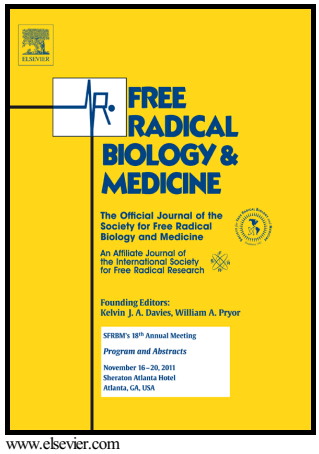


www.elsevier.com

A Photo-Triggered and Photo-Calibrated Nitric Oxide Donor: Rational Design, Spectral Characterizations, and Biological Applications

Haihong He, Yuxin Liu, Zhongneng Zhou, Chunlei Guo, Hong-Yin Wang, Zhuang Wang, Xueli Wang, Ziqian Zhang, Fu-Gen Wu, Haolu Wang, Daijie Chen, Dahai Yang, Xiaowen Liang, Jinquan Chen, Shengmin Zhou, Xin Liang, Xuhong Qian, Youjun Yang

PII: S0891-5849(18)30740-8
DOI: https://doi.org/10.1016/j.freeradbiomed.2018.04.563
Reference: FRB13731

To appear in: *Free Radical Biology and Medicine*

Received date: 31 January 2018
Revised date: 29 March 2018
Accepted date: 19 April 2018

Cite this article as: Haihong He, Yuxin Liu, Zhongneng Zhou, Chunlei Guo, Hong-Yin Wang, Zhuang Wang, Xueli Wang, Ziqian Zhang, Fu-Gen Wu, Haolu Wang, Daijie Chen, Dahai Yang, Xiaowen Liang, Jinquan Chen, Shengmin Zhou, Xin Liang, Xuhong Qian and Youjun Yang, A Photo-Triggered and Photo-Calibrated Nitric Oxide Donor: Rational Design, Spectral Characterizations, and Biological Applications, *Free Radical Biology and Medicine,* https://doi.org/10.1016/j.freeradbiomed.2018.04.563

A Photo-Triggered and Photo-Calibrated Nitric Oxide Donor: Rational Design, Spectral Characterizations, and Biological Applications

Haihong He,[a] Yuxin Liu,[g] Zhongneng Zhou,[c] Chunlei Guo,[a] Hong-Yin Wang,[e] Zhuang Wang,[a] Xueli Wang,[c] Ziqian Zhang,[f] Fu-Gen Wu,[e] Haolu Wang,[b] Daijie Chen,[d] Dahai Yang,[a] Xiaowen Liang,[b,*] Jinquan Chen,[c,*] Shengmin Zhou,[a,*] Xin Liang,[a,*] Xuhong Qian[a], Youjun Yang[a,*]

[a]. State Key Laboratory of Bioreactor Engineering, Shanghai Key Laboratory of Chemical Biology, School of Pharmacy, Shanghai Key Laboratory of New Drug Design, School of Pharmacy, East China University of Science and Technology, Shanghai, 200237, China. [b]. Therapeutics Research Centre, The University of Queensland Diamantina Institute, The University of Queensland, Translational Research Institute, Woolloongabba, QLD 4102, Australia. [c]. State Key Laboratory of Precision Spectroscopy, East China Normal University, Shanghai, 200062, China. [d]. School of Pharmacy, Shanghai Jiao Tong University, Shanghai, 200240, China. [e]. State Key Laboratory of Bioelectronics, School of Biological Science and Medical Engineering, Southeast University, Nanjing, Jiangsu 210096, China. [f] Guangxi Scientific Research Center of Traditional Chinese Medicine, Guangxi University of Chinese Medicine, Nanning, Guangxi 530200, China. [g]. School of Environment and Biological Engineering, Nanjing University of Science and Technology, Nanjing, Jiangsu 210094, China.

Abstract: Nitric oxide (NO) donors are valuable tools to probe the profound implications of NO in health and disease. The elusive nature of NO bio-relevance has largely limited the use of spontaneous NO donors and promoted the development of next generation NO donors, whose NO release is not only stimulated by a trigger, but also readily monitored via a judiciously built-in self-calibration mechanism. Light is without a doubt the most sensitive, versatile and biocompatible method of choice for both triggering and monitoring, for applications in complex biological matrices. Herein, we designed and synthesized an N-nitroso rhodamine derivative (**NOD560**) as a photo-triggered and photo-calibrated NO donor to address this need. **NOD560** is essentially non-fluorescent. Upon irradiation by green light (532nm), it efficiently release NO and a rhodamine dye, the dramatic fluorescence turn-on from which could be harnessed to conveniently monitor the localization, flux, and dose of NO release. The potentials of **NOD560** for in vitro biological applications were also exemplified in *in vitro* biological models, i.e. mesenchymal stem cell (MSC) migration suppression. **NOD560** is expected to complement the existing NO donors and find widespread applications in chemical biological studies.

Keywords: Nitrc oxide donors; photo-triggered; photo-calibrated; mesenchymal stem cell migration.

