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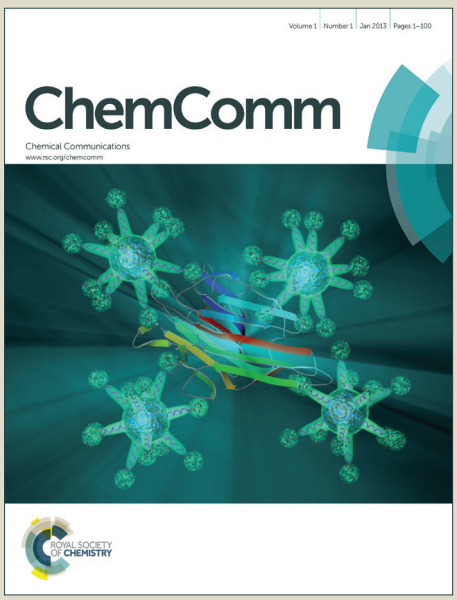

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Carbon dot-based fluorescence turn-on sensor for hydrogen peroxide with a photo-induced electron transfer mechanism

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A carbon dot-based fluorescence turn-on sensor for hydrogen peroxide (H_2O_2) with a photo-induced electron transfer mechanism was developed. The sensor exhibits good selectivity, sensitivity, and rapid response to H_2O_2 with a detection limit of 84 nM. The sensor maintains excellent sensing performance in a wide pH range.

Hydrogen peroxide (H_2O_2) is a major reactive oxygen species (ROS) in living organisms. Recent studies have demonstrated that H_2O_2 poses a substantial threat to living cells when its concentration exceeds 0.7 μM.[1] An abnormal level of H_2O_2 in the biological system is an indicator of health issues.[2] H_2O_2 is also extensively applied in chemical fields, biological fields, food and environmental disinfection, and so on.[3] High H_2O_2 concentration could be a great threat to the environment and human health; for example, it may lead to the generation of acid rain and could induce a number of diseases, including Parkinson's disease, senile dementia, and even cancer.[4] Obviously, sensitive and reliable detection of H_2O_2 is of particular importance in biological and toxicological diagnosis.

Approaches, including chemical titrimetric determination,[5] electrolytic analysis,[6] High-performance liquid chromatography,[7] and spectrophotometry,[8] are available to measure H_2O_2. However, these approaches generally require expensive instruments and entail low sensitivity and/or sophisticated sample preparation. Fluorescent sensors can be a powerful assay in this area because of their simplicity, high sensitivity, rapid response, and capacity for real-time and in situ monitoring of dynamic processes.[9] Considerable research effort has been devoted to the development of highly selective and sensitive fluorescent sensors to trace H_2O_2 and perform quantitative detection; many sensors based on semiconductor quantum dots or organic dyes have been reported.[10] However, these probes often involve complex preparation procedures and exhibit poor biocompability and water solubility, issues that hamper their further practical application.

As a promising fluorescent material, carbon dots (CDs) have elicited much research interest recently because of their outstanding properties, such as easy preparation, readiness for modification, good water solubility, chemical inertness, favorable biocompatibility, and tunable fluorescence (FL).[11] Moreover, CDs are suitable electron donors or acceptors.[12] These merits make CDs especially useful for fluorescent bioimaging and biosensing.[13] Shan *et al*. demonstrated that the FL of boron-doped CDs (B-CDs) can be quenched by H_2O_2 through charge transfer from H_2O_2 to the B atom in B-CDs. However, 25 μM of H_2O_2 is required to induce FL spectrum quenching.[14] Using formic acid as the precursor, Sadhukhan *et al*. synthesized CDs by implementing a microwave-assisted thermal method.[15] The FL intensity of these CDs was quenched with H_2O_2 at ~700 nM limit of detection (LOD); meanwhile, their selectivity to other ROS needs to be further studied. The FL behavior of the CDs was found to be sensitive to the solvent and pH of the medium. Zhang *et al*. constructed a fluorescence resonance energy transfer (FRET)-based FL turn-off sensor to detect H_2O_2 with the assistance of horseradish peroxidase (HRP). Although LOD was calculated to be 200 nM, the response time was as long as 18 min.[16] Wei *et al*. recently reported another FL turn-off CD-based sensor for H_2O_2 with LOD of approximately 50 nM.[3, 17] The FL intensity of the CDs was easily quenched by H_2O_2 in the presence of Fe^{2+}. The researchers inferred that the hydroxyl radical ($OH^{\bullet}$) produced from H_2O_2 and Fe^{2+} was the cause of quenching. However, it only worked in the strong acid environment (pH 2~5), and the response time was as long as 10 min. Table S1 summarizes the sensing performance of several recently reported CD-based H_2O_2 FL sensors. All of the sensors provide an FL quenching signal. The response time, pH stability, and selectivity toward other reactive oxygen species of these FL turn-off sensors need to be further improved for practical applications.

