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From curiosity to applications. A personal perspective on inorganic photochemistry

Peter C. Ford

Over the past several decades, the photochemistry and photophysics of transition metal compounds has blossomed from a relatively niche topic to a major research theme. Applications arising from the elucidation of the fundamental principles defining this field now range from probing the rates and mechanisms of small molecules with metalloproteins to light activated molecular machines. Offered here is a personal perspective of metal complex photochemistry drawn from this author’s long involvement with this field. Several examples are described. Topics include characterizing key excited states and tuning these to modify chemical reactivity and/or photoluminescence properties, as well as using photoreactions as an entry to reactive intermediates relevant to homogeneous catalysts. This is followed by discussions of applying these concepts to developing precursors and precursor-antenna conjugates for the photochemical delivery of small molecule bioregulators to physiological targets.

Introduction

The interaction of light and matter has long intrigued humans of all ages. Indeed this author’s personal interest in the photochemistry of transition metal complexes can be traced to the qualitative observation that certain metal compounds left on the benchtop for several days looked very different than those kept in the dark for a comparable period. This was during a postdoctoral fellowship in Henry Taube’s Stanford U. laboratory, where as a new convert to inorganic chemistry, I became fascinated by the bright colors of transition metal complexes. The compounds of interest were the pentaammineruthenium(II) complexes Ru(NH₃)₅L²⁺ of various aromatic nitrogen heterocycles L. The absorption bands that dominate the visible absorption spectra of these Ru(NH₃)₅L²⁺, e.g., Fig. 1, are markedly dependent on the nature of L. For example, if L is a substituted pyridine py-X, this band shifts markedly to the red when X is an electron accepting substituent and to the blue when X is an electron donor. This and related observations drove me to study C. K. Jorgensen’s book on metal complex spectroscopy² and to learn sufficient group and ligand field theory to interpret these absorption bands as metal-to-ligand charge transfer (MLCT) transitions.¹

It was with these materials that I first noted light induced color changes and learned to store my samples in the dark. Furthermore, curiosity about the underlying causes led me to begin investigating the photoreactions of the Ru(II) ammine complexes, once I joined the chemistry faculty of the University of California, Santa Barbara (UCSB). I was fortunate that several UCSB colleagues were studying gas phase photoreactions and, moreover, were willing to teach this novice some nuances of photochemical science as it was then practiced. To set the context, I should emphasize that there had already been significant activity investigating photoreactions of metal complexes. Much of this work has been summarized in books by Balzani and Carassiti (1970)³ and by Adamson and Fleischauer (1974),⁴ both of which remain valuable resources. In addition, it would be remiss not to draw special attention to the iron(III) oxalate-based actinometry developed by Hatchard and Parker in the UK (1956).⁵ This provided a convenient and sensitive method for measuring the intensities of the light at the location where solution phase photochemical reactions occur. It is a tool that continues to be in use many decades later.

Transition metal photochemistry continues to evolve, especially as new tools or potential new applications are realized. Offered here is a personal and not very comprehensive perspective of this evolution organized around the author’s focus on the reactions and mechanisms that are triggered by light.

Fig. 1. Absorption spectrum of Ru(NH₃)₅(py)³⁺ ion in aqueous solution.

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Fundamental mechanistic photochemistry

**Excited states:** The first question one might ask when considering the solution photochemistry of a complex ion like Ru(NH$_3$)$_6$(py-X)$_2^{2+}$ is "what excited states (ES) are involved?" From the orbital parentage of these ES, one might develop a qualitative view of expected reaction trajectories. Given the power of density functional theory (DFT) computations now accessible, such visualization might seem primitive, but it does provide intuition of the type that has made the practice of chemistry a very human activity. Stepping back to our earliest photochemical investigations, neither DFT nor laser flash photolysis were readily available. As a consequence, mechanistic conclusions were largely built on determining photoreaction products and quantum yields and on comparing these data to the electronic spectroscopic properties.

Fig. 2 illustrates the types of one-electron transitions that are commonly used to assign absorption bands and excited states in mononuclear metal complexes. Those most characteristic of transition metal complexes with partially filled d-orbitals are absorption bands representing excitation between "d" orbitals that are split by mixing with s-donor and p-donor and acceptor orbitals of the ligands. These "d-d" ligand field (LF) transitions involve molecular orbitals (MOs) that are largely metal in character, hence the resulting ES are often referred to as metal-centered excited states (MC*). In a centrosymmetric complex, LF bands are Laporte forbidden and typically have relatively low extinction coefficients. In contrast, metal-to-ligand charge transfer (MLCT) and ligand-to-metal charge transfer (LMCT) bands are much more intense owing to the dipole moment change between the ground and excited states. The fourth type denoted in this figure is a ligand centered or intraligand (IL) transition, represented here as π$_i$ $\rightarrow$ π$_i^*$ excitation. While these one-electron designations provide a convenient framework for addressing the nature of the relevant ES, theoretical calculations have long shown that there is much more mixing of the excited state characters than implied by Fig. 2.

For Ru(NH$_3$)$_6$(py-X)$_2^{2+}$ and related complexes such as Ru(NH$_3$)$_6$(pz)$_2^{2+}$ (pz = pyrazine), the strong absorption bands ($\varepsilon \approx 10^4$ M$^{-1}$ cm$^{-1}$) seen in the visible spectra were assigned as spin-allowed (singlet to singlet) MLCT transitions, while those in the ultraviolet region (UV) as IL (π$_i$ $\rightarrow$ π$_i^*$) in character. Where would one expect to find the LF absorption bands of these low spin d$^6$ complexes? As an empirical model, one might consider the spectrum of Ru(NH$_3$)$_6^{3+}$, which does not include any π-unsaturated ligands. The electronic spectrum of this octahedral complex displays two relatively weak absorption bands at 390 nm ($\varepsilon \approx 35$ M$^{-1}$ cm$^{-1}$) and 275 nm (640 M$^{-1}$ cm$^{-1}$) as predicted by group theory ($^3$A$_{1g}$ $\rightarrow$ $^1$T$_{1g}$ and $^3$A$_{1g}$ $\rightarrow$ $^3$T$_{2g}$). Note: the higher intensity of the second band has been attributed to mixing with a charge transfer to solvent transition, CTTS). Analogous LF bands might be expected at comparable energies for the Ru(NH$_3$)$_6$(py-X)$_2^{2+}$ ions, but they are obviously obscured by the much stronger MLCT bands.

**Quantum yields and ES dynamics:** The quantum yield is basically an efficiency measurement and can be defined for a specific photoprocess $i$ as shown in eq. 1. Note that $\Phi_i$ is unit-less. An efficient process has a quantum yield near 1 (or higher if a chain reaction results); however, as discussed below, there are many reasons why $\Phi_i$ might be much smaller. A key point here is the nature of the denominator, since photoreactions will not occur unless light is absorbed. Quantum yields are measured at a specific wavelength of irradiation ($\lambda_o$), so this concept is less precise when a broadband excitation source such as sunlight is used.

With certain applications, for example, the uncaging of a drug at a physiological target, the overall rate of the photochemical reaction is of key interest. The photoreaction rate equals $\Phi_i \times I_o$, where $I_o$ is the intensity of the light absorbed, typically in units of Einsteins/unit time (1 Einstein = 1 mole of photons) or in Einsteins per unit volume/unit time. For a solution where the photoreactant is the only species absorbing the incident light, $I_o = I_o(1-10^{-\alpha o b s}(\lambda))$, where $\alpha o b s(\lambda)$ is the solution absorbance at $\lambda_{avr}$ and equals the product of the molar concentration of the photoactive species (c), the molar extinction coefficient (ε, in L moles$^{-1}$ cm$^{-1}$) at that λ and the path-length of the cell (in cm). There is no unimolecular rate constant associated with this process.

Taking for example Ru(NH$_3$)$_6$(py)$_2^{2+}$, continuous photolysis of this ion with 405 nm light in ambient temperature, aqueous solution resulted in the photossubstitution reactions depicted in eq. 2. The respective quantum yields for pyridine and ammonia substitution $\Phi_{py}$ and $\Phi_{NH_3}$ were measured as 0.045 and 0.063 for $\lambda_{avr} = 405$ nm. This excitation wavelength is close to the $\lambda_{max}$ of the spin-allowed MLCT band (407 nm), so one can be assured that the vast majority of the photons absorbed lead to MLCT excitation.

