

# Selective and Real-Time Detection of Nitric Oxide by a Two-Photon Fluorescent Probe in Live Cells and Tissue Slices

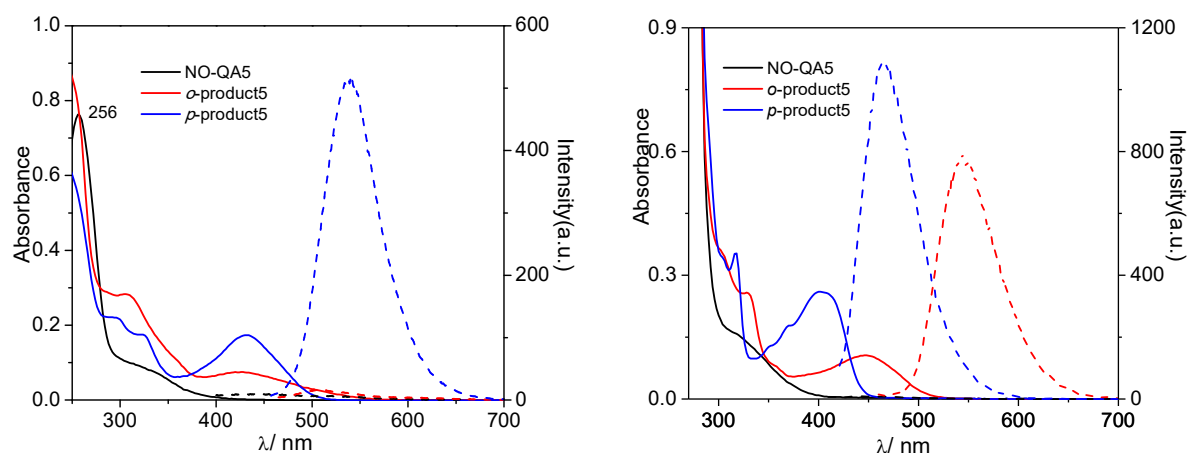
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## I. UV/vis absorption and fluorescence spectra of **NO-QA5** and its products



**Figure S1.** UV/vis absorption (bold) and fluorescence spectra (dash) of 20  $\mu$ M **NO-QA5** and its products in 0.1 M PBS buffer solution (pH 7.4) (left), or toluene (right).

**Table S1. Photophysical properties of the probe and its products**

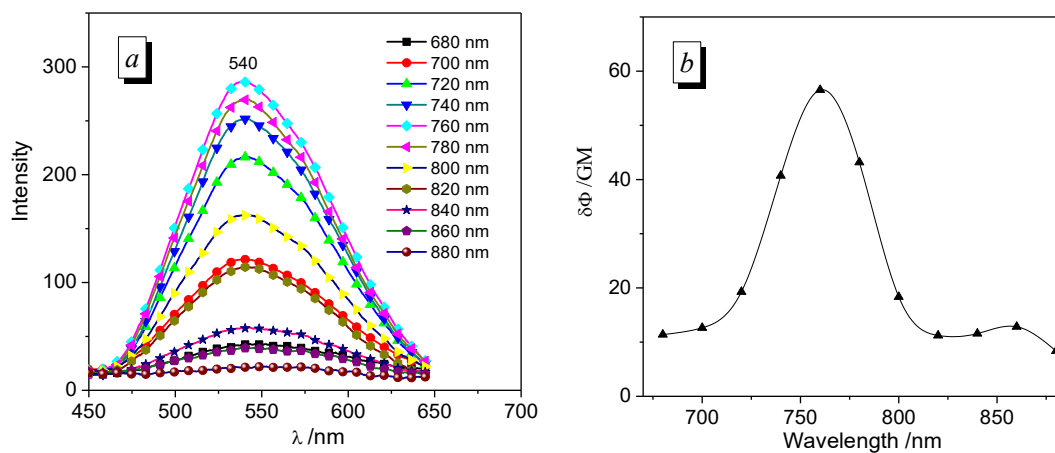
Compds	$\Phi_f / 10^{-3}$	$\lambda_{abs}$	$\lambda_{em}$	$\delta\Phi^b$
	(PBS/Tol) <sup>a</sup>	(PBS/Tol)	(PBS/Tol)	/GM
<b>NO-QA5</b>	<sup>c</sup>	256/-	<sup>c</sup>	<sup>c</sup>
<i>p</i> -product	14.9/93	432/404	540/465	57
<i>o</i> -product	0.52/61	426/448	508/545	<sup>c</sup>

<sup>a</sup> Measured in 0.1 M PBS buffer or toluene, respectively.

