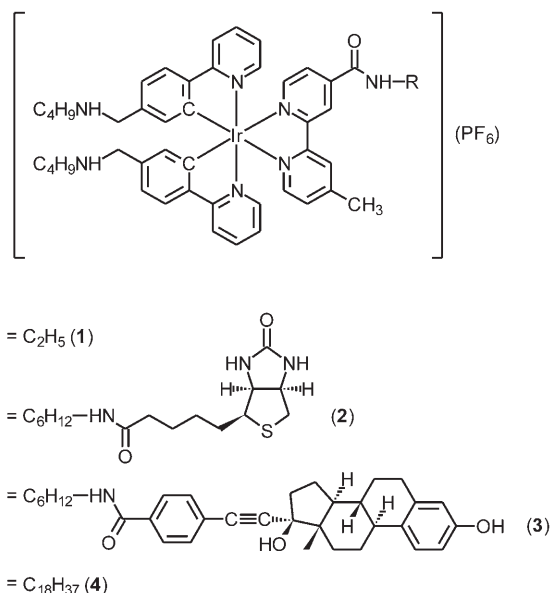


Exploitation of the Dual-emissive Properties of Cyclometalated Iridium(III)–Polypyridine Complexes in the Development of Luminescent Biological Probes**

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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-CH₂NH-C₄H₉)₂(bpy-CONH-C₂H₅)](PF₆) (**1**; Hppy-CH₂NH-C₄H₉ = 2-(4-(*N*-(*n*-butyl)aminomethyl)phenyl)pyridine; bpy-CONH-C₂H₅ = 4-(*N*-(ethyl)aminocarbonyl)-4'-methyl-2,2'-bipyridine; Scheme 1) was synthesized from the reaction of the aldehyde complex [Ir(ppy-CHO)₂(bpy-CONH-C₂H₅)](PF₆) (Hppy-CHO = 4-(2-pyridyl)benzaldehyde) with *n*-butylamine in refluxing methanol, followed by reduction with NaBH₃CN. 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 ($\tau_o = 1.1\text{--}2.5\ \mu\text{s}$) and a low-energy (LE) broad band/shoulder at approximately 593–619 nm ($\tau_o = 0.1\text{--}0.3\ \mu\text{s}$; 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 $[\text{Ir}(\text{ppy})_2(\text{bpy-CONH-C}_2\text{H}_5)](\text{PF}_6)_3$ (**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 $^3\text{MLCT}$ ($d\pi(\text{Ir}) \rightarrow \pi^*(\text{N}^{\wedge}\text{N})$) and ligand-to-ligand

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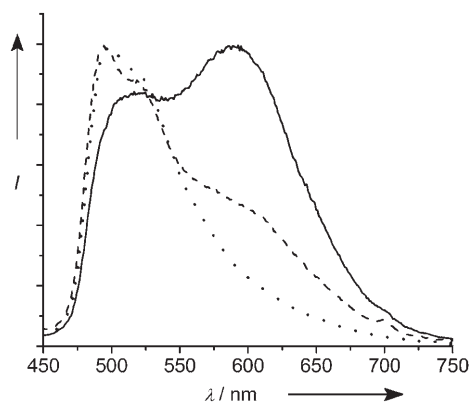
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Table 1: Photophysical data of complexes **1**, **1a**, and **2–4** in degassed solvents at 298 K and in alcohol glass at 77 K.

