Supporting information

Reaction-based Indicator displacement Assay (RIA) for the colorimetric and fluorometric detection of hydrogen peroxide†

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Contents

1. General methods

The UV-Vis spectra and fluorescent titrations with hydrogen peroxide were carried out at 25 °C in pH 7.30 PBS buffer (KH_2PO_4, 1/15 M; Na_2HPO_4, 1/15 M). The electrochemical data was obtained in pH 7.30 PBS buffer (KCl, 100 mM; KH_2PO_4, 1/15 M; Na_2HPO_4, 1/15 M).

2. ROS preparation

- $^1O_2$ was generated by the reaction of H_2O_2 with NaClO. Drop the solution of H_2O_2 into the aqueous NaClO and stir for 2 min.
- ROO• was generated from 2, 2'-azobis (2-amidinopropane) dihydrochloride. AAPH (2, 2'-azobis (2-amidinopropane) dihydrochloride, 10 M) was added in de-ionized water, and then stirred at 37 ºC for 30 min.

- Superoxide was generated from KO_2 with a saturated solution of KO_2 in DMSO (~1 mM).
- Hydroxyl radical was generated by Fenton reaction. To prepare •OH solution, ferrous chloride (1 M) was added in the presence of 10 equiv of H_2O_2 (37.0 wt%).
- The concentration of ^-OCl was determined from the absorption at 292 nm (ε = 350 $M^{-1}cm^{-1}$).[1] The concentration of H_2O_2 was determined from the absorption at 240 nm (ε = 43.6 $M^{-1}cm^{-1}$).

All other chemicals were from commercial sources and of analytical reagent grade, unless indicated otherwise.

3. Supplementary spectra

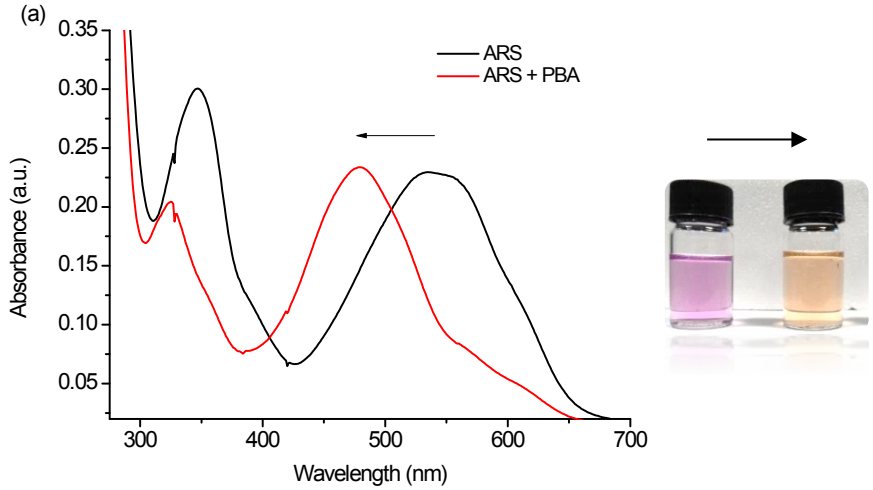

Figure S1. UV-Vis spectra of **ARS** (50 μM) and addition of phenylboronic acid (**PBA**, 200 μM) and visible color change from purple to orange. The data was obtained in 1/15 M PBS buffer with 2.0 mM CTAB at 25 °C.

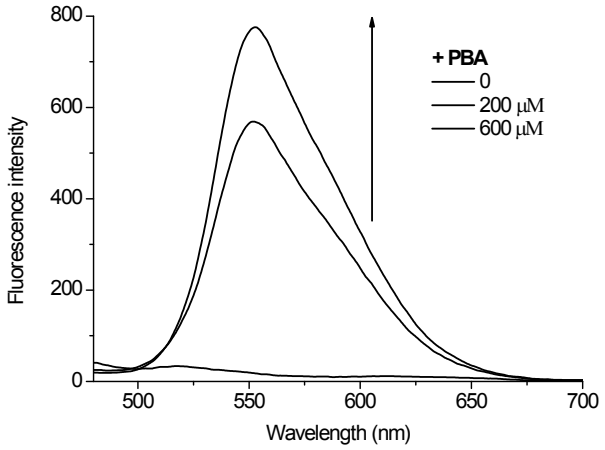

Figure S2. Fluorescence spectra (λ_{ex} = 460 nm) of **ARS** (50 μM) in the presence of phenylboronic acid (**PBA**, 0, 200 μM, 600 μM). The data was obtained in 1/15 M PBS buffer with 2.0 mM CTAB at 25 °C.

