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A colorimetric and fluorescent turn-on probe for carbon monoxide and imaging in living cells

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ABSTRACT

A colorimetric and fluorescent dual probe for CO is designed and synthesized. The probe is capable of sensitive and specific detection of CO with naked-eye through colorimetric assay in phosphate buffer from colorless to yellow, which also features 75-fold turn-on fluorescence enhancement upon exciting at 480 nm with the detection limit of 26.3 nM. The probe shows great potential for sensing intracellular CO without the interference in living cell imaging. On the other hand, the probe could also be utilized as an efficient and convenient visual indicator for CO gas in air by naked eyes.

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It is well known that biological activity of carbon monoxide (CO) is closely dependent on its concentration in living beings. High concentration of CO is toxic to humans and animals because of its strong affinity for hemoglobin.^{1,2} In human body, the formation of endogenous CO is one of the most common chemical processes through the endogenous degradation of heme and inducible heme oxygenase enzymes,³⁻⁵ which appears to control the sinusoidal tone in the hepatic circulation, to regulate the proliferation of vascular smooth muscle cells and to suppress the rejection of transplanted hearts.⁶ Recently, some fluorescent probes for imaging small molecules have been one of the most powerful tools to visualize the complicated biological processes.^{7,8} A few fluorescent probes for CO detection, especially a major breakthrough in protein CO probe,^{9,10} have been newly reported,¹¹⁻¹⁵ but these probes are only fluorescence response except a chromo-fluorogenic CO probe based on bimetallic rhodium.¹³ More importantly, most of the probes are soluble in organic solvent, and the application suitable for the biological samples is still at a very early stage. Therefore, the quantitative analytical methods with simple and portable point-of-care testing are highly desired for various applications, particularly in environmental gas and complicated living systems.

The colorimetric and fluorescent probe has many advantages such as real-time monitoring and the use of

simple and inexpensive instrumentation as well.¹⁶ Moreover, it is equipment-free and rapid to detect analyte by the naked eye. **NBDA** (4-amino-7-nitro-2,1,3-benzoxadiazole) derivative is the typical intra-molecular charge transfer (ICT) fluorophore with excellent optical properties such as high fluorescence quantum yield, low molecular weight, good biological tolerance and so on,¹⁷⁻¹⁹ which is widely used in the bioassay.²⁰⁻²³ With this context, a new chromo-fluorogenic probe **NBD-APC** [4-allyl (propyl)carbamate-7-nitro-2,1,3-benzoxadiazole] for CO detection based on **NBD** fluorophore has been designed and investigated. This probe not only displays colorimetric and fluorogenic dual response to CO in phosphate buffer, but shows high sensitive and selective response to CO over other biologically relevant species in living cells as well.

Scheme 1. The proposed mechanism of **NBD-APC**.

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Figure 1. A) Absorption spectra of **NBD-APC** (10 μM) and the absorption spectra of **NBD-APC** (10 μM) + PdCl₂ (20 μM) upon adding **CORM-3** (0, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 60, 80, 100 μM) in PBS buffer solutions. Inset: the pictures of visual color change: (a) **NBD-APC** (10 μM) + PdCl₂ (20 μM); (b) **NBD-APC** (10 μM) + PdCl₂ (20 μM) + **CORM-3** (50 μM). B) The fluorescence spectra of **NBD-APC**, **NBD-APC** (10 μM) + PdCl₂ (20 μM), **NBD-APC** (10 μM) + **CORM-3** (50 μM), **NBD-APC** (10 μM) + PdCl₂ (20 μM) + **CORM-3** (50 μM). C) Fluorescence spectra of **NBD-APC** (10 μM) + PdCl₂ (20 μM) upon adding **CORM-3** (0, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 60, 80, 100 μM) in PBS buffer solutions. Inset: the pictures of fluorescence change: (a) **NBD-APC** (10 μM) + PdCl₂ (20 μM); (b) **NBD-APC** (10 μM) + PdCl₂ (20 μM) + **CORM-3** (50 μM). D) The fluorescence intensity at 549 nm versus equivalents of **CORM-3** added.

