Electronic Supplementary Information

Carbon nanodots as peroxidase mimetics and their applications to glucose detection

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Experimental Section

Materials: Glucose, fructose, lactose, and maltose were purchased from Sinopharm Chemical Reagent Co. (Shanghai, China). 3,3,5,5-tetramethylbenzidine (TMB), horseradish peroxidase (HRP, EC1.11.1.17, 300 U mg^{-1}) and glucose oxidase (GOx, EC 1.1.3.4. 47, 200 U mg^{-1}) were purchased from Sigma-Aldrich (St. Louis, MO) and stored in a refrigerator at 4 °C. H_2O_2, acetic acid, sodium acetate and sulfuric acid were obtained from Chongqing Pharmaceutical Co., Ltd. Keyi Assay Glass Branch (Chongqing, China). Serum samples were obtained from local hospital (the ninth hospital of Chongqing). Dialysis bags (with cutoff molecular weight of 8000–14,000 Da) were purchased from Shanghai Green Bird Science & Technology Development Co. (Shanghai, China). Ultra-filtration tubes (with cutoff molecular weight of 30 kDa and 5 kDa) were purchased from Millipore Corporation (Billerica, MA 01821, USA). All other chemicals were of analytical reagent grade and used without further purification, and all solutions were prepared using ultra-pure water.

Preparation of C-Dots: The C-Dots were prepared according to our previous method [1] and the method reported by Liu et al.[2] Briefly, after being washed with acetone and dried at 110 °C, 0.5 g of candle soot was added into 150 mL of HNO_3 (5 M), and the mixture was refluxed at 140 °C for 12 h. The C-Dots were purified by neutralizing the resulting black solution with sodium carbonate, and loading it into dialysis bags for dialysis against ultra-pure water for 2 d. Acetone was added to the purified solution and the C-Dots were collected by centrifugation at 16,000 rpm for 15 min. The solid pellet was dried under nitrogen flow, dispersed in ultra-pure water, and then ultra-filtered (with 30 KDa) to remove large particles. The brown C-Dots solution obtained was stored at 4 °C until use. The zeta potential of C-Dots solution was measured by Zetasizer Nano ZS90 apparatus (Malvern, UK). The size of the as-synthesized C-Dots was characterized by TEM on a Tecnai G^2 20 transmission electron microscopy (FEI, Netherlands). The size distribution was determined by counting 105 particles. X-ray photoelectron spectroscopy (XPS) spectra were

measured by a XSAM-800 X-ray photoelectron spectroscope (KRATOS, UK). The near-infrared (NIR) spectra were recorded with a model U-4100 UV-Vis-NIR spectrophotometer (Hitachi, Japan).

Electron spin resonance: 330 μL samples were prepared at room temperature by adding 200 μL of 0.2 M NaAc buffer (pH=3.5), 10 μL of 3% H_2O_2, and 20 μL of 0.2 M DMPO and proper amount of ultra-pure water into a 1 mL plastic tube. The prepared sample solution was transferred to a quartz capillary tube and placed in the ESR cavity. DMPO was used to trap the ·OH radicals to form the DMPO/·OH spin adduct. Each sample was UV-irradiated at 355 nm for 7 min, and spectra were recorded afterwards. The ESR spectra were obtained on a Bruker ESR 300E with microwave bridge (receiver gain, 1×10^5; modulation amplitude, 2 Gauss; microwave power, 10 mW; modulation frequency, 100 kHz).

Bioassay: Kinetic measurements were carried out in time course mode by monitoring the absorbance change at 652 nm [3] on a UV-2450 UV-Vis spectrophotometer (Shimadzu, Japan). Experiments were carried out using 1 μg mL^{-1} C-Dots in 10 mL of buffer solution (0.2 M NaAc buffer, pH 3.5, 35 °C) with 0.8 mM TMB as substrate. The H_2O_2 concentration was 50 mM, unless otherwise stated. The Michaelis–Menten constant was calculated using Lineweaver–Burk plots of the double reciprocal of the Michaelis–Menten equation, $1/v=K_m/V_{max}\cdot(1/[S]+1/K_m)$.

