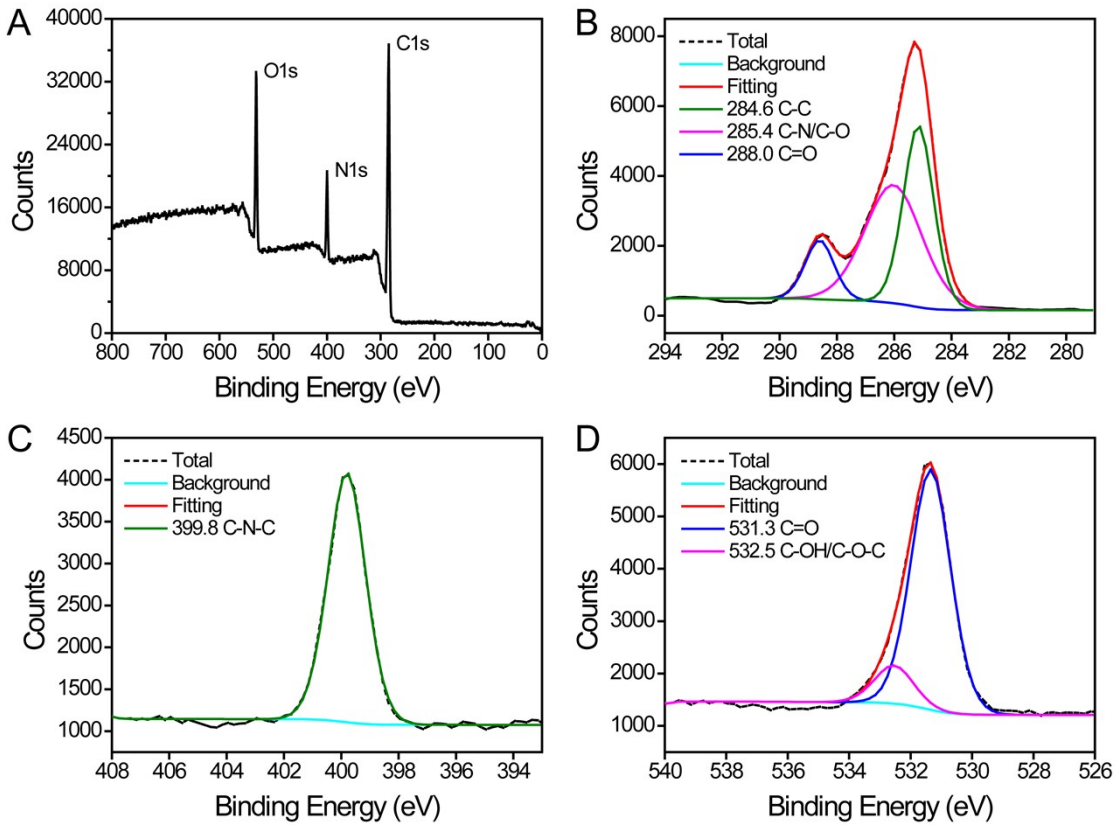

Fig. S1 Survey (A), high-resolution C 1s (B), N 1s (C), and O 1s (D) XPS spectra of CDs.

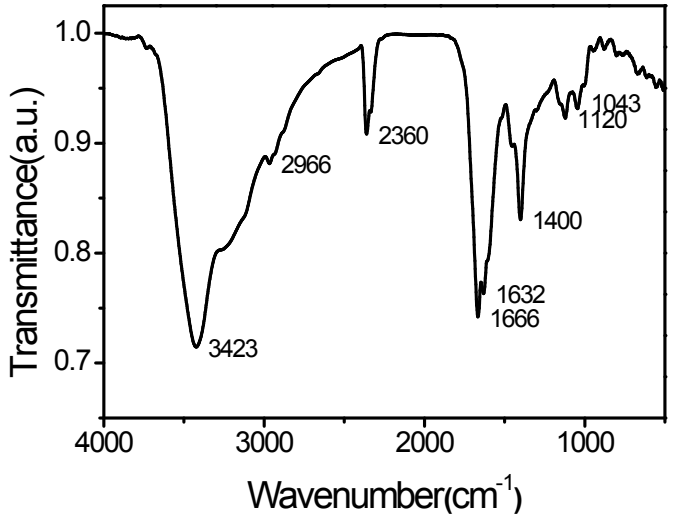

Fig. S2 FT-IR spectrum of the as-prepared CDs.

