Electronic Supplementary Information

Light-activated Carbon Dots Nanozyme with Scandium for Highly Efficient and pH-Universal Bio-Nanozyme Cascade Colorimetric Assay

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Reagents and Materials.

Citric acid monohydrate, ascorbic acid (AA), 3,3’,5,5’-Tetramethylbenzidine (TMB), scandium chloride hexahydrate ($ScCl_3·6H_2O$), cerium chloride heptahydrate ($CeCl_3·7H_2O$), vanadium chloride (VCl_3), chromium trichloride ($CrCl_3$), manganese chloride ($MnCl_2$), iron chloride ($FeCl_3$), cobalt chloride ($CoCl_2$), nickel chloride ($NiCl_2$), copric chloride dihydrate ($CuCl_2·2H_2O$), zinc chloride ($ZnCl_2$), dichlorvos standard (DDV), parathion-methyl solution (PM), profenofos (PF), Glucose oxidase (GOx), Lysozyme, Trypsin, γ-Globulins, sulfamethoxazole, amoxicilin and penicillin were obtained from Aladdin (Shanghai, China). Tris(hydroxymethyl)aminomethane was obtained from Damas-beta (Shanghai, China). Superoxide dismutase bovine, acetonitride-d3 (99.8 atom %D), deuterium oxide (D, 99.9%) , Pepsin, Papain, Protamine and Bovine serum albumin (BSA) were obtained from Sigma-Aldrich (Shanghai, China). Mannite, tryptophan, catalase, acetylcholine (ATCh), acetylcholinesterase (AChE), sodium L-ascorbyl-2-phosphate (AA2P), and alkaline phosphatase (ALP) were obtained from Yuanye Biotechnology Co. Ltd. (Shanghai, China). Hydrochloric acid, ethylenediamine, acetic acid, sodium acetate, sodium hydroxide, dimethyl sulfoxide (DMSO), and methanol were obtained from Kelong Reagent Co. (Chengdu, China). Column chromatography silica (300–400 mesh) was obtained from Jiangyou Co. (Shandong, China). All chemicals were of analytical grade, OPs were dissolved in methanol originally and the Milli-Q water (18.2 MΩ·cm) was used for all of these experimental solutions. The concentration of C-dots in this work was 10 μg/mL. The wavelength of LED light used in all experiments was 365 nm (3 V, 3 W, XP-3535, Kiwi Optoelectronics Co. Shenzhen).

Instruments.

The absorption spectra were collected by using a UV-Vis spectrophotometer (PerkinElmer Lambda365). The fluorescence spectra were obtained with FluoroMax-4 spectrofluorometer (Horiba Jobin Yvon). The FT-IR spectra (KBr pellets) was taken on a Nicolet IS50 Fourier transform infrared spectrometer (ThermoFisher Inc.). The phosphorescence spectra of singlet oxygen were carried out using a Fluorolog-3 spectrofluorometer (Horiba Jobin Yvon) with a Near-Infrared detector. The fluorescence lifetimes were measured on a Fluorolog-3 spectrofluorometer (Horiba Jobin Yvon) with DeltaDiode (Ex: 405 nm) as the excitation source and a picosecond photon detection module (PPD-850, Horiba Jobin Yvon) as the detector, respectively. Zeta-potential was get using particle size analyzer ZEN3690 (Malvern Instruments Ltd.). All pH values were obtained with a pH meter (FiveEasy plus, METTLER TOLEDO) with a combined glass–calomel electrode. The absorbance spectra at 650 nm were recorded by a microplate reader BioTek Instrument Co. Ltd. (H1M). The mass concentration of C-dots was obtained with a freeze dryer (Scientz-10N/A). Transmission electron microscopy (TEM) images of C-dots was obtained using a Tecnai G2 F20 S-TWIN transmission electron microscope at an accelerating voltage of 200 kV (FEI Co., USA).

Synthesis of Photooxidation C-dots.

UV-responsive photooxidation C-dots were synthesized by the common hydrothermal method.[1] Specifically, ethylenediamine (335 μL) and citric acid monohydrate (1.15 g) were dissolved in Milli-Q water (10 mL), and then transferred the mixed solution into a polytetrafluoroethylene (Teflon)-lined autoclave (20 mL) and heated to 240°C for 5 h. After complete carbonization and cooling to room temperature, purification was carried out by using column chromatography silica (300 ~ 400 mesh) to get water-soluble homogeneous C-dots (water as the eluent). Eventually, C-dots powder was obtained by freezing-drying calculated concentration was 10 mg/mL (original concentration).

Interactions between C-dots and Sc^{3+}.

The UV-vis absorption spectra, fluorescence spectra, and fluorescence lifetimes of C-dots, C-dots with Sc^{3+} (1 mM or 5 mM) were obtained at pH = 4.5. C-dots with 1 mM Sc^{3+} in a CD_3CN-D_2O mixed solvent (v/v = 15:1) were prepared to get phosphorescence emission spectra of $^1O_2$ produced by the photosensitization reaction (pH = 7.0 buffer solution). The zeta potential was determined in the presence and absence of Sc^{3+}. The absorption spectra of pre-light and post-oxidation of TMB were carried out at pH = 4, which meant after 100 s of C-dots/Sc^{3+} irradiation, the light was switched off and TMB was added immediately. To examine the need for C-dots and Sc^{3+} incubation, pH = 4.0, 7.0 and 10.0 were chosen as representatives, and the above concentration of C-dots and Sc^{3+} were incubated for 0, 15, 30, 120, 360, 600 minutes, respectively. The 1 mM Sc^{3+}, V^{3+}, Cr^{3+}, Mn^{2+}, Fe^{3+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+} were added to 10 mM pH 7.0 buffer solution to explore the complexation ability among transition metal ions.

Sensing selectivity of ALP, AchE and Ops based on C-dots/Sc^{3+} photocatalytic oxidation system.

The GOx, Lysozyme, Trypsin, γ-Globulins, Pepsin, Papain, Protamine and BSA were utilized to examine the selectivity of the enzyme-nanozyme cascade reaction for sensing ALP and AChE when ATCh and AA2P as substrate, respectivrly. The concentration of ALP and AChE were 30 U/L and 10 U/L, respectively. The final concentration of other proteins were 20 μg/mL. Three kinds of OPs including dichlorvos (DDV), parathion-methyl (PM), profenofos (PF) and three kinds antibiotics including sulfamethoxazole, amoxicilin, penicillin were examined for studying the determination selectivty of OPs upon inhibitors-induced competition reaction of photocatalytic oxidation enzyme-nanozyme cascade.

