Coordination Chemistry Reviews 255 (2011) 737–756

Contents lists available at ScienceDirect

Coordination Chemistry Reviews

journal homepage: www.elsevier.com/locate/ccr

Review

Photochemical reagents for the study of metalloproteins by flash photolysis

Federico I. Rosell, A. Grant Mauk*

Department of Biochemistry and Molecular Biology and the Centre for Blood Research, University of British Columbia, Canada

Contents

ARTICLE INFO

Article history:
Received 28 September 2010
Accepted 17 November 2010
Available online 25 November 2010

This article is dedicated to Harry B. Gray, a great practitioner and ambassador of chemistry, on the occasion of his 75th birthday.

Keywords:
Flash photolysis
Metalloproteins
Caged reagents
pH-jump

ABSTRACT

Flash photolysis has been used extensively in the advancement of our understanding of the electron transfer reactivity of metalloproteins and in investigation of the kinetic complexities of electron transfer-initiated protein folding. Additional opportunities for the use of flash photolysis to understand the functional properties of metalloproteins have been afforded through the use of photoactive caged complexes. This review surveys the uses of caged complexes to the study of metalloproteins that have been reported and considers the potential for expanded use of photoactive caged complexes that have not yet been applied in this manner.

1. Introduction

Following the initial reports of Norrish and Porter [1–3] that a brief, highly intense light burst can be used to initiate photochemical reactions and enable the spectroscopic detection of transient chemical species, this method was soon applied to the investigation of biological processes. Early biological applications of flash photolysis concerned the visual pigment rhodopsin [4], the photosynthetic pigment chlorophyll [5,6], and rebinding of carbon monoxide to hemoglobin [7,8]. Subsequently, DeVault and Chance used flash photolysis of photosynthetic proteins at cryogenic temperature to provide the first experimental evidence for electron tunneling in a biological electron transfer reaction [9,10]. Together, these pioneering studies demonstrated the value of flash photoly-

* Corresponding author at: Department of Biochemistry and Molecular Biology, Life Sciences Centre, University of British Columbia, 2350 Health Sciences Mall, Vancouver, BC V6T 1Z3, Canada.
E-mail address: mauk@interchange.ubc.ca (A.G. Mauk).

0010-8545/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.ccr.2010.11.025

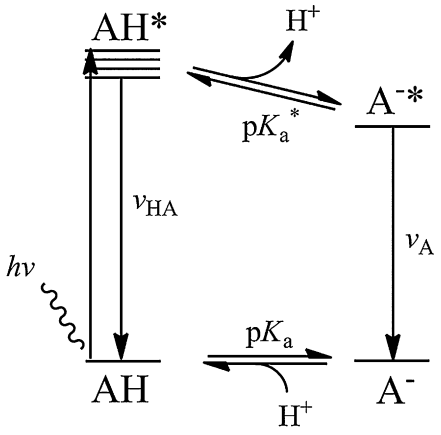
sis spectroscopy as a means of characterizing the kinetics of rapid chemical processes in biological systems and led to development of increasingly sophisticated instrumentation and chemical strategies for use in biological studies. These improvements in technologies in turn resulted in the application of flash photolysis to investigation of a broader range of biological phenomena.

While these pioneering studies focused on chemical systems that are inherently photosensitive, the use of small, photoactive molecules to initiate chemical reactions involving photolytically unreactive biological systems made flash photolysis far more generally applicable. Thus, flavins [11,12], inorganic coordination compounds [13], and reduced pyridine nucleotides (NADH) [14] were useful reagents for the study of metalloprotein electron transfer kinetics and have been used by a number of investigators to characterize an increasing number of biological systems. The use of photoactive reagents in this way has been refined subsequently by Gray and colleagues through the design and synthesis of enzyme substrate analogues with appended photoactive functional groups. These molecular "wires" enable photo-initiated electron transfer to structurally inaccessible active sites that otherwise exhibit little or no photochemical response to exogenous reagents [15–17].

Another category of small, photoactive reagents that has been developed over the past 30 years for use in flash photolysis experiments is comprised of compounds that undergo rapid photolytic release of a chemical species to initiate a chemical reaction. Following the terminology introduced by Kaplan et al. [18], reagents of this type are generally referred to as "caged" compounds, a term that has had a variety of alternative chemical meanings in other contexts. The first reports of caged compounds of this type concerned caged cAMP [19] and caged ATP [18]. Since then, a great number of photoactive caged complexes has been reported that permit the rapid but controlled release of a wide range of biologically active chemical species that vary in complexity from protons (vide infra) to proteins [20–24]. The current review surveys those caged compounds that have been used in or may have the potential for use in mechanistic studies of metalloproteins in aqueous solution. From this survey, it should be apparent that although the value of this experimental approach has been well established, many new and informative opportunities remain to be developed through application of this technology.

2. pH-jump reagents

Among the earliest type of flash photolysis reagents to be reported are those capable of inducing rapid changes in solution pH. This early start can be credited in part to Weber, who in 1931 observed that the absorption and fluorescence emission spectra of 1-naphthylamine-4-sulfonate (**1a**) exhibit different dependences on pH [25]. This report sparked considerable activity concerning investigation of the acid–base equilibria of molecules in the excited



$$pK = pK_a - pK_a^* = \frac{0.625}{T} \times (\bar{\nu}_{HA} - \bar{\nu}_A)$$

Fig. 1. Simplified Jablonski diagram illustrating a thermodynamic cycle for excited-state proton transfer.

state (Fig. 1). One consequence of this work and related studies concerning excited-state proton transfer by Förster [26] and Weller [27] among others was recognition by the groups of Winn [28], Gutman [29] and Irie [30] that this phenomenon can be used as a means to perturb chemical and biological systems.

In principle, any molecule that exhibits a pK_a shift upon electronic excitation could be used for pH-jump studies, but the suitability of a reagent for this purpose depends on many factors [31,32]. As with all reagents reviewed here (Plate 1), pH-jump reagents must be sufficiently soluble in water. The magnitude of photo-initiated pH change will depend on the photochemical quantum yield (Φ_{pc}), the concentration of reagent and the change in pK_a between the ground and the excited states. These reagents should also be chemically inert with respect to the analyte. Reactions between analyte and reagent should be limited to proton transfer reactions unless, as in the studies by Gutman et al. [33–36], the experimental results are derived from absorbance and fluorescence changes of the reagents that are modulated by their non-covalent interactions with the analyte. Next, reagents should exhibit either mildly alkaline or acidic pK_as in the ground-state and a substantial pK_a shift upon excitation ($\Delta pK_a \sim 3$–7 pH units; Table 1) so that proton transfer reactions are energetically favourable. The compilation by Ireland and Wyatt [37] of ground- and excited-state pK_a values of a wide range of organic compounds which exhibit photo-induced pK_a shifts is a valuable source of such information. This database has been augmented over the years [38–40] and, more recently, it has been expanded by Vos to include a number of $[Ru(bipy)_2L]^{2+}$ complexes [41]. In cases where these data are unavailable for a

Table 1
Kinetic and thermodynamic parameters of reagents used in pH- and pOH-jump experiments.

Compound	Jump[a]	λ_{ex} (nm)	pK_a	pK_a^*	k_{act}[b] ($\times 10^9$ s^{-1})	k_{recomb} ($\times 10^9$ M^{-1} s^{-1})	Refs.
Pyranine (8-hydroxypyrene-1,3,6-trisulfonate)	pH-	347.2	7.7	0.5	10	160	[33]
				1.4	32		[252]
							[253]
2-Naphthol-3,6-disulfonate	pH-	347.2		0.5	31	70	[253]
							[254]
2-Naphthol-6-sulfonate	pH-	347.2	9.1	1.6	1.02	90	[37]
							[28]
Triphenylmethane leucohydroxide	pOH-						
6-Methoxy-quinoline	pOH		5.18	5.2			[37]
Acridine	pOH		5.5	10.6			[37]

[a] pH- and pOH- denote, respectively, the photoacidic and photobasic character of the reagent.
[b] k_{act} is the rate constant for proton dissociation of photoacids or proton abstraction for photobases.

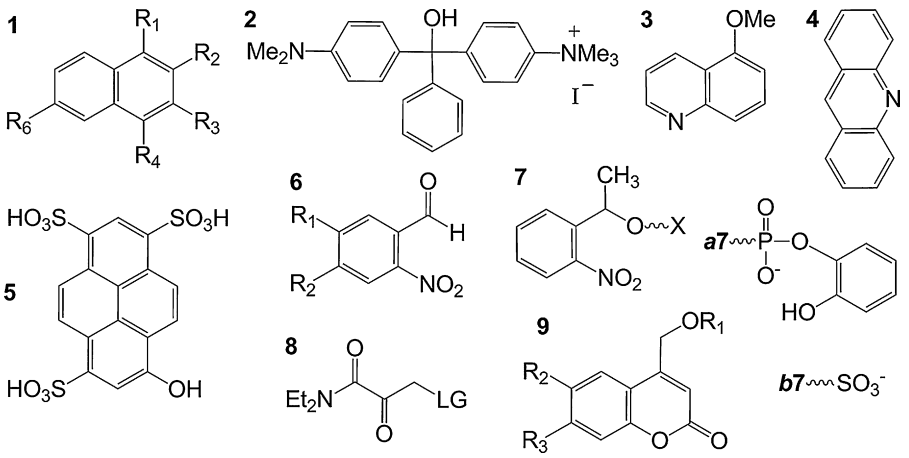


Plate 1. Reagents for pH-jump experiments. (**1**) Naphthalene derivatives: (**a**) 1-naphthylamine-4-sulfonate ($R_1 = NH_2$, $R_4 = SO_3^-$), (**b**) 2-naphthol-6-sulfonate ($R_2 = OH$, $R_6 = SO_3^-$), (**c**) 2-naphtol-3,6-disulfonate ($R_2 = OH$, $R_3 = R_6 = SO_3^-$), (**d**) 1-(2-nitroethyl)-2-naphthol ($R_1 = CH_2CH_2NO_2$, $R_2 = OH$); (**2**) 4-(dimethylamino),4′-(trimethylammonium)triphenylmethanol iodide; (**3**) 6-methoxy-quinoline; (**4**) acridine; (**5**) 8-hydroxypyrene-1,3,6-trisulfonate (pyranine); (**6**) (**a**) *ortho*-nitrobenzaldehyde ($R_1 = R_2 = H$) and its derivatives: (**b**) 4-formyl-6-methoxy-3-nitrophenoxyacetic acid (FMNA: $R_1 = OCH_3$, $R_2 = OCH_2CO_2H$), (**c**) 4,5-methoxy-2-nitrobenzaldehyde (DMNBA: $R_1 = R_2 = OCH_3$), (**d**) 4-chloro-2-nitrobenzaldehyde (CNBA: $R_1 = H$, $R_2 = Cl$); (**7a**) 2-hydroxyphenyl-1-(2-nitrophenyl)ethyl phosphate (HPP, R = 2-hydroxyphenyl-phosphate), (**7b**) 1-(2-nitrophenyl)ethyl sulfate (caged sulfate, $R = SO_3^-$; see table 2 for more derivatives); (**8**) *N*,*N*-diethyl α-ketomide (LG = CH_3CO_2H, $(C_6H_6)CHCO_2$, BocNHCH$(CH_3)CO_2$ or 4-$NCC_6H_4CO_2$); (**9**) (coumarin-4-yl)methyl esters ($R_1 = SO_3^-$, SO_2CH_3, or $PO(OEt)_2$; R_2 and R_3 = H, OCH_3, $N(CH_3)_2$).

particular reagent, the ΔpK_a has been estimated with the Förster equation (Eq. (1); Ref. [42]) on the basis of the known pK_a of the ground-state at a desired temperature (T in K) and the 0–0 transition frequencies of the conjugate acid/base ($\bar{\nu}_{HA}$ and $\bar{\nu}_{A^-}$ (cm^{-1}), respectively).

$$\Delta pK = pK_a - pK_a^* = \frac{0.625}{T} \times (\bar{\nu}_{HA} - \bar{\nu}_{A^-}) \quad (1)$$

This approach is a practical alternative to fluorescence pH titration or life-time measurements [43], but the results can be subject to significant error. This problem stems in part from complications in the identification of 0–0 transitions in fluorescence spectra that often lack sufficient fine structure to permit accurate determination of emission maxima. Ireland and Wyatt cite a deviation of ~1 pK unit when the combined uncertainty is as small as 4 nm in the near-UV region of the spectrum. Nevertheless, many investigators consider the sign of the result (i.e., of ΔpK_a) sufficient to learn whether a compound will act as a photoacid or base. Moreover, the magnitude of the pH-jump is generally measured empirically with pH indicators, and, ultimately, the rate of photoactivation is a more important consideration for time-resolved measurements than thermodynamics. The foremost requirement is that proton/hydroxide ion release must be competitive with radiative and non-radiative fluorescence decay processes of the reagent because these compounds act as strong photoacids or bases only while in the excited state (compare cases 1–3 starting on page 66 of Ref. [44]). Upon relaxation to the ground state, the pK_as shift to their pre-pulse value and reverse the pH-jump following second-order kinetics so that the pH change lasts up to a few hundreds of microseconds.

2.1. pH-jump up

Relatively few studies have featured flash photolysis to increase solution pH. Irie proposed using triphenylmethane leucohydroxide for this purpose because it exhibits a Φ ~0.25 in water and enhanced solubility upon derivatization (e.g., 4-(dimethylamino),4′-(trimethylammonium) triphenylmethanol iodide, (**2**) [30]). Concentrations of these reagents in the millimolar range can increase solution pH substantially (~4.5 pH units) [30] following the photo-induced heterolytic cleavage of the reagent into a hydroxide ion and a coloured, highly stable triaryl carbocation ($k > 1 \times 10^8\ s^{-1}$ [45]). Abbruzzetti et al. subsequently demonstrated that multi-exponential return to the pre-pulse pH consists of a fast, pH-independent phase associated with a type of geminate recombination ($k \sim 1 \times 10^7\ s^{-1}$) and two slower heterogeneous recombination processes ($k \sim 2.7 \times 10^4$ and $3.0 \times 10^3\ s^{-1}$)[45]. When monitoring the pOH-jump with an indicator (bromoxylenol), the pH rose within ~30 μs to a value that was stable for at least 20 ms. Considering the intense absorbance of the triaryl carbocation, however, it should also have been possible to evaluate the hydroxide pulse based on spectrophotometric monitoring of the carbocation.

An alternative mechanism to excited-state hydroxide ion release for the rapid alkalinization is provided by compounds such as 6-methoxiquinoline (**3**) that generate a hydroxide ion *in situ*. With an excited-state lifetime and pK_a^* of 1.6 ns and 11.9, respectively [46], Gutman and Nachliel reasoned that collisional proton transfer is too improbable within the lifetime of the excited state and at mildly alkaline pH to account for the observed protonation time constant of 3 ns. Therefore, these authors suspected that the reagent actively abstracts a proton from the water that solvates the compound in solution to generate a hydroxide ion that escapes electrostatic pairing with the protonated quinoline [46,47]. Upon return to the ground state, the proton recombines with the hydroxide ion by hopping between any suitable proton acceptor, thus reversing the pH change. In a study conducted on halorhodopsin, however, this relaxation did not happen before the pOH pulse effectively deprotonated the Schiff base of retinal and triggered the unfolding of the protein [46]. One important *caveat* concerning the use of 6-methoxyquinoline (and the analogous acridine (**4**)) is that depending on the pre-pulse pH relative to the pK_a of indicators or other analytes present in the solution, this reagent can appear to acidify the solution by undergoing collisional proton transfers to these reporting agents [47].

2.2. pH-jump down

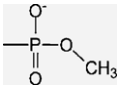
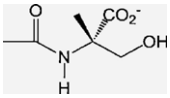
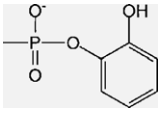
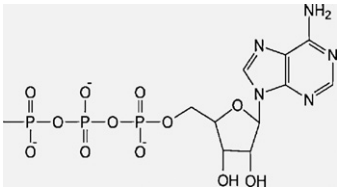
Gutman and co-workers have also conducted extensive and systematic studies on the dynamics of proton transfer reactions that acidify solutions using predominantly hydroxyarenes like 8-hydroxypyrene-1,3,6-trisulfonate (pyranine, (**5**)) and other, sulfonated naphthols (e.g., (**1b**) and (**c**), reviewed in [48]). Establishing at each step the physical foundations for the interpretation of results, these authors extended their studies to biological systems.

Of particular interest was the manner in which microenvironments immediately around and within proteins [33,34,46,49–52], within ion channels [36], and at the surface of lipid bilayers [35] influence protonation reactions to reveal information about the microenvironments themselves. In one study, for example, the authors found that one pyranine molecule binds tightly and reversibly within the heme pocket of apo-myoglobin [34]. It was estimated that the pH within this cavity reached a value ~0 upon illumination. Under conventional equilibrium conditions, this pH is sufficiently acidic to denature the protein. However, because this acidic pH was generated within the confines of the heme pocket rather than in bulk solution, the rate for recombination was enhanced to restore native conditions before the slower unfolding events could occur. In water, photo-induced proton release from this dye is fast ($k_{diss} = 1 \times 10^{10}\ s^{-1}$) and capable of reducing the solution pH by 3–5 units [31]. In contrast, proton release from the myoglobin-bound pyranine was 93% slower ($k_{diss} = 6.9 \times 10^{8}\ s^{-1}$), reflecting a low water activity in this environment ($a_w = 0.67$, which is equivalent to the activity of water in a concentrated salt solution) and providing a measure of the strong interaction of (~60, closely-packed) water molecules with residues lining the cavity. By comparison, the water activity determined from a similar study with a pyranine–albumin complex was $a_w = 0.85$ [33]. In a different study, Gutman and Huppert encapsulated 2-naphthol-3,6-disulfonate ((**1c**); rate for proton dissociation from this reagent = $1.02 \times 10^{9}\ s^{-1}$ [28]) within liposomes [29] and determined that although this reagent cannot permeate the lipid membrane, photolysis results in a ~10^4-fold increase in proton concentration within the liposome. Thus, intense photolysis poises a proton-motive force ($\Delta\mu H^+$) estimated to be 180–240 mV which was proposed to be adequate to perturb the energy-coupled reactions of oxidative phosphorylation.

