SUMMARY
Bioorthogonal chemistry offers powerful tools to study and manipulate biological systems without interfering with the biological processes. Of paramount interest is the possibility to exploit bioorthogonal tools to deliver novel therapeutic applications. Bioorthogonal prodrug activation is one of the most exciting areas of research and is currently progressing toward phase I clinical trials. Despite their limitations in biological media, transition-metal catalysts have been engineered to enact abiotic cofactors and trigger bond-breaking reactions widely employed for the release of small molecules from masking groups. Here, we highlight the challenges and opportunities of metal-mediated bioorthogonal cleavage reactions for the release of cargo molecules and other emerging applications such as protein activation and RNA degradation.

Bioorthogonal chemistry has the potential to deliver myriad, exciting biomedical applications. Over the past 20 years, several techniques have been established to manipulate biological systems. The biggest challenge in chemical biology is to successfully transition bioorthogonal chemistries into humans. Bertozzi and co-workers—and subsequently many other scientists—identified bioorthogonal ligation as an excellent tool to study biological systems. These findings have been applied to murine models, and several approaches are rapidly progressing toward the clinic. Bioorthogonal reactions that uncage or trigger bioactive molecules are also extremely useful in biomedicine. Many different approaches have been adopted for the release of drug molecules, such as pericyclic reactions, transition metals, and photo-triggered reactions. This review focuses on metal-mediated reactions that result in the bioorthogonal bond cleavage of cargo-bearing masking groups.

Nature has evolved to integrate earth-abundant metals inside living systems. Life’s catalytic machinery depends on these metals. Nevertheless, precious metals, such as ruthenium, gold, palladium, and platinum, are active under physiological conditions and have been extensively studied for dissociative reactions. The idea is to have access to abiotic, highly specific reactions that enable the gain of function in a biocompatible environment. This type of reaction relies on the potential of transition-metal catalysts to “uncage” a cargo of interest (e.g., a drug molecule) through specific bond cleavage (Figure 1). The cargo of interest is initially made inactive by a masking group which allows control and localization of a chemical event upon addition of the metal. This strategy recalls the concept of antibody-directed enzyme prodrug therapy (ADEPT) introduced in the 1970s, in which enzymes are directed to the site of a tumor to catalyze the bioorthogonal activation of prodrugs. Similarly, and without immunogenicity issues faced by the ADEPT strategy in the clinic, the metal catalyst exerts its activity specifically toward the masking group within a biological system. Metal complexes have therefore led to numerous applications in prodrug activation and protein gain of function. Here, we discuss the several strategies
employed for bioorthogonal release, their current limitations, and future perspectives of transition metal triggered uncaging chemistry in biology and medicine.

METAL COMPLEXES

This section gives an overview on the metal complexes used in bioorthogonal uncaging reactions. These focus primarily on five transition metals: Ru, Pd, Au, Pt, and Cu. Figure 2 displays the most prominent strategies developed for the metal-mediated bioorthogonal release of cargoes.

Deallylation

Streu and Meggers introduced in 2006 the first Ru-catalyzed deallylation reaction by means of a [Cp*Ru(cod)-Cl] catalyst (Figure 2Aa).23 The catalyst required the presence of thiophenols to work well, but optimization of the catalysts with a quinoline-based ligand (Figure 2B) led to full uncaging and high turnover numbers.24 This seminal study led to the intracellular release of allyloxycarbonyl (alloc) protected rhodamine by an organometallic Ru(II) complex in HeLa cells. Later, Völker et al. reported Ru-mediated deallylation to uncage bioactive agents.25 Besides prodrug activation, Ru-triggered deallylation proved to be a useful tool for site-specific protein modification in Escherichia coli by Schultz and coworkers,26 whereas Mascaren˜as and colleagues used the same reaction for DNA binder activation in mammalian cells.27 Deallylation reactions allow localization of specific compartments of the cell and thus enhance spatiotemporal control. Mascaren˜as and colleagues used a ruthenium complex based on 2-quinolinecarboxylate ligands bearing a pyrenephosphonium group (Figure 2B) that accumulates in the mitochondria.28 Addition of 2-(allyloxy)-1,4-dinitrobenzene generates the uncoupling agent 2,4-dinitrophenol, which stops ATP production. This study shows how bioorthogonal deallylation affects a specific compartment without interfering with the rest of the cell.

