Dual-Emissive Probes

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Exploitation of the Dual-emissive Properties of Cyclometalated Iridium(III)–Polypyridine Complexes in the Development of Luminescent Biological Probes**

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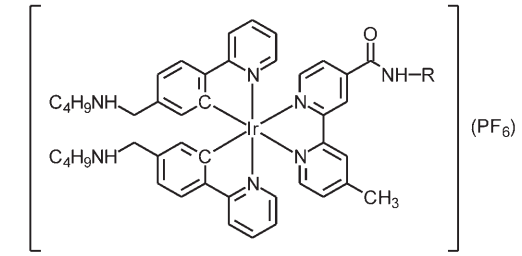
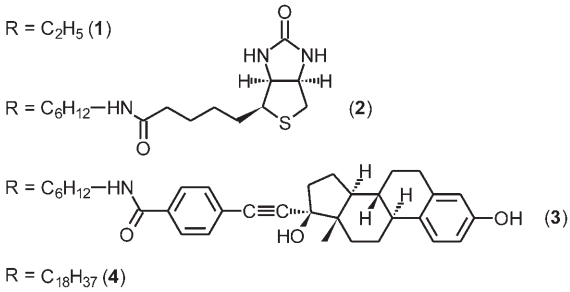
There has been fast-growing interest in utilizing iridium(III)–polypyridine complexes as new luminescent sensors for analytes, including protons,[1] halide ions,[2] metal cations,[3] oxygen,[4] and biomolecules.[5] These complexes display changes in their emission intensities and lifetimes upon analyte binding. Although the emission maxima exhibit small shifts in some cases, the emission profiles and spectral characteristics of the luminescent probes basically remain the same. Compared to common metal-to-ligand charge-transfer (MLCT) emitters, such as the ruthenium(II)– and osmium(II)–polypyridine systems, iridium(III)–polypyridine complexes exhibit emissive states that are very sensitive to their ligands and local environment, resulting in distinct emission features. However, it appears that this behavior has not been utilized in the current array of sensors available. Whilst dual emission is not uncommon for iridium(III)–polypyridine complexes in glass at low temperature, it is very rare in fluid solutions under ambient conditions.[5b,6] We believe that an attractive approach to the development of new iridium(III)-based luminescent probes would be the utilization of novel complexes that display environment-responsive dual-emissive properties.

Herein we report a series of novel dual-emissive cyclometalated iridium(III)–polypyridine complexes that serve as luminescent sensors for various biological receptors. The complex $[Ir(ppy\text{-}CH_2NH\text{-}C_4H_9)_2(bpy\text{-}CONH\text{-}C_2H_5)](PF_6)$ (**1**; $Hppy\text{-}CH_2NH\text{-}C_4H_9$ = 2-(4-(*N*-(*n*-butyl)aminomethyl)phenyl)pyridine; $bpy\text{-}CONH\text{-}C_2H_5$ = 4-(*N*-(ethyl)aminocarbonyl)-4′-methyl-2,2′-bipyridine; Scheme 1) was synthesized from the reaction of the aldehyde complex $[Ir(ppy\text{-}CHO)_2(bpy\text{-}CONH\text{-}C_2H_5)](PF_6)$ ($Hppy\text{-}CHO$ = 4-(2-pyridyl)benzaldehyde) with *n*-butylamine in refluxing methanol, followed by reduction with $NaBH_3CN$. Upon irradiation, **1** exhibited intense and long-lived luminescence (Table 1).





Scheme 1. Structures of complexes **1**–**4**.

