A Two-Channel Responsive Fluorescent Probe with AIE-Property and Its Application for Selective Imaging of Superoxide Anion in Living Cells

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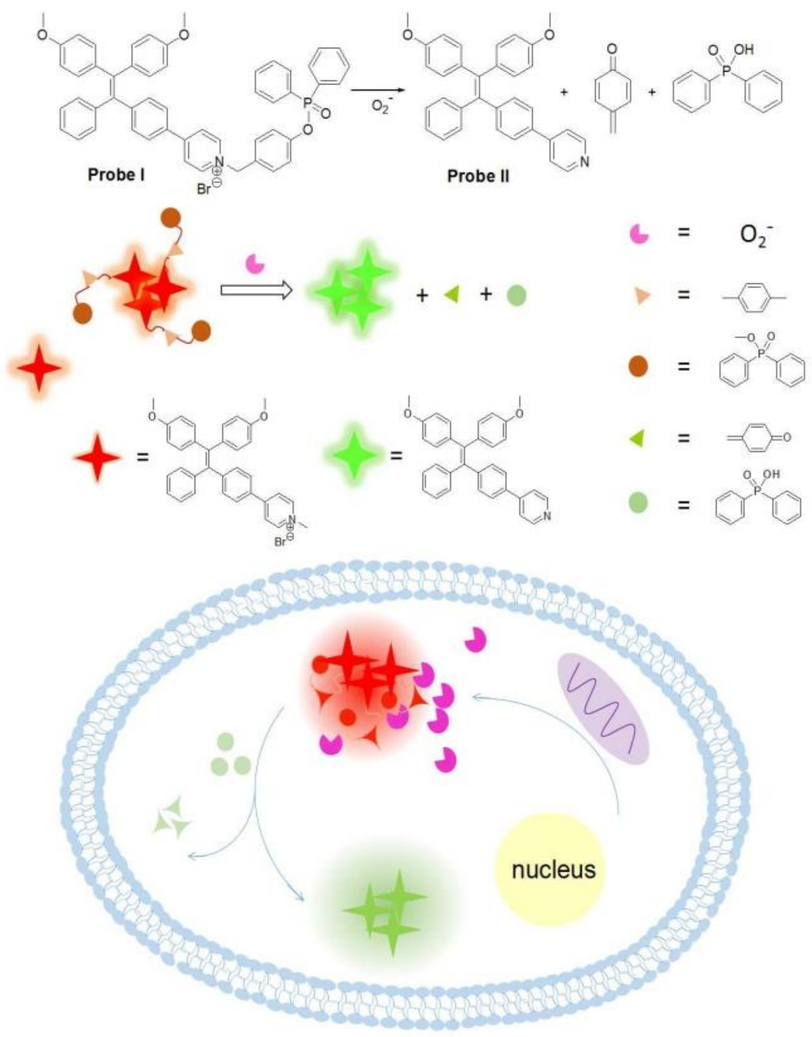
INTRODUCTION

Reactive oxygen species (ROS) is closely related to the function and health of cells. Endogenous ROS produced in normal level plays an important role in signalling and immune processes and superoxide anion is the precursor of other ROS. In this work, A two-channel responsive and AIE-active fluorescent probe was developed to selectively detect superoxide anion in living cells, which can be used to track endogenous superoxide anion level when cells undergo apoptosis and inflammation.

RESULTS AND DISCUSSION



Scheme 1 Chemical structure of the two-channel fluorescent probe and its working mechanism in selective reaction with superoxide anion. A schematic illustration of the two-channel (red and green) emission responses of the AIE-active fluorescent probe.

Figure 1. (A) Fluorescence (FL) spectra of Probe I (10 μM) in PBS buffer solution (10 mM, containing 10% DMSO). (B) Time-dependent FL spectra of probe I (10 μM) in PBS buffer solution (10 mM, pH = 7.8) upon addition of potassium superoxide (KO_2) at 25 °C. KO_2 concentration: 100 μM; excitation wavelength (λ_{ex}): 390 nm.