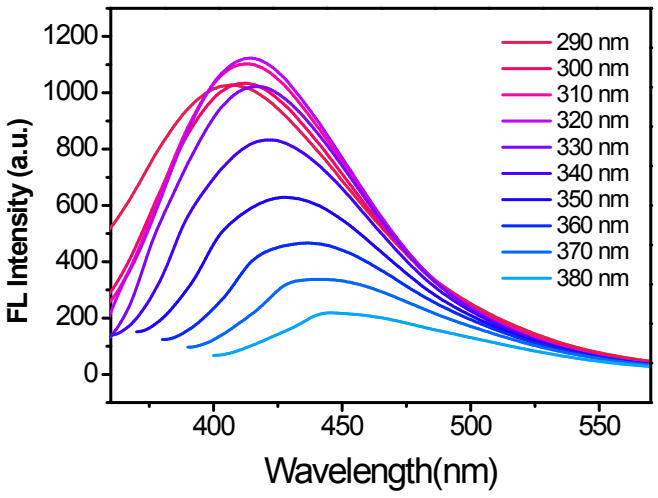

Fig. S3 Fluorescent emission spectra of the CDs at different excitation wavelengths ranging from 290 nm to 380nm with increments of 10 nm.

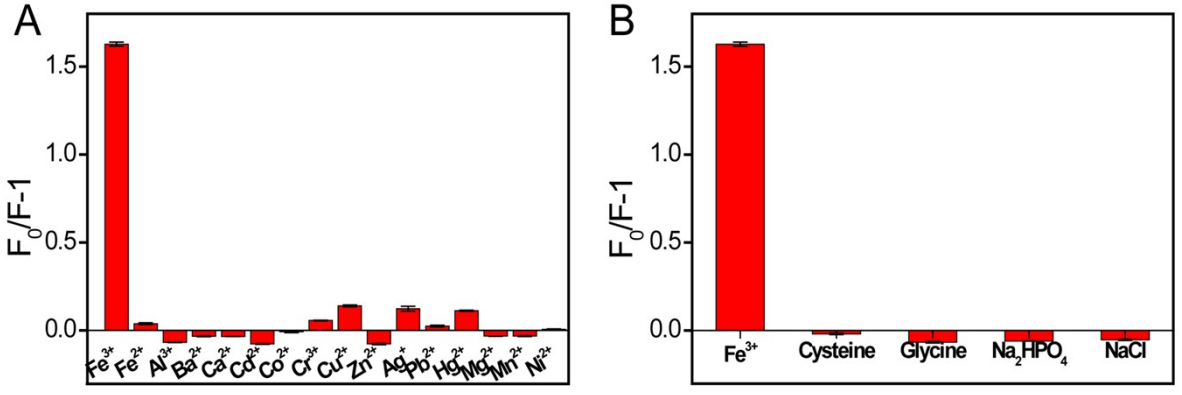

Fig. S4 Fluorescence response of CDs in the presence of different metal ions (A) and small compounds (B). (The concentration of metal ions and compounds were 100 μM)

Table S1 Fluorescence lifetimes obtained with three-exponential fit of the fluorescence decay curves of the CDs alone, CDs/Fe(III), and CDs/Fe(III)/AA, respectively.

Samples	Lifetimes (s)			B1	B2	B3	τ_{avg}(s)
	τ_1	τ_2	τ_3				
CD	9.49E-10	4.65E-09	1.27E-08	28.63	42.48	28.90	9.48E-09
CD/Fe(III)	1.09E-09	4.86E-09	1.24E-08	21.37	42.39	36.24	9.76E-09
CD/Fe(III)/AA	1.26E-09	5.02E-09	1.29E-08	26.67	43.12	30.20	9.62E-09

Note: The fluorescence lifetimes of the CDs under different conditions were measured. The fluorescence decays could be fit to the three-exponential Equation (1):

$$f(\tau)= A+B_1e\text{^}(\textbf{Error!})+B_2e\text{^}(\textbf{Error!}) +B_3e\text{^}(\textbf{Error!})$$

Where τ_1, τ_2, τ_3 are the three components of the lifetimes, A is the background offset (a fitting parameter), and B1, B2, B3 are pre-exponential functions which are related to the emission intensity. The average lifetime τ_{avg} can be calibrated according to Equation (2):

