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Abstract

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Visible Light Photoredox Catalysis Using Ruthenium Complexes in Chemical Biology

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ABSTRACT: The development of bioorthogonal reactions have had a transformative impact in chemical biology and the quest to expand this toolbox continues. Herein we review recent applications of ruthenium-catalyzed photoredox reactions used in chemical biology.

INTRODUCTION

The pioneering work of Bertozzi and co-workers demonstrating chemoselective functionalization of cell-surface oligosaccharides[1-2] inspired the development of a growing repertoire of so-called bioorthogonal reaction.[3-4] A quintessential consideration in the design of bioorthogonal reactions is to leverage the chemistry on functional groups that are inert to a biological environment yet react at high rate in order to enable a reaction at low substrate concentrations. Within this context, transition metals hold a special place in organic chemistry for opening an array of reactivities and transformations that have no equivalent in natural systems. While transition metals are often associated with the well-known toxicity of anticancer drugs such as cis-platins, a number of publications have demonstrated that there is an interesting window of opportunity.[5-7] While the term bioorthogonal chemistry was initially coined for ligation reactions, bond cleavage reactions are also of interest in chemical biology to uncage substrates, inhibitors or a functional group of interest.[8] Light is an attractive external stimulant to trigger such cleavage events since it can be controlled both in time and space. Not surprisingly, photolabile groups to unmask the function of a molecule with spatiotemporal control has been extensively used in chemical biology.[9] Likewise, photoactivated crosslinkers also have a long history. Herein, we summarize exciting developments using the photoredox properties of ruthenium complexes to achieve both bond-forming reactions and bond cleavage reactions in biological environments, including live organisms. The ground-state stability of these catalysts coupled to their photoexcited state reactivity make them a candidate of choice for spatiotemporal-controlled bioorthogonal reactions.

Trisbipyridine-type ruthenium complexes have been extensively used in applications ranging from solar cells, water splitting, imaging and photodynamic therapy.[10-13] More recently the chemistry of $Ru(bpy)_3Cl_2$ and related analogs have enjoyed a renewed interest in synthetic chemistry for their unique single electron transfer (SET) photocatalysis with visible light.[14] Photoexcitation of the complex leads to a metal-to-ligand charge-transfer (MLCT), followed by an intersystem crossing (ISC), yielding a triplet excited state (Figure 1).

For $Ru(bpy)_3Cl_2$, the MLCT λ_{max} at 452 nm; however, many ligands besides bipyridine have been investigated and they can have a significant impact on the λ_{max} of the complex. For instance $[Ru(biq)_2phen]^{2+}$ has a MLCT λ_{max} at 550 nm.[15] In the absence of a reactant, the excited state $[Ru(bpy)_3]^{2+*}$ will relax to the ground state with phosphorescence emission (τ: 1100 ns, λ_{max} = 615 nm for $Ru(bpy)_3Cl_2$; varies with the nature of the ligands). While the luminescent properties of the polypyridyl complex have been used for imaging of DNA in living cells,[16] the brightness of such complex is much lower than traditional organic fluorescent dyes due to the weaker absorption coefficient (typically 5-10 lower than organic fluorophores) coupled to the poorer quantum yield (typically 5-20 times lower than organic fluorophores). This restrict the detection threshold to low micromolar concentrations as opposed to organic dyes that can be easily detected at the low nanomolar concentration in most confocal microscopes. In the presence of a reactant with a suitable redox potential, the

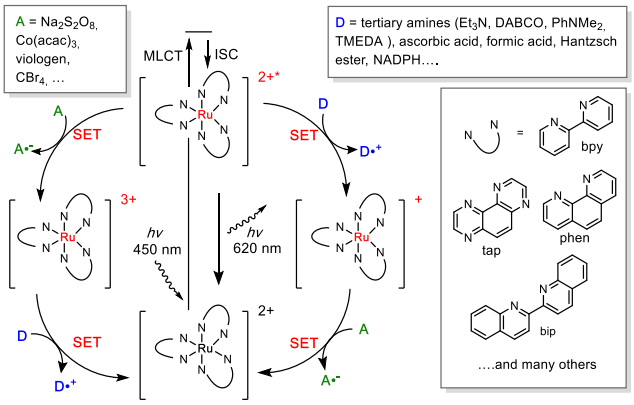

Figure 1. Mechanism of photocatalysis of $[Ru(bpy)_3]^{2+}$ and related complexes.

complex will either donate an electron to become a strong oxidant or accept an electron to become a strong reducing agent. The fact that this chemistry requires visible light provides a spatiotemporal control akin the use of a photocaging group however, the scope of the chemistry is vastly different.

