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Bioorthogonal Chemistry

Bioorthogonal Synthetic Chemistry Enabled by Visible-Light Photocatalysis

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Abstract: The field of bioorthogonal chemistry has revolutionized our ability to interrogate and manipulate biological systems at the molecular level. However, the range of chemical reactions that can operate efficiently in biological environments without interfering with the native cellular machinery, remains limited. In this context, the rapidly growing area of photocatalysis offers a promising avenue for developing new type of bioorthogonal tools. The inherent mildness, tunability, chemoselectivity, and external controllability of photocatalytic transformations make them particularly well-suited for applications in biological and living systems. This minireview summarizes recent advances in bioorthogonal photocatalytic technologies, with a particular focus on their potential to enable the selective generation of designed products within biologically relevant or living settings.

1. Introduction

Bioorthogonal chemistry refers to the toolbox of chemical reactions that can be performed in live settings without interfering with native biochemical processes.[1] To be considered as bioorthogonal, reactions should be rapid, compatible with aqueous media and with native pHs and temperatures, inert to biological components, and physiologically benign (Figure 1, top).[2] These reactions can be used for different purposes that range from the in vivo labelling of specific biomolecules or cells, to the production of desired products at selected biological sites. Therefore, they encompass an impressive potential for interrogating and manipulating biological systems or for the development of new types of biomedical tools.[3]

The idea of interfacing synthetic chemical reactions with cellular biology has been present in the scientific community for decades. However, the perception of the cell as a highly complex and challenging reaction vessel has delayed research efforts in this field. Indeed, it was not until the beginning of this century that the concept of bioorthogonal chemistry was established, after the demonstration by Bertozzi and co-workers that azide-containing sugars embedded in cellular membranes can be selectively labelled using Staudinger ligations.[4] The parallel development of click chemistry,[5] spearheaded by the copper-catalyzed azide-alkyne cycloaddition reaction (CuAAC), contributed to further nurture the field.[6] A major advance in the area came with the development of strain-driven cycloadditions (Figure 1A).[7] Reactions like the SPAAC (strain-promoted azide-alkyne cycloaddition) and specially, the cycloaddition between tetrazines and strained alkenes (IEDDA, inverse electron demand Diels-Alder reaction), have found an impressive number of applications.[8] Despite the impact and significance of these reactions, they are based on high-energy reagents, which can be problematic in terms of stability and orthogonality, and the range of products that can be formed is inherently limited by the imprinted heterocyclic cores resulting from the cycloadditions. Thus, there is a need of new bioorthogonal reactions that can mimic better the transformative potential of organic synthesis. In this context, catalytic reactions are especially attractive, as they are based on stable substrates that do not react until meeting a suitable catalyst. Indeed, this is the approach followed by Nature, as most metabolic transformations depend on the catalytic action of enzymes, natural catalysts that promote the reaction of otherwise inert reactants.[9]

While enzymes are magnificent catalysts for bioorthogonal chemistry, their versatility and scope tend to be very limited, as they have evolved to perform very specific tasks, and exhibit a rather limited mechanistic variability. In this context, catalysts based on transition-metal complexes represent a highly promising alternative to enzymes, because they can promote a much broader range of reactions that proceed by a vast variety of new-to-nature organometallic mechanisms.[10] Curiously, the potential of transition-metal catalysis in the bioorthogonal arena was ignored for many years, likely because of the perception of water incompatibility and presumable cytotoxicity of the metal complexes. This vision shifted after the demonstration that CuAACs

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can be carried out in bacteria[11] and, especially, with the report by Meggers and co-workers in 2006 of a cell-compatible ruthenium-promoted deallylation reaction (Figure 1B).[12] The field experienced a slow start, but in recent years it has been extensively demonstrated that appropriately crafted transition-metal catalysts can drive designed abiotic transformations within cellular environments.[13] In this context, most reactions developed so far are based on deprotection processes, both because of their relative simplicity and their potential for uncaging active products from inert precursors.[14] Nevertheless, a crescent number of examples involving other types of synthetic transformations, from cross-couplings and cyclizations to carbene-transfer or redox processes, have also been published.[13,15] Despite this progress, performing metal-promoted reactions in cellular environments still presents numerous challenges such as the deactivation of the metal catalysts, poor turnovers and rates, and bioorthogonality deficiencies.[16] While numerous research groups are tackling these challenges using a variety of strategies, further fostering the field of bioorthogonal synthetic chemistry requires the discovery of new reactions that can be translated into the biological arena. In this context, reactions that can be promoted by light are especially attractive because of their spatial and temporal controllability. Indeed, a number of photo-click reactions based on the direct photoactivation of reactants such as tetrazoles have been reported (Figure 1C).[17] Some other new-to-nature transformations promoted by light have been performed in the presence of live cells over the past decade, such as a free-radical polymerization of alkenes.[18] Light has also been used for the in situ activation of metal catalysts.[19] For example, tailored ruthenium sandwich complexes bearing photoresponsive arene ligands [Cp*Ru(II)arene] have been employed to catalyze cycloadditions between azides and thioalkynes (RuAtAC) upon UV-light irradiation (Figure 1D).[20] This ruthenium-based technology can be com-

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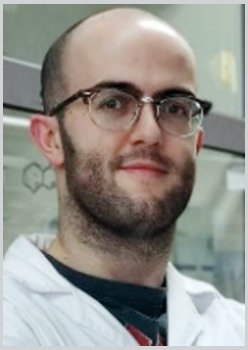
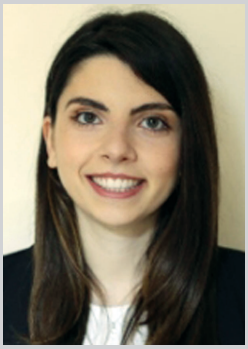


Xulián Fernández González studied Chemistry at the University of Santiago de Compostela (USC). In 2018 he joined the group of Prof. J. L. Mascareñas and Dr. M. Tomás-Gamasa at the same university, first as undergraduate student and then as Master student. He is currently conducting his doctoral research, focused on the development of photocatalytic bioorthogonal reactions.



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María Tomás-Gamasa completed her Ph.D. in 2011 under the supervision of Profs. J. Barluenga Mur and C. Valdés at the University of Oviedo. She undertook two predoctoral stays with Profs. V. K. Aggarwal and A. Charette, and completed a postdoctoral stay with Prof. T. Carell (2011–2014). In 2015, she joined the University of Santiago de Compostela as a "Juan de la Cierva" fellow. Since 2022, she is a Ramón y Cajal researcher at USC, working on the design and development of new bioorthogonal processes, and exploring the synthetic bioorthogonal photocatalysis field.

José Luis Mascareñas completed his PhD at the University of Santiago de Compostela (USC). He carried out postdoctoral work at Stanford University and Harvard University, and is full professor at the USC since 2005. He is scientific director of CIQUS since 2014. In 2015 he received the gold medal of the Royal Spanish Society of Chemistry, and in 2016 was appointed a member of the European Academy of Sciences. His research combines discovering novel methods based on metal catalysis and the development of bioorthogonal synthetic tools for biological intervention.