Glucose detection was performed as follows: a) 0.1 mL of 1 mg mL^{-1} GOx and 0.1 mL of glucose of different concentrations in 0.5 mL of 10 mM Na_2HPO_4 buffer (pH 7.0) were incubated at 37 °C for 30 min; b) 0.1 mL of 5 mM TMB, 0.2 mL of the C-Dots stock solution (50 μg mL^{-1}) and 9 mL of 0.2 M NaAc buffer (pH 3.5) were added to the above glucose reaction solution (0.7 mL); and c) the mixed solution was incubated at 35 °C for 30 min and then for standard curve measurement.

For glucose determination in serum, the samples were first treated by ultra filtration with 5 kDa Amicon cell at 3000 rpm for 30 min. Then 1 mL of the filtrate was added into 1 mL of 10 mM Na_2HPO_4 buffer (pH 7.0) and 0.1 mL of 1 mg mL^{-1}

GOx. The obtained mixed solution was incubated at 37 °C for 30 min. After the reaction, 0.1 mL of 5 mM TMB, 0.2 mL of the C-Dots stock solution (50 μg mL^{-1}) and 7.6 mL of 0.2 M NaAc buffer (pH 3.5) were added into the above reaction solution (2.1 mL). The mixed solution was incubated at 35 °C for 30 min and then used for the glucose measurement. In control experiments, 5 mM maltose, 5 mM lactose, and 5 mM fructose were used instead of glucose.

Reference

1 X. J. Mao, H. Z. Zheng, Y. J. Long, J. Du, J. Y. Hao, L. L. Wang and D. B. Zhou, *Spectrochim. Acta A*, 2010, **75**, 553-557.

2 H. P. Liu, T. Ye and C. D. Mao, *Angew. Chem. Int. Edit.*, 2007, **46**, 6473-6475.

3 L. Z. Gao, J. Zhuang, L. Nie, J. B. Zhang, Y. Zhang, N. Gu, T. H. Wang, J. Feng, D. L. Yang, S. Perrett and X. Y. Yan, *Nat. Nanotechnol.*, 2007, **2**, 577-583.

Table S1. Reproducibility between different batches of C-Dots using the same preparation method.

Batch No.	1	2	3	RSD (%)
Catalytic activity (%)	100±3.2 [a]	88.0±3.5 [a]	95.8±2.6 [a]	6.1

[a] RSD for three duplicate determinations.

Method for calculation of initial reaction rate: Absorbance data were back-calculated to concentration by the Beer–Lambert Law using a molar absorption coefficient of 39000 M^{-1} cm^{-1} for TMB-derived oxidation products. Apparent steady-state reaction rates at different concentrations of substrate were obtained by calculating the slopes of initial absorbance changes with time (Absorbance determination was performed in 5 min at the frequency of one time per minute), as shown in following **Table S2**.

Table S2. An illustration for calculation of initial reaction rate

A (Average)	Time (s)	The concentration of TMB-derived oxidation products (M^{-1}) (c=A/εb, where ε=39000 M^{-1} cm^{-1}, b=1 cm)	Rate (M^{-1} s^{-1})
0.054	60	1.37949E-06	
0.057	120	1.46062E-06	
0.059	180	0.00000152	1.13×10^{-9}
0.061	240	1.5641E-06	
0.065	300	1.66667E-06	

Table S3. Comparison of the apparent Michaelis-Menten constant (K_m) and maximum reaction rate (V_{max}) between C-Dots and HRP.

Catalyst	Substance	K_m [mM] [a]	V_{max} [10^{-8} M · s^{-1}] [a]
C-Dots	TMB	0.039±0.001	3.61±0.012
C-Dots	H_2O_2	26.77±2.94	30.61±0.38
HRP	TMB	0.155±0.012	52.9±3.67
HRP	H_2O_2	0.276±0.057	12.1±2.6

[a] Mean value ± standard deviation (n=3)