The probe **NBD-APC** is easily prepared and well characterized by NMR and HR-MS spectra (see Supporting Information). The probe displays stable emission from 4.0 to 9.0 in pH (Fig. S1). The absorption spectrum of **NBD-APC** in Figure 1 exhibits a maximal absorption peak at 370 nm ($\epsilon = 12400 \text{ M}^{-1} \text{ cm}^{-1}$) in PBS buffer (10 mM, pH = 7.4), which transfers to 363 nm ($\epsilon = 12300 \text{ M}^{-1} \text{ cm}^{-1}$) when PdCl₂ is added up to 20 μM . Upon adding **CORM-3** [Ru(CO)₃Cl(glycinate)] into the mixed solution of **NBD-APC** and PdCl₂ (20 μM), a new absorption peak at 480 nm ($\epsilon = 21000 \text{ M}^{-1} \text{ cm}^{-1}$) gradually forms with two clear isosbestic points at 358 nm and 415 nm, respectively (Fig. 1A). A remarkable red shift of the absorption peak is obviously observed from 370 nm to 480 nm ($\Delta\lambda = +110 \text{ nm}$), which results in a vivid colour change from colorless to yellow (Fig. 1A, Inset).

In the fluorescence study, free **NBD-APC** is weakly fluorescent ($\lambda_{\text{em}} = 549 \text{ nm}$, $\Phi = 0.0038$) in PBS buffer upon exciting at 480 nm. The allyl formate may weaken the potential of ICT through the electron-withdrawing competition with nitro group. The fluorescent intensity of **NBD-APC** slightly increases when mixing with PdCl₂ (20 μM). Upon adding **CORM-3** into the mixed solution of **NBD-APC** (10 μM) and PdCl₂ (20 μM), the fluorescent intensity significantly increases (Fig. 1B). The possible mechanism is proposed: CO can reduce Pd(II) to Pd(0)¹⁵,

and Pd(0) subsequently reacts with the probe **NBD-APC** to produce **NBD-PPA** ($\lambda_{\text{em}} = 549 \text{ nm}$, $\Phi = 0.53$) through the Tsuji-Trost reaction, which leads to the colorimetric and fluorescent change. The mechanism is identified by the fact that **NBD-PPA** is found in the reaction solution according to HPLC analysis (Fig. S2). Within the titration experiments of **CORM-3**, the emission intensities at 549 nm progressively increase, and the enhancement is positively correlated with the concentration of **CORM-3** (Fig. 1C). Upon treating the solution of **NBD-APC** (10 μM) + PdCl₂ (20 μM) with **CORM-3** (50 μM), the fluorescence is enhanced promptly and nearly reaches a plateau at 30 minute (Fig. S3). The maximal fluorescence enhancement is up to 75-fold (Fig. 1D).

To evaluate the selectivity of **NBD-APC**, various physiologically relevant species are examined. As shown in Figure 2, the probe **NBD-APC** shows a negligible response to physiologically relevant species (50 μM) in presence or absence of PdCl₂ (20 μM) (Figs. 2a and 2b). By comparison, noticeable color change and fluorescence enhancement are obtained when treating the mixed solution of **NBD-APC** (10 μM) and PdCl₂ (20 μM) with **CORM-3** (50 μM) (Figs. 2a and 2c). These results suggest that **NBD-APC** has excellent selectivity for CO over other biological species. The detection limit is calculated to be $2.63 \times 10^{-8} \text{ M}$, indicating that the probe is highly sensitive to CO (Fig. S4). Taking all

these results together, the probe **NBD-APC** exhibits great potential in colorimetric and fluorescent detection of CO with excellent selectivity and sensitivity.

