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Supporting Information

Real-time fluorescence turn-on assay for acetylcholinesterase activity based on the controlled release of a perylene probe from the MnO_2 nanosheets

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EXPERIMENTAL SECTION

Materials

P-4C+ was prepared as previously described.[S1] AChE (from Electrophorus electricus) and trypsin were purchased from Sigma (St. Louis, MO, USA). Acetylthiocholine iodide, donepezil hydrochloride and 3-hydroxycarbofuran were purchased from J&K Scientific Ltd. (Beijing, China). S1 nuclease was purchased from Fermentas Inc. (MBI, Canada). Lysozyme was purchased from Dingguo Biotechnology Co., Ltd. (Beijing, China). Alkaline phosphatase (ALP) and exonuclease I (Exo I) were purchased from Takara Biotechnology Co., Ltd. (Dalian, China). All stock and buffer solutions were prepared using water purified with a Milli-Q A10 filtration system (Millipore, Billerica, MA, USA).

Instrumentation

UV-Vis absorption spectra were obtained using a Cary 50 Bio Spectrophotometer (Varian Inc., CA, USA) equipped with a xenon flash lamp. Emission spectra were recorded using a Fluoromax-4 spectrofluorometer (Horiba Jobin Yvon Inc., USA). Excitation wavelength was 442 nm. Quartz cuvettes with 10 mm path length and 2 mm window width were used for UV-vis and emission measurements. Zeta potential measurements were performed with a Zetasizer NanoZS (Malvern Instruments, USA). Transmission electron microscopy (TEM) images were obtained using a JEM-2100F high resolution transmission electron microscope (Philips, The Netherlands) operated at 200 kV. Atomic force microscopy (AFM) characterization was performed on a Multimode-V (Veeco Instruments, USA) using a tapping mode. Unless specified, all

concentrations of **P**-4C+, ATCh, MnO_2 nanosheets and buffer were those in the final assay solutions (total sample volume, 400 μL), and all spectra were taken at 37 ºC in 5 mM phosphate buffer at pH 7.4.

Preparation of the MnO_2 nanosheets

The MnO_2 nanosheets were prepared following the reported procedures.[S2] In short, an aqueous solution comprised 20 mL of tetramethylammonium hydroxide (0.6 M) and H_2O_2 (3 wt%) was first prepared, and 10 mL of 0.3 M $MnCl_2$ was quickly mixed. The resulting mixture was stirred vigorously overnight in open air. The bulk MnO_2 was obtained though centrifugation, cleaned with double-distilled water and methanol and dried in an oven. MnO_2 nanosheets were obtained via ultrasonication of the aqueous solution of the bulk manganese dioxide.

Fluorescence quenching of P-4C+ by the MnO_2 nanosheets

A series of concentrations of the MnO_2 nanosheets (0, 8.75, 17.5, 26.25, 35, 43.75, 52.5, 61.25, and 70 μg/mL) were mixed with **P**-4C+ (5 μM) in 5 mM phosphate buffer (pH 7.4). Samples were mixed completely and maintained at 37 ºC for 3 min. The emission intensity was then recorded.

ATCh concentration optimization

Thiocholine was prepared following the reported procedures[S3] and characterized by ^{1}H-NMR (Figure S5). Different amounts of thiocholine were mixed with 5 mM phosphate buffer solution (pH 7.4) comprising 26.25 μg/mL MnO_2 nanosheets and 50 μM PPi. The samples were

incubated at 37 ºC for 60 min. 5 μM of **P**-4C+ was added. Samples were mixed and maintained at 37 ºC for 3 min. The emission intensity was then recorded. Pyrophosphate was introduced to complex with the Mn^{2+} ions, since the in situ generated Mn^{2+} ions could attach to the surface of the MnO_2 nanosheets and caused aggregation of the nanosheets.

