Catalysis Concepts In Medicinal Inorganic Chemistry

Silvia Alonso-de Castro,[#[a]] Alessio Terenzi,[#[a]] Juan Gurruchaga-Pereda,[[a,b]] and Luca Salassa*[[a,c]]

[a] Dr. S. Alonso-de Castro, Dr. A Terenzi, J. Gurruchaga-Pereda, Prof. L. Salassa
Donostia International Physics Center, Paseo Manuel de Lardizabal 4, Donostia, 20018 (Spain) E-mail: lsalassa@dipc.org
[b] J. Gurruchaga-Pereda
CIC biomaGUNE, Paseo de Miramón 182, Donostia, 20014 (Spain)
[c] Prof. L. Salassa
Ikerbasque, Basque Foundation for Science, Bilbao, 48011 (Spain)
[#] Authors contributed equally

Abstract: Catalysis has strongly emerged in the medicinal inorganic chemistry field as a suitable tool to deliver new drug candidates and overcome drawbacks associated to metallodrugs. In this concept article, we discuss representative examples of how catalysis has been applied in combination with metal complexes to deliver new therapy approaches. In particular, we explain key achievements in the design of catalytic metallodrugs that damage biomolecular targets and in the development of metal catalysis schemes for the activation of exogenous organic prodrugs. Moreover, we discuss our recent discoveries on the flavin-mediated bioorthogonal catalytic activation of metal-based prodrugs; a new catalysis strategy in medicinal inorganic chemistry in which metal complexes are unconventionally employed as substrates rather than catalysts.

Introduction

The capacity of catalysis to amplify chemical signals and its intrinsic selectivity are features that can be harnessed in drug development to lower drug dosage and limit off-target effects. These potential advantages are of particular significance in the case of metallodrugs for which concerns on the safety of transition metals are regularly brought up. Additionally, different mechanisms of action are normally associated to catalytic drugs, which can in principle help avoiding drug resistances.[1]
So far, two general strategies have been adopted in the implementation of catalysis for drug design (Figure 1).

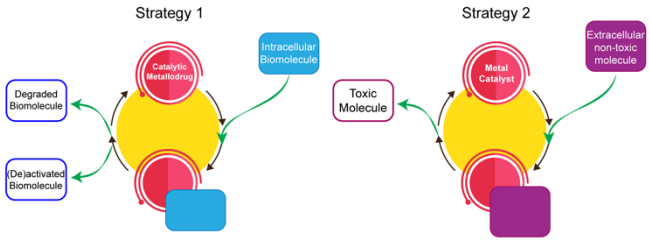

Figure 1. Catalysis approaches used in drug design.

Traditionally, researchers have conceived metallodrugs to function as enzyme mimics for the catalytic cleavage or modification of targeted biomolecules.[2] However, more recently, metal-based catalysis and bioorthogonal chemistry have merged to deliver new approaches that enable the activation of exogenous substrates, such as organic prodrugs, in biological environments.

Bioorthogonal reactions are abiotic chemical modifications that can be performed within living biological systems, such as cultured mammalian cells or even complex organisms (e.g. zebrafish or mice) without interfering with their host biochemistry. The term bioorthogonal was coined 15-years ago by Bertozzi to

describe the use of Staudinger ligation reactions for the selective modification of glycoproteins with biochemical and biophysical tags.[3,4] In drug development, bioorthogonality principally refers to the capacity of a catalytic process to occur selectively with minimal o without unwanted side reactions, yet ultimately triggering a biological response and inducing therapeutic effects.

A number of recent review articles revise the achievements of catalytic metallodrugs and metal-based bioorthogonal catalysis.[1,5–11] In this Concept article, we instead aim at picking out key examples and concepts that have laid the foundations for the use of catalysis in medicinal inorganic chemistry and relate them to recent discoveries obtained in our laboratory. To this end, we put our focus on how innovative catalyst design hypotheses have opened to new chemistries for application in therapy.
The nature of substrates determines the biological target and the biological mechanism of action of the drug candidate, *i.e.* damaging of nucleic acids or proteins, induction of oxidative and reductive stress and activation of synthetic prodrugs. However, fundamental advances in this area have come from the introduction of efficient and robust catalysts. These need to survive and perform in extremely demanding environments, where high concentrations of biomolecules and reactive chemical species can easily interfere and deactivate the catalytic process.

We have organized this brief discussion in three sections that illustrate the different approaches adopted in the implementation of catalysis schemes in the context of therapy:

- Catalytic metallodrugs;
- Transition metal-mediated bioorthogonal catalysis;
- Metal substrate bioorthogonal catalysis.

1. Catalytic metallodrugs

The great majority of catalytic metallodrugs are either (a) metal compounds that induce direct catalytic degradation of biomacromolecules such as proteins, nucleic acids and oligosaccharides, eventually provoking a lethal biological response (Figure 2); or (b) metal complexes that prompt a mismatch in the cellular concentration of endogenous oxidizing and reducing agents, ultimately causing cell death by either oxidative or reductive stress (Figure 3).

