Cyclometalated Iridium-Coumarin Ratiometric Oxygen Sensors: Improved Signal Resolution and Tunable Dynamic Ranges

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Abstract. In this work we introduce a new series of ratiometric oxygen sensors for hypoxic environments based on phosphorescent cyclometalated iridium centers partnered with organic coumarin fluorophores. Three different cyclometalating ligands and two different pyridyl-containing coumarin types were used to prepare six target complexes with tunable excited-state energies. Some of the complexes exhibit only phosphorescence originating from the cyclometalated Ir moiety, as a result of excited-state energy transfer from the coumarin to the Ir-centered excited states. Three of the complexes display dual emission, with fluorescence arising from the coumarin ligands and phosphorescence from the cyclometalated iridium synthons, and hence function as ratiometric oxygen sensors. Oxygen quenching experiments on these complexes demonstrate that the iridium centered phosphorescence is quenched under \( \ce{O_2} \) while fluorescence is unaffected. These sensors have good signal resolution, and the sensitivity and dynamic range, measured with Stern-Volmer analysis, span two orders of magnitude. This work demonstrates that this simple, modular approach for conjoining fluorescent and phosphorescent molecules can produce effective oxygen sensors with a wide range of attributes.

Introduction

Chemical and biochemical mechanisms of aerobic metabolism largely depend on molecular oxygen.\(^1\)\(^-\)\(^3\) Tumor hypoxia is associated with a variety of common diseases\(^4\) and therefore the sensing of triplet oxygen has been a recent area of interest.\(^5\)\(^-\)\(^10\) The level of molecular oxygen in tumors is a good indicator of metabolic state and can help guide therapy for cancer treatment. Beyond applications in pathology, accurate oxygen sensing with good spatial and temporal resolution is critical for monitoring physiological responses to extreme environments, e.g. subsea, artic, and outer space.\(^11\) Luminescent sensing of oxygen is particularly appealing, where the oxygen concentration is analyzed via the color, intensity, and/or lifetime of photoluminescence. In principle any phosphorescent compound can serve as an oxygen sensor, since molecular triplet states are efficiently quenched by \( \ce{O_2} \).\(^12\) However, phosphorescent compounds give a “turn-off” response to oxygen, so their use requires either accurate measurement of absolute luminescence intensity, which is prone to issues with calibration and reproducibility, resulting from variable excitation power, optical path length, and in biological applications heterogeneous cellular environments.\(^13\) Measurement of the phosphorescence lifetime attenuation works well in principle for sensing oxygen but is more technically complex. Luminescent ratiometric oxygen sensors avoid these limitations by incorporating two emission signals which are differentially modulated by \( \ce{O_2} \), and hence the ratio of the two emission intensities can provide a simple and accurate readout of oxygen concentration.

Classical ratiometric oxygen sensors combine a fluorescent moiety with a molecular phosphor. These constructs have quenched phosphorescence in the presence of \( \ce{O_2} \) while fluorescence is unaffected. Previous designs of ratiometric \( \ce{O_2} \) sensors include quantum dots with phosphorescent metal complexes tethered to the surface\(^14\) and metal-organic frameworks (MOFs) that combine fluorescent and phosphorescent components.\(^15,16\) Polymer nanocomposites are also quite common as ratiometric oxygen sensors; these include polymer beads embedded with both molecular phosphors and fluorophores,\(^17\)\(^-\)\(^20\) fluorescent polymers with covalently attached or physically blended phosphorescent molecules,\(^21,22\) and TADF copolymers where the ratio of prompt to delayed fluorescence is used to measure oxygen.\(^23\) There are also single-component compounds with dual emission and ratiometric oxygen response, such as bimetallic lanthanide complexes from metal-centered excited states\(^24\) and boron clusters\(^25\) or lutetium porphyrin compounds\(^26\) with dual fluorescence/phosphorescence emission.
Among potential phosphorescent molecules for ratiometric sensing applications, cyclometalated iridium complexes, which have dominated the electroluminescence field,\textsuperscript{27,28} have perhaps the most desirable attributes. They are chemically robust, can be engineered to luminesce in any part of the visible or near-infrared spectrum, often with high quantum yields, and their phosphorescence lifetimes (on the order of microseconds) are well-suited for detecting typical atmospheric or physiological concentrations of $O_2$. Nevertheless, only a few previously reported ratiometric oxygen sensors included cyclometalated iridium complexes. Cyclometalated iridium complexes have been linked to conjugated polymers which then function as ratiometric oxygen sensors,\textsuperscript{29} and most relevant to the present work, red-phosphorescent cyclometalated iridium complexes have been paired with blue-fluorescent coumarins to access biocompatible ratiometric $O_2$ sensors.\textsuperscript{5,30} This latter design is effective, although the synthetic strategy involves upwards of 12 steps, owing primarily to the complex polyproline-substituted acetylacetone spacer between the iridium center and the coumarin. One of our primary goals in this area of research has been to design simpler, more generalizable synthetic strategies for preparing ratiometric oxygen sensors featuring cyclometalated iridium. In doing so, it would be much easier to modify the sensor attributes and optimize them for a specific application. The spectral profile, the resolution between the phosphorescence and fluorescence signals, the photoluminescence quantum yield, and the triplet lifetime, which is the critical determinant of the oxygen sensing dynamic range, can all in principle be quickly modified. Our initial foray into this area presented a series of bis-cyclometalated iridium complexes joined with pyridyl-substituted BODIPY fluorophores.\textsuperscript{31} The complexes are prepared in a few simple synthetic steps, the last one involving the generation of a substitutionally labile cyclometalated iridium that “snaps” together with the pyridyl-substituted BODIPY under mild conditions. This same synthetic strategy has been used in our group to access other multi-component photoactive structures, which are not ratiometric sensors but nonetheless are valuable platforms for studying excited-state energy transfer pathways.\textsuperscript{12,33} Some of the Ir-BODIPY compounds prepared in this way functioned as effective ratiometric oxygen sensors, with dynamic ranges that spanned hypoxic levels of $O_2$ ($pO_2 \leq 150$ mmHg).\textsuperscript{31}