Introduction

The bio-relevance of nitric oxide was unambiguously established with the identification of NO as the vasodilator.[1-4] This event not only rationalizes the use of NO releasing molecules (NO donors) for hypertensive emergencies[5], but also celebrates the birth of NO biology[6,7]. Over the years, NO has been found to be produced *in vivo* via facile pathways and plays key roles in numerous physiological or pathological processes other than vasodilation.[6,7] It inhibits platelet aggregation[8,9] and is involved in wound healing[10,11]. It is a neurotransmitter, augmenting post-synaptic plasticity and hormone secretion.[12,13] Therefore NO is implicated in cognition[14], motor coordination[15], and sleep-wake cycle[16], etc. Recently, the involvement of NO in stem cell proliferation[17,18], differentiation[17,18] and migration[19] has received tremendous attentions.[20] The elusive biology of NO and profound implications in health and diseases has promoted the development of NO donors for mechanistic chemical biological studies and translational pharmaceutical therapies.[21-26]

A variety of NO-releasing compounds has been reported. Many spontaneously release NO in a biological milieu, e.g. organic nitrates, organic nitrites, nitrosothiols, diazeniumdiolates, N-nitrosamines, metal-nitrosyl complexes, (benzo-/)furoxans.[24-26] The biological outcome of NO is a function of a multitude of parameters, such as

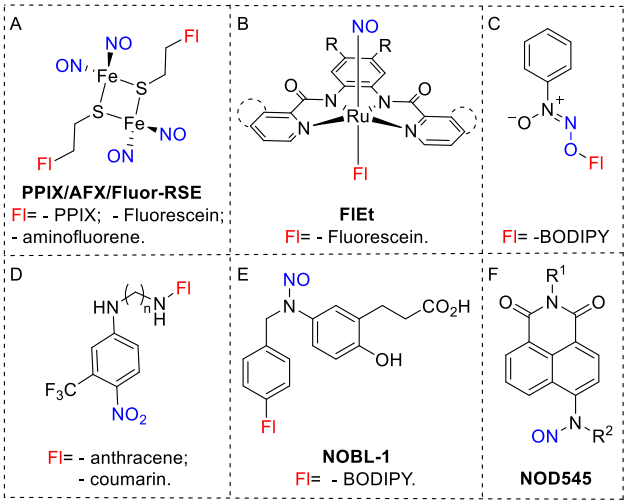


Figure 1. Structures of visible light triggered NO donors. (A) **PPIX/AFX/Fluor-RSE**; (B) **FlEt**; (C) BODIPY labled NONOates ; (D) *o*-trifluoromethyl nitrobenzene; (E) **NOBL-1** and (F) **NOD545**.

its localization, flux, and dose.[27,28] Lack of strict spatiotemporal control over the NO release from the spontaneous NO donors might be a contributing factor for many dichotomous reports of the NO involvement in similar biological settings. The development of light-triggered NO donors[29-35] has attracted tremendous attention, with the *o*-nitrobenzyl protected NONOate by Tsien et al. as the first example[29]. Other notable examples are N-nitrosamines[30], nitrobenzene[31], and metal-nitrosyl complexes[34,35]. Currently, UV-light is required to trigger the NO release in most of these aforementioned molecular systems. Moving to the less cytotoxic longer-wavelength spectral region is keenly desired. Recent progresses in the field include visible light controlled NO donors, e.g. **PPIX/AFX/Fluor-RSE**[36-38], **FlEt**[39], BODIPY labled NONOates[33], *o*-trifluoromethyl nitrobenzene[40,41] and **NOBL-1**[42](Figute 1).

We recently proposed to develop NO donors with both a robust photo-trigger to render a spatiotemporal control of NO release, and a built-in calibration mechanism in form of a concomitant fluorescence turn-on to allow convenient monitoring of NO release. The first embodiment of such photo-triggered and photo-calibrated NO donors is N-nitrosated naphthalimides (**NOD545**)[43], whose scope of applications is however limited by the fact that a UV light at 365 nm was in need to trigger NO release. Ideally, a photo-triggered and photo-calibrated NO donor should exhibit high chemostability to avoid unintended NO release, easy photo-activation by visible light to avoid cytotoxicity, a large fluorescence turn-on ratio to facilitate monitoring of NO release, clean photo-decomposition pathway, high cell permeability, and low cytotoxicity. We wish to report our recent progress in this line of research.

Results and discussion

The design rationale of NO donors. N-nitrosated secondary arylamines, especially electron-deficient arylamines, readily homolyze photolytically to release NO.[44-46] Also, dyes are typically electron-deficient scaffolds, due to intramolecular charge transfer. This inspired us to search for photo-triggered and photo-calibrated donors, from N-nitrosated push-pull dyes. Our initial success with **NOD545** verifies the feasibility of this design rational (Figure 1F). However, it requires UV-activation and precludes use in UV-sensitive biological systems. Therefore, we have also set to develop donors with potentially red-shifted triggering light and synthesized **NOD560** (Figure 2).

Spectral properties of donors and fluorescent products. The spectral properties of **NOD560** and its corresponding rhodamine product (**3**) were studied in phosphate buffer (50 mM, pH = 7.4) with 1% DMSO as a co-solvent. **NOD560** absorbs maximally at 500 nm (ε = 3,100 $M^{-1}cm^{-1}$) with two shoulder peaks at 470 nm (ε = 2,400 $M^{-1}cm^{-1}$) and 533 nm (ε = 2,300 $M^{-1}cm^{-1}$) respectively, suggesting the lactone ring of **NOD560** has

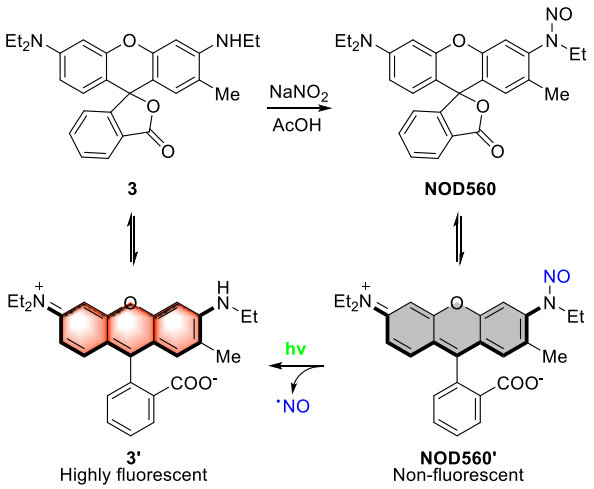


Figure 2. Synthesis and mechanism of photo-triggered and photo-calibrated NO donor, **NOD560**.

