Electronic supplementary information

Effect of protein on the oxidase-like activity of CeO_2 nanozymes for immunoassays

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Chemicals and animals for antibody production.

Lactoferrin (LF) from bovine milk was purchased from FUJIFILM Wako Pure Chemical Co. Ltd. (Japan). Complete and incomplete Freund's adjuvants, bovine serum albumin (BSA), FITC-BSA, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC), N-Hydroxysuccinimide (NHS) were obtained by Sigma-Aldrich. Pierce™ Rapid Antibody Isotyping Kit plus Kappa and Lambda-Mouse was obtained from Thermo Fisher. Bal b/c female mice were supplied by the Guangdong Medical Experimental Animal Centre and raised at South China Agriculture University Animal Centre (license: SYXK (Yue) 2019–0136). The animal experiment was carried out in a laboratory with a license for experiment animal, which was conformed to the welfare principle (ethical approval number: 2019054, **Fig. S1**). All the other reagents were of analytical reagent grade or higher purity.

Hapten synthesis

The synthetic route of fenitrothion haptens was shown in **Fig. S1**. The following detailed characterization including mass and NMR spectrograms are shown in **Fig. S2 and S3**.

Synthesis of Hapten-1, isopropyl 2-((chloro(methoxy)phosphorothioyl)oxy) benzoate. 3.28 g methyl O-methyl phosphorodichloridothioate (20 mmol) was mix with 1.8 g isopropyl salicylate (10 mmol) in 10 mL dichloromethane. 0.25 g NaOH and 0.5 g tetrabutylammonium bromide was dissolve in 10 mL H_2O and then add to the above dichloromethane solution with vigorous stirring for mixing aqueous phase and organic phase. The mixture was kept vigorous stirring for 12 h at room temperature. The organic phase was separated and purified with 300-400 mesh chromatography silica gel. ESI-MS (negative) m/z 309 $[M+H]^+$; ^1H NMR (600 MHz, DMSO) δ 7.71 (d, J = 7.7 Hz, 1H), 7.60 – 7.54 (m, 1H), 7.36 (d, J = 8.3 Hz, 1H), 7.27 (t, J = 7.5 Hz, 1H), 5.10 (dt, J = 12.5, 6.3

Hz, 1H), 3.72 – 3.64 (m, 3H), 1.30 (t, *J* = 5.1 Hz, 7H).

Synthesis of Hapten-2, 6-(((2-(isopropoxycarbonyl)phenoxy)(methoxy) phosphorothioyl)amino)hexanoic acid. Hapten-1 (0.4 g) was dissolved in 5 mL 1,4-dioxane, while 6 g of 6-aminocaproic acid and 2 g of NaOH were dissolved in 10 mL H_2O. The 1,4-dioxane solution was added to the 6-aminocaproic acid solution with stirring. The mixture was stirred for 12 h and then adjust pH to 4~5 by HCl. The product was extracted by ethyl acetate and then evaporated. The crude product was purified with 300-400 mesh chromatography silica gel. ESI-MS (negative) m/z 342 $[M-H]^-$; ^1H NMR (600 MHz, MeOD) δ 7.76 (d, *J* = 7.6 Hz, 1H), 7.52 (d, *J* = 3.8 Hz, 2H), 7.25 (dd, *J* = 7.9, 3.6 Hz, 1H), 5.20 (dd, *J* = 12.5, 6.3 Hz, 1H), 3.75 (d, *J* = 14.0 Hz, 3H), 3.05 (ddd, *J* = 13.8, 7.7, 6.3 Hz, 2H), 2.27 (t, *J* = 7.4 Hz, 2H), 1.63 – 1.55 (m, 2H), 1.49 (dd, *J* = 14.7, 7.4 Hz, 2H), 1.41 – 1.32 (m, 8H).

Synthesis of immunogens and coating antigens. The synthetic method of artificial antigen is showed in **Fig. S4**. For immunogen synthesis, hapten-2 was conjugated to LF by the NHS ester method. For coating antigen, hapten-1 and hapten-2 were respectively conjugated to BSA. For hapten-1, it was dissolved in 1,4-dioxane and was added to BSA solution, which was dissolved in carbonate buffer (50 mM, pH 9.6) and stirred overnight. For hapten-2, it was conjugated to BSA by the NHS ester method. UV-vis spectrometry was used to characterize all the final conjugates of the artificial antigens (**Fig. S5**).

Animal immunization

All the female Bal b/c mice were housed and maintained at the South China Agriculture University Animal Center (license: SYXK (Yue) 2019–0136). All the animal experiments were performed incompliance with the protective and administrative laws for laboratory

animals of China and conducted with the approval of the Institutional Authority for Laboratory Animal Care, South China Agricultural University, Guangzhou, China. The animal experiments were carried out in a laboratory with a license for experiment animals, which was conformed to the welfare principle (ethical approval number: 2019054, **Fig. S6**).

All other reagents were of analytical reagent grade or higher purity.

For the first immunization, each mouse (7-week-old) was intradermally and intramuscularly immunized with 0.1 mL of an emulsion containing 0.5 mL of an immunogen in PBS (1 mg/mL) and 0.5 mL complete Freund's adjuvant. The following four booster immunizations using the same amount of immunogen emulsified in incomplete Freund's adjuvant were every three weeks. One week after the fourth booster injection, the serum was collected from the tail tip from each mouse for ciELISA. After serum characterized and coating antigen screening, the mouse that exhibited the best inhibition (%) for 1-NAP was chosen as the donor of spleen cells for hybridoma production. Serum was collected from the tail tip from each mouse prior to the first immunization and used as the negative controls. The mouse anti-serum characterization is shown in **Table S1**.