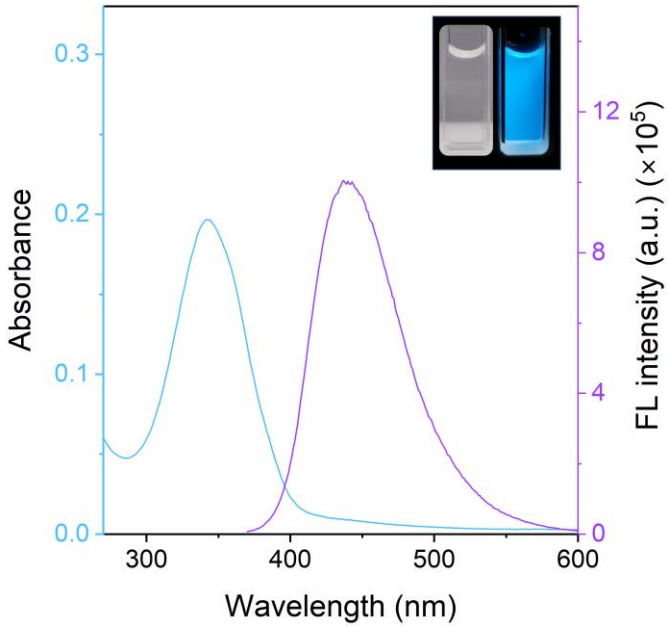

Fig. S1. The absorbance and fluorescence spectra (Ex: 365 nm) of C-dots (10 μg/mL) (in UP water) in this work. The inset are the photographs of C-dots solution in natural light (left) and 365 nm UV-light excitation (right).

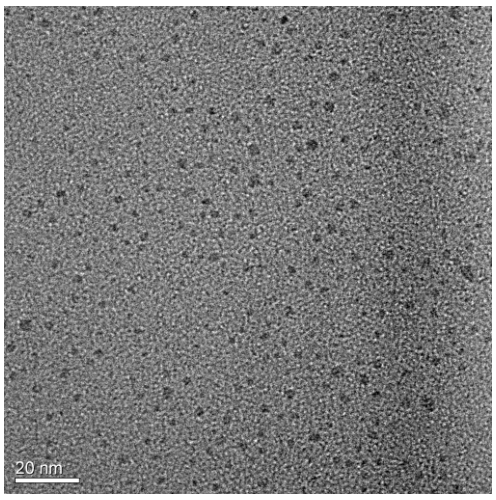

Fig. S2. TEM micrograph of synthesized C-dots in this work. The C-dots have a homogeneous dispersion with no significant aggregation and particle diameters of ~2 nm.

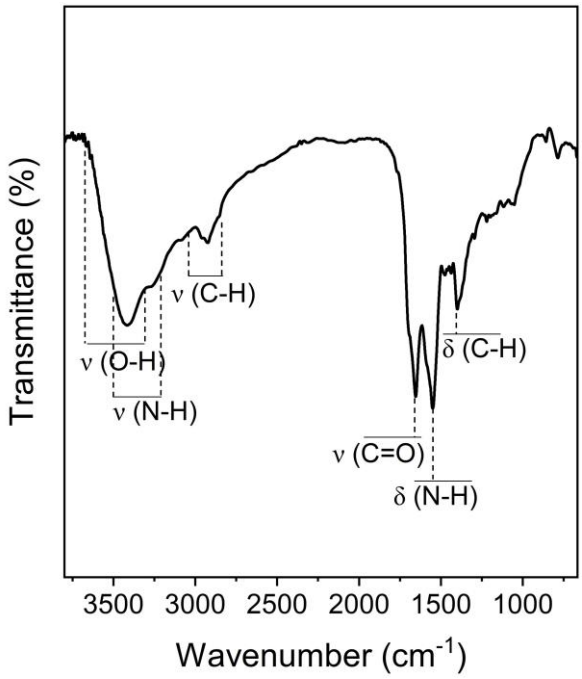

Fig. S3. FTIR spectra of C-dots. It mainly contains -OH, -C=O, NH, and CH groups.

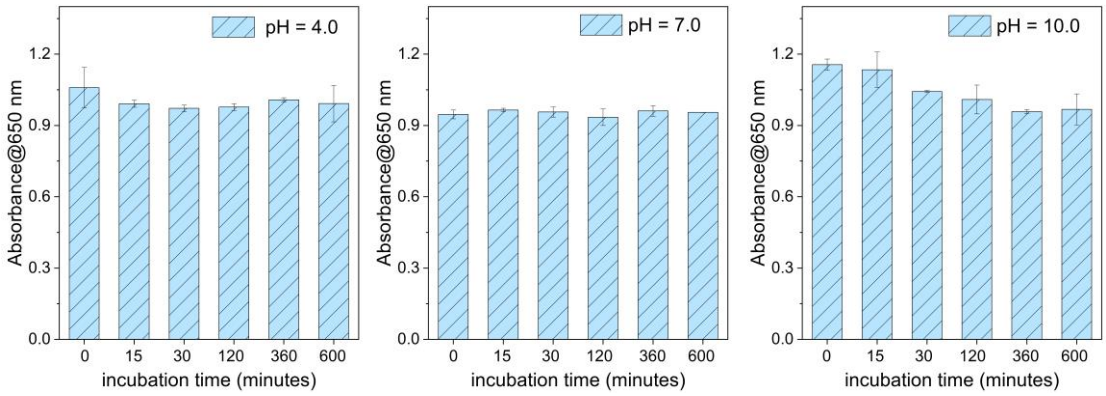

Fig. S4. The incubation time of Sc^{3+} and C-dots at pH = 4.0, 7.0, and 10.0, respectively. (1 mM Sc^{3+} for pH 4.0-7.0, 5 mM Sc^{3+} for pH 10.0)

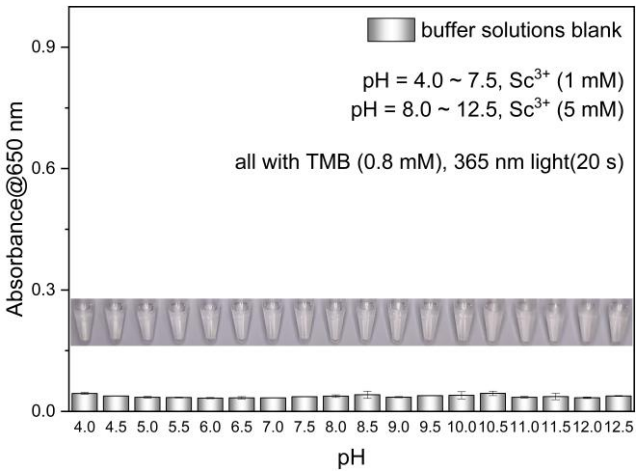

Fig. S5. Absorbance@650 nm of TMB (0.8 mM) oxidation when only buffer solutions exist at broad pH ranges, the inset are corresponding photographs. (pH 4.0–7.5: 10 mM NaAc-HAc buffer, pH 8.0 ~ 10.5: 10 mM Tris-HCl buffer, pH 11.0–12.5: NaOH solution.)