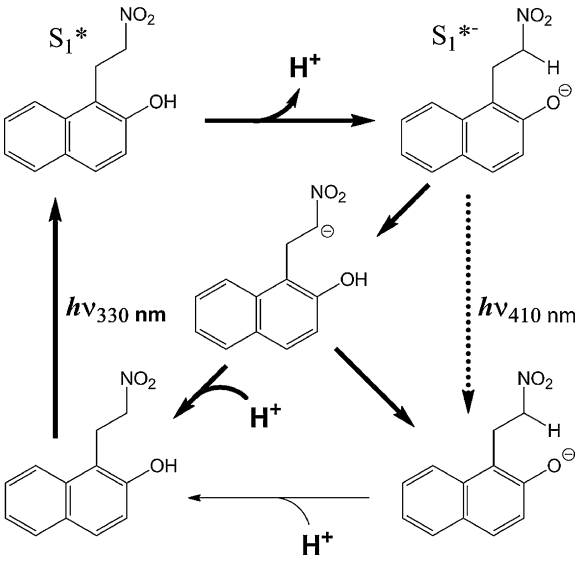
Modification of hydroxyarenes has been explored in an effort to improve various aspects of their performance. 2-Naphtol-3,6-disulfonate, for example, exhibits greater aqueous solubility than does 2-naphthol, but a sulfonate group is not as strongly electron-withdrawing as a cyano group. This realization inspired Tolbert and coworkers to investigate the properties of various cyanonaphthols. Much of the work to characterize these so-called "super photoacids" (reviewed in [39]) was carried out in organic solvents where modifications of this type were seen to increase the acidity of the naphthol group. Unfortunately, water quenches the fluorescence of these compounds and thereby counteracts the enhancements gained by the modifications [53].

More recently, Nunes et al. reported a persistent and reversible acidification of aqueous solutions with 1-(2-nitroethyl)-2-naphthol (NO2nH, (**1d**), Scheme 1) [32]. This derivative of 2-naphthol was synthesized following a rational design strategy based on Formosinho's Interacting-State Model [54–56] to complement the rapid rate of proton dissociation from the naphthol hydroxyl group (k_{diss} ~$2 \times 10^{9}\ s^{-1}$) with the propensity from a neighboring nitroalkane to transfer a proton to the resulting hydroxide while still in the excited-state (k_{iESPT} ~$3 \times 10^{8}\ s^{-1}$; Scheme 1). The carbanion that forms on the aliphatic side chain is very stable, so it reprotonates relatively slowly (k_p ~$5 \times 10^{4}\ M^{-1}s^{-1}$). Overall, these steps compete efficiently with the fluorescence lifetime of the naphtholate (k_{fl} ~$5 \times 10^{7}\ s^{-1}$) and with the ion recombination that follows immediately to regenerate the starting reagent (k_{recomb} ~$3 \times 10^{12}\ M^{-1}\ s^{-1}$). Whereas

Table 2
Leaving group of 1-(2-nitrophenyl)ethyl caged compounds and physical parameters associated with their light-induced acidification of solutions.

Cage name	Leaving group R	Φ	pK_a			Ref.
			gs[a]	*aci*-[b]	eq[c]	
1-(2-Nitrophenyl)ethyl sulfate (NPE sulfate)		0.47		3.69	1.92	[60,69]
1-(2-Nitrophenyl)ethyl phosphate (caged phosphate)		0.54				[255]
1-(2-Nitrophenyl)ethyl methyl phosphate (caged methyl phosphate)						
N-1-(2-nitrobenzyl) ethoxycarbonyl-L-serine [d]		0.65				[77]
2-Hydroxyphenyl 1-(2-nitrophenyl) ethyl phosphate (caged HPP)		0.095–0.29	10.1		5.3	[77]
1-(2-Nitrophenyl)ethyl adenosine-5′-triphosphate (caged ATP)		0.63				[255] [71]

[a] Ground state pK_a of caged reagent prior to photolysis.
[b] pK_a of leaving group after photolysis.
[c] Other amino acids have been protected with a 4,4-dimethoxy-nitrophenyl group according to Patchornik et al. [256].



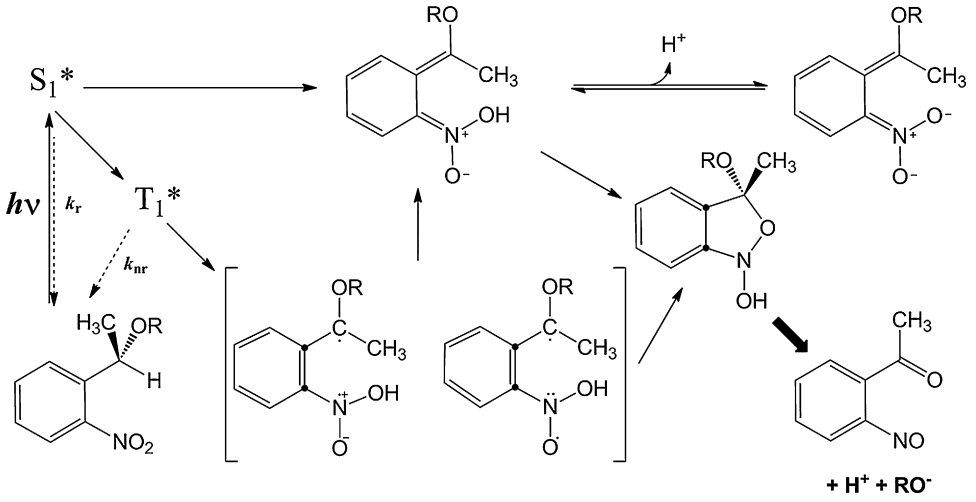
Scheme 1. Mechanism for the photolysis 1-(2-nitroethyl)-2-naphthol and the persistent but reversible change in solution pH-jump.
Adapted from Ref. [32].

the pH-jump generated with ~4 mM nitrobenzaldehyde (NBA, (**6**)) solution persisted ~500 μs before reverting to nearly the initial pH by ~1 ms, flash photolysis of a 200 μM solution of NO2nH with 10 μM bromocresol green in a 2% methanol/water solution remained acidic for a considerably longer period (>5 ms). It is reasonable to assume that sulfonation of NO2nH can improve its solubility in water (thus obviating the need for methanol) to permit its use in studies of proteins without exerting a significant deleterious effect on the other properties of the reagent.

A popular class of pH-jump reagent that fulfills more closely the role of a "caged" complex as first envisioned by Hoffman and coworkers [18] is based on 2-nitrobenzaldehyde (*o*-NBA; (**6**)). This reagent and its derivatives have been used extensively in studies by the groups of Viappiani [57–68], Corrie [60,61,69], Terazima [70–72], Krishnamoorthy [73,74] and others [32,75–82]. *o*-NBA in fact affords a versatile platform for caging and delivering a myriad of other bio-effectors upon photolysis, often with the release of an accompanying proton (e.g., caged ATP [83]; Table 2). For pH-jump applications, a noteworthy feature of these reagents is that the chemical alterations of the cage that accompany proton release render the acidification irreversible. Non-photolytic changes in pH that follow activation result from the mixing of the photoactivated volume with the surrounding solution that did not undergo photolysis and the buffering by any indicator, buffer and analyte molecules present in the solution.

The mechanism of proton release from *o*-NBA (illustrated in Scheme 2 for 1-(2-nitrophenyl)ethyl caged analogues) has been studied extensively by time-resolved techniques (e.g., [57,75,81]). The chain of reactions (first observed as early as 1901 [84]) begins from the first excited singlet state with an intramolecular proton transfer from the aldehyde to the nitro group (k_{diss} ~2×10^7 s^{-1} in water; [85]). This step deactivates the excited state through π-electron reorganization [57], and it generates an *o*-quinonoid [81], also referred to as an *aci*-nitro intermediate. Depending on the solution pH, the protonated form of this intermediate, nitronic acid, either deprotonates reversibly to its conjugate nitronate base (pK_a = 2–3.5; [57,85]), or it proceeds via a bicyclic benzisoxazolidine intermediate to the final photoproduct, a nitrosobenzoic acid [75]. Yip et al. have reported [81] a parallel pathway (for *o*-nitrobenzyl *p*-cyanophenyl ether in acetonitrile) in which internal conversion of the excited singlet to the triplet state leads to a similar intramolecular proton abstraction that yields a biradical species with a maximum at ~460 nm. This intermediate then re-enters the first pathway through conversion to either the nitronic acid and/or the bicyclic intermediate. In the absence of other species that may buffer the solution, the pK_a of the final photoproduct, nitrosobenzoic acid (pK_a ~3.5 [57]), controls the final pH of the solution; the stability of the nitrosobenzoic acid renders the pH change irreversible over longer periods (ms-s [57]) than those observed with excited-state proton transfer mechanisms alone. In aqueous solution, Φ_{pc} for this reagent is 0.40–0.45.

The performance of *o*-NBA derivatives has undergone considerable study in an effort to modulate various aspects of proton release from this reagent. The oxyacetate group of 4-formyl-6-methoxy-3-nitrophenoxyacetic acid (**6b**) [76], for example, enhances the solubility of the caged proton relative to the parent *o*-NBA. Moreover, this modification renders the reagent impermeable to lipid bilayers and, therefore, amenable to compartmentalization and the generation of electromotive gradients. The methoxy moiety, on the other hand, results in a bathochromic shift in the absorption maximum and increases the molar absorptivity as in 4,5-methoxy-2-nitro-benzaldehyde (**6c**) [57]. A greater two-photon absorption cross section in this case is a desirable property for the activation



Scheme 2. Mechanism for light-induced solution acidification with *o*-NBA-based reagents.
Adapted from Refs. [69,81].

of proton release with near-IR radiation [86]. However, detrimental consequences of the introduction of these electron-donating groups into the phenyl ring are a ~0.5 unit increase in the pK_a of the *aci*-nitro intermediate, a 50–80% decrease in the Φ_{pc}, and a reduction in the conversion rate to nitrosobenzoate that delays pH stabilization from tens of nanoseconds to a few microseconds [57]. In contrast, an electron-donating chlorine at the 4-position of the phenyl ring (**6d**) fails to perturb appreciably either Φ_{pc} or the pK_a, but it appears to accelerate the formation of the nitrosobenzoate photoproduct beyond the detection limit of the instrument used in the study [57]. George and Scaiano [75] have reported similar studies on additional phenyl ring derivatives of *o*-NBA, but these studies have been conducted entirely in organic solvents and, therefore, are not considered further here.

The aldehyde group of *o*-NBA is the coupling site for the encapsulation of other bio-effectors by this cage. Consequently, modifications of the aldehyde group have also been investigated in an effort to modulate functionality and, for pH-jump experiments, to enhance their proton-releasing characteristics. The highest pK_a of all the photoproducts generated limits the lowest solution pH achievable by photolysis. This property is illustrated in the comparison of caged-"HPP" and "sulfate" (2-hydroxyphenyl-1-(2-nitrophenyl)ethyl phosphate (**7a**) [77] and 1-(2-nitrophenyl)ethyl sulfate (**7b**), respectively) [69]. In these compounds, an ethyl ether group replaces the aldehyde to link either a 2-phosphophenol moiety or a sulfate group to the aromatic ring at the position *ortho* to the nitro group. Breakdown of the bicyclic intermediate following photolysis yields 2-nitrosoacetophenone rather than nitrosobenzoate, so the spent cage does not buffer the final solution. Instead, the other photoproducts that accompany proton release, namely a 2-phosphophenol group or a sulfate, act in this capacity. With a pK_a of 5.3 [69], the 2-phosphophenol leaving group buffers the solution to a final pH that approaches this value whereas the caged sulfate has been reported capable of protonating compounds with pK_as as low as ~2 [69] thanks to the strongly acidic pK_a of the sulfate group (~1.92).

Despite the wealth of information available concerning *o*-NBA-based pH-jump reagents, their use in metalloprotein research has been surprisingly limited to protein folding studies of myoglobin [58,61,69,78] and mitochondrial cytochrome *c* [63]. Abbruzzetti et al. used time-resolved photoacoustic detection to monitor volume changes associated with conversion of native apo-myoglobin to its acidic intermediate I following an *o*-NBA-induced pH-jump in 200 μM guanidine hydrochloride [58]. This method allowed the authors to distinguish between the fast neutralization of two to six carboxylate groups depending on the pH (3.4 mL/mol expansion; $125 \times 10^9\ M^{-1}s^{-1}$) and the slower protonation of two His residues (presumably His24 and 119 based on Barrick et al. [87]; 82 mL/mol contraction; $7.7 \times 10^9\ M^{-1}\ s^{-1}$). Both processes were observed with a pre-pulse pH = 7.0, but reaction with the carboxylate groups was observed almost exclusively when the starting pH was 4.5. More importantly, the magnitude of the slower expansion was unusually large for the protonation of two His residues, leading the authors to conclude that this expansion included the volume change associated with the conformational transition of myoglobin to its acidic intermediate [58].

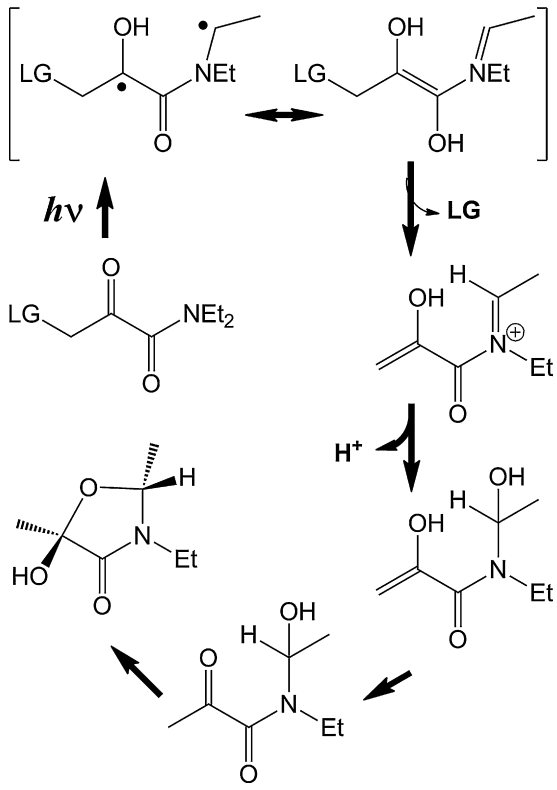
Mikšovská and Larsen also used *o*-NBA to investigate further the acid denaturation of apo-myoglobin by photoacoustic calorimetry and photothermal beam deflection but in the absence of denaturants [78]. In this study, the contraction associated with the protonation of the His residues and the conformational transition of the protein was ~25% that reported by Abbruzzetti et al., and all reaction rates were ~4-fold faster. These differences were attributed to the lack of denaturant and the higher temperatures used for the experiment, but the mildly acidic pre-pulse pH (5.5) used for these measurements played a role. Also, there was no evidence of further volume changes occurring over the next 5 ms after the pH-jump, a time-point easily accessed with conventional rapid mixing techniques.

Abbruzzetti et al. subsequently revisited the acid unfolding of myoglobin [61] by focusing on the heme-protein complex, which allowed them to monitor the progress of the reaction by time-resolved spectrophotometry. On this occasion, the authors used the caged sulfate *o*-NBA derivative (1-(2-nitrophenyl)ethyl sulfate) to achieve a more acidic pH as shown by Barth and Corrie in their pH titration of myoglobin using the same photo-activated caged sulfate to acidify the solution [69]. In Abbruzzetti et al., monitoring the kinetics of acidification spectrophotometrically revealed the bi-exponential formation of an unfolded intermediate (k = 55 and 1.86 s^{-1}), which decayed on a longer time scale (k = 1.06 s^{-1}). These events are slower than those exhibited by apo-myoglobin, but these steps reflected the changes in the heme environment that followed earlier protein unfolding events, which presumably were also influenced by the interaction of the polypeptide with the heme group.

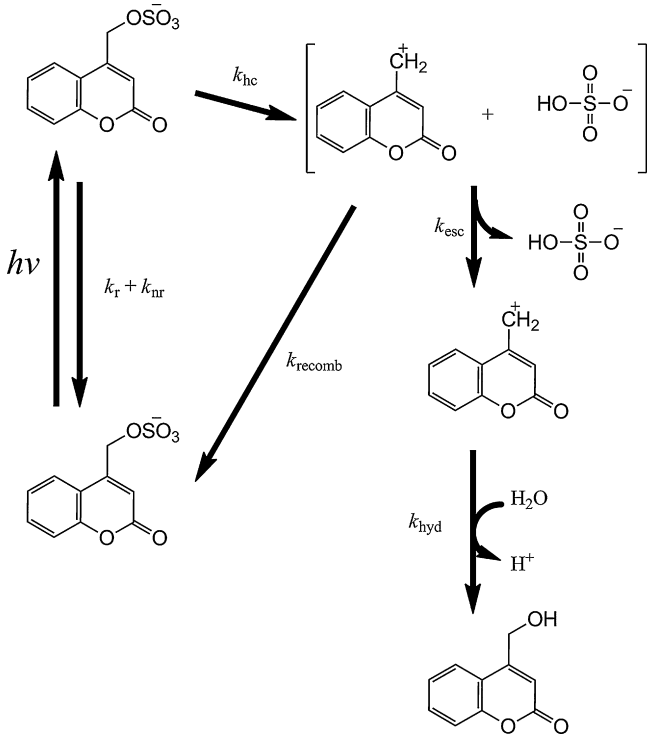
Finally, Viappiani and co-workers again used time-resolved spectrophotometry and *o*-NBA to follow the effect of rapid solution acidification on the axial coordination of mitochondrial cytochrome *c* in 4.5 M guanidine hydrochloride [63], and of the *N*-terminal (residues 1–56) heme-containing fragment of the protein [62]. In both studies, the kinetic data were best described with a bi-exponential model reflecting the scission of the non-native bond between the heme iron and one of two His residues (His26 and 33). While this process occurred at room temperature over a timescale accessible with conventional rapid mixing techniques, use of flash photolysis to achieve the pH-jump allowed the authors to access much higher temperatures from which they extracted activation energies. In both cases, these non-native conformations were seen to pose kinetic traps to the unfolding of the protein, but the differences in these activation parameters were a result of the different behaviors of the polypeptide in the presence or absence of the denaturant or the missing polypeptide fragment.

Emerging proton-emitters that undergo similar chemical modification as part of the photolytic process to make pH perturbations irreversible include compounds based on either α-ketoamides (**8**) [88] or (coumarin-4-yl)methyl esters (**9**) [89]. Reaction by-products of α-ketoamide photolysis (Scheme 3) consist of a five member hemiacetal ring originating from the ketoamide backbone and a generic leaving group (typically a carboxylate or a phenolate ion) that, depending on its composition, could buffer the solution in a manner similar to that described above for some *o*-NBA photoproducts. The reported Φ_{pc} of these reagents is high (~0.8), and indicator (bromocresol green) bleaching was observed within ~100 μs. As with many of the studies to characterize pH-jump reagents, the investigation on these ketoamides was performed in 50% acetonitrile, so it is unclear how these reagents perform under conditions that are more biocompatible.

Similarly, kinetic analyses on the photochemistry of (coumarin-4-yl)methyl esters (**9**) has been carried out for the most part in aqueous organic solvents [90,91], but Hagen and co-workers also showed that these compounds are soluble and functional in water [89]. Photolysis (Scheme 4) begins with excitation of the reagent, which then undergoes heterolytic cleavage (k_{hc} ~$2 \times 10^{10}\ s^{-1}$) that competes effectively with radiative and non-radiative fluorescence decay processes. The resulting ion pair then either recombines to regenerate the starting ground state reagent, or the ions separate completely to leave an anion (e.g., a sulfate or phosphate group) and a (coumarin-4-yl)methyl cation. The former photoproduct (in effect a buffer) limits the final acidity of the solution while the latter intermediate hydrolyses water to release a proton and (coumarin-4-yl)methyl alcohol [89]. The overall apparent proton dissociation rate is fast (k_{diss} ~$5 \times 10^8\ s^{-1}$) and can be observed by



Scheme 3. Photolysis mechanism leading to solution acidification with α-ketoamides.
Adapted from Refs. [88].