AChE assay procedures

0.3 mM ATCh (20 μL) was injected to 380 μL of 5 mM phosphate buffer solution (pH 7.4) comprising 26.25 μg/mL MnO_2 nanosheets, 50 μM PPi, 5 μM **P**-4C+ and AChE of different concentrations. The final concentrations of AChE were 0, 5, 10, 25, 50, 100, 250, 500 and 1000 mU/mL, respectively. The temperature was stabilized at 37 ºC and the emission intensity of **P**-4C+ at 488 nm was recorded with data points taken every 5 seconds.

Selectivity of the AChE assay

Different enzymes including lysozyme, alkaline phosphatase (ALP), S1 nuclease, trypsin, Exo I (10.0 U/mL each) and AChE (500 mU/mL) were mixed with the solution of 0.3 mM ATCh, 50 μM PPi and 26.25 μg/mL MnO_2 nanosheets in 5 mM phosphate buffer (pH 7.4). The solutions were incubated at 37 ºC for 60 min. 5 μM of **P**-4C+ was added. Samples were mixed completely and maintained at 37 ºC for 3 min. The emission intensity was then recorded.

AChE assay in biological fluid

Various quantities of AChE were mixed with the sample solution of 26.25 μg/mL MnO_2 nanosheets, 0.3 mM ATCh, 50 μM PPi and 2.5% human serum in 5 mM phosphate buffer (pH

7.4). Samples were kept at 37 ºC for 15 min. 5 μM of **P**-4C+ was then mixed with the solution. Samples were mixed completely and maintained at 37 ºC for 3 min. The emission intensity was then recorded.

AChE inhibitor screening

Various concentrations of the inhibitors (donepezil or 3-hydroxycarbofuran) were mixed with the sample solution of 26.25 μg/mL MnO_2 nanosheets, 0.3 mM ATCh, 50 μM PPi and 100 mU/mL AChE in 5 mM phosphate buffer (pH 7.4). The samples were kept at 37 ºC for 15 min. 5 μM of **P**-4C+ was added. Samples were mixed thoroughly and maintained at 37 ºC for 3 min. The emission intensity was then recorded.

The inhibition efficiency (IE) is given by: $IE = [I - I_i]/[I - I_0]$

The emission intensity values of **P**-4C+ at 488 nm in the absence and presence of the inhibitor are symbolized as I and I_i, I_0 is the emission intensity of **P**-4C+ at 488 nm in the absence of AChE.

REFERENCES

S1. J. Chen, H. Jiao, W. Li, D. Liao, H. Zhou and C. Yu, *Chem. Asian J.*, 2013, **8**, 276-281.

S2. K. Kai, Y. Yoshida, H. Kageyama, G. Saito, T. Ishigaki, Y. Furukawa, and J. Kawamata, *J. Am. Chem. Soc.* 2008, **130**, 15938-15943.

S3. P. Thanei-Wyss, and P. Waser, *Helv. Chim. Acta*, 1983, **66**, 2198-2205.

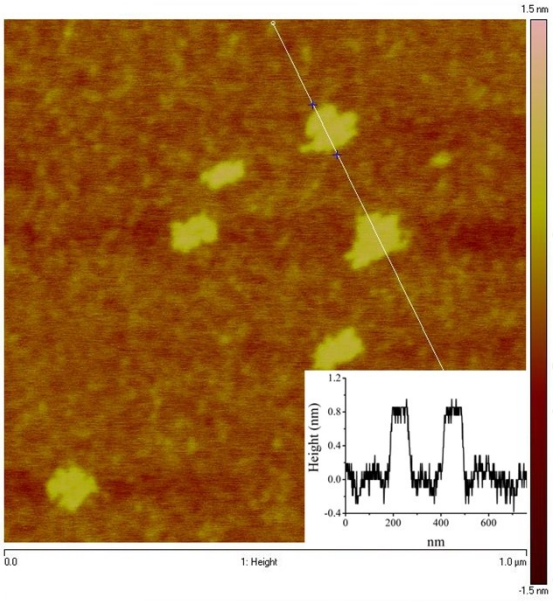

Figure S1. Tapping-mode of the AFM image of the MnO_2 nanosheets. Inset: the height profile of the section labeled with the white line.

