# **Supporting Information**

### SI text

#### **S1** Principles of Fluorescence Quenching

If the quenching mechanism belongs to the dynamic quenching, it should follow the well-known Stern-Volmer equation, <sup>28</sup>

$$F_0 / F = 1 + K_{sv}[Q] = 1 + K_q \tau_0[Q]$$
(1)

where  $F_0$  and F denote the steady-state fluorescence intensities in the absence and presence of quencher, respectively.  $K_q$  is the quenching rate constant of the biological macromolecule;  $K_{sv}$  is the Stern–Volmer quenching constant; [Q] is the concentration of quencher;  $\tau_0$  is the average lifetime of the molecule without any quencher and the fluorescence lifetime of the biopolymer is  $10^{-8}$  s.<sup>28</sup>

If not, the fluorescence quenching of CAT should be analyzed using the modified Stern–Volmer equation: <sup>18</sup>

$$\frac{F_0}{\Delta F} = \frac{1}{f_a K_a} \frac{1}{[Q]} + \frac{1}{f_a}$$
(2)

In that case,  $\Box F$  is the difference in fluorescence intensity between the absence and presence of the quenching compound at concentration [Q]; K<sub>a</sub> is the effective quenching constant for the accessible fluorophores, and f<sub>a</sub> is the fraction of accessible fluorescence. The dependence of F<sub>0</sub>/ $\Box F$  on the reciprocal value of the quenching compound concentration [Q] <sup>-1</sup> is linear, with slope equal to the value of (f<sub>a</sub>K<sub>a</sub>)<sup>-1</sup>.

#### S2 Analysis on Thermodynamic Parameters and Binding Modes

If the temperature only changes a little, the enthalpy change ( $\Delta H$ ) can be regarded

as a constant in the formula below:  $^{\rm 28}$ 

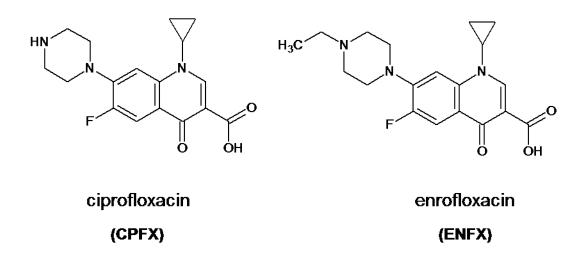
$$\ln\left(\frac{K_2}{K_1}\right) = \frac{\Delta H}{R} \left(\frac{1}{T_1} - \frac{1}{T_2}\right)$$
(3)  
$$\Delta G = \Delta H - T\Delta S = -RT \ln K$$
(4)

where 
$$K_1$$
 and  $K_2$  are the binding constants (analogous to  $K_a$  in Equation 2) at  $T_1$  and  $T_2$ , and R is the universal gas constant.

For a static quenching interaction, the binding constant ( $K_b$ ) and the number of binding sites (n) can be determined using the following formula, <sup>18</sup>

$$\log \frac{F_0 - F}{F} = \log K_b + n \log[Q]$$
(5)

where  $F_0$ , F, and [Q] are the same as in Equation 2;  $K_b$  is the binding constant and n is the number of binding sites per molecule.



Scheme S1 Molecular structure of CPFX and ENFX.

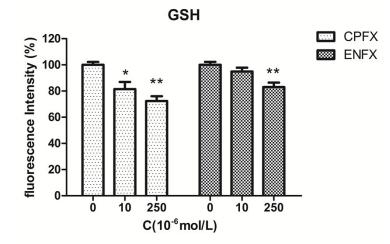


Fig. S1 Average amount of GSH in single erythrocytes incubated with CPFX and ENFX.<sup>18</sup>

Statistical significance vs control group: \*p<0.05, \*\*p<0.01; The values  $\pm$  standard deviations were estimated from 180 individual cells.<sup>18</sup>