Production of monoclonal antibody

The mouse that exhibited the best inhibition (%) for the immunogen was chosen as the donor of spleen cells for hybridoma production. Through the cell fusion technology, the above spleen cells were fused with SP2/0 murine myeloma cells to form hybridomas by PEG 4000 at 37 °C. The hybridoma was cultured in five 96 well plates for preliminary screening by icELISA method in the 10th day. Briefly, the coating antigens (1 μg/mL, 100

μL/well) in carbonate buffer were added to 96-well polystyrene ELISA plates and incubated at 37 °C overnight, and then the wells were washed twice with PBST solution prior to adding 5% skimmed milk in PBST (100 μL/well) to block the uncoated sites for 3 h at 37 °C and dried at 37 °C for 1 h. Fenitrothion standards in PBS (50 μL, 1 μg/mL) and the diluted culture fluid from 96 well plates (50 μL, 1 μg/mL) were added to each well and incubated at 37 °C for 40 min, and then the wells were washed five times with PBST. The secondary antibody (HRP conjugated goat anti-mouse IgG) was diluted 1:5000 in PBST (100 μL/well) was then added to the wells and incubated for 40 min at 37 °C. The wells were washed again five times with PBST before the TMB solution was added to the wells (100 μL/well) and incubated for 10 min. Finally, 50 μL of 10% H_2SO_4 was added to quench the reaction and the optical density was measured at 450 nm. The percent inhibition of antibody binding used to characterize the binding ability of antibodies was expressed as follow: inhibition= $[(B_0 – B)/B_0)] \times 100\%$; B_0 was the mean absorbance of the wells in the absence of a competitor; B was the mean absorbance of wells in the presence of a competitor. The highest inhibition rate and titer antibody-producing clone (4C6) was sub-cloned five times by limiting dilution with lower concentration of MT in each sub-cloning (from 1 μg/mL to 0.01 μg/mL). Then hybridomas secreting MT specific antibodies were expanded and selected to produce ascitic antibodies. The obtained ascitic mAb were purified by ammonium sulphate precipitation method and stored at -20 °C. The purified mAb concentration was detected by NanoDrop 2000c. The subtype of the obtained mAb was IgG1 (**Fig. S7**).

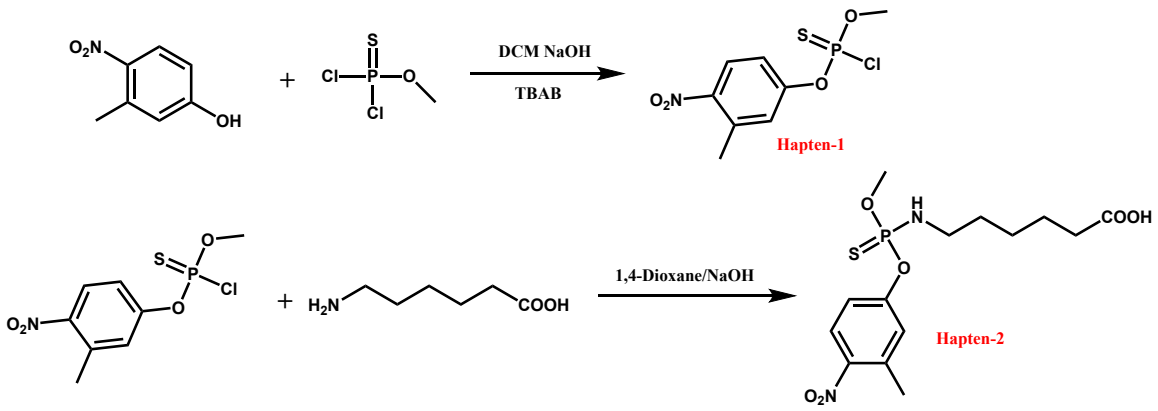
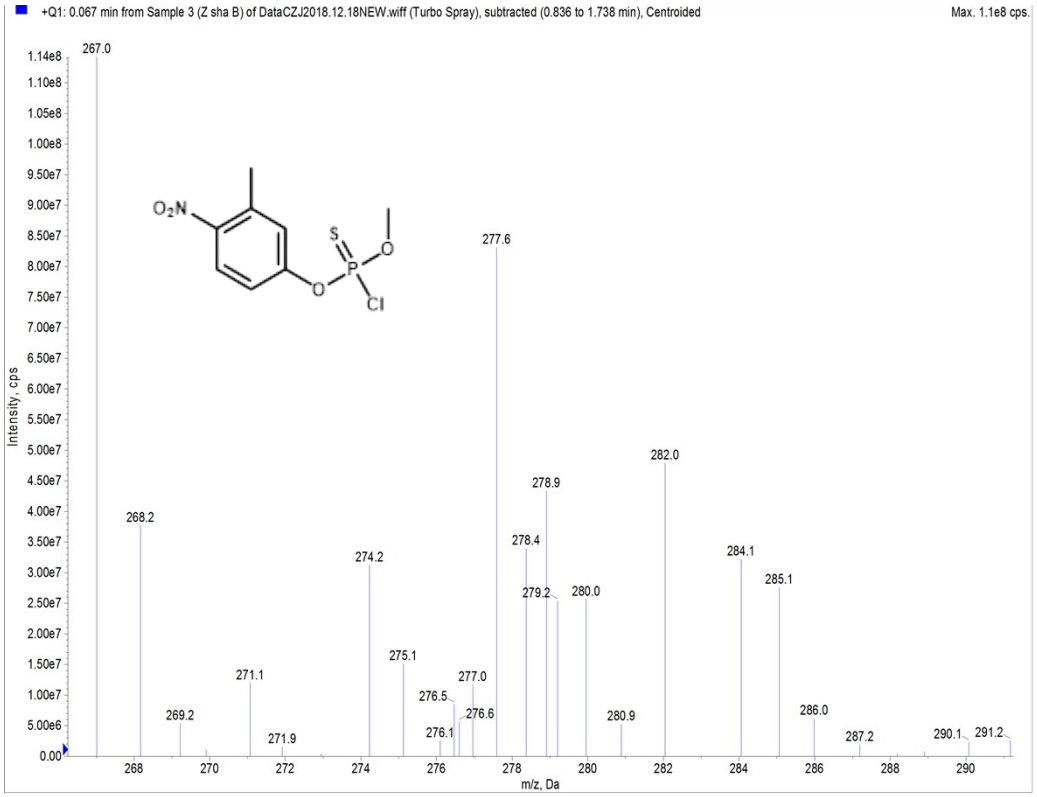


