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A mitochondria-targeted ratiometric fluorescent probe to monitor endogenously generated sulfur dioxide derivatives in living cells

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ABSTRACT

Sulfur dioxide (SO_2) can be endogenously produced by enzymes in mitochondria during oxidation of H_2S or sulphur-containing amino acids, and plays important roles in several physiological processes. However, the design and synthesis of fluorescent probes which can detect mitochondrial SO_2 and its derivatives in living cells still remain unresolved. Herein, we report the preparation of a lipophilic cationic dye **1** (**Mito-Ratio-SO_2**), which targets the mitochondria in living cells and is sensitive to the presence of SO_2 derivatives. The ratiometric probe **Mito-Ratio-SO_2** displays a 170 nm blue-shift in emission with two well-resolved emission bands upon addition of sulfite. Mechanistic studies indicate that three probe-SO_2 adducts coexist after reaction, as supported by liquid chromatography and density function theory investigations. Importantly, the ratiometric probe is highly selective for sulfite over other bio-species including H_2S. Fluorescence co-localization studies indicate that the probe localizes solely in the mitochondria of HeLa cells. Last but not least, fluorescent imaging of HeLa cells successfully demonstrates the detection of intrinsically generated intracellular SO_2 derivatives in living cells.

1. Introduction

Sulfur dioxide (SO_2), has long been recognized as merely a toxicant, waste, or environmental pollutant with little or no physiological significance, resulting from increased industrial activity over the past several decades [1,2]. Chronic or acute exposure to SO_2 is associated with increased risks of respiratory and cardiovascular diseases, lung cancer, as well as many neurological disorders [1–4]. However, SO_2 can also be endogenously produced enzymatically in cytosols and mitochondria of cells (e.g. cardiovascular system) during oxidation of hydrogen sulfide (H_2S), sulphur-containing amino acids and decomposition of sulfinylpyruvate to pyruvate in mammals [5–9], which equilibrates with aqueous sulfites (HSO_3^- and SO_3^{2-}) in biological environments. Endogenous SO_2, like other gaseous signaling molecules such as nitric oxide (NO), carbon monoxide (CO) and hydrogen sulfide (H_2S), has also been recognized to mediate a wide range of physiological processes, including increasing anti-oxidative capacity [5–15] and regulation of cardiovascular structure and function (lowering blood pressure, relaxing blood vessels and a negative inotropic effect in the heart) [6,7,16–18]. However, the abnormal endogenous SO_2 level is related to a number of diseases and neurological disorders such as cardiovascular diseases, lung cancer [15,19–22]. Thus, SO_2 displays dichotomy of signal/stress. Nonetheless, many aspects of its roles in biological systems remain unclear.

Mitochondria is a vital organelle in eukaryotic cells that plays a major role in a variety of critical processes involved in cell survival and death [23–25]. It is reported that endogenous SO_2 can be enzymatically generated by AAT2 (aspartate aminotransferase 2) in mitochondria [6–9]. In addition, mitochondrial SO_2 has been recognized to markedly attenuate myocardial injury and ISO-

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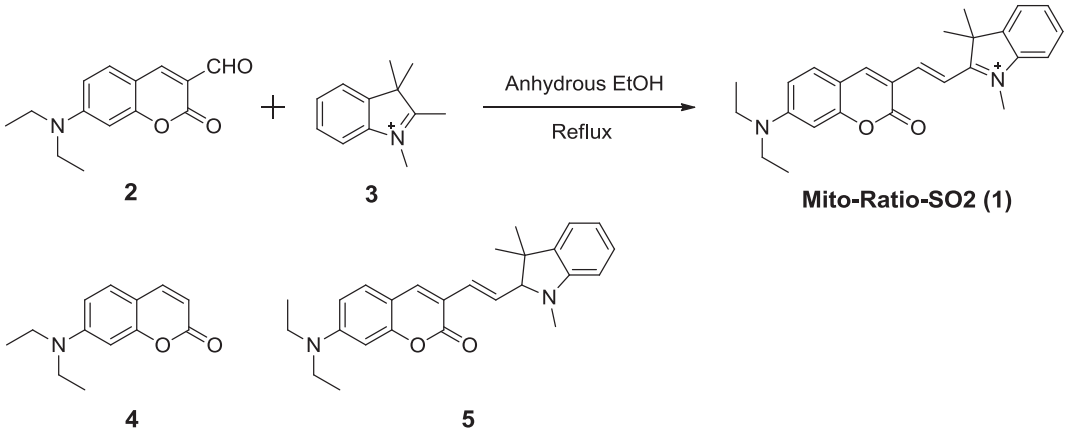

Scheme 1. . The structures of probe **Mito-Ratio-SO$_2$** (**1**) and control compounds **4** and **5**.

induced mitochondrial swelling and deformation [22,26,27]. In the brain, mitochondrial biogenesis increases after exposure to SO_2 [27]. Thus, to understand the production, biological and pathological roles of SO_2 and its derivatives in mitochondria, a targetable molecular tool for monitoring SO_2 and its derivatives is highly sought after.