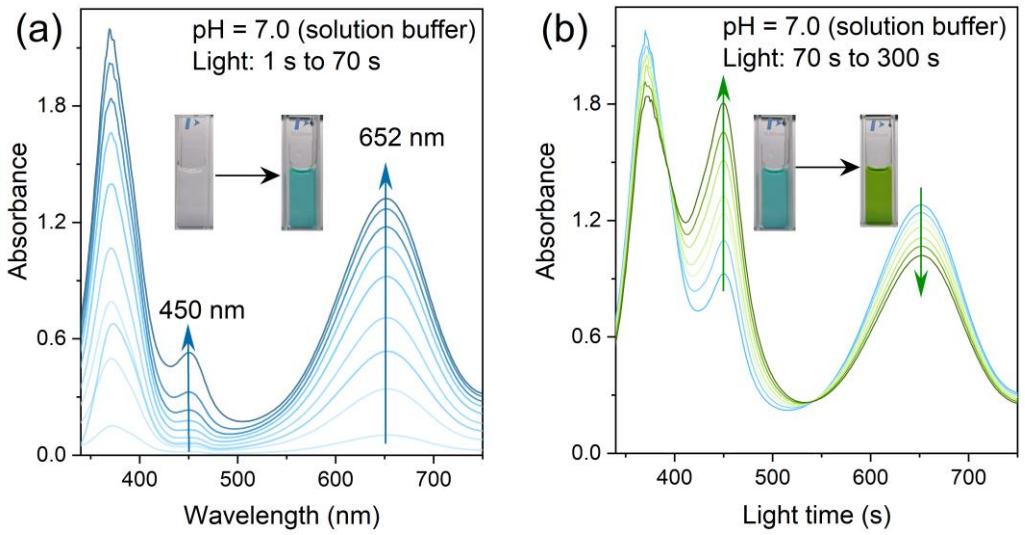

Fig. S6. (a) The absorption spectra of oxTMB with 1 s ~ 70 s irradiation time. (b) The absorption spectra of oxTMB with 90 s ~ 300 s irradiation time (TMB: 0.1 mM).

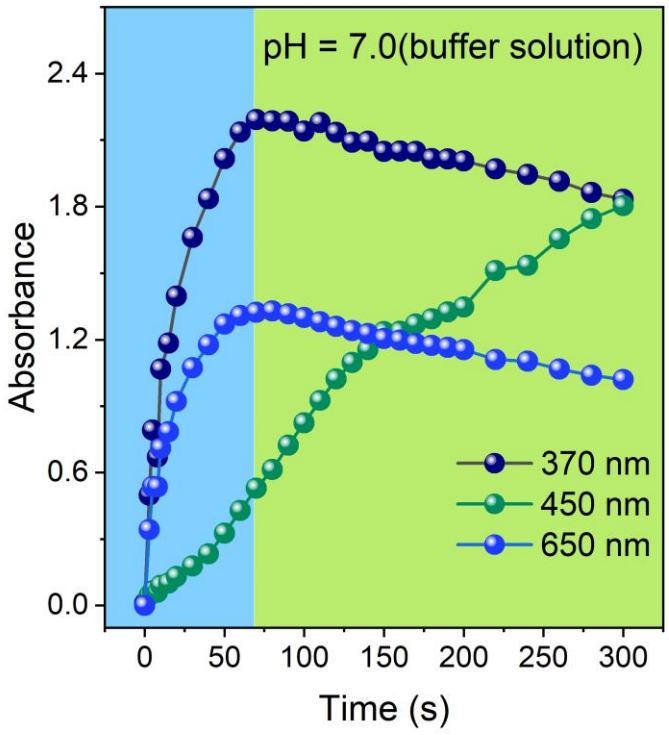

Fig. S7. Kinetics of TMB (0.1 mM) photooxidation monitored at 370 nm, 450 nm and 650 nm in the presence of the C-dots (10 μg/mL) and Sc^{3+} (1 mM) at pH = 7.0 buffer solution.

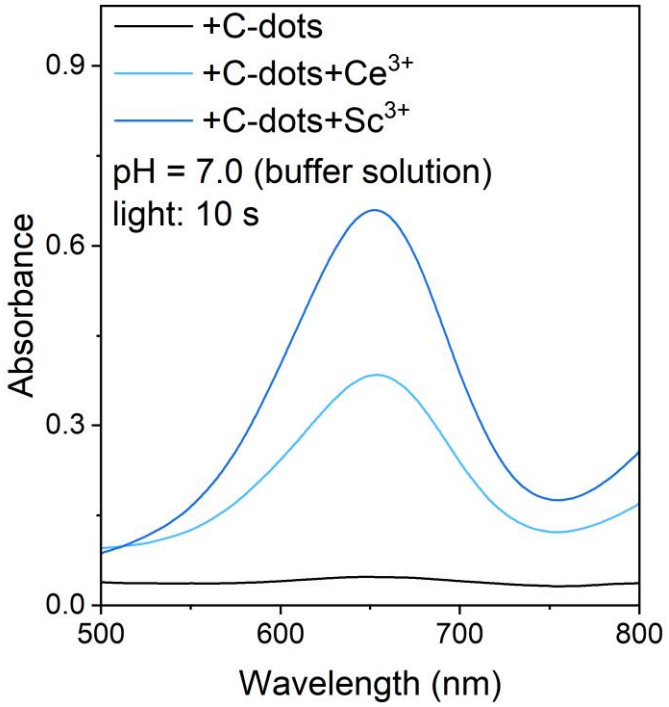

Fig. S8. Compare the TMB oxidation degree of C-dots, C-dots + Sc^{3+} (1 mM), and C-dots + Ce^{3+} (1 mM). All in pH = 7.0 Tris-HCl buffer (10 mM) solution, and light for 10 s.