Fig. S1 The hapten synthesis route of fenitrothion.

A



B

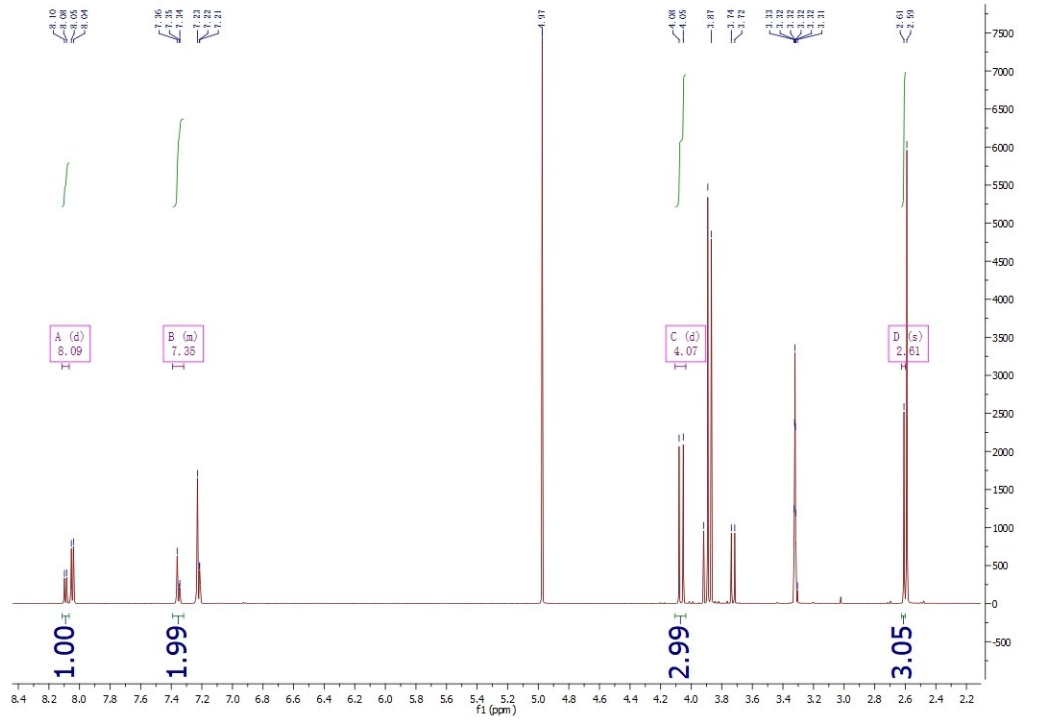
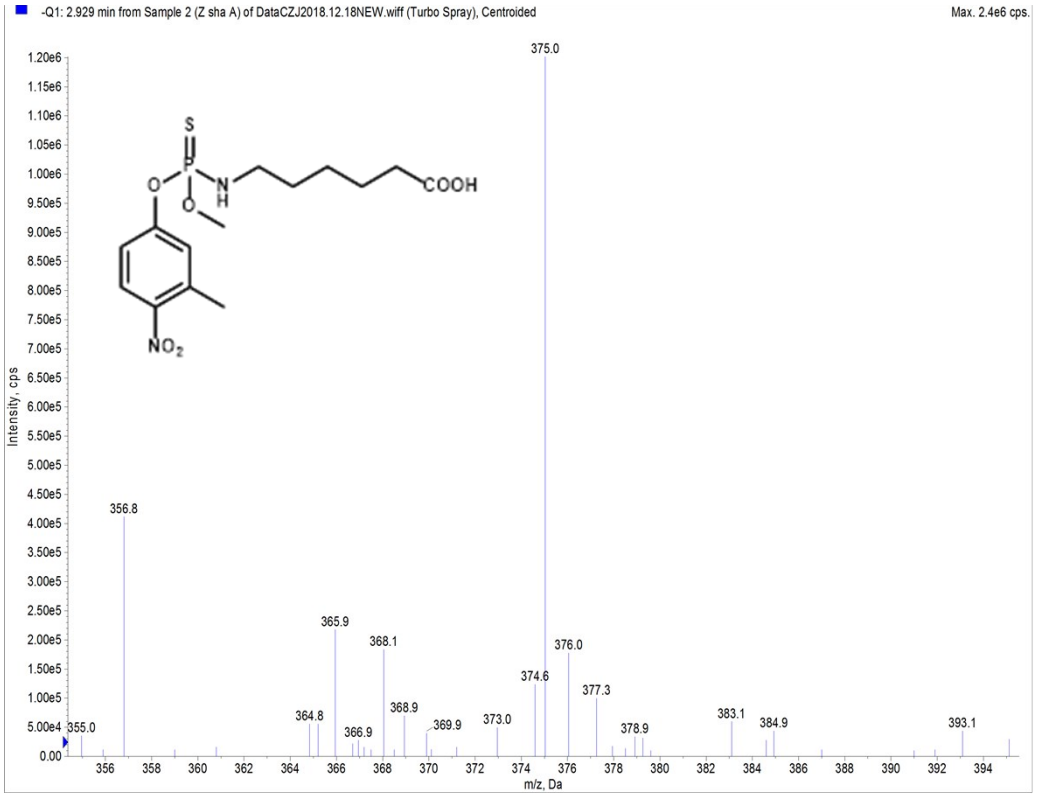


