Electronic Supplementary Information for:

An unprecedented strategy for selective and sensitive fluorescence detection of nitric oxide based on its reaction with a selenide

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Apparatus and Materials

NMR spectra were determined on a Bruker DMX-300 spectrometer at 300 MHz in $CDCl_3$ with tetramethylsilane as the internal standard. Electrospray ionization (ESI) mass spectra were measured on an LC-MS 2010A (Shimadzu) instrument. Elemental analyses were carried out with a Flash EA 1112 instrument. High-resolution Fourier transform ion cyclotron resonance mass spectrum (FTICR-MS) was recorded on an APEX II mass spectrometer (Bruker, Daltonics). A Delta 320 pH-meter [Mettler-Toledo Instruments (Shanghai) Co., China] was used for pH measurements. Fluorescence spectra were obtained with a Hitachi F-2500 spectrofluorimeter. The absorption spectra were recorded with a TU-1900 spectrophotometer (Beijing Purkinje General Instrument Co. LTD). Fluorescence imaging experiments were performed on a FV 1000-IX81 confocal laser scanning microscope (Olympus, Japan) with FV5-LAMAR for excitation at 515 nm and a variable bandpass emission filter set to 530-630 nm through a 40 × 0.9 NA objective. Optical sections were acquired at 0.8 μm.

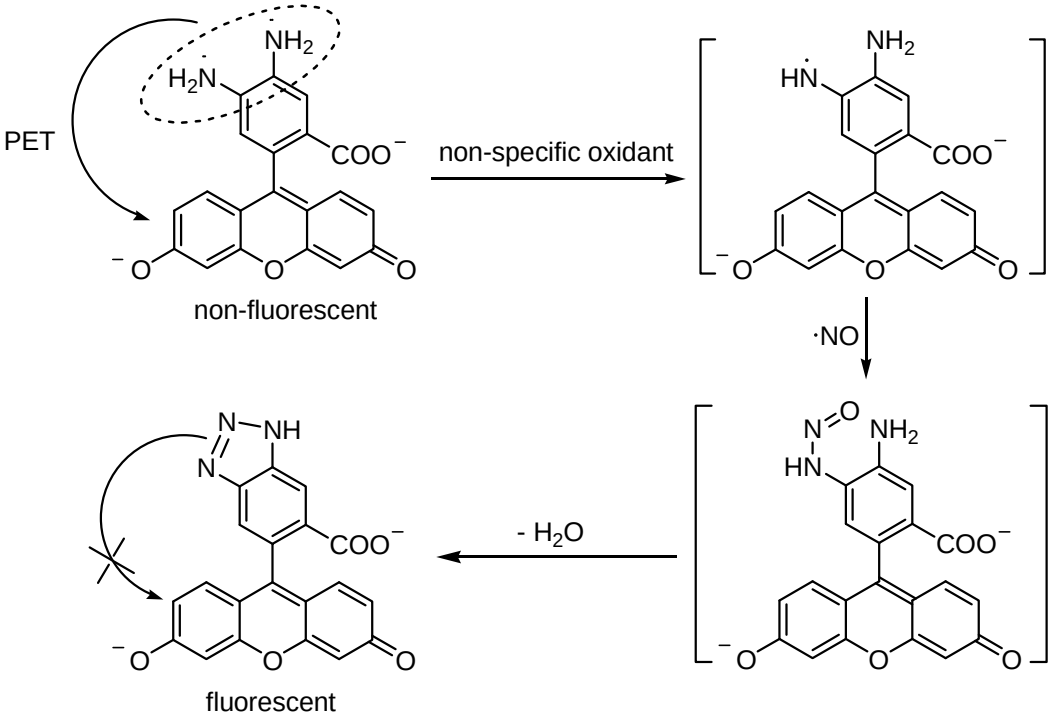
Rhodamine B and selenium powder were obtained from Beijing Chemical Company. 3-(Aminopropyl)-1-hydroxy-3-isopropyl-2-oxo-1-triazene (NOC 5), 2-(4-carboxy-phenyl)-4,4,5,5,-tetramethylimidazoline-1-oxyl-3-oxide (sodium salt) (PTIO),

