DOI: 10.1002/anie.201002542

Photochemistry

Keys for Unlocking Photolabile Metal-Containing Cages

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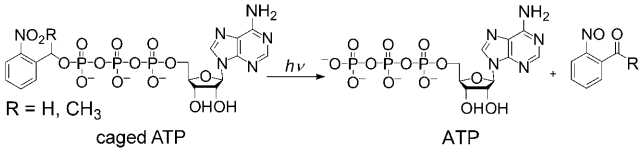
cage compounds · chelates · photochemistry · photolysis · transition metals

Photolabile metal-containing cages are metal complexes that undergo a change in coordination environment upon exposure to light of an appropriate wavelength. The light-responsive functionality can either be a component of the encapsulating ligand or a property of the metal complex itself. The altered coordination properties of light-responsive complexes can result in release of the coordinated metal ion into its surroundings, a differential reactivity of the metal center, or the liberation of a reactive molecule that had been passivated by binding to the metal center. These triggerable agents can be useful tools for manipulating the bioavailability of metals or their coordinating ligands in order to study biological pathways or for potential therapeutic purposes.

1. Introduction

The ability to control the spatial distribution and temporal release of specific biologically active molecules allows researchers to interrogate how various chemical events mediate biological processes. Caged or light-modulated molecules enable the use of light as a noninvasive trigger to turn on such events. The term "caged" was coined by Kaplan, Forbush, and Hoffman in 1978 to describe their light-activated ATP molecule (Scheme 1).[1] In this sense, caged means that the biological activity of a molecule is blocked by the covalent attachment of a photolabile protecting group onto a key functional group of the molecule. The molecule is thereby rendered inactive (i.e., caged) until irradiation with light of an appropriate wavelength alters the photolabile component to reveal the functionality of the molecule and restore its biological activity. This strategy has been used to cage various biomolecules, including peptides, proteins, nucleic acids, effectors that regulate gene expression, secondary messengers, and nucleotide cofactors.[2–4]

Metal ions can also elicit profound biological responses,[5] and it would be desirable to control these responses by either releasing metal ions or by altering their reactivity "on-demand" in a similar fashion to the light-induced uncaging of organic molecules. Caging metal ions, however, requires a different strategy from the covalent-protecting-group method used for organic molecules. In this Minireview, we explore various methods to cage metal ions, including transition-metal ions. We define two types of cages for metal ions: "locked" and "unlockable". In this context, a locked metal cage refers to a conventional definition of a cage compound wherein a metal ion is completely encapsulated in a three-dimensional cavity provided by a macropolycyclic ligand.[6] In contrast, unlockable cages refer specifically to photocages in which the coordination environment around the metal center changes upon exposure to light.



Scheme 1. Nitrobenzyl-protected adenosine triphosphate: the first example of a "caged" molecule.

2. Locked Metal Cages

The utility of locked metal cages in biology and medicine relies on the principle that the metal ion remains both isolated from and unreactive toward other species in its environment. Some examples are shown in Scheme 2. In these cases, the three-dimensional scaffold of the ligand provides all of the

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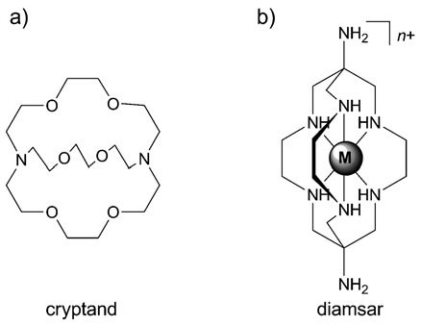

Scheme 2. Examples of locked cages: a) cryptands are examples of preassembled cages that can encapsulate a variety of cations; b) diamsar is an example of a clathrochelate that can be assembled around a kinetically inert metal ion such as Co^{3+} by template synthesis, followed by replacement with a different metal ion of choice.

donor atoms that coordinatively saturate the metal, and thus imposes further steric and electronic restraints beyond those of corresponding two-dimensional scaffolds. This cryptate effect provides impressive thermodynamic and kinetic stability to caged metal complexes.[7–9] The synthesis of preassembled ligands such as cryptands (Scheme 2a) is often tedious and low-yielding.[10] However, the templated synthesis of molecules such as diamsar (Scheme 2b) and other clathrochelates provides efficient access to encapsulating ligands.[11]

The ease of synthesis and ability to vary the molecular scaffold of clathrochelates, as well as their kinetic and thermodynamic stability, mean that these complexes have both biochemical and medicinal applications. Some uses of these molecules include complexation of radioactive metal ions for radiotherapy and diagnostics, encapsulation of paramagnetic ions for magnetic resonance tomography, coordination of metals for HIV therapy, and membrane transporters for metal ions.[12] A comprehensive survey of all of the applications of locked metal cages is beyond the scope of this Minireview, but further information on this subject can be found in references [6], [11], and [12].

3. Unlockable Metal Cages

The distinction between a locked metal cage and an unlockable metal cage is that the unlockable cage can be opened. As we are specifically discussing photocages, light is the key that opens the cage, and the process of opening the cage entails a change in coordination environment around the metal ion. Whereas the utility of locked metal cages relies on the unreactivity of the metal ion toward its environment, the utility of unlockable metal cages is that their reactivity with their surroundings changes upon selective stimulation by light of an appropriate wavelength.

The following sections describe two types of photocages that rely on light to alter the coordination chemistry of a metal complex. In the case of Type 1 photocages, light reacts with a photoactive component of the ligand to cause a change in reactivity at the metal center, whereas in the case of Type 2 photocages, light reacts with a photoactive metal center to cause a change in reactivity of a ligand that was once coordinated to the metal. This distinction is shown in Figure 1: light is used as the key to unlock the reactivity of either the metal ion or a component of the cage. It should be noted that the following examples fit into these two narrowly defined terms that are part of a much broader field of inorganic and bioinorganic photochemistry.[13] Examples such as photoactive metal complexes that generate diffusible radical species that react with DNA,[14] or photosensitizers that generate singlet oxygen for photodynamic therapy,[15,16] are not included within the scope of this Minireview.