Oxidative crosslinking reactions: The Kodadek group first reported the crosslinking of proteins using the photocatalytic properties of $[Ru(bpy)_3]^{2+}$ under oxidative conditions with ammonium persulfate ($(NH_4)_2S_2O_8$) almost twenty years ago (Figure 2A).[17] High yields of cross-linked products were obtained with irradiation times of < 1 s using a 150 w xenon arc lamp (>380 nm emission) using 125 μM of $Ru(bpy)_3Cl_2$ and 2.5 mM of ammonium persulfate. The authors demonstrated the crosslinking of UvsY, a native hexamer involved in phage T4 recombination, with 60% yield. The same procedure was used to crosslink transcription factors known to form homodimers or tetramers. The proposed mechanism of the crosslinking involves the oxidation of the photoexcited state, $[Ru(bpy)_3]^{2+*}$, by the persulfate to yield $[Ru(bpy)_3]^{3+}$ and a sulfate radical. SET from a tyrosine residue to $[Ru(bpy)_3]^{3+}$ yields a reactive tyrosyl radical that engages in a reaction with a neighboring nucleophile (tyrosine, tryptophan, methionine, or cysteine), followed by hydrogen abstraction by the sulfate radical to afford the crosslinked product.[18] This method clearly differentiates itself from classical azide- or benzophenone-based crosslinkers since it harnesses the reactivity of a native amino acid residue and proceeds with visible light rather than ultraviolet. The technology was also shown to work in crude cell lysates.[19] This methodology was further extended by Bonnafous and coworkers demonstrating the crosslinking between GPCRs and peptidic ligands containing a tyrosine. To facilitate detection of crosslinked product, the tyrosyl residue was radio-iodinated (Figure 2B).[20] The labeling studies were conducted on intact cells using 0.5 mM of $Ru(bpy)_3Cl_2$ and 0.5 mM of ammonium persulfate with 3 s irradiation (200 W tungsten lamp). Concurring with the previous observation by Kodadek and coworkers, the authors found that the addition of tryptophan, tyrosine and cysteine to the reaction inhibited the crosslinking (phenylalanine, histidine and lysine did not), adding support to the fact that the crosslinking proceeds through a tyrosyl radical which undergoes a radical coupling with a Trp, Tyr, or Cys disulfide residue on the target protein. The methodology was validated with agonists and antagonists of bradykinin receptor (B_1 and B_2), an angiotensin II receptor (AT1), the vasopressin receptor (V1a), and the oxytocin receptor, illustrating the broad applicability to the protocol.

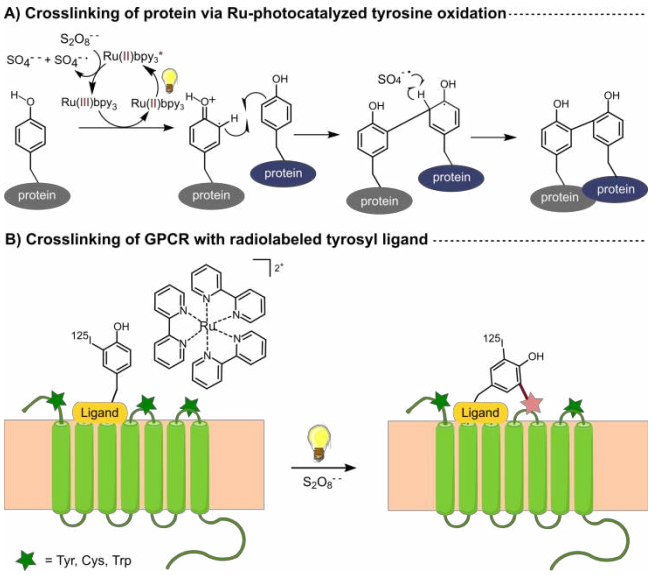

Figure 2. Ruthenium-photocatalyzed oxidative crosslinking reactions.

Oxidative receptor labeling reactions:
The ability to functionalize a ligand that selectively targets a protein of interest with a catalyst to direct chemistry has attracted significant attention.[21-22] The Nakamura group investigated the use of $[Ru(bpy)_3]^{2+}$ conjugates to direct the oxidation of tyrosine residues and coupling with tyrosyl radical trapping agents (Figure 3). It was shown that a phenylenediamine performed best (25 alternatives tested) and it could be functionalized with rhodamine or biotin for in-gel visualization of the labeled protein.[23] Model reactions showed that an external oxidant (ammonium persulfate) dramatically accelerated the process. The addition of superoxide dismutase instead of ammonium persulfate also accelerated the reaction. This

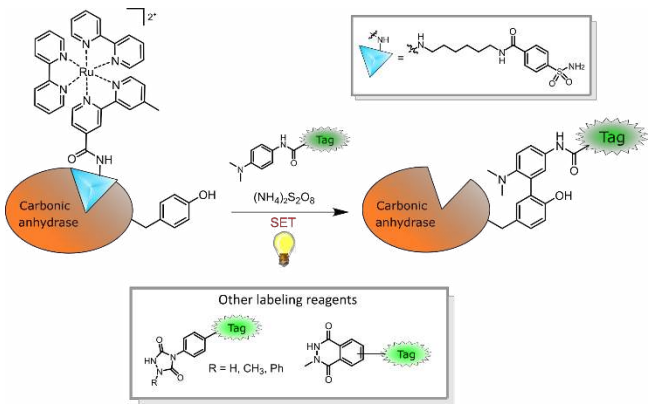

Figure 3. Proximity-induced ruthenium-photocatalyzed oxidative labeling reactions.

is attributed to the fact that the photoexcited $[Ru(bpy)_3]^{2+*}$ sensitizes the formation of $^1O_2$ which can serve as acceptor to yield $[Ru(bpy)_3]^{3+}$ and $O_2^{\cdot-}$; however, this reaction is reversible.[24] In the absence of a driving force to displace this equilibrium, very low yields of Ru(III) are obtained.

