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Heat Stroke in Cells Tissues related Sulfur Dioxide level Precisely Monitored by Light-controlled Fluorescent Probes

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ABSTRACT: Heat stroke (HS)can cause serious organism damage or even death. Early understanding of the mechanism of heat cytotoxicity can prevent or treat heat stroke related diseases. In this work, probe **Ly-NT-SP** was synthesized, characterized and used for sulfur dioxide (SO_2) detection in lysosomes. PBS solutions of probe **Ly-NT-SP** at pH 5.0 presents a marked broad emission band in the green zone (535 nm). After UV irradiation the spiropyran group in **Ly-NT-SP** isomerizes to the merocyanine form (**Ly-NT-MR**) which presented a weak red-shifted emission at 630 nm. Besides, photo-controlled isomerization of **Ly-NT-SP** to **Ly-NT-MR** generated a C=C─C=N^+ fragment able to react, through a Michael addition, with SO_2 yielding a highly emissive adduct with a marked fluorescence in the green channel (535 nm). *In vitro* studies showed a remarkable selectivity of photo-activated **Ly-NT-MR** to SO_2 with a limit of detection as low as 4.7 μM. Besides, MTT viability assays demonstrated that the **Ly-NT-SP** is non-toxicity to HeLa cells and can be used to detect SO_2 in lysosomes. Taking advantage of this, the sensor is successfully applied to image increasing SO_2 values in lysosomes during heat shock for the first time. Moreover, we also confirmed that the increased SO_2 can protect the small intestine against damage induced by heat shock through regulating oxidative stress in cells and mice.

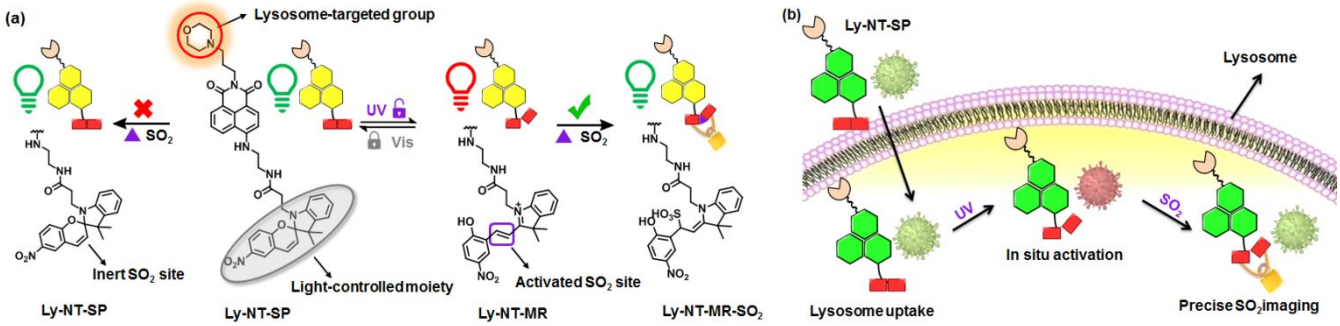
INTRODUCTION

Fluorescent imaging offers great opportunities to monitor and analyze disease-related bio-molecules in live system with a high spatiotemporal resolution.[1-6] Comparing with the traditional used fluorophores, light responsive dyes encountere their best ages because of their diverse physical and chemical properties changes upon light irradiation.[7-9] Spiropyran, as one species of light responsive dyes featuring reversibly switch between two isomer states with distinct fluorescent signals changes.[10-12] Their readily modified structures bring various derivatives as fluorescent probes to detect a various of analytes. Specifically, the merocyanine form of spiropyran hold polarized unsaturated C=C, caged in the spiropyran form, which could act as nucleophilic reaction site for nucleophilic reagent such as sulfur dioxide (SO_2).[13-14] The above unit constructed probes permit artificially activation *in situ* during their bio-labeling and imaging applications which promoted them as supra candidate to be designed organelle targetable probes being able to eliminate the false signals output before they accumulated in organelle. Additionally, the light-control over photochromic probes is mild and non-invasive with high spatiotemporal resolution.

Recently, our group reported a light-controlled fluorescent probe for highly selective response of SO_2 based on spiropyran group and accurate detection of SO_2 in complex biological systems.[14] Thus; it will provide an effective tool to study the physiology role of SO_2 in living system Among all kinds of diseases, there is an unimpressive one heat stroke (HS) as a heat-related pathology which invokes a series of cellular responses, including metabolic disorders, inflammatory responses, and apoptosis.[15-16] Recently studies have shown that heat stroke is closely related to central nervous system dysfunction, which always results in a higher mortality rate.[17-19] Besides, it has been also evidenced that small intestine damage caused by heat stroke is a major factor resulting in heat stroke-related morbidity and mortality.[20] In spite of these facts, however the molecular and organelle behaviors during a heat shock process is still poorly understood.[21] Lysosomes, as one of the vital organelles, store a large number of enzymes to regulate cell homeostasis and thus protect cells from stress.[22-26] In fact, SO_2 as an antioxidant plays crucial roles in maintaining the cell homeostasis or inducing cell apoptosis.[27-28] Abnormal SO_2 will affect the amount and activities of lysosomal enzymes in macrophages, which will further reduce impair local immune function.[29-30] A question worth considering is the relationship between lysosome temperature and SO_2 fluctuation? Thus it is highly desirable for researchers to develop effective and reliable tools to study the relevance between SO_2 level and lysosome temperature in lysosomes.

