



## Communication

pH and H<sub>2</sub>O<sub>2</sub> dual-responsive carbon dots for biocatalytic transformation monitoringWenxin Lv<sup>1</sup>, Xin Wang<sup>1</sup>, Jiahui Wu, Haiyin Li\*, Feng Li\*

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## ABSTRACT

Development of sensitive biosensors for biocatalytic transformations monitoring is in high demand but remains a great challenge. It is ascribed to the current strategies that focused on the single metabolite detection, which may bring about the relatively low sensitivity and false diagnosis result. Herein, we report the design and fabrication of novel carbon dots (CDs) with strong orange light emission, pH and H<sub>2</sub>O<sub>2</sub> dual-responsive characteristics. The fluorescence quenching of CDs by H<sup>+</sup> and H<sub>2</sub>O<sub>2</sub> enables the highly sensitive detection of H<sup>+</sup>/H<sub>2</sub>O<sub>2</sub>-generating biocatalytic transformations. This is exemplified by the glucose oxidase-mediated catalytic oxidation reaction on glucose, in which H<sup>+</sup> and H<sub>2</sub>O<sub>2</sub> would be formed. As compared to the case in which glucose is present, significant fluorescence reduction is detected, and the fluorescence intensity is negatively proportional to glucose concentration. Thus, highly sensitive detection of glucose was readily achieved with a detection limit down to 10.18 nmol/L. The prepared CDs not only realize the highly sensitive detection of glucose, but also allows the probing other substances by changing the enzymes, thus providing a versatile platform, and demonstrating good potential to be used for biocatalytic transformations effective monitoring.

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Enzyme-assisted biocatalytic transformations, play key regulatory mechanism in human life activities and medical interventions, and have attracted ever-increasing attentions [1–3]. Accumulating information has witnessed that it is favorable for people to guard against, recognize, and treat diseases through monitoring the biocatalytic transformations between different enzymes and substances [4–6]. For example, glucose, the amount of which was closely related with human health, can be catalyzed and hydrolyzed into H<sub>2</sub>O<sub>2</sub> and gluconic acid with the aid of glucose oxidase (GOx) [7]. For the sake of comprehending the process of biocatalytic transformations, substantial efforts have been made. Heinen *et al.* reported two antagonistic enzymes with a pH-modulating effect in a feedback controlled biocatalytic reaction system [1]. Su's group demonstrated a label-free fluorescence sensing platform for specifically monitoring H<sub>2</sub>O<sub>2</sub>-generated biocatalytic processes [8]. Evidently, the strategies mainly focused on the analysis of biocatalytic products. Among them, H<sub>2</sub>O<sub>2</sub> and H<sup>+</sup> are the primary metabolites, and generated through the catalyzed oxidation of enzymes toward the corresponding substances with the aid of O<sub>2</sub>. Despite the enhanced analytical performance,

thereported sensing strategies suffered from the single metabolite (H<sub>2</sub>O<sub>2</sub> or H<sup>+</sup>) detection, which is bad for increasing the sensitivity and improving the diagnosis accuracy in the early stage of biocatalytic transformations [9,10]. Moreover, it is well known that the sensing performance of the proposed biosensor basically concentrated on the properties of the chosen signal materials [11–15]. **With the aim to the above issues, we, herein, try to design and develop a H<sub>2</sub>O<sub>2</sub> and pH dual-responsive biosensor, which favored the better comprehension of biocatalytic transformations.**

Carbon dots (CDs) are a broad class of carbon-based materials with ultra-small size, strong stability, high fluorescence quantum yield, and excellent biosafety [16–22]. These unique characteristics make CDs as ideal alternatives to previously reported optical materials (organic/polymeric dyes, quantum dots, metal clusters, metal complexes, etc.) for different analytes probing [23–25]. In view of the distinguished properties, we believe that CDs could realize the sensitive motoring of the enzyme-assisted biocatalytic transformations through assaying the expression level of the related metabolites (H<sub>2</sub>O<sub>2</sub> and H<sup>+</sup>). However, to our knowledge, most of the reported CDs commit themselves to single metabolite detection, which does not favor us to monitor the enzymatic reactions. In addition, their excitation wavelengths suffered from the ultraviolet light or blue light, which easily resulted in significant background noise, subsequently reducing the sensitivity and diagnosis accuracy. From this context, it is highly desirable to design and prepare H<sub>2</sub>O<sub>2</sub>