Scheme 4. Competing pathways for the relaxation of coumarin derivatives following photoexcitation and resulting in solution acidification
Adapted from Refs. [89].

monitoring the fluorescence of the (coumarin-4-yl)methyl alcohol photoproduct as the cage is only weakly fluorescent prior to photoactivation [89]. With high molar absorptivity and Φ_{pc} (as high as 17,000 M^{-1} cm^{-1} and 0.79, respectively), the photosensitivity of these reagents also makes them appropriate for two-photon activation with Ti-sapphire lasers. Finally, this reagent is similar to *o*-NBA insofar as it provides a versatile platform for caging of a multitude of bio-effectors (which are released as the anionic photoproduct) such as capsaicin [92], GTP, amino acids and cyclic mononucleotides (e.g., AMP) [91].

The range of reagents available for pH-jump experiments is rich in its variety and functionality, yet these compounds remain largely underused in metalloprotein research. This deficiency may result in part from an assumption that these reagents are solely for kinetics studies in the nano to microsecond time regimes. As the groups of Viappiani and Hagen have shown, however, caged proton reagents can also be used to perturb the pH of a solution persistently (e.g., in a sealed vessel) and in a controlled fashion with pulses of light rather than through addition of a titrant. When used for kinetics studies, repetition may be desirable to accumulate and average multiple transients, and this capability can be achieved even with the irreversible reagents by incorporating a flow-flash strategy (e.g., [14]). In this respect, reversible pH-jump reagents such as the hydroxyarenes and triphenylmethane leucohydroxides described above may be superior as long as the duration and magnitude of the pH pulse are compatible with the kinetics under investigation. Another situation in which these reversible pH-jump agents may prove useful is in the study of reversible processes such as metal ion binding to proteins or in the study of reversible protein conformational changes. Solutions of the protein(s) of interest and the pH-jump agent in a suitable buffer cocktail could be subjected to measurements analogous to saturation transfer difference NMR spectroscopy experiments [93,94], for example, in which a pulse of light is used rather than a saturation pulse from an RF emitter to perturb the analyte (Fig. 2). The resulting difference spectrum could be used to identify residues affected by metal ion binding or conformational changes induced by the change in pH. Alternatively, all these pH-jump reagents could be used in tandem with metal ion chelators that are not otherwise photoactive. Upon subjecting the chelated-metal ion complex to the pH change, such an agent would release the metal ion(s) into the solution so that, upon restoration of the pre-pulse pH, the chelators would compete with the protein to bind the metal ion. In the end, the applications and uses of these pH-jump reagents is only limited by imagination and experimental design.

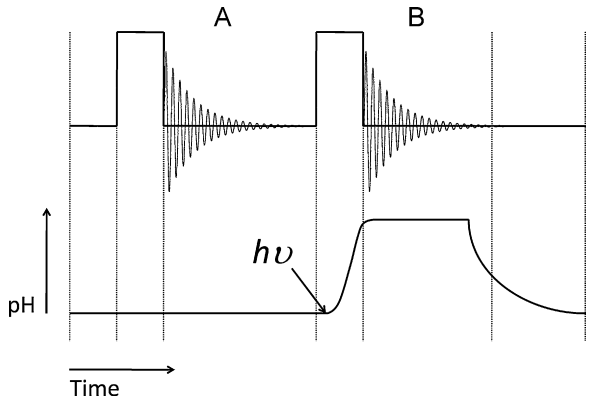


Fig. 2. Difference NMR experiment pulse sequence for the observation of pH-dependent protein structural changes induced by flash photolysis of pH-jump reagent(s) in solution.

3. Caged complexes

3.1. Metal ions

3.1.1. Calcium

Initial efforts to design a caged metal ion complex involved synthesis of photoactive derivatives of established chelators for calcium, the photoproducts of which exhibited significantly lower affinity for the metal ion. The focus of research concerning these reagents has been development of tools for investigation of cellular events initiated by the release of calcium as monitored by optical microscopy or electrophysiological methods [95]. While many of these cellular processes are mediated by calcium binding to proteins, these reagents have not been used directly in the study of calcium binding proteins. As the availability and photochemical characterization of these reagents grows, the opportunity to initiate calcium-triggered changes in protein structure or catalytic activity rapidly by flash photolysis provides an attractive means of investigating the kinetics and mechanisms by which these proteins function.

The first example of this approach was reported by Tsien and Zucker [96] in which the calcium-selective chelator BAPTA [97] was modified by introduction of a nitropiperonyl group (**10**) (Plate 2) that upon photolysis (365 nm) was proposed to produce a nitrosobenzophenone group that would decrease the affinity of the ligand for calcium. Determination of the stability constant of the ligand before and after photolysis established that the photoproduct exhibited an affinity for calcium that is 30–40 fold lower than that of the parent ligand. The rate constant for calcium release from this ligand was relatively slow ($\tau \sim 210\ s^{-1}$) owing to the involvement of a rate-limiting thermal step, and the quantum yield for the nitr-2-Ca^{2+} complex (0.05) was slightly greater than that for nitr-2 alone (0.01) [98]. Subsequently, a series of related ligands (nitr-1 through nitr-7) was reported by this group [98,99] that retained selectivity for calcium (relative to magnesium) and relatively low quantum yields, but that exhibited time constants as short as ~1.8 ms for release of calcium.

At about the same time, Ellis-Davies and Kaplan reported a related but photochemically different chemical strategy for calcium cage complexes through preparation of *o*-nitrophenyl-substituted derivatives of EGTA and EDTA [83,100], which are referred to in the literature as NP-EGTA (**11**) and DM-nitrophen (**12**), respectively. These ligands differ from those described above in that photolysis results in destruction of the ligand. As a result, the stability constant for binding of calcium to the photoproduct of the ligand is much lower than that of the photoproducts of the nitr-family of ligands. The quantum yields for release of Ca^{2+} following photolysis (347 nm) of the complex formed with DM-nitrophen (~0.18 [83]) and nitrophenyl-EGTA (~0.23 [101]) are also greater. While the affinity of DM-nitrophen for Mg^{2+} is just 500-fold lower than that for Ca^{2+}, NP-EGTA exhibits greater selectivity for Ca^{2+} with an affinity for this metal ion that is ~12,500-fold greater than for Mg^{2+} [101]. The half-life ($\tau_{1/2}$) for photo-dissociation of Ca^{2+} from DM-nitrophen was reported as ≤180 μs [102], and indirect evidence led to the expectation that Ca^{2+} release from the complex formed with NP-EGTA is also rapid [101].

Subsequently, the photolysis of both of these caged Ca^{2+} complexes was studied in greater kinetic detail to demonstrate that both form transient intermediates that exhibit bi-exponential decay processes and that photolysis of NP-EGTA is at least six times faster than photolysis of DM-nitrophen overall [103]. While the origin of the bi-exponential decay kinetics remains to be fully clarified, a calcium indicator dye was used to estimate the rate of photolytic release of Ca^{2+} from NP-EGTA ($6.5 \times 10^4\ s^{-1}$) and DM-nitrophen ($4.1 \times 10^4\ s^{-1}$). These slight differences in rate constants probably reflect minor differences in experimental conditions used for their determination. It is also likely that these rate constants are at the limit of detection by the indirect nature of this method of measure-

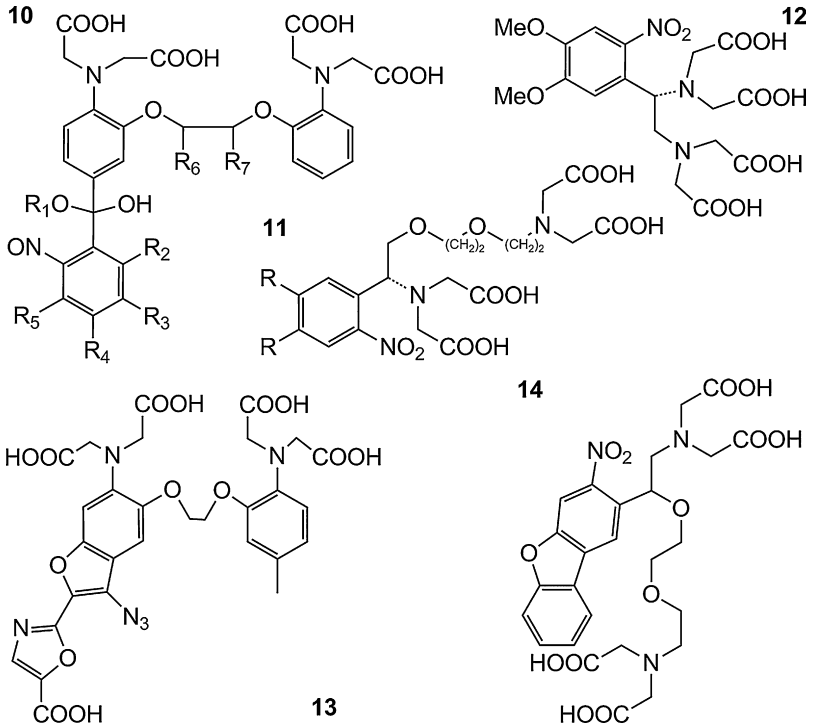


Plate 2. Ca2+-releasing photo-reagents. (**10**) BAPTA derivatives: (**a**) Nitr-1 ($R_1 = CH_3$), (**b**) Nitr-2: Nitr-5 with $R_1 = CH_3$, (**c**) Nitr-3 ($R_1 = CH_3$, $R_2 = OCH_3$, $R_5 = NO_2$), (**d**) Nitr-4: Nitr-2 with R_6–R_7 = *cis*-$(CH_2)_3$, (**e**) Nitr-5 (R_3–$R_4 = OCH_2O$), (**f**) Nitr-6: Nitr-5 with $R_1 = COCH_3$, (**g**) Nitr-7: Nitr-5 with R_6–R_7 = *cis*-$(CH_2)_3$; (**11**) (**a**) *o*-nitrophenyl-EGTA (NP-EGTA, R = H), (**b**) dimethoxynitrophenyl-EGTA-4 (DMNPE-4: $R = OCH_3$); (**12**) DM-nitrophen; (**13**) Azid-1; (**14**) NDBF–EGTA. Dashed lines denote bonds that are severed upon photolysis.

ment. More recently, Faas et al. reported [104] a detailed analysis of the kinetics of Ca^{2+} release from NP-EGTA and DM-nitrophen under conditions different from those reported previously and found that in each case the process was best analyzed in terms of two time constants. Clearly, on the basis of these results and other considerations [105], the selection of calcium cage complex and experimental conditions must be evaluated carefully in the design of experiments using these reagents.

Six new photolabile derivatives of EGTA, most of which involve derivatization with a dimethoxynitrophenyl group were subsequently reported by Ellis-Davies and co-workers [106]. Of these reagents, dimethoxynitrophenyl-EGTA-4 (DMNPE-4) (**11b**) exhibited selectivity for calcium (relative to magnesium) that was comparable to that of NP-EGTA, more than a 5-fold increase in molar absorptivity (5140 M^{-1} cm^{-1} at 347 nm) and comparable quantum yield (~0.2). While DMNPE-4 has proved to be amenable to 2-photon excitation, the resulting photoproducts retain a greater affinity for Ca^{2+} than those of DM-nitrophen or NP-EGTA, so the amount of Ca^{2+} liberated by photolysis is ~45% of that observed for complexes formed by these latter ligands [106]. Although chelators closely related to DMNPE-4 have been synthesized and partially characterized [107], all appear to exhibit photochemical properties inferior to those of DMNPE-4.

All of the calcium cage complexes discussed so far are based on rendering established chelators photochemically active by their derivatization with 2-nitrobenzyl or dimethoxy-nitrophenyl groups. The success of this approach is demonstrated by the widespread use of these reagents in the literature. Nevertheless, alternative strategies for the preparation of photosensitive chelators have been developed in an effort to improve the functional properties of the resulting cage complexes and to increase their range of use. For example, Tsien and co-workers reported the 3-azido derivatization of the fura calcium sensors [108] to produce the calcium cage azid-1 (**13**) [109], a calcium complex with a molar absorptivity (342 nm) of 33,000 M^{-1} cm^{-1} and a quantum yield of 1. The affinity of azid-1 for calcium is 230 nM, and this value decreases to 120 μM (500-fold decrease) following photolysis. Notably, azid-1 exhibits a cross-section for two-photon excitation (~1.4 GM) that is two orders of magnitude greater than that for DM-nitrophen (the cross-section for NP-EGTA is negligible) and that has permitted use of this reagent for photolytic reagent in femtoliter volumes [86]. However, detailed kinetic analysis similar to that described above for NP-EGTA and DM-nitrophen of photo-initiated release of calcium from the complex formed with azid-1 has not been reported.

Another approach to improved photochemical performance has involved the nitrodibenzofuran derivatization of EGTA to afford the ligand NDBF–EGTA (**14**) [110], the calcium complex of which exhibits a molar absorptivity (330 nm) of 18,400 M^{-1} cm^{-1} and a quantum yield of 0.7 (347 nm). The affinity of this ligand for Ca^{2+} is ~14 nM, but the affinity of the photoproduct is 140,000-fold lower. The photolytic release of Ca^{2+} is monophasic with a rate constant of 20,000 s^{-1}, is ~7-fold more efficient than its release from DM-nitrophen, and is amenable to two-photon photolysis [110]. While the calcium complex of azid-1 exhibits a greater quantum yield than the corresponding complex of NDBF–EGTA, the latter complex exhibits much greater affinity for Ca^{2+}, a greater differential in affinity before and after photolysis, and faster release of Ca^{2+}.

3.1.2. Iron

As reviewed by Balzani and Carassati, the photosensitivity of chelated iron complexes such as the oxalate complex of Fe^{3+} has been recognized for about 180 years [111] and was studied extensively during the first half of the previous century. This work led to the general recognition that aerobic photolysis of the ferric trisoxalate complex leads to reduction of the iron and decomposition of the ligand with the production of CO_2 and other products. In related work, illumination of the Fe^{3+}–EDTA complex under a variety of conditions (as indicated) results in the formation of CO_2 (250–400 nm) [112], formaldehyde (350 nm) [113], and a variety of other larger, partially degraded forms of the ligand [114] with a quantum yield in the range of ~0.02–0.06 [113]. While the number of photoproducts formed during this reaction would greatly complicate any attempt to determine the stability of Fe^{2+} binding to the ligand following photolysis (a measure of "uncaging"), these early reports demonstrate a fundamental difference between the reactivities of photoactive iron and the photoactive calcium complexes in that Fe^{3+} in such complexes typically undergoes photoreduction.

The abundance of iron in the earth's crust combined with the remarkable chemical reactivity of iron has led to evolution of a metabolic requirement for iron by nearly all life forms. In development of this dependence on iron, a variety of chemical processes have been engaged to optimize the biological use of its catalytic potential while controlling its potential toxicity and competing successfully for its acquisition. In recent years, Butler and co-workers have discovered that some marine bacteria acquire iron through secretion of a variety of siderophores, some of which are not photosensitive (hydroxamate siderophores [115]), some of which are photoreactive in the absence of bound iron (catecholate siderophores [115]), and some of which become photosensitive upon binding Fe^{3+} (α-hydroxy carboxylate siderophores (e.g., **15a**, **b**) (Plate 3) [115–120]). Although most of the latter group of siderophores releases bound iron as Fe^{2+} following photolysis (the synechobactins A–C seem to be an exception [121]), the photooxidized ligand in many cases retains sufficient affinity for Fe^{3+} formed by re-oxidation of the photolytically released Fe^{2+} that the iron is quickly rebound [116,118,122]. In the case of vibrioferrin (**16**), however, introduction of two α-hydroxy acid functionalities renders the Fe^{3+} complex of the siderophore sufficiently photoreactive that the photoproducts of the siderophores have no affinity for iron [120]. As a result, it could be argued that at least in the case of vibrioferrin, a naturally-occurring iron cage complex has evolved that may provide insight into the design of a synthetic iron cage for use in mechanistic studies of iron-requiring proteins.

Recently, Baldwin and co-workers have used this approach in the development of five new ligands for possible use in the preparation of iron cage complexes that are based on the principles derived from studies of photosensitive siderophores [123]. As a result, this group has prepared α-hydroxy acid-containing ligands that incorporate a bidentate salicylidene group [123]. The first of these complexes to be structurally characterized, $Na[Fe_3(3,5\text{-diCl-Sal-AHA})_3(\mu_3\text{-}OCH_3)]\cdot 3.5H_2O$ (**17**), crystallized as a trinuclear complex and was photoreactive upon exposure to sunlight. Initial results suggest that 50–60% of the iron from these complexes is released following 2–3 h of exposure to an Hg lamp in the presence of the Fe(II) chelator bathophenanthroline disulfonate (BPDS), a result that compares with ~70% release of iron from aquachelin (**18**) under similar conditions [116]. The presence of BPDS in these experiments protects the Fe(II) produced during photolysis from reoxidation to Fe(III) by dioxygen and is not required for photoreduction of the iron [123]. At present, however, full photochemical characterization of these iron complexes and their photoproducts remain to be determined.