In this work, we present two significant advances in the design of ratiometric oxygen sensors featuring cyclometalated iridium, showing that the simple synthetic approach we have developed facilitates modification and optimization of the sensor attributes. One limitation of our first-generation Ir-BODIPY oxygen sensors is a significant overlap between the BODIPY’s green fluorescence and the broad red phosphorescence from the iridium center. This poor signal resolution between the two luminescence channels means the effective ratiometric response never reaches zero, limiting the dynamic range. Here we study complexes that replace the green-fluorescent coumarin with a blue-fluorescent coumarin, and in one such complex we also shift the phosphorescence deeper to the red, achieving greatly improved signal resolution showing no overlap between fluorescence and phosphorescence. This improved signal resolution contributes to a much larger dynamic range for oxygen sensing. A second major discovery in this work, enabled by the ability to quickly “mix and match” organic fluorophores and bis-cyclometalated iridium phosphors, is the preparation of sensors that are sensitive to much lower concentrations of $O_2$. For certain applications it would be desirable to have sensors that show large responses to very low levels of oxygen as opposed to a gradual response over a wider range, and in this work, we show that is possible with some of our coumarin-based sensors. In total, this paper describes the preparation and characterization of six new cyclometalated iridium-coumarin complexes, and the three that show dual luminescence are subjected to an in-depth quantification of their oxygen-sensing attributes in abiological solutions.

Results and Discussion

Synthesis of Ir-Coumarin Complexes

The general synthetic preparation of the complexes is outlined in Scheme 1, and in most cases follows closely with the procedure used to prepare our first-generation Ir-BODIPY sensors.\textsuperscript{31} Three precursor classes were used to prepare the six compounds in this study. Two pyridyl-substituted coumarin compounds, abbreviated C-1 and C-2, were synthesized using known procedures.\textsuperscript{34,35} Chloro-bridged cyclometalated iridium dimers $[Ir(C^\text{N})_2(\mu-\text{Cl})]_2$ (1a–c $C^\text{N} =$
cycloometalating ligand) are ubiquitous precursors accessed by the method of Nonoyama. In all iridium complexes described here, the letter in the numerical abbreviation denotes the cycloometalating ligand used, 2-(2,4-difluorophenyl)pyridine (F₂ppy, a), 1-phenylisoquinoline (piq, b), and 6-phenylphenanthridine (pphen, c), which normally produce blue, red, and deep red phosphorescence when chelated to iridium(III), respectively. The bis-cycloometalated iridium precursors 2a–c of the type Ir(C^N)₂(CNArdmp)(Cl) (CNArdmp = 2,6-dimethylphenylisocyanide) were synthesized as previously described by our group, cleaving the chloro-bridged dimers with isocyanides. Finally, the cycloometalated iridium-coumarin dyads 3a–c and 4a–b were prepared using a simple, one-pot reactions between the respective coumarin and the isocyanide precursors 2a–c in the presence of AgPF₆. Here we also introduce a new structural class where the bis-cycloometalated iridium center is coordinated to two pyridyl-coumarins, represented by complex 5a. This latter compound is prepared directly from the chloro-bridged cycloometalated iridium dimer 1a and coumarin C-1, also in the presence of AgPF₆. These reactions are reasonably high-yielding based on crude NMR spectra but following multiple rounds of rigorous purification low to moderate isolated yields (20–60%) were obtained. ¹H and ¹⁹F NMR spectroscopy was performed on all the target complexes to affirm their identity and bulk purity, and ¹³C{¹H} NMR was also collected on complexes 3b, 3c and 4b which lack fluorination in the C^N ligands. ¹⁹F NMR spectra were particularly useful for determining the C₁ point group symmetry of 3a and 4a and the C₃ symmetry of 5a; the former show four distinct ¹⁹F resonances for the F₂ppy ligands, whereas the latter only shows two. The NMR spectra of the complexes can be found in Fig. S1–S15 in the ESI.

