Electronic Supplementary Information

N-Carbamoylmaleiimide-treated carbon dots: stabilizing the electrochemical intermediate and extending for the ultrasensitive detection of organophosphate pesticides

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1. Preparation of CDs and N-MAL-CDs

In the present work, N-MAL-CDs was synthesized based on N-MAL functionalized CDs, which was divided into three steps and shown in Fig. S1.

Step 1: CDs was synthesized following a standard hydrothermal procedure.[1] Citric acid (1.15 g) was dissolved in 15 mL water and then this solution was sealed into a Teflon-lined autoclave and heated at 200 °C for 4 h. After the autoclave was cooled to room temperature, the aqueous solutions were filtered and centrifuged at 6000 rpm for 10 min to separate them from agglomerated larger particles.

Step 2: N-MAL was used to amidate with CDs through EDC/NHS catalysis to form derivatives of citrazinic acid. Finally the resulted solution was obtained and named N-MAL-CDs solution.

Step 3: Most unreacted reagents were removed through dialysis bag of 1000 D for 24 h to purify N-MAL-CDs.

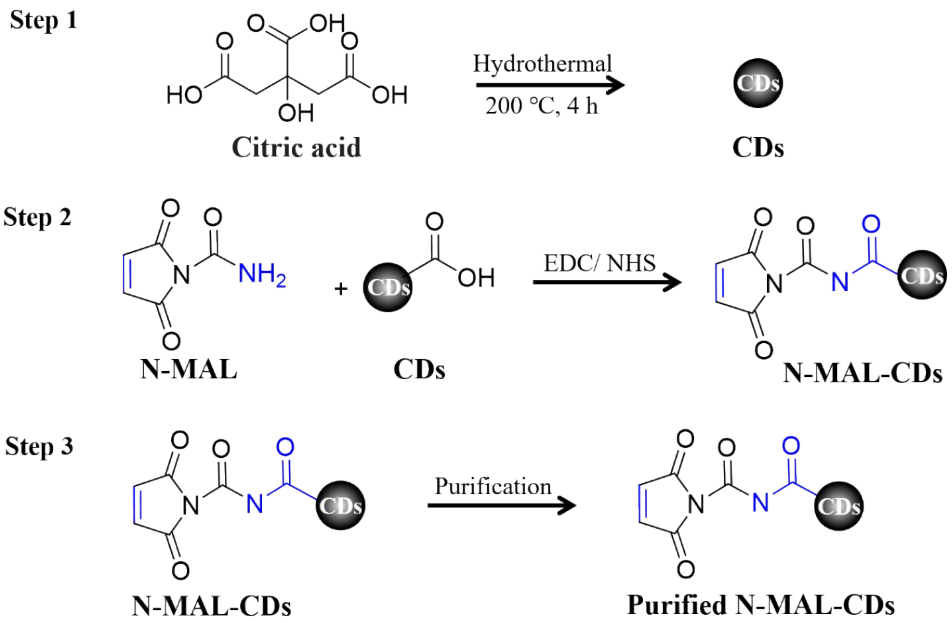


Fig. S1 The process of forming CDs and N-MAL-CDs. Step 1: Citric acid was used for producing CDs with hydrothermal method. Step 2: N-MAL was used to amidate with CDs through EDC/NHS catalysis. Step 3: Purification by dialysis (24 h) for N-MAL-CDs.

2. Characterization of CDs and N-MAL-CDs

Purification of the prepared CDs was implemented by using filtration, centrifugation and dialysis in turn. CDs was filtered and centrifuged at 6000 rpm for 10 min to separate it from agglomerated larger particles, and then the product was concentrated by the evaporation of water using rotary evaporation system. Afterwards, the concentrated sample was put in dialysis of 1000 Da against deionized water for 24 h in order to remove most unreacted reagents.

As shown in Fig. S2, black line is corresponding to 24 h dialysis and red line is corresponding to 7 d dialysis. By comparing the results of 24 h and 7 d dialysis, undesired impurities would be removed completely by 24 h dialysis. As shown in Fig. S3, obvious changes about the fluorescent intensity can be noticed from the very beginning to 18 h. However, no obvious fluorescent changes can be observed for the purified CND after 1 d dialysis, as well as for the following every 24 h, until 168 h. The fluorescence intensity is stable around 330 a.u. after 24 h dialysis.