Together with our continuing effort in the exploration of fluorescent CDs for bioimaging and biosensing,[18] we report on the development of a novel CD-based FL turn-on sensor for H_2O_2 monitoring in aqueous solutions. A photo-induced electron transfer (PET) mechanism was utilized in the design of the proposed sensor

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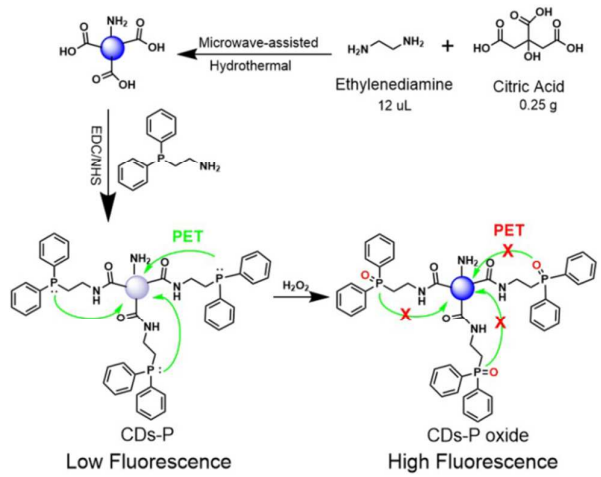
(CDs-P).[19] Diphenylphosphine moiety was covalently coupled to the surface of the CDs, and they served as the PET donor and acceptor, respectively. A schematic of CDs-P selective FL turn-on sensor for H_2O_2 is shown in Scheme 1. The detailed experiments are provided in the supporting information. Blue emission CDs were prepared through a microwave-assisted hydrothermal method with citric acid and ethylenediamine as the precursors. After being modified with 2-(diphenylphosphino) ethylamine, the FL of the obtained CDs-P was quenched through PET. However, in the presence of H_2O_2, diphenylphosphine can selectively react with H_2O_2 to produce diphenylphosphine oxide. The PET derived from the lone electron pair of the phosphorus atom to the CDs was cancelled and FL intensity increased.[20] The developed sensor has several advantages. First, it is the first CD-based FL turn-on sensor for H_2O_2 that can ensure reliable signal variation and accurate detection with a linear range of detection from 0 to 2 μM and detection limit of 84 nM. The LOD of the sensor satisfies the safety concentration (0.5–0.7 μM). Second, the sensor is based on a PET mechanism through a specific chemical reaction; thus, the sensor exhibits a rapid response and excellent selectivity to H_2O_2. It also exhibits good sensing performance in a wide pH range (from 4 to 12). Moreover, the synthesis approach of the sensor is more cost-effective than that of sensors applied to synthesize other fluorescent materials, such as organic dyes, noble metal nanoparticles, and semiconductor QDs.



Scheme 1 Schematic of the fabrication and sensing process of CDs-P for H_2O_2.