Complex	Medium (T [K])	λ_{em} [nm]	τ_0 [μ s]	Φ_{em}
1	CH ₂ Cl ₂ (298)	498 sh, 521 593 (max)	1.06 0.28	0.11
	CH ₃ CN (298)	495, 523 sh 612 sh	1.43 0.14	0.065
	CH ₃ OH (298)	498, 523 sh 619 sh	1.89 0.09	0.057
	buffer ^[a] (298)	500, 525 sh	2.48	0.17
	glass ^[b] (77)	495 sh, 525, 566 sh, 615 sh	5.72	
1a	CH ₂ Cl ₂ (298)	609	0.47	0.12
	CH ₃ CN (298)	614	0.27	0.061
	CH ₃ OH (298)	620	0.11	0.038
	buffer ^[a] (298)	632	0.04	0.0084
	glass ^[b] (77)	540	3.86	
2	CH ₂ Cl ₂ (298)	494 (max), 523 574	1.92 0.57	0.24
	CH ₃ CN (298)	492, 518 sh 601 sh	2.06 0.34	0.11
	CH ₃ OH (298)	492, 517 sh 603 sh	2.06 0.16	0.089
	buffer ^[a] (298)	492, 517 sh	2.61	0.13
	glass ^[b] (77)	482, 523 (max), 564 sh	5.20	
3	CH ₂ Cl ₂ (298)	495, 522 sh 600 sh	1.18 0.23	0.061
	CH ₃ CN (298)	490, 520 sh 598 sh	1.13 0.12	0.037
	CH ₃ OH (298)	492, 518 sh 612 sh	1.53 0.09	0.028
	buffer ^[a] (298)	496, 523 sh	1.74	0.072
	glass ^[b] (77)	481 sh, 524, 567 sh, 617 sh	5.71	
4	CH ₂ Cl ₂ (298)	491 (max), 521 596	1.73 0.35	0.14
	CH ₃ CN (298)	491, 521 sh 610 sh	1.98 0.24	0.093
	CH ₃ OH (298)	492, 521 sh 622 sh	2.14 0.11	0.064
	buffer ^[a] (298)	494, 521 sh	2.42	0.13
	glass ^[b] (77)	482, 523 (max), 569 sh, 615 sh	5.30	

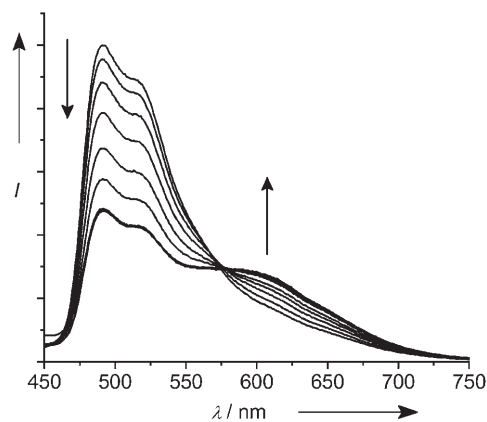
[a] Potassium phosphate (50 mM, pH 7.4) containing 30% DMSO.

[b] EtOH/MeOH (4:1 v/v).

**Figure 1.** Normalized emission spectra of **1** in degassed CH₂Cl₂ (—), CH₃CN (---), and phosphate buffer (.....) at 298 K.

charge-transfer ³LLCT (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 ³IL (π → π*)(N[^]N or N[^]C) excited state, and 2) the LE feature to an excited state with high ³MLCT/³LLCT character. The latter should also possess substantial triplet amine-to-ligand charge-transfer ³NLCT (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 ³IL and ³CT 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-CH₂NH-C₆H₅)₂(bpy-CONH-C₆H₄-NH-biotin)](PF₆) (**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 μ s) whilst a new LE shoulder at about 608 nm (τ = 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 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-CH₂NH-C₄H₉)₂(bpy-CONH-C₆-Ph-est)](PF₆) (**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

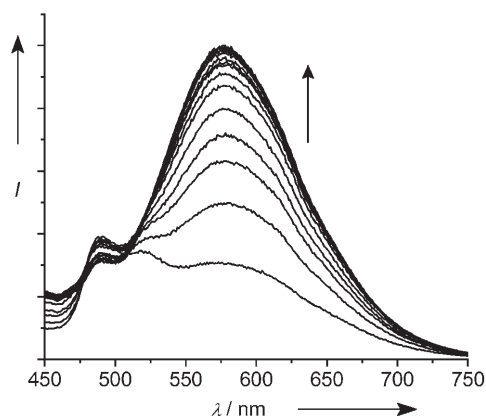


Figure 3. Emission spectra of **3** in aerated phosphate buffer at 298 K upon addition of ER α .