Interestingly, it showed dual emission in fluid solutions at room temperature, with a high-energy (HE) structured band at about 500 nm (τ_o = 1.1–2.5 μs) and a low-energy (LE) broad band/shoulder at approximately 593–619 nm (τ_o = 0.1–0.3 μs; Table 1). The possibility of emissive impurities in the samples was excluded on the basis of the characterization data. In degassed nonpolar solvents such as CH_2Cl_2, the emission intensity of the LE band was higher than or comparable to that of the HE band, whilst in more polar solvents such as CH_3CN and CH_3OH, it became much weaker; in aqueous buffer the spectrum was dominated by the HE band (Figure 1).[7] The intensities of both the HE and LE emission features were reduced in aerated solutions, with the former being more sensitive to quenching by oxygen. As a result, the LE band became dominant in aerated solutions, except in the case of aqueous buffer. Addition of trifluoroacetic acid (TFA) to an aerated solution of the complex in CH_2Cl_2 shifted the LE emission band to a shorter wavelength (ca. 574 nm) and the HE feature was eventually embedded into the broad LE band. Interestingly, the amine-free analogue complex $[Ir(ppy)_2(bpy\text{-}CONH\text{-}C_2H_5)](PF_6)$ (**1a**; Hppy = 2-phenylpyridine) did not display dual emission in fluid solutions (Table 1). The only broad band of this complex at around 609–632 nm was insensitive to the presence of TFA and has been assigned to a charge-transfer (CT) state of mixed ^{3}MLCT ($d\pi(Ir)\rightarrow\pi^*(N\text{^}N)$) and ligand-to-ligand

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Table 1: Photophysical data of complexes **1**, **1a**, and **2**–**4** in degassed solvents at 298 K and in alcohol glass at 77 K.

Complex	Medium (T [K])	λ_{em} [nm]	τ_o [μs]	Φ_{em}
1	CH_2Cl_2 (298)	498 sh, 521	1.06	0.11
		593 (max)	0.28	
	CH_3CN (298)	495, 523 sh	1.43	0.065
		612 sh	0.14	
	CH_3OH (298)	498, 523 sh	1.89	0.057
		619 sh	0.09	
	buffer[a] (298)	500, 525 sh	2.48	0.17
	glass[b] (77)	495 sh, 525, 566 sh, 615 sh	5.72	
1a	CH_2Cl_2 (298)	609	0.47	0.12
	CH_3CN (298)	614	0.27	0.061
	CH_3OH (298)	620	0.11	0.038
	buffer[a] (298)	632	0.04	0.0084
	glass[b] (77)	540	3.86	
2	CH_2Cl_2 (298)	494 (max), 523	1.92	0.24
		574	0.57	
	CH_3CN (298)	492, 518 sh	2.06	0.11
		601 sh	0.34	
	CH_3OH (298)	492, 517 sh	2.06	0.089
		603 sh	0.16	
	buffer[a] (298)	492, 517 sh	2.61	0.13
	glass[b] (77)	482, 523 (max), 564 sh	5.20	
3	CH_2Cl_2 (298)	495, 522 sh	1.18	0.061
		600 sh	0.23	
	CH_3CN (298)	490, 520 sh	1.13	0.037
		598 sh	0.12	
	CH_3OH (298)	492, 518 sh	1.53	0.028
		612 sh	0.09	
	buffer[a] (298)	496, 523 sh	1.74	0.072
	glass[b] (77)	481 sh, 524, 567 sh, 617 sh	5.71	
4	CH_2Cl_2 (298)	491 (max), 521	1.73	0.14
		596	0.35	
	CH_3CN (298)	491, 521 sh	1.98	0.093
		610 sh	0.24	
	CH_3OH (298)	492, 521 sh	2.14	0.064
		622 sh	0.11	
	buffer[a] (298)	494, 521 sh	2.42	0.13
	glass[b] (77)	482, 523 (max), 569 sh, 615 sh	5.30	

[a] Potassium phosphate (50 mM, pH 7.4) containing 30% DMSO. [b] EtOH/MeOH (4:1 v/v).

charge-transfer ^{3}LLCT (ppy→π*(N^N)) character. On the basis of the spectral profiles, emission wavelengths, and lifetimes of the two bands of **1**, we have tentatively assigned 1) the HE band to a triplet intraligand ^{3}IL (π→π*)(N^N or N^C) excited state, and 2) the LE feature to an excited state with high ^{3}MLCT/^{3}LLCT character. The latter should also possess substantial triplet amine-to-ligand charge-transfer ^{3}NLCT (amine→π*(N^N)) character in view of the pH-dependent emission energy and the electron-donating properties of the secondary amine of the cyclometalating ligand.[8] The emission spectra of **1** and **1a** in glass at 77 K featured vibronically structured and broad bands, respectively, thus indicating that the emission originates from ^{3}IL and ^{3}CT states, respectively. Our reasons for using derivatives of **1** as luminescent biosensors are illustrated in the following examples.

