## **Supporting Information**

## A novel electrochemical sensor based on microporous polymeric nanospheres for measuring peroxynitrite anion released by living cells and studying the synergistic effect of antioxidants

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Figure S1. UV-visible spectrum of ONOO<sup>-</sup> at wavelength 302 nm.



Figure S2. Effect of loading amount of CTS–MPNS in ONOO<sup>-</sup> detection.



**Figure S3.** Cyclic voltammograms of bare GCE, CTS/GCE, MPNS/GCE and CTS–MPNS/GCE in 1.0 mM [Fe  $(CN)_6$ ]<sup>3-/4-</sup> solution containing 0.1 M KCl, the number of trials (n = 5), at 50 mV/s.

Cyclic voltammograms of Fig.S3 showed the current responses GCE, CTS/GCE, MPNS/GCE and CTS–MPNS/GCE in 1.0 mM [Fe  $(CN)_6$ ]<sup>3–/4–</sup> solution containing 0.1 M KCl at a scan rate of 50 mV s<sup>-1</sup>. CTS–MPNS/GCE electrode had faster electron transfer rate than GCE indicating that it had the excellent electrochemical properties.



**Figure S4.** Cyclic voltammograms of (A) bare GCE and (C) CTS–MPNS/GCE in 1.0 mM [Fe  $(CN)_6$ ]<sup>3-/4-</sup> solution containing 0.1 M KCl with various scan rates 20, 30, 50, 80, 100, 150, 200, 250 and 300 mV s<sup>-1</sup>. Plots between the square root of the scan rate ( $v^{1/2}$ ) and current responses (Ipa and Ipc) of (B) bare GCE and (D) CTS–MPNS/GCE.

The electroactive surface area of CTS–MPNS/GCE was calculated by the Randles– Sevcik equation  $(1)^1$ . Thus, the scan rate studies were performed in [Fe (CN)<sub>6</sub>]<sup>3-/4–</sup> by changing the scan rates from 20 to 300 mV s<sup>-1</sup>.

$$Ip = 2.69 \times 10^5 n^{3/2} A D^{1/2} C v^{1/2}$$
<sup>(1)</sup>

Where, D is the diffusion coefficient of  $[Fe (CN)_6]^{3-/4-} (cm^2 s^{-1})$ , Ip is the anodic or cathodic peak current (A), C is the concentration of the  $[Fe (CN)_6]^{3-/4-}$  (mol cm<sup>-3</sup>), A is the electroactive area (cm<sup>2</sup>), n is the number of transferred electrons and u<sup>1/2</sup> is the square root of scan rate (mV s<sup>-1</sup>). The electroactive surface areas of CTS–MPNS/GCE and the bare GCE were calculated as 0.0950 cm<sup>2</sup> and 0.0810 cm<sup>2</sup>. Therefore, CTS–MPNS modified electrode can furnish a better electrochemical sensing performance.



**Figure S5.** (A) Cyclic voltammogram of CTS–MPNS/GCE for 10.0  $\mu$ M ONOO<sup>-</sup> in PBS (pH = 7.0) with various scan rates 20, 30, 50, 80, 100, 120, 150, 200, 250 and 300 mV s<sup>-1</sup>. (B) Plot between the square root of the scan rate ( $\nu^{1/2}$ ) and current response (Ipa) (n = 5).



**Figure S6.** (A) Amperometric responses of the CTS–MPNS/GCE in a 0.2 M PBS (pH = 7.0) with the successive addition of 30.0  $\mu$ M of ONOO<sup>-</sup>, GLU, NO<sub>3</sub><sup>-</sup>, AA, UA, X, H<sub>2</sub>O<sub>2</sub>, Cl<sup>-</sup>, and NO<sub>2</sub><sup>-</sup> (The concentration of the interfering substances was 600  $\mu$ M) with potential at 1.1 V vs SCE. (B) Columnar current responses of the interfering substances in Fig. 6A. (C) Column graph of CV responses of recorded ONOO<sup>-</sup> at CTS–MPNS/GCE: 1 to 5 column graphs of CV signals of ONOO<sup>-</sup> (10.0  $\mu$ M) at five different electrodes prepared under the same conditions. (D) Stability measurements of the CTS–MPNS/GCE for 15 days.



**Figure S7.** Bright–field images of U87 cells: (A) before the experiment; (B) after incubated with the CTS–MPNS/GCE for about 3 h; (C) stimulated by 500  $\mu$ M Mn<sup>2+</sup>; Images of U87 cells stimulated by 500  $\mu$ M Mn<sup>2+</sup> in the presence of: (D) ALA; (E) GSH; (F) ALA + GSH.

