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A targetable fluorescent probe for real-time monitoring of fluoride ions in mitochondria

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ABSTRACT

Fluorion are pivotal anions in biology because they play an important role in dental care, treating osteoporosis, preventing tooth decay and promoting the healthy growth of bone. Studies have shown that high levels of fluoride will lead to the inactivation of the mitochondria. Therefore, it is urgent to develop a method to detect the fluoride anions in the mitochondria. Herein, we have developed a novel mitochondrial-target fluorescent probe for detecting F^- in living cells. The probe exhibited excellent sensitivity and high selectivity for F^- over the other relative species. With changing fluoride ions, the fluorescence spectrum of the probe changed significantly with a large turn-on fluorescence signal. Cell imaging indicated that the probe can penetrate viable cell membranes and rapidly detects and images fluorion over other anions in the mitochondria.

1. Introduction

Fluoride ions, the smallest and most negative anions, are of particular interest due to their important role in dental care and promoting the healthy growth of bone [1–3]. However, excessive fluoride can cause various diseases such as, metabolic disturbances, tooth mottling, skeletal fluorosis, immune damage, neurotoxicity, and kidney disease [4,5]. Additionally, NaF, as an effective activator of G protein and inhibitor for Ser/Thr phosphatase, affects plenty of essential cell signaling elements [6]. At the cell organelles level, studies have shown that high levels of fluoride can cause oxidative stress on mitochondria and reduce mitochondrial respiratory chain efficiency, and leading to mitochondrial dysfunction, which is an earliest event in most delayed neurodegenerative diseases [7–10]. In order to fully elucidate the toxicity of fluoride ions, it is very necessary to develop a sensitive, specific and accurate method for the detection of its distribution and dynamic fluctuation in biological systems. For traditional methods, including ^{19}F NMR spectroscopy [11], ion chromatography [12] and ion-selective electrodes [13], complicated pre-treatments of samples, relatively cumbersome equipment, and professional operators are require ineluctably, which hinders their application in the effective and convenient detection of F^-. In the various analytical methods available, fluorescence sensing is regarded as an ideal technology due to its advantages of simplicity of operation, high specificity and sensitivity [14–19]. Although significant progresses have been made in the development of plenty fluorescent fluoride ions probes [20–26], there are still some deficiencies in practical applications. For example, some B-F complexing sensors are often sensitive to oxygen and moisture and involve complex equilibria due to the formation of various fluoroborate species. Furthermore, some desilylation F^- probes usually require an excess of F ions to reach saturation and the response time required is often longer. Currently, fluorescent F^- probes based on H-bond interaction are more fascinating due to their satisfactory response time and stable chemical property. Although several fluoride ion fluorescence sensors based on F-induced deprotonation through H-bonding with protonic units have been reported [27–31], rare of them can be used for the detection and imaging of fluorinion levels in cellular mitochondria.

Herein, we designed and synthesized a new intramolecular charge transfer (ICT) based, mitochondrial-targeted fluorescent probe (**Mito-FV**) by linking imidazole (donor, D) and hemicyanine (acceptor, A) through a phenyl ring. The sensor **Mito-FV** exhibited excellent sensitivity and high selectivity for F^- over the other relative species and can display strong turn-on (~204-fold) fluorescence in response to F^-. Furthermore, **Mito-FV** was able to penetrate the membranes of living cells, rapidly detect and image the F^- in mitochondria.

2. Experimental

2.1. Materials and Instruments

Unless otherwise noted, all reagents were obtained from commercial suppliers, and used directly without further purification. Solvents used in experiment were purified by standard methods prior to use. The water used in all experiments refers to the twice-distilled water.

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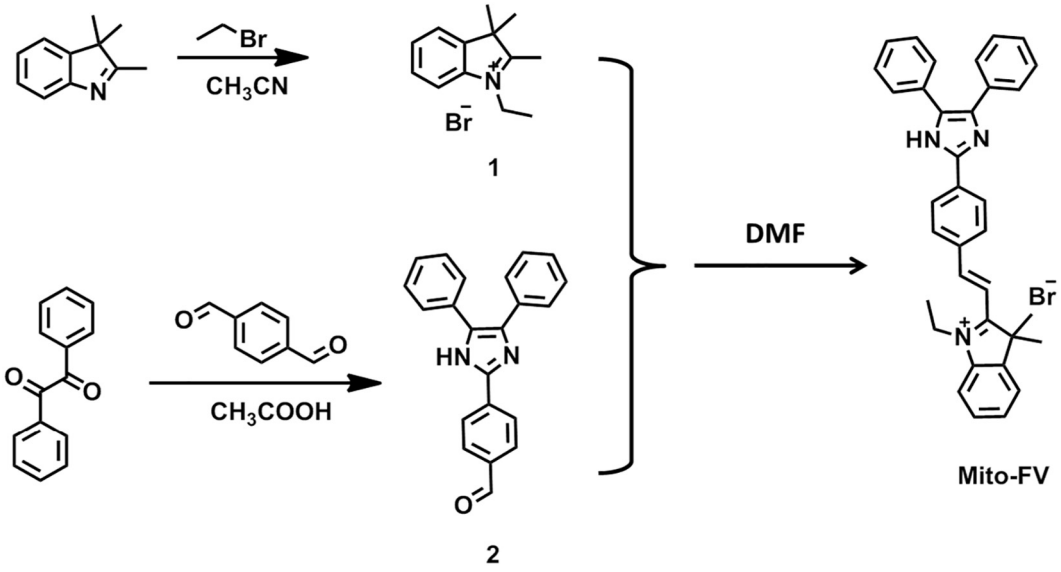


Scheme 1. Synthesis route of probe **Mito-FV**.

