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Review

# Cyclometalated iridium(III) complexes for life science

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ABSTRACT

Photoluminescent cyclometalated iridium(III) complexes are now a well established class of organometallic compounds with advantageous potential applications in biology and life science. While these complexes, along with other luminescent transition metals and lanthanoid complexes, were initially proposed as alternative markers to organic fluorophores in the staining of cells, it is now evident that their specific biological behavior makes this class of compounds useful in broader areas of life science such as imaging, sensing and therapy. The critical factors for the effective design of cyclometalated iridium(III) complexes with specific biological properties are still rather difficult to rationalize, and often mainly rely of aspects such as the intrinsic charge of the complex, its lipophilicity and its aqueous solubility. This review overviews the area of cyclometalated iridium(III) complexes in biology, with an emphasis on comparing the various conditions that these compounds have been assessed for their biological potential, such as the specific tested cells lines, concentration of internalization, incubation time, and mechanism of cellular entrance.



**Contents**



## 1. Introduction

Cellular imaging has become a very powerful tool in life science [1–4]. The technique allows the direct visualization of cells at subcellular resolution, and therefore it can be conveniently exploited to image the cell as a whole, to image specific subcompartments and organelles (such as nucleus, mitochondria, and lysosomes, for example), and even the presence and concentration of key biological and metabolic species [5–11].

While label-free techniques that exploit the autofluorescence of endogenous molecular species have been extensively developed [12–17], optical imaging commonly relies on the incubation of luminescent markers (often referred to as molecular probes or cellular imaging agents). The area of organic fluorophores for cellular imaging is certainly well advanced and in continuous evolution, and many probes that are now of fundamental importance for optical imaging have been developed for various applications within a cellular environment [18–23]. While these markers are indeed well

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established and essential in the field, for specific imaging conditions they might be associated with drawbacks that are intimately linked to the organic nature of these compounds.

Typically, luminescent organic molecules have rather small Stokes shifts, often resulting in a significant overlap between the absorption and emission spectral profiles [24,25]. Therefore, as the probe accumulates within a specific cellular compartment and its local concentration effectively increases, its brightness (defined as the product of molar absorptivity and photoluminescent quantum yield) can be reduced due to concentration quenching phenomena. As mentioned above, cells are inherently fluorescent due to the presence of photoactive endogenous compounds such as flavins, nicotinamides, and indole side groups of tryptophan amino acids, for example [26–31]. If the excitation and emission profiles of the marker in use are in the same region as those of autofluorescent endogenous species, it might be difficult to discriminate between the two signals. Lastly, but potentially one of the most limiting drawbacks, is represented by the tendency of some organic molecules to undergo photobleaching [32–34]. This process occurs once the compound is excited to higher energy states, from which unwanted reactivity can occur destroying the emissive properties of the marker. It should be noted that some molecular probes can undergo extensive photobleaching within a very short amount of time (less than one minute), limiting their usefulness for longer-time experiments in live cells.

In the event that one or more of the previously listed drawbacks are encountered, depending on the specific conditions used for the imaging experiment, it is essential to have alternative markers to overcome them. To fulfil this goal, luminescent transition metal complexes of low spin $d^6$ electronic configuration such a ruthenium(II), rhenium(I), and iridium(III), or $d^8$ electronic configuration such as gold(I) and platinum(II) [10,35–37], as well as lanthanoid complexes of visible emitters, such as Eu(III) and Tb(III), or NIR emitters, such as Yb(III) [38–42], have been investigated. All these complexes display favourable chemical characteristics and advantageous photophysical properties that make them ideal candidates for the development of probes complementary to organic fluorophores for applications in cellular imaging [43].

The transition metal complexes listed above are triplet state emitters, which makes them phosphorescent given that their ground state is of singlet spin multiplicity [44,45]. The energy stabilization on passing from a singlet to a triplet excited state, promoted by the strong spin–orbit coupling of the metal centre, ensures that the Stokes shift is much larger compared to fluorescent molecules. This larger shift results in a lack of overlap between the absorption and emission profiles, therefore limiting the extent of concentration quenching. The lanthanoid complexes also have quite large (“apparent”) Stokes shifts [46,47], derived from the fact that their efficient excitation is obtained *via* the antenna effect, where a coordinated chromophoric ligand is photoexcited to its singlet manifold, undergoes intersystem crossing, and eventually transfers the energy to the respective emissive state of the lanthanoid cation [48,49].