In forward-moving work, we found an effective sensing strategy based on spiropyran group as a specific light-controlled site to more efficiently target lysosomes SO_2 compared with other no light-controlled sensors. The detailed sensing strategy was described in Scheme 1. Lysosomes usually displayed an acidic environment of pH 3.8–5.0.[31-33] Morpholine moiety of **Ly-NT-SP** was easily protonated, and once probe **Ly-NT-SP** entered cells, it would accumulate in the lysosome. However, it could not response to SO_2 due to the sensing activity controlled by

Scheme 1. (a) Molecular design of Ly-NT-SP and proposed sensing mechanism towards SO_2. (b) Schematic illustration of lysosome-target Ly-NT-SP and in suit response of SO_2 in controlled by UV irradiation.



UV irradiation. After UV irradiation, the structure of spiropyran (SP) can be reversibly switched to the merocyanine (MR) state. And a "red-shift" fluorescence response from green fluorescence to red fluorescence could be observed owning to Förster resonance energy transfer (FRET) process from naphthalimide donor to the MR acceptor. In particular, the UV-activated MR state contains a special C=C double bonds, which was a proposed recognition site and attacked by SO_2 *in situ*. In this regard, this *in situ* UV-activated strategy would allow us accurately monitor SO_2 in lysosomal with ratiometric manners, which largely avoided the interference of false positive signals during the transit of probes in cells. Furthermore, taking advantage of this, we showed for the first time that the lysosome SO_2 rises with the increase of temperature. Moreover, tissue imaging suggested that SO_2 could protect small intestinal injury against heat shock. This might have a guiding significance to deeply study the pathologic pathway of certain diseases and cause of death initiated by heat stroke.

RESULTS AND DISCUSSION

Photochemical Properties of Ly-NT-SP in PBS Buffer Solution. We initially investigated the basic chemical and photochemical features of probe **Ly-NT-SP** upon UV/Vis irradiations in buffer solution. Since the lysosomal pH value is ranging from 3.8 to 5.5, all these spectra measurement was carried out at pH 5.0 in this study. As could be seen in Figure 1a, probe **Ly-NT-SP** displayed maximum emission at 535 nm in PBS. Notably, we can find that the emission maxima at 535 nm shifted to 630 nm with UV irradiation for 120 s. Synchronously, under UV irradiation, a new band at 540 nm appeared and its intensity growth progressively with time yielding the maximum absorbance after 120 s (Figure 1b). Importantly, probe shows good reversibility between UV and Vis light controlled absorbance (Figure 1c) and fluorescence switch (Figure 1d). These observed changes are ascribed to the transformation of the spiropyran conformation of probe **Ly-NT-SP** to the merocyanine form (**Ly-NT-MR**). As a consequence a FRET process from naphthalimide donor to the merocyanine acceptor is active which account for the observed emission band shifts. And the FRET efficiency of Ly-NT-MR was calculated to be 68.5% (E=1-F_{DA}/F_D, Figure S1).[34]

SO_2 detection using Ly-NT-SP probe. After we assessed the UV light controlled transformation of probe **Ly-NT-SP** to **Ly-NT-MR**, then we tested the fluorescence emission and UV-visible behaviours of the probe **Ly-NT-SP** in the presence of SO_2 after exposure to UV light. Upon the addition of Na_2SO_3 (as a SO_2 donor), the emission band of the probe at 630 nm shifts forward to 535 nm (Figure 1e), and the absorption band of **Ly-NT-MR** at 540 nm gradually decreased concomitantly (Figure 1f). Moreover, ESI-MS of the final product exhibited a peak at m/z = 849.2901 corresponding to [**Ly-NT-MR**-SO_3^{2-} + H]$^+$ (Figure S2). Thus confirming that the Michael addition of SO_2 to **Ly-NT-MR** probe contributing to the observed optical changes. Besides, the plot of the fluorescence intensity ratios between the two emissions at 630 nm and 535 nm (F_{630}/F_{535}) of the system exhibited a linear relationship with the increased concentration of Na_2SO_3 (from 0 to 50 μM). Determined from 10 blank solutions, the detection limit of **Ly-NT-MR** toward SO_2 was calculated to be 4.7 μM based on IUPAC (CDL = $3S_b/m$) (Figure S3).[35]

To evaluate the selective of probe, some representative analytes such as thiols (Cys, Hcy, GSH and NAC) and common biomolecules (SO_4^{2-}, SCN^-, F^-, Cl^-, Br^-, I^-, AcO^-, NO_2^-, N_3^-, formaldehyde) etc. were treated with **Ly-NT-SP** under pH 5.0. It was found that **Ly-NT-SP** showed negligible fluorescence response on other tested species (see Figure S4). By contrast, significant fluorescence intensity ratio change was observed in the presence of Na_2SO_3, indicating that **Ly-NT-SP** showed high selectivity for SO_2 over bio-relevant species. Afterward, the time-dependent fluorescence emission change of **Ly-NT-SP** with Na_2SO_3 was explored. The fluorescence signal at 535 nm increased and reached a maximum equilibrium within 5 min (Figure S5), indicating that the MR state exhibit highly reactivity towards SO_2 derivatives.

