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Phosphorescent iridium(III) complexes as multicolor probes for specific mitochondrial imaging and tracking

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ABSTRACT

In the present study, four phosphorescent iridium(III) complexes $[Ir(C\text{–}N)_2(PhenSe)]^+$ (**Ir1**–**Ir4**, in which C–N = 2-(2,4-difluorophenyl)pyridine (dfppy), dibenzo[*f*,*h*]quinoxaline (dbq), 2-phenylquinoline (2-pq) and 2-phenylpyridine (ppy), PhenSe = 1,10-phenanthrolineselenazole) with tunable emission colors were developed to image mitochondria and track the dynamics of the mitochondrial morphology. In comparison with commercially available mitochondrial trackers, **Ir1**–**Ir4** possess high specificity to mitochondria in live and fixed cells without requiring prior membrane permeabilization or the replacement of the culture medium. Due to the high resistance of **Ir1**–**Ir4** to the loss of mitochondrial membrane potential as well as the appreciable tolerance to environmental changes, these complexes are applicable for the imaging and tracking of the mitochondrial morphological changes over long periods of time. In addition, **Ir2**–**Ir4** exhibited superior photostability compared to the commercially available mitochondrial trackers. These colorful iridium(III) complexes may contribute to the future development of staining agents for organelle-selective imaging in living cells.

1. Introduction

Mitochondria are essential organelles required for cellular energy production and are involved in many other cellular activities, such as lipid modification, maintenance of redox balance, maintenance of calcium balance, and controlled cell death [1–3]. One of the prominent functions of the mitochondria is to produce reactive oxygen species (ROS), which leads to mitochondria-mediated apoptosis [4–6] as well as the selective autophagic degradation of the mitochondria, a process called mitophagy [7]. It is believed that dysfunction in the mitophagy pathway causes several neurodegenerative disorders, including Parkinson's disease and Alzheimer's disease [8–10]. Further work has revealed that the multifunctional mitochondrion is motile and highly dynamic in shape and form; this dynamism is caused by the ability of mitochondria to undergo fission and fusion with other mitochondria. Reports also link the proteins participating in apoptosis to the morphology of the mitochondria [11,12]. Thus, tracking the mitochondrial morphological changes is of importance in further understanding the variety of cellular functions that the mitochondria participate in.

Fluorescence microscopy using molecular probes allows for highly sensitive imaging techniques to be utilized in various *in vitro* and *in vivo* studies for detecting and tracing purposes and offers a approach for visualizing morphological details in tissues that cannot be resolved by other imaging technologies as this technique has a resolution of several hundred nanometers [13]. Several organic dyes are used as commercial mitochondria imaging agents. The representative examples are the fluorescent dyes Rhodamine 123 (λ_{ex} = 505 nm, λ_{em} = 560 nm, Rh123), MitoTracker® Green FM (λ_{ex} = 490 nm, λ_{em} = 516 nm, MTG) and MitoTracker® Red FM (λ_{ex} = 581 nm, λ_{em} = 644 nm, MTR). Although the extinction coefficients and quantum yields (Φ_{em}) of these dyes are very high, they are easily washed out of cells once the mitochondria experience a loss in membrane potential. This characteristic limits the use of such conventional stains in experiments that require cells to be treated with aldehyde fixatives or with other agents that affect the energetic state of the mitochondria [14]. In addition, because diluted solutions are used in the imaging process, the photostability of these probes leaves much to be desired [15]. Using higher fluorophore concentrations cannot improve their photostability because these probes tend to stain other cellular structures. Moreover, the aggregate formations at higher concentrations often quench light emission [16]. Thus, organic dyes exhibiting aggregation-induced emission (AIE), which would primarily resolve the problem of fluorescence quenching that resulting from aggregation, have attracted much attention. However, it should be noted that reports on compounds exhibiting AIE behaviors in the field of bioimaging are rare,