<sup>b</sup> Measured in ethanol-PBS buffer mixture (v/v 9:1).

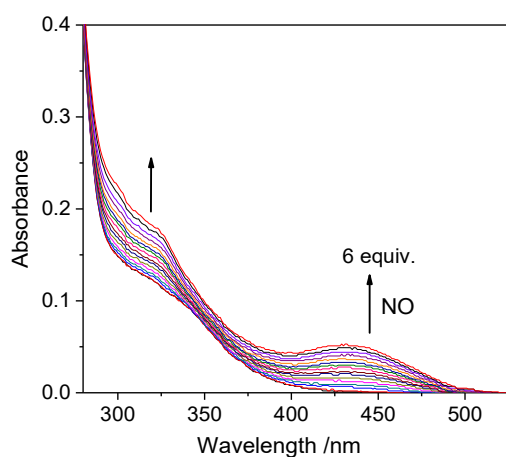
<sup>c</sup> No detectable fluorescence.

## II. Measurements of two-photon absorption cross section ( $\delta\Phi$ )



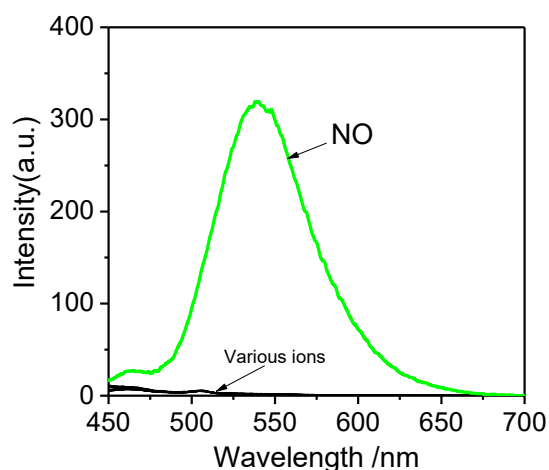
**Figure S2.** (a) Two-photon fluorescence spectra at various excitation wavelengths, (b) Two-photon excitation spectrum, of 1 mM *p*-product in the mixture solution of acetonitrile-PBS buffer (pH 7.4) (v/v 9:1).

## III. Absorption response toward different amounts of NO



**Figure S3.** UV/vis absorption spectra of 20  $\mu$ M **NO-QA5** upon addition of different amounts of NO (0-6 equiv.) in PBS buffer solution (pH 7.4) recorded after 5 min.

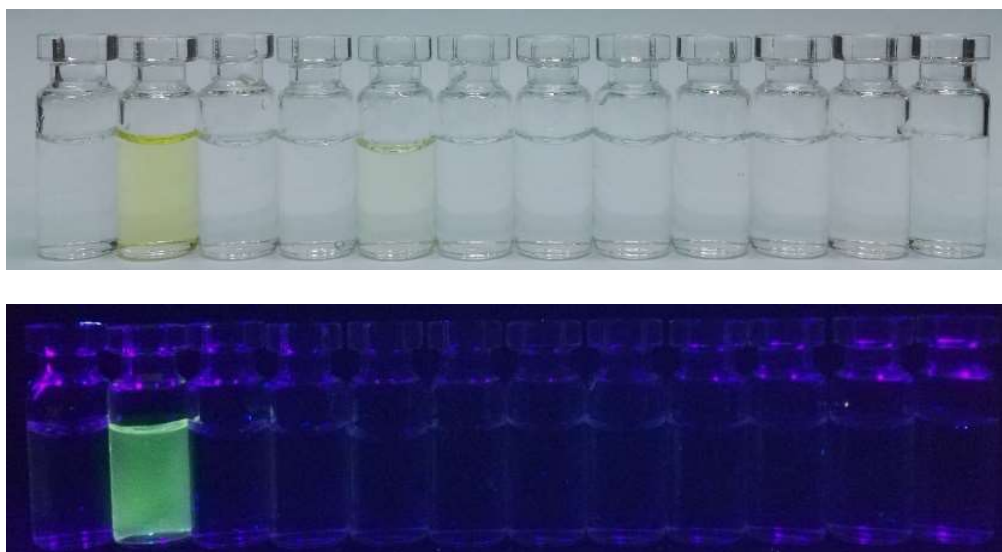
#### IV. The selectivity of **NO-QA5** to NO over various ions



