Supporting information

Graphene Oxide-based Colorimetric Detection of Organophosphorus Pesticides via a Multi-enzyme Cascade Reaction

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Experimental Section

Materials and Instruments.

All chemicals from commercial sources are of analytical grade. Acetylcholinesterase (AChE, from Electrophorus electricus) and cholin oxidase (CHO, from Alcaligenes sp.) were obtained from Sigma-Aldrich. Acetylcholine chloride (ACh), 3,3,5,5-tetramethylbenzidine (TMB) and horseradish peroxidase (HRP) were purchased from Aladdin Reagent Co., Ltd (Shanghai, China). The dimethoate, methyl paraoxon and chlorpyrifos were purchased from Tianjin Zhongyi Jiaxin Technology Development Co., Ltd. The UV-Vis absorbance measurements were obtained on a UV-2450 spectrometer (Shimadzu). TEM images were recorded with a JEM-2010 (JEOL Co., Japan) operated at an accelerating voltage of 200 kV. The samples for TEM measurements were prepared by depositing GO dispersion (1 mg/mL) on carbon-coated copper grids. AFM in tapping model was performed with a SPI 3800N Probe Station (Seiko Instruments Inc., Japan). The samples for AFM measurements were prepared by spin-coating GO dispersion (0.1 mg/mL) onto mica substrates at 2000 rpm for 2 mins. XPS measurements were carried out using a VG Escalabmkll spectrometer (UK) with an Al-Kα irradiation source (1486.7 eV) under ultra-high vacuum conditions ($<10^{-10}$ Torr).

Synthesis of graphene oxides (GO)

(1) Synthesis of large-sized GO-1:

1 g of graphite powders (Beijing Yuceda Trade Co., Ltd (thickness < 40 μM)) was added to 46 mL sulfuric acid (98%) under stirring, followed by the addition of 1.2 g potassium nitrate at room temperature. 6 g $KMnO_4$ was slowly added to the mixture under stirring below 20 °C. The mixture was then heated to 35 °C for 6 h under stirring. Subsequently, 80 mL of de-ionized water was added dropwise to the mixture under vigorous stirring, leading to a quick rise of temperature to ~80 °C. The mixture was further stirred for another 30 mins, and then 200 mL of water and 6 mL of H_2O_2 solution (30%) were added in sequence to the reaction vessel. The resulting graphite oxide suspension was washed repeatedly by a large amount of water until the solution pH reached ~4.0, and then the suspension was diluted to 600 mL with water. Finally, the resultant dispersion in water was ultrasonicated for 1 h, followed by centrifugation at 2000 rpm for 10 mins to obtain GO-1.

(2) Synthesis of small-sized GO-2:

0.5 g of graphite powders (Nanjing XFNANO Materials Tech Co., Ltd (lateral size < 40 μM)) was added to 25 mL sulfuric acid (98%), followed by the addition of 0.5 g sodium nitrate at room temperature. Afterwards, the mixture was cooled down to 0 °C. 6 g of $KMnO_4$ was then slowly added to the mixture under vigorously stirring below 20 °C. The mixture was stirred at 35 °C for 30 mins, then stirred at 90 °C for 90 mins. The reaction was ended by addition of 80 mL distilled water and 10 mL H_2O_2 solution (30%). The resulting graphite oxide suspension was washed consecutively with 1 M HCl solution and de-ionized water. 200 mL of water were added to the graphite oxide product. Finally, the resultant dispersion in water was ultrasonicated for 1 h, followed by centrifugation at 10000 rpm for 30 mins to obtain GO-2.

Kinetic analysis of GO

The steady-state kinetics of GO (20 μg/mL GO-1 or GO-2) were performed by varying the concentration of H_2O_2 (5 mM~50 mM) and fixing the concentrations of TMB (0.2 mM, 0.4 mM, and 0.6 mM), or varying the concentration of TMB (80 μM~400 μM) and fixing the concentrations of H_2O_2 (25 mM, 50 mM, and 75 mM). All the reactions were monitored by measuring the UV-vis absorbance change of TMB at 652 nm in 0.2 M, pH = 4.0 acetate buffer solution at 35 °C.

