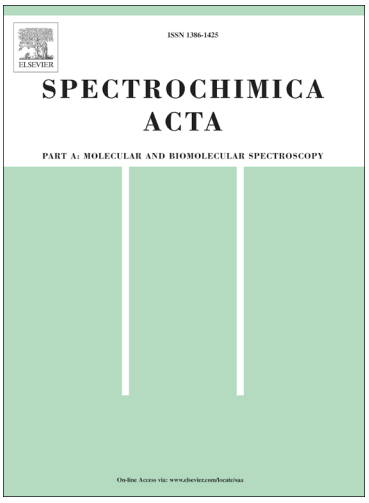
Journal Pre-proofs



An endoplasmic reticulum targetable turn-on fluorescence probe for imaging application of carbon monoxide in living cells

Yunyan Zhang, Yonghe Tang, Xiuqi Kong, Weiying Lin

PII: S1386-1425(20)31129-X
DOI: https://doi.org/10.1016/j.saa.2020.119150
Reference: SAA 119150

To appear in: *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*

Received Date: 13 May 2020
Revised Date: 23 October 2020
Accepted Date: 26 October 2020

Please cite this article as: Y. Zhang, Y. Tang, X. Kong, W. Lin, An endoplasmic reticulum targetable turn-on fluorescence probe for imaging application of carbon monoxide in living cells, *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy* (2020), doi: https://doi.org/10.1016/j.saa.2020.119150

An endoplasmic reticulum targetable turn-on fluorescence probe for imaging application of carbon monoxide in living cells

Yunyan Zhang [a], Yonghe Tang [b], Xiuqi Kong [a], and Weiying Lin [a, b*]

[a] Institute of Fluorescent Probes for Biological Imaging, School of Chemistry and Chemical Engineering, School of Materials Science and Engineering, University of Jinan, Jinan, Shandong 250022, P.R. China.

[b] Institute of Optical Materials and Chemical Biology, School of Chemistry and Chemical Engineering, Guangxi University, Nanning, Guangxi, 530004, P. R. China

*Corresponding author. Tel.: +86 53182769108; Fax: +86-531-82769031

E-mail address: weiyinglin2013@163.com.

Abstract

Carbon monoxide (CO) is a significant mediator in regulating endoplasmic reticulum (ER) stress, and its level may play a potential role in the treatment of vascular diseases combined with ER stress. *In-situ* visualization of CO in the ER helps to elucidate its physiological and pathological mechanistic behavior. Herein, a novel CO fluorescent probe (**Na-CM-ER**) with ER-targeting characteristics was structured. **Na-CM-ER** with naphthalimide as a fluorescent group, under the trigger of CO, an ICT (Intramolecular Charge Transfer) mechanism was constructed by converting a nitro group to an amino group and showed dazzling green fluorescence. **Na-CM-ER** exhibited satisfactory response speed, selectivity, photo-stability and sensitivity to CO *in vitro*. Furthermore, biological imaging experiments demonstrated that **Na-CM-ER** could monitor the changes of exogenous/endogenous CO in living cells and possess an ER-targeting property. To sum up, we hope that **Na-CM-ER** can be as a serviceable molecular tool for imaging CO in cellular ER.

Key Words: Carbon monoxide; Fluorescent probe; Endoplasmic Reticulum; Turn-on; Imaging.

1. Introduction

Carbon monoxide (CO) is known as an odorless and colorless gas. Medical studies have shown that CO can bind the same site to hemoglobin as oxygen does, and has higher affinity to hemoglobin (around 210 times) than oxygen (O_2) [1]. After entering the organism, CO is easily combined with hemoglobin to produce carboxyhemoglobin, which causes hemoglobin to lose its ability and function to carry oxygen, causing tissue asphyxia [2]. Recent years, CO has been found to be an endogenous gas signaling molecule in plants and animals, which shoulders a major role in maintaining the normal operation of animal and plant life [3-5]. In organisms, heme is catalyzed by heme oxygenase (HO) to produce endogenous CO [6]. In addition, related studies have found that abnormal endogenous CO concentration is closely related to the occurrence and deterioration of various diseases, including neurodegenerative diseases, cardiovascular diseases, lung diseases, inflammation, obesity, sepsis and cancer [7-9]. Therefore, it is important to design new methods to detect and perceive the physiological/pathological functions of carbon monoxide selectively and visually in living systems [10,11].

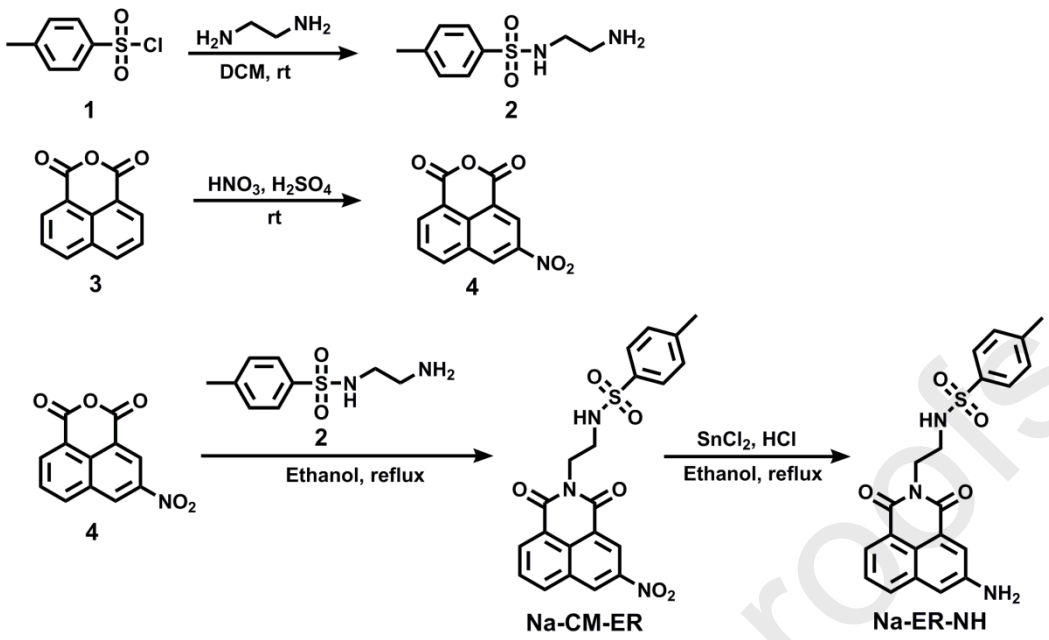
Endoplasmic reticulum (ER) is the largest organelle with single membrane structure in eukaryotic cells [12]. The membrane area of ER accounts for at least half of the total membrane system of eukaryotic cells. ER is involved in important physiological functions, including synthesizing and modifying protein, stabilizing the Ca^{2+} and K^{+} level [13]. ER participates in life activities with other organelles such as mitochondria, cell membranes, Golgi apparatus, and lipid droplets in cells [14,15]. It is noteworthy that the HO which produces endogenous CO is mainly distributed in ER of the living cells. Accumulation of unfolded proteins, hypoxia and viral infections could cause ER stress, which leads to ER dysfunction. Exogenous or endogenous CO could inhibit associated proteins (X-box binding protein 1 and protein kinase R-like endoplasmic reticulum kinase, PERK) expression, and make endoplasmic reticulum to restore homeostasis and maintain normal physiological function [16-18]. However, the detail regulatory mechanisms of CO for ER have not

been well defined. Thus, it is in an urgent need for develop effect tools for tracing CO in ER.