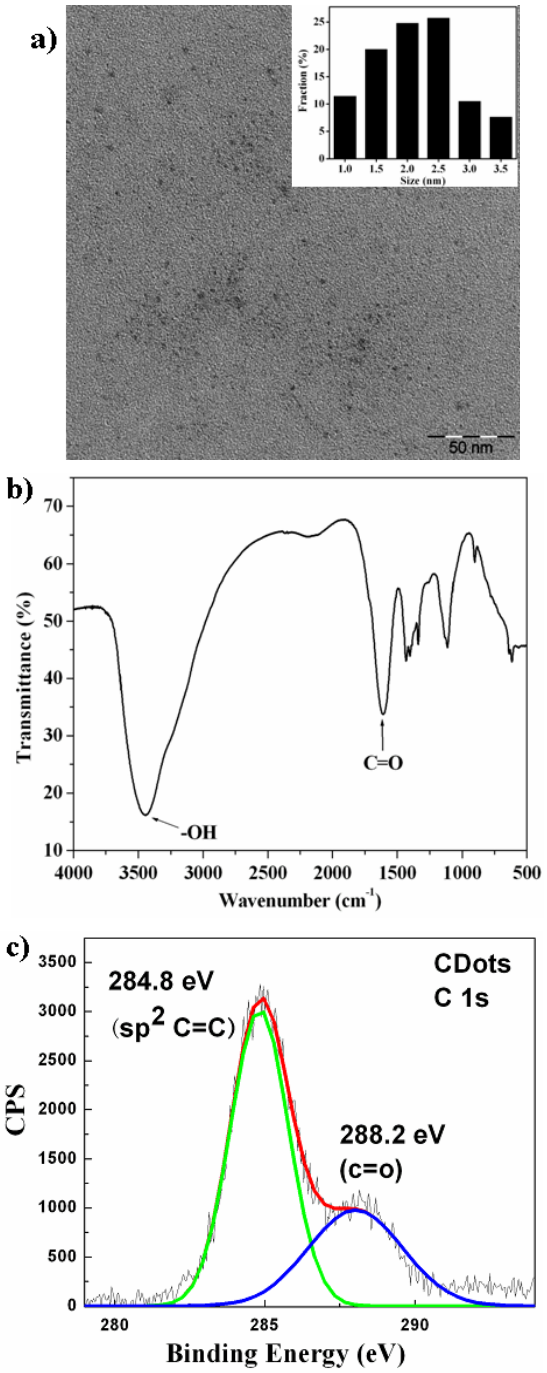

Fig. S1 a) TEM image and size distribution (inset) of the C-Dots. b) FTIR spectrum of the C-Dots. c) XPS of the C-Dots.

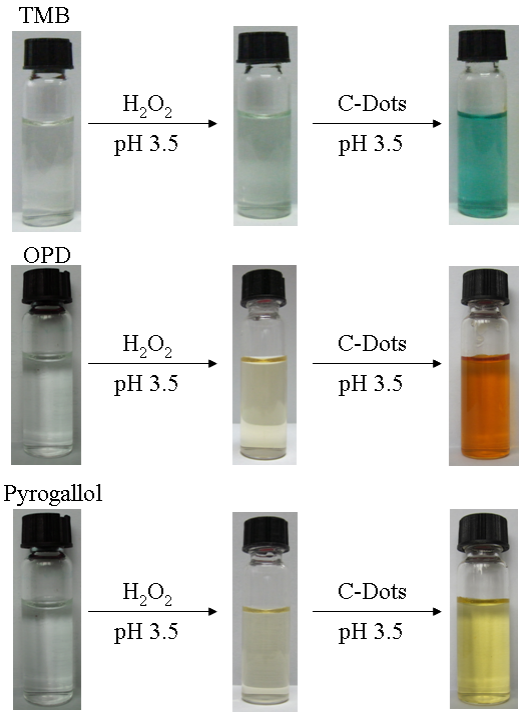

Fig. S2 Images of oxidation color reaction of TMB, OPD, and Pyrogallol by H_2O_2 after catalyzing by C-Dots at pH 3.5 NaAc buffer solution.