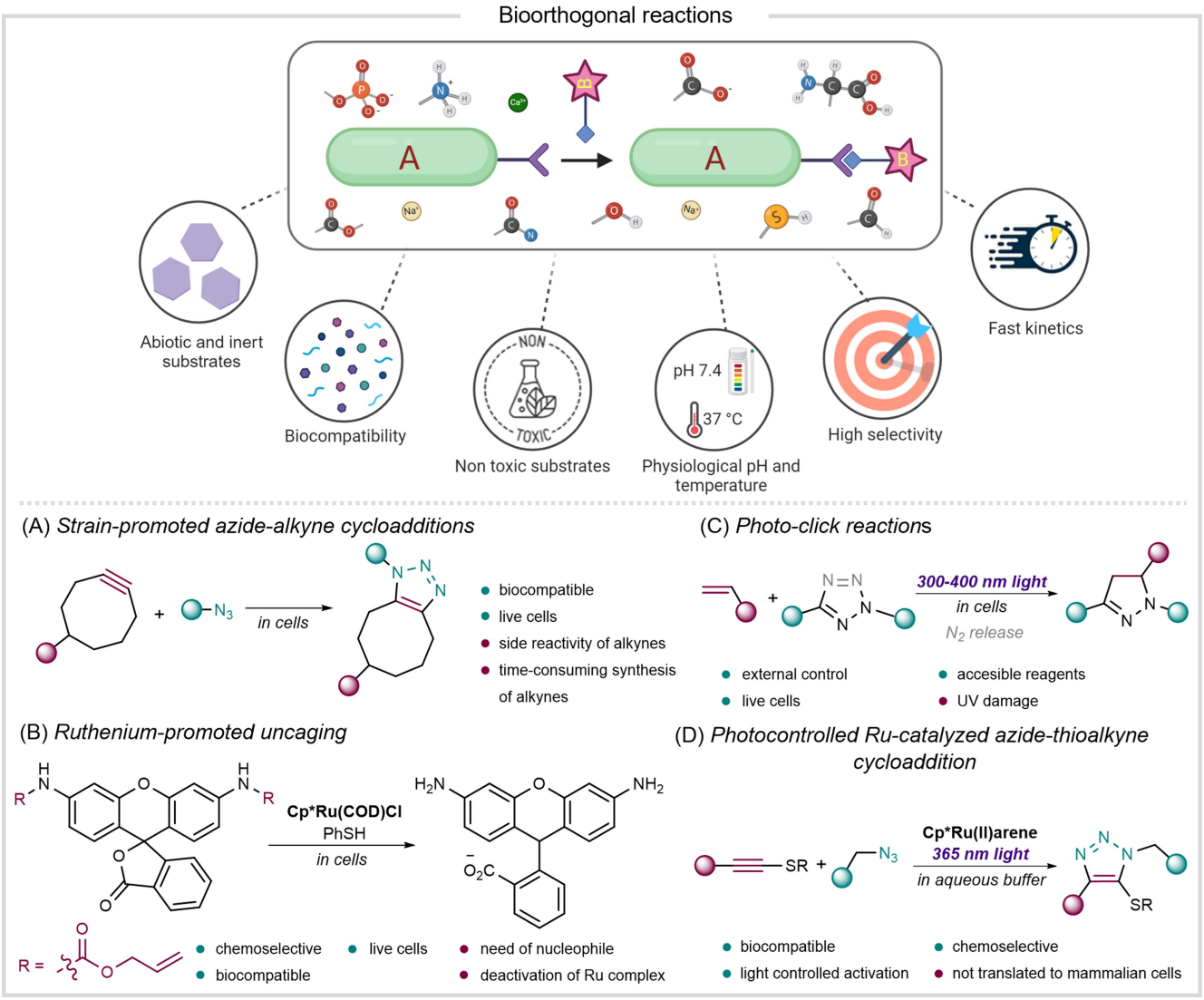

Figure 1. Top: Overview of general reaction features and requirements in bioorthogonal chemistry. Bottom: (A) Strain-promoted azide-alkyne cycloadditions; (B) Metal-mediated deprotections reactions: uncaging of fluorophores by Ru-mediated deallylation; (C) Click reactions with tetrazoles promoted by UV-light irradiation; (D) Light-activated biocompatible metal catalysts: photocontrolled Ru-catalyzed azide-thioalkyne cycloadditions. COD = 1,5-cyclooctadiene; Cp* = pentamethylcyclopentadienyl.

bined with the standard copper-catalyzed azide-alkyne cycloaddition (CuAAC) in a mutually orthogonal and bioorthogonal manner. Unfortunately, these photo-click tools are very difficult to translate into cellular settings, largely because of the requirement of cytotoxic high-energy light for the direct photoactivation step. Therefore, the use of photosensitizers that can absorb light at longer wavelengths and trigger chemical reactions by transferring the resulting extra energy to otherwise unreactive substrates is certainly appealing. This is even more tempting if one considers the impressive developments on photocatalytic synthetic chemistry along the last two decades (see Section 2), and the possibility of obtaining a much higher spatiotemporal control on the reactivity, which is especially relevant for biological applications.[21] However the development of bioorthogonal photocatalytic processes is not trivial, because of the delicate nature of excited-state intermediates and the photophysical requirements of the reactions, among other reasons.

2. Photocatalysis in Organic Synthesis

The discovery and evolution of photocatalysis marks a revolutionary paradigm shift in the field of synthetic chemistry. Its renaissance over the past decades represents a transition from a simple scientific curiosity to a fundamental pillar of modern synthesis.[22] After Ciamician and Silber laid the groundwork at the beginning of the 20th century,[23] the field remained relatively dormant for a period dominated by the direct excitation of organic molecules with high-energy UV light.[24] The area of synthetic photocatalysis experi-

enced an explosive surge at the beginning of the 21st century, especially after key simultaneous contributions from the groups of Yoon, MacMillan and Stephenson.[25] These groups streamlined the synthetic use of $[Ru(bpy)_3]Cl_2$ (bpy = 2,2'-bipyridine) as a photoredox catalyst that responds to visible light.[26] These developments marked the beginning of a new era that has now elevated photocatalysis as a widely embraced tool among synthetic chemists worldwide.[27]

Most photocatalytic processes (Figure 2A) are based on the absorption of visible light (*hv*) by a photocatalyst (PC), which evolves from its electronic ground state (S_0) into a short-lived singlet excited state (S_1) before it undergoes intersystem crossing (ISC) into a triplet excited state (T_1). The triplet states of common photocatalysts are long-lived enough (ns-μs) to interact bimolecularly with organic molecules through different pathways, triggering chemical reactions upon regeneration of the ground-state photocatalyst. Mechanistically, photocatalytic reactions are often classified in two general types depending on their physicochemical nature. Energy-transfer photocatalysis (EnT) or photosensitization refers to the process of transferring the excited-state energy from an excited donor (PC*) to a ground-state acceptor (R), leading to an excited acceptor species (^{3}R*) which may engage in further reactivity.[28] Alternatively, the excited-state photocatalyst can undergo a single-electron-transfer process (SET), donating or accepting a single electron to or from a substrate or reactant R_1, generating highly reactive radical-ion species (photoredox catalysis).[27] An illustrative example with the prototypical photocatalyst $[Ru(bpy)_3]^{2+}$ is outlined in Figure 2B. Upon absorption of a visible-light photon and ISC, the photosensitizer reaches a relatively long-lived triplet state which can engage in EnT processes (E_T = 2.12 eV, 49 kcal/mol) or act either as a comparably strong single-electron reductant (oxidative quenching) or oxidant (reductive quenching). In both cases, these modes of activation often lead to intermediates or reactivity pathways that cannot be productively harnessed under thermal conditions.[29]

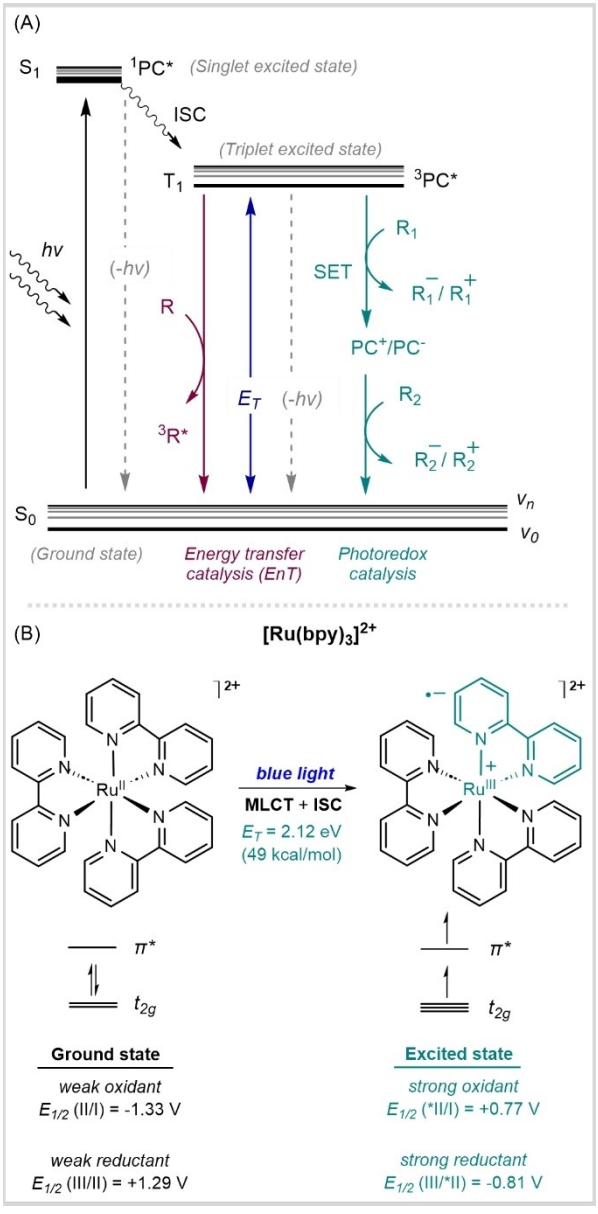

Figure 2. (A) General representation of the photophysical and photochemical processes behind photocatalysis, and (B) Simplified photochemical properties of $[Ru(bpy)_3]^{2+}$. MLCT = metal-to-ligand charge transfer; ISC = intersystem crossing; SET = single-electron transfer. E_T = triplet energy. $E_{1/2}$ = half-wave potential (in V vs SCE).

Beyond the ability to trigger chemical reactions that are not achievable using other protocols, one of the key features responsible for the success of photocatalysis is the mildness and chemoselectivity displayed by these reactions. These characteristics have been exploited to solve an enormous variety of synthetic challenges,[27] not only through pure photocatalytic approaches, but also merging them with organocatalysis,[30] or transition-metal catalysis.[31] More importantly, this inherent mildness, together with the relatively low toxicity of visible light, and the conceivable water-compatibility of reactive intermediates, make of photocatalysis an ideal tool to be used in biological environments.[32] Indeed, some well-known biomedical tools like photodynamic therapy (PDT), which has been used in clinical settings,[33] rely on the use of visible-light photosensitizers (see next section). During the past decade, the number of reported protocols merging the area of photocatalysis with biomolecular and biological chemistry has steadily increased. These approaches range from bioconjugation reactions or biopolymer modifications to the uncaging of bioactive products.[19] However, the field of bioorthogonal photocatalysis is yet under construction, especially with regard to the development of biocompatible synthetic transformations.