The traditional CO detection methods, including chromogenic detection, absorption spectroscopy, chromatography, electrochemical assays [19-21], need to destroy the tested samples and cannot realize the *in-situ* detection of CO. In contrast, fluorescence microscopy imaging is a frontier crossover technique for *in situ* detection of bioactive molecules [22-25]. Although several CO fluorescent probes have been developed in recent years [26], to our best knowledge, there is no CO fluorescent probe targeting ER up to date, thus disclosing the physiological function of CO in the ER remains very challenging.

Herein, we firstly developed an intelligent ER-specific fluorescent probe (**Na-CM-ER**) for imaging of cellular CO. The classic dye naphthalimide dye in **Na-CM-ER** was selected as the fluorescence platform, and the labeled methyl sulfonamide segment as the ER-targeting moiety. Nitro group was employed as a smart identification site due to the selective "turn-on" response to CO. **Na-CM-ER** could selectively detect CO *in vitro* by reducing nitro group to a highly fluorescent amino functional derivative (**Na-ER-NH**). Furthermore, biological imaging experiments demonstrated that **Na-CM-ER** could monitor the changes of exogenous/endogenous CO in living cells and possessed an ER-targeting property. We expect **Na-CM-ER** to be a serviceable molecular tool for exploring the physiologic action of CO in living cellular ER.

2. Experimental section



Scheme 1. Synthesis routes for **Na-CM-ER.**

2.1. Synthesis

Na-CM-ER was synthesized by four steps reaction (Scheme 1). The compound **2** was synthesized according to the previous synthesis method of our research group [27]. The 1,8-naphthalic anhydride underwent the nitration reaction yielding the compound **4** [28], which reacted with compound **2** underwent a condensation reaction obtaining the probe **Na-CM-ER**. The Structural characterization data, including ^{1}H NMR, ^{13}C NMR and HR-MS (High Resolution Mass Spectrometry) were provided in Supporting Information.

*2.1.1. Synthesis of the compound **Na-CM-ER***

Compound **4** (0.216 mmol) and compound **2** (0.216 mmol) were dissolved in 2 mL ethanol, the mixture was reacted at 80 °C for 2 h with nitrogen protection. Then the mixture was dissipated to 0 °C and the precipitate was filtered out and washed by methanol and cyclohexane, and dried to obtain compound **Na-CM-ER** with a yield of 81 %. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.48 (s, 1H), 8.91 (s, 1H), 8.77 (d, J = 8.0 Hz, 1H), 8.64 (d, J = 4.0 Hz, 1H), 8.05 (t, J = 8.0 Hz, 1H), 7.80 (t, J = 4.0 Hz, 1H), 7.57

(d, *J* = 8.0 Hz, 2H), 7.23 (d, *J* = 4.0 Hz, 2H), 4.12 (t, *J* = 8.0 Hz, 2H), 3.11 (q, *J* =8.0 Hz, 2H), 2.25 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 164.1, 163.6, 147.1, 139.0, 137.5, 135.1, 132.1, 130.9, 130.8, 130.7, 130.5, 127.6, 125.4, 124.0, 123.9, 22.1. HR-MS m/z calculated for $C_{21}H_{17}N_3O_6S$ $[M+H]^+$: 440.0916; found 440.0914.

*2.1.2. Synthesis of the compound **Na-ER-NH***

The compound **Na-CM-ER** (88 mg, 0.2 mmol) and $SnCl_2$ (135 mg, 0.6 mmol) was dispersed in ethanol (10 mL), then added concentrated hydrochloric acid, the mixture reacted at 80 °C for 3 h under nitrogen protection. After completing the reaction, the pH was adjusted with 40% NaOH and then extracted with ethyl acetate. Finally, the mixture was concentrated under vacuum to afford orange solid of the compound **Na-ER-NH** with a yield of 53 %. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.04 (s, 1H), 8.03 (d, *J* = 4.0 Hz, 1H), 7.93 (d, *J* = 4.0 Hz, 1H), 7.76 (s, 1H), 7.58 (t, *J* = 6.0 Hz, 3H), 7.28 (d, *J* = 4.0 Hz, 1H), 7.25 (d, *J* = 8.0 Hz, 2H), 6.01 (s, 2H), 4.08 (t, *J* = 8.0 Hz, 2H), 3.05 (s, 2H), 2.28 (s, 3H). HR-MS m/z calculated for $C_{21}H_{19}N_3O_4S$ $[M+H]^+$: 410.1163; found 410.1173.

2.2. Optical studies

Unless otherwise specified, all optical tests were conducted in PBS (10 mM, pH=7.4, 10% DMSO as co-solvent) with **Na-CM-ER** (10 μM). CORM-2 (A commercial CO release reagent) was prepared in dimethyl sulfoxide (DMSO), and the analytes stock solutions were prepared in the ultrapure water at the appropriate concentration. HITACHI F4600 fluorescence spectrophotometer was used to perform fluorescence spectrum test. Excitation wavelength was set to 430 nm, and the slit widths of excitation and emission were set to 5 nm.

