Cationic iridium(III) complexes for phosphorescence staining in the cytoplasm of living cells†

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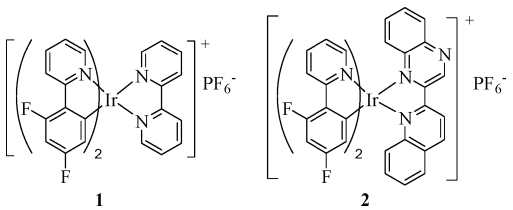
Two cationic iridium(III) complexes with bright green and red emissions were demonstrated as phosphorescent dyes for living cell imaging. In particular, their exclusive staining in cytoplasm, low cytotoxicity and reduced photobleaching, as well as cell membrane permeability, make the two complexes promising candidates for the design of specific bioimaging agents.

Fluorescence imaging in combination with fluorescent probes offers a unique approach for visualizing morphological details in tissue with subcellular resolution that cannot be resolved by ultrasound or magnetic resonance imaging.[1] One obvious prerequisite for *in cellulo* imaging is that the luminescent probe must be non-toxic and cell-permeable, preferably with a distinctive compartmentalization profile, *i.e.* a preference to localize in a given organelle.[2,3] Predicting cellular compartmentalisation profiles for low-MW luminescent species, is not straightforward. Therefore, it is necessary to develop new luminescent probes for staining cellular compartmentalisation profiles.

Compared with pure organic fluorophores,[2] phosphorescent heavy-metal complexes based on metal-to-ligand charge-transfer transition (^{3}MLCT) exhibit advantageous photophysical properties (such as relatively long lifetimes and significant Stokes shifts for easy separation of excitation and emission),[4] and thus seem to be appealing targets for avoiding autofluorescence in bioimaging. However, at least to our knowledge, very limited reports of the use of phosphorescent heavy-metal complexes for staining cellular compartmentalisation profiles have been published, although increasingly, certain phosphorescent heavy-metal complexes have recently been applied in bioimaging[5] and flow cytometry analysis.[6]

Being among the best class of phosphorescent heavy-metal complexes, iridium(III) complexes exhibit high luminescent quantum yield ($\sim$0.7 in organic solvents), tunable luminescent color (from blue to red), and remarkable structure–function relationships, and have been used as highly efficient emitters in organic light-emitting diodes,[7] biolabeling[8] and phosphorescent chemosensing[9] systems. To date, no luminescent staining of living cells using iridium(III) complexes has been reported. In this communication, we present two cationic iridium(III) complexes $[Ir(dfpy)_2(bpy)]^+PF_6^-$ (**1**) and $[Ir(dfpy)_2(quqo)]^+PF_6^-$ (**2**) [dfpy = 2-(2,4-difluorophenyl)pyridine;[10] Scheme 1] as phosphorescent dyes for exclusively staining in the cytoplasm of living cells.



Scheme 1 Chemical structures of iridium(III) complexes **1** and **2**.

Complexes **1** and **2** were synthesized by introducing diimine N^N ligands[11] with different conjugated lengths, and then were characterized by means of ^{1}H NMR, MALDI-TOF-MS, and elemental analyses (ESI†). As shown in Fig. 1, **1** and **2** exhibited intense green (λ_{max}^{em} = 517 nm) and red (λ_{max}^{em} = 623 nm) photoluminescence with emission lifetimes of 0.90 and 1.10 μs, respectively (Table S1, ESI†). Such long emission lifetimes on the microsecond time scale are indicative of the phosphorescent nature of the emissions.[3] More interestingly, the absorption spectral profiles of the two complexes were retained in DMSO/phosphate buffer solution (PBS) (pH 7, 1 : 49, v/v), while the emissions peaks at 530 and 643 nm were observed for **1** and **2** with quantum efficiencies of above 3% in such a solution (Table S1, ESI†). Thus, their tunable emission

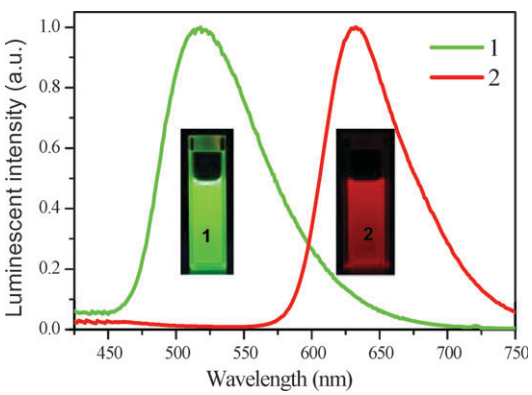


Fig. 1 Room-temperature photoluminescence spectra (λ_{ex} = 360 nm) and a photograph showing the luminescences (under 365 nm UV excitation; inset) of 20 μM **1** and **2** in CH_2Cl_2.