hemoglobin, and 2-[4-(2-hydroxyethyl)-1-piperazinyl]ethanesulfonic acid (HEPES) were purchased from Sigma-Aldrich. Cu/Zn superoxide dismutase was obtained from Fluka (Belgium). Reduced glutathione and pyruvic acid were purchased from Acros. All other chemicals were of analytical grade and used without further purification, unless otherwise noted. Distilled-deionized water was employed throughout. Rhodamine B selenolactone (RBSe) was synthesized according to our procedure described previously,[S1] and its stock solution (1.0 mM) was prepared by dissolving the appropriate amount of it in ethanol, which should be used freshly. Nitric oxide (NO) was prepared by treating metallic copper with 3 M nitric acid and its stock solution (2.057 mM)[S2] was prepared by bubbling NO into deoxygenated de-ionized water for 2 h. Superoxide radical anion (O_2^-) was generated from KO_2, hydroxyl radical (·OH) from H_2O_2 and $FeSO_4$, and singlet oxygen ($^1O_2$) from ClO^- and H_2O_2.[S3] Peroxynitrite ($ONOO^-$) was generated from butyl nitrite and H_2O_2 following the known procedure.[S4] Stock solutions (1.0 mM) of other substances were prepared by dissolving their compounds in water. All reactions were performed at 37 ^oC in 20 mM HEPES buffer solution (pH 7.2) under anaerobic conditions.

Fluorescence Imaging of NO

The Hela cells were grown on glass-bottom culture dishes (MatTek Co.) in Dulbecco's Modified Eagle Media (DMEM) supplemented with 10% (v/v) fetal bovine serum, penicillin (100 μg/mL), and streptomycin (100 μg/mL) at 37 ºC. Before use, the adherent cells were washed three times with phenol red-free DMEM. For NO imaging, the cells were first loaded with a 10 μM RBSe solution in DMEM at 37 ºC for 20 min, washed 3 times with 0.1 M phosphate buffered saline (PBS) solution (pH 7.4) to remove the free RBSe, and then incubated in PBS media with 20 μM NOC 5 for 30 or 60 min. To examine the effect of the NO scavenger PTIO, the RBSe-loaded Hela cells were pre-treated with PTIO (100 μM) for 20 min, washed 3 times with PBS solution, and then incubated in PBS media with NOC 5 (20 μM) for 60 min. Prior to imaging, all cells were rinsed 3 times with 0.1 M PBS buffer.

