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Polychromophoric Metal Complexes for Generating the Bioregulatory Agent Nitric Oxide by Single- and Two-Photon Excitation

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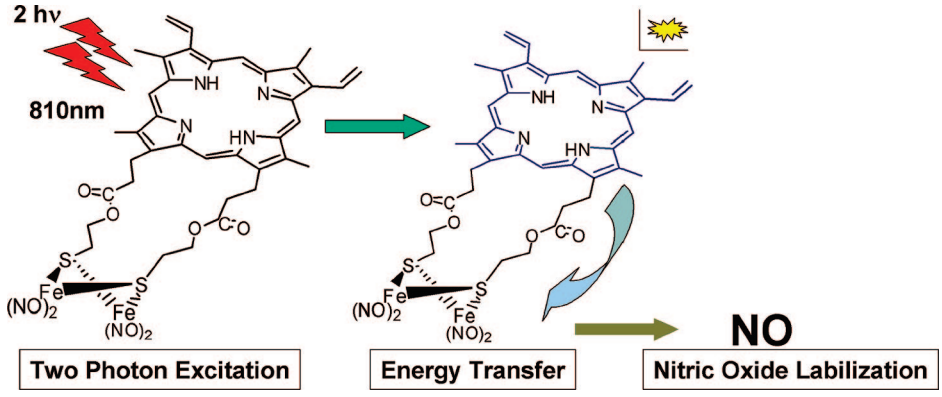

In order to deliver a bioactive agent to a physiological location, it is important to be able to regulate precisely the location and the dosage. Such exquisite control can easily be envisioned for a photochemical drug that is active toward release of the desired bioactive agent upon irradiation of a specific tissue site. These materials should be thermally stable but reactive under excitation at visible (vis) or near-infrared (NIR) wavelengths where tissue transmission is optimal. Two photon excitation (TPE) is of special interest, since the use of focused laser pulses to activate release could provide 3D spatial control in therapeutic applications.

This Account describes the preparation and photochemistry of a series of transition metal complexes designed to release the simple bioregulatory compound nitric oxide upon vis or NIR excitation. In order to enhance the light gathering capability of such compounds, we have attached chromophores with high single- or two-photon absorption cross sections to several photochemical NO precursors. For example, the iron nitrosyl clusters $Fe_2(\mu\text{-}SR)_2(NO)_4$ (Roussin's red esters) have been prepared with various chromophores as pendant groups, an example being the protoporphyrin XI derivative illustrated here. Direct excitation into the vis absorbing Q bands of the porphyrin leads to enhanced rates of NO generation from the Fe/S/NO cluster owing to the larger rate of light absorption by that antenna. Furthermore, femtosecond pulsed laser NIR excitation of the same compound at 810 nm (a spectral region where no absorption bands are apparent) leads to weak emission at 630 nm and generation of NO, both effects providing evidence of a TPE mechanism. Roussin's red esters with other chromophores described here are even more effective for TPE-stimulated NO release.

Another photochemical NO precursor discussed is the Cr(III) complex *trans*-$Cr(L)(ONO)_2^+$ where L is a cyclic tetraamine such as cyclam. When L includes a chromophore tethered to the ligand backbone, excitation of that functionality results in energy transfer to the spin-forbidden ligand field double states and light-stimulated release of NO. We are working to develop systems where L is attached to a semiconductor nanoparticle as the antenna. In this context, we have shown that electrostatic assemblies are formed between the anionic surface of water-soluble CdSe/ZnS core/shell quantum dots (QDs) and $Cr(L)(ONO)_2^+$ cations via an ion-pairing mechanism. Photoexcition of such modified QDs leads to markedly enhanced NO generation and suggests promising applications of such nanomaterials as photochemical drugs.

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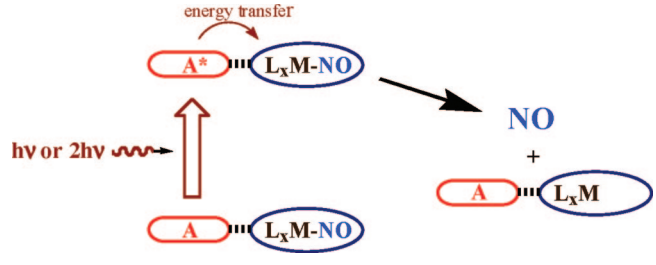
Introduction

This Account describes our continuing effort to design photoactive metal complexes for controlled release of "caged" bioactive agents at physiological targets. Although we have focused primarily on the delivery of nitric oxide,[1] these concepts should apply to other chemotherapeutic molecules. The photochemical strategy allows one to control the location, the timing, and the dose of NO release with an external signal, namely, the light that is delivered to tissue. Desirable properties for the NO precursors would be thermal stability, target specificity, and photochemical lability at wavelengths convenient for light delivery to the target tissue. Photochemical NO precursors studied in this laboratory include various metal nitrosyl (M(NO))[2,3] and metal nitrito (M(ONO)) complexes[4] and iron/sulfur/nitrosyl clusters.[5–7] Direct excitation of these compounds results in NO release, and as described below, this NO can directly affect various biological functions and have other therapeutic effects. However, many of these metal nitrosyl and nitrito complexes display relatively low absorptivity at the longer visible and near-infrared (NIR) wavelengths that have optimal tissue penetration properties. Described here is research that addresses this problem by attaching other, more strongly absorbing, chromophores to the precursor to enhance photochemical NO release.

Scheme 1 idealizes this approach. The second chromophore acts as an antenna (A) to harvest light at desirable wavelengths. A may be a dye or even a quantum dot. Absorption of light gives the excited antenna (A*). Energy transfer from A* to the NO precursor (illustrated as $L_xM(NO)$) photosensitizes release of NO from the latter. Such a system would be more effective than the precursor alone. More light is absorbed; thus the rate of NO delivery is faster. The NO dose is still controlled by the amount of light delivered to the target.

