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Ratiometric sensor with the selective fluorescence enhancement effect based on photonic crystals for the determination of acetylcholinesterase and its inhibitor

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Fabrication of AuNCs/FL and AuNCs/FL-PhCs

The AuNCs were synthesized by chemical reduction of $HAuCl_4$ according to the previous literature with a litter change [S1, S2]. All glass containers in this experiment were cleaned in a bath of freshly prepared aquaregia (Caution!), and rinsed thoroughly with ethanol and ultrapure water prior to use. $HAuCl_4$ solution (5 mL, 10 mM) was added to an equal volume of aqueous BSA solution (50 mg/mL) under vigorous stirring for 2 min at 37 ℃. Afterward, 0.5 mL of 1 M NaOH solution was introduced, the mixture solution was under continuous stirring at 37 ℃ for 12 h for the purpose of incubation. The role of BSA in the synthesis is to act as both reducing agent and stabilizing ligand. The color of the solution changed from light yellow to light brown after reaction for 12 h. Then, the mixture solution was dialyzed in ultrahigh-purity water for 48 h (changing the water every 8 h). At last, the collected AuNCs solution was stored at 4 ℃ for further used.

Fluorescein (FL) powder was first dissolved in 10 mL ethanol by stirring to a final

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concentration of 0.4 mM. In addition, the prepared FL solution was stored at 4℃ in a dark environment for further used.

FL (200 μL) and AuNCs (800 μL) were added to 1.5 mL centrifugal tube, which was then sonicated for 30 min in an ice bath by covering with aluminum paper to avoid light. This probe was then centrifuged at 10 000 rpm for 5min and filtered using 0.22 μm syringe filters, the supernatant was collected and used for further experiment. This is the fabrication process of AuNCs/FL.

The fabrication process of AuNCs/FL-PhCs was as follows, 10 μL of AuNCs/FL ratiometric probe solution was mixed with 10 μL solvent of ethanol/water of 2:1 (v/v), and the above mixture solution was dripped onto the surface of PhCs film.

Quenching the fluorescence of AuNCs caused by different groups

Different concentration of kind of quenchers, such as ATCh and AChE mixture solution, ACh and AChE mixture solution, $N(CH_3)_4Br$, $HSC_2H_4NH_2$, HSC_2H_4COOH, were separately added into the 8 μL AuNCs solution. And then, 10 mM PBS (pH = 7.4) added to the above solution to a final volume of 500 μL. The final solution was incubated at 37 ℃ for 50 min. The fluorescence spectra were recorded at room temperature by an FS-5 fluorescence spectrometer. The quenching efficiency (*QE*) of kinds of quenchers was calculated by using the equation, where I_0 is the fluorescence intensity of AuNCs in the absence of quencher, and *I* refers to the fluorescence intensity of AuNCs in different kind of quenchers, respectively. The numerical value of *I* is the fluorescence intensity of AuNCs at the wavelength of 670 nm.

$$QE = \frac{I_{0(no\ quencher)} - I_{(quecener)}}{I_{0(no\ quencher)}}$$

Detection of sensitivity and selectivity of paraoxon

AChE (0.8 U/mL) solution of 5 μL was incubated with different concentrations of organophosphate (in 10 mM PBS, pH=7.4) in a final volume of 15 μL at 37 ℃ for 60 min. Then, 5 μL of ATCh (1.6 mM) was added, and the reaction solution was incubated at 37 ℃ for 36 min. The reaction solution of 10 μL was then added to AuNCs/FL ratiometric probe solution to a final volume of 20 μL (in ethanol /water

2:1 v/v, 10 mM PBS, pH 7.4). And then, the resulting mixture was all dripped onto the surface of PhCs, and that was scanning by fluorescence spectroscopy using the same step as the section of "Assay of activity of AChE". The Inhibition efficiency (*IE*) of paraoxon on AChE was calculated by the following equation [S3], in which $(I_{670}/I_{515})_0$ refers to the fluorescence intensity ratio of AuNCs/FL-PhCs in the absence of AChE and inhibitor, $(I_{670}/I_{515})_x$ (inhibitor) and (I_{670}/I_{515}) (no inhibitor) are the fluorescence intensity ratio of AuNCs/FL during the hydrolysis reaction with AChE in the presence and the absence of inhibitor, respectively. The I_{670} and I_{515} are the maximum fluorescence intensity of AuNCs and FL, respectively.