Fig. S2 Mass (A) and ^{1}H NMR (B) spectrogram of hapten-1.

A



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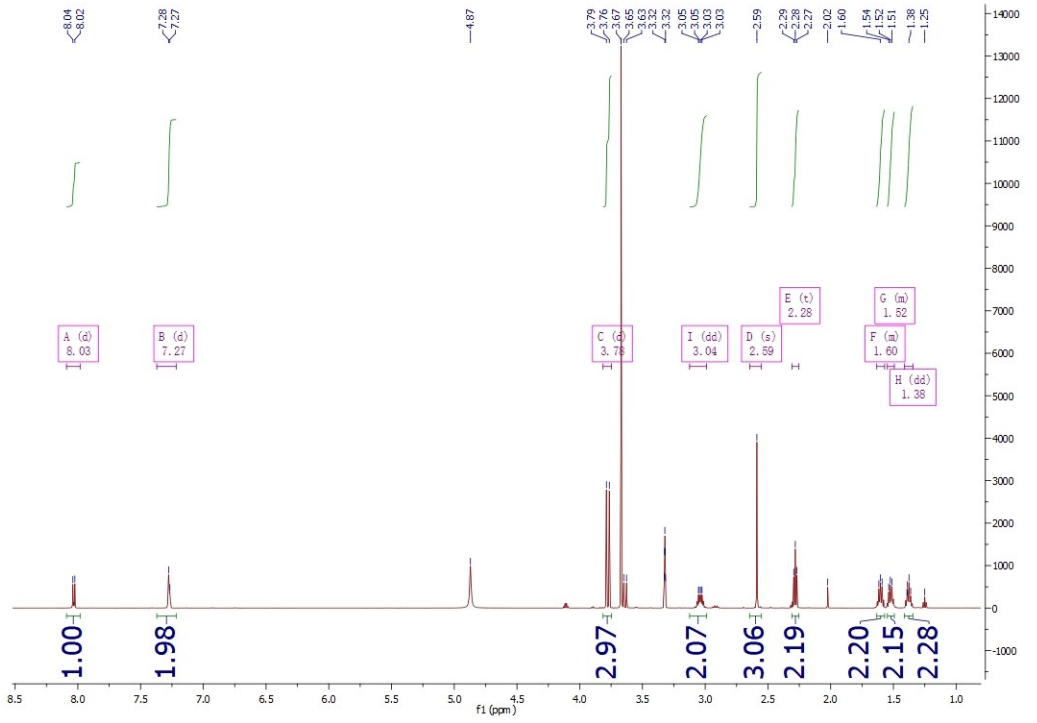

Fig. S3 Mass (A) and ^{1}H NMR (B) spectrogram of hapten-2.

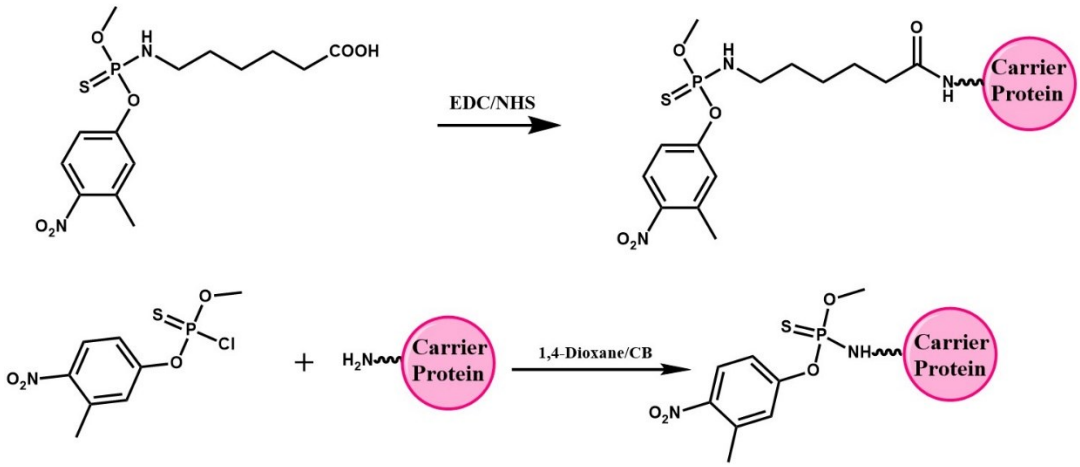

Fig. S4 Synthesis of the artificial antigen.