Scheme 1. Synthesis of cycloometalated iridium-coumarin compounds.

Single-crystal X-ray diffraction further confirms the molecular structures of the three F₂ppy complexes 3a–5a, which are shown in Figure 1. Refinement and diffraction data for these three complexes are summarized in Table S1 of the ESI. All the complexes show a distorted octahedral geometry centered on the Ir atom. The pyridyl nitrogen atoms from the F₂ppy ligands are in a trans orientation, with the other two cis-oriented coordination sites are occupied by one CNArdmp and one pyridyl coumarin ligand for complexes 3a and 4a, and two pyridyl coumarins in 5a. The C₁ symmetry of 3a and 4a and the approximate C₂ symmetry of 5a, readily apparent
from their NMR spectra, are confirmed by the crystal structures. The Ir center is covalently bound to the coumarin via the N atom in the pyridyl moiety, with Ir–N bond distances ranging between 2.172 and 2.190 Å, slightly longer than the Ir–N bond distances between the F2ppy nitrogen atom and the iridium center, which range from 2.046 to 2.079 Å.

**Figure 1.** X-ray crystal structures of complexes 3a–5a. Ellipsoids are drawn at the 50% probability level with hydrogen atoms, solvent molecules and counterions omitted.

**Photophysical Properties**

Figure 2 displays the overlaid UV-Vis absorption and photoluminescence emission spectra of free coumarins C-1 and C-2. The absorption spectra of both coumarins show two near-UV absorption peaks between 300–350 nm, which can be assigned to $S_0 \rightarrow S_1$ and $S_0 \rightarrow S_2$ transitions. Both C-1 and C-2 display deep-blue fluorescence emission with maxima at $\lambda = 406$ nm and 420 nm, respectively, with moderate Stokes shifts of 6100 cm$^{-1}$ for both compounds.

**Figure 2.** Overlaid UV-vis absorption (black dashed line) and photoluminescence (red solid line) spectra of coumarins C-1 and C-2.

Figure 3 shows overlaid UV-vis absorption and photoluminescence spectra of each iridium-coumarin compound, with the numerical photophysical data summarized in Table 1. The iridium-coumarin complexes have near-UV absorption in the same regions as free coumarins C-1 and C-2, overlapped with other strong absorption bands that are likely $\pi \rightarrow \pi^*$ transitions from the $C^\wedge N$ ligands chelated to iridium. Metal-to-ligand charge transfer (MLCT) bands originating from an Ir(5d)$\rightarrow C^\wedge N(\pi^*)$ transitions are clearly observed in the complexes with the more conjugated $C^\wedge N$ ligands piq and pphen. These broad bands occur at ca. 410 nm in piq complexes 3b and 4b and ca. 420 nm in pphen complex 3c.
Figure 3. Overlaid UV-vis absorption and photoluminescence spectra of the iridium-coumarin complexes. UV-vis absorption (solid black line) and room-temperature photoluminescence (blue solid line) were both recorded in CH$_2$Cl$_2$ at 293 K, and photoluminescence at 77 K (red dashed line) was measured in 1:3 (v/v) CH$_2$Cl$_2$/toluene. All spectra were recorded in CH$_2$Cl$_2$, while absorption and room temperature emission were taken at 293 K.

Emission spectra of all cyclometalated Ir coumarin complexes are recorded both at room temperature in CH$_2$Cl$_2$ and at 77K in CH$_2$Cl$_2$/toluene (1:3) glass, which are presented in Figure 3 with the photophysical data summarized in Table 1. Among the six compounds, there are three distinct patterns for the room-temperature photoluminescence:

1. In ppiq complexes 3b and 4b, the only appreciable photoluminescence is phosphorescence from the bis-cyclometalated iridium fragment. The emission both at 298 and 77 K is almost identical to the previously described model complex [Ir(piq)$_2$(CNArdmp)(pyridine)](PF$_6$)$_3$. The lifetimes of 3b and 4b, c.a. 6 µs, are likewise similar to the model complex, and photoluminescence quantum yields ($\Phi_{PL}$) are about a factor of two higher, 0.17 (3b) and 0.22 (4b), vs. 0.096 in the model complex. Because these two compounds only exhibit phosphorescence and no fluorescence, they are not suitable candidates for ratiometric oxygen sensing.