Superoxide dismutase drives the reaction by converting $O_2^{\cdot-}$ to H_2O_2.[19] However, it is not clear at this stage whether the reaction is initiated with the oxidation of the phenyldiamine, which then reacts with tyrosine, or *vice versa*.[25] The methodology was showcased with the selective labeling of carbonic anhydrase in crude cell lysates using a sulfonamide ligand that binds specifically to this protein. The reaction was performed with 1 μM concentration of the ligand-$[Ru(bpy)_3]^{2+}$ conjugate and 500 μM of the tyrosyl trapping reagents to yield a selective labeling of carbonic anhydrase. A further refinement of this methodology was recently reported with the use of 1-methyl-4arylazole derivatives.[25] This tyrosyl radical trapping reagent was shown to have a shorter radical lifetime than previously reported phenylenediamine-based substrates. This translated into smaller labeling radius than the phenyldiamine-based substrate in the same experiment. Specifically, using the sulfonamide-$[Ru(bpy)_3]^{2+}$ conjugate that targets carbonic anhydrase, labeling was confined to the tyrosine residue closest to the ligand binding site (out of six residues on the protein).

Selective photo-inactivation of proteins:

The general idea to harness a photosensitizer that generates reactive oxygen species (ROS) upon light irradiation in order to degrade a protein of interest has been demonstrated for genetically encoded proteins as well as organic fluorophores.[26-28] When a sensitizer is conjugated to a protein of interest and excited, the local concentration of ROS inactivates the protein function or induces its degradation (chromophore-assisted light inactivation: CALI). Using an assay specifically designed to evaluate the efficacy of different chromophores/sensitizers, the Kodadek group identified a $[Ru(bpy)_3]^{2+}$ derivative as significantly more efficient than fluorescein for CALI, achieving 50% inactivation over 30 min irradiation (150 W xenon arc lamp) with the $[Ru(bpy)_3]^{2+}$ conjugate *vs* 20% with fluorescein.[29] $Ru(bpy)_3Cl_2$ is an efficient sensitizer for the formation of singlet oxygen but singlet oxygen does not diffuse more than 40-80 Å, restraining the reaction to the local environment of the target protein. It is well appreciated that singlet oxygen reacts with electron rich aromatic side chain (Trp and Thy, His) and sulfur (Met, Cys), however, the rate of reaction is highly dependent on the accessibility as well as local environment of these residues.[30] The Kodadek group further showed

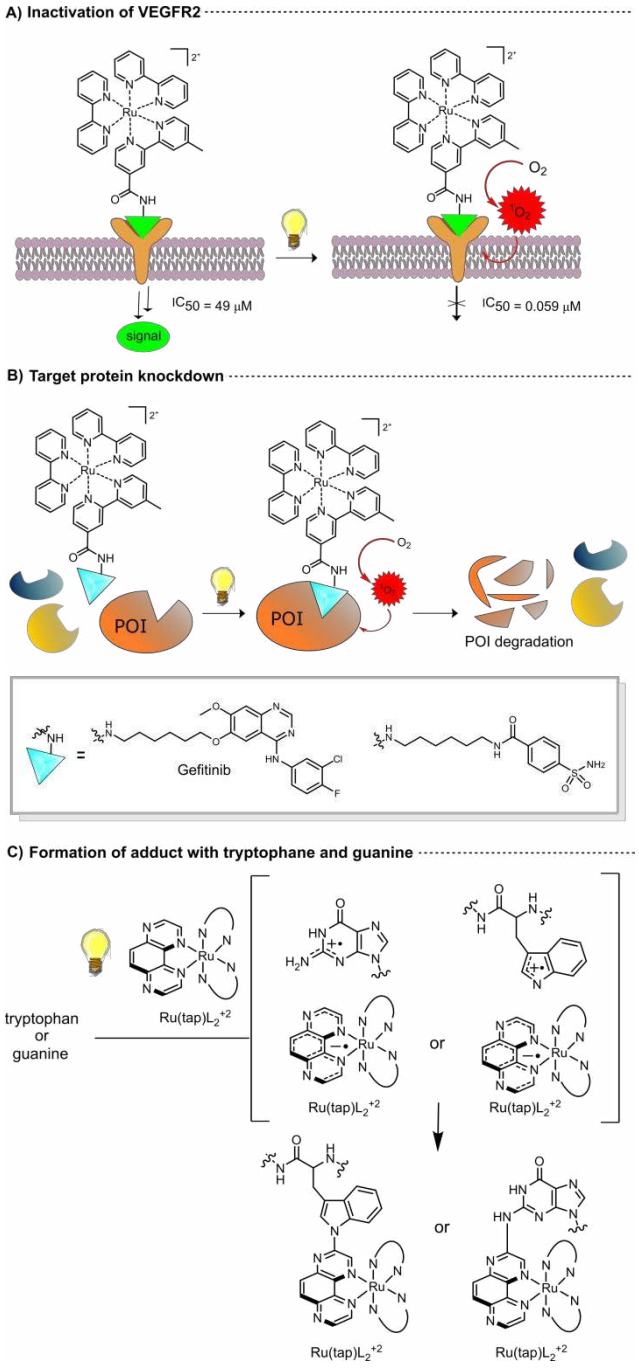

Figure 4. Ruthenium-photocatalyzed degradation of a protein of interest (POI).

