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Review

Interaction of dioxygen with the electronic excited state of Ir(III) and Ru(II) complexes: Principles and biomedical applications

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ABSTRACT

Luminescent transition metal complexes are enjoying a growing interest because of their ubiquitous applications in, e.g., the fields of material science, sensors and (biomedical) diagnostics, and iridium(III) and ruthenium(II) complexes are among the best studied. Due to their long-living excited states, these complexes can have a strong interaction with dioxygen, resulting in luminescence quenching. This oxygen quenching might be regarded as an unwanted effect in luminescence imaging, but, on the other hand, it can be exploited for diagnostic and therapeutic applications as well. After a theoretical introduction concerning the dioxygen quenching mechanism and the parameters involved, in the second part of this review we focus on the possibility of tailoring this quenching by modifying selected properties of a complex (triplet energy, oxidation potential, localization and shielding of the excited state) in order to obtain systems with higher or lower oxygen quenching compared to the archetypical complexes. In the third part of this review an overview of the applications of oxygen quenching of luminescence is offered, with particular attention to biomedical use: diagnostic oxygen sensing, imaging and therapy.

1. Introduction

1.1. Overview

Luminescent transition metal complexes have recently shown to be a potentially good alternative to the more widespread organic luminophores (e.g. phenalenone, fluorescein, rhodamine and Alexa® dyes) in diagnostic imaging [1–5]. However, the interaction of the relatively long-lived excited state of transition-metal luminophores with dioxygen readily results in luminescence quenching and production of highly reactive species, i.e. singlet oxygen and/or superoxide radical. Dioxygen quenching of excited states does not necessarily represent a disadvantage that has to be avoided, and can, in fact, also be exploited, for instance in luminescence imaging for sensing and quantification of dioxygen levels. In particular the generation of singlet oxygen has been an attractive

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field of research since the first observations of the phenomenon back in the 1960s [6,7]. In more recent times, the study of oxygen quenching of the excited state of fluorophores like porphyrins and phthalocyanines with the consequent generation of the cytotoxic singlet oxygen, has even demonstrated potential applications in so-called PhotoDynamic Therapy (PDT) [8,9]. Herein the local interaction of photoactivated transition metal complexes and dioxygen is used to damage e.g. DNA and cell membranes in tumor tissue [2].

Ruthenium(II), and iridium(III) complexes have been extensively studied for their rich photochemistry and remarkable photophysical properties. Due to their chemical stability and the possibility of inducing different cell localization by changing the substituents on the (usually chelating di- or tridentate) ligands these two types of complexes have also been studied for biological applications [1]. In fact, diagnostic-related applications of dioxygen sensing have been recently described and many of them are based on Ru(II) or Ir(III) complexes [10], and their dioxygen quenching has been studied both from a theoretical point of view and for its potential applications. In this review we aim to offer a comprehensive summary of the different strategies that can be used to either increase or decrease oxygen quenching of Ru(II) and Ir(III) complexes, depending on the application, in order to provide a guideline for the future development of optimized luminophores. After a theoretical introduction summarizing the dioxygen quenching mechanisms and the parameters involved in this processes (this section), we describe the different strategies used for tailoring the oxygen quenching of ruthenium(II) and iridium(III) complexes, and finally report some relevant examples of applications of oxygen quenching in the biomedical field: oxygen sensing, imaging and therapy.

1.2. Theoretical considerations

Luminescent molecules can interact with a quencher molecule, thus altering their photophysical properties. In this section the oxygen quenching mechanism and the molecular parameters involved in this process are described. Both singlet and triplet excited states might be quenched by molecular oxygen [11], however, the oxygen quenching of triplet states is usually more efficient than the quenching of singlet states. Moreover, Ru(II) and Ir(III) complexes usually show excited states with a pronounced triplet character due to the high degree of spin–orbit coupling usually observed for heavy metals. Therefore, in this review only the quenching mechanism of triplet excited states will be considered.

A quencher molecule (viz. dioxygen) colliding with a luminophore in its excited state causes a collision-dependent decrease of luminescence. The quenching process can be illustrated by using a Jablonski diagram (Fig. 1) and it can be described by the Stern–Volmer equation (Eq. (1)):

$$\frac{I_0}{I} = \frac{\tau_0}{\tau} = 1 + K_{SV}[O_2] = 1 + k_q\tau_0[O_2] \quad (1)$$

where I_0 and I are the luminescence intensities without and with quencher (dioxygen), respectively, and τ_0 and τ are the corresponding luminescence lifetimes. K_{SV} is the Stern–Volmer constant and k_q is the kinetic quenching constant [12], that reflects the efficiency of the quenching process and can be used to compare luminophores with different lifetimes but with similar size.

The kinetic constant k_q is proportional to the diffusion-controlled rate constant k_d as shown in Eq. (2):

$$k_q = f_Q k_d \quad (2)$$

In which f_Q is the quenching efficiency, and k_d is defined according to the Smoluchowski equation (Eq. (3)):

$$k_d = \frac{4\pi N}{1000}(R_f + R_q)(D_f + D_q) \quad (3)$$

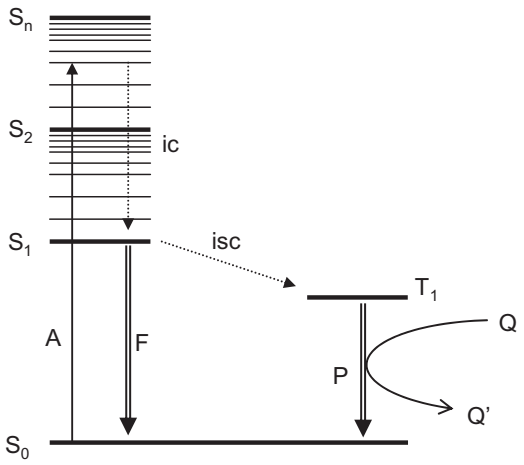


Fig. 1. Simplified Jablonski diagram for a generic luminophore showing the possible pathways of excitation and relaxation. S_0, S_1, S_2, S_n = singlet states, T_1 = triplet state, ic = internal conversion, isc = intersystem crossing, *A* = absorption, F = fluorescence, and P = phosphorescence. The collisional quenching process by a generic quencher *Q* – giving the product *Q*′ – is shown. For sake of clarity, only the quenching of a triplet state is depicted.

where N is the Avogadro number, and R_f and R_q are the radii of the luminophore and quencher, respectively, and D_f and D_q the diffusion coefficients of the luminophore and quencher, respectively. The diffusion coefficients can be obtained from the Stokes–Einstein equation (Eq. (4)):

$$D = \frac{k_B T}{6\pi\eta R} \quad (4)$$

where k_B is the Boltzman's constant, T the temperature, η the viscosity of the solvent and R the molecular radius. It should be pointed out that the diffusion constants evaluated through Eq. (4) are frequently underestimated (up to a factor of 3) when the quencher species has a smaller radius than the solvent molecules, which is usually the case for molecular oxygen [12].

In heterogeneous media, fluorophores are usually located in different environments, which causes different quencher accessibility, i.e. different quenching degrees. Under these conditions, the Stern–Volmer plot is not linear anymore. Several models have been elaborated in order to treat these cases. We concisely report here about Lehrer's and Demas' models.

Lehrer reported first a modified Stern–Volmer equation which takes into account the different environment of each fluorophore [13]. For a series of n fluorophores (for instance bound to a protein), the modified Stern–Volmer equation in the quantum yield form is reported in Eq. (5)

$$\frac{\phi_0}{\Delta\phi} = \left[\sum_{i=0}^{n} \frac{K_{SVi}[Q](\phi_{0i}/\phi_0)}{1 + K_{SVi}[Q]}\right]^{-1} \quad (5)$$

ϕ_0 is the fluorescence quantum yield in the absence of quencher, ϕ_{0i} is the quantum yield of the *i*th fluorophore in absence of quencher, K_{SVi} the Stern–Volmer quenching constant of the *i*th fluorophore and [Q] the molar concentration of quencher. Plots of the ratio $\phi_0/\Delta\phi$ vs. [Q] are often linear in the case of fluorophores in different environments, whereas in these conditions the classic Stern–Volmer plot (according to Eq. (1)) usually fails.

A similar approach has been developed by Demas et al. This model is based on the analysis of the multiexponential decays usually shown by fluorophores with different environments [14]. A

preexponential weighted mean lifetime τ_M can be defined according to Eq. (6)

$$\tau_M = \frac{\sum_{i=0}^{n} \alpha_i \tau_i}{\sum_{i=0}^{n} \alpha_i} \quad (6)$$

α_i is the preexponential factor of the ith fluorophore and τ_i is the lifetime of the ith fluorophore in presence of the quencher. By using this parameter, a new Stern–Volmer equation for etherogeneous systems can be defined (Eq. (7))

$$\frac{\tau_{M0}}{\tau_M} = \left[\sum_{i=0}^{n} \frac{\alpha_i \tau_{0i} / \sum_{i=0}^{n} \alpha_i \tau_{0i}}{1 + K_{SVi}[Q]} \right]^{-1} \quad (7)$$

τ_{M0} is the preexponential weighted mean lifetime in absence of quencher and τ_{0i} is the lifetime of the ith fluorophore in absence of quencher. In order to apply Eq. (7) to practical data it is necessary to estimate τ_M, which is usually not trivial, but Demas et al. have reported a method based on the numerical fit of the decay to a sum of exponentials by using non-linear least squares algorithm [14]. Details about the calculations methods are out of the scope of this review and the interested reader is referred to the original papers [15,14]. Lehrer's or Demas' models are often necessary for the analysis of data concerning the oxygen quenching of fluorophores encapsulated in polymeric membranes or mesoporous materials.