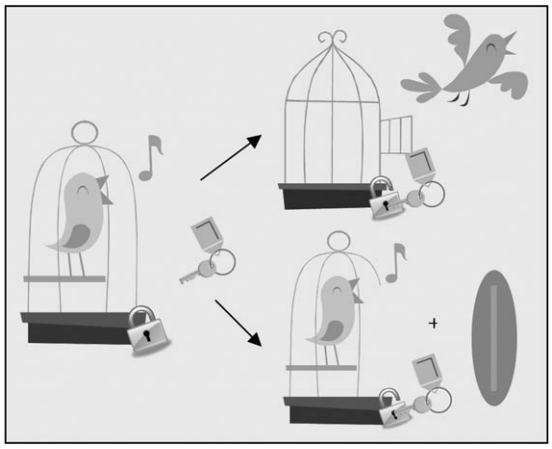

Figure 1. Cartoon of unlockable metal cages: the bird represents a metal ion and the key represents light. Unlocking the cage can either cause the metal ion to escape and become reactive (top, Type 1 cage), or cause a piece of the cage to be released and become reactive (bottom, Type 2 cage).

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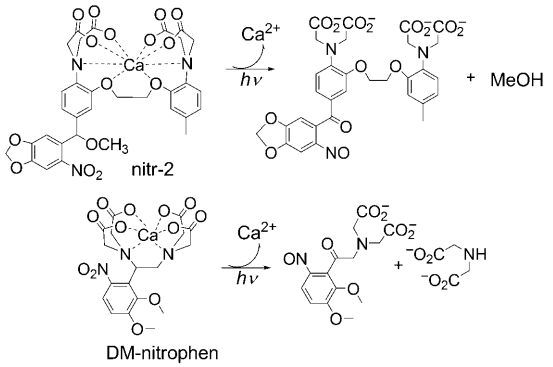
Katherine Franz received a bachelor's degree from Wellesley College and a PhD from MIT, where she worked with S. J. Lippard. She was a post-doctoral fellow with B. Imperiali at MIT before starting at Duke University in 2003, where she is an Associate Professor of chemistry. Her research interests include designing triggerable chelating agents to manipulate and probe the concentration, reactivity, and distribution of metal ions in biological systems.

3.1. Type 1 Unlockable Metal Cages: Photoactive Ligands

A potential application for caged metal complexes is their use as reagents to alter metal-ion bioavailability. Incorporation of a photoactivatable agent into a chelating ligand introduces a switch that can control the release of the coordinated metal in a spatial and temporal fashion. Such reagents could be valuable tools for studying cellular processes related to metal-ion uptake, distribution, storage, usage, and trafficking.

3.1.1. Calcium

Calcium cages are the most prominent example of a Type 1 unlockable metal cage. In this design, a photolabile, high-affinity ligand chelates Ca^{2+} ions to render them biologically unavailable. Bioavailability is restored when the ligand backbone undergoes a photochemical change, which is caused by UV irradiation and liberates products with a diminished affinity for Ca^{2+} ions. In the late 1980s, the first calcium cages were developed independently by two research groups.[17–19] These cages (Scheme 3) became commercially

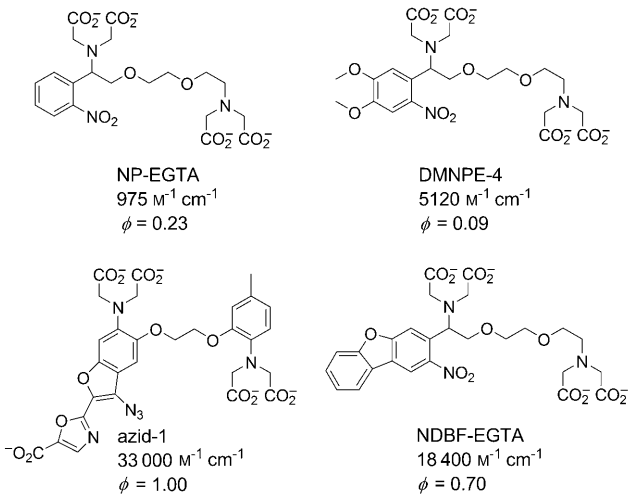


Scheme 3. Photochemistry of nitr-2 and DM-nitrophen caged Ca^{2+} complexes and their modes of light activation for Ca^{2+} release.

available because of their applicability to biological systems. Both compounds utilize the same nitrobenzyl photochemical reactions, although the resulting photoproducts are quite different. When nitr-2 is photolyzed, methanol is released and a nitroso photoproduct forms. The presence of a benzylic carbonyl group *para* to the conjugated nitrogen atom decreases the affinity for calcium from a dissociation constant (K_d) of 160 nM to approximately 8 μM.[19] On the other hand, when DM-nitrophen is exposed to UV light, the ligand backbone is cleaved, changing the binding affinity from a K_d value of 5 nM to 3 mM after photolysis.[20] This dramatic post-photolysis change in binding affinity means that light can be used to control the time, location, and amplitude of calcium release.

Most calcium cages utilize a 2-nitrophenyl group as the photolabile component of the molecule. These photoactive moieties were first used as protecting groups in organic synthesis and remain the most prevalent groups in photolabile cages today. The popularity of the 2-nitrophenyl group stems in part from its compatibility with a wide variety of functional groups, including amides, amines, carboxylates, hydroxy groups, and phosphates.[21,22] In addition, there are a number of derivatives that are commercially available or relatively easy to synthesize. Other photolabile groups that undergo intramolecular photoreactions that lead to bond cleavage are also becoming more prevalent. A recent tutorial review surveys the structure, mechanism, and properties of some common photolabile protecting groups, including nitrobenzyl, coumarin, and hydroxyphenacyl derivatives.[23]

The nitrophenyl compounds can be photolyzed with near-UV light centered at 350 nm, which lies in the UVA range (315–400 nm). Unlike the UVB (280–315 nm) and UVC (100–280 nm) ranges, UVA is not absorbed by DNA and therefore does not directly cause DNA damage. Depending on the intensity of the light source, duration of exposure and cell type, however, UVA light can indirectly damage DNA and other cellular components by formation of reactive oxygen species.[24,25] Light toxicity can therefore be a serious limitation of these compounds, so it is essential that their photolysis be as efficient as possible. Some of the later-generation calcium cages designed to improve the efficiency of photolysis are shown in Scheme 4.