that a $[Ru(bpy)_3]^{2+}$ complex conjugated to a weak but selective binder to the VEFGR receptor potently inhibited its function (autophosphorylation) upon irradiation with a high-intensity lamp (Figure 4A).[31] Remarkably, the IC_{50} of the conjugate (measuring the level of autophosphorylation) was 49 µM without irradiation and 59 nM under irradiation. This represent an 800-fold increase in potency. As comparison, a fluorescein conjugate of the same ligand had an IC_{50} of roughly 2 µM under the same irradiation procedure. Moreover, using a higher affinity ligand with an IC_{50} of 500 nM in the absence of light, an IC_{50} of 0.590 nM under irradiation was measure (1700-fold increase). While these ligands bound the extracellular domain of VEGFR, the authors also demonstrated the target-inactivation of an intracellular protein. Using a proteasome agonist which enhances proteasomal activity in the absence of light, an inhibition was observed under irradiation, with an IC_{50} that improved with longer irradiation time (IC_{50} = 300 nM at 10 min, IC_{50} = 85 nM at 20 min irradiation). The selectivity of this target inactivation was evaluated by measuring the activity of a luciferase expressed in the same cellular environment. While the proteasome was inhibited under the experimental conditions, no change in luciferase activity was observed, supporting the fact that the production of singlet oxygen has an effect only in the direct vicinity of the target protein. Furthermore, no ROS were detected using a general ROS-sensitive probe (H_2DCFDA), consistent with the premise that only a local concentration is generated. This selective inactivation of protein was further studied by Nakamura and coworkers (Figure 4B).[32] Mass spectrometry analysis of carbonic anhydrase treated with the sulfonamide-$[Ru(bpy)_3]^{2+}$ conjugate (carbonic anhydrase ligand), showed a distinct pattern for the residue in the vicinity of the ligand binding site when comparing samples with and without irradiation. Selective degradation of EGFR was also demonstrated with a gefitinib-$[Ru(bpy)_3]^{2+}$ conjugate. Compared to the aforementioned EGFR ligand, gefitinib binds to the nucleotide binding site of the kinase which is located intracellularly. Interestingly, analysis of EGFR degradation by gel revealed that protein degradation was accompanied by oxidative crosslinking between EGFRs and associated proteins. However, addition of phenylenediamine derivatives or other tyrosyl radical trapping reagents (reagents shown in Figure 3) led to reduced protein degradation, presumably by acting as a singlet oxygen scavengers.[33]

While the photoexcited complex $[Ru(bpy)_3]^{2+*}$ does not have the oxidizing potential to react directly with amino acids or nucleobases, the use of a complex with more electron deficient ligands (such as $Ru(tap)_2bpy^{2+}$) affords a photoexcited state that abstract an electron from guanine (Figure 4C).[34] Radical recombination and proton transfer leads to an adduct between the ruthenium complex and guanine (Figure 4). Such ruthenium complexes can thus crosslink DNA. Likewise, tryptophan is oxidized by similar complexes and also forms photo-adducts.[35]

Photouncaging of ligand on ruthenium complexes: Ruthenium complexes of the general type $[Ru(bpy)_2X_2]^{2+}$ where X is a monodentate ligand such as an amine, nitrile, pyridine or thioether, are stable in the ground state, however, they undergo facile ligand exchange upon photoexcitation to $[Ru(bpy)_2X_2]^{2+*}$ (Figure 5). While the reaction is mechanistically reversible, carrying out the reaction in water drives the equilibrium to the loss of the ligand.[36] Accordingly, bioactive compounds such as 4-aminopyridine, tryptamine, serotonin and γ-amino butyric acid have been caged with $Ru(bpy)_2$ complexes.[37-38] The uncaging of ligand under visible light photoirradiation (450 nm, blue light) has been harnessed to release drugs in a cellular environment. A recent example includes the use of two pyridine ligands to force a hairpin in an antisense morpholino oligonucleotide (MO).[39] The cyclized MO was ineffective in inhibiting translation but upon photoirradiation, the MO opens to a linear conformation that can bind the target mRNA. This technology was used to uncage the activity of MO in zebrafish at 24 hours post-fertilization. In the absence of irradiation, no phenotype was detected whereas irradiation afforded an activity comparable to the MO alone, indicating that the caged MO was stable in the zebra fish and uncaging was high yielding. Notwithstanding the importance of photo-uncaging bioactive molecules with visible light, the bond cleavage in these examples do not make use of ruthenium's photocatalysis as discussed in the next section.