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 (ER α), 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 μ s and 56 ns to 0.84 μ s and 0.21 μ s, 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 ER α . Although metal complexes that show ER α -induced emission enhancement have been reported,^[5c,11] **3** is the first probe that exhibits a change in the emission profile upon binding to ER α as a consequence of the selective enhancement of the LE emission.

Thirdly, we have prepared a hydrophobic complex [Ir(ppy-CH₂NH-C₄H₉)₂(bpy-CONH-C₁₈H₃₇)](PF₆) (**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 (τ = 0.12 μ s), whose intensity was even higher than that of the HE band (τ = 0.55 μ s; 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

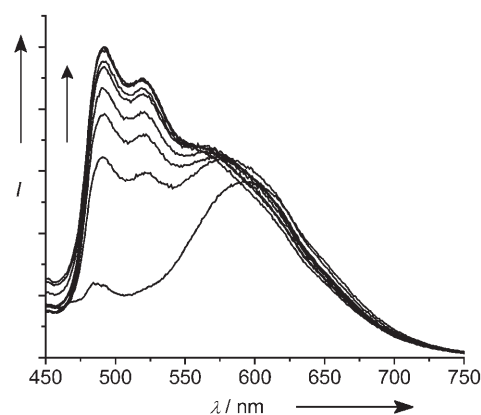


Figure 4. Emission spectra of **4** in aerated phosphate buffer at 298 K upon addition of HSA.

intensity of the HE emission band at about 492 nm increased approximately 4.4 fold (τ = 1.22 μ s), whereas the LE emission band did not show any difference and eventually became a shoulder at about 576 nm (τ = 0.19 μ s) (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 ($I_{\text{HE}}/I_{\text{LE}}$ = 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 ($I_{\text{HE}}/I_{\text{LE}}$ = ca. 0.3:1). This observation is in sharp contrast to the green emission of the complex (λ_{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 $[\text{Ir}(\text{ppy-CHO})_2(\text{bpy-CONH-C}_6\text{H}_5)](\text{PF}_6)_3$ (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 $\times$ 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. ^1H NMR (300 MHz, $[\text{D}_6]\text{acetone}$, 298 K, relative to Me_4Si): δ = 9.06 (s, 1 H, H3 bpy), 8.81 (s, 1 H, H3' bpy), 8.21–8.16 (m, 4 H, bpy-4-CONH, H6 bpy, H3 pyridyl ring ppy), 7.97–7.91 (m, 5 H, H6' bpy, H4 pyridyl ring, H6 phenyl ring ppy), 7.86–7.64 (m, 3 H, H5 bpy, H6 pyridyl ring ppy), 7.55 (d, J = 5.6 Hz, 1 H, H5' bpy), 7.11 (dt, J = 6.2, 1.2 Hz, 2 H, H5 pyridyl ring ppy), 7.03 (d, J = 7.9 Hz, 2 H, H5 phenyl ring ppy), 6.38 (s, 1 H, H3 phenyl ring ppy), 6.35 (s, 1 H, H3 phenyl ring ppy), 3.50–3.39 (m, 4 H, ppy-4- CH_2NH), 2.63 (s, 3 H, CH_3 bpy), 2.43–2.32 (m, 6 H, NHCH_2CH_3 , $\text{NHCH}_2\text{C}_2\text{H}_4\text{CH}_3$), 1.41–1.17 (m, 8 H, $\text{NHCH}_2\text{C}_2\text{H}_4\text{CH}_3$), 0.85 ppm (t, J = 7.1 Hz, 9 H, NHCH_2CH_3 , $\text{NHCH}_2\text{C}_2\text{H}_4\text{CH}_3$); 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 $[\text{M}-\text{PF}_6]^+$; elemental analysis calcd (%) for $\text{IrC}_{46}\text{H}_{53}\text{N}_7\text{O}_2\text{PF}_6$: 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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