opened due to the high polarity of the solvent system. **NOD560** was found not to be fluorescent. In comparison, **3** exhibits a red-shifted absorption maximum at 535 nm, with a *ca.* 12-fold increase of the extinction coefficient (ε = 38,800 $M^{-1}cm^{-1}$). Compound **3** is also intensely fluorescent at 560 nm with a quantum yield of 0.43 in phosphate buffer (pH = 7.4). Therefore, a fluorescent enhancement from a dark background is expected, which is highly favorable for sensitive detection. The photophysical properties of **NOD560** and its corresponding rhodamine product (**3**) are summarized in Table S1.

The solution of **NOD560** (10 μM) in aqueous phosphate buffer (50 mM, pH = 7.4) with 1% DMSO as a co-solvent was irradiated with green laser (532 nm) and UV-Vis absorption and fluorescence emission spectral changes were recorded intermittently. We note that the light within a broad spectral range from UV to ~ 570 nm can induce photolysis of **NOD560**. The light at 532 nm was chosen because it overlaps with a main peak of the absorption spectrum of **NOD560** ($\varepsilon_{532\ nm}$ = 2,300 $M^{-1}cm^{-1}$) and the 532 nm laser line is commonly available in microscopic instruments. Photo-irradiation of **NOD560** induced the decrease of the donor absorption (highlighted in blue, Figure 3A) and the increase of the absorption of **3** (highlight in red, Figure 3A). The sharp isosbestic point at ~475 nm suggests a clean conversion from **NOD560** to **3**, without formation of other chromophoric side-products. The formation of the corresponding rhodamine dye (**3**) from photolysis of **NOD560** was further supported by ^{1}H-NMR and MS spectra. (Figure S1, S2).

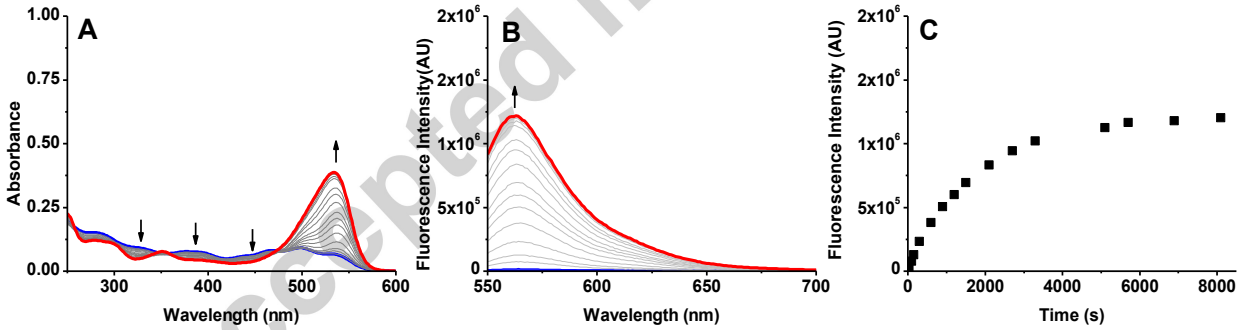


Figure 3. UV-Vis absorption spectral (A) and fluorescence emission spectral (B) changes of **NOD560** solution upon photo-irradiation by 532 nm. (C) The enhancement of emission intensity at 560 nm of **NOD560** solution with respect to the duration of photo-irradiation.

Correspondingly, the fluorescence emission enhancement of **3** at 560 nm was also observed to rise from a dark background and saturate within ca. 6000 s (Figure 3B). We also wish to note that other commonly available laser lines, e.g. 375 nm, 405 nm, 450 nm, 488 nm, 520 nm, and 561 nm could also be employed to trigger NO release. This compatibility of **NOD560** with such a broad selection of commonly available laser lines has greatly promoted its scope in different applications. The NO release from **NOD560** could be conveniently followed by monitoring the fluorescence turn-on of **3**. It can be seen from Figure 3C that the NO release from **NOD560** was faster within the first 2000 s and gradually level off in the next 4000 seconds under the experimental conditions, i.e. 200 mW. It is anticipated that the kinetics could be modulated by the laser intensity.

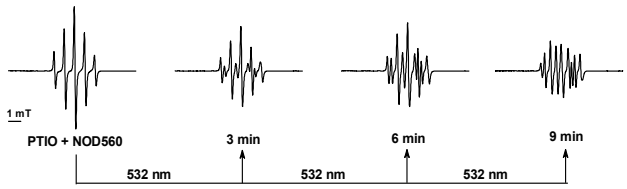


Figure 4. EPR signals of a solution containing **NOD560** (10 μM) and **PTIO** (10 μM) in aqueous phosphate buffer (50 mM, pH = 7.4) with DMSO, 20% of which was used to keep **PTIO** and **PTI** from precipitating.

The release of NO was further confirmed by EPR with **PTIO**, a spin-trap that reacts with NO to yield **PTI** as the final product.[47] Both **PTIO** and **PTI** are stable radical with characteristic EPR signals for convenient identification. The EPR spectrum of a solution containing a stoichiometric amount of both **NOD560** (10 μM) and **PTIO** (10 μM) was monitored (Figure 4). Prior to photo-irradiation by light, a clean EPR signal of **PTIO** was obtained. Upon irradiation, the EPR signal of **PTIO** gradually decreased with the appearance and gradual enhancement of the characteristic signal of **PTI**. After irradiation for 9 min, a clean conversion from **PTIO** to **PTI** was completed as suggested by the EPR signal.