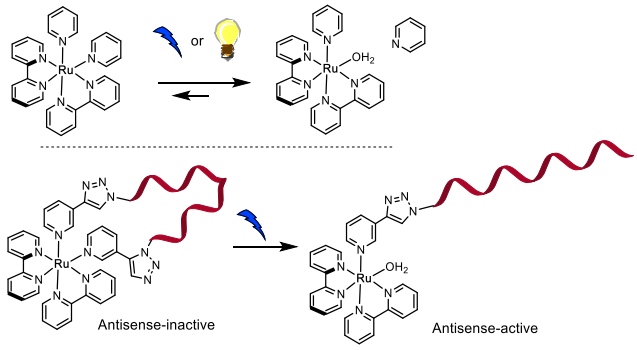

Figure 5. Ruthenium complex as photocaging agents.

Bond cleaving reactions under reductive ruthenium photocatalysis: Falvey's group designed the first photolabile group that can be reductively cleaved with $[Ru(bpy)_3]^{2+}$ photocatalysis.[40] Following a standing interest in the fragmentation of N-methyl-4-picolinium esters[41] through photoinduced electron transfer, they

identified 2-cyanopyridinium as having the appropriate reduction potential and reasoned that a 4-carboxymethyl analog would undergo cleavage upon SET from $[Ru(bpy)_3]^{1+}$. However, this substrate was found to be easily deprotonated at the benzylic position and sensitive to base such as DABCO or dimethylaniline. Nonetheless, the excellent deprotection yields were obtained using ascorbic acid as a stoichiometric reducing agent and catalytic quantities of $Ru(bpy)_3Cl_2$ (Figure 6A). The rate constant for the reaction of $[Ru(bpy)_3]^{2+*}$ with ascorbic acid was found to be near diffusion control (3.84×10^7 $M^{-1}s^{-1}$) whereas the rate constant for reduction of the pyridinium by $Ru(bpy)_3^{1+}$ was 1.77×10^9 $M^{-1}s^{-1}$. The quantum yield of the reaction was calculated to be 1%. The scope of this protecting group was extended to simpler picolinium carbonate for amines[42] and picolinium ethers for phenols.[43] We have made extensive use of these advances in templated reactions (see discussion below).

The photosensitization of alkyl and aryl azides is well established and has been extensively used since its discovery in the late 1960s.[44] More recently, it has been revisited with ruthenium-complexes as sensitizers.[45] This reaction proceeds through an energy transfer rather than SET resulting in a nitrene intermediate. Using a DNA-templated reaction screen, Liu and coworkers identified $Ru(bpy_3)Cl_2$-photocatalyzed coupling reactions between an aryl azide and several functionalities (alkene, sulfone, phenol and nitrile).[46] Mechanistic investigation led to the hypothesis that azides were good substrates for the reduced ruthenium intermediate $[Ru(bpy_3)]^{1+}$ resulting in an aminyl radical that underwent radical addition. Performing the reaction with sacrificial reducing agents / hydrogen donors such as Hantzsch ester, formic acid, ascorbic acid or NADPH led to excellent reduction yields (Figure 6B). Importantly, it was also demonstrated that the reaction was compatible with biomolecules. Namely, oligonucleotides functionalized with an aryl or alkyl azide as well as a disulfide were reduced with >95% efficiency without reduction of the disulfide. This example clearly established the orthogonality of this $Ru(bpy_3)Cl_2$-photocatalyzed azide reduction and its complementarity to the Staudinger reaction, which had been extensively used in bioorthogonal reactions, but also reduces disulfide bonds. Furthermore, performing the reaction in the presence of RNAse A did not alter the activity of the enzyme. Taken together these results established the biocompatibility of the reductive cycle of ruthenium photocatalysis with biomolecules. The biocompatibility of this reaction is remarkable considering the aforementioned use of $[Ru(bpy_3)]^{2+}$ as CALI reagents. However, it is important to note that the photoexcited ruthenium complex reacts with ascorbate at a rate close to diffusion control. Thus, as long as the redox buffer is at higher concentration than oxygen, the ruthenium can be driven towards the reductive cycle. Furthermore, if singlet oxygen is produced in the presence of ascorbate, it is rapidly reduced ($k = 3 \times 10^8$ M^{-1} s^{-1}, this reaction proceeds close to diffusion control rate).[47-48] Our group had developed azide-reduction triggered immolative groups[49-50] which were subsequently used in ruthenium photocatalyzed cleavage (Figure 6B).