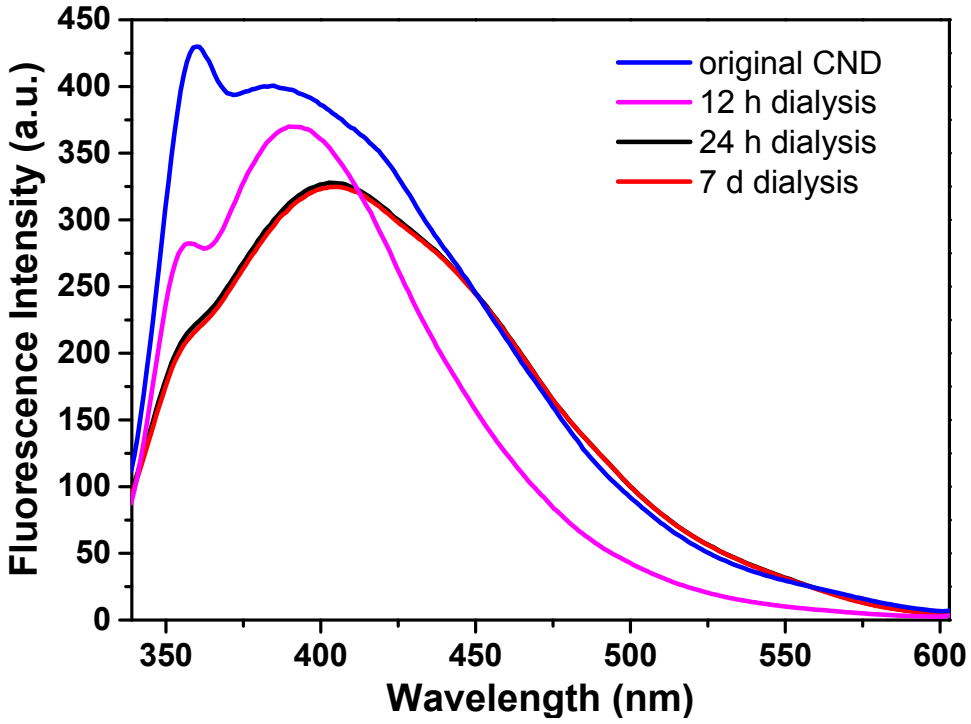


Fig. S2 Fluorescence emission spectra of CND that without dialysis (blue) and subjected to dialysis for 12 h (magenta), 24 h (black) and 7 d (red) in aqueous solutions.

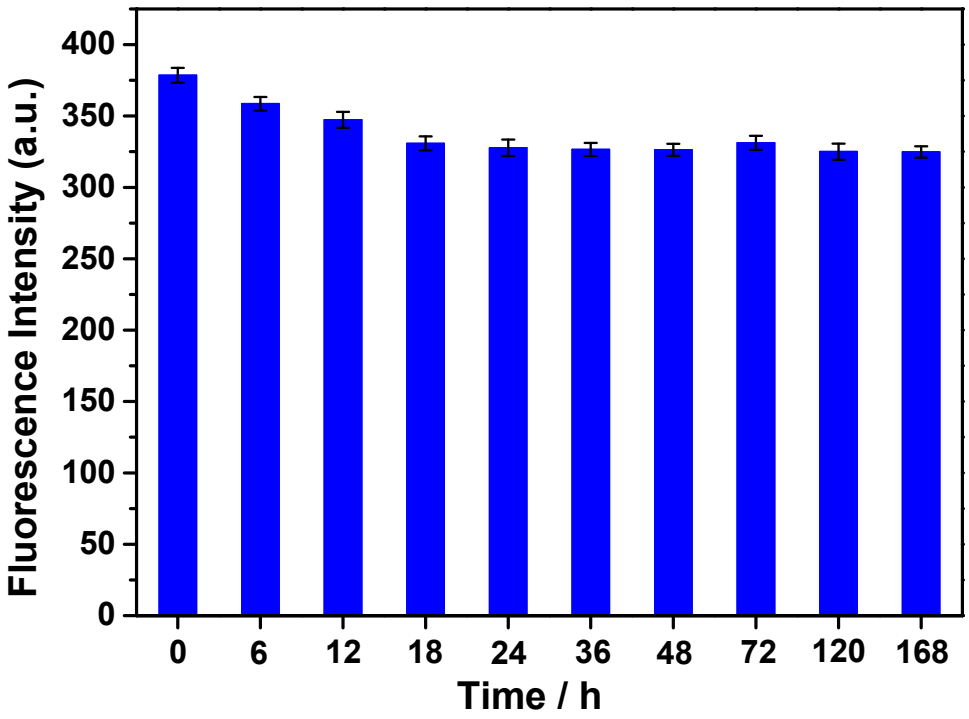


Fig. S3 The fluorescence intensities of the CND under different dialysis time at the fluorescent emission peak position of 405 nm.