Activity assays for AChE/CHO/GO system

Various concentrations of AChE were incubated with 50 μL ACh (0.6 M) and 25 μL CHO (2.0 U/mL) in 100 μL Tris-HCl buffer (0.1 M, pH=8.0) for 30 mins at 37 °C. Subsequently, 25 μL GO (400 μg/mL), and 50 μL TMB (1 mg/mL) in 225 μL HAc-NaAc buffer (0.2 M, pH=4.0) were added to the above mixture. The UV-vis absorbance of the final solution was recorded on an ultraviolet spectrophotometry at 652 nm after 10 mins.

Colorimetric analysis of OPs.

The concentration of OPs was determined according to the deceased color intensity as the activity of AChE can be inhibited by OPs in the multienzyme-cascade color reaction. First, AChE (20 mU/mL) was mixed with different concentrations of dimethoate, methyl paraoxon, or chlorpyrifos in the presence of 50 μL ACh (0.6 M) and 25 μL CHO (2.0 U/mL) in 100 μL Tris-HCl buffer (0.1 M, pH=8.0) at 37 ℃. After 30 mins incubation, 25 μL GO (400 μg/mL) and 50 μL TMB (1 mg/mL) in 225 μL HAc-NaAc buffer (0.2 M, pH=4.0) were then added to the above mixtures. The UV-vis absorbance of the final solution was recorded on an ultraviolet spectrophotometry at 652 nm after 10 mins.

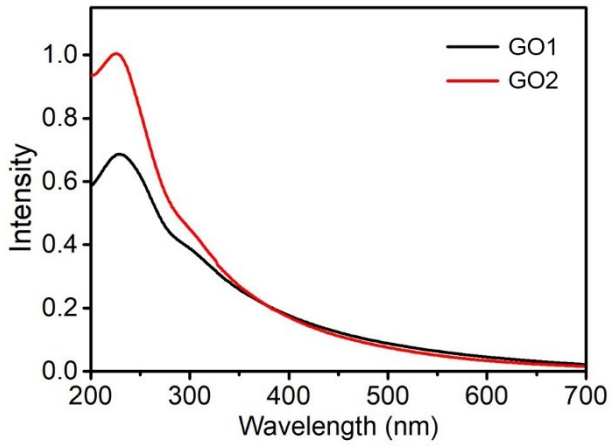

Figure S1 UV−Vis absorption spectra of GO-1 and GO-2 solutions.

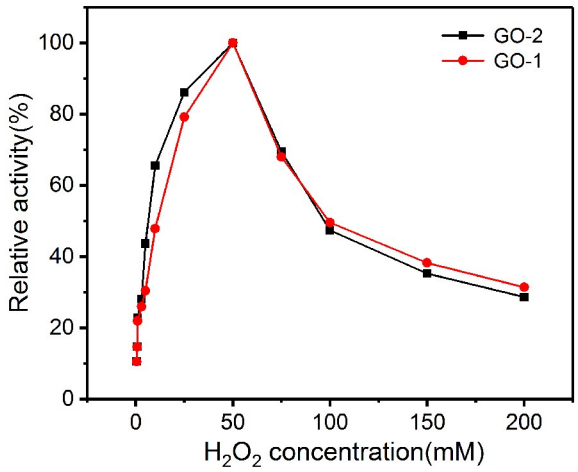

Figure S2 The effect of H_2O_2 concentration on the relative activity of GO.