Subsequently, the pH effect of **Ly-NT-MR** for SO_2 detection was investigated. As shown in Figure S6, **Ly-NT-SP** was silent in a broad pH range (3.0-8.0) after UV irradiation, and an obvious enhancement of ratiometric signals (F_{630}/F_{535}) was achieved in both neutral and weak acid pH range (4.0-7.0). Finally, the influence of temperature (from 37° to 45°C) on the fluorescence intensity ratio of **Ly-NT-MR** was also studied (Figure S7). The results showed that fluorescence ratio (F_{630}/F_{535}) of PBS solutions (10 mM, pH 5) of **Ly-NT-MR** in the absence of Na_2SO_3 remained unchanged when the temperature increased from 37° to 45°C. All the above mentioned results indicated that probe **Ly-NT-SP** was suitable for accurate measurement of SO_2 concentration changes in lysosomes.

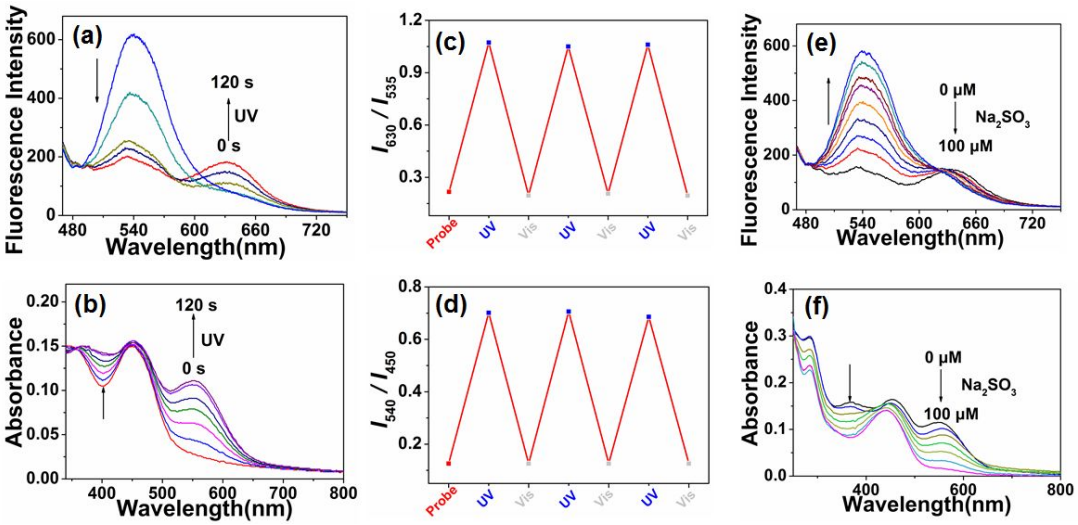


Figure 1. (a) Emission and (b) absorption spectra changes of PBS (10 mM, pH 5) solutions of probe **Ly-NT-SP** (10 μM) after irradiation with UV light for 120 s (for the emission spectra excitation was set at 450 nm). Fluorescence emission (c) and UV-Vis absorbance (d) photo-switching of **Ly-NT-SP** in PBS (10 mM, pH 5). a) Absorption and emission spectra change of **Ly-NT-MR** (10 μM) upon addition of Na_2SO_3 (0-100 μM) in PBS buffer solution (pH = 5.0, 10 mM). λ_{ex} = 450 nm, slit: 5 nm/5 nm.

Imaging of exogenous and endogenous SO_2 in living cells. Firstly, we assessed cytotoxicity of **Ly-NT-SP** via MTT assays using HeLa cell line. MTT assay showed no significant cytotoxicity of **Ly-NT-SP** at the level up to 10 μM (Figure S8). Next, the cell viability was checked under different UV irradiations time (0, 50, 100, 200, 300 s), and these results showed that cells were perfectly viable (>70 %) upon 200 s UV irradiation (Figure S9), indicating a low photo-toxicity of UV irradiation procedure in this experiment.

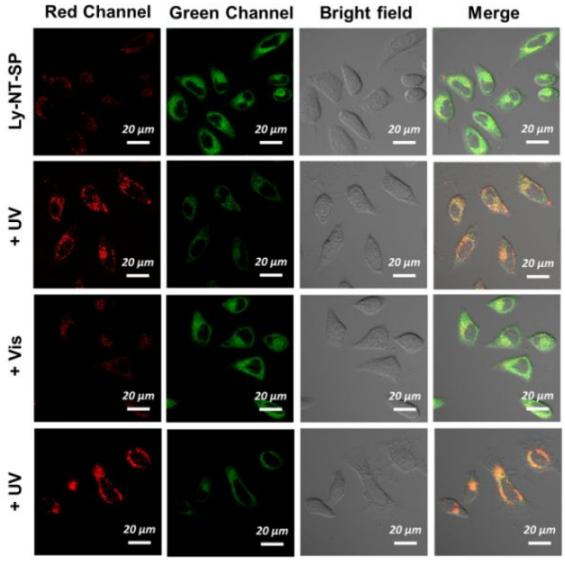


Figure 2. Confocal microscopy images of **Ly-NT-SP** (10 μM) with alternate UV/Vis irradiation. From top to bottom, probe **Ly-NT-SP**, UV irradiation, Vis irradiation, UV irradiation. From left to right, Red channel, λ_{em} = 610 ± 30 nm (λ_{ex} = 561 nm), Green channel, λ_{em} = 530 ± 20 nm (λ_{ex} = 458 nm), Bright field, Overlay images, Scale bar: 20 μm.