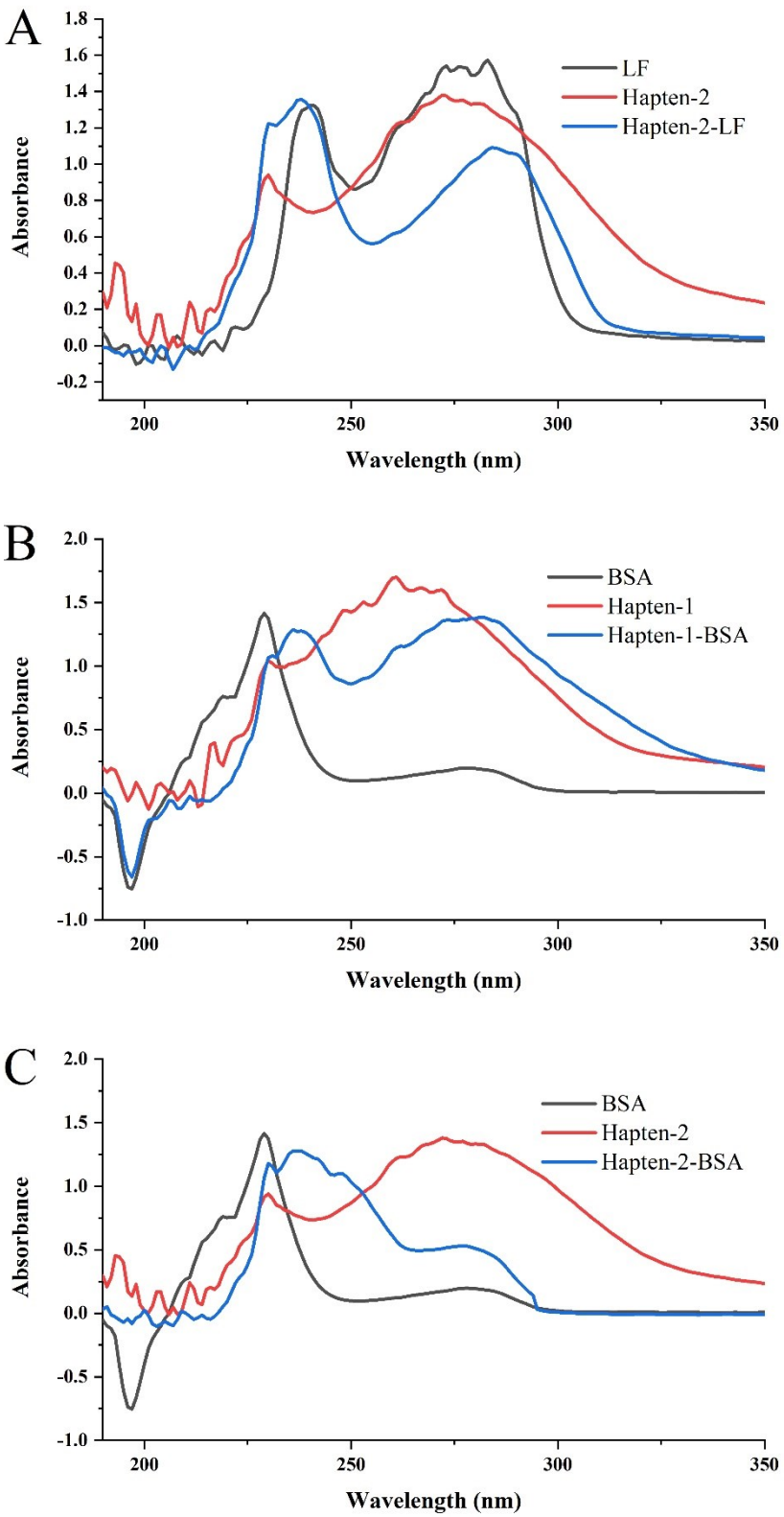

Fig. S5 UV spectra of the synthesized antigens of fenitrothion.

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Results of ethical review of animal experiments

No：2019054

Experiment Item	The preparation of monoclonal antibody for pesticides and their metabolite			
Application number	2019B054			
Comments on conservation of experimental animals	All the experimental mice used in this experiment came from experimental animal centre legal license. The type, quantity and grouping of mice were conformed to the 3R principle.			
Comments on welfare assessment of experimental animals	This experiment was carried out in a laboratory with a license for experiment animal, which was conformed to the welfare principle.			
Comments on ethical and moral	The animals were euthanized after the experiment.			
Comments on comprehensive scientific evaluation	This experimental study has scientific significance.			
Time of experiment animal type and quantity	Date: 2019-05-15 to 2019-07-30. Experimental animal: SPF BAL B/c female mice. Quantity: 70.			
Comments of ethical reviewer	Agree			
	Reviewer	Zhonghua Liu	Review Date	2019-05-08
Comments of ethical reviewer	Agree			
	Reviewer	Wei Huang	Review Date	2019-05-08
Final comments of director (or deputy director)	Agree			
	Reviewer	Ming Liao	Review Date	2019-05-08

Experimental Animal Ethics Committee of
South China Agricultural University

Date 2019-05-08

Fig. S6 Ethical review of animal experiments.