The capacity of metal ions and complexes to catalytically degrade biomacromolecules via hydrolytic or oxidative cleavage has been well documented.[12,13] Sigman and co-workers pioneered this approach in the late 70's discovering that $[Cu(phen)_2]^+$ (phen = 1,10-phenanthroline) could induce oxygen-dependent catalytic cleavage of DNA.[14,15] Following this example, numerous monometallic and bimetallic artificial nucleases have been synthesized over the years as biological tools (e.g. footprinting, gene editing) and candidates for therapy.[16–19] Similarly, demonstration of the hydrolytic activity towards peptide bonds of complexes such as Co^{III}-trien/en and Fe^{III}-EDTA complexes (trien = triethylenetetramine, en = ethylenediamine and EDTA = ethylenediaminetetraacetic acid),[20,21] stimulated the design of artificial proteases to tackle protein-related diseases.[22]
Nevertheless, breakthroughs in the development of catalytic metallodrugs were obtained introducing the concept of target-selective artificial enzyme, that is synthetic catalysts hydrolyzing oligopeptides and proteins in a selective manner.[2] Agents of this kind are characterized by a metal-based catalytic center coordinated by a ligand, bearing an organic moiety that recognizes the target biomacromolecule.
In the 90s', Sigman and collaborators redirected the nuclease activity of their Cu^{II} complexes to the targeted cleavage of enzymes. Representative is the example of **1** (Figure 2), a compound coordinating a phen ligand which incorporated a benzenesulfonamide inhibitor of the carbonic anhydrase enzyme. This inhibitor-Cu^{II} conjugate showed sub-μM affinity for human and bovine carbonic anhydrase. In the presence of sodium ascorbate, **1** catalytically cleaved the enzyme within the active site to yield discrete fragments.[23]
Suh and coworkers developed a few years later artificial proteases combining the $[Co^{III}(cyclen)(OH_2)_2]^{3+}$ scaffold with targeting vectors selected among hundreds through a combinatorial approach. For example, complex **2a** catalyzed the cleavage of peptide deformylase (k_{cat} = 0.05 h^{-1}), whereas **2b** mediated the deactivation of amyloid ß oligopeptides with a 30% cleavage yield. The proteolytic activity of these synthetic enzymes is of relevance for the development of new antibiotics and drugs against Alzheimer's disease, respectively.[24,25]

The amino-terminal Cu^{II}- and Ni^{II}-binding motif (ATCUN) is a metal-binding site found in the N-terminus of several naturally occurring proteins. Cowan's laboratory demonstrated the versatility of this peptide fragment for the coordination of several transition metal ions and the development of metallodrug candidates able to target and catalytically cleave nucleic acids, enzymes and sugars (**3a-d**, Figure 2). For example, **3a** functionalized with the inhibitor lisinopril catalytically cleaved and inactivated the somatic angiotensin-converting enzyme (k_{cat} = 150 min^{-1}) in the presence of redox co-reagents (ascorbate or H_2O_2).[26] The same Cu derivative cleaved the hepatitis C virus RNA (k_{cat} = 0.53 min^{-1})[27] when tagged with a YrFK-amide peptide (**3b**) or the HIV1 RNA when the Rev peptide was employed instead (**3c**). In this latter case, catalytic cleavage was also demonstrated in *Escherichia Coli* and in mammalian Jurkat cell lines.[28] Complex **3d** is an artificial glycosidase and was obtained attaching the fucose-selective odorranalectin binding domain to the Cu-ATCUN unit. The conjugate exhibited selective cleavage reactivity toward L-fucose over D-glucose in H-trisaccharides (k_{cat} 1.49 min^{-1}). This process may find application in the removal of specific antigens from erythrocytes and is of relevance for the conversion of regular human type-O blood into a blood substitute for rare blood types.[29]
Cells maintain a delicate redox balance by fine-tuning the concentration of oxidizing and reducing agents. This balance can be disrupted by oxidative or reductive stress, that is inducing a mismatch between reactive oxygen and nitrogen species (ROS and RNS) and intracellular antioxidants.[30] As a result, the structure of cellular components may be damaged, and the normal functioning of the cell altered.[1] Thus, the modulation of the cellular redox equilibrium is an attractive strategy in which applying metal-based catalysts for therapy. The capacity of catalysis to boost reaction kinetics is determinant in this type of strategy since cells have efficient machinery capable to cope with oxidative and reductive stress.

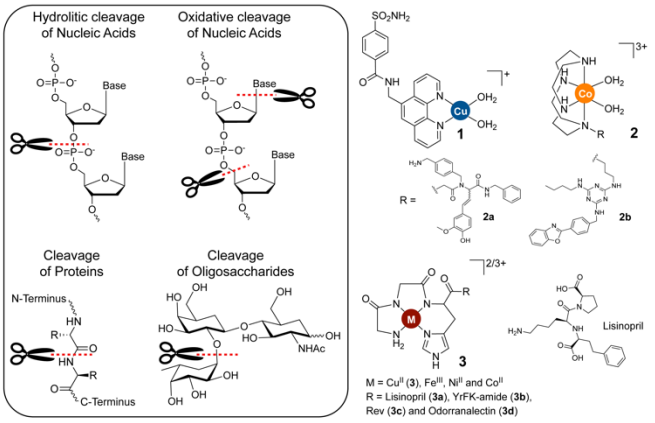

Figure 2. Cleavage reactions and chemical structures of catalytic metallodrugs employed in the degradation of biomolecules.