Then, we tested the intracellular light-controlled manners of **Ly-NT-SP** in HeLa cells. For this purpose, HeLa cells were incubated with **Ly-NT-SP** (10 μM) for 20 min at 37 °C, then irradiated with UV light (100 s) and with visible light (10 min) consecutively. As expected, HeLa cells treated with **Ly-NT-SP** showed a significant green fluorescence in the green channel ascribed to probe internalization. Meanwhile, negligible emission was observed in the red channel (Figure 2). However, after UV irradiation, the fluorescence of the green channel vanished and a marked red emission appeared in the red channel as a consequence of the transformation of inactive **Ly-NT-SP** to the SO_2-active **Ly-NT-MR**. Then, upon irradiation with visible light, a marked increase in green fluorescence together with a reduction in the red channel was observed. These results clearly validated the light-controlled strategy of probe **Ly-NT-SP** for intracellular photochromic actions.

Having demonstrated the favorable optical performances of **Ly-NT-SP** in living cells, the ability of the **Ly-NT-SP** for imaging lysosomes SO_2 was carried out in HeLa cells. To investigate the lysosome-targeting ability of **Ly-NT-SP** in living cells, the co-localization study of **Ly-NT-SP** with commercial available lysosome red (Lyso-Tracker Red) was conducted in HeLa cells. HeLa cells was incubated with 10 μM **Ly-NT-SP** at 37 °C for 20 min, and further incubated with 500 nM Lyso-Tracker Red for another 20 min. As shown in Figure 3, both the green and red channels displayed strong emission, and the fluorescence images overlapped well (Figure S10) with high Pearson's colocalization 0.82. These results showed that **Ly-NT-SP** can well accumulated in lysosomes of living cells.

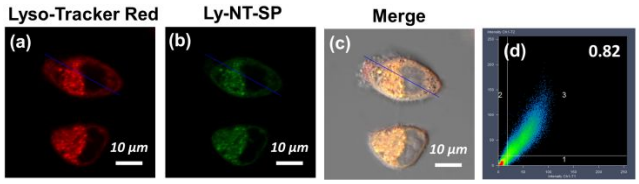


Figure 3. Confocal microscopy images of **Ly-NT-SP** (10 μM) and Lyso-Tracker in living cells. (a) Confocal microscopy image of Lyso-

Tracker in the red channel. (b) Confocal microscopy image of **Ly-NT-SP** in the green channel. (c) Overlay image. (d) The correlation of Lyso-Tracker and **Ly-NT-SP** intensities. Red channel, λ_{em} = 610 ± 30 nm (λ_{ex} = 561 nm), Green channel, λ_{em} = 530 ± 20 nm (λ_{ex} = 458 nm), Scale bars = 10 μm.

Furthermore, we investigated the sensing performance of probe **Ly-NT-SP** for exogenous and endogenous SO_2 detection in living cells. HeLa cells were pretreated with **Ly-NT-SP** for 15 min, and then further treated with UV irradiation for two minutes. As shown in Figure 4a, strong red fluorescence was observed in red channel, while negligible signal captured in green channel after UV irradiation. By contrast, after addition of exogenous SO_2 (Na_2SO_3), the fluorescence signals in green channel largely increased along with decrease red fluorescence in the red channel. To evaluate the ability of **Ly-NT-SP** for endogenous SO_2 detection, HeLa cells were pretreated with 1 μg/mL lipopolysaccharide (LPS), which can induce the inflammatory response of cells to produce low-level sulfite endogenously.[13] In comparison with cells treated with **Ly-NT-SP** and irradiated with UV light, LPS pretreated HeLa cells exhibited strong green fluorescence accompanied with relative weak red fluorescence (Figure 4b). Taken together, probe **Ly-NT-SP** was capable of tracking both exogenous and endogenous SO_2 in living cells.

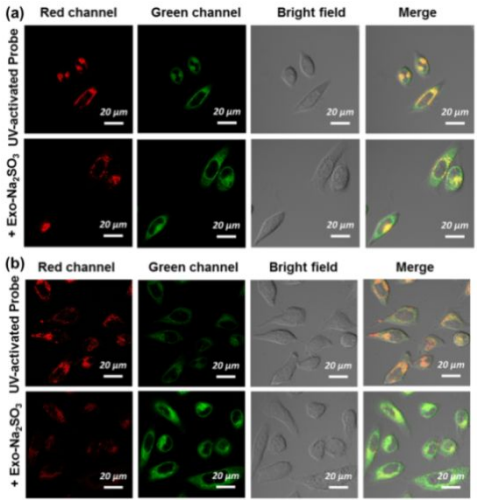


Figure 4. (a) Confocal microscopy images of **Ly-NT-MR** with exogenous Na_2SO_3 (50 μM). (b) Confocal microscopy images of **Ly-NT-MR** with endogenous Na_2SO_3 (pretreating the cells with 1 μg/mL lipopolysaccharide). Red channel, λ_{em} = 610 ± 30 nm (λ_{ex} = 561 nm), green channel, λ_{em} = 530 ± 20 nm (λ_{ex} = 458 nm), bright field, overlapped images, ratio images. Scale bar: 20 μm.