2. In complexes 3c and 4a, there is dual emission involving fluorescence from the coumarin and phosphorescence from the [Ir(C\(^{\text{N}}\)]$_2$] moity. The fluorescence occurs at similar wavelengths as the free coumarins (see Fig. 2), and the phosphorescence is dictated by the cyclometalating ligand. In 4a the coumarin fluorescence overlaps strongly with the phosphorescence, appearing as a shoulder on the high-energy side of the phosphorescence vibronic progression. This substantial overlap between fluorescence and phosphorescence means that 4a would not function well as a ratiometric sensor. The phosphorescence spectrum and lifetime (1.6 µs) in 4a strongly resemble those of an [Ir(Fppy)$_2$(CNArdmp)(pyridine)](PF$_6$)$_3$ model complex, further confirming this assignment. In pphen complex 3c, the blue fluorescence from the coumarin ($\lambda_{\text{max}} = 413$ nm) is well-separated from the deep red phosphorescence ($\lambda_{\text{max}} = 650$ nm), with no appreciable overlap between the two signals. The good resolution
between the two signals allowed us to separately integrate the fluorescence and phosphorescence bands and determine quantum yields for each. With excitation at 310 nm, the fluorescence quantum yield is 0.022, and the phosphorescence quantum yield is 0.086. The large separation between the two bands also makes complex 3c ideally suited for ratiometric sensing, as described below.

(3) In the other two F2ppy complexes 3a and 5a, dual luminescence with some blue fluorescence from the coumarin is likewise observed. However, the phosphorescence in these compounds, with two vibronic maxima at ca. 550 and 590 nm, occurs at much longer wavelength than typically observed in [Ir(F2ppy)2]+ complexes. This structured luminescence in 3a and 5a is ascribed to phosphorescence originating from a coumarin-centered triplet state and is similar to the luminescence observed in iridium complexes with coumarin-based cyclometalating ligands.\textsuperscript{38,39} In these compounds the [Ir(F2ppy)2]\textsuperscript{+} triplet state is higher in energy than the coumarin-centered state, resulting in population of this lowest-energy state prior to any phosphorescence occurring.

Overlaid UV-vis absorption with excitation spectra of each complex are shown in Figure 4, and they reveal additional insights into the excited-state dynamics. In cases where coumarin fluorescence is observed (all but 3b and 4b), the excitation spectrum when monitored at the coumarin fluorescence maximum does not overlay the UV-vis absorption of the complex, but more closely matches the UV-vis absorption spectrum of the respective free coumarin. This observation suggests that the coumarin fluorescence is only observed when the coumarin moiety is directly excited. When collecting excitation spectra monitored at the phosphorescence maximum, reasonably good overlap between the excitation spectrum and the UV-vis absorption spectrum of the complex is observed. This observation suggests that the relevant triplet state in each compound, be it the [Ir(C^N)2]\textsuperscript{+} triplet state (all piq and pphen complexes, and 4a) or the coumarin-centered triplet state (3a and 5a), is generated at any excitation wavelength. In other words, for the T\textsubscript{1} state Kasha’s rule is followed and, in all complexes, there is a pathway to generate the relevant T\textsubscript{1} state following excitation anywhere in the absorption window. One interesting observation noted above is that in F2ppy complexes 3a and 5a, which both use the 3-pyridyl-substituted coumarin, phosphorescence occurs from the coumarin triplet state, whereas in 4a, where the longer carboxy-pyridine linker is used, phosphorescence occurs from the iridium center. This phenomenon underscores the importance of the linker between the fluorophore and phosphor in determining the energy-transfer dynamics and suggests that with the longer linker in 4a triplet energy transfer to the coumarin is not as rapid, and all phosphorescence occurs from the iridium center.
Figure 4. Overlaid absorption and excitation spectra of each target complex. Excitation wavelengths were chosen based upon the location of emission bands for each compound. In some cases (for 3a, 3c and 4a) absorption spectra of C-1 and C-2 were included for notable overlap.

Table 1. Summary of photophysical properties of each iridium-coumarin complex. UV-vis absorption and room-temperature photoluminescence spectra were measured in CH₂Cl₂ at 293 K. Photoluminescence at 77 K were obtained in a mixture of CH₂Cl₂/toluene (1:3 v/v). Samples for photoluminescence measurement were excited at 310 nm.