(1) *ortho* aromatic diamine mechanism



(2) transition-metal complex mechanism

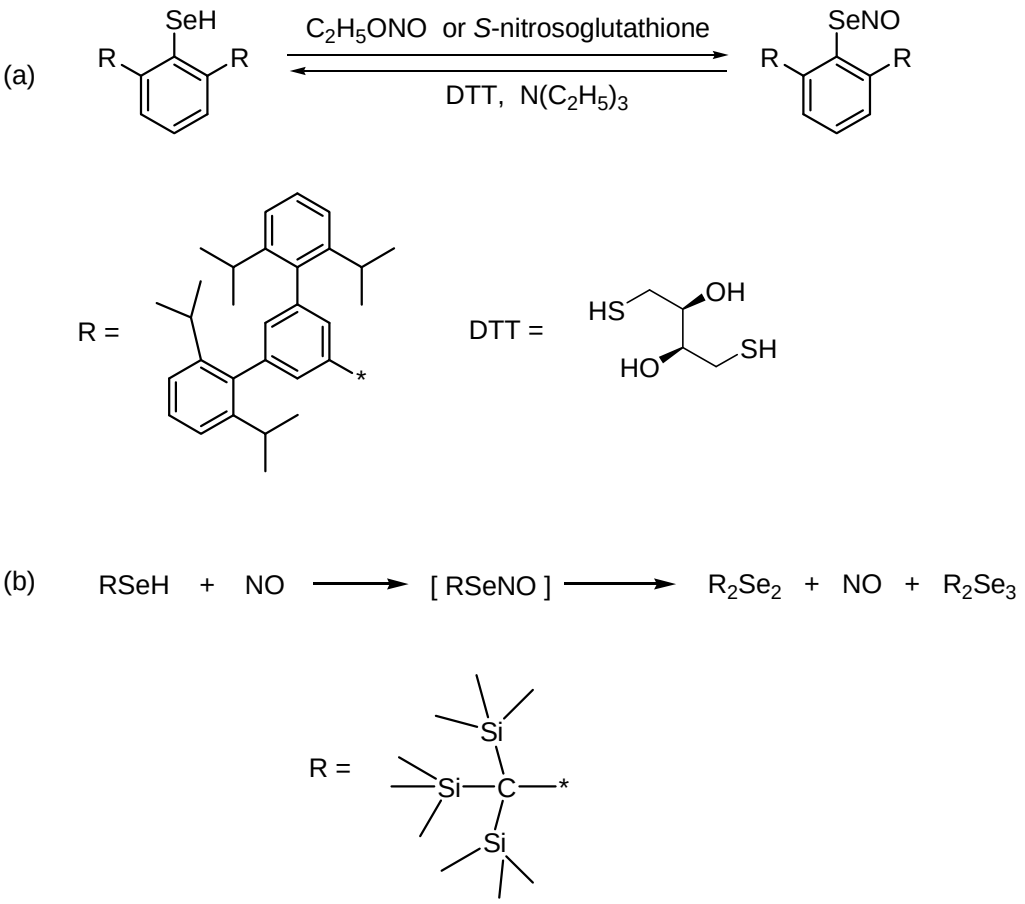
(a) $FL\text{-}M^{n+}L$ (non-fluorescent) $\rightleftharpoons$ (NO) $NO\text{-}M^{n+}L + FL$ (fluorescent)

(b) $FL\text{-}M^{n+}L$ (non-fluorescent) $\xrightarrow[ROH\ or\ water]{NO}$ $FL\text{-}M^{(n-1)+}L + RONO + H^{+}$ (fluorescent)

(c) $FL\text{-}M^{n+}L$ (non-fluorescent) $\rightleftharpoons$ (NO) $M^{(n-1)+}L + FL\text{-}NO$ (fluorescent)

(FL = Fluorophore, M = Metal, L = Ligand)

Scheme S1. Two typical mechanisms for NO detection.[S5,S6]



Scheme S2. The reactions between NO and Se-H groups.[S7,S8]

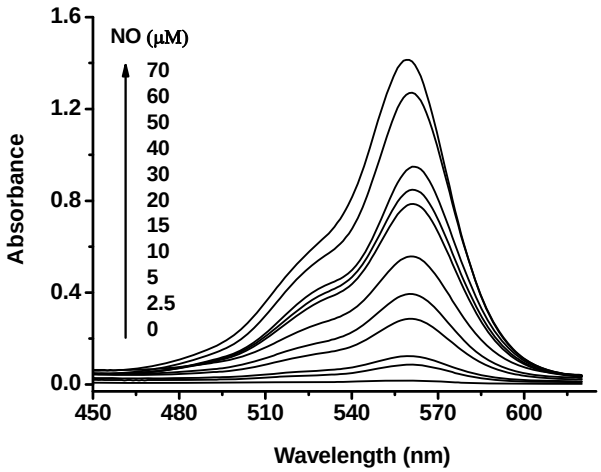


Fig. S1 Absorption spectra of RBSe (20 μM) with varied concentrations of NO from 0 to 70 μM in 20 mM HEPES buffer (pH 7.2) containing 20% (v/v) of ethanol at 37 ^{o}C.

