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A novel fluorescent probe with a large Stokes shift for real-time imaging mitochondria in different living cell lines

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ABSTRACT

Fluorescent dyes with large Stokes shift play a key role in avoiding self-quenching and scattered light of dyes in the process of biological imaging. In this work, a novel mitochondria-targetable fluorescent dye (**PI-C2**) with large Stokes shift (e. g. Maximum value is 219 nm in DMSO) have been developed. Compared to the commercial mitochondria probes MTR and MTG (Less than 30 nm in various solution), the newly constructed **PI-C2** has a much larger Stokes shift in various solutions (169–219 nm in various solutions). Furthermore, the probe can successfully be applied for sensing mitochondria, and exhibited excellent photostability in different living cell lines. The novel fluorescent platform with the large Stokes may be extended to construct powerful fluorescent probes with large Stokes shift for detecting a wide variety of biomolecules in mitochondria.

Introduction

Fluorescent dyes with large Stokes shift play a key role in avoiding self-quenching and scattered light of dyes in the process of biological imaging.[1] Especially, most of commercial fluorescent dyes possess some disadvantageous properties to biological applications, including short fluorescence maxima emission wavelength and small Stokes shift[2,3] in various solutions. These commercial fluorescent dyes with small Stokes shifts may cause self-quenching and the measurement error of the excitation light and scattered light.[4] It will directly cause the low sensitivity and accuracy of the imaging of fluorescent dyes in the process of biological imaging.[5] Therefore, it is highly desirable to utilize the advantages of large Stokes shift for developing fluorescent dyes.

Mitochondria, as a major organelle, plays a key role in energy production through respiratory chains,[6,7] cell signaling *via* reactive oxygen species production[8,9], regulation of Ca^{2+} homeostasis,[10,11] and triggering cell death.[12,13] Mitochondrial dysfunction was associated with serious diseases such as neurodegenerative diseases,[14,15] Alzheimer's disease,[16,17] cancer and diabetes.[18,19] These findings promoted us to search for a method for locating mitochondria in different living cells lines.

Fluorescent probes have become powerful tools for detecting various biological targets in the living system, due to its high sensitivity, high selectivity, and unique applications in biological images fields.[20–22] More recently, the development of mitochondria probes shows an increasing trend.[23] More and more mitochondria probes, including commercial mitochondria probes (e. g. MitoTracker Red (MTR) and MitoTracker Green (MTG)), have been developed.[24] However, up to present, little attention had been paid on construction of mitochondria fluorescent probes with large Stokes shifts.[25] Thus, the goal of our work was to design a mitochondria fluorescent probe with a large Stokes shift for imaging mitochondria in different living cell lines.

Phenanthrenequinone imidazole-based dyes have received high attention due to their attractive optical properties.[26] For example, a phenanthrenequinone imidazole-based amino acid probe have been reported by us.[27] Its Stokes shift reach 150 nm in buffer solution. Thus, on the basis of our long-term interest on phenanthrenequinone imidazoles,[28] we further exploited mitochondria fluorescent probe with large Stokes shift. Pyridinium salt was chosen as the targeting unit for mitochondria.[23] As shown in Fig. 1, introduction of pyridinium salt unit on the phenanthrenequinone imidazole-core to provide a new compound **PI-C2**. **PI-C2** may

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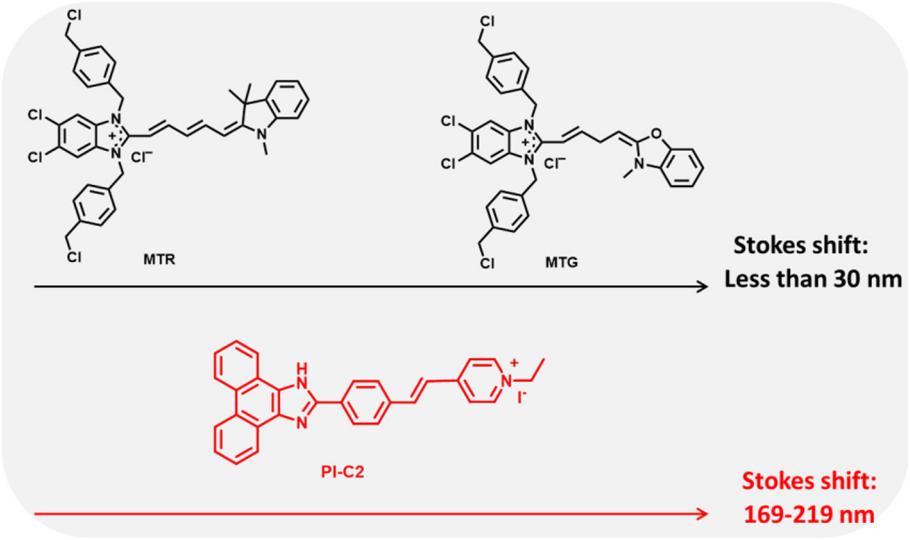


Fig. 1. Structures and Stokes shift of commercial mitochondria probes MTR and MTG and PI-C2.

exhibit large Stokes shift and image mitochondria in different living cell lines.