$$IE = \frac{(\frac{I_{670}}{I_{515}})_{x(inhibitor)} - (\frac{I_{670}}{I_{515}})_{(no\ inhibitor)}}{(\frac{I_{670}}{I_{515}})_0 - (\frac{I_{670}}{I_{515}})_{(no\ inhibitor)}}$$

Moreover, the detection selectivity of this ratiometric sensor for paraoxon was examined including to potential interferes of 100 ng/mL glucose (Glu), vitamin C (Vc), Na^+, K^+, Mg^{2+}, Ca^{2+}, Ba^{2+}, Zn^{2+}, Fe^{3+}, Cl^- and PO_4^{3-}, which were compared with 20 ng/mL of paraoxon.

Analysis of paraoxon in real samples

Apple, a kind of common fruit, was chosen to evaluate the potential of this assay for paraoxon detection in real samples. Firstly, the apple samples were shattered homogenously in a blender. And then, 0.5 g fragment was mixed with 50 mL phosphate buffer solution (PBS, 10 mM, pH 7.4) for 20 min under vigorous stirring. Then, the resulting mixture was filtered with filter paper to remove the insoluble materials. Different concentrations of paraoxon were mixed with the filtrate respectively to cause the final concentrations of paraoxon at a fixed range of 2-20 ng/mL. The following operation was the same as the section of "Detection of sensitivity and selectivity of paraoxon".

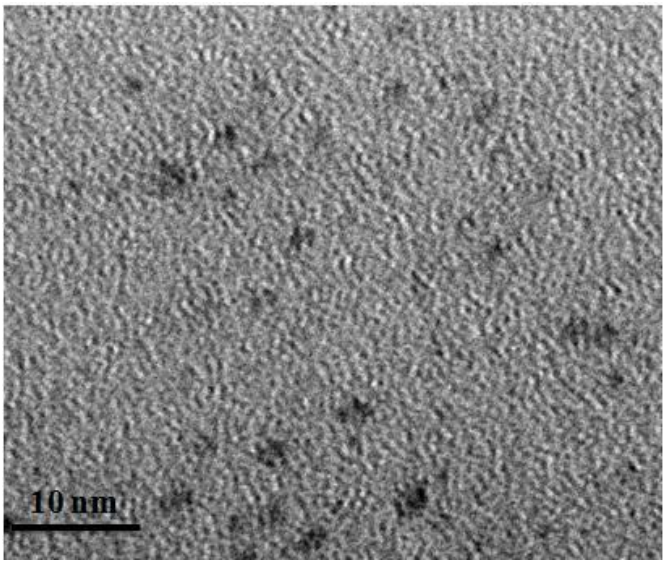

Fig.S1 TEM image of AuNCs.