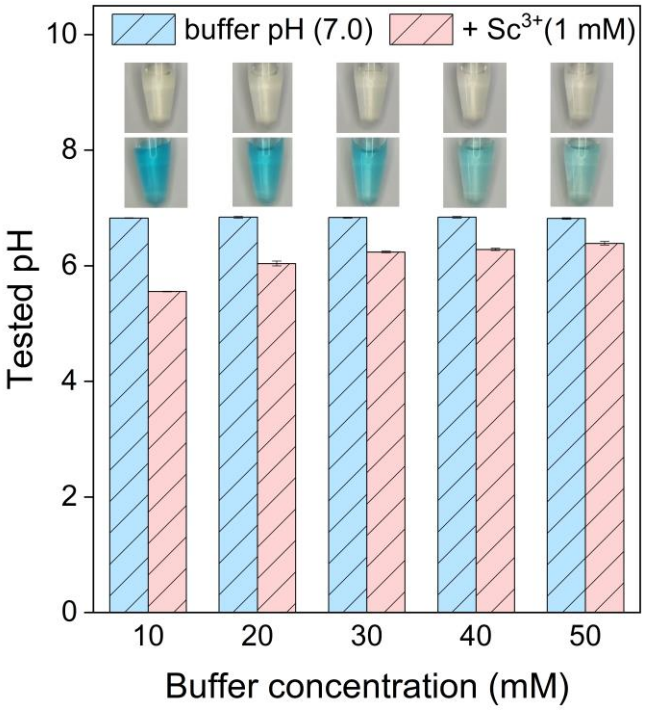

Fig. S9. The actual pH values of different final concentration buffer solution when added with 1 mM Sc^{3+}. The insets are photographs of photocatalytic oxTMB under different buffer solutions in the presence (bottom) and absence (top) of Sc^{3+}.

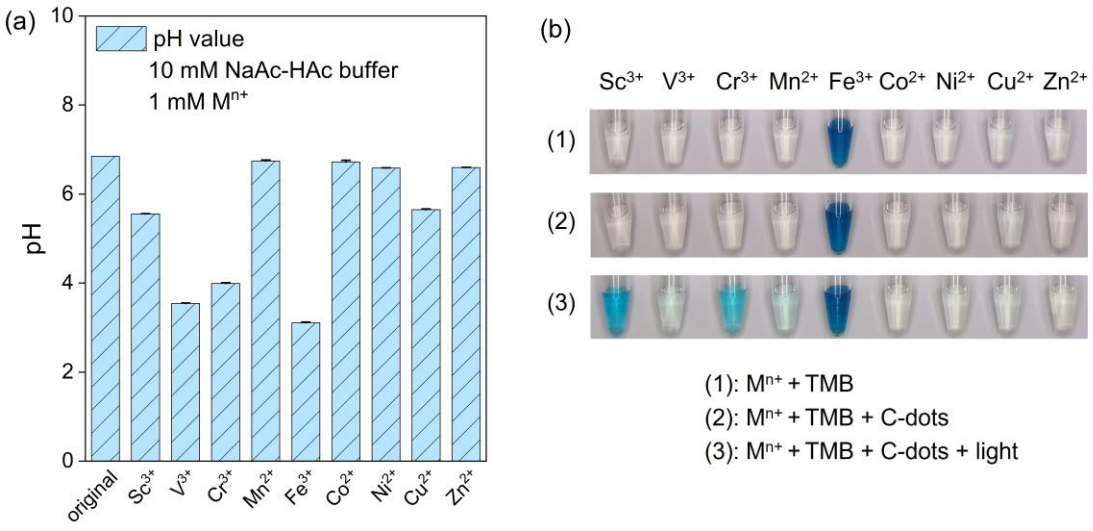

Fig. S10. (a) The actual pH values when added with 1 mM different transition metal ions. (b) The photographs of TMB oxidation at three varied condition. (10 mM NaAc-HAc buffer solution, TMB: 0.8 mM, C-dots: 10 μg/mL, light: 20 s)

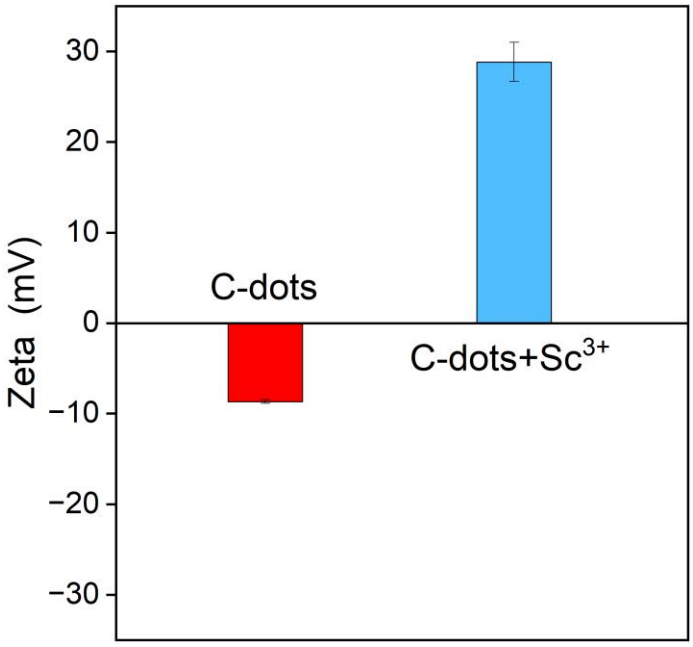

Fig. S11. The zeta-potential change of C-dots (10 μg/mL) in the presence and absence of Sc^{3+} (1 mM) in UP water.

