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Design of cyclometalated iridium(III) polypyridine complexes as luminescent biological labels and probes*

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Abstract: The interesting emission properties of cyclometalated iridium(III) polypyridine complexes, originated from a range of excited states, have been well documented. The intense and long-lived emission of these complexes has been exploited in various areas of research including photovoltaic cells, chemosensors, and light-emitting devices. Additionally, there is an emerging interest in the applications of these luminescent complexes in various biological studies. In this paper, we summarize our recent work on the utilization of luminescent cyclometalated iridium(III) polypyridine complexes as biomolecular and cellular probes.

Keywords: cellular uptake; iridium; luminescence; polypyridine; probes.

INTRODUCTION

The design of molecular probes is dominated by fluorescent compounds since they offer high detection sensitivity and photophysical parameters such as emission wavelengths, intensities, and lifetimes can all be conveniently monitored by modern analytical equipment with high accuracy [1]. Fluorescent organic molecules play a key role in the area of biological probes owing to their high absorptivity and fluorescence quantum yields, which lead to a low detection limit and high sensitivity [2]. Additionally, a number of lanthanide chelates have been used as biological labels since they show intense and extraordinarily long-lived luminescence with large Stokes shifts and narrow emission bands [3]. In the past decade, there is an increasing interest in the development of luminescent transition-metal complexes as probes for biomolecules and cellular structures. The main reason is that these complexes possess the advantages of both organic compounds (tunable and intense emission) and lanthanides chelates (high photostability, long emission lifetimes, and large Stokes shifts). The use of various transition-metal centers, ligands, and functional groups results in a range of luminescent probes that have been successfully utilized in different sensing applications [4].

Many transition-metal polypyridine complexes with a d^6 electronic configuration [such as ruthenium(II), osmium(II), and rhenium(I)] display emission originated from a triplet metal-to-ligand charge-transfer (^{3}MLCT) state [5]. In contrast, the nature of the emissive states of iridium(III) polypyridine complexes is much more diverse, resulting in very rich photophysical properties which are distinctly different from those of common ruthenium(II), osmium(II), and rhenium(I) polypyridine com-

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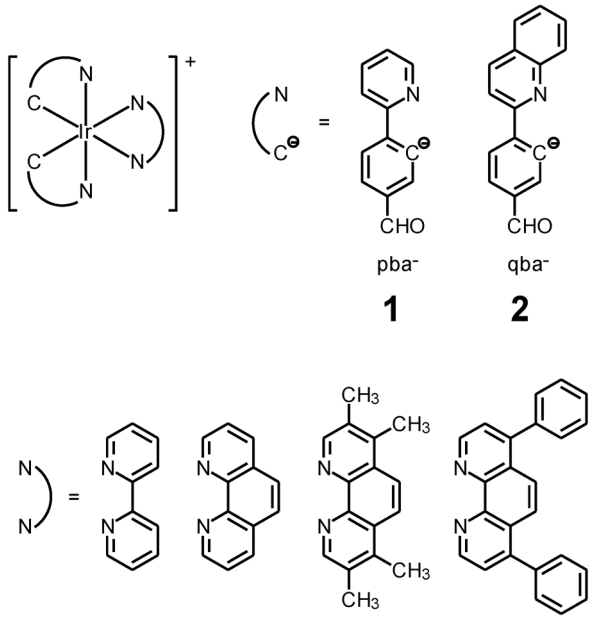
plexes; for example, the cyclometalated complex, $[Ir(ppy)_3]$ (Hppy = 2-phenylpyridine) shows ^{3}MLCT emission [6], whereas the coordination complexes $[Ir(bpy)_3]^{3+}$ (bpy = 2,2'-bipyridine), $[Ir(phen)_3]^{3+}$ (phen = 1,10-phenanthroline), and $[Ir(tpy)_2]^{3+}$ (tpy = 2,2':6',2"-terpyridine) are typical triplet intraligand (^{3}IL) emitters [7]. For the mixed-ligand cyclometalated iridium(III) polypyridine complexes, ^{3}MLCT, triplet ligand-to-ligand charge-transfer (^{3}LLCT), and sigma-bond-to-ligand charge-transfer (^{3}SBLCT) emissive states have been identified [8–11]. These different excited states render iridium(III) polypyridine complexes to show interesting emission with a range of emission wavelengths, quantum yields, and lifetimes [12]. For example, whilst common ruthenium(II) and osmium(II) polypyridines emit in the red or NIR region, and rhenium(I) polypyridines from green to red, the emission of iridium(III) complexes can occur from the blue to red region, depending on the nature of the ligands. In most cases, the emission lifetimes of these complexes are longer than those of their ruthenium(II), osmium(II), and rhenium(I) counterparts. Most importantly, the photophysical properties of many iridium(III) complexes are highly dependent on their local environment, enabling the development of environment-sensitive chemical and biological probes. In this article, we summarize our recent work on the utilization of luminescent cyclometalated iridium(III) polypyridine complexes as biomolecular and cellular probes.

RESULTS AND DISCUSSION

Covalent biomolecular labels

Since the isothiocyanate and iodoacetamide groups are reactive toward the primary amine and sulfhydryl groups, respectively, many fluorescent organic compounds have been functionalized with these units in the development of biological labels [13]. We have linked these groups to cyclometalated iridium(III) polypyridine complexes and used them as luminescent labels for protein molecules [14]. Upon excitation, all the conjugates emit in the orange to yellow region with a long lifetime. We have also designed a series of iridium(III) bis(pyridylbenzaldehyde) diimine complexes $[Ir(pba)_2(N\hat{}N)]^+$(Hpba = 4-(2-pyridyl)benzaldehyde) (**1**) [15]. All the complexes show yellow intense and long-lived emission with vibronically structured bands at very similar energy. The independence of emission energy on the nature of the diimine indicates an emissive state of ^{3}IL ($\pi \rightarrow \pi^*$) (pba) character. These complexes have been used to cross-link amino acid and proteins through reductive amination of the aldehyde groups. Interestingly, upon the conversion of the aldehyde units to secondary amines, the emissive states of the conjugates become ^{3}MLCT [$d\pi(Ir) \rightarrow \pi^*(N\hat{}N)$] in nature. Thus, the use of various diimine ligands enables tuning of the emission colors of the cross-linked conjugates. Recently, we have replaced the cyclometalating ligand with a more π-conjugated ligand 4-(2-quinolyl)benzaldehyde (Hqba), yielding complexes (**2**) with cross-linking properties [16]. These complexes show orange–yellow ^{3}IL ($\pi \rightarrow \pi^*$) (qba)/^{3}MLCT [$d\pi(Ir) \rightarrow \pi^*$(qba)] emission with indistinguishable energy. They have been used to cross-link the protein bovine serum albumin (BSA). Interestingly, these luminescent qba conjugates, unlike their pba counterparts, show very similar emission energy despite the different diimine ligands. We have ascribed this finding to an emissive state of predominant ^{3}MLCT [$d\pi(Ir) \rightarrow \pi^*$(qba)] character. Additionally, we have studied the cellular-staining properties of these qba complexes and their aldehyde-free counterparts. Confocal microscopy images of human cervix epitheliod carcinoma cells (HeLa) stained by these complexes show intense emission. However, after the cells have been fixed and successively washed with methanol or formaldehyde, only the cells stained with the qba complexes remain emissive, suggestive of covalent modification of the cellular structures by the complexes.