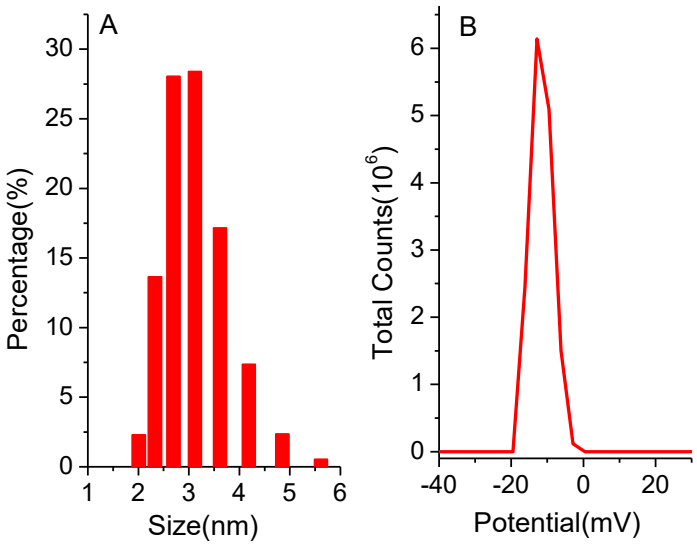

Fig.S2 The size distribution (A) and Zeta potential (B) of AuNCs solution

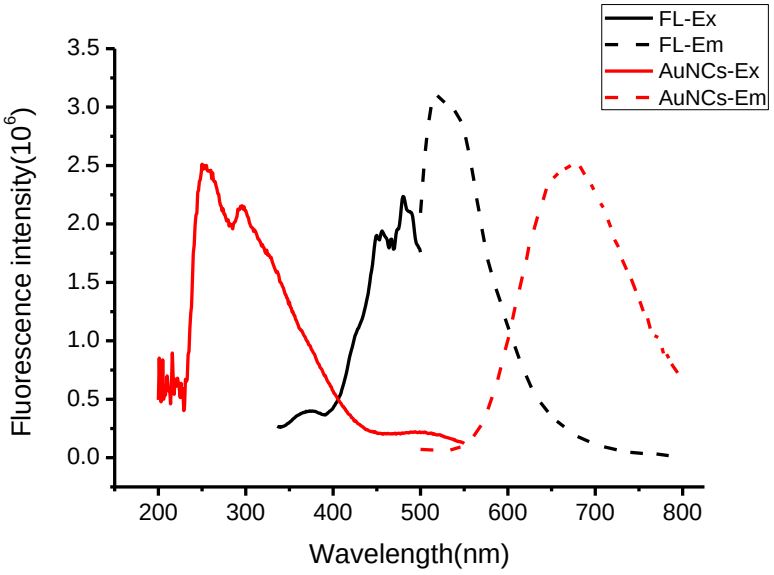

Fig.S3 The excitation (Ex) and emission (Em) spectra of AuNCs and FL.

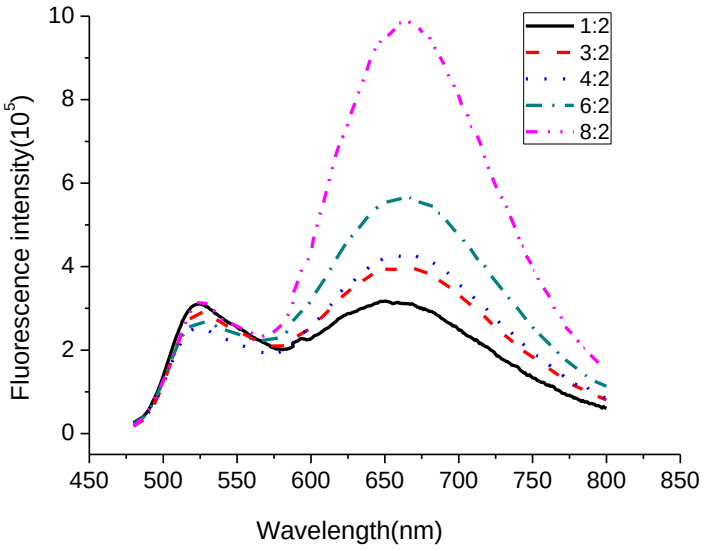

Fig.S4 Fluorescence spectra of the AuNCs/FL-PhCs with different concentration of AuNCs in a certain amount of fluorescein. The ratio value is the volume ratio of AuNCs to FL.

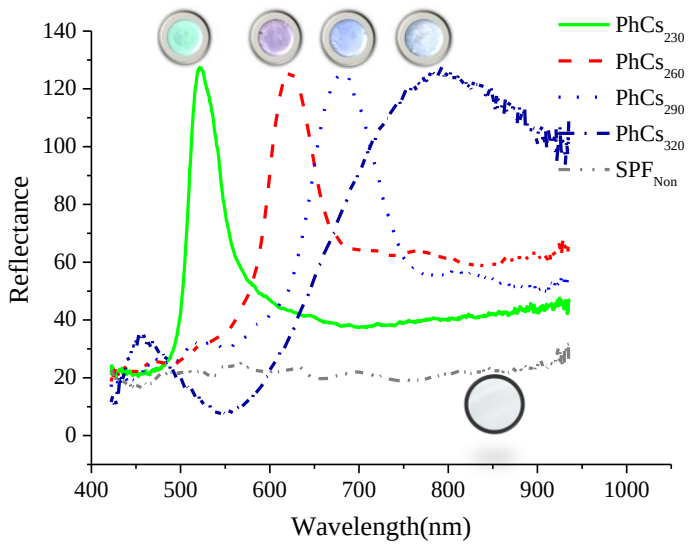

Fig.S5 Reflectance spectra of photonic crystals with different SiO_2 particle sizes.