The optimization of the ligand often boosts the catalytic activity, leading to higher turnover numbers in biocompatible media; however, the Chen group demonstrated that deallylation reactions can also occur with simpler Pd-based catalysts, such as allyl2Pd2Cl2 and Pd(dba)2, albeit in the presence of a nucleophile that restores the catalytic cycle.29 Our group has reported on Pd(II) salts that can be reduced “on

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**Reaction:**

(a) Deallylation

\[ \text{[Pd] [Au]} \]

(b) Depropargylation

\[ \text{[Pd] [Ru]} \]

(c) Deallenylation

\[ \text{[Pd]} \]

(d) Intramolecular Cyclisation

\[ \text{[Pt] [Au]} \]

**Catalysts (reactions):**

- Pd(PPh3)4 \((\text{ai, bi})\)
- PdCl2(TFP)2 \((\text{aii, bii})\)
- Pd (0) Resin \((\text{ai, bi})\)
- Na2PdCl4 + NaAsc \((\text{ai, aii})\)
- K2[PtCl4] \((\text{bii, di, dii})\)
- Pd(TPPTS)4 \((\text{ci})\)
- Cu(I)-BTTA \((\text{bii})\)
- CisPt \((\text{di})\)
- NaAuCl4 \((\text{dii})\)
- Au-Nanoparticles \((\text{ai, bi, bii})\)

**Figure 2.** Masking groups and metal triggers used in bioorthogonal uncaging reactions

(A) Different reaction types including (a) deallylation, (b) depargarlylation, (c) deallenylation, and (d) intramolecular cyclization.

(B) Main metal catalysts used for the bioorthogonal release of compounds.
demand” to the active species Pd(0) using sodium ascorbate (NaAsc). The in cellulo Pd reduction offers a further degree of control in bioorthogonal prodrug activation.

**Depropargylation**

The propargyl group can also be removed upon coordination of a variety of precious metals (Figure 1Abi–iii). Similarly to alloc, propargyloxy carbonyl (proc) can be used as a bioorthogonal masking group. By using the proc group, Chen and coworkers reported in 2014 the first metal-mediated protein activation in living cells. In this study, a genetically encoded lysine bearing the proc moiety was effectively removed by allylPdCl2 to restore the enzymatic activity of the bacterial phosphothreonine lyase OspF (Figure 3A). More recently, Mascareñas and coworkers demonstrated that a synthetic metallopeptide formed by Pd(II) and a bis-histidine peptide scaffold could efficiently promote depropargylation reactions inside mammalian cells (Figure 3B). In this work, Pd(II) was induced to bind the two histidine residues at i and i+4 positions of bZIP transcription factor to generate a stable peptide-palladium complex with cell penetration ability. The catalytic activity of the peptide-palladium complex in living cells relied on a suitable Pd source, such as [Pd(COD)Cl2] (COD = 1,8-cyclooctadiene), and on the efficient cell internalization of bis-histidine peptide, which could be improved with abundant arginine residues. The peptide scaffold acted as a protective shield to stabilize and protect the Pd center and also facilitated the translocation of the whole catalytic complex into living mammalian cells.