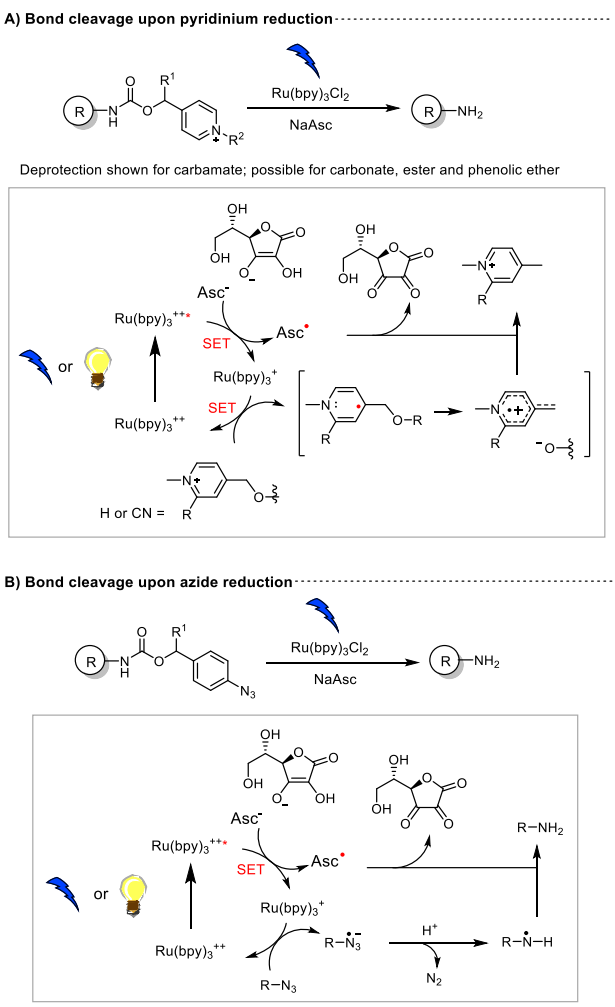

Figure 6. Ruthenium-photocatalyzed bond cleaving reactions.

Templated bond-cleaving reactions: The functional group orthogonality of these reductive bond cleaving reactions inspired us to explore their utility in systems designed to sense proteins and nucleic acids. For simplicity of conjugation we used $[Ru(bpy)_2phen]^{2+}$ conjugates that can be readily prepared using commercially available $Ru(bpy)_2(phen\text{-}NCS)Cl_2$. This reagent cleanly reacts with an amine in a substrate of interest.[51] The complex is stable to TFA, making conjugates accessible by SPPS. We showed that ligands targeting proximal binding sites on a protein of interest could be used to bring the ruthenium photocatalyst and substrate in close proximity, thus promoting the bond cleaving reaction and the uncaging of a fluorophore (Figure 7A).[52]

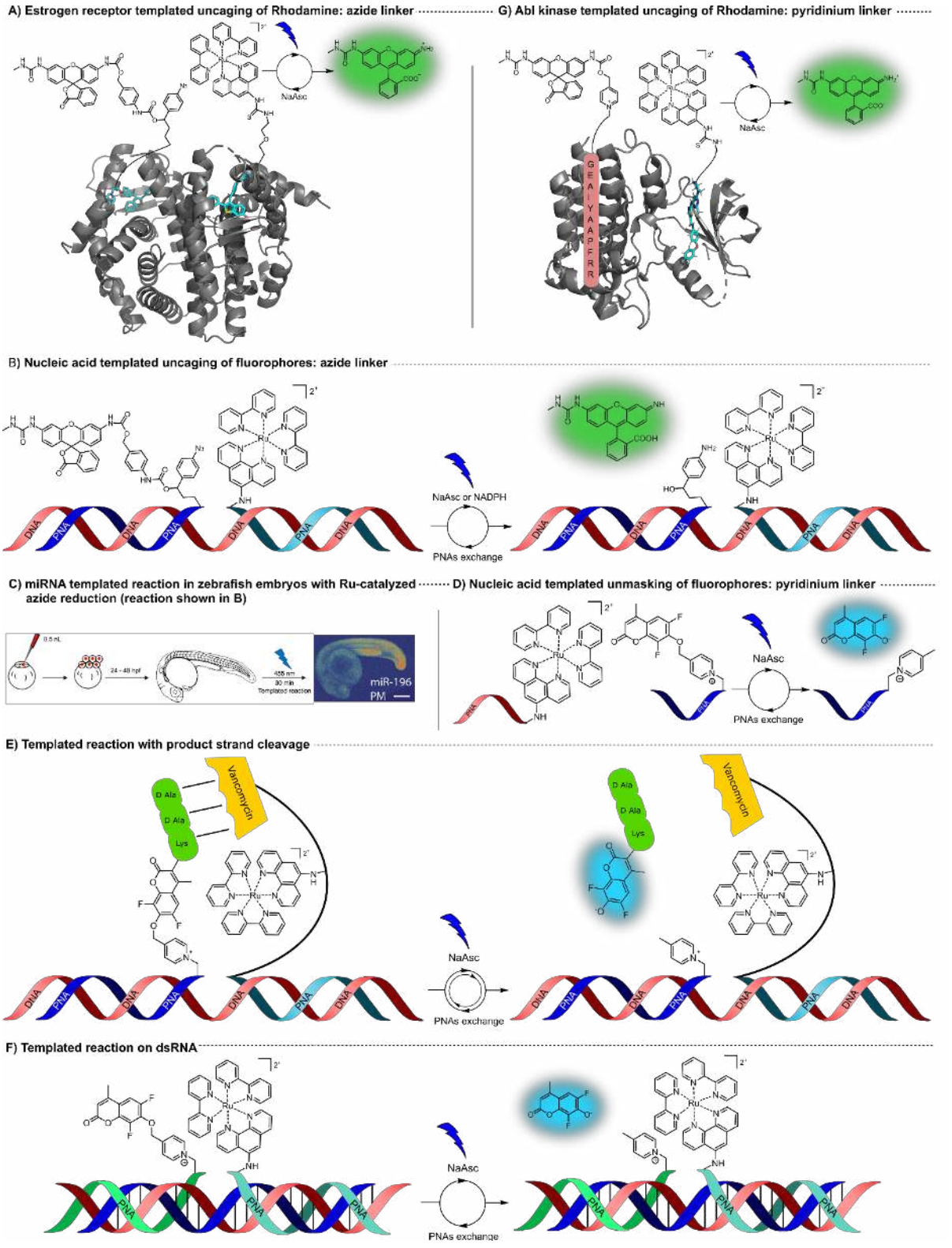

Figure 7. Templated reactions using ruthenium photocatalysis.