$$\tau_{avg} = \textbf{Error!}$$

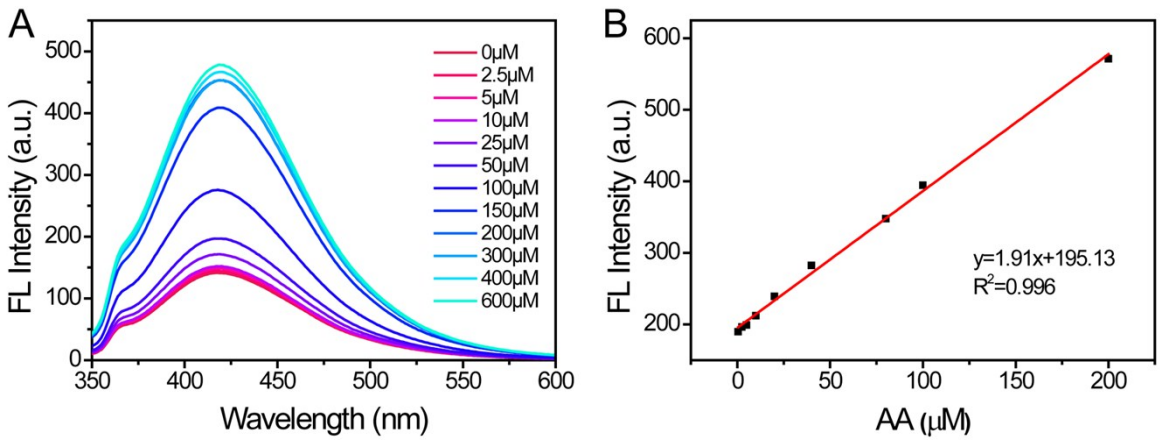

Fig. S5 (A) Fluorescence spectra of CDs in the presence of 300 μM Fe(III) and different concentration of AA from 0 to 600 μM. (B) Relationship between fluorescence intensity and the concentration of AA.

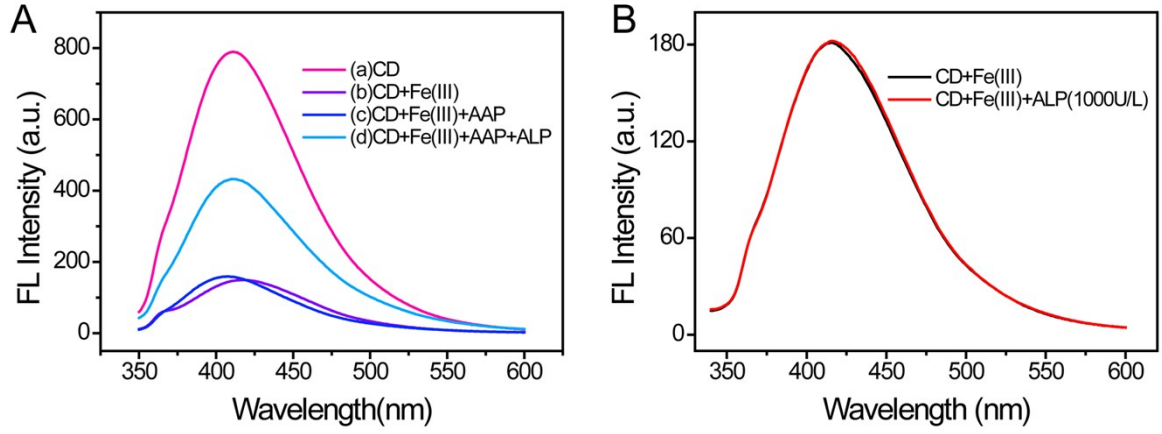

Fig. S6 (A) The changes of fluorescence spectra of CDs in the presence of different composition: (a) mere CDs ; (b) CDs + Fe(III); (c) CDs + Fe(III) + AAP; (d) CDs + Fe(III) + AAP + ALP. All of the CDs concentrations were 1 μL/mL; Fe(III) concentrations were 300 μM in (a), (b) and (c); AAP concentrations were 50 mM in (c) and (d); ALP concentration was 1000 U/L in (d). Excitation: 320 nm and emission: 412 nm. (B) The change of fluorescence spectra of CDs in the presence of Fe(III) and ALP.

