Electronic Supplementary Material (ESI) for Analyst.


# Electronic Supplementary Information

## $MnO_2$/DNAzyme mediated ratiometric fluorescence assay of acetylcholinesterase

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## Materials and Chemicals

Potassium permanganate ($KMnO_4$), manganese sulphate ($MnSO_4$), dopamine (DA), glutathione (GSH) and benactyzine hydrochloride were purchased from Aladdin Reagent Co., Ltd. (Shanghai, China). Acetylcholinesterase (AChE) from electrophorus electricus, butyrylcholinesterase (BChE) from equine serum, acetylcholine iodide (ATCh, ⩾98%), glucose oxidase (GOX), and alkaline phosphatase (ALP) were obtained from Sigma-Aldrich Company (St. Louis, Mo). DNA oligonucleotides, N,N,N',N'-Tetramethylethylenediamine (TEMED), ammonium persulfate (APS), and 4S Red Plus nucleic acid dye were prepared by Sangon Inc. (Shanghai, China). The sequences were provided in Table S1. Human whole blood samples were collected from the healthy volunteers at the Hospital of Northeastern University. All the other chemical reagents were of analytical reagent grade and provided by standard reagent suppliers. Ultrapure water with conductivity of ⩾18.2 MΩ·cm (25℃) was obtained on a Milli-Q water purification system (Millipore) and used throughout the experiments.

## Instrumentation

X-ray diffraction (XRD) patterns were collected on an X'Pert Pro (PANalytical BV, Holland) diffraction meter with Cu Kα, λ=0.154 nm. Fourier transform infrared spectra (FT-IR) were recorded in the solid powder using potassium bromide pellets on a VERTEX 70 FT-IR spectrophotometer (Brooker, Germany) from 4000 to 500 $cm^{-1}$. The scanning electron microscopy (SEM) images were recorded on a SU8010 scanning electron microscope (Hitachi High Technologies, Japan). The transmission electron microscopy (TEM) images were obtained by a field emission high resolution 2100 F transmission electron microscope (JEOL, Japan) operating at 200 kV. X-ray photoelectron spectroscopy (XPS) spectra are recorded on an ESCALAB 250 surface analysis system (Thermo Scientific, USA). Fluorescence spectra were recorded on an RF-6000 fluorescence spectrophotometer (Shimadzu, Japan) equipped with a quartz cell. UV-vis absorption spectra were recorded on a U-3900 double-beam UV-vis

absorption spectrophotometer (Hitachi High-Techonologies Corporation, Japan). Fluorescence lifetime spectra were obtained by using a FluoroMax-4 TCSPC spectrofluorometer (HORIBA Jobin Yvon, USA). pH measurement and regulation were conducted on a SevenCompact Multi-parameter tester S220 METTLER TOLEDO Instruments (Co., Ltd., Shanghai, China). Electrochemical measurements were carried out on a CHI 660D electrochemical workstation (CH Instruments, China). Gel electrophoresis was performed using the GelDoc XR$^+$ Imaging System (Bio-Rad, USA).

**Preparation of $MnO_2$ Nanowires**

$MnO_2$ nanowires were prepared by the hydrothermal reaction according to the reported literature with minor modifications.[1] 2 g $KMnO_4$ and 1 g $MnSO_4$ were dissolved in 40 mL ultrapure water under magnetic stirring for 30 min. The above solutions were transferred into a 100 mL Teflon-lined stainless-steel autoclave and maintained at 180°C for 12 h. After cooling at room temperature, the obtained material was centrifuged at 5000 rpm for 8 min and washed with ultrapure water for three times at room temperature. Finally, the obtained product was dried at 60°C for 5 h in oven, and then crushed with a mortar and pestle and stored for future use.