Biotin binds to the glycoprotein avidin with a very high affinity, and the biotin–avidin interaction has been widely utilized in immunology, in situ hybridization, and affinity chromatography.[9] We have reported luminescent conjugates formed between transition-metal complexes and biotin that show enhanced emission and longer lifetimes upon binding to avidin.[10] In this study, we have used an iridium(III)–biotin complex [Ir(ppy-CH_2NH-C_4H_9)$_2$(bpy-CONH-C6-NH-biotin)](PF_6) (**2**; Scheme 1). Similar to **1**, this complex exhibited dual emission in organic solvents, with only one structured HE band at about 492 nm in buffer at 298 K under both degassed and aerated conditions. The photophysical data are summarized in Table 1. The binding of **2** to avidin has been confirmed by the 2-(4′-hydroxyazobenzene)benzoic acid (HABA) assay. Remarkably, in the presence of avidin, the HE emission band of the complex in aerated buffer displayed a 52% decrease in intensity (τ increased from 0.54 to 0.91 μs) whilst a new LE shoulder at about 608 nm ($\tau = 66$ ns) appeared in the spectrum, thereby resulting in a sharp isoemissive point at 574 nm (Figure 2). Similar changes were not observed using **1** or when unmodified biotin was present in excess from the outset. In contrast to the avidin-induced emission enhancement of other luminescent transition-metal–biotin systems,[10] **2** showed a decrease in its HE emission intensity and the development of an LE emission feature

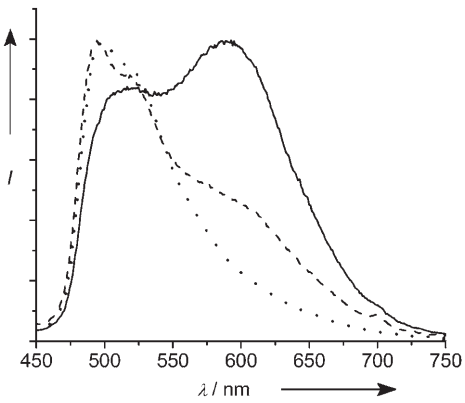

Figure 1. Normalized emission spectra of **1** in degassed CH_2Cl_2 (——), CH_3CN (-----), and phosphate buffer (•••••) at 298 K.

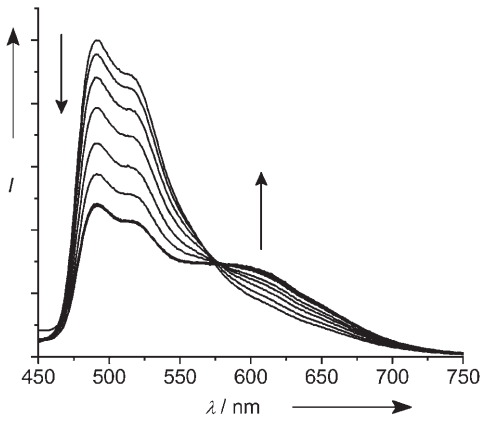

Figure 2. Emission spectra of **2** in aerated phosphate buffer at 298 K upon addition of avidin.

upon binding to the protein. On the basis of the dual-emissive properties of this complex, this observation has been attributed to the increased hydrophobicity of the local environment of the complex upon protein binding.

The second example is an iridium(III)–estradiol complex $[Ir(ppy\text{-}CH_2NH\text{-}C_4H_9)_2(bpy\text{-}CONH\text{-}C6\text{-}Ph\text{-}est)](PF_6)$ (**3**; Scheme 1) that showed similar dual-emissive properties. This complex displayed a structured HE band at 496 nm in degassed buffer (Table 1). However, under aerated conditions, it exhibited an additional LE band of comparable intensity at around 580 nm, as a result of the more efficient quenching of the HE band by oxygen (Figure 3). We reason

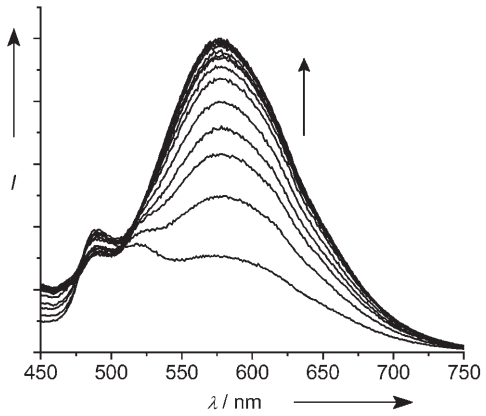

Figure 3. Emission spectra of **3** in aerated phosphate buffer at 298 K upon addition of ERα.