Also, we confirmed that **NOD560** releases NO quantitatively under green light illumination by DAN assay (Figure S3).

The aforementioned spectroscopic studies, i.e. UV-Vis, fluorescence, NMR, MS and EPR, together unambiguously established the photo-triggered decomposition pathway of **NOD560** to nitric oxide and **3**, which is in agreement with the design rationale.

Femtosecond transient absorption (TA) spectroscopy. To gain mechanistic insights on the photolysis of our NO donor, femtosecond transient absorption measurements with **NOD560** was carried out. To facilitate spectral analysis, the femtosecond TA spectra of compound **3** was first collected for comparison purposes.

The TA spectra of **3** (in CH_3CN) were collected in the first place for comparison purposes (Figure 5A) since it is the photolysis product of **NOD560**. Upon excitation by 365 nm, a negative bleach band (500-700 nm) with a peak at 545 nm, and two shoulder bands at 575 nm and 630 nm was observed at 1.66 ps. From 1.66 ps to 75.7 ps, the peak at 545 nm and the shoulder at 630 nm were essentially unchanged, while the shoulder at 575 nm enhanced slightly. From 75.7 ps, this bleach signal gradually recovered to the baseline (Figure 5B). Global fitting of the kinetic traces at different wavelengths by a two-exponential decay function yielded two lifetimes of 14 ± 3 ps and 1.0 ± 0.1 ns, respectively (Figure 5C).

The TA spectra of **NOD560** (in CH_3CN) were then collected. As shown in Figure 5D, there is an initial narrow negative bleach signal centered at 545 nm as well as two broad positive absorption band in the range of 450-520 nm and 565-700 nm, respectively. While the negative signal could originate from the ground state bleaching or stimulated emission, the positive absorption signal is assigned to the excited state absorption of **NOD560**. From 1.58 ps to 67.58 ps, the observed positive bands gradually decayed while the bleach signal at 545 nm became stronger with a significant broadening and the band feature of this bleach signal now resembles that of **3**. Then, it gradually recovered with the same band feature and ended up with a broad positive absorption band

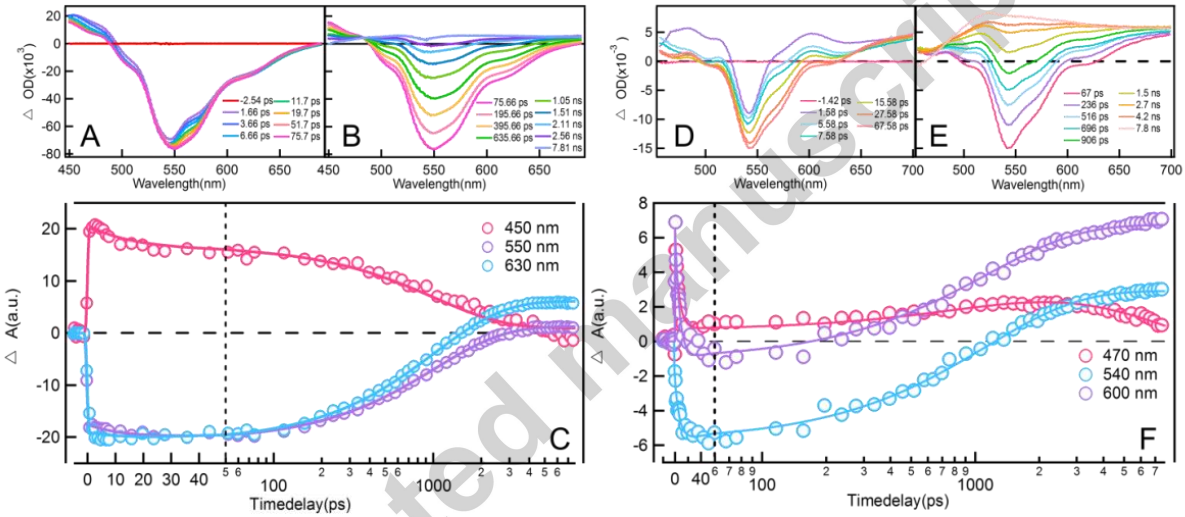


Figure 5. (A-B) Femtosecond time-resolved transient absorption spectra of **3**; (C) individual kinetic traces of **3c** at representative wavelength; (D-E) femtosecond time-resolved transient absorption spectra of **NOD560**; (F) individual kinetic traces of **NOD560** at representative wavelengths.

with a maximum at 530 nm by 8 ns (Figure 5E). This positive absorption band is attributed to the S_0-S_1 absorption of **3**, generated *in situ* from photolysis of **NOD560**. Figure 5F shows the representative kinetic traces obtained from the TA spectra. A two-exponential decay with a non-decay offset was applied to globally fit the data and two lifetimes, of 7.0 ± 0.7 ps and τ_2=1.1 ± 0.1 ns, were determined.

By comparing the decay kinetics between **NOD560** and **3**, we found that the fast 7 ps decay component in **NOD560** is missing in compound **3** while the long-lived 1.1 ns lifetime in **NOD560** agreed well with that in **3**. This indicates that the short-lived 7 ps decay component should be assigned to the photolysis of **NOD560**, and the 1.1 ns lifetime is coming from **3**. Since the photolysis of **NOD560** reaction is a multistep cascade commencing from homolysis of the N-N bond, NO release should also be completed within the same time range. Thus, NO release should be a fast and efficient process with a life- time shorter than 7 ps after photo-excitation.