The oxygen quenching of luminescence for a generic fluorophore is usually a complex process which can involve different kind of interactions and transitions. In the following section we present an overview of the relevant processes involved in the luminescence quenching of transition metal complexes. We refer the interested reader to several papers and reviews where it is possible to find theoretical details concerning the mechanisms involved in the oxygen quenching of organic luminophores [11,16]. Dioxygen quenching of excited triplet states of transition metal complexes can basically follow two different mechanisms: energy transfer and electron transfer [11,17]. In the first mechanism (Eq. (8)) a ground state triplet oxygen molecule $O_2(X^3\Sigma_g^-)$ interacts with the triplet excited state of the luminophore *F yielding the ground state of the luminophore F and singlet oxygen in one of its two excited possible states $O_2(^1\Delta_g)$ or $O_2(^1\Sigma_g)$:

$$^*F + O_2(X^3\textstyle\sum_g^-) \rightarrow F + O_2(^1\Delta_g) \quad \text{or} \quad O_2(^1\textstyle\sum_g) \quad (8)$$

The singlet oxygen produced during this reaction is an extremely reactive species: it readily reacts with organic molecules giving peroxides, in which the O–O bond can be easily cleaved to generate highly reactive oxygen radicals like the hydroperoxide •OH [18]. The two possible states of singlet oxygen differ only in the spin and occupancy of the two degenerate antibonding π_g^* orbitals: in the case of $O_2(^1\Delta_g)$ (the excited state with lowest energy) two electrons are paired in the same π_g^* orbital, whilst in the case of $O_2(^1\Sigma_g)$ two electrons with opposite spin occupy the two degenerate antibonding π_g^* orbitals.

The free energy (ΔG_{en}) for an energy transfer quenching is related to the triplet energy of the luminophore, which can be approximated by the energy of the 0–0 transition E_{00} according to Eq. (9):

$$\Delta G_{en} = -(E_{00} - E_{O_2*}) \quad (9)$$

where E_{00} is the energy of the 0–0 transition (i.e. the energy of the triplet excited state) and E_{O_2*} the energy of the (excited state) singlet oxygen: $E_{O_2*}(^1\sum_g) = 1.63$ eV and $E_{O_2*}(^1\Delta_g) = 0.98$ eV depending on which excited state of dioxygen is initially produced during quenching [19–21]. The observed quenching constant k_q^{en} is given by Eq. (10)

$$k_q^{en} = \frac{k_d}{1 + e^{\Delta G_{en}/RT}} \quad (10)$$

where ΔG_{en} is the free energy of the energy transfer (which can be calculated according to Eq. (9)). This equation is valid only under the approximation of minimally distorted excited states ("vertical" energy transfer). A more general equation has been reported by Balzani et al. and the interested reader is referred to the original paper [21].

In the electron transfer mechanism (Eq. (11)) a dioxygen molecule interacts with *F generating an oxidised ground state of the luminophore F^+ and the superoxide radical $O_2^{\bullet -}$:

$$^*F + O_2 \rightarrow F^+ + O_2^{\bullet -} \quad (11)$$

The superoxide radical $O_2^{\bullet -}$ is a toxic species, because it can react with biomolecules, e.g. DNA [22]. The free energy for electron transfer quenching (ΔG_{el}) is related to the oxidation potential and to the energy of the 0–0 transition of the fluorophore (Eq. (12)):

$$\Delta G_{el} = F(E_F^{ox} - E_{O_2}^{red}) - E_{00} + C \quad (12)$$

where F is the Faraday's constant, E_F^{ox} is the oxidation potential of the fluorophore (in volt), $E_{O_2}^{red}$ is the reduction potential of oxygen (−0.78 V), E_{00} is the energy of the 0–0 transition (in electron volts) and C a Coulomb term (usually neglected in polar solvents) [23].

A more refined description of the electron transfer mechanism has been proposed by Marcus et al. [24,25]. In this model, the interacting species, an electron donor D and an electron acceptor A, are approximated as two charged spheres embedded in a continuous medium. The mechanism of the electron transfer reaction can be depicted as in Eq. (13).

$$D + A \underset{k_{-d}}{\overset{k_d}{\rightleftarrows}} (DA) \xrightarrow{k_{el}} D^+ + A^- \quad (13)$$

where k_d is the previously defined diffusion-controlled rate constant, k_{-d} is the rate constant of dissociation and k_{el} is the rate constant of the irreversible electron transfer step. The two species D and A diffuse together with a rate constant k_d and interact forming a collision complex (AD). In order to reach the transition state, the energy of the system is increased upon bond reorganization (e.g. bond stretching/compression and angle deformations) and solvent (e.g. changes in the electrostatic environment). The actual electron transfer takes place after such reorganization, and after the electron transfer, the transition state relaxes and eventually dissociates into the products. On the basis of this mechanism, an expression for the free energy of activation (ΔG^*) can be derived, according to Eqs. (14) and (15)

$$\Delta G^* = \frac{z_1 z_2 e^2 f}{\varepsilon r_{12}} + \frac{\lambda}{4}\left(1 + \frac{\Delta G_0'}{\lambda}\right)^2 \quad (14)$$

$$\Delta G_0' = \Delta G_{el} + (z_1 - z_2 - 1)\frac{e^2 f}{\varepsilon r_{12}} \quad (15)$$

where z_1 and z_2 are the charges of D and A, e is the elementary charge, f the ionic strength, ε the dielectric constant, r_{12} the distance between the centres of the reacting spheres (which is usually assumed to be equal to the sum of the hydrodynamic radii of the two species), ΔG_{el} is the free energy change of the electron transfer (which can be calculated according to Eq. (12)) and λ is the reorganization energy. Eq. (15) can be combined with the Eyring equation, thus relating ΔG^* with the rate constant k (Eq. (16))

$$k = \chi Z e^{-\Delta G^*/RT} \quad (16)$$

χ is the transmission coefficient ($\chi = 1$ for adiabatic reactions), Z is an universal frequency factor (for solution reactions usually is assumed $Z = 6 \times 10^{11}\,s^{-1}$), T is the temperature and R is the gas constant. Applying Eqs. (15) and (16) to the kinetic scheme shown in Eq. (13), in a steady state approximation, it is possible to derive Eq. (17), which describes the relationship between the experimentally accessible rate constant for electron transfer k_q^{el} and $\Delta G'_0$

$$k_q^{el} = \frac{k_d}{1 + (k_d/K_d Z)e^{((z_1 z_2 e^2 f/\varepsilon r_{12}) + (\lambda/4)(1+(\Delta G'_0/\lambda))^2)/RT}} \quad (17)$$

K_d is the equilibrium constant of the formation of the encounter complex (DA). Both k_d and K_d can be evaluated on the basis of D, r_1, r_2, z_1 and z_2. If $k_d \gg k_{el}$ then $k_q \sim k_{el}$ and Eq. (17) can be simplified as shown in Eq. (18) (assuming that at least one of the interacting species is neutral, and not taking into account the diffusion controlled step [26]):

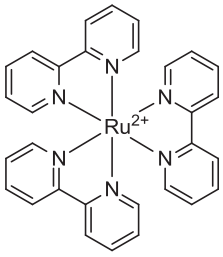
$$k_q^{el} = Fe^{-((\lambda/4)(1+(\Delta G'_0/\lambda))^2)/RT} \quad (18)$$

The reorganization energy λ is the energy change which is necessary to reach the excited state both by structural changes (bond and angle changes) and by reorganization of the solvent molecules. On the basis of the calculated λ for homonuclear reactions (i.e. reaction in which the same species with different charge act both as donor and acceptor) it is possible to derive some empirical rules for a qualitative evaluation of λ [26]. Low values of λ are usually observed in solvents with low polarity. Systems with a high degree of electronic delocalization (e.g. extended conjugation), with polarisable groups or ligands and with restrained conformational flexibility usually show low values of λ. More quantitative methods for the estimation of λ, based on computational techniques, have been developed, but their description is out of the scope of this review.

From Eqs. (9) and (12) it is possible to deduce that, in order to modulate the dioxygen quenching of a fluorophore, one can basically tune two crucial parameters: the triplet energy state (E_{00}) and the oxidation potential of the luminophore (E_F^{ox}). Generally speaking, both energy and electron transfer contribute to define the degree of oxygen quenching. A further parameter to be considered is the shielding effect provided by the environment (e.g. matrix or suitable shielding species in solution) or by the structure itself (e.g. bulky molecules). Such shielding can decrease the degree of quenching by physically preventing the collision between dioxygen and the luminophore in its excited state. Eventually, when the electron transfer plays a significant role, the effect of the reorganization energy λ should be taken into account. In the following section we will provide examples of applications of these general principles.