**$MnO_2$/DNAzyme Sensing Platform**

First, the locked strands were obtained by mixing the DNAzyme strands and the DNA H2 strands at a molar ratio of 1:1. The locked strands were heated to 95°C for 5 min and gradually cooled to 25°C. Then, the locked strands, FAM-labeled signal probe DNA-F and NR at a molar ratio of 2:1:10 were mixed and stirred at room temperature in PB buffer (10 mM, pH 6.0), storing at 4°C atmosphere for future use.The displacement of locked strands through H2 strands was studied by non-denatured polyacrylamide gel electrophoresis (PAGE). The same quantities of DNAzyme strands and H2 strands were mixed and annealed according to the above mentioned temperature procedure. After introducing H2 strands, the mixture was adjusted to 20 μLby PB buffer. It was incubated at 37°C for 1 h, then 10 μL mixture solution was loaded onto 15% polyacrylamide gel. Electrophoresis was performed in 5×TBE buffer at 120 V for 90 min. The polyacrylamide gel stained for 20 min by 4S Red Plus

nucleic acid dye and then applied for de-staining 20 min by ultrapure water. Finally, the polyacrylamide gel imaging was recorded by UV imaging system (Junyi Instrument Co., Beijing, China).

**AChE Assay with the Sensing Platform**

50 μL AChE solution of various concentrations were mixed with 50 μL of 6 mmol $L^{-1}$ ATCh and incubated at 37°C for 30 min to generate the enzymatic hydrolysate TCh. 50 μL mixture of $MnO_2$ nanowires and H1 strands were introduced into the solution and incubated at 37°C for 40 min. Then $MnO_2$ nanowires were reduced to $Mn^{2+}$ by TCh. After 100 μL of above solution was introduced into the mixture of 50 μL DNA-F (20 μmol $L^{-1}$), NR (200 μmol $L^{-1}$) and DNAzyme-H2 strands and the final volume was set to 200 μL with PB (pH 6.0). The fluorescence emission spectra were recorded at 518 nm and 632 nm at $\lambda_{ex}$=487 nm.

The real samples of human whole blood and hemocyte were collected from healthy individuals at the Hospital of Northeastern University. The human whole blood was centrifuged at 3500 rpm for 10 min to gather the hemocyte of substratum. The obtained whole blood and hemocyte were diluted 1000-fold and stored at -20°C for the following use. The obtained real samples were applied for AChE assay according to the proposed protocol. In order to eliminate the BChE in real samples, 10 μL inhibitor of BChE solution, i.e., benactyzine hydrochloride of 2 mmol $L^{-1}$, was added for facilitating the assay of AChE.