It includes four kinds of periodic PhCs with single SiO_2 diameters of 230, 270, 290, 320 nm and one kind of non-periodic solid phase film (SPF_{Non}) with mixed SiO_2 particles.

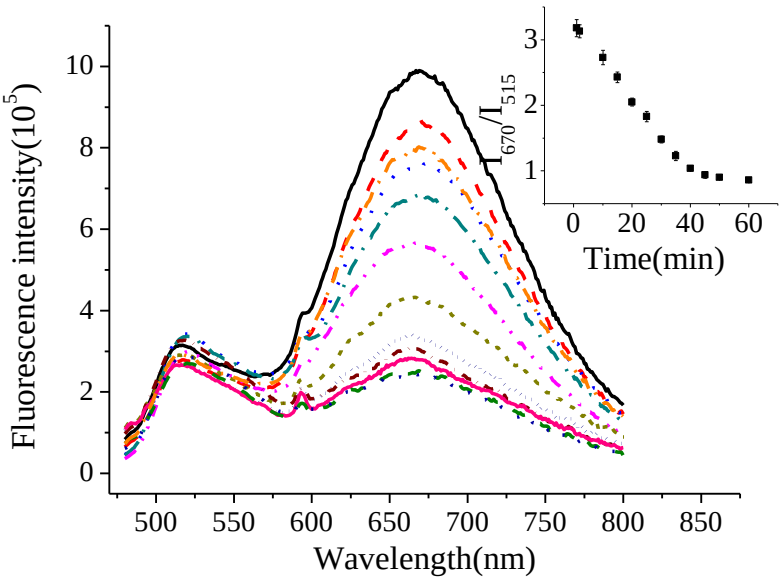

Fig. S6 Fluorescence spectra of AuNCs/FL with 200 μM ATCh and 100 mU/mL AChE at different reaction time. The insert is the I_{670}/I_{515} value versus reaction time.

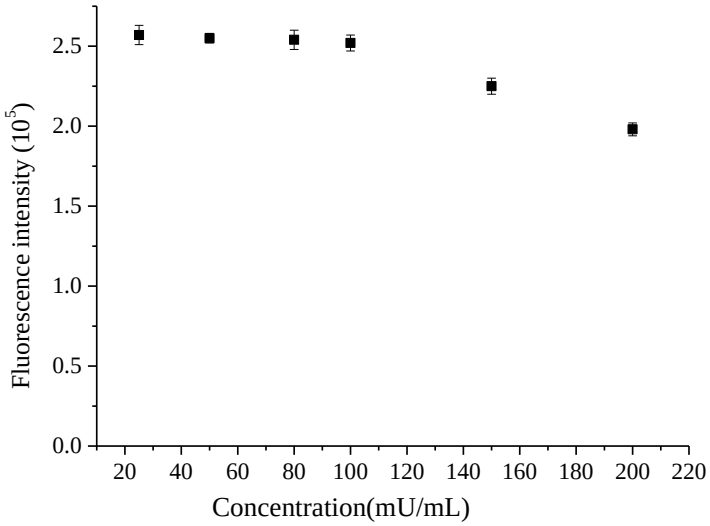

Fig. S7 The I_{670}/I_{515} value of AuNCs/FL in different concentration of AChE with pre-incubated 20 ng/mL paraoxon. The concentration of ATCh was 200 μM.