Scheme 4. Comparison of later-generation ligands developed as calcium cages along with their extinction coefficients at 350 nm and quantum yields of photolysis (ϕ) in the presence of saturating calcium.

Two defining qualities of a photolabile group are its quantum efficiency and its extinction coefficient at the irradiation wavelength. If a photolabile compound can absorb light efficiently, then lower exposure times are needed and, in turn, cellular damage is reduced. One strategy used to improve the quantum efficiencies of these compounds is to increase their extinction coefficients at 350 nm. This aim was accomplished in calcium cages by incorporating methoxy substituents at the 4- and 5-positions of the *ortho*-nitrophenyl group. The addition of methoxy groups to NP-EGTA to form DMNPE-4 increases the extinction coefficient from $975\,M^{-1}cm^{-1}$ to $5120\,M^{-1}cm^{-1}$ (Scheme 4).[26] Unfortunately, this increase in the extinction coefficient is not accompanied

by an increase in quantum efficiency, which decreases from 0.23 for NP-EGTA to 0.09 for DMNPE-4.[26] Interestingly, DMNPE-4 was only the second calcium cage reported that is capable of undergoing efficient two-photon uncaging. The first such cage was azid-1 (Scheme 4), which is a member of the nitr family and a derivative of fura-2, a fluorescent calcium sensor. The fluorescent properties of azid-1 result in an extinction coefficient of $33000\,M^{-1}cm^{-1}$ and allow it to photolyze with an impressive quantum yield of unity to produce an amidoxime photoproduct.[27] Similarly, the incorporation of a nitrodibenzofurane chromophore into the ligand backbone of EGTA increases the extinction coefficient to $18400\,M^{-1}cm^{-1}$, as seen in NDBF-EGTA. This value is significantly higher than the extinction coefficient of NP-EGTA ($975\,M^{-1}cm^{-1}$).[28] NDBF-EGTA also has a very high quantum yield of photolysis (0.70), and is capable of two-photon uncaging.[28]

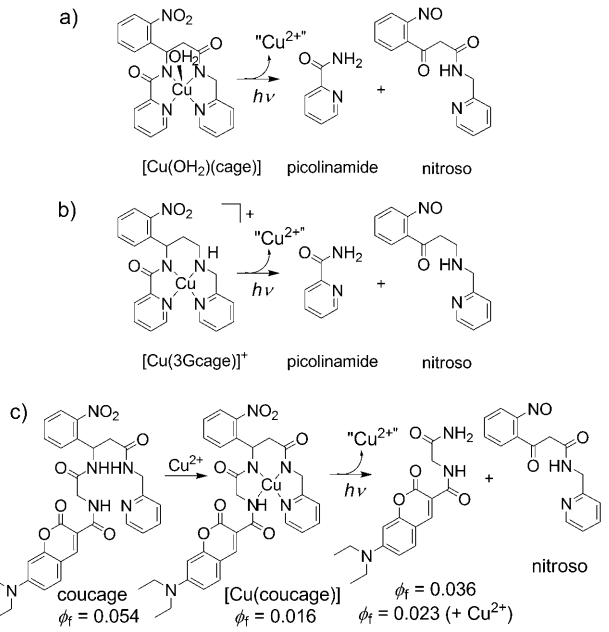
Calcium cages have been used successfully in many types of physiological experiments and have been useful for studying the roles of calcium in biological processes.[20] This strategy has also been implemented to cage Sr^{2+}, Ba^{2+}, Mg^{2+}, Cd^{2+}, Mn^{2+}, and Co^{2+} ions.[29–31] However, there are few reported examples of these compounds for biologically relevant d-block metals. The following sections give an overview of the existing caged transition- and d-block-metal compounds, and their applications.

3.1.2. d-Block Metal Cages

When developing a caging ligand for transition-metal ions, it is important to consider the preferences of the donor atoms, the size of the chelate rings, and the geometry of the complex. Binding affinity can be tuned by selecting donor atoms in accordance with the hard–soft acid–base (HSAB) principle and by optimizing the number and size of the chelate rings compared to the size of the cation. In addition, the binding geometry can aid in metal-ion selectivity as transition-metal ions vary in their number of valence d electrons, and, in turn, their preference for a certain binding geometry. Finally, careful consideration of the implications of the parameters discussed above for photolysis efficiency and on the desired reactivity pre- and post-photolysis is required.

3.1.3. Copper

In an effort to expand the concept of caged metal complexes beyond Ca to biologically relevant transition metals, our research group recently introduced a photosensitive caged Cu complex [$Cu(OH_2)$(cage)].[32] Our caging ligand H_2cage has a nitrophenyl group incorporated into a tetradentate ligand composed of two amide and two pyridyl nitrogen atoms. H_2cage binds Cu^{2+} ions with an apparent K_d value of 16 pM at pH 7.4.[32] Irradiation with 350 nm UV light cleaves the ligand backbone within 4 minutes to release bidentate photoproducts with a lower affinity for Cu^{2+} ions (Scheme 5a). The high efficiency of photolysis of H_2cage (0.73) is lowered to 0.32 upon coordination to Cu^{2+} ions, thus indicating that Cu impairs photolysis but does not prevent it.[32] In the absence of light, [$Cu(OH_2)$(cage)] was shown to



Scheme 5. Copper cages.

inhibit hydroxyl radical formation in an in vitro assay. However, following light-induced uncaging, copper-catalyzed hydroxyl radical formation increased by 160%.[32] This photoactive caged Cu complex is the first example of the use of light to alter the availability and reactivity of a biologically relevant transition metal.

One drawback of H_2cage is that its K_d value of 16 pM for Cu^{2+} ions is not strong enough to prevent competition with copper-binding proteins. To address this problem, we developed a series of second-generation chelators to better understand the effects of changes to the ligand backbone of H_2cage on the Cu^{2+} ion binding affinity and photolysis efficiency. By using the results of this study, we developed a third-generation Cu cage, namely 3Gcage, which has an apparent K_d value of 0.18 fM for Cu^{2+} ions at pH 7.4;[33] this value is a remarkable improvement, and should be strong enough to keep Cu^{2+} ions sequestered in the presence of endogenous copper-binding proteins. Like H_2cage, 3Gcage coordinates Cu^{2+} ions in a tetradentate binding site until activation with UV light cleaves the ligand backbone to release bidentate photoproducts that have a diminished affinity for Cu^{2+} ions (Scheme 5b). In the dark, 3Gcage inhibits hydroxyl radical formation, while exposure to UV light increases hydroxyl radical formation by more than 300%. [Cu(3Gcage)]$^+$ may be a useful tool for the delivery of metal ions to study metal trafficking pathways or to induce oxidative stress as a chemotherapy strategy, although the application of [Cu(3Gcage)]$^+$ in a cellular system has not yet been validated.