An alternative approach to development of a photocage for Fe(III) was attempted by Burdette and colleagues [124] through use of the *N*-phenyl-1-oxa-4,10-dithia-7-azacyclododecane ($AT_2$12C4) macrocycle that had previously been used [125] as the metal ion binding component of a highly selective fluorescent sensor for Fe(III) in biological applications. The resulting cage complex, which

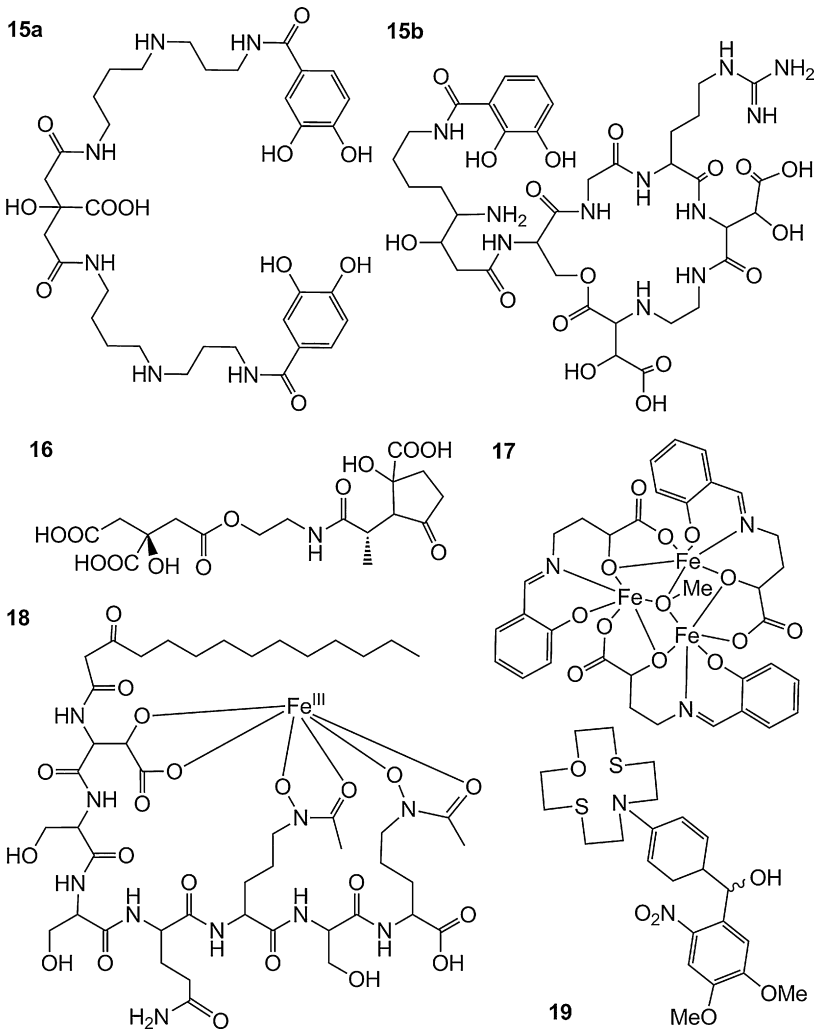


Plate 3. Ligands for iron-releasing photo-reagents. (**15**) (**a**) Petrobactin, (**b**) Alterobactin A; (**16**) Vibrioferrin; (**17**) Fe_3(3,5-diCl-Sal-AHA)$_3$(μ-OCH_3); (**18**) Aquachelin B; (**19**) FerriCast.

the authors named FerriCast (**19**), possesses the $AT_2$12C4 macrocycle with an appended 4,5-dimethoxy-2-nitrophenyl group. While characterizing the solution behavior of FerriCast, the authors discovered that use of reagents based on the selective coordination chemistry of $AT_2$12C4 is restricted to non-aqueous solvents lacking strong oxygen donor atoms (e.g., alcohols, water) under which conditions this ligand does not bind iron.

3.1.3. Zinc

Development of zinc photocages has begun only recently through the activities of two groups. Burdett and co-workers have focused on use of the nitrobenzhydrol caging group variously coupled with a 4,5-dimethoxy-2-nitrophenyl group to develop the reagent ZinCast-1 (Plate 4) (**20**), which exhibits a diminished affinity for zinc following photolysis [126], and the reagent ZinCleav-1

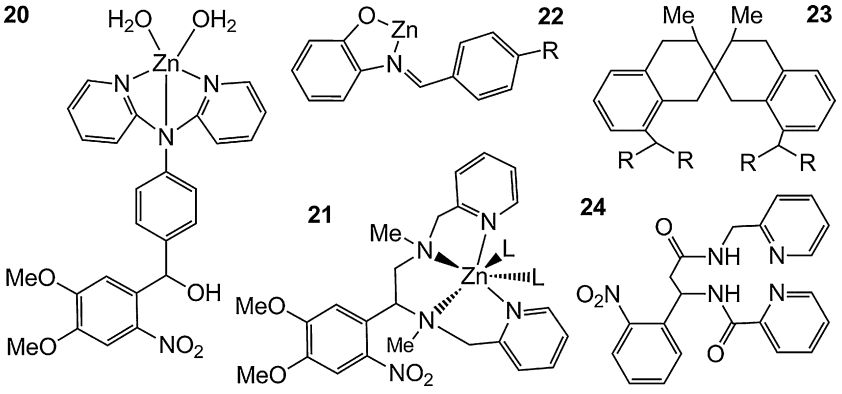


Plate 4. Ligands and complexes for delivery of Zn^{2+} or other metal ions by flash photolysis. (**20**) $[Zn(ZinCast\text{-}1)(H_2O)]^{2+}$; (**21**) ZinCleav-1; (**22**) (**a**) (*E*)-2-((4-methoxybenzylidene) amino)phenol (R = OCH_3), (**b**) R = a second methanylidineaminophenol group; (**23**) bisBenzospiropyran (R = $(CH_2)_nCH_3$ where n = 1 (**a**) or 2 (**b**)); **24**) *N*-(1-(2-nitrophenyl)-3-oxo-3-(pyridine-2-ylmethylamino)propyl)picolinamide.

(**21**) that undergoes fragmentation as the result of photolysis [127]. ZinCast-1 exhibits an increase in dissociation constant (K_d) for zinc from 14.3 μM prior to photolysis to a value of 5.5 mM afterwards and a quantum yield of 1 ± 2% for release of zinc. On the other hand ZinCleav-2 exhibits an increase in corrected apparent dissociation constant for zinc of 0.023 ± 0.04 pM prior to photolysis to a value of >150 μM for the photoproducts with a quantum yield of 0.55% for the release of zinc. An alternative, simpler approach proposed by Zhang and Chen involves use of a phenolic Schiff base that forms a benzoxazole upon photolysis of the zinc complex (**22a**) [128]. This ligand is presumably bidentate, and the stability of the zinc complex that it forms is quite low ($K_d = 3.4 \times 10^{-4}$ M). An extended version of this ligand (**22b**) [129] with two bidentate binding regions forms a zinc complex with similar stability, $K_d = 3 \times 10^{-4}$ M, and photoproducts related to the benzoxazole proposed to form by photolysis of the original compound. The lack of detailed characterization of the speciation, quantum yields, and kinetics of zinc release related to the zinc complexes formed by these reagents and the limited stability and specificity of these complexes limits the use of these reagents in the study of biological systems.

3.1.4. Other metal ions

The effort invested in development of photoactive cage complexes for the release of calcium, zinc, and iron relative to other metal ions is not surprising in view of the abundance of these metal ions in biological systems. Nevertheless, other metal ions also contribute critically important biological functions, and some attention has been paid to development of reagents to study some of them as well. In fact, in their original report of DM-nitrophen, Kaplan and Ellis-Davis reported its use in the photo-release of Mg^{2+} in addition to its use in release of Ca^{2+} described above [83]. The stability constant for the DM-nitrophen–Mg^{2+} complex (2.5×10^{-6} M^{-1}, pH 7.1, 22 °C) was about three orders of magnitude lower than that for the corresponding Ca^{2+} complex, but characterization of the properties of the complex formed with Mg^{2+} or for several other metals with which DM-nitrophen has been used to a limited extent in cellular studies [130,131] has not been pursued in detail.

McCurdy and co-workers have proposed that the photoisomerization of bisbenzospiroyan [132] derivatives may be useful in the release of Mg^{2+}, Ca^{2+} and Sr^{2+}. Bisbenzospiroyan modified with two aminodiacetate substituents (**23a**) forms a 1:1 complex with Mg^{2+} (5.6×10^2 M^{-1}) and Ca^{2+} (6.7×10^3 M^{-1}) while modification with two aminodipropionate substituents (**23b**) increases the stability constants for the complex formed with Mg^{2+} ($3.7(3) \times 10^3$ M^{-1}) relative to that for the complex formed with Ca^{2+} ($5.6(7) \times 10^3$ M^{-1})(HEPES buffer (10 mM), pH 9.8). As indicated, however, the stability constants for complex formation are relatively low, and the selectivity is somewhat limited. The quantum yields for the bisbenzospiroyan bearing two aminodipropionate or two aminodiacetate substituents are also relatively low (0.0083(5) and 0.0057(6)) for photolysis at 310 nm, respectively (HEPES buffer (10 mM) pH 9.8)).

More recently, a caged copper complex has been reported by Franz and co-workers in work that involved development of the first ligand for photo-initiated release of a *d*-block metal ion [133]. The bispyridylamide ligand designed by this group (*N*-(1-(2-nitrophenyl)-3-oxo-3-(pyridine-2-ylmethylamino)propyl) picolinamide; **24**) binds Cu^{2+} with a stability constant of 16 pM in phosphate buffer at pH 7.4 and releases copper with a quantum yield of 0.32 following irradiation at 350 nm. As photolytic release of copper occurs with partial degradation of the ligand through release of picolinamide, the photoproducts have minimal residual binding affinity for copper. The authors note that the oxidation state of the copper released was not determined, but that Cu^{+} was probably the initial product and that subsequent prompt oxidation to Cu^{2+} was likely under aerobic photolysis.

3.1.5. Caged chelators

The logical complement to photo-initiated release of metal ions is the photoinitated sequestration of metal ions. The strategy underlying design of most of the reagents of this type that have been reported involves increasing the affinity of a ligand for metal ions through photo-isomerization. While a large number of ligands of this type have been reported, few that are soluble in aqueous solution have been identified. The first example of this type was based on the photo-isomerization of the planar *trans*-4,4″-bis(α-iminodiacetic acid)azotoluene (or azo-IDA; **24a**) (Plate 5), which does not bind metal ions, to the non-planar *cis* stereoisomer that does [134]. Upon irradiation at 320 nm, ~80% of the *trans* isomer converts to the *cis* isomer, which forms a 1:1 complex with Zn^{2+} with a stability constant of $\sim 1 \times 10^5$ M^{-1} in unbuffered aqueous solution, but the quantum yield and kinetics of Zn^{2+} binding and the ability of this compound to bind other divalent metal ions were not reported. Subsequently, Shinkai et al. studied the Zn^{2+} binding properties of azo-IDA and the related compound azo-ED (**24b**) in which the iminodiacetate groups were replaced with ethylenediamine groups [135]. These authors found that in buffered aqueous solution at pH 9 the affinity of *trans*-azo-IDA for Zn^{2+} is much greater ($\sim 6 \times 10^9$ M^{-1}) than in unbuffered solution and that of *trans*-azo-ED ($\sim 3 \times 10^7$ M^{-1}), and that both bind Cu^{2+} with slightly greater affinity than that exhibited with Zn^{2+} ($\sim 6.5 \times 10^9$ M^{-1} for *trans*-azo-IDA and 5×10^7 M^{-1} for *trans*-azo-ED). The affinity of

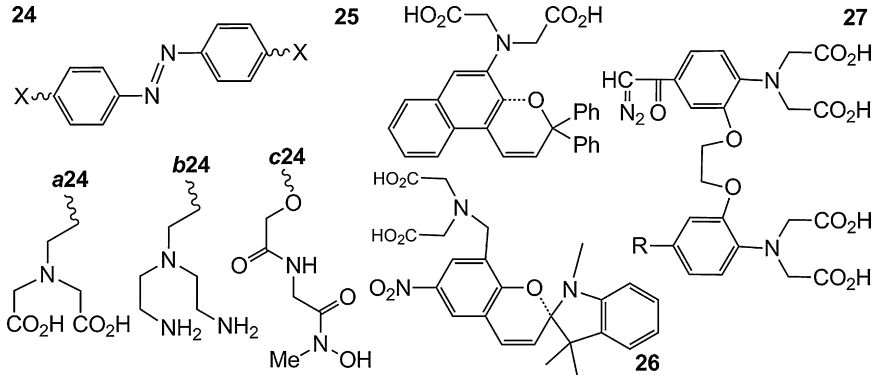


Plate 5. Photoactivatable caged ligands. (**24**) *trans*-4,4″-bis(R)azotoluene (*n* = 1, 2): (**a**) azo-IDA (R = α-iminodiacetic acid), (**b**) azo-ED (R = ethylenediamine), (c) Azo-Gly; (**25**) 2,2′-(3,3diphenyl-3H-benzo[f]chromen-5-ylazanediyl)diacetic acid; (**26**) NiroBIPS-8-DA; (**27**) (**a**) Diazo-2 (R = CH_3), (**b**) Diazo-4 (R = $COCHN_2$). Dashed lines depict photoclevable bonds.

trans-azo-IDA for Zn^{2+} contrasts with the previous observation that the *trans* isomer does not bind zinc possibly because the earlier study was performed in unbuffered aqueous solution. Shinkai et al. also found that photoisomerization of either caged chelator increases its affinity for Zn^{2+} or Cu^{2+} just 1.4–4.7-fold. Although the quantum yields for photoisomerization and the kinetics of metal ion binding were not reported, the relatively small difference in affinity exhibited by the *cis* and *trans* isomers of these ligands for either metal ion limits their usefulness in the study of metalloproteins.

Just as siderophores have provided a model for synthesis of caged iron complexes, they have also suggested an approach for design of a caged chelator. Nagasaki and colleagues developed a synthetic trihydroxamate "siderophore" with an azobenzene framework, azo-Gly (**24c**), that undergoes photoinitiated isomerization that increases affinity of the ligand for iron [136]. While the photochemical and thermodynamic properties of this reagent were not reported and it has limited aqueous solubility, the *cis* isomer promotes bacterial growth while the *trans* isomer does not [137]. The photochemical and thermodynamic properties of azo-Gly remain to be elucidated.

More recently, photochemical studies of naphthopyran derivatized with a single aminodiacetate substituent (**25**) have suggested that this ligand undergoes photoisomerization that increases the affinity for Ca^{2+} 77-fold at pH 7.6 [138]. This process is reversible such that metal ion binding occurs upon illumination with conversion of the ligand to the "open" form and metal ion dissociation occurs in the dark with re-formation of the "closed" form of the ligand. Reversible cycling of this interconversion of the ligand was demonstrated for at least five cycles. Although the quantum yield for the napthopyran derivative with a single aminodiacetate substituent was not reported, the derivative with two such substituents exhibited a quantum yield of 0.006 in HEPES buffer (10 mM, pH 9.8). A nitrobenzospiropyran derivatized with a single aminodiacetate substituent, nitroBIPS-8-DA (**26**), exhibits two states with differential affinity for Gd^{2+} that are inter-converted by irradiation at different wavelengths [139]. Irradiation at 365 nm or 720 nm (2-photon excitation) converts the spiro (SP) form of nitroBIPS-8-DA into the merocyanine (MC) form, which binds Gd^{2+} with a dissociation constant of 5.2 μM in water, and irradiation at 543 nm converts the MC form to the SP form. Although the stabilities of the complexes formed with other metal ions were not reported, the affinity of this reagent for Ca^{2+} is apparently poor [139].

Tsien and co-workers reported an approach to a caged chelating agent that does not rely on photoisomerization and that is based on derivatization of the BAPTA ligand developed previously by this group for binding of Ca^{2+}. By introducing a diazoketone substituent on one or both phenyl groups *para* to the aminodiacetate groups of BAPTA, the caged chelators diazo-2 and diazo-4 (**27a** and **b**), respectively, were synthesized [140]. In this way, the affinity of diazo-4 for Ca^{2+} could be modified by electronic effects such that upon photolysis (365 nm), the K_d of diazo-4 for Ca^{2+} changes from 8.9×10^{-5} to 5.5×10^{-8} M, an increase in affinity of ~1600-fold. The quantum yield for this process, however, was just 0.015 for photolysis of both diazoketone substituents. While the quantum yield for 2-diazo was double this value owing to the presence a single diazoketone substituent, the photo-induced increase in affinity for Ca^{2+} for this reagent was much smaller (~30-fold).

A related approach has been described by Pourzand and co-workers in an effort to design photo-activated iron chelators for therapeutic use and thus ameliorate the pathological consequences of inappropriate distribution of iron *in vivo* while avoiding the side effects that result from chronic exposure to chelators with high affinity for iron [141].

3.2. Caged di- and tri-atomic gases

As noted in the Introduction, flash photolysis has been used since the 1950s for photodissociation of diatomic gases (i.e., carbon monoxide, dioxygen, nitric oxide) that are coordinated to heme iron at the active site of heme proteins to permit analysis of the kinetics of re-association of these ligands with the heme iron in the dark. The great number of publications in which this approach has been used is evidence of the effectiveness of this method. Nevertheless, the application of this approach is limited to proteins that bind such ligands and that can release them upon photoexcitation. As a result, cage reagents that enable the photo-initiated delivery of these gases for use in the study of proteins under other circumstances afford a variety of useful and attractive experimental options.

3.2.1. O_2

The use of caged dioxygen for the study of metalloproteins was first proposed by MacArthur et al. in a report that provided proof of principle by demonstrating the photo-induced generation of oxyhemoglobin from an anaerobic solution of deoxyhemoglobin and the perchlorate salt of $[(\mu\text{-}O_2)(\mu\text{-}OH)(Co(bpy)_2)_2]^{3+}$ (HPBC, **28a**) (Plate 6) following irradiation at 355 nm [142]. The quantum yield for this process was 0.04, and the irreversible release of dioxygen from the cage is complete within no more than 40 ns as determined from the change in electronic spectrum of the complex. This group then applied this approach to the study of the reaction of fully reduced bovine heart cytochrome *c* oxidase with dioxygen [143] to eliminate any potential complications that might arise from the flow-flash method normally used for studying this reaction . In the course of this work, the authors determined that a dioxygen concentration of ~90 μM could be achieved with a single laser pulse by this method ([HPBC], 7.5 mM).

Subsequently, Lübben and co-workers prepared the nitrate salt of HPBC to improve the solubility and avoid potential toxicity of the perchlorate as part of a study concerning the reaction of cytochrome bo_3 oxidase with dioxygen. The revised procedure yielded a preparation of HPBC with a both improved solubility and greater purity that permitted crystallographic structure determination [144]. As well, the quantum yield of the improved product following irradiation at 308 nm was significantly greater than that observed previously presumably as the result of removal of impurities and the use of an excitation wavelength closer to that of the absorption maximum. In fact, the quantum yield varies with concentration of HPBC owing to the influence of a significant inner filter effect, but at concentrations of 0.5–1 mM, it was approximately an order of magnitude greater than reported for the less pure preparation. As with the previous studies of the mammalian cytochrome oxidase, HPBC provides a valid means of studying the reaction of dioxygen with the bacterial cytochrome bo_3 oxidase. The

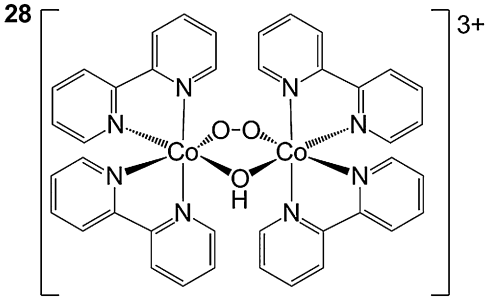


Plate 6. Photoinducible dioxygen cages. (**28**) μ-peroxo dinuclear cobalt cages. (**a**) HPBC (μ-peroxo)(μ-hydroxo) bis-[bis(bipyridyl)Co(III)] and (**b**) HPPC with phenanthroline instead of bipyridyl (not illustrated).

photolysis of this HPBC salt at cryogenic temperatures [145] and the thermodynamic parameters related to photolysis of the perchlorate salt of HPBC and the related $[(\mu\text{-}O_2)(\mu\text{-}OH)(Co(phen)_2)_2]^{3+}$ (**28b**) perchlorate salt [146] have been reported.