**Excited states**

$\Phi_i = \frac{\text{number of molecules undergoing process } i}{\text{number of photons absorbed}}$ (1)

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Fig. 2. Representation of one-electron transitions between the MOs of a mononuclear metal complex ML$_6$ in O$_3$ symmetry. LF: ligand field transition; LMCT: ligand to metal charge transfer; MLCT: metal to ligand charge transfer; IL: intraligand or ligand centered.

```latex
\begin{align*}
\text{Ru(NH}_3\text{)}_6\text{H}_2\text{O}^{2+} + \text{py} & \rightarrow \text{Ru(NH}_3\text{)}_6(\text{H}_2\text{O})\text{py}^{2+} + \text{NH}_3 \\
\text{Ru(NH}_3\text{)}_6(\text{py})^{2+} & \rightarrow \text{Ru(NH}_3\text{)}_6\text{H}_2\text{O}^{2+} + \text{py}\quad \text{(2)}
\end{align*}
```

Are the observed photossubstitution reactions consistent with that expected for an MLCT excited state, which can simply be represented as having an oxidized metal center and a reduced pyridine, [Ru$^{n+}$(py)]$^{2+}$? Given that low spin d$^5$ Ru(III) complexes are not very thermally labile, this behavior is not what one might expect for the MLCT$^*$ state. In contrast, the MC$^*$ states, which involve promoting an electron from a non-bonding or even σ-bonding orbital to one which is σ* relative to the metal-ligand bonds would appear to be logical precursors of ligand photossubstitutions. Consistent with this argument, the hexaaamine complex Ru(NH$_3$)$_6^{3+}$ is comparably labile towards photodissociation of NH$_3$ ($\Phi_{NH_3} = 0.27$ for $\lambda_{avr} = 405$ nm). Drawing such a conclusion does, however, presume that the mechanism for ES ligand labilization is predominantly dissociative in character. In support of this view, a
recent TD-DFT calculation on the metal centered $^3MC^*$ state of Ru(NH$_3$)$_5$(py)$_2$ confirms that this state is nearly dissociative in character.9

This reasoning led to our proposal that the ES responsible for the reactions noted in eq. 2 was the result of rapid internal conversion/intersystem crossing from MLCT states populated by excitation to spectrally unobserved MC* states as illustrated in Fig. 3.10 The observation that photolabilization of aq. Ru(NH$_3$)$_5$(py)$_2$ is largely independent of the excitation wavelength suggested that a common reactive excited state, presumably the lowest energy excited state(s) (LEES), is responsible for these photo-reactions. Another consequence of this model is the prediction that we can use ligand substituents to tune the excited state energies to where the $^3$MLCT is the LEES. In that case, there should be marked decreases in the observed photolability.

Our ES “tuning model” was thus determined by examining the photoreactions of a series of Ru(NH$_3$)$_5$(py-$X$)$^{2+}$ complexes.11 Electron-withdrawing substituents that shift the MLCT band to $\lambda_{\text{max}}$ values $>$470 nm led to dramatically less photolability than those with $\lambda_{\text{max}}$ values at shorter wavelengths. Similar MLCT/MC state tuning approaches have since been used with a number of systems including those based on the Ru(II) polypyridyl complexes of interest with regard to solar energy conversion devices12 and similar Ir(III) phenylpyridyl complexes of interest for organic light emitting diodes (OLED) applications.13

The observation that photoreaction quantum yields $\Phi_\text{r}$ for aq. Ru(NH$_3$)$_5$(py)$_2$ and Ru(NH$_3$)$_5$(py)$_2$ described are considerably less than unity is explained by the Jablonski diagram shown in Fig. 3. Radiative and nonradiative pathways for ES deactivation to the ground state (GS) compete with the chemical pathways leading to products. In this simplified diagram, all three pathways are represented as occurring from the lowest energy ES of metal complex. However, one should keep in mind that with organic molecules and even complexes of lighter metals, intersystem crossing (ISC) rates are often not competitive with direct radiative (fluorescence) and non-radiative deactivation to the GS. Furthermore, Franck-Condon states formed directly by excitation from the GS may undergo processes, including ISC, faster even than vibrational relaxation.14 Thus, the ES kinetics can be quite complex owing to the numerous states and deactivation pathways potentially involved. (Note: For compounds of lighter elements, e.g., organic dyes, internal conversion to the lowest energy ES of a particular spin state is typically much faster than the forbidden ISC to an ES of a different spin state. However, the much greater spin orbit coupling with complexes of heavier elements tends to break down this pattern.)

For illustrative purposes, we will continue discussion of quantum yields based on the simple model described by Fig. 3, where a single state (or collection of ES) is populated by rapid and highly efficient internal conversion and ISC from states initially formed by excitation. For such a system, where decay occurs only by first order ES processes, $\Phi_\text{r}$ can also be described (eq. 3), where $k_\text{r}$ is the rate constant for photoproduct formation, while $k_e$ and $k_n$ represent those for nonradiative deactivation to the ground state and radiative deactivation (emission), respectively. In the case of aq. Ru(NH$_3$)$_5$(py)$_2$ emission is at best very weak, and the photoprodut quantum yield $\Phi_\text{r}$ is less than 0.5, so the dominant LEES pathway must be nonradiative deactivation.

$$\Phi_\text{r} = k_\text{r} / (k_e + k_n + k)$$ (3)

Excited state lifetimes can be measured either by transient absorption, transient bleaching or luminescence methods. If the decay pathways from the LEES are unimolecular as implied by Fig. 3, then the observed lifetime $\tau$ equals $\langle \Delta k \rangle^{-1}$, where in this case $\tau = (k_e + k_n + k)$. Thus $\Phi_\text{r}$ can be rewritten eq. 4, and excited state rate constants for internal pathways can be calculated from $k_\text{r} = \Phi_\text{r} \cdot \tau$. However, it must be emphasized that, while this approach often applies to complexes of the heavier transition metals, the kinetics are more complicated when more than one ES is responsible for the chemical or photoluminescence behavior.

$$\Phi_\text{r} = k_\text{r} \cdot \tau$$ (4)

It was long thought that the Ru(NH$_3$)$_5$(py-$X$)$^{2+}$ complexes did not display any emission either in solution or as solids at. This conclusion is a matter of detection sensitivity given the Einstein principle that every excited state undergoes spontaneous emission.15 The inherent intensities of such luminescence are determined by the competing processes that deplete that ES. Ultrafast flash photolysis studies by Winkler et al.16 and others17 did not detect emission, but with transient bleaching measurements did demonstrate ES lifetimes from <20 ps to ~200 ps in ambient temperature solution. The longer values of $\tau$ were seen for those complexes for which the LEES are MLCT in character. Recently, Endicott and coworkers16 detected very weak emissions ($\Phi$, ~5 x 10$^{-6}$) from several of these complexes in 77 K organic glasses. Under these conditions, lifetimes were found to be on the 0.1-3 $\mu$s timescale, so the weak emissions were attributed to very low values of $k_\text{r}$ rather than to short lifetimes under these conditions.

The reaction dynamics implied by Fig. 3 and eq. 3 assumes that the lowest energy ES lies on potential surfaces with clearly defined minima. Such states are “bound” ES, for which there is an energy barrier larger than $k_\text{r}T$, ($k_\text{r}$ being the Boltzmann constant) to decay along any relevant reactive coordinate (Fig. 4). It is also possible to have a “dissociative” state, where the barrier is less than $k_\text{r}T$. The latter would have exceedingly short lifetimes, and it is not correct to treat their dynamics by classical kinetics methods.

Fig. 3. Proposed ES diagram to explain photolabilization of aq. Ru(NH$_3$)$_5$(py)$_2$ upon irradiation of MLCT bands. $k_\text{r}$ is the rate constant for reactions to photoproducts. $k_\text{ic}$/isc, $k_e$ and $k_n$ are rate constants for internal conversion/ intersystem crossing to a lowest energy metal centered excited state, nonradiative deactivation to the ground state $^1$GS and radiative deactivation (emission), respectively.
Coordination compounds:

There have been a number of quantitative photochemical studies of coordination compounds, although the earlier studies primarily focused on those that were thermally stable in solution.

Consequently, d⁴ (primarily Cr(III) complexes), low spin d⁴ (Fe(II), Ru(II) Os(II), Co(II), Rh(III), Ir(III) and some Pt(IV) complexes) and low spin d⁵ complexes (e.g., Rh(II), Ir(I) and Pt(III)) received the most attention, although this list is not comprehensive. Described here are several Rh(III) ammine complexes for which the detailed ligand photosubstitution dynamics and mechanisms were studied in this laboratory. These provide a valuable case study on the reactions of the metal-centered excited states of low spin d⁴ complexes, an electronic configuration of metal systems often found in applications such as dye sensitized solar cells (DSSCs) and OLEDs.

Rhodium(III) ammine complexes: The importance of MC* states in defining the photostability of the Ru(II) species even when initial excitation is MLCT led us to focus attention on the analogous photochemistry of the isoelectronic Rh(III) ammine complexes such as the Rh(NH₃)₆⁶³⁺ and Rh(NH₃)₅Cl⁺² ions. The absorption spectra of these rhodium ammine complexes are dominated by the spin-allowed LF bands (¹A₁ → T₂, ¹A₁ → T₁) for the O₆ complex Rh(NH₃)₆⁶³⁺, Fig. 5) predicted by group theory, while the emission spectra are also characteristic of that expected from the lowest energy MC* states (¹T₁ → ¹A₁ for Rh(NH₃)₅Cl⁺²). Our entry into these studies also corresponded to our collaborations with R. J. Watts and D. S. Magde, with whom we were able to conduct ns and ps pulsed laser experiments that allowed determining for the first time the emission and transient absorption lifetimes of the lowest energy MC* states in fluid solutions. In this manner we were also able to probe the MC* state ligand substitution and nonradiative decay mechanisms with hydrostatic pressure effects in collaboration with R. van Eldik.