3. Synthetic Photocatalysis in Biological and Living Settings

3.1. Initial Applications. Photodynamic Therapy (PDT)

Whereas the synthetic exploitation of photocatalysis is relatively recent, light-promoted reactions have been widely used in the biomedical arena for many years. It all began with the direct use of light to treat diseases, a practice that goes back to ancient civilizations.[34] The term "phototherapy" was adopted at the end of the 19th century by Danish scientist Niels Finsen, who was awarded a Nobel Prize in 1903 for the development of clinical uses of concentrated light irradiation.[35] By the same time, the first observations were made on the cytotoxic effects resulting from the combination of light and certain small molecules, like acridine, describing them as "photodynamic actions".[36] Nowadays, this type of techniques are classified within the field of photodynamic therapy (PDT), and represent powerful biomedical tools with clinical indications, especially for the treatment of cancers.[37] In PDT, photosensitizers excited by light generate different reactive oxygen species (ROS) such as $O_2^{\bullet-}$ or $HOO^{\bullet}$ via SET processes (Type I), or singlet oxygen via energy transfer (Type II) (Figure 3A). These highly reactive intermediates are responsible for altering biochemical processes, usually producing cytotoxic responses.[38] The most used photosensitizers in PDT are organic dyes like porphyrins. However, metal-based complexes have also been employed and shown to provide certain chemical and photophysical advantages, such as a higher stability and longer-lived excited states.[39] Despite the clinical utility, PDT suffers from several problems. Especially relevant is the dependence of oxygen to cause biological effects, which limits the efficacy in hypoxic settings.[40] In this context, alternative strategies based on the photocatalytic modification of natural metabolites are highly attractive. For instance, Sadler and co-workers have reported the use of metal-based photocatalysts in biological and cellular settings to modify redox balances and thereby generate biological (usually cytotoxic) responses. They designed Ir(III) photocatalysts that accumulate in mitochondria and are capable of oxidizing NADH (reduced nicotinamide adenine dinucleotide) upon light irradiation, generating a redox imbalance in the cell (Figure 3B). These complexes can also work under hypoxia conditions.[41] While not a catalytic process, it is also worth highlighting the use of light for the release of bioactive agents attached to photosensitizers. This strategy, often referred to as photoactivated chemotherapy (PACT),[42] has been carefully explored with ruthenium polypyridine complexes. Overall, these examples have been developed within biomedical contexts and therefore represent a solid groundwork for translating abiotic photocatalysis into biological settings.[43]

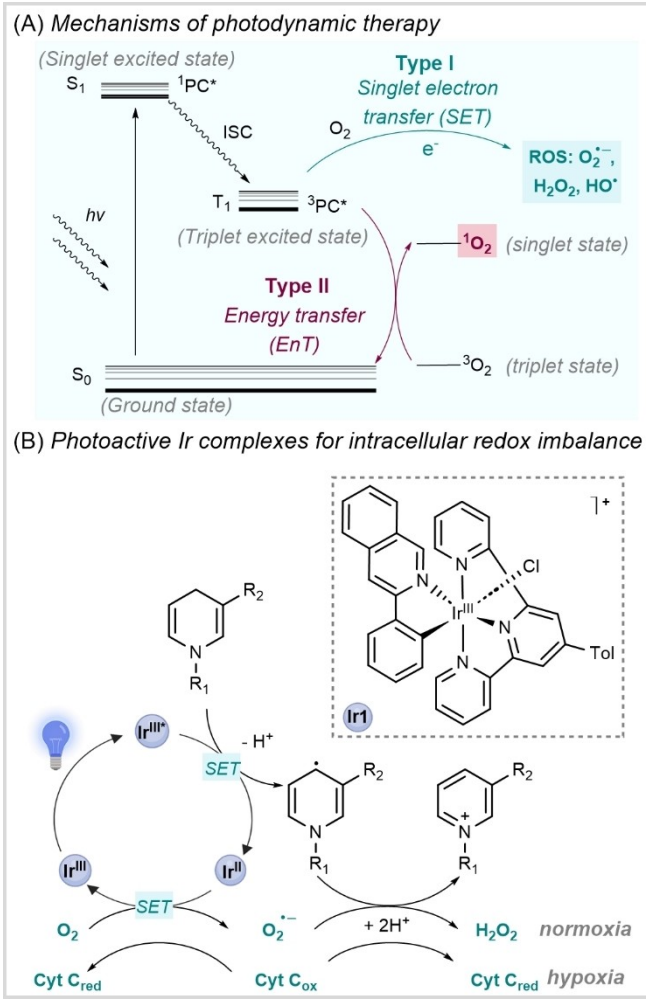

Figure 3. (A) Mechanisms of photodynamic therapy; (B) Photocatalytic activation of Ir(III) complexes to promote intracellular redox imbalances. In the presence of the photocatalyst, light irradiation leads to lower levels of NADH and higher levels of H_2O_2. Cyt C = cytochrome C.

3.2. Photocatalytic Net-Oxidative and Net-Reductive Processes in Biological Environments

Most of the processes described in the previous section were designed to alter cellular redox balances rather than to promote specific chemical reactions of exogenous substrates. Following the explosive progress in the field of photocatalytic synthetic organic chemistry, it is pertinent to question whether some of these well-defined chemical reactions could be exported to the biological field.[44] Among *net-oxidative transformations*, a significant contribution towards this goal was reported by Chen and co-workers in 2019.[45] Building on their previous work with H_2O_2-sensing boronic-acid probes, they demonstrated the viability of performing photocatalytic deboronative hydroxylations in phosphate buffers (PBS) and in biological media. They used this strategy to uncage bioactive molecules in bacteria and even in mammalian cells (Figure 4A). Key for the success of the approach was the selection of the appropriate dyes as photocatalysts (fluorescein or rhodamine), that favor the

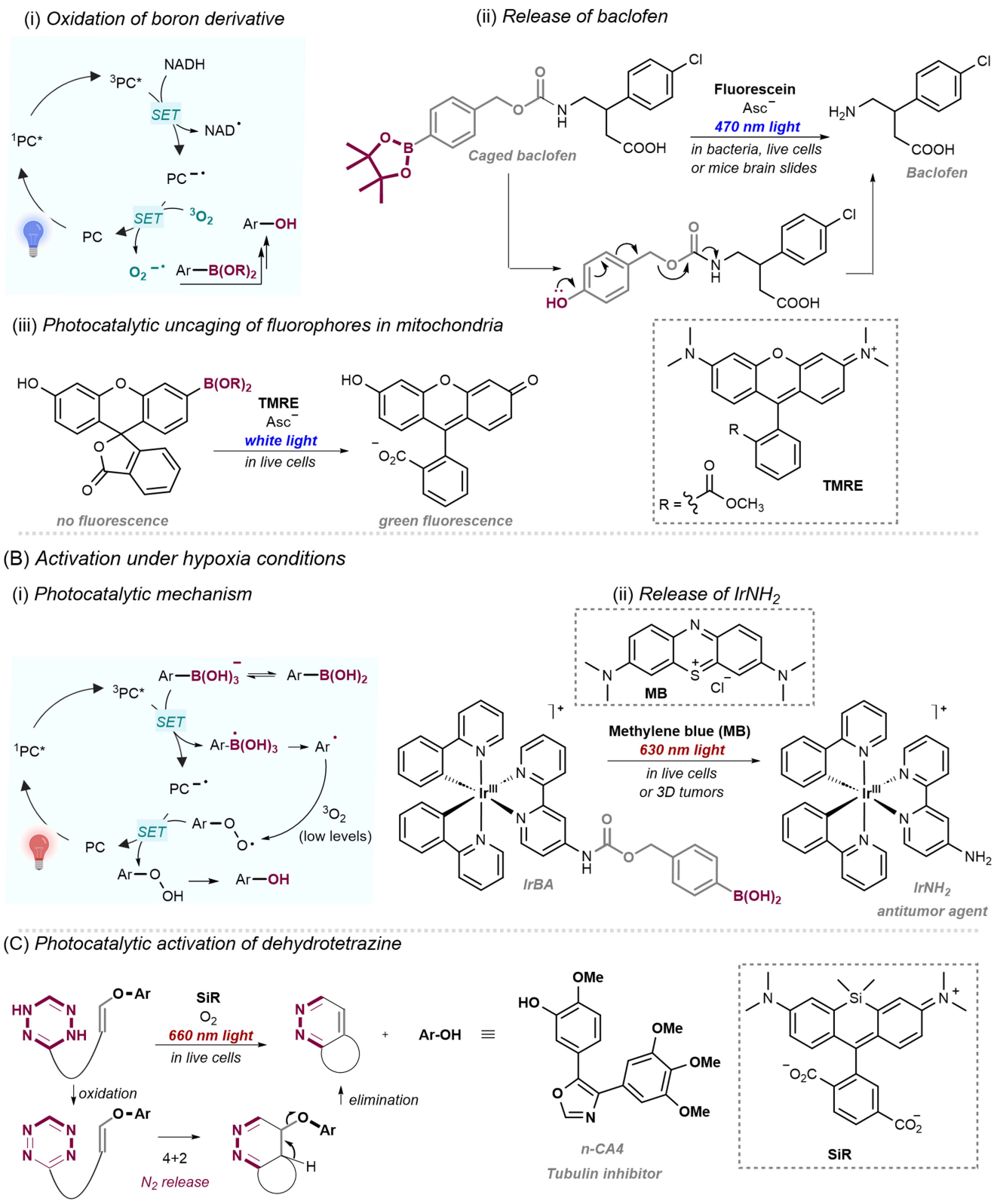

Figure 4. Net-oxidative photocatalytic transformations and uncaging in live environments. (A) Deboronative hydroxylations under normoxia conditions, and (B) under hypoxia conditions; (C) Uncaging through a sequential photocatalyzed dihydrotetrazine oxidation, cycloaddition, nitrogen extrusion and phenol-drug elimination. Asc^- = ascorbate; TMRE = tetramethylrhodamine ethylester.

production of $O_2^{\bullet-}$ (superoxide radical) and H_2O_2 via SET mechanisms. The reaction was proposed to involve the reductive quenching of the excited organophotocatalyst with endogenous reductants (e.g.: ascorbate or NADH). The reduced dye undergoes SET to oxygen, generating superoxide radical anions that react with the aryl boronic acid to give the corresponding phenol (Figure 4A.i). The strategy can also be extended to the uncaging of other alcohols and amines from protected precursors exhibiting a self-immolating group. For example, a benzyl-carbamate boronic ester precursor was used for the release of baclofen, an agonist of neuronal GABA B, inside neuronal cells. In this process, phenol formation triggers the subsequent release of the uncaged bioactive compounds (Figure 4A.ii). The same group also demonstrated the viability of a subcellular photorelease of fluorescent molecules by using mitochondria-localized photocatalysts like TMRE (Figure 4A.iii). They also succeeded in linking genetically encoded SNAP proteins to organic photosensitizers. The resulting constructs could be targeted to mitochondria, nucleus, membrane, cytosol, and endoplasmic reticulum, where they can promote the photocatalyzed oxidation of organoborate precursors and the subsequent uncaging of diverse drugs, such as doxorubicin, DNP (a mitochondrial uncoupler), or baclofen.[46] Moreover, this genetically encoded photocatalysis method was applied for modulating the neuronal activity in cultured cells, brain slices and live mice.