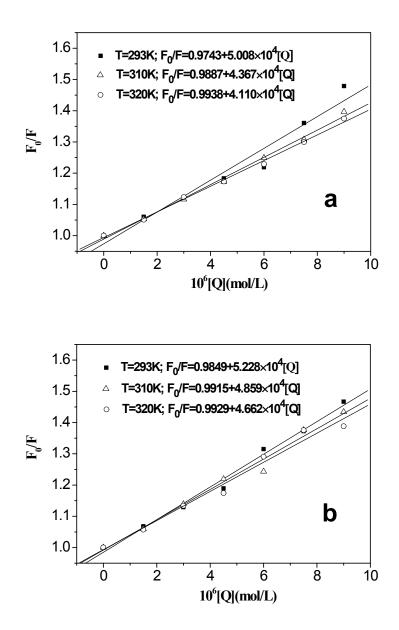


Fig. S2 Stern–Volmer plots of CPFX (a) and ENFX (b) quenching with Cu/Zn-

## SOD at three different temperatures

Conditions: Cu/Zn-SOD: 3×10<sup>-6</sup> mol/L; Buffer: NaH<sub>2</sub>PO<sub>4</sub>/Na<sub>2</sub>HPO<sub>4</sub>, pH=7.4.

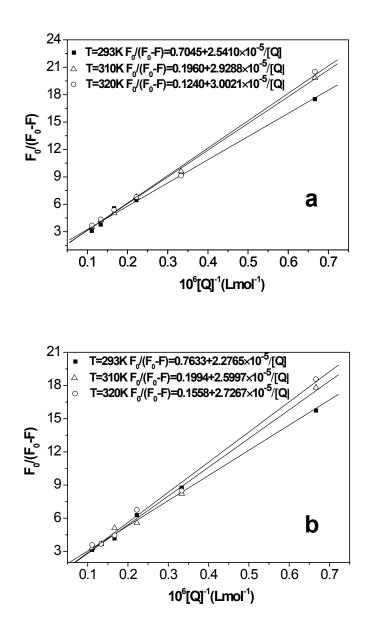


Fig. S3 Modified Stern–Volmer plots for the quenching of Cu/Zn-SOD by CPFX

## (a) and ENFX (b) at different temperatures

Conditions: Cu/Zn-SOD: 3×10<sup>-6</sup> mol/L; Buffer: NaH<sub>2</sub>PO<sub>4</sub>/Na<sub>2</sub>HPO<sub>4</sub>, pH=7.4.

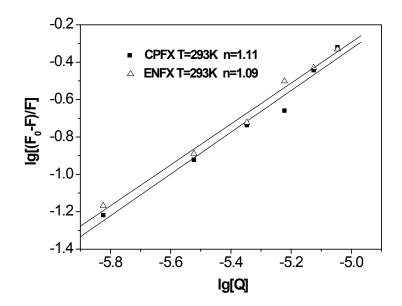


Fig. S4 Plots of log [(F<sub>0</sub> - F)/F] vs log [Q] for the binding of CPFX (■) and ENFX

(
 to Cu/Zn-SOD at 293K.

	T(K)	$K_{sv}$ (×10 <sup>4</sup> Lmol <sup>-1</sup> )	k <sub>q</sub> (×10 <sup>12</sup> Lmol <sup>-1</sup> s <sup>-</sup> <sup>1</sup> )	R	SD
CPFX	293	5.008	5.008	0.9790	0.0376
	310	4.367	4.367	0.9976	0.0108
	320	4.110	4.110	0.9982	0.0087
ENFX	293	5.228	5.228	0.9949	0.0189
	310	4.859	4.859	0.9927	0.0209
	320	4.662	4.662	0.9895	0.0242

Table S1 Stern–Volmer quenching constants for the interaction of both CPFX and ENFX

with Cu/Zn-SOD at different temperatures

	T(K)	K <sub>a</sub> (×10⁴ Lmol <sup>-1</sup> )	R	∆H KJ⋅mol <sup>-1</sup>	∆G KJ⋅mol <sup>-1</sup>	∆S J⋅mol <sup>-1</sup>
	293	2.76	0.9974		-24.91	-127.41
CPFX	310	0.68	0.9995	-62.24	-22.75	-127.39
	320	0.41	0.9967		-22.14	-125.31
	293	3.35	0.9974		-25.39	-136.31
ENFX	310	0.77	0.9964	-65.33	-23.07	-136.32
	320	0.57	0.9964		-23.01	-132.25

Table S2 Binding parameters for FQ/SOD interactions at pH 7.4