2. Tailoring oxygen quenching of transition metal complexes

Because of their unique electronic structure, luminescent coordination compounds usually show a large Stokes shift (i.e. a well-separated excitation/emission energy) and allow for tuning of their ground-state and excited-state energy by changing the structure of the ligands [27,28]. The large Stokes shift is particularly interesting for biological applications: the separated excitation/emission enables to label a biological molecule with multiple fluorophores without reduction of luminescence intensity due to self-quenching [29]. For the same reason, the large Stokes shift permits to realize macromolecules with high brightness [30,31]. The emissive state of transition metal complexes is usually an admixture of singlet and triplet states, with concomitant longer luminescence lifetimes (τ) compared to organic dyes, and their excited states have therefore a higher probability to interact with molecular oxygen, resulting in a remarkable quenching of luminescence [27,32]. Ru(II) complexes, in particular, have been most widely studied and therefore their description is predominant in this review. However, the luminescence of these complexes can be tuned only from yellow to near IR by changing the ligand structure [33]. On the other hand, Ir(III) cyclometallated complexes are more attracting due to the possibility of selective fine tuning their energy from near UV to the near IR by changing the chemical structure of the ligands [34]. However, the synthesis of Ir(III) trisphenylpyridine derivatives usually requires more harsh conditions with concomitant more restrictions on the ligand design. Demas et al. first reported about the dioxygen quenching of Ru(II) and Ir(III) complexes and proposed an interpretation based on the formation of singlet oxygen, which was proved by photooxygenation of trimethylethylene [35–37]. Other authors proved the mechanism of electron transfer to be efficient in complexes with low oxidation potential [38]. The mechanism of singlet oxygen photogeneration has been widely investigated especially in view of the interest of singlet oxygen for therapeutical applications (*vide infra*) [11,18,39,40]. The photophysical properties of the two archetypical compounds $[Ru(bpy)_3]^{2+}$ (**1**) and $[Ir(ppy)_3]$ (**2**) are reported in Table 1 together with their oxygen quenching constant (k_q).



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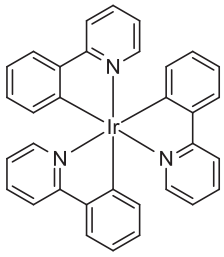

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In the following section we summarize the different strategies which have been exploited in order to modify oxygen quenching of Ru(II) and Ir(III) complexes [11], i.e., modification of triplet energy states or tailoring of the oxidation state, structural modification of ligand to create steric environments, and supramolecular shielding. Both energy and electron transfer are usually involved in the oxygen quenching mechanism. In order to discriminate between the two effects, one should ideally compare systems with the same triplet energy or oxidation potential, i.e. with the same degree of energy or electron transfer. However, this is, in practice, often compromised as changes in electronic states often regard both. Likewise, other factors like size (i.e. diffusion-controlled rate constant k_d) and reorganization energy should are hard to compare. In the following four sections we aim to discuss several examples from literature, in which it is possible to discriminate between the factors which mainly contribute to the oxygen quenching, subdivided by modification of electronic states of the luminophore, covalent functionalization of the luminophore's ligand structure to affect accessibility of relevant orbitals, non-covalent interactions influencing accessibility of orbitals, and solvent or matrix effects.

2.1. Dioxygen quenching tailoring by triplet-state energy or oxidation potential modification

According to theoretical models (*vide supra*) the easiest strategy to tailor the oxygen quenching of a luminophore, particularly in the case of quenching occurring by energy transfer, is changing the oxidation state of the complex or the triplet energy of the luminophore's excited state, E_{00} [16].

By increasing the triplet energy of the luminophore's excited state it is possible to decrease the dioxygen quenching and, conversely, luminescent complexes with low triplet energy show

Table 1
Molecular structures and photophysical properties and of the archetypical $[Ru(bpy)_3]^{2+}$ (**1**) and $[Ir(ppy)_3]$ (**2**).

	λ_{em} (nm)	ϕ	ϕ_{air}	τ (ns)	τ_{air} (ns)	E_{00} (eV)	E_{ox} (V)	k_q (M^{-1} s^{-1})
1[a]	620	0.042	0.028	650	390[b]	2.13[c]	1.28[c]	3.1×10^{9}[c]
2[d]	523	0.71	0.03	1873	82	2.51[e]	0.31[f]	2.4×10^{10}

[a] Solution in H_2O. From Ref. [41] unless otherwise stated.
[b] Solution in H_2O. From Ref. [42].
[c] Solution in CH_3CN. Potential vs. SCE. From Ref. [43].
[d] Solution in DMF. From Ref. [44] unless otherwise stated.
[e] Solution in MeOH. From Ref. [34].
[f] Potential vs. ferrocene/ferrocenium. From Ref. [45].

usually a more pronounced oxygen quenching. This effect has been initially experimentally proven for series of aromatic hydrocarbons, which show a high oxidation potential which makes the contribution of electron transfer negligible (*vide infra*) [46,47]. The dependence between triplet energy and oxygen quenching degree has been observed also for transition metal complexes, but in this case the oxidation potential is usually low enough to make also the electron transfer feasible. Therefore, in the case of transition metal complexes, usually both energy and electron transfer give a contribution to the observed oxygen quenching. However, it is possible to discriminate between the two mechanisms by comparing systems in which one of the two parameters (i.e. triplet energy or oxidation potential) remains basically unchanged. For instance, when comparing the series of Ru(II) derivatives **3**, **4** and **5** (Fig. 2) in acetonitrile, the complexes show a triplet energy E_{00} = 1.99 eV, E_{00} = 1.94 eV and E_{00} = 1.92 eV respectively and a quenching constant $k_q = 3.0 \times 10^9$ M^{-1} s^{-1} and $k_q = 3.65 \times 10^9$ M^{-1} s^{-1} and $k_q = 3.86 \times 10^9$ M^{-1} s^{-1}. Significantly, complexes **3** and **4** have the same oxidation potential (E_{ox} = 1.31 eV vs. Saturated Calomel Electrode (SCE)), whilst the oxidation potential of **5** is slightly higher (E_{ox} = 1.34) [20]. The size difference (i.e. the diffusion-controlled rate constant k_d) is expected to be negligible for **3** and **4**, whilst the value of k_d of **5** could be slightly higher.

An easily oxidizable molecule can give oxygen quenching *via* photoinduced electron transfer (PET) interaction. This effect has initially been observed in ruthenium(II) complexes by Tan-Sien-Hee et al. [38], who reported that upon substituting a bpy ligand for a 1,4,5,8,9,12-hexaazatriphenylene (hat) ligand (**6**, Fig. 3) a large decrease of quenching is obtained in acetonitrile from $k_q = 3.1 \times 10^9$ M^{-1} s^{-1} for $[Ru(bpy)_3]^{2+}$ to $k_q = 0.5 \times 10^8$ M^{-1} s^{-1} for $[Ru(hat)_3]^{2+}$. The analysis of the oxidation potential (E_{ox}, measured vs. SCE unless otherwise stated) shows a significant increase of the potential compared to $[Ru(bpy)_3]^{2+}$ (+2.07 V vs +1.28 V, respectively) whilst the 0–0 energy (E_{00}) basically does not change. The higher oxidation potential, according to Eqs. (12) and (17), results in a lower k_q, proving the importance of electron transfer in dioxygen quenching of $[Ru(bpy)_3]^{2+}$. More recent results corroborate the possibility of tuning the oxygen quenching by varying the oxidation potential of the complex with an adequate choice of ligands. Abdel-Shafi et al. described the different behaviour towards oxygen quenching of several Ru(II) complexes containing bpy ligands functionalized with different electron-withdrawing and electron-donor groups. For instance, by using amide substituents (**7**, Fig. 3) the authors were able to obtain a complex with a quenching constant $k_q = 1.2 \times 10^9$ M^{-1} s^{-1} in acetonitrile. The complex shows an increased oxidation potential (E_{ox} = +1.45 V) and a more favourable ΔG_{et} compared to the vinyl substituted complex **8** (Fig. 3), which shows an E_{ox} = +1.26 V and a quenching constant $k_q = 2 \times 10^9$ M^{-1} s^{-1} in acetonitrile. The two complexes **7** and **8** have the same E_{00}, thus the observed difference of k_q likely derives only from the oxidation potential E_{ox} [23]. However, for a correct comparison of the observed quenching constants k_q, the impact of the reorganization energy (λ) and of the diffusion-controlled rate constant (k_d), which is described in Eq. (17), should also be taken into account. For instance in their pioneering work about oxygen quenching of Ru(II) complexes *via* electron trans-