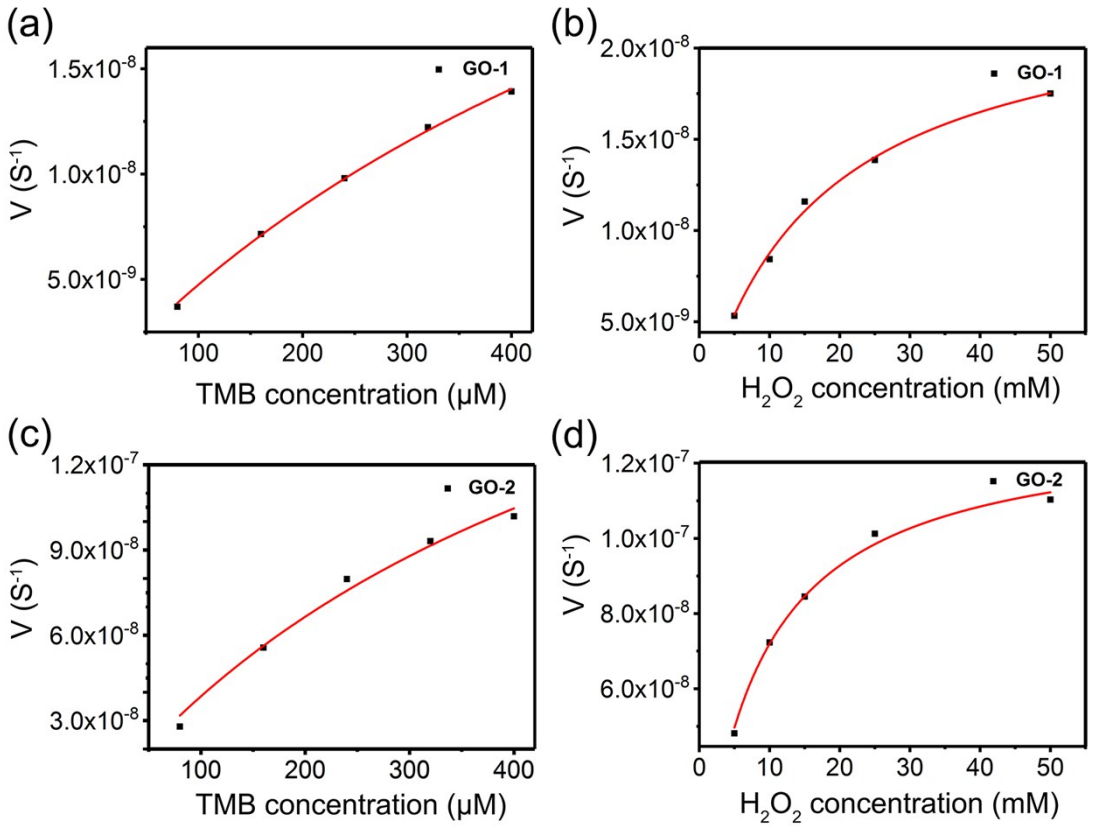

Figure S3 Steady-state kinetic assay and catalytic mechanism of GO (a-d). The velocity (v) of the reaction was measured using 400 μg/mL GO in HAc-NaAc buffer (0.2 M, pH=4.0) at 35 °C. The concentration of H_2O_2 was fixed to 50 mM with the varied TMB concentration for GO-1 (a) and GO-2 (c), respectively. The concentration of TMB was fixed to 400 μM with the varied H_2O_2 concentration for GO-1 (b) and GO-2 (d), respectively.