To further demonstrate the purification results, we chose Agilent 1200 Infinity

chromatograph to carry out the purification. Chromatographic separation of the prepared CDs was performed at 30 °C on Diamonsil C-18 column (5 μm, 250 ×4.6 mm) using isocratic elution of acetonitrile-water (20:80, v/v) as mobile phase. Injection volume was 10 μL, and the flow rate was set at 0.6 mL/min. Chromatograms of original CDs (red line), 24 h dialysis CDs (black line) and 7 d dialysis CDs (blue line) are shown in Fig. S4. Only one chromatographic peak was observed for both 24 h and 7 d dialysis CDs, demonstrating the successful purification of CDs.

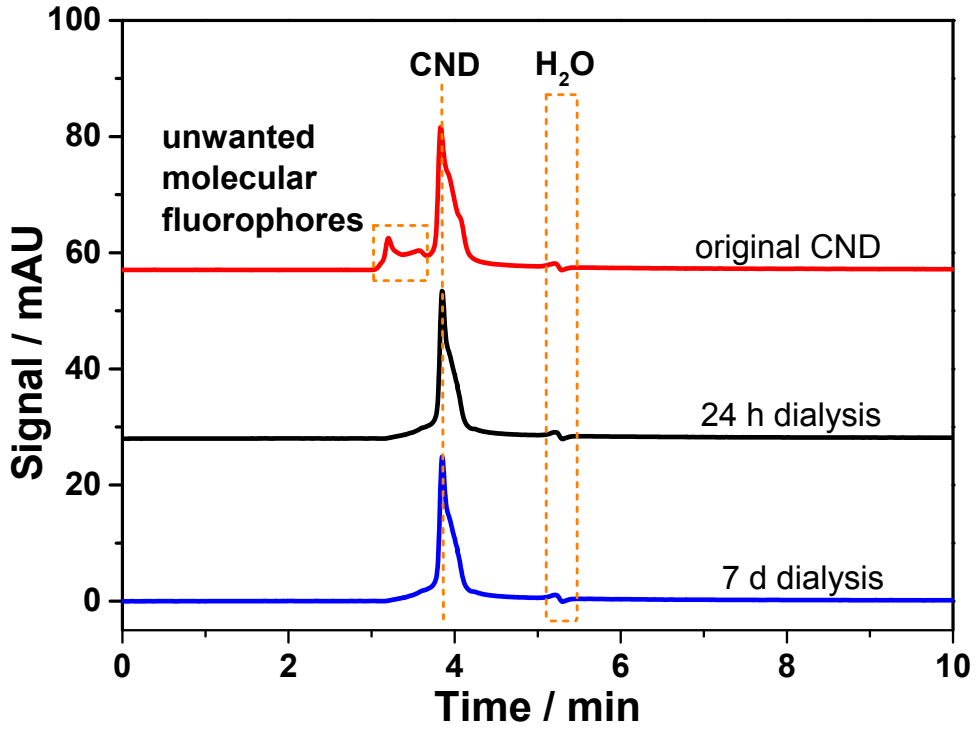


Fig. S4 HPLC chromatograms of CND that subjected to dialysis (1000 Da cut off) for 0 h (black), 24 h (red) and 168 h (blue).

The CDs formation occurs through carbonization of the precursors at 200 °C and yields small particles, as the TEM images shown in Fig. S5A, indicating the carbon core was formed at the expense of the molecular fluorophores. N-MAL itself has a fluorescent phenomenon (Fig. S5B, black). When the amino group of N-MAL and the carboxyl group on the surface of CDs were amidated through EDC/NHS catalysis, organic fluorophores were introduced to the surface of CDs. So by comparing the emission spectra of those three kinds of materials (Fig. S5B), the fluorescence signal of N-MAL-CDs emitted is the synergistic effect of carbon core and organic fluorophores.[2-8] Briefly, the fluorescence signal of N-MAL-CDs emitted is the synergistic effect of

carbon core and organic fluorophores. So N-MAL-CDs is N-MAL functionalized CDs. There is organic fluorophore in the surface of N-MAL-CDs.

XRD patterns of the N-MAL-CDs and purified N-MAL-CDs (Fig. S7) displayed a broad peak centered at $2\theta = 24.2°$, which is also attributed to highly disordered carbon atoms.[1,9] In summary, the material is amorphous.