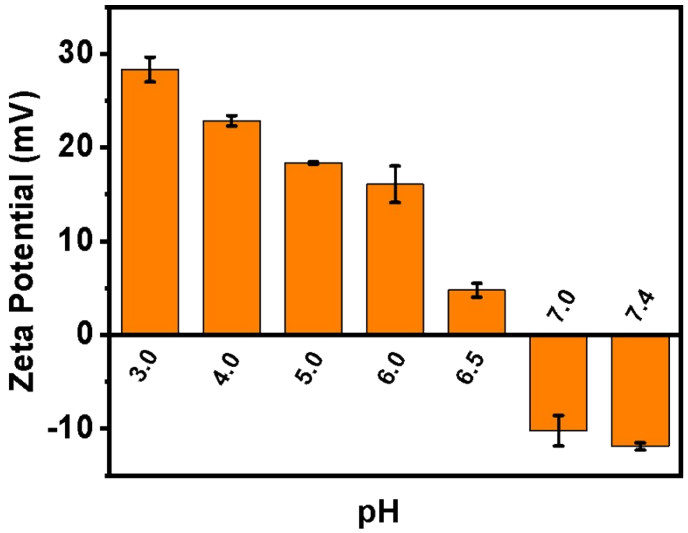


**Fig. S1.** Zeta potentials of NR at different pH values.

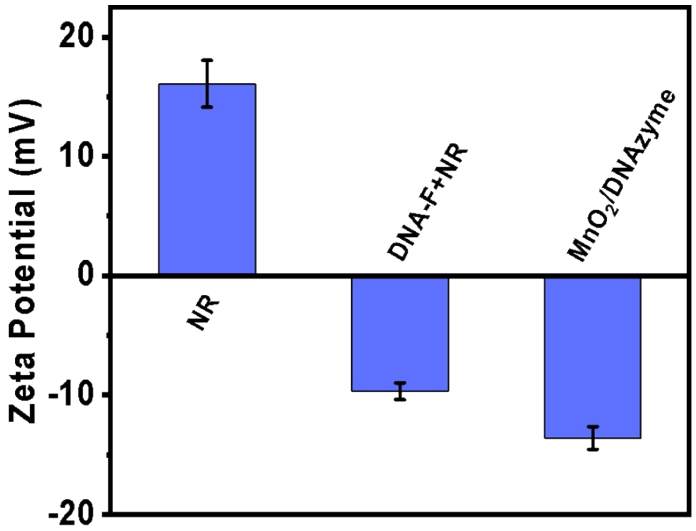


**Fig. S2.** Zeta potentials of NR, DNA-F+NR, $MnO_2$/DNAzyme systems in PB buffer solution (10 mM, pH 6.0).

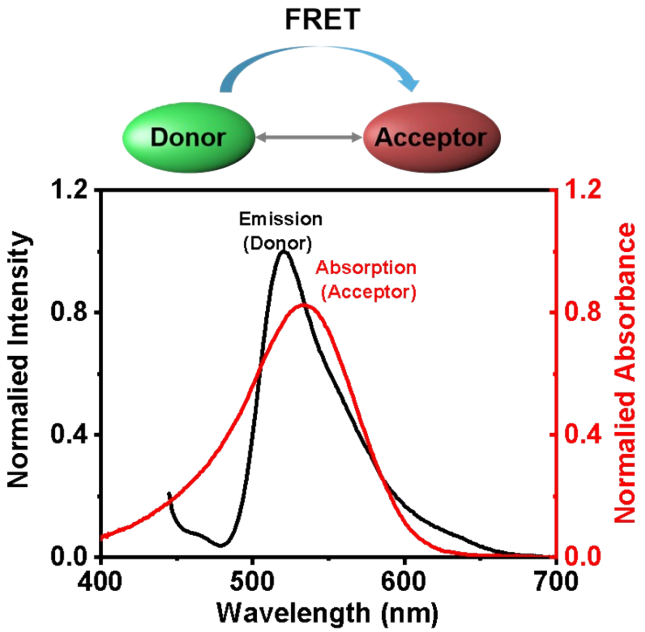


**Fig. S3.** Schematic illustration of the FRET process between DNA-F (donor) and NR (acceptor).

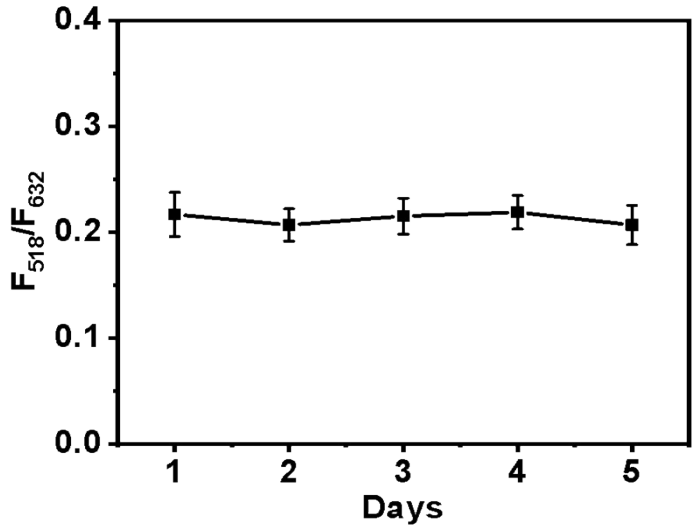


**Fig. S4.** The stability of DNA-F+NR in PB buffer (10 mM, pH 6.0) for 5 days.

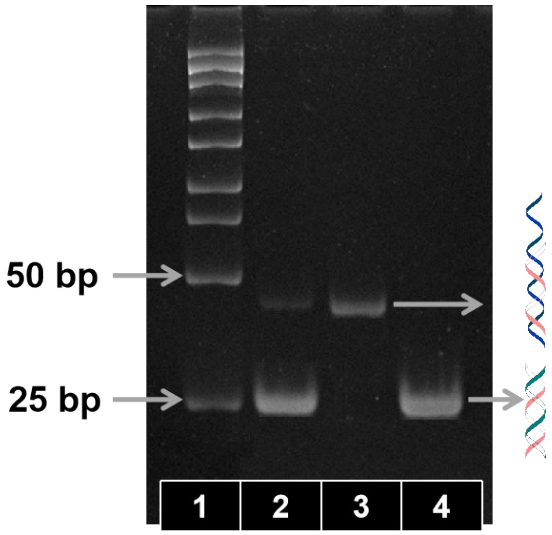


**Fig. S5.** Native PAGE for strands displacement. Lane 1, Marker, Lane 2, DNAzyme+H1+H2. Lane 3, DNAzyme+H2. Lane 4, H1+H2.

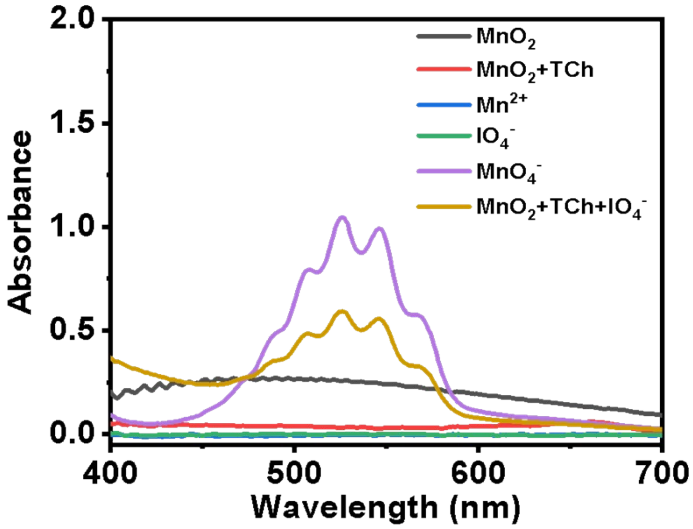


**Fig. S6.** UV-vis absorption spectra of $MnO_2$, $MnO_2$+TCh, $Mn^{2+}$, $IO_4^-$, $MnO_4^-$, and $MnO_2$+TCh+$IO_4^-$ ($MnO_2$: 0.1 mg $mL^{-1}$, $Mn^{2+}$: 10 mmol $L^{-1}$, $IO_4^-$: 10 mmol $L^{-1}$, $MnO_4^-$: 10 mmol $L^{-1}$, ATCh: 10 mmol $L^{-1}$, AChE: 20 U $mL^{-1}$).

The specific reaction between $Mn^{2+}$ and $IO_4^-$ is given in the following equation:

$2\ Mn^{2+} + 5\ IO_4^- + 3\ H_2O \rightarrow 2\ MnO_4^- + 5\ IO_3^- + 6\ H^+$

UV-vis absorption spectra were matched well with that of $MnO_4^-$ at 450-600 nm. This further indicated the presence of $Mn^{2+}$ after the reduction of $MnO_2$ nanowires with TCh.