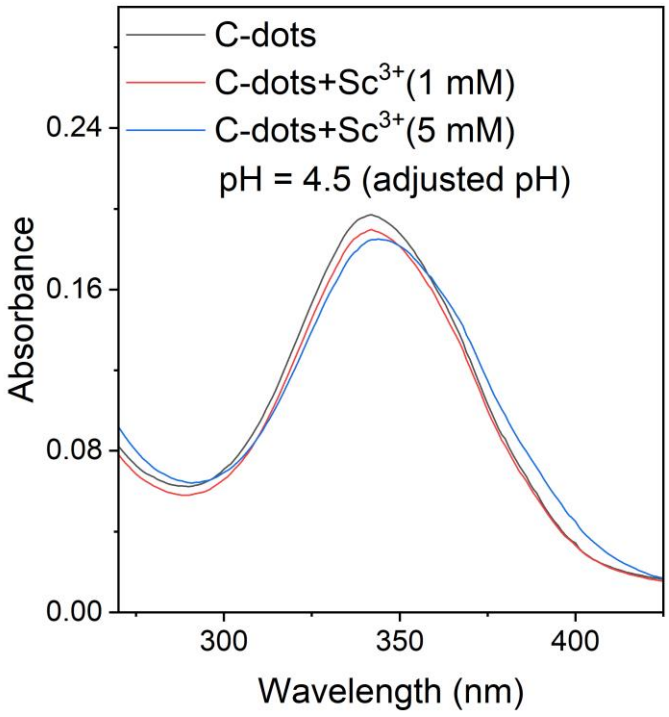

Fig. S12. Absorption spectra of C-dots (10 μg/mL) in the presence and absence of Sc^{3+} (1 mM or 5 mM), respectively. (All groups are adjusted to same pH condition, pH = 4.5).

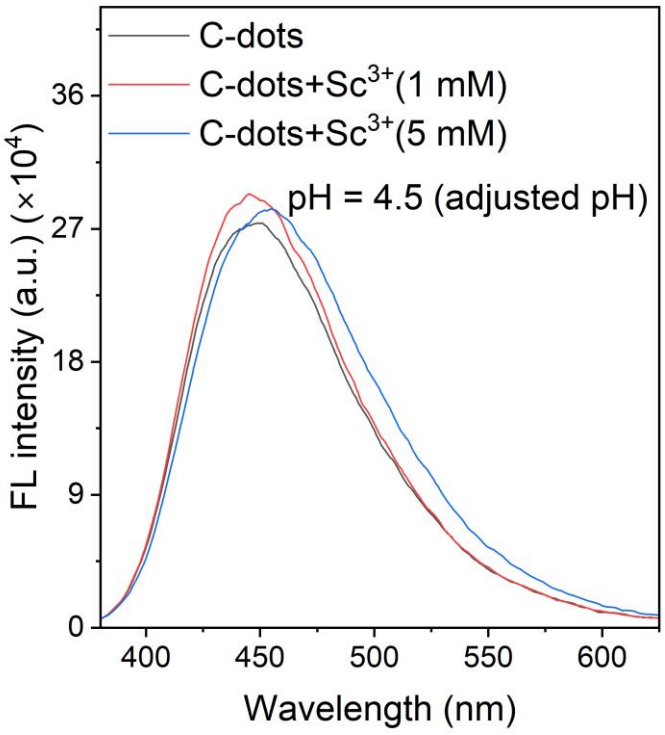

Fig. S13. Fluorescence spectra of C-dots (10 μg/mL) in the presence and absence of Sc^{3+} (1 mM or 5 mM), respectively. (All groups are adjusted to same pH condition, pH = 4.5)

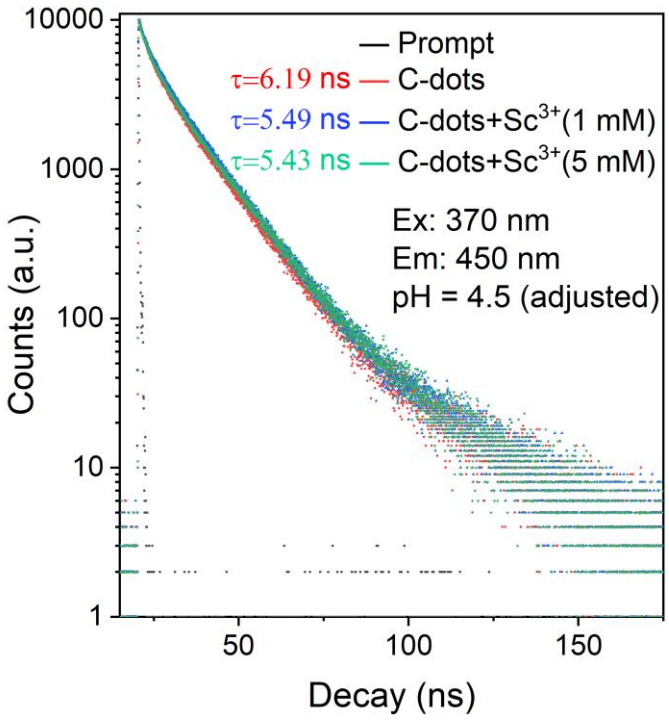

Fig. S14. Fluorescence lifetime of C-dots (10 μg/mL) in the presence and absence of Sc^{3+} (1 mM or 5 mM), respectively. (All groups are adjusted to same pH condition, pH = 5.5)

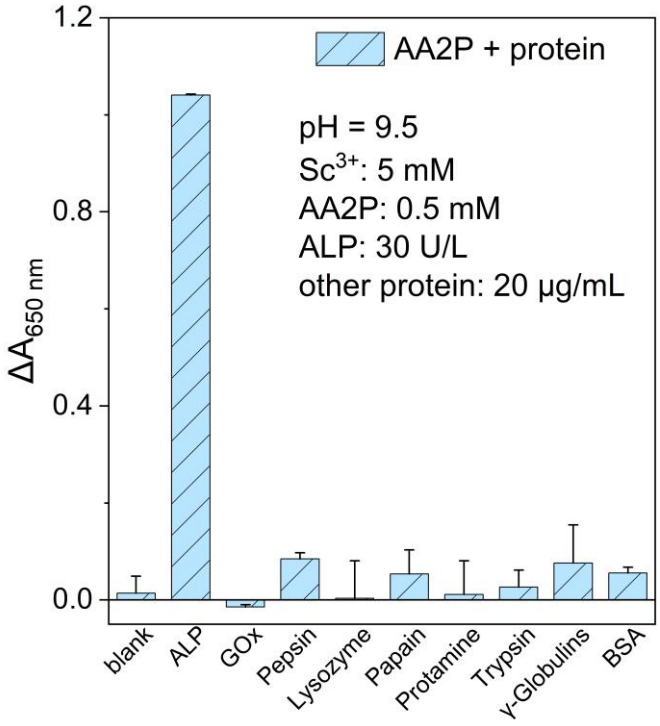

Fig. S15. ΔA of TMB photocatalytic oxidation when substrate AA2P with different types of proteins. (10 μg/mL C-dots, 0.8 mM TMB, light 20 s)