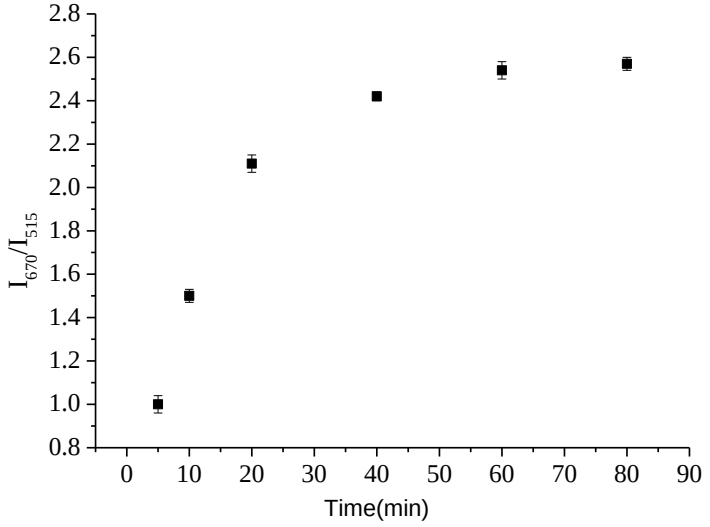

Fig. S8 The I_{670}/I_{515} value of AuNCs/FL in 100 mU/mL AChE with 20 ng/mL paraoxon at different incubation time. The concentration of ATCh was 200 μM.

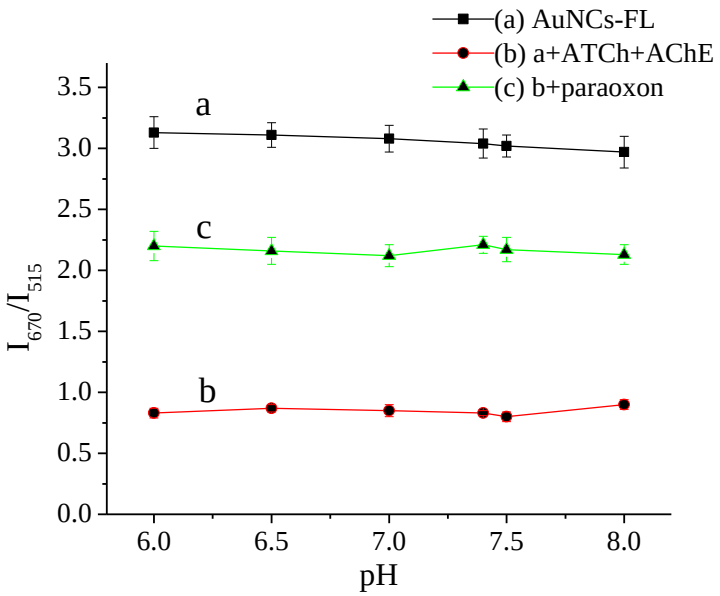

Fig. S9 The I_{670}/I_{515} value at different pH value of AuNCs/FL (a), AuNCs/FL with both 200 μM ATCh and 100 mU/mL AChE (b), AuNCs/FL in both 200 μM ATCh and 100 mU/mL AChE with pre-incubated 20 ng/mL paraoxon (c) in the PBS.

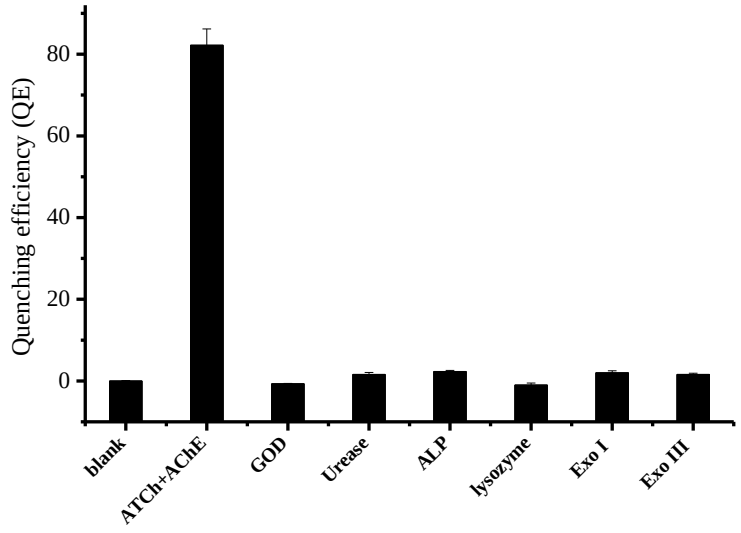

Fig.S10 The selectivity of this ratiometric probe, the concentration of AChE was 100 mU/mL, whereas the concentration of GOD, Urease, ALP, lysozyme, Exonuclease I (Exo I) and Exo III were all 500 mU/mL.