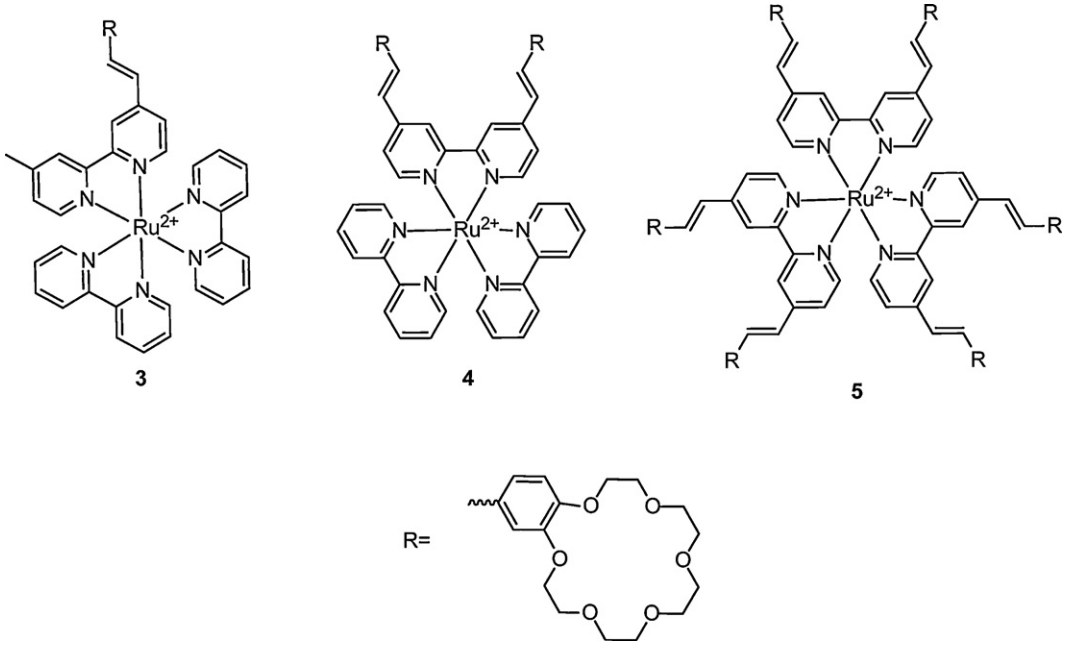

Fig. 2. Examples of Ru(II) and Ir(III) complexes showing tailored oxygen quenching deriving from the control of the energy of the excited triplet state.

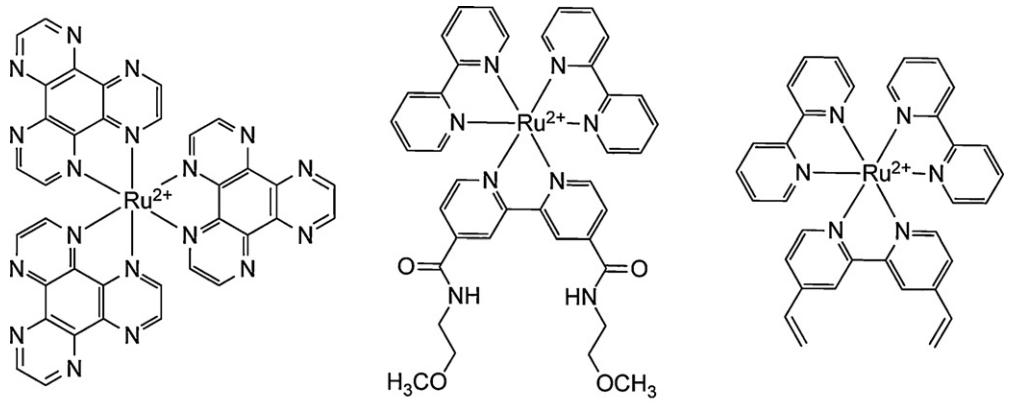

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Fig. 3. Examples of Ru(II) and Ir(III) complexes showing tailored oxygen quenching deriving from the control of the oxidation potentials.

fer, Tan-Sien-Hee et al. showed that the quenching constant k_q observed for many $[Ru(bpy)_3]^{2+}$ and $[Ru(phen)_3]^{2+}$ (phen = 1,10-phenanthroline) derivatives is under diffusion control, thus shows only little changes with E_{ox} [38]. On the other hand, low values of reorganization energies might make the energy transfer process more feasible (thus giving higher values of k_q) even when the value of ΔG_{el} suggests an opposite behaviour.

In summary, by increasing the triplet energy it is possible to obtain luminophores with a lower degree of dioxygen quenching. Conversely, a luminescent complex with low triplet energy usually presents a higher dioxygen quenching. However, it should be pointed out that the structural modification required in order to change the triplet energy usually results also into a change of the oxidation potential of the complex, thus in a change in the electron transfer feasibility. Moreover, for a quantitative study of the relationship between triplet energy and oxygen quenching degree, the contribution given by the diffusion-controlled rate constant k_d (see Eq. (12)) should also be taken into account. Regarding the strategies to design systems with tailored triplet energy, a number of reviews have been published recently and we remand the interested reader to those reviews [34,48].

By increasing the oxidation potential of Ru(II) and Ir(III) complexes it is possible to obtain systems with a lower degree of oxygen quenching and, conversely, luminophores with a low oxidation potential usually show a higher oxygen quenching due to the more favourable electron transfer mechanism. For a more quantitative description, the effect of the diffusion constant (k_d) and of the reorganization energy (λ) should also be taken into account. The oxidation potential can generally be tuned by using suitable substituents, for instance the addition of electron-withdrawing groups usually leads to an increase of the oxidation potential. We refer the interested reader to excellent reviews on this topic [34,48].

2.2. Dioxygen quenching tailoring by structural modification

The effect of ligand size on dioxygen quenching has been investigated since the first observations of the quenching effect on transition metal complexes. Demas et al. [37] observed only very small differences in dioxygen quenching constant between $[Ru(bpy)_3]^{2+}$, $[Ru(phen)_3]^{2+}$ and $[Ru(PhPhen)_3]^{2+}$ (PhPhen = 4,7-diphenyl-1,10-phenanthroline); these compounds give oxygen quenching constants in methanol (k_q) of $1.8 \times 10^9\ M^{-1}\ s^{-1}$, $3.3 \times 10^9\ M^{-1}\ s^{-1}$ and $2.5 \times 10^9\ M^{-1}\ s^{-1}$, respectively. More interestingly, Abdel-Shafi et al. reported that the functionalization of bpy ligands with bulky benzo-crown ethers (**9**, Fig. 4) basically does not change the oxygen quenching constant in acetonitrile compared to $[Ru(bpy)_3]^{2+}$ ($k_q = 2.21 \times 10^9\ M^{-1}\ s^{-1}$ and $k_q = 2.75 \times 10^9\ M^{-1}\ s^{-1}$, respectively) [20]. Despite the seemingly high steric hindrance given by the benzo-crown ethers, the small dioxygen molecule can still easily interact with the excited state yielding an efficient quenching. However, $[Ru(bpy)_3]^{2+}$ complexes functionalized with bulky dendritic substituents (**10**, Fig. 4) do give a higher steric hindrance and therefore an effective shielding against dioxygen quenching. This results in an almost 90% decrease of quenching ($k_q = 0.22 \times 10^9\ M^{-1}\ s^{-1}$ in acetonitrile) [49]. Despite the latter example is quite impressive, the introduction of bulky moieties on the ligand *via* covalent bonds usually results into an alteration of the triplet energy and/or oxidation potential. Therefore, analogously to what observed in Section 2.1, the impact of these changes should be taken into account and the archetypical compounds are not always by default the best comparison in order to obtain a quantitative indication of the change of oxygen quenching upon structural modification.

Alternatively, caged ligands have been successfully explored in order to decrease oxygen quenching. A caged ruthenium(II) complex by Barigelletti et al. (**11**, Fig. 5) with an 80% decrease of oxygen quenching in acetonitrile ($k_q = 0.94 \times 10^9\ M^{-1}\ s^{-1}$) compared to $[Ru(bpy)_3]^{2+}$ [43]. A similar effect has been recently reported also for an iridium(III) caged complex (**12**, Fig. 5), which has an oxygen quenching constant $k_q = 5.2 \times 10^9\ M^{-1}\ s^{-1}$ in DMF with an 80% decrease of quenching compared to the archetypical $[Ir(ppy)_3]$ (ppy = 2-phenylpyridine) ($k_q = 2.4 \times 10^{10}\ M^{-1}\ s^{-1}$ in DMF) [44]. The reason of the lower quenching of these caged complexes is not fully understood, yet, but it has been hypothesized that the caged structure can provide some shielding on the excited state (LUMOs) of the complexes, by sterically impeding the approach of molecular oxygen and therefore preventing efficient collisions.

Summarizing, only adequate steric hindrance can keep molecular oxygen from interacting with the excited states, adding large steric substituents or caging the metal ion being the best examples. The former is a brute force strategy and rather straightforward, whereas the latter is more subtle, but also requires a more profound understanding of the electronic density distribution in the ligand system in the excited states of the molecule.

