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Ruthenium polypyridyl phototriggers: from beginnings to perspectives

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Octahedral Ru(II) polypyridyl complexes constitute a superb platform to devise photoactive triggers capable of delivering entire molecules in a reliable, fast, efficient and clean way. Ruthenium coordination chemistry opens the way to caging a wide range of molecules, such as amino acids, nucleotides, neurotransmitters, fluorescent probes and genetic inducers. Contrary to other phototriggers, these Ru-based caged compounds are active with visible light, and can be photolysed even at 532 nm (green), enabling the use of simple and inexpensive equipment. These compounds are also active in the two-photon regime, a property that extends their scope to systems where IR light must be used to achieve high precision and penetrability. The state of the art and the future of ruthenium polypyridyl phototriggers are discussed, and several new applications are presented.

1. Introduction

Ruthenium(II) polypyridyl complexes are widely known to the inorganic chemistry community. The photophysical properties of the $[Ru(bpy)_3]^{2+}$ cation and many of its derivatives have been profusely investigated [1], aiming at their use as light-harvesting components in different applications [2]. These complexes show very interesting photophysical properties, including excited triplet states that can be easily populated [3]. Many of them are good emitters even at room temperature, showing acceptable emission quantum yields and high photostability.

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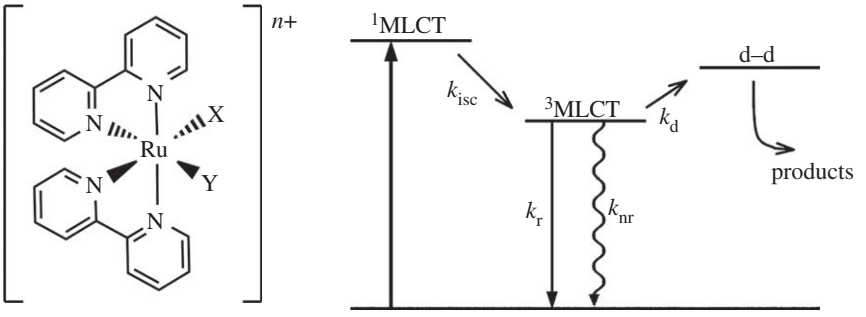


Figure 1. Basic structure and electronic states diagram of a $[Ru(bpy)_2XY]^{n+}$ complex.

The analogous complexes bearing two bipyridines and two monodentate ligands, i.e. $[Ru(bpy)_2XY]^{n+}$, share some of the photophysics of $[Ru(bpy)_3]^{2+}$ complexes, but their photochemistry is dominated by the photodissociation of one or both of the monodentate ligands, in a well-defined pathway. Although the photoreactivity of $[Ru(bpy)_2XY]^{n+}$ was reported by Dwyer *et al.* [4], the first systematic study on the photochemistry of these compounds was performed by Pinnick & Durham [5] two decades later. They measured the quantum yield of photosubstitution of several complexes, showing that it correlates with the absorption maxima of the bands involved and with the redox potential ($E_{1/2}$) of the Ru(II)/Ru(III) couple.

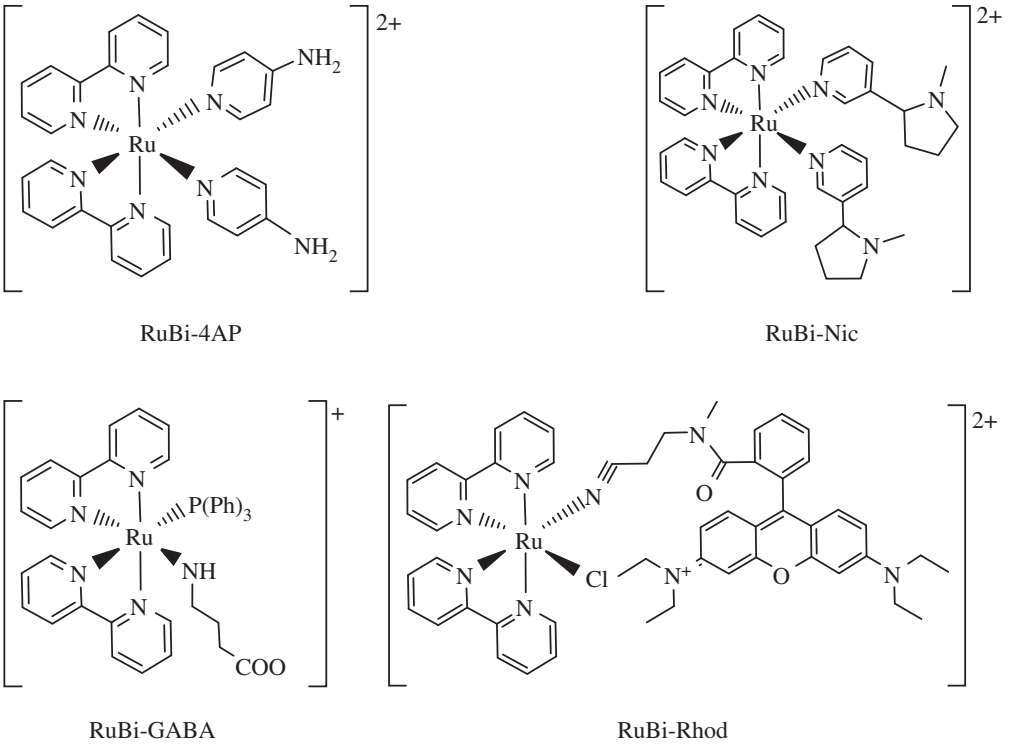
The photochemistry of this family of complexes can be modelled in a simple fashion by the state diagram shown in figure 1. The metal-to-ligand charge transfer (MLCT) band, whose maximum usually lies between 400 and 500 nm, populates the singlet ^{1}MLCT band, that can be formally depicted as a Ru^{III}–bpy^- state. An efficient intersystem crossing populates the triplet ^{3}MLCT band, which can be deactivated through emission, non-radiative pathways or population of the non-bonding d–d state, which could lead to labilization of a ligand.