Fluorescent sensors for Ca^{2+},[34] Zn^{2+},[35,36] and Cu^{+}[37,38] ions have been widely used to investigate metal-ion distribution. A common strategy is to design a probe such that coordination to a particular metal ion increases the fluorescence intensity of the probe, that is, a "turn-on" sensor. The development of this type of probe for Cu^{2+} ions is challenging, since they are paramagnetic and quench the probe fluores-

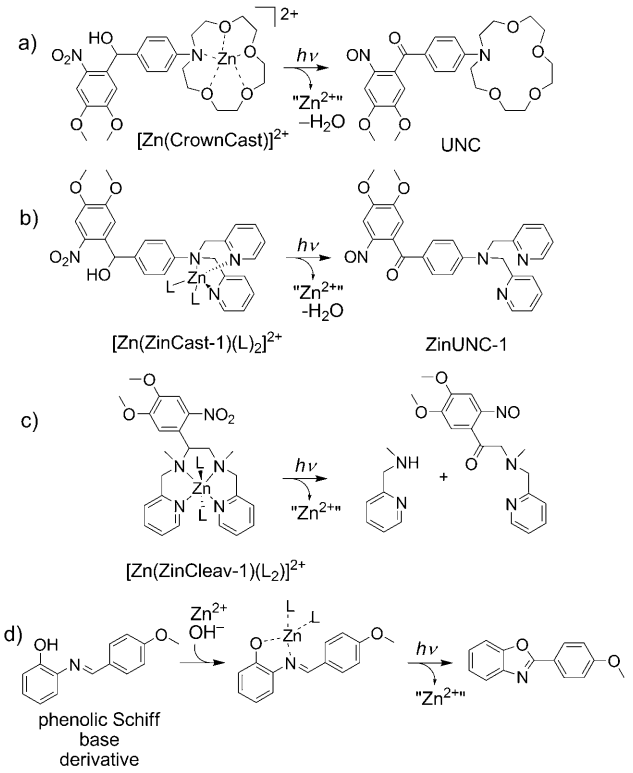
cence. Therefore, many Cu^{2+} probes have a "turn-off" mechanism, which offers less accuracy and sensitivity. We have developed a new type of turn-on sensor, coucage, in which a photoactive nitrophenyl group is incorporated into the backbone of a coumarin-tagged tetradentate ligand.[39] Coordination of Cu^{2+} ions reduces the fluorescence output until irradiation with UV light cleaves the ligand backbone, thus removing the copper-induced quenching and providing a turn-on response (Scheme 5c). Coucage displays a selective fluorescence response for Cu^{2+} ions over other biologically relevant metal ions. Experiments in live MCF-7 cells show that coucage can be used for detecting changes in intracellular Cu^{2+} ions upon the addition of a high concentration of exogenous Cu^{2+} ions, thus indicating that coucage could be a useful tool for visualizing the cellular distribution of labile Cu to gain insight into the mechanisms of Cu trafficking.[39] However, since the probe is destroyed upon UV exposure, this strategy does not provide real-time monitoring of cellular Cu^{2+} ion fluctuations. The probe instead reports the locations where Cu^{2+} ions were available for chelation. This method could be useful for monitoring endogenous pockets of Cu^{2+} ions, provided that adjustments can be made to the ligand to increase its copper-binding affinity and to improve its quenching efficiency prior to light activation.

3.1.4. Zinc

Burdette and co-workers have set out to develop caged zinc complexes to facilitate research into Zn^{2+} ion signaling.[40–42] For example, these tools can be used to address discrepancies about the relevance of synaptic zinc in neurotransmission. One current method for releasing Zn^{2+} ions into a biological system is exogenous application in the form of a Zn^{2+} salt. This method has the risk of overwhelming the system with biologically irrelevant concentrations of Zn^{2+} ions that may induce a response not seen under more appropriate biological concentrations.

One of the first photoactive cages reported to chelate Zn^{2+} was CrownCast (Scheme 6a).[40] CrownCast has a crown ether receptor and photochemical properties similar to that of the nitr-2 calcium cage reported by Adams and Tsien.[21] Upon photolysis, a nitrosobenzophenone (UNC) that has lower metal-binding capabilities is formed. This decrease in binding affinity results from donation of the lone pair of electrons on the aniline nitrogen atom to the oxygen atom on the benzophenone carbonyl group. The ligand is capable of binding Cd^{2+}, Ba^{2+}, Sr^{2+}, Ca^{2+}, Mg^{2+}, and Zn^{2+} ions. The highest binding affinity was observed for Ca^{2+} ions and the lowest binding affinity for Zn^{2+} ions, with dissociation constants of 14 μM and 161 μM, respectively.[40]

The next-generation chelator ZinCast-1 (Scheme 6b) binds Zn^{2+} ions in its tridentate binding pocket with a K_d value of 14.3 μM.[41] Upon exposure to UV light, ZinCast-1 operates by the same photochemical mechanism as CrownCast. The formation of a ketone at the benzylic position results in decreased electron density on the aniline nitrogen atom to produce a weaker Zn–N bond. After irradiation, the resulting photoproduct has a Zn^{2+} binding affinity of 5.5 mM.[41] ZinCast-1 has also been shown to bind Cu^{2+} ions



Scheme 6. Zinc cages. L = coordinating solvent or unidentified supporting ligands.

with a binding affinity of 4.5 μM pre-photolysis and 1.6 μM post-photolysis, thus indicating very little change in binding strength.[41]