When a system is irradiated by a continuous lamp or even a nanosecond pulsed laser, normal single-photon excitation (SPE) is expected. In that case, the photoreaction rate is proportional to the concentration and extinction coefficient of the compound excited and to the reaction quantum yield. It is also linearly proportional to the intensity of the excitation source

SCHEME 1



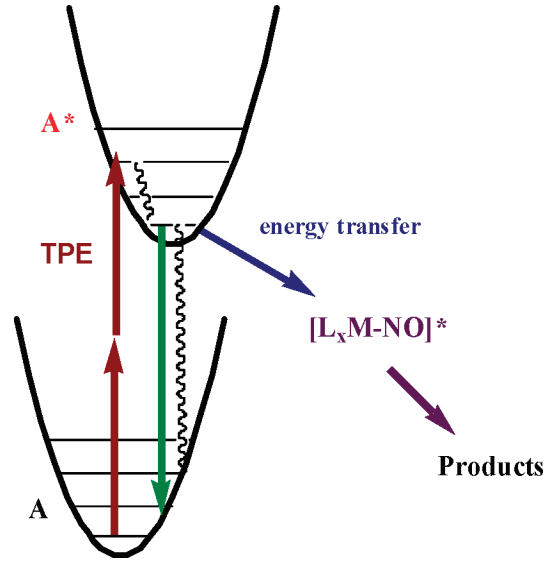


FIGURE 1. Illustrating TPE into an antenna followed by energy transfer to reactive center.

(units being einstein L^{-1} s^{-1}). In contrast when a system is irradiated with very intense excitation sources, typically when pulsed picosecond or femtosecond pulsed lasers are used, simultaneous two-photon excitation (TPE) (Figure 1) can occur. (With ultrafast lasers, the photons are delivered in a very short time interval, so pulse powers are extremely high.) For TPE, the excitation rate is proportional to the intensity squared. Owing to this quadratic relationship, TPE can also provide additional spatial resolution,[8] since excitation occurs primarily at the focal point of the light source, not throughout the irradiated volume. This feature has found applications in imaging and photodynamic therapy (PDT),[9] and it would be of interest to exploit this spatial resolution for the photochemical delivery of bioactive agents.

For physiological applications, it is very desirable to extend the effective excitation range of the photoactivated precursors to red or near-infrared wavelengths.[10] NIR frequencies are especially attractive owing to optimal tissue penetration properties, illustrated in Figure 2.[10] However, compounds that are photolabile when subjected to single-photon NIR excitation may not be sufficiently thermally stable for physiological applications. This concern has drawn our attention to antennae that are responsive to two-photon excitation. TPE at NIR wavelengths would prepare excited states of the antennae that are not otherwise accessible by lower intensity irradiation at such frequencies (Figure 1). Energy transfer from A* to the NO precursor would generate NO at the targeted tissue as the result of more penetrating NIR excitation and with exquisite spatial control. This Account summarizes recent studies directed toward using such polychromophoric systems to access NO

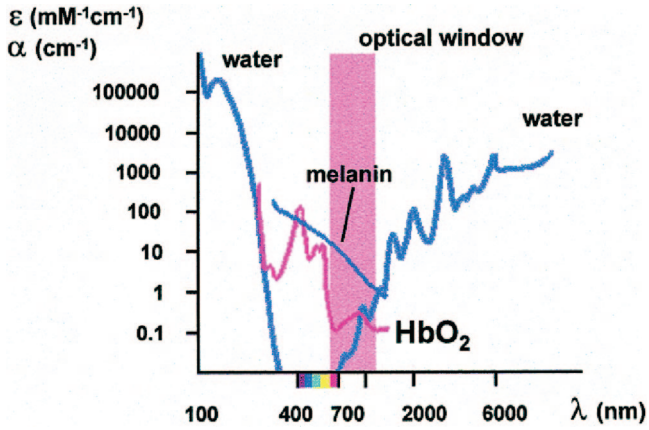


FIGURE 2. Absorption properties of major intracellular chromophores. Reproduced from ref 10c with permission from Blackwell Publishing.

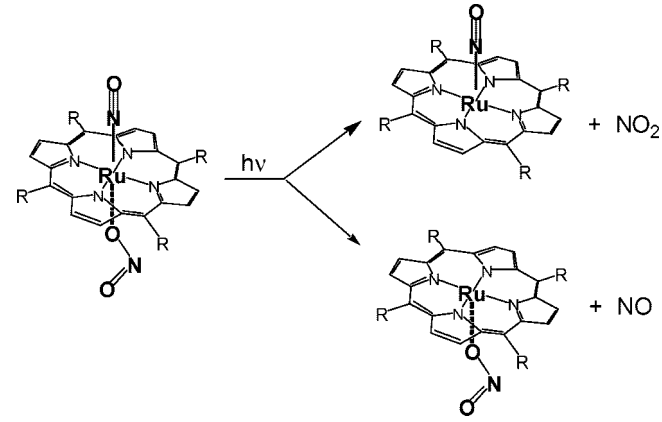
release from stable precursors at longer visible and NIR wavelengths.

Nitric oxide is an endogenous mediator in numerous physiological processes including vasodilation and neurotransmission.[11] Given that its bioregulatory action is triggered at nanomolar concentrations,[12] using a photochemical methodology to modify local NO concentrations and alter certain biological functions is quite feasible. For example, such NO delivery has been shown to trigger vasodilation.[13] Another property attributed to NO is that it increases tumor sensitivity to radiation therapy.[14,15] Malignant tumors have hypoxic regions of low oxygen tension that are much more radioresistant than normoxic tissue. Cells that survive radiation in these regions are likely to result in tumor reoccurrences that are even less amenable to treatment. Hypoxia cell resistance may be alleviated by introducing a radiation sensitzer or a vasodilator to increase dioxygen flow; both are roles played by NO. Radiation sensitization requires NO concentrations approaching 1 μM,[15] while vasodilation occurs at much lower NO concentrations. Photochemical activation of an appropriate precursor provides a procedure for controlling the site, timing, and dosage of NO delivery.

Photoreactions of NO Precursors

Metalloporphyrins. We initiated photochemical studies of metal nitrosyls in the early 1990s by using flash photolysis to generate reactive intermediates from nitrosyl adducts of ferriheme proteins and models to probe relaxation kinetics back to the equilibrium states (eq 1, $k_{obs} = k_{on}[NO] + k_{off}$, Por^{2-} = porphyrinato dianion).[16] Although others had generated similar intermediates,[17] our studies, partly with Hoshino[16a] and with van Eldik,[16c] represented the first systematic determinations of activation parameters $\Delta H^{\ddagger}$, $\Delta S^{\ddagger}$, and $\Delta V^{\ddagger}$ for the NO "on" and "off" reactions with heme proteins and models.

