Electronic Supplementary Information

The antioxidant mechanism of nitroxide TEMPO: scavenging with glutathionyl radicals

Hui Yu, Linying Cao, Feifei Li, Qingfeng Wu, Qiang Li, Shuai Wang and Yong Guo*

Fig. S1
Kinetic study of the R-NO• probe in the presence or absence of GS•. The probe had a fast response to GS• with a marked fluorescence enhancement. Meanwhile, the probe had good photostability for its fluorescence intensity remained unchanged during the detection process.

Fig. S2
Development of fluorescence intensity with the effect of L-tyrosine in HRP-catalyzed reaction. L-tyrosine as a phenolic compound to catalyze the oxidation of GSH was also tested, and fluorescence increase was observed. Incubation conditions: (black line) 5 µM R-NO•, 20 µM L-tyrosine, 5 µM GSH, 5 µM H₂O₂ and 0.0625 U/mL HRP; (red line) same as above, but minus GSH.
**Fig. S3**

Development of fluorescence intensity with the effect of cysteine in HRP-catalyzed reaction. As one of small thiol compounds, cysteine can effectively quench the phenoxy radical to generate thyl radicals, and then significantly scavenged by R-NO• with highly fluorescence increase. Incubation conditions: (black line) 5 µM R-NO•, 20 µM phenol, 5 µM cysteine, 5 µM H₂O₂ and 0.0625 U/mL HRP; (red line) same as above, but minus cysteine.

**Fig. S4**

The linear relationship between the relative fluorescence intensity and concentrations of GS•. There was a good linearity between the relative fluorescence intensity (F–F₀) and GSH concentrations in the range from 0 to 3.75 µM.
Fig. S5
Dependence of reaction time on phenol concentration. With the increase of phenol concentration, the reaction time was significantly shortened, which also confirmed the fact that phenol was recycled in the HRP-catalyzed reaction.

Fig. S6
Mass spectra of the reaction mixture of R-NO• with •OH and GSH, respectively. (a) MS spectrum of R-NO• with •OH; (b) MS spectrum of R-NO• with GSH. The main molecular ion peak was still R-NO• with m/z 597.
Fig. S7
Mass spectra of the reaction mixture of R-NO•. (a) MS spectrum of R-NO• with H₂O₂ and GSH, and the main molecular ion peak was still R-NO• (m/z 597); (b) MS spectrum of R-NO• with Fe²⁺ and GSH, compared R-NO• (m/z 597), a new ion peak m/z 598 was found, which was regarded as R-NOH. That was because redox-active iron would react with nitroxide when [nitroxide] >> [H₂O₂], to produce hydroxylamine derivative.
Fig. S8

Mass spectra of R-NO• and its derivative: (a) MS spectrum of R-NO• standard with m/z 597; (b) MS spectrum of HRP-catalyzed oxidation of GSH; (c) MS spectrum of metal-catalyzed oxidation of GSH, and the peak at m/z 582 represents secondary amine of R-NO• derivative.