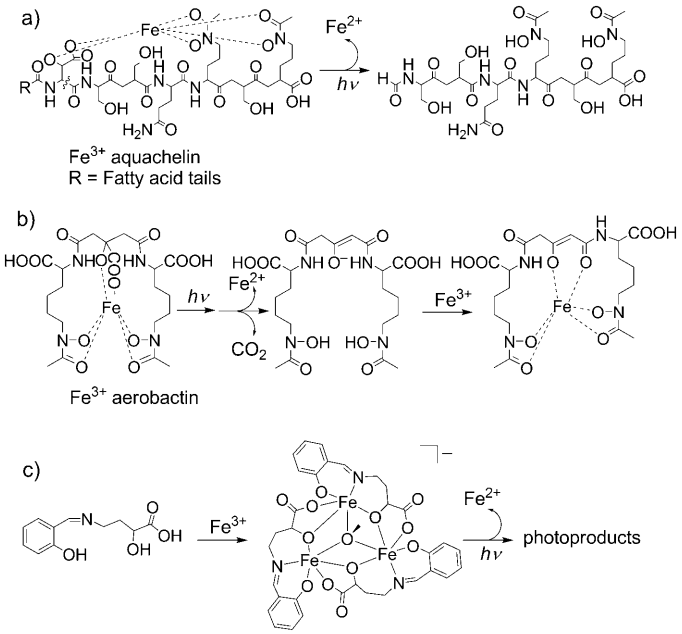
ZinCleav-1 (Scheme 6c) is different to the two previously discussed Zn^{2+} ion chelators in that the lower binding affinity after photolysis results from cleavage of the ligand backbone. Of the three chelators, ZinCleav-1 has the highest affinity for Zn^{2+} ions, with a K_d value of 0.23 pM.[42] After photolysis, it was estimated that the resulting photoproducts have Zn^{2+} ion binding affinities greater than 150 μM for the 1:1 metal–ligand complexes and in the millimolar range for the 2:1 metal–ligand complexes.[42] In addition, ZinCleav-1 is photolyzed most efficiently, with a quantum yield of 0.024[42] compared to 0.007[41] for ZinCast-1 and 0.005[40] for CrownCast.

Zhang and Chen have developed a different scaffold for a zinc cage, in which a phenolic Schiff base derivative is employed as the caging molecule. When this molecule is dissolved in an organic solvent in the presence of stoichiometric amounts of base, the binding affinity for Zn^{2+} ions was estimated to be $2.93 \times 10^3\,M^{-1}$ ($K_d = 0.34$ mM).[43] Upon irradiation with 365 nm UV light, a 2-arylbenzoxazole photoproduct is formed with a decreased affinity for Zn^{2+} ions (Scheme 6d). This system is different to those previously discussed since a bidentate chelate cyclizes upon irradiation so that metal binding is no longer possible. It was also demonstrated that this phenolic Schiff base derivative binds other divalent metal ions, such as Fe^{2+} ($1.34 \times 10^3\,M^{-1}$), Ca^{2+} ($6.28 \times 10^2\,M^{-1}$), Cu^{2+} ($2.73 \times 10^3\,M^{-1}$), and Hg^{2+} ions ($2.23 \times 10^3\,M^{-1}$).[43] However, the reactivity of these metal ions

in vitro and in vivo both pre- and post-photolysis has yet to be determined.

3.1.5. Iron

Examples of naturally occurring unlockable cages include some classes of siderophores, which are high-affinity Fe^{3+} chelators produced by bacteria to enable iron acquisition.[44–46] Butler and co-workers have investigated the photochemical properties of marine siderophores known as aquachelins to gain insight into the biochemistry of iron in the upper ocean.[47] Aquachelins contain various fatty acid tails attached to a peptidic headgroup that is responsible for coordinating Fe^{3+} ions through two hydroxamate groups and one β-hydroxyaspartate group to form a 1:1 Fe^{3+}–aquachelin complex.[48] In the presence of UVA light, Fe^{3+} is reduced to Fe^{2+} ions and the siderophore backbone is cleaved at the β-hydroxycarboxylate site (Scheme 7a).[47] As a result, the conditional binding affinity for Fe^{3+} ions decreases from $10^{12.2}\,M^{-1}$ ($K_d = 0.63$ pM) pre-photolysis to $10^{11.5}\,M^{-1}$ ($K_d = 3.16$ pM) post-photolysis.[47] These findings show that siderophores not only mediate bacterial iron transport but also that their photolytic properties may affect the bioavailability of iron.

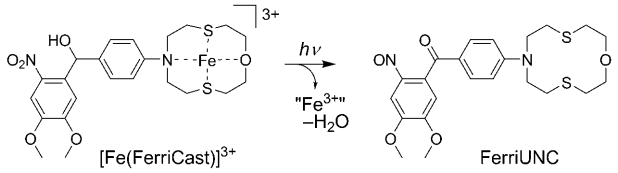


Scheme 7. Photolabile siderophore (a, b) and siderophore model (c) complexes.

A second class of photoreactive siderophores that are structurally similar to aquachelins is aerobactin. Aerobactin contains an α-hydroxy acid in the form of citric acid.[49–51] It is proposed that Fe^{3+} ions are coordinated through two hydroxamate groups and the hydroxycarboxylic acid.[52,53] Irradiation with UV light into the LMCT results in the reduction of Fe^{3+} ions and the release of CO_2 (Scheme 7b). Unlike aquachelin, photolyzed aerobactin retains a similar affinity for Fe^{3+} ions as its unphotolyzed counterpart and promotes iron acquisition.[52] Since photolysis does not result in a change in binding affinity, the repercussions of photolysis on biological function remain to be resolved.

Inspired by photoresponsive marine siderophores, Baldwin and co-workers prepared a series of mixed-donor chelates that contain an α-hydroxy acid as a light-sensitive unit, together with phenol and imine donor groups.[54] One of these ligands is shown in Scheme 7c. These ligands bind Fe^{3+} ions with high affinity in methanolic solution to form trinuclear clusters in which each ligand spans two iron centers, with the alkoxo oxygen atoms from the hydroxy acid groups bridging the iron centers (Scheme 7c). A capping methoxy group completes the structure. The α-hydroxy acid group is not inherently photosensitive, but coordination to Fe^{3+} ions creates a LMCT band that is sensitive to sunlight. Although the photoproducts have not yet been fully identified, Fe^{2+} ions are released upon photolysis. Importantly, this initial study demonstrates the feasibility of tuning the kinetics of Fe^{2+} ion release by modulating ancillary functional groups on chelate structures that contain α-hydroxy acids as light-sensitive units.[54]

Caged iron complexes could be useful to gain insight into iron homeostasis and the role of iron in pathological processes. Burdette and co-workers developed Ferricast,[55] which is a photosensitive macrocyclic compound that binds Fe^{3+} ions in organic solvents. In a manner similar to the first-generation zinc cages developed by the same research group, Ferricast is converted into the weaker-binding FerriUNC upon UV irradiation (Scheme 8). The quantum yield of



Scheme 8. Iron cage FerriCast forms both 1:1 and 2:1 ligand–metal complexes.

photolysis for this conversion is 0.01 for the apo ligand and 0.04 for the metal complex.[55] Both the 1:1 and 2:1 FerriCast–Fe^{3+} complexes are present in organic solution.[55] Unfortunately, complexes of FerriCast decompose in aqueous solution, thus limiting their utility under biologically relevant conditions.