<table>
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<th></th>
<th>λ&lt;sub&gt;abs&lt;/sub&gt;/nm (ε x 10⁻⁴/M⁻¹/cm⁻¹)</th>
<th>λ&lt;sub&gt;em&lt;/sub&gt;/nm (298 K)</th>
<th>λ&lt;sub&gt;em&lt;/sub&gt;/nm (77 K)</th>
<th>Φ&lt;sub&gt;PL&lt;/sub&gt;</th>
<th>τ/µs&lt;sup&gt;c&lt;/sup&gt;</th>
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<tr>
<td>3a</td>
<td>254 (5.2), 308 (3.3), 337 (2.1)</td>
<td>395, 447, 477, 547, 592, 648</td>
<td>396, 443, 467, 525, 567, 613</td>
<td>N.D.</td>
<td>N.D.</td>
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<tr>
<td>3b</td>
<td>289 (7.9), 338 (4.0), 407 (1.1)</td>
<td>587, 627</td>
<td>576, 624, 677</td>
<td>0.17</td>
<td>6.4</td>
</tr>
<tr>
<td>3c</td>
<td>258 (5.3), 293 (3.9), 335 (2.5), 387 (1.1), 411 (0.8)</td>
<td>413, 650</td>
<td>406, 614, 655</td>
<td>0.022&lt;sup&gt;a&lt;/sup&gt;, 0.086&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.7</td>
</tr>
<tr>
<td>4a</td>
<td>256 (4.6), 302 (3.2), 346 (1.3)</td>
<td>415, 448, 478, 506</td>
<td>407, 443, 489, 516, 556</td>
<td>0.027</td>
<td>1.6</td>
</tr>
<tr>
<td>4b</td>
<td>287 (4.8), 350 (2.0), 407 (1.0)</td>
<td>591, 626</td>
<td>577, 625, 679</td>
<td>0.22</td>
<td>6.5</td>
</tr>
<tr>
<td>5a</td>
<td>298 (3.2), 338 (2.3)</td>
<td>407, 550, 592, 650</td>
<td>406, 444, 475, 521, 565, 614</td>
<td>0.0061&lt;sup&gt;a&lt;/sup&gt;, 0.016&lt;sup&gt;b&lt;/sup&gt;</td>
<td>N.D.</td>
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<sup>a</sup> Fluorescence quantum yield. <sup>b</sup> Phosphorescence quantum yield. <sup>c</sup> Excited at 330 nm; only phosphorescence lifetime is shown.
Ratiometric Oxygen Sensing

Fppy complexes \(3a\) and \(5a\) and pphen complex \(3c\) exhibit clear dual emission and hence were chosen for ratiometric oxygen sensing. A qualitative assessment of all six complexes’ response to oxygen was performed by preparing each sample in a nitrogen-filled glovebox, the photoluminescence spectra observed, then the spectra taken again after equilibration in air. The spectra of each complex in N2-saturated versus aerated environments can be found in Fig. S16 – S21 of the ESI.† For the dual-emitting compounds there is no change in coumarin fluorescence upon exposure to air, while in all compounds, phosphorescence is quenched. A quantitative assessment was performed on dual-emitting compounds \(3a\), \(5a\) and \(3c\) by taking photoluminescence spectra at increasing \(pO_2\) levels, either until phosphorescence was completely quenched or until atmospheric levels of oxygen (ca. 160 mmHg) were present. Figure 5 (left column) shows the spectral change for each complex with increasing \(pO_2\) levels, which clearly shows quenching of the longer-wavelength phosphorescence with no change in the coumarin’s fluorescence intensity. All three complexes are capable of sensing partial pressures of oxygen below atmospheric content (\(pO_2 \leq 160\) mmHg), meaning they can be used in hypoxic environments.

![Spectral response to increasing oxygen concentration (left column), calibration curves showing the ratio of phosphorescence to fluorescence as a function of \(pO_2\) (center column), and Stern-Volmer oxygen quenching studies (right column) for \(3a\), \(3c\) and \(5a\). All data were recorded in CH2Cl2 at 293 K.](image)

A more quantitative description of the oxygen-sensing comes from Stern-Volmer analysis. Equation 1 shows the Stern-Volmer relationship, where \(K_{SV}\) is the Stern-Volmer constant, \(pO_2\) is the oxygen partial pressure, \(k_q\) is the quenching rate constant, and \(\tau_0\) and \(\tau\) are the lifetimes.
without and with oxygen present, respectively. In complexes 3a and 5a the photoluminescence is too weak and the lifetimes too long to determine accurate values on our instrumentation, so we analyzed the data with a previously reported modified Stern-Volmer equation that circumvents the need to measure accurate lifetimes. In this method $R_l$ describes the intensity ratio of phosphorescence to fluorescence, with $R_l^0$ describing this ratio in the absence of O$_2$. Equation 2 shows this modified Stern-Volmer relationship, using $R_l$ as the dependent variable with $K_{SV}$ and $pO_2$ defined the same as in Equation 1.

$$\frac{\tau_0}{\tau} = 1 + K_{SV}pO_2 = 1 + k_q\tau_0pO_2$$ (1)

$$\frac{R_l^0}{R_l} = 1 + K_{SV}pO_2$$ (2)