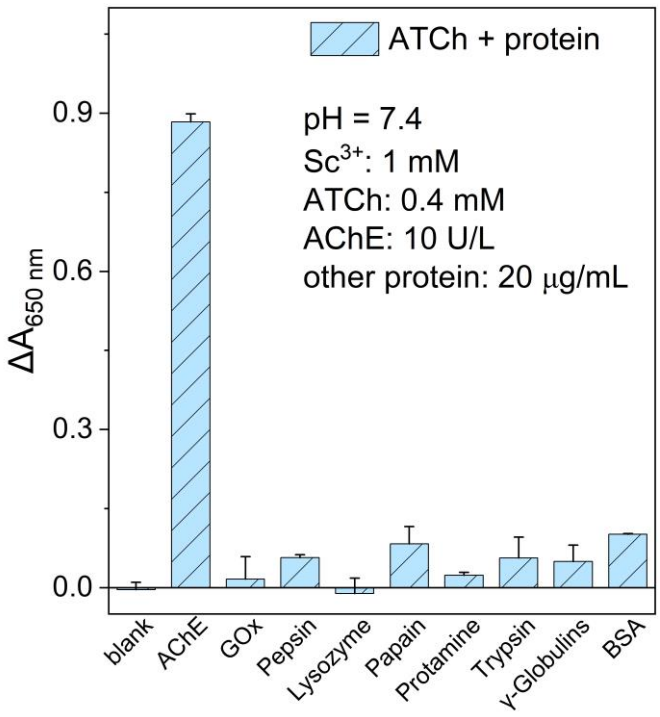

Fig. S16. ΔA of TMB photocatalytic oxidation when substrate ATCh with different types of proteins. (10 μg/mL C-dots, 0.8 mM TMB, light 20 s)

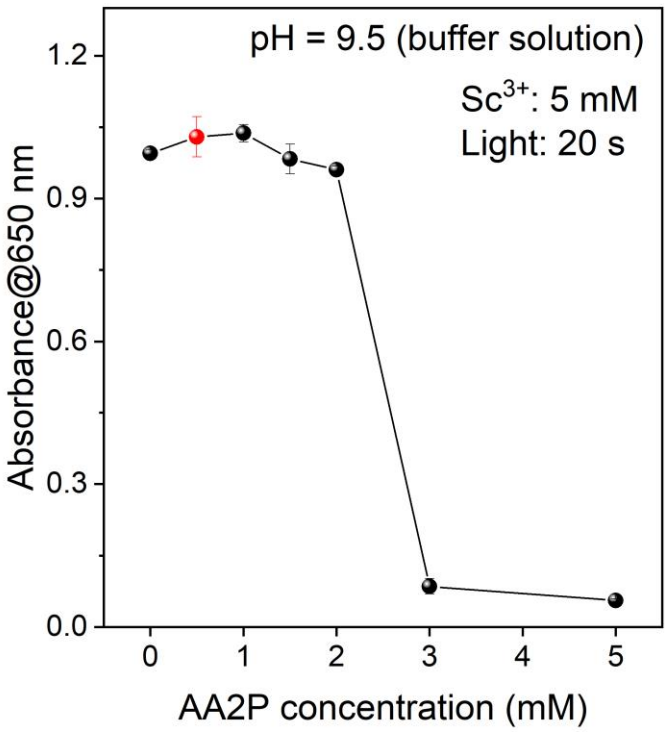

Fig. S17. Optimization of AA2P concentration.(pH = 9.5 buffer solution, 5 mM Sc^{3+}, 10 μg/mL C-dots, 0.8 mM TMB, light 20 s)

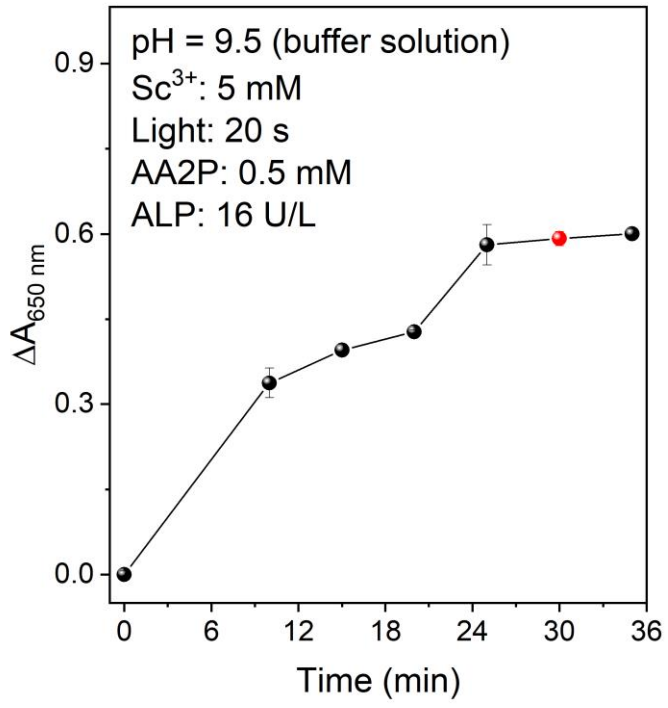

Fig. S18. Optimization of the bathing time of ALP and AA2P at 37℃. (pH = 9.5 buffer solution, 5 mM Sc^{3+}, 10 μg/mL C-dots, 0.8 mM TMB, light 20 s, 0.05 mM AA2P, 16 U/L ALP)