Photodynamic therapy (PDT) is the prototypical example of catalysis-based therapeutic approach affecting the cell redox balance. This clinically approved treatment relies on the photoinduced catalytic production of $^1O_2$ and ROS in tumors (Figure 3). Despite several PDT agents have been approved for clinical use,[31] there is an established interest in pursuing the discovery of new photosensitizers and extending the use of metal complexes in PDT. Few have emerged as promising PDT agents and have been tested in clinical trials including the polypyridyl Ru complex TLD1433 (**4**) which recently completed phase Ib trials.[31]
Metal-based catalysts have also been explored as analogues of the enzyme superoxide dismutase (SOD) for the management of oxidative stress under physiological and pathological conditions. SOD is an endogenous and first-line-of-defense enzyme that eliminates the superoxide anion O_2^- into O_2 and H_2O_2 via disproportionation reactions (Figure 3).[32] Among the numerous SOD mimics,[30] the Mn^{II} complexes Calmangafodipir (**5**) and M40403 (**6**) underwent clinical trial for metastatic colorectal cancer[33] and metastatic melanoma and renal carcinoma,[34] respectively.

Another way by which metal complexes can alter the redox balance of cells is interfering with the activity of biological thiols that are key components in the control of the cellular redox homeostasis.[35] The tripeptide glutathione (GSH) is the most important among the biological thiols[35] and exerts its antioxidant activity

through conversion into glutathione disulfide (GSSG, Figure 3). Besides, GSH detoxifies endogenous and exogenous toxins, including metal complexes, converting them into GS-X adducts.[36]
Sadler and co-workers first reported catalytic drugs whose activity directly influenced cellular GSH/GSSG levels. In the catalytic oxidation of GSH, the phenylazopyridinato-Ru^{II} catalysts **7a** and **7b** (Figure 3) achieved turnover numbers (TON) and frequency (TOF) of approx. 40 and 0.32 h^{-1}, respectively. The interplay between the metal center and the ligand azo group had a crucial role in the catalysis mechanism since free ligands were not able to catalyze the reaction. Cell viability of A2780 human ovarian and A549 human lung cancer cells treated with **7a** and **7b** was significantly affected (IC_{50} 2–6 μM), as result of the depletion of GSH and increase of ROS levels.[37]

The NADH/NAD^+ (nicotinamide adenine dinucleotide) couple is critical in the maintenance of cellular redox balance and antioxidant defences of cells. Variation of the NADH/NAD^+ equilibrium is a potential strategy to kill cancer cells, in which high levels of oxidizing species are produced due to their intrinsic high metabolic rate. Transition metal complexes catalyse transfer hydrogenation reactions, in which a hydride ion from a donor molecule (e.g. formate) reduces NAD^+ to produce NADH.
Steckhan and coworkers reported at end of the 80's the first examples of metal catalyst capable of regenerating NAD(P)H via $NAD(P)^+$ reduction in a buffered solution. In the presence of formate, their $[(Cp^*)Rh(bpy)Cl]^+$ (where Cp* = pentamethylcyclopentadienyl and bpy = 2,2′ -bipyridine) complex catalysed the reduction of $NAD(P)^+$ with TOF approaching 80 h^{-1}.[38] Several years later, the Sadler's group proved that NAD^+/NADH conversion could modulate the redox status of cells and prompt cancer cell death. In their study, Soldevilla *et al.* employed water-soluble Noyori-type Ru^{II} sulfonamido ethyleneamine complexes such as **8** (Figure 3) that were able to reduce NAD^+ to NADH, although with much lower TOF values (0.2–7 h^{-1}) compared to $[(Cp^*)Rh(diimine)Cl]^+$ catalysts.[39] These Ru^{II} complexes were moderately active against A2780 cells (IC_{50} = 11–14 μM), however up to a 50-fold decrease in survival was observed as result of a NAD^+/NADH conversion when co-incubated with 2 mM formate.[40]

The NAD^+/NADH ratio can also be perturbed in the other direction for therapeutic purposes. Liu *et al.* demonstrated that the Ir^{III} complexes $[(Cp^{Xbiph})Ir(phpy)Cl]$ and $[(Cp^{Xbiph})Ir(phpy)(pyridine)]^+$ (**9a** and **9b**, Figure 3) catalytically produce H_2O_2 by electron transfer from NADH to O_2 inducing oxidative stress and cell death in a number of cancer cell lines. The pyridine-derivative **9b** showed nM potency in the NCI-60 screening. Its promising therapeutic index compared to the chloride-analogue could reasonably be associated to slower hydrolysis and deactivation by GSH.[41]
Likewise, Do and co-workers employed nontoxic $[(Cp^*)Ir(diimine)Cl]^+$ complexes to alter the NAD^+/NADH ratio and achieve selective chemosensitization of cancer cells toward carboplatin. Co-administration of the Pt agent and Ir catalysts increased the anti-proliferative activity of the drug by up to 30–50% in cancer cells, as a result of a disrupted cellular redox balance.[42] The same group also showed that NADH and Cp*-Ir catalysts could be combined for the catalytic conversion of low-molecular-weight aldehydes in the biological environment. Several aldehydes are implicated in different metabolic diseases and hydrogenation transfer catalysts of this type are proposed as enzymes mimic for the remediation of aldehyde toxicity.[43]

Cancer cells can be threatened by catalyzing the interconversion of key metabolites. This concept was demonstrated by Sadler using an exogenous catalyst and hydride source for the cellular transformation of pyruvate into lactate. This reaction is catalyzed by the enzyme lactate dehydrogenases (LDH), which is overexpressed in cancer cells. Interfering with the process employing an artificial catalyst potentially menaces the capacity of cancer cells to harness energy and essential metabolites.