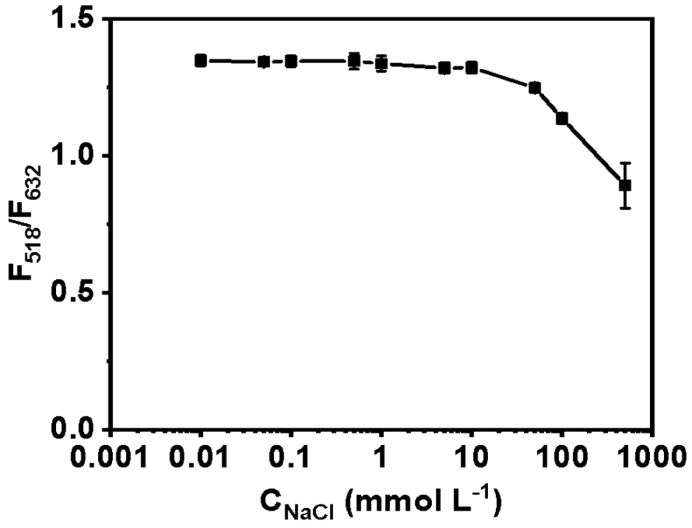


**Fig. S7.** Ratiometric fluorescence $F_{518}/F_{632}$ toward different concentrations of NaCl (DNA-F: 5 μmol $L^{-1}$, NR: 50 μmol $L^{-1}$, DNAzyme, H1, H2 strands: 10 μmol $L^{-1}$, ATCh: 1 mmol $L^{-1}$, AChE: 1 U $mL^{-1}$, $MnO_2$: 0.05 mg $mL^{-1}$, $\lambda_{ex}$=487 nm).

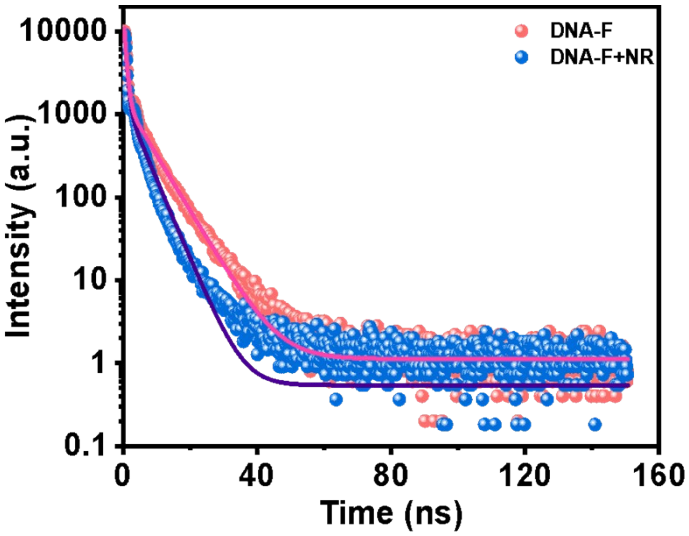


**Fig. S8.** The fluorescence lifetime curves of DNA-F in the absence and presence of NR.

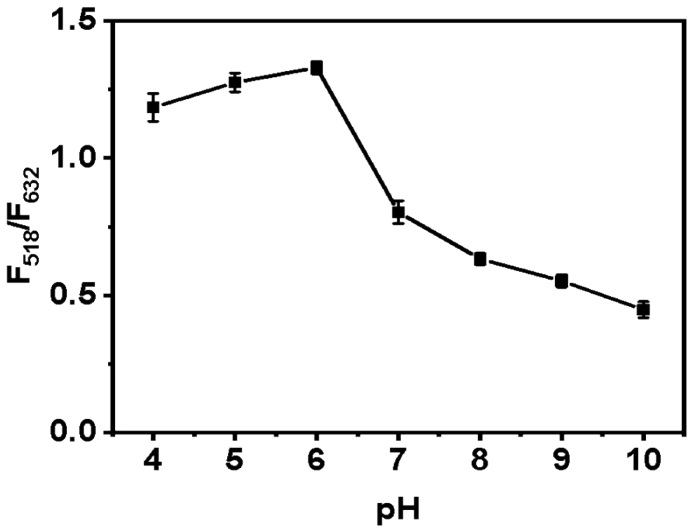


**Fig. S9.** Ratiometric fluorescence $F_{518}/F_{632}$ toward different pH values (DNA-F: 5 μmol $L^{-1}$, NR: 10 μmol $L^{-1}$, DNAzyme, H1, H2 strands: 10 μmol $L^{-1}$, ATCh: 1 mmol $L^{-1}$, AChE: 1 U $mL^{-1}$, $MnO_2$: 0.05 mg $mL^{-1}$, $\lambda_{ex}$=487 nm).