As the radiative decay of transition metal complexes is forbidden by the spin selection rule, given the fact that there is a change in spin multiplicity on decaying from a triplet excited state to a singlet ground state, the characteristic excited state lifetime of these species is typically longer compared to organic fluorophores [50,51]. Transition metal complexes usually decay within a time range between hundreds of nanoseconds up to microseconds, whereas spin-allowed fluorescence from organic species typically occurs within few nanoseconds. Also in the case of lanthanoid complexes [47], *f–f* interconfigurational electronic transitions are parity and often spin forbidden, resulting in elongated excited state lifetimes ranging between microseconds and milliseconds. A long excited state lifetime can be exploited to significantly reduce unwanted background autofluorescence using time-gated detection associated with microscopy techniques [52,53]. In fact, a time delay of few hundreds of nanoseconds would ensure that the luminescence signal coming from the cells is exclusively belonging to the metal probe, because autofluorescence processes are already fully completed. This aspect is especially useful when the excitation and emission profiles of the probe overlap with those of endogenous fluorescent species.

Depending on the specific chemical nature of the coordinated ligands, metal complexes of ruthenium(II), rhenium(I), iridium (III), gold(I) and platinum(II) can be quite kinetically inert, which favours lack of reactivity through ligand exchange that can potentially lead to cytotoxicity. On the other hand, the typical lability of lanthanoid cations is usually overcome by coordination with high denticity ligands, making the resulting complexes stabilized *via* the chelate effect. These design factors can also indeed aid in reducing photobleaching, thus making metal complexes viable molecular probes for longer timescale imaging with live cells without significant loss of photoluminescence [37,39,51,54].

While phosphorescent metal complexes certainly possess advantageous photophysical properties, especially in the case of phosphorescent transition metals, it is essential to consider their ability to sensitize singlet oxygen [55–57]. Production of singlet oxygen within live cells can lead to extensive photocytotoxicity, even in cases when the metal complex is non cytotoxic when incubated within live cells that are kept in the dark. In fact, this very characteristic has sparked the investigation of many metal complexes from cellular markers to phototherapeutic agents in the field of photodynamic therapy (PDT) [58–62].

Amongst the various metal complexes, cyclometalated iridium (III) species [63–69] have received extensive investigation for applications in life science. Typically, these complexes comprise an iridium(III) centre bound to two cyclometalated ligands such as phenylpyridine, and one bidentate ligand such as a diimine (although it is not uncommon to find two monodentate ligands). The chemical nature of this family of complexes can be readily tuned by chemical variations of the coordinated ligands, thus allowing tuning of properties such as charge, lipophilicity, and solubility, as well as photoluminescent characteristics. In fact, it is known that iridium complexes can be tuned to be emissive across the entire range of the visible spectrum, from blue to red, and further in the near-infrared region. Given this versatility, it is not surprising that a large number of iridium complexes have been investigated for their potential application as cellular markers and therapeutic agents.

The strategies adopted to design iridium(III) complexes for life science can be broadly divided into two categories. Complexes can be bioconjugated to biological or targeting vectors, with the aim of inducing a specific biological behavior. For example, the complex could be bioconjugated to target localization within a specific organelle such as mitochondria, to interact with specific biological species such as a proton in the measurement of intracellular pH, or to sense and quantify the presence of specific analytes. The complexes can also be designed to exhibit a photophysical response that is able to be modulated by the targeted event, as for example in the case of fluorogenic turn-ON species or ratiometric complexes. On the other hand, the complexes might lack bioconjugation, and their biological behavior can be investigated to elucidate a structure–activity profile. In this case, it becomes immediately apparent how a rationalization between structure and activity is quite complex to achieve, despite the fact that it would be extremely beneficial for the design of advanced and superior iridium complexes for targeted applications in life science. The main critical aspects that are typically invoked in structure–activity studies are charge, lipophilicity and solubility [70]. These are indeed very useful, but in general these considerations seem to be