2.3. Cell cytotoxic assays

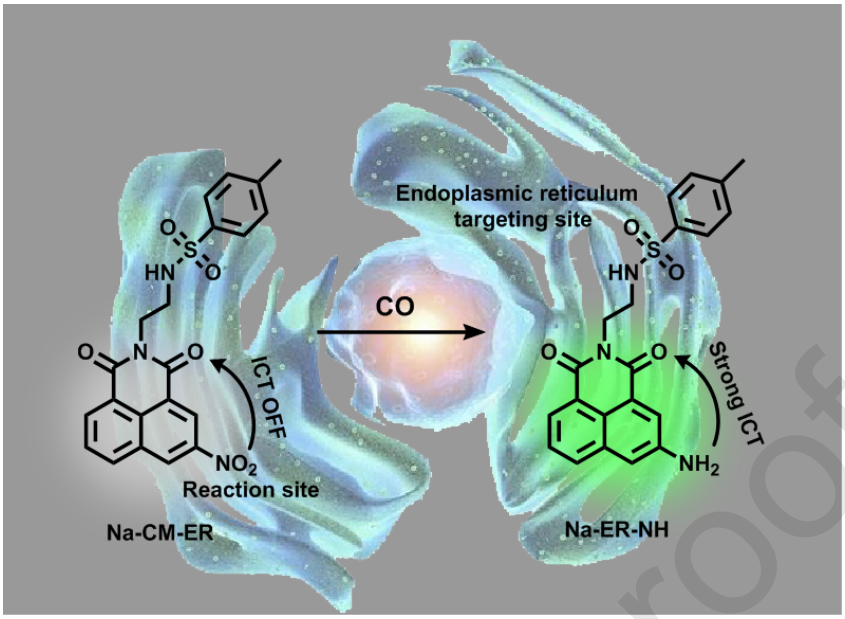
HeLa cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) provided with 10% FBS in an atmosphere of 5% CO_2 at 37 °C. HeLa cells were planted into aseptic 96-well plates, and then 0-30 μM of **Na-CM-ER** and **Na-ER-NH**

was added respectively. Subsequently, cells were incubated for 24 h in the same environment. Finally, the absorbance of the solution was acquired by using the microplate reader at 570 nm. The cell viability was calculated by the following formula: Cell viability (%) = (OD_{570}(sample)-OD_{570}(blank))/(OD_{570}(control)-OD_{570}(blank))*100%. OD_{570} sample denotes the cells incubated with various of concentrations of **Na-CM-ER** and **Na-ER-NH**, OD_{570} control denotes the cells without the probes, OD_{570} blank denotes the wells containing only the culture medium.

3. Results and discussion

*3.1. Design of probe **Na-CM-ER***

Nitro group has electron-withdrawing feature, which could hold electron distribution as an electron acceptor for fluorescent switch, and easily reduced by the reducing agents such as CO [29]. By installing a nitro group and an ER targeting group, the methyl sulphonamide group, on to the 1,8-naphthalimide fluorescent platform, a ER-targetable turn-on fluorescent probe (**Na-CM-ER**) was constructed. **Na-CM-ER** exhibited non-fluorescent due to the strong electron-withdrawing ability of the nitro group, the electron structure performed as "A-π-A", which suppressed the intramolecular charge transfer (ICT) effect. When CO was introduced, the nitro group was reduced to amino group due to the reducibility of CO, the electronic structure was transformed into D-π-A. Then the ICT process was recovered, and the fluorescent signal was generated (Scheme 2).



Scheme 2. The recognition mechanism of CO by ER-targeted CO fluorescent probe **Na-CM-ER.**

*3.2. Photophysical properties of **Na-CM-ER***

With the ER-target CO fluorescent probe **Na-CM-ER** in hand, we firstly conducted fluorescence titration experiments with **Na-CM-ER** (10 μM) and various concentrations of CORM-2 (the concentration range of CORM-2 was 0-250 μM) in PBS (10 mM, pH=7.4, 10% DMSO as co-solvent). At first, as expected, the fluorescent intensity of **Na-CM-ER** showed no fluorescence because of the strong electron-withdrawing ability of the nitro group. Satisfactorily, when CO was added, the fluorescence was generated at 520 nm due to the ICT effect appeared (Fig. 1A), and the intensity gradually enhanced with the increasing of CO concentrations (Fig. 1B). Fluorescence of probe reached saturation after addition with 210 μM CORM-2, and the signal enhancement enhanced 143-fold. Besides, fluorescent intensity of **Na-CM-ER** at 520 nm enhanced linearly with CORM-2 ranging from 0-100 μM, and the detection limit of probe for CORM-2 was measured to be 0.42μM (3σ/slope). The results indicate that the novel probe **Na-CM-ER** is ultrasensitive to CO with high signal-to-noise ratio, which is suitable for detecting CO.

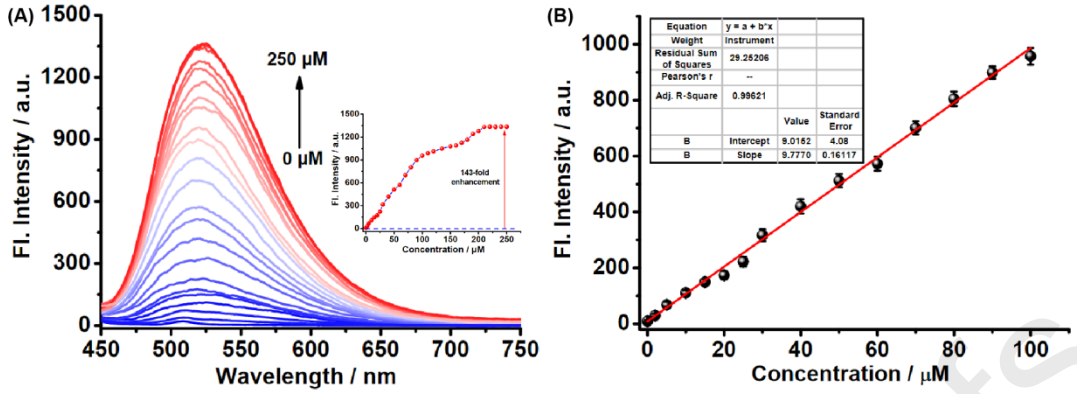


Fig. 1. (A) Fluorescent curves of **Na-CM-ER** (10 μM) encountering with CORM-2 (0-250 μM) in 10 mM PBS buffer (pH 7.4, 10 % DMSO v/v). Inset shows the fluorescent intensity changes of **Na-CM-ER** (10 μM) at I_{520} with CORM-2 (0-250 μM). (B) Linear relationship of between intensity and CORM-2 concentration. λ_{ex} = 430 nm. Error bars represent standard deviation (±S.D.), n =3.