The Stern-Volmer constant $K_{SV}$ can be obtained from the slope of the linear fit, and all three compounds in Figure 5 give very different $K_{SV}$ values, indicating different sensitivities to O$_2$. $K_{SV}$ for pphen complex 3c is 1.3 x 10$^{-2}$ mmHg$^{-1}$, slightly smaller than those of our first-generation sensors ($K_{SV} = 3.0–8.1 \times 10^{-2}$ mmHg$^{-1}$), but same order of magnitude. For complexes 3a and 5a where the phosphorescence arises from the coumarin triplet state, the oxygen sensitivity is much higher, and $K_{SV}$ is one or two orders of magnitude higher than that of 3c, measured at 0.26 (5a) and 2.5 (3a) mmHg$^{-1}$. For complex 3c the inherent phosphorescence lifetime $\tau_0$ is known (2.7 μs, see Table 1), allowing us to use Equation 1 to determine a bimolecular quenching rate constant, $k_q$, of 4.8 x 10$^3$ s$^{-1}$mmHg$^{-1}$ equivalent to 5.2 x 10$^9$ s$^{-1}$M$^{-1}$ using the known solubility of oxygen gas in dichloromethane. This $k_q$ value approaches the diffusion limit which suggests that the diffusion limit which suggests that 3c is efficiently quenched by O$_2$, and is within the range of $k_q$ values observed in our first-generation Ir-BODIPY sensors. Without available lifetime values we cannot determine $k_q$ values for 3a and 5a, but in most cases oxygen quenching of triplet states is near the diffusion limit, so if we assume that $k_q$ spans the range of 10$^{-9}$–10$^{-10}$ s$^{-1}$M$^{-1}$, we can estimate that the excited-state lifetime for 3a is in the range of 10$^{-3}$ to 10$^{-3}$ s, and that of 5a about an order of magnitude shorter in the range of 10$^{-3}$ to 10$^{-4}$ s. Both of these are substantially longer than that of 3c, consistent with the organic 3(π→π*) nature of the emissive state in 3a and 5a, versus the significant Ir(5d)→C^N(π*) 3MLCT character in 3c.

To better quantify and compare the dynamic ranges of the sensors, here we define a parameter abbreviated as $p(O_2)_{90\%}$, the value of $p(O_2)$ needed to reach 90% quenching. In these sensors the good signal resolution allows $R_l$ to approach 0, so $p(O_2)_{90\%}$ is defined as the pressure where $R_l^0/R_l = 10$. Using the best-fit Stern-Volmer lines in Figure 5, we extrapolate $p(O_2)_{90\%}$ values of ca. 4 mmHg (3a), 36 mmHg (5a), and 690 mmHg (3c). Thus, all three sensors operate under very different dynamic ranges, giving good sensitivity to small (3a), intermediate (5a), and large ranges of $pO_2$ (3c) The slightly smaller $K_{SV}$ value and much improved signal resolution in 3c combine to give a much larger dynamic range when compared to our previous Ir-BODIPY sensors, by at least a factor of 10 in terms of the estimated $p(O_2)_{90\%}$. The superior signal resolution in 3c is critical, as the smaller $K_{SV}$ value accounts for only about a factor of 2.5 difference in dynamic range. All these metrics show that, using our synthetic approach that allows us to easily join different fluorophores and phosphors, we can dial in a wide range of sensor attributes, allowing us to optimize the characteristics for a specific application.

Conclusions
To summarize, we have synthesized a set of cyclometalated iridium-coumarin dyads applied as ratiometric oxygen sensors. The complexes are prepared using relatively simple synthetic methods, allowing ready access to several structural variants. The compounds have diverse photoluminescence profiles, determined by the choice of cyclometalating ligand on iridium and the linker between the coumarin and the iridium center. In some cases only phosphorescence from the cyclometalated iridium center is observed, and in others dual luminescence involving blue fluorescence from the coumarin and longer-wavelength phosphorescence occurs, the latter arising either from an iridium-centered 3MLCT state or a coumarin-centered 3(π→π*) state. Three of the six complexes exhibit well-resolved dual emission, and their oxygen sensing attributes are distinctly improved in relation to our first-generation sensors. In all cases clear
resolution between the fluorescence and phosphorescence signals is observed, with no overlap. The sensitivities and dynamic ranges of the sensors span two orders of magnitude, showing that the structural modifications enabled by our synthetic approach can have dramatic consequences on the sensing profile. In complex 3c, where phosphorescence arises from a 3MLCT state centered on the [Ir(pphen)]³⁺ phosphor, the comparatively small Stern-Volmer constant (K_{SV}) and large separation between fluorescence and phosphorescence enables oxygen detection over a wide range of partial pressures, substantially improved over our first-generation sensors where the green fluorescence and red phosphorescence overlapped. In compounds 3a and 5a, the coumarin-centered phosphorescence is apparently much longer lived, leading to much higher sensitivity to low O₂ levels. Thus, these sensors are ideally suited to applications where accurate detection of small O₂ partial pressures is needed. This work shows the versatility of our approach in designing effective ratiometric oxygen sensors. Future modifications will target improved photoluminescence quantum yields, which are all less than 0.1 in this class of sensors, by leveraging our group’s previous insights into the design of red and near-infrared phosphors with high quantum yields.⁴⁴,⁴⁵

Experimental Section

Materials

All chemicals were purchased from commercially available sources and were used without further purification unless otherwise specified. Solvents were deoxygenated and dried using a Grubbs Solvent Purification System. Iridium precursors Complexes 1a, 1b, 2a, and 2b were synthesized following previously reported methods.³²,³⁷ Complexes 1c and 2c were prepared using analogous approaches.