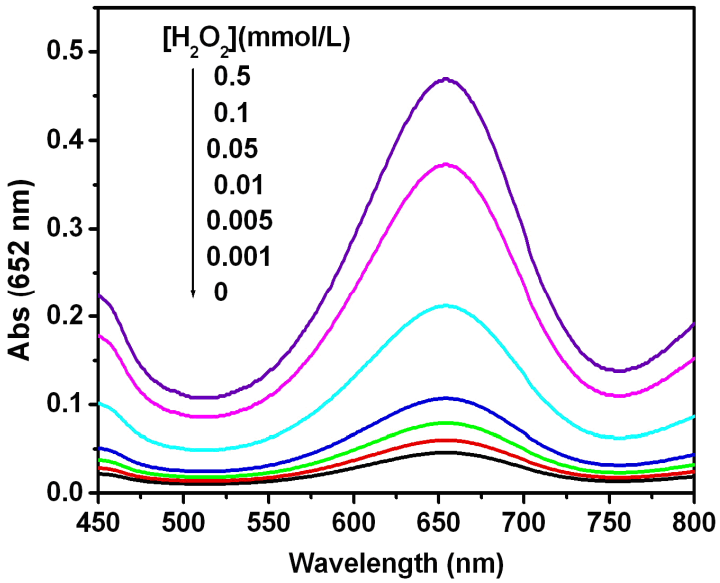

Fig. S3 UV/Vis spectra for a solution of TMB-H_2O_2-C-Dots in 0.2 M NaAc buffer (pH 3.5) at 35 °C. The C-Dots and TMB concentrations were 1 μg mL^{-1} and 50 μM, respectively.

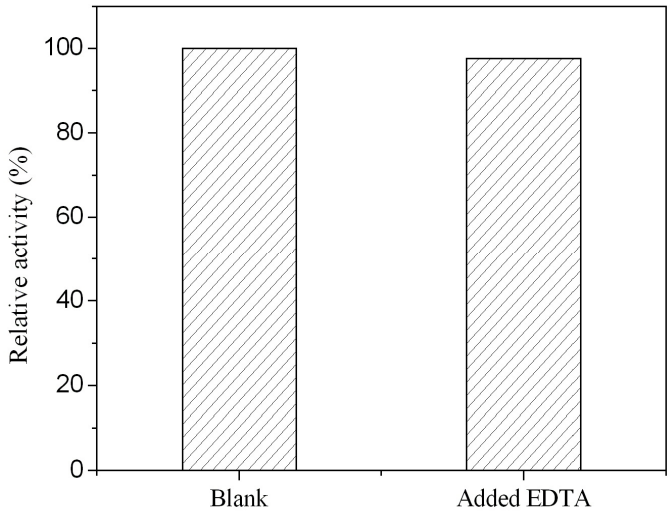

Fig. S4 The absorbance of the reaction solution before and after addition of EDTA. Conditions: 1 mM H_2O_2, 1 μg mL^{-1} C-Dots, 50 μM TMB, and 1 mM EDTA.