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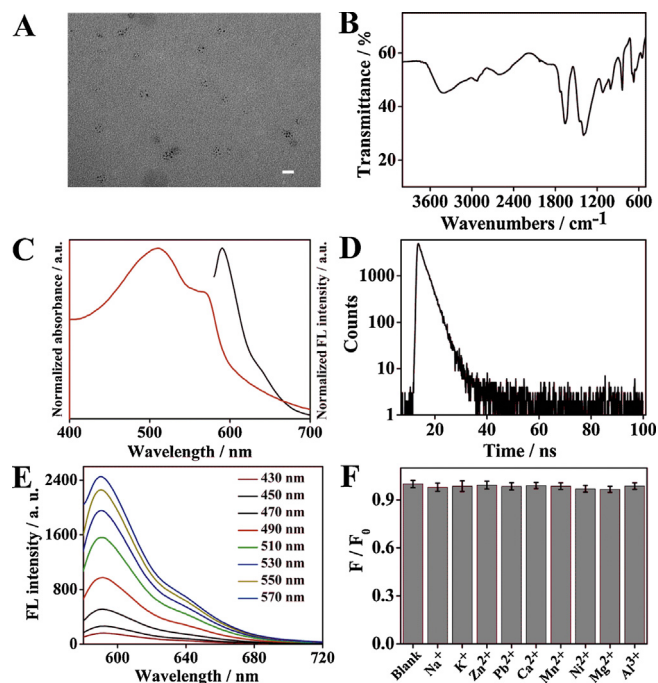
and pH dual-responsive CDs with long excitation wavelength, which are competent for better assessing the biocatalytic transformations.

To develop CDs-based biosensor for biocatalytic transformations monitoring, it is prerequisite to fabricate CDs with strong fluorescence, long emission wavelength, and dual responsive capability. Very recently, it is reported that arylamines can be used as ideal precursors to fabricate CDs with unique optical properties [18,21]. Inspired by these studies, we infer that CDs with ideal properties and dual responsive capability could be fabricated via using their analogous as precursors. In the present study, *p*-phenylenediamine was selected as the raw material to fabricate CDs via the hydrothermal carbonization in acidic solutions for 10.0 h, and the diagram was depicted in Fig. S1 (Supporting information). The identity of CDs was firmly verified by TEM (Fig. 1A). Evidently, the as-prepared CDs are uniform in size with a diameter of about 5 nm. After that, zeta potential was carried out to study their stability in water solution, and the results demonstrated that they enjoy exceptional water stability with zeta value of about +34.5 mV. This also justified the positive groups on CDs surface. Further, the positive groups can also be confirmed by FT-IR peaks located at 3420 and 1653  $\text{cm}^{-1}$  corresponding to  $-\text{NH}_2$  (Fig. 1B). This is not surprise to us that the  $-\text{NH}_2$  come from the carbon source *p*-phenylenediamine.