that in such a highly polar environment, the hydrophobic estradiol group approaches the complex core, thereby creating a local hydrophobic region which results in the appearance of the LE emission band. Upon addition of estrogen receptor α (ERα), the natural biological host for estradiol, the LE band showed an approximately 3.3-fold increase in the emission intensity (Figure 3). The lifetimes of the HE and LE bands increased from 0.63 μs and 56 ns to 0.84 μs and 0.21 μs, respectively. A control experiment using **1** did not give similar changes, which indicates that the observation resulted from the specific binding of **3** to a hydrophobic pocket of ERα. Although metal complexes that show ERα-induced emission enhancement have been reported,[5c,11] **3** is the first probe that exhibits a change in the emission profile upon binding to ERα as a consequence of the selective enhancement of the LE emission.

Thirdly, we have prepared a hydrophobic complex $[Ir(ppy\text{-}CH_2NH\text{-}C_4H_9)_2(bpy\text{-}CONH\text{-}C_{18}H_{37})](PF_6)$ (**4**), which contains a C18 chain (Scheme 1). As expected, this complex showed dual-emissive properties (Table 1). Similar to **3**, in aerated aqueous buffer, **4** displayed dual emission with an LE emission band at about 593 nm ($\tau = 0.12$ μs), whose intensity was even higher than that of the HE band ($\tau = 0.55$ μs; Figure 4). This LE emission feature has been attributed to the wrapping of the complex by the very hydrophobic octadecyl chain in the highly polar buffer medium. We have examined the interaction of **4** with the lipid-binding protein human serum albumin (HSA).[12] Upon addition of this protein, the

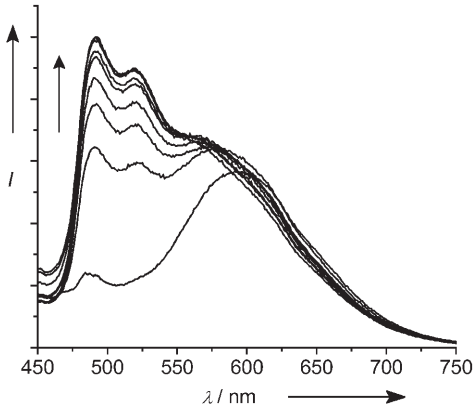

Figure 4. Emission spectra of **4** in aerated phosphate buffer at 298 K upon addition of HSA.

intensity of the HE emission band at about 492 nm increased approximately 4.4 fold ($\tau = 1.22$ μs), whereas the LE emission band did not show any difference and eventually became a shoulder at about 576 nm ($\tau = 0.19$ μs) (Figure 4). This was not observed using denatured albumin samples, thus revealing that the complex interacted only with the intact protein. It is conceivable that the octadecyl chain of the complex binds to the hydrophobic lipid-binding cavity of the protein, thereby increasing the exposure of the iridium(III)–polypyridine to the polar buffer and leading to the predominant HE emission. Interestingly, a similar emission profile (I_{HE}/I_{LE} = ca. 1.3:1) was observed when β-cyclodextrin (β-CD), which binds long aliphatic chains strongly, was added to an aerated solution of the complex in buffer. Additionally, we have prepared vesicles from 1,2-distearoyl-*sn*-glycero-3-phosphocholine (DSPC) and **4**. In degassed buffer, the vesicle sample exhibited strong orange-yellow emission as a result of a strong LE band at approximately 587 nm; the HE band at about 504 nm was much less intense (I_{HE}/I_{LE} = ca. 0.3:1). This observation is in sharp contrast to the green emission of the complex ($\lambda_{em} = 494$ nm) in degassed buffer without the vesicles (Figure 5). These findings indicate that **4** is localized in the hydrophobic region of the vesicles and would serve as an excellent probe for lipid bilayers, micelles, and lipoproteins.

Figure 5. Solutions of **4** only (left) and incorporated into DSPC vesicles (right) in degassed phosphate buffer at 298 K upon irradiation.