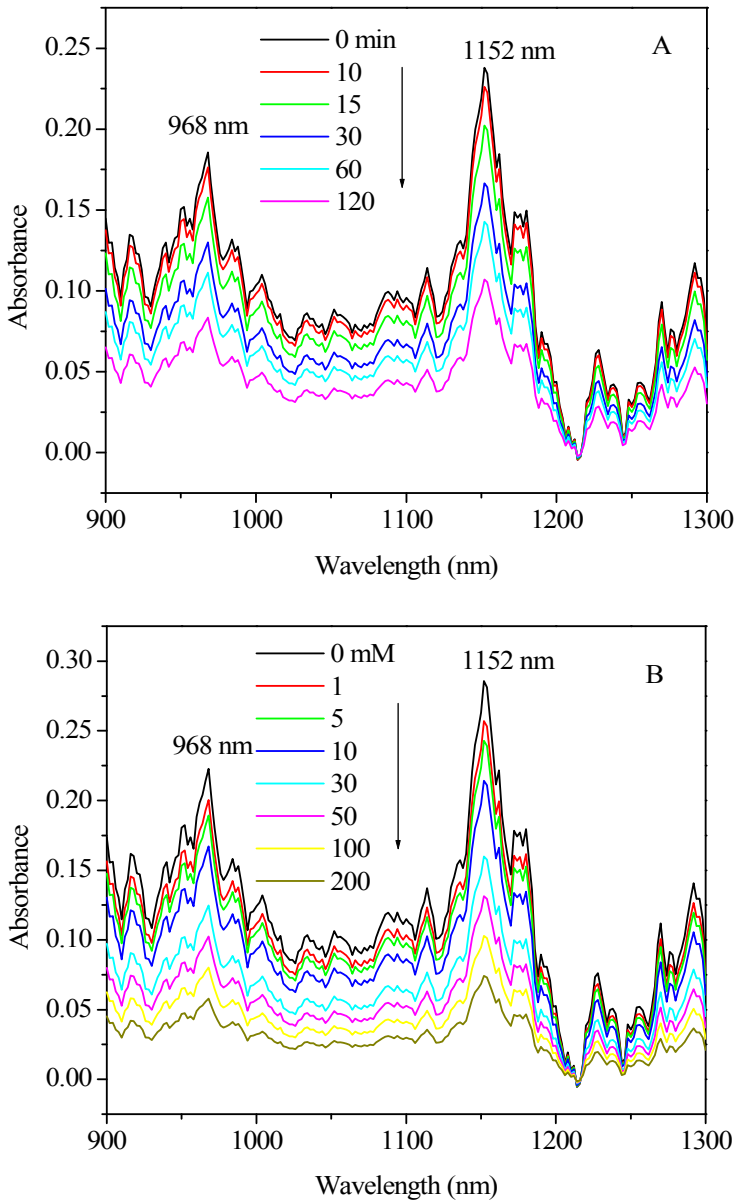

Fig. S5 (A) Absorption spectra of 20 μg mL^{-1} C-Dots in a pH 3.5 acetate buffer solution change as a function of time after addition of 20 mM H_2O_2; (B) Absorption spectra of 20 μg mL^{-1} C-Dots in a pH 3.5 acetate buffer solution change as a function of H_2O_2 concentration after 60 min.

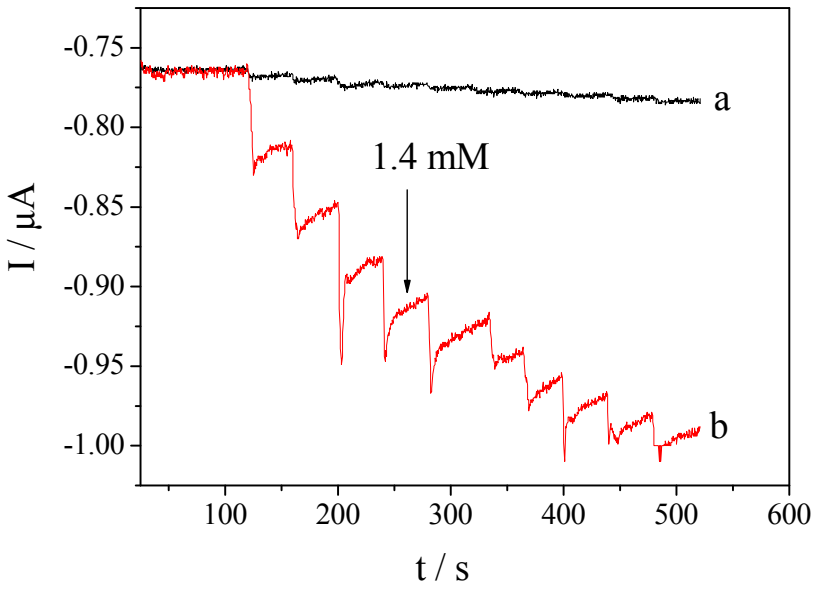

Fig. S6 Amperometric response of (a) bare GCE and (b) the C-Dots modified GCE in 0.1 M PBS (pH 7.0) at applied potential of -0.4 V upon successive additions of 1.4 mM H_2O_2 in the time intervals of 40 s.