Noncovalent biomolecular probes

The emission properties of cyclometalated iridium(III) polypyridine complexes are very sensitive to their surroundings, such as the hydrophobicity of the local environment, which can be exploited for biosensing applications. We have attached different biologically relevant molecules to iridium(III) polypyridine complexes and used the resultant complexes as luminescent noncovalent probes for specific biological receptors. Binding of the complexes to the proteins leads to a change of the local environment of the complexes, which is reflected by different emission properties of the complexes. Three different examples are described in more detail below.

Estradiol is the most important estrogen that binds to estrogen receptors (ER) and regulates the reproductive and sexual functioning in females [17]. In the first example, we have incorporated an estradiol unit to a bipyridine ligand using a spacer-arm and isolated the complexes $[Ir(N^C)(N^N)]^+$ (HN^C = Hppy, 1-phenylpyrazole Hppz, 7,8-benzoquinoline Hbzq, 2-phenylquinoline Hpq, 2-[(1,1'-biphenyl)-4-yl]benzothiazole Hbsb; N^N = 5-[4-(17α-ethynylestradiolyl)phenyl]-2,2'-bipyridine bpy-est, 4-(*N*-{6-[4-(17α-ethynylestradiolyl)benzoylamino]hexyl}aminocarbonyl)-4'-methyl-2,2'-bipyridine bpy-C6-est) (**3**) [18]. Upon irradiation, these complexes exhibit intense and long-lived ^{3}MLCT $[d\pi(Ir) \rightarrow \pi^*(N^N)]/^3$IL ($\pi \rightarrow \pi^*$) (N^C and N^N) emission. The specific binding of these iridium(III) estradiol complexes to ERα results in emission enhancement (I/I_o ~ 1.3–4.8 fold) and lifetime elongation (τ/τ_o ~ 2.4–7.0 fold), which have been ascribed to a less polar and more rigid local environment of the complex upon the binding event. In order to increase the detection sensitivity, we have used ferricyanide as a quencher to selectively suppress the emission of the free complexes. Results of the emission titration experiments showed that ERα-induced emission enhancement factors (I/I_o) are significantly amplified to ~ 7.7–48.7 fold (Fig. 1).

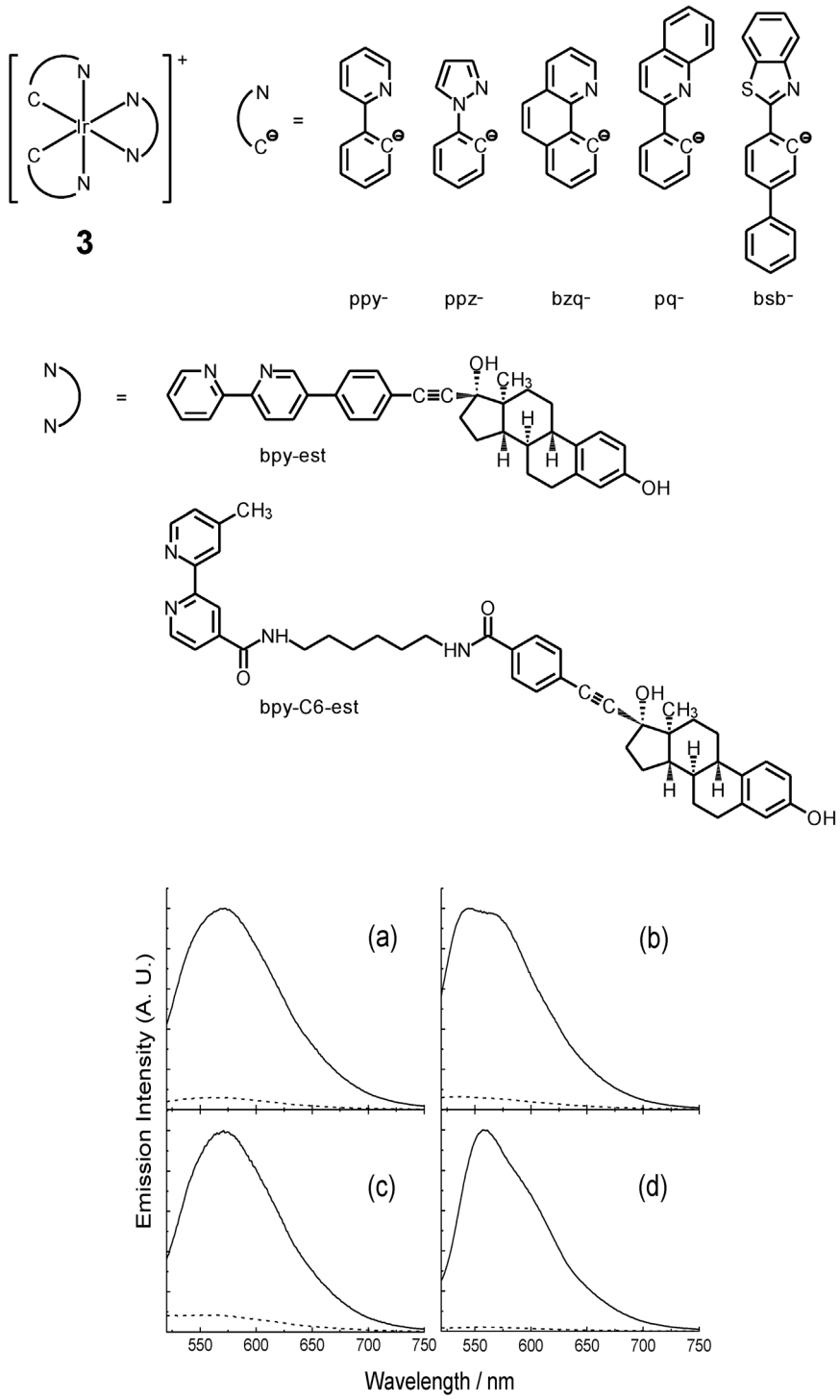
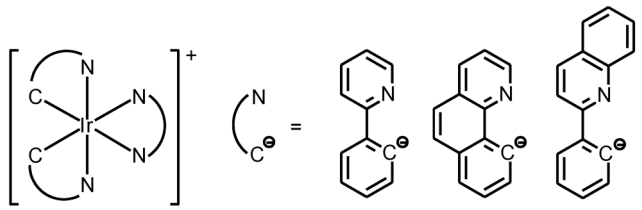