Next, as a proof of concept, we tested the ability of probe **Ly-NT-SP** for imaging of SO_2 concentration changes in HeLa cells under heat shock. 39 °C, 41 °C and 43 °C were used as model temperatures of heat stroke, whereas 37 °C was used as control group. HeLa cells were firstly incubated with **Ly-NT-SP** (10 μM) for 20 min at 37°C and then irradiated with UV light. Then, in a second step, cells were further incubated at 37, 39, 41, and 43 °C for another 30 min and confocal microscopy images were finally taken. As could be seen in Figure 5a, upon temperature increase the red emission markedly decreased whereas the in the green channel a moderate fluorescence enhancement was observed. Also the green-to-red emission ratio increased with temperature (Figure 5b). The marked emission changes (reduction and enhancement in the red and green channel respectively) are in agreement with the isomerization of **Ly-NT-SP** to the merocyanine form **Ly-NT-MR** and subsequent reaction with lysosome SO_2. For the control experiment, FA (formaldehyde), which known as an inhibitor of bisulfite, was used for SO_2 imaging during heat shock.[36] **Ly-NT-SP** located HeLa cells was incubated with FA (200 μM) for 20 min at 37 °C, and then irradiated with UV light and further incubated at 43 °C for another 30 min. As expected, the presence of FA enhanced the fluorescent signals in the red channel, and the green-to-red emission ratio was significantly reduced. To investigate the role of SO_2 in heat shock, **Ly-NT-SP** located HeLa cells were pretreated with NAC (N-acetyl-L-cysteine, a well-known antioxidant, 200 μM), and then cultured at 43 °C for another 30 min. Conspicuously, after NAC treatment, the expression levels of SO_2 was down-regulated. Possible reasons are as follows; HS-treated cells can induce the over-generation of intracellular reactive oxygen species (ROS), while the presence of NAC can significantly inhibit ROS generation. As a result, the oxidative stress-dependent increased of SO_2 was suppressed. These results pointed to a direct relationship between cell temperature and SO_2 concentration in lysosomes, strongly suggested that SO_2 plays a crucial role as an antioxidant to regulating redox homoeostasis, and up-regulation of SO_2 in the lysosomes was dependent on the oxidative stress induced by heat shock.

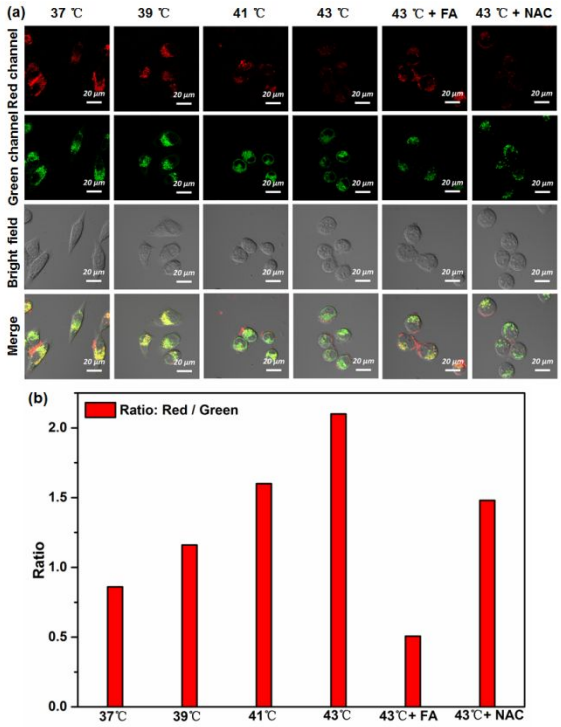


Figure 5. (a) Confocal microscopy images of HeLa cells incubated with **Ly-NT-MR** (10 μM), **Ly-NT-MR** (10 μM) with FA (200 μM) and **Ly-NT-MR** (10 μM) with NAC (200 μM) at different temperatures (37°, 39°, 41° and 43°C) after UV irradiation. Red channel, λ_{em} = 610 ± 30 nm (λ_{ex} = 561 nm), green channel, λ_{em} = 530 ± 20 nm (λ_{ex} = 458 nm), bright field, overlapped images. Scale bar: 20 μm. (b) Ratio of green/red fluorescence intensity at different temperatures.

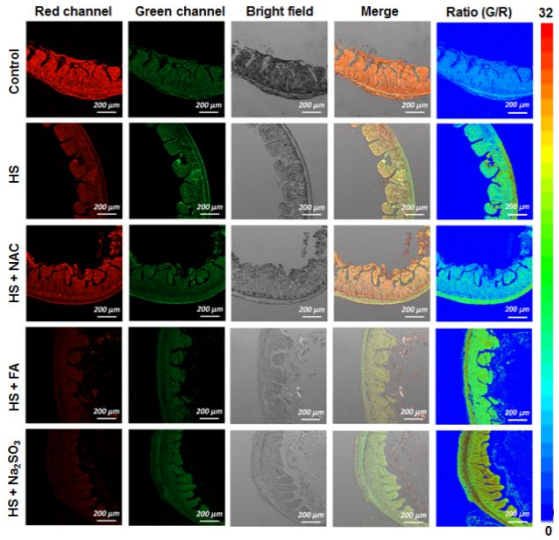


Figure 6. Confocal microscopy images of SO_2 in HS pre-treated intestinal tissues. From top to bottom: control (normal), HS, HS + NAC, HS + HCHO, HS + Na2SO3 and ratio Green/Red. Red channel, λ_{em} = 610 ± 30 nm (λ_{ex} = 561 nm), green channel, λ_{em} = 530±20 nm (λ_{ex} = 458 nm), Scale bar: 200 μm.