In 2023, Zou and co-workers reported a different platform geared towards the photo-release of bioactive products within hypoxic tumor microenvironments (Figure 4B.i).[47] This strategy enabled the uncaging of antitumor complex **$IrNH_2$** from boronic acid-derivative **IrBA** (Figure 4B.ii), among other bioactive molecules such as baclofen or vorinostat. This photoredox process is enabled by the equilibrium between the boronic acid $ArB(OH)_2$ and the corresponding hydroxyl boronate complex $ArB(OH)_3^-$. Whereas the formal B(III)/B(IV) oxidation for the former is difficult, (E_{red} $[ArB(OH)_2]^+/ArB(OH)_2] = 2.23$ V vs Ag/AgCl), the latter (E_{red} $[ArB(OH)_3]/ArB(OH)_3^-] = 0.85$ V) can easily transfer an electron to an excited photocatalyst. Depending on the excitation wavelength, the photooxidant can be either external methylene blue (under red-light irradiation; E_{red} MB*/$MB^- = 1.6$ V) or the iridium complex itself (under blue-light irradiation; [E_{red} Ir(III)*/[Ir(II) = 1.02 V). The oxidized boron-radical intermediate can undergo fragmentation, releasing $B(OH)_3$ and generating reactive aryl radicals that can trap even the extremely low levels of oxygen characteristic of tumor cells. Oxygen trapping, followed by another SET from the reduced photocatalyst, results in aryl-peroxide species that can oxidize another unit of **IrBA**, to give the corresponding phenol. Again, phenol formation triggers the self-immolating group to release the cytotoxic **$IrNH_2$**. Importantly, the authors applied this technique even in live mice, observing a significant tumor-growth inhibition. Another approach to harness a photocatalytic oxidation in the context of bioorthogonal chemistry has been disclosed by Fox and co-workers.[48] The method is based on the oxidation of dihydrotetrazines with oxygen using a far-red light-promoted photosensitization with a silarhodamine catalyst. A subsequent cycloaddition of the resulting tetrazine with an ancillary strained dienophile leads to the concomitant release of an uncaged phenol drug inside cells, such as tubulin-inhibitor **n-CA4** (Figure 4C). The reaction can be performed in specific locations of live cells by conjugating the photosensitizer to suitable targeting agents.

On the other hand, photoredox reactions entailing *net-reductive transformations* have also been used in biological contexts. Indeed, as early as in 2011, Liu and co-workers developed a photoredox-promoted azide to amine reduction in buffered solutions, using ascorbate as stoichiometric reductant (Figure 5A).[49] The authors found that the mild reaction conditions allow the orthogonal reduction of azide-containing oligonucleotide and oligosaccharide substrates in the presence of proteins. This reaction was also applied to the photo-uncaging of carboxylic acids using 4-azidobenzyl ester as protecting/caging group. The authors proposed a mechanism entailing the reductive quenching of triplet-state $[Ru(bpy)_3]^{2+}$* by ascorbic acid or NADPH. The resulting $[Ru(bpy)_3]^+$ is reducing enough to transfer an electron to the azide and generate the azide radical anion, which undergoes nitrogen extrusion and further reduction to give the corresponding aniline. Stimulated by these pioneering results, Winssinger and co-workers have demonstrated that the photocatalyzed reduction of azides can be used in the context of nucleic acid sensing and imaging, both in vitro and in living cells. For example, in 2012, they demonstrated the viability of reducing azide-containing substrates linked to a PNA (peptide nucleic acid) using a photoredox process mediated by another PNA conjugated with a Ru(II) photocatalyst. The photoreduction process results in the release of rhodamine or other fluorophores.[50] The reaction efficiency is dependent on the presence of a nucleic-acid unit that works as a template to bring the photocatalyst and the azide substrate into proximity. These initial studies were performed in buffered solutions, but later the same group demonstrated the applicability of this type of templated photoreduction for *in cellulo* uncaging of fluorescent molecules, using different oligomeric protein templates.[51] They also showed that it is possible to promote photocatalyzed azide reduction in live cells with subcellular localization, by using a specific ligand for the target protein. For example, they could direct this reactivity towards the cell nucleus by using a Ru photocatalyst linked to an estrogen agonist (raloxifene). This process was monitored by the release of a caged precipitating dye, QNP (Figure 5B).[52] They also demonstrated the viability of using RNA as template to promote the photocatalytic unmasking of fluorophores. The reaction was shown to proceed in live cells with signal amplification, and it was selective for double-stranded RNA over DNA and single-stranded RNA. The generality of the triplex formation was enabled by non-canonical nucleobases that extend the Hoogsteen base-pairing repertoire.[53,54] A similar type of release mechanism (but displaying faster kinetics) could be developed by replacing the azide with a 4-(phenoxymethyl)pyridinium group as self-immolating linker, which can release the phenoxy group (a fluorophore) upon light-promoted single-electron reduction from *Ru(II)/as-

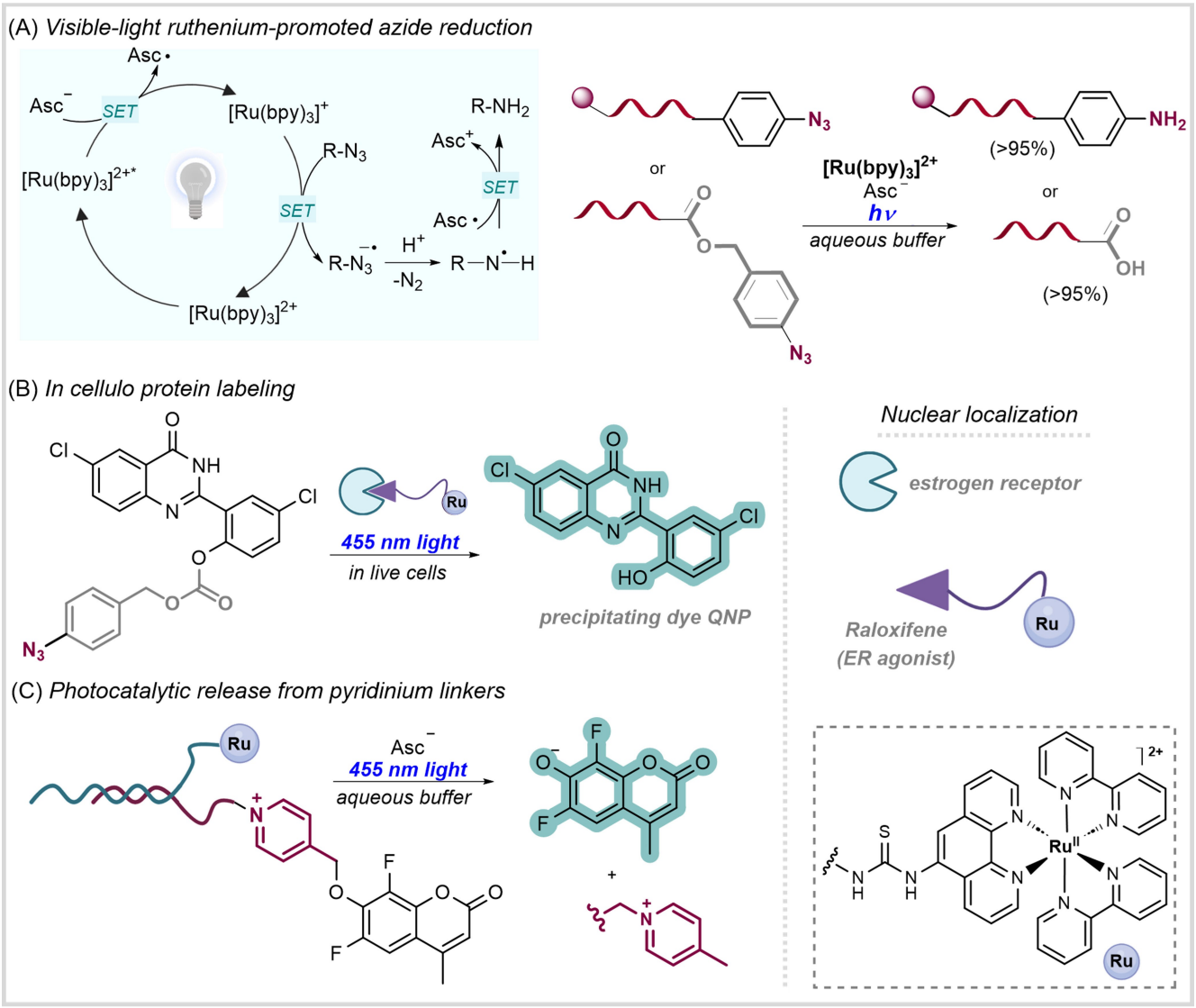

Figure 5. Net-reductive photocatalytic transformations and uncaging in biorelevant environments. (A) Ru-catalyzed photoredox reduction of azides in buffer solutions; (B) *In cellulo* protein labelling at the cell nuclei via photocatalyzed self-immolative targeted release of fluorophores; (C) Photoredox reduction of pyridinium self-immolative linkers. Asc^- = ascorbate; $h\nu$ = CLF bulb; ER = estrogen receptor.