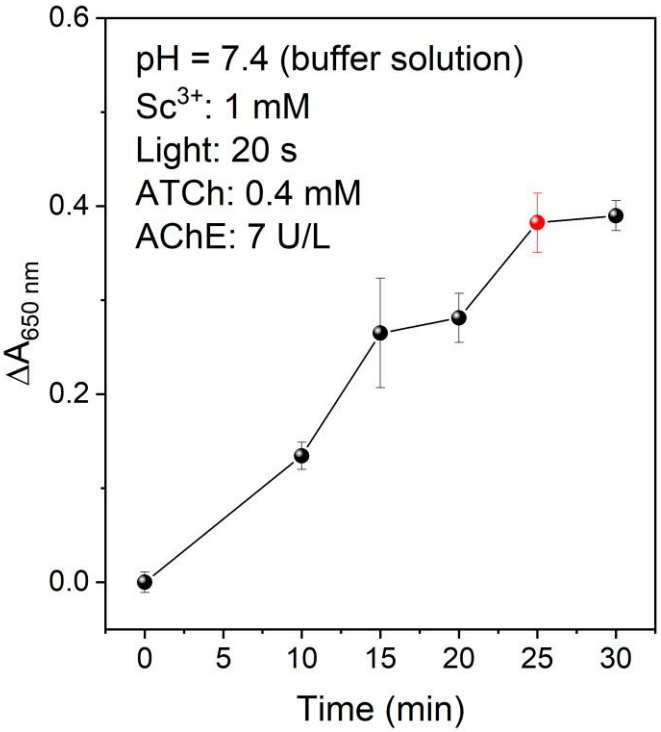

Fig. S19. Optimization of the bathing time of ATCh and AChE at 37 ℃. (pH = 7.4 buffer solution, 1 mM Sc^{3+}, 10 μg/mL C-dots, 0.8 mM TMB, light 20 s, 0.4 mM ATCh, 7 U/L AChE)

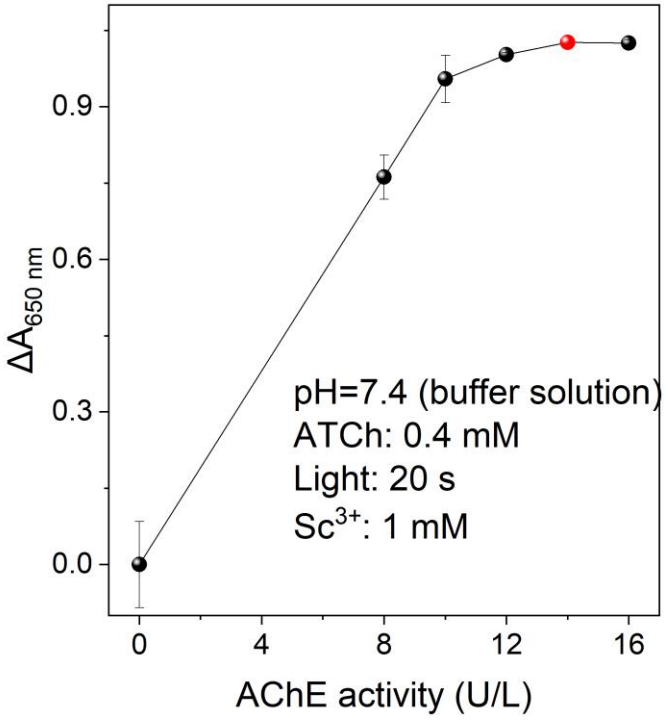

Fig. S20. Optimization of the activity of AChE reacted with OP. (pH = 7.4 buffer solution, 1 mM Sc^{3+}, 0.8 mM TMB, light 20 s, 0.4 mM ATCh, 3 μM DDV as OP model)

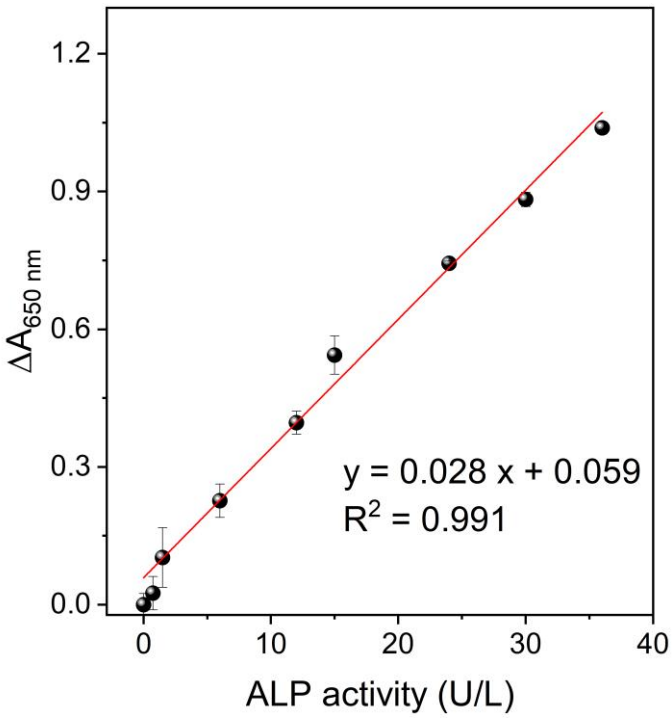

Fig. S21. Calibration curve for the ALP detection. (pH = 9.5 buffer solution, 5 mM Sc^{3+},0.8 mM TMB, light 20 s, 0.5 mM AA2P, ΔA means A_0 - A)

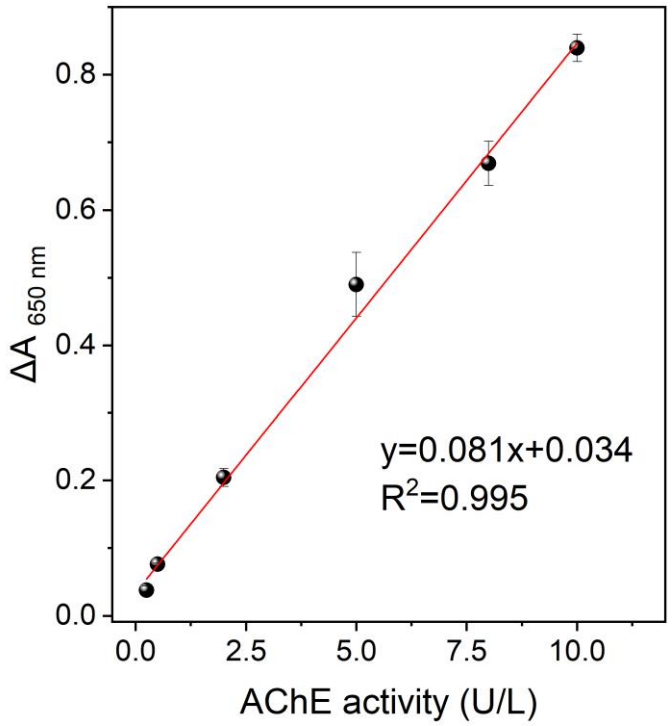

Fig. S22. Calibration curve for the AChE detection. (pH = 7.4 buffer solution, 1 mM Sc^{3+}, 0.8 mM TMB, light 20 s, 0.4 mM ATCh)