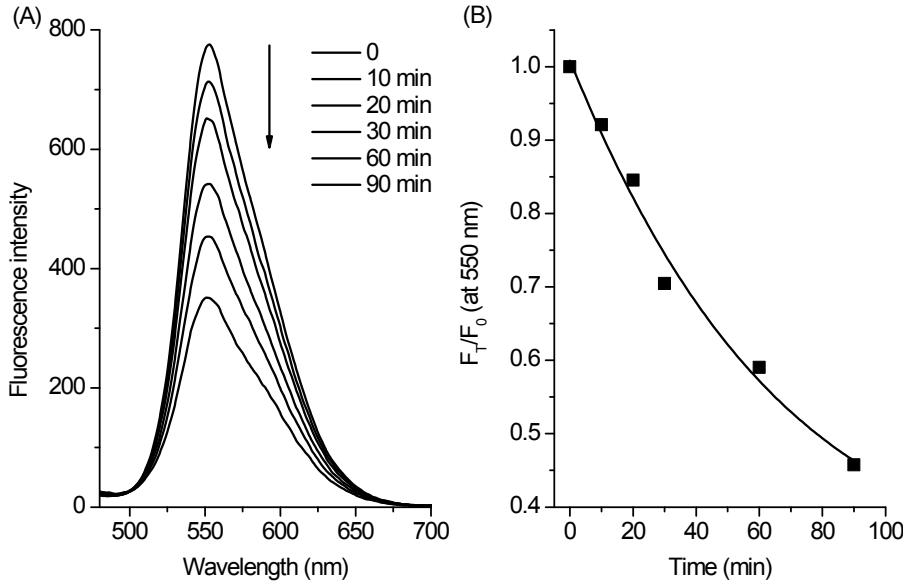

Figure S3. Fluorescence spectra (λ_{ex} = 460 nm) of **ARS-PBA** (**ARS**: 50 μM, **PBA**: 600 μM) in the presence of hydrogen peroxide (H_2O_2, 500 μM). (A) Emission spectral in different time; (B) Time curve and non-fitting correlation. The data was obtained in 1/15 M PBS buffer with 2.0 mM CTAB solution at 25 °C.

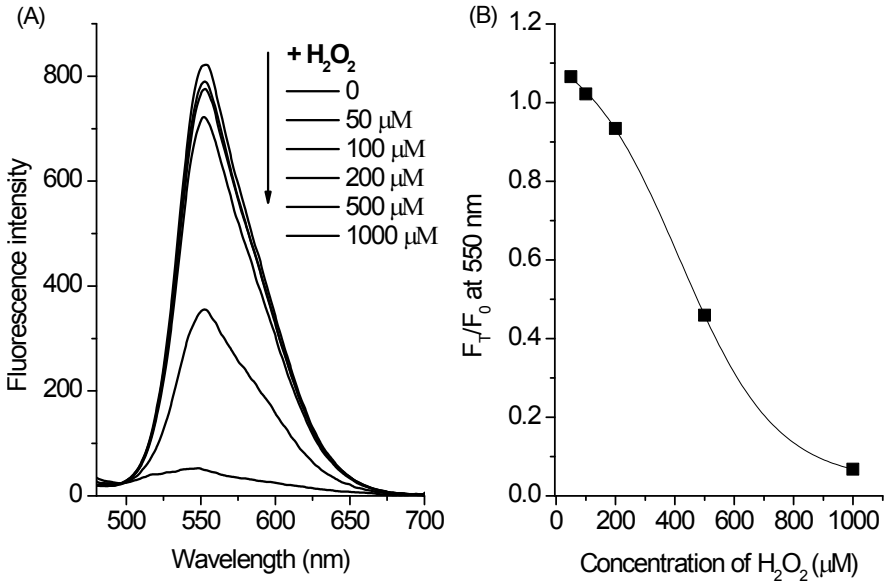

Figure S4. Fluorescence spectra (λ_{ex} = 460 nm) of **ARS-PBA** (**ARS**: 50 μM, **PBA**: 600 μM) in the presence of hydrogen peroxide (H_2O_2, 0, 50 μM, 100 μM, 200 μM, 500 μM, 1000 μM), then stirred for 60 min. (A) Emission spectral in different dose of H_2O_2; (B) Dose-dependent curve and non-fitting correlation. The data was obtained in 1/15 M PBS buffer with 2.0 mM CTAB solution at 25 °C.