Fig. 1 Emission spectra of $[Ir(N^C)(bpy\text{-}est)]^+$ (N^C = ppy (a), ppz (b), bzq (c) and pq (d)) in the absence (dashed line) and presence (solid line) of ERα (375 nM) in potassium phosphate buffer (50 mM, pH 7.4)/methanol (9:1) at 298 K containing 100 μM $[Fe(CN)_6]^{3-}$. Reproduced from ref. [18], with permission of the Wiley-VCH.

Indole is an interesting molecule as it is the skeleton of many important biomolecules including tryptophan, the plant hormone auxin (indolyl-3-acetic acid), serotonin, and melatonin. Also, there are a large number of indole alkaloids reported to date [19]. Thus, the identification and purification of the biological receptors of indole and its derivatives have received much attention. We isolated luminescent cyclometalated iridium(III) polypyridine complexes appended with an indole moiety (**4**) [20]. In the presence of an indole-binding protein BSA, all the indole complexes show emission enhancement (I/I_o up to 12.6 fold) and lifetime elongation (τ/τ_o up to 2.4 fold). Fitting of the photophysical data obtained from emission titrations indicates that the binding constants are on the order of 10^4 M^{-1}, which is similar to that of unmodified indole [21]. This means that modification of indole by the iridium polypyridine units does not appear to affect the protein-binding affinity of indole.



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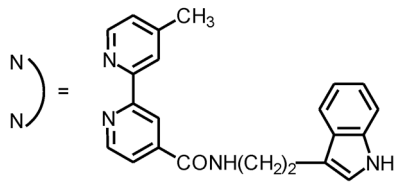
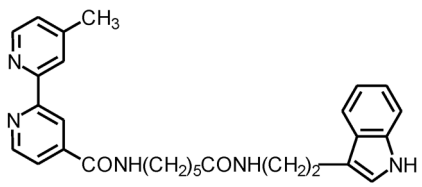
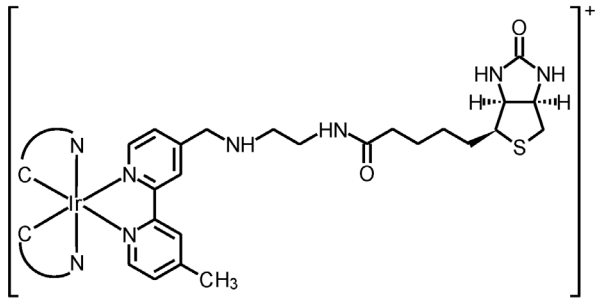




Since biotin (vitamin H) binds to the glycoprotein avidin with a very high affinity [22], the avidin-biotin system has been widely employed in the purification, recognition, and immobilization of biomolecules [23]. Fluorescent organic biotin compounds have been designed and most of them show substantial emission quenching upon binding to avidin [24]. Luminescent transition-metal biotin complexes, in contrast, do not display similar quenching because of their large Stokes shifts [25]. We have isolated a class of luminescent cyclometalated iridium(III) polypyridine complexes containing a biotin moiety $[Ir(N^C)_2(bpy\text{-}CH_2\text{-}NH\text{-}C2\text{-}NH\text{-}biotin)]^+$ (HN^C = Hppy, 2-(4-methylphenyl)pyridine Hmppy, 3-methyl-1-phenylpyrazole Hmppz, Hppz, Hbzq, Hpq; bpy-CH_2-NH-C2-NH-biotin = 4-{*N*-[2-(biotinamido)ethyl]aminomethyl}-4'-methyl-2,2'-bipyridine) (**5**) [26]. Upon excitation, all the complexes show intense and long-lived ^{3}MLCT [$d\pi(Ir) \rightarrow \pi^*$(bpy-$CH_2$-NH-C2-NH-biotin)] emission in fluid solutions under ambient conditions. Emission titrations show that the complexes display enhanced emission intensities (I/I_o ~ 1.5–3.3 fold) and longer emission lifetimes (τ/τ_o ~ 1.5–3.3 fold) upon binding to avidin, which have been ascribed to the increased hydrophobicity and rigidity of the local environments of the complexes after their binding to avidin. We have also designed another class of biotin complexes $[Ir(N^C)_2(bpy\text{-}CO\text{-}NH\text{-}C6\text{-}NH\text{-}biotin)]^+$ (HN^C = 2-phenylbenzothiazole Hbt, Hbsb, 2-(2-thienyl)benzothiazole Hbtth, 2-(1-naphthyl)benzothiazole Hbsn; bpy-CO-NH-C6-NH-