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† Electronic supplementary information (ESI) available: Experimental procedures and characterization of the iridium(III) complexes. Movie of relative rates of photobleaching of DAPI and **2**. See DOI: 10.1039/b800939b

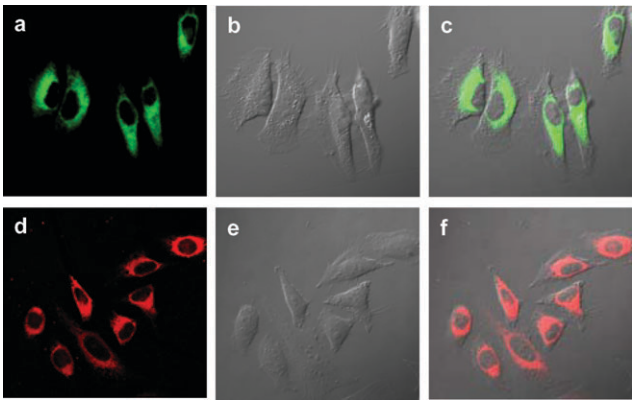

Fig. 2 Confocal luminescence (a and d) and brightfield images (b and e) of living HeLa cells incubated with 20 μM **1** (top) or **2** (bottom) in DMSO/PBS (pH 7, 1 : 49, v/v) for 10 min at 25 °C. Overlays of luminescence and brightfield images are shown in (c) and (f) for **1** or **2**, respectively (λ_{ex} = 405 nm).

colors and moderate luminescence efficiencies in buffer solution imply that **1** and **2** can serve as novel luminescent dyes for bioimaging.

By virtue of a confocal laser scanning microscope, practical application of **1** and **2** was investigated in luminescence imaging of living cells. HeLa cells showed negligible background fluorescence (data not shown). However, after incubation with 20 μM **1** or **2** in DMSO/PBS (pH 7, 1 : 49, v/v) for 10 min at 25 °C, intense intracellular luminescence with high signal-to-noise ratio (I_2/I_1 > 50) was observed between the cytoplasm (region 2) and background (region 1) (Fig. 2 and 3). Spectrum scan experiments showed that maximum emission wavelengths of the luminescence were at 512 and 617 nm after incubation with **1** and **2**, respectively (Fig. S1, ESI†). This result revealed that the intracelluar luminescence should be attributed to the iridium(III) complexes **1** and **2**. Additionally,

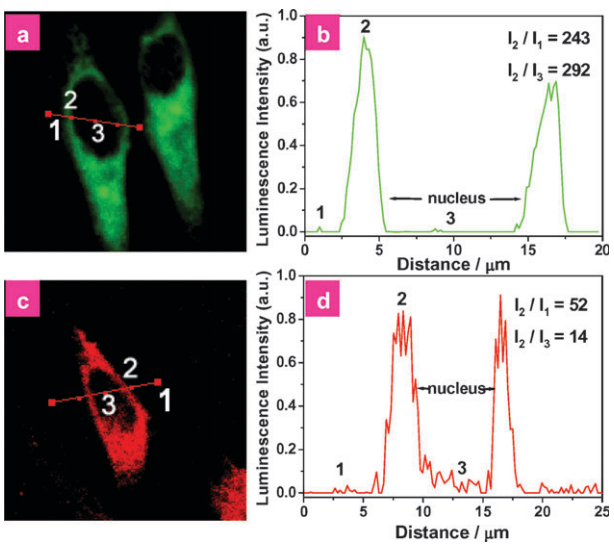

Fig. 3 Confocal luminescence images and luminescence intensity profile (across the lines shown in (a) and (c) of HeLa cells incubated with 20 μM **1** or **2** in DMSO/PBS (pH 7, 1 : 49, v/v) for 10 min at 25 °C. The signal-to-noise ratios (I_2/I_1) and cytoplasm-to-nucleus ratios (I_2/I_3) were shown in the right column (λ_{ex} = 405 nm).

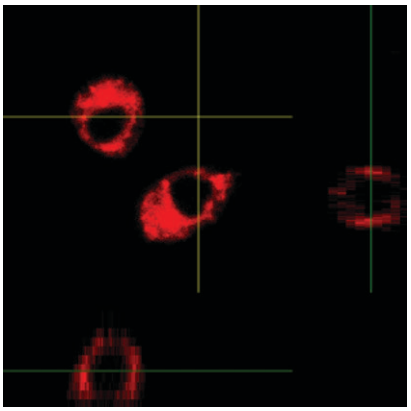

Fig. 4 The overlap *Z*-scan confocal image of the living HeLa cells incubated with 20 μM **2** in DMSO/PBS (pH 7, 1 : 49, v/v) for 10 min at 25 °C (λ_{ex} = 405 nm).

brightfield measurements after the treatment with **1** and **2** confirmed that the cells were viable throughout the imaging experiments (Fig. 2(b) and (e)). Overlays of confocal luminescence and brightfield images further demonstrated that the luminescence was evident in the cytoplasm over the nucleus and membrane (Fig. 2(c) and (f)), suggesting that **1** and **2** were internalized into the cells rather than merely staining the membrane surface. Further quantization by line plots (Fig. 3) revealed large signal ratios (I_2/I_3 > 10) between the cytoplasm (region 2) and nucleus (region 3), implying that weak nuclear uptake occurred for the complexes. These results indicated the exclusive staining in the cytoplasm of **1** and **2**, which was also confirmed by *Z*-scan luminescence imaging of HeLa cells stained with the two complexes (Fig. 4 and S2, ESI†). To the best of our knowledge, this is the first example of the use of iridium(III) complexes as cell-permeable dyes for exclusive luminescence staining in the cytoplasm of living cells. Their facile internalization by cells may be correlative with the positive charge and lipophilicity (fluorination) of the two cationic complexes.