For Rh(NH₃)₆⁶⁺, the photosubstitution chemistry (eq. 5, Φₐ = 0.075 in 296 K eq. solution, A = NH₃) and the luminescence properties proved to be largely independent of the excitation wavelengths. These observations, combined with early sensitization experiments, led to the conclusion that initial LF excitation to form MC* states was followed by efficient internal conversion/intersystem crossing to the lowest energy MC* state (i.e. the ³T₁) from which emission or reaction largely occurred. (MC*: kₖc values > 5 x 10⁹ s⁻¹ have been measured for Rh(III) ammine complexes.) This progression is envisioned by the Jablonski diagram shown in Fig. 6. Accordingly, one can treat the photoreaction and emission quantum yields in terms of the excited state rate constants as in eq. 3. Thus, for example, Φₐ = kᵣ / (kₙ + kᵣ). As seen for the ruthenium complexes, the modest quantum yield for eq. 5 and the very weak emission clearly indicate that the dominant pathway from the ³T₁ is nonradiative deactivation, thus kₙ > kᵣ. If that were the case, then inhibiting kₙ would have the potential to increase Φₐ.

\[
\text{Rh(NH₃)₆}^{3+} \xrightarrow{\text{hv}} \text{Rh(NH₃)₅H₂O}^{3-} + \text{NH₃}
\]

(S)

The question was: How to test this idea by perturbing kₙ? To do so, we drew upon studies by Glen Crosby and coworkers, who had shown that the perdeuterated complex Rh(D₄)₆⁶⁺ had dramatically longer luminescence lifetimes in frozen solutions at 77 K than did the perproto analog. Since no photochemistry occurred under those conditions and the emission was a minor deactivation pathway, this meant that exchanging the N-H's for N-D's significantly decreased the rate of nonradiative deactivation. This, in turn, would carry over to studies in fluid solutions. Indeed, we were able to show that exchanging the amines with D₂O to give Rh(D₄)₆⁶⁺, led to roughly doubled values of Φₐ in ambient T solutions, consistent with this premise.

However, given that eq. 3 interprets the quantum yield as a ratio of rate constants, the increased Φₐ is only indirect evidence in support of the conclusion that we were suppressing kₙ partially by perdeuterating the complex. One could instead draw the seemingly less likely conclusion that the amine perdeuteration leads to an increase in the photoreaction rate constant kᵣ. This conundrum led us to increase efforts to measure excited state lifetimes under conditions directly relevant to the photoreaction pathways. Initially these studies were with Doug Magde at UC San Diego, but this quest was reinforced by access to one of the first ns pulsed Nd/YAG lasers commercially available. We were thus able to measure lifetimes of the faint phosphorescence from the MC* states of various perpropto and perdeuterio Rh(III) ammine complexes in fluid aqueous solutions.
If X is a stronger α-donor than is NH₃, then the 3A₂ state is the LEES, and the excited electron is primarily in the equatorial α-antibonding (dₓz) orbital. Ligand withdrawal would then be expected along the axes perpendicular to the principal axis. Consistent with this argument, the cyano complex undergoes NH₃ labilization of the equatorial ammine to give cis-Rh(NH₃)₅(H₂O)CN⁻ with a Φ₄₃ of 0.09 in ambient temperature aqueous solution (eq. 7).\(^{20}\) Furthermore, selective isotopic labeling demonstrated that the labilized NH₃ occurs specifically from the equatorial sites as predicted.\(^{21}\)

\[
\Phi_A = 0.02 \quad \text{and} \quad \Phi_C = 0.18 \quad \text{in 298 K aq. solution.}^{21b}
\]

The quantum yields for eq. 6 are Φ₄ = 0.02 and Φ₅ = 0.18 in 298 K aq. solution.\(^{21b}\) We measured the emission quantum yield Φ₅ for the 3MC* state of Rh(NH₃)₅Cl⁻ as 3 x 10⁻⁵ under these conditions,\(^{21b}\) so this compound also represents another case where nonradiative decay is the principal deactivation pathway from the 3MC* state. Accordingly, the emission lifetime (35 ns) of the perdeuterio complex Rh(ND₃)₅Cl⁻ is significantly longer than that (14 ns) of Rh(NH₃)₅Cl⁻. By using eq. 4, the respective excited state rate constants for the latter ion calculated from the quantum yields and lifetimes are k₇ = 1.4 x 10⁻⁵ s⁻¹, k₈ = 0.14 x 10⁻⁵ s⁻¹, k₉ = 2.3 x 10⁻⁵ s⁻¹ and k₁₀ = 5.6 x 10⁻⁵ s⁻¹. By measuring the quantum yields and lifetimes at several temperatures, we calculated an activation energy (Eₗ) of 25 kJ/mol for k₉. This result clearly indicates that the potential well for the reactive MC* state of Rh(NH₃)₅Cl⁻ is well defined. In other words, it is a "bound" state as described in Fig. 4.

For comparison, heating an acidic, aqueous solution of Rh(NH₃)₅Cl⁻ leads to Cl⁻ aquation only.\(^{22}\) This displays a first-order rate law, and extrapolation to ambient temperature gives k₇ = 5.4 x 10⁻⁵ s⁻¹ for the ground state reaction. Thus, the 3e triplet ES is >14 orders of magnitude more reactive toward chloride labilization than is the ground state. Furthermore, the Eₗ (120 kJ mol⁻¹) for k₇ is much higher than that for the analogous photoreaction.

These comparisons do not differentiate the possible "unimolecular" mechanisms by which the MC* state might undergo cleavage of the metal-ligand bonds. For example, is this reaction the result of a dissociative substitution mechanism, where M-L bond breaking is the predominant contribution to the activation energy of that step, as might be implied by the LEES (t₈) electronic configuration for a hexaammine complex? Alternatively, the t₈ vacancy (relative to the t₉) ground state) might enhance a more associative pathway. We explored this by determining the ES reaction rate constants k₇ and k₈ in different solvents.\(^{31}\) The key result was that changing the solvent polarity (and nucleophilic character) had a modest impact on k₇ but major influence on k₈, reducing the latter dramatically in the less polar solvents. This argues against a major associative component to the exchange of the coordinated NH₃ with the solvent molecule. While it could suggest such a component to the Cl⁻ labilization, a more likely explanation is that the barrier to dissociation is strongly affected by the solvation of the developing charge separation as the \((\text{NH₃})_5\text{Rh}^{3+} \rightarrow \text{Cl}^- \) bond dissociates.

In this context, a collaborative investigation with Rudi van Eldik\(^{23}\) measured the photoreaction quantum yields and lifetimes for aq. Rh(NH₃)₅Cl⁻ as a function of hydrostatic pressure up to 200 MPa, and activation volumes ΔVₗ were determined. These results are self-consistent with the solvent effects on the analogous reactions in the context that the solvation of the developing charge...
separation as Cl\(^-\) dissociates from the Rh(III) center has a major impact on the barrier for the \(k_\text{q}\) pathway. Although no experiment proves a mechanism, these results are clearly consistent with the model presented by Huisgen et al.\(^{12}\)

\[\Phi_{\text{NO2}} = 0.045\]

\[\text{hv} (355 \text{ nm}) \rightarrow \text{Mn}^{4+}(\text{TPP})(\text{NO}_2)\]  

\[\text{Mn}^{4+}(\text{TPP}) + \text{NO}_2\]

\[
(8)
\]

dissociative labilization mechanisms from the \(^{3}\text{MC}^*\) state.

**Bimolecular processes:**

Our discussion has so far focused on unimolecular processes that occur subsequent to excitation. For transition metal complexes these processes will be dominated by the competing nonradiative deactivation, emission and “unimolecular” events such as ligand solvent, isomerization or redox reactions like eq. 8 (TPP\(^{2+}\) = tetraphenyl-porphyrinato dianion).\(^{34}\)

However, excited state complexes may also participate in bimolecular events such as energy or electron transfer to another chromophore.