Given that the energy of the d–d state is somewhat higher than that of the ^{3}MLCT band, this last transition is thermally activated. At a given temperature, therefore, a higher energy ^{3}MLCT band (which corresponds to a higher energy ^{1}MLCT and a blue-shifted MLCT absorption band) has a higher quantum yield for the population of the d–d state and thus has a higher yield of photosubstitution. Moreover, a higher $E_{1/2}$ corresponds to a higher energy of the Ru^{3+}–bpy^- MLCT state and hence the quantum yield of photosubstitution, the ruthenium redox potential and the energy of the MLCT band are all highly and positively correlated.

These interesting properties make the complexes $[Ru(bpy)_2XY]^{n+}$ excellent candidates for the design of phototriggerable cores. The fact that the MLCT bands lie in the visible region of the spectrum allows for the ligands to be photoreleased using visible rather than UV light, diminishing the risk of harming tissues in biological specimens and increasing beam penetrability in the sample. The ligands, which are coordinated to the Ru centre, are detached as entire molecules, with no further dark reactions that could introduce unwanted side products. Some examples of these kinds of phototriggers obtained in our laboratory are depicted in scheme 1.

The first caged compound based on Ru-bpy chemistry was $[Ru(bpy)_2(4AP)_2]^{2+}$ (RuBi-4AP in scheme 1), in which two coordination positions were occupied by 4-aminopyridine (4AP), a blocker of K^+ channels [6]. The sequential release of the 4AP ligands was followed by NMR and the biological activity was monitored by the activation of neuronal firing in a leech ganglion. Further research showed that the complex was also active in the two-photon (2P) regime, allowing photouncaging of 4AP with 800 nm IR light using a Ti–Sa laser [7]. Another compound of this kind was $[Ru(bpy)_2(Nic)_2]^{2+}$ (RuBi-Nic in scheme 1), with the cholinergic agonist nicotine as the photoreleasable ligand [8].

Similar complexes of the form $[Ru(bpy)_2(L)_2]^{2+}$ were synthesized for caging several aliphatic amines, including the neurotransmitter γ-aminobutyric acid (GABA) [9,10]. All these compounds present almost the same UV–visible spectrum, with maxima around 500 nm and quantum yields of uncaging of around $\phi_{PR} \cong 0.03$. An exhaustive theoretical calculation for the bis-amine



Scheme 1. Examples of phototriggers based on ruthenium–bipyridine cores. RuBi-4AP: $[Ru(bpy)_2(4AP)_2]^{2+}$, a photodeliverer of 4-aminopyridine, a potassium channel blocker. RuBi-Nic: the same configuration can deliver nicotine. RuBi-GABA: the presence of triphenylphosphine (PPh_3) as auxiliary ligand increases the quantum yield of photorelease. RuBi-Rhod: a complex bearing a rhodamine ligand as antenna for enhanced activity at longer wavelengths.

complexes was reported by Gobetto and co-workers [11]. In that work, the relative position of the energy states of the molecules was calculated, yielding a complete panorama of the photosubstitution pathways.