Chemostability. Mechanistically, denitrosation may occur via transnitrosation[48] or reductive denitrosation[46] (Scheme S2). Therefore, **NOD560** (10 μM) was incubated with biological thiols and bio-relevant reducing agents, i.e. cysteine, glutathione, and ascorbic acid, in phosphate buffer (50 mM at pH = 7.4) with 1% DMSO for 24 h in the dark at room temperature. The absorbance values of the solution at 535 nm were measured and plotted in Figure S5. Physiological levels of these three reagents did not find to induce any spectral changes, suggesting sufficient chemostability of **NOD560** for practical biological applications.

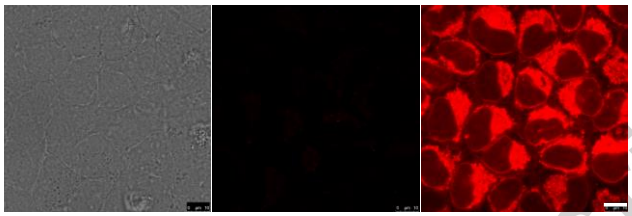


Figure 6. Confocal fluorescence imaging of HeLa cells incubated with **NOD560** (20 μM), for 20 min. (A) Bright-field image. (B) Fluorescence image before photo-irradiation. (C) Fluorescence image after photo-irradation by green light in confocal microscopy for 60 s. Scale bar: 10 μm.

***In vitro* studies.** The capability of **NOD560** to release NO *in vitro* was showcased with HeLa cells. The HeLa cells were incubated with **NOD560** at 20 μM for 20 min in the dark before the cell culture medium was rinsed off. Cells were imaged immediately by confocal microscopy at the excitation of 532 nm. As we expected, the image is essentially dark (Figure 6B) since the NO donor is not fluorescent. Upon irradiation by the 532 nm laser for 60 s,the fluorescence within the cells was significantly enhanced (Figure 6C), suggesting NO donor have been photo-activated to release NO and the corresponding rhodamine fluorophore *in vitro*. Signals are strong in the cytosolic region, but not in the nuclei, suggesting **NOD560** cannot enter cell nuclei.

MSC migration in the presence of NO donors. Mouse mesenchymal stem cells (MSCs) have attracted tremendous research interests due to their immunomodulatory capabilities and therapeutic potentials for tissue engineering.[49,50] The feasibility of **NO560** in MSC cells was first studied. MSCs were incubated with

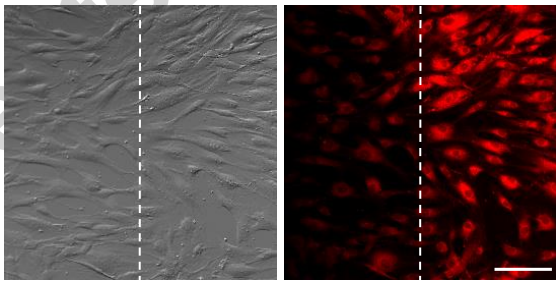


Figure 7. Confocal fluorescence imaging of MSCs incubated with 15 μM **NOD560** for 1 h. (A) Bright-field image. (B) Fluorescence image. Image was recorded with 540 nm as excitation wavelength, emission in the range of 555-585 nm were collected. Scale bar: 100 μm.

NOD560 (15 μM) for 1 h in dark. The cells in the left half of the field of view (FOV) were blocked from irradiation, leaving the cells in the right half exposed to irradiation for 30 s. Then, the cover was removed and the entire FOV was exposed to irradiation for another 30 s. A strong fluorescence turn-on was observed, which hasbeen harnessed to confirm that NO was released in the cell cytosolic region. The fluorescence intensity could be semi-quantitative over the dose of NO release (Figure S9). Further, the fluorescence intensity of MSCs in two different halves of FOV showed different fluorescence intensity (Figure 7). This proves that *in vitro* release of NO can be achieved, and a spatiotemporal control over NO release can be rendered by controlling the site and duration of light-irradiation.

Quick migration of MSCs from blood to injury sites can be regulated by NO release.[51] NO functions as a stop-migration signal by disordering the cytoskeletal

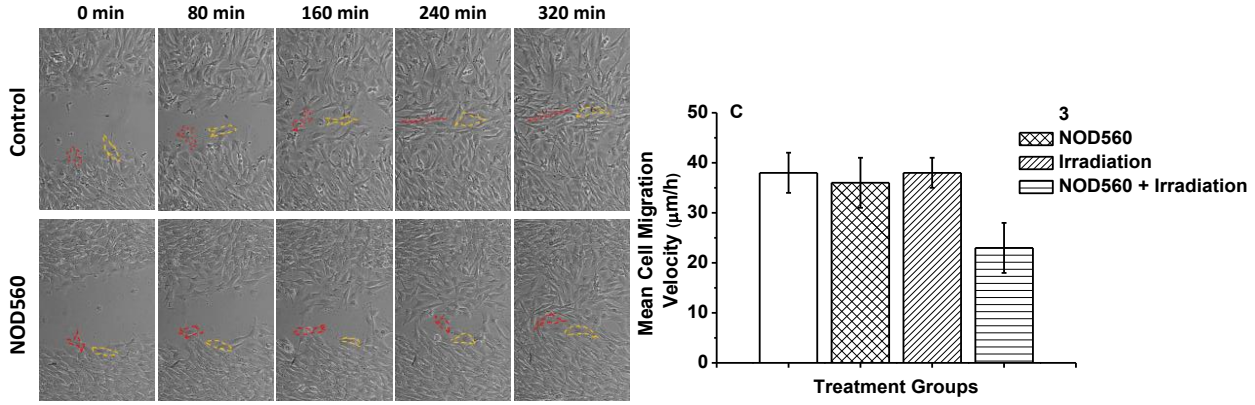


Figure 8. MSC migration in a wound model. (A) Control MSCs exhibited cellular movement directly into the wound zone. The red and yellow outlines represent two cells. (B) Pretreatment of the MSCs with **NOD560** (15 μM) and under irradiation for 2 h prior to wound formation significantly suppressed MSC movement into the open wound zone. Scale bar: 100 μm. (C) The mean migration velocity of MSCs under different conditions. Values are the mean ± s.d. for n = 50 cells; * $p < 0.05$, compared with control groups.