Subsequently, the time-dependent study of **Na-CM-ER** with CO was conducted. In the presence of CORM-2 (210 and 40 μM), the fluorescent intensity of **Na-CM-ER** at 520 nm increased gradually and reached the equilibrium point after 70 min (Fig. 2 and Fig. S1). Besides, the photo-stability study of **Na-CM-ER** was also carried out with or without CORM (210 and 40 μM). After continuous irradiation for 60 min, the fluorescence intensities of free **Na-CM-ER** (10 μM) and coexisting CORM-2 (210 and 40 μM) at 520 nm showed little changes (Fig. 3 and Fig. S2). What is more, the stability of probe **Na-CM-ER** in serum was also performed. The results in Fig. S3 showed that the fluorescence had satisfactory stability in 10% serum, which was the concentration of the general cell culture. This offers a well property for further biological applications. These data indicate that **Na-CM-ER** has fast recognition speed for CO and excellent photo-stability.

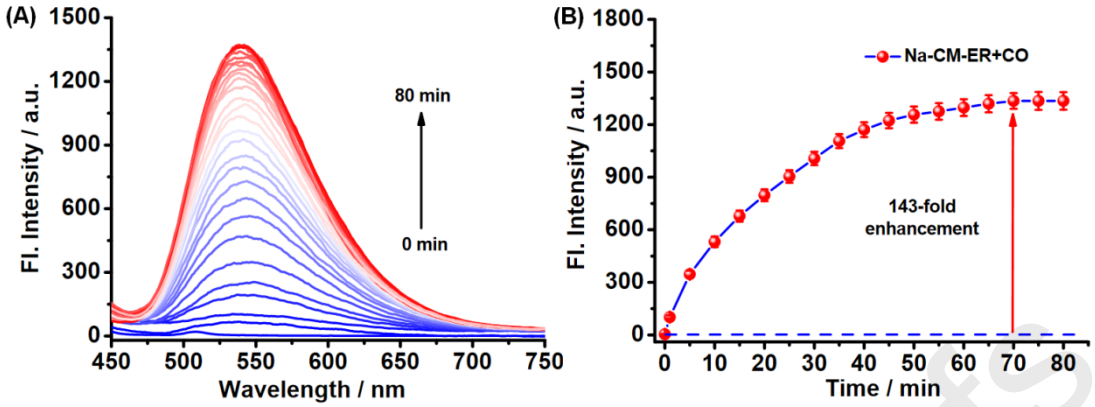


Fig. 2. (A) Time-dependent fluorescence spectra of **Na-CM-ER** (10 μM) treated with CORM-2 (210 μM). (B) Fluorescent intensity changes of **Na-CM-ER** at I_{520} treated with CORM-2 (210 μM). Error bars represent standard deviation (±S.D.), n =3. λ_{ex} = 430 nm..

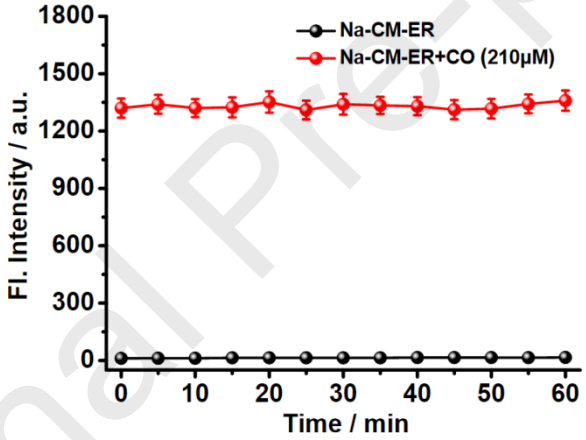


Fig. 3. Photo-stability profiles of **Na-CM-ER** (10 μM) in the absence or presence of CORM-2 (210 μM). Error bars represent standard deviation (±S.D.), n =3. λ_{ex} = 430 nm.

Then the reactive mechanism was investigated. As above-mentioned, the assumed reaction between **Na-CM-ER** and CO could produce **Na-ER-NH**. Thus, the HRMS and optical septra were used to test this assumption. Firstly, the HRMS was obtained after the reaction of **Na-CM-ER** (10 μM) in the presence of CO. As shown in the Fig.

S4, the data showed primary signal at 410.1051 (calcd 410.1096), which could be assigned to [**Na-CM-ER**+H]$^+$. Secondly, the fluorescence spectra of the reaction between **Na-CM-ER** and CO (CORM-2) in PBS (10 mM, pH=7.4, 10% DMSO as co-solvent) was carried out. After **Na-CM-ER** interacting with CO, an obvious fluorescence emission appeared at 520 nm, which was consistence with the maximum emission peak of the compound **Na-ER-NH** (Fig. S5). These results directly confirm our hypothesis that **Na-CM-ER** can interact with CO through the nitro-reduction reaction to form **Na-ER-NH**.

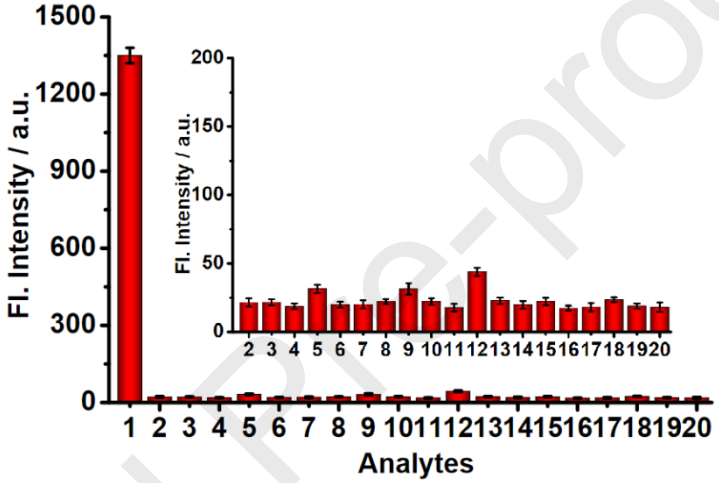


Fig. 4. Fluorescent intensity (I_{520}) changes of **Na-CM-ER** (10 μM) in the presence of different biological relevant analytes. The numbers represent: (1) CORM-2，(2) NaHS, (3) Cys, (4) $CaCl_2$, (5) GSH, (6) NaClO, (7) $NaNO_2$, (8) $NaHSO_3$, (9) KCl, (10) N-Acetylglycine, (11) free probe, (12) *tert-butyl* hydroperoxide, (13) N-Acetyl-L-cysteine, (14) $ONOO^-$, (15) NO, (16) sodium pyruvate, (17) formaldehyde, (18) trichloracetic aldehyde, (19) H_2O_2, (20) acetone. Error bars represent standard deviation (±S.D.), n =3. λ_{ex} = 430 nm.