2.3. Oxygen quenching tailoring by exploiting supramolecular shielding

A fluorophore hosted in a bulky molecule has less interaction with external agents. For instance the luminescence of tryptophan in proteins has varying quenching constants depending on its position on the protein viz. being on the surface or buried in

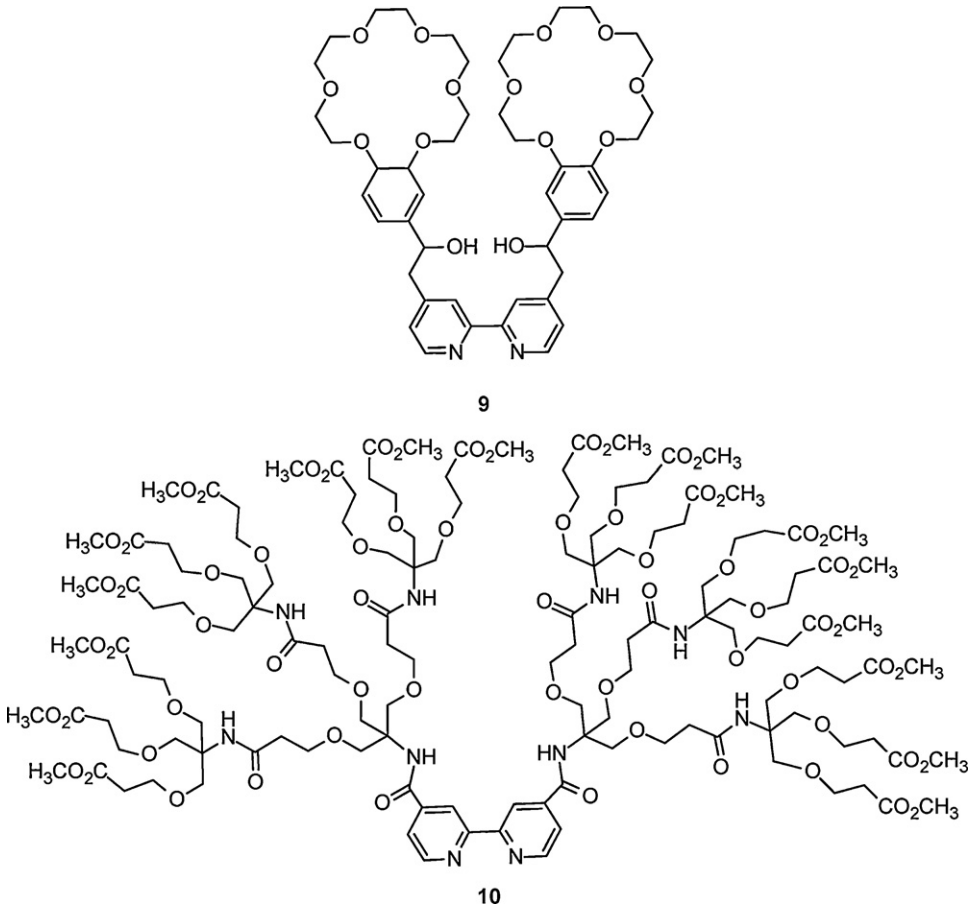

Fig. 4. Bulky ligands used for the synthesis of $[RuL_3]^{2+}$ complexes (L = **9**, **10**) showing different oxygen quenching deriving from structural effects.

the interior. This difference is explained in terms of steric shielding; the protein structure physically does not allow the quencher to approach the excited state of the fluorophore, resulting in a decrease of quenching [12]. A similar "shielding" effect can be artificially obtained by using supramolecular encapsulation of Ru(II) and Ir(III) complexes. For instance, Xu et al. showed that by modifying a $[Ru(phen)_3]^{2+}$ complex with some extra phenyl units (**13**, Fig. 6) it is possible to induce the binding of the Ru(II) complex to a polymer chain functionalized with several β-cyclodextrins (CDs) [50]. This interaction leads to a consequent shielding of the excited state and reduces oxygen quenching up to the 90%, depending on the length of the polymeric chain. In the absence of poly-CD the degree of oxygen quenching is similar as found for the reference compound $[Ru(phen)_3]^{2+}$.The same principle can be applied to other "host" molecules. In fact, recently a zeolite (L) was used to reduce the oxygen quenching of the luminescence of a $[Ru(bpy)_3]^{2+}$ in which one of the bpy ligands was functionalized with a tetraphenyl chain (**14**, Fig. 6) [51]. The zeolite structure sterically prevents the approach of oxygen and therefore results in a significant decrease of oxygen quenching in CH_2Cl_2 ($k_q = 0.29 \times 10^9\ M^{-1}\ s^{-1}$ and $k_q = 4.09 \times 10^9\ M^{-1}\ s^{-1}$ in presence or absence of zeolite L, respectively). More interestingly, the quenching decrease was also discussed in terms of orbital shielding: by using computational methods, the authors calculated the electron density distribution of the LUMO, which is mostly located along the "tail". Since only the tetraphenyl tail is small enough to penetrate into the Zeolite L, this makes the shielding very effective (Fig. 6). This effect still occurs, whilst the $Ru(bpy)_2$ "head" resides on the zeolite surface and it is virtually not shielded. This result constitutes a hallmark for the design of systems with low oxygen quenching. Herein, it is possible to decrease the degree of oxygen quenching of a fluorophore by sterically shielding the region of the molecule where the LUMO is localized.

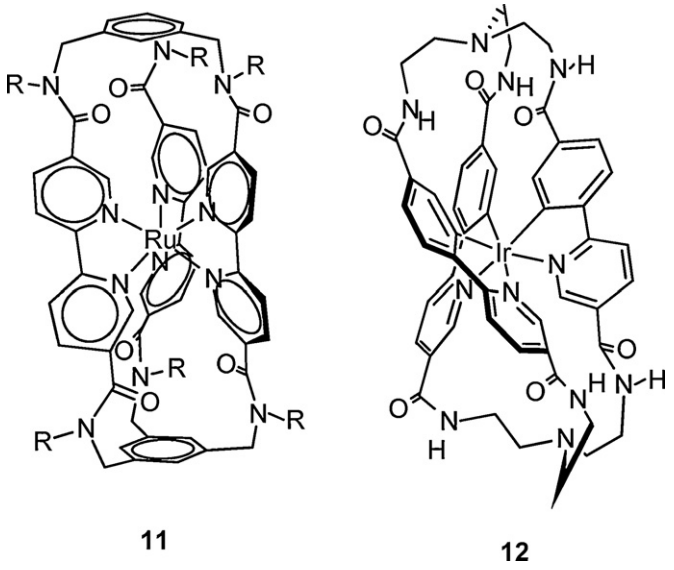

Fig. 5. Ru(II) and Ir(III) caged-complexes showing different oxygen quenching deriving from structural effects. R = CH_2Ph.

In summary, supramolecular shielding of complexes is an elegant and most effective tool to diminish the luminescence quenching by dioxygen. Looking at Eqs. (2) and (3), the main param-

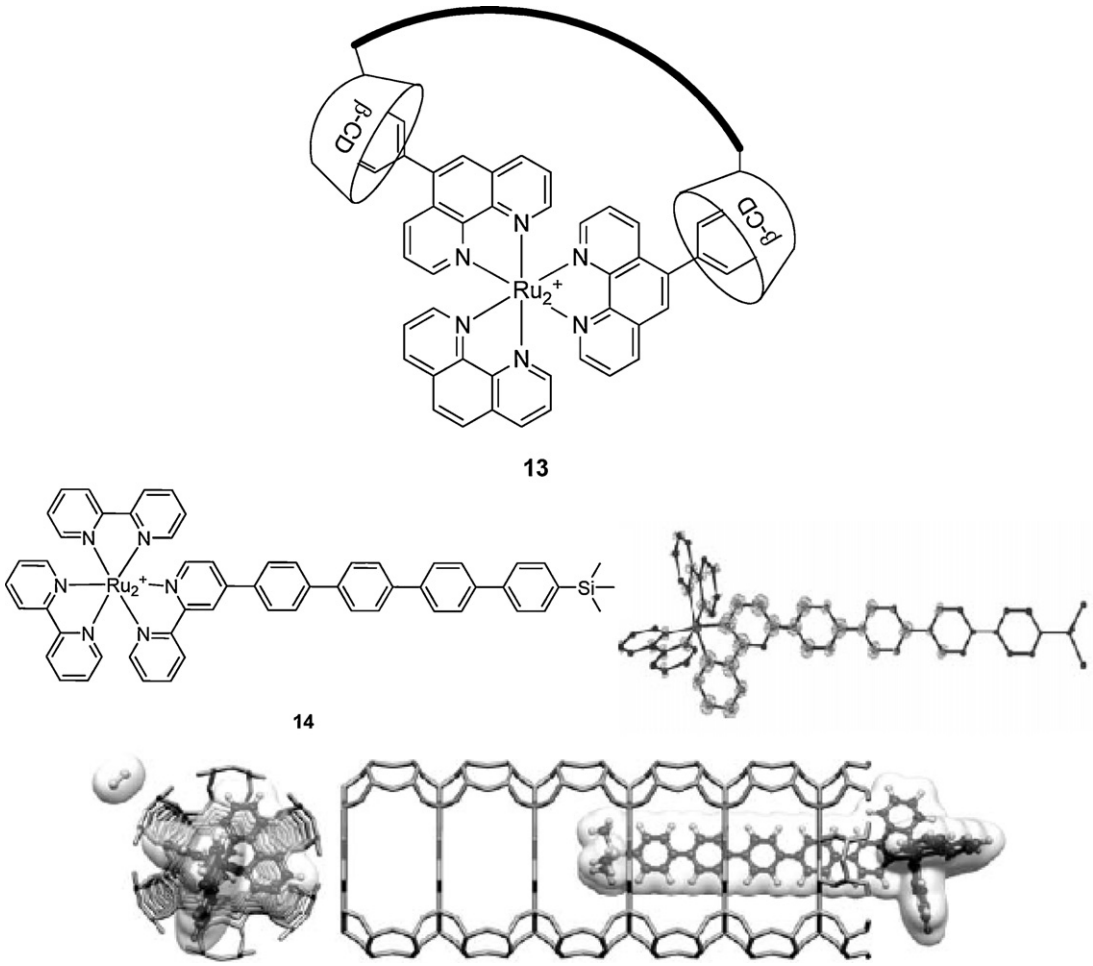

Fig. 6. Ru(II) complexes showing reduced oxygen quenching as consequence of supramolecular shielding against molecular oxygen. Reproduced from Ref. [51] with permission.

eter being affected is the diffusion-controlled bimolecular rate constant k_0, as the shielding decreases the collisional quenching of the compound, independently of the individual diffusion constants.

