

Supporting Information for

**Multi-doped carbon dots with ratiometric pH sensing property for
monitoring enzyme catalytic reactions**

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Experimental Section

Materials. 3-Aminophenylboronic acid hydrochloride (3-APBA), 4-carboxyphenylboronic acid, glucose oxidase (GOx), acetylcholinesterase (AChE), urease and acetylthiocholine (ATCh) were purchased from Sigma-Aldrich China (Shanghai, China). 4-Mercaptophenylboronic acid, 3-carboxyphenylboronic acid and phenylboronic acid were obtained from J&K Chemicals (Beijing, China). Ethanol, acetic acid, sodium acetate, boric acid, phosphoric acid, glucose, urea, NaOH and phenylamine were obtained from Sinopharm Group Chemical Reagent Co., Ltd. (Shanghai, China). All the chemicals were used as received without further purification. MilliQ water (18.2 M Ω ·cm) was used throughout the experiment.

Instruments and Measurements. Absorption spectra were measured with a Cary 60 UV–vis spectrophotometer (Agilent Technologies, Palo Alto, CA, USA). Fluorescence spectra were measured with a Cary Eclipse spectrofluorophotometer (Agilent Technologies, Palo Alto, CA, USA) at room temperature. Fourier transform infrared (FTIR) spectra were measured with a Thermo Nicolet 380 FTIR spectrometer (Thermo Nicolet Co., USA). X-ray photoelectron spectroscopy (XPS) measurements were performed on ESCALab 220-Xi (VG, UK). Raman spectra were recorded with a Renishaw InVia micro-Raman (Renishaw, UK) with excitation wavelength of 532 nm. Transmission electron spectroscopy (TEM) images were collected with JEM-2100F microscopy (JEOL Ltd., Tokyo, Japan).

Synthesis of the multi-doped C-dots. Typically, 0.1 g of 3-aminophenylboronic acid was dissolved in 50 mL of ethanol to form a clear solution. Then the solution was transferred to the Teflon-lined autoclave chamber and heat to 180 °C for 12 h. After cooling to room temperature, the C-dots-containing solution was centrifuged at 10 000 rpm for 10 min to remove large aggregates. The resulting C-dots in the supernatant were then purified by using silica column chromatography with a mixture of methanol and methylene chloride as the eluent. The purified C-dots were redispersed in ethanol for optical measurements or in the mixture of the buffer and ethanol (10 vol%) for pH sensing.

Silica column chromatography. Firstly, a thin layer chromatography (TLC) experiment was carried out to optimize the eluents. Silica gel (300–400 mesh) was used as the stationary phase for the column chromatography. A gradient of CH₂Cl₂ and methanol at a volume ratio of 16/1 to 10/1 was used as the eluent. The purity of each fraction was verified by using TLC measurement. The

solvents were evaporated and the products were redissolved in ethanol. Finally, three different fractions of 3-APBA-based C-dots were obtained, and the third fraction showing three dominant fluorescence emissions was used for pH sensing.

The C-dots synthesized with 4-carboxyphenylboronic acid, 4-mercaptophenylboronic acid, 3-carboxyphenylboronic acid, phenylboronic acid or phenylamine was all purified by using silica column chromatography as described above.

Glucose, ATCh and urea sensing with the C-dots. To demonstrate the potential of the C-dots for glucose, ATCh and urea sensing, the concentrations of GOx, AChE and urease were fixed at 1.0, 0.5 and 0.5 mg/mL, respectively. Upon addition of the substrates, the mixtures were incubated at 37°C (25°C for urease) for 1 h to complete the catalytic reactions. Afterwards, the C-dots were added as the fluorescence probe.

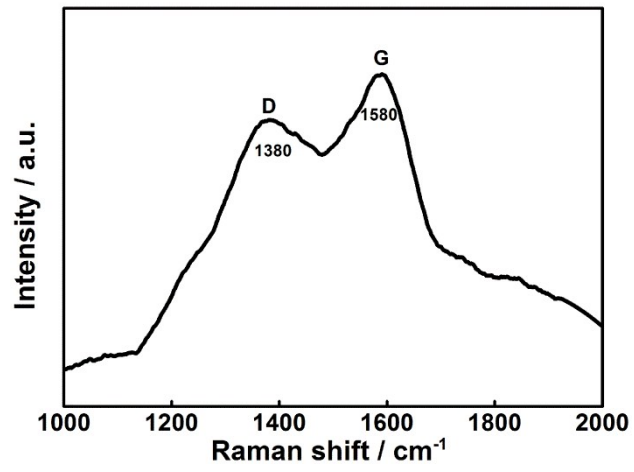


Fig. S1 Raman spectra of the C-dots prepared based on 3-APBA.