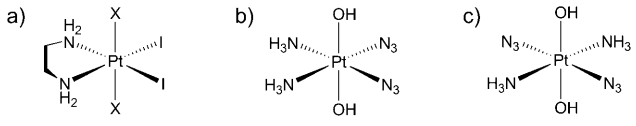
3.1.6. Platinum

The anticancer activity of Cisplatin has been well established since the discovery of its biological effects in the 1960s.[56] Because of the overall success of Cisplatin, thousands of analogues have also been synthesized. The clinical effectiveness of many of these agents is unfortunately restricted since they are toxic to both healthy and cancerous tissue. Drawbacks include dose limitations that are necessary because of severe side effects, as well as intrinsic and acquired resistance to the drug. Therefore, a different approach to Pt

drug design is needed. In this section, we will outline photoactive drugs that can deliver Pt intracellularly in a site- and time-specific manner, which could potentially alleviate the limitations of the current drugs.

Photoactive drugs have been employed in photodynamic therapy (PDT), which uses a photosensitizing drug and light of a specific wavelength to generate singlet oxygen. The benefit of this method is that cytotoxic species are localized at the site of a tumor and that healthy tissue is unaffected. PDT has been successful in the treatment of numerous cancers. The drawback of this method is that oxygen is required for the cytotoxic mechanism, and yet many tumors are hypoxic.[57] An advantage of the potentially phototherapeutic agents discussed in this section is that they do not require oxygen for cytotoxicity.

The first photoactivatable platinum druglike molecule was the Pt^{4+} complex $[PtCl_2I_2(en)]$ introduced by Bednarski and co-workers (Scheme 9a).[58] Pt^{4+} complexes are more



Scheme 9. Photoactive Pt^{4+} compounds. a) *trans*,*cis*-$[PtX_2I_2(en)]$ ($X = Cl^-$ or OAc^-), b) *cis*,*trans*,*cis*-$[Pt(N_3)_2(OH)_2(NH_3)_2]$, and c) *trans*,*trans*,*trans*-$[Pt(N_3)_2(OH)_2(NH_3)_2]$.

inert to ligand substitution than their Pt^{2+} counterparts, and therefore must be reduced to their active Pt^{2+} form by extracellular and/or intracellular agents prior to reaction with DNA.[59,60] If the rate of reduction of Pt^{4+} to Pt^{2+} can be increased at or around a tumor relative to the rate in normal tissue, then the effectiveness of the drug could be maximized. The $[PtCl_2I_2(en)]$ complex can be photoreduced with visible light. While the photoproducts were not characterized, the resulting complex was shown to bind DNA. However, the unphotolyzed complex was also able to bind DNA and there was no difference in cytotoxicity observed for cells kept in the dark compared to those exposed to light. The lack of stability of this first-generation complex was attributed to its high positive reduction potential of 75 mV.[58]

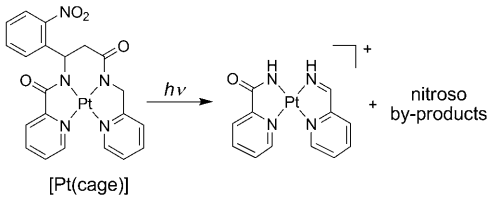
Next-generation complexes were designed to have increased stability in the dark. One such complex, *trans*,*cis*-$[Pt(OAc)_2I_2(en)]$ (where $X = OAc^-$; Scheme 9a), has a reduction potential of −65 mV.[61] Unfortunately, the cytotoxicity difference between cells kept in the dark and those exposed to light was less significant than expected. It was determined that Pt^{4+} diiodo complexes are easily reduced in vivo by biological thiols to their toxic Pt^{2+} counterparts, thus making them inadequate as photolabile drugs. These compounds may not have operated as desired, but importantly, they did reveal the possibility of releasing a toxic platinum complex with light. By irradiating the LMCT band of the complex, a reductive elimination reaction is initiated.[62,63] The iodide leaving group is oxidized to a radical and Pt^{3+} is formed. This platinum species will either recombine with the iodide radical anions or initiate the oxidation of another iodide group to form a Pt^{2+} complex. This class of compounds is different than the photocages previously discussed since the metal is the photosensitive component of the cage, as opposed to a photolabile component of the ligand. In this respect, they could be cross-classified as Type 2 photocages.

In order to build Pt^{4+} complexes that are more stable in the presence of cellular reductants, Sadler and co-workers replaced the iodide groups with azides, since platinum azide complexes are known to undergo photoreductive elimination.[63] Upon irradiation with UVA light centered at 365 nm, the Pt^{4+} complexes shown in Scheme 9b,c undergo photoreduction to form Pt^{2+} species. The mechanism of photoreduction is similar to that described above, except the azide radicals rapidly decompose into molecular nitrogen, thus preventing the reoxidation of the platinum center.[62]

Cis,*trans*,*cis*-$[Pt(N_3)_2(OH)_2(NH_3)_2]$[64] (Scheme 9b) is soluble in aqueous solution, stable in the presence of glutathione, and photolyzes into a complex that binds DNA and 5′-GMP. In addition, the photolyzed complex inhibits the growth of human bladder cancer cells as well as Cisplatin-resistant cells, while cells treated with the complex and kept in the dark showed very little growth inhibition.[65] Changing the NH_3 ligands does not significantly affect the stability of these complexes, therefore derivatives with various amines have been synthesized and characterized.[66]