The transmission electron microscope (TEM) image (Fig. 1a) shows that the as-prepared CDs are spherical and disperse uniformly. The inset size distribution analysis demonstrates that the diameter of the CDs is around 2.7 nm. The high-resolution TEM (HRTEM) image clearly shows the crystalline structure of the CDs with a lattice spacing of ~0.21 nm (Fig. 1a, inset), which is in agreement with the value of the (100) planes of graphitic carbon.[21] The topographic morphology of CDs was further measured by atomic force microscopy (AFM), as shown in Fig. 1b and Fig. S1, the CDs are uniform in size with a height ranging from 2 nm to 3 nm. The X-ray diffractometer (XRD) pattern of the CDs (Fig. S2) displayed a broad peak centered at ~18°, which may be due to the small size of the CDs.[22] The X-ray photoelectron spectroscopy (XPS) and Fourier transform infrared (FTIR) spectra of the CDs were recorded to analyze the surface composition and elements of the CDs and CDs-P. As shown in Fig. 1c, the characteristic peaks of C1s, N1s, and O1s at about 284.5, 399, and 530 eV are presented in the XPS survey spectrum of CDs. The C/N/O atom ratio of the CDs was calculated to be 64.0/9.3/26.7. The high-resolution XPS spectrum of C1s in the inset of Fig. 1c was resolved into three peaks with binding energies of approximately 284.5, 285.6, and 288 eV corresponding to C-C, C-N, and C=O, respectively. The XPS analysis of CDs-P was provided in the supporting information (Fig. S3). Weak Phosphorus signals (P2p ~132 eV and P2s ~189 eV) were observed in the XPS survey spectrum, and the high-resolution P2p spectrum in the inset of Fig. S3 could be resolved to two peaks corresponding $P2p_3$ and $P2p_1$.

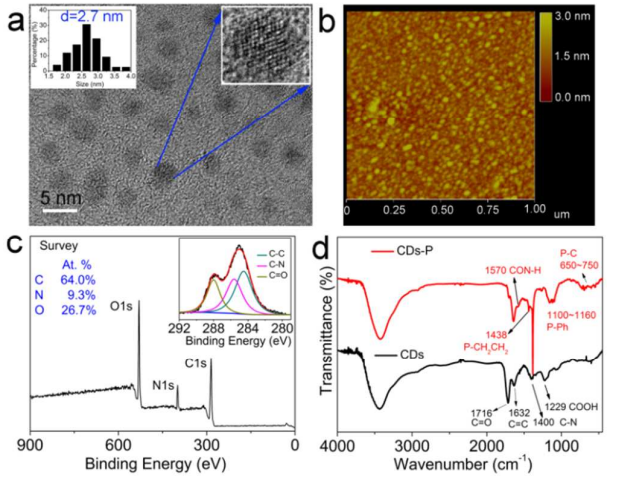


Fig. 1 (a) TEM image of the CDs. The insets show the particle size distribution histogram (n=60) and HRTEM image of the CDs. (b) AFM image of the CDs on a silica substrate. (c) XPS survey spectrum of the CDs. The inset in (c) shows the high-resolution C1s spectrum. (d) FTIR spectra of the CDs (black line) and CDs-P (red line)

Fig. 1d (black line) shows the FTIR spectrum of the CDs. The peaks at ~1400 and 1632 cm^{-1} is attribution to C-N and C=C bonds, respectively. The two peaks at 1229 and 1716 cm^{-1} confirm the existence of COOH group. In the FTIR spectrum of CDs-P (Fig. 1d, red line), the stretching vibration of C-N at 1400 cm^{-1} was strengthened significantly compared with that in the CDs. The absorption band around 1100-1160 cm^{-1} is corresponding to the P-Ph stretch mode. In addition, two new peaks at ~1438 and 1570 cm^{-1} which represent P-CH_2CH_2 and CO-NH bonds appeared. The absorption band around 650~750 cm^{-1} is matching with the P-C stretch mode. Since the most of the COOH group was converted into a CO-NH group, the peak at ~1229 cm^{-1} disappeared. These results suggest that the surface of the CDs were functionalized with amino, hydroxyl, and carboxylic moieties that originated from citrate acid and ethylenediamine, and 2-(diphenylphosphino) ethylamine were successful coupled onto the surface of the CDs.