Optimization of Experimental Variables

Effect of pH

The effect of pH has been investigated. As is shown in Fig. S2, the fluorescence intensity of both the probe and its reaction products is hardly affected by the pH change in the range of about pH 6.8-7.8, indicating that RBSe is suited for physiological environment applications. Thus, a HEPES buffer solution of pH 7.2 may be used in our experiments.

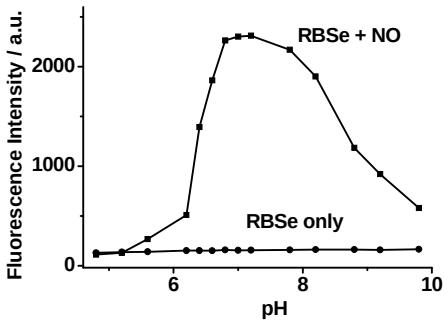


Fig. S2 Effect of pH on the fluorescence intensity of RBSe (20 μM) in the absence and presence of NO (100 μM) in 20 mM HEPES buffer with different pH values containing 20% (v/v) of ethanol at 37 ^{o}C. The fluorescence data were recorded at $\lambda_{ex/em}$ = 520/580 nm 30 min later after addition of NO.

Effect of time

Time course studies (Fig. S3) show that the reaction is very fast; the fluorescence intensity rises to a plateau in 15 min, and then increases only slowly.

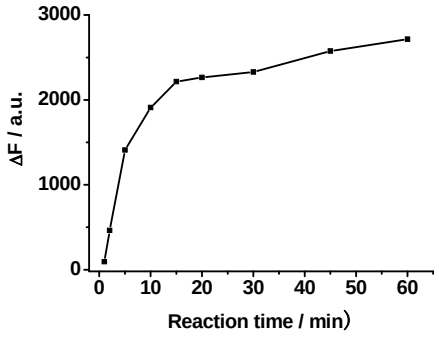


Fig. S3 The effect of reaction time on the fluorescence change of RBSe (20 μM) with NO (100 μM) in 20 mM HEPES buffer (pH 7.2) containing 20% (v/v) of ethanol at 37 ^{o}C. $\lambda_{ex/em}$ = 520/580 nm.

Table S1. Recovery of 20 μM NO in the presence of various coexisting species.

Species	Concentration (mM)	Molar ratio of the added species to NO	Recovery (%)
Na^+	150	7500	106
K^+	150	7500	95
Ca^{2+}	4	200	102
Mg^{2+}	4	200	93
Cu^{2+}	0.1	5	99
Zn^{2+}	0.1	5	94
Fe^{3+}	0.01	0.5	91
Ag^+	2.0×10^{-5}	0.001	102
Hg^{2+}	2.0×10^{-5}	0.001	100
Cl^-	150	7500	102
SO_4^{2-}	2	100	94
NO_3^-	4	200	106
NO_2^-	4	200	102
ClO^-	0.05	2.5	107
H_2O_2	0.1	5	96
O_2^-	0.1	5	95
$^1O_2$	0.1	5	103
$ONOO^-$	0.1	5	108
·OH	0.1	5	103
L-cysteine	0.1	5	95
pyruvic acid	0.1	5	105
reduced glutathione	0.1	5	104
superoxide dismutase	0.02	1	102
hemoglobin	0.02	1	92

Exploration for Reaction Mechanism

In order to explore the reaction mechanism of the present system, high-resolution mass spectra of the reaction products of RBSe with NO were obtained immediately after 5 min (Fig. S4) and 30 min (Fig. S5).