SCHEME 2

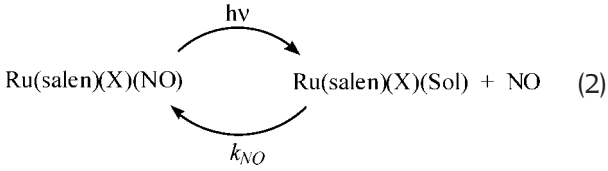


$$M(Por) + NO \underset{k_{off}}{\overset{k_{on}}{\rightleftharpoons}} M(Por)(NO) \qquad (1)$$

Within the same time frame, we also began efforts to utilize photochemical techniques for site-directed NO delivery. The ideal would be a thermally stable NO precursor that releases NO only when triggered by light. It was soon established that the ferro- and ferriheme models were too unstable to be good NO precursor candidates. The ferric complexes are thermally labile toward NO dissociation, while Fe^{II}(Por)(NO) analogs are susceptible to air oxidation.[18]

To address these issues, our attention turned to the ruthenium analogs Ru(Por)(X)(NO) (X = various anions),[2] which are generally robust toward thermal NO release. The photochemistry of these porphyrinato complexes is quite rich, perhaps too rich for a good NO donor. For example, flash photolysis of Ru(TPP)(NO)(ONO) (TPP^{2-} = tetraphenylporphyrin dianion) demonstrated two short-lived intermediates, one from NO labilization to give Ru(Por)(ONO) and the other from NO_2 dissociation to give the transient species Ru(Por)(NO) (Scheme 2).[2]

The salen complexes Ru(salen)(X)(NO) (salen = *N*,*N*′-ethylenebis(salicylideneiminato) dianion) and related species provide a more versatile synthetic platform and have been the focus of continuing studies. These undergo reversible NO photolabilization (eq 2, Sol = solvent),[3] and the back reactions are strongly solvent sensitive, with k_{NO} values ranging from 4×10^7 M^{-1} s^{-1} in toluene to $\sim 5 \times 10^{-4}$ M^{-1} s^{-1} in acetonitrile (298 K). Positive volumes of activation $\Delta V^{\ddagger}$ indicate a dissociative ligand substitution mechanism.[19] Ru salen nitrosyls are also photoactivated catalyst precursors for asymmetric epoxidations etc.,[20] and our results explain the photobehavior and solvent dependence of such systems.



(2)

In the interest of developing solid materials as implants for photochemical NO delivery, we have also worked with Elia Tfouni to incorporate [Ru(salen)(H_2O)(NO)]PF_6 into porous silica sol–gels,[21] which release NO upon photolysis. Surprisingly, the encapsulated Ru(salen)(H_2O)$(NO)^+$ can be regenerated by treating the spent solid with aqueous NO_2^- and a reductant, suggesting that the photoactive nitrosyl in the implant might be regenerable *in vivo* from endogenous nitrite.[21]

Ruthenium nitrosyls have also drawn attention from others interested in NO delivery,[22–27] beginning with early studies involving photochemical release from $K_2[RuCl_5(NO)]$ solutions to an *in vitro* target.[13a] Also of note are publications from Franco, Tfouni, and Santana da Silva in Brazil concerning thermally and photoinduced NO release from amine and polypyridyl nitrosyl complexes[23–25] and Mascharak[26] regarding photoreactions of RuL(NO) (L = polydentate pyridyl ligand).

Roussin's Salts and Esters. The Roussin's black salt anion, $Fe_4S_3(NO)_7^-$ (RBS) was the first of these iron/sulfur/nitrosyl clusters[28] to be exploited as photochemical NO donors in early qualitative studies of vascular relaxation.[13b,29,30] These observations inspired our decision to address the quantitative photochemistry of RBS and the red salt anion $Fe_2S_2(NO)_4^{2-}$ (RRS).[5,31]

As illustrated in Figure 3, the water-soluble Roussin's salts have broad absorptions into the visible, although the relevant excited states are not well characterized. RBS and RRS are both photoactive toward NO labilization; however, net photoreaction yields depend on trapping the iron-based intermediates by O_2, since this competes against the back reaction to reform the original cluster. Scheme 3 summarizes flash photolysis experiments with aqueous RRS.[31] The quantum yield for NO production from RRS ($\Phi_{NO} \approx 0.07$ in aerated aqueous solution confirmed electrochemically) proved to be nearly independent of the excitation wavelength λ_{irr};[6,7] however, the net efficiency of NO production is poor. Only one NO is released per 2 equiv of RRS consumed, since intermediates are self-trapped by RRS to generate the less photoactive tetranuclear cluster RBS.[5]

As noted above, NO sensitizes hypoxic cells to γ-radiation damage.[15] In this context, we conducted collaborative studies at the NCI Radiation Biology Branch to test the viability of photochemically delivered NO as a radiosensitizer. First, hypoxic cultures of Chinese hamster V79 cells were treated with RRS (<1 mM), and it was found that survival rates were

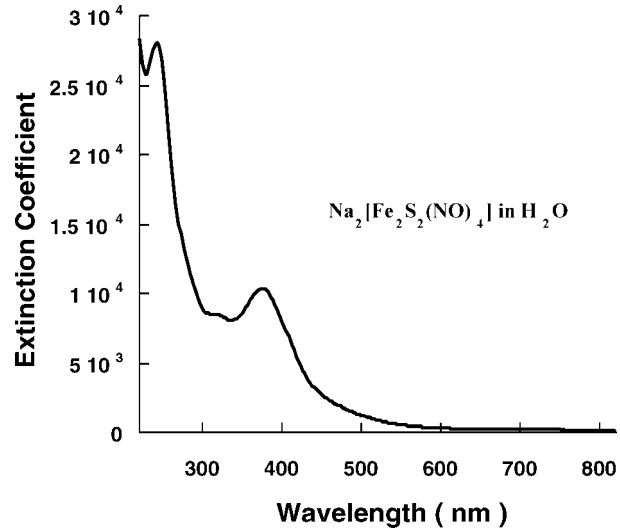
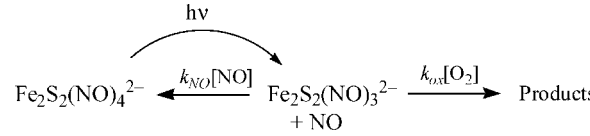


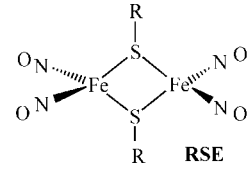
FIGURE 3. Optical spectrum of $Na_2[Fe_2S_2(NO)_4]$ in aqueous solution.