Physical methods

¹H, ¹⁹F, and ¹³C {¹H} NMR spectra were recorded at room temperature on a JEOL ECA-400, ECA-500 or ECA-600 NMR spectrometer. UV–vis absorption spectra were recorded in screw-capped 1 cm quartz cuvettes using an Agilent Cary 8454 UV–Vis spectrophotometer. Emission and excitation spectra were obtained using a Horiba FluoroMax-4 spectrofluorometer. Room-temperature emission studies were housed in 1 cm quartz cuvettes with septum-sealed screw caps and the low-temperature emission spectra were recorded in a custom quartz EPR tube with high-vacuum valve immersed in liquid nitrogen using a finger Dewar. Samples for emission and excitation measurements were prepared inside a nitrogen-filled glovebox using dry and deoxygenated solvents to exclude air. Luminescence lifetimes were measured with a Horiba DeltaFlex Lifetime System, using pulsed diode excitation and excited at 330 nm. Emission wavelengths were selected by using appropriate long-pass filters, and the decay trace was fit using the instrument’s analysis software or the software Origin 2020b. Emission quantum yields for complex 4a were measured with respect to a standard of quinine sulfate in 0.05 M sulfuric acid having a reported quantum yield (Φ_F) of 0.52⁴⁶, while quantum yields for complexes 5a, 3b, 4b and 3c were measured relative to tetraphenylporphyrin, which has a reported Φ_F of 0.11⁴⁷. The quantum yield of the Ir-coumarin conjugates (Φ_x) was calculated using Equation 5 below, where Φ_{st} is the quantum yield of the standard, m_x is the slope of emission intensity versus absorbance for the samples, m_{st} is the slope of emission intensity versus absorbance for the standard compound, and η_x and η_{st} are the refractive indexes of the solvents of the sample and standard, respectively.

\[
\Phi_x = \Phi_{st} \left( \frac{m_x}{m_{st}} \right)^2 \frac{n_x}{n_{st}}
\]

Syntheses

Synthesis of coumarin C-1. This compound was prepared as previously described.³⁴ A mixture of 3-pyridylacetic acid (1.10 g, 6.4 mmol), salicylaldehyde (0.5 mL, 3.5 mmol), acetic anhydride (1.2 mL, 11 mmol) and triethylamine (1 mL) was heated at 185 ºC for 3 hours. Afterwards, the reaction was cooled down and washed with DI-water and diethyl ether obtaining the final product as white powder. The spectral data matches that previously reported for this compound. (Yield: 505 mg, 65%) ¹H NMR (600 MHz, CDCl₃): δ = 8.88 (s, 1H), 8.65 (s, 1H), 8.15 (d, 1H, J = 7.8 Hz), 7.90 (s, 1H), 7.58 (t, 2H, J = 9.0 Hz), 7.39–7.41 (m, 2H), 7.34 (t, 1H, J = 7.5 Hz).
**Synthesis of coumarin C-2.** This product was prepared as previously described. Inside the glovebox, a solution containing coumarin-3-carboxylic acid (100 mg, 0.53 mmol), 4-hydroxypyridine (53 mg, 0.56 mmol) and dimethylaminopyridine (6 mg) in anhydrous DCM (100 mL) was added EDC·HCl (110 mg, 0.57 mmol) and was allowed to stir at room temperature overnight. Afterwards, the mixture was washed with DI water and the organic layer was collected and dried over MgSO₄, which was then filtered, and the filtrate was dried under vacuum. The obtained white solid was washed with ether and ethanol and recrystallized from chloroform and diethyl ether. (Yield: 73 mg, 51%) ¹H NMR (400 MHz, CDCl₃): δ = 8.88 (s, 1H), 7.87 (d, 2H, J = 7.2 Hz), 7.72–7.76 (m, 2H), 7.42–7.47 (m, 2H), 6.68 (d, 2H, J = 7.6 Hz).