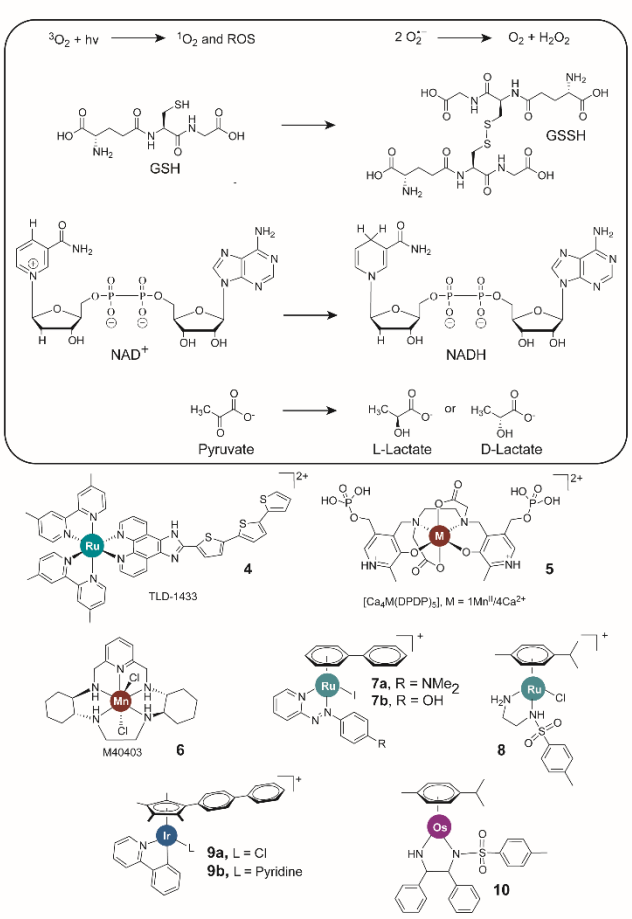

Figure 3. Chemical structures of catalytic metallodrugs affecting the cellular redox balance through the modulation of natural redox reactions (black box).

Chiral 16-electron Os^{II} complexes such as (*R,R*)-**10** (Figure 3) were capable to carry out the enantioselective reduction of pyruvate into D-lactate (e.e. 83%) in A2780 human ovarian cancer cells using either sodium formate or N-formylmethionine as hydride source.[44] The latter is a non-toxic precursor that is converted to formate intracellularly by the peptide deformylase, an enzyme overexpressed in several cancer cells.[44] Complex **10** displayed a potentiated antiproliferative activity in the presence of hydride donors indicating that they acted as catalytic drugs inside cancer cells. D-Lactate is present in extremely low concentration in cells and its production is unlikely to be the direct cause of cell death. Nevertheless, perturbation of the concentration of either lactate enantiomer or depletion of pyruvate might have had a major impact on the cell dynamics. The activity of these drugs is independent of DNA, hence they act through new mechanisms that are valuable for overcoming chemotherapy resistance.

The scope and therapeutic potential of catalytic metallodrugs described in this section may greatly benefit integrating some of the strategies introduced in the design of artificial metalloenzymes. Embedding transition metal catalysts in host protein scaffolds via covalent, dative or supramolecular interactions provides a well-defined second coordination sphere environment for the metal catalysts. This would aid improving the catalytic efficiency and selectivity as well as to protect the catalysts from deactivation by biological agents. Besides, it is plausible to envisage that protein scaffolds with inherent functionalities (e.g. substrate binding, redox properties) may be exploited to enhance and widen the drug action through secondary mechanisms.[45]

2. Transition metal-mediated bioorthogonal catalysis

In cellulo catalytic generation of organic drugs from caged compounds or synthesis precursors holds promise to control the biological activity of anticancer agents and potentially reduce their systemic side effects. These processes, to date mostly cleavage or cross-coupling reactions, use abiotic substrates and metal-based catalysts and can occur with remarkable efficiency and bioorthogonal selectivity.[5,6,10] Catalysts are administered at low concentrations, thus moderating metal toxicity concerns.

