Supporting information

A rhodamine-based fast and selective fluorescent probe for monitoring exogenous and endogenous nitric oxide in live cells

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General methods

DEA/NONOate (diethylamine NONOate), ascorbic acid (AA), KO₂, H₂O₂, NaClO, NaNO₂, NaNO₃, LPS and N⁶-monomethyl-L-arginine (L-NMA) were obtained from commercial sources and used without additional purification. Hydroxyl radicals (·OH) were generated by reaction of Fe²⁺ with H₂O₂.¹ Peroxynitrite (ONOO⁻) was generated from amyl nitrite and H₂O₂ following literature procedures and the concentration of the ONOO⁻ stock solution was determined by measuring the absorbance at 302 nm (ε = 1670 M⁻¹ cm⁻¹).²

The stock solution of NO was produced by adding H₂SO₄ (20 %) to sodium nitrite solutions and bubbling NO into water for 20 min.³ The concentration of the NO solution was determined by the Griess method.⁴ Aliquots (50 μL) of this solution were added to 1 mL of potassium phosphate buffer (0.1 mM, pH 7.4) containing sulfanilamide solution (17 mM) and N-(1-naphthyl)ethylenediamine (0.4 mM). The solution was immediately mixed by inversion and incubated at room temperature for 5 min. The colorimetric product was measured at 496 nm by use of a UV/Vis spectrophotometer. The NO concentration of the solution was calculated according to Beer’s law using an extinction coefficient of 5400 M⁻¹ cm⁻¹ as determined from experiments using chemiluminescence standardization. Based on this method, the concentration of the NO stock solution is 1.2 mM.
<table>
<thead>
<tr>
<th>Probes</th>
<th>Structure</th>
<th>References</th>
<th>pH usage value</th>
<th>Response time</th>
<th>Detection limit</th>
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<tr>
<td>DAN</td>
<td><img src="image" alt="DAN structure" /></td>
<td><em>Anal. Biochem.</em> 1993, 214, 11.</td>
<td>In acidic condition</td>
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<td>DAR-n</td>
<td><img src="image" alt="DAR-n structure" /></td>
<td><em>Anal. Chem.</em>, 2001, 73, 1967</td>
<td>&gt; 4</td>
<td>-</td>
<td>7 nM (DAR-4M)</td>
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<td>RB-NO</td>
<td><img src="image" alt="RB-NO structure" /></td>
<td><em>Org. Lett.</em>, 2008, 10, 2357</td>
<td>&gt; 5</td>
<td>30 min</td>
<td>3.0 nM</td>
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<td>SiRB-NO</td>
<td><img src="image" alt="SiRB-NO structure" /></td>
<td><em>Chem. Eur. J.</em>, 2016, 22, 5649</td>
<td>Fl intensity changed with pH</td>
<td>&gt; 30 min</td>
<td>32.6 nM</td>
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<td>ROPD</td>
<td><img src="image" alt="ROPD structure" /></td>
<td>This paper</td>
<td>4.0-9.3</td>
<td>2.5 min</td>
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**Fig. S1** HRMS spectrum of the solution of ROPD after the addition of 10 equiv. of NO. The peak (m/z) at 517.2245 corresponds to the triazole derivative 4 (Calcd: 517.2239).

**Fig. S2** The absorption a) and fluorescence emission b) spectra of ROPD (5 μM) in the presence of 20 equivalents of NO solution (black line) and the triazole 4 (5 μM) (red line) in PBS (100 mM, pH 7.4).
Fig. S3 Measurement of fluorescence dissociation constant ($K_d$) of ROPD with NO.

Fig. S4 The detection limit of ROPD (2.5 μM) towards NO by 3σ/k in PBS buffer (100 mM, pH = 7.4).

The detection limit was calculated based on the fluorescence titration. The emission intensity of the probe ROPD without nitric oxide was measured by 8 times, and the standard deviation of blank measurements was determined. The detection limit is then calculated with the following equation:

$$LOD = \frac{3\sigma}{k}$$

Where $\sigma$ is the standard deviation of the blank solution measured by 8 times; $k$ is the slope of the calibration curve.