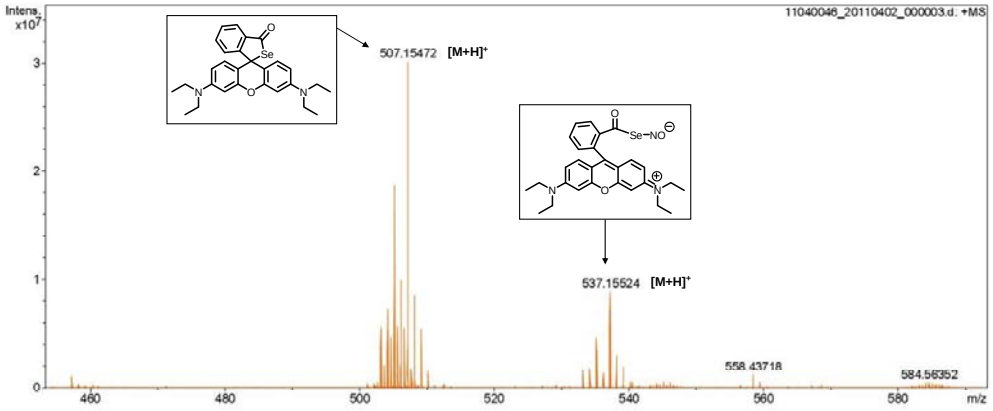


Fig. S4 High-resolution FTICR-MS of the reaction products of RBSe (20 μM) with NO (80 μM) after 5 min.

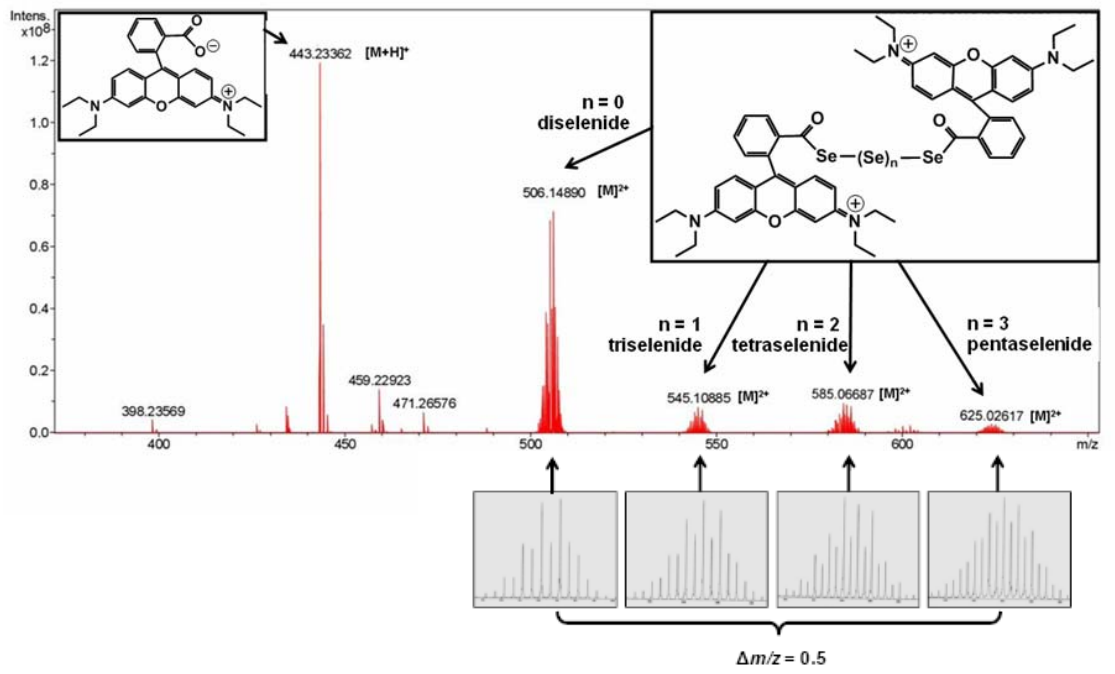


Fig. S5 High-resolution FTICR-MS of the reaction products of RBSe (20 μM) with NO (80 μM) after 30 min. The four signals with higher molecular weights are proven to be doubly charged ion peaks, because the mass differences (Δm/z) between the neighboring isotopic peaks all are 0.5.