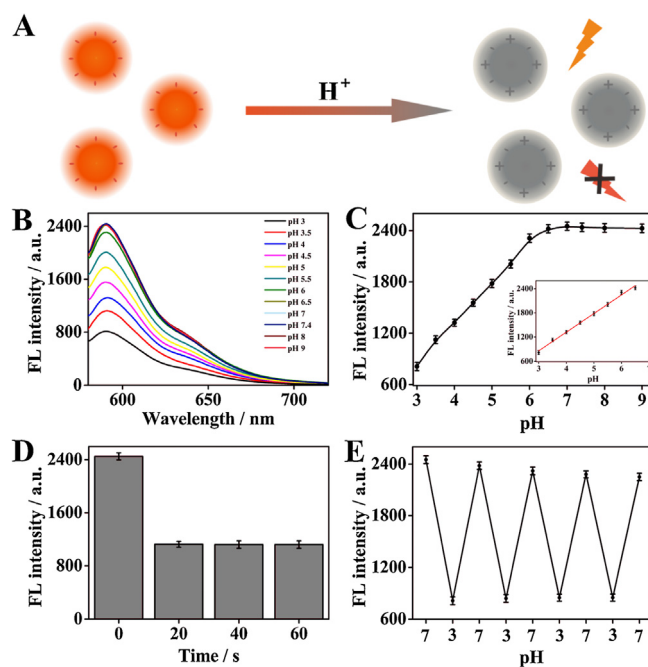
To evaluate the optical behavior of the prepared CDs, we carried out UV-vis/fluorescence characterizations on CDs in phosphate buffer (PB). As manifested in Fig. 1C, the CDs possess two obvious absorption peaks located at 510 nm and 570 nm, respectively, corresponding to the conjugated systems from part to whole. In addition, the prepared CDs emit strong fluorescence with the wavelength located at 590 nm, which is more favorable to reduce the background interference than the short emission wavelength. Further, the time-resolved emission decay behavior of the CDs was studied, and the curve and parameters were depicted in Fig. 1D and

Table S1 (Supporting information), respectively. It is evident that two relaxation pathways existed in the decay, implying that the prepared CDs possess two irrelevant emissions from different conjugation segments. The weighted mean lifetime ( $\tau$ ) was calculated to be 2.60 ns, corresponding well to typical fluorescence decay level for CDs. Changing the excitation wavelength from 430 nm to 570 nm demonstrated that the fluorescence peaks of the CDs are excitation-wavelength-independent (Fig. 1E). Prior to application in the field of bioanalysis, the stability of the prepared CDs was investigated by adding different metal ions into CDs' solution to evaluate the fluorescence changes. As depicted in Fig. 1F, the fluorescence intensities changed slightly in the presence of different metal ions, even their concentrations increased to 2 mmol/L. This successfully verified the excellent stability of CDs in complex solution, and thus, CDs can be used as ideal biosensor to detect target analyte in real sample.

Based on the unique properties of the prepared CDs, we adopt them as probes to detect different pH value in the sensing system, and the detection diagram was manifested in Fig. 2A. When the pH value reduced, the fluorescence intensity rapidly decreased. It is not surprise and can be attributable to the amino protonation on the surface of CDs, which increased the intramolecular electron transfer ability between  $-\text{NH}_3^+$  and CDs, subsequently reducing the fluorescence intensity. The detailed fluorescence curves of CDs in different pH solutions were illustrated in Fig. 2B. Evidently, in the pH value range of 7.0–9.0, the intensity changed negligibly, due to the low protonation of  $-\text{NH}_2$  in the slightly alkaline and neutral solutions. However, as the pH value reduced from 7.0 to 3.0, their fluorescence intensities decreased accordingly. To quantitatively evaluate the responsive capability of CDs toward different pH value, the working curve was drafted using fluorescence intensity ( $F$ ) as the vertical coordinate and pH value as the horizontal coordinate (Fig. 2C). The result evidently demonstrated that the  $F$  is linearly relevant to pH value in the range of 6.5–3.0. The linear equation was determined to be  $F = -530.15\text{pH} + 462.69$  with correlation coefficient of 0.9946.



**Fig. 1.** (A) TEM image of the CDs (scale bar: 20 nm). (B) FT-IR spectrum of the CDs. (C) Normalized absorption (red curve) and fluorescence spectra (black curve) of the CDs. (D) Fluorescence lifetime of the CDs. (E) Fluorescence spectra of CDs with different excitation wavelength. (F) Stability studies of the CDs in the presence of different metal ions. ( $F_0$  and  $F$  represented the intensity in the absence/presence of metal ions, respectively).