SCHEME 3



the same as the hypoxic cell controls, both in the absence of radiation and when subjected to equivalent doses of γ-radiation.[5] However, when the RRS incubated cells were subjected to white (visible) light irradiation simultaneous with γ-radiation, there were up to 100-fold enhancements of radiation-induced cell death. In contrast, visible light irradiation alone had little effect on cell survival in RRS incubated cultures. This experiment provided clear demonstration that NO delivered photochemically sensitizes living cells toward simultaneous γ-radiation and further incentive for designing molecular systems for targeted NO delivery.

The Roussin's red esters, $Fe_2(\mu\text{-SR})_2(NO)_4$ (RSE), are prepared by alkylating the bridging sulfides of RRS.[32] Of particular interest is the opportunity to manipulate reactivity, solubility, optical spectral profile, or biological specificity by varying R. Another advantage is that all four NOs are released upon RSE photodecomposition in aerated solutions (eq 3).[6,33]



$\xrightarrow[O_2]{h\nu}$ $2\,Fe^{2+} + 2\,RS^- + \sim 4\,NO$ (3)

Flash photolysis studies of RSEs show the primary photochemical step to be NO dissociation. The presumed intermediate $Fe_2(\mu\text{-SR})_2(NO)_3$ reacts with NO to regenerate $Fe_2(\mu\text{-SR})_2(NO)_4$ with a high k_{NO} (1.1×10^9 M^{-1} s^{-1} for R =

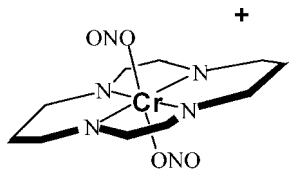
$-CH_2CH_2SO_3^-$ in aqueous solution)[6] in competition with trapping by O_2 ($k_{O_2} = \sim 1.1 \times 10^7\ M^{-1}\ s^{-1}$) to give oxidized products. The remaining NO's are apparently released subsequently as the cluster decomposes. RRS esters with saturated R groups have electronic spectra similar to that of RRS itself and do not have the strong absorbances at the longer wavelengths desirable for *in vivo* studies. To address this issue, we initiated the preparation of RRE compounds with pendant dye chromophores to serve as intramolecular photosensitizers (see below).

Several other groups have explored the photochemistry and potential biological applications of non-heme iron nitrosyls, systems including Roussin's clusters,[34] sodium nitroprusside,[7,35] and iron nitrosyls with polydentate ligands.[36]

Nitrito Complexes. Another strategy is to use a nitrito complex, M−ONO, as the photochemical NO precursor. It was reported that certain metalloporphyrin nitrito complexes undergo photolysis-induced β-cleavage of nitrite to the oxo species (eq 4),[37] although homolytic cleavage of the M−ONO bond to give the NO_2 radical is common.[38] We reasoned that an oxophilic metal such as Cr(III) would favor NO over NO_2 release and that using neutral ligands would give water-soluble cationic complexes. This thinking led to our studies of the *trans*-Cr(cyclam)$(ONO)_2^+$ cation (**I**, cyclam = 1,4,8,11-tetraaza-cyclotetradecane).[4,38b] A key point is that the *trans*-Cr(cyclam)X_2^+ cations (X = various anions), unlike other Cr(III) amine complexes, show little tendency toward photoaquation of axial ligands.[39]

$$L_xM(ONO) \xrightarrow{h\nu} L_xM{=}O + NO \qquad (4)$$

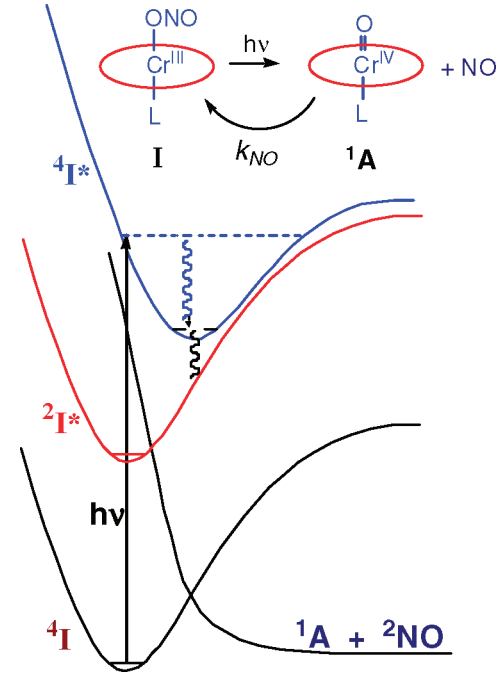
When **I** was photolyzed in aerated aqueous solutions at various λ_{irr} from 365 to 546 nm, NO was released. The primary photoreaction product was identified provisionally as the Cr(IV) intermediate *trans*-Cr(cyclam)(O)$(ONO)^+$, which is trapped by O_2 to give a Cr(V) complex with an overall Φ_{NO} of 0.27 at 436 nm. Comparable quantum yields were found at other λ_{irr}.



(I)

Flash photolysis studies demonstrated that NO photodissociation is reversible with a moderately fast back reaction

SCHEME 4. Model for *trans*-Cr(cyclam)$(ONO)_2^+$ Photochemistry[a]



[a] ^{4}**I** is ground state.