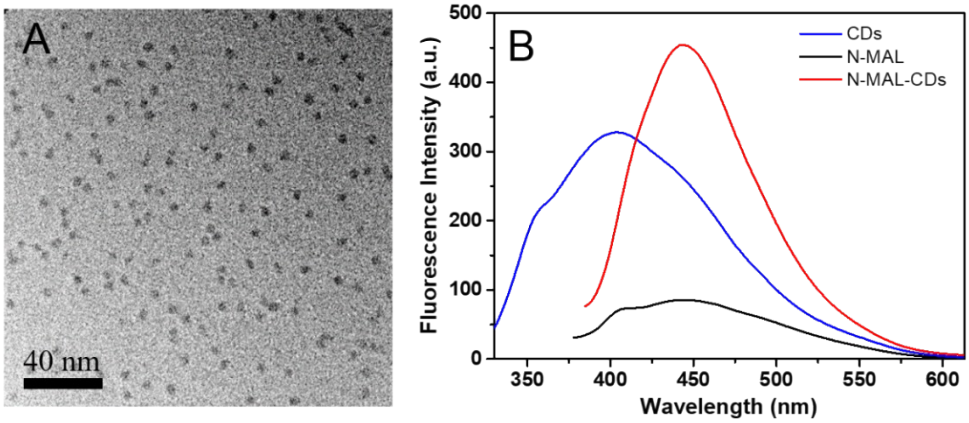


Fig. S5 (A) TEM images of CDs. (B) Fluorescence emission spectra of N-MAL (black), CDs (blue) and N-MAL-CDs (red) in aqueous solutions.

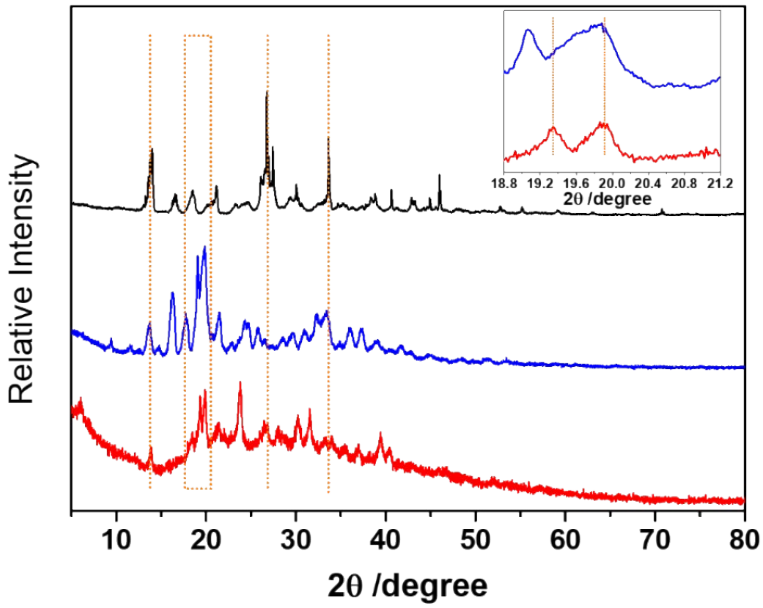


Fig. S6 XRD patterns of N-MAL (black), CDs (blue), and N-MAL-CDs (red). The dotted line is a guide for the eyes.

