

Electronic Supplementary Information

A Highly Sensitive Electrochemiluminescence Biosensor for Detection of Organophosphate Pesticides Based on Cyclodextrin Functionalized Graphitic Carbon Nitride and Enzyme Inhibition

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Experimental section

Apparatus

The ECL signal was monitored with a model MPI-A electrochemiluminescence analyzer (Xi'an Remax Electronic 65 Science & Technology Co. Ltd., Xi'an, China). The voltage of photomultiplier tube (PMT) was set at 800 V and the potential scan was from 0 to 1.5 V. The electrochemical signal was detected with a CHI660D electrochemical workstation (Shanghai CH Instruments Co., China). Scanning electron micrographs (SEM) were performed with a scanning electron microscope (SEM, S-4800, Hitachi, Japan). Transmission electron micrographs (TEM) were conducted on TECNAI 10 (Philips Fei Co., Hillsboro). UV-visible (UV-vis) absorption spectra were performed with an UV-2450 UV-vis spectrophotometer (Shimadzu, Japan). Fourier transform infrared (FT-IR) spectra were detected using a Nexus 670 FT-IR spectrophotometer (Nicolet Instruments). A conventional three electrode system contained a platinum wire as auxiliary electrode, an Ag/AgCl as the

reference electrode and the bare or modified glassy carbon electrode (GCE, $\Phi = 4.0$ mm) as working electrode.

Reagent and Materials

Acetylcholinesterase (AChE, type C2888-1KU), Triethylamine (Et_3N , 99%), N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimidehydrochloride (EDC), N-hydroxysuccinimide (NHS) and acetylthiocholine (ATCl) were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Melamine (2,4,6-triamino-1,3,5-triazine, 99%) and ethyl paraoxon were obtained from Aladdin Ltd. (Shanghai, China). β -cyclodextrin (CD) and ferrocenecarboxylic acid (Fc-COOH) were purchased from Shanghai Chemical Reagent Co. (Shanghai, China). Cabbage, lettuce and lotus white were bought from the local supermarket. The phosphate buffered salines (PBS) with various pH were prepared with the stock solution of 0.10 M KH_2PO_4 and Na_2HPO_4 . 0.10 M KCl was used as a supporting electrolyte. Doubly distilled water was used throughout the experiment.

Synthesis of g- C_3N_4 -CD nanocomposite

g- C_3N_4 was obtained by calcining of melamine in an alumina crucible¹. Then, 1 g bright yellow g- C_3N_4 powder was stirred with 10 mL 37% hydrochloric acid (HCl) for 3 h at room temperature. Then, the obtained suspension was centrifugated and washed with doubly distilled water until neutral condition. Then 20 mg protonated g- C_3N_4 was mixed with 10 mL 0.2 M KOH and 10 mL 80 $\text{mg}\cdot\text{mL}^{-1}$ CD. The solution was kept stirring for 24 h, followed by washing with doubly distilled water until neutral condition and drying at 60 °C for overnight.

Synthesis of g- C_3N_4 -CD-Fc-COOH nanocomposite

The g-C₃N₄-CD-Fc-COOH was prepared by the following method². Firstly, 10 mg g-C₃N₄-CD and 10 mg Fc-COOH were dissolved in 100 mL ethylene glycol with continually stirring for 24 h at room temperature. Then, 150 mL acetone was put into above solution for stirring 15 min. Finally, g-C₃N₄-CD-Fc-COOH was obtained by centrifugation and washing with ethanol for three times to remove residual Fc-COOH.

Fabrication of ECL biosensor

The GCE was polished carefully by 0.3 μm and 0.05 μm alumina slurry to obtain a mirror. Subsequently, the electrode was sonicated successively in doubly distilled water and ethanol for 3 min, respectively. Next, the pretreated GCE was coated with 10 μL g-C₃N₄-CD-Fc-COOH suspension and dried in air at room temperature for 8 h. Then, 15 μL EDC/NHS solution was placed onto the electrode to activate carboxyl. Subsequently, 5 μL 250 KU $\cdot\text{L}^{-1}$ AChE was dropped onto the electrode surface for 8 h. Non-chemisorbed species were removed by washing the electrode with PBS before next modification step. Finally, the modified electrode abbreviated to AChE/g-C₃N₄-CD-Fc-COOH/GCE was stored at 4 $^{\circ}\text{C}$ in a refrigerator for further using. The procedure for constructing the biosensor was shown schematically in Scheme 1.