3.2.2. NO

A substantial number of caged-NO complexes suitable for flash photolysis experiments [147] has arisen as the result of efforts to develop photo-responsive agents for the site-specific, therapeutic delivery of this potent but unstable diatomic signaling agent. The originally intended uses of these reagents include, among other applications, the treatment of acute hypertension [148,149], the enhancement of chemotherapy [150,151] and the eradication of protozoan and metazoan infections [152–154]. For *in vitro* studies on the kinetics of NO interactions with metalloproteins following flash photolysis, issues such as reagent and photoproduct toxicity are less of a concern unless such toxicity translates into undesired or ill-defined side reactions with the analyte. These side reactions include, for example, the release of cyanide ligands from sodium nitroprusside ($Na_2[Fe(CN)_5(NO)]$) that then compete with NO for coordination of the metal center(s) of interest or electron transfer reactions between the spent cage and the metal center. Instead, high quantum yields (Φ_{NO}) and rates (k_{NO}) for NO release are required along with high solubility in aqueous, buffered solutions.

As part of a program to develop reagents for the elucidation of cellular metabolic processes, Makings and Tsien [155] combined the photo-responsiveness of *o*-NBA with the NO-releasing capabilities of the diazeniumdiolate, $Na_2[Et_2N(N_2O_2)]$, described by Keefer and coworkers [156–158]. The resulting family of reagents (CNO-1 through CNO-5, **29**) (Plate 7) incorporate methoxy, acetoxy and longer ester groups at positions 4 and 5 of the nitrophenyl ring to enhance the absorptivity of the reagent and to modulate its permeability across lipid bilayers. More importantly, these compounds are thermally stable in solution, and they release NO within 5 ms following exposure to 365 nm light (300 μs pulse) as monitored by absorbance changes of hemoglobin reacting with the NO released (ΔA_{430}). Based on this criterion, the quantum yield, Φ_{NO}, was 0.05 for CNO-1 while decomposition of this complex monitored at A_{365} yielded $\Phi_{diss} = 0.09$.

The rapidity of NO release observed relative to the reported half-life of the diazeniumdiolate at 25 °C ($t_{1/2}$ = 2.1 min) [157] suggests that the mechanism of NO release from this case is more complex than the simple decomposition of the diazeniumdiolate after it is photolyzed from the NBA cage. This situation prompted the authors to suggest that the proton released as a by-product of NBA photolysis catalyzes the decomposition of the diazeniumdiolate. Toscano and coworkers have since investigated this process more closely (reviewed in [159]) and found that photolysis of caged complexes containing a diazeniumdiolate moiety can proceed through a mechanism that produces multiple species. Using time-resolved FT-IR spectroscopy to follow the formation of photoproducts, for example, Srinivasan et al. found [160] that photolysis of O^2-benzyl 1-(*N*,*N*-diethylamino)diazen-1-ium-1,2-diolate (**30a**) in acetonitrile results predominantly in the cleavage of the N═N bond of the diazeniumdiolate to yield a nitrosamine and an oxygen-substituted oxynitrene; secondary photolysis of the nitrosamine results in NO release [158,159,161]. A less important photolytic pathway leads to the production of nitrous oxide along with aminyl and alkoxy radicals that react further. In a different study focusing on the products of sodium 1-(*N*,*N*-diethylamino)-diazen-1-ium-1,2-diolate photolysis in aqueous solution [157], the authors determined that releasing the NO-donor from its photo-active protecting group as the diazeniumdiolate anion leads predominantly to the same products observed upon solvation of the reagent and thermal decomposition, namely two molecules of NO and a diethylamine. Following photoexcitation of sodium diazeniumdiolate and intersystem crossing to the triplet state, the resulting intermediate dissociates into NO and nitrosamine anion radical followed promptly by a secondary photochemical event in which a second NO molecule and diethylamine are produced. This finding thus set the stage for investigations in which benzyl [162] and naphthol derivatives [163] bearing a diazeniumdiolate were subjected to similar analyses to identify modifications of the cage framework that can lead predominantly

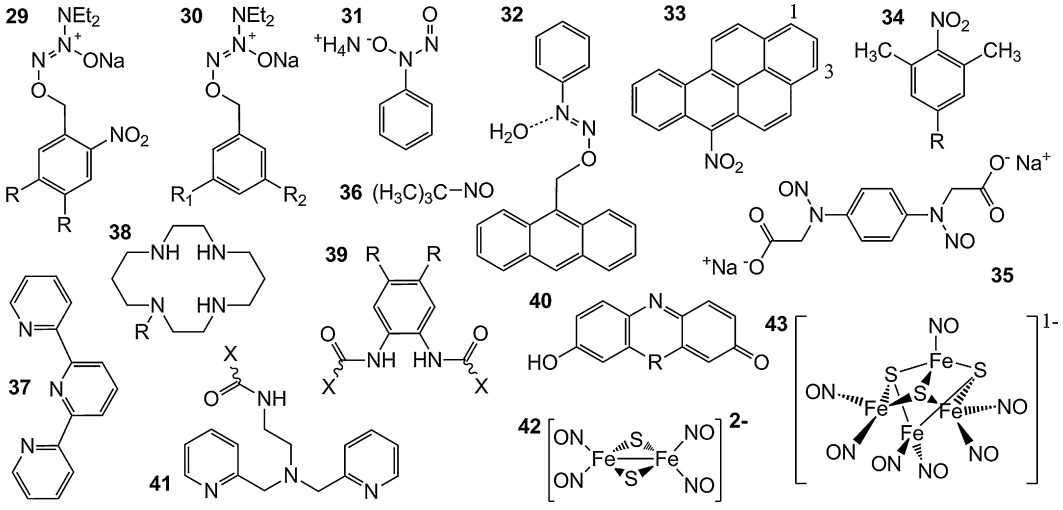


Plate 7. Photoinducible NO cages and ligands for the preparation of photoactive metal nitrosyl complexes. (**29**) 1-[(2′-nitrophenyl)methoxy]-2-oxo-3,3-diethyl-l-triazenes. (**a**) CNO-1 (R = H) and its derivatives, (**b**) CNO-2 (R = OCH_3), (**c**) CNO-3 (R = OCH_2CO_2Et), (**d**) CNO-4 (R = OCH_2CO_2K) and (**e**) CNO-5 (R = $OCH_2CO_2CH_2O_2CCH_3$); (**30a**) e^2-benzyl 1-(*N*,*N*-diethylamino)diazen-1-ium-1,2-diolate ($R_1 = R_2 = H$) and derivatives, (**b**) $R_1 = (CH_3)_2N$, $R_2 = H$ and (**c**) $R_1 = R_2 = (CH_3)_2N$; (**31**) Cupferron (*N*-nitroso-*N*-phenylhydroxylamine ammonium salt); (**32**) *O*-anthranyl-9-methyl-cupferron; (**33**) 6-nitrobenzo[a]pyrene (6-nitroBAP); (**34**) 1,3-dimethyl-2-nitrobenzene; (**35**) sodium *N*,*N*′-dinitroso-phenylenediamine-*N*,*N*′-diacetic acid (BNN5Na); (**36**) 2-methyl-2-nitroso-propane; (**37**) 2,2′:6′,2″-terpyridine (terpy); (**38**) (**a**) 1,4,8,11-tetraazacyclotetradecane (cyclam, R = H) and its derivative, (**b**) 1-(3-propyl-ammonium)-cyclam (1-pramcyH, R = $CH_2CH_2CH_2NH_3^+$); (**39**) 1,2-bis(pyridine-2-carboxamido)benzene (H_2Me_2bpb, R = H) and its analogues, (**b**) 1,2-bis(quinaldine-2-carboxamido)-4,5-dimethylbenzene (H_2Me_2bQb, R = CH_3) and (**c**) 1,2-bis(quinaldine-2-carboxamido)-4,5-dimethoxybenzene ($H_2(OMe)_2bQb$, ligand **39b** with R = OMe); (**40**) (**a**) 7-hydroxy-3H-phenoxazin-3-one (Resorufin, Resf, R = O) and its homologuese, (**b**) Thionol (Thnl, R = S) and (**c**) Selenophore (Seln, R = Se); (**41**) (**a**) *N*,*N*-bis(2-pyridylmethyl)amine-*N*-ethyl-2-pyridine-2-carboxamide ($PaPy_3H$) and its derivative, (**b**) *N*,*N*-bis(2-pyridylmethyl)amine-*N*-ehtyl-2-quinaldine-2-carboxamide (PaPyQH); (**42**) Roussin's Red salt; (**43**) Roussin's black salt.

to the formation of the diazeniumdiolate ion products followed by NO in the manner summarized above. Whereas attachment of a phenyl ring to the diazeniumdiolate leads to the undesirable nitrosamine and oxynitrene photoproducts, incorporation of dimethylamine groups at the *meta* positions lead to the higher Φ_{NO} in aqueous media (0.66 and 0.51 for the 3-dimethylamine- (**30b**) and 3,5-bis-dimethylamine (**30c**) derivatives, respectively) [162]. Similarly, incorporation of a methoxy group at position 5 of the O^2-1-naphthylallyl-substituted diazeniumdiolate led predominantly to the formation of the anionic diazeniumdiolate and a Φ_{NO} of 0.66 [163]. However, the solubility of this NO-cage is low. More importantly, acidic conditions that may be incompatible with many proteins are required to achieve rapid NO release from the nitrosamine anion radical produced with either cage framework (~20 s [162]).

More recently, Sortino and co-workers [164] combined the thermal stability of the diazeniumdiolate analogue cupferron (*N*-nitroso-*N*-phenylhydroxylamine ammonium salt, **31**) through *O*-alkylation [165] as above with the known antineoplastic activity of 9-anthrylmethyl carbocation to create a reagent (**32**) that fulfills two roles. The first is to enhance the absorptivity of the cupferron in the near-UV region of the spectrum, while the second is to provide a vehicle for both the carbocation and the cupferron that are released upon photoactivation. Within tens of seconds of continuous irradiation, NO concentration in the nanomolar range is produced in 50% methanolic phosphate buffer solution (pH 7.4), reflecting the relatively slow release of NO that limits the use of this and similar NO-donors in fast kinetics studies involving flash photolysis.

Fukuhara et al. investigated the potential of 6-nitrobenzo[*a*]pyrene (6-nitroBaP, **33**) as a photoinducible source of NO [166]. Unlike its analogues with the nitro group at positions 1 or 3, 6-nitroBaP exhibited evidence of NO release upon illumination with visible light, presumably at wavelengths longer than 350 nm. NO-detection was based on the reaction of NO with the spin-traps $(MGD)_2$-Fe^{2+} and carboxyPTIO, which were transformed, respectively, to the [$(MGD)_2$-Fe^{2+}(NO)] complex and carboxyPTI according to ESR spectroscopy analysis. Further confirmation of NO release was obtained from the observation of various BaP quinones that resulted from oxidation of the 6-oxyBaP radical. Based on semi-empirical MO calculations, the authors proposed that 90° torsion of the 6-nitro group with respect to the macrocycle plane increases the susceptibility of the reagent to intramolecular rearrangement of the nitro group to form nitrite, thereby making NO release possible from this compound only. Similarly, the structural conditions that make 6-nitroBaP susceptible to photolytic release of NO were introduced into nitrobenzene by flanking the nitro group with methyl groups at both *ortho*-positions (**34**) [167]. Nevertheless, release of NO was not detected even after prolonged exposure of the resulting 1,3-dimethyl-2-nitrobenze, its 4-hydroxy or its 4-dimethylamine derivatives to light. It was only for 1,3-dimethyl-2-nitrobenze rings bearing extended π-electron systems at this *para*-position that any NO release was detected by ESR spectroscopy following introduction of the spin traps above or from Griess assay results. Unfortunately, NO yields or NO release rates were not included, making it difficult to compare the performance of this reagent to other NO-cages.

One NO-cage that seems to be suitable for flash photolysis experiments is sodium *N*,*N*′-dinitroso-phenylenediamine-*N*,*N*′-diacetic acid (BNN5Na) [168,169]. BNN5Na is one of several related compounds bearing two photolyzable NO groups. Like its ester derivative, BNN5Na is soluble and stable in aqueous solution for prolonged periods and, more importantly, it releases NO in high yield (Φ_{NO} ~1.87) within ~100 μs following irradiation (20 ns laser pulse, 300 nm). The high efficiency of this reaction is attributed to the formation of a diimine in the spent cage to yield an extended aromatic photoproduct. Based on the influence of free NO on the process and based on the kinetics of NO trapping by TPPCo (mesotetraphenylporphinatocobalt(II)), the authors concluded that NO release occurs in two sequential steps with a $BNN5^{\bullet-}$ anion radical intermediate. The release of the second NO molecule ($k_d = 2.96 \times 10^4\ s^{-1}$) competes with the second order re-association of free NO with the $BNN5^{\bullet-}$ intermediate ($k_f = 1.38 \times 10^8\ M^{-1}\ s^{-1}$) [168]. In a re-examination of the kinetics of NO release from BBN5Na, Cabail et al. obtained different values for the rate constants of the dissociation and re-association steps ($500\ s^{-1}$ and $1.1 \times 10^9\ M^{-1}\ s^{-1}$, respectively) [170] and identified conditions that would generate up to 10 μM NO *in situ* (50 mM phosphate buffer, pH 7.4) with 308 nm excitation from a XeCl excimer laser. However, in subsequent flash photolysis experiments using this compound to study the interaction of NO with *Nitrosomonas europaea* hydroxylamine oxidoreductase (HAO), these authors determined that the greatest free NO concentration generated in this case was ≤2.4 μM [171]. This factor became an issue because their kinetic analysis revealed that NO affected the 1-electron reduction of one or two *c*-type heme group(s) in the protein based on the appearance of absorption maxima at 552, 523 and 420 nm and decreased absorbance at 404 nm. Given that only two of the seven heme groups in the protein have any exposure to solvent, a 6-coordinate *c*-type heme and a P_{460} heme, the authors reasoned that reduction was mediated by the 5-coordinate P_{460} heme group followed by intramolecular electron transfer to account for the rapid reduction observed. While this argument is consistent with results obtained by these authors for reduction of the protein in a solution equilibrated with a p_{NO} of ~0.68 atm, it is inconsistent with previous evidence that NO (at ~1 mM) failed to reduce the protein [172].

Pacheco and co-workers have since carried out additional studies on the chemical reactivity of HAO with NO released by flash photolysis of BNN5Na. In one of these studies, Cabail et al. reported that the $BNN5^{\bullet-}$ intermediate oxidized deoxy-Mb unless a sacrificial electron donor (e.g., $[M(NH_3)_6]^{2+}$ (where M = Fe or Ru)) was also present [173]. Thus, in the presence of $[Ru(NH_3)_6]^{2+}$, Cabail et al. quenched the radical intermediate within 20 μs and concluded that just two of the four electrons involved in the HAO-catalyzed conversion of hydroxylamine to nitrite are transferred initially to the *c*-type heme "pool" in the protein while the other two remain at the P_{460} $\{Fe(NO)\}^7$ center [174]. In the presence of methyl viologen, HAO can also mediate the 5-electron reduction of NO released from BNN5Na to ammonia in two steps [175]. Throughout these studies, the authors were able to quantify the NO released (by now up to 60 μM) based on colorimetric control experiments performed with deoxy-myoglobin as an NO indicator.

Many other types of organic NO-donors release NO as a result of thermal and/or photo-induced decomposition (e.g., the *C*-nitroso compound 2-methyl-2-nitrosopropane (**36**) [176] and others reviewed in [177]). Additional reagents have been designed specifically for use in photodynamic therapy [178–180], and some have been adapted for deployment on solid supports or nanoparticles (reviewed in [181]). The NO-releasing properties of many of these compounds may be appropriate for use in flash photolysis experiments, but photochemical characterization and optimization of these reagents is required before they can be used in this capacity.

Metal nitrosyl complexes comprise another group of NO-delivery agents that have attracted much attention. Following the original comprehensive review of the subject by Richter-Addo and Legzdins [182], updates [183,184] and reviews of their photochemistry [185–188] have been published. Cox and Wallace reported the photolytic release of NO from $[Ru(Cl)_n(NO)]^{3+}$ (n = 3 or 5) as early as 1971 [189]. With discovery of physiological roles for NO, the possible clinical potential of these reagents stimulated a substantial effort in characterization of this group of metallonitrosyls [190]. Rose and Mascharak [191] have recently provided a comprehensive review of the resulting literature concerning the photochemistry

Table 3
Selected ruthenium nitrosyl complexes that release NO in aqueous media near neutral pH upon exposure to UV light [191].

Complex	Φ (λ_{irr})	pH	λ_{max} (ε M^{-1} cm^{-1})	Reference	k_{NO}[a] (s^{-1})
trans-$[Ru(NH_3)_4(NO)(py)](BF_4)_3$	0.18(330)	6.4	324(160)	[194,205]	0.060
trans-$[Ru(NH_3)_4(NO)(4\text{-pic})](BF_4)_3$	0.40(330)	6.0	325(220)	[194,205]	0.070
trans-$[(cyclam)Ru(NO)(Cl)](PF_6)_2$	0.16(355)	7.4	263(3011)	[195]	–
$[(PaPy_2Q)Ru(NO)](BF_4)_2$	0.20(355)	7.4	420(1030)	[213]	–

[a] From Ref. [206].

of these compounds that is not repeated here. Instead, we focus on these reagents as photolytic sources of NO and provide a brief update.

Table 3 lists four ruthenium nitrosyl complexes from the compilation of Rose and Mascharak [191] that exhibit the best combination of aqueous solubility near neutral pH and relatively high quantum yield (ϕ) for release of NO along with the associated rate constants for NO release reported by Toledo et al. [190]. Other ruthenium complexes exhibit comparable or greater quantum yields (e.g., $[Ru(terpy)(NH_2\text{-}NH_2ph)(NO)](PF_6)_3$, $\phi_{355} = 0.47$ in CF_3COOH solution, pH 2.01 (terpy, **37**) [192]), but the NO photolysis of the reagents highlighted here was investigated at mild to strongly acidic pH. These conditions may appear to be counterproductive in light of the observation that ϕ_{NO} increases with pH ~6 (e.g., *trans*-[Ru(1-pramcyH)(NO)Cl]$^{3+}$ (pramcyH, **38b**) [193]; *trans*-$[Ru(NH_3)_4(NO)L]^{3+}$ where L = a series of *N*-linked heterocyclic molecules [194]; *trans*-[Ru(cyclam)(NO)(Cl)]$^{2+}$ (cyclam, **38a**) [195]) because deprotonation of the water ligand that aquates NO yields a hydroxyl ligand that renders the complex less susceptible to ligand exchange in a back-reaction [196]:

$$cis\text{-}[Ru^{II}(bpy)_2(NO^+)Cl]^{2+} + h\upsilon \rightarrow cis\text{-}[Ru^{III}(bpy)_2(NO^0)Cl]^{2+}$$

$$H_2O \downarrow\uparrow NO$$

$$cis\text{-}[Ru^{III}(bpy)_2(H_2O)Cl]^{2+} \leftrightarrow cis\text{-}[Ru^{III}(bpy)_2(OH^-)Cl]^{+} + H_2O$$

Nevertheless, ruthenium nitrosyl complexes are often characterized under acidic conditions or in solvents such as acetonitrile to overcome solubility issues [196]. Use of these compounds at low pH also circumvents complications with nucleophilic attack by hydroxide, which reacts reversibly with the nitrosyl to yield a nitro group [197,198]:

$$[Ru(bipy)_2(NO)L]^{2+} + 2OH^- \leftrightarrow [Ru(bipy)_2(NO_2)(L)]^{+} + H_2O$$

The nitrosyl and nitro forms are present in equal amounts (based on the absorption maximum at ~440 nm associated with the nitro complex) at pH 3.8 and 9.2 when L = pyridine and Cl^-, respectively [198]. For the *cis*-isomer with $L = NH_3$, this condition is observed at pH ~5.7 [199], while *trans*-$[Ru(NH_3)_4(NO)(L)]^{3+}$ complexes are stable in solution for >24 h at 25 °C up to pH 8 [200]. The equilibrium constant (K_{eq}) for the spectrochemical series comprised of various *N*-linked heterocyclic ligands varies between 2.2×10^5 and 4.6×10^{13} dm^6 mol^{-2} depending on the π-acidity of the coordination sphere [200,201] as discussed by Winter [202] and Bottomley [203,204] and more recently by others [190,205,206].