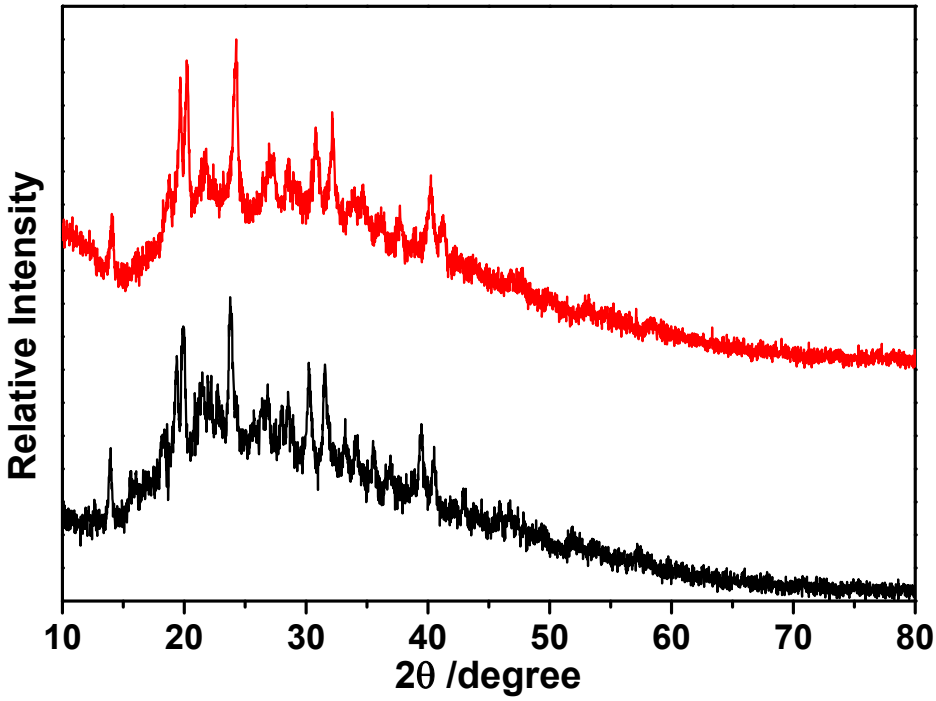
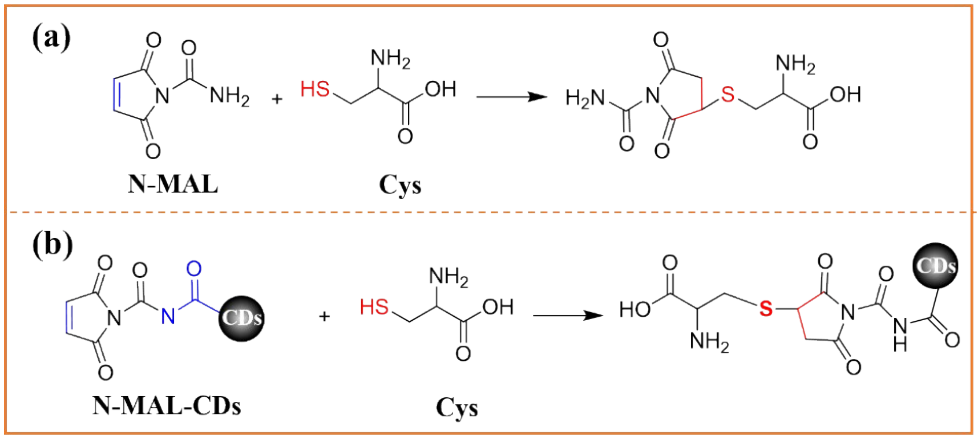


Fig. S7 XRD patterns of N-MAL-CDs (red) and purified N-MAL-CDs (black).

3. Fluorescence investigation of the occurrence of Michael addition

The Michael addition reaction is a facile reaction between nucleophiles and activated olefins or alkynes in which the nucleophile adds across carbon-carbon multiple bonds. Therefore, Cysteine (Cys) that can provides a live -SH as a reactant were chose to demonstrate the mechanism of Michael addition in this work. The reaction schemes of N-MAL and Cys (a), N-MAL-CDs and Cys (b) toward the formation of C-C-S based on Michael addition was shown in Scheme R1. As all known, the change of fluorescence signals is due to the change of molecular structure. So the fluorescence experiments were carried out to verify the mechanism, shown as Fig. S8. It can be seen that a much higher fluorescence is observed for both N-MAL and N-MAL-CDs upon addition of Cys, which means stable complexes were formed. The good stability was considered from the chemical covalent-binding between C=C on N-MAL-CDs derived from N-MAL and -SH from Cys to form C-C-S bond through

Michael addition. Moreover, there is no obvious change of fluorescence intensity of CDs upon addition of Cy. By using fluorescence tests, Michael addition between N-MAL-CDs and –SH containing group has been demonstrated.



Scheme. S1 Schematic illustration of C-C-S formation based on Michael addition between N-MAL and Cys (a), and N-MAL-CDs and Cys (b).