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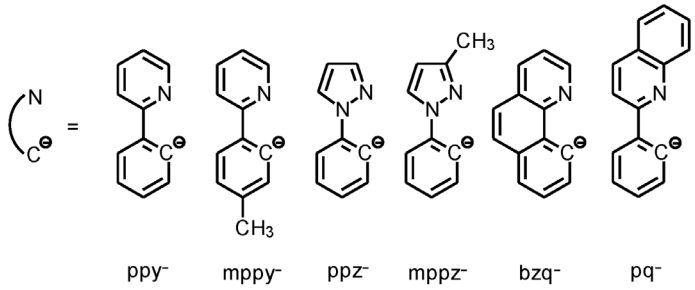
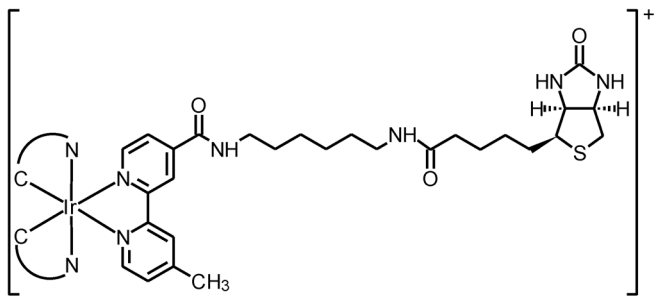


biotin = 4-{*N*-[(6-biotinamido)hexyl]aminocarbonyl}-4'-methyl-2,2'-bipyridine) with arylbenzothiazoles as the cyclometalating ligand (**6**) [27]. The very long-lived excited state and rich structural features of the emission spectra indicate that these complexes are typical ^{3}IL ($\pi \rightarrow \pi^*$) (N^C) emitters (Fig.



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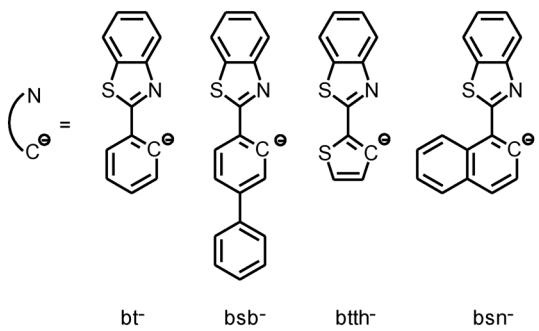


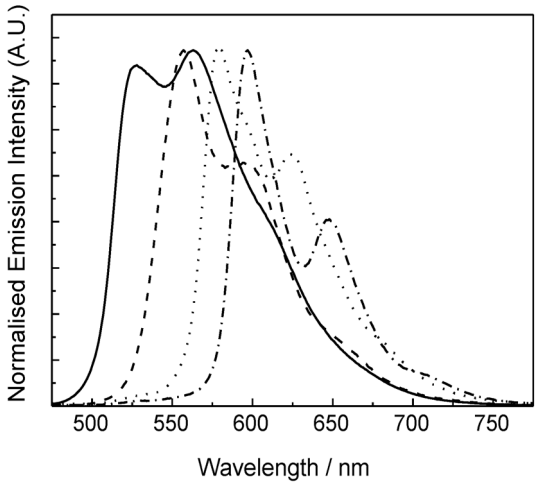
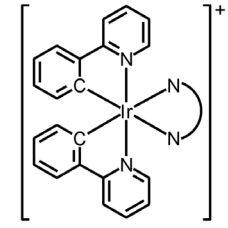


Fig. 2 Emission spectra of $[Ir(N^C)_2(bpy\text{-}CO\text{-}NH\text{-}C6\text{-}NH\text{-}biotin)]^+$ (N^C = bt (solid line), bsb (dashed line), btth (dotted line) and bsn (dash-dotted line)) in degassed CH_2Cl_2 at 298 K. Reproduced from ref. [27], with permission of the American Chemical Society.

2). As expected, these complexes display enhanced emission intensities and longer emission lifetimes upon binding to avidin. Most importantly, the more hydrophobic bsb and bsn complexes reveal more significant emission enhancement (~ 8.1 and 5.8 fold, respectively). Additionally, we have synthesized two luminescent biotin complexes containing the dipyrido[3,2-*f*:2',3'-*h*]quinoxaline (dpq) and dipyrido[3,2-*a*:2',3'-*c*]phenazine (dppz) ligands (**7**) [28]. These complexes display long-lived green to orange luminescence in nonpolar aprotic solvents, but become non-emissive in aqueous buffer due to



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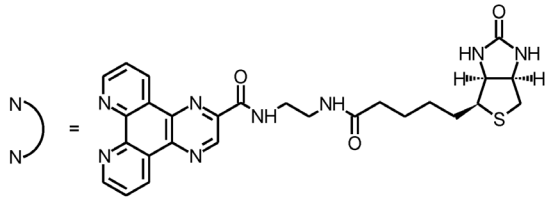
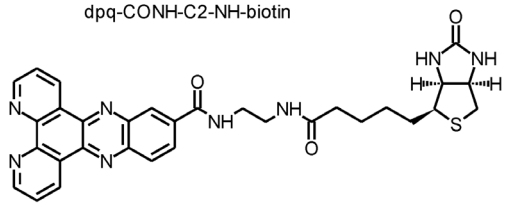