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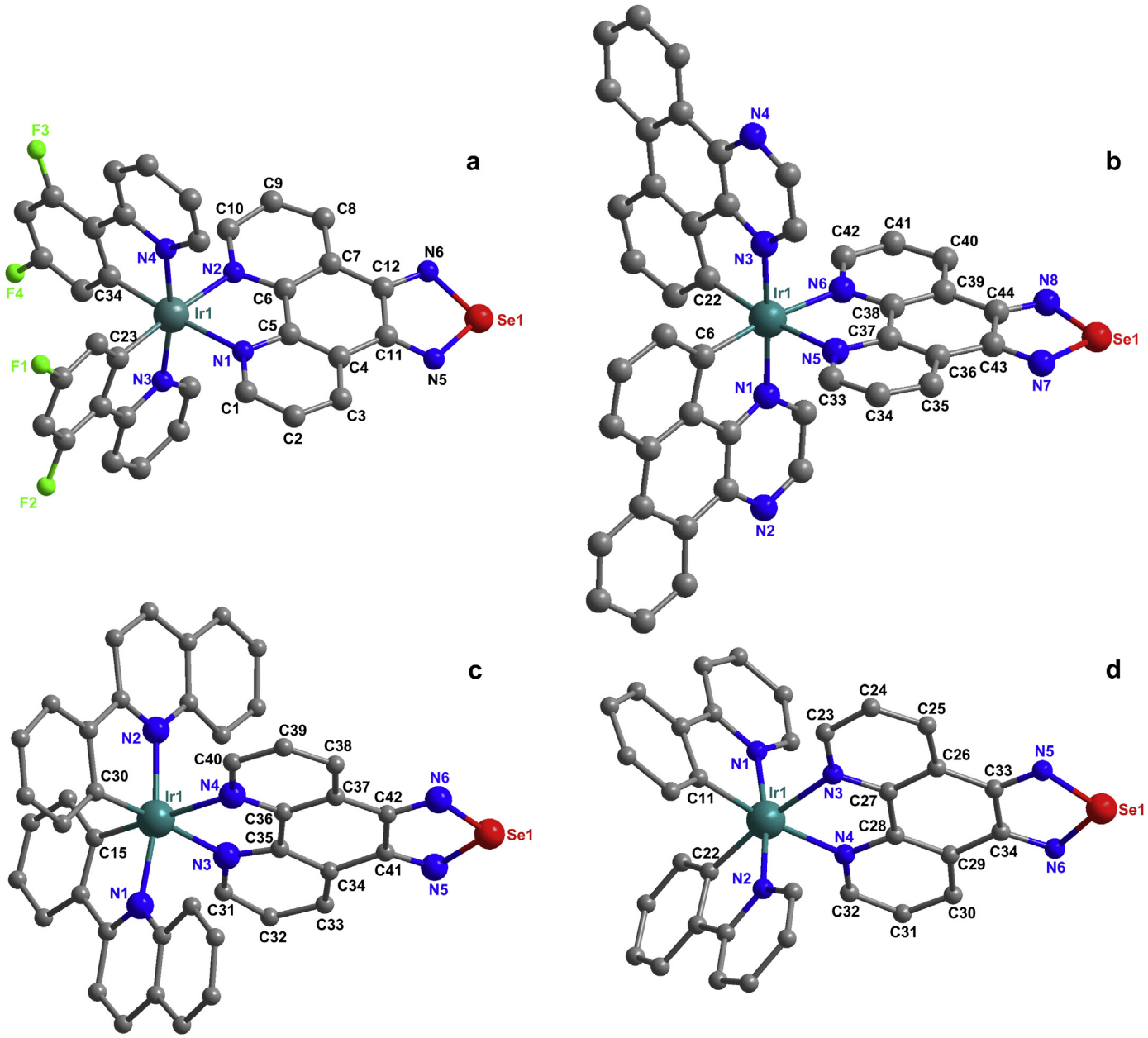

Fig. 1. ORTEP diagrams of the Ir(III) complexes **Ir1** (a), **Ir2** (b), **Ir3** (c) and **Ir4** (d). For clarity, the solvent molecules, the hydrogen atoms, and the counteranions are omitted.

especially reports regarding their use in mitochondrial staining. Recently, one example of an AIE-active organic dye, reported by Tang et al., has been successfully applied for the imaging and tracking of mitochondria [15].

In comparison to organic dyes, metal-based emissive dyes are interesting alternatives because they display many superior physicochemical properties for bioimaging, such as large Stokes shifts (hundreds of nm), long luminescence lifetimes (100 ns to ms), and enhanced photostabilities (lower photobleaching) [16]. Several recent notable reviews have shown that phosphorescent iridium(III) complexes have emerged as promising candidates for wide use in chemosensors, biolabeling, *in vivo* tumor imaging, and live cell compartmentalization staining, such as in the nucleus, in the cytoplasm and in the Golgi apparatus [16–19]. However, only a few examples of iridium(III) complexes have been successfully applied for the imaging of mitochondria to date. Recently Zhou and Fei have developed an iridium(III)-labeled peptide for mitochondrial imaging [20], and Yang achieved specific murine mitochondrial imaging by using a d–f heteronuclear iridium–gadolinium complex [13]. However, no results for tracking mitochondrial morphological changes were found in these reports, and the properties of the iridium(III) complexes need to be further improved to satisfy the requirements of biological applications. Hence, in the present study, we designed and synthesized a series of phosphorescent iridium(III) complexes $[Ir(C\text{–}N)_2(PhenSe)]^+$ (**Ir1**–**Ir4**, in which C–N = 2-(2,4-difluorophenyl)pyridine (dfppy), dibenzo[*f*,*h*]quinoxaline (dbq), 2-phenylquinoline (2-pq) and 2-phenylpyridine (ppy), PhenSe = 1,10-phenanthrolineselenazole, Fig. 1) as mitochondria probes. The results of this study confirm that these four iridium(III) complexes with different emission colors can specifically illuminate mitochondria in live cells with low cytotoxicity and superior photostability, which enabled the observation of mitochondrial morphological changes. Furthermore, the cellular uptake and the biodistribution of **Ir1**–**Ir4** were investigated using inductively coupled plasma mass spectrometry (ICP-MS) analyses. Finally, the