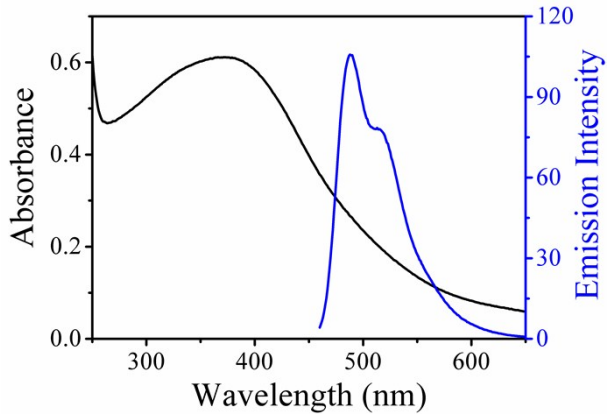

Figure S2. UV-vis absorption spectrum of the MnO_2 nanosheets (black curve) and the fluorescence emission spectrum of **P**-4C+ (blue curve).

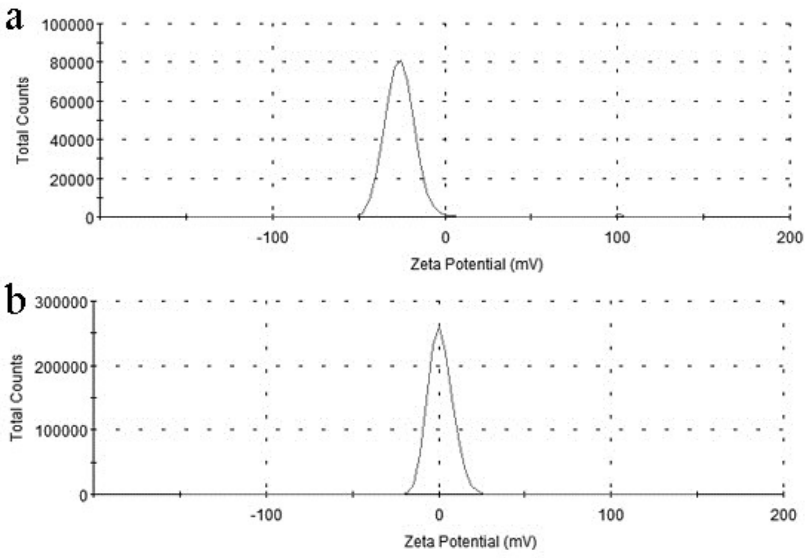

Figure S3. Zeta potential analysis: the MnO_2 nanosheets possess a potential value of −26.2 mV before (a) and 1.29 mV after (b) mixed with **P**-4C+.

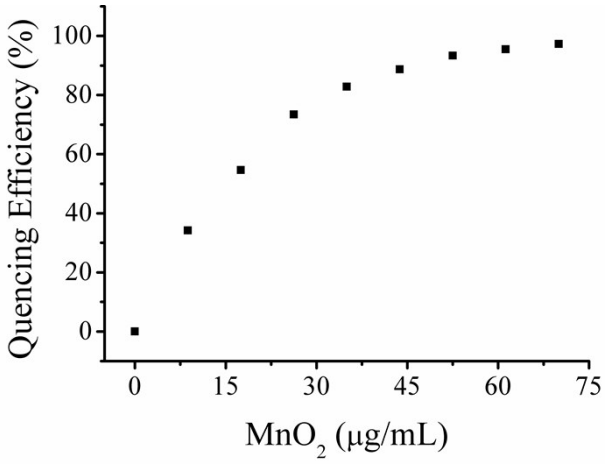

Figure S4. Fluorescence quenching efficiency versus MnO_2 nanosheets concentration (0, 8.75, 17.5, 26.25, 35, 43.75, 52.5, 61.25, and 70 μg/mL). Buffer: 5 mM phosphate buffer, pH 7.4.