Specifically, biotin and raloxifen conjugates were used to sense acetyl-CoA carboxylase (an enzyme that uses biotin as cofactor) and the estrogen receptor.[53] It was shown that below 1 μM of conjugate, the bi-molecular reactions had negligible rates in the absence of the targets but fast reactions were observed in the presence of the target proteins (>30 fold rate acceleration) using a 1W LED. The reaction was shown to proceed in live cells without any external reducing agent and without apparent cytotoxicity. As discussed below, templated reactions using the same chemistry were also found to proceed in live zebra fish without any apparent toxicity.

The cytosol of cells is known to be reductive with a ratio of NADP/NADPH largely in favor of NADPH. Considering that NADPH is an excellent reducing agent for the photoexcited state of ruthenium complexes, the redox buffer of cells can account for the observed reductive cycle of ruthenium photocatalysis. The same reaction manifold was also found to be highly productive for nucleic acid-templated reactions and as a means to sense microRNA in live cells (Figure 7B).[54-55] In vitro, the reaction was shown to respond to just 5 pM of DNA with probes at 250 nM and 50 nM for the substrate and ruthenium conjugates respectively. This represent a turnover in excess of 4000-fold. In live cells, the probes were found to be stable for extended time (up to 16h investigated). The reaction still afforded strong response to a target sequence with conjugates at 10 nM of azide substrate and 2 nM of ruthenium complex.[56] In 30 min reaction time, it was possible to observe a response to 100 pM of target DNA. Based on the temporal control in the reaction, this system was use to image the localized expression of three different microRNA in live zebrafish. The probes were injected at the one cell stage and the embryo was allowed to develop to 24 hpf or 36 hpf before triggering the reaction (1 W LED lamp, Figure 7C).[56] Notably, the treated embryo lived for up to 5 days (maximum duration of the protocol) and showed no morphological phenotype following a reaction. The demonstration that the ruthenium-photocatalyzed azide reduction can be performed in a live vertebrate adds a valuable reaction to the chemical toolbox for *in vivo* chemistry.

Templated reactions are promoted by the high effective concentration of reagent achieved upon interaction of the probes with the template. The rate limiting step in turnover of the template is either limited by the rate of the reaction or the dissociation rate of spent reactants. In nucleic acid templated reactions, the rate of dissociation can be easily adjusted by the length of the oligonucleotide probes. We showed that dramatically faster templated reactions (>200 fold) were possible using a ruthenium-photocatalyzed pyridinium reduction rather than azide reduction to trigger the immolation (Figure 7D).[43] To facilitate turn-over, a system inspired by a three-way junction that yields a product with faster dissociation than the substrate was designed (Figure 7E).[57] This chemistry requires that one component of the templated reaction acts catalytically. The same chemistry also proved useful for the detection of dsRNA using triplex forming probes (Figure 7F).[58]

The faster kinetics of pyridinium reduction compared to azide reduction also proved essential for kinase templated reaction.[59] Using two probes that target the nucleotide-binding site and substrate binding site of a target kinase respectively, the reagents appended on the probes are brought within reactive distance, thereby enabling the chemical transformation. Notably, the templated reaction only proceeded with the pyridinium reduction chemistry, not with the azide reduction chemistry (Figure 7G). The failure of the latter reaction was attributed to the fast dissociation (k_{off}) of the peptide substrate, a dissociation rate that exceeded the rate of azide reduction but not pyridinium reduction. The combined selectivity of a nucleotide binding site pharmacophore and a ligand binding the substrate site delivered a kinase-specific reaction with 6-fold amplification for the target kinase relative to a closely related kinase (ex. Abl *vs* Src). The selectivity of the reaction was also demonstrated in cells.

In cellulo protein tracking with Ru-conjugates: Proximity-induced chemistry using the reductive cycle of ruthenium photocatalysis was also demonstrated. To this end, a caged precipitating dye was prepared with an azide reduction trigger (Figure 8).[60] Ligand targeting EGFR and the estrogen receptor were conjugated to $[Ru(bpy)_2phen]^{2+}$ and used in ruthenium photocatalyzed uncaging reaction yielding a distinct labeling at the membrane (exclusively for cells expressing EGFR) and in the nucleus, consistent with the localization of the target proteins. The reaction required 5-20 μM of substrate to afford the precipitate. While this is a higher concentration than in templated reaction, the concentration remains compatible with live cell experiments. Owing to the fact that the dye precipitates once uncaged, a subcellular resolution can be achieved in the imaging of protein localization.