corbate and subsequent fragmentation (Figure 5C).[55] More recently, the same type of process was used to demonstrate the viability of a BRET (bioluminescence resonance energy transfer)-induced photoreductive uncaging of small-molecule drugs within cell systems. The reaction is promoted by an energy-transfer process from a bioluminescent protein (Nluciferase) to a $[Ru(bpy)_2(phen\text{-}NH_2)]^{2+}$ photocatalyst, which triggers the uncaging of a pyridinium-linked substrate in the presence of an appropriate nucleic-acid template.[56]

Finally, it is worth highlighting that photocatalytic reductions have also been used for the light-controlled stoichiometric generation of chemotherapeutic agents, like cytotoxic Pt(II) complexes, from inactive Pt(IV) precursors. For this, Salassa and co-workers employed riboflavin as photoredox catalyst, which can be excited under blue-light irradiation and subsequently reduced by NADH or MES (2-(*N*-morpholino)ethanesulfonic acid). The reduced riboflavin then transfers electrons to the Pt(IV) prodrug, releasing Pt(II) cisplatin upon regeneration of ground-state riboflavin (Figure 6).[57] The process was used in human prostate cancer cells leading to an antiproliferative effect.

Furthermore, this type of net-reductive bioorthogonal photoredox processes can also be coupled to other reactions. In 2018, Ward, Wenger and co-workers demonstrated that it is possible to merge enzymatic reactions with the visible-light-driven reduction of cyclic imines to amines. This reaction was carried out in *E. coli* cells that express a monoamine oxidase (MAO–N-9), using 405 nm light, an Ir photocatalyst, and ascorbic acid (AscH) as reducing agent (Figure 7A).[58] The photocatalyst mediates the reduction of the imines to α-amino alkyl radicals that are intercepted through hydrogen-atom transfer (HAT) from ascorbate or thiol donors to afford the corresponding amine. One of the enantiomers of the resulting amine is accumulated, whereas

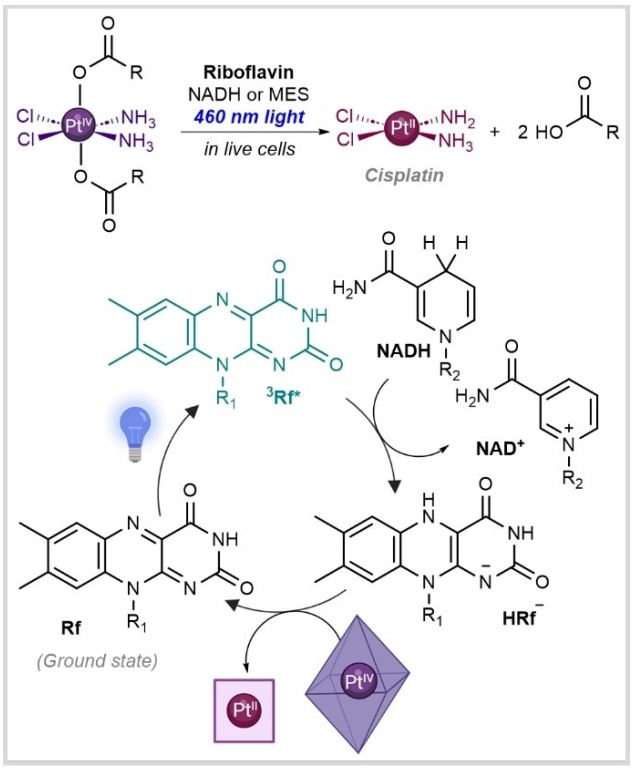

Figure 6. Photocatalytic in situ generation of anticancer Pt(II) complexes. Rf = riboflavin.

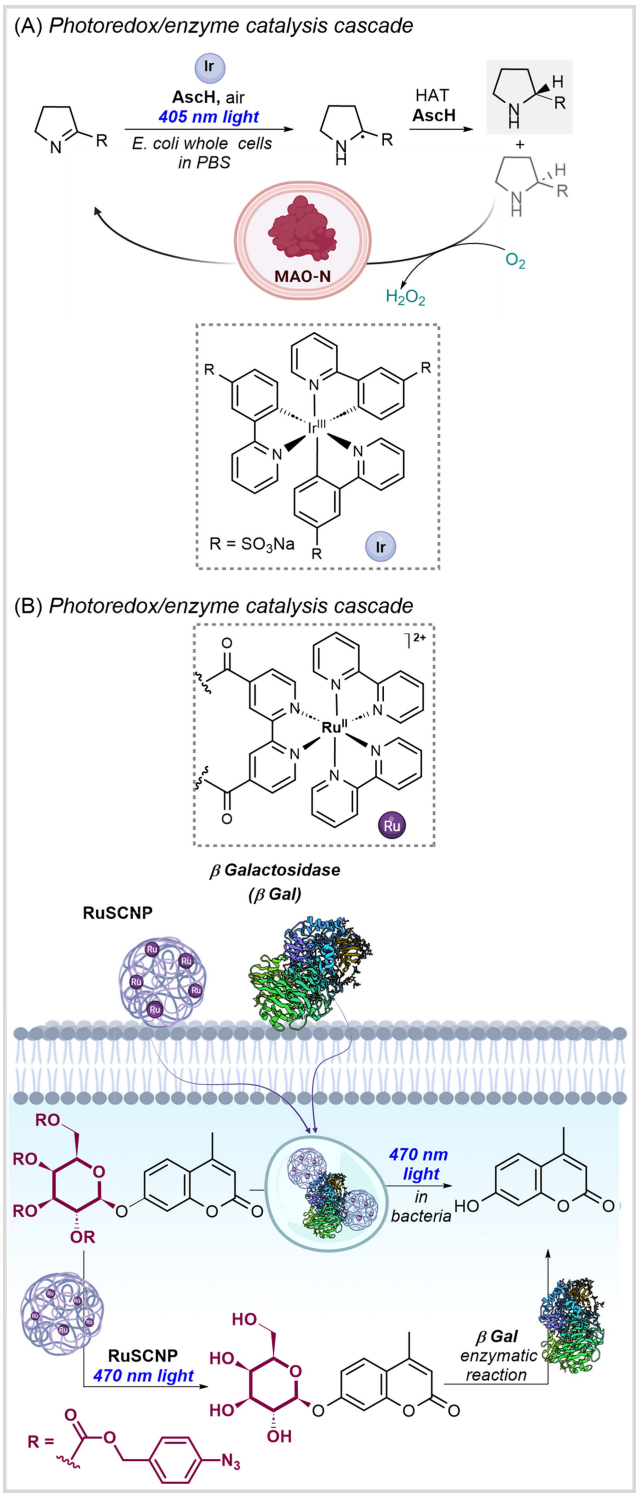

Figure 7. Combination of enzymatic reactions and photocatalysis. (A) Formal enantioselective reduction of cyclic imines to amines; (B) Concurrent or tandem uncaging processes in live cells. HAT = hydrogen-atom transfer; MAO = monoamine oxidase; SCNP = single-chain nanoparticles.

the other one is processed by MAO–N-9, regenerating the starting imine, resulting in a dynamic kinetic-resolution process. Later, a one-pot whole-cell cascade combining photocatalysts and ketoreductases was described for the decarboxylative carbonylation of carboxylic acids and the subsequent bio-reduction to generate valuable chiral alcohols.[59] Using this approach, various chiral alcohols with complementary (*R*)- or (*S*)-configurations were prepared with good yields (up to 93 %) and excellent stereoselectivity (up to 99 % ee). In 2020, Zimmerman and co-workers demonstrated that single-chain nanoparticles (SCNP) containing a Ru-based photosensitizer and an exogenous β-Gal enzyme can promote concurrent and/or tandem bioorthogonal reactions in HeLa cells (Figure 7B).[60] The system can process engineered azide-caged probes that release active agents only after the reduction of the azide to the amine, and the subsequent action of the enzyme β-Gal. This tandem reaction could be performed in *E. coli* but was not translated successfully to live HeLa cells.