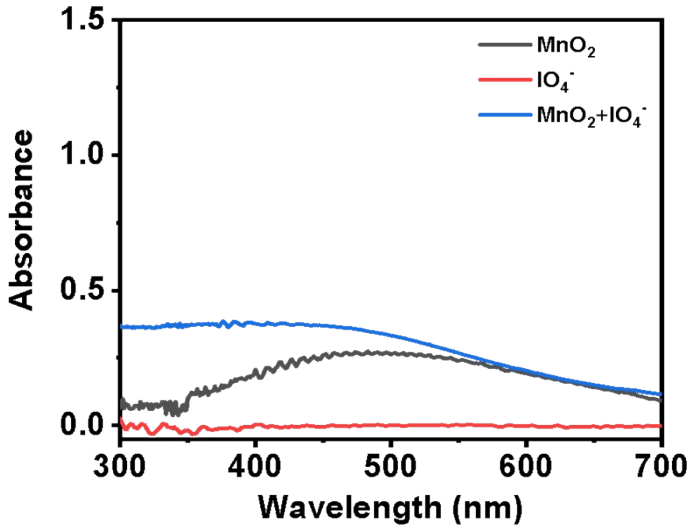


**Fig. S10.** UV-vis absorption spectra of $MnO_2$, $IO_4^-$, and $MnO_2$+$IO_4^-$ in PB buffer (10 mM, pH 6.0) ($MnO_2$: 0.1 mg $mL^{-1}$, $IO_4^-$: 10 mmol $L^{-1}$).