The simplest case is one in which there is a single state or set of states in thermal equilibrium that is largely responsible for the photochemical and photophysical behavior of the metal complex. Such is the case for the extensively investigated Ru(bpy)\(^{2+}\) ion.\(^{12}\)

For this species, the situation described in Fig. 3 is reversed, i.e., the lowest energy ES is the \(^{3}\text{MLCT}\) ES, which is relatively unreactive towards ligand substitution. As a consequence, Ru(bpy)\(^{2+}\) salts are strongly luminescent even in ambient temperature solutions (\(\Phi_{\text{em}} = 0.063\) in 298 K deaerated H\(_2\)O, 0.040 in aerated H\(_2\)O).\(^{35}\)

Although the major decay pathway remains the non-radiative deactivation which is largely attributed to the back population from \(^{3}\text{MLCT}\) state to the lowest energy \(^{3}\text{MC}\) state. The roughly microsecond lifetimes and the sensitivity of luminescence detection make such a species ideal for probing bimolecular quenching mechanisms either by energy transfer or by electron transfer. Notably, excited states are both stronger oxidants and stronger reductants than the respective ground state, and this feature has been extensively exploited in applications such as solar energy conversion,

\(^{36,37}\) organic synthesis,\(^{38}\) and new photo-initiated cancer drugs.\(^{39}\)

From a kinetics perspective, quenching may have a major impact on the luminescent lifetime \(\tau\). The quenching rate constants \(k_q\) in solution can be determined by measuring \(\tau\) as a function of quencher concentrations [Q] using Stern-Volmer plots of \(\tau/\tau_0\) vs. [Q] according to eq. 9 where \(\tau_0\) is the lifetime in the absence of quencher. The value of \(k_q\) will depend on the viscosity of the solvent (hence the rate of diffusion) and the operating quenching mechanism(s). If the latter involves energy transfer, the rate will depend on the spectral overlap between the donor and acceptor pair as well as the energies of the relevant excited states. If it involves electron transfer, then the key issue will be the relative redox potentials of the excited state species and of the quencher.

We illustrate these points with photo-luminescent Cu(I) complexes.

\[\tau/\tau_0 = 1 + k_q[\text{Q}]\]

\[
(9)
\]

**The Cu(I) cluster Cu\(_{4}\)tpy\(_4\).** Luminescence in first row transition metal complexes is frequently bypassed by the presence of lowest energy MC states that are too short-lived to have measurable emissions. Thus, filled shell d\(^{10}\) metal systems offer an opportunity to observe other excited states.\(^{40}\) Excellent examples are the Cu(I) complexes of the type Cu(DMPT)\(^+\) ion (DMPT = 2,9-dimethyl-1,10-phenanthroline) described by McMillin et al.\(^{40a,b}\) In the absence of MC\(^*\) states these moieties display MLCT emissions. Here we shall focus on the photophysical/chemical behavior of a different type of Cu(I) complexes, tetraneuronic clusters such as Cu\(_{4}\)tpy\(_4\) that consist of Cu\(_4\) tetrahedra with short Cu-Cu distances. Bridging iodides and terminal pyridine ligands complete the unit. Related polynuclear copper(I) clusters have drawn recent interest for potential applications in OLEDs.\(^{41}\)

The photophysical properties of Cu\(_{4}\)tpy\(_4\) demonstrate marked environmental sensitivity, i.e., the luminescence spectra change reversibly by varying the temperature and the rigidity of the medium\(^{42}\) owing to the presence of multiple emitting excited states. In ambient solutions, Cu\(_{4}\)tpy\(_4\) displays two distinct emission bands that also demonstrate different lifetimes (Fig. 7). This behavior is in obvious contrast to the patterns seen for the Rh(NH\(_3\))\(_6\)\(^{3+}\) and Ru(bpy)\(^{2+}\)\(^{3+}\) ions described above, where single emissions from lowest energy MC and MLCT states were respectively recorded.

The strong, longer-lived (\(\tau = 10.6 \mu s\)) lower energy (LE) emission (\(\lambda_{\text{max}} = 690\) nm) from Cu\(_{4}\)tpy\(_4\) in 296 K toluene solution was assigned to a triplet cluster-centered (\(^{3}\text{CC}\)) ES, resulting from a combination of delocalized iodide-to-copper charge transfer and d-s transitions.\(^{42}\) The weaker, shorter-lived (\(\tau = 0.45\) \(\mu s\)) higher energy (HE) band (\(\lambda_{\text{max}} = 460\) nm) was assigned to a triplet halide-to-ligand (pyridine) charge transfer (\(^{3}\text{XLCT}\)) ES.

Notably, when the pyridines are replaced by the aliphatic amine piperidine, the LE band is still present, but the HE band is not.\(^{43}\) Relative to the GS, the \(^{3}\text{CC}\) and \(^{3}\text{XLCT}\) ES of Cu\(_{4}\)tpy\(_4\) are distorted along different trajectories, since the former ES is formed by promoting an electron from filled orbitals that are non-bonding (or even anti-bonding) to orbitals that are bonding with respect to the Cu\(_4\) cluster. As a result the surfaces of the \(^{3}\text{CC}\) and \(^{3}\text{XLCT}\) states overlap poorly, and there is an energy barrier for internal conversion from the \(^{3}\text{XLCT}\) state to the lower energy \(^{3}\text{CC}\) excited state (Fig. 8). This is especially apparent at lower temperatures.

Fig. 7. Emission spectrum from Cu\(_{4}\)tpy\(_4\) in 296 K toluene solution. The excitation wavelength was 350 nm.

where the \(^{3}\text{XLCT}\) emission is much stronger. Computational studies clearly support these assignments.\(^{44}\)
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ARTICLE

Flash photolysis probes of reactive intermediates.

Within the anatomy of a photoreaction, there are potentially a number of steps between the initial ground state and the final product(s) as qualitatively illustrated in Fig. 10. The first is the initial absorption of light that prepares a Franck-Condon state (FC*) that is both electronically and vibrationally excited. The FC* may undergo reaction if oriented along a particular trajectory, or it may undergo other reactions due to internal conversion. Thus, the final products observed may result from secondary pathways of the intermediate(s) I.

There are several ways to probe such intermediates. For example, in low temperature matrices, the activation energies for surmounting the barriers illustrated in Fig. 10 may be sufficient to inhibit further reaction. In that case, photolysis can be used to generate I, which then can be characterized by conventional spectroscopy. An alternative (and complementary) approach is to use flash photolysis to generate such species and fast detection techniques to characterize their spectroscopy and reaction dynamics as they evolve to the final products.

Our first flash photolysis apparatus was a "conventional" flash lamp system with a design not altogether that different from that first built by Norrish and Porter, who were awarded the 1966 Nobel Prize in Chemistry for their contributions to fast reaction kinetics. This system operated by discharging hundreds of Joules of electrical energy from a capacitor bank through 10-20 cm long cylindrical...
quartz flashlamps filled with xenon gas. The white light flash lasted about 10 μs and irradiated a sample cell with a path of similar length. If one were sufficiently careful to avoid saturating the PMT detector with scattered light, it was possible to record flash-induced temporal changes in the transient spectra.

**Flash photolysis of Ru(NH$_3$)$_3$(py)$^{2+}$**: The first system that we used this flash photolysis apparatus to study was the highly colored Ru(NH$_3$)$_3$(py)$^{2+}$ ion that had first stirred our interest in photochemistry. Our early flash studies indicated the formation of a transient species that partially returned to the starting material on a millisecond time scale. In acidic solution, the recovery was slower and less of the starting ion reformned. These observations paralleled our finding that pyridine photoaquation quantum yields are higher and transient species that partially returned to the starting material on photochemistry. Our early flash studies indicated the formation of a transient species by which the Ru ion that had first stirred our interest in the catalytic cycle was concluded to be the most stable intermediate, although Ru(II) coordination at a pyridine C–H bond has isomerized from monodentate coordination to a π complex such as the η$^2$-py species illustrated in Scheme 2. It is notable that a recent study using laser flash photolysis techniques and DFT computations confirmed the formation of such an intermediate, although Ru(II) coordination at a pyridine C–C bond was concluded to be the most stable η$^2$-pyridine complex.

![Scheme 2. Possible scenario for flash photolysis dynamics of Ru(NH$_3$)$_3$(py)$^{2+}$ (A = NH$_3$).](image)

Flash photolysis also provides a valuable tool for probing the mechanisms of thermal reactions where the presence of a key reactive intermediate is suspected. We will give several examples to illustrate this approach.

**Scheme 3. Proposed mechanism for hydrogenation of alkenes by Wilkinson’s catalyst (L = PPh$_3$).**

**Wilkinson’s catalyst**: One such study was concerned with the reactivity of intermediates proposed in the homogeneous hydrogenation of olefins by the rhodium(I) species Rh(PPh$_3$)$_2$Cl, first reported by Wilkinson and Osborn. Those workers and Halpern had suggested that the key intermediate in the catalytic cycle was the “three coordinate” species Rh(PPh$_3$)$_2$Cl formed by spontaneous dissociation of PPh$_3$ (Scheme 3). One way to access this intermediate is by flash photolysis induced CO dissociation from the carbonyl analog trans-Rh(PPh$_3$)$_2$(CO)Cl (Scheme 4). We studied this reaction first with the conventional flash system, then later using laser flash photolysis. In benzene solution, this substrate undergoes reversible photo-dissociation of CO (Scheme 4, $k_{CO} = 7.8 \times 10^7$ M$^{-1}$ s$^{-1}$). By carrying out the experiment in the presence of reactants such as PPh$_3$, H$_2$, and ethylene, it was possible to determine the second order rate constants for trapping the Rh(PPh$_3$)$_2$Cl intermediate.