elements required for cell movement and proliferation of MSCs.[52] Herein, we showcased the feasibility of our NO donor in modulating MSC migration. In the wound model of cellular migration, cells at the margin of the wound preferentially migrated into the cell-free zone (wound) without the addition of attractants. The typical migration track of control MSCs (Figure 8A and Video S1) exhibited primarily directed movement into the cell-free zone, with a migratory velocity of ca. 1.20 μm/min. Treating the MSCs with **3** or **NOD560** alone, or green light irradiation alone did not alter the migratory velocities of MSCs (Figure 8C). In comparison, MSCs incubated with **NOD560** (15 μM) followed by irradiation (green LED light, 16 W, 2 h) exhibited a reduced migratory capability, with a mean velocity of 0.73 μm/min, upon wound formation (Figure 8B, C and Video S2). This would indicate that the cellular pathways responsible for the velocity of movement can be affected by the exogenous NO controlled-released from **NOD560**. Similar inhibitory results on cellular migration have been observed in MSCs and cancer cells that were treated with other NO donors such as SNAP.[52]

Conclusions

We have developed a novel NO donor (**NOD560**) via N-nitrosation of a rhodamine dye. It is efficiently photoactivated by UV, blue, green and yellow light to release nitric oxide and a bright rhodamine dye, as evidenced by UV-Vis absorption, fluorescence emission, NMR and EPR spectra. The concomitant fluorescence turn-on can be harnessed for convenient monitoring of the flux and dose of NO release from **NOD560**, even in the complex biological milieu. **NOD560** readily crosses the plasma membrane and is suitable to release NO intracellularly. The potentials of **NOD560** for biological studies are exemplified in MSC migration suppression.

ASSOCIATED CONTENT

Supporting Information

General methods, experimental, synthesis, characterizations, tabulated photophysical data, additional spectral data, crystal structure of **NOD560**, chemostability study, quantification of NO by DAN assay, cell cytotoxicity, ^{1}H-NMR, ^{13}C-NMR and HRMS spectra.

The following files are available free of charge.
Supporting information (PDF)
MSCs migration video (AVI)

AUTHOR INFORMATION

Corresponding Author

1. Email: youjunyang@ecust.edu.cn (Yang, Y.);
2. Email: x.liang@uq.edu.au (Liang, X);
3. Email: jqchen@lps.ecnu.edu.cn (Chen, J.);
4. Email: zhoushengmin@ecust.edu.cn (Zhou, S.);
5. Email: xin.liang@ecust.edu.cn (Liang, X.).

Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

ACKNOWLEDGMENT

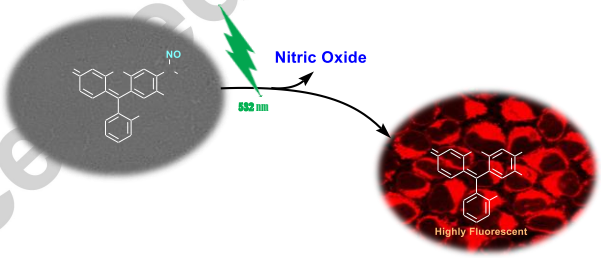
The work is supported by the Fundamental Research Funds for the Central Universities (Nos. WY1514053, WY1516017), Shanghai Pujiang Program (16PJ1402500) and the National Natural Science Foundation of China (Nos. 21372080, 21572061, 11674101, 21236002, and 81502540).