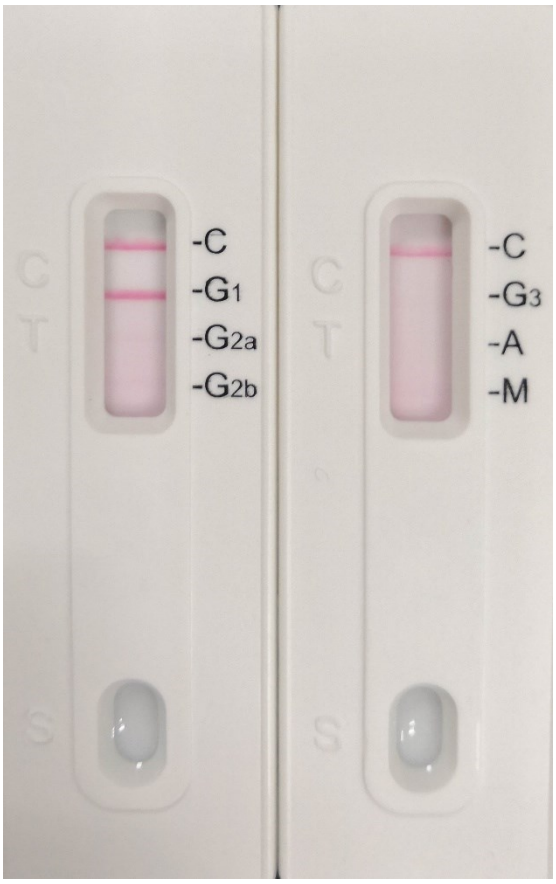

Fig. S7 The isotyping of anti-fenitrothion mAb was IgG_1.

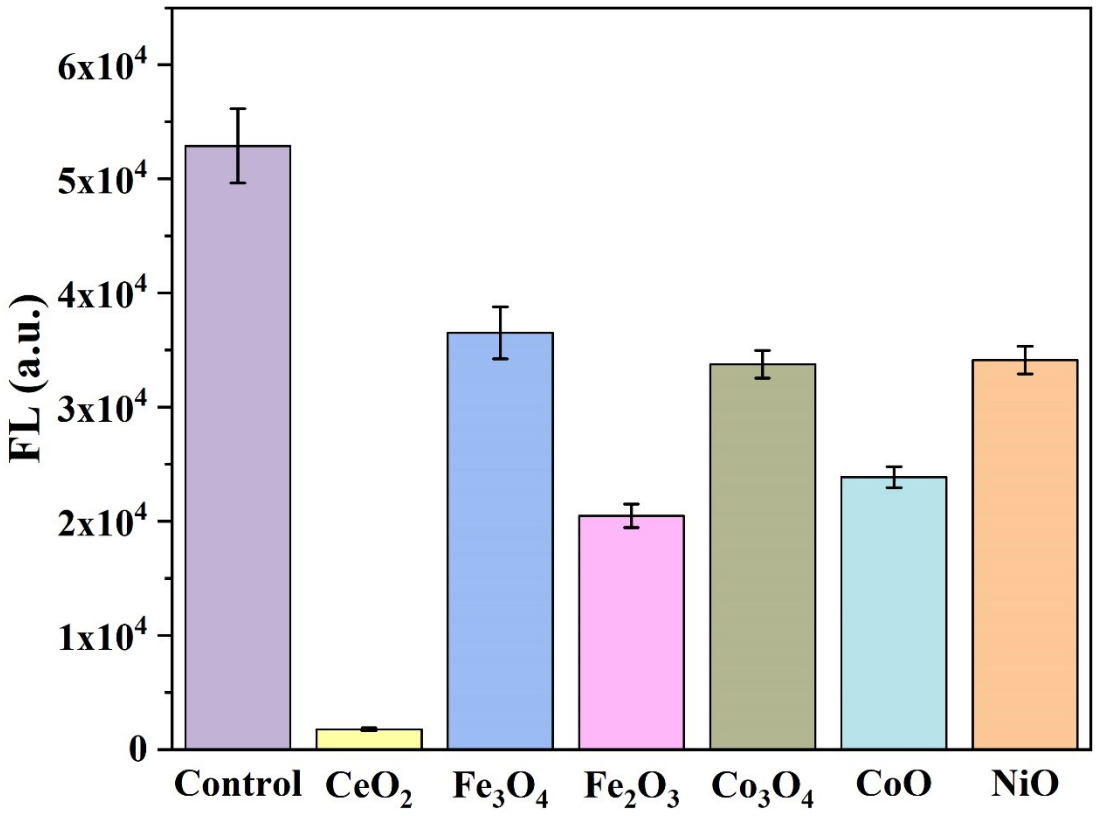

Fig. S8 FITC-BSA adsorption on the metal oxide nanoparticles. 450 μL of FITC-BSA (100 μg/mL) was mixed with 3 μL of 100 mg/mL metal oxide nanoparticles for 5 min incubation and centrifuged at 15000 rpm for 5 min. The florescence intensity values of supernatants were subsequently measured (Ex 490 nm; Em 525 nm).