Gold species are also known to perform depropargylation reactions. Au(III) species embedded on a TentaGel resin can efficiently remove propargyl groups in PBS buffer. Copper-mediated cleavage of alkynes extended the applicability of depropargylation reactions for the rescue of amino or phenol groups. Chen and co-workers used a Cu(I)-BTTAA catalytic system to release cytotoxic payloads from antibody-drug conjugates (ADCs) and site-specifically regulate ligand-receptor interactions via reversible modification. Internal alkynes also offer the possibility of site-selective protein modification and bioorthogonal drug release, as demonstrated by our group. We reported on a linker containing the prodrug Proc-doxorubicin, which is easily appended to cysteine-bearing proteins to form an anti-HER-2 nanobody conjugate. Upon addition of [Pd(COD)Cl2], cytotoxic doxorubicin is released and selectively kills cancer cells that overexpress the HER2 antigen (Figure 3C). More examples of depropargylation reactions with heterogeneous catalysts will be given in the section on nanodevices.

**Deallenylation**

Inspired by the mechanism of transition-metal-mediated depropargylation, the Chen group reasoned that allenyl groups might be susceptible to palladium catalysts and could be used to uncage hydroxy groups (Figure 1Aci). The chemistry was demonstrated for different allene derivatives, and a Pd(TPPTS)4 catalyst was used to uncage modified tyrosine residues. Although this is a welcome addition to the bioorthogonal toolkit, it is still to be determined whether deallenylation reactions add further value to uncaging reactions or can be simply used as a replacement to deallylation or depropargylation reactions.

**Intramolecular cyclization**

Besides the alkyl deprotections, researchers have explored different ways to unleash small molecules by using transition-metal catalysis. In recent years new masking groups were introduced, which lead to intramolecular reactions that follow cargo release upon introduction of the transition metal. Our group reported Pt complexes
(II and IV) that cleave alkyne-bearing masking groups in living systems that can be used to release secondary amines as active drugs or cleave ADCs (Figure 2Adi).37 The carboxamide acts as an internal nucleophile that cyclizes and displaces the secondary amine following a hydration step. The chemotherapeutic drug cisplatin (a Pt(II) complex) activated 5-fluoro-1-propargyl-uracil (pro-5FU), which resulted in tumor shrinkage in a zebrafish xenograft model when the drug (cisplatin) and prodrug (pro-5FU) were added together. Similarly, Tanaka and coworkers used N-heterocyclic carbene (NHC)-Au(I) complexes to release anticancer drugs as secondary

Figure 3. Examples of metal-mediated bioorthogonal cleavage in chemical biology
(A) Protein modification/activation by selective cleavage of masked amino acids. (B) In cellulo depropargylation via bis-histidine metallopeptide. Pd(II) binds the two histidine residues at i and i+4 positions of bZIP transcription factor to generate a stable peptide-palladium complex with cell penetration ability. (C) Targeted delivery of produgs to selectively kill cancer cells. An anti-HER2 nanobody with an internal propargyl thioether is cleaved upon coordination with Pd(COD)Cl₂ metal, which releases doxorubicin in the proximity of HER2-positive cancer cells. (D) Cu(I)-mediated RNA degradation. The metal binds the imidazole ligand covalently attached to the tagged RNA, which is degraded via transition-metal-induced generation of ROS that leads to oxidative damage.
amines upon intramolecular nucleophilic attack of a 2-alkynylbenzamide moiety followed by hydrolysis (Figure 2Adii). Besides deallylation, ruthenium can also trigger uncaging reactions through olefin metathesis. While cross-metathesis has been used for bioorthogonal ligations and protein modifications, ring-closing metathesis can release small-molecule cargoes following an aromatization step as demonstrated by Ward and coworkers (Figure 2Adiii). This “close-to-release” technology has good potential, as the masking group can be further derivatized and employed in heterogeneous catalysis or bioconjugation.