**Figure S4.** Fluorescence spectra of 20  $\mu$ M NO-QA5 (PBS buffer pH 7.4) in the presence of 6 equiv. analytes: blank, NO,  $\text{NO}_3^-$ ,  $\text{Ca}^{2+}$ ,  $\text{Mn}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Zn}^{2+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Fe}^{3+}$ , recorded after 10 min.

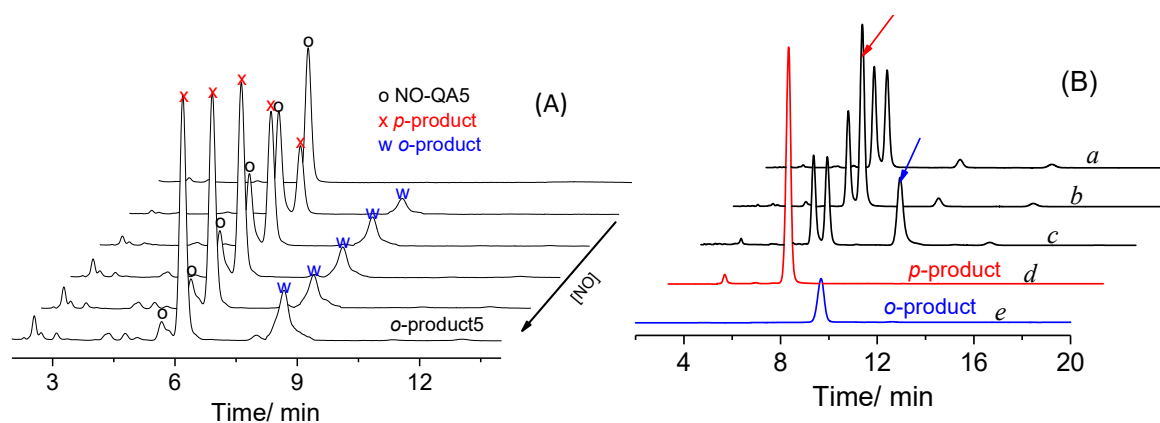
\* The following salts were used to prepare above metal ion stock solutions,  $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ ,  $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ ,  $\text{Mg}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ ,  $\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ ,  $\text{Cu}(\text{NO}_3)_2 \cdot 3\text{H}_2\text{O}$ , and  $\text{Fe}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$ .