REFERENCES

1. Arnold, W. P.; Mittal, C. K.; Katsuki, S.; Murad, F. Nitric oxide activates guanylate cyclase and increases guanosine 3': 5'-cyclic monophosphate levels in various tissue preparations. Proc. Nat. Acad. Sci. USA 74: 3203-3207; 1977.
2. Furchgott, R. F.; Zawadzki, J. V. The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. Nature 299:373-376; 1980.
3. Ignarro, L. J.; Buga, G. M.; Wood, K. S.; Byrns, R. E.; Chaudhuri, G. Endothelium-derived relaxing factor produced and released from artery and vein is nitric oxide. Proc. Nat. Acad. Sci. USA 84:9265-9269; 1987.
4. Palmer, R. M.; Ferrige, A. G.; Moncada, S. Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. Nature 327:524–526; 1987.
5. Friederich, J. A.; Butterworth, J. F. Sodium nitroprusside: twenty years and counting. Anesth. Analg. 81:152-162; 1995.
6. Ignarro, L. J. Nitric Oxide: Biology and Pathobiology, Academic Press, San Diego, California, London; 2000.
7. Ignarro, L. J. Nitric Oxide: Biology and Pathobiology, 2nd ed., Academic Press, San Diego, California, London; 2009.
8. Radomski, M. W.; Palmer, R. M. J.; Moncada, S. The role of nitric oxide and cgmp in platelet adhesion to vascular endothelium. Biochem. Biophys. Res. Commun. 148:1482-1489; 1987.
9. Sogo, N.; Magid, K. S.; Shaw, C. A.; Webb, D. J.; Megson, I. L. Inhibition of human platelet aggregation by nitric oxide donor drugs: relative contribution of cgmp-independent mechanisms. Biochem. Biophys. Res. Commun. 279:412-419; 2000.
10. Schäffer, M. R.; Tantry, U.; Gross, S. S.; Wasserkrug, H. L.; Barbul, A. Nitric oxide regulates wound healing. J. Surg. Res. 63:237-240; 1996.
11. Witte, M. B.; Barbul, A. Role of nitric oxide in wound repair. Am. J. Surg. 183:406-412; 1988.
12. Garthwaite, J.; Charles, S. L.; Chess-Williams, R. Endothelium-derived relaxing factor release on activation of nmda receptors suggests role as intercellular messenger in the brain. Nature 336:385-388; 1988.
13. Garthwaite J.; Boulton, C. L. Nitric oxide signaling in the central nervous system. Annu. Rev. Physiol. 57:683-706; 1995.
14. Paul, V.; Ekambaram, P. Involvement of nitric oxide in learning & memory processes. Indian J. Med. Res. 133:471-478, 2011.
15. Del Bel, E. A.; Guimarães, F. S.; Bermúdez -Echeverry, M.; Gomes, M. Z.; Schiaveto-de-Souza, A.; Padovan-Neto, F. E.; Tumas, V.; Barion-Cavalcanti, A. P.; Lazzarini, M.; Nucci-da-Silva, L. P.; de Paula-Souza, D. Role of nitric oxide on motor behavior. Cell. Mol. Neurobiol. 25:371-392; 2005.
16. Burlet, S.; Cespuglio, R. Voltammetric Detection of nitric oxide (no) in the rat brain: its variations throughout the sleep-wake cycle. Neurosci. Lett. 226:131-135; 1997.
17. Gibbs, S. M. Regulation of neuronal proliferation and differentiation by nitric oxide. Mol. Neurobiol. 27:107-120; 2003.
18. Cheng, A.; Wang, S.; Cai, J.; Rao, M. S.; Mattson, M. P. Nitric oxide acts in a positive feedback loop with bdnf to regulate neural progenitor cell proliferation and differentiation in the mammalian brain. Dev. Biol. 258:319-333; 2003.
19. Segal, M. S.; Shah, R.; Afzal, A.; Perrault, C. M.; Chang, K.; Schuler, A.; Beem, E.; Shaw, L. C.; Calzi, S. L.; Harrison, J. K.; Tran-Son-Tay, R. Nitric oxide cytoskeletal-induced alterations reverse the endothelial progenitor cell migratory defect associated with diabetes. Diabetes 55:102-109; 2006.
20. Bonafè, F.; Guarnieri, C.; Muscari, C. Nitric oxide regulates multiple functions and fate of adult progenitor and stem cells. J. Physiol. Biochem. 71:141-153; 2015.
21. Miller, M. R.; Megson, I. L. Recent developments in nitric oxide donor drugs. Br. J. Pharmacol. 151:305-321; 2007.
22. Seabra, A.; Durán, N. Nitric oxide-releasing vehicles for biomedical applications. J. Mater. Chem. 20:1624-1637; 2009.
23. Carpenter, A. W.; Schoenfisch, M. H. Nitric oxide release: part ii. therapeutic applications. Chem. Soc. Rev. 41:3742-3752; 2012.
24. Wang, P. G.; Xian, M.; Tang, X.; Wu, X.; Wen, Z.; Cai, T.; Janczuk, A. Nitric oxide donors: chemical activities and biological applications. J. Chem. Rev. 102:1091-1134; 2002.
25. Hrabie, J. A.; Keefer. L. K. Chemistry of the nitric oxide-releasing diazeniumdiolate ("nitrosohydroxylamine") functional group and its oxygen-substituted derivatives. Chem. Rev. 102:1135-1154; 2002.
26. Wang, P. G.; Cai, T. B.; Taniguchi, N. *Nitric* oxide donors: for pharmaceutical and biological applications. Wiley-VCH, New York; 2005.
27. Ridnour, L. A.; Thomas, D. D.; Donzelli, S.; Espey, M. G.; Roberts, D. D.; Wink, D. A.; Isenberg, J. S. The biphasic nature of nitric oxide responses in tumor biology. Antioxid. Redox Signaling 8:1329-1337; 2006.
28. Calabrese, V.; Mancuso, C.; Calcani, M.; Rizzarelli, E.; Butterfield, D. A.; Stella, A. M. G. Nitric oxide in the central nervous system: neuroprotection versus neurotoxicity. Nat. Rev. Neurosci. 8:766-775; 2007.
29. Makings L. R.; Tsien, R. Y. Caged nitric oxide. J. Biol. Chem. 269:6282-6285; 1994.
30. Namiki, S.; Arai, T.; Fujimori, K. High-performance caged nitric oxide: a new molecular design, synthesis, and photochemical reaction. J. Am. Chem. Soc. 119:3840-3841; 1997.
31. Fukuhara, K.; Kurihara, M.; Miyata, N. Photochemical generation of nitric oxide from 6-nitrobenzo[a]pyrene. J. Am. Chem. Soc. 123:8662-8666; 2001.
32. Ruane, P. H.; Bushan, K. M.; Pavlos, C. M.; D'Sa, R. A.; Toscano, J. P. Controlled photochemical release of nitric oxide from o2-benzyl-substituted diazeniumdiolates. J. Am. Chem. Soc. 124:9806-9811; 2002.
33. Blangetti, M.; Fraix, A.; Lazzarato, L.; Marini, E.; Rolando, B.; Sodano, F.; Fruttero, R.; Gasco, A.; Sortino, S. A nonmetal-containing nitric oxide donor activated with single-photon green light. Chem. Eur. J. 38:9026-9029; 2017.
34. Fry, N. L.; Mascharak, P. K. Photoactive ruthenium nitrosyls as no donors: how to sensitize them toward visible light. Acc. Chem. Res. 44:289-298; 2011.
35. Ford, P. C. Photochemical delivery of nitric oxide. Nitric Oxide 34:56-64; 2013.
36. Conrado, C. L.; Wecksler, S.; Egler, C.; Magde, D.; Ford, P. C. Synthesis and photochemical properties of a novel iron-sulfur-nitrosyl cluster derivatized with the pendant chromophore protoporphyrin IX1. Inorg. Chem. 43:5543-5549; 2004.
37. Wecksler, S. R.; Mikhailovsky, A.; Korystov, D.; Ford, P. C. A two-photon antenna for photochemical delivery of nitric oxide from a water-soluble, dye-derivatized iron nitrosyl complex using NIR light. J. Am. Chem. Soc. 128:3831-3837; 2006.
38. Wecksler, S. R.; Mikhailovsky, A.; Korystov, D.; Buller, F.; Kannan, R.; Tan, L.-S.; Ford, P. C. Single- and two-photon properties of a dye-derivatized roussin's red salt ester (Fe2(μ-RS)2(NO)4) with a large TPA cross section. Inorg. Chem. 46:395-402; 2007.