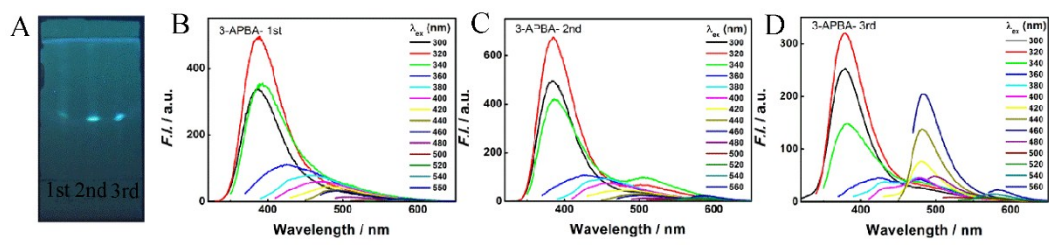


Fig. S2 (A) TLC examination of the three fractions of the 3-APBA-based C-dots purified with a silica chromatography. (B-D) The fluorescence spectra of (B) the 1st fraction, (C) 2nd fraction; (D) 3rd fraction.

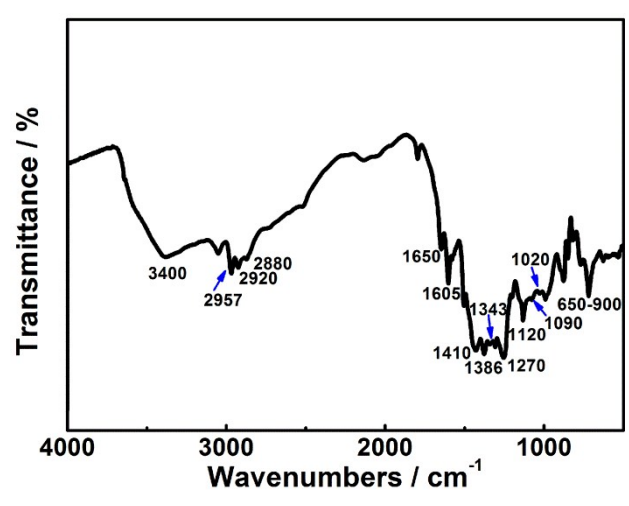


Fig. S3 FTIR spectra of the C-dots synthesized with 3-APBA.