3.3. Covalent Modification of Biopolymers in Biologically Relevant Settings

The surge in the synthetic applications of photocatalysis has also reached the field of biopolymers.[61] Indeed, a substantial number of light-mediated approaches for the chemoselective modification of peptides, proteins or oligonucleotides have been unlocked.[62,63] This type of late-stage modifications, which can be described as part of the discipline of bioconjugation (defined as the covalent modification of biomolecules), may enable new ways to inter-

rogate and manipulate biology.[19,64] While early examples of photochemical labelling of biopolymers in vitro date back to the 1980s,[65] the field of covalent bioconjugation truly emerged over the last decade, in parallel to the explosive development of photocatalytic methods and tools. The group of Kodadek pioneered the use of $[Ru(bpy)_3]^{2+}$ as photocatalyst for the modification of proteins at tyrosine residues in aqueous solutions (Figure 8A). Specifically, they demonstrated the viability of performing cross-linking reactions of proteins with either other proteins or different molecules.[66] These processes rely on the oxidation of photoexcited $[Ru(bpy)_3]^{2+}$ with persulfate ($S_2O_8^{2-}$), and a subsequent selective oxidation of the phenol ring of tyrosine by proton-coupled electron transfer (PCET) to $[Ru(bpy)_3]^{3+}$. This process results in a tyrosyl radical that can react with another nucleophilic residue (tyrosine, tryptophan, methionine, or cysteine) to finally give the cross-linked product. Importantly, this type of photocatalytic cross-linking strategy was found to be compatible with complex cellular environments,[67] and can also be used to label proteins using exogenous coupling agents such as 1-methyl-4-aryl-urazole (MAUra).[68]

After these pioneering demonstrations, a number of photocatalytic modifications of peptides and proteins have been described, although very few of these bioconjugation strategies have been used in biological settings.[61] For example, the group of MacMillan reported a photoredox-based approach for the decarboxylative macrocyclization of peptides in aqueous buffers, through an intramolecular Giese addition of highly reactive alpha-amino alkyl-radical intermediates.[69] An intermolecular version of this Giese-type approach was employed shortly after for the bioconjugation of endogenous peptides and proteins. These reactions operate through a reductive-quenching photoredox cycle with riboflavin, generating α-amino alkyl radicals upon oxidative decarboxylation at the terminal carboxylate of peptide chains. This is followed by addition of the radical into a Michael acceptor and a subsequent SET reduction/protonation sequence (Figure 8B).[70] This and other photoredox strategies have evolved into standard tools for the selective functionalization (bioconjugation) of residues such as tyrosine,[68a,71] tryptophan,[72] methionine,[73] cysteine,[74] dehydroalanine,[75] or histidine,[76] generally in vitro. Among those, some of the most appealing modifications are based on the chemoselective introduction of fluorescent tags in order to track the biological pace of the tagged biopolymers.[74,77] Another illustrative in vivo application of a photocatalytic cross-linking strategy was reported by Fox and co-workers, based on their aforementioned oxidation of dihydrotetrazines to tetrazines.[48] They demonstrated the viability of performing photocatalytic cross-linking of aqueous solutions of hyaluronic acid polymers in live mice through an injection of the photocatalyst and the hydrogel precursor.[78]

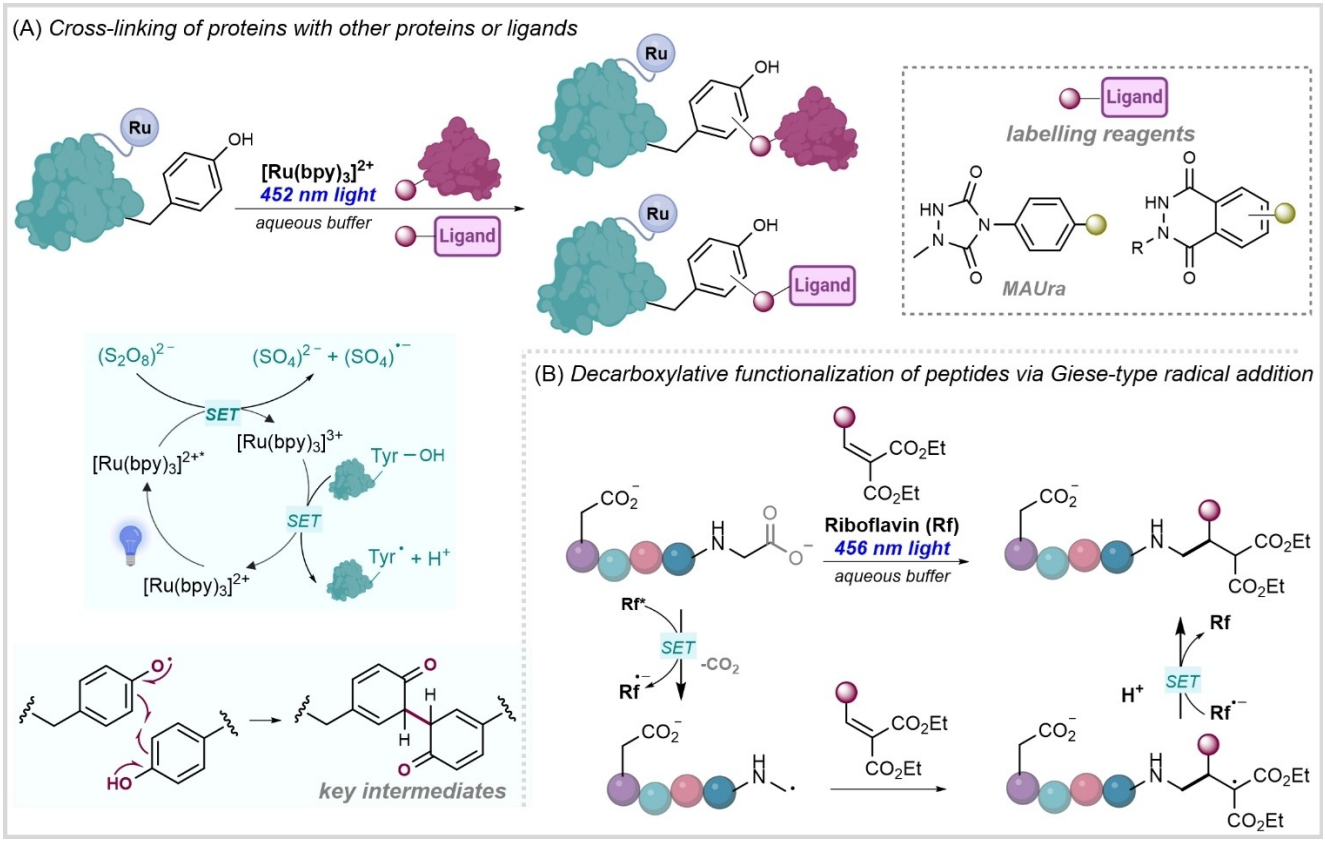

Figure 8. (A) Photocatalytic oxidative cross-linking of tyrosine; (B) Photocatalytic intermolecular Giese-type functionalization of peptides. Rf = riboflavin.

Further relevant applications of bioorthogonal bioconjugation using photocatalysis are related to the profiling of biomolecular interactions using proximity-labeling techniques. This strategy has emerged as one of the most powerful and versatile tools for mapping proteins and their interactions in biological environments, and for studying subcellular trafficking and basic cellular physiology.[79] Proximity labeling methods have traditionally relied on enzyme-based systems that activate an inert probe to tag a proximal target protein.[80] The combination of photocatalysis with spatial proteomics displays features similar to enzyme-based approaches, but can be superior in terms of precision or resolution. Most importantly, it allows for a considerable expansion of the labelling repertoire offered by enzymes.[62] A cutting-edge demonstration of the concept was reported in 2020 in a joint work between Merck and the group of MacMillan. They described a microenvironment mapping platform that exploits the photocatalytic generation of short-lived carbenes from diazirines to identify protein-protein interactions on cell membranes (Figure 9A).[81] The use of an antibody-conjugated iridium-based photocatalyst allows for the spatially precise labelling of protein-interaction networks. Subsequently, other proximity photo-labelling platforms to profile intracellular interactomes,[82] intercellular processes,[83] or even cell-virus interactions have been designed.[84]

The field of photocatalysis has also found a broad range of applications for the modification of oligonucleotides.[61] For instance, the group of Famulok described early examples for a photosensitized RNA cleavage at G-U base pairs.[85] Furthermore, several groups have employed photocatalytic techniques to promote the formation of DNA-protein cross-links.[86] Interstrand DNA cross-linking has also been achieved using furan as linking agent. Singlet oxygen (generated by photosensitization) reacts with the furan unit, much faster than with DNA, to give electrophilic 1,4-dicarbonyl intermediates.[87] The group of Shi built upon this idea to develop the first red-light-mediated RNA cross-linking system, with high potential applicability in cancer imaging and therapy. The furan probe features a NIR-fluorescent dye (Cyanine 7, Cy) as signal reporter, and a cyclic RGD peptide for tumor targeting (Figure 9B). The authors revealed how the covalent cross-linking of the probe to cytoplasmic RNA can induce severe apoptosis of cancer cells, causing tumor suppression.[88] In 2018, the group of Flanagan at Pfizer reported the first covalent bond-forming reaction between DNA-tagged Michael acceptors and alkyl radicals in degassed DMSO/water mixtures. These radicals are generated upon single-electron oxidation of α-amino acids by an excited Ir(III) photocatalyst, followed by decarboxylation.[89] These novel bioconjugation strategies have been applied for the preparation of drug-DNA conjugates to be used in screening platforms based on DNA-encoded libraries (DEL).[90] Other illustrative approaches towards the photocatalytic construction of DNA-encoded conjugates include the open-air (in vitro) metallaphotoredox reductive coupling of DNA-bound aryl halides with alkyl-radical precursors reported by Molander and co-workers,[91] or the photocatalytic [2 + 2] cycloaddition between DNA-tagged styrenes with cinnamates and enones via energy-transfer sensitization (EnT) developed by Pfizer (also in degassed solvent systems),[92] among many others.[61] It is also worth commenting that photosensitization techniques have also found applications in mapping the spatial