The distinct anticancer mechanism of *trans* Pt^{2+} compared to *cis* Pt^{2+} complexes has been known for some time,[67–69] and the *trans* photoactive Pt^{4+} complexes also appear to show distinct anticancer properties compared to the *cis* isomers. The all-*trans* isomer, *trans*,*trans*,*trans*-$[Pt(N_3)_2(OH)_2(NH_3)_2]$[70] (Scheme 9c) has enhanced water solubility compared to the all-*cis* version. In addition, the LMCT band of the all-*trans* isomer is shifted to longer wavelengths with a larger extinction coefficient. This isomer is stable in the dark in the presence of glutathione, and is not cytotoxic to human cancer cells. The all-*trans* isomer binds 5′-GMP after exposure to light and is as cytotoxic as Cisplatin, albeit through a different toxicity mechanism. Derivatives of this complex have been studied in depth.[66] Importantly, a derivative where NH_3 is replaced by a pyridine ligand is 90 times more cytotoxic than Cisplatin.[71]

As an alternative to photoactive Pt^{4+} compounds, our research group recently introduced a Pt^{2+} photocaged complex, [Pt(cage)], in which the Pt^{2+} ion is coordinated to the photoactive ligand H_2cage through two amide and two pyridyl nitrogen atoms.[72] The intact complex is unreactive toward ligand-exchange reactions until activation with UV light (350 nm) uncages a Pt complex that more readily exchanges its ligands (Scheme 10). When MCF-7 human breast carcino-

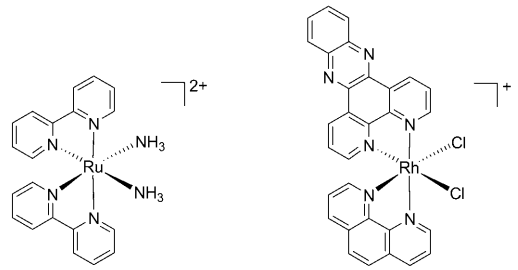


Scheme 10. Photolysis of [Pt(cage)].

ma cells were treated with up to 200 μM [Pt(cage)] in the absence of light, 20% or fewer cells were dead after 96 hours of treatment. However, the cytotoxicity increased by 65% with 2 minutes of irradiation and approached the toxicity response of Cisplatin.

3.1.7. Ruthenium and Rhodium

Ru^{2+} complexes such as *cis*-$[Ru(bpy)_2(H_2O)_2]^{2+}$ and *cis*-$[Ru(phen)_2Cl_2]$ are known to be cytotoxic as they bind to DNA,[73–76] although, like the case of Cisplatin, cytotoxicity is not localized to cancer cells. Therefore, Singh and Turro developed the photolabile octahedral Ru^{2+} complex *cis*-$[Ru(bpy)_2(NH_3)_2]^{2+}$ (Scheme 11).[77] Similar to the Pt^{4+} compounds discussed above, the metal acts as the photosensitive component of the cage. Irradiation of the complex in water results in the loss of the NH_3 ligands and the formation of *cis*-$[Ru(bpy)_2(OH_2)(OH)]^+$. The photolyzed complex binds to 9-methyl- and 1-ethyl-guanine as well as single-stranded and double-stranded DNA. In contrast, no DNA binding was observed in the absence of light.



cis-$[Ru(bpy)_2(NH_3)_2]^{2+}$ DDP$_2$PHEN

Scheme 11. Ru and Rh cages.

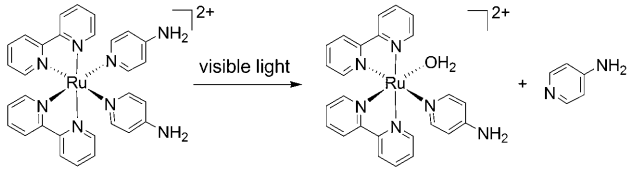
Similarly, Loganathan and Morrison developed octahedral Rh^{3+} complexes[78] such as DDP$_2$PHEN (Scheme 11). These complexes are thermodynamically stable in the dark but are susceptible to hydrolysis when exposed to UV light to form species that readily bind DNA. DPP$_2$PHEN was shown to be cytotoxic to cancer cells and to inactivate an intracellular alphavirus Sindbis (SINV) in vivo following irradiation.[79]

3.2. Type 2 Unlockable Metal Cages: Photoactive Metals

In the second type of unlockable cage, the metal acts as the photoprotecting group that cages a biologically active molecule. In this case, the concept is that irradiation changes the coordination around the metal center to release (i.e., uncage) a molecule that has biological activity. A key difference from Type 1 cages is that the bioactivity of the released ligand is the focus, as opposed to changes at the metal center.

3.2.1. Ruthenium

Etchenique and co-workers recognized that the $[Ru^{2+}(bpy)_2]^{2+}$ core is photosensitive, and coordinated amine-containing neurochemicals to the remaining sites of the octahedral Ru^{2+} center as a means of keeping the molecules unavailable for their normal biological functions.[80,81] Irradiation into the MLCT band of the $[Ru^{2+}(bpy)_2]^{2+}$ center induces decomposition of the $[Ru(bpy)_2(X)_2]$ complex, where X is a neurochemical, to release $[Ru(bpy)_2(X)]$ and one equivalent of neurochemical (Scheme 12). Ru was employed to cage various amine-containing neurotransmitters including 4-aminopyridine (4AP), serotonin, butylamine, tryptamine, and tyramine.[80] The compound where X is 4AP has been shown to operate in a biological system when $[Ru(bpy)_2(4AP)_2]$ is irradiated with visible light above 480 nm and one equivalent of 4AP is released. The free 4AP promotes the activation of a leech neuron by blocking its K^+ ion channels.[81]



cis-$[Ru(bpy)_2(4AP)_2]^{2+}$ *cis*-$[Ru(bpy)_2(OH_2)(4AP)]^{2+}$

Scheme 12. Photolysis of $[Ru(bpy)_2(X)_2]^{2+}$.