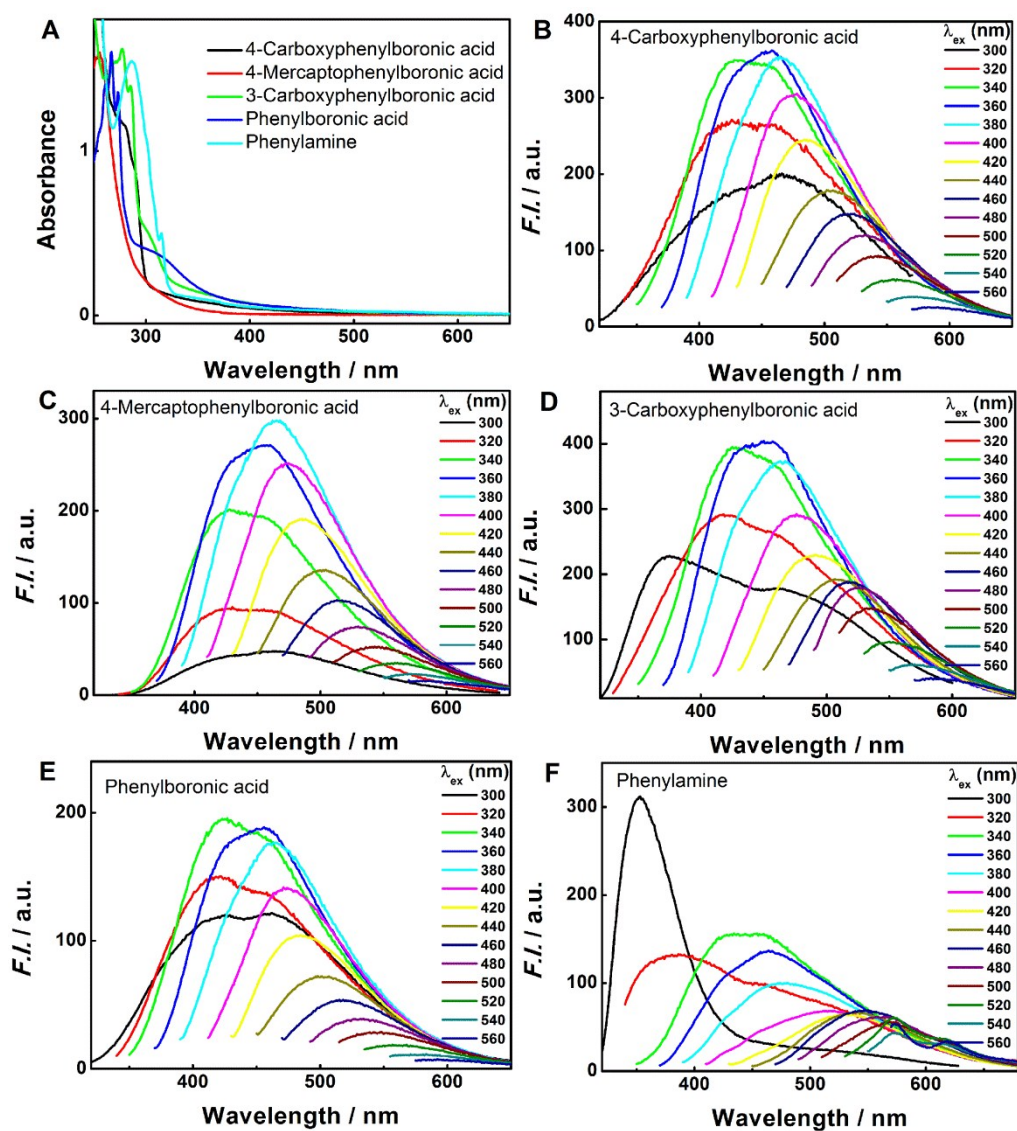


Fig. S4 The absorbance (A) and excitation-dependent fluorescence spectra (B-F) of the C-dots prepared with (B) 4-carboxyphenylboronic acid, (C) 4-mercaptophenylboronic acid, (D) 3-carboxyphenylboronic acid, (E) phenylboronic acid or (F) phenylamine. All the C-dots were synthesized in the Teflon-lined autoclave chamber at 180 °C for 12 h. These C-dots displayed excitation-dependent emission and the intensities decrease rapidly with the red-shift of the excitation wavelength.

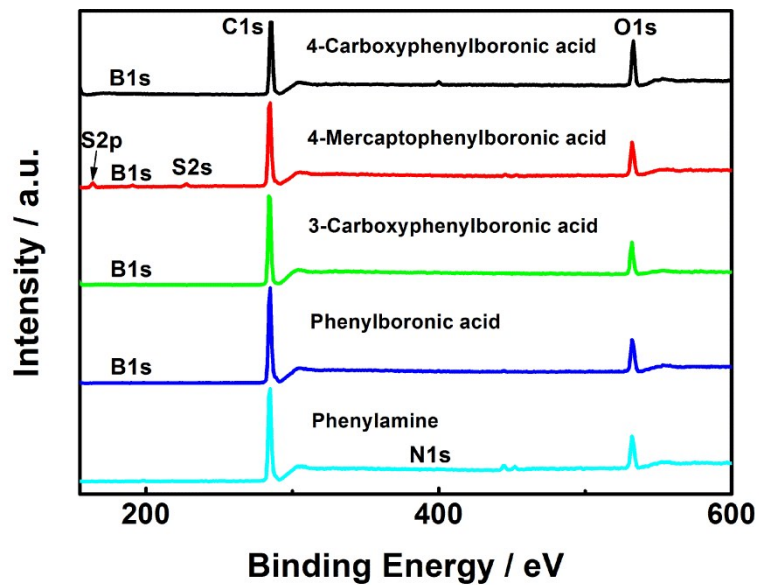


Fig. S5 XPS analysis of the purified C-dots prepared with 4-carboxyphenylboronic acid, 4-mercaptophenylboronic acid, 3-carboxyphenylboronic acid, phenylboronic acid or phenylamine.