#### V. Photos for the selectivity of **NO-QA5**



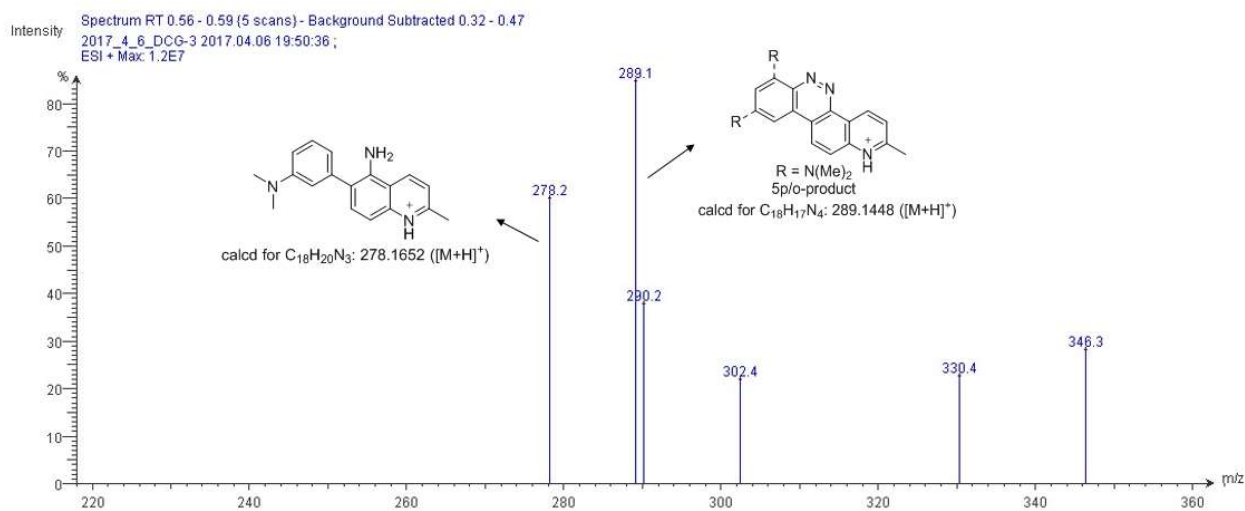
**Figure S5.** Photos for the color (up) and fluorescence (down) of **NO-QA5** (20  $\mu$ M) after additions of 6 equiv. analytes for 10 min in the PBS buffer solutions (pH 7.4). Analytes from left to right: blank, NO,  $\text{ClO}^-$ ,  $\text{H}_2\text{O}_2$ ,  $\text{OH}^\bullet$ ,  $\text{O}_2^{\bullet-}$ ,  $^1\text{O}_2$ , MGO, AA,  $\text{NO}_2^-$ ,  $\text{NO}_3^-$ ,  $\text{ONOO}^-$ .

## VI. HPLC analysis for the sensing products



**Figure S6.** (A) HPLC profiles of **NO-QA5** upon additions of different amounts of NO in PBS buffer (pH 7.4), (B) HPLC analysis of co-injection with *p*-product/*o*-product: (a) the reaction mixture of **NO-QA5** with NO; (b) *p*-product co-injection with (a); (c) *o*-product co-injection with (a); (d) neat *p*-product; (e) neat *o*-product. Analysis condition: methanol/water (70:30, v/v) as eluent, monitored at 376 nm.

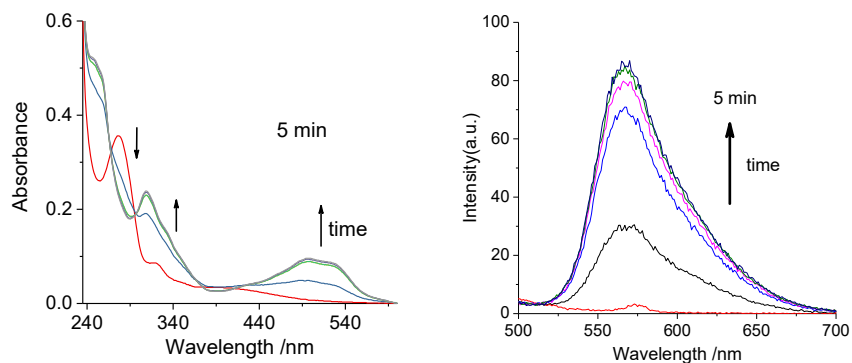
## VII. MS evidence of the sensing products of **NO-QA5** with NO



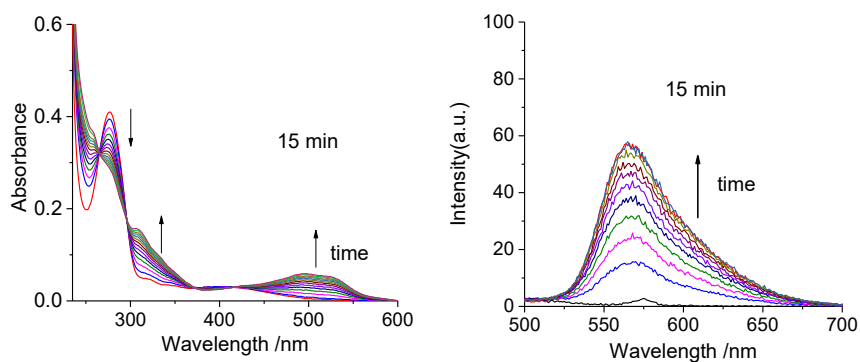
**Figure S7.** Mass spectrum of the reaction mixture of **NO-QA5** and NO in the PBS buffer solution (pH 7.4).