For example, the $k_f$ for the reaction with PPh$_3$ was determined to be $~6 \times 10^7$ M$^{-1}$ s$^{-1}$ in 296 K benzene while with H$_2$ was nearly a factor of 30 slower. In addition, $k_{CO}$ values in cyclohexane and dichloromethane were “2-fold and 5-fold lower, respectively, suggesting that the intermediate is not three-coordinate, but is weakly bound by solvent in the position vacated by CO.

**Metal carbonyls and the migratory insertion reaction.** While flash induced changes in the optical spectra are often particularly valuable for following the dynamics of excited states and reactive intermediates, the interpretable structural information from the transient spectra is often limited by the broadening of the absorption and emission bands in solution. In this regard, other time-resolved spectroscopic techniques can provide new dimensions. Time-resolved infrared (TRIR) spectroscopy is especially effective in probing fast reactions of ES and intermediates generated by the photo excitation of metal carbonyls. These compounds display strong IR absorption bands, the positions of which provide insight into the electronic nature of the metal center in the transient species. In order to access such information we constructed a TRIR apparatus that (initially) used a XeCl Excimer laser as the pulsed excitation source, tunable lead salt IR diodes as the detection source and a Hg/Cd/Te solid state fast response IR detector. This allowed pump-probe experiments on the ns to μs timescale with detection in the ν$_{CO}$ region of the IR spectrum.
An example is shown in Fig. 11, which describes the temporal IR difference spectra in the νCO region upon 308 nm flash photolysis of CH₃Mn(CO)₅ in cyclohexane solution. The IR spectrum can be interpreted in terms of CO photodissociation from this entity to give primarily cis-CH₃Mn(CO)₅(Sol) (eq. 11, Sol being the solvent). Homolytic fragmentation of the Mn-CH₃ bond was a minor (<10%) photochemical pathway as well.

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\text{(11)}
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As other studies have shown, the empty site generated by CO photodissociation is often filled by coordination to a solvent molecule, even ones as weakly binding as a saturated hydrocarbon. ⁶³,₆⁴ In the presence of excess CO this transient undergoes exponential decay back to CH₃Mn(CO)₅ at rates linearly dependent on [CO] and strongly affected by the nature of the solvent. In cyclohexane, the second order rate constant \(k_{CO}(295 \text{ K})\) was \(4.5 \times 10^{7} \text{ M}^{-1} \text{ s}^{-1}\), while the value in THF \((1.4 \times 10^{7} \text{ M}^{-1} \text{ s}^{-1})\) was more than 6 orders of magnitude smaller. For reactions in THF, the temperature dependence of \(k_{CO}\) was investigated over the range 232–293 K, and an Eyring plot gave \(\Delta H^\ddagger = 76 \text{ kJ mol}^{-1}\) and \(\Delta S^\ddagger = 59 \text{ J mol}^{-1} \text{ K}^{-1}\). Although these data do not clearly differentiate between associative and dissociative pathways, the positive \(\Delta S^\ddagger\) value suggests that considerable Mn–Sol bond breaking is occurring in the rate-limiting step of this substitution pathway.

Another reaction of interest from the catalysis perspective is the "migratory insertion" of carbon monoxide into metal alkyl bonds (eq. 12). This is the key carbon-carbon bond formation pathway in catalytic carbonylations such as acetic acid synthesis from methanol and alkene hydroformylation. ⁶⁵ Alkyl manganese carbonyl complexes such as CH₃Mn(CO)₅ were extensively probed as mechanistic models for this important class of organometallic reactions. ⁶⁶ Those studies suggest that alkyl migration to a cis carbonyl leads to a reactive intermediate in a step promoted by more polar solvents.

Our strategy for characterizing the structure and reactivity of such intermediates started with the acyl complex. Photodissociation of a ligand L' from the acyl complex M-C(O)R prepares a reactive species I (eq. 13) with the same composition as the intermediate proposed for migratory insertion. Time-resolved optical and IR spectral studies were then used to interrogate I and the dynamics of the reactions with various L' to give the stable acyl products and of reverse alkyl migration to give the metal alkyl complex M-R.

Comparisons of rates measured for I in this manner to the competitive reactivities deduced for intermediates in the thermal migratory insertion kinetics provides guidance relevant to mechanisms of these reactions. We studied such migratory insertion pathways by starting with Mn(CO)₅(C(O)R), ⁶⁵,₆⁶

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\text{(12)}
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\[
\text{(13)}
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The temperature dependence of these rate constants over the range 298 - 318 K, gave the $\Delta H^f$ and $\Delta S^f$ values for the NO "on" and "off" pathways of Fe$^{III}$(TPPS) as 69 $\pm$ 10 $mol^{-1} K^{-1}$ for $k_{on}$ and 76 $\pm$ 10 $mol^{-1} K^{-1}$ for $k_{off}$, respectively.

Another parameter is the activation volume $\Delta V^f$, which reflects the sensitivity of the rate constants to pressure ($\Delta V^f = -RTd(ln k)/dP_{hydr}$), where $P_{hydr}$ is the applied hydrostatic pressure. Pressure effects on laser flash photolysis kinetics were measured in these laboratories using the laser flash photolysis cell illustrated in Fig 13, which was designed to function from 0.1 to 400 MPa. The experiment involved measuring $k_{obs}$ over a range of [NO] to determine $k_{on}$ and $k_{off}$ for each hydrostatic pressure investigated. Plots of $\ln(k_{on})$ and of $\ln(k_{off})$ vs P were linear in this pressure region and from these $\Delta V^f = +9 cm^3 mol^{-1}$ and $\Delta V_{act} = +18 cm^3 mol^{-1}$ were determined.

These activation parameters suggest the reaction pathways envisioned in eqs. 16 and 17 that requires water exchange for
NO, formed endogenously by several isoforms of the enzyme nitric oxide synthase (NOS), has numerous roles in mammalian biology including cardiovascular regulation and immune response. Among potential therapeutic applications of controlled NO delivery are cardiovascular treatment, antibacterial control of biofilms and toxic effects on cancer cells. With regard to tumors, precise spatiotemporal control is essential, since high levels of NO can kill tissue by inducing apoptosis, but low levels may instead be proliferative. Our initial interest in NO delivery derived from its role as a radiation sensitizer. Although gamma-radiation is a common treatment of malignant tumors, the hypoxic regions of tumors are more radiation-resistant than normoxic tissues. Therefore, increasing the radiation-sensitivity of a targeted site will reduce collateral damage to healthy tissue. NO is also a potent vasodilator, so release at a specific site should enhance oxygenation and, correspondingly, sensitivity of the targeted tissue. Two themes have guided our photochemical studies of metal nitrosyls. One, described above, used flash photolysis to investigate the mechanisms of ferri- and ferro-heme reactions with NO. The other was to develop new photochemical precursors for NO delivery to biological targets. Early collaborative experiments with researchers at the Radiation Biology Branch of the US National Cancer Institute (NG) involved probing γ-radiation effects on hypoxic Chinese hamster lung (V79) cells treated with the compound Na₂[Fe₃S₂(NO)]₄, also know as Roussin’s red salt (RRS). In the dark, RRS treatment had little effect on radiation survival rates; however, simultaneous white light irradiation led to markedly enhanced cell death attributed to radiation-sensitization by photochemically released NO (eq. 18). Stimulated by these results, we have since explored NO photo-uncaging from other Fe/S/NO clusters. With the exception of a qualitative application of Roussin’s black salt (RBS) as a light-activated NO donor for vascular relaxation, these were the first photochemical studies to focus on NO uncaging.

With regard to carbon monoxide, it has long been known that CO is formed endogenously during heme catabolism by the enzyme heme oxygenase. More recently, it was shown that exogenously introduced CO is cytoprotective during inflammation and promotes wound healing. Moreover, it is anti-bacterial and disrupts biofilms. In addition, CO donors called “CORMs” (CO releasing moieties) were shown in animal studies to be effective in alleviating ischemia/reperfusion (I/R) injury with various organs and tissues. Thus, for both NO and CO, there is considerable interest in developing methodologies for controlled release at targeted sites. Successful application of photo-uncaging requires elucidating both the fundamental photochemistry and photophysics of the SMB precursors and the mechanisms for transporting these species to the sites of interest. One must also recognize that mammalian tissue is a poor transmitter of shorter visible and UV wavelengths. Tissue penetration improves for longer visible wavelengths and reaches its deepest values in the near infrared (NIR) spectral region (~700-1100 nm).

Applying what we know and learning new tricks: Photochemical uncaging of nitric oxide and other bioactive molecules.