Figure 2. The fluorescent response of the **NBD-APC** (10 μM) + PdCl_2 (20 μM) to various relevant species (50 μM) in pH 7.4, 10 mM PBS buffer solutions for 30 minutes. Black bar: the probe treat with relevant species without PdCl_2 (20 μM); Red bar: the probe treat with relevant species and in the presence of PdCl_2 (20 μM). Inset: the color images of **NBD-APC** (10 μM) treated with the biologically relevant analytes (50 μM) in the presence (a) and in the absence (b) of PdCl_2 (20 μM), and the fluorescence images (c) of **NBD-APC** treated with the biologically relevant analytes in the presence of PdCl_2 (20 μM) under UV irradiation at 365 nm.

In order to evaluate the ability of **NBD-APC** to detect CO in living beings, the cellular imaging is performed in the human cervical cancer cells (HeLa cells) by confocal laser scanning microscopy. The HeLa cells incubated with the probe **NBD-APC** alone (20 μM) or with the mixture of **NBD-APC** (20 μM) and PdCl_2 (40 μM) for 30 minutes at 37 $^\circ\text{C}$ exhibit very weak fluorescence at the emission window (Figs. 3a-h). However, HeLa cells are incubated with **CORM-3** (100 μM) for 30 minutes at 37 $^\circ\text{C}$, and treated with the mixture of **NBD-APC** (20 μM) and PdCl_2 (40 μM) for other 30 minutes at 37 $^\circ\text{C}$. The results display strong fluorescence (Figs. 3i-l). These results indicate that the probe **NBD-APC** is an efficient candidate for tracking intracellular CO.

Figure 3. The cellular imaging of HeLa cells for CO detection with incubating of (a-d) **NBD-APC** (20 μM); (e-h) **NBD-APC** (20 μM) + PdCl_2 (40 μM); (i-l) **NBD-APC** (20 μM) + PdCl_2 (40 μM) + **CORM-3** (100 μM) in the PBS buffer for 30 min at 37 $^\circ\text{C}$.

Motivated by the favorable chromo-fluorogenic sensing features of the probe observed in PBS solution, the system is developed to explore the potential use for CO detection in the air. The solution of **NBD-APC** and PdCl_2 is adsorbed on silica gel. The silica gel is exposed to the mixed gas ($V_{\text{air}}:V_{\text{CO}} = 2:1$) in the bottle, and then the bottle is shaken for 30 seconds up and down. The color of silica gel dramatically changes (Figs. 4a and 4b) from white to yellow, and it is simultaneously found to display a remarkable turn-on fluorescence under the UV light (Figs. 4c and 4d). On the other hand, this approach is also conducted on some strips of cellulose paper, and the turn-on fluorescence signal in the presence of CO could also be easily observed with naked eye (Fig. S5). These results suggest that the probe could be an efficient and convenient visual indicator for CO detection in air.

Figure 4. Summary of visual color and fluorescence changes observed by the naked eye: adsorbed on silica gel and detected in the absence (a, c) and in the presence (b, d) of CO (10ppm) for the probe by color changes and fluorescence emission under UV irradiation at 365 nm.

In summary, this study reports a colorimetric and fluorescent dual probe **NBD-APC** for *in vitro* and intracellular sensing CO, and its response to CO gas in the air as well. Taking advantage of the distinct color change and significant turn-on fluorescence response to CO, the probe **NBD-APC** can be utilized as an efficient and convenient visual indicator for CO gas in air by naked eyes. **NBD-APC** exhibited excellent selectivity and sensitivity for CO over other biologically relevant analytes with a detection limit of 2.63×10^{-8} M. Moreover, living-cell imaging results demonstrate that **NBD-APC** could be applied to track intracellular CO. Therefore, this probe **NBD-APC** can be appreciated as a promising candidate in CO research field. This work will shed light on searching for new probes for CO detection based on the Tsuji-Trost reaction.