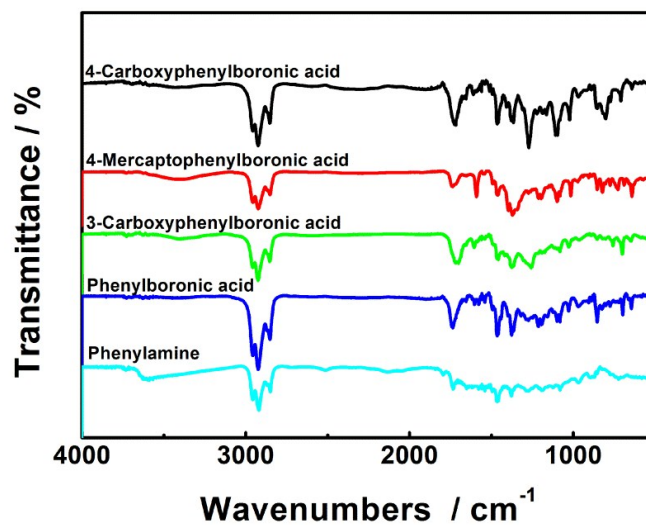


Fig. S6 FTIR spectra of the purified C-dots prepared with 4-carboxyphenylboronic acid, 4-mercaptophenylboronic acid, 3-carboxyphenylboronic acid, phenylboronic acid or phenylamine.

Table S1 Elemental contents of the C-dots determined by XPS measurement.

C-dots from various raw materials	C (%)	O (%)	N (%)	B (%)	S (%)
3-APBA-3rd	77.4	20.1	1.8	0.7	/
4-Carboxyphenylboronic acid	73.7	25.1	/	1.2	/
4-Mercaptophenylboronic acid	81.2	13.6	/	3.2	2.1
3-Carboxyphenylboronic acid	82.9	14.6	/	2.4	/
Phenylboronic acid	83.9	15.0	/	1.2	/
Phenylamine	87.8	11.7	0.5	/	/