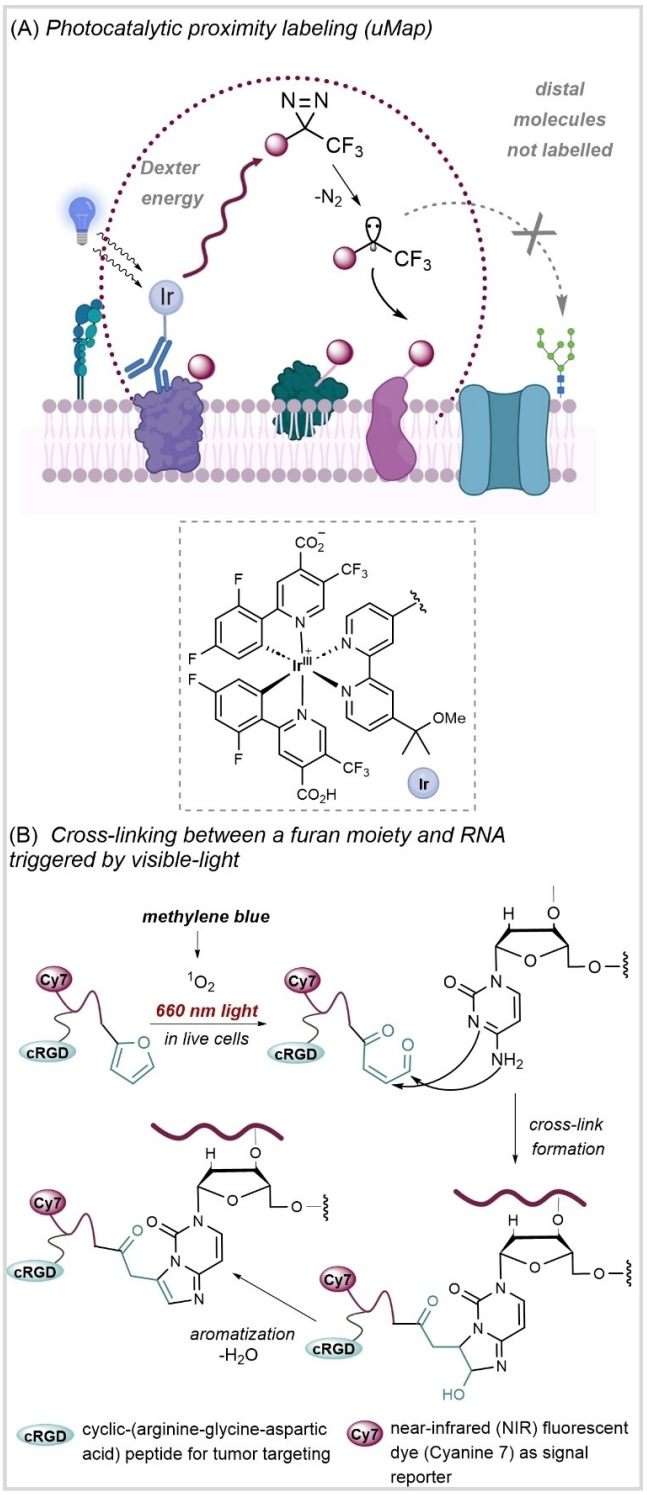

Figure 9. (A) Photocatalytic proximity labelling through carbene intermediates; (B) Furan RNA cross-linking promoted by red light via singlet-oxygen generation.

organization of RNA in living cells. This was achieved via photocatalytic ROS generation and oxidation of nucleobases, which occurs with high spatial specificity.[93] Besides biomacromolecules, visible-light photocatalysis has also played an important role in the in vitro modification of carbohydrate derivatives.[94]

Overall, the advent of photocatalysis has brought a significant revolution in our ability to introduce late-stage modifications in different types of biopolymers, especially proteins or nucleic acids. However, except for the mapping technologies, most of the bioconjugation reactions described herein have been carried out in vitro, and are still far from being fully biocompatible. A significant amount of work and optimization remains to be done in order to translate these methods into complex biological settings and living cells, which would truly unfold the field of *bioorthogonal bioconjugation.*

3.4. Synthesis of Small Molecules in Living Habitats

Despite all the above-mentioned advances in the field of bioorthogonal photocatalysis, the photocatalytic bond-forming synthesis of specific products in cellular environments remains to be truly developed. Adding this type of transformations to the toolbox of life-compatible synthetic reactions would broaden considerably the field of abiotic biological chemistry and should provide further opportunities to investigate and manipulate biology. However, progress in this endeavor has been very slow. In a initiating effort in this direction, Chen and co-workers developed a series of photocatalytic coupling reactions that can operate in aqueous buffers or cell lysates. Their studies were centered on the use of hypervalent iodine(III) species as oxidants to activate different radical precursors under photoredox conditions. Thus, a chemoselective cross-coupling reaction between alkyl trifluoroborates and alkynyl iodine(III) reagents (alkynyl benziodoxoles), was enabled by a catalytic system composed of hydroxybenziodoxol **I(III)–OH** as oxidative initiator, and $[Ru(bpy)_3](PF_6)_2$ under visible-light irradiation. As mentioned, the reaction could be performed in buffered aqueous conditions and in bacterial cell lysates (Figure 10A.i).[95] The authors propose as initiation step the homolytic cleavage of **I(III)–OH**, to give an I(II) radical that is able to oxidize photoexcited $[Ru(bpy)_3]^{2+}$, upon release of 2-iodophenyl benzoate. The resulting $[Ru(bpy)_3]^{3+}$ can oxidize the corresponding alkyl trifluoroborate to release an alkyl radical upon regeneration of $[Ru(bpy)_3]^{2+}$. Then, the alkyl radical can undergo an α–addition to the alkynyl I(III) substrate, giving an α-I(III) alkenyl radical. Subsequent elimination of I(II) results in the formation of the alkyne reaction product. The released I(II) would then engage in another SET oxidation with photoexcited $[Ru(bpy)_3]^{2+}$, closing the catalytic cycle. Later, the same group extended this approach for the development of a decarboxylative C–C coupling reaction between alkynyl iodonium reagents and benzoyl or carbamoyl ketoacids that can also operate in bacterial cell lysates (Figure 10A.ii).[96]

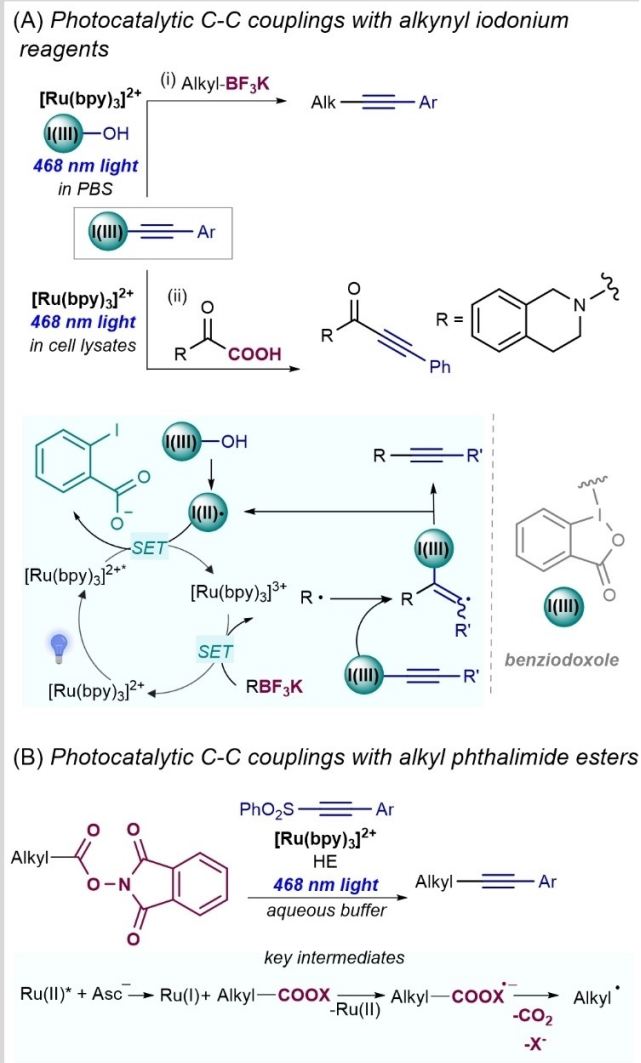

Figure 10. Photocatalytic C–C cross-coupling reactions in biologically relevant media using: (A) alkynyl iodonium reagents via oxidative quenching, and (B) alkyl phthalimide esters via reductive quenching. HE = Hantzsch ester.

By replacing benziodoxoles with redox-active alkyl phthalimide esters, the same group described a visible-light-induced decarboxylative $C(sp^3)$–C(sp) cross-coupling reaction using alkynyl sulfones as radical-acceptor partners (Figure 10B). The authors proved compatibility with a range of biomolecules, including nucleosides, cell lysates, or even enzymes such as human carbonic anhydrase II (HCA II), which retained catalytic activity after the reaction.[97] Contrastingly to the two previous examples, this reaction involves the reductive quenching of the photoexcited Ru(II)* with HE (Hantzsch ester), DIPEA or ascorbates, to give a reducing Ru(I) species, which engages in SET to a redox-active ester. The resulting radical anion readily undergoes irreversible fragmentation, generating reactive alkyl radicals upon release of CO_2. The alkyl-radical fragments are trapped by the acceptor alkynes, giving the reaction product after C–S bond scission.