Heat stress-related oxidative stress has been shown to cause small intestines damage of both rats and pigs.[37-38] Thus, we further examined the level of SO_2 change as well as theantioxidant effect of SO_2 in heats stroke effected mice. HS-induced small intestines injury was modeled according to previously reported methods as described in supporting information. Pathogen-free 6 to 8 week old male BALB/c mice were randomly assigned to five experimental groups, control, HS, HS + NAC, HS + HCHO and HS + Na_2SO_3. Firstly, the UV irradiation for converting **Ly-NT-SP** to the **Ly-NT-MR** was tested in intestinal tissues. As shown in Figure S11, the ileum slice incubated with **Ly-NT-SP** (20 μM) displayed slight red emission in the red channel. Then, the tissue was irradiated with UV light in darkroom for 100 s. After that, we observed a clearly increased red fluorescence in the red channel, and the green fluorescence decreased simultaneously. These results suggested the transformed **Ly-NT-MR** could potentially response to SO_2 in organ tissues. Then HS group pre-treated ileum slices were incubated with **Ly-NT-SP** (20 μM) for 30 min at 37 °C, and exposed to UV light for 3 min, followed by culturing another 30 min at 37 °C. As expected, compared with control group (first row of images in Figure 6), remarkable reduction in the red channel together with an increased fluorescent signals in the green fluorescence was noticed in HS treated intestines tissues (second row of images in Figure 6). And the emission ratio (green/red) of HS-treated mice increase by 4.1-fold compared with control group (Figure S12). However, due to fact that suppressed of SO_2 generation, the fluorescent ratio of NAC and FA pre-treated mice only increased 1.85-fold and 2.78-flod compared with control group, respectively (Figure S12. As for Na_2SO_3 treated mice, the obtained tissues images showed significant fluorescence enhancement in green channel, and the image from red channel showed rather weak fluorescent signals (last row of images in Figure 6). Besides, compared with control group, we can notice that the injection of Na_2SO_3 (100 mg/kg) triggered most obvious fluorescent ratio changes up to 4.6-fold (Figure S12). These results indicated that heat stroke induced SO_2 upregulation in small intestines of mice, which confirming the advantage of **Ly-NT-SP** for precise SO_2 imaging. Apart from this, reduced heat stroke-induced intestinal damage was observed in Na_2SO_3 and NAC pre-treat mice. By contrast, marked intestinal damage such as villous stroma broadening and focal necrosis was noticed in HS and HS + HCHO treated mice. These results suggested the increased SO_2 could largely reduce heat stroke-induced intestinal damage by defense of over-generated ROS.

CONCLUSIONS

In summary, we have successfully developed a lysosome-targeted ratiometric fluorescent probe for the evaluation of changes in the SO_2 concentration in lysosomes. **Ly-NT-SP** probe displays excellent lysosome-targeting ability and is able to detect SO_2 upon UV irradiation. This UV-activation enables precision imaging of SO_2 in a spatial and temporal manner. Furthermore, **Ly-NT-SP** was successfully used for the visualization of exogenous and endogenous SO_2 in HeLa cells. Additionally, **Ly-NT-SP** is used for the first time to tack lysosomal SO_2 changes during heat shock. What's more, cell and intestinal tissue imaging studies have clearly revealed the elevation of endogenous SO_2 play an important role in regulating oxidative stress, and increased SO_2 could protect small intestine from heat stroke induced damage. We believe that the present studies may fuel the development of targeting photo-actable probes for the detection of small molecules, such as SO_2, which might related to certain pathologies and treatments as for instance those related with heat shock.

ASSOCIATED CONTENT

Supporting Information

Generally information; experimental section; additional photophysical spectra; NMR and ESI-MS spectra.

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Author Contributions

§ Weijie Zhang and Fangjun Huo contributed equally.

Notes

The authors declare no competing financial interest.

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REFERENCES

(1) Li, H. Y.; Shi, W.; Li, X. H.; Hu, Y. M.; Fang, Y.; Ma, H. M. Ferroptosis accompanied by •OH generation and cytoplasmic viscosity increase revealed via dual-functional fluorescence probe. *J. Am. Chem. Soc.* **2019**, *141*, 18301-18307.

(2) Chen, Z. Y.; Mu, X. L.; Han, Z.; Yang, S. P.; Zhang, C. L.; Guo, Z. J.; Bai, Y.; He, W. J. An optical/photoacoustic dual-modality probe: ratiometric in/ex vivo imaging for stimulated H_2S upregulation in mice. *J. Am. Chem. Soc.* **2019**, *141*, 17973-17977.

(3) Chan, J.; Dodani, S. C.; Chang, C. J. Reaction-based small-molecule fluorescent probes for chemoselective bioimaging. *Nat. Chem.* **2012**, *4*, 973-984.

(4) Stich, M. I. J.; Fischer, L. H.; Wolfbeis, O. S. Multiple fluorescent chemical sensing and imaging. *Chem. Soc. Rev.* **2010**, *39*, 3102-3114.

(5) Sun, W.; Guo, S. G.; Hu, C.; Fan, J. L.; Peng, X. J. Recent development of chemosensors based on cyanine platforms. *Chem. Rev.* **2016**, *16*, 7768-7817.