In summary, we have discovered a new class of luminescent cyclometalated iridium(III)–polypyridine complexes that show interesting environment-responsive dual emission. The use of these complexes as biosensors is not limited; incorporation of biological substrates into this system will lead to new luminescent probes that would display pronounced changes in their emission profiles and spectral characteristics upon binding to their specific receptors.

Experimental Section

A mixture of $[Ir(ppy\text{-}CHO)_2(bpy\text{-}CONH\text{-}C_2H_5)](PF_6)$ (0.11 mmol), *n*-butylamine (0.44 mmol), and triethylamine (200 μL) in methanol (50 mL) was heated to reflux under an inert atmosphere of nitrogen in the dark for 2 h. After the solution was cooled to room temperature, solid $NaBH_3CN$ (1.76 mmol) was added to the solution. The solution was stirred under an inert atmosphere of nitrogen for 2 h and then evaporated to dryness to give a yellow solid. The solid was dissolved in CH_2Cl_2 (50 mL) and the solution was washed with distilled water (30 mL × 3). The CH_2Cl_2 layer was collected, dried over anhydrous $MgSO_4$, and evaporated to dryness to give an orange solid. The product was recrystallized from CH_2Cl_2/diethyl ether to give **1** as orange crystals in 81 % yield. ^{1}H NMR (300 MHz, [D$_6$]acetone, 298 K, relative to Me_4Si): $\delta = 9.06$ (s, 1H, H3 bpy), 8.81 (s, 1H, H3′ bpy), 8.21–8.16 (m, 4H, bpy-4-CON*H*, H6 bpy, H3 pyridyl ring ppy), 7.97–7.91 (m, 5H, H6′ bpy, H4 pyridyl ring, H6 phenyl ring ppy), 7.86–7.64 (m, 3H, H5 bpy, H6 pyridyl ring ppy), 7.55 (d, $J = 5.6$ Hz, 1H, H5' bpy), 7.11 (dt, $J = 6.2$, 1.2 Hz, 2H, H5 pyridyl ring ppy), 7.03 (d, $J =$ 7.9 Hz, 2H, H5 phenyl ring ppy), 6.38 (s, 1H, H3 phenyl ring ppy), 6.35 (s, 1H, H3 phenyl ring ppy), 3.50–3.39 (m, 4H, ppy-4-CH_2NH), 2.63 (s, 3H, CH_3 bpy), 2.43–2.32 (m, 6H, NHCH_2CH$_3$, NHCH_2C$_2$H$_4$CH$_3$), 1.41–1.17 (m, 8H, NHCH$_2$C$_2$$H_4CH_3$), 0.85 ppm (t, $J = 7.1$ Hz, 9H, NHCH$_2$CH_3, NHCH$_2$C$_2$H$_4$CH_3); IR (KBr): $\tilde{\nu} =$ 3432 (N-H), 1653 (C=O), 846 cm^{-1} (PF_6^-); positive-ion ESI-MS ion clusters at *m*/*z* 911 $[M-PF_6]^+$; elemental analysis calcd (%) for $IrC_{46}H_{53}N_7OPF_6$: C 52.26, H 5.05, N 9.27; found: C 52.01, H 4.99, N 9.07. The synthetic procedures and characterization data for other complexes, ionic strength dependence and temperature dependence of the emission of **1**, and details of emission titrations and vesicle preparation are included in the Supporting Information.

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[7] The absence of an LE band for **1** in aqueous buffer could be due to the high polarity of the solvent and hydrogen-bonding interactions of the amine moieties with water.
[8] Protonation of the amine by TFA would make it impossible for the ^{3}NLCT to occur. However, the highest occupied molecular orbital (HOMO) also contains some ppy character because of the contribution from the LLCT state. Thus, upon protonation of the electron-donating amine group, the HOMO becomes lower in energy, thereby resulting in a blue-shift of the LE emission band. In contrast, the ppy ligand of **1a** does not possess the secondary amine, and thus the MLCT/LLCT emission is insensitive to the addition of TFA. Another reason for the TFA-induced blue-shift of the LE band of **1** is the increased polarity of the CH_2Cl_2 solution because of the presence of trifluoroacetate ions. Studies on the effects of ionic strength and temperature on the emission of **1** are included in the Supporting Information.
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