The University of Hong Kong

DEVELOPMENT OF A MITOCHONDRIA-TARGETING RATIOMETRIC PROBE FOR HYDROGEN PEROXIDE DETECTION AND IMAGING

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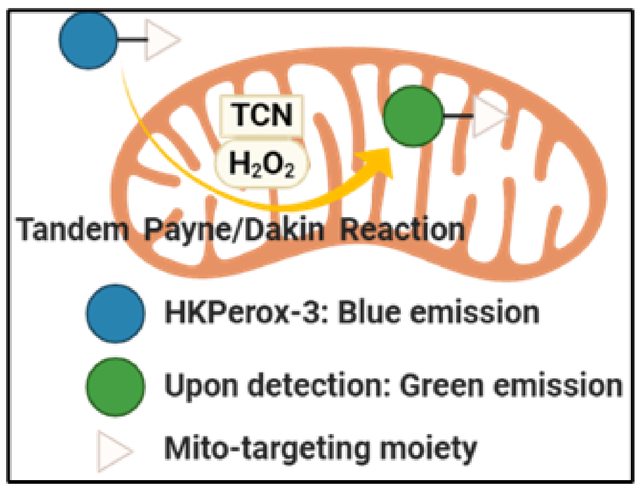
ABSTRACT



Hydrogen peroxide (H_2O_2) been demonstrated by previous research as an emerging redox signaling molecule in various physiological and pathological signaling pathways, such as cell differentiation, proliferation, tumor metastasis and immune response. Mitochondria, the organelle where oxidative phosphorylation takes place, is regarded as a major source of cellular H_2O_2. Current small molecule-based probes for H_2O_2 detection are arguably less reliable due to slow kinetics or poor specificity against other reactive oxygen species. In this project, a mitochondria-targeting ratiometric probe for H_2O_2 detection, based on Tandem Payne/Dakin reaction, was designed and synthesized.

INTRODUCTION

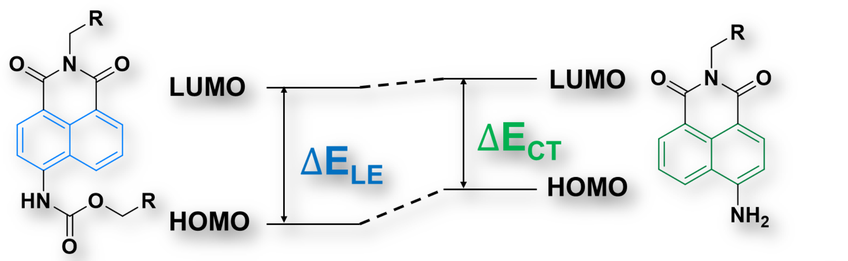
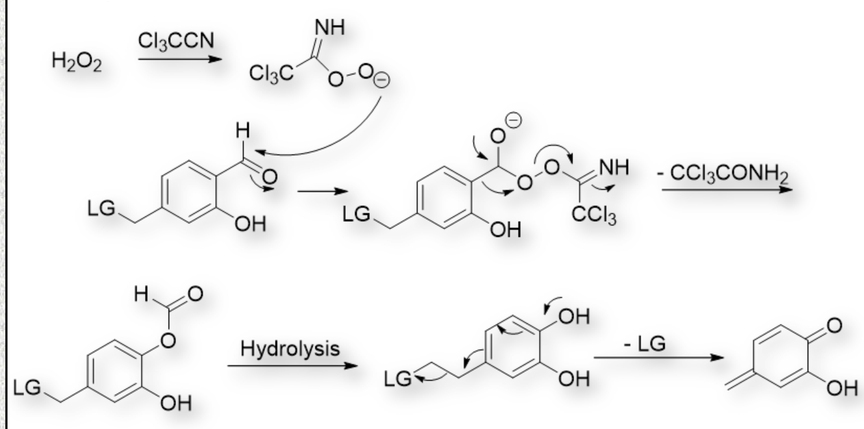


Figure 1. A ratiometric probe based on modulating the internal charge transfer on 1,8-naphtahlimide fluorophore.