In the past decade, fluorescence sensing and imaging has emerged as one of the most powerful techniques to monitor the level, localization, and transportation of bio-molecules within the context of living systems [28–32]. Thus, fluorescence sensing and imaging have been viewed as a powerful and versatile toolbox in diverse fields including biology, clinical diagnosis, and drug discovery. Moreover, fluorescent probes are able to provide information on analytes in an organelle of interest, which serves as a powerful tool to study the biology of analytes in certain organelles such as mitochondria [33–42]. Unfortunately, the development of fluorescent probes for SO_2 and its derivatives suitable for biological settings is largely lagged. Till date, few fluorescent probes, which

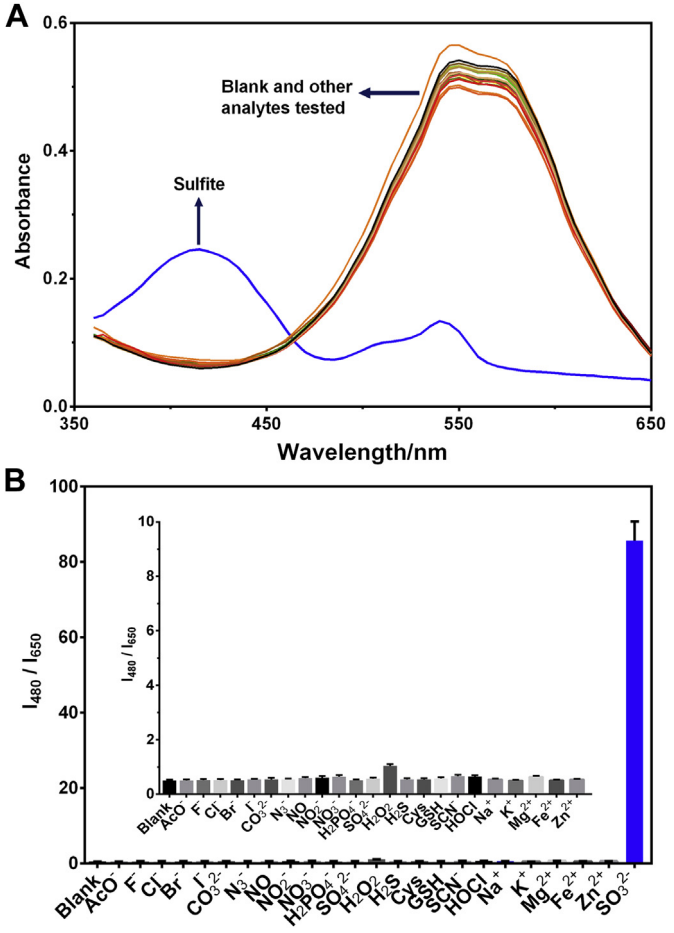

Fig. 1. Absorption (a) and emission ratio (I_{480}/I_{650}) (b) responses of **Mito-Ratio-SO$_2$** (5 μM) to various species (1 mM for Cysteine, 5 mM for GSH, 0.1 mM for other analytes tested) in pH 7.4 PBS (containing 15% EtOH as a co-solvent). The spectra were recorded after incubation of the probe with each species for 30 min.

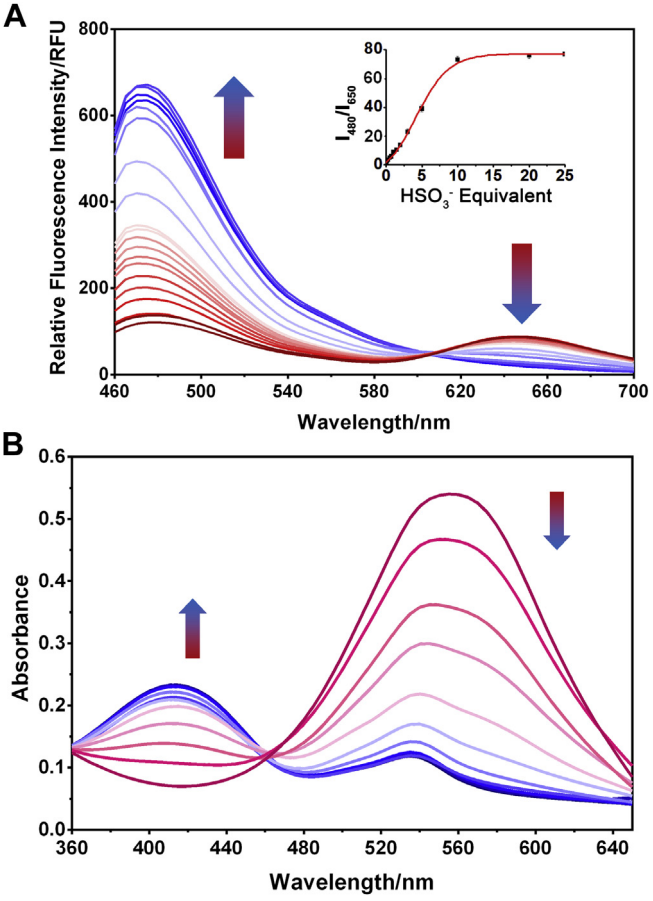

Fig. 2. Fluorescence (a) and absorption (b) spectral changes of **Mito-Ratio-SO$_2$** (5 μM) upon addition of sulfite (0–25 equiv.) in pH 7.4 PBS (containing 15% EtOH as a co-solvent) excited at 405 nm. The spectra were recorded after incubation of the probe with sulfite for 30 min.