It is important to note that ruthenium nitrosyl complexes have been extensively explored as nitric oxide donors and/or photoreleasers [12–15]. However, the mechanism of this process is very different, involving a photoredox reaction that oxidizes the Ru(II) complex to Ru(III) prior to the photorelease. The parameters that control this reaction are very different from the ones relevant to other Ru-bpy complexes and hence this group of compounds can be considered as a different family.

2. Different ligands, different properties and different opportunities

In the octahedral Ru-bpy caged compounds, one coordination position is occupied by the caged molecule to be released. The characteristics of the other five ligands can be modified in order to tune the properties of the resulting caged compounds. Two positions are required for the coordination of a planar bidentate ligand like a substituted 2,2′-bipyridine or 1,10-phenanthroline which generates the MLCT long-lived excited state. The $Ru(bpy)_2$ core is usually present due to the readily available $Ru(bpy)_2Cl_2$ as a starting material. The remaining fifth position can be used to tune the physical and chemical properties of the phototrigger [16].

As an example of the relevance of this tuning, the complexes $[Ru(bpy)_2(GABA)_2]$ and $[Ru(bpy)_2(PPh_3)(GABA)]^+$ (the latter, RuBi-GABA in scheme 1) are two caged compounds of the neurotransmitter GABA which differ only in one ligand. The replacement of one GABA ligand

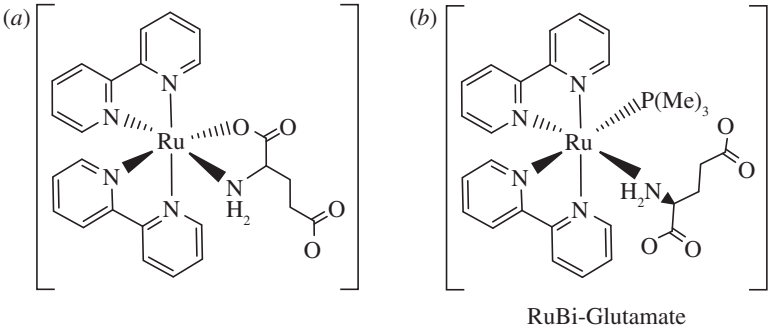


Figure 2. Ruthenium bipyridyl complexes of glutamate in bidentate (*a*) and monodentate (*b*) forms. While the first one does not present photoreactivity due to recaptation, the second one releases glutamate upon irradiation with visible light.

by triphenylphosphine (PPh_3) brings very important differences in the properties of these two complexes. The phosphine group is a much weaker σ-donor and a stronger π-acceptor [17] than the amine group in GABA. As a consequence, the Ru(II) becomes electronically depleted and a higher potential is observed for the Ru(III)/Ru(II) couple. Also the position of the ^{1}MLCT band shifts from 489 nm for the bis-amino complex to 424 nm for the amino-phosphino complex. As expected, the increase in energy of the MLCT band results in a sevenfold increase of the quantum yield of photorelease of GABA, from 0.03 to 0.21 [9,18].

The incorporation of PPh_3 and the resulting lower electron density on the ruthenium also has a deep impact on the ligand substitution kinetics of the complexes. While the complex $[Ru(bpy)_2(GABA)_2]$ presents some GABA leakage due to thermal substitution at 37°C, the phosphine analogue is completely inert, not showing traces of decomposition in physiological preparations. Not all the effects of the incorporation of PPh_3 are beneficial. The presence of the phenyl rings makes the complex much more lipophilic, increasing the affinity of the caged compound for the cell membrane. At high concentrations of the caged compound, unwanted effects have been observed due to a lowered membrane resistance. Lipophilicity can be diminished by replacing PPh_3 with the less hydrophobic trimethylphosphine (PMe_3). The GABA photoreleaser $[Ru(bpy)_2(PMe_3)(GABA)]^+$ can be used at high concentrations even with *in vivo* preparations [19]. An intermediate behaviour can be achieved using phosphines of the family PPh_nMe_{3-n}.

The inert character of the phosphine ligands can also be exploited to force bidentate ligands such as amino acids to coordinate through only one position. This is the case of the complex $[Ru(bpy)_2(PMe_3)(Glu)]$ (RuBi-Glutamate) [20], capable of photoreleasing the excitatory neurotransmitter glutamate, shown in figure 2.

Figure 2*a* shows the normal bidentate coordination of α-amino acids, in which both the amine and the α-carboxylate group are involved. In such a configuration, even if the photoreaction proceeds, very fast recaptation will occur and the overall photorelease yield will be very low. In fact, no photolysis can be observed in solutions of $[Ru(bpy)_2(Glu)]^+$ under visible light. On the other hand, the complex depicted in figure 2*b* is the most active caged glutamate known to the present day, and the only one that can be photolysed with 532 nm light, showing a quantum yield of 0.13 even at such a long wavelength.