Generally, the cytotoxic, cell-permeable and photobleaching characteristics of fluorescent materials are critical to the application of such materials as bioprobes. Herein, the effect of the two complexes on cell proliferation was determined using two cell lines, MCF-7 and HCT-8, by means of a MTS

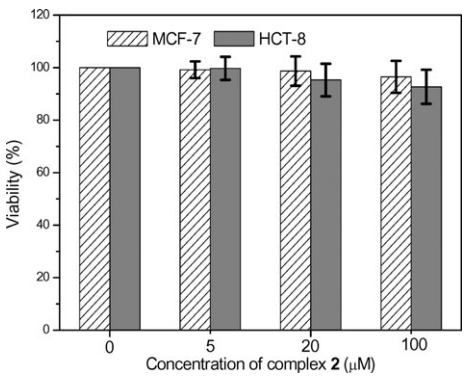

Fig. 5 Cell viability values (%) estimated by MTS proliferation test *vs.* incubation concentrations of **2**. Two cell lines, MCF-7 and HCT-8, were cultured in the presence of 5–100 μM **2** at 37 °C for 24 h.

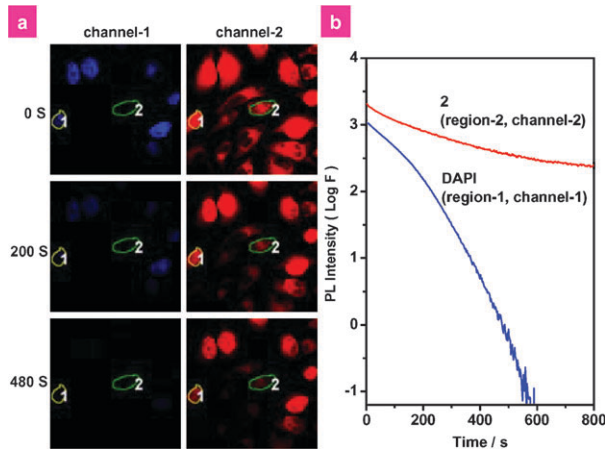

Fig. 6 Comparison of **2** and DAPI for resistance to photobleaching. (a) Confocal luminescence images of fixed HeLa cells stained with **2** and DAPI under continuous excitation at 405 nm with different laser scan times (0, 200, 480 s). (b) Luminescence decay curves of **2** and DAPI during the same period. The signals of DAPI and **2** were collected from the region 1 of channel 1 (460 ± 20 nm) and region 2 of channel 2 (620 ± 20 nm), respectively.

assay at 24 h. The results for **2** are shown in Fig. 5. No significant differences in the proliferation of the cells could be observed in the absence and presence of 5–100 μM **2**. The cellular viabilities were estimated to be greater than 90%. A similar result was obtained for complex **1** (Fig. S3, ESI†). These data show that the iridium(III) complexes **1** and **2** ($\leq$100 μM) can be considered to have low cytotoxicity.

Furthermore, the photostability of **2** (as an example) was compared with that of organic dye under the same excitation conditions. To distinguish their luminescent wavelengths and staining regions, **2** (λ_{max}^{em} = 623 nm) and DAPI (λ_{max}^{em} = 460 nm)[2,12] were chosen to stain the cytoplasm and nuclei of fixed HeLa cells, respectively. To quantify the luminescence decay rates of **2** and DAPI, the luminescence intensity (after background subtraction) of each dye was integrated, normalized, and plotted as a function of time. After continuous excitation at 405 nm for 480 seconds, the luminescence intensity of DAPI (460 ± 20 nm, region 1, channel 1) decreased to 1% of its initial value (owing to photobleaching) (Fig. 6 and movie in ESI†). In contrast, the luminescence intensity of **2** (620 ± 20 nm, region 2, channel 2) stayed at essentially one-eighth of the original value during the same period of excitation (Fig. 6). This result establishes that iridium(III) complexes show reduced photobleaching and higher photostability than the organic dye.

In conclusion, we have presented two cationic iridium(III) complexes as phosphorescent dyes for living cell imaging. The two complexes with bright green and red phosphorescence emissions was synthesized by variation of the diimine ligand structure. In particular, the exclusive staining in cytoplasm, low cytotoxicity, and reduced photobleaching observed for the two iridium(III) complexes, as well as their cell membrane permeability and moderate luminescence efficiencies in buffer solution, make them promising candidates for the design of specific phosphorescence bioimaging agents.

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