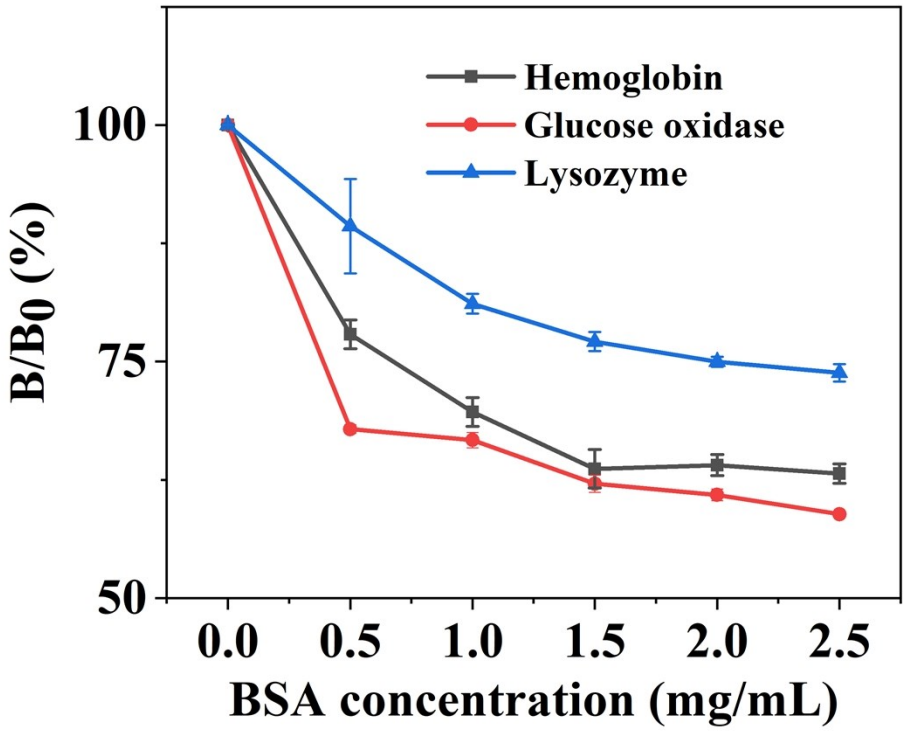

Fig. S9 The oxidase-like activity of CeO_2 inhibited by hemoglobin, glucose oxidase and lysozyme. B_0 is the mean absorbance of the wells of TMB^{2+} (5 mM, in 20 mM pH 4.0 citrate buffer, 10% H_2SO_4 was added before measuring) in the absence of BSA; and B is the mean absorbance of the wells in the presence of various concentrations of BSA.

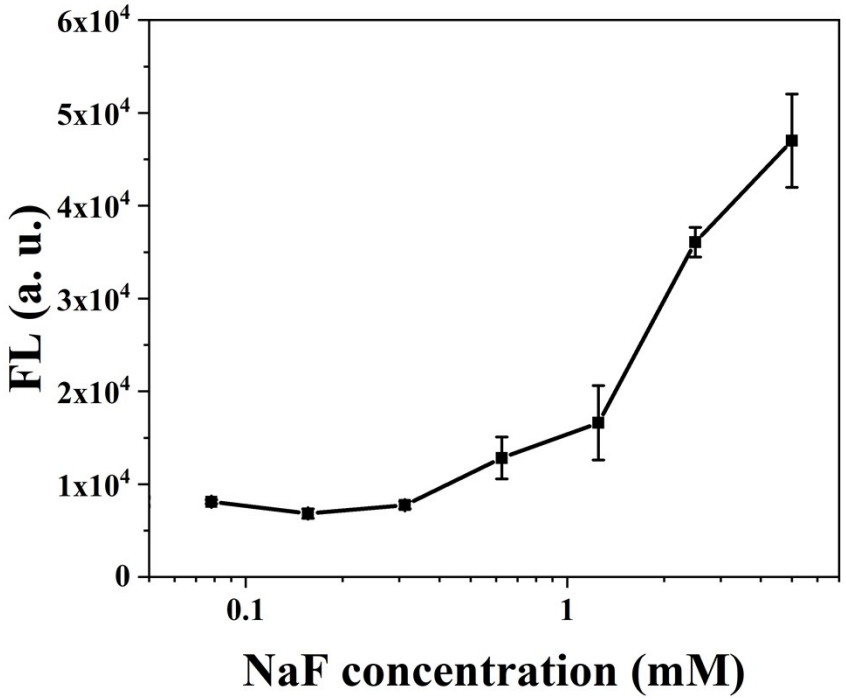

Fig. S10 F^- concentration dependent desorption of FITC-BSA from the CeO_2 nanoparticles. For physisorbed BSA, a low concentration (31 μM) of F^- can avoid desorption of FITC-BSA.