Measurement procedure

The AChE/g-C₃N₄-CD-Fc-COOH/GCE was employed for detection of OPs with different concentrations using ECL technique. The proposed electrode was incubated in OPs solution with different concentrations for 15 min. Then, the obtained electrode was transferred into the detecting cell with 3.0 mL PBS (0.1 M, pH 7.0) containing 50 mM Et₃N and 0.1 mM ATCl for ECL detection.

Preparation of real-life samples

The real-life samples were prepared according to the literature with some modifications³. The cabbage, lettuce and lotus white were washed with doubly distilled water and then crushed. 5 g of samples was added to 1 mL of acetone and 9 mL of PBS (0.1 M, pH 7.0). Then, the suspension was sonicated for 15 min and centrifuged (10 min, 10000 rpm). The supernatant was directly detected without extraction or further concentration.

Results and discussion

Characterizations of different nanocomposites

The UV-vis absorption spectroscopy (Fig. S1A) was used to demonstrate the successful synthesis of g-C₃N₄-CD-Fc-COOH. The UV-vis spectra of g-C₃N₄ (curve a) exhibited a strong characteristic absorption peak at 320 nm, corresponding with literature⁴. The characteristic absorption peak of g-C₃N₄-CD shifted from 320 nm to 310nm, and the result might be attributed to the protonation of HCl in the formation of g-C₃N₄-CD⁵. Three strong optical absorption peaks belonged to Fc-COOH (curve c) were observed at 310 nm, 260 nm, 220 nm, which were consistent with literature⁶. As seen in curve d, the UV-vis spectra of the g-C₃N₄-CD-Fc-COOH nanocomposite presented the characteristic peaks of both g-C₃N₄-CD and Fc-COOH, indicating the successful synthesis of g-C₃N₄-CD-Fc-COOH nanocomposite.

The FT-IR was used to further support the successful synthesis of g-C₃N₄-CD-Fc-COOH nanocomposite. As shown in Fig. S1B, the vibration of triazine ring of g-C₃N₄ at 810 cm⁻¹ could be observed (curve a), and the peaks at 1200-1650 cm⁻¹ were attributed to the stretching vibration of connected units of C-N(-C)-C (full

condensation) or C-NH-C (partial condensation). Meanwhile, the peaks at 3000-3500 cm^{-1} were assigned to the N-H stretching and hydrogen-bonding interactions of g- C_3N_4 ⁷. Compared with g- C_3N_4 , the g- C_3N_4 -CD presented three characteristic peaks of CD at 1080 cm^{-1} , 1418 cm^{-1} and 3179 cm^{-1} (curve b), which were assigned to the coupled C-O/C-C stretching/O-H bending vibrations, C-H/O-H bending vibrations and O-H stretching vibrations, respectively. And the O-H stretching vibration at 3179 cm^{-1} was blue shift than that of the free OH mode at 3700 cm^{-1} , indicating that there existed the strong hydrogen bond between CD and N-H of g- C_3N_4 ⁸. The FT-IR spectra of g- C_3N_4 -CD-Fc-COOH (curve c) had no obviously change compared with that of g- C_3N_4 -CD, indicating that Fc-COOH successfully entered into the hydrophobic cavity of CD molecules.

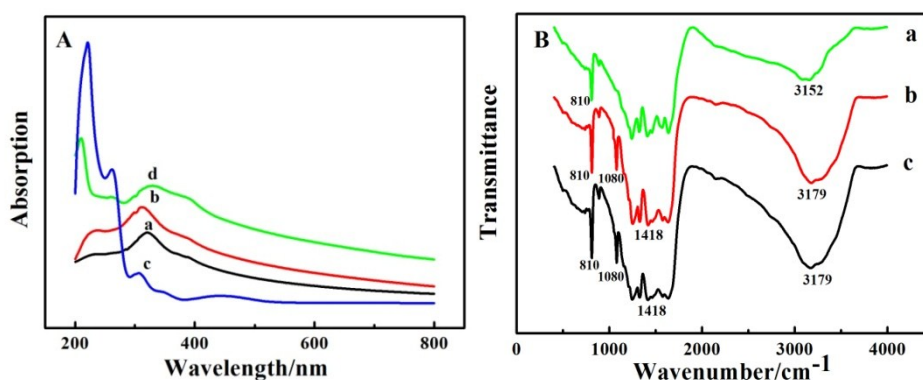


Fig. S1 (A) The UV-vis spectra of (a) g- C_3N_4 (b) g- C_3N_4 -CD (c) Fc-COOH (d) g- C_3N_4 -CD-Fc-COOH. (B) The FT-IR spectra (a) g- C_3N_4 (b) g- C_3N_4 -CD (c) g- C_3N_4 -CD-Fc-COOH.