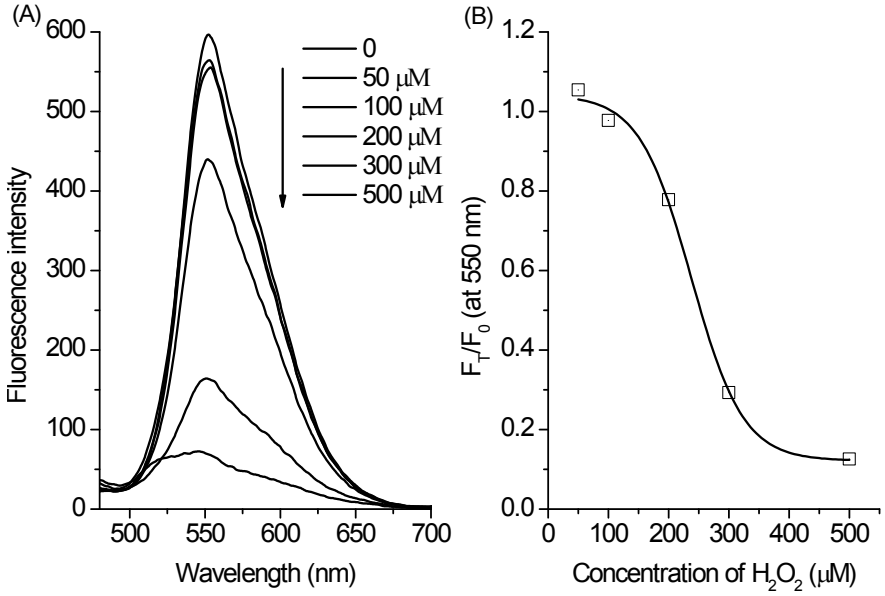

Figure S5. Fluorescence spectra (λ_{ex} = 460 nm) of **ARS-PBA** (**ARS**: 50 μM, **PBA**: 200 μM) in the presence of hydrogen peroxide (H_2O_2, 0, 50 μM, 100 μM, 200 μM, 500 μM), then stirred for 60 min. (A) Emission spectral in different dose of H_2O_2; (B) Dose-dependent curve and non-fitting correlation. The data was obtained in 1/15 M PBS buffer with 2.0 mM CTAB solution at 25 °C.

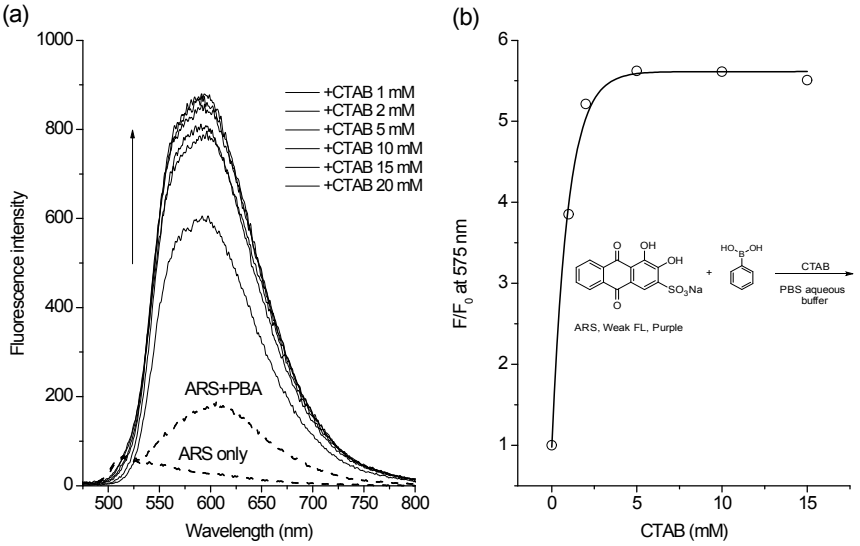

Figure S6. (a) Fluorescence spectra of **ARS (**50 μM), **ARS-PBA** (**ARS**: 50 μM, **PBA**: 200 μM) and **ARS-PBA** (**ARS**: 50 μM, **PBA**: 200 μM) with different concentrations of CTAB (1 mM, 2 mM, 5 mM, 10 mM, 15 mM, 20 mM). (b) Dose-dependent curve for ARS-PBA with CTAB. The data was obtained in 1/15 M PBS buffer at 25 ^{o}C. Fluorescence intensity were measured with λ_{ex} = 460 nm with Ex slit: 5 nm and Em slit: 5 nm.