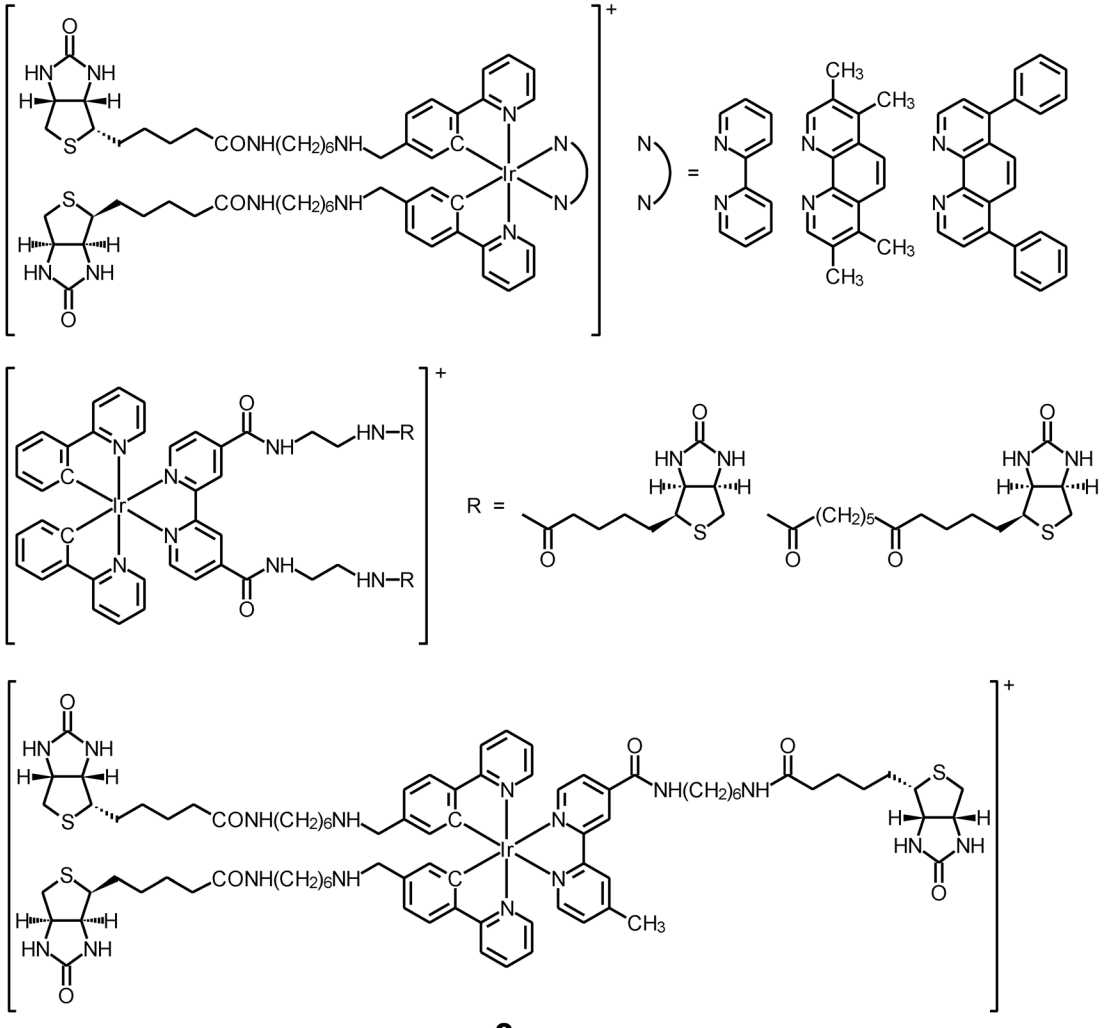
dpq-CONH-C2-NH-biotin



dppz-CONH-C2-NH-biotin

hydrogen-bonding interaction between the quinoxaline/phenazene nitrogen atoms (and amide) and water molecules. Addition of avidin to the buffer solution of the complexes results in the appearance of an emission band at 490 and 520 (sh) nm, which is assigned to a ^{3}IL ($\pi \rightarrow \pi^*$) (N^N) excited state. At [Ir]:[avidin] = 4, the emission intensities of the iridium(III) dpq and dppz biotin complexes at 490 nm are increased by ~ 31 and 8 fold, respectively. These results demonstrate that suppressing the emission intensity of the free complexes can yield larger avidin-induced emission enhancement.

Luminescent multibiotin complexes are attractive biological tools as they can serve as cross-linkers for avidin. This would allow the design of new avidin-biotin assays with enhanced sensitivity, as the number of luminescent units can be significantly increased [29]. We have synthesized and characterized luminescent cyclometalated iridium(III) polypyridine bis- and tris-biotin complexes (**8**), which can effectively cross-link avidin to give luminescent protein dimers, trimers, and oligomers [30,31]. Microsphere assays (involving avidin-coated green-fluorescent microspheres) and size-exclusion high-performance liquid chromatography (HPLC) analyses reveal that the degree of avidin cross-linking is similar among the bis-biotin complexes, but considerably higher for the tris-biotin complex. In another experiment, avidin-coated non-fluorescent microspheres are successively incubated with a



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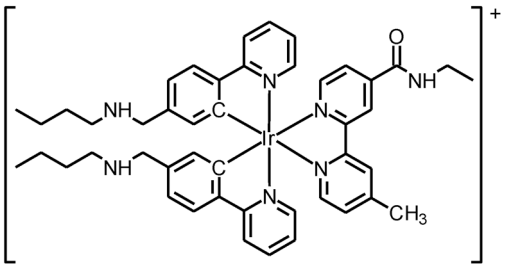
luminescent iridium(III) bis-biotin complex, with stringent washing steps between each incubation. The emission intensity of the microspheres, as measured by confocal microscopy, is directly proportional to the number of layers of metal complex immobilized. This suggests that these luminescent multibiotin complexes and avidin system can yield amplified emission intensity on a solid phase. We believe that single-stranded DNA and an antigen can also be immobilized similarly. The combined use of biotinylated DNA and a biotinylated antibody, respectively, with these luminescent multibiotin complexes and avidin would lead to the development of ultrasensitive heterogeneous assays for DNA hybridization and immunoreactions.

In the search for new luminescent systems, we have discovered a novel dual-emissive iridium(III) polypyridine complex $[Ir(ppy\text{-}CH_2\text{-}NH\text{-}C_4H_9)_2(bpy\text{-}CONH\text{-}C_2H_5)]^+$ (ppy-CH_2-NH-C_4H_9 = 2-{4-[(*N*-*n*-butyl)aminomethyl]phenylpyridine}; bpy-$CONH\text{-}C_2H_5$ = 4-ethylaminocarbonyl-4'-methyl-2,2'-bipyridine) (**9**) [32]. This complex, containing a secondary amine in the cyclometalating ligand and an amide group in the diimine ligand, shows dual emission in fluid solutions, with a high-energy (HE) structured band at ~ 495–500 nm (τ_o ~ 1.06–2.48 μs) and a low-energy (LE) broad band/shoulder at ~ 593–619 nm (τ_o ~ 0.09–0.28 μs) (Fig. 3). In degassed CH_2Cl_2, the emission intensity of the LE band is higher than that of the HE band, whereas in aqueous buffer the spectrum is dominated by the HE band. On the basis of the emission wavelengths, spectral profiles, and lifetimes, the HE band has been tentatively assigned to a ^{3}IL ($\pi \rightarrow \pi^*$) (ppy-CH_2-NH-C_4H_9) excited state, and the LE feature to an excited state with high ^{3}CT [$d\pi$(Ir)/π(ppy)/amine $\rightarrow \pi^*$(bpy-$CONH\text{-}C_2H_5$)] character. In view of the environment-dependent emission profile of this complex, biotin, estradiol, and an octadecyl chain have been covalently linked to this dual-emissive system. Upon binding to their specific biological hosts (i.e., avidin and ERα, respectively), the biotin and estradiol complexes show a decrease in the HE band emission intensity and an increase in the LE band intensity. These changes are reasonable as the local environments of the complexes become less polar. Interestingly, the octadecyl complex displays a predominant LE band even in aqueous buffer. This has been ascribed to wrapping of the iridium(III) polypyridine core with the hydrophobic C18 chain, leading to a very nonpolar local surrounding. Upon binding of the aliphatic chain to a lipid-binding protein human serum albumin (HSA), the complex is unwrapped and the iridium polypyridine core is more exposed to the polar buffer, giving rise to a significant increase in the HE emission intensity. This interesting environment-dependent emission profile enables the C18 complex to function as a luminescent probe for the lipid bilayer of vesicles.