2.4. Solvent and matrix effect

As described above, the quenching of luminescence of Ru(II) and Ir(III) complexes by dioxygen can occur through different mechanisms, and different strategies have been exploited in order to tailor this, i.e., modification of triplet energy state or the oxidation state, structural modification of ligand to create steric environments, and supramolecular shielding. Besides these 'molecular strategies', also the compound's environment is a factor strongly influencing the amount of luminescence quenching by dioxygen. Solvent polarity in particular, has a major impact on the oxygen quenching sensitivity. Generally speaking, a low solvent polarity usually induces a lower degree of oxygen quenching of both Ru(II) and Ir(III) complexes. Conversely a higher solvent polarity, generally results in a higher quenching; in the case of $[Ru(bpy)_3]^{2+}$ quenching constants $k_q = 3.3 \times 10^9\ M^{-1}\ s^{-1}$ and $k_q = 1.9 \times 10^9\ M^{-1}\ s^{-1}$ have been reported in water and methanol, respectively [52–54]. A similar behaviour has been described for $[Ir(ppy)_3]$ [55].

Since most of the transition metal-based oxygen sensors consist of luminophores incorporated into an oxygen permeable polymeric matrix (*vide infra*), the effect of the matrix on the oxygen quenching has been studied by many authors. In general, analogously to what is observed for solvents, the quenching of $[Ru(phen)_3]^{2+}$ changes greatly with the polymer polarity whereby a lower polarity of the polymer induces a lower oxygen quenching [14]. Amao et al. reported that fluorinated polymers induce a higher oxygen quenching of $[Ir(ppy)_3]$ because of their higher oxygen permeability [56]. However, the permeability of the matrix to oxygen often induces a nonhomogeneous response, i.e. a non linear Stern–Volmer plot, which makes the interpretation of quenching data quite challenging. Carlson et al. reported that Ir(III) derivatives dissolved in fluoroacrylic polymers show an uniform behaviour towards oxygen quenching, as evident from the linearity of the Stern–Volmer plot. Therefore, fluoroacrylic polymers are very good candidates for the realization of oxygen sensors with uniform response [57]. The non-covalent incorporation of oxygen sensitive luminophore in a polymeric matrix often shows leaching of the dyes from the matrix, thus reducing the lifetime of the devices. This problem can be solved by functionalizing the luminophores with suitable dendrons [58]. An aluminium oxide matrix has also been used for the realization of oxygen sensors. Since the capillary forces are high, oxygen is quickly driven into the nanopores, resulting in a high sensitivity [59]. The covalent functionalization of polymers or silicon-based materials is another strategy to avoid the leaching of luminophores out of the matrix. Moreover, the quenching behaviour can be modulated by functionalizing the polymeric chains of the matrix with the transition-metal complex. For instance polysiloxane covalently functionalized with $[Ir(ppy)_2(vpy)Cl]$ (vpy = 4-vinylpyridine) yields a higher degree of oxygen quenching compared to a dispersion of the com-

plex in the same polymer [60]. A Langmuir–Blodgett monolayer functionalized with $[Ru(bpy)(dpphen)_2]^{2+}$ (dpphen = 4,7-diphenyl-1,10-phenanthroline) on glass also shows an increased oxygen quenching compared to the same dye in solution [61]. Mesostructured silicates with covalently grafted $[Ru(bpy)_2(phen)]^{2+}$ show an increased oxygen quenching with respect to the same silicated in which the dye has been only physically incorporated. Moreover, the covalently grafted silicates show a uniform response to oxygen, whilst physically incorporated dyes show nonhomogeneous response, resulting from different accessibility of the luminophores to the quencher [62]. A similar increased oxygen sensitivity has been reported for mesoporous molecular sieves covalently functionalized with $[Ru(bpy)_3]^{2+}$. However, in this case the response to oxygen is not linear, thus indicating a microheterogeneous environment [63]. Nonhomogeneous response to oxygen is shown also by organically modified silicates (ormosil) grafted with $[Ru(phen)_3]^{2+}$ derivatives [64].

3. Applications

In this section we will focus on the bio-related applications of luminescent transition metal-based complexes and the interaction with dioxygen with their electron-excited states, i.e. oxygen sensing *in vitro* and *in vivo*, biomedical imaging, singlet oxygen generators for therapeutical use (PDT and DNA cleavage).

3.1. Dioxygen sensing

The determination of oxygen concentrations is required in many different fields like environmental chemistry, industrial processes, biotechnology and medicine. Common methods for oxygen quantification are based on titration and amperometric techniques [65]. Most of these methods are time consuming and/or difficult to miniaturize. Moreover, they usually cannot be used for a continuous determination of the oxygen concentration. Optical oxygen sensors based on the dioxygen quenching of luminophores are a possible solution to these problems. Concentrations can be quantified by recording the variation of luminescence intensity or lifetime [66,67].

Optical sensors are usually made of an oxygen-sensitive dye incorporated into an dioxygen permeable polymer (silicone, polystyrene, poly(methylmethacrylate), fluoropolymer and cellulose derivatives) [68]. Due to their high oxygen sensitivity and the relatively unsophisticated equipment required for the measurement, detection methods based on the lifetime variations of luminophores with a long lifetime (in the order of μs) are particularly attractive. From this point of view, transition metal-based luminophores are highly eligible for the realization of optical dioxygen sensors. The first example of application of transition metal complexes to the realization of dioxygen sensors has been reported by Bacon et al., who exploited the oxygen quenching of $[Ru(Ph_2Phen)_3]^{2+}$ (PhPhen = 4,7-diphenyl-1,10-phenanthroline) immobilized in a silicone film for a sensor for the determination of oxygen in breath [69]. The advantages achieved by the use of transition metal complexes are summarized by the authors as: (1) high chemical stability, (2) low interference by other chemicals (e.g. anaesthetics or industrially relevant gases) and (3) fast response. The same luminophore has been incorporated also in a polysulfone matrix, which improved the mechanical stability and allowed sterilization in an autoclave. This latter sensor has been used for oxygen detector in bioreactors [70].

More recently iridium(III)-based luminophores have been used for the realization of oxygen sensors [60]. By functionalizing a silicone chain with $[Ir(ppy)_2(vpy)Cl]$ an oxygen sensor was obtained with high sensitivity and low response time. A further improvement can be obtained by blending the functionalized polymer with polystyrene. The resulting polymer is more sensitive and more robust compared to the unblended functionalized silicone. As extension of this technology, a fibre optic fluorescent oxygen sensor based on a Ru(II) complex has been recently reported (Fig. 7) [71]. This fibre technology has been used both for *in vivo* and *in vitro* oxygen determination in blood. The changes of physiological parameters (pH, osmolarity, viscosity) do not affect the sensor response. Furthermore, the authors proved that the sensor can be used for continuous monitoring (up to 8 h) without any serious complex degeneration. Nowadays optical oxygen sensors based on ruthenium(II) complexes are even commercially available [68]. Other systems based on Pt-porphyrin quenching have been developed but their description is beyond the scope of this review [72,73]. Moreover, several dioxygen sensors based on lanthanide complexes have also been reported by Parker et al. [74,75]. In these sensors, detection is based on the dioxygen quenching of the organic antenna, which usually act as a sensitizer of the lanthanide ion. The deactivation of the triplet state of the antenna by dioxygen results into a proportional decrease of lanthanide sensitization and therefore into a lower luminescence.

3.2. In vitro and in vivo imaging (of dioxygen levels)

The potential of using transition metal complexes in luminescence cell imaging has recently boosted the interest in such complexes. The possibility of tuning the emission and the cell uptake by changing the ligands around the metal-centre makes these luminescent transition-metal complexes extremely versatile imaging agents [1,3,76,77].