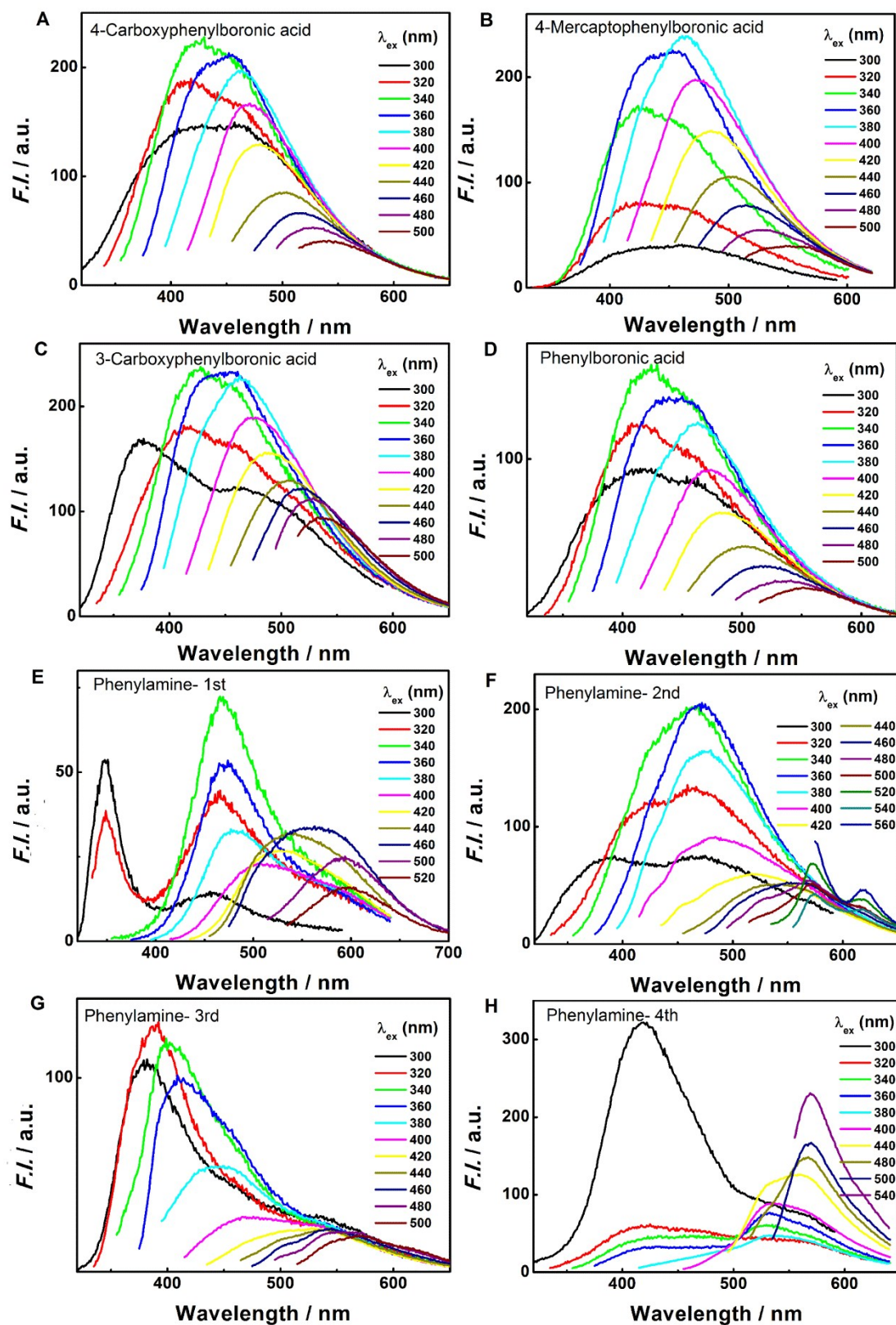


Fig. S7 Fluorescence spectra of the C-dots after purification prepared with different raw materials.

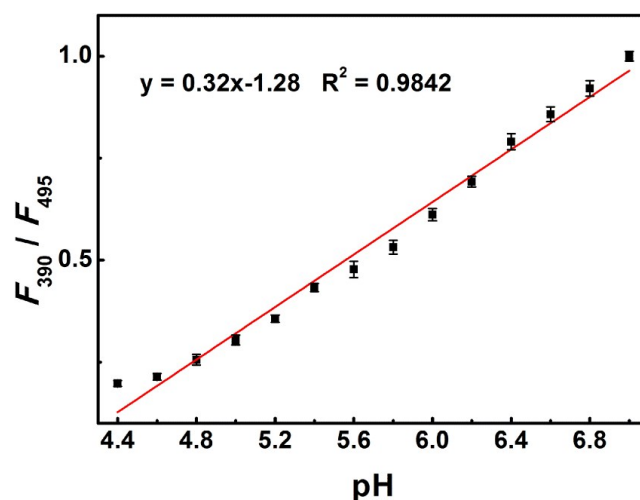


Fig. S8 Plots of F_{390}/F_{495} versus pH in B-R buffer.

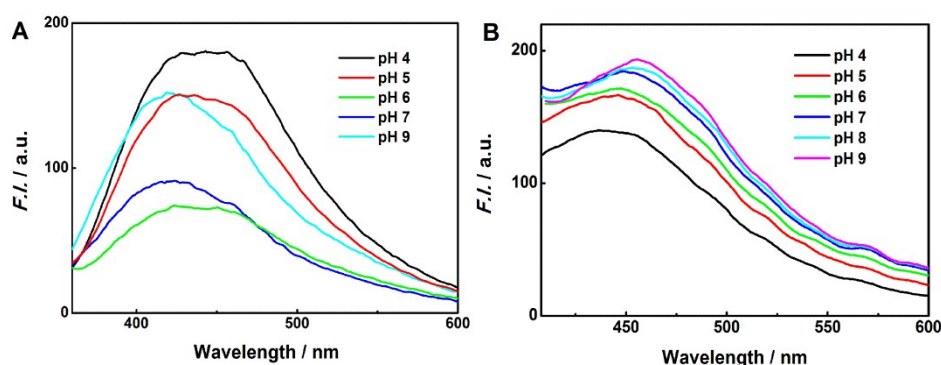


Fig. S9 The influence of buffer pH on the fluorescence spectra of the C-dots synthesized with (A) phenylamine and (B) phenylboronic acid, respectively.