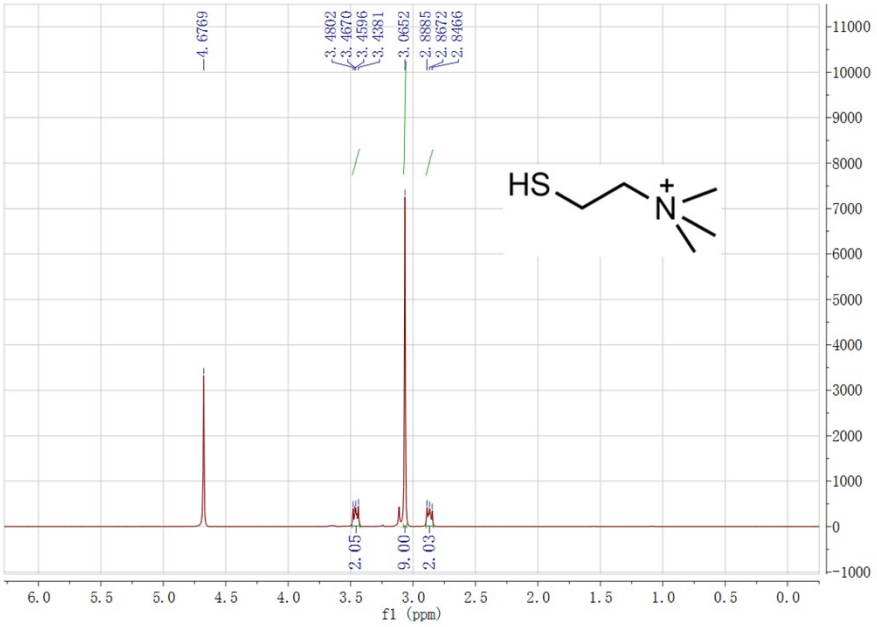

Figure S5. ^{1}H-NMR spectrum of thiocholine.

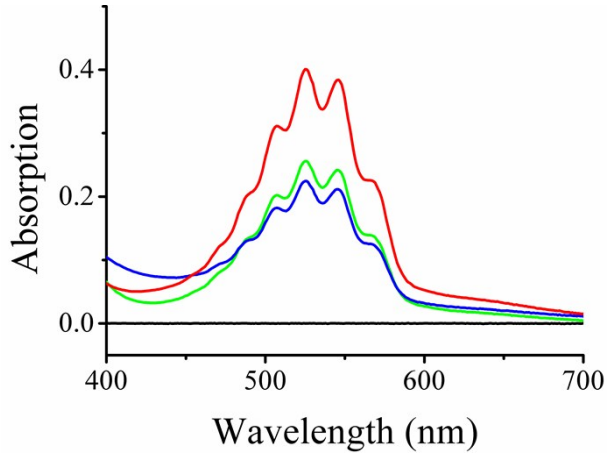

Figure S6. UV-vis absorption spectra: Blue curve: the reaction mixture of MnO_2 nanosheets and thiocholine was centrifuged, the supernatant was taken and treated with sodium periodate; Green curve: the reaction mixture of sodium periodate and $MnCl_2$; Red curve: $KMnO_4$ aqueous solution; Black curve (blank control): the MnO_2 nanosheets containing sample solution was centrifuged, the supernatant was taken and mixed with sodium periodate.