In addition to prodrug activation and protein modification, transition metals play other significant roles in chemical biology. Our lab has recently unraveled the role of Cu(I) in RNA degradation. We developed an RNA modulator that catalyzes the cleavage of RNA phosphodiester bonds in a copper-dependent manner. The strategy relies on an imidazole ligand covalently attached to tagged RNA strands. When the Cu(I) is bound to the imidazole, the complex triggers the generation of reactive oxygen species (ROS), leading to formation of radical nucleotide species causing oxidative damage and degradation of the proximal RNA (Figure 3D). These click-degraders are important tools for the study of RNA modification and can be further exploited in precision therapy.

Several transition metals as complexes or simple salts are effective tools for the bioorthogonal release of cargo molecules and protein modification. Future research needs to provide better strategies that benefit from the catalytic efficiency of transition metals in a biocompatible environment. A better mechanistic insight into these reactions in complex media will help achieve faster release kinetics. While the metals guarantee an excellent degree of bioorthogonality and specificity toward masking groups, there are some concerns with regard to the stability and toxicity of these metals in biological systems. In particular, soft transition metals are easily poisoned via covalent binding with free thiols in the form of glutathione, which is ubiquitous and present in high concentrations (mM) in the cytoplasm of mammalian cells. Further efforts will focus on keeping the catalytic species active in biological media for an extended period of time.

NANODEVICES
Heterogeneous catalysts also offer potential for bioorthogonal uncaging reactions in living systems. The added value of nanodevices lies in the optimization of delivery systems that can be integrated in a biological system and at the same time protect the metal from the hostile biological media. The first examples of bioorthogonal uncaging mediated by a metal-loaded nanodevice were described by Bradley and coworkers, who performed intracellular deallylation (Figure 2Aai) and Suzuky-Miyaura cross-coupling for the activation of profluorescent probes with Pd nanoparticles (Pd-NPs) captured at the surface of polystyrene beads. These early works sparked interest in the potential of transition metals as heterogeneous catalysts to unmask bioactive molecules. Further optimization highlighted the capacity of Pd-NPs to mediate depropargylation reactions for the in vivo activation of clinical anticancer drugs (Figure 4A).

The Unciti-Broceta group expanded the applicability of NPs to gold-catalyzed depropargylation, reporting the first controlled in vivo release of a fluorescent dye into the brains of zebrafish. In a follow-up study recently published, the researchers coated the outer layer of a polymer scaffold with Au-NPs to yield a brain-penetrating catalyst able to uncage the anxiolytic agent fluoxetine in a zebrafish model.
Figure 4. Overview of nanodevices employed for the bioorthogonal release of small molecules

(A) Pd(0)-mediated prodrug activation through depropargylation.
(B) Pd(0)-nanosheet hydrogel releases anticancer drug paclitaxel.
(C) Fe-based azide reduction of nanozymes.
(D) Light-triggered activation of Pd(0)-NPs.
(E) Pd-NPs loaded into cancer-derived exosomes.
(F) Dual nanoparticles strategy with encapsulated prodrug and Pd(0)-NPs to release monomethyl auristatin E (MMAE) or doxorubicin.
Au-microimplants are truly catalytic in biocompatible media and can be recycled to achieve an overall turnover number of 40 in depropargylation reactions. This is the first example of bioorthogonal decaging known to affect the neural networks of a living system.

Toward the development of biodegradable, implantable catalytic systems, Perez-López et al. reported the use of natural hydrogels (agarose and alginate) to entrap ultrathin Pd nanosheets in solid polymeric frameworks that allow the internal diffusion of small molecules (Figure 4B). This strategy provides a catalytic system that temporarily promotes local bioorthogonal reactions, which would be ideal for short-term applications (e.g., neoadjuvant chemotherapy). Interestingly, the authors designed a Pd-activatable prodrug of paclitaxel by using a protecting group tailor-made for the aliphatic –OH.