**Fig. 2.** (A) Schematic illustration of the CDs-based biosensor for different pH value. (B) Fluorescence spectra of the CDs corresponding to different pH values. (C) Fluorescence intensity of CDs versus different pH values. Inset: Linear plot of FL intensity versus pH value. (D) Fluorescence intensity of CDs versus different incubation time. (E) Reversibility of fluorescence between pH 3 and pH 7.

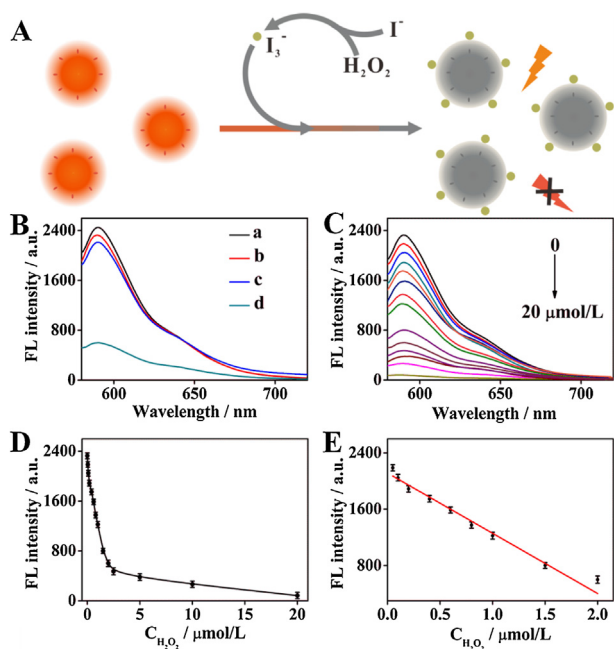
Subsequently, the responsive time was discussed (Fig. 2D). The result demonstrated that 20 s was adequate for completing the analytical experiment. Extending the incubation time to 40 s or 60 s would not influence the fluorescence intensity. Next, the reversibility of CDs against different pH was investigated by changing the pH value of the sensing system to 3.0 and 7.0 four times repeatedly. As shown in Fig. 2E, the fluorescence response presents an excellent reproducibility between highly acidic system and neutral system, demonstrating the prepared CDs can be used as ideal optical material for rapid/effective pH detection. Furthermore, the UV-vis and time-resolved emission spectra of CDs demonstrated that the fluorescence is static quenching toward  $H^+$  (Figs. S2 and S3, Table S2 in Supporting information).

$H_2O_2$  is another important metabolite generated from the enzyme-assisted biocatalytic transformations, and acts a key role in diagnosis and management of various diseases [2]. In view of this, the responsive ability of the prepared CDs toward  $H_2O_2$  was investigated with the help of  $I_3^-$ , and the scheme was manifested in Fig. 3A. Upon the addition of  $H_2O_2$ ,  $I^-$  would be oxidized into  $I_2$ , which reacted with  $I^-$  to produce  $I_3^-$ .  $I_3^-$  enjoyed strong negative charge and oxidation ability, which benefitted them to be adsorbed on the surface of CDs through the electrostatic interaction, simultaneously decreasing the CDs' fluorescence via the catalyzed oxidation reaction. Such fluorescence changes can be justified by the fluorescence measurements (Fig. 3B). The CDs gave a strong orange emission with the intensity of 2450 a.u. After the addition of  $I^-$ , the fluorescence intensity changed slightly with the value of only 123 a.u., successfully suggesting that  $I^-$  made little influence on the fluorescence of CDs. Then, when target  $H_2O_2$  was further added into the sensing solution, the fluorescence signal significantly reduced. This obvious decrease firmly justified the feasibility of CDs-based assay for highly sensitive detection of  $H_2O_2$ . Meanwhile, hardly changes in UV-vis spectra and lifetime were observed (Table S3, Figs. S4 and S5 in Supporting information), implying the static fluorescence quenching of CDs by  $H_2O_2$ . Subsequently, when ascorbic acid (AA) or glutathione (GSH) was added into the reaction system (Fig. S6 in Supporting