The reduction of MnO_2 to Mn^{2+} by thiocholine could be proven by a highly specific reaction of Mn^{2+} with sodium periodate, and the resulting sample solution shows clear color changes:

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

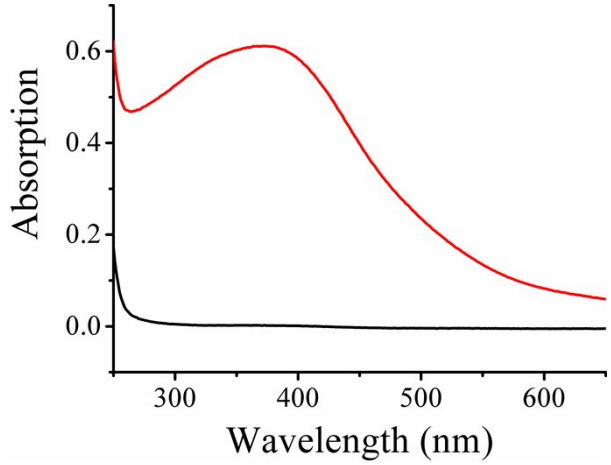

Figure S7. UV-vis absorption spectra of the MnO_2 nanosheets before (red curve) and after (black curve) the enzymatic reaction of AChE with the substrate.

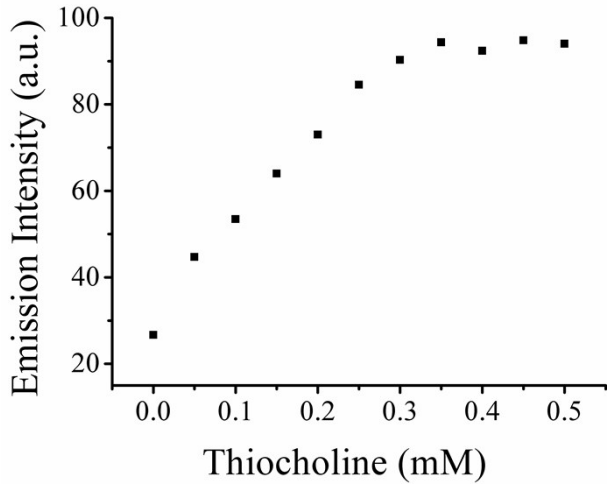

Figure S8. Changes in emission intensity of **P**-4C+ (5 μM) at 488 nm upon the addition of

increasing concentrations of free thiocholine. Conditions: 5 mM phosphate buffer (pH 7.4), 26.25 μg/mL MnO_2 nanosheets and 50 μM PPi.

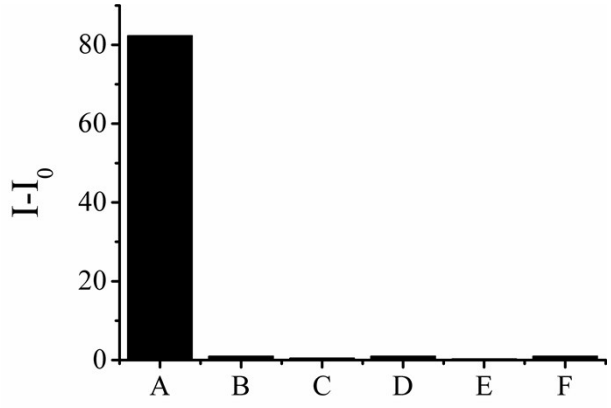

Figure S9. Selectivity study. Columns A–G: AChE, lysozyme, ALP, S1 nuclease, trypsin and Exo I nuclease. AChE: 500 mU/mL; the other enzymes: 10 U/mL each. 50 μM EDTA was used instead of pyrophosphate. I and I_0 represent the emission intensity of **P**-4C+ in the presence and absence of the enzyme.