The instruments used in this work were same as those in our previously reported articles [32].

2.2. Synthesis of *Mito-FV*

The synthesis route of **Mito-FV** was outlined in Scheme 1. Compound **1** and **2** were prepared referencing the reported literature [33,34]. The desired compound **Mito-FV** was readily obtained in one simple step. Dissolved the crude product **1** (200 mg, 0.75 mmol, 1.0 eq) and compound **2** (245 mg, 0.75 mmol, 1.0 eq) in 4 mL *N*,*N*-dimethylformide (DMF), and the mixture was refluxed for 6 h at 90 °C under a nitrogen atmosphere. After cooled to room temperature, the mixture was poured into water and extracted with dichloromethane. Subsequently, the organic layer was combined, washed with water and brine, dried over sodium sulfate, filtered, and then concentrated in vacuo. The residue was purified by column chromatography on silica gel (petroleum ether to MeOH/DCM = 1/20, v/v) to afford the target compound **Mito-FV** (210 mg purple powder, yield: 48%). ^{1}H NMR (400 MHz, DMSO) δ 13.34 (s, 1H), 8.53 (d, J = 16.2 Hz, 1H), 8.42 (d, J = 8.9 Hz, 2H), 8.39 (d, J = 8.8 Hz, 2H), 7.98 (dd, J = 5.2, 3.7 Hz, 1H), 7.94–7.90 (m, 1H), 7.82 (d, J = 16.3 Hz, 1H), 7.67–7.62 (m, 2H), 7.60–7.58 (m, 2H), 7.58–7.55 (m, 2H), 7.46 (t, J = 7.3 Hz, 2H), 7.41 (d, J = 7.2 Hz, 1H), 7.34 (t, J = 7.4 Hz, 2H), 7.26 (t, J = 7.3 Hz, 1H), 4.81 (q, J = 6.9 Hz, 2H), 1.84 (s, 6H), 1.48 (t, J = 7.2 Hz, 3H)·^{13}C NMR (101 MHz, DMSO) δ 181.74 (s), 153.81 (s), 144.94 (s), 144.51 (s), 140.91 (s), 138.91 (s), 135.37 (s), 134.83 (s), 134.42 (s), 131.90 (s), 131.06 (s), 130.29 (s), 129.90 (s), 129.62 (s), 129.09 (d, J = 6.3 Hz), 128.73 (s), 128.52 (s), 127.75 (s), 127.32 (s), 126.02 (s), 123.61 (s), 115.65 (s), 112.64 (s), 52.75 (s), 42.72 (s), 26.09 (s), 14.35 (s). HRMS (ESI): m/z calculated for $C_{35}H_{32}N_3^+$ 494.2591 $[M]^+$, found: 494.2592 (Scheme 2).

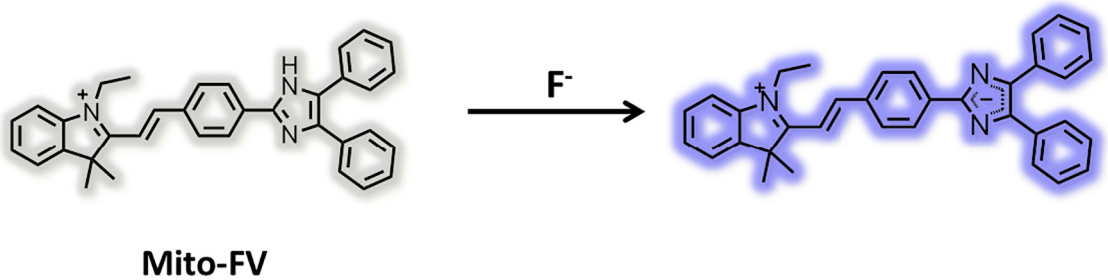
3. Results and Discussion

3.1. Design and Synthesis of *Mito-FV*

Imidazole derivatives were wildly applied in different areas due to their favorable properties such as high stability, flexible photophysical properties, high extinction coefficients, and ease synthesis [35–37]. Considering its susceptible to detect anions either by deprotonation of potential —NH fragment or H-bonding interaction, imidazole was selected as the recognition site for F^-. Hemicyanine, as a strong electron withdraw group, was widely applied to the synthesis of dyes and fluorescent probes [38–41]. Moreover, hemicyanine can act as a mitochondrial targeting group and rapidly enter the mitochondria in cells in a short time [42]. Thus, we constructed a mitochondra-target fluorescent probe **Mito-FV** by linking carbazole and hemicyanine through the benzene ring. The target sensor **Mito-FV** was readily synthesized in one simple step. Treatment of compound **1** with the compound **2** in *N*,*N*-dimethylformide afforded probe **Mito-FV** in good yield. The synthesis route of **Mito-FV** was shown in Scheme 1, and the structure was fully characterized by standard ^{1}H NMR, ^{13}C NMR and mass spectrometry in Supporting information.

3.2. Optical Response of *Mito-FV* to F^-

With the probe **Mito-FV** in hand, we initially investigated its optical responses to fluoride ions (TBAF as F^- donor) by absorption and



Scheme 2. Proposed response mechanism of **Mito-FV** to F^-.