(6) Li, M. L.; Xia, J.; Tian, R. S.; Wang, J. Y.; Fan, J. L.; Du, J. J.; Long, S. R.; Song, X. Z.; Foley, J. W.; Peng, X. J. Near-infrared light-initiated molecular superoxide radical generator: rejuvenating photodynamic therapy against hypoxic tumors. *J. Am. Chem. Soc.* **2018**, *140*, 14851-14859.

(7) Szymanski, V.; Beierle, J. M.; Kistemaker, H. A. V.; Velema, W. A.; Feringa, B. L. Reversible photocontrol of biological systems by the incorporation of molecular photoswitches. *Chem. Rev.* **2013**, *113*, 6114-6178.

(8) Shao, Q.; Xing, B. Photoactive molecules for applications in molecular imaging and cell biology. *Chem. Soc. Rev.* **2010**, *39*, 2835-2846.

(9) Zou, Y.; Xiao, S. Z.; Li, F. Y.; Li, C. Y.; Gao, X.; Wu, J. C.; Yu, M. X.; Huang, C. H. Amphiphilic diarylethene as a photoswitchable probe for imaging living cells. *J. Am. Chem. Soc.* **2008**, *130*, 15750-15751.

(10) Klajn, R. Spiropyran-based dynamic materials, *Chem. Soc. Rev.* **2014**, *43*, 148-184.

(11) Samanta, D.; Galaktionova, D.; Gemen, J.; Shimon, L. J. W.; Diskin-Posner, Y.; Avram, L.; Kral, P.; Klajn, R. Reversible chromism of spiropyran in the cavity of a flexible coordination cage. *Nat. Commun.* **2018**, *9*, 641-650.

(12) Zou, Y.; Xiao, S. Z.; Li, F. Y.; Li, C. Y.; Gao, X.; Wu, J. C.; Yu, M. X.; Huang, C. H. Amphiphilic diarylethene as a photoswitchable probe for imaging living cells. *J. Am. Chem. Soc.* **2008**, *130*, 15750-15751.

(13)Zhang, J. J.; Fu, Y. X.; Han, H. H.; Zang, Y.; Li, J.; He, X. P.; Feringa, B. L.; Tian, H. Remote light-controlled intracellular target recognition by photochromic fluorescent glycoprobes. *Nat. Commun.* **2017**, *8*, 987-996.

(14) Zhang, W. J.; Huo, F. J.; Yin, C. X. Photocontrolled single-/dual-site alternative fluorescence probes distinguishing detection of H_2S/SO_2 in vivo. *Org. Lett.* **2019**, *21*, 5277-5280.

(15) Patz, J. A.; Campbell-Lendrum, D.; Holloway, T.; Foley, J. A. Impact of regional climate change on human health. *Nature* **2005**, *438*, 310-317.

(16) Wen, Y.; Zhang, W. J.; Liu, T.; Huo, F. J.; Yin, C. X. Pinpoint diagnostic kit for heat stroke by monitoring lysosomal pH. *Anal. Chem.* **2017**, *89*, 11869-11874.

(17) Argaud, L.; Ferry, T.; Le, Q. H. Short- and Long-term Outcomes of Heatstroke Following the 2003 Heat Wave in Lyon, France. *Arch. Intern. Med.* **2007**, *167*, 2177-2183.

(18) Epstein, Y.; Yanovich, R. Heatstroke. *N. Engl. J. Med.* **2019**, *380*, 2449-2459.

(19) Bouchama. A.; Knochel, J. P. Heat stroke. *N. Engl. J. Med.* **2002**, *346*, 1978-1988.

(20) Yi, G.; Li, L.; Luo, M.J.; He, X.; Zou, Z.M.; Gu, Z.T.; Su, L. Heat stress induces intestinal injury through lysosome- and mitochondria-dependent pathway in vivo and in vitro. *Oncotarget*, **2017**, *8*, 40741-40755.

(21) Kourtis, N.; Nikoletopoulou, V.; Tavernarakis, N. Small heat-shock proteins protect from heat-stroke-associated neurodegeneration. *Nature* **2012**, *490*, 213-218.

(22) Yue, Y. K.; Huo, F. J.; Yue, P.; Meng, X. M.; Salamanca, J. C.; Escobedo, J. O.; Strongin, M. R. Yin, C. X. In situ lysosomal cysteine-specific targeting and imaging during dexamethasone-induced apoptosis. *Anal. Chem.* **2018**, *90*, 7018-7024,

(23) Casey, J. R.; Grinstein, S.; Orlowski, J. Sensors and regulators of intracellular pH. *Nat. Rev. Mol. Cell Biol.* **2010**, *11*, 50-61.

(24) Balce, D. R.; Allan, E. R. O.; McKenna, N.; Yates, R. M. γ-Interferon-inducible lysosomal thiol reductase (GILT) maintains phagosomal proteolysis in alternatively activated macrophages. *J. Biol. Chem.* **2014**, *289*, 31891-31904.

(25) Wu, L.; Li, X.; Ling, Y. C.; Huang, Jia, N. Morpholine derivative-functionalized carbon dots-based fluorescent probe for highly selective lysosomal imaging in living cells. *ACS Appl. Mater. Interfaces* **2017**, *9* (34), 28222-28232.

(26) Settembre, C.; Fraldi, A.; Medina, D. L.; Ballabio, A.; Signals from the lysosome: a control centre for cellular clearance and energy metabolism. *Nat. Rev. Mol. Cell Biol.* **2013**, *14*, 283-296.