One of the bpy ligands was replaced by triphenylphosphine PPh_3 to form $[Ru(bpy)_2(PPh_3)(X)]$. By incorporating only one equivalent of an amine-containing neurochemical, the quantum yield of photolysis increased from 0.03 for $[Ru(bpy)_2(X)_2]$ to 0.21 for $[Ru(bpy)_2(PPh_3)(X)]$.[82] When X is γ-aminobutyric acid (GABA), which is the main inhibitory neurotransmitter in the brain, exposure to light at 450 nm has been shown to activate GABA ion channels in frog oocytes.[82] When the complex was applied in the dark, no cellular changes were observed. The system in which nonleaving groups occupy five of the six coordination sites has been used to cage amino acids such as glutamate.[83] In this case, one of the bpy ligands and the PPh_3 group are replaced by a tridentate chelator, tris(pyrazolyl)amine. To the best of our knowledge, ruthenium-caged amino acids have not yet been tested in vivo.

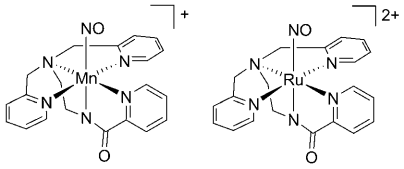
There are a number of light-activated molecules that cage neurochemicals in a similar fashion to the light-activated ATP molecule reported by Kaplan, Forbush, and Hoffman. Like many of the examples discussed in this Minireview, these strategies require UV light with a wavelength of approximately 300 nm to break a covalent σ bond; however, light of this wavelength may cause cellular photodamage. Therefore, an advantage offered by ruthenium-based caging groups is their photoreactivity with light of longer wavelengths.

3.2.2. Metal Complexes that Cage Nitric Oxide

Nitric oxide is a signaling molecule that is implicated in various physiological responses including blood pressure regulation, neurotransmission, immune response, and cell death.[84] Metal complexes that contain a metal–nitrosyl (M–NO) bond have been implemented to liberate NO as a

therapeutic agent or to investigate the roles of NO in biological pathways. Although the first metal-containing NO-releasing drug $Na_2[Fe(NO)(CN)_5]$ is clinically useful for the reduction of hypertension, it is not without drawbacks as the loss of its ancillary ligands causes cyanide toxicity.[85,86] As a result, much effort has been dedicated to the development of new metal complexes with improved stability to prevent the loss of supporting ligands and for the controlled delivery of NO. In particular, the known photosensitivity of many M–NO compounds makes light an ideal trigger for the release of NO with spatial and temporal selectivity.

Mascharak and co-workers developed octahedral metal complexes containing $PaPy_3H$ (*N*,*N*-bis(2-carboxamide; a pentadentate ligand) and NO. Both the Ru[87] and Mn complexes[88] (Scheme 13) were found to be stable at phys-



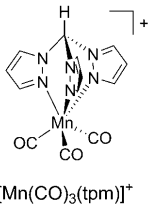
Scheme 13. Light-activated NO-releasing compounds.

iologically relevant pH and are activated with light to release NO. Even though the Ru analogue is activated with UV light and the Mn analogue is activated with visible light (500–650 nm), both have biological application in the activation of soluble guanylate cyclase in vitro, trigger a concentration-dependent increase in cGMP in vascular smooth muscle cells, and elicit vasorelaxant activity in a rat thoracic ring.[89]

Variation of the coordinating ligands has resulted in red-shifts of the wavelengths needed for photoactivation. The coordination of a dye to the metal center not only alters the photochemical properties of the metal complex but also provides a means to monitor cellular distribution. Ford and co-workers incorporated chromophores such as AFchromophore,[90] fluorescein,[91,92] and protoporphyrin IX[93] into metal complexes to increase light absorption and improve NO release at longer wavelengths. Although NO release is not very efficient, these complexes have a two-photon cross section that enables NO release with two-photon excitation with 810 nm femtosecond pulsed light.[94]

3.2.3. Metal Complexes that Cage Carbon Monoxide

Carbon monoxide is another important signaling molecule that is implicated in some of the same pathways as NO, as well as other physiological processes.[95] Like NO, the controlled release of CO can be used to study the roles of CO in various pathways or administered as a therapeutic agent. The development of CO-releasing molecules, or CORMS, has therefore become an active area of research.[96,97] Whereas most CORMs release their CO as a result of hydrolysis or ligand exchange reactions, Schatzschneider and co-workers have introduced photolabile CORMS in an effort to control the release of high concentrations of CO.[14,98,99] The octahedral Mn complex $[Mn(CO)_3(tpm)]^+$ (Scheme 14), has been shown to release two equivalents of CO to myoglobin upon activation with 365 nm light.[99] This compound is nontoxic in vivo to human colon cancer cells in the dark, however, in the presence of light it displays photoinitiated cytotoxicity.[99] An intrinsic benefit of these CO-containing molecules is the ability to use Raman microspectroscopy to image their cellular uptake and distribution.[100]



Scheme 14. Light-activated CO-releasing compound.

4. Summary and Outlook

Photolabile metal cages can be used as tools for manipulating the bioavailability of metals or their coordinating ligands in order to study biological pathways or for potential therapeutic purposes. The scope of different biological applications for photolabile transition-metal cages is particularly exciting. As this field of research continues to expand, questions of light toxicity are likely to increase. Future developments will therefore need to focus on increasing the wavelengths of activation while maintaining photolysis efficiency. It will be interesting to see how these compounds will be developed over the next few years to aid in the study of metal trafficking and in the discovery of new applications for caged complexes in biology and medicine.

5. List of Abbreviations

4AP	4-aminopyridine
ATP	adenosine triphosphate
bpy	2,2′-bipyridine
CO	carbon monoxide
CORM	carbon monoxide releasing molecule
EGTA	ethylene glycol tetraacetic acid
en	ethylenediamine
GABA	gamma aminobutyric acid
GMP	cyclic guanosine monophosphate
HSAB	hard soft acid base
LMCT	ligand-to-metal charge transfer
MLCT	metal-to-ligand charge transfer
NO	nitric oxide
PDT	photodynamic therapy
phen	phenanthroline
PPh_3	triphenylphosphine

tpm	tris(pyrazolyl)methane
UV	ultraviolet
UVA	315–400 nm
UVB	280–315 nm
UVC	100–280 nm

We thank the Sloan Foundation, the Camille and Henry Dreyfus Foundation, the National Science Foundation, and Duke University for supporting various aspects of our work in this area.

Received: April 28, 2010
Published online: December 17, 2010

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