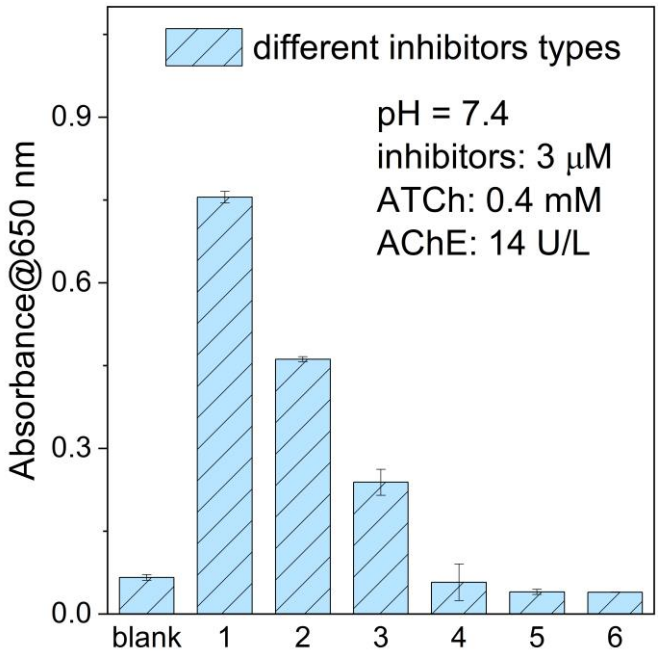

Fig. S23. Absorbance of photocatalytic TMB oxidation when substrate ATCh and AChE enzyme with different types of inhibitors (1: DDV, 2: PM, 3: PF, 4: sulfamethoxazole, 5: amoxicilin, 6: penicillin). (10 μg/mL C-dots, 0.8 mM TMB, light 20 s)

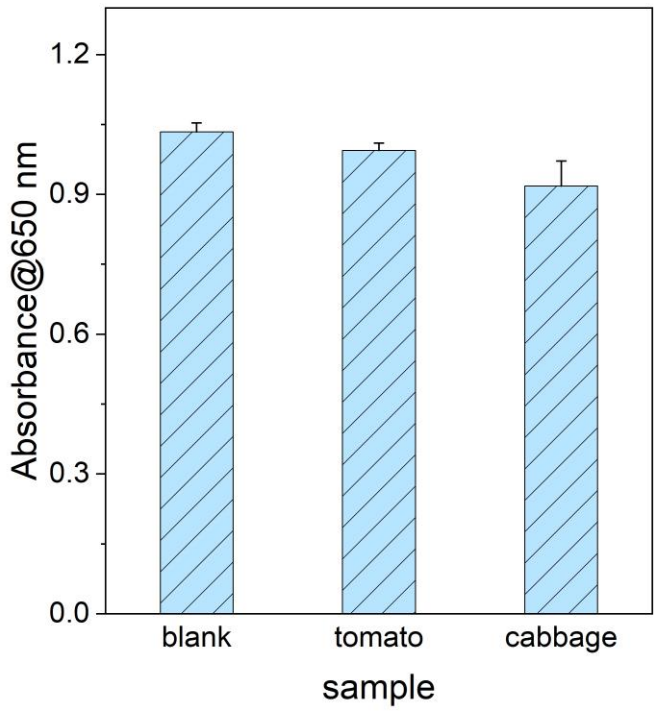

Fig. S24. Compare the influence of tomato and cabbage spraying liquid with blank UP water.

Table S1. Listed the one-pot catalytic cascade colorimetric methods for targets detection.

Expressed signal	Analyte	pH	Time (min)	Linear range	LOD	Ref.
AuNCs@ZIF-8	OPs	7.4 → 4.0	60 + 15	0.8 ~ 10^5 ng/mL	0.4 ng/mL	[2]
H_2O_2/TMB	OPs	7.4 → 5.0	30 + 20	10 ~ 10^3 ng/mL	4 ng/mL	[3]
	AChE		20	2 ~ 14 U/L	0.5 U/L	
CeGONRs	OPs	7.5 → 4.0	35 + 15	12 ~ 3500 ng/mL	3.43 ng/mL	[4]
$NiFe_2O_4$/NiO	OPs	up → 3.8	15 + 15	5×10^3 ~ 2.5×10^3	10^3 ng/mL	[5]
MIL-88B(Fe)-NH_2@ GLOX	Glutamate	9.8 → 4.0	30	1 ~ 100 μM	2.5 μM	[6]
Cu/NC NS	Lactose	7.2 → 4.0	60 + 10	100 ~ 1400 μM	30 μM	[7]
	β-galactosidase	7.2 → 4.0	60 + 10	25 ~ 200 U/L	10 U/L	
Cu/Fe_3O_4@FeOOH	Cholesterol	6.5	20 + 20	10 ~ 400 μM	8.2 μM	[8]
ATP/Fe_3O_4 NPs	Glucose	7.4	30	50 ~ 4000 μM	50 μM	[9]
CuO NPs	ALP	9.1 → 7.0	30 + 20	1.2 ~ 14.4 U/L	0.058 U/L	[10]
Sc^{3+}/C-dots	**AChE**	**7.4**	**25**	**0.25 ~ 10 U/L**	**0.14 U/L**	**This**
	ALP	**9.5**	**30**	**0 ~ 36 U/L**	**0.35 U/L**	**work**
	OPs	**7.4**	**35**	**0.25 ~ 3 μM**	**0.06 μM**	

Table S2. The tested and recovery analysis of vegetable samples upon Sc^{3+}-boosted photocatalytic oxidation system.

Sample	Content (μM)	Spiked DDV (μM)	Founda) (μM)	Recovery (%)
Tomato	ND	0.50	0.54 ± 0.02	108
		1.50	1.40 ± 0.03	93
		2.00	2.10 ± 0.02	105
		2.50	2.60 ± 0.02	104
Cabbage	ND	0.50	0.59 ± 0.02	118
		1.50	1.53 ± 0.03	102
		2.00	2.10 ± 0.03	105
		2.50	2.64 ± 0.03	106

ND means not detected; a) ± means standard deviation (n = 3).

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