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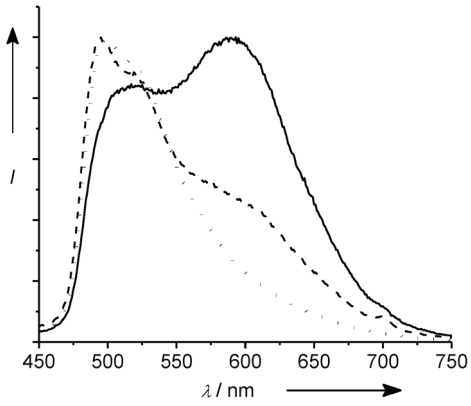


Fig. 3 Normalized emission spectra of $[Ir(ppy\text{-}CH_2\text{-}NH\text{-}C_4H_9)_2(bpy\text{-}CONH\text{-}C_2H_5)]^+$ in degassed CH_2Cl_2 (solid line), CH_3CN (dashed line) and phosphate buffer (dotted line) at 298 K. Reproduced from ref. [32], with permission of the Wiley-VCH.

Cellular probes

In the past few years, there has been a fast-growing interest in the cellular uptake properties and cytotoxicity of luminescent transition-metal complexes [33]. The important results are expected to contribute to the development of effective cellular imaging probes and therapeutic agents. We have extended the studies of our luminescent cyclometalated iridium(III) polypyridine systems with an emphasis on their possible exploitation as cellular probes for in vivo applications. The most important considerations in these studies are the cellular uptake efficiency, intracellular transport and subsequent localization, organelle-specificity, and cytotoxicity of these complexes.

We have examined the lipophilicity and studied the cytotoxicity and cellular uptake properties of the iridium(III) polypyridine indole (**4**) [20] and biotin (**8**) [31] complexes described above using HeLa as a model cell line. We found that the lipophilicity of the complexes is strongly influenced by the relatively hydrophobic indole and hydrophilic biotin units. The lipophilicity also has a direct effect on the cytotoxicity of the complexes; for example, the IC_{50} values of the lipophilic indole complexes range from 1.1 to 6.3 μM, whereas the hydrophilic biotin complexes are basically noncytotoxic (IC_{50} > 400 μM). It is likely that the high cytotoxicity of the indole complexes originates from more efficient cellular uptake by the HeLa cells (due to the high lipophilicity of the complexes), which subsequently leads to extensive staining and labeling of subcellular structures [31]. Indeed, confocal microscopy images confirm that indole complexes are heavily localized in the perinuclear region after internalization (Fig. 4). Additionally, temperature-dependence experiments reveal that the cellular uptake is an energy-requiring process, which is probably endocytosis. In the case of the biotin complexes, emissive cytoplasmic granules are observed in the confocal microscopy images. This should be a result of endosomal labeling and/or aggregation of the luminescent complexes inside the cells. Similar endosomal staining is also exhibited by another luminescent iridium(III) biotin complex that has been conjugated to a BSA molecule [34]. Interestingly, the microscopy images of HeLa cells treated with one of the bioconjugates and an endocytic marker reveal partial overlap, pointing to the involvement of endosomes in the intracellular trafficking of the luminescent bioconjugate.

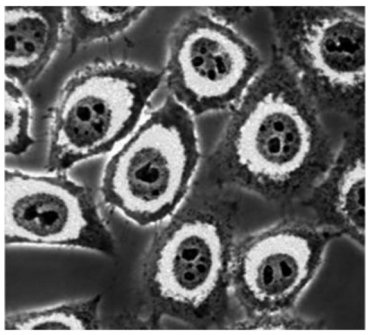
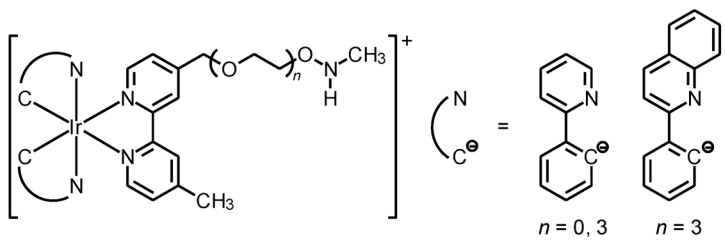


Fig. 4 Laser-scanning confocal microscopy image of live HeLa cells incubated with $[Ir(pq)_2(bpy\text{-}spacer\text{-}indole)]^+$ (5 μM) at 37 °C for 1 h. Reproduced from ref. [20], with permission of the American Chemical Society.

Since reducing sugars, carbohydrates, and glycoproteins play an important role in various biological areas, the development of luminescent labels for these molecules has attracted much interest [35]. The amino-oxy unit has captured our attention as it reacts readily with the aldehyde and ketone groups of reducing sugars with high product yields [36]. Thus, we have designed cyclometalated iridium(III) bipyridine complexes appended with an *N*-methylamino-oxy group (**10**) and utilized them as luminescent labels for reducing sugars including glucose, galactose, lactose, and maltose [37]. While the iridium-galactose conjugate is isolated as both the α and β anomers, the other three iridium sugar conjugates are produced as the β form exclusively. All the sugar conjugates exhibit bright yellow luminescence upon excitation and the emission quantum yields are as high as 85 %. The internalization of the iridium(III) complexes and their sugar conjugates by HeLa cells has been studied by ICP-MS and confocal microscopy, which shows extensive perinuclear staining. Interestingly, the galactose, lactose, and maltose conjugates reveal less efficient cellular uptake and lower cytotoxicity compared to their glucose counterpart. It is likely that glucose transporters (GLUT) on the cell membrane can enhance the transport efficiency of the glucose conjugate.