In many fundamental investigations, there is often later recognition that the knowledge gained can be utilized for practical applications not perceived at the onset. This is especially true in Chemistry, a discipline that lies at the interface between truly fundamental science and the chemical, materials and pharmaceutical industries. Indeed, many practitioners of transition metal photochemistry have for some time been interested in developing methods for converting solar radiation to chemical potential energy, for designing new photo-optical materials and for improving the efficiency of lighting with new types of OLEDs. Biomedical applications include photodynamic therapy, new photoactivated anti-cancer drugs and the photochemical delivery (photo-uncaging) of bioactive substances to physiological targets.

Our focus in this regard has been on the photo-uncaging of the small molecule bioregulators (SMBs) nitric oxide and carbon monoxide. This technique has the potential to provide unprecedented control, not only of the location and timing, but also of the dosage for SMB delivery. Thus, photo-uncaging has value both as an investigative tool in physiology and for its ability to affect the progression of specific disease states.

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\begin{align*}
    \text{Fe}^{II}(\text{TPPS})(\text{H}_2\text{O})_2 & \rightarrow k_i \rightarrow \text{Fe}^{II}(\text{TPPS})(\text{H}_2\text{O}) + \text{H}_2\text{O} \quad (16) \\
    \text{Fe}^{II}(\text{TPPS})(\text{H}_2\text{O}) + \text{NO} & \rightarrow k_i \rightarrow \text{Fe}^{II}(\text{TPPS})(\text{H}_2\text{O})(\text{NO}) \quad (17)
\end{align*}
\]
There are several approaches to the problem of limited tissue transmission at certain wavelengths. For example, one might design a small polymeric device attached to an implantable optical fiber.\textsuperscript{107} Excitation through the fiber could then provide a mechanism for delivering the caged compound upon demand. However, a less invasive approach would be to develop precursors or precursors that are responsive to single- or multi-photon excitation by tissue-transmitting light (Scheme 7).

In order to simplify terminology we will use “photoCORM”, a term we coined several years ago,\textsuperscript{107} as shorthand for photo-activated CO releasing moiety, and analogously, “photoNORM” for photo-activated NO releasing moiety. In designing new photoCORM and photoNORM systems, certain design guidelines are particularly attractive. Among these are (i) reasonable stability under physiological conditions (e.g. aerated, aqueous media at 37 °C) and (ii) the absence of undesirable toxicity of the photochemical precursor or of the residual photoproduct once the SMB is released. Also desirable are (iii) the ability to activate SMB release using longer wavelength, esp. NIR, excitation, and (iv) to track the location of these species in the organism and to determine whether the system has indeed undergone photo-activated release. In this regard, photoluminescence (PL) is a particularly sensitive imaging method. Lastly, (v) spatio-temporal control of SMB release is essential, and while this is partially achieved by using light as the trigger, developing delivery mechanisms that can target specific sites will enhance efficiency and reduce undesirable side effects.

PhotoNORMs. Following the earlier studies referenced above, we and others developed a number of transition metal containing complexes and materials that release NO when activated by light. These studies have been summarized in various reviews,\textsuperscript{109} so the discussion here will be confined to systems studied in our laboratory that illustrate key aspects of the design guidelines given.

**CrONO**: The chromium(III) O-nitrito complex trans-Cr(cyclam)(ONO)$_2$ (“CrONO”, cyclam = 1,4,8,11-tetrazacyclododecane) meets several of the desired criteria. It is photoactive with quantum yields measured by spectral changes of 0.25-0.30 moles Einstein over the λ<sub>irr</sub> range 365-536 nm and NO is a photoproduct.\textsuperscript{110,111} Furthermore, CrONO is stable under physiologically relevant conditions (normoxic pH 7.4 aq. buffer at 37 °C), and cell culture studies indicate no acute toxicity. Direct measurement of NO release using a Sievers Nitric Oxide Analyzer (NOA) gave Φ<sub>NO</sub> = 0.25 when the photoreaction was carried out in the presence of added glutathione (GSH). Photolysis of low concentration CrONO solutions released sufficient NO to activate the key enzyme soluble-guanylate cyclase (s-GC) and, in separate experiments, to contract relaxed endothelium-free porcine arteries with an IC$_{50}$ concentration ~ 320 nM.\textsuperscript{112}

The discovery of CrONO’s photochemistry was not accidental. There were previous indications that a Cr(III) nitrito complex might undergo Cr-O-NO photofragmentation to give NO plus a Cr(IV)(O) species.\textsuperscript{113} However, photolysis of a simple complex such as Cr(NH$_3$)$_5$(ONO)$_3$, while showing some NO release, primarily led to ligand photolabilization.\textsuperscript{112} This is where knowing the photochemical literature proved valuable, since earlier studies by Kutal and Adamson had demonstrated that trans-Cr(cyclam)Cl$_2$ is relatively inert to photosubstitution, although other chlorido complexes such as Cr(NH$_3$)$_5$Cl$^+$ are not.\textsuperscript{113} These differences have been interpreted in terms of the ligand substitution from Cr(III) MC excited states requiring substantial distortion along reaction channels involving the equatorial ligands.\textsuperscript{7} The relatively rigid coordination of the tetradentate cyclam ligand prevents this distortion, thereby in the case of CrONO, allows the competitive NO fragmentation pathway to dominate. DFT studies suggest that the latter occurs from the lowest energy ‘MC state of the Cr(III) center.’\textsuperscript{110}

The Cr$^{IV}$ oxo intermediate is also quite reactive. For example, flash photolysis studies showed that it reacts with NO via second order kinetics (k<sub>NO</sub> = 3.1 x 10$^{9}$ M$^{-1}$ s$^{-1}$ in 298 K aq. solution) to regenerate CrONO. Permanent photochemistry is only observed when the NO is removed by entraining the solution with air or helium, or if a trapping agent intercepts the Cr$^{IV}$O, thereby preventing the back reaction. O$_2$ serves this purpose. Thus photolysis of CrONO in aerated aq. solution leads to permanent photochemistry, presumably via oxidation of Cr(IV) to Cr(V). However, the net NO production is inefficient owing to scavenging by the superoxide presumably also generated in this step. On the other hand, when mM concentrations of the common cellular reductant GSH are present, reduction back to a Cr(III) product dominates (Scheme 8).\textsuperscript{110}

**Antennas**: A key problem with using CrONO as a photo-NORM is that its longer wavelength absorptions are ligand field bands with very low extinction coefficients (ε); they are Laporte-forbidden transitions for this centro-symmetric complex. Thus, the rate of NO generation is relatively slow unless the CrONO concentration and/or excitation light intensity is high. To address this issue, we decided to attach various organic chromophores to the CrONO-type platform to serve as antennas to enhance light absorption and, correspondingly, NO production (Scheme 9).\textsuperscript{114}
While these new constructs are proof-of-principle examples of the antenna effect, they do not extend light absorption to the longer visible wavelengths needed for biological systems. For this reason we turned to semiconductor quantum dots (QDs) as photosensitizers, since QD absorption and emission energies can be tuned through the visible spectrum by varying the nanoparticle diameters. Accordingly, we prepared water-soluble CdSe:ZnS core:shell QDs by decorating the QD surface with dihydrolipoic acid (Scheme 10). Adding CrONO salts to solutions of such nanoparticles with 3.8 nm core diameters led to progressive quenching of the QD PL centered at 570 nm with increasing CrONO concentrations. More importantly, substantially more NO was photogenerated than from solutions containing only CrONO. Subsequent studies confirmed that the PL quenching and the sensitized NO production are due to Förster resonance energy transfer (FRET) from the excited QDs to CrONO. However, these electrostatic CrONO:QDs assemblies are unlikely to be stable in physiological fluids, so we are developing protocols to attach CrONO and other photoNORMs to QD surfaces via covalent bonds.

In parallel studies, we investigated the antenna effect with Roussin’s red salt esters (RSE, Fe$_2$(μ-RS)$_2$(NO)$_4$), prepared from the RRS anion by the reaction shown in eq. 19. These RSE are more stable than is RRS and upon photolysis in aerated solution release all 4 NO (eq. 20). Variations of this synthesis were used to build RSE’s with strongly absorbing chromophores, an example being PPIX-RSE (Fig. 14). Excitation of the pendant protoporphyrin IX antenna (PPIX) photosensitizes NO release. The enhanced rate of NO release from PPIX-RSE versus that from a simpler RSE such as Fe$_2$(μ-SET)$_2$(NO)$_4$ (ET-RSE) is largely due to more efficient light absorption ($I_{abs}$) by the tethered antenna. Energy transfer from PPIX to the cluster is also evidenced by the quenching of the characteristic PPIX PL (Fig. 14). Similar behavior was seen with Fluor-RSE, where the pendant anions are fluorescein derivatives. Also recent study described using carbon quantum dots to photosensitize NO release from the iron sulfur nitrosyl cluster Na[Fe$_5$S$_5$(NO)$_3$] in a nanocarrier made from carboxymethyl chitosan; however, the excitation wavelength was the near UV.