Bright-field microscope images of the U87 cells (in Fig. S7) were obtained under different experimental conditions. In Fig. S7A, the living U87 cells showed a typical shuttle pattern before the experiment. After incubated with the CTS–MPNS/GCE for about 3 h, the cells had no changes in morphology and quantity (in Fig. S7B), indicating that the CTS–MPNS/GCE electrode had good biocompatibility. However, when the cells were stimulated by 500  $\mu$ M Mn<sup>2+</sup>, the number of cells dramatically decreased and the morphology became round as shown in Fig. S7C, indicating the excessive amounts of ONOO<sup>-</sup> released by cells will result in cell death.

In the presence of antioxidants, the morphology and quantity of cells were further studied under strong stimulation. Cells were incubated for 30 minutes with antioxidants (the concentration of each antioxidant was 5.0 mM). As shown in Fig. S7D, the number of cells was decreased in the presence of ALA. However, the number of cells decreased a lot and a small number of round cells appeared in the presence of GSH (in Fig. S7E), illustrating ALA had stronger antioxidant capacity than GSH. More important, there were no obvious change in the morphology and number of cells in the presence of ALA + GSH in Fig. S7F. Taken together, above results were consistent with the electrochemical experiments (Fig. 5A and Fig. S8).

![](_page_8_Figure_1.jpeg)

**Figure S8.** Amperometric responses of U87 cells induced by 500  $\mu$ M of Mn<sup>2+</sup> in the presence of (A) single antioxidant, (C) antioxidant mixture. Insert of Fig. S8A represents without antioxidants. The histograms of currents (B and D) correspond to the currents in Fig. S8A and Fig. S8C.

Amperometric responses of Fig. S8 showed that U87 cells stimulated with 500  $\mu$ M of Mn<sup>2+</sup> in the presence of antioxidants (the concentration of each antioxidant was 5.0 mM). As shown in Fig. S8A, in the presence of single antioxidant, the current responses of curves (a, b and c) at 100 s were lower than in the absence of antioxidants (insert of Fig. S8A). Instead, at 150 s, the currents were higher than insert of Fig. S8A. The histogram of Fig. S8B provided a clearer view of the responses at 100 s and 150 s. The result indicated that although single antioxidant had clearing capacity to a certain extent, strong stimulation (500  $\mu$ M Mn<sup>2+</sup>) will result in excess of ONOO<sup>-</sup> released from cells, which damage or even kill cells.

Similarly, amperometric responses and the corresponding column current of the

antioxidant mixtures were shown in Fig. S8C and Fig. S8D. By comparison, antioxidant mixtures had greater protective effect on cells than single antioxidant, which were also consistent with the curve b in Fig. 5B.

![](_page_9_Figure_1.jpeg)

**Figure S9.** (A) Amperometric responses of U87 cells induced by 30  $\mu$ M of Zymosan A, L-Arg, CHAPS, and ADP. (B) The histogram of currents corresponds to the current in Fig. S9A. (C) Amperometric responses of U87 cells in the presence of antioxidants. (D) The histogram of currents showed the current difference caused by antioxidants. Insert represented the current difference in the presence of single antioxidant.

The drugs such as Zymosan A, L-Arg, CHAPS, and ADP were chosen to stimulate U87 cells released ONOO<sup>-</sup>. As shown in Fig. S9A, when drugs (the concentration of each drug was 30  $\mu$ M) were added in the U87 cells solution, the current responses of curve b to e increased greatly compared to curve a (U87 cells only), indicating that cells can quickly release ONOO<sup>-</sup> under drugs stimulation. Columnar currents of Fig. S9B showed the current responses of ONOO<sup>-</sup> produced by cells under different drugs stimulation. Zymosan A generated the highest current signal, so it was selected as

the optimal stimulus.

Similarly, we further studied the scavenging ability and synergistic effect of different antioxidants under Zymosan A stimulation. Firstly, cells were incubated for 30 minutes with antioxidants (the concentration of each antioxidant was 1.0 mM). Next, 30 µM of Zymosan A was used to stimulate cells. As shown in Fig. S9C, the current signals of curves a to f were significantly lower than curve e (in Fig. S9A). Moreover, the current signals of curves d, e and f (antioxidant mixture) significantly lower than curves of a, b and c (single antioxidant). The results were consistent with the experimental phenomenon of Fig. 4, and the changing trend of columnar current in Fig. S9D was also similar to Fig.4D. Thus, we can draw a conclusion, that is, Zymosan A was selected as the optimal stimulus, and ALA had the strangest ability to clear ONOO<sup>-</sup>. Also, ALA + GSH had stronger clearing capacity among the antioxidant mixtures.

## References

1 Bard, Allen J and Larry R. Faulkner, *Electrochemical Methods*, 2001, **1**, 482.