(27) Tamima, U.; Santra, M.; Song, C. W.; Reo, Y. J.; Ahn, K. H. A benzopyronin-based two-photon fluorescent probe for ratiometric imaging of lysosomal bisulfite with complete spectral separation. *Anal. Chem.* **2019**, *91*, 10779-10785.

(28) Wu, M. Y.; Wang, Y.; Liu, Y. H.; Yu, X. Q. Dual-site lysosome-targeted fluorescent probe for separate detection of endogenous biothiols and SO_2 in living cells. *J. Mater. Chem. B* **2018**, *6*, 4232-4238.

(29) Wan, Q. Q.; Chen, S. M.; Shi, W.; Li, L. H.; Ma, H. M. Lysosomal pH rise during heat shock monitored by a lysosome-targeting near-infrared ratiometric fluorescent probe. *Angew. Chem. Int. Ed.* **2014**, *53*, 10916-10920.

(30) Wu, L. L.; Wang, Y.; James, T. D.; Jia, N. Q.; Huang, C. A hemicyanine based ratiometric fluorescence probe for mapping lysosomal pH during heat stroke in living cells. *Chem. Commun.* **2018**, *54*, 5518-5521.

(31) Yu, H. B.; Xiao, Y.; Jin, L. J. A lysosome-targetable and two-photon fluorescent probe for monitoring endogenous and exogenous nitric oxide in Living cells. *J. Am. Chem. Soc.* **2012**, *134*, 17486-17489.

(32) Yuan, L.; Wang, L.; Agrawalla, B. K.; Park, S. J.; Zhu, H.; Sivaraman, B.; Peng, J. J.; Xu, Q. H.; Chang, Y. T. Development of targetable two-photon fluorescent probes to image hypochlorous acid in mitochondria and lysosome in live cell and inflamed mouse model. *J. Am. Chem. Soc.* **2015**, *137*, 5930-5938.

(33) Liu, C. L.; Zhang, R.; Zhang, W. Z.; Liu, J. P.; Wang, Y. L.; Du, Z. B.; Song, B.; Xu, Z. P.; Yuan, J. L. "Dual-key-and-lock" ruthenium complex probe for lysosomal formaldehyde in cancer cells and tumors. *J. Am. Chem. Soc.* **2019**, *141*, 8462-8472.

(34) Sapsford, K.E.; Berti, L.; Medintz, I.L. Materials for fluorescence resonance energy transfer analysis: Beyond traditional donor-ccceptor combinations. *Angew. Chem. Int. Ed.* **2006**, *45*, 4562-4588.

(35) Zhang, W. J.; Liu, T.; Huo, F. J.; Ning, P.; Meng, X. M.; Yin, C. X. Reversible Ratiometric Fluorescent Probe for Sensing Bisulfate/H_2O_2 and Its Application in Zebrafish. *Anal. Chem.* **2017**, *89*, 8079-8083.

(36) Ma, Y.Y.; Gao, W.J.; Zhu, L.L.; Zhao, Y.P.; Lin, W.Y. Development of a unique reversible fluorescent probe for tracking endogenous sulfur dioxide and formaldehyde fluctuation in vivo. *Chem. Commun.* **2019**, *55*, 11263-11266.

(37) Gao, Z.M.; Liu, F.H.; Yin, P.; Wan, C.R.; He, S.S.; Liu, X.X.; Zhao, H.; Liu, T.; Xu, J.Q.; Guo, S.N. Inhibition of heat-induced apoptosis in rat small intestine and IEC-6 cells through the AKT signaling pathway. *BMC Vet Res.* **2013**, *9*, 241-249.

(38) Liu, F.; Cottrell, J.J.; Furness, J.B.; Rivera, L.R.; Kelly, F.W.; Wijesiriwardana, U.; Pustovit, R.V.; Fothergill, L.J.; Bravo, D.M.; Celi, P.; Leury, B.J.; Gabler, N.K.; Dunshea, F.R. Selenium and vitamin E together improve intestinal epithelial barrier function and alleviate oxidative stress in heat-stressed pigs. *Exp Physiol.* **2016**, *101*, 801-810.

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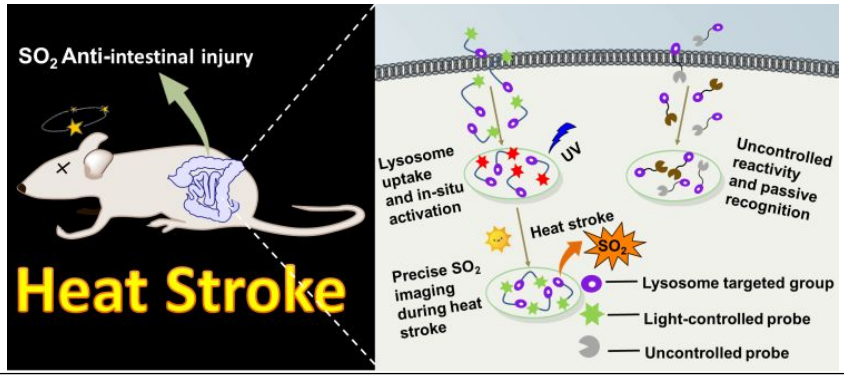
SO_2 Anti-intestinal injury
Heat Stroke
Lysosome uptake and in-situ activation
UV
Uncontrolled reactivity and passive recognition
Heat stroke
SO_2
Precise SO_2 imaging during heat stroke
Lysosome targeted group
Light-controlled probe
Uncontrolled probe