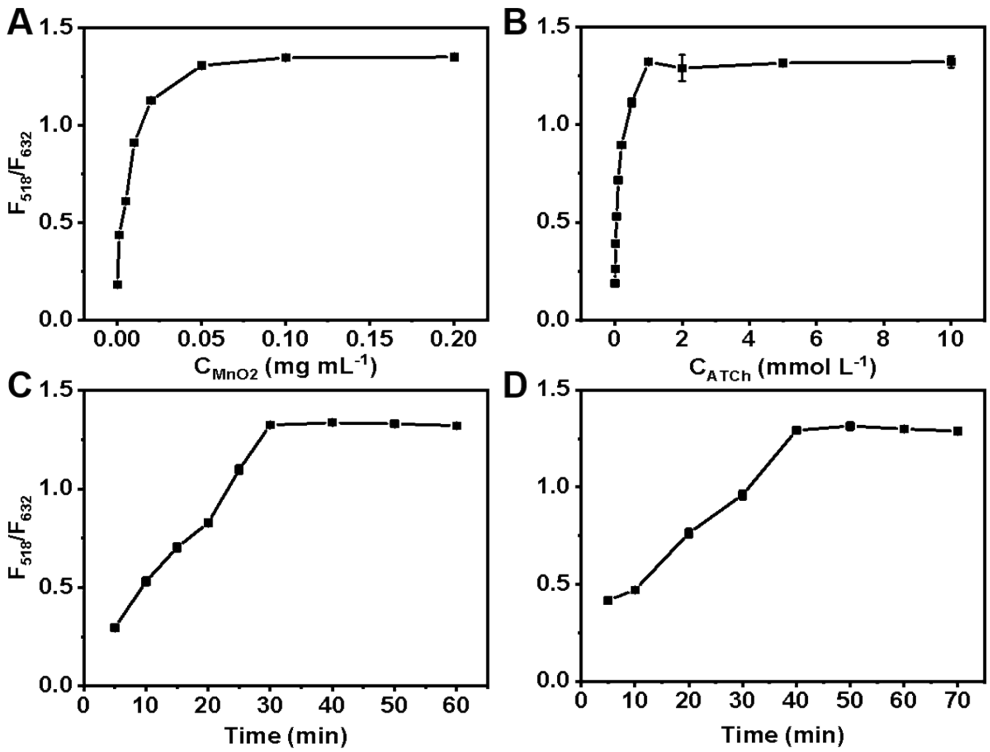


**Fig. S11.** The variation of ratiometric fluorescence at $F_{518}/F_{632}$ by changing (A) the concentration of $MnO_2$, (B) the concentration of ATCh, (C) the reaction time between AChE and ATCh, and (D) the reaction time of TCh and $MnO_2$ (DNA-F: 5 μmol $L^{-1}$, NR: 50 μmol $L^{-1}$, DNAzyme, H1, H2 strands: 10 μmol $L^{-1}$, AChE: 1 U $mL^{-1}$, $\lambda_{ex}$=487 nm).

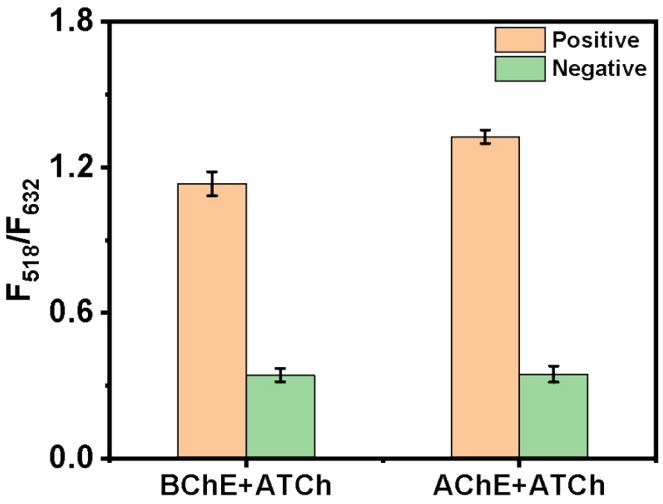


**Fig. S12.** The fluorescence responses of BChE and AChE with substrate ATCh (DNA-F: 5 μmol $L^{-1}$, NR: 50 μmol $L^{-1}$, DNAzyme, H1, H2 strands: 10 μmol $L^{-1}$, $MnO_2$: 0.05 mg $mL^{-1}$, ATCh: 1 mmol $L^{-1}$, BChE and AChE: 1 U $mL^{-1}$, $\lambda_{ex}$=487 nm).

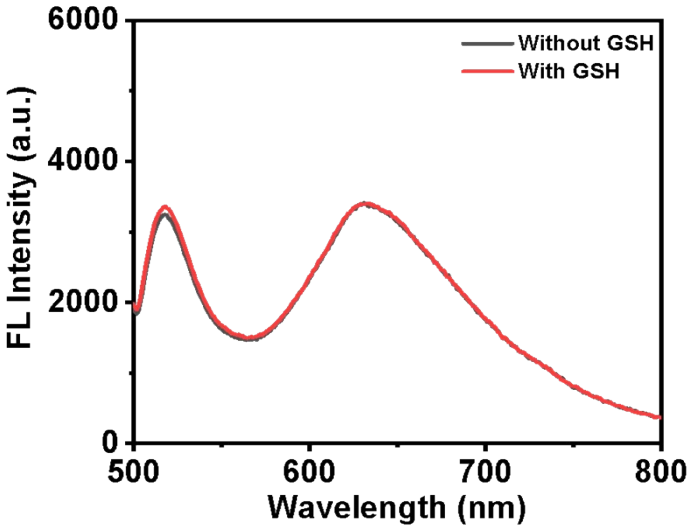


**Figure S13.** Fluorescence responses in the absence and presence of GSH (DNA-F: 5 μmol $L^{-1}$, NR: 50 μmol $L^{-1}$, DNAzyme, H1, H2 strands: 10 μmol $L^{-1}$, $MnO_2$: 0.05 mg $mL^{-1}$, ATCh: 1 mmol $L^{-1}$, AChE: 1 U $mL^{-1}$, GSH: 2 μmol $L^{-1}$, $\lambda_{ex}$=487 nm).