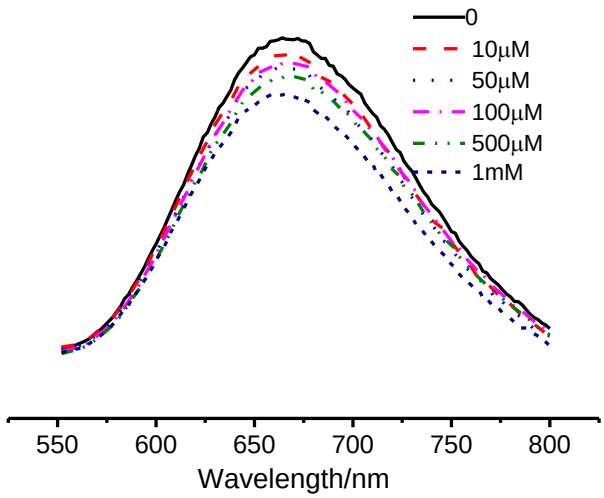

Fig.S11 Fluorescence spectra of the AuNCs with different concentrations of acetic acid.

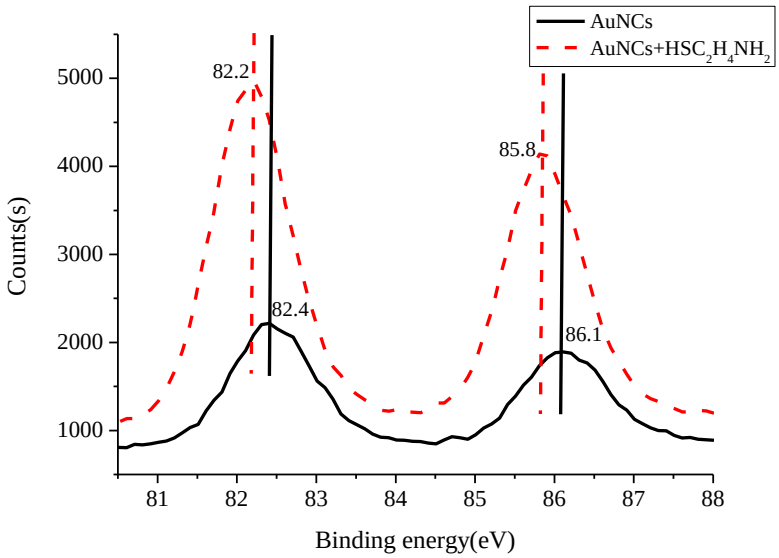

Fig. S12 XPS figure of Au in the AuNCs before and after adding mercaptoethylamine

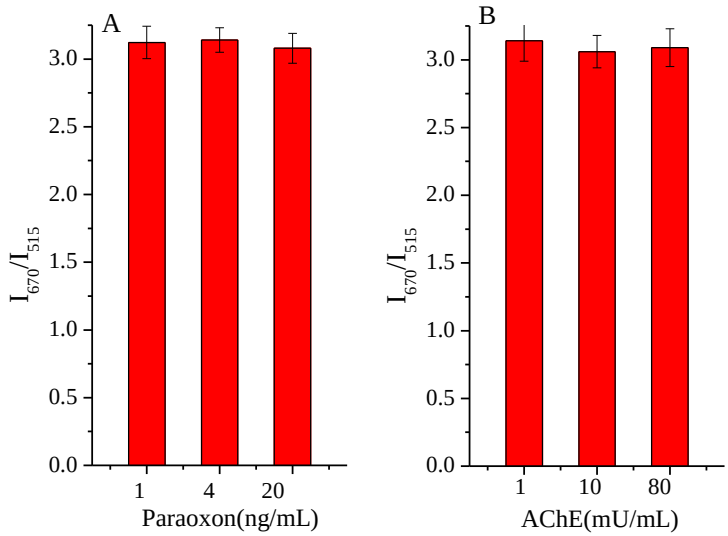

Fig.S13 Fluorescence I_{670}/I_{515} ratio of AuNCs/FL in the absence of ATCh upon addition of different concentration of paraoxon(A), a mixture of 20 ng/mL paraoxon with different contration AChE (B).