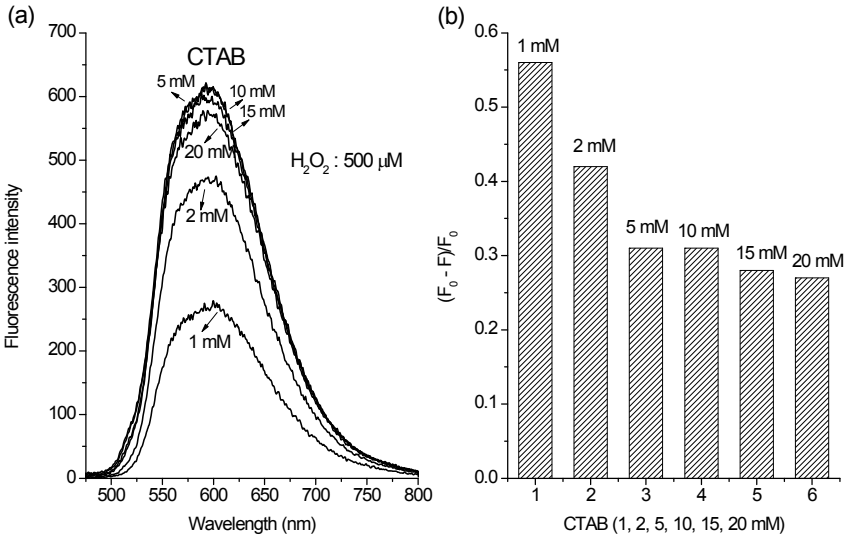

Figure S7: (a) Fluorescence spectra and (b) intensity changes (before and after addition of H_2O_2) of **ARS-PBA** (**ARS**: 50 μM, **PBA**: 200 μM) with different concentrations of CTAB (1, 2, 5, 10, 15, 20 mM) in the presence of hydrogen peroxide (H_2O_2, 500 μM) in 1/15 M PBS buffer at 25 ^{o}C. Each measurement was made after 30 min. Fluorescence intensity were measured with λ_{ex} = 460 nm/ λ_{em} = 590 nm with Ex slit: 5 nm and Em slit: 5 nm.