Asiedu et al., reported the use of $[Ru(phen)_3]^{2+}$ for optical intracellular dioxygen detection [78]. The authors used the Ru(II) luminophore to monitor the changes of oxygen concentration in macrophages monitoring the cells response to external oxygen level changes. The variation of lifetime upon oxygen exposure has also been used to monitor oxygen concentration in living cells by using Fluorescence Lifetime Imaging Microscopy (FLIM) [10,79,80]. Since the transition metal complex lifetimes are up to 3 orders of magnitude longer than the lifetime of organic dyes, amino acids, etc. it is possible to "filter out" the autofluorescence by using time-gated imaging [81,82]. For example, a $[Ru(bpy)_3]^{2+}$ complex functionalized with a pyrenyl unit with a lifetime of 58 μs and a high stability towards photodissociation was reported by Ji et al.; the complex is more stable towards photodissociation than the parent unsubstituted $[Ru(bpy)_3]^{2+}$ and therefore is suitable for *in vivo* applications [83]. More recently, zeolites [84] and polymer-based nanoparticles [85] functionalized with $[Ru(bpy)_3]^{2+}$ derivatives have been used for dioxygen quantification in cells. $[Ru(phen)_3]^{2+}$ complexes have also been used for the *in vivo* determination of oxygen concentration in liver and brain [86,87]. Since ruthenium(II) complexes are known to be phototoxic (*vide infra*), the dye concentration is a crucial parameter both for cellular and for *in vivo* imaging. A limit concentration of 0.2 mM has been reported by Dobrucki. At this concentration both $[Ru(bpy)_3]^{2+}$ and $[Ru(phen)_3]^{2+}$ are internalized in endosomes and do not cause any cell damage. Higher extracellular concentrations (in the range of 1 mM) result in higher phototoxicity upon excitation, causing a loss of membrane integrity [88].

We recently reported an alternative method to make Ru(II)-based luminophores suitable for *in vitro* imaging [31]. The decoration of a PAMAM dendrimer with negatively charged ruthenium(II) luminophores (**16**, Fig. 8) causes a partial internalization of the dyes into the dendritic structure, resulting in a negligible phototoxicity towards cells, despite the production of singlet oxygen upon irradiation. On the other hand, PAMAM dendrimer

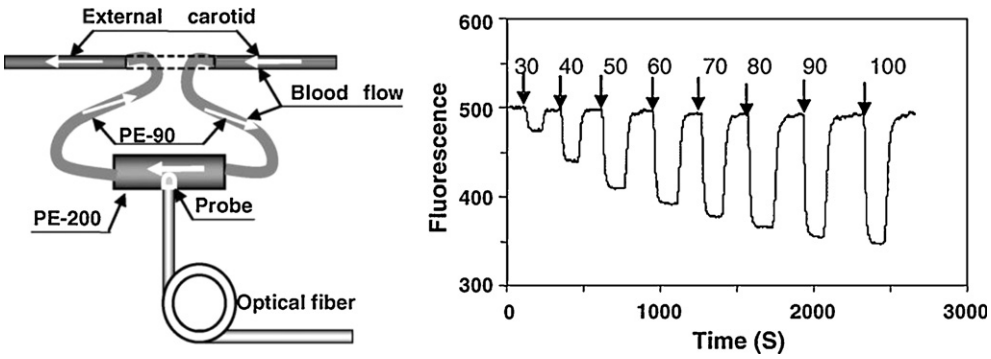

Fig. 7. Scheme of a Ru(II)-based oxygen sensor (left) and his typical response to different concentration of oxygen in time. Reproduced from Ref. [71].

functionalized with a $[Ru(bpy)_3]^{2+}$ (**15**, Fig. 8) shows the expected phototoxicity towards cells.

The measurement of dioxygen concentration levels is extremely important for the determination of hypoxia, a pathological condition of low oxygen concentration in cells and tissues. Hypoxia has been recognised as a critical factor in malignancy progression and metastasis in tumors and direct measurement of tumor oxygenation is a well-established marker for the prognosis of solid tumors [89–91]. An optical method for the direct determination of oxygen concentration would be extremely valuable, also considering a possible application of in intraoperative imaging during e.g. tissue or organ transplantations [92,93]. Zhang et al. recently reported about the application of an iridium(III) complex (**17**, Fig. 9) and performed hypoxia imaging in a tumor-bearing mice [94]. The red emission of the Ir(III) complex shows dioxygen quenching and, as expected, hypoxic tumor tissues show a lower concentration of oxygen than healthy tissues and therefore are clearly visible upon blue-light excitation (Fig. 9).

3.3. Therapy

As mentioned above, ruthenium(II) complexes are known to be phototoxic [88]. The hypothesized mechanism of this phototoxicity involves the production of singlet oxygen ($^1O_2$), a highly reactive

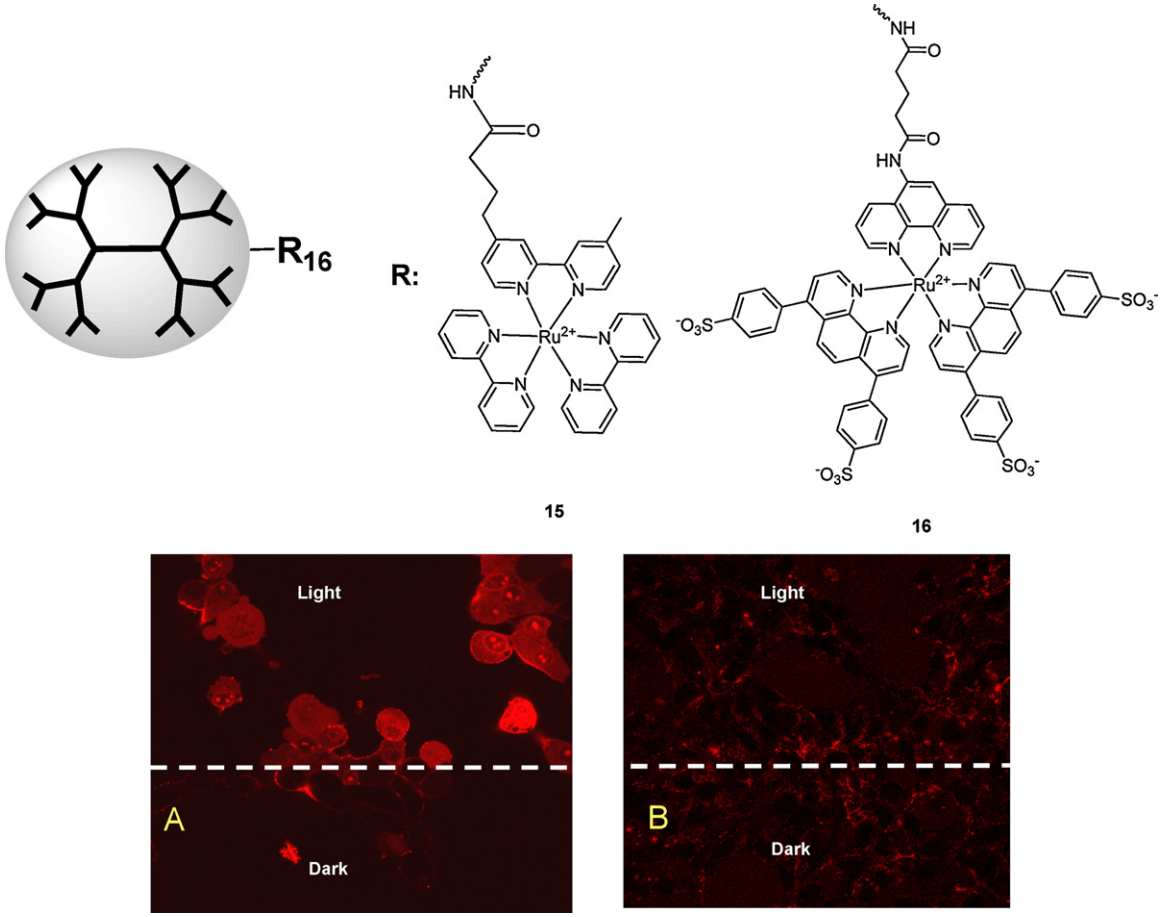

Fig. 8. A PAMAM dendrimer decorated with positive (**15**) or negative (**16**) Ru(II) complexes showing different phototoxicity: tumoral cells incubated with **15** show apoptosis upon irradiation (A) whilst the same cells incubated with **16** do not show any change upon irradiation (B).
Modified from Ref. [31].