Ratiometric probes provide advantages over traditional single-peak intensity measuring probes by performing self-calibration, minimizing errors arising from probe concentration, and uneven distribution in cellular environment. Internal charge transfer (ICT) is a common mechanism utilized in ratiometric fluorescent chemosensors. By changing the electron donating/withdrawing pattern of 4-amino group on the 1,8-naphtahlimide fluorophore, both ICT and emission color change. It should be desirable that the off-state probe consists of a carbamate masked 4-amino group and by reacting with H_2O_2, the free 4-amino-1,8-naphtahlimide fluorophore is released as the on-state probe.



Scheme 1. The mechanism of H_2O_2 detection based on Tandem Payne/Dakin reaction. LG = leaving group.

Contemporary small molecule-based probes for hydrogen peroxide detection mostly utilize H_2O_2-mediated boronate oxidation, with problems of slow kinetics and poor selectivity against other reactive oxygen species. Recently, a novel detection mechanism was developed by Prof. Dan Yang's group, based on Tandem Payne/Dakin reaction was reported with higher specificity and kinetics.

PROBE DESIGN & RETROSYNTHETIC ANALYSIS

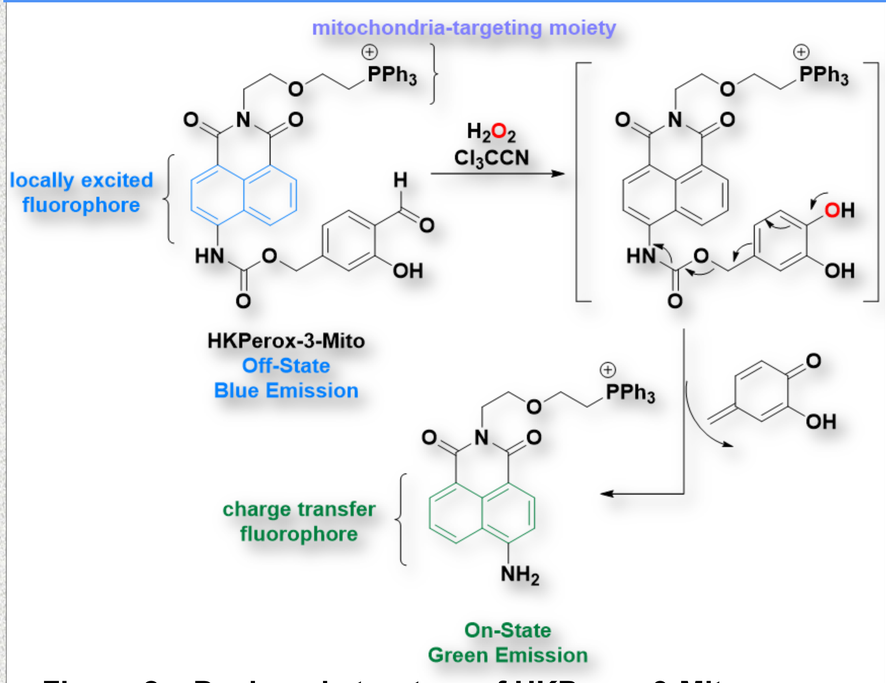
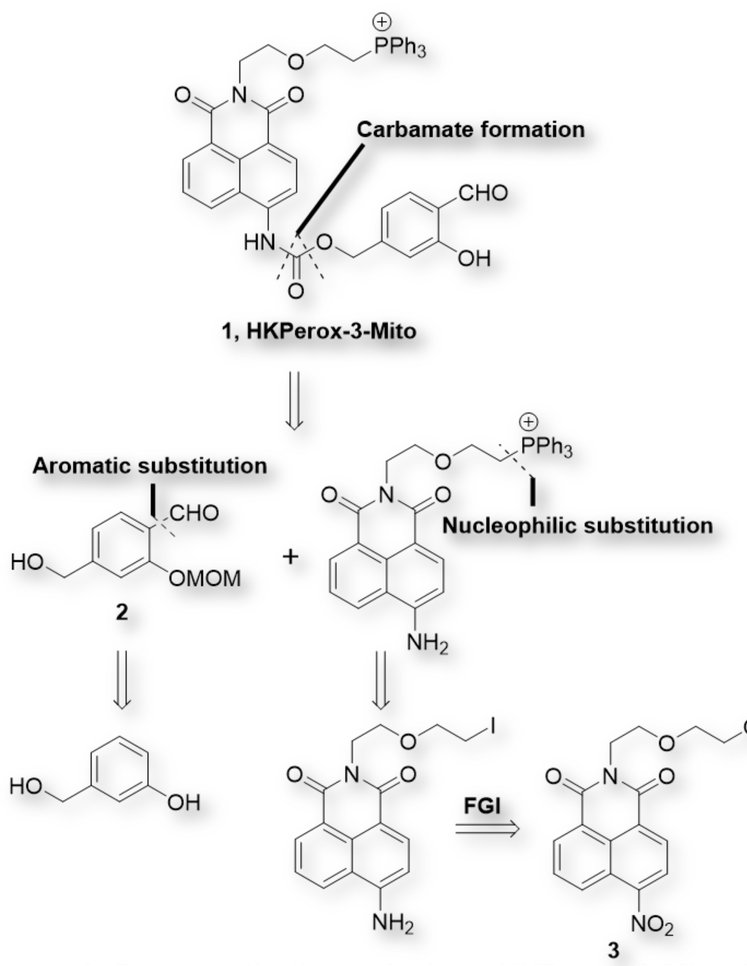