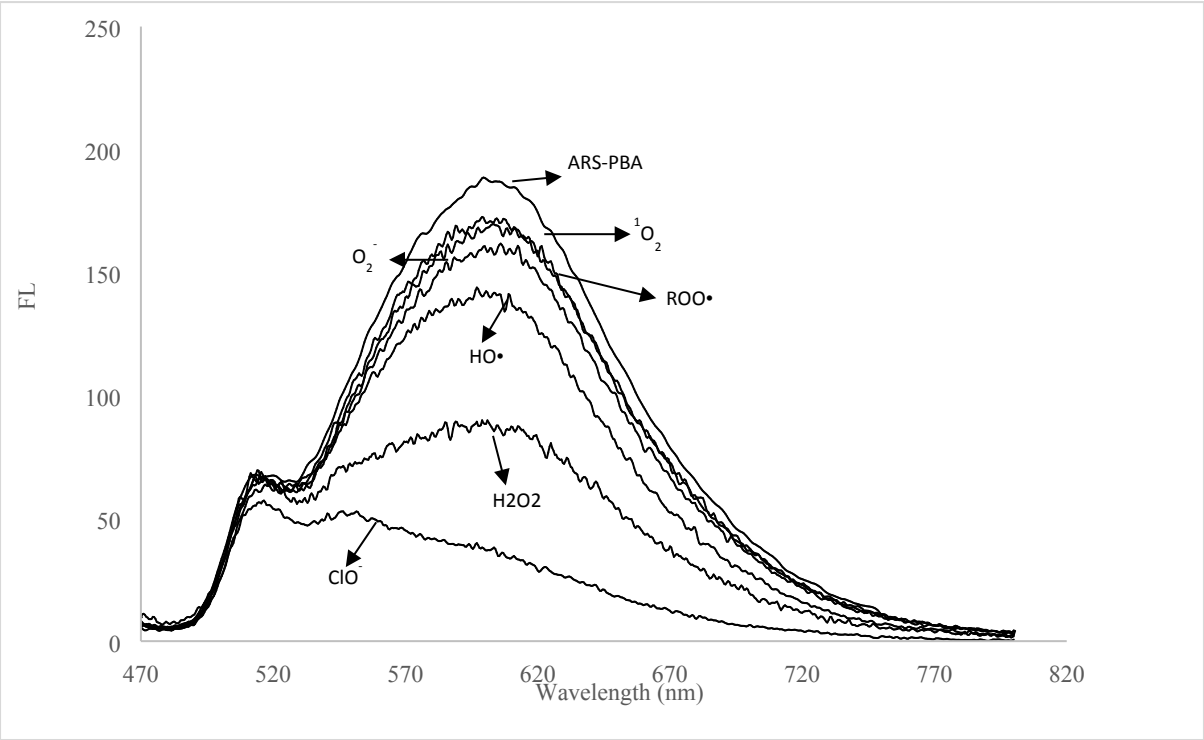

Figure S8. Fluorescence spectra of **ARS-PBA** (**ARS**: 50 μM, **PBA**: 200 μM) in the presence of various ROS: $^-$OCl (500 μM), H_2O_2 (500 μM), ROO· (500 μM), $^-O_2$ (500 μM), ·OH (500 μM), $^1O_2$ (500 μM). The data was obtained in 1/15 M PBS buffer at 25 °C. Fluorescence intensity were measured with λ_{ex} = 460 nm with Ex slit: 5 nm and Em slit: 5 nm.