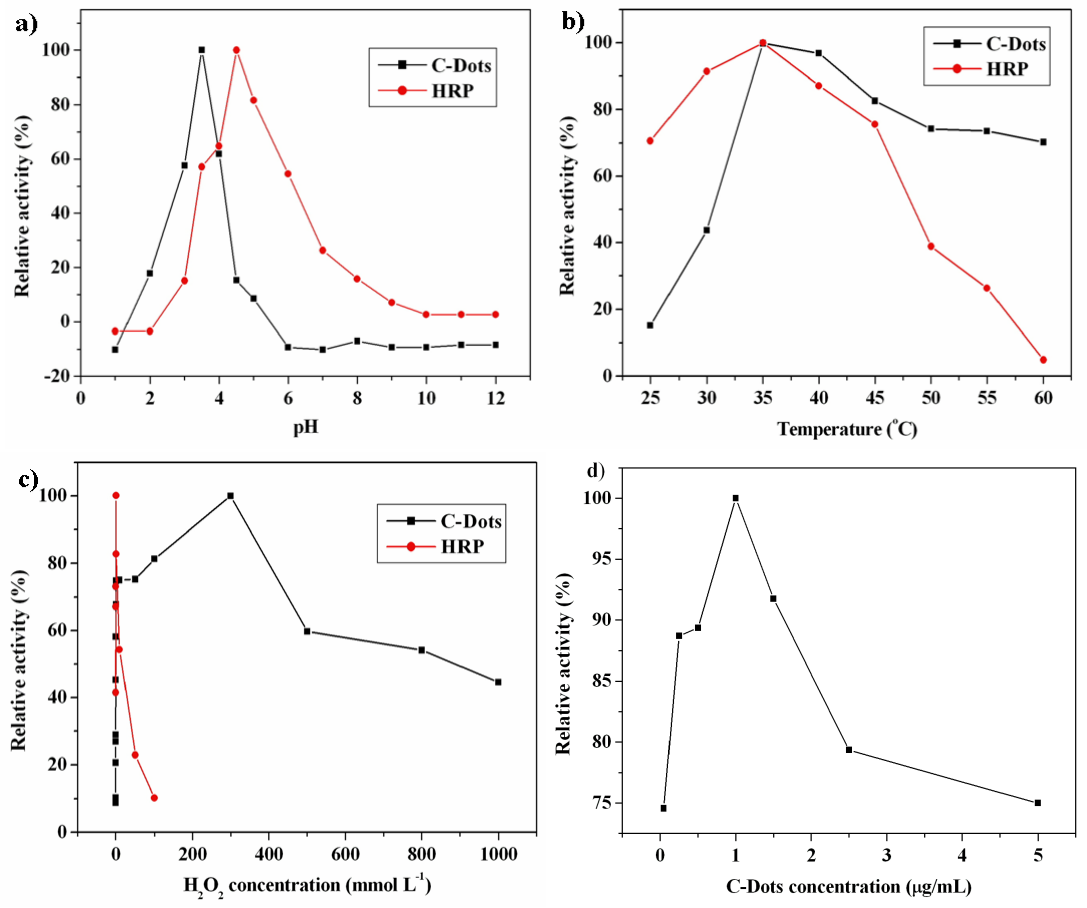

Fig. S7. Dependency of the C-Dots peroxidase-like activity on a) pH, b) temperature, c) H_2O_2 concentration and d) C-Dots concentration. Experiments were carried out using 1 μg mL^{-1} C-Dots or 1 ng mL^{-1} HRP in 10 mL of 0.2 M NaAc buffer with 50 μM TMB as substrate. The H_2O_2 concentration was 1 mM at pH 3.5 and 35 °C unless otherwise stated. The maximum point in each curve was set as 100 %.