ECL and CV characterization of stepwise fabrication of the electrode

The stepwise fabrication of the electrode was investigated by ECL in PBS (0.1 M, pH 7.0) containing 50 mM Et_3N . As depicted in Fig. S2A, no ECL emission was observed at the bare GCE (curve a). When g- C_3N_4 -CD-Fc-COOH was modified on GCE, a strong ECL signal was presented due to the luminescence property of g- C_3N_4

and signal amplification of CD (curve b). Then, AChE/g-C₃N₄-CD-Fc-COOH/GCE (curve c) exhibited a decreased ECL response for the reason that biomacromolecules AChE would obstruct the electron transfer.

The assembly process was also confirmed by cyclic voltammetry (CV) in PBS (0.1 M, pH 7.0). As shown in Fig. S2B, compared with the bare GCE (curve a), well-defined oxidation and reduction peaks of g-C₃N₄-CD-Fc-COOH/GCE were observed (curve b) due to the conversion of Fe(II) and Fe(III). When AChE was dropped onto the electrode, a decrease in the peak current was noticed (curve c) because of hindrance of non-conductive AChE. All above results indicated that the stepwise fabrication of the ECL biosensor was successful.

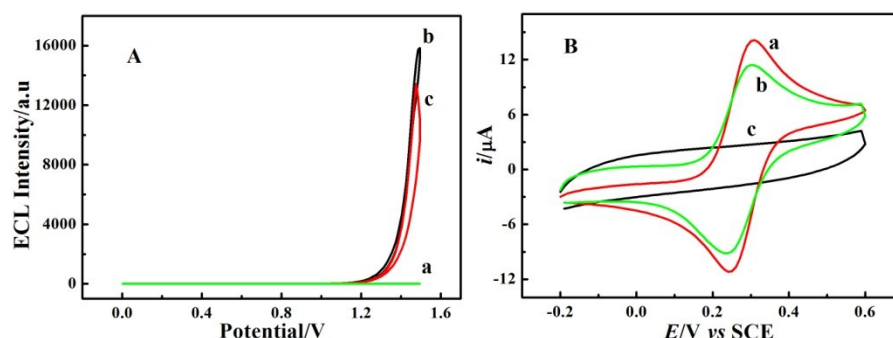


Fig. S2 ECL (A) and CV (B) response of (a) bare GCE (b) g-C₃N₄-CD-Fc-COOH/GCE (c) AChE/g-C₃N₄-CD-Fc-COOH/GCE.

Optimization of experimental conditions

In order to obtain optimum ECL response, the concentration of coreactant Et₃N was investigated in PBS (0.1 M, pH 7.0). As shown in Fig. S3A, the ECL intensity increased with the augment of Et₃N concentration from 10 mM to 50 mM and then decreased after 50 mM. The reason might be that excess coreactant would react readily with positively charged g-C₃N₄ (g-C₃N₄⁺), inhibiting the production of the

excited-state $\text{g-C}_3\text{N}_4$ ($\text{g-C}_3\text{N}_4^*$). Thus, the optimal coreactant concentration was 50 mM in this ECL system.

The pH played an important role in the ECL biosensor. The pH dependence of the ECL response was investigated over the pH from 5.5 to 8.5 in 0.1 M PBS containing 50 mM Et_3N . As shown in Fig. S3B, the ECL intensity showed a rapid increase with increasing of pH from 5.5 to 7.0 and decrease when the value of pH exceeded 7.0. At low pH, it is difficult for the cation Et_3N^{++} to produce a radical $\text{Et}_3\text{N}^\bullet$ by deprotonation which was crucial for ECL intensity^{9,10}. At high pH, the competitive reaction, OH^- and positively charged $\text{g-C}_3\text{N}_4$ ($\text{g-C}_3\text{N}_4^{++}$), inhibited the formation of the excited-state $\text{g-C}_3\text{N}_4$ ($\text{g-C}_3\text{N}_4^*$). Therefore, pH 7.0 was selected as the optimal pH and used in subsequent work.

The concentration of ATCl was vital factor for OPs detection. It was surveyed in PBS (0.1 M, pH 7.0) containing 50 mM Et_3N with various ATCl concentrations from 0 μM to 120 μM . As seen from Fig. S3C, the ECL intensity decreased with increasing the ATCl concentration and then reached a relatively stable value when the concentration of ATCl exceeded 100 μM , since the hydrolyzation product (HAc) increased with the increase of ATCl concentrations, which consumed more coreactant Et_3N and induced a decrease ECL signal before saturation of enzyme. Therefore, 100 μM ATCl was chosen as the optimum concentration.