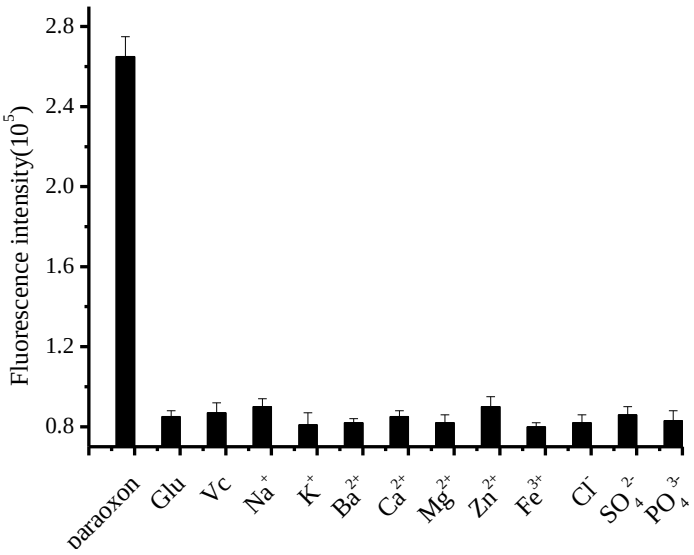

Fig. S14 The selectivity of this ratiometric sensor for paraoxon contain other relevant common ions and organic compounds, the concentration of paraoxon was 20 ng/mL, whereas the concentrations of glucose (Glu), vitamin C (Vc), Na^+, K^+, Mg^{2+}, Ca^{2+}, Ba^{2+}, Zn^{2+}, Fe^{3+}, Cl^-, PO_4^{3-} were all 100 ng/mL.

Table S1 Comparison of the determination of AChE by fluorescence method

Probe	Linear range	Limitation of detect	Ref.
AgNCs	0 – 4 U/L	0.071 U/L	S4
carbon quantum dots	14.2 – 121.8 U/L	4.25 U/L	S5
CuNCs	3 – 200 mU/mL	1.38 mU/mL	S6
Phosphorus Quantum Dots	0.2 – 5.0 U/L	0.04 U/L	S7
Nile red	0.5 – 50.0 mU/mL	0.2 mU/mL	S8
AuAgNCs	0.4 – 25 mU/mL	0.15 mU/mL	S9
ratiometric fluorescence	0.1 – 25 mU/mL	0.027 mU/mL	This work

Table S2 Comparison of the determination of paraoxon by different methods

Analytical method	Linear range	Detection limit	IC_{50}	Ref.
Colorimetric method	---	3.72×10^{-8} mol/l	4.00×10^{-6} mol/l	S10
Colorimetric method	3.3 – 66.7 ng/mL	3.3 ng/mL	--	S11
Electrochemical method	2 – 2500 ppb	2 ppb	--	S12
Electrochemical method	0.2 – 8 μmol/L	0.12 μmol/L	--	S13
Electrochemical method	2 – 20 ng/mL	2 ng/mL	--	S14
Electrochemical method	0 – 25 ng/mL	3 ng/mL	--	S15
Fluorometric method	1 – 100 ng/mL	1 ng/mL	--	S16
Fluorometric method	7 – 300 ng/mL	5.0 ng/mL	49 ng/mL	S17
Fluorometric method	0.25 – 50.0 ng/mL	0.1 ng/mL	--	S18
Fluorometric method	2 – 300 ng/mL	1.9 ng/mL	60 ng/mL	S19
Fluorometric method	0.1 – 25 ng/mL	--	1.9 ng/mL	S9
Fluorometric method	0.06 – 25 ng/mL	0.025 ng/mL (0.091 nmol/L)	1.75 ng/mL (6.36 nmol/L)	This work

Table S3 The results of paraoxon detection in real samples by this method (n=3)

Samples	Detection (ng/mL)	Added (ng/mL)	Found (ng/mL)	Recovery (%)	RSD (%)
1	--	2.00	1.94	97.0	3.6
2	--	6.00	6.22	103.7	4.7
3	--	12.00	12.25	102.1	2.9
4	--	20.00	19.79	98.95	3.2

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