## VIII. pH effects on the diazotization reaction of **NO-QA5** with $\text{NaNO}_2$

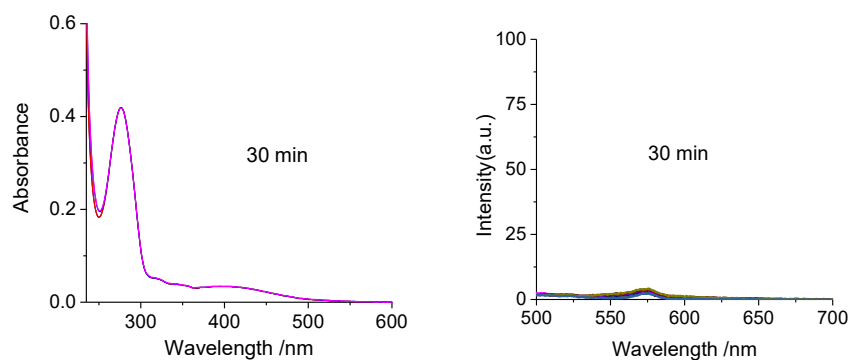
pH=0



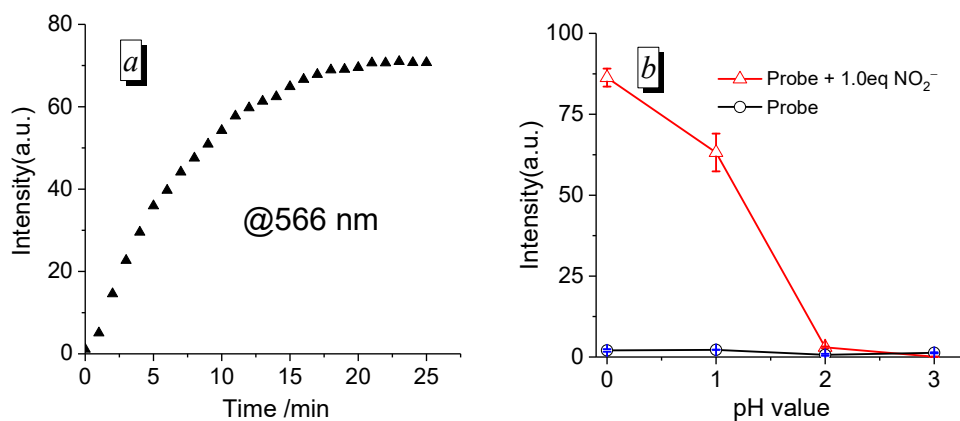
pH=1



pH=2



**Figure S8.** UV/vis absorption (left) and Fluorescence (right) spectra of 10  $\mu\text{M}$  **NO-QA5** in acidic aqueous solution (pH = 0, 1, 2) upon addition of 10  $\mu\text{M}$   $\text{NaNO}_2$ , recorded once every minute for 5, 15 or 30 min, respectively, excitation at 480 nm.



**Figure S9.** (a) Time-dependent fluorescence intensity of 10  $\mu\text{M}$  NO-QA5 upon addition of NaNO<sub>2</sub> (1.0 equiv.) in HCl (pH = 1) solution,  $\lambda_{\text{ex}} = 480$  nm. (b) Fluorescence intensities at 566 nm of 10  $\mu\text{M}$  NO-QA5 and its reaction mixture with 1.0 equiv. NaNO<sub>2</sub> in different pH solutions (pH 0-3).

## IX. Copies of NMR spectra of new compounds