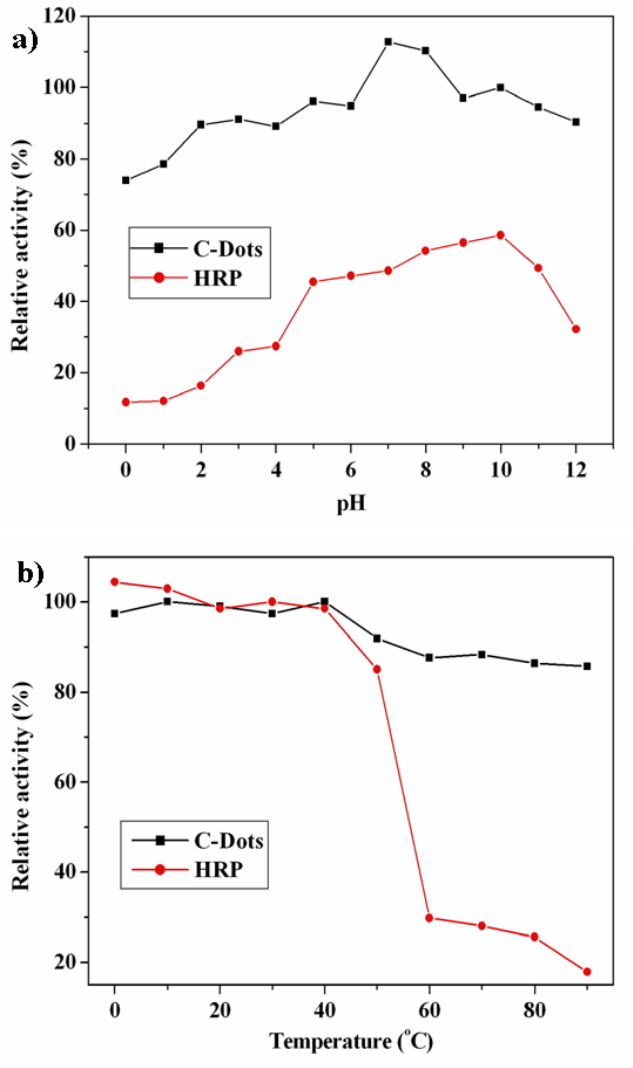

Fig. S8. Comparison of the stability of C-Dots and HRP. a) C-Dots and HRP were first incubated at pH 0–12 for 2 h and then their peroxidase activities were measured under standard conditions. b) C-Dots and HRP were first incubated at 0–90 °C for 2 h and then the peroxidase activity was measured under standard conditions.