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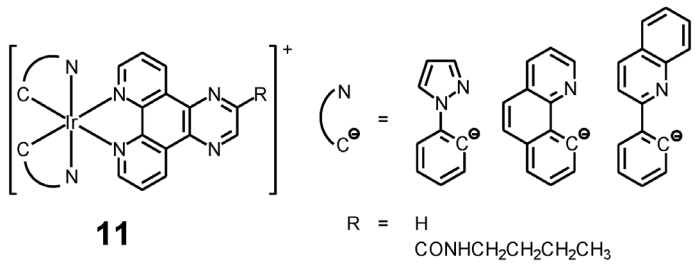
On the basis of our previous work on DNA metallointercalators [28], we have synthesized a related class of cyclometalated iridium(III) complexes coordinated with an extended planar dpq and 2-*n*-butylaminocarbonyldipyrido[3,2-*f*:2',3'-*h*]quinoxaline (dpqa) ligand (**11**) and studied their biological properties [38]. These complexes show intense and long-lived ^{3}MLCT or ^{3}IL emission in organic solvents but become weakly emissive or even non-emissive in aqueous solution. Interestingly, the emission can be turned on by addition of double-stranded DNA or the protein BSA. SDS-PAGE gels containing various proteins are brightly stained by the complexes. Confocal microscopy shows that incubation of Madin-Darby canine kidney (MDCK) cells with the complexes results in staining of subnuclear structures (Fig. 5). Costaining experiments confirm that the labeled organelles are nucleoli.



11

It is interesting to note that nucleolar staining still occurs when cells are fixed with methanol or pretreated with RNase, suggesting that the staining is due to the affinity of the complexes to cellular structures instead of a consequence of active sequestration by the living cells or complex-RNA interactions. Despite the intense DNA-induced emission of these complexes, the nucleoplasm of the MDCK cells is largely unstained. From all these results, we have concluded that the emission observed in the nucleoli originates from nucleolar protein-bound complexes.

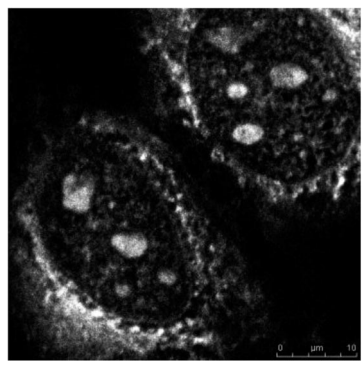
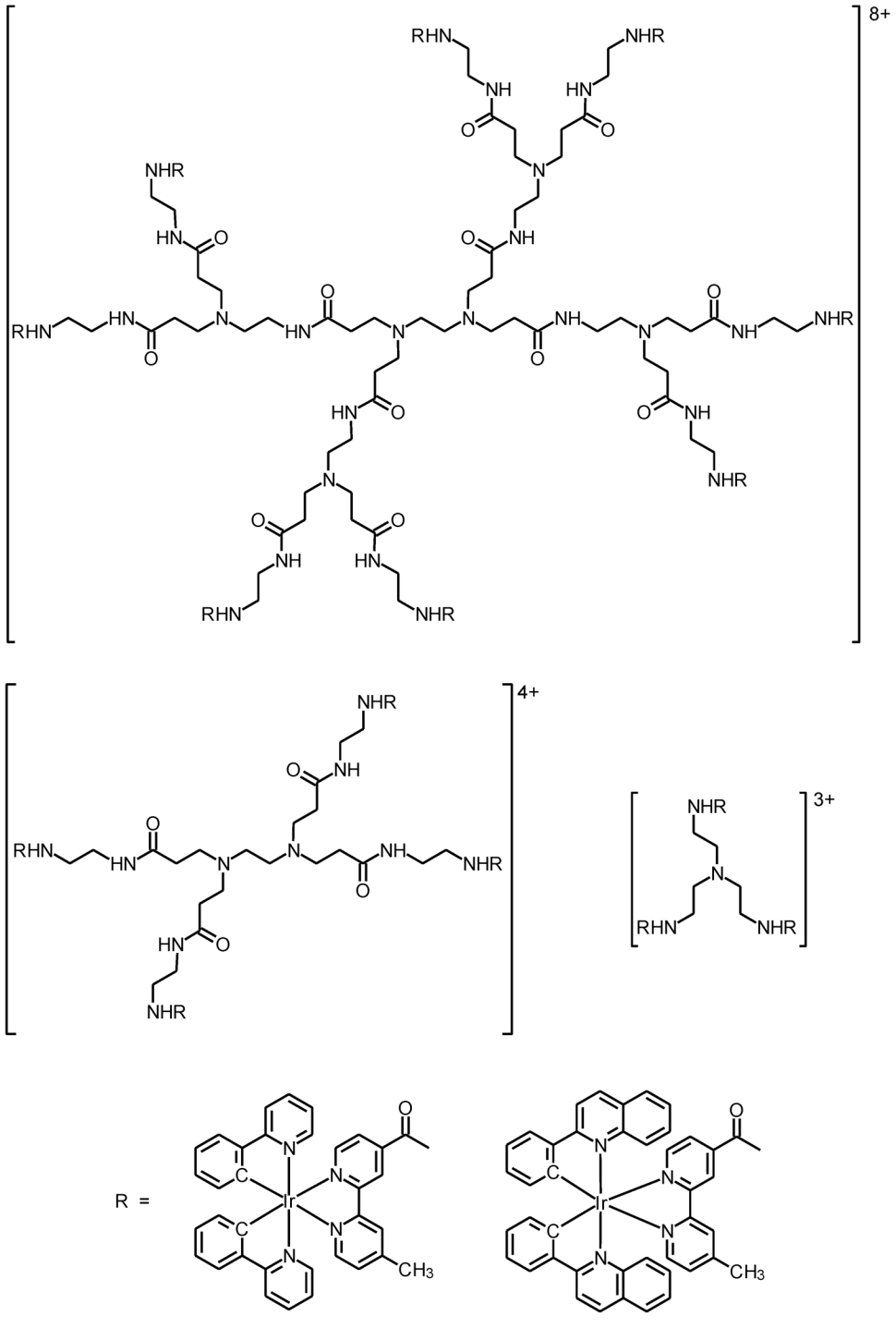


Fig. 5 Laser-scanning confocal microscopy image of fixed MDCK cells treated complex $[Ir(pq)_2(dpq)]^+$ (5 μM, 30 min). Reproduced from ref. [38], with permission of the American Chemical Society.

