Supporting information

Reaction-based Indicator displacement Assay (RIA) for the selective colorimetric and fluorimetric detection of peroxynitrite†

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1. Supplementary spectra

Figure S1. (a) UV-Vis absorption spectra and (b) Fluorescence spectra ($\lambda_{ex} = 460$ nm) of ARS only (50 $\mu$M), ARS-PBA (ARS, 50 $\mu$M; PBA, 200 $\mu$M), ARS-BBA (ARS, 50 $\mu$M; BBA, 200 $\mu$M), ARS-NBA (ARS, 50 $\mu$M; NBA, 200 $\mu$M). The data was obtained in 1/15 M PBS buffer (pH 7.30) solution at 25 °C.

Figure S2. Color images for ARS (50 $\mu$M), ARS-PBA (ARS, 50 $\mu$M; PBA, 200 $\mu$M), ARS-BBA (ARS, 50 $\mu$M; BBA, 200 $\mu$M) and ARS-NBA (ARS, 50 $\mu$M; NBA, 200 $\mu$M). The pictures were taken in 1/15 M PBS buffer (pH 7.30) at 25 °C.
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Figure S3. (a) Fluorescence spectra ($\lambda_{ex} = 460$ nm) and (b) Absorption spectra for ARS only (50 μM), ARS-PBA (ARS, 50 μM; PBA, 200 μM), ARS-BBA (ARS, 50 μM; BBA, 200 μM). The complexes were formed in situ. The data was obtained in 52.1% MeOH/H$_2$O PBS buffer (pH 8.10) at 25 °C.

Figure S4. Color images for ARS-NBA (ARS, 50 μM; NBA, 200 μM), ARS-BBA (ARS, 50 μM; BBA, 200 μM), ARS-PBA (ARS, 50 μM; PBA, 200 μM), ARS (50 μM). The pictures were taken in 52.1% MeOH/H$_2$O PBS buffer (pH 8.10) at 25 °C.
Figure S5. (a) UV-Vis absorption titration spectra of ARS (50 μM) and addition of various concentrations of NBA (0 – 200 μM). (b) Curve fitting and binding constant calculation between ARS and NBA. The data were taken in 52.1% MeOH/H$_2$O PBS buffer (pH 8.10) at 25°C.

Binding constant calculation:

$$Y = \frac{(1 + k_Y X)}{(1 + kX)}$$  \hspace{1cm} \text{Equation 1}

By fitting the relationship curve between absorption intensity and concentration of NBA using equation 1, we resulted:

$$k = 7200 \pm 92 \text{ M}^{-1}$$

$$Y_{\text{lim}} = 0.37 \pm 0.04$$

Figure S6. $^1$H NMR for (a) NBA (10 mM); (b) in the presence of ARS (10 mM); (c) drop addition of NaOH (10 N) in MeOD/D$_2$O = 1:4.
By using the UV-Vis data, we calculated the LOD (3σ/k), where σ = \[\Sigma(y - y')^2/(n - 2)\]/2; k = slope of the linearity curve obtained by regression analysis; n = number of points; y = experimental response; y' = calculated response; By using the UV-Vis data, we calculated the LOD (3σ/k) = 5.4 µM.
**Figure S9.** Time dependent response for probe ARS-NBA (ARS, 50 µM; NBA, 200 µM) complex (a) UV-Vis Absorption at $\lambda_{\text{max}} = 500$ nm and (b) Fluorescence ratio change $F/F_0$ at $\lambda_{\text{max}} = 550$ nm in the presence of peroxynitrite (0.5 mM). The data were recorded in 52.1% MeOH/H$_2$O PBS buffer (pH 8.10) at 25°C.

**Figure S10.** Reaction rate constant calculation between fluorescence intensity $\ln(F/F_0)$ and time (0 – 500 seconds) for probe ARS-NBA (ARS, 50 µM; NBA, 200 µM) complex in the presence of peroxynitrite (0.5 mM). The reaction rate constant can be obtained through processing the data following a simple 1st order rate equation;

$$F/F_0 = \exp(-kt')$$

We observed that a good linear relationship formed between $\ln(F/F_0)$ and time (0 – 500 seconds), hence,

It is calculated the $k' = 4.39 \text{ s}^{-1}$ from the slope.

Considering rate $= k \cdot [A][B]$ where $A =$ concentration of ARS-NBA (50 µM), and $B =$ concentration of ONOO (500 µM), $[B] >>$
Therefore, rate = $k' [A]$, where $k' = k_2 [B]$. We obtained $k_2 = 4.39 \text{s}^{-1}/0.5 \text{mM} = 8.78 \times 10^3 \text{s}^{-1} \text{M}^{-1}$.
mM), hypochlorite (0.5 mM) and peroxynitrite (0.5 mM). The data was obtained in 52.1% MeOH/H2O PBS buffer (pH 8.10) at 25 °C.

**Figure S13.** (a) Absorption and (b) Fluorescence response of ARS (50 μM) complex towards H2O2 (0.1 mM), hypochlorite (0.1 mM), ONOO⁻ (0.1 mM) for 60 min. The data was obtained in 52.1% MeOH/H2O PBS buffer (pH 8.10) at 25 °C.

**Figure S14.** Selectivity test of probe ARS-NBA (ARS, 50 μM; NBA, 200 μM) complex towards various ROS/RNS species. (a) column of UV-Vis absorption intensity A500nm/A465nm; (b) Column of fluorescence intensity (F – F0)/F0 at 550 nm in the presence of blank (1), H2O2 (2, 0.5 mM), NO (3, 0.5 mM), O2⁻ (4, 0.5 mM), AAPH (5, 0.5 mM), O2 (6, 0.5 mM), ONOO⁻ (7, 0.5 mM) for 60 min. The data...
was obtained in 52.1% MeOH/H₂O PBS buffer (pH 8.10) at 25 °C.

2. Notes and references

3. NMR spectra
Nuclear magnetic resonance (NMR) spectra were obtained in methanol-D. Where a Bruker AVANCE 300 was used, ¹H spectra were recorded at 300 MHz, ¹³B spectra at 96 MHz and ¹³C at 75 MHz. Chemical shifts (δ) are expressed in parts per million and are reported relative to the residual solvent peak as an internal standard in ¹H and ¹³C spectra. The multiplicities and general assignments of the spectroscopic data are denoted as: singlet (s), doublet (d), unresolved multiplet (m), and broad (br).

![NMR spectrum](image)

Figure S15. ¹H NMR of compound BBA in MeOD.
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Figure S16. $^{13}$C NMR of compound BBA in MeOD.

Figure S17. $^{11}$B NMR of compound BBA in MeOD.