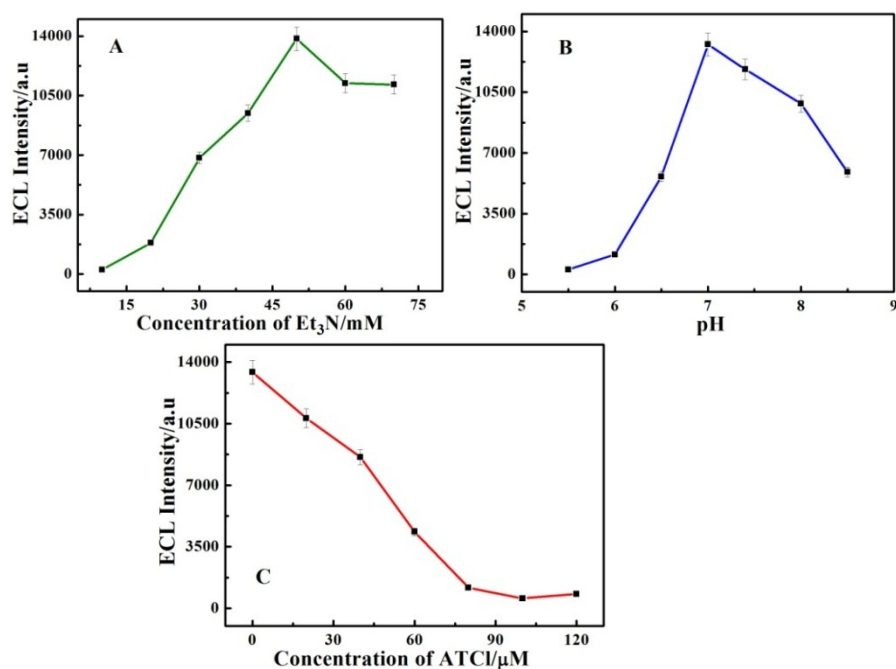


Fig. S3 Optimization of (A) concentration of Et_3N (B) pH (C) concentration of ATCl.

Stability and reproducibility

The stability of the proposed biosensor was tested in PBS (0.1 M, pH 7.0) containing 50 mM Et_3N and 0.1 mM ATCl for successive measurements ($n=14$) with 0.5 μM OPs. As shown in Fig. S4, the relative standard deviation (R.S.D.) was 0.9 %. The reproducibility was measured using five modified electrode in PBS (0.1 M, pH 7.0) containing 50 mM Et_3N and 0.1 mM ATCl with 0.5 μM OPs, and the R.S.D. was 4.52%. As a whole, the ECL biosensor exhibited an excellent stability and reproducibility.

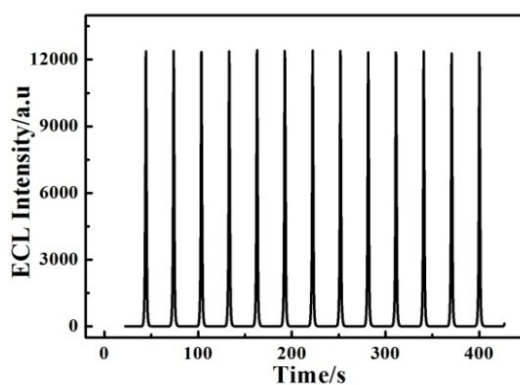


Fig. S4 The stability of AChE/g- C_3N_4 -CD-Fc-COOH/GCE incubated with 0.5 μM OPs.

Table S1 Comparison of response characteristics of different modified electrode for determination of ethyl paraoxon

Electrode materials	Determine method	Linear range /nM	Detection limit /pM	Reference
AChE/PVA-AWP	Amperometry	5-500	5000	[11]
Au-ZrO ₂ -SiO ₂ /GCE	square wave voltammetric	3.63-1820	1820	[12]
AChE/Au-PPy-rGO	Amperometry	1-5000	500	[13]
AChE/MWCN	Amperometry	0.01-10	0.9	[14]
AChE/g-C ₃ N ₄ -CD-Fc-COOH/GCE	ECL	0.001-500	0.3	This work

Table S2 Recoveries test of ethyl paraoxon in different vegetable samples

Sample	C_{Added} /nM	$C_{Detected}$ /nM	Recovery /%	RSD /%
Cabbage 1	5	4.775	95.5	1.96
Cabbage 2	0.5	0.498	99.6	5.01
Cabbage 3	0.05	0.051	102.4	5.90
Lotus White 1	5	5.21	104.2	0.89
Lotus White 2	0.5	0.471	94.2	5.28
Lotus White 3	0.05	0.052	104	1.71
Lettuce 1	5	4.825	96.5	4.15
Lettuce 2	0.5	0.542	108.4	1.51
Lettuce 3	0.05	0.049	98.9	6.41

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