The constructed probe should have exclusive selectivity so that the target molecule can be detected effectively, thus the selective experiments of **Na-CM-ER** (10 μM)

with different biological relevant analytes were conducted. Other related analytes including typical representative amino acid (1 mM GSH, 210 μM Cys, 210 μM N-Acetylglycine, 210 μM N-Acetyl-L-cysteine (NAC)), inorganic salts (210 μM NaHS, 210 μM $NaNO_2$, 210 μM $NaHSO_3$, 210 μM KCl, 210 μM $CaCl_2$), reactive oxygen species (210 μM H_2O_2, 210 μM *tert-butyl* hydroperoxide (TBHP), 210 μM NaClO, reactive nitrogen species (210 μM NO, 210 μM $ONOO^-$), reactive carbonyl species (210 μM sodium pyruvate, 210 μM trichloracetic aldehyde, 210 μM acetone, 210 μM formaldehyde (FA)), 210 μM CORM-2 were added into the mixture containing **Na-CM-ER** for 70 min. As shown in the Fig. 4, fluorescent signals caused by other analytes had little changes, and were almost the same as that of the free probe. However, the signal had distinct enhanced coexisting with CO. Therefore, **Na-CM-ER** has well selectivity with CO, which is suitable for detecting exclusive CO in complex biological environments.

To evaluate whether **Na-CM-ER** is applicable to detecting CO at physiological pH, the fluorescence emission spectra of **Na-CM-ER** in the presence and absence of CO (210 and 40 μM) in PBS with different pH values (4.5-10.0) were measured. Fluorescent intensities of **Na-CM-ER** in different pH (7.0-8.0) buffer reached maximum, and the fluorescent intensities were consistent with that of concentration titration results. Thus, **Na-CM-ER** is favorable for tracing CO in physical environments (Fig. S6 and Fig. S7).

3.3. Confocal fluorescence imaging

Fluorescent spectra tests show that the probe **Na-CM-ER** has high sensitivity and selectivity, well photo-stability, which are benefit for further biological imaging research. The cellular cytotoxicity of **Na-CM-ER** was firstly tested. MTT assay showed **Na-CM-ER** and **Na-ER-NH** had no obvious toxic in living HeLa cells at the different concentrations (0-30 μM), indicating that **Na-CM-ER** could be used in biological sample for tracking CO (Fig. S8). Next, we investigated the ability for detecting exogenous CO in a complex cellular environments. HeLa cells were

incubated with culture media containing 10 μM **Na-CM-ER** for 30 min, then imaged using a fluorescence confocal microscope. As shown in Fig. 5a-c, cells showed no fluorescence excited at 405 nm. In stark contrast to phenomenon of free **Na-CM-ER** in cells, strong fluorescent signals were observed in HeLa cells further treated with CORM-2 (40 μM) (Fig. 5d-f). Quantified mean fluorescence intensities were further confirmed these profiles (Fig. 5g). The cellular imaging results are the same as those of spectral experiments, which show that the **Na-CM-ER** has ability for tracking exogenous CO.

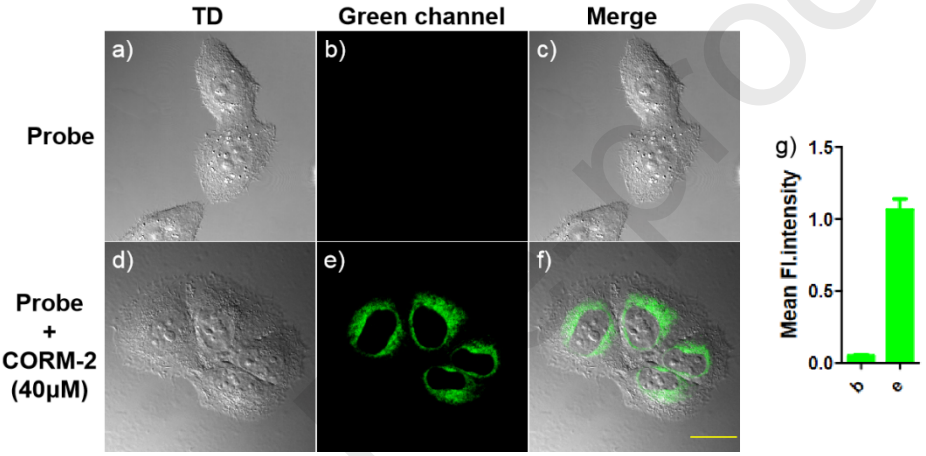


Fig. 5. Fluorescent images of probe **Na-CM-ER** in HeLa cells for detecting exogenous CO. (a-c) Cells were treated with free **Na-CM-ER** (10 μM) for around 30 min; (d-f) Cells were treated with **Na-CM-ER** (10 μM) for 30 min and further incubated with CORM-2 (40 μM) for another 30 min; (g) Quantified mean fluorescence intensities of cells in the green channel. λ_{ex} = 405 nm, λ_{em}= 500-550 nm, scale bar = 20 μm.

Next, we tried to test the feasibility of monitoring CO with time-resolved mode using our probe **Na-CM-ER**. After addition of CORM-2 (40 μM), imaging was immediately capatured with confocal microscopy. The results were exhibited in Fig. S9, observable signals was detected within 2 min at first, demonstrating that probe can quickly react with CO in cells. With the extension of time, the fluorescence in cells gradually became much brighter and reached saturation at around 30 min.

Moreover, after another 40 min visualization, the fluorescence had little fluctuations, suggesting that **Na- ER-NH**, the product of reaction between **Na-CM-ER** and CO, has satisfactory photostabilty in living cells. Thus, probe **Na-CM-ER** can detect CO with time-solved manner.

After acquiring achievements that **Na-CM-ER** is capable of tracking exogenous CO in living cells, we continued to verify the applications of visualizing endogenous CO in living cells. It is well known endogenous CO is generated by an HO-2 mediated heme, thus, heme is an inducer of endogenous CO [30, 31]. Like the behaviors of cells treated with free **Na-CM-ER** (10 μM) in exogenous CO detecting experiments, cells pretreated with only **Na-CM-ER** (10 μM) for 30 minutes produced dull green fluorescence (Fig. 6a-c). However, after the stimulation of 100 μM heme for 2 h (4 h), cells then loaded with 10 μM **Na-CM-ER** for further 30 min showed marked green fluorescence, and the intensities increased with extension of incubation time of heme (Fig. 6d - i).

After achieving in tracing endogenous CO by stimulation using heme, we next turned our attention on capturing CO triggered by hypoxia, which is another effective manner for generation of endogenous CO. During the experiments, the cells were cultured in hypoxia incubator with 5%. The results were shown as Fig. S10. At 5% hypoxia incubator, cells firstly cultured for 12 h, then treated with **Na-CM-ER** (10 μM) for 30 min displayed marked green fluorescence excited at 405 nm, indicating that **Na-CM-ER** can detect endogenous CO under hypoxia conditions. Therefore, all results clearly suggest that **Na-CM-ER** is capable of tracing endogenous CO in living cells.