Selection of the complexes included in Table 3 from the results summarized by Rose and Mascharak [207] is based on the unambiguous solubility and stability of these reagents in aqueous solution. Insufficient information is available for all of the compounds compiled by these authors to determine whether some of the other reagents should also be included here. For example, *trans*-[(Me_2bpb)Ru(NO)(Resf)] (Me_2bpb, **39a**; Resorufin, Resf, **40a**) exhibits similar photolytic characteristics in water and DMF (quantum yield of ~0.052 when illuminated at 500 nm, $t_{1/2}$ ~5 min [208]). This complex illustrates two other features that, if implemented together with suitable modifications to enhance solubility in water, may enable use in fast kinetics experiments. First, the coordination of the ruthenium by one or more carboxamide nitrogen atoms from a multidentate ligand generally stabilizes higher oxidation states of metal ions [209]. As shown above in the reaction scheme for the photolysis of *cis*-$[Ru^{II}(bpy)_2(NO^+)Cl]^{2+}$, photolysis of NO is accompanied by the oxidation of the ruthenium center; consequently, carboxamido nitrogen coordination from a ligand such as $PaPy_3$ (**41a**) increases quantum yields [210–216]. To characterize the ability of these reagents to serve as photolytic NO donors, cytochrome *c* oxidase and myoglobin form NO complexes without interference from the spent cage [210,212]. The second noteworthy feature is that with judicious selection of the equatorial ligands and with direct coordination of a suitable dye to ruthenium at the position *trans* to NO, the absorption maximum for the $d_\pi(Ru) \rightarrow \pi^*(NO)$ transition and the absorption maximum of the complex can be made to converge. Improving this resonance condition in turn enhances the extent of energy transfer upon illumination to improve the photolytic efficiency of the complex. Mascharak and co-workers have demonstrated how modulation of the tetradentate, equatorial ligand shifts the absorption maximum (Me_2bpb *vs.* Me_2bQb (**39b**)) or changes the molar absorptivity (Me_2bQb *vs.* $(OMe)_2$bQb (**39c**)) [207,211]. Similarly, these authors showed how a series of Resorufin dye homologues with sulfur or selenium in place of the oxygen atom in the middle ring, systematically shifted the absorption maximum to longer wavelengths (λ_{max} = 500, 530 and 535 nm for Resf (O, **40a**), Thnl (S, **40b**) and Seln (Se, **40c**), respectively; [217]). Therefore, given the interest in developing effective photo-inducible NO-donors, it is very likely that a soluble complex or complexes combining all these desirable properties for flash photolysis experiments will be synthesized in the near future.

The photolytic potential of bi-nuclear [218,219] and tri-nuclear [220] ruthenium nitrosyl complexes has also been considered. The binuclear complexes exhibit relatively low Φ_{NO} (0.02–0.04 [219]), but they are soluble in aqueous solution (acetate buffer, pH 4.5). More recently, De Candia et al. characterized another soluble binuclear complex, *trans*-$[(NC)Ru(py)_4(\mu\text{-}CN)Ru(py)_4(NO)]^{3+}$, that exhibits an intense maximum at 518 nm that is attributed to charge transfer from the $[(NC)_2Ru(py)_4]$ nucleus to $[Ru(py)_4(NO)]^{3+}$ [221]. Despite the enhanced absorptivity of this complex, the Φ_{NO} is considerably lower (0.06×10^{-3}) owing to the low-spin d^6 electronic structure of the $\{Ru^{II}\text{-}NO^{\bullet}\}^7$ complex, which is regarded as inert.

Although not directly applicable in kinetics experiments, Doro et al. recently reported a method to immobilize $[(isn)Ru(NH_3)_4(NO)]^{3+}$ on a silica gel support [222]. More importantly, irradiation (334 nm) of this immobilized complexes releases NO [222]. NO released in this manner in a chromatography column during elution of an NO-binding protein may represent a simple

means of preparing protein nitrosyl complexes for downstream flash photolysis experiments or other spectroscopic analysis. An especially attractive feature of this approach is that the NO-donor trapped in the resin can be regenerated once the NO supply is depleted by reaction with a nitrite solution at pH 7.4 without significant loss in nitrosylation performance. The feasibility of this method could be increased by designing a donor that releases NO upon irradiation at longer wavelengths that are less susceptible to inner filter effects arising from interfering materials within the chromatography column.

The first $\{Mn\text{-}NO\}^6$ system known to release NO photolytically, $[Mn(PaPy_3)(NO)]ClO_4$, was reported by Mascharak and co-workers [223] shortly after they designed the pentadentate ligand $PaPy_3$ (**41a**). Spectroscopic and electrochemical analysis define this complex as a low-spin, diamagnetic $\{Mn^{II}\text{-}NO^{\bullet}\}$ species. The reactivity of this Mn center with NO is attributable to coordination of the Mn(II) center by the carboxamido nitrogen atom that also stabilizes the Mn(III) oxidation state. The Mn(III) that results following photolysis cannot react with NO, so NO release is effectively irreversible. In water, NO release occurs with a rate constant of $8700\,s^{-1}$ following irradiation with weak, visible light [223]. Szundi et al. reported that NO released from this reagent by flash photolysis (355 nm) reacts with cytochrome *c* oxidase with a lifetime of ~77 μs (k_{obs} ~13,000 s^{-1}), which was estimated to be 10 to 20-fold faster than could be detected by conventional stopped-flow spectroscopy [212]. Afshar exploited these properties of $[Mn(PaPy_3)(NO)]ClO_4$ to inactivate papain through *S*-nitrosylation of the cysteine residue (ostensibly) at position 25, [224]. The quantum yield Φ_{NO} for this release of NO from this complex has been determined to be 0.39 and 0.40 in water (λ_{irr} = 500 and 550 nm, respectively) [225]. By exchanging the pyridyl group in the carboxamido arm of $PaPy_3$ with a quinoline moiety, the expanded π-system of the resulting ligand ($PaPy_2Q$) almost doubled the Φ_{NO} values in water (0.74_{500} and 0.69_{550}). Both of these reagents have been incorporated into sol–gel matrices successfully to elicit NO release into the adjacent liquid media even when the sol–gel surface is protected with a coat of polyurethane [225–227]. The motivation for the production of this assembly was its potential use as a light-activated bactericidal agent in wound dressings [215].

Ford and co-workers have prepared and characterized a variety of organometallic nitrosyl complexes. Among these, Cr(III) complexes such as *trans*-Cr(III)(cyclam)$(ONO)_2^+$ (cyclam = 1, 4, 8, 11-tetraaza-cyclotetra-decane, **38a**) undergo one of two competing reactions upon irradiation (λ_{irr} = 436 nm) [228]. In one reaction, irreversible photoaquation is promoted under anaerobic conditions with the release of nitrite (Φ_{aq} 0.0092). In the other reaction, NO undergoes reversible, homolytic dissociation from a coordinated nitrite to yield a Cr(IV) intermediate. In the presence of dioxygen, this intermediate oxidizes further to *trans*-Cr(V)(cyclam)(O)$(ONO)^{2+}$ with the production of superoxide anion that reacts with NO at low levels to produce peroxynitrite and nitrite as secondary products [229]. NO release nevertheless occurs under anaerobic conditions in the presence of glutathione to mimic the reducing environment of a tissue or cell. The NO concentrations generated under these conditions are sufficient to activate soluble guanylyl cyclase [230]. The overall photolytic quantum efficiency (Φ_{NO}) for NO release as determined by electrochemical NO detection with an NO-sensitive electrode was ~0.27. However, the weak near-UV absorbance of these complexes (267 and 40 $M^{-1}\,cm^{-1}$ at 336 and 476 nm, respectively) was considered to be a serious limitation to the performance of this and other organometallic complexes. DeRosa et al. addressed this shortcoming by appending so-called light-gathering antennae or photosensitizing groups to the cyclam ligand to increase the absorption cross-section of the complex [231]. While attachment of a benzyl group did not influence NO release appreciably, anthracyl and pyrenyl groups increased NO release ~8- and 13-fold, respectively, albeit in a 50% aqueous solution of acetonitrile. Prolonged exposure of these complexes to light elicited the escape of a second NO molecule but with a reduced quantum efficiency (~10% of $\Phi_{NO\text{-}1}$). Consistent with this observation, analysis of the photoproducts by mass spectrometry revealed changes of −29 and −58 amu after brief and prolonged light exposure, respectively, which was attributed to the binding of a proton coupled to NO release. More recently, Neuman et al. reported the photosensitization of the same Cr(III) complex resulting from electrostatic interaction with ZnS-coated CdSe quantum dots (QD; ~6 ZnS monolayers over a ~3.8 nm diameter CdSe core) [232]. As in the case of the cyclam derivatives, association of the Cr(III) complex with the QD quenched the fluorescence of this photosensitizer (i.e., the QD) without reduction of the metal center, and NO release increased ~6-fold upon excitation leading to the conclusion that photolysis in these cases is enhanced by excitation energy transfer [232] from the photosensitizer to the Cr(III) center. QDs have the advantage over the aromatic modifications to coordinating ligands in that the QD can be custom-tailored to target different tissues and for excitation with light from the visible to the near-IR without decreasing solubility.

The iron nitrosyl reagent sodium nitroprusside ($[Fe(CN)_6(NO)]Na$) has been known to release NO upon exposure to near-UV and visible light [233] since the early investigations of Mitra et al. on the ligand exchange reactions of the parent $[Fe^{III}(CN)_6]$ complex [234]. Despite the good yields of NO observed (0.18–0.35 [235]), the release of CN^- anion that accompanies this photolysis and the slow thermal equation reaction of NO have motivated efforts to formulate suitable replacements for flash photolysis experiments and for use in all but the most extreme of medical emergencies (e.g., acute cardiovascular challenges). Roussin's red salt ($[Fe_2S_2(NO)_4]^{2-}$, **42**) is also susceptible to photolysis, releasing ~0.5 mol NO per mol complex [185,236]), which can re-associate with the co-product $[Fe_2S_2(NO)_3]^{2-}$ ($k_{NO} = 9.1 \times 10^8\,M^{-1}\,s^{-1}$) [237]. Alternatively, and depending on dioxygen and proton availability, the dianionic species can also react with dioxygen ($k_{Ox} = 5.6 \times 10^7\,M^{-1}\,s^{-1}$) to yield a secondary intermediate that Bourassa and Ford believe may be a precursor to the formation of the black salt ($[Fe_4S_3(NO)_7]^{2-}$, **43**). This 4-iron, 3-sulfur nitrosyl form of the complex decomposes photolytically with the release of 5.9 NO equivalents per mol of complex [236]. Ford and co-workers recently presented interesting advances in the enhancement of the photolytic performance of Roussin's red salt [238–240]. In these reports, the authors described the synthesis and photophysical properties of Roussin's red salt derivatives with two pendant fluorescein groups [239,241] or a protoporphyrin IX group [238], both of which served to increase the absorption cross-section of the complex considerably for two-photon excitation. Photolysis of the former reagent at 436 nm results in the release of 3.2 NO equivalents, each with Φ_{NO} = 0.0036. Based on their experimentally determined two-photon cross-section (δ = 63 GM), the authors estimated an accumulation of 0.21 nM NO s^{-1} in solution. A similar analysis of the porphyrin-based compound was not possible owing to analytical difficulties, but based on empirical observations it was estimated that as much as 2.5% of the complex undergoes photolysis within 1 min of irradiation at 810 nm. While this level of performance may not compete with those of the Mn nitrosyl complexes described above, further modification of the pendant groups to improve the solubility of these reagents in aqueous media and the NO quantum yield should be possible.

3.2.3. *CO and CO_2*

Despite the similarity of CO and NO and their proposed roles as biological signaling agents, the effort invested in development of caged CO reagents for biological applications has been negligible

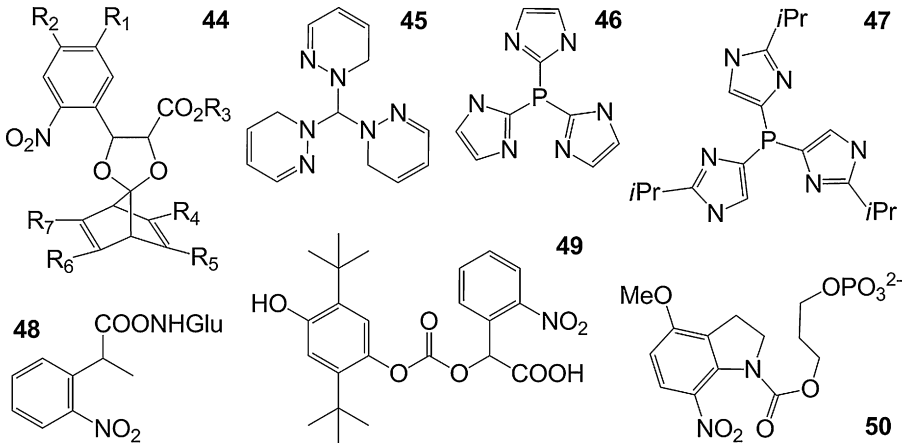


Plate 8. Photoinducible CO-cages. (**44**) Nitrophenyl acetal derivative of bicyclo[2,2,1]hepta-2,5-diene-7-one (norbornadienone); (**45**) tris(pyrazolyl)methane (tmp); (**46**) tris(imidazol-2-yl)phosphane (2-TIPH); (**47**) tris[2-isopropylimidazol-4(5)-yl)]phosphane (4-TIPiPr); (**48**) caged-glutamate; (**49**) Nmoc-protected caged di(tert-butyl)hydroquinone (Nmoc-DBHQ); (**50**) 2-((4-methoxy-7-nitroindoline-1-carbonyl)oxy)ethyl phosphate.

compared to the intense effort spent in development of caged NO. In view of the considerable number of inorganic and organometallic carbonyl complexes that have been reported in the literature, this situation may seem surprising, but as most if not all of this work has been undertaken in non-aqueous solvents, the solubility, stability and behavior of these complexes in aqueous solution are presumably problematic with respect to their use for the study of metalloproteins. Nevertheless, Motterlini and colleagues found that iron pentacarbonyl ($Fe[(CO)_5]$) and dimanganese decarbonyl ($[Mn_2(CO)_{10}]$) would release CO to deoxymyoglobin (phosphate buffer, pH 6.8) only after exposure to light [242]. While iron pentacarbonyl formed a precipitate upon continued illumination and was not used in subsequent experiments, dimanganese decarbonyl (0–100 μM) was found not to be toxic to vascular smooth muscle cells following exposure for 24 h under the conditions studied. Nevertheless, the stoichiometry, kinetics, and quantum yield for of CO release was not reported for either compound.

The first example of caged CO reagents developed for biological applications were those of Kao and Keitz [243]. The approach taken by these investigators was to build on the known photo-decomposition of norbornadienone to benzene and CO through synthesis of nitrophenyl-bearing acetal derivatives of this compound (**44**) (Plate 8) with increased photosensitivity and a red-shifted absorption maximum so that CO can be released by photolysis at wavelengths between 300 and 400 nm. The authors' goals in the preparation of these compounds was their use in electrophysiological or microscopy studies rather than the study of metalloproteins, so the kinetics of CO release and the quantum yields of these compounds have not been characterized.

More recently Schatzschneider and colleagues have developed a number of manganese and, in some cases, rhenium carbonyl complexes for possible use in photodynamic therapy of cancer. The first reagent from this group to afford relatively prompt, albeit kinetically undefined, photo-release of CO was the tris-carbonyl complex $[Mn(CO)_3$(tris(pyrazolyl)methane)$]PF_6$ (tmp, **45**) [244]. While solutions of this compound in phosphate buffer (pH undefined) released no CO in the dark, irradiation at 365 nm resulted in the release of two equivalents of CO as monitored by the formation of carbonyl-myoglobin [244]. This group then designed and synthesized a pentapeptide framework (Thr-Phe-Ser-Asp-Leu) onto which a reactive derivative of the tris-carbonyl Manganese complex was conjugated either at the *N*-terminus or the Phe side chain [245]. The resulting peptide conjugates of this manganese triscarbonyl complex retained the same ability to release CO photolytically as the parent compound to the extent that this reaction was characterized. Subsequently, this same group prepared the triscarbonyl adducts of the manganese and rhenium tris(imidazol-2-yl)phosphane (**46**) and tris[2-isopropylimidazol-4(5)-yl)]phosphane (**47**) complexes, both of which are soluble and stable in buffer [246]. While the rhenium compounds exhibited no absorption maximum in the near UV that made them of potential interest, the manganese complexes were, again, stable to thermal release of CO and released ~2 equiv. of CO following irradiation at 365 nm. Currently, the photophysics of CO release by all of these reagents remain to be determined.

Carbon dioxide is liberated by a variety of photochemical reactions but is generally a by-product and not of primary interest. In principle, therefore, any cage reagent that liberates CO_2 in the process of releasing the molecule of immediate concern could be considered also as a CO_2 cage as long as the protein under study does not react with the molecule that is released to solution concomitant with the release with CO_2. In aqueous solution, of course, the speciation of CO_2 is relatively complex and dependent on pH, among other variables [247], so it is important to consider experimental conditions carefully when designing experiments in which photo-initiated release of CO_2 is a critical concern. While the applications of photo-generated CO_2 are more limited than those for the other gases discussed here, metalloproteins such as transferrin and carbonic anhydrase exemplify systems for which photolytic CO_2 release might be useful.