These complexes can also be used to protect surfaces. In order to attach them to a glass surface, the complex $[Ru(bpy)_2(PMe_3)(APTS)]^{2+}$ (APTS = (3-aminopropyl)triethoxysilane) was synthesized. This complex exhibits a silane tail that can be covalently attached to SiO_2 surfaces as depicted in figure 3 [21].

In this case, a glass surface homogeneously covered with a monolayer of the Ru-bpy phototrigger is irradiated with visible light (460 nm) or with IR light (900 nm) in the 2P regime. Irradiation results in the release of the Ru-bpy complex and the exposure of the amine

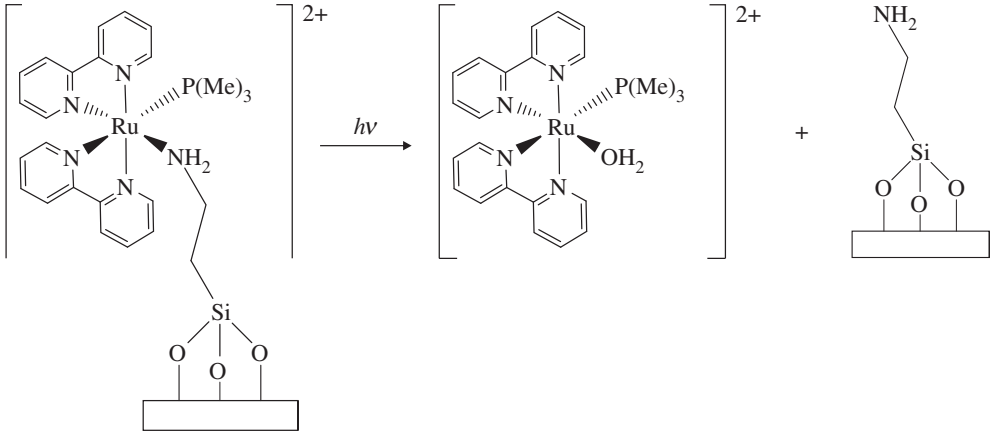


Figure 3. The complex $[Ru(bpy)_2(PMe_3)(APTS)]^{2+}$ (APTS = (3-aminopropyl)triethoxysilane) attaches on any glassy surface, allowing the precise assembly of photoactive complexes in monolayers. This approach can be used to define cell-adhesion areas in order to perform microstructured cell cultures.

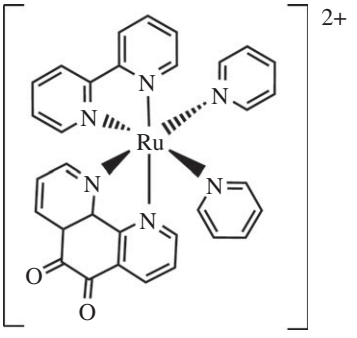


Figure 4. Surface-sticky Ru-bpy complex bearing a phen-dione, capable of attaching to TiO_2 surfaces while retaining its photoreactivity towards its monodentate ligand.

functionality. By means of spatial and temporal control of the irradiation, it is possible to generate a defined pattern on the treated surface. Reaction with Alexa Fluor 488 enables the detection of this monolayer pattern using fluorescence microscopy. The strategy can be used to derivatize surfaces with amine groups following a given pattern, allowing for further chemical reactions to proceed with high spatial resolution.

Functional groups can also be incorporated on the rings of bipyridine ligands. Several strategies have been reported for the stepwise incorporation of bipyridine ligands with different substituents. In this case, the main purpose has been to extend the absorbance of the resulting complexes in order to capture as much energy as possible from the visible spectrum, pursuing the so-called black absorbers [22]. A similar strategy can be used to introduce functional groups in one bipyridine ligand, keeping the other one intact and hence minimizing the effects on the excited state properties of the complex. The example in figure 4 shows the complex $[Ru(bpy)(phen\text{-}dione)Py_2]^{2+}$ (phen-dione = 1,10-phenanthroline-5,6-dione).

The $[Ru(bpy)(phen\text{-}dione)Py_2]^{2+}$ complex retains the ability to photorelease the pyridine ligands, whereas the phen-dione ligand allows its addition to a TiO_2 surface. The incorporation of phototriggers onto surfaces opens the possibility of using them in more complex units. For example, a phototrigger can be combined with a photosensitizer to improve the near-infrared light absorption properties of the group.