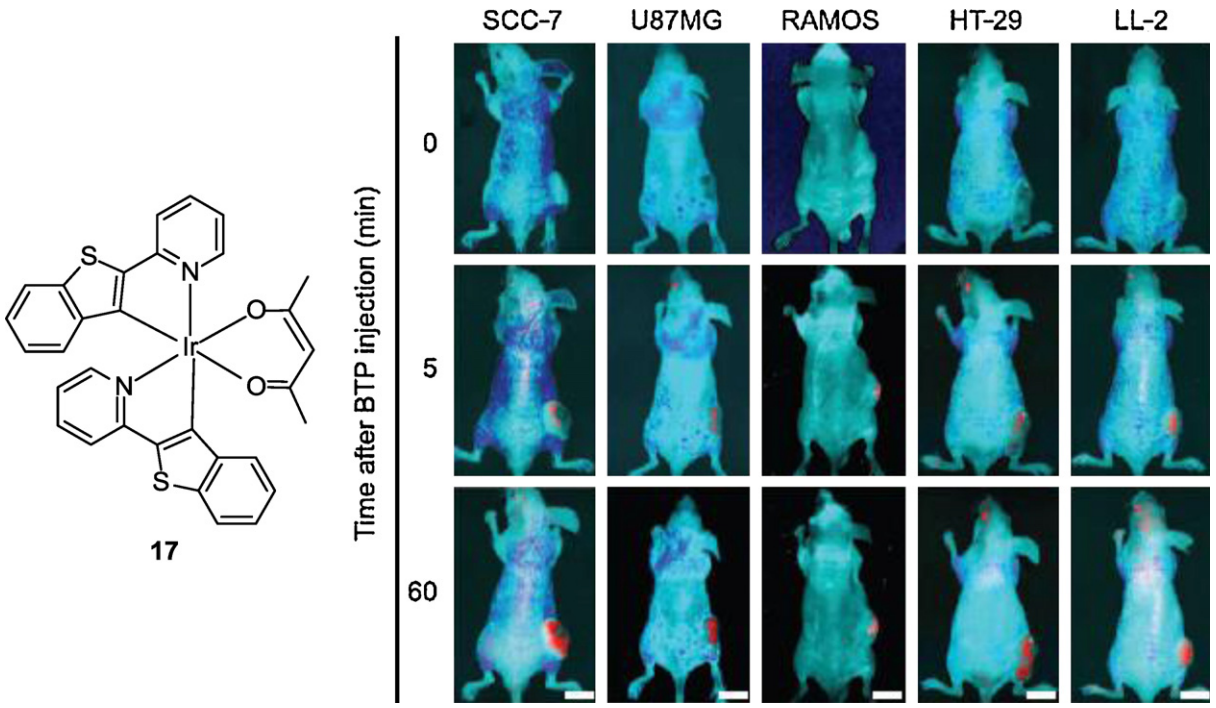

Fig. 9. Ir(III) complex used for imaging of tumor-induced hypoxia. Red luminescence indicating the localization of the tumor upon labelling with complex **17** (BTP) [94]. Reprinted by permission from Macmillan Publishers Ltd.: Nature Biotechnology, copyright 2004.

and cytotoxic species, which is produced by Ru(II) luminophores upon irradiation and is responsible for the loss of plasma membrane integrity [35]. Another proposed mechanism for toxicity is based on the strong oxidizing properties of Ru(II) excited state towards DNA bases, resulting in DNA cleavage [95]. In fact, this DNA cleavage mechanism is more complex and it has been proved that four different pathways can be involved: (1) direct electron transfer from guanine to metal complex; (2) oxo-transfer to the sugar and DNA bases; (3) long range DNA mediated electron transfer; and (4) interaction of singlet oxygen (produced *via* metal photosensitization) with DNA [96]. The latter pathway has been widely exploited for therapeutic applications, known as PhotoDynamic Therapy (PDT) [97]. Traditional drugs used in PDT are based on hematoporphyrin derivatives (e.g. Photofrin®) with or without (transition) metal ions, but more recently some Ru(II) complexes functionalized with polypyridine ligand have been reported as possible photosensitizers for PDT [40]. Most of the Ru(II) complexes discussed in Section 2 produce singlet oxygen as a "side product" of dioxygen quenching and, therefore, have potential as sensitizer for PDT [23,35,52,54]. For instance, Mulazzani et al. reported that the complexes $[Ru(bpm)_3]^{2+}$ and $[Ru(bpz)_3]^{2+}$ (bpm = 2,2′-bipyrimidine and bpz = 2,2′-bipyrazine) show a yield of singlet

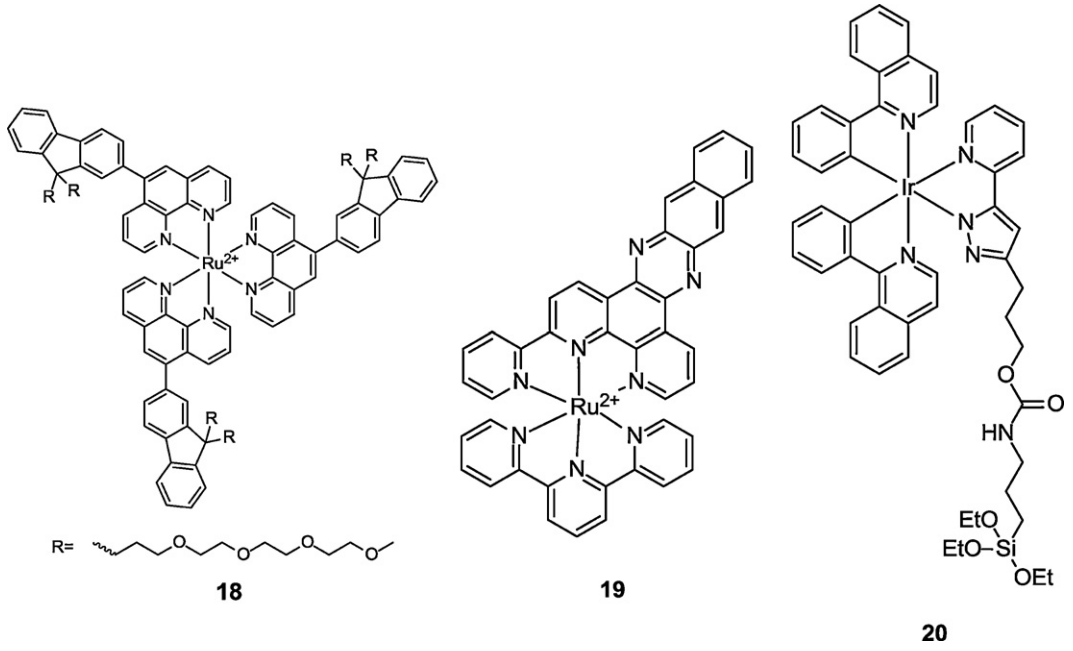

Fig. 10. Ru(II) and Ir(III) complexes with therapeutical applications: singlet oxygen photosensitization (**18**, **20**) and DNA cleavage (**19**).

oxygen as high as that of tetrakis(4-sulfonatophenyl)porphyrin, a well-known sensitizer used for PDT [17]. On the other hand, most of those early works just show the efficiency of Ru(II) complexes as singlet oxygen sensitizers but they do not corroborate the effective photoxicity of the sensitizers with cell studies. The study of Ru(II) and, more recently, Ir(III) complexes as sensitizers for PDT is still a very active field [2]. For example, Boca et al. have described a $[Ru(phen)_3]^{2+}$ complex functionalized with fluorene moieties (**18**, Fig. 10) which can be used as sensitizer for two-photon PDT [98]. The advantage of the two-photon technique resides in the possibility of using higher wavelength radiations (e.g. in the near IR region), which are less energetic (and therefore less harmful to healthy tissues) than traditional visible-light radiations. In addition NIR light can penetrate deeper in the tissues, expanding the use of PDT to deeper lying lesions.

A Ru(II) complex with two *mer* tridentate N-donor chelators (**19**, Fig. 10) with improved yield of singlet oxygen has recently been reported [99]. This complex shows a 20 μs lifetime, a unitary singlet oxygen yield and an impressive efficiency in DNA photocleavage. Very high singlet oxygen yields have also been reported for cyclometallated Ir(III) complexes (e.g. the well known $[Ir(ppy)_3]$ shows a singlet oxygen yield of 90%) [100]. A cyclometallated Ir(III) complex has even been applied to the decoration of magnetic nanoparticles (**20**, Fig. 10), and the resulting multimodal system has been used as imaging agent and PDT sensitizer, i.e. combining optical imaging and therapeutical applications. The possibilities shown by this multimodal system possibly opens up a field suitable for future applications of transition-metal complexes [101].

4. Conclusions

The study of the oxygen quenching of transition metal complexes' luminescence is a very active field, with important applications in sensing and biomedical sciences. Considering the examples described in the previous sections, it is evident that the oxygen quenching of the luminescence is not only an unwanted effect. Obviously, a low degree of oxygen quenching is a prerequisite for the development of highly luminescent dyes; on the other hand, the quenching of the luminescence by oxygen can be fruitfully exploited for the quantification of oxygen in tissues, which has a capital importance in diagnostics. Therapeutic applications of oxygen quenching are even more valuable: the efficient quenching of luminescence by oxygen, and the concomitant formation of highly reactive species (i.e. singlet oxygen and superoxide radical) is nowadays widely used for photodynamic therapy. The understanding of the physical processes regulating the interaction of the excited state of transition metal complexes with molecular oxygen allows the tailoring of the quenching degree and, consequently, it makes possible to design luminophores with a degree of quenching tuned up the desired application. Unfortunately, a simple roadmap for chemical development in this direction is difficult to set up, as quenching depends on many parameters, e.g. (substitutions on) ligands affecting the triplet energy and/or oxidation potential of the compounds, covalent or non-covalent functionalization influencing accessibility of relevant orbitals, solvent or matrix effects, all of which can have higher or lower impact depending on the application. Nonetheless, the application of oxygen quenching of transition metal luminophores in biomedicine, like oxygen sensing *in vitro* and *in vivo*, diagnostic imaging of oxygen distribution *in vivo* and singlet oxygen photosensitization for therapeutic applications, make this field very attracting and stimulating. In this review several promising applications with Ru(II) and Ir(III) complexes are described that have been reported in recent years, and we expect this field to be significantly further explored in the coming decades, driven by common sense and practical approaches as well as by an increasing understanding of the theoretical fundamentals of the different factors influencing the quenching mechanisms.

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