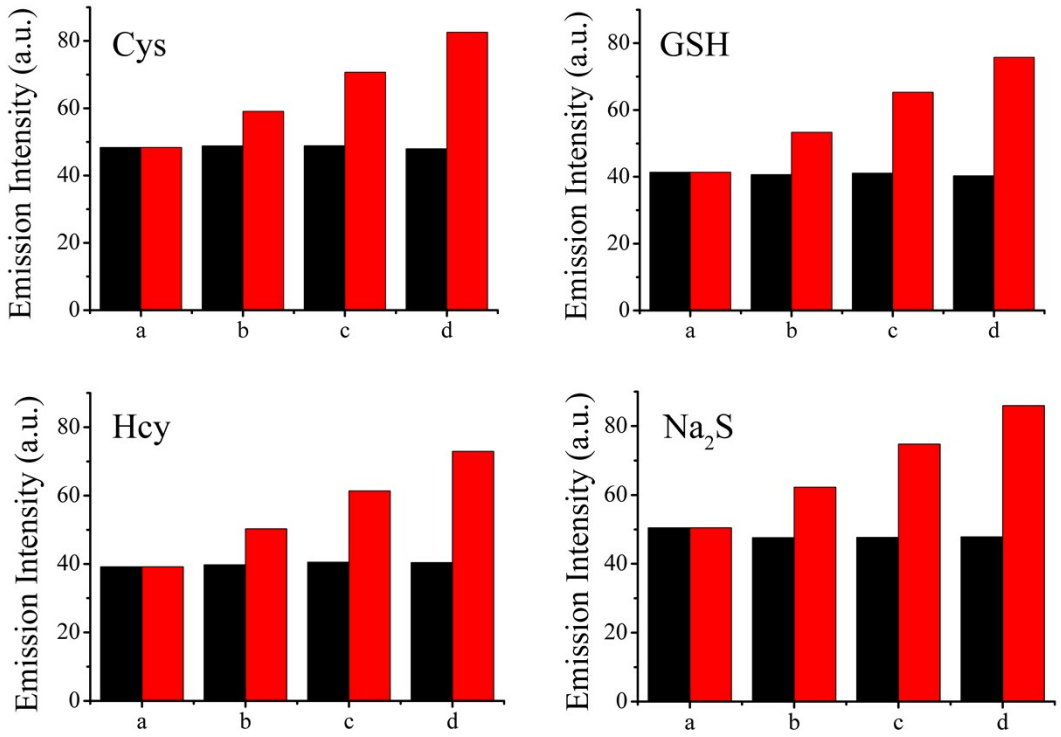

Figure S10. Probing AChE activity in the presence of different biothiols (Cys, GSH, Hcy and Na_2S). Black columns: no AChE added. Red columns: all contains 100 mU/mL AChE. Columns a – d: different concentrations of ATCh were added (0, 50, 100, 150 □M, respectively). All samples contain 5 mM phosphate buffer (pH 7.4), 5 μM **P**-4C+, 26.25 μg/mL MnO_2 nanosheets, 50 μM biothiol (Cys, GSH, Hcy, or Na_2S), and the emission intensity changes of **P**-4C+ at 488 nm were monitored.

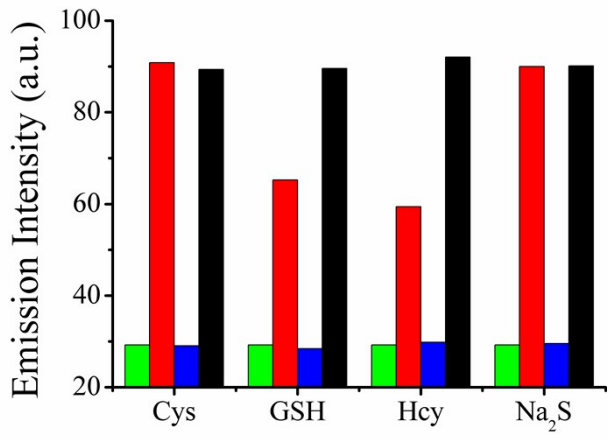

Figure S11. Changes in emission intensity of **P**-4C+ at 488 nm (5 μM) in the presence of the reductive substances. Green column: background emission of **P**-4C+ in the presence of the MnO_2 nanosheets and ATCh only; Red column: emission of **P**-4C+ in the presence of the MnO_2 nanosheets, ATCh, and Cys (or GSH, Hcy, Na_2S); Blue column: samples of Cys, GSH, Hcy, and Na_2S were passed through a filter (MWCO 30K), wash with water, the supernatant was taken, and mixed with **P**-4C+, MnO_2 nanosheets, and ATCh; Black column: samples of Cys, GSH, Hcy, and Na_2S were mixed with AChE, passed through the filter, and mixed with **P**-4C+, MnO_2 nanosheets, and ATCh. Conditions: 5 mM phosphate buffer (pH 7.4), 26.25 μg/mL MnO_2 nanosheets, 0.3 mM ATCh, 50 μM PPi, 0.3 mM Cys (or GSH, Hcy, Na_2S), 0.5 U/mL AChE.