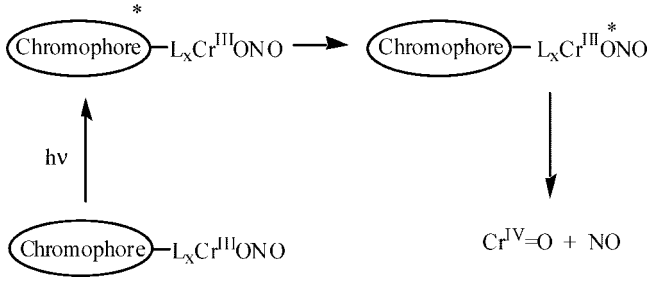
($k_{NO} = 3.1 \times 10^6\ M^{-1}\ s^{-1}$). Hence, this is another case where facile photoreaction is seen under continuous photolysis conditions only when O_2 is present to trap reactive intermediates. This water-soluble and thermally stable system, which demonstrates a large Φ_{NO} release with visible light, would seem very promising. However, the absorption cross section of **I** in the visible is very small, since these transitions are Laporte forbidden ligand field (d−d) bands. As described below, we addressed this challenge by attaching antennae to serve as intramolecular photosensitizers.

Scheme 4 proposes an excited-state reaction mechanism for **I**. The insensitivity of Φ_{NO} to λ_{irr} suggests that the reactive state is the lowest quartet or doublet ligand field ES, and direct sensitization experiments[40] show (at least) a substantial fraction of NO generation occurs from the latter. From ^{2}**I***, there would be no spin restriction in dissociating to the primary photoproducts, singlet Cr^{IV}=O plus doublet NO.

Polychromophoric Complexes

A consistent problem for most of the above systems, the metalloporphyrins excepted, is low absorbance at visible wavelengths. Since UV light transmission through tissue is poor,[10] this would limit photochemical applications to topical treatment or to encapsulation in a solid matrix[21,22,41] connected to an optical fiber and implanted. An alternative would be to construct molecules that harvest tissue-penetrating light by attaching antennae sensitive to the appropriate wavelengths.

SCHEME 5



Energy transfer from antenna to precursor would activate the latter to release NO or another bioactive agent as illustrated in Scheme 1.

Derivatives of I. The Cr(III) nitrite complexes are a good starting point for synthetic designs for antenna attachment, since, although absorptivities are low, direct excitation gave good Φ_{NO} at visible wavelengths. Thus, one would expect energy transfer from antenna to Cr(III) to generate reactive ligand field ES and NO generation (Scheme 5).

With this goal, Frank DeRosa prepared Cr(III) complexes of cyclam ligands modified by covalent attachment of polycyclic aromatics such as anthracene and pyrene.[42] Although not displaying the desired absorptions at longer visible wavelengths (ongoing studies are addressing this issue),[40] these compounds demonstrated that pendant chromophores can serve as antennae to gather light and to sensitize reactions localized at the Cr(III) center. The anthracene-tethered complex *trans*-[Cr(mac)$(ONO)_2$]BF_4 (**II**, mac = 5,7-dimethyl-6-anthracyl-cyclam, Figure 4) illustrates this behavior.

The first question is whether excitation of the anthracene leads to sensitization of the Cr(III) center. This was tested by examining the photophysical properties of **II** and its dichloro analog *trans*-Cr(mac)Cl_2^+. Excitation (365 nm) of free mac led to a strong fluorescence with the characteristic structured emission spectrum of the anthracene. In contrast, excitation of the *trans*-Cr(mac)X_2^+ salts under identical conditions (50 μM in aqueous solution) gave only very weak emission, at least 1000-fold less intense (Figure 5). For *trans*-Cr(mac)Cl_2^+, this was accompanied by weak phosphorescence from the Cr(III) doublet states (~700 nm), consistent with energy transfer from the anthracene to the metal center. With the dinitrito analog, the emission was too weak to detect. Instead, photolysis of aqueous **II** led to modest absorption changes with $\Phi_d = 0.17$ in aerated solution but much smaller ($<3.0 \times 10^{-3}$) in deaerated solution as expected for the reversible reaction illustrated in Scheme 4. That these spectral changes are due to NO photogeneration from nitrito ligands was confirmed electrochemically. Thus, attachment to the dinitrito

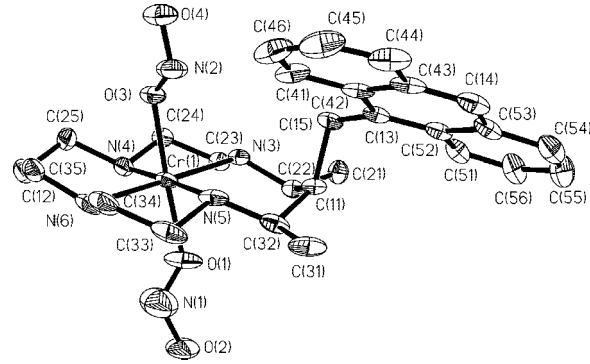


FIGURE 4. Molecular structure for cation of *trans*-[Cr(mac)$(ONO)_2$]BF_4 (**II**). Reproduced from ref 42. Copyright 2005 American Chemical Society.

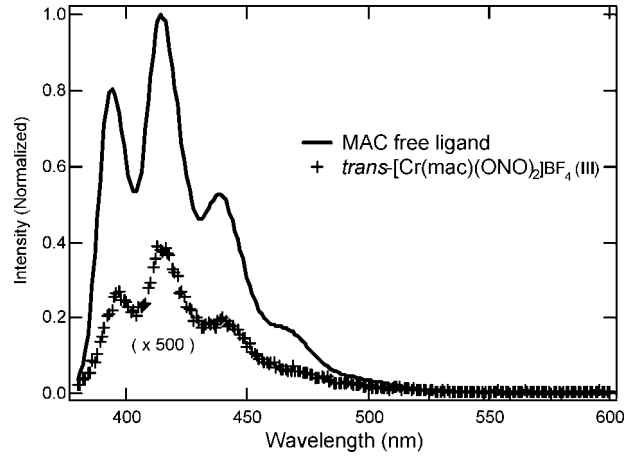


FIGURE 5. Emission spectra of a dilute aqueous solutions of *trans*-[Cr(mac)$(ONO)_2$]BF_4 (**II**) (50 μM) and the mac free ligand (50 μM). λ_{ex} = 360 nm. Reproduced from ref 42. Copyright 2005 American Chemical Society.

chromium(III) center quenches emission from the mac ligand >99.9%, presumably via energy transfer from the $\pi-\pi^*$ states of the pendant anthracene to metal-centered LF states followed by CrO−NO cleavage to generate NO.