The capability of the as-prepared CDs as an electron acceptor was studied by monitoring the fluorescence spectra of the CDs in the presence of strong electron donors, such as dopamine (at acid aqueous solution) and *N,N*-diethylaniline (DEA). As shown in Fig. S4a and b, the fluorescence of CDs was quenched by the addition of electron donors. The observed Stern-Volmer quenching constants

by dopamine and DEA were calculated to be 122 M^{-1} and 56 M^{-1}, respectively (Fig. S4c). However, under alkaline conditions, dopamine could act as an electron acceptor to quench the fluorescence of the CDs with a quenching constant of 114 M^{-1}. In addition, we found that the fluorescence of the CDs could also be quenched by strong electron acceptors, 2,4,6-trinitrotoluene (TNT) and 2,4-dinitrotoluene (DNT), as shown in Fig. S4d and e. The corresponding observed Stern-Volmer quenching constants were calculated to be 1633 M^{-1} and 1222 M^{-1}, respectively (Fig. S4f). The above results demonstrated that the as-prepared CDs in our experiments could act as either an electron donor or an acceptor, agreeing with the previous reports.[12]

The absorption spectra of the CDs and CDs-P are presented in Fig. 2a. The absorption band of the CDs at ~343 nm exhibited a blue shift to ~340 nm after surface modification with 2-(diphenylphosphino) ethylamine. A new strong peak at 263 nm was observed for the CDs-P sample; this peak is due to the typical absorption of the aromatic π system coming from the diphenyl moieties.[23] Fig. 2b shows the excitation and emission spectra of the CDs and CDs-P. The CDs aqueous solution displayed a strong FL peak at around 437 nm under 350 nm excitation. The FL quantum yield was calculated to be 0.73 with quinine sulfate as the reference. Its corresponding excitation spectrum exhibited a broad peak with the maximum at 345 nm. However, after modification with 2-(diphenylphosphino) ethylamine, the FL peak of CDs-P blue shifted to 430 nm, and the FL quantum yield decreased to 0.12. The excitation spectrum of CDs-P also blue shifted to 339 nm. Furthermore, the fluorescence lifetime of CDs and CDs-P was calculated to be 13.25 ns and 10.36 ns, respectively (Fig. S5). This FL quenching and a shorter fluorescence lifetime may be due to the effectiveness of the PET process, in which the lone electron pair of the P atom was transferred to the excited CDs and diminished the FL of the CDs. However, it is expected that the PET efficiency could be improved by using a stronger electron donor (triphenylphosphine).[20d, e] The FL spectra of both CDs and CDs-P exhibited a typical excitation wavelength-dependent behavior. As shown in Figs. S6a and S6b, the FL wavelengths red shifted under longer excitation wavelengths. However, the exact mechanism that resulted in the excitation wavelength-dependent behavior of the CDs needs to be further investigated.[24]

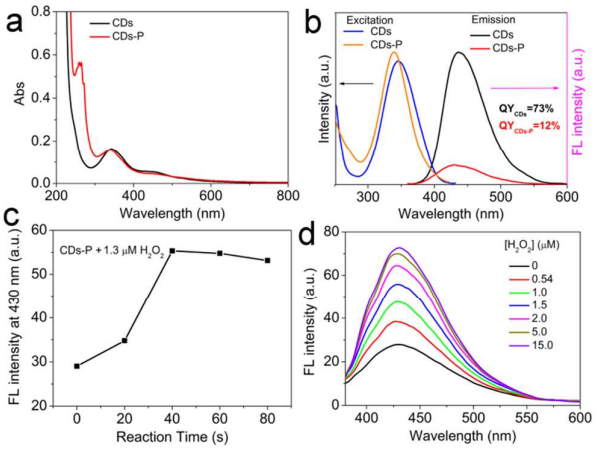


Fig. 2 (a) UV-vis absorption spectra of the CDs (black line) and CDs-P (red line) dispersed in an aqueous solution. (b) FL excitation and emission spectra (λ_{ex}=350 nm) of the CDs and CDs-P dispersed in an aqueous solution. (c) Time course of the FL intensity of CDs-P at 430 nm in the presence of 1.3 μM of H_2O_2 in HEPES buffer solution; the observation time interval is 20 s. (d) FL titration spectra of CDs-P in HEPES buffer solution with the gradual addition of H_2O_2 from 0 to 15 μM, λ_{ex} = 350 nm.