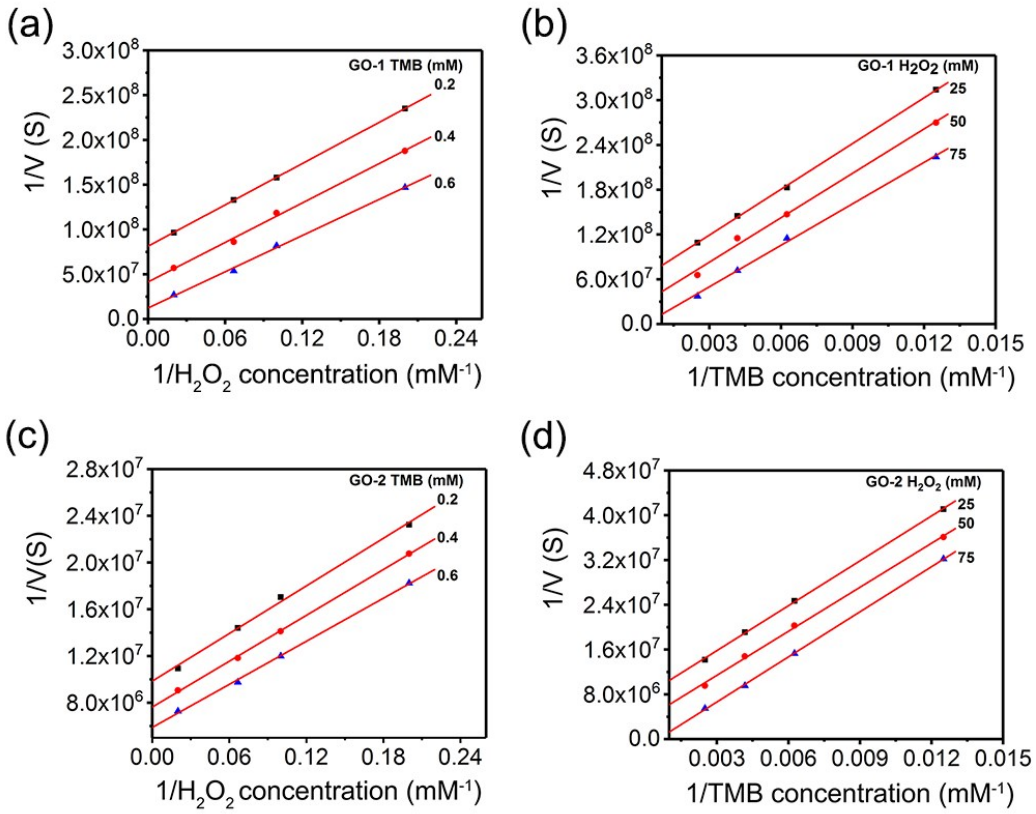

Figure S4 Double reciprocal plots of activity of GO at a fixed concentration of one substrate versus different concentration of the second substrate for TMB or H_2O_2. (a,b) GO-1, (c,d) GO-2.

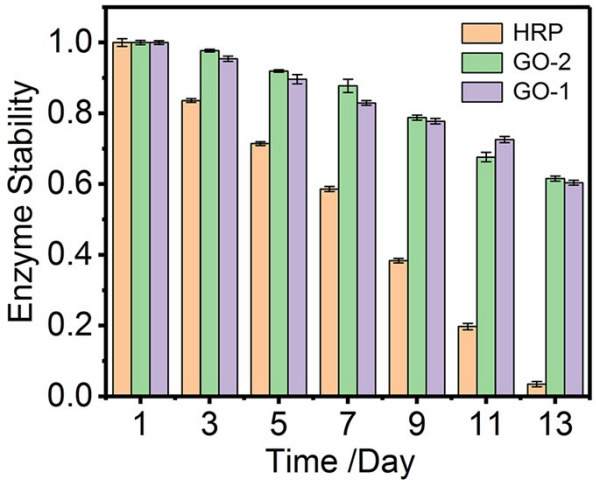

Figure S5 Comparison of the catalytic stability of the commercially available HRP, GO-1, and GO-2 at 35 °C.

Figure S6 The color intensity decreased gradually with increasing the concentration of OPs. (a) Dimethoate, (b) Methyl paraoxon, (c) Chlorpyrifos.

Table S1. The fitted results (%) of C1s XPS spectra of GO-1 and GO-2.

Samples	C-C/C=C	C-O/C-O-C	C=O/COOH
GO-1	50.73	47.46	1.81
GO-2	41.12	54.89	3.99

Table S2. Comparison of the kinetic parameters of different catalysts.

Catalyst	Substance	K_m [mM]	V_{max} [×10^{-8} M/s]
GO-1	H_2O_2	16.69 ± 0.183	2.27 ± 0.056
GO-1	TMB	0.86 ± 0.014	4.30 ± 0.160
GO-2	H_2O_2	8.03 ± 0.481	12.58 ± 0.532
GO-2	TMB	0.75 ± 0.078	28.47 ± 2.825
HRP	H_2O_2	0.61 ± 0.042	2.35 ± 0.087
HRP	TMB	0.15 ± 0.018	4.53 ± 0.300