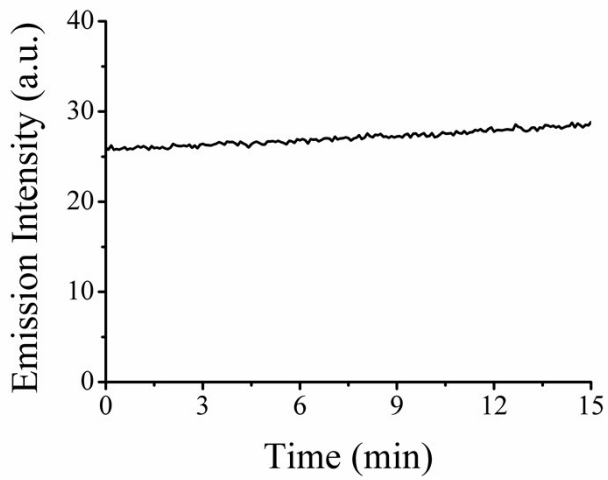

Figure S12. Emission intensity changes of **P**-4C+ (5 μM) at 488 nm in dilute human serum (2.5%) with time. Conditions: 5 mM phosphate buffer (pH 7.4), 50 μM PPi, 26.25 μg/mL MnO_2 nanosheets. The results show that the assay mixture is stable enough for the enzyme activity quantification (Figure 3).

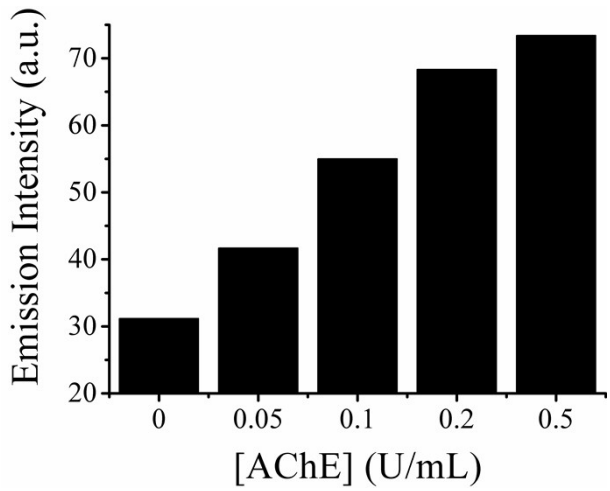

Figure S13. Changes in emission intensity of **P**-4C+ at 488 nm (5 μM) with AChE concentration in dilute human serum (2.5%). Conditions: 5 mM phosphate buffer (pH 7.4), 26.25 μg/mL MnO_2 nanosheets, 0.3 mM ATCh, 50 μM PPi.