In 2006, Streu and Meggers described the use of the Ru(II) half-sandwich complex [Cp*Ru(COD)Cl] (**11**, Figure 4) as catalyst for the switch-on activation of an allylcarbamate-caged rhodamine in HeLa cells.[46] Few years later, Meggers reported that Ru(IV)-quinoline catalysts **12a** and **12b** (Figure 4) could perform the same reaction employing weaker nucleophiles such as GSH, rather than exogenous aromatic thiols as in the case of **11**.[47,48] Complexes **12a** and **12b** also greatly increased the catalytic efficiency compared to **11**, providing TONs up to 270. Furthermore, substitution of quinoline-2-carboxylato ligand of **12a** and **12b** with the 8-hydroxyquinolinate ligand to afford **13** (Figure 4) resulted in the most efficient catalyst of the series (TON > 300, reaction rate = 580 $M^{-1}s^{-1}$), a complex that also displayed significant catalytic activity in blood serum. Besides fluorophore deprotection, these catalysts were effective in transforming the anticancer prodrug N-(allyloxycarbonyl)doxorubicin inside HeLa cells, inducing cell death via apoptosis.[47,48] For example, co-incubation of **13** (1 μM) with alloc-doxorubicin in cells afforded an almost quantitative conversion of the prodrug inside the cell and an IC_{50} value which practically corresponded to direct administration of doxorubicin.[48]
As shown by the Mascareñas group using profluorescent probes, this type of Ru-catalyzed deprotection reactions could be targeted to specific cellular organelles by functionalizing Ru catalysts with the triphenylphosphonium (TPP) cation (**14**, Figure 4), that is known to promote accumulation in mitochondria.[49]

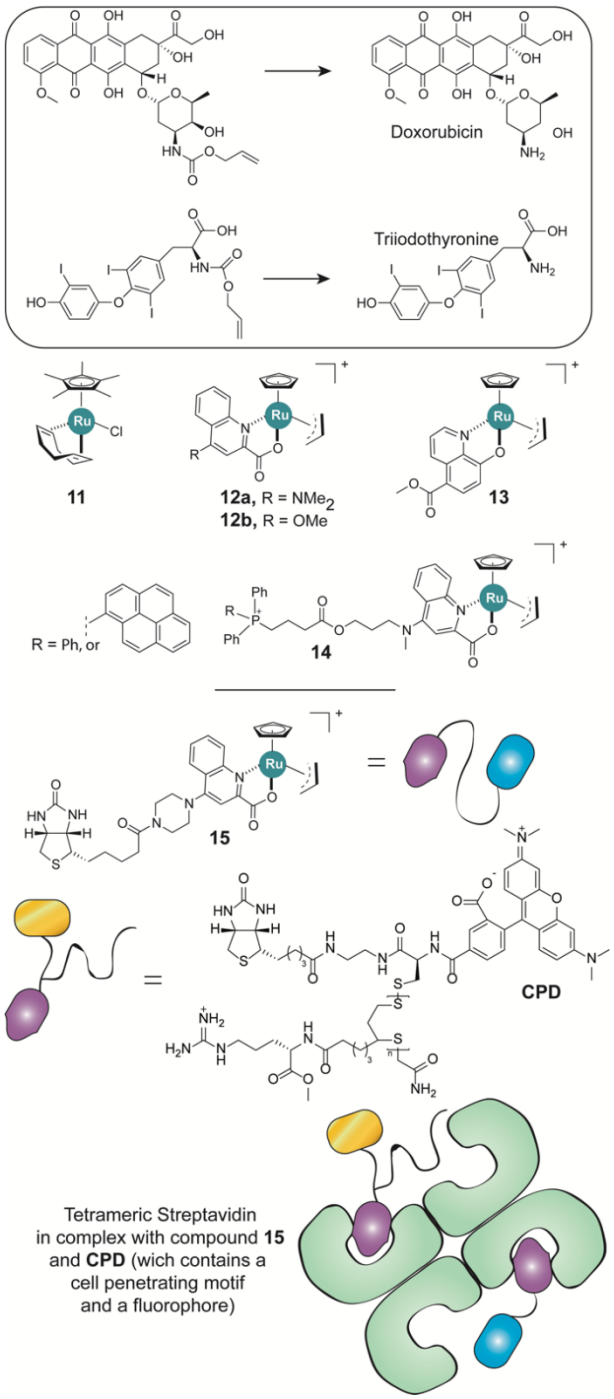

Figure 4. Ru bioorthogonal catalysts and catalytic deprotection reactions of therapeutical use.

Complex **12a** was employed by Ward in combination with the biotin–streptavidin technology to develop an artificial enzyme capable to penetrate into HEK-293T cells and catalyze uncaging of allyl-carbamate-protected thyroid hormone triiodothyronine, a gene switch known to affect thermogenesis, carbohydrate metabolism, and lipid homeostasis in all tissues.

In their artificial metalloenzyme, Ward and co-workers used the homotetrameric nature of streptavidin and its affinity for biotin to simultaneously load the biotinylated catalyst (**15**, Figure 4) and the cell-penetrating

moiety poly(disulfide) (CPD) attached to the TAMRA fluorophore, thus increasing the cell uptake of the catalyst and enabling the monitoring of its localization.[50]

The application of Pd-catalyzed reactions in biological settings and the use of nanostructured Pd^0 catalysts marked another fundamental step in the field of bioorthogonal catalysis and its application to therapy. In their pioneering work, Bradley and Unciti-Broceta described the capacity of Pd^0 nanoparticles (5 nm) supported on amino-functionalized polysterene microspheres to catalytically uncage non-fluorescent carbamate-protected rhodamines in complex biological environments, including *in vivo* (i.e. zebrafish).[51,52]

The same catalytic reactions were used for activation of a variety of anticancer drugs (Figure 5), including the extracellular generation of 5-fluorouracil (5FU) from propargyl caged precursors in colorectal and pancreatic cancer cells[52], as well as the activation of carbamate-protected gemcitabine in both buffered solution and cell culture medium.[53]