Figure 6 illustrates the desired antenna effect for photolyses in dilute aqueous solutions of *trans*-Cr(mac)$(ONO)_2^+$, the phenyl analog *trans*-Cr(mbc)$(ONO)_2^+$ (replace the anthracene of **II** with C_6H_5-) and a related cyclam complex with a pyrene chromophore *trans*-Cr(pbc)$(ONO)_2^+$. When excited at 360−440 nm, **II** produced ~8× as much NO as did the mbc complex under identical conditions. This difference is attributed to the much larger absorption cross section for **II**; at 365 nm, the absorbance of 1.0 μM **II** is 5.9×10^{-3} while that of *trans*-Cr(mbc)$(ONO)_2^+$ is ~2×10^{-4}, so **II** absorbs ~30× more light at that wavelength. The larger NO value for *trans*-Cr(pbc)$(ONO)_2^+$ can be attributed to even stronger absorbance by the pyrene. In very dilute solutions, the principal differences are the absorptivities due to the different antennae.

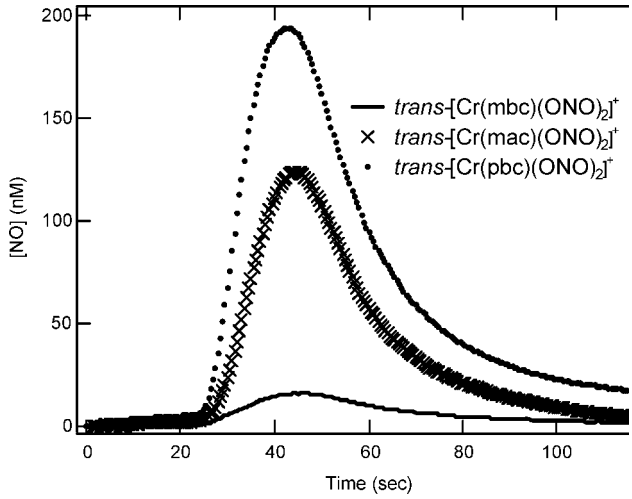
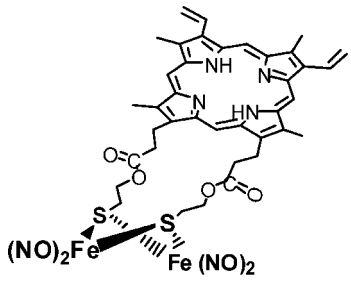


FIGURE 6. Electrochemical NO detection upon photolysis of 1.0 μM aq *trans*-Cr(L)$(ONO)_2^+$ complexes irradiated with a Hg short-arc lamp (360−440 nm bandpass filter). The solution was added at 20 s. Reproduced from ref 42. Copyright 2005 American Chemical Society.

Roussin's Red Salt Esters with Strongly Absorbing Chromophores. In parallel studies, we prepared RSEs modified with pendant antennae. The first generation was PPIX-RSE prepared by Christa Conrado, where the chromophore is a derivative of protoporphyrin-IX (PPIX) attached to the $Fe_2S_2(NO)_4$ cluster via $-CH_2CH_2-$ links.[43]



PPIX-RSE

The absorption spectrum of PPIX-RSE is close to the summed spectra of equal molar solutions of RRS and PPIX, so there is little ground-state electronic coupling between these chromophores. Qualitatively, the emission spectrum of PPIX-RSE is similar to PPIX or its dimethyl ester PPIX-DME. Quantitatively, however, the emission intensity from PPIX-RSE is ~10% that from PPIX; thus, PPIX fluorescence is quenched when linked to the iron cluster. This conclusion was supported by picosecond studies that demonstrated two lifetimes for PPIX-RSE ($\tau_1 = 0.22$ ns; $\tau_2 = 10.7$ ns).

A likely explanation of these data would be the presence of two PPIX-RSE conformers, the shorter lived one being folded along the flexible linkages to bring the porphyrin ring closer to the metal cluster. The presence of (at least) two such conformers was confirmed by ion-mobility mass spectrophotometry experiments.[44]

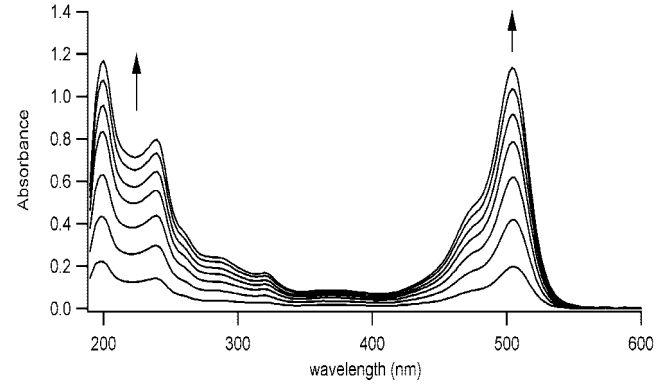
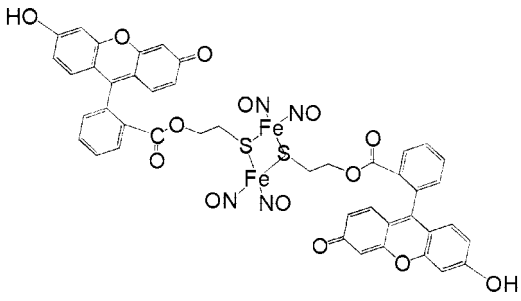


FIGURE 7. Spectral changes upon photolysis ($\lambda_{irr} = 436$ nm) of Fluor-RSE in 50/50 CH_3CN/phosphate aerated buffer (pH 7.4, 10 mM). (Figure provided by S. Wecksler).

The photochemistry of PPIX-RSE clearly demonstrated the antenna effect. For dilute solutions, photo-decomposition of PPIX-RSE (10 μM) occurred at much higher rates compared with the simpler ester $Fe_2(\mu\text{-SEt})_2(NO)_4$ under otherwise identical conditions. The effect was largely attributed to greater light absorption by the tethered PPIX. The *good news* was that attaching an antenna to enhance the visible light photoreaction worked as intended; the *bad news* was that quantum yields for both esters proved to be relatively low at this excitation wavelength.