A new class of nanodevices called nanozymes are used for selective intracellular activation (Figure 4C) through endogenous activation of bioorthogonal nanozymes. Nanozymes comprise Au-NP cores and self-assembling monolayers to facilitate the incorporation of hydrophobic Ru, Pd, and Fe complexes within its lipophilic monolayer. Rotello and coworkers reported on nanozymes that are initially made inactive by tethered supramolecular anchors (cucurbiturils). Displacement of the anchors through host-guest interactions with 1-adamantylamine yields the active nanozyme that can accommodate prodyes and prodrugs to undergo dealkylation reactions. Nanozymes were also tested as potential antibiotic treatment. Iron-based nanoparticles were assembled in polyzemes able to penetrate into biofilms and kill bacteria upon activation of a proantibiotic via Fe-mediated reduction of aryl azides to the corresponding self-immolating amine (Figure 4C). Further research by the Rotello group has led to the intracellular activation of nanozymes using protein corona. The authors observed that hard “irreversible” corona non-incorporating tetraethylene glycol (TEG) deactivated nanozymes through aggregation and steric blocking. The catalytic activity of the nanozymes was restored after proteolytic degradation of the protein corona through endogenous proteases present in the endosome and lysosome. Hence, a selective intracellular activation system (without TEG) was obtained by engineering the monolayer of ligands on nanoparticles. Tuning the formation of the protein corona on nanozymes leads to specific activation of a profluorescent dye via Ru-based bioorthogonal deallylation. This strategy could reduce off-target effects and extend on-demand generation of imaging agents and localized therapeutics. Similar, “on-demand” activation was achieved by Wang et al. by using silica-based Pd-NPs (Figure 4D). In this case, spatiotemporal control was achieved by UV activation with Pd-NPs made inactive by β-cyclodextrin. The azobenzene groups that are complexed to the Pd-NPs trigger the activation under UV light.

The development of Pd nanodevices that can perform uncaging chemistries inside cells requires robust vectors that are able to enter cells with high efficacy and are compatible with the complex intracellular environment (e.g., redox and pH). Recently, the therapeutic potential of metal-based nanodevices has been improved by combining the catalytic activity of Pd nanodevices and the targeting capabilities of delivery systems, such as exosomes. Sancho-Albero et al. engineered cancer-derived exosomes with Pd nanosheets (Pd-exo) for site-selective delivery and drug activation (Figure 4E). Pd-exo have the unique ability to enter exclusively the cancer cells from where they were derivatized and perform bioorthogonal uncaging reactions while leaving other cell types unaffected. The authors demonstrated the selectivity of Pd-exo549 by activation of the anticancer drug panobinostat in lung...
cancer A549 cells, but not in U87 (a glioma cell line), which shows preferential tropism for their progenitor cells. Opposite results were observed in the reverse situation: Pd-exo originating from U87 cells displayed a drug-mediated cytotoxic effect in U87 cells superior to that in A549 cells.

One of the key advantages of using heterogeneous or encapsulated transition-metal catalysts in nanoscale carriers is the potential to achieve intratumoral accumulation in vivo through enhanced permeability and retention effects. Miller et al. demonstrated this possibility by using poly(lactic-co-glycolic acid)-polyethylene glycol (PLGA-PEG) polymeric micelles to encapsulate PdCl2(TFP)2 and deliver the catalyst into tumor xenografts (Figure 4F).57 Separate administration of an alloc-protected prodrug of doxorubicin58 (also encapsulated in PLGA-PEG polymeric micelles) achieved site-selective delivery of both formulations, which upon reaction elicited anticancer activity in a fibrosarcoma model in mice. The versatility of this approach was expanded in vivo with the delivery of an encapsulated prodrug of the extremely potent toxin, monomethyl auristatin E.

Nanodevices are extraordinary tools for drug delivery. Such systems allow the encapsulation of transition-metal catalysts, which are protected from the surrounding environment while being directed to the target area. The nanocompartments provide an environment in which prodrugs can easily diffuse and be subjected to activity exerted by the metals. The great advantage of these nanodevices is the potential to reach and accumulate into solid tumors owing to their tunable permeability and retention properties.