Multi-photon excitation using NIR light. None of the photoNORMs described above displayed significant absorbance at the NIR wavelengths where tissue transmission is optimized. In principle, this limitation can be addressed by using multi-photon excitation, where the summed energy of several NIR quanta is sufficient to populate the higher energy excited states from which the desired uncaging occurs. See, e.g., the report by Castellano et al [111] that NIR excitation from a fs laser triggers visible emission from Ru(II) bipyridyl complexes. In this laboratory, we are investigating two multi-photon approaches: simultaneous two-photon excitation (TPE) and energy transfer upconversion (ETU), which functions by sequential photon absorptions. Unlike the single photon excitation (SPE) we have been discussing, the rates of TPE and ETU processes have a non-linear dependence on the incident light intensity $I_0$. For example, the probability of TPE is proportional to $I_0^2$, if a single source is used. Thus, TPE induced photoreaction occurs mostly at the focal point of the excitation source, offering three-dimensional spatial resolution, a property widely exploited in imaging. Such resolution would provide unprecedented definition of the uncaging location. However, the high intensities needed for TPE require light sources such as pulsed lasers where very high peak powers (photons/unit time) are readily achieved. This may change with the recent description of a lower intensity mechanism for NIR to visible upconversion.

The selection rules for TPE are different from those for SPE. The former is allowed only between two states that have the same parity; the latter requires a parity change. Chromophores with high TPE cross sections in the NIR include π-conjugated molecules with electron-donor and -acceptor units arranged symmetrically with respect to the center. Semi-conductor QDs are also TPE chromophores with two photon absorption cross-sections ($δ$) as large as 10$^5$ GM (Goeppert-Mayer units, 1 GM = 10$^{20}$ cm$^2$ photon$^{-1}$) seen for CdSe nanoparticles.

![Diagram](image-url)
Notably, aqueous solutions of PPIX-RSE that show no absorbance at NIR wavelengths, display a weak PL ($\lambda_{\text{max}}$ 632 nm) when subjected to 810 nm excitation with 100 fs pulses from a Ti:sapphire laser. This PL is accompanied by NO generation. These observations thus provide clear evidence that the higher energy states responsible were being accessed by multi-photon excitation, even though the $\delta$ value for PPIX is quite low (~2 GM).

Fluorescein has a higher $\delta$ value, which was one rationale for preparing Fluor-RSE. NIR excitation of this species using an ultrafast laser gave the same PL as seen upon SPE at 436 nm, suggesting that analogous emissive excited states are formed by both methods. The Fluor-RSE, the PL from Fluor-RSE was much less than that from the chromophores free of the Fe/S/NO cluster owing to energy transfer to the latter. The log/log plot of the NO generated vs. laser intensity at 800 nm light gave a slope of 1.8 ± 0.2 consistent with the $(l_0^2)^\frac{1}{n}$ dependence predicted for a photochemical process initiated by TPE.

Subsequent studies have confirmed TPE with pulsed NIR lasers for other RSE derivatives with chromophores having large two photon cross-sections. Several other molecular platforms for TPE-induced NO release have also been recently described. Multi-photon NIR excitation of caged NO and other bioactive molecules can also be achieved by energy transfer upconversion. The advantage of ETU is that it involves sequential, rather than simultaneous excitation, so lower $l_0$ values are needed. Instead of pulsed lasers, such NIR to visible/UV upconversion can be achieved with relatively inexpensive continuous wave (CW) NIR diode lasers. If the desired upconversion involves the net of two photons, the intensity dependence can vary from second order at low $l_0$ to first order at very high $l_0$ (where the initial excitation step becomes rate limiting). This depends upon the rates of the competing energy transfer and deactivation pathways. The typical situation is between these limits, and as a consequence, the potential for 3-D resolution using light source focusing remains.

In order to exploit ETU, we collaborated with Fan Zhang and Dongyuan Zhao from Fudan U. (China) and UCSB colleague Galen Stucky to test lanthanide ion-doped upconverting nanoparticles (UCNPs) as NIR antennas for NO photo-uncaging. With such UCNPs, a Ln ion such as Yb is the sensitizer that absorbs the NIR light. This energy is transferred to an activator such as Er as shown in Figure 15. Further excitation of the Yb and energy transfer to the long-lived excited state of the activator populates higher energy states from which visible/UV emission occurs. If an appropriate solid matrix is used and the core is coated with a shell of host lattice, silica or polymer, deactivation pathways are suppressed and stronger upconverted emissions result.

There was limited precedent for using such UCNPs for uncaging bioactive substances with NIR excitation. In 2010, Carling et al. used 980 nm photolysis of NaYF$_4$:Yb/Tm UCNPs to uncage acetate from 3′,5′-(carboxymethoxy)benzoin acetate loaded on the surface, and in 2012 Y. Yang et al. utilized similar UCNPs to uncage d-luciferin in C6 glioma cells and in living mice. Notably, the latter studies showed that NaYF$_4$, (Yb$_{20}$/Er$_{2}$) UCNPs are non-toxic, their components are eventually excreted, and their location in the organism can be imaged by PL.

Our first UCN uncaging study, also reported in 2012, showed that 980 nm excitation with a CW diode laser of NaYF$_4$:Yb/Er:NaYF$_4$ core:shell UCNPs triggers NO release from the anion of Roussin’s black salt Na[Fe$_6$S$_6$(NO)$_4$] (RBS). Solutions of RBS are photoactive under visible excitation (eq. 21) but show no such activity when irradiated in the NIR. This first example used UCNPs coated with a silica layer (~10 nm) and surface modified with cationic -NH$_3$ groups to render the nanoparticle water-soluble and to facilitate ion pairing with the RBS anions. As shown in Figure 16, the strong absorptions of RBS overlay the visible emissions from the NaYF$_4$:Yb/Er UCNPs, so the emitted light can be reabsorbed by RBS to facilitate the photoreaction. The NO output as measured with the NOA was linear with the irradiation time at constant power but non-linear in response to systematic increases in excitation intensity ($\sim l_0^{1.5}$).
A more robust nanocarrier is illustrated in Scheme 11.\textsuperscript{134} The UCNP core was coated with silica then with a mesoporous silica shell to give a porous layer that was subsequently impregnated with RBS. Coating with poly(allylamine hydrochloride) encapsulated the photoNORM. NIR irradiation released NO, which unlike RBS can diffuse out of the nanocarrier. These proof-of-concept experiments showed that UCNPs offer the opportunity to deliver NO to specific targets using NIR light generated by a relatively inexpensive diode laser.

Scheme 11. Nano-carrier with UCNP core and mesoporous silica shell impregnated with RBS (green dots) and coated with poly(allylamine). 980 nm irradiation leads to upconversion and NO release.

Although the UCNP emission/ photoNORM absorption may not be the most efficient, this pathway simply requires a precursor having strong absorption bands that overlap with the UCNP emissions. This concept led our laboratory to develop biocompatible polymer-based carriers in which the UCNP and the photoNORM are simply co-dissolved. The first examples are mm-sized polymer disks consisting of polydimethylsiloxane (PDMS) containing RBS and NaYF\textsubscript{4}:Yb/Er UCNPs.\textsuperscript{135} NO release was effected by 980 nm irradiation with a CW diode laser. Furthermore, analogous NIR excitation triggered photo-uncaging from these devices even after passing through a tissue filter. Subsequent experiments used the hydrophobic forms of Ru(salen-R)[NO]X and CrONO derivatives as photoNORMS in disks of various NuSIL silicone formulations.\textsuperscript{136} One can envision using such materials as drug delivering implants. It is also possible to miniaturize these polymer-based mini-carriers to micron and sub-micron sizes. Our ongoing studies are addressing how to deliver the resulting micro- and nanocarriers with analogous formulations to physiological targets.

Another key issue in therapeutic NO delivery is detection and analysis. The Sievers NOA mentioned above measures NO that has been entrained into the gas phase from a solution or an organism. Although very accurate and sensitive, this method is transport-limited and not very useful for real time studies. There are also sensitive NO specific electrodes that are effective in solution media, although in our hands there were issues with reproducibility.\textsuperscript{108} Some years ago, derivatives of diaminofluorescein (DAF) were developed to provide sensitive detection of NO generation in cellular media,\textsuperscript{137} but it should be noted that this method actually senses the NO autoxidation product, \( NO_2 \). More direct NO detection is now available using copper(II) complexes with chelating ligands that have pendant luminophores.\textsuperscript{138} The Cu(I) largely quenches the emission from these species owing to its low energy LF transitions, but reaction with NO both modifies the ligand to enhance its PL and reduces the metal center to Cu(II), thereby deleting these low lying transitions.\textsuperscript{139}

PhotoCORMS: Although our investment in developing photoCORMs has been less than with NO releasing analogs, the design principles carry over as do some problems, among them the need for reliable detection methods.\textsuperscript{103d,e} The standard \textit{in vitro} test for CO is the carboxyhemoglobin (Mb-CO) assay, where CO is added to a solution of deoxy-Mb and formation of Mb-CO is followed via changes in the absorption spectrum.\textsuperscript{143} However, this test is not effective in aerobic media. Several fluorescent sensors have recently been reported, and this is an area of growing development.\textsuperscript{144,145} In our laboratory, we have determined photoCORM CO release by headspace analysis using both gas-phase infra-red spectroscopy and gas chromatography techniques, but found the latter to be the more reliable quantitatively.\textsuperscript{148} Schiller and coworkers\textsuperscript{150} have described headspace analysis using a standard CO gas detector. These methods were reviewed recently,\textsuperscript{103d,e,142} and will not be discussed further here.