The second generation of Roussin's esters with pendant antennae is represented by Fluor-RSE, with two pendant fluorescein dye units. This compound was prepared[33] by alkylating RRS and is moderately soluble in buffered aqueous solutions. Like PPIX-RSE, fluorescence characteristic of the fluorescein is ~85% quenched upon linking to the iron cluster. However, unlike the PPIX derivative, Fluor-RSE displays emission with a single exponential decay ($\tau = 3.3$ ns) and is a single conformer in the ion-mobility MS experiment. The photochemistry in aqueous media parallels that seen for other esters (Figure 7). All four NOs were released with a Φ_{NO} of 0.014.

Two-Photon Excitation of Certain Roussin'S Red Salt Esters. As discussed in the Introduction, TPE offers interesting possibilities in physiology, the opportunities to use NIR excitation wavelengths and to achieve spatial resolution.[8–10] One can imagine a scenario where TPE generation of the radiation sensitizer NO with focused NIR pulses is coordinated to pulsed γ-radiation at dosages less damaging to normal tissue in order to destroy small regions of specific tissues in the organism. However, doing so requires NO precursors coupled



Fluor-RSE

to chromophores with high two-photon absorption (TPA) cross sections (δ) as well as the appropriate stability, solubility, etc. Although a long way from finding practical solutions to all issues, we have made encouraging advances that will be summarized.

With the goal of exploiting such nonlinear effects, we embarked upon a search for polychromophoric systems that would demonstrate TPE-stimulated NO release. Steve Wecksler initiated this approach by examining the TPE photochemistry of the Roussin's ester PPIX-RSE. Despite the poor two-photon absorption (TPA) typical of porphyrin systems (for PPIX, $\delta = 2$ GM at 790 nm, where 1 GM $= 10^{-50}$ cm^4 s photon^{-1}),[45] he found that 810 nm NIR excitation of PPIX-RSE with 100 fs pulses leads to weak emission with a λ_{max} of 632 nm and to photochemical NO generation.[46] The emission occurs at an energy above that of the excitation source, so a nonlinear photolytic process is apparent. Analogous excitation of the free PPIX gave much stronger emission. In other words, the behavior under TPE paralleled that seen under single-photon excitation.

The next system tested was Fluor-RSE, which was actually prepared with TPE in mind since the fluorescein chromophore has a larger TPA cross section ($\delta = 38$ GM at 782 nm at pH 11).[47] Excitation of Fluor-RSE with NIR pulses gave a weak emission centered at ~530 nm, about 20-fold weaker than that seen for fluorescein or the model compound Fluor-ET under the same conditions (Figure 8).[48] This behavior was analogous to that seen under SPE with higher energy light.[33]

The quantitative emission of Fluor-RSE and of fluorescein upon TPE were used to calculate the cross section δ according to eq 5 (where I is the integrated fluorescence intensity, c is the concentration, Φ is the fluorescence quantum yield, P is the excitation power, K is the solution refractive index ratio, and ref designates the reference).[47] When the sample and reference are under identical conditions, $P = P_{ref}$ and $K = 1$, further simplifying the equation. These experiments demonstrated that for Fluor-RSE δ (63 ± 5 GM) is nearly twice that

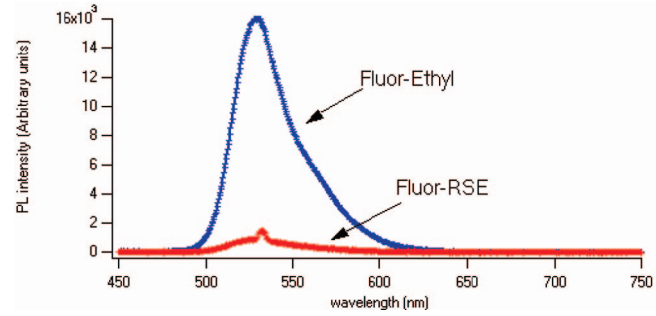


FIGURE 8. TPE photoluminescence spectra of Fluor-RSE and Fluor-Et in 50/50 CH_3CN/phosphate buffer (pH 7.4). Reproduced from ref 48. Copyright 2006 American Chemical Society.

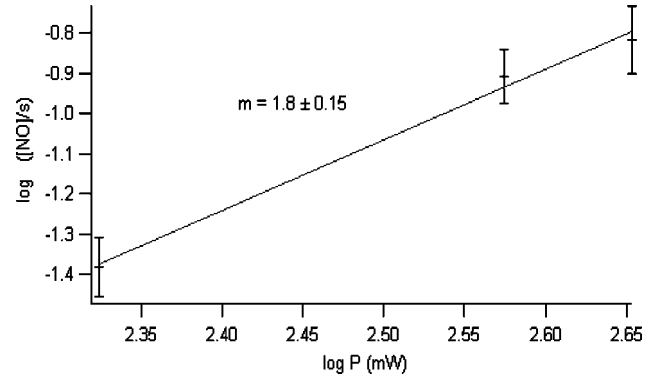


FIGURE 9. Log of NO released under 800 nm excitation vs log of averaged power of the pulsed laser (100 fs pulses at 80 MHz) for 30 intervals.BF_4 (**II**). Reproduced from ref 42. Copyright 2005 American Chemical Society.

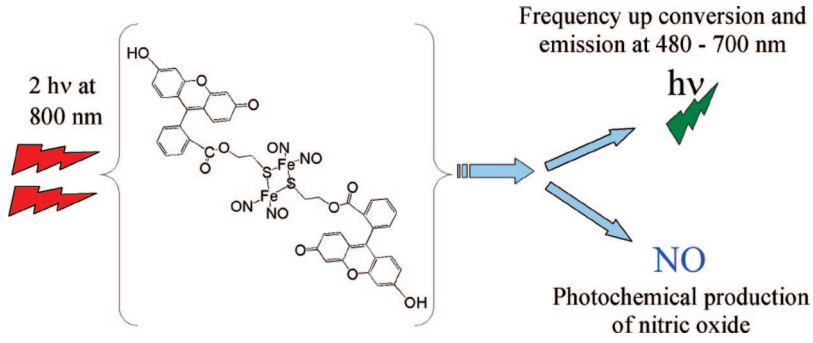
of fluorescein at 800 nm, an observation that is not surprising given that Fluor-RSE includes two fluorescein chromophores.

$$\delta = \frac{\Phi_{ref}\delta_{ref}c_{ref}P_{ref}^2 I}{\Phi c P^2 I_{ref}} K \qquad (5)$$