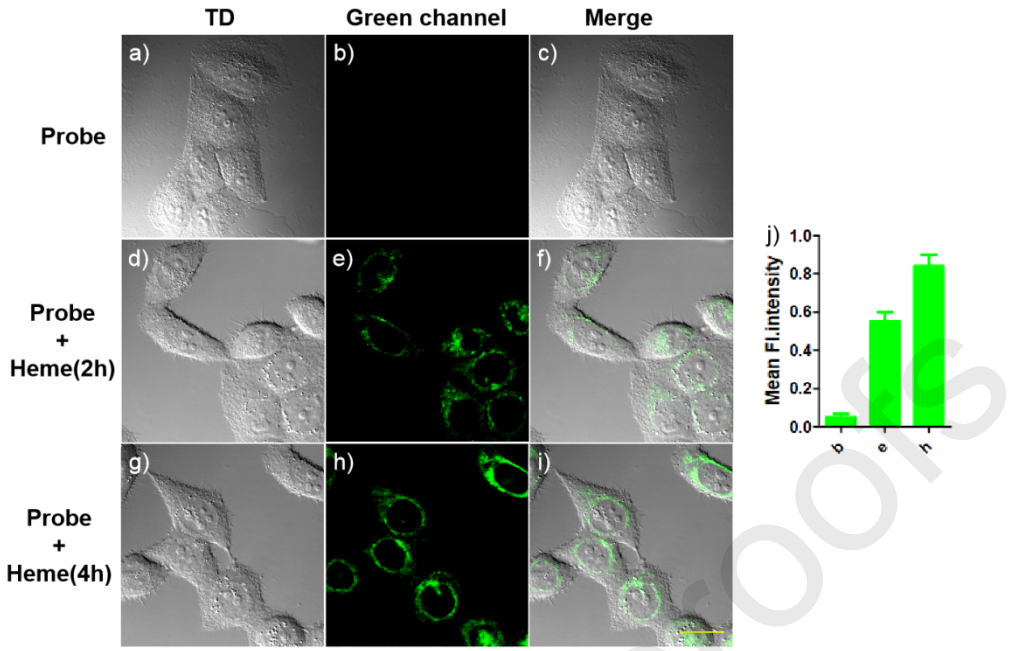


Fig. 6. Fluorescent images of probe **Na-CM-ER** in living HeLa cells for detecting endogenous CO. (a-c) Cells were treated with **Na-CM-ER** (10 μM) for 30 min; (d-f) Cells were firstly treated with heme (100 μM) for 2 h, and then further incubated with **Na-CM-ER** (10 μM) for another 30 min; (g-i) Cells were firstly treated with heme (100 μM) for 4 h, and further incubated with **Na-CM-ER** (10 μM) for another 30 min. (j) Quantified mean fluorescence intensities of cells in the green channel. λ_{ex} = 405 nm, λ_{em} = 500-550 nm. Scale bar = 20 μm.

Inspired by successful results in imaging endogenous CO using **Na-CM-ER**, we further performed co-localization experiments to determine the distribution of **Na-CM-ER** in cells. Firstly, cells were incubated with 10 μM **Na-CM-ER** for 30 min, then CORM-2 (40 μM) was added into cells and further incubated for another 30 min. After that, ER-Tracker Red (a commercial ER indicator) was put into media for incubation with 10 min according to protocols. The images in Fig.7 showed that the fluorescence of ER-Tracker Red in the red channel overlapped well with the fluorescence of **Na-CM-ER** in the green channel. Moreover, the Pearson's correlation coefficient between them was calculated to be 0.97. Therefore, the results of the co-localization imaging show that **Na-CM-ER** has a property for anchoring endoplasmic reticulum in living cells, and can detect CO in endoplasmic reticulum.

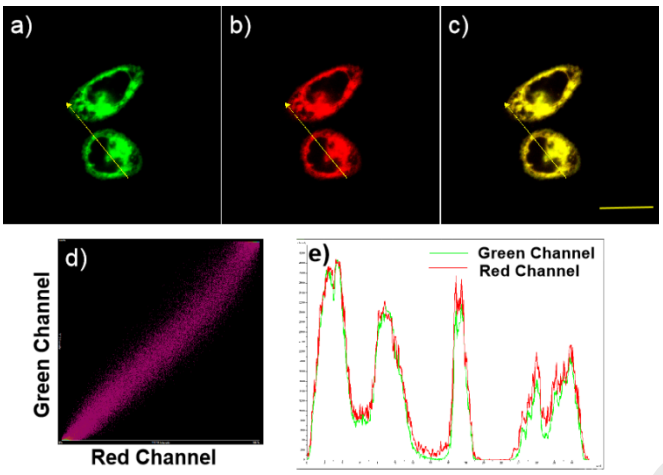


Fig. 7. Co-localization imaging between probe **Na-CM-ER** and ER-Tracker Red. a) Fluorescence imaging of cells incubated with **Na-CM-ER** (10 μM) for 30 min, then further loaded with CORM-2 (40 μM) for another 30 min in green channel; b) Fluorescence imaging of cells treated with ER-tracker Red in red channel; c) The merged imaging of a and b; d) Intensity scatter plot of the green and red channels; e) Intensity profile changes of red channel of ER-Tracker Red and green channel of probe. The green channel: λ_{ex} = 405 nm, λ_{em}= 500-550 nm; the red channel: λ_{ex} = 561 nm, λ_{em}= 570-620 nm. Scale bar: 20 μm.

4. Conclusions

In conclusion, we have rationally designed and synthesized the first ER-targeted turn-on CO fluorescent probe, named as **Na-CM-ER**. Based on the nitro reduction, the probe **Na-CM-ER** emits a strong fluorescence after interacting with CO. **Na-CM-ER** possesses high sensitivity and selectivity to CO during the spectrum testing. Furthermore, **Na-CM-ER** has the ability to detect exogenous and endogenous CO in living cells. Importantly, **Na-CM-ER** can effectively accumulate in cellular ER verified by the co-localization imaging experiments. Taken together, **Na-CM-ER** is a powerful molecule tracking reagent for imaging CO, especially CO in ER, and will play vital roles in further exploring the physiological and pathological functions related to ER CO.