From the graph we get slope ($k$) = 15055830, and $\sigma$ value is 0.3423

Thus we get the Limit of Detection (LOD) = $3\sigma/k$ = 68.2 nM.
**Fig. S5** a) Histogram of the fluorescence enhancement ratio \( \frac{(F_i - F_0)}{F_0} \) of ROPD (5 μM) at 581 nm in the presence of various metal ions (100 μM). 0: ROPD, 1: NO, 2: AgNO₃, 3: Al(NO₃)₃, 4: Ca(NO₃)₂, 5: Cd(NO₃)₂, 6: Co(NO₃)₂, 7: Cr(NO₃)₃, 8: Cu(NO₃)₂, 9: FeCl₂, 10: FeCl₃, 11: Hg(ClO₄)₂, 12: KNO₃, 13: Mg(NO₃)₂, 14: MnCl₂, 15: NaNO₃, 16: Ni(NO₃)₂, 17: Pb(NO₃)₂, 18: Zn(NO₃)₂; b) Change ratio \( \frac{(F_i - F_0)}{F_0} \) of fluorescence intensity (581 nm) of ROPD (5 μM) upon addition of each metal ions (100 μM) followed by NO (100 μM) in PBS buffer solution (100 mM, pH = 7.4). 1: probe ROPD after addition of NO alone, and in the presence of 2: AgNO₃, 3: Al(NO₃)₃, 4: Ca(NO₃)₂, 5: Cd(NO₃)₂, 6: Co(NO₃)₂, 7: Cr(NO₃)₃, 8: Cu(NO₃)₂, 9: FeCl₂, 10: FeCl₃, 11: Hg(ClO₄)₂, 12: KNO₃, 13: Mg(NO₃)₂, 14: MnCl₂, 15: NaNO₃, 16: Ni(NO₃)₂, 17: Pb(NO₃)₂, 18: Zn(NO₃)₂; λₑₓ: 505 nm. Slit: 10 nm, 10 nm.
Fig. S6 $^1$H NMR spectrum of 2 (400 MHz, CDCl$_3$).

Fig. S7 $^{13}$C NMR spectrum of 2 (100 MHz, CDCl$_3$).
Fig. S8 HRMS of 2. HRMS: m/z [M + H+] = 508.1884; Calcd for [C_{30}H_{25}N_3O_5 + H^+]: 508.1873.

Fig. S9 ¹H NMR spectrum of 3 (400 MHz, CDCl₃).
Fig. S10 $^{13}$C NMR spectrum of 3 (100 MHz, CDCl$_3$).

Fig. S11 HRMS of 3. HRMS: m/z [M - Cl$^-$] = 536.2196; Calcd for C$_{32}$H$_{30}$N$_3$O$_5^+$ = 536.2186.
Fig. S12 $^1$H NMR spectrum of probe ROPD (400 MHz, CDCl$_3$).

Fig. S13 $^{13}$C NMR spectrum of probe ROPD (100 MHz, CDCl$_3$).
Fig. S14 HRMS of probe ROPD. HRMS: m/z [M - Cl⁺ + H⁺]/2 = 253.6264, [M - Cl⁺] = 506.2443; Calcd for [C₃₂H₃₂N₃O₃⁺ + H⁺]/2 = 253.6261; [C₃₂H₃₂N₃O₃⁺] = 506.2444.

Fig. S15 ¹H NMR spectrum of the triazole 4 (400 MHz, CDCl₃).
Fig. S16 $^{13}$C NMR spectrum of triazole 4 (100 MHz, CDCl$_3$).

Fig. S17 HRMS of triazole 4. HRMS: m/z [M - Cl$^-$] = 517.2253; Calcd for [C$_{32}$H$_{29}$N$_4$O$_3$]$^+$ = 517.2240.

References