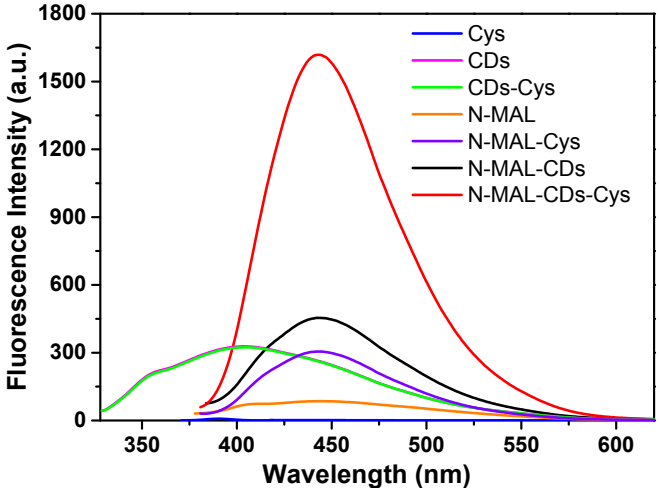


Fig. S8 Fluorescence emission spectra of CD (green), N-MAL (violet) and N-MAL-CDs (red) in the presence of 1.0 m M Cys in aqueous solutions (denoted as CDs-Cys, N-MAL-Cys, N-MAL-CDs-Cys). Compared to the spectra of pure Cys aqueous solution (blue), CDs aqueous solution (magenta), N-MAL aqueous solution (orange) and N-MAL-CDs aqueous solution (black).

4. Comparison of electrochemical performances of N-MAL-CDs and CDs for OPs detection

The electrochemical performances of N-MAL-CDs and CDs for OPs detection were compared and the results are shown in Fig. S9. Fig. S9 displays a well-defined oxidation peak (Ep_a 0.66 V) on AChE/N-MAL-CDs/SPE. The operation potential was negatively shifted 80 mV, and the electrochemical signals greatly increased with AChE/N-MAL-CDs modified SPE compared to AChE/CDs. The lower operation potential of 0.66 V and higher electrochemical current of 4.68 μA demonstrated that the effect of N-MAL-CDs/SPE was stronger than CDs/SPE. When incubated with 1.6×10^{-8} M parathion-methyl for 14 min firstly and then the same amount of ATCh as above was added, AChE would be inhibited and resulted in a decrease in the current response, and the results demonstrate that AChE/N-MAL-CDs shown higher sensitivity than AChE/CDs.

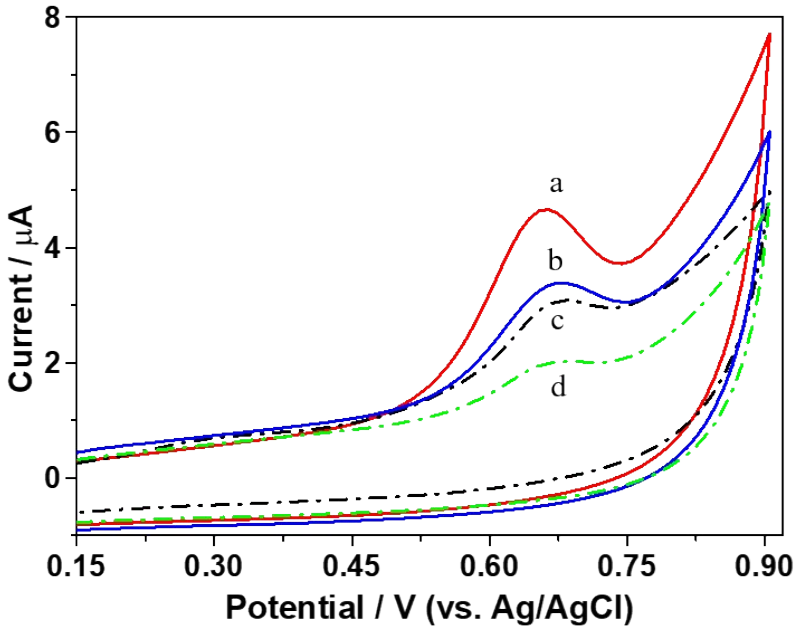


Fig. S9 CVs of AChE/N-MAL-CDs (a), AChE /CDs (c), on the SPE in 0.01 M PBS containing 2 mM ATCh. CV of AChE /N-MAL-CDs/SPE (b) and AChE/CDs/SPE (d) in the presence of 1.6×10^{-8} M parathion-methyl in 0.01 M PBS.

Table S1 Determination of parathion-methyl and paraoxon in tap water samples.

Sample (tap water)	Taken (M)	Found (M)	Recovery (%)
Parathion-methyl			
1	1.9×10^{-14}	2.1×10^{-14}	110.5
2	3.8×10^{-12}	3.9×10^{-14}	102.6
3	3.8×10^{-10}	4.1×10^{-14}	107.9
Paraoxon			
1	3.6×10^{-14}	3.5×10^{-14}	97.2
2	3.6×10^{-12}	3.7×10^{-12}	102.8
3	3.6×10^{-10}	3.9×10^{-10}	108.3

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