In order to design new phototriggers based in the Ru-bpy system, it is convenient to have a rationale that indicates which of the ligands will be photolysed upon irradiation. In general terms, a complete analysis of the photoreactive pathways must be done in order to ensure a given behaviour. For complexes bearing only one bipyridine attached to the ruthenium centre plus four identical monodentate ligands, it has been demonstrated that the axial ligands are selectively photolysed as long as they are less basic (weaker σ-donors) than the bipyridine nitrogens [23]. In such a case, the stronger σ-donor ability of the bpy nitrogens determines the orbital ordering and therefore the nature of the lowest lying ^{3}d–d state responsible for the photochemistry. This finding provides an explanation to the *cis*-to-*trans* photoisomerization of $[Ru(bpy)_2(H_2O)_2]^{2+}$ and analogues, which has been reported and used as a synthetic tool [24,25].

Even in the absence of a precise calculation of the photochemical paths, we have found some simple rules applicable to Ru-bpy phototriggers having the structure *cis*-$[Ru(bpy)_2(X)(Y)]^{n+}$. If X and Y are different, the photosubstitution occurs on the weaker donor ligand. Therefore, nitriles, phosphites, thioethers and tetrazoles are photolysed in a clean way in mixed complexes that have a pyridine, amine or phosphine as fifth ligand. Pyridines are photolysed in amine–pyridine mixed complexes, but in some cases release of both ligands was observed. As a general rule, we have found that phosphines behave as inert ligands and are never released, although an exception is the extremely poor base triphenylphosphine trisulfonate, which can be photocleaved. In the case of the complexes $[Ru(bpy)_2(L)_2]^{n+}$, having two identical monodentate ligands, the obvious photochemistry pathway is the loss of one of them to form the aquo (or solvent) complex. The photoreaction will proceed further only in the case that the σ-donor ability of the remaining ligand is lower than that of the solvent. While aqueous solutions of $[Ru(bpy)_2(AN)_2]^{2+}$ (AN = acetonitrile) undergo complete photolysis yielding two free AN molecules and $[Ru(bpy)_2(H_2O)_2]^{2+}$, the similar complex $[Ru(bpy)_2(4AP)_2]^{2+}$ yields just one free aminopyridine molecule and $[Ru(bpy)_2(4AP)(H_2O)]^{2+}$.

3. Excited states and dependence on temperature

The efficiency of ligand photorelease in Ru-bpy complexes depends on the ability of the system to reach the d–d metal-centred state. Direct excitation from the ground state is forbidden, and the corresponding absorption band cannot be detected in the UV–visible spectrum of the complex. It is the highly absorptive MLCT band, the one responsible for the capture of the photons, that will be funnelled to the d–d state [5].

If the d–d states are higher in energy than the ^{3}MLCT state, the efficiency for populating the d–d states—and thus the photorelease quantum yield—becomes temperature dependent. Pinnick & Durham [26] investigated this fact immediately after their report on the band positions and the quantum yield for photorelease of the ligands. Surprisingly, they found a very small temperature dependence, just above the experimental error.

Given the biological interest of Ru-bpy phototriggers, the temperature dependence of their efficiency becomes a relevant issue. We have thus conducted a brief investigation on the activation processes and temperature dependence of ligand photorelease for RuBi-Glutamate.

Following the nomenclature of figure 1, once the ^{3}MLCT state is reached, and using a simple model for the activated process, the quantum yield can be calculated as

$$\phi_{PR} = \frac{k_d}{k_d + k_{r1} + k_{nr1}} \quad \text{with} \quad k_d = A e^{-E_a/RT}, \tag{3.1}$$

where E_a is the energy gap between the ^{3}MLCT state and the d–d state. Reordering gives

$$\ln\left(\frac{1}{\phi_{PR}} - 1\right) = \frac{E_a}{R}\frac{1}{T} - \ln\frac{A}{k_{r1} + k_{nr1}}. \tag{3.2}$$

Density functional theory calculations of some Ru-bpy complexes show that the observed MLCT band is in fact the overlap of several transitions [11,23,27] and several different excited states, with somewhat different energies, can be populated. As the distribution of the populated excited