Unciti-Broceta and Leung demonstrated that Pd^0-microdevices have the capacity to carry out *ex vivo* carbamate cleavage of masked doxorubicin in tumor tissues. The Pd catalysts showed promising biocompatibility when implanted in mice bearing prostate tumors and studies on zebrafish revealed that caged doxorubicin avoided the cardiotoxicity typically produced by the drug alone.[54] A propargyl-masked prodrug of the histone deacetylase inhibitor vorinostat was also activated via Pd^0 catalysis.[55] The deprotection proceeded at physiological pH via a tandem mechanism triggered by the Pd-catalyzed depropargylation. Vorinostat has reached the clinics for the treatment of coetaneous T cell lymphoma and is investigated for a series of malignancies, however it suffers for systemic side effects and poor pharmacokinetics that might be addressed with this bioorthogonal catalysis approach.

The same uncaging approach was used to activate a prodrug derived from the active metabolite of the anticancer agent irinotecan. The propargyl-masked prodrug displayed reduced cytotoxic effects, yet its anticancer activity was restored in the presence of a Pd^0 catalyst. Furthermore, a combination strategy where the irinotecan prodrug was administered with the propargyl caged precursor of 5FU, demonstrated to elicit a superior cytotoxic activity toward different cancer cell lines in the presence of heterogenous Pd catalysts.[56]

Besides Pd^0 catalysis, the Unciti-Broceta group developed biocompatible Au resins for the catalytic deprotection of a range of propargyl-masked therapeutics *in vitro*, including floxuridine, vorinostat and doxorubicine.[57]

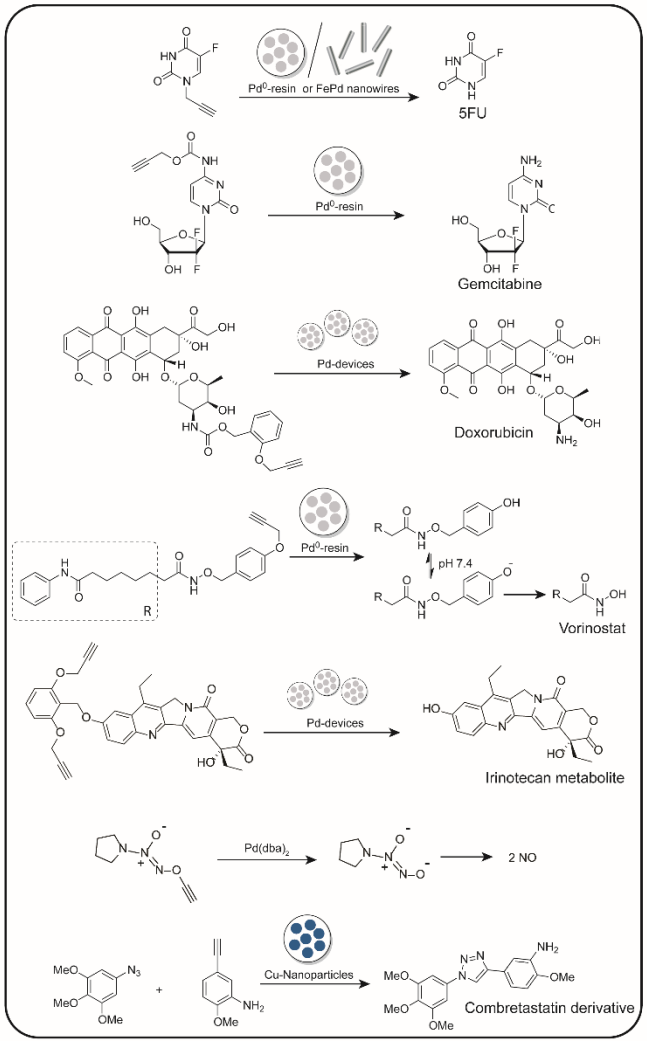

Figure 5. Catalytic deprotection of prodrugs by Pd and Cu nanocatalysts.

Organopalladium catalysis was employed by the Huang group to activate a newly designed propargyl-masked NO releasing agent (Figure 5). This masked pyrrolidinyl diazeniumdiolate was stable in plasma and exhibited potent antiproliferative activity against human colon carcinoma HCT116 cells, only when cells were pretreated with the $Pd(dba)_2$ catalyst (dba = dibenzylideneacetone).[58]

Pané and collaborators designed hybrid FePd magnetic nanowires responding to magnetic fields and capable of performing the deprotection of propargyl-caged 5FU (Figure 5). Placing a magnet under specific regions of a Petri-dish, they could attract the magnetic catalysts in defined areas where they triggered the activation of the prodrug and induced cell death with spatial control. FePd nanowires injected in tumors and intraperitoneal administration of 5FU significantly reduced tumor growth in mice models.[59]

The Bradley group proposed a diverse bioorthogonal catalysis concept applying copper-catalyzed azide–alkyne cycloaddition (CuAAC) for the *in situ* synthesis of a cytotoxic Cobrestatin derivative in the extracellular milieu. Combretastatin A4 is a tubulin polymerization inhibitor and a highly cytotoxic agent against a variety of cancer cell lines. The triazole analogue synthesized *in situ* via CuAAC from two non-toxic azide and alkyne precursors (Figure 5)[60] is known to inhibit K562 leukemia cancer cell growth with an IC_{50} value of 1.3 μM.[61] Polymeric microspheres supporting Cu nanoparticles were employed to generate catalytically the Cobrestatin analogue *in vitro* in SKOV-3 and HeLa cells. Moreover, application of the Cu nanostructures in zebrafish embryos triggered the synthesis of a fluorophore and proved the usability of this approach in a living organism.