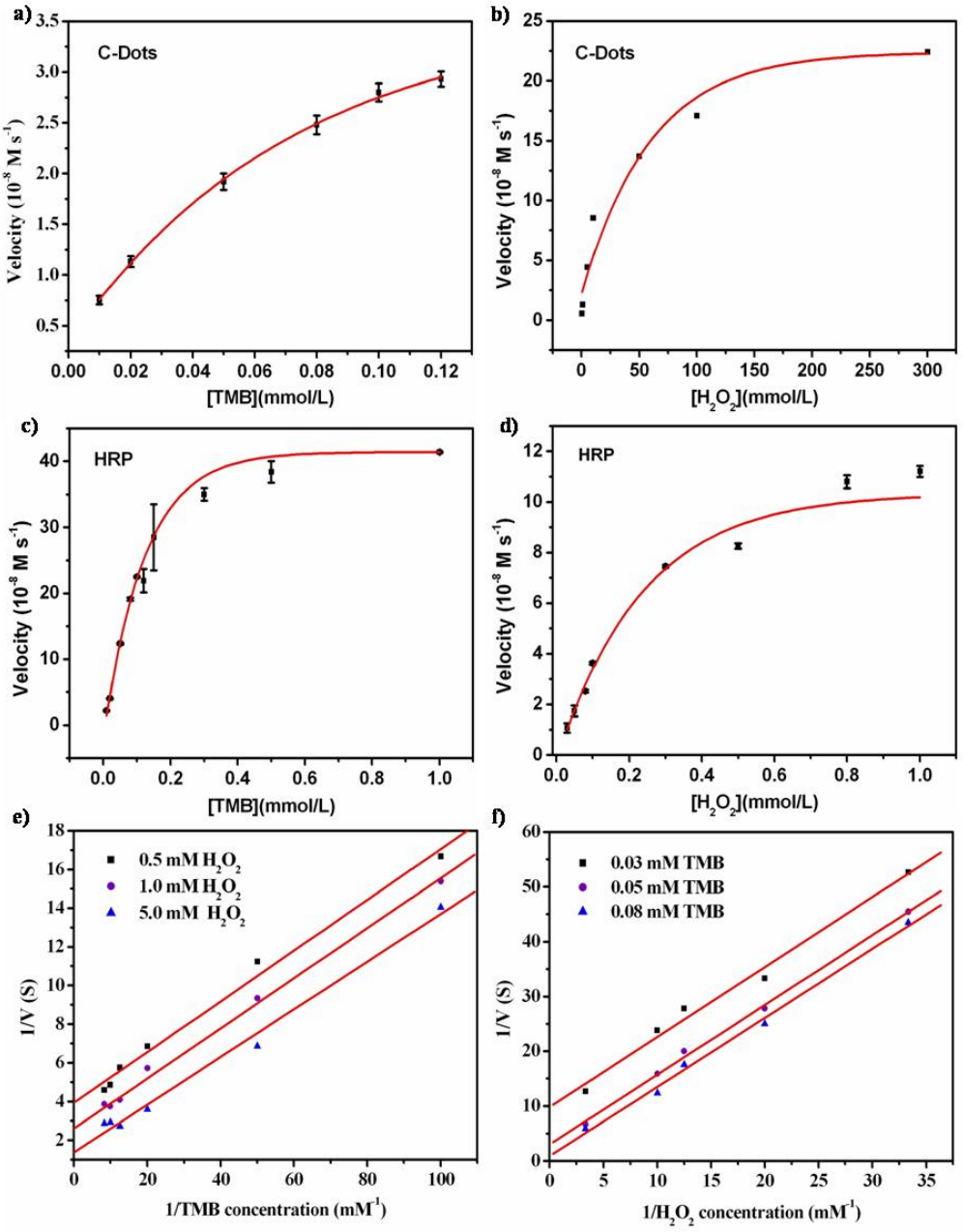

Fig. S9. Steady-state kinetic assay and catalytic mechanism of C-Dots (a–d). The velocity (v) of the reaction was measured using 1 μg mL^{-1} C-Dots (a, b) or 1 ng mL^{-1} HRP (c, d) in 10 mL of 0.2 M NaAc buffer at pH 3.5 and 35 °C. The error bars represent the standard error derived from three repeated measurements. a, c) The concentration of H_2O_2 was 50 mM for C-Dots or 1 mM for HRP and the TMB concentration was varied. (b, d) The concentration of TMB was 0.8 mM and the H_2O_2 concentration was varied. (e, f) Double reciprocal plots of activity of C-Dots with the concentration of one substrate (H_2O_2 or TMB) fixed and the other varied. Details are included in the Experimental Section.

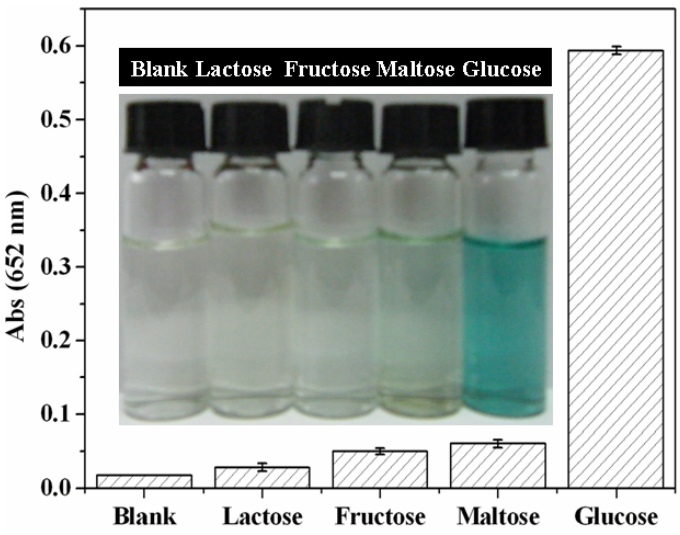

Fig. S10. Determination of the selectivity of glucose detection with 5 mM lactose, 5 mM fructose, 5 mM maltose, and 1 mM glucose. The error bars represent the standard deviation of three measurements. Inset: The color change with the different solutions.

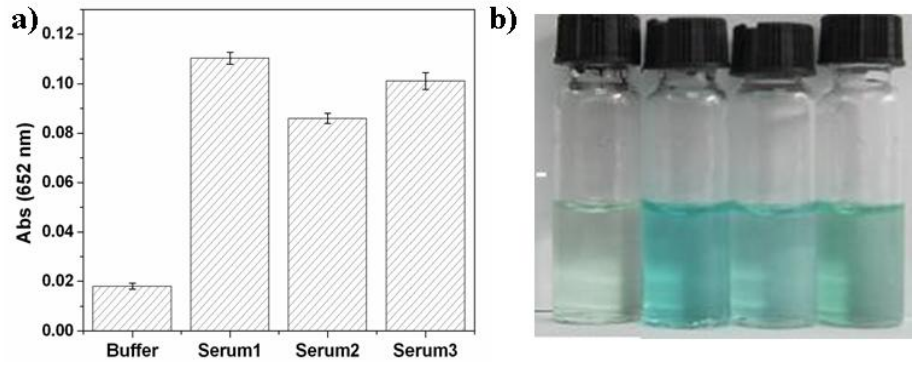

Fig. S11. a) The absorbance at 652 nm for buffer solution and a 10-fold serial dilution of a serum sample, Error bars represent the standard deviation for three measurements. b) Images of production of colored product for serum samples.