Figure 2. Designed structure of HKPerox-3-Mito.



Scheme 2. Retrosynthetic analysis of HKPerox-3-Mito. MOM = methoxymethyl, FGI = functional group interchange.

RESULTS

Scheme 2. Reagents and conditions: a) Et_3N (3.8 equiv), $MgCl_2$ (1.5 equiv), $(CH_2O)n$ (6.9 equiv), MeCN, reflux, overnight, 12% ; b) NaH (1.1 equiv), DMF, 0 °C, 25 min, then MOMBr (1.2 equiv), 0 °C, 30 min, 56%; c) HNO_3, AcOH, r.t., 4 h, 70%; d) $Na_2Cr_2O_7$ (3.0 equiv), AcOH, reflux, 5 h, 60 %; e) i) 2-(2-aminoethoxy)ethanol (1.1 equiv), EtOH, reflux, 2 h, 81 %; ii) $SOCl_2$ (1.0 equiv), pyridine (2.0 equiv), 0 °C to reflux, 5 h, 73%; f) Zn (50 equiv), NH_4Cl (100 equiv), acetone/water (4:1), r.t., 15 min, 95%; g) i) NaI (10 equiv), acetone, reflux, 22 h; ii) PPh_3 (1.2 equiv), MeCN, reflux, 5 h, 49%; h) triphosgene (1.0 equiv), Et_3N (3.0 equiv), CH_2Cl_2, 0 °C to r.t., 6 h; then 2 (1.2 equiv), r.t., overnight; HCl/MeOH, r.t..

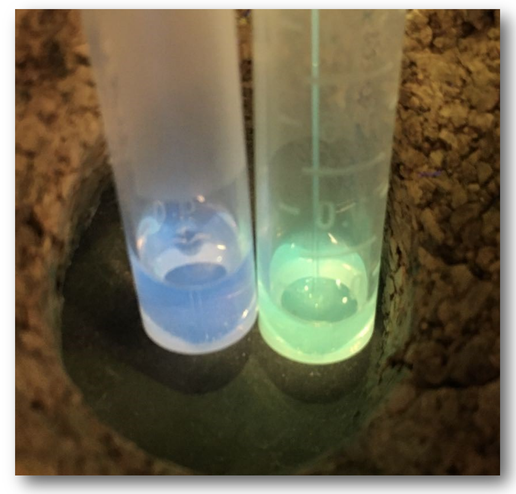


Figure 3. Blue fluorescence of the off-state probe (left) and the green fluorescence of the on-state probe (right).

FUTURE PLAN

Upon the successful synthesis of HKPerox-3-Mito, its properties are to be determined under physiological like conditions, including:

1. maximum absorption with or without H_2O_2,
2. the ratio of on- to off-state emission intensities,
3. the kinetics of H_2O_2 detection
4. the specificity among other reactive oxygen species.

The final goal is to apply HKPerox-3-Mito to for mitochondria targeting, H_2O_2 detection and imaging in live cells.

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