Acknowledgements

This work was financially supported by NSFC (21672083, 21877048, 21807039, 22077048), Taishan Scholar Foundation (TS 201511041) and the startup fund of University of Jinan (309-10004, 160100137)

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

[1] L. D. Prockop, R. I. Chichkova, Carbon monoxide intoxication: an updated review, J. Neurol. Sci. 262 (2007) 122-130.

[2] L. K. Weaver, Clinical practice. Carbon monoxide poisoning, N. Engl. J. Med. 360 (2009) 1217-1225.

[3] J. M. Fukuto, S. J. Carrington, D. J. Tantillo, J.G. Harrison, L.J. Ignarro, B.A.Freeman, A. Chen, D.A.Wink, Small molecule signaling agents: the integrated chemistry and biochemistry of nitrogen oxides, oxides of carbon, dioxygen, hydrogen sulfide, and their derived species, Chem. Res. Toxicol. 25 (2012) 769-793.

[4] Y. Han, J. Qin, X. Chang, Z. Yang, J. Du, Hydrogen sulfide and carbon monoxide are in synergy with each other in the pathogenesis of recurrent febrile seizures, Cell. Mol. Neurobiol. 26 (2006) 101-107.

[5] R. Motterlini, L. E. Otterbein, The therapeutic potential of carbon monoxide, Nat. Rev. Drug Discovery 9 (2010) 728-743.

[6] L. Wu, R. Wang, Carbon Monoxide: Endogenous production, physiological functions, and pharmacological applications, Pharmacol. Rev. 57 (2005) 585-630.

[7] S. H. Heinemann, T. Hoshi, M. Westerhausen, A. Schiller, Carbon monoxide -physiology, detection and controlled release, Chem. Commun. 50 (2014) 3644-3660.

[8] I. T. Lee, S.F. Luo, C.W. Lee, et al., Overexpression of HO-1 protects against TNF-α-mediated airway inflammation by down-regulation of TNFR1-dependent oxidative stress, Am. J. Pathol. 175 (2009) 519-532.

[9] S. W. Ryter, A. M. K. Choi, Targeting heme oxygenase-1 and carbon monoxide for therapeutic modulation of inflammation, Transl. Res. 167 (2016) 7-34.

[10] J. Marhenke, K. Trevino, C. Works, The chemistry, biology and design of photochemical CO releasing molecules and the efforts to detect CO for biological applications, Coord. Chem. Rev. 306 (2016) 533-543.

[11] L. Yuan, W. Lin, L.Tan, K. Zheng, W. Huang, Lighting up carbon monoxide: fluorescent probes for monitoring CO in living cells, Angew. Chem. Int. Ed. 52 (2013) 1628-1630.

[12] P. Quinn, G. Griffiths, G. Warren, Density of newly synthesized plasma membrane proteins in intracellular membranes II. Biochemical studies, J. Cell Biol. 98 (1984) 2142-2147.

[13] S. Carrasco,T. Meyer, STIM proteins and the endoplasmic reticulum-plasma membrane junctions, Annu. Rev. Biochem. 80 (2011) 973-1000.

[14] C.L. Chang, T.S. Hsieh, T. T. Yang, K.G. Rothberg, D.B. Azizoglu, E.Volk, J.C. Liao, J. Liou, Feedback regulation of receptor-induced Ca^{2+} signaling mediated by E-Syt1 and Nir2 at endoplasmic reticulum-plasma membrane munctions, Cell Rep. 5 (2013) 813-825.

[15] M. A. De Matteis, L. R. Rega, Endoplasmic reticulum-Golgi complex membrane contact sites, Curr. Opin. Cell Biol. 35 (2015) 43-50.

[16] J. Chung, D.Y. Shina, M. Zheng, Y. Joe, H.-O. Pae, S. W Ryter, H.-T. Chung, Carbon monoxide, a reaction product of heme oxygenase-1, suppresses theexpression of C-reactive protein by endoplasmic reticulum stress through modulation of the unfolded protein response, Mol. Immunol. 48 (2011) 1793-1799.

[17] M. Zheng, S.K. Kim, Y. Joe, S.H. Back, H.R. Cho, H.P. Kim, L.J. Ignarro, H.T. Chung, Sensing endoplasmic reticulum stress by protein kinase RNA-like endoplasmic reticulum kinase promotes adaptive mitochondrial DNA biogenesis and cell survival via heme oxygenase-1/carbon monoxide activity, FASEB J. 26 (2012)

2558-2568.

[18] K.M. Kim, H.O. Pae, M. Zheng, R. Park, Y.M. Kim, H.T.Chung, Carbon monoxide induces heme oxygenase-1 via activation of protein kinase R-like endoplasmic reticulum kinase and inhibits endothelial cell apoptosis triggered by endoplasmic reticulum stress, Circ. Res. 26 (2007) 919-927.

[19] D..Benito-Garagorri, M..Puchberger, K..Mereiter, K..Kirchner, Stereospecific and reversible CO binding at iron pincer complexes, Angew. Chem., Int. Ed. 47 (2008) 9142-9145.

[20] H. Kitagishi, S. Minegishi, A.Yumura, S. Negi, S. Taketani, Y. Amagase, Y. Mizukawa, T. Urushidani, Y. Sugiura, K. Kano, Feedback response to selective depletion of endogenous carbon monoxide in the blood, J. Am. Chem. Soc. 138 (2016) 5417-5425.

[21] S. S. Park, J. Kim, Y. Lee, Improved electrochemical microsensor for the real-time simultaneous analysis of endogenous nitric oxide and carbon monoxide generation, Anal. Chem. 84 (2012) 1792-1796.

[22] K. Liu, X. Kong, Y. Ma, W. Lin, Rational design of a robust fluorescent probe for the detection of endogenous carbon monoxide in living zebrafish embryos and mouse tissue. Angew. Chem. Int. Edit. 56(2017) 13489-13492.

[23] Z. Guo, S. Park, J. Yoon, I. Shin, Recent progress in the development of near-infrared fluorescent probes for bioimaging applications, Chem. Soc. Rev. 43 (2014) 16-29.

[24] J. Li, Y. Liu, Y. Xu, L. Li, W. Huang, Recent advances in the development of NIR-II organic emitters for biomedicine. Coordin. Chem. Rev. 415, (2020) 213318-213331.

[25] L. Tua, Y. Xu, Q. Ouyang, X. Li, Y. Sun, Recent advances on small-molecule fluorophores with emission beyond 1000 nm for better molecular imaging in vivo. Chin. Chem. Lett. 30 (2019) 1731-1737.

[26]Y. Tang, Y. Ma, J. Yin, W. Lin, Strategies for designing organic fluorescent probes for biological imaging of reactive carbonyl species, Chem. Soc. Rev. 48 (2019) 4036-4048.