Our goals with photoCORMs are similar to those with the photoNORMs, namely to develop strategies to effect CO delivery to specific physiological targets. Initial studies at UCSB focused on water-soluble metal carbynols and on improving methods for quantifying CO release.\textsuperscript{108} For selected group \( 6 \) carbynols in aerated media, multiple CO’s were released per complex, and the quantum yield for overall CO release \( (\Phi_{\text{CO}}) \) in some cases exceeded 1.0. The high yields of CO release can be attributed to subsequent reactions of initial photoproducts with \( \text{O}_2 \) and/or the aqueous solvent (Scheme 12). In such instance, the primary photoproduct could be considered a “proCORM”, a molecular species that leads to slow CO release after the initial photoreaction.

\[
\text{Scheme 12. Generating a proCORM intermediate}
\]

A very interesting photoCORM developed at UCSB is the rhodium(I) complex \( \text{ReL}(\text{CO})_y(bpy)^+ \) \((L = \text{P}(\text{CH}_2\text{OH})_3)_2\).\textsuperscript{149} This complex ion is strongly luminescent \((\tau = 400 \text{ ns})\) in solution and releases one CO upon photolysis (eq. 22, \( \Phi_{\text{CO}} = 0.11 \)).
photoprodut Re(\(\text{L})(\text{CO})_3(\text{H}_2\text{O})(\text{bpy})^+\)) is also luminescent. Neither Re(\(\text{L})(\text{CO})_3(\text{bpy})^+\)) nor its photoprodut displayed any acute cellular toxicity up to 100 \(\mu\text{M}\). Thus, we were able to use confocal microscopy to observe the PL of the rhenium species incorporated into cells (human prostatic carcinoma cell line PPC-1) before and after photolysis (Fig. 17). The ability to image the photoCORM in the cells and to determine whether it has undergone photoreaction is a highly desirable trait for uncaging. A major disadvantage, however, is the relatively short visible wavelengths needed to photolabilize the CO.

![Fig. 17. Confocal fluorescence microscopy images of PPC-1 cells incubated with \(\text{Re(PI(CH}_3\text{OH})_2\text{CO})_3(\text{bpy})^+\)) (50 \(\mu\text{M}\) (A) and after photoysis at 405 nm (B). (Figure adapted from ref. 149)](image)

The task remains therefore to develop a mechanism to release CO with NIR light. In this context, the photophysics of metal carboneyls offers a challenge. The stronger lower energy absorptions and the PL of dimine complexes such as Re(\(\text{L})(\text{CO})_3(\text{bpy})^+\)) can be attributed to metal-to-dimine charge transfer states. However, CO photolability occurs primarily from metal-centered ligand field states. Substituents can be used to tune the MLCT absorptions of the Re(I) complexes to the red, but excitation at those longer wavelength bands does not lead to significant CO release, since this does not populate the higher-energy MC states necessary for CO labilization. In fact, the story is quite analogous to the photoreactivity of the Ru(\(\text{NH}_3)_3\text{L})^+ complexes discussed above.

We have addressed these issues by designing a nanocarrier that incorporates both a photoCORM and a UCNP antenna to mediate multi-photon NIR labilization of CO. The photoCORM is a Mn(I) complex similar to the Re(I) complex just described. Manganese, however, has smaller d-orbital splitting than the heavier transition element analogs. Thus, it has inherently lower energy LF states, thereby making CO photodissociation energetically more accessible.

![Scheme 13. Water-soluble photoCORM nano-carriers with diameters 16–30 nm according to dynamic light scattering.](image)

For example, the Mn(\(\text{CO})_3(\text{PPh}_3)_2(\text{bpy})^+\) displays strong visible range absorptions, photolysis of which leads to facile CO release. This spectrum is well suited for matching the upconverted emissions from a \(\text{NaYF}_3(\text{Yb}_{20}\text{Er}_{10})\) UCNPs. In collaboration with Nanfeng Zheng of Xiamen U (China), we assembled nano-carriers consisting of a UCNPs core coated with a phospholipid-functionalized with poly-(ethylene glycol) (DSPE-PEG 2000) (Scheme 13). The amphiphilic polymer confers water solubility to the nano-carrier while providing a lipid-like interior into which hydrophobic compound trans-

\[\text{Mn(\text{CO})}_3(\text{PPh}_3)_2(\text{bpy})^+(\text{CF}_3\text{SO}_3)\]

is readily infused. NIR excitation (980 nm) of an aq. solution containing the loaded nano-carrier leads to CO release. With these hydrophobic components, leaching is minimized, while encapsulation isolates them from the medium, thereby reducing potential toxicity and promoting higher loading. This remarkable nano-carrier brings together the UCNPs NIR antenna and a hydrophobic photoCORM in a water-soluble ensemble.

We are extending these studies to examine the efficacies of such nano-carriers with other photoCORMs and photoNORMs and with UCNPs responsive to 800 nm excitation. In addition, surface modifications should be well suited for the control of targeting and other properties of these units.

**Better Targeting:** While photo-excitation allows one to define location and timing of SMB uncaging in tissue, a key remaining task is to improve transport of the precursor-antenna conjugates to the desired targets. Although one can envision implanting a device such as the mm-sized PDMS mini-disks described above, a more elegant strategy would be to employ biological mechanisms for this purpose. We are embarking on this quest. One approach is to use targeting peptides that, depending on the amino acid sequence, can direct water-soluble nano-carriers to specific cell types both in vitro and in vivo. For example, Tirrell et al. used micelles constructed of peptide terminated polymers for the targeted delivery of chemotherapeutic agents to malignant gliomas, thereby minimizing other side effects.

In collaboration with UCSB colleague Norbert Reich, we have constructed nano-carriers with hollow gold nanonanoparticle (HGN) cores (~40 nm diameters). The surfaces of these HGN cores were decorated with thiocupferron (TCF), a molecule that when heated releases one equivalent of NO. Also decorating the surface was a 5-kDa thiolated polyethylene glycol (TPEGGR), which was terminated by a C-end Rule peptide, RPAPAR that targets cells with the Neuropilin-1 receptor common to certain cancers. The TCF-HGN-TPEGGR conjugates prepared in this manner undergo Neuropilin-1 receptor mediated endocytosis by PPC-1 and 22RV1 prostate cancer cells but not with HeLa cells, which lack that receptor. NIR irradiation with a pulsed laser at a wavelength (800 nm) resonant with the surface plasmon of the gold nanoparticles rapidly heats the nanoparticle. In solution the resulting NO release (Scheme 14) was quantified using the NOA, while intracellular NO release in 22RV1 cells was demonstrated by using the DAF-2 assay.

Based on this precedent, our premise is that decorating photoCORM and photoNORM nano-carriers with a targeting...
Concluding remarks:

This article illustrates the evolution of research into the photochemistry of transition metal chemistry by tracing the author’s interest and involvement in this area over four decades. Initial studies focused on correlating reaction product characterizations and quantum yields with spectroscopic properties to identify the excited states responsible for specific photochemical processes. This information was then used to tune excited state energies in order to correspondingly direct the resulting photoreactivities. Within the same time frame, related studies were directed toward elucidating specific mechanisms for excited state reactions and deactivation pathways. The availability of flash methods provided access to the dynamics and trajectories of excited state pathways as well as of subsequent reactions of transient intermediates formed. Notably, such species are often analogous to intermediates proposed for certain thermochemical processes, so pulsed photolysis offers an important mechanistic tool in catalysis and bioinorganic chemistry. Our personal journey in this area has turned to using photochemical methodologies for the uncaging of bioactive small molecules, with the eventual goal of establishing guidelines for therapeutic application.

Another researcher would undoubtedly provide a different perspective, given that individual’s choice of other research problems and tools employed. Regardless, it is clear that the successful use of inorganic photochemistry, whether in energy science, biomedicine, or other foreseen or unforeseen application, needs to be based on a sound understanding of the fundamental principles that define the mechanisms of these systems. I am convinced that, as new tools and ideas are introduced, fascinating and unexpected developments in the photochemistry and photophysics of transition metal compounds will continue to be forthcoming.

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Notes and references.


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