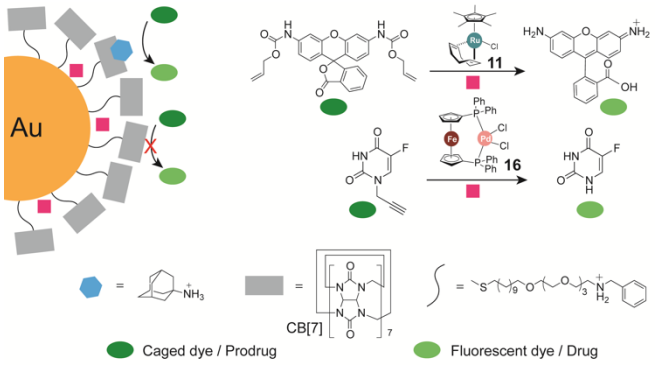

Figure 6. Schematic representation of Rotello's Au nanozymes and the catalytic activation of a profluorophore and prodrug.

Rotello and coworkers used Ru- and Pd-mediated bioorthogonal catalysis conceiving Au nanozymes capable of unmasking caged substrates in cancer cells (Figure 6). These Au nanoparticles had a protein-sized core (2 nm) and their surface was decorated with functional thiol ligands. The hydrophobic chain of the ligands served for the encapsulation of the organometallic Ru and Pd catalysts, the central tetra(ethylene glycol) unit for improving biocompatibility and the terminal dimethyl benzylammonium group for imparting water solubility. Host-guest interactions between the ligand head group and the non-toxic curcubit[7]uril (CB[7]) transformed the nanocarrier in a 'gate keeper' nanozyme. When bound, CB[7] inhibited the catalytic activity of Ru and Pd catalysts blocking the accessibility of substrates by steric hindrance. However, catalysis was restored as soon as the CB[7] competitive guest 1-adamantylamine (ADA) was added. Under these controlled conditions, the Ru-catalyst [Cp*Ru(COD)Cl] (**11**) turned on the fluorescence of the allylcarbamate-caged rhodamine 110 dye and the Pd catalyst **16** cleaved the propargyl protecting group of pro-5FU in HeLa cells. Intracellular conversion of pro-5FU into 5FU using this gated activation approach resulted in a significant reduction of cell viability.[62]
The same group recently demonstrated that changes in the capping ligand head groups of these nanozymes dictated either extra- or intracellular catalysis. They generated nanozyme capable of penetrating cancer cells by employing a positively charged quaternary ammonium ligand, while zwitterionic sulfobetaine heads provided zwitterionic nanoparticles with minimal cell uptake. Encapsulation of **11** in the nanozymes and catalytic activation of protected dyes and doxorubicin demonstrated that these systems could selectively activate substrates in a localized manner and modulate the therapeutic activity of drugs.[63]

3. Metal substrate bioorthogonal catalysis

In the described examples, and in catalysis at large, metal complexes act as catalysts, that is, mediators that facilitate the transformation of organic substrates in molecules of added value increasing the rate of chemical reactions. Our group has recently proposed a paradigm shift, devising an innovative prodrug photoactivation strategy (Figure 7) where metal complexes are unconventionally employed as substrates.[64] A wide range of metal-based agents can serve as substrates in catalytic reactions and their efficient and selective light-activation may help reducing some of the unwanted biological effects characteristic of metallodrugs. Catalytic transformations of metal complexes remain up to date practically unknown.

In the last two years, we demonstrated that flavins, including flavoproteins, are capable to perform photocatalytic transformations of Pt^{IV} and Ru^{II}-arene prodrug complexes in biological environments (Figure 7).[64–66] In this new type of catalysis, the rich photoredox chemistry of flavins triggers metal reduction and ligand dissociation reactions in the Pt^{IV} and Ru^{II} complexes, respectively. Pt^{IV} and Ru^{II}-arene complexes are families of anticancer compounds that have shown promising features as chemotherapeutic agents upon light activation, yet have poor absorption properties and often require damaging UVA light for triggering the formation of their biologically active species. According to our current comprehension of the catalysis mechanism, the active catalyst is the two-electron reduced flavin generated by the photooxidation

of electron donor molecules. Reduced flavins are likely to interact with the metal substrates forming transient adducts which favor electron transfer to the metal center.[64]
For instance, we reported in our first work that riboflavin (Rf, vitamin B2) under irradiation with low doses of 460-nm light behaved simultaneously as photosensitizer and photocatalyst converting *cis,cis,trans*-[$Pt^{IV}(NH_3)_2(Cl_2)(O_2CCH_2CH_2CO_2H)_2$]) (**17a**) to cisplatin. The catalysis was efficient and TTN and TOF as high as 38 and 13.2 min^{-1} were obtained in the presence of sacrificial agents. The reaction was dependent on the electron donor concentration, which in early studies was MES buffer. Notably, the reaction also displayed outstanding selectivity since it occurred practically unaltered in cell culture medium containing low concentrations of the zwitterionic buffer molecule.[64]