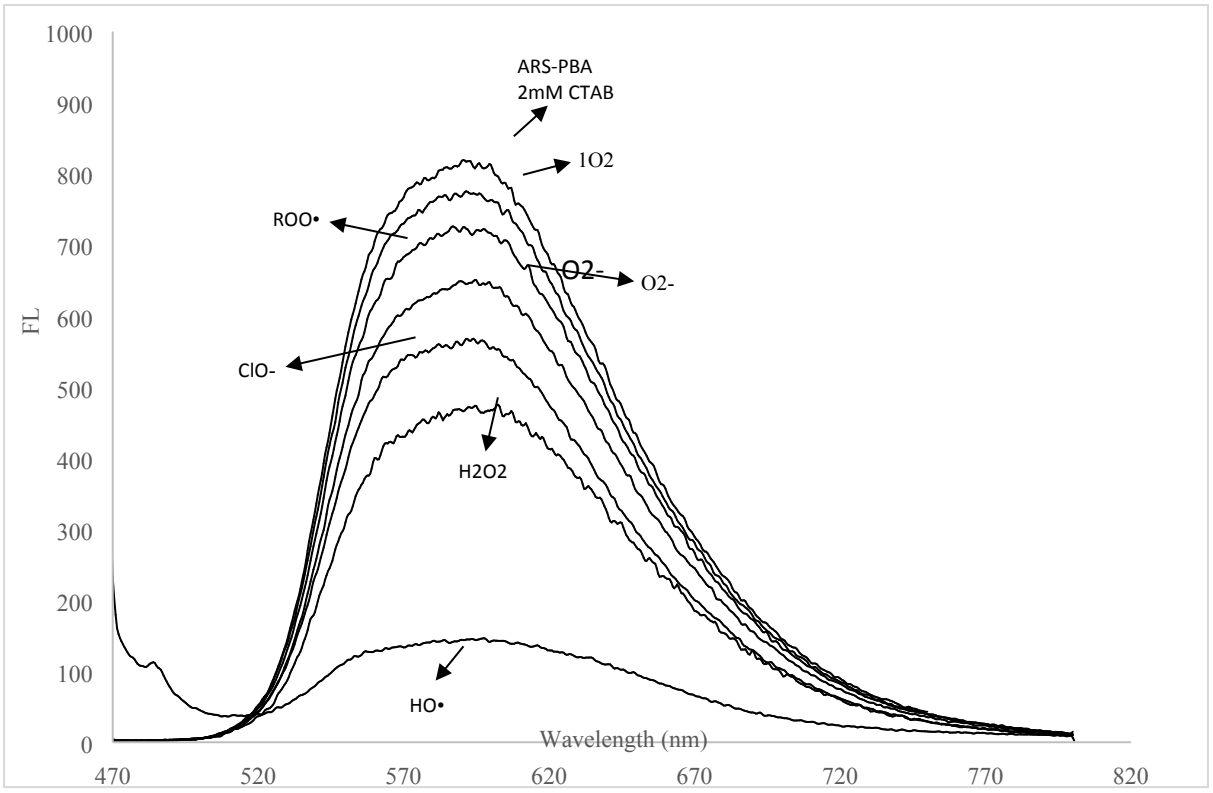

Figure S9. Fluorescence spectra of **ARS-PBA** (**ARS**: 50 μM, **PBA**: 200 μM) with 2 mM CTAB in the presence of various ROS: $^{-}$OCl (500 μM), H_2O_2 (500 μM), ROO$^{\cdot}$ (500 μM), $^{-}O_2$ (500 μM), OH (500 μM), $^1O_2$ (500 μM). The data was obtained in 1/15 M PBS buffer at 25 ^{o}C. Fluorescence intensity were measured with λ_{ex} = 460 nm with Ex slit: 5 nm and Em slit: 5 nm.

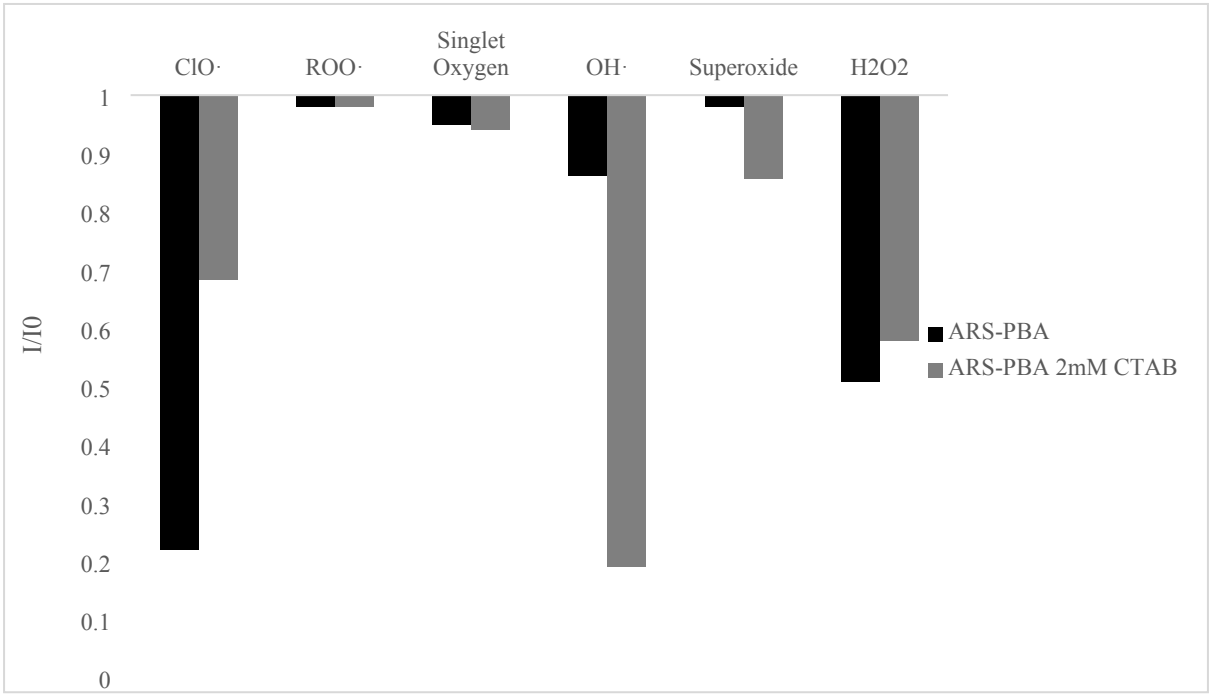

Figure S10. Selectivity of **ARS-PBA** (**ARS**: 50 μM, **PBA**: 200 μM) without and with CTAB (2 mM) in the presence of various ROS: $^{-}$OCl (500 μM), H_2O_2 (500 μM), ROO$^{\cdot}$ (500 μM), $^{-}O_2$ (500 μM), OH (500 μM), $^1O_2$ (500 μM). The data was obtained in 1/15 M PBS buffer at 25 ^{o}C. Fluorescence intensity were measured with λ_{ex} = 460 nm/ λ_{em} = 590 nm with Ex slit: 5 nm and Em slit: 5 nm.

4. References

(1). M. Abo, Y. Urano, K. Hanaoka, T. Terai, T. Komatsu and T. Nagano, *J. Am. Chem. Soc.*, 2011, **133**, 10629-10637.