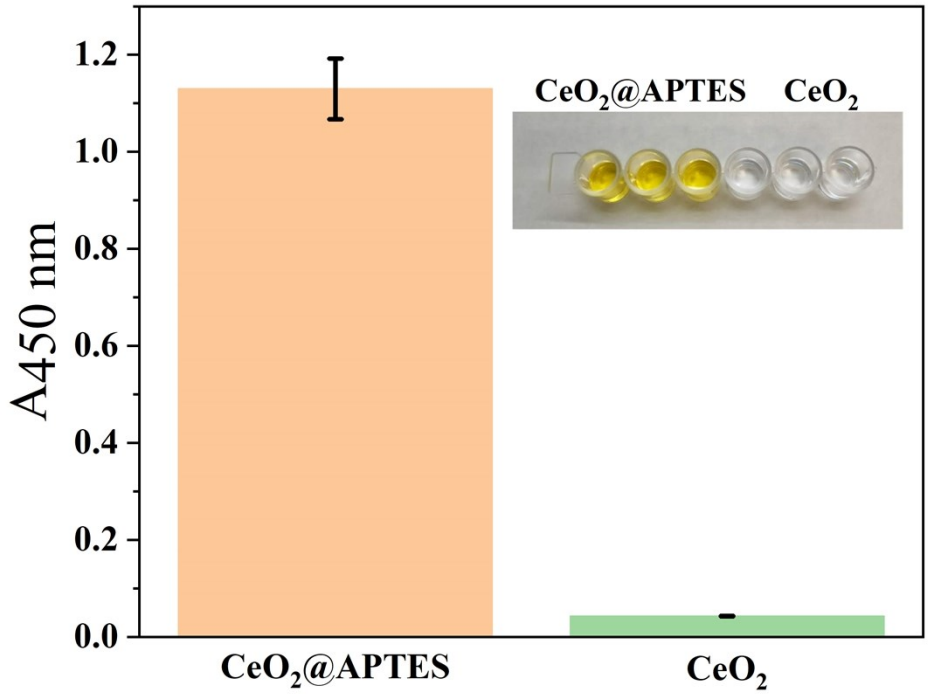

Fig. S11 ELISA titer of the CeO_2@APTES conjugated antibody and the bare CeO_2 labeled antibody. The absorbance was measured 1 h after adding TMB (0.5 mM, in 0.25% H_2SO_4 with 40 mM NaF).

Table S1 Characterization of mice antiserum against fenitrothion with homologous and heterologous coating antigen.

Coating antigen	Immunogen Hapten2-LF					
	Mouse 1		Mouse 2		Mouse 3[c]	
	Titer[a] ($\times 10^3$)	Inhibition[b] (%)	Titer ($\times 10^3$)	Inhibition (%)	Titer ($\times 10_3$)	Inhibition (%)
Hapten 1 -BSA	128	9	64	0	16	17
Hapten 2 - BSA	16	72	16	76	32	79

[a]Titer is defined as the dilution factor of antiserum with the absorbance at 450 nm being situated at about 1.0~1.5 at a coating concentration of 100 ng/mL.

[b]Percentage inhibition is expressed as follow: inhibition (%) = $[1 - (B/B_0)] \times 100$. B_0 is the mean absorbance of the wells in the absence of a competitor; and B is mean absorbance of the wells in the presence of a certain concentration of a competitor.

[c]The mouse produced highest inhibition of anti-serum was chosen for mAb production.

Table S2 Characterization information of various metal oxide nanoparticles

Materials	Size[a] (d, nm)	Hydrodynamic Size (d, nm)	ζ-Potential (mV)	Density (g/mL)	Protein adsorption[b] (ng/nmol)	Protein adsorption[c] (ng/nm^2, 10^{-6})	Vendor	Catalog Number
CeO_2	5	5.9±0.4	34.5±2.36	7.22	0.85±0.002	1527±3	Sigma	289744
CoO	50	591±145	22.6±1.13	6.44	24.7±0.48	5.0±0.1	US Research Nano.	US3051
Co_3O_4	10-30	128±22	-1.85±1.12	6.11	1.21±0.06	25±2	US Research Nano.	US3056
NiO	10-20	434±82	28.0±2.47	6.67	0.8±0.03	63.6±2.4	US Research Nano.	US3356
Fe_2O_3	20	322±28	-9.56±4.3	5.24	2.94±0.12	71±3	US Research Nano.	US3200
Fe_3O_4	50	534±24	12.5±3.79	5.17	3.87±0.27	0.97±0.07	Sigma	637106

[a] The information provided the vendors.

[b] The FITC-BSA adsorption (ng) of various metal oxide nanoparticles (nmol). The molar concentration of various metal oxide nanoparticles was calculated based on the average diameter and density.

[c] The FITC-BSA adsorption (ng) of unit surface area of various metal oxide nanoparticles (nm^3).

Table S3 Parameters of GC-MS/MS

Injector temperature	270 ℃
Injection volume	1 μL
Carrier gas	Helium
Constant flow rate	1.2 mL/min
Temperature programming	40℃, 1.5min; 40℃ to 90 ℃, 25 ℃/min; 90 ℃, 1.5min; 90 ℃ to 180℃,25℃/min; 180℃ to 280 ℃,5 ℃/min; 280 ℃ to 300℃, 10℃/min; 300℃, 5 min
EI mode	MRM
Ion source temperature	280℃
Quadrupole temperature	150℃
Quantitative fragment ion (collision energy)	*m/z* 277.0>260.0（5 V）
Qualitative fragment ion (collision energy)	*m/z* 277.0>109.0（15 V）