Rf-Pt^{IV} catalyst-substrate pairs reduced cell viability in different types of cancer cells upon light activation.[65] In Capan-1, Rf-activated **17a** displayed antiproliferative profiles resembling cisplatin, and indicating that extremely low doses (0.36 $J \cdot cm^{-2}$) of light are sufficient to liberate lethal concentrations of drug. Cisplatin-related DNA damage is the cause of cell death in the case of light-activated Rf-**17a** (10% catalyst), as demonstrated by solution and *in vitro* experiments. Whereas cisplatin upregulated the major vault protein (MVP, also known as lung resistance protein LRP), Capan-1 cells treated with Rf-**17a** and light reduced MVP levels, suggesting that flavin photocatalytic activation strategy may help overcoming MVP-mediated cisplatin resistance.[65]

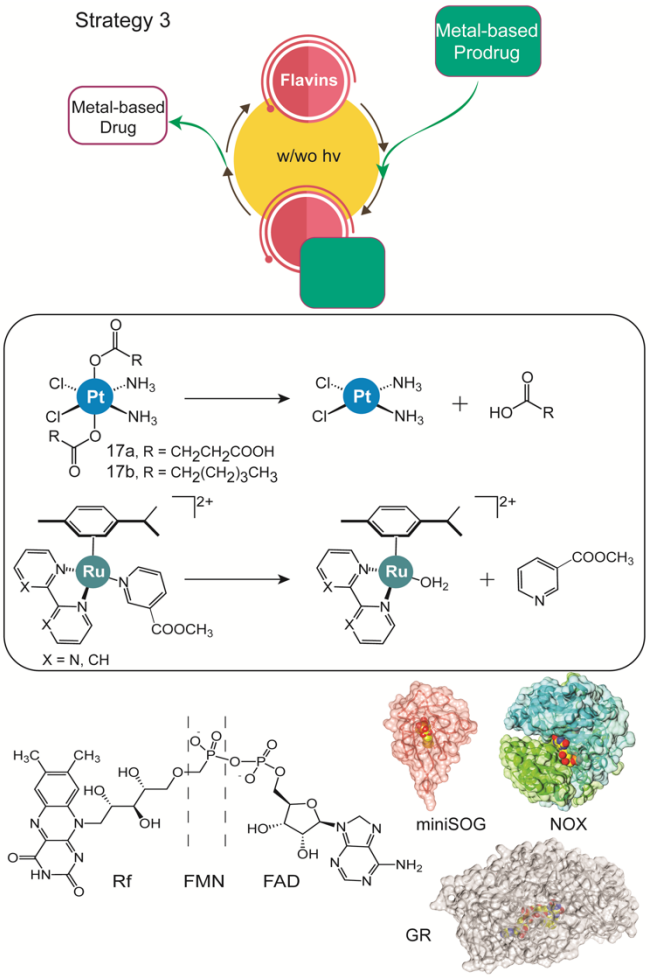

Figure 7. Flavin-mediated bioorthogonal catalytic photoactivation of anticancer metal complexes.

An intriguing aspect of this chemistry is that also flavoproteins and flavoenzymes can act, like naked flavins, as photocatalysts in the redox activation of Pt^{IV} prodrugs. We showed how the flavoprotein miniSinglet Oxygen Generator (miniSOG) efficiently photoconverted **17a** and **17b** to their Pt^{II} analogues under light irradiation and in the presence of electron donors such as MES or NADH. When in the presence of NADH, the flavoenzymes NADH Oxidase (NOX) could carry out the same reaction also in the dark and in cell culture medium. Both proteins were robust catalysts that could generate lethal concentration of Pt^{II} drugs. Their efficiency, in terms of TOFs, is in some cases comparable to the naked flavins (4.3–8.6 min^{-1}), depending on the condition used. Protein scaffolds play a crucial role in governing transformation of Pt^{IV} substrates. Indeed, enzymes such as Glucose Oxidase (GOX) and Glutathione Reductase (GR) showed no or little catalytic activity because of the limited accessibility of their flavin catalytic site.

Summary and outlook

The implementation of catalysis concepts to improve the potential of (metallo)drugs has been pursued for quite some time. Efforts in the field have been motivated by the capacity of catalysis to enhance the selectivity of drug candidates and by the possibility to discover new mechanisms suitable to reduce side effects and drug resistance. Nevertheless, researchers have focused on a relatively small number of catalytic reactions, a fact that has somehow hampered the development of this area. In fact, the most recent breakthroughs demonstrated that widening the pool of catalytic reaction is mandatory to make this field thrive and ultimately find new strategies for the treatment of diseases. Despite all the challenges, application of catalysis in the biological environment also promises to deliver fundamental understanding in biology and innovative chemical biology tools.
In this context, the bioorthogonal catalytic transformation of Pt and Ru complexes opens unique opportunities, potentially giving access to a mine of inorganic compounds whose biological chemistry can be enhanced or changed by flavin catalysis.

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Keywords: Bioorthogonal catalysis • Metallodrugs • Prodrug activation • Photochemistry • Bioinorganic chemistry

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