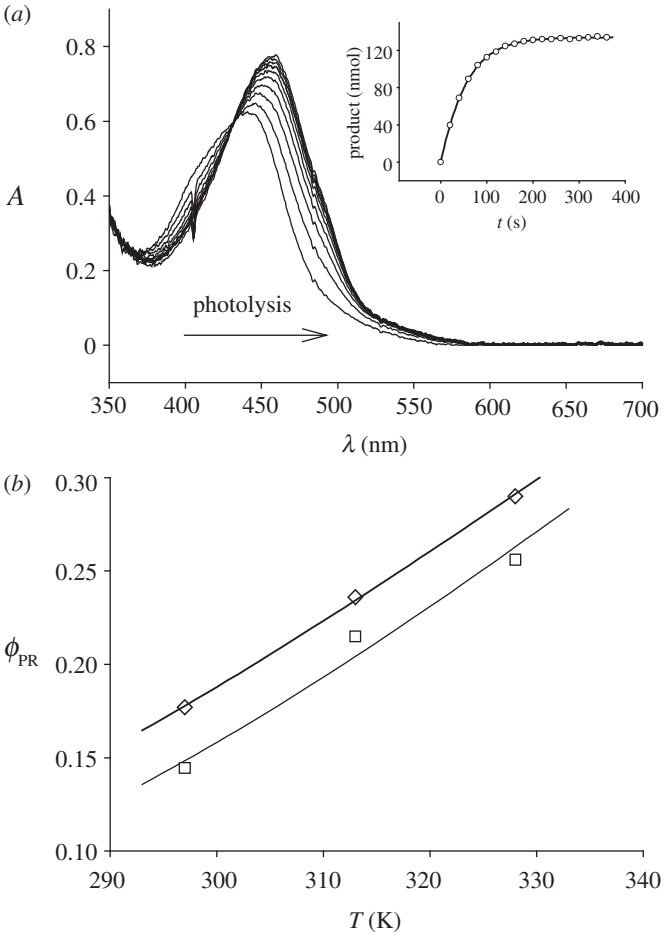


Figure 5. (*a*) UV–visible spectra of a solution of RuBi-Glutamate during irradiation at 405 nm at pH = 3 and 24°C. One of every 10 spectra is shown, with an interval of 20 s between them. Inset: time plot of the photolysis corresponding to the same experiment. Experimental data, 1 every 20 measurements (circles), and fitted data (line) are shown together. (*b*) Quantum yield of photoreaction at three different temperatures under irradiation at 405 nm (squares) and 532 nm (diamonds). The lines show the best fit using equation (3.2).

states depends on the irradiation energy, it is not surprising that some complexes also exhibit a dependency of the activation energy E_a on the irradiation wavelength.

To explore the activation energy of the ligand photorelease at different temperatures and irradiation wavelengths, the following procedure was used. Solutions of the complex RuBi-Glutamate, at about 100 μM and brought to pH = 3 by addition of HCl, were placed in a four-face cuvette with stirring and were thermostatized using a Peltier element. Once constant temperature was reached, a laser beam of a selected wavelength was directed through the cuvette, while absorption spectra were recorded every 2 s at an angle of 90°. The obtained spectra under 405 nm irradiation at 24°C are shown in figure 5*a*.

Given the power of the incident beam, and the volume and concentration of the complex solution, it is possible to calculate the differential amount of product as

$$\frac{dn_P}{dt} = I_{beam} \cdot (1 - 10^{-A_T}) \cdot \frac{A_R}{A_T} \cdot \phi_{PR}, \tag{3.3}$$

where n_P are the moles of uncaged product, I_{beam} is the intensity of the incident light in einsteins per second, A_T and A_R are the solution's total absorbance and the reactant's absorbance,

respectively, and ϕ_{PR} is the photoreaction quantum yield. The integration of equation (3.3) is done by a finite differences approach. The experimental data and the fit to the integrated equation (3.3) are shown in the inset of figure 5. The photorelease quantum yield at the desired temperature and wavelength is obtained by this procedure. The quantum yield at different temperatures can be measured in the same way (figure 5*b*) and according to equation (3.2) it is possible to estimate activation energy $E_a = 18.6\,\mathrm{kJ\,mol^{-1}}$ for the irradiation at 405 nm. Similar procedures were conducted using a 532 nm DPSS laser, and the corresponding activation energy was found to be similar: $E_a = 16.9\,\mathrm{kJ\,mol^{-1}}$.

The strong dependence of ϕ_{PR} on the temperature for this kind of complex is consistent with the accepted photolysis mechanism described in figure 1. At 532 nm and 15°C, RuBi-Glutamate presents a value of $\phi_{PR} = 0.15$, while it rises to $\phi_{PR} = 0.22$ at 37°C, a 50 per cent higher activity. This change in the photorelease efficiency of the Ru-bpy complexes should be taken into account when working with different biological preparations.