**Synthesis of 3a.** Inside the glovebox, complex 2a (100 mg, 0.14 mmol) was dissolved in 10 mL of CH₂Cl₂ and combined with AgPF₆ (34 mg, 0.13 mmol) and 3-(3-pyridyl)coumarin (C-1) (31 mg, 0.14 mmol), which turned into a cloudy yellow suspension. The mixture was stirred overnight at room temperature. Then the reaction mixture was filtered, and the solvent was removed under vacuum to obtain an oily yellow material. The final product was obtained after silica gel column chromatography eluting with CH₂Cl₂/Et₂O as light-yellow powder. (Yield: 52 mg, 36%) ¹H NMR (600 MHz, CD₃CN): δ = 9.40 (d, 1H, J = 5.4 Hz), 9.11 (s, 1H), 8.69 (s, 1H), 8.43 (t, 2H, J = 8.7 Hz), 8.34 (d, 1H, J = 9.0 Hz), 8.26 (d, 1H, J = 7.8 Hz), 8.13 (t, 1H, J = 8.1 Hz), 8.08 (t, 1H, J = 7.8 Hz), 7.97 (s, 1H), 7.66 (t, 2H, J = 7.5 Hz), 7.55 (t, 1H, J = 6.3 Hz), 7.45 (t, 1H, J = 6.6 Hz), 7.40 (t, 3H, J = 7.8 Hz), 7.25 (t, 1H, J = 7.8 Hz), 7.13 (d, 2H, J = 7.2 Hz), 6.64–6.71 (m, 2H), 5.85 (d, 1H, J = 7.8 Hz), 5.74 (d, 1H, J = 6.6 Hz), 2.09 (s, 6H). ¹⁹F NMR (564 MHz, CD₃CN): δ = –72.35 (d, 6F, J = 704.2 Hz, PF₆).
**Synthesis of 4b.** Inside the glovebox, complex 2b (120 mg, 0.16 mmol) was dissolved in 10 mL DCM and combined with AgPF₆ (40 mg, 0.16 mmol) and an excess amount of C-2 (50 mg, 0.19 mmol). The mixture was allowed to react at room temperature for 2 days. The reaction mixture was then filtered and the solvent was removed under vacuum. The crude product was purified through alumina column chromatography using CH₂Cl₂/ethyl acetate (4:1) and crystallization from CH₂Cl₂/Et₂O, obtaining the final product as orange powder. (Yield: 65 mg, 36%). ¹H NMR (600 MHz, CD₃CN): δ = 9.28 (d, 1H, J = 6.6 Hz), 9.04 (d, 1H, J = 8.4 Hz), 8.93 (d, 1H, J = 8.4 Hz), 8.88 (s, 1H), 8.78 (s, 2H), 8.34 (d, 1H, J = 8.4 Hz), 8.29 (d, 1H, J = 7.8 Hz), 8.26 (d, 1H, J = 6.0 Hz), 8.13–8.17 (m, 2H), 7.74–7.90 (m, 8H), 7.38–7.43 (m, 4H), 7.21 (t, 1H, J = 7.5 Hz), 7.14–7.15 (m, 1H), 7.07–7.10 (m, 3H), 6.97 (t, 1H, J = 7.0 Hz), 6.83 (t, 1H, J = 7.8 Hz), 6.40 (d, 1H, J = 7.8 Hz), 6.24 (d, 1H, J = 7.8 Hz), 2.06 (s, 6H, CH₃).

**19F NMR (564 MHz, CD₃CN):** δ = –72.51 (d, 6F, J = 695.4 Hz, PF₆).

**Synthesis of 5a.** A mixture of 1a (41 mg, 0.034 mmol) and AgPF₆ (17 mg, 0.068 mmol) was dissolved in 15 mL of DCM in the glovebox. After stirring for 2 hours, 3-(3-pyridyl)coumarin (C-1) (30 mg, 0.136 mmol) was added and the reaction mixture was stirred for 48 h at room temperature. The AgCl was filtered off and the solvent was removed under vacuum. The yellow solid was then washed three times with diethyl ether and purified by precipitation from CH₂Cl₂/diethyl ether, followed by washing with toluene three times and recrystallization from CH₂Cl₂/hexane and chloroform/hexane/diethyl ether. (Yield: 25 mg, 32%). ¹H NMR (400 MHz, CDCl₃): δ = 8.87 (s, 2H), 8.78 (d, 2H, J = 5.6 Hz), 8.46 (d, 2H, J = 5.6 Hz), 8.23 (d, 2H, J = 8.4 Hz), 8.07 (d, 2H, J = 8.0 Hz), 7.89 (t, 4H, J = 8.4 Hz), 7.50–7.60 (m, 8H), 7.26–7.31 (m, 4H), 6.43–6.49 (m, 2H), 5.80–5.83 (dd, 2H, J = 2.0 Hz). ¹⁹F NMR (564 MHz, CD₂CN): δ = –72.91 (d, 6F, J = 679.6 Hz, PF₆), –107.61 (q, 2F, J = 10.3 Hz, F₂ppy), –110.18 (t, 2F, J = 13.0 Hz, F₂ppy).

**Conflicts of interest**
There are no conflicts to declare.

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**Notes and references**