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References and notes

1. Ryter, S.W.; Alam, J.; Choi, A.M.K. *Physiol. Rev.* **2006**, 86, 583.
2. Wu, L.Y.; Wang, R. *Pharmacol. Rev.* **2005**, 57, 585.
3. Sjostrand, T. *Scand. J. Clin. Lab. Inv.* **1949**, 1, 201.
4. Mann, B.E.; Motterlini, R. *Chem. Commun.* **2007**, 41, 4197.
5. Clark, J.E.; Naughton, P.; Shurey, S.; Green, C.J.; Johnson, T.R.; Mann, B.E.; Foresti, R.; Motterlini, R. *Circ. Res.* **2003**, 93, e2.
6. Sato, K.; Balla, J.; Otterbein, L.; Smith, R.N.; Brouard, S.; Lin, Y.; Csizmadia, E.; Seigny, J.; Robson, S.C.; Vercellotti, G.; Choi, A.M.; Bach, F.H.; Soares, M.P. *J. Immunol.* **2001**, 166, 4185.
7. Kobayashi, H.; Ogawa, M.; Alford, R.; Choyke, P.L.; Urano, Y.; *Chem. Rev.* **2010**, 110, 2620.
8. Vendrell, M.; Zhai, D.; Er, J.C.; Chang, Y.T. *Chem. Rev.* **2012**, 112, 4391.
9. Wang, J.; Karpus, J.; Zhao, B.S.; Luo, Z.; Chen, P.R.; He, C. *Angew. Chem. Int. Ed.* **2012**, 51, 9652.
10. Michel, B.W.; Lippert, A.R.; Chang, C.J. *J. Am. Chem. Soc.* **2012**, 134, 15668.
11. Yuan, L.; Lin, W.Y.; Tan, L.; Zheng, K.B.; Huang, W.M. *Angew. Chem. Int. Ed.* **2013**, 52, 1628.
12. Zheng, K.B.; Lin, W.Y.; Tan, L.; Chen, H.; Cui, H.J. *Chem. Sci.* **2014**, 5, 3439.
13. Moragues, M.E.; Toscani, A.; Sancenón, F.; Martínez Máñez, R.; White, A.J.P.; Wilton-Ely, J.D.E.T. *J. Am. Chem. Soc.* **2014**, 136, 11930.
14. Cao, Y.; Li, D.W.; Zhao, L.J.; Liu, X.Y.; Cao, X.M.; Long, Y.T. *Anal. Chem.* **2015**, 87, 9696.
15. Pal, S.; Mukherjee, M.; Sen, B.; Mandal, S.K.; Lohar, S.; Chattopadhyay, P.; Dhara, K. *Chem. Commun.* **2015**, 51, 4410.
16. Li, X.H.; Gao, X.H.; Shi, W.; Ma, H.M. *Chem. Rev.* **2013**, 114, 590.
17. Crampton, M.R.; Pearce, L.M.; Rabbitt, L.C. *J. Chem. Soc., Perkin Trans. 2.* **2002**, 257.
18. Uchiyama, S.; Santa, T.; Imai, K. *J. Chem. Soc., Perkin Trans. 2.* **1999**, 2525.
19. Uchiyama, S.; Santa, T.; Imai, K. *Anal. Chem.* **2001**, 73, 2165.
20. Jiang, Q.; Guo, Z.J.; Zhao, Y.; Wang, F.Y.; Mao, L.Q. *Analyst*, **2015**, 140, 197.
21. Lee, D.Y.; Kim, G.; Yin, J.; Yoon, J.Y. *Chem. Commun.* **2015**, 51, 6518.
22. Qian, F.; Zhang, C.L.; Zhang, Y.M.; He, W.J.; Gao, X.; Hu, P.; Guo, Z.J. *J. Am. Chem. Soc.* **2009**, 131, 1460.
23. Xie, Z.J.; Wang, K.; Zhang, C.L.; Yang, Z.H.; Chen, Y.C.; Guo, Z.J.; Lu, G.Y.; He, W.J. *New J. Chem.* **2011**, 35, 607.