ARTIFICIAL METALLOENZYMES

Transition metals that are heavily exposed to the biological environment have a higher risk of generating inactive species, which results in limited activity. To harness metal-mediated bioorthogonal transformations toward biomedical applications, it is crucial to develop strategies that protect the catalyst. Researchers have developed artificial metalloenzymes (ArMs) in the attempt to mimic the extraordinary efficiency of natural enzymes.59,60 ArMs are hybrid catalysts composed of an organometallic moiety embedded into a protein scaffold by using different anchoring strategies.61 Ward and coworkers developed several uncaging strategies with artificial metalloenzymes based on supramolecular anchoring that exploited the exceptional affinity between biotin and streptavidin.62 Notably, Okamoto et al. engineered an ArM with a cell-penetrating poly(disulfide) that triggered the uncaging of the thyroid hormone triiodothyronine in live mammalian cells via Ru-triggered deallylation (Figure 5A).63 The same group developed another artificial metalloenzyme based on dative anchoring between arylsulfonamides and carbonic anhydrase IX to release fluorescent probes by Ir-based asymmetric transfer hydrogenation (Figure 5B).64 Samanta et al. used encapsulated ArMs to investigate the lifelike behavior of DNA protocells with a metathesis-triggered uncaging reaction (Figure 5C).65 Tanaka and coworkers have recently developed an albumin-based ArM for improved biocompatibility in metathesis reactions.66 The ArM tolerates the “poisonous” glutathione, which offers the prospect of metathesis-based in vivo uncaging reactions.

Artificial metalloenzymes present biomimetic properties that could help to overcome some of the limitations of metal-mediated uncaging reactions. Directed evolution will certainly play an important role in the optimization of such systems and confer biomimetic properties. Although the use of ArMs in bioorthogonal cleavage reactions is a very recent development, they have already found several interesting
applications. One of the key limitations remains the lack of intracellular activity of ArMs as a result of their poor cell penetration or thiol-mediated metal poisoning.

CONCLUSIONS AND OUTLOOK

The latest advances in bioorthogonal chemistry have shown great promise and progress in the use of transition-metal catalysis for new biomedical applications. Metal-mediated bioorthogonal release is a well-established strategy for the selective activation of therapeutically relevant molecules. To date, most in cellulo studies have focused on deprotection reactions of allylic or propargylic functionalities. Although these groups are easily cleaved by a variety of metals in biological media, derivatization of the masking group is more challenging. Recently, two uncaging
strategies developed from the Tanaka group and the Ward group aimed to address the limitations in derivatization of the masking groups. Such improvements allow one to covalently append a variety of (bio)molecules to the masking group. New approaches to perform bioorthogonal release in biological systems are currently being investigated, and future developments will likely focus on fine-tuning target selectivity and improving long-lasting catalytic activity in vivo to maximize abiotic catalysis with precious metals. Nanodevices and artificial metalloenzymes are proven effective approaches to address the limitations of bare metal catalysts. Although they still present some limitations (e.g., poor cell penetration, low catalytic activity in cells), they present some excellent features such as target selectivity and enhanced drug delivery. Moreover, they actively shield the transition-metal catalysts from direct contact with intercellular/intracellular environments, which is essential to prolong a catalyst’s lifespan such that bioactive levels of drug release at the disease site can be achieved. Other than prodrug activation, precious metals are exceptional chemical biology tools for bond-cleavage reactions to study and modulate biological processes.67 Using these tools to unravel hitherto unknown biological mechanisms will be extremely useful for the validation of new therapeutic targets. Although very challenging, there is hope that in the close future transition metals will catalyze abiotic bond-breaking reactions in vivo and with enzyme-like properties.

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AUTHOR CONTRIBUTIONS

V.S. organized the literature and wrote the manuscript. V.B.U. edited the figures and revisited the literature. G.J.L.B. supervised the work and co-wrote the manuscript. All authors agreed to the final version of the manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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