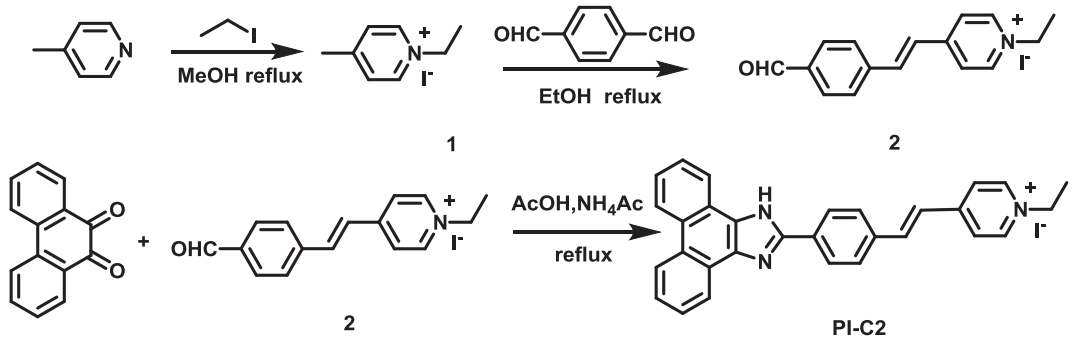
In 2017, our group firstly found a novel mitochondria probe (**CA-C2**) with large Stokes Shift.[29] Maximum Stokes shift of **CA-C2** was 161 nm in water solution. Compared with **CA-C2**, it is firstly found that **PI-C2** allows for efficient separation of absorbance and emission and exhibited larger Stokes shift in various solutions (169–219 nm in various solutions). In this work, the newly constructed **PI-C2** has much larger Stokes shift in various solutions than the commercial mitochondria probes MTR and MTG (Less than 30 nm in various solution). Furthermore, the probe **PI-C2** is capable of real-time imaging mitochondria in different living cell lines.

Results and discussion

Chemical synthesis of the compound **PI-C2** was accomplished in a total of three steps (Scheme 1). The compound *N*-(2-ethyl)-4-methylpyridinium iodide (1) was prepared by alkylation reaction. Compound (2) was synthesized by condensation reactions between compound (1) and terephthalaldehyde. The objective molecule was obtained by a Knoevenagel condensation reaction between compound (2) and 9, 10-phenanthroquinone. Synthetic details of the products were given in the Supporting Information.

With the probe **PI-C2** in hand, to demonstrate the potential utility as suitable tracers for live-cell imaging, optical photophysical properties of probe was investigated, as comparison, the same properties of commercial mitochondrial tracker MitoTracker Red (MTR) and MitoTracker Green (MTG) were evaluated. As shown in Fig. 2, it is found that the absorbance and emission spectra of the probe were different from MTR and MTG in various solutions. For the compound **PI-C2**, the maximum absorption and emission peak of the probe emerge at 400 and 600 nm in various solutions (Fig. 2a and b), respectively. For the MTR and MTG, the maximum absorption were 580 nm and 490 nm (Fig. 2c and e), respectively. The emission peaks of both probes appear at 610 nm and 520 nm in various solutions, respectively (Fig. 2d and f). The above results demonstrate that the probe **PI-C2** should possess larger Stokes shift than commercial mitochondrial probes MTR and MTG.

To further demonstrate **PI-C2** is fluorescent probe with large Stokes shift, we set out to investigate Stokes shift of **PI-C2** in various solutions. Stokes shift of **PI-C2**, MTR and MTG in H_2O, PBS, DMSO, DCM, DMF, MeCN, THF and MeOH have been list (Fig. 3a and Fig. S1). The results demonstrated that probe **PI-C2** has large Stokes shift in various solutions, especially in DMF, DMSO, and



Scheme 1. Synthesis of the probe **PI-C2**.