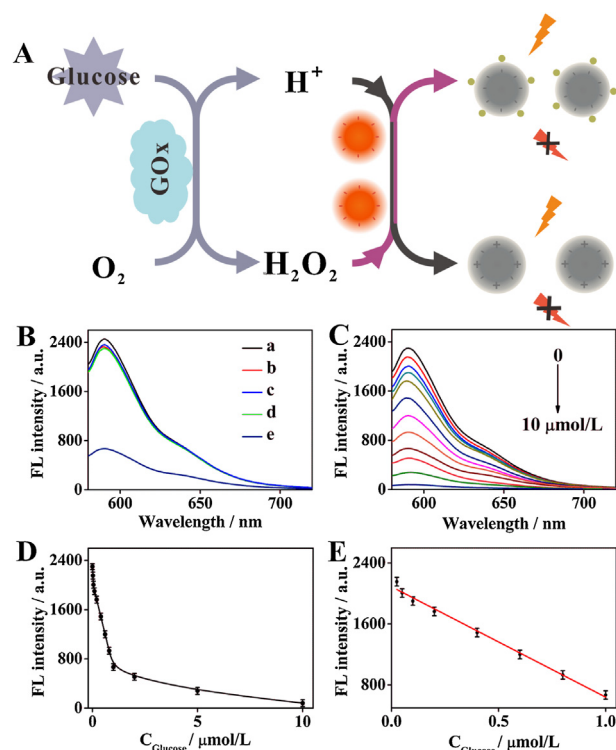
information), the intensity changed negligibly, justifying the irreversible interaction of  $I_3^-$  on the prepared CDs.

Under the optimization of correlative factors (Figs. S7 and S8 in Supporting information), the fluorescence responses of the sensing system toward different concentrations of  $H_2O_2$  were studied. As shown in Fig. 3C, the fluorescence intensity of CDs gradually decreased with the increase of  $H_2O_2$  concentrations from 0 to 20  $\mu\text{mol/L}$ . Evidently, the fluorescence intensity (F) presented a negative relationship with the increased  $H_2O_2$  concentrations. Furthermore, the F is linearly relevant with  $H_2O_2$  concentrations ( $C_{H_2O_2}$ ) in the range from 0.05  $\mu\text{mol/L}$  to 2  $\mu\text{mol/L}$ . The linear equation is  $F = -855.44 C_{H_2O_2} + 2113.77$  with correlation coefficient of 0.9860 (Fig. 3E). Additionally, the detection limit was calculated to be 19.24 nmol/L based on three sigma rule ( $3\sigma$ ), which, as shown in Table S4 (Supporting information), was comparable or superior to those of existing fluorescent methods for  $H_2O_2$  probing. These results demonstrated that the prepared CDs exhibited excellent responsive ability toward  $H_2O_2$ , and can be used as ideal sensor for monitoring biocatalytic transformations.

As is mentioned above, enzymes could catalyze oxidation of their substrates to produce metabolites ( $H_2O_2$  and  $H^+$ ), which can be applied as biomarkers for biocatalytic transformations. Based on the unique properties of the prepared CDs, the enzyme-assisted biocatalytic transformations, such as glucose/glucose oxidase, cholesterol/cholesterol oxidase, sarcosine/sarcosine oxidase, lactic acid/lactic acid oxidase, uric acid/uric acid oxidase, can be sensitively monitored [2]. In the present study, the GOx/glucose system was studied as the proof-of-concept target, and the diagram was illustrated in Fig. 4A. Glucose was hydrolyzed into gluconic acid and  $H_2O_2$  by GOx, which would significantly decrease the CDs' fluorescence. Thus, glucose can be sensitively detected



**Fig. 3.** (A) Schematic illustration of the CDs-based biosensor for  $H_2O_2$  biosensing. (B) Fluorescent spectra of CDs under different conditions: (a) CDs, (b) CDs + KI, (c) CDs +  $H_2O_2$ , (d) CDs + KI +  $H_2O_2$ . (C) Fluorescence spectra of CDs corresponding to  $H_2O_2$  concentrations. (D) Fluorescence intensity of CDs versus different  $H_2O_2$  concentrations. (E) Linear plot of FL intensity versus  $H_2O_2$  concentrations.