With pH increase, the emission intensity of the C-dots prepared with phenylamine decreases firstly and then increases, which is thought to be caused by the deprotonation of the functional groups related to nitrogen. The results agree with the response of the 495 nm emission to pH in case of the C-dots synthesized with 3-APBA. The slight difference of the pH turn point of the emission decrease to increase is probably attributed to the discrepancy of the N-doping contents in two kinds of C-dots. As for the C-dots synthesized with phenylboronic acid, the fluorescence intensity increases with pH increase and then becomes stable. Elemental boron is electron-deficient. With pH increase, the dissociation of the functional groups correlated with B is influenced, leading to the corresponding variation of fluorescence emission. This is in line with the response of the 390 nm emission to pH in case of the C-dots synthesized with 3-APBA. The simultaneous doping of N and B endows the C-dots with unique fluorescence performance.

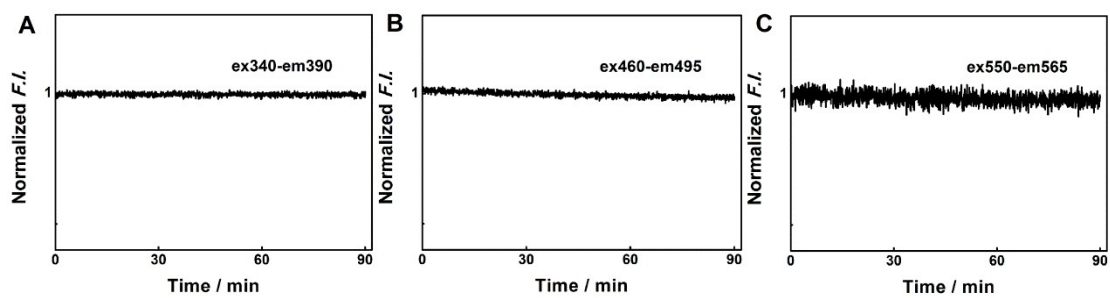


Fig. S10 Fluorescence photostability of the 3-APBA-based C-dots in aqueous solution (10 vol% of ethanol).

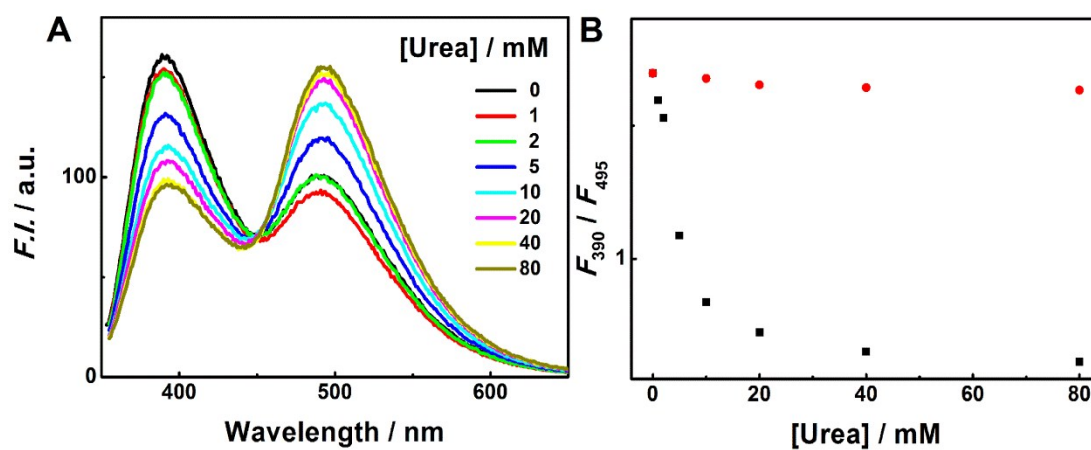


Fig. S11 (A) Fluorescence spectra of the C-dots for monitoring the hydrolysis of urea catalyzed by urease (0.5 mg/ml) from 0 to 80 mM in a 0.01 mM B-R buffer (pH 7). (B) Plot of F_{390}/F_{495} versus the concentration of urea (black dot). No change of F_{390}/F_{495} was detected in the absence of urease as shown by the red dots.