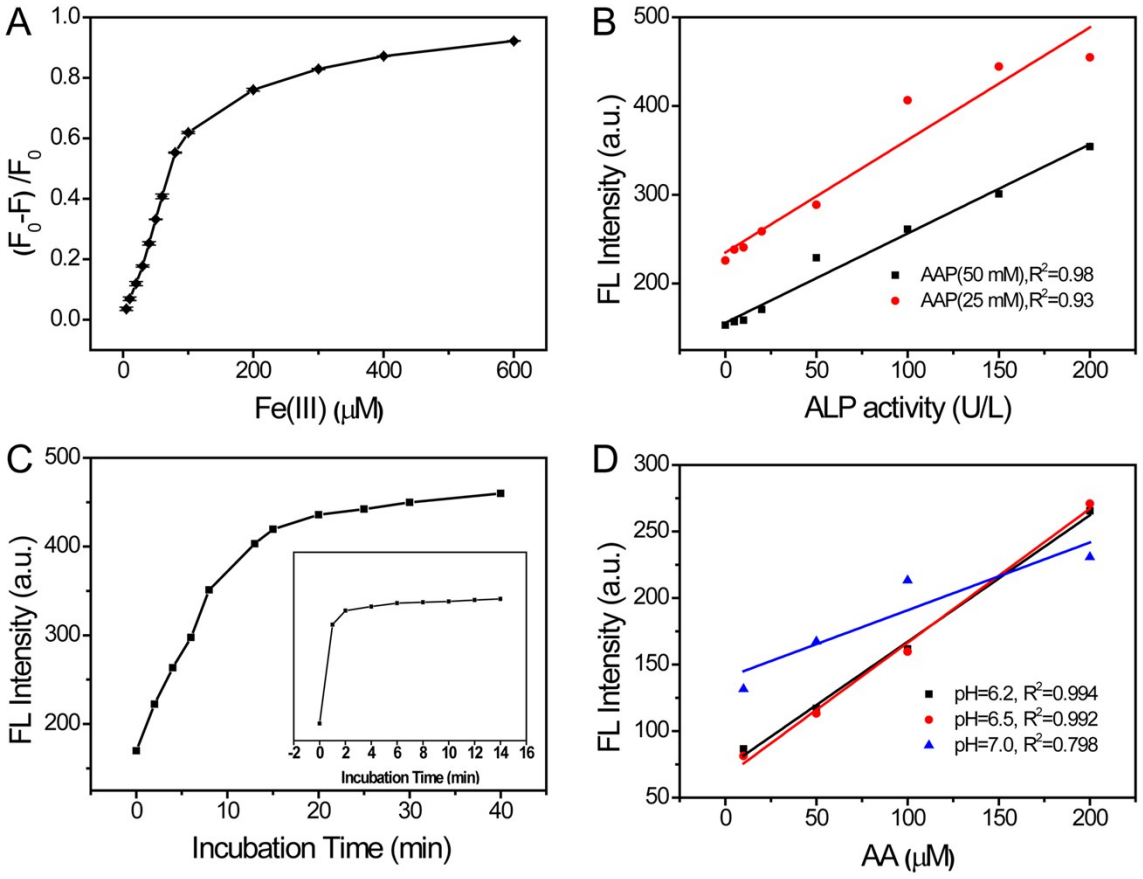

Fig. S7 Optimization of the experimental conditions for sensing ALP. (A) $(F_0\text{-}F)/F_0$ versus concentrations of Fe(III) from 0 to 600 μM. (B) The fitting curve between fluorescence intensity of the system and ALP level. Fe(III) of 300 μM and 50 mM AAP (black curve) or 25 mM AAP (red curve) were present. (C) Incubation time of the mixture containing CDs, Fe(III) (300 μM), AAP (50 mM) and ALP (1000 U/L). Inset: The variation of relative FL intensity of mixture containing CDs, Fe(III) (300 μM) and AA (200 μM) at different time interval. (D) The fitting curve between fluorescence intensity of the system and AA level from 5 to 200 μM at different pH (Fe(III): 300 μM).

Table S2 Comparison of other CDs-based sensors for the determination of ALP.

Composition of CDs-based sensors	Linear range (U/L)	LOD (U/L)	Reference
β-CD-CDs + p-Nitrophenol phosphate disodium	3.4–100	0.9	(3)
CDs + Cu^{2+} + PPi	16.7–782.6	1.1	(4)
CDs + Ce^{3+} + ATP	4.6–383.3	1.4	(5)
CDs + Fe^{3+} + AAP	5–200	0.8	This work

Table S3 Determination of ALP in human serum samples.

Added (U/L)	Found (U/L)	Recovery (%, n=3)	RSD (%, n=3)
30	28.0	93.3	6.3
60	65.6	109.3	5.0
100	106.4	106.4	6.0

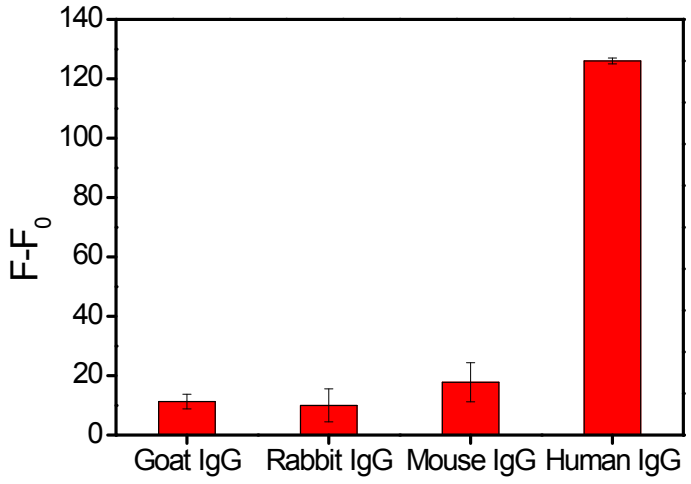

Fig. S8 Selectivity of the fluorescence immunoassay to human IgG (500 ng/mL) compared with other interfering proteins at the concentration of 500 ng/mL.

References

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