Early examples of organic reagents that undergo photodecomposition in aqueous solution to products that include CO_2 are provided by 3- and 4-nitrophenylacetate and 4-nitrophthalate [248]. The quantum yields for photolysis of these reagents following irradiation at 367 nm are ~0.6 although 2-nitrophenylacetate and 2,4-dinitrophenylacetate exhibit much lower values (~0.04). 4-nitrophenylacetate and 4-nitrophthalate both release CO_2 after formation of *aci*-nitro intermediates, the decomposition of which is not readily detected by electronic spectroscopy. Although generation of CO_2 during photolysis of various cage reagents is not uncommon (e.g., caged glutamate (**48**) [249], caged di(*tert*-butyl)hydroquinone (DBHQ, **49**) [250]), this release of CO_2 is rarely studied in its own right. As reported for 4-nitrophenylacetate, however, the release of CO_2 typically results from the decomposition of a carboxylated intermediate at a rate that may be limiting. The kinetics of this decomposition to form CO_2 can be difficult to monitor spectroscopically, and so are generally not characterized. One exception to this generalization is the work by Papageorgiou et al. in which time-resolved IR spectroscopy was used to monitor the rate of CO_2 release following flash photolysis of a nitroindoline (**50**)

[251]. Although the quantum yield for photolysis of this compound was quite low (~0.04), the alkoxycarbonate intermediate produced by photolysis was sufficiently stable that its decomposition could be monitored by this method.

4. Concluding remarks

While most of the reagents discussed in this review were not developed specifically for kinetic studies of metalloproteins, the reactions that they undergo upon photolysis should afford valuable means of characterizing various functions of a wide range of these proteins. Nevertheless, it can be difficult if not impossible to assess the suitability of a specific reagent in the design of an experiment because the manner in which these reagents have been characterized has been highly variable. Although we have attempted to provide fundamental parameters such as excitation wavelengths, quantum yields, and kinetic parameters wherever possible, these parameters were not always of relevance or interest to the authors who reported many of these reagents. As well, experimental conditions used in evaluating the photochemistry of many of these reagents often involve broad band excitation (e.g., sunlight, irradiation from arc lamps) with few if any details concerning intensity or duration of irradiation. Despite these limitations and our resulting inability to provide a truly comprehensive and systematic review that allows unambiguous selection of the "ideal" photo-reagent for any specific experimental occasion, we believe it is useful to highlight the potential value of these reagents in the investigation of metalloproteins. As this potential is increasingly recognized and implemented, these limitations in our understanding will diminish.

References

[1] R.G.W. Norrish, G. Porter, Nature 164 (1949) 658.
[2] G. Porter, Proc. R. Soc. (London) Ser. A 200 (1950) 284.
[3] G. Porter, Disc. Faraday Soc. 9 (1950) 60.
[4] W.A. Hagins, Nature 177 (1956) 989.
[5] R. Livingston, G. Porter, M. Windsor, Nature 173 (1954) 485.
[6] E. Fujimori, R. Livingston, Nature 180 (1957) 1036.
[7] Q.H. Gibson, J. Physiol. 134 (1956) 112.
[8] Q.H. Gibson, J. Physiol. 134 (1956) 123.
[9] D. DeVault, B. Chance, Biophys. J. 6 (1966) 825.
[10] D. DeVault, J.H. Parkes, B. Chance, Nature 215 (1967) 642.
[11] S.P. Vaish, G. Tollin, J. Bioenerg. 2 (1971) 61.
[12] M.A. Cusanovich, G. Tollin, Biochemistry 19 (1980) 3343.
[13] K.M. Yocom, J.B. Shelton, J.R. Shelton, W.A. Schroeder, G. Worosila, S.S. Isied, E. Bordignon, H.B. Gray, Proc. Natl. Acad. Sci. U. S.A. 79 (1982) 7052.
[14] Y. Orii, Biochemistry 32 (1993) 11910.
[15] J.J. Wilker, I.J. Dmochowski, J.H. Dawson, J.R. Winkler, H.B. Gray, Angew. Chem. Int. Ed. 38 (1999) 90.
[16] I.J. Dmochowski, B.R. Crane, J.J. Wilker, J.R. Winkler, H.B. Gray, Proc. Natl. Acad. Sci. U.S.A. 96 (1999) 12987.
[17] S.M. Contakes, Y.H.L. Nguyen, H.B. Gray, E.C. Glazer, A.-M. Hays, D.B. Goodin, Struct. Bond. 123 (2007) 177.
[18] J.H. Kaplan, B. Forbush III, J.F. Hoffman, Biochemistry 17 (1978) 1929.
[19] J. Engels, E.J. Schlaeger, J. Med. Chem. 20 (1977) 907.
[20] G. Marriott, Biochemistry 33 (1994) 9092.
[21] G. Marriott, P. Roy, K. Jacobson, Methods Enzymol. 360 (2003) 274.
[22] G. Mayer, A. Heckel, Angew. Chem. Int. Ed. Engl. 45 (2006) 4900.
[23] H.M. Lee, D.R. Larson, D.S. Lawrence, Chem. Biol. 4 (2009) 409.
[24] K.L. Haas, K.J. Franz, Chem. Rev. 109 (2009) 4921.
[25] K. Weber, Z. Phys. Chem. B 15 (1931) 18.
[26] T. Förster, Naturwissenschaften 36 (1949) 186.
[27] A. Weller, in: G. Porter (Ed.), Progress in Raction Kinetics, Pergamon Press, New York, 1961, p. 189.
[28] J.H. Clark, S.L. Shapiro, A.J. Campillo, K.R. Winn, J. Am. Chem. Soc. 101 (1979) 746.
[29] M. Gutman, D. Huppert, J. Biochem. Biophys. Methods 1 (1979) 9.
[30] M. Irie, J. Am. Chem. Soc. 105 (1983) 2078.
[31] M. Gutman, D. Huppert, E. Pines, J. Am. Chem. Soc. 103 (1981) 3709.
[32] R.M.D. Nunes, M. Pineiro, L.G. Arnaut, J. Am. Chem. Soc. 131 (2009) 9456.
[33] M. Gutman, D. Huppert, E. Nachliel, Eur. J. Biochem. 121 (1982) 637.
[34] M. Gutman, E. Nachliel, D. Huppert, Eur. J. Biochem. 125 (1982) 175.
[35] M. Gutman, E. Nachliel, S. Moshiach, Biochemistry 28 (1989) 2936.
[36] M. Gutman, Y. Tsfadia, A. Masad, E. Nachliel, Biochim. Biophys. Acta 1109 (1992) 141.
[37] J.F. Ireland, P.A.H. Wyatt, Acid–base properties of electronically excited states of organic molecules, in: V. Gold, D. Bethell (Eds.), Advances in Physical Organic Chemistry, Academic Press, New York, 1976, p. 131.
[38] L.G. Arnaut, S.J. Formosinho, J. Photochem. Photobiol. A 75 (1993) 1.
[39] L.M. Tolbert, K.M. Solntsev, Acc. Chem. Res. 35 (2002) 19.
[40] P. Wan, D. Shukla, Chem. Rev. 93 (1993) 571.
[41] J.G. Vos, Polyhedron 11 (1992) 2285.
[42] T. Förster, Elektrochem. Angew. Phys. Chem. 54 (1950) 42.
[43] M.R. Loken, J.W. Hayes, J.R. Gohlke, L. Brand, Biochemistry 11 (1972) 4779.
[44] S.G. Schulman, Fluorescence and Phosphorescence Spectroscopy: Physicochemical Principles and Practice, Pergamon Press, New York, 1977.
[45] S. Abbruzzetti, M. Carcelli, P. Pelagatti, D. Rogolino, C. Viappiani, Chem. Phys. Lett. 344 (2001) 387.
[46] M. Gutman, E. Nachliel, FEBS Lett. 190 (1985) 29.
[47] E. Nachliel, Z. Ophir, M. Gutman, J. Am. Chem. Soc. 109 (1987) 1342.
[48] M. Gutman, E. Nachliel, Biochim. Biophys. Acta-Bioenergetics 1015 (1990) 391.
[49] E. Nachliel, M. Gutman, S. Kiryati, N.A. Dencher, Proc. Natl. Acad. Sci. U.S.A. 93 (1996) 10747.
[50] E. Nachliel, N. Pollak, D. Huppert, M. Gutman, Biophys. J. 80 (2001) 1498.
[51] E. Shimoni, Y. Tsfadia, E. Nachliel, M. Gutman, Biophys. J. 64 (1993) 472.
[52] R. Yam, E. Nachliel, M. Gutman, J. Am. Chem. Soc. 110 (1988) 2636.
[53] L.M. Tolbert, J.E. Haubrich, J. Am. Chem. Soc. 116 (1994) 1593.
[54] L.G. Arnaut, A.A.C.C. Pais, S.J. Formosinho, M. Barroso, J. Am. Chem. Soc. 125 (2003) 5236.
[55] M. Barroso, L.G. Arnaut, S.J. Formosinho, J. Phys. Chem. A 111 (2007) 591.
[56] M. Barroso, L.G. Arnaut, S.J. Formosinho, ChemPhysChem 6 (2005) 363.
[57] S. Abbruzzetti, M. Carcelli, D. Rogolino, C. Viappiani, Photochem. Photobiol. Sci. 2 (2003) 796.
[58] S. Abbruzzetti, E. Crema, L. Masino, A. Vecli, C. Viappiani, J.R. Small, L.J. Libertini, E.W. Small, Biophys. J. 78 (2000) 405.
[59] S. Abbruzzetti, E. Grandi, C. Viappiani, S. Bologna, B. Campanini, S. Raboni, S. Bettati, A. Mozzarelli, J. Am. Chem. Soc. 127 (2005) 626.
[60] S. Abbruzzetti, S. Sottini, C. Viappiani, J.E.T. Corrie, J. Am. Chem. Soc. 127 (2005) 9865.
[61] S. Abbruzzetti, S. Sottini, C. Viappiani, J.E.T. Corrie, Photochem. Photobiol. Sci. 5 (2006) 621.
[62] S. Abbruzzetti, C. Viappiani, F. Sinibaldi, R. Santucci, Protein J. 23 (2004) 519.
[63] S. Abbruzzetti, C. Viappiani, J.R. Small, L.J. Libertini, E.W. Small, J. Am. Chem. Soc. 123 (2001) 6649.
[64] R. Bizzarri, R. Nifosi, S. Abbruzzetti, W. Rocchia, S. Guidi, D. Arosio, G. Garau, B. Campanini, E. Grandi, F. Ricci, C. Viappiani, F. Beltram, Biochemistry 46 (2007) 5494.
[65] G. Bonetti, A. Vecli, C. Viappiani, Chem. Phys. Lett. 269 (1997) 268.
[66] E. Polverini, G. Cugini, F. Annoni, S. Abbruzzetti, C. Viappiani, T. Gensch, Biochemistry 45 (2006) 5111.
[67] C. Viappiani, S. Abbruzzetti, J.R. Small, L.J. Libertini, E.W. Small, Biophys. Chem. 73 (1998) 13.
[68] C. Viappiani, G. Bonetti, M. Carcelli, F. Ferrari, A. Sternieri, Rev. Sci. Instrum. 69 (1998) 270.
[69] A. Barth, J.E.T. Corrie, Biophys. J. 83 (2002) 2864.
[70] J. Choi, N. Hirota, M. Terazima, J. Phys. Chem. A 105 (2001) 12.
[71] J. Choi, M. Terazima, J. Phys. Chem. B 107 (2003) 9552.
[72] J. Choi, M. Terazima, Rev. Sci. Instrum. 74 (2003) 319.
[73] R. Mallik, J.B. Udgaonkar, G. Krishnamoorthy, Proc.-Indian Acad. Sci. Chem. Sci. 115 (2003) 307.
[74] A.M. Saxena, J.B. Udgaonkar, G. Krishnamoorthy, Prot. Sci. 14 (2005) 1787.
[75] M.V. George, J.C. Scaiano, J. Phys. Chem. 84 (1980) 492.
[76] K. Janko, J. Reichert, Biochim. Biophys. Acta 905 (1987) 409.
[77] S. Khan, F. Castellano, J.L. Spudich, J.A. McCray, R.S. Goody, G.P. Reid, D.R. Trentham, Biophys. J. 65 (1993) 2368.
[78] J. Mikšovská, R.W. Larsen, J. Protein Chem. 22 (2003) 387.
[79] H. Schupp, W.K. Wong, W. Schnabel, J. Photochem. 36 (1987) 85.
[80] P. Wan, K. Yates, J. Org. Chem. 48 (1983) 136.
[81] R.W. Yip, Y.X. Wen, D. Gravel, R. Giasson, D.K. Sharma, J. Phys. Chem. 95 (1991) 6078.
[82] S. Nag, A. Bandyopadhyay, S. Maiti, J. Phys. Chem. A 113 (2009) 5269.
[83] J.H. Kaplan, G.C.R. Ellis-Davies, Proc. Natl. Acad. Sci. U.S.A. 85 (1988) 6571.
[84] G. Ciamician, P. Silber, Berichte 34 (1901) 2040.
[85] M. Schworer, J. Wirz, Helv. Chim. Acta 84 (2001) 1441.
[86] E.B. Brown, J.B. Shear, S.R. Adams, R.Y. Tsien, W.W. Webb, Biophys. J. 76 (1999) 489.
[87] D. Barrick, F.M. Hughson, R.L. Baldwin, J. Mol. Biol. 237 (1994) 588.
[88] C. Ma, M.G. Steinmetz, E.J. Kopatz, R. Rathore, Tetrahedron Lett. 46 (2005) 1045.
[89] D. Geissler, Y.N. Antonenko, R. Schmidt, S. Keller, O.O. Krylova, B. Wiesner, J. Bendig, P. Pohl, V. Hagen, Angew. Chem. Int. Ed. 44 (2005) 1195.
[90] R. Schmidt, D. Geissler, V. Hagen, J. Bendig, J. Phys. Chem. A 109 (2005) 5000.
[91] R. Schmidt, D. Geissler, V. Hagen, J. Bendig, J. Phys. Chem. A 111 (2007) 5768.
[92] D. Gilbert, K. Funk, B. Dekowski, R. Lechler, S. Keller, F. Moehrlen, S. Frings, V. Hagen, ChemBioChem 8 (2007) 89.
[93] B. Meyer, T. Peters, Angew. Chem. Int. Ed. Engl. 42 (2003) 864.
[94] M. Pellecchia, I. Bertini, D. Cowburn, C. Dalvit, E. Giralt, W. Jahnke, T.L. James, S.W. Homans, H. Kessler, C. Luchinat, B. Meyer, H. Oschkinat, J. Peng, H. Schwalbe, G. Siegal, Nat. Rev. Drug Discov. 7 (2008) 738.
[95] G.C.R. Ellis-Davies, Chem. Rev. 108 (2008) 1603.