Owing to the interesting biological properties of dendrimers [39], we have designed a new class of luminescent polynuclear cyclometalated iridium(III) bipyridine complexes with a dendritic skeleton $[\{Ir(N^C)_2(bpy)\}_n(\text{dendritic-core})]^{n+}$ (N^C = ppy, pq; n = 8, 4, 3) (**12**) [40]. Electrochemical and photophysical studies reveal that there are no electronic communications between the iridium bipyridine moieties. Agarose gel retardation assays show that all the dendritic iridium(III) complexes, in contrast to their mononuclear counterparts, exhibit DNA-condensation ability, which leads to the formation of dendriplex. Interestingly, HeLa cells loaded with the monomeric complexes display extensive perinuclear staining, whereas specific organelle staining is observed in the case of the dendritic complexes (Fig. 6). From colocalization studies, these organelles have been identified to be the Golgi apparatus. Results of the lipophilicity, uptake efficiency, and cytotoxicity experiments reveal that the dendritic complexes are internalized essentially by an energy-dependent endocytic process, whilst the mononuclear complexes exhibit an additional passive-diffusion pathway. These different uptake pathways are most likely due to the different formal charge and molecular size of the complexes.



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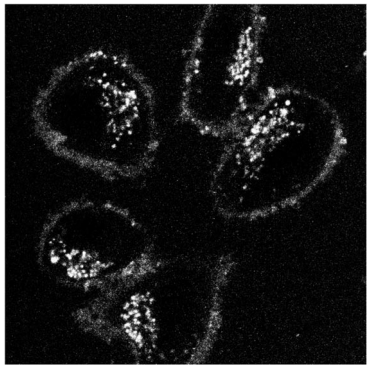
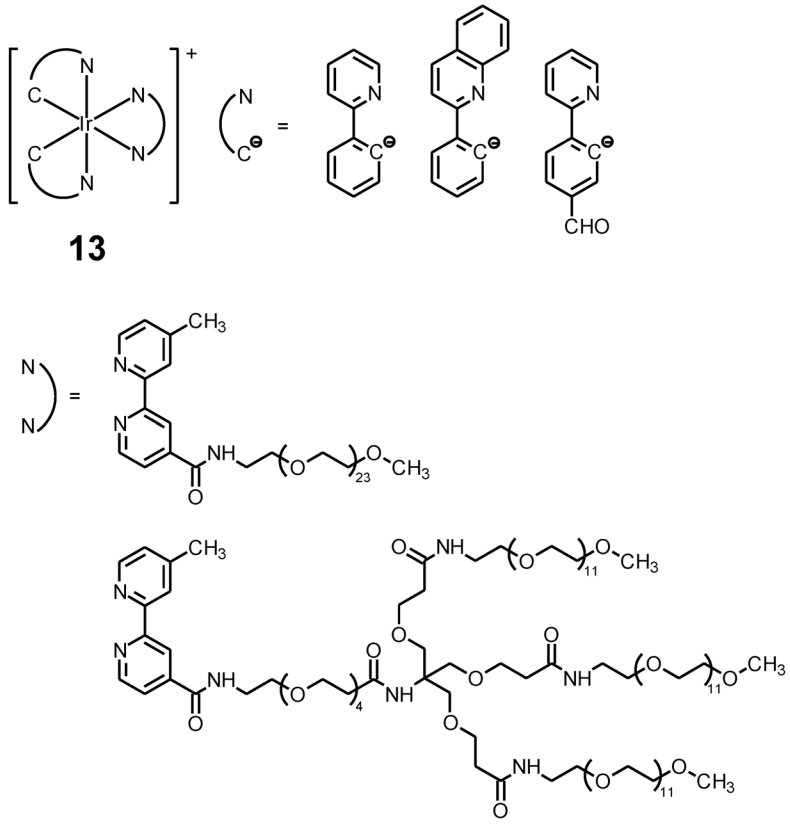


Fig. 6 Laser-scanning confocal microscopy image of live HeLa cells incubated with $[\{Ir(ppy)_2\}_8(bpy\text{-}8)]^{8+}$ (250 nM, 2 h). Reproduced from ref. [40], with permission of the American Chemical Society.

With an aim to improve their water-solubility and biocompatibility, we have modified cyclometalated iridium(III) complexes with discrete poly(ethylene glycol) (PEG) chains [41]. The resultant complexes (**13**) show intense and long-lived emission upon photoexcitation. Unexpectedly, the PEG pendants perturb the emission properties of the complexes in aqueous buffer, which could be due to wrapping of the complex cores by the PEG chains. Importantly, MTT assays using HeLa cells as a model reveal that the IC_{50} values of these PEG complexes (~ 286.5–1180.0 μM) are significantly higher



13

than those of their PEG-free counterparts and cisplatin (~ 4–15 μM). The low cytotoxicity and high water-solubility of these PEG complexes allow them to be highly biocompatible, which has been demonstrated by the fact that HeLa cells remain viable even after treatment with a high concentration of complex (200 μM) for a long incubation time (2 h) in the cellular uptake experiments. No significant cell death is observed in post-staining incubation for another 72 h. The pba-PEG complex can be considered as a novel luminescent PEGylation reagent, which has been used to label BSA and a transfection reagent poly(ethyleneimine) (PEI) to yield luminescent conjugates. We have studied DNA-binding, intracellular delivery, and transfection properties of the PEG-Ir-PEI conjugate by gel retardation assays, particle size measurements, confocal microscopy, and luciferase expression assays. The results conclude that this conjugate serves as a luminescent vector, which is the first of its kind.

CONCLUSIONS

Recent studies have revealed that many luminescent inorganic and organometallic transition-metal complexes possess high structural diversity and rich photophysical properties that can be exploited in the development of probes to determine biological structures and to understand molecular recognition and cellular processes. In particular, it can be seen in this article that luminescent iridium(III) polypyridine complexes are attractive candidates for biological applications owing to their intriguing structural, spectroscopic, and emission properties. We have demonstrated that the water solubility, lipophilicity, cytotoxicity, cellular uptake, and intracellular localization can all be tuned by using various cyclometalating and polypyridine ligands. To further extend the potential of this type of probes, however, basic designs of related complexes with other ligands and functional groups with more advanced properties are still required. We anticipate that luminescent biological sensors and probes derived from luminescent iridium(III) polypyridine complexes with new functions will continue to appear, and they will ultimately become more sensitive and specific sensing molecules for both in vitro and in vivo applications.

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