4. Applications of Ru-bpy complexes in neurophysiology

Although the capability of Ru-bpy complexes of losing ligands upon visible light irradiation has been known since the 1980s, this was considered just a synthetic tool or even a major drawback of these Ru light-harvesting molecules. Many efforts were made to achieve more robust and photoinert Ru-bpy complexes to take advantage of their photophysics, leaving their interesting photochemistry almost unexplored.

Since the first Ru-bpy-based neuroactive caged compound was used to control neuronal firing in a leech ganglion in the 1P [6] or in mice in the 2P regime, these new phototriggers have found their place as a powerful tool for neurophysiologists. The design of RuBi-GABA [18] and RuBi-Glutamate [20] opened a wide spectrum of possibilities to experiment with 1P and 2P active caged versions of the most ubiquitous inhibitory and excitatory neurotransmitters. RuBi-Glutamate appears to be a superb tool to determine circuitry in dense neuronal systems [28–32]. On the other hand, the physiology of neuronal inhibition can be studied with the use of RuBi-GABA, the first caged GABA with 2P capabilities, necessary to address single dendritic spines [33–36]. Direct photoactivation of RuBi-GABA was shown to be capable of decreasing the activity of the anaesthetized mouse cortex [19], and even to stop epileptic seizures [37], indicating promising therapeutic applications.

Recently, RuBi-GABA, RuBi-Glutamate, caged nicotine and caged 4AP have become commercially available [38], a fact that will probably produce an important increase in the utilization of these phototriggers by the biosciences community.

In other fields, the same strategy has been adopted for the photorelease of protease inhibitors [39] and anti-cancer drugs [40,41] suitable for visible light photodynamic therapies. In the latter case, a nitrile-modified analogue of an oncological drug was used in order to obtain a stable complex. Conversely, the use of the $[Ru(bpy)_2PMe_3L]^{n+}$ structure could lead to the caged form of intact tested pharmaceutical drugs, an attractive option which could facilitate the adoption of these compounds in realistic therapeutic situations.

5. Ru-bpy complexes, poor donor ligands and genetic inducers

It is known that Ru-bpy complexes can coordinate thioethers [42]. Recently, we found that these ligands can also be photoreleased with very high efficiency. This is not surprising, because thioethers are very weak donors and hence high energy MLCT bands are expected. Methylthiogalactoside (MTG) is an S modified sugar that acts as an inducer of a genetic regulatory mechanism known as the Lac operon. The Lac operon is a set of genes that code for proteins which together regulate the transport and metabolism of lactose in prokaryotes like *Escherichia coli*. It is inducible in the sense that the genes are normally not expressed until a small molecule (like lactose or MTG) binds to a DNA-binding protein and induces the synthesis of the regulated proteins [43]. This genetic construct has been adapted to other organisms beyond *E. coli* and it

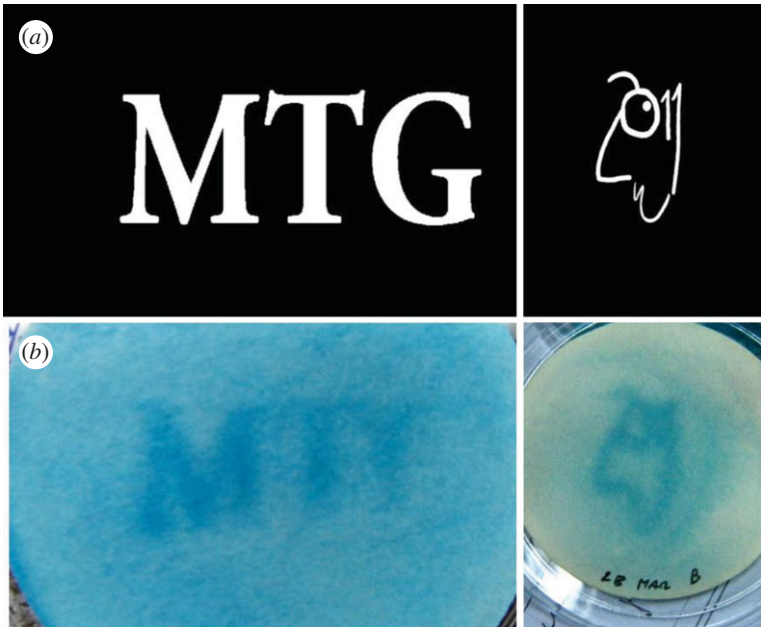


Figure 6. Demonstration of gene induction by a caged gene expression regulator ($[Ru(bpy)_2(PMe_3)(MTG)]^{2+}$). (*a*) Templates for 3M LED-powered pocket projector. (*b*) The image template was projected on a culture of *E. coli* in the presence of the complex, inducing the expression of a reporter protein which results in a blue precipitate. (Online version in colour.)