The aforementioned SET-based reactions can be considered bioorthogonal, but they have yet to be implemented in live settings. Similarly, photocatalytic bond-forming synthetic transformations involving energy transfer (EnT) have also been only scarcely explored in biocompatible settings. Glorius and co-workers disclosed a water-compatible chemoselective anti-Markovnikov hydrothiolation of alkenes and alkynes promoted by triplet-triplet energy-transfer activation of disulfides, using an Ir(III) complex as photosensitizer (Figure 11A).[98] Importantly, they demonstrated that the reaction can be performed in a physiologically compatible Tris-HCl buffer (0.2 M, pH 7.4) in the presence of a wide variety of biomolecules, including amino acids, saccharides, nucleosides, single-stranded DNA, RNA, and even in human-cell lysates. A related photocatalytic thiol-ene reaction was reported by Schlau-Cohen and co-workers using a biohybrid photocatalyst **RPE-(Ru)** (Figure 11B).[99] The system was designed by covalent attachment of the photosynthetic red-light-harvesting protein R-phycoerythrin (RPE) to blue-light-active photocatalyst $[Ru(bpy)_3]^{2+}$. Upon red-light irradiation, both separated fragments are inactive in the thiol-ene coupling, but the biohybrid system was able to harvest low-energy light productively. The RPE can absorb light up to 630 nm, and transfers the energy to $[Ru(bpy)_3]^{2+}$, which can then promote the transformation. Although this system works in aqueous buffers, this bond-forming reaction has yet to be evaluated in biological environments. In this regard, while not involving a covalent-bond formation, the group of Winssinger has reported a biocompatible photocatalytic *E*-to-*Z* isomerization of stilbenes based on energy-transfer processes, using $[Ru(bpy)_3]Cl_2$ as photosensitizer.[100] Importantly, they demonstrated that this photocatalyzed isomerization can be performed in HeLa cells, generating biologically active *Z*-stilbenes (such as *Z*-resveratrol derivatives Res-3M or CA-4) *in cellulo*, resulting in increased cytotoxic activities or in controlled perturbations of microtubule dynamics.

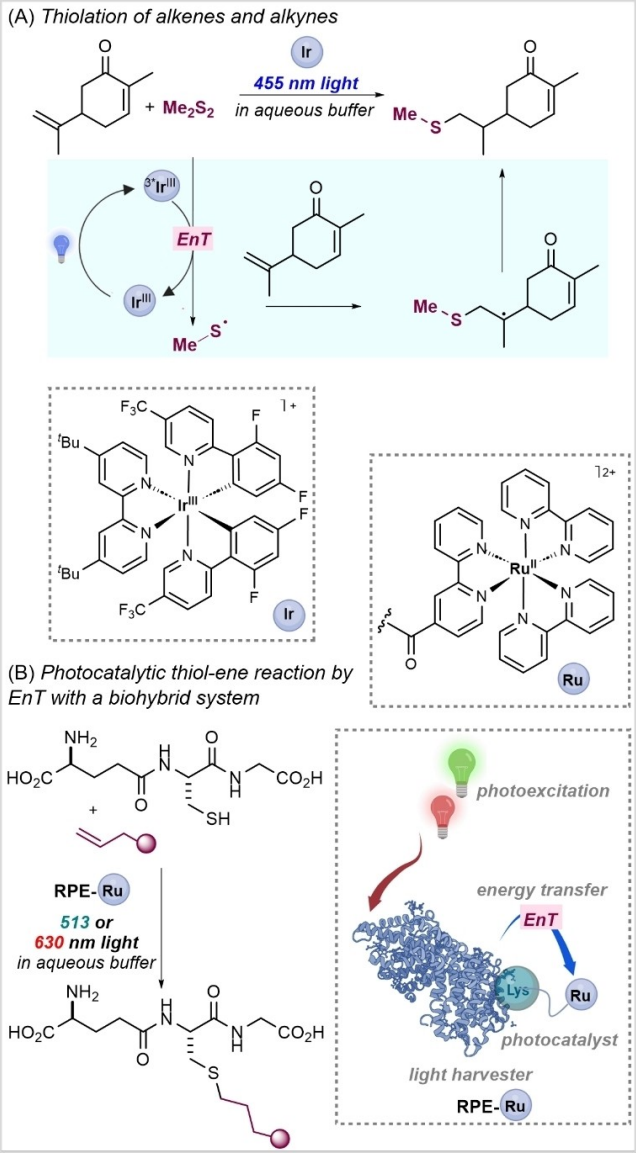

Figure 11. (A) Thiolation of alkenes and alkynes by triplet energy transfer; (B) Thiol-ene reaction via energy transfer within a biohybrid system. RPE = photosynthetic light-harvesting protein R-phycoerythrin.

Recently, the group of Mascareñas described the first abiotic photocatalytic bond-forming reaction that can take place in live cells (Figure 12A).[101] Specifically, the authors demonstrated that exogenous styryl azides can be converted into indoles inside living mammalian cells under photocatalytic conditions. Moreover, they could develop cell-selective photocatalytic reactions, using targeted photocatalysts equipped with RGD (arginine-glycyne-aspartic acid), a tripeptide that can target integrins in cell surfaces (Figure 12A,B). The authors demonstrated that only cells expressing high levels of integrins in their membrane (such as HeLa cells) were able to internalize enough amount of the **Eosin-RGD** photocatalyst, which was then capable of promoting the intracellular indole formation upon green-light excitation (blue fluorescence signal).

4. Conclusions and Outlook

The enormous progress in synthetic photocatalysis over the past two decades calls for its translation into the field of bioorthogonal chemistry. Photocatalysis exhibits a series of inherent features that fulfill key requirements for the development of biocompatible reactions, including mildness, chemoselectivity, water-compatibility and spatiotemporal controllability. Indeed, an increasing number of processes involving photocatalytic reactions are being performed in biologically relevant environments. Among others, these processes include redox transformations of exogenous or endogenous molecules, release of bioactive products from caged precursors, bioconjugation and modification of biopolymers, or mapping of biological regions using proximity-labelling techniques. Bioorthogonal bond-forming synthetic chemistry using photocatalytic approaches has been only scarcely explored, but it represents a promising area for further research. Beyond unlocking fundamental new knowledge in synthetic chemistry, these tools could pave the

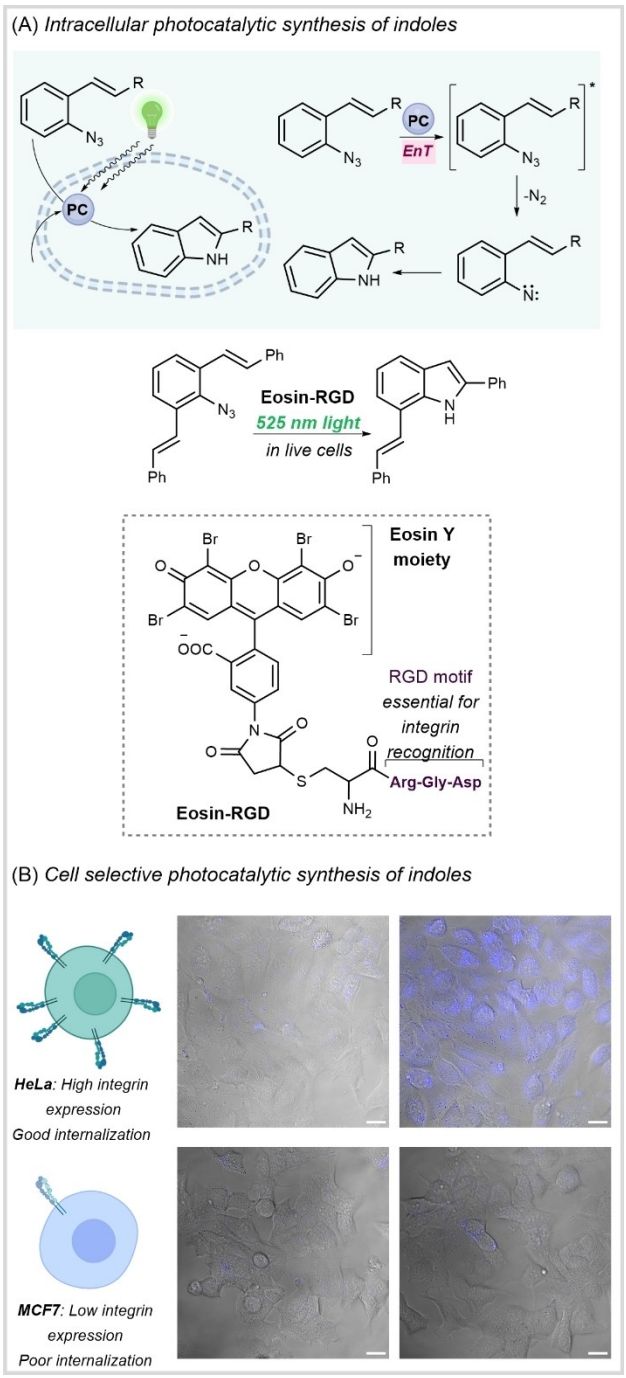

Figure 12. Synthetic bioorthogonal photocatalysis. (A) Schematic mechanistic proposal for the intracellular photocatalytic synthesis of indoles; (B) Cell-selective intracellular reactions.

way for new targeted drug-delivery strategies or enable the tailored modification of biomolecules to eventually manipulate cellular functions. However, the development of this field will require overcoming significant challenges, such as ensuring the orthogonality of processes, precisely localizing reagents and reactants in cellular environments, and managing secondary reactions with oxygen that could generate undesirable reactive oxygen species (ROS). Future progress may be driven by utilizing low-energy red/NIR light (which is less cytotoxic and offers greater tissue penetration), developing artificial photoenzymes that function within various cell types, or introducing nano-photoreactors into living systems, which could enhance energy or electron transfer through confinement effects.

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Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Keywords: bioorthogonal chemistry · photocatalysis · photoredox · in vivo synthesis · biomolecule modification

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