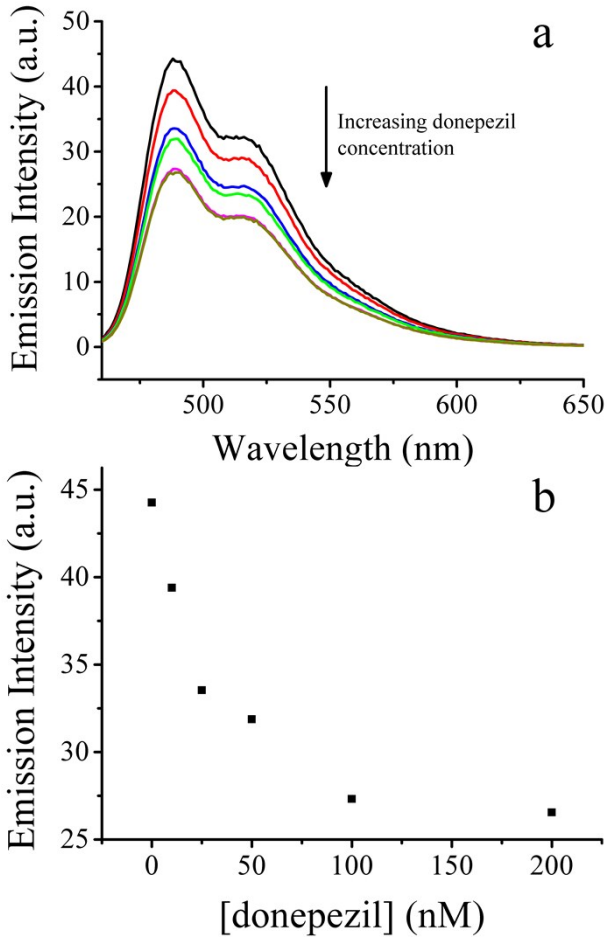

Figure S14. Changes in emission spectrum (a) and the corresponding emission intensity at 488 nm (b) of **P**-4C+ upon the addition of increasing concentrations of donepezil (0 – 200 nM). Conditions: 5 mM phosphate buffer (pH 7.4), 26.25 μg/mL MnO_2 nanosheets, 0.3 mM ATCh, 50 μM PPi and 100 mU/mL AChE.

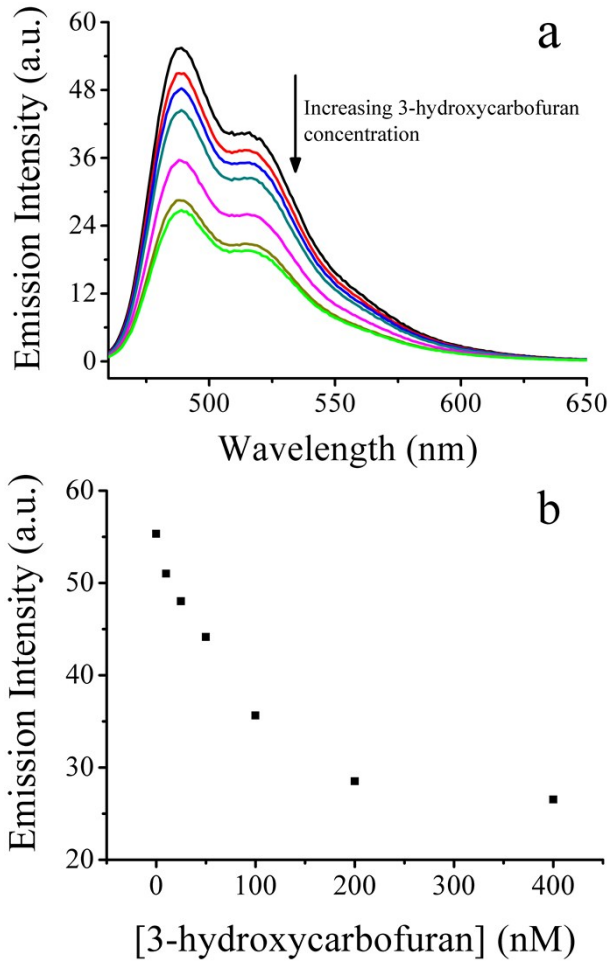

Figure S15. Changes in emission spectrum (a) and the corresponding emission intensity at 488 nm (b) of **P**-4C+ upon the addition of increasing concentrations of 3-hydroxycarbofuran (0 – 400 nM). Conditions: 5 mM phosphate buffer (pH 7.4), 26.25 μg/mL MnO_2 nanosheets, 0.3 mM ATCh, 50 μM PPi and 100 mU/mL AChE.