**Fig. 4.** (A) Schematic illustration of the CDs-based biosensor for glucose biosensing. (B) The fluorescence spectra of the CDs under different conditions: (a) CDs, (b) CDs + KI, (c) CDs, KI and glucose, (d) CDs, KI and glucose oxidase, (e) CDs, KI, glucose oxidase and glucose. (C) The fluorescence spectra of the CDs corresponding to different concentrations of glucose. (D) Fluorescence intensity of CDs versus different glucose concentrations. (E) Linear plot of FL intensity versus the different concentrations of glucose.

through the fluorescence quenching based on the prepared CDs. As shown in Fig. 4B, when glucose or GOx was severally added into the sensing system, hardly change in fluorescence intensity was observed compared with that of CDs. However, when both glucose and GOx were added into the sensing system, the fluorescence significantly decreased. As demonstrated in Figs. 4C and D, a regular decrease in fluorescence intensity was detected along with the increase of glucose concentration from 0 to 10  $\mu\text{mol/L}$ . This is consistent with the fact that more glucose would be catalyzed and hydrolyzed into more  $\text{H}^+$  and  $\text{H}_2\text{O}_2$ , thus more significantly decreasing the fluorescence intensity. It can be clearly found that the fluorescence intensity of the sensing system is negatively proportional to glucose concentrations ranging from 0.025  $\mu\text{mol/L}$  to 1  $\mu\text{mol/L}$  (Fig. 4E). The linear equation was  $F = -1447.54C_{\text{Glu}} + 2088.27$  with correlation coefficient of 0.9910 and detection limit of 10.18 nmol/L. It should be noted that the LOD was lower than that of previously reported methods (Table S5 in Supporting information), which can be mainly ascribed to the pH and  $\text{H}_2\text{O}_2$  dual-responsive properties of CDs.

To evaluate the selectivity of the proposed method, maltose, lactose, galactose, sucrose, mannose, and xylose with their structures similar to that of glucose, were selected as the interferences (Fig. S9 in Supporting information). The results indicated that in the presence of one of the interferences, the fluorescence intensity changed negligibly compared with that of CDs. Only in the presence of glucose, the fluorescence intensity reduced significantly. These results successfully confirmed the excellent specificity of the CDs to discriminate glucose from interfering saccharides. To further demonstrate the potential practical application of the prepared CDs, the pretreated serum and urine are analyzed. 1.0 mmol/L glucose was added into them. The samples were diluted with PB until glucose concentration was in the range of the CDs-based probe. As shown in Tables S6 and S7 (Supporting information), the recoveries ranged from 95.6% to 103.4% with all RSDs below 4.50%, indicating the good practicability of CDs-based biosensor.

In summary, we presented a highly sensitive biosensor for biocatalytic transformations using CDs as fluorophore. The CDs with orange emission were prepared via the hydrothermal reaction using *p*-phenylenediamine as the carbon resource, and enjoyed pH and  $\text{H}_2\text{O}_2$  dual-responsive characteristics. Based on the excellent properties, the CDs were used as nanosensors for probing biocatalytical transformations, using glucose/GOx as the model target. The CDs' emission was quenched by  $\text{H}^+$  and  $\text{H}_2\text{O}_2$  generated from the catalytic oxidation of glucose by GOx. Thus, highly sensitive detection of glucose was realized with the detection limit

of 10.18 nmol/L, which is lower than that of previously reported fluorescence methods. Moreover, highly sensitive detection of other enzyme-mediated biocatalytic transformations can also be simply achieved through identifying the expression level of  $\text{H}^+$  and  $\text{H}_2\text{O}_2$ , thus presenting a versatile platform for better monitoring the biocatalytic transformations.

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## Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.cclet.2019.06.029>.

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