is routinely used to achieve inducible gene expression in animals ranging from fruit flies [44] to mice [45]. Visible (as opposed to UV) light sensitivity becomes particularly important in this application, as the time course of cellular responses to UV-induced oxidative stress and DNA damage is on the same scale as that of gene expression control [46,47]. We have synthesized the first visible light phototriggered gene inducer, $[Ru(bpy)_2(PMe_3)(MTG)]^{2+}$ (RuBi-MTG), which presents maximum absorbance at 420 nm, blue and green light sensitivity, excellent stability in the dark either dry or in physiological solution, and a quantum efficiency of about 0.3 at 25°C. We used it to induce gene expression in a predefined spatial pattern within a homogeneous cell culture. A culture of *E. coli* cells growing on a filter paper soaked in LB medium containing RuBi-MTG was illuminated with a given pattern by means of a light-emitting diode (LED)-powered pocket projector, resulting in the localized expression of the target reporter β-galactosidase gene and the formation a blue precipitate from the hydrolysis of the substrate X-Gal (figure 6). As the reporter gene is replaceable by any other set of genes of therapeutic value, the capacity to induce gene expression in some cells and not in their neighbours is a valuable tool in biotechnology and tissue engineering.

6. Perspectives at longer wavelengths

The photochemical mechanism of Ru-bpy imposes some restrictions regarding the energy of the photons capable of eliciting the ligand release [5]. The energy–quantum yield relationship implies that it is difficult—if not impossible—to design a phototrigger with both high absorption at long wavelengths and high photodissociation quantum yield. This fact limits the useful wavelength to a limit around 540 nm. RuBi-Glutamate presents green light photoreactivity, but its molar absorptivity at 532 nm is 760 M^{-1} cm^{-1}, only one-seventh of its maximum at 450 nm.

However, it is possible to circumvent this limitation by means of a ligand capable of harvesting light with high efficiency and transferring that energy to the Ru MLCT state.

The complex $[Ru(bpy)_2Cl(RhodB\text{-}MAPN)]^{2+}$ (RuBi-Rhod in scheme 1) uses a rhodamine B ligand to harvest light. In order to coordinate this fluorescent dye, a derivatization using a tail of *N*-methylamine propionitrile (MAPN) has been obtained through amidation [48].

Its analogue complex $[Ru(bpy)_2Cl(MAPN)]^{2+}$ presents the usual behaviour of a Ru-bpy complex bearing a nitrile ligand: it releases MAPN upon irradiation at 405 and 532 nm. Molar absorptivity of $[Ru(bpy)_2Cl(MAPN)]^{2+}$ at 532 nm is rather high due to the presence of the negative-charged chloride ligand: $\varepsilon_{532} = 1700\,M^{-1}\,cm^{-1}$. Its photorelease quantum yield at this wavelength is $\phi_{PR} = 0.145$, which results in an overall photoactivity efficiency of $\varepsilon_{532}.\phi_{PR} = 246\,M^{-1}\,cm^{-1}$.

On the other hand, the RhodB-MAPN ligand presents very high absorption at 532 nm, and its complex $[Ru(bpy)_2Cl(RhodB\text{-}MAPN)]^{2+}$ has $\varepsilon_{532} = 84\,500$ with $\phi_{PR} = 0.070$, increasing 24-fold the photoactivity efficiency to $\varepsilon_{532}.\phi_{PR} = 5920\,M^{-1}\,cm^{-1}$, the highest known figure for a Ru-bpy complex at such a long wavelength. This increase in the overall photoactivity can be explained by a Förster resonance energy transfer mechanism in which most of the photons are captured by the rhodamine ligand and transferred to the Ru-bpy core with high efficiency owing to its very close proximity [49]. This short distance is primal to counteract the small overlap between donor (rhodamine) emission and acceptor (Ru-bpy core) absorption. The use of fluorescent ligands to harvest light with an increased cross section will open a wide range of new applications requiring long wavelengths to ensure deep penetration, such as *in vivo* studies of physiology and photodynamic therapies.

7. Conclusions

In the last decade, since the first Ru-bpy-based caged 4AP, quite a lot of progress has been made. Although still in its beginnings, the strategy of using these coordination complexes to cage molecules has already rendered concrete achievements in the field of neurosciences and surface engineering. The incipient work in anti-cancer drugs and gene inducers is also very promising, and new perspectives will be opened as the design of these molecular devices improves.

Ru-bpy phototriggers offer simplicity of synthesis, a rational design, a wide spectrum of cageable functional groups and a fast, clean and efficient way to photorelease entire molecules for a broad set of applications.

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