39. Fry, N. L.; Wei, J.; Mascharak, P. K. Triggered dye release via photodissociation of nitric oxide from designed ruthenium nitrosyls: turn-on fluorescence signaling of nitric oxide delivery. Inorg. Chem. 50:9045-9052; 2011.
40. Vittorino, E.; Sciortino, M. T.; Siracusano, G.; Sortino, S. Light-activated release of nitric oxide with fluorescence reporting in living cells. Chem. Med. Chem. 6:1551-1554; 2011.
41. Marino, N.; Perez-Lloret, M.; Blanco, A. R.; Venuta, A.; Quaglia, F.; Sortino, S. Photo-antimicrobial polymeric films releasing nitric oxide with fluorescence reporting under visible light. J. Mater. Chem. B. 4:5138-5143; 2016.
42. Ieda, N.; Hotta, Y.; Miyata, N.; Kimura, K.; Nakagawa, H. Photomanipulation of vasodilation with a blue-light-controllable nitric oxide releaser. J. Am. Chem. Soc. 136:7085-7091; 2014.
43. Zhang, Z.; Wu, J.; Shang, Z.; Wang, C.; Cheng, J.; Qian, X.; Xiao, Y.; Xu, Z.; Yang, Y. Photocalibrated NO release from n-nitrosated napthalimides upon one-photon or two-photon irradiation. Anal. Chem. 88:7274-7280; 2016.
44. Grover, T. A.; Ramseyer, J. A.; Piette, L. H. Photolysis of nitrosamines and nitrosamides at neutral ph: a spin-trap study. Free Radical Biol. Med. 3:27-32; 1987.
45. Piech, K.; Bally, T.; Sikora, A.; Marcinek, A. Mechanistic aspects of the oxidative and reductive fragmentation of N-nitrosoamines: a new method for generating nitrenium cations, amide anions, and aminyl radicals. J. Am. Chem. Soc. 129:3211-3217; 2007.
46. Tanno, M.; Sueyoshi, S.; Miyata, N.; Umehara, K. Characterization of the cytotoxic activity of nitric oxide generating n-nitroso compounds. Chem. Pharm. Bull. 45:595-598; 1997.
47. Akaike, T.; Yoshida, M.; Miyamoto, Y.; Sato, K.; Kohno, M.; Sasamoto, K.; Miyazaki, K.; Ueda, S.; Macda, H. Antagonistic action of imidazolineoxyl n-oxides against endothelium-derived relaxing factor/•no through a radical reaction. Biochem. 32:827-832; 1993.
48. Williams, D. L. H. Nitrosation reactions and the chemistry of nitric oxide. Elsevier, London; 2004.
49. Aggarwal, S.; Pittenger, M. F. Human mesenchymal stem cells modulate allogeneic immune cell responses. Blood 105:1815-1822; 2005.
50. Uccelli, A.; Moretta, L.; Pistoia, V. Mesenchymal stem cells in health and disease. Nat. Rev. Immunol. London 8:726-736; 2008.
51. Li, N.; Lu, X.; Zhao, X.; Xiang, F-L.; Xenocostas, A.; Karmazyn, M.; Feng, Q. Endothelial nitric oxide synthase promotes bone marrow stromal cell migration to the ischemic myocardium via upregulation of stromal cell-derived factor-1α. Stem Cell 27:961-970; 2009.
52. Fuseler, J. W.; Valarmathi, M. T. Nitric oxide modulates postnatal bone marrow-derived mesenchymal stem cell migration. Front. Cell Dev. Biol. 4:133; 2016.

Authors are required to submit a graphic entry for the Table of Contents (TOC) that, in conjunction with the manuscript title, should give the reader a representative idea of one of the following: A key structure, reaction, equation, concept, or theorem, etc., that is discussed in the manuscript. Consult the journal's Instructions for Authors for TOC graphic specifications.



Video S1. The migration of control MSCs (Video of Figure 8A).

Video S2. The migration of MSCs incubated with NOD560 upon green light irradiation (Video of Figure 8B).

Highlights

NOD560 releases NO upon activation by green-light.

NOD560 is non-fluorescent with excellent chemostablity.

A rhodamine dye is co-generated stoichiometrically with respective to NO.

The potentials of **NOD560** as a NO donor were showcased with MSC cells.