CDs-P shows much better photostability as compared with the organic dyes such as fluorescein disodium. The fluorescence intensity showed no obvious quenching after 1 h irradiation while the fluorescence of fluorescein disodium was quenched by about 50%. In addition, it was revealed that a high concentration of NaCl did not affect the CDs-P's fluorescence intensity (Fig. S7). These outstanding fluorescence properties enable CDs-P a promising fluorescent probe. The response velocity of CDs-P to H_2O_2 was investigated by monitoring the time-dependent FL intensity of the probe against 1.3 μM H_2O_2 in HEPES buffer solution (pH 7.2) at room temperature. As shown in Fig. 2c, the FL intensity of the CDs-P aqueous solution sharply increased from 0 to 40 s and saturated at 40 s after the addition of H_2O_2. CDs-P can perform as a FL turn-on sensor for the detection of H_2O_2 with a rapid response. Based on this condition, the following spectra were recorded at 60 s after the addition of H_2O_2. Fig. 2d shows the FL titration spectra of CDs-P upon the addition of H_2O_2 with a concentration range of 0 to 15 μM. An enhancement in FL intensity by approximately threefold was observed after adding 15 μM of H_2O_2. These results reveal that the PET process of CDs-P was effectively cancelled by H_2O_2. This PET on/off switching mechanism fluorescent sensor was further confirmed by the time-resolved fluorescence decay curves. As shown in Fig. S5, the fluorescence lifetime of the CDs-P aqueous solution was increased to 11.29 ns after addition of H_2O_2. The longer lifetime of CDs-P/H_2O_2 mixture indicates that the PET process was interrupted. To assess the sensitivity of the proposed sensor, FL intensity at 430 nm was plotted against H_2O_2 concentrations. As shown in Fig. S8, the FL intensity of CDs-P at 430 nm increased gradually when the concentration of H_2O_2 was raised from 0 to 2 μM and then reached a plateau. Only a slight change in FL intensity was observed when the concentration of H_2O_2 was larger than 2 μM. A good linear relationship was noted between FL intensity and H_2O_2 concentration, i.e., Y=28.2+19.1X, R^2=0.998. LOD was calculated to be 84 nM.[25] This result indicates that CDs-P can be applied as an FL turn-on senor for H_2O_2 with high sensitivity.

The effect of pH on the FL response of CDs-P to H_2O_2 was also investigated to further evaluate the practical applicability of the sensor. As shown in Fig. S9a, the FL intensity of CDs-P at 430 nm showed minimal changes in the pH range from 4 to 12 (black line) and was constantly maintained in the value range of 27 a.u. to 29 a.u. However, when 1.3 μM H_2O_2 was added to the samples, FL intensity was enhanced by about twofold (red line), indicating that CDs-P can effectively interact with H_2O_2 in a wide pH range. To investigate the ability of the sensor to selectively detect H_2O_2 in a complex system, CDs-P was made to react with other ROS (including $^1O_2$, ROO$^\bullet$, $O_2^{\bullet-}$, NO_2^-, OCl^-, $^\bullet$OH, $ONOO^-$) in HEPES buffer solution. As depicted in Fig. S9b, the FL intensities at 430 nm fluctuated between 20 and 30 a.u. in the presence of these ROS (2 μM) except H_2O_2. After the addition of 1.3 μM H_2O_2, FL intensity obviously increased as in the previous results (about twofold of the original value). Moreover, the selectivity of the CDs-P towards H_2O_2 and

other metal ions have been further investigated, as shown in Fig. S10. The addition of these metal ions showed no obvious effect on the fluorescence intensity of the CDs-P. These findings show the good selectivity of CDs-P to H_2O_2.

Conclusions

The PET mechanism was applied in the design of a CDs-based FL turn-on sensor for the detection of H_2O_2 in an aqueous solution. Blue emission CDs were synthesized through a microwave-assisted hydrothermal method with citric acid and ethylenediamine as the precursors. After surface functionalization of the CDs with 2-(diphenylphosphino) ethylamine, a PET mechanism-based sensor was fabricated. CDs and diphenylphosphine moiety served as the PET acceptor and donor, respectively. The PET process of CDs-P can be disturbed by H_2O_2, which oxides CDs-P into its oxidation state. CDs-P was thus demonstrated to be selective and sensitive to H_2O_2 with a detection limit of 84 nM. The sensor exhibited excellent pH stability and a rapid response to H_2O_2. We believe this strategy may provide a new approach for the construction of PET-based FL turn-on systems for analyte sensing.

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