[27] A. M. Tafesh, J. Weiguny, A review of the selective catalytic reduction of aromatic nitro compounds into aromatic amines, isocyanates, carbamates, and ureas using CO, Chem. Rev. 96 (1996) 2035-2052.

[28] Y. Tang, Y. Ma, A. Xu, G. Xu, W. Lin, A turn-on fluorescent probe for endogenous formaldehyde in the endoplasmic reticulum of living cells, Methods Appl. Fluoresc. 5 (2017) 024005-024012.

[29] K.R. Wang, F. Qian, X.M. Wang, X.-M. Wang，G.-H. Tan，R.-X. Rong，Z.-R. Cao，H. Chen，P.-Z. Zhang，X.-L. Li, Cytotoxic activity and DNA binding of naphthalimide derivatives with amino acid and dichloroacetamide functionalizations, Chin. Chem. Lett. 25 (2014) 1087-1093.

[30] X. Tian, X. Liu, A. Wang, C. Lau, J. Lu, Bioluminescence imaging of carbon monoxide in living cells and nude mice based on Pd^0-mediated Tsuji-Trost reaction, Anal. Chem. 90 (2018) 5951-5958.

[31] W. Feng, D. Liu, S. Feng, G. Feng, A readily available fluorescent probe for carbon monoxide imaging in living cells, Anal. Chem. 88 (2016) 10648-10653.

Biographies

Yunyan Zhang obtained her B.S. degree at University of Jinan in 2018. Currently, she is a graduate student in the Institute of Fluorescent Probes for Biological Imaging, University of Jinan, and her supervisor is Professor Weiying Lin. Her research interests focus on the design and synthesis of novel functional fluorescent probes and their biological applications.

Yonghe Tang got his doctor's degree at University of Jinan in 2019. Now, he is a lecturer in the Institute of Optical Materials and Chemical Biology, School of Chemistry and Chemical Engineering, Guangxi University. His research interests mainly include the design and synthesis of fluorescent probes.

Xiuqi Kong got her doctor's degree at Nankai University in 2015. Now, she is a lecturer in the Institute of Fluorescent Probes for Biological Imaging, University of Jinan. Her research interests mainly including the design, synthesis and biological applications of fluorescent probes.

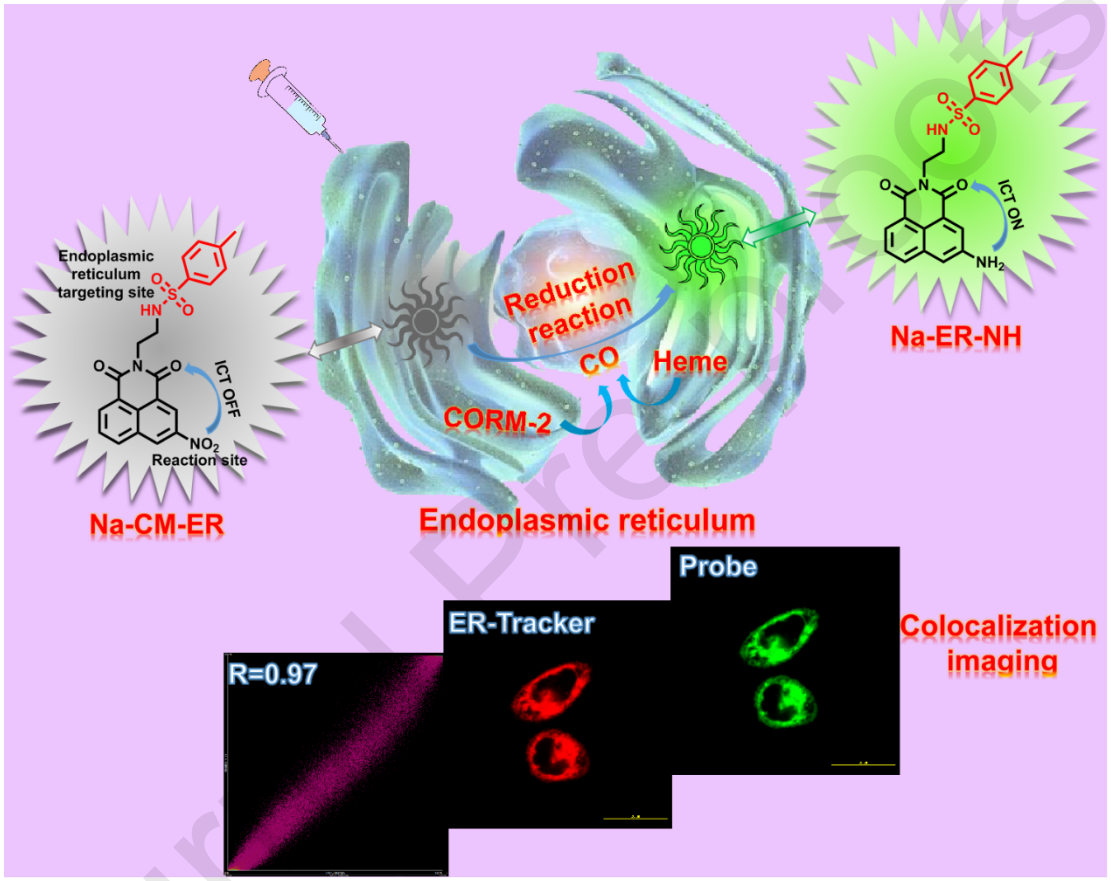
Professor Weiying Lin received his Ph.D. from the University of Kansas in 2000. After completing postdoctoral research at Massachusetts Institute of Technology, in 2005, he joined the faculty at Hunan University. Subsequently, he moved to the University of Jinan as the Dean and the Distinguished Professor of the Institute of Fluorescent Probes for Biological Imaging. His research interests cover the interdisciplinary areas of molecular recognition, photochemistry, analytical chemistry, and chemical biology.

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Graphic Abstract



The first endoplasmic reticulum-targeted fluorescent probe (**Na-CM-ER**) was designed and synthesized for monitoring carbon monoxide (CO) in endoplasmic reticulum of the living cells.

Highlights

1) The probe **Na-CM-ER** is the first endoplasmic reticulum-targeted fluorescent probe for CO.
2) The probe **Na-CM-ER** shows high selectivity and sensitivity to CO.
3) The probe **Na-CM-ER** is applied for sensing exogenous/endogenous CO in the living cells.
4) The probe **Na-CM-ER** is successfully employed to monitor CO in subcellular endoplasmic reticulum.