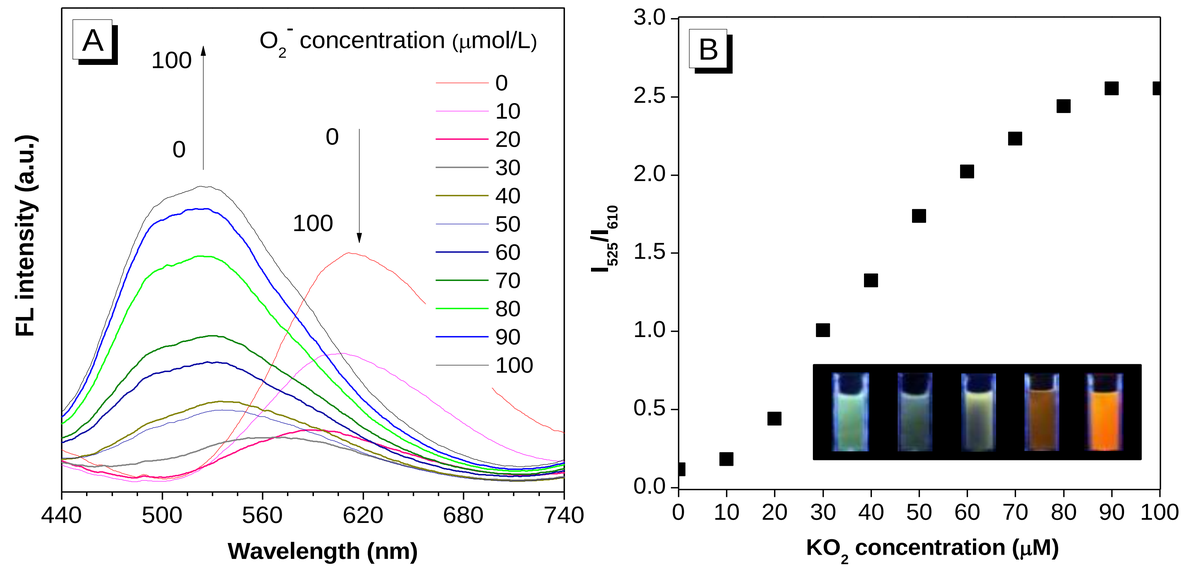


Figure 2. (A) FL spectra of probe I (10 μM) in PBS buffer solution (pH = 7.8, 10 mM, containing 1% DMSO) in the presence of different concentrations of KO_2 at 25°C; (B) Relative FL intensity (I_{525}/I_{615}) of probe I (10 μM) in PBS (pH = 7.8) incubated with different concentrations of KO_2 at 25 °C. λ_{ex}:390 nm. The inset shows the photographs under UV lamp in the presence of different concentrations of KO_2.

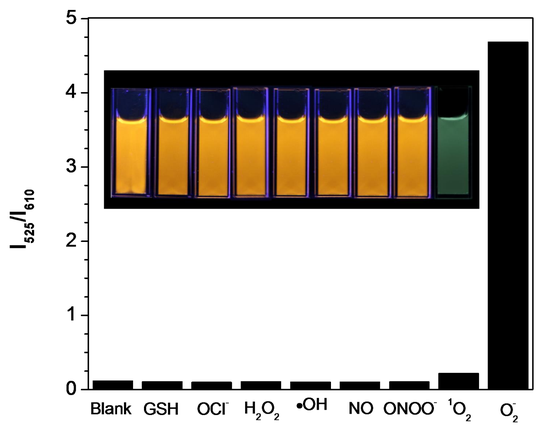


Figure 3. Relative fluorescence intensity response (I525/I615) of probe I (10 μM) to KO2 (100 μM) and 500 μM of other ROS/RNS species. Excitation wavelength: 390 nm; incubation time: 10 min. The inset shows the photographs under UV lamp in the presence of different ROS/RNS species.

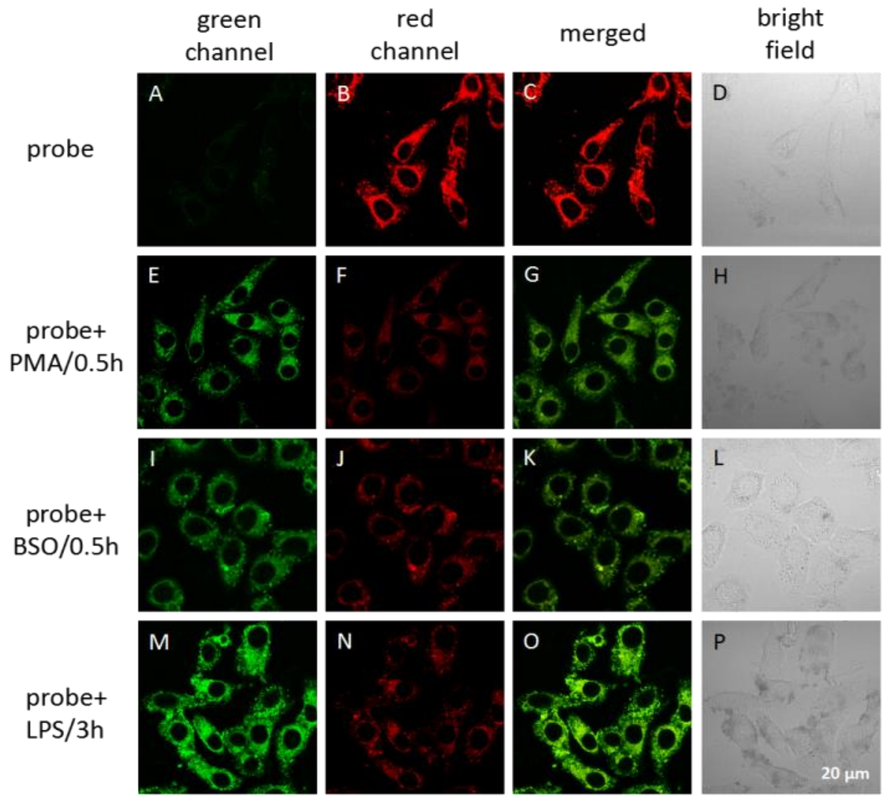


Figure 4. Confocal lasing scanning microscopic (CLSM) and bright field images of HepG2 cells with different treatments. (A-D) HepG2 cells treated with probe I (10 μM) for 1 h at 37 °C; (E-H) HepG2 cells loaded probe (10 μM) treated with PMA (1 μg /mL) for 30 min; (I-L) cells loaded probe (10 μM) treated with BSO (5 mM) for 30 min; (M-P) cells loaded probe (10 μM) treated with LPS (5 μg/mL) for 3h. Green channel: λ_{ex}: 405 nm; collection wavelength 525-550 nm. Red channel: λ_{ex}: 405 nm, collection wavelength ≥560 nm.

SUMMARY

An AIE-active probe working in a two-channel responsive manner has been designed, synthesized and used for detecting superoxide anion with high selectivity and sensitivity. The probe can be also applied to image endogenous superoxide anion in living cells, thus differentiating apoptotic and inflamed cells from normal cells.