Table S2. Summary of the calculated and observed *m/z* values for different species.

Species		Observed *m/z*	Calculated *m/z*
RBSe	$[M+H]^+$	507.15472	507.15508
Compound 1	$[M+H]^+$	537.15524	537.15306
Rhodamine B	$[M+H]^+$	443.23362	443.23347
Diselenide	$[M]^{2+}$	506.14890	506.14725
Triselenide	$[M]^{2+}$	545.10885	545.10591
Tetraselenide	$[M]^{2+}$	585.06687	585.06417
Pentaselenide	$[M]^{2+}$	625.02617	625.02243

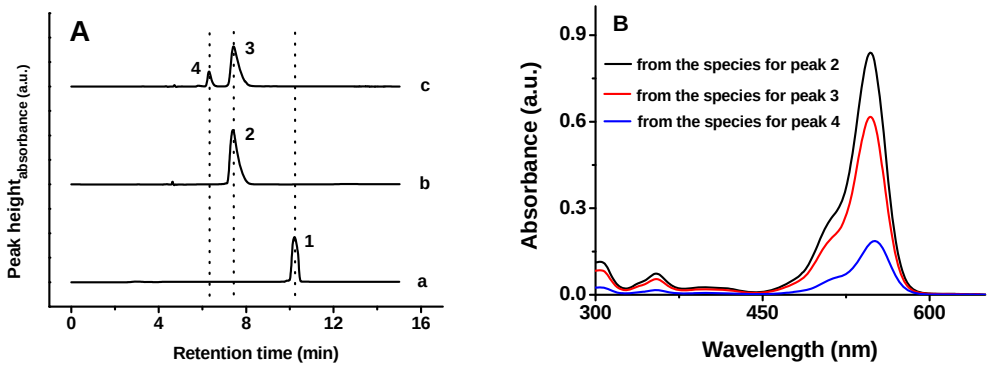


Fig. S6. (A) HPLC chromatograms of different reaction systems. (a) 50 μM RBSe; (b) 50 μM rhodamine B; (c) 50 μM RBSe + 100 μM NO. HPLC analyses were carried out with LC-20AT pumps, SPD-20A UV–vis detector at 254 nm (Shimadzu, Japan) and Inertsil ODS-SP column (5 μm, 4.6 mm×250 mm, GL Sciences Inc.) with methanol-water (6:1, v/v) as eluent (flow rate, 0.7 mL/min). As can be seen, RBSe and rhodamine B have a retention time of 10.36 min (peak 1 in curve a) and 7.40 min (peak 2 in curve b), respectively. However, the reaction solution of RBSe with NO (curve c) no longer gives the peak at 10.36 min, but produces two main new peaks at 7.40 min (peak 3) and 6.30 min (peak 4). This indicates that after reaction the probe is transformed to two main products. The product corresponding to the major peak 3 is characterized as rhodamine B, because it has the same retention time (7.40 min) as rhodamine B. By combining with the above mass spectral data (Fig. S5), the product corresponding to the peak 4 at 6.30 min might be assigned

as the diselenide (see Scheme 1), though other minor multi-selenides cannot be ruled out. (B) Absorption spectra of the corresponding species for peaks 2-4 in Fig. S6(A). These spectra were recorded by a diode array detector in the Shimadzu HPLC system. As can be seen, both of the two species corresponding to the peaks 3 and 4 display the characteristic absorption spectrum of rhodamine B (peak 2), further supporting that the two products contain the rhodamine skeleton. With respect to rhodamine B with λ_{max} = 547 nm, the slight red shift (to 550 nm) of the maximum absorption of the species for peak 4 (blue curve) might be ascribed to the change in the corresponding molecular structure.

References

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