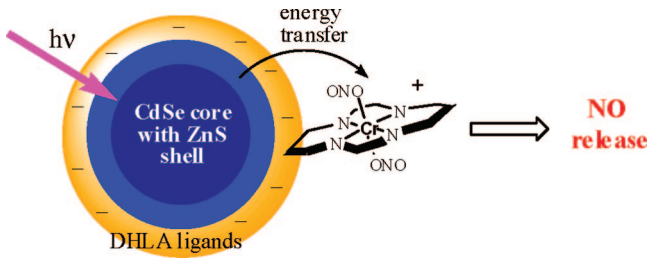
Electrochemical studies showed the relationship between the quantity of NO generated and the intensities of the laser pulses to be nonlinear. A log–log plot of the NO signal versus excitation power was linear with a slope of 1.8 ± 0.2 (Figure 9) indicating that the rate of NO production is proportional to the excitation power squared. This clearly points to a TPA mechanism as illustrated in Scheme 6).

These studies have demonstrated that TPE using NIR light can activate a model prodrug such as Fluor-RSE. We have now prepared another RSE with a much larger TPA cross section in collaboration with Loon-Seng Tan[49] and plan to attach various TPE dyes to other NO precursors. Although we have just begun to exploit the options, it is clear that two-photon NIR excitation offers some very interesting possibilities.

SCHEME 6



SCHEME 7



Even Bigger Chromophores, CdSe Quantum Dots

While continuing investigations with pendant SPE and TPE dye chromophores, we have also initiated efforts to establish the feasibility of using quantum dots as light-gathering antenna. Nanocrystal QDs offer important advantages including very high SPE[50] and TPE[51] cross sections and the opportunity to tune the optical properties by varying the QD diameter.[52] One may also take advantage of multiple coordination sites on the QD surface to impart properties such as solubility and biological specificity[53] and to attach the NO precursor. In preliminary studies, Dan Neuman and Alexis Ostrowski recorded enhanced NO photogeneration from electrostatic assemblies of water-soluble CdSe/ZnS core/shell QDs and the cationic complex *trans*-Cr(cyclam)$(ONO)_2^+$ (**I**) as illustrated in Scheme 7.[54]

This behavior was indicated both by quenching of the QD photoluminescence by **I** (Figure 10) and by increased photochemical NO generation from solutions of **I** when the water-soluble CdSe/ZnS core/shell QDs were present (Figure 11). Although either energy or electron transfer from the excited QD to the Cr(III) cations at the surface might account for the quenching, the photostimulated release of NO parallels the excited-state chemistry of **I** (Scheme 5).

The effect of QDs in enhancing photochemical NO production can be attributed to the dramatically higher QD extinction coefficients (about 3 orders of magnitude higher than **I**). Ongoing studies are concerned with building nanoparticle assemblies directly coordinated to the NO precursor. Given the multiplicity of functions that can be incorporated onto QD surfaces, one may anticipate NO precursors having QD antennae can be targeted to specific tissues *in vivo*.

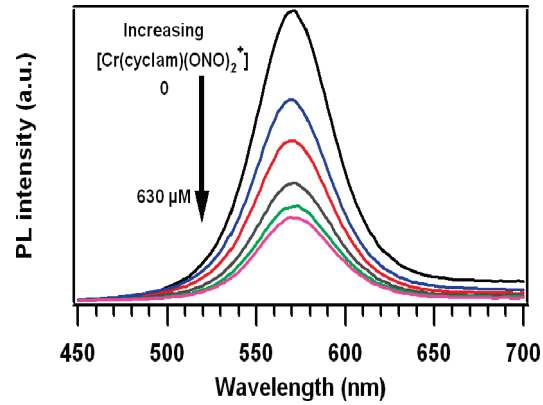


FIGURE 10. Photoluminescence quenching of water-soluble QDs (~130 nM) in phosphate buffer (15 mM, pH 8.2) with added **I** (0 to 630 μM). Reproduced from ref 54. Copyright 2007 American Chemical Society.

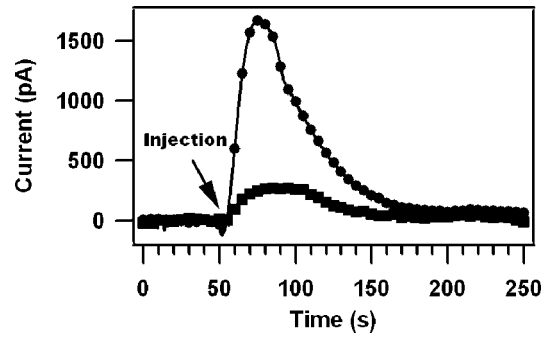


FIGURE 11. NO photochemically produced from **I** (200 μM) in stirred buffer solutions (15 mM, pH 8.2) with (●) and without (■) added QDs (100 nM). NO was detected with a nitric oxide specific electrode (sensitivity 1 nM = ~100 pA). QDs alone elicited no response. Reproduced from ref 54. Copyright 2007 American Chemical Society.

Summary

This Account described several strategies for photochemical generation of the bioregulator and γ-radiation sensitizer nitric

oxide from metal-based precursors. Initial studies focused on direct excitation of the nitrosyl and nitrito complexes, but these were challenged by the relatively low extinction coefficients at visible or NIR wavelengths where tissue penetration is more favorable. While such systems might be adaptable for topical applications or with solid implants, we have addressed this issue by building molecular constructs with light-gathering antennae that increase absorption cross sections. Energy transfer from the antenna to the NO precursor serves to photosensitize reactions at the latter chromophore. Of particular interest are antennae with high cross sections for two-photon absorption at NIR wavelengths. The use of NIR TPE to generate NO release has now been demonstrated for several cases, and studies with pendant dyes and QD antennae continue. While the emphasis of the present Account has been on NO precursors, these same strategies should apply to the photoinduced delivery of other bioregulators or drugs to targeted sites with doses and timing controlled by using light to trigger their release.

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BIOGRAPHICAL INFORMATION

Peter C. Ford is a Professor at UC Santa Barbara where he was appointed to the faculty after earning a Ph.D. at Yale University with K. B. Wiberg and serving as a Postdoctoral Fellow with Henry Taube at Stanford University.

FOOTNOTES

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