[96] R.Y. Tsien, R.S. Zucker, Biophys. J. 50 (1986) 843.
[97] R.Y. Tsien, Biochemistry 19 (1980) 2396.
[98] S.R. Adams, J.P.Y. Kao, G. Grynkiewicz, A. Minta, R.Y. Tsien, J. Am. Chem. Soc. 110 (1988) 3212.
[99] A.M. Gurney, R.Y. Tsien, H.A. Lester, Proc. Natl. Acad. Sci. U.S.A. 84 (1987) 3496.
[100] G.C.R. Ellis-Davies, J.H. Kaplan, J. Org. Chem. 53 (1988) 1966.
[101] G.C.R. Ellis-Davies, J.H. Kaplan, Proc. Natl. Acad. Sci. U.S.A. 91 (1994) 187.
[102] J.A. McCray, N. Fidler-Lim, G.C.R. Ellis-Davies, J.H. Kaplan, Biochemistry 31 (1992) 8856.
[103] G.C.R. Ellis-Davies, J.H. Kaplan, R.J. Barsotti, Biophys. J. 70 (1996) 1006.
[104] G.C. Faas, K. Karacs, J.L. Vergara, I. Mody, Biophys. J. 88 (2005) 4421.
[105] G.C.R. Ellis-Davies, Methods Enzymol. 360 (2003) 226.
[106] F. DelPrincipe, M. Egger, G.C.R. Ellis-Davies, E. Niggli, Cell Calcium 25 (1999) 85.
[107] G.C.R. Ellis-Davies, R.J. Barsotti, Cell Calcium 39 (2006) 75.
[108] G. Grynkiewicz, M. Poenie, R.Y. Tsien, J. Biol. Chem. 260 (1985) 3440.
[109] S.R. Adams, V. Lev-Ram, R.Y. Tsien, Chem. Biol. 4 (1997) 867.
[110] A. Momotake, N. Lindegger, E. Niggli, R.J. Barsotti, G.C.R. Ellis-Davies, Nat. Methods 3 (2006) 35.
[111] V. Balzani, V. Carassiti, Photochemistry of Coordination Compounds, Academic Press, New York, 1970.
[112] L.H. Hall, J.L. Lambert, J. Am. Chem. Soc. 90 (1968) 2036.
[113] J.H. Carey, C.H. Langford, Can. J. Chem. 51 (1973) 3665.
[114] H.B. Lockhart Jr., R.V. Blakeley, Environ. Sci. Technol. 9 (1975) 1035.
[115] K. Barbeau, E.L. Rue, C.G. Trick, K.W. Bruland, A. Butler, Limnol. Oceanogr. 48 (2003) 1069.
[116] K. Barbeau, E.L. Rue, K.W. Bruland, A. Butler, Nature 413 (2001) 409.
[117] K. Barbeau, G. Zhang, D.H. Live, A. Butler, J. Am. Chem. Soc. 124 (2002) 378.
[118] F.C. Küpper, C.J. Carrano, J.-U. Kuhn, A. Butler, Inorg. Chem. 45 (2006) 6028.
[119] J.D. Martin, Y. Ito, V.V. Homann, M.G. Haygood, A. Butler, J. Biol. Inorg. Chem. 11 (2006) 633.
[120] S.A. Amin, D.H. Green, F.C. Kupper, C.J. Carrano, Inorg. Chem. 48 (2009) 11451.
[121] Y. Ito, A. Butler, Limnol. Oceanogr. 50 (2005) 1918.
[122] R.J. Abergel, A.M. Zawadzka, K.N. Raymond, J. Am. Chem. Soc. 130 (2008) 2124.
[123] H. Sayre, K. Milos, M.J. Goldcamp, C.A. Schroll, J.A. Krause, M.J. Baldwin, Inorg. Chem. 49 (2010) 4433.
[124] D.P. Kennedy, C.D. Incarvito, S.C. Burdette, Inorg. Chem. 49 (2010) 916.
[125] J.L. Bricks, A. Kovalchuk, C. Trieflinger, M. Nofz, M. Buschel, A.I. Tolmachev, J. Daub, K. Rurack, J. Am. Chem. Soc. 127 (2005) 13522.
[126] C. Gwizdala, D.P. Kennedy, S.C. Burdette, Chem. Commun. (2009) 6967.
[127] H.M.D. Bandara, D.P. Kennedy, E. Akin, C.D. Incarvito, S.C. Burdette, Inorg. Chem. 48 (2009) 8445.
[128] X. Zhang, Y. Chen, ChemPhysChem 10 (2009) 1993.
[129] X. Zhang, Y. Chen, Phys. Chem. Chem. Phys. 12 (2010) 1177.
[130] E. Grell, E. Lewitzki, H. Ruf, E. Bamberg, G.C.R. Ellis-Davies, J.H. Kaplan, P de Weer, Cell. Mol. Biol. 35 (1989) 515.
[131] T. Kishimoto, T.-T. Liu, Y. Ninomiya, H. Takagi, T. Yoshioka, G.C.R. Ellis-Davies, Y. Miyashita, H. Kasai, J. Physiol. 533 (2001) 627.
[132] S. Kumar, C. Chau, G. Chau, A. McCurdy, Tetrahedron 64 (2008) 7097.
[133] K.L. Ciesienski, K.L. Haas, M.G. Dickens, Y.T. Tesema, K.J. Franz, J. Am. Chem. Soc. 130 (2008) 12246.
[134] M. Blank, L.M. Soo, H.N. Wassermann, B.F. Erlanger, Science 214 (1981) 70.
[135] S. Shinkai, S. Naramura, M. Nakashima, O. Manabe, M. Iwamoto, Bull. Chem. Soc. Jpn. 58 (1985) 2340.
[136] T. Masuda, S. Asari, S. Tamagaki, T. Nagasaki, Terahedron Lett. 41 (2000) 2411.
[137] T. Masuda, M. Azuma, H. Ooshima, S. Tamagaki, T. Nagasaki, Chem. Lett. (2000) 872.
[138] S. Kumar, D. Hernandez, B. Hoa, Y. Lee, J.S. Yang, A. McCurdy, Org. Lett. 10 (2008) 3761.
[139] T. Sakata, D.K. Jackson, S. Mao, G. Marriott, J. Org. Chem. 73 (2008) 227.
[140] S.R. Adams, J.P.Y. Kao, R.Y. Tsien, J. Am. Chem. Soc. 111 (1989) 7957.
[141] A. Yiakouvaki, J. Savovic, A. Al-Qenaei, J. Dowden, C. Pourzand, J. Invest. Dermatol. 126 (2006) 2287.
[142] R. MacArthur, A. Sucheta, F.F. Chong, O. Einarsdottir, Proc. Natl. Acad. Sci. U.S.A. 92 (1995) 8105.
[143] N. Van Eps, I. Szundi, O. Einarsdottir, Biochemistry 39 (2000) 14576.
[144] C. Ludovici, R. Frohlich, K. Vogtt, B. Mamat, M. Lübben, Eur. J. Biochem. 269 (2002) 2630.
[145] A.R. Howard-Jones, V. Adam, A. Cowley, J.E. Baldwin, D. Bourgeois, Photochem. Photobiol. Sci. 8 (2009) 1150.
[146] J. Miksovska, R.W. Larsen, Inorg. Chim. Acta 355 (2003) 116.
[147] A. Wanat, M. Wolak, L. Orzel, M. Brindell, R. van Eldik, G.Y. Stochel, Coord. Chem. Rev. 229 (2002) 37.
[148] S.A. Waldman, F. Murad, Pharmacol. Rev. 39 (1987) 163.
[149] B.F. de Barros, J.C. Toledo Jr., D.W. Franco, E. Tfouni, M.H. Krieger, Nitric Oxide 7 (2002) 50.
[150] B. Bonavida, S. Khineche, S. Huerta-Yepez, H. Garbán, Drug Resist. Updat. 9 (2006) 157.
[151] K. Karidi, A. Garoufis, A. Tsipis, N. Hadjiliadis, H. den Dulk, J. Reedijk, Dalton Trans. (2005) 1176.
[152] P. Ascenzi, A. Bocedi, L. Gradoni, IUBMB Life 55 (2003) 573.
[153] J.J.N. Silva, A.L. Osakabe, W.R. Pavanelli, J.S. Silva, D.W. Franco, Br. J. Pharmacol. 152 (2007) 112.
[154] J.J.N. Silva, W.R. Pavanelli, J.C.M. Pereira, J.S. Silva, D.W. Franco, Antimicrob. Agents Chemother. 53 (2009) 4414.
[155] L.R. Makings, R.Y. Tsien, J. Biol. Chem. 269 (1994) 6282.
[156] J.A. Hrabie, J.R. Klose, D.A. Wink, L.K. Keefer, J. Org. Chem. 58 (1993) 1472.
[157] C.M. Maragos, D. Morley, D.A. Wink, T.M. Dunams, J.E. Saavedra, A. Hoffman, A.A. Bove, L. Isaac, J.A. Hrabie, L.K. Keefer, J. Med. Chem. 34 (1991) 3242.
[158] J.A. Hrabie, L.K. Keefer, Chem. Rev. 102 (2002) 1135.
[159] C.M. Pavlos, H. Xu, J.P. Toscano, Free Radic. Biol. Med. 37 (2004) 745.
[160] A. Srinivasan, N. Kebede, J.E. Saavedra, A.V. Nikolaitchik, D.A. Brady, E. Yourd, K.M. Davies, L.K. Keefer, J.P. Toscano, J. Am. Chem. Soc. 123 (2001) 5465.
[161] C.M. Pavlos, H. Xu, J.P. Toscano, Curr. Top. Med. Chem. 5 (2005) 637.
[162] P.H. Ruane, K.M. Bushan, C.M. Pavlos, R.A. D'Sa, J.P. Toscano, J. Am. Chem. Soc. 124 (2002) 9806.
[163] K.M. Bushan, H. Xu, P.H. Ruane, R.A. D'Sa, C.M. Pavlos, J.A. Smith, T.C. Celius, J.P. Toscano, J. Am. Chem. Soc. 124 (2002) 12640.
[164] E. Vittorino, E. Cicciarella, S. Sortino, Chem. Eur. J. 15 (2009) 6802.
[165] Y. Hou, W. Xie, A.J. Janczuk, P.G. Wang, J. Org. Chem. 65 (2000) 4333.
[166] K. Fukuhara, M. Kurihara, N. Miyata, J. Am. Chem. Soc. 123 (2001) 8662.
[167] T. Suzuki, O. Nagae, Y. Kato, H. Nakagawa, K. Fukuhara, N. Miyata, J. Am. Chem. Soc. 127 (2005) 11720.
[168] S. Namiki, T. Arai, K. Fujimori, J. Am. Chem. Soc. 119 (1997) 3840.
[169] S. Namiki, F. Kaneda, M. Ikegami, T. Arai, K. Fujimori, S. Asada, H. Hama, Y. Kasuya, K. Goto, Bioorg. Med. Chem. 7 (1999) 1695.
[170] M.Z. Cabail, P.J. Lace, J. Uselding, A.A. Pacheco, J. Photochem. Photobiol. A: Chem. 152 (2002) 109.
[171] M.Z. Cabail, A.A. Pacheco, Inorg. Chem. 42 (2002) 270.
[172] M.P. Hendrich, A.K. Upadhyay, J. Riga, D.M. Arciero, A.B. Hooper, Biochemistry 41 (2002) 4603.
[173] M.Z. Cabail, V. Moua, E. Bae, A. Meyer, A.A. Pacheco, J. Phys. Chem. A 111 (2007) 1207.
[174] M.Z. Cabail, J. Kostera, A.A. Pacheco, Inorg. Chem. 44 (2005) 225.
[175] J. Kostera, M.D. Youngblut, J.M. Slosarczyk, A.A. Pacheco, J. Biol. Inorg. Chem. 13 (2008) 1073.
[176] S.J. Pou, D.E. Anderson, W. Surichamorn, L.L. Keaton, M.L. Tod, Mol. Pharmacol. 46 (1994) 709.
[177] P.G. Wang, M. Xian, X. Tang, X. Wu, Z. Wen, T. Cai, A.J. Janczuk, Chem. Rev. 102 (2002) 1091.
[178] Y.-C. Hou, A. Janczuk, P.G. Wang, Curr. Pharm. Des. 5 (1999) 417.
[179] A. Gasco, R. Fruttero, G. Sorba, Farmaco 51 (1996) 617.
[180] D.M. Gooden, H. Chakrapani, E.J. Toone, Curr. Top. Med. Chem. 5 (2005) 687.
[181] S. Sortino, Chem. Soc. Rev. 39 (2010) 2903.
[182] G.B. Richter-Addo, P. Legzdins, Metal Nitrosyls, Oxford University Press, New York, 1992.
[183] P.C. Ford, I.M. Lorkovic, Chem. Rev. 102 (2002) 993.
[184] T.W. Hayton, P. Legzdins, W.B. Sharp, Chem. Rev. 102 (2002) 935.
[185] P.C. Ford, J. Bourassa, K. Miranda, B. Lee, I. Lorkovic, S. Boggs, S. Kudo, L. Laverman, Coord. Chem. Rev. 171 (1998) 185.
[186] W. Macyk, A. Franke, G. Stochel, Coord. Chem. Rev. 249 (2005) 2437.
[187] U. Schatzschneider, Eur. J. Inorg. Chem. (2010) 1451.
[188] E. Tfouni, Coord. Chem. Rev. 196 (2000) 281.
[189] A.B. Cox, R.M. Wallace, Inorg. Nucl. Chem. Lett. 7 (1971) 1191.
[190] J.C. Toledo Jr., B.S. Lima Neto, D.W. Franco, Coord. Chem. Rev. 249 (2005) 419.
[191] M.J. Rose, P.K. Mascharak, Coord. Chem. Rev. 252 (2008) 2093.
[192] R.G. de Lima, M.G. Sauaia, D. Bonaventura, A.C. Tedesco, L.M. Bendhack, R.S. da Silva, Inorg. Chim. Acta 359 (2006) 2543.
[193] K.Q. Ferreira, E. Tfouni, J. Braz. Chem. Soc. 21 (2010) 1349.
[194] R.M. Carlos, A.A. Ferro, H.A.S. Silva, M.G. Gomes, S.S.S. Borges, P.C. Ford, E. Tfouni, D.W. Franco, Inorg. Chim. Acta 357 (2004) 1381.
[195] F. de Souza Oliveira, K.Q. Ferreira, D. Bonaventura, L.M. Bendhack, A.C. Tedesco, S. de, P. Machado, E. Tfouni, R.S. da Silva, J. Inorg. Biochem. 101 (2007) 313.
[196] V. Togniolo, R.S. da Silva, A.C. Tedesco, Inorg. Chim. Acta 316 (2001) 7.
[197] J.B. Godwin, T.J. Meyer, Inorg. Chem. 10 (1971) 2150.
[198] F.R. Keene, D.J. Salmon, J.L. Walsh, H.D. Abruna, T.J. Meyer, Inorg. Chem. 19 (1980) 1896.
[199] Z. Assefa, D.M. Stanbury, J. Am. Chem. Soc. 119 (1997) 521.
[200] S.D.S.S. Borges, C.U. Davanzo, E.E. Castellano, J. Z-Schpector, S.C. Silva, D.W. Franco, Inorg. Chem. 37 (1998) 2670.
[201] M.G. Gomes, C.U. Davanzo, S.C. Silva, L.G.F. Lopes, P.S. Santos, D.W. Franco, J. Chem. Soc. Dalton Trans. (1998) 601.
[202] E.R.S. Winter, J. Catal. 22 (1971) 158.
[203] F. Bottomley, Acc. Chem. Res. 11 (1978) 158.
[204] F. Bottomley, Reactions of nitrosyls, in: P.S. Braterman (Ed.), Reactions of Coordination Ligands, Plenum, New York, 1989, p. 115.
[205] E. Tfouni, M. Krieger, B.R. McGarvey, D.W. Franco, Coord. Chem. Rev. 236 (2003) 57.
[206] J.C. Toledo Jr., H.A.S. Silva, M. Scarpellini, V. Mori, A.J. Camargo, M. Bertotti, D.W. Franco, Eur. J. Inorg. Chem. (2004) 1879.
[207] M.J. Rose, N.L. Fry, R. Marlow, L. Hinck, P.K. Mascharak, J. Am. Chem. Soc. 130 (2008) 8834.
[208] M.J. Rose, M.M. Olmstead, P.K. Mascharak, J. Am. Chem. Soc. 129 (2007) 5342.
[209] D.S. Marlin, P.K. Mascharak, Chem. Soc. Rev. 29 (2000) 69.
[210] A.K. Patra, P.K. Mascharak, Inorg. Chem. 42 (2003) 7363.
[211] A.K. Patra, M.J. Rose, K.A. Murphy, M.M. Olmstead, P.K. Mascharak, Inorg. Chem. 43 (2004) 4487.
[212] I. Szundi, M.J. Rose, I. Sen, A.A. Eroy-Reveles, P.K. Mascharak, O. Einarsdottir, Photochem. Photobiol. 82 (2006) 1377.
[213] M.J. Rose, M.M. Olmstead, P.K. Mascharak, Polyhedron 26 (2007) 4713.

[214] M.J. Rose, A.K. Patra, E.A. Alcid, M.M. Olmstead, P.K. Mascharak, Inorg. Chem. 46 (2007) 2328.
[215] G.M. Halpenny, P.K. Mascharak, Inorg. Chem. 48 (2009) 1490.
[216] N.L. Fry, M.J. Rose, D.L. Rogow, C. Nyitray, M. Kaur, P.K. Mascharak, Inorg. Chem. 49 (2010) 1487.
[217] M.J. Rose, P.K. Mascharak, Inorg. Chem. 48 (2009) 6904.
[218] M.G. Sauaia, R.G. de Lima, A.C. Tedesco, R.S. da Silva, J. Am. Chem. Soc. 125 (2003) 14718.
[219] M.G. Sauaia, R.G. de Lima, A.C. Tedesco, R.S. da Silva, Inorg. Chem. 44 (2005) 9946.
[220] H.E. Toma, A.D.P. Alexiou, A.L.B. Formiga, M. Nakamura, S. Dovidauskas, M.N. Eberlin, D.M. Tomazela, Inorg. Chim. Acta 358 (2005) 2891.
[221] F. Roncaroli, L.M. Baraldo, L.D. Slep, J.A. Olabe, Inorg. Chem. 41 (2002) 1930.
[222] F. Gorzoni Doro, U. Rodrigues-Filho, E. Tfouni, J. Colloid Interface Sci. 307 (2007) 405.
[223] K. Ghosh, A.A. Eroy-Reveles, B. Avila, T.R. Holman, M.M. Olmstead, P.K. Mascharak, Inorg. Chem. 43 (2004) 2988.
[224] R.K. Afshar, A.K. Patra, P.K. Mascharak, J. Inorg. Biochem. 99 (2005) 1458.
[225] A.A. Eroy-Reveles, Y. Leung, C.M. Beavers, M.M. Olmstead, P.K. Mascharak, J. Am. Chem. Soc. 130 (2008) 4447.
[226] A.A. Eroy-Reveles, Y. Leung, P.K. Mascharak, J. Am. Chem. Soc. 128 (2006) 7166.
[227] G.M. Halpenny, K.R. Gandhi, P.K. Mascharak, Med. Chem. Lett. 1 (2010) 180.
[228] M. De Leo, P.C. Ford, J. Am. Chem. Soc. 121 (1999) 1980.
[229] A. Nemes, O. Pestovsky, A. Bakac, J. Am. Chem. Soc. 124 (2002) 421.
[230] A.D. Ostrowski, S.J. Deakin, B. Azhar, T.W. Miller, N. Franco, M.M. Cherney, A.J. Lee, J.N. Burstyn, J.M. Fukuto, I.L. Megson, P.C. Ford, J. Med. Chem. 53 (2010) 715.
[231] F. DeRosa, X. Bu, P.C. Ford, Inorg. Chem. 44 (2005) 4157.
[232] D. Neuman, A.D. Ostrowski, R.O. Absalonson, G.F. Strouse, P.C. Ford, J. Am. Chem. Soc. 129 (2007) 4146.
[233] C.M. Pavlos, H. Xu, J.P. Toscano, Release of second messengers—caged nitric oxide, in: M. Goeldener, R. Givens (Eds.), Dynamic Studies in Biology: Phototriggers, Photoswitches and Caged Biomolecules, Wiley-VCH Verlag, Weinheim, 2005, p. 178.
[234] R.P. Mitra, D.V.S. Jain, A.K. Banerjee, K.V.R. Chari, J. Inorg. Nucl. Chem. 25 (1963) 1263.
[235] S.K. Wolfe, J.H. Swinehart, Inorg. Chem. 14 (1975) 1049.
[236] S. Kudo, J.L. Bourassa, S.E. Boggs, Y. Sato, P.C. Ford, Anal. Biochem. 247 (1997) 193.
[237] J.L. Bourassa, P.C. Ford, Coord. Chem. Rev. 200–202 (2000) 887.
[238] S. Wecksler, A. Mikhailovsky, P.C. Ford, J. Am. Chem. Soc. 126 (2004) 13566.
[239] S.R. Wecksler, A. Mikhailovsky, D. Korystov, P.C. Ford, J. Am. Chem. Soc. 128 (2006) 3831.
[240] P.C. Ford, Acc. Chem. Res. 41 (2008) 190.
[241] S.R. Wecksler, J. Hutchinson, P.C. Ford, Inorg. Chem. 45 (2006) 1192.
[242] R. Motterlini, J.E. Clark, R. Foresti, P. Sarathchandra, B.E. Mann, C.J. Green, Circ. Res. 90 (2002) E17.
[243] J.P.Y. Kao, P.F. Keitz, Photosensitive organic compounds that release carbon monoxide upon illumination, U.S.A. U.S. Patent 5,670,664 (1997).
[244] J. Niesel, A. Pinto, H.W.P. N'Dongo, K. Merz, I. Ott, R. Gust, U. Schatzschneider, Chem. Commun. (2008) 1798.
[245] H. Pfeiffer, A. Rojas, J. Niesel, U. Schatzschneider, Dalton Trans. (2009) 4292.
[246] P.C. Kunz, W. Huber, A. Rojas, U. Schatzschneider, B. Spingler, Eur. J. Inorg. Chem. (2009) 5358.
[247] J.N. Butler, Carbon Dioxide Equilibria and their Applications, CRC Press, Boca Raton, 1991.
[248] J.D. Margerum, C.T. Petrusis, J. Am. Chem. Soc. 91 (1969) 2467.
[249] J.E.T. Corrie, A. DeSantis, Y. Katayama, K. Khodakhah, J.B. Messenger, D.C. Ogden, D.R. Trentham, J. Physiol. 465 (1993) 1.
[250] F.M. Rossi, J.P.Y. Kao, J. Biol. Chem. 272 (1997) 3266.
[251] G. Papageorgiou, A. Barth, J.E.T. Corrie, Photochem. Photobiol. Sci. 4 (2005) 216.
[252] R. Barnadas-Rodriguez, J. Estelrich, J. Photochem. Photobiol. A 198 (2008) 262.
[253] K.K. Smith, K.J. Kaufmann, D. Huppert, M. Gutman, Chem. Phys. Lett. 64 (1979) 522.
[254] M. Gutman, E. Nachliel, E. Gershon, R. Giniger, E. Pines, J. Am. Chem. Soc. 105 (1983) 2210.
[255] J.W. Walker, G.P. Reid, J.A. McCray, D.R. Trentham, J. Am. Chem. Soc. 110 (1988) 7170.
[256] A. Patchornik, B. Amit, R.B. Woodward, J. Am. Chem. Soc. 92 (1970) 6333.