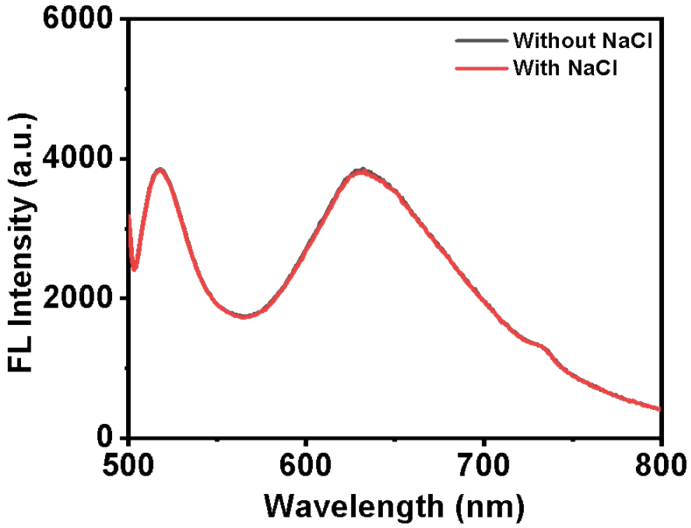


**Figure S14.** Fluorescence responses in the absence and presence of NaCl (DNA-F: 5 μmol $L^{-1}$, NR: 50 μmol $L^{-1}$, DNAzyme, H1, H2 strands: 10 μmol $L^{-1}$, $MnO_2$: 0.05 mg $mL^{-1}$, ATCh: 1 mmol $L^{-1}$, AChE: 1 U $mL^{-1}$, NaCl: 200 nmol $L^{-1}$, $\lambda_{ex}$=487 nm).

**Table S1.** Sequences of oligonucleotides designed in this work.

| Name | Sequence (5'-3') |
|---|---|
| DNAzyme strand | TAG AAC CGA ATT TGT GTC TCT TCT CCG AGC CGG TCG AAA TAG T |
| H1 strand | TAC CCT GTA GAA CCG AAT TTG TG |
| H2 strand | AGA GAC ACA AAT TCG GTT CTA CAG GGT A |
| Signal probe | CCA CCA CTA TGA AAT TGA CCC ACT AT rA GGA AGA GAT GTT ACG AGG CGG TGG TGG-FAM |

**Table S2**. The comparison of various procedures based on ratiometric fluorescence for the assay of AChE.

| Sample | Linear range ($\times 10^{-4}$ U mL$^{-1}$) | LOD ($\times 10^{-4}$ U mL$^{-1}$) | Ref. |
|---|---|---|---|
| Human blood | 5-800 | 1.6 | 2 |
| Human blood | 5-90 | 1.9 | 3 |
| Human blood | 0.3-40 | 0.021 | 4 |
| Human blood | 5-300 | 0.3 | 5 |
| Human blood | 20-700 | 5.6 | 6 |
| Human blood | 5-150 | 3.6 | 7 |
| Human blood | 30-500 | 10.8 | 8 |
| Human blood | 5-10000 | 2.7 | This work |

**Table S3.** Spiking recovery of AChE in real samples.

| Sample | Spiked (U $mL^{-1}$) | Found (U $mL^{-1}$) | Recovery (%) | RSD (%) (n=5) |
|---|---|---|---|---|
| Whole blood | 0 | 6.13 ± 0.11 | — | 2.4 |
| | 5 | 10.95 ± 0.37 | 97 | 3.2 |
| | 10 | 16.28 ± 0.28 | 102 | 1.3 |
| Hemocyte | 0 | 5.26 ± 0.19 | — | 4.8 |
| | 5 | 10.03 ± 0.15 | 95 | 1.6 |
| | 10 | 15.01 ± 0.23 | 95 | 2.9 |

## References


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