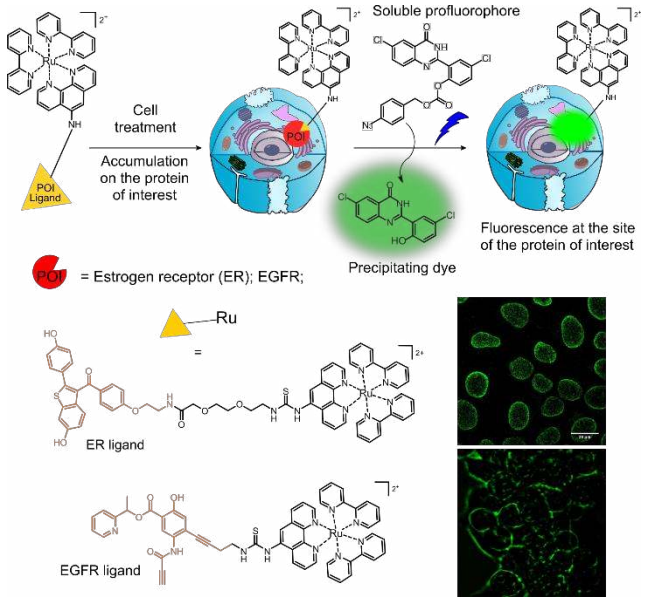

Figure 8. Localized uncaging of precipitating dye.

Cross coupling reactions in the presence of biomolecules: The Chen group reported a cross coupling reaction between alkyltrifluoroborates

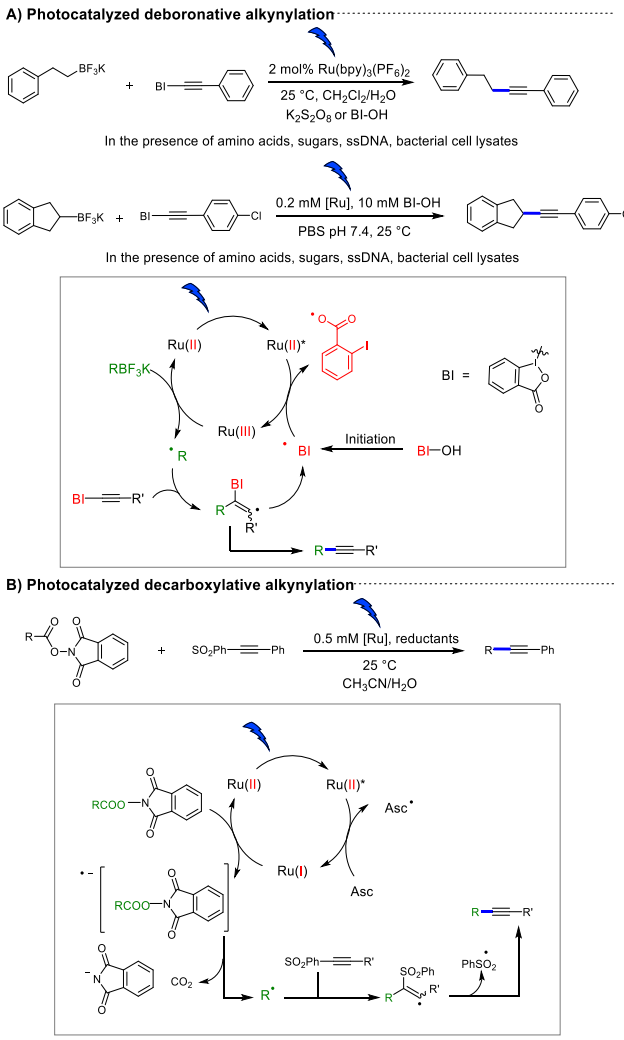

Figure 9. Ruthenium-photocatalyzed reductive bond forming reaction (BI= benziodoxole).

or boronic acids and benziodoxole-alkyne via an oxidative deboronation catalyzed by $Ru(bpy)_3Cl_2$ using a blue LED (Figure 9).[61] Using catalytic quantities of hydroxybenziodoxole (BI-OH), the photoexcited $[Ru(bpy)_3]^{2+*}$ is oxidized to $[Ru(bpy)_3]^{3+}$ and promotes the oxidative deboronation yielding an alkyl radical. This radical is trapped by the benziodoxole-akyne (α-addition onto the alkyne, elimination of the hydroxybenziodoxole radical) to propagate the oxidative deboronation. The hydroxybenziodoxole generated served as the oxidant for Ru(II)*. While the reaction proceeded fastest with catalytic quantities of hydroxybenziodoxole (5 mol%), air (oxygen) was sufficient to generate $[Ru(bpy)_3]^{3+}$ and promote the oxidative deboronation, thus triggering the catalytic cycle. The reaction was shown to proceed in excellent yield (10 mM of substrate) in the presence of DNA, BSA or even crude cell lysate. The same group also reported a decarboxylative alkynylation under reductive ruthenium phototocatalysis.[62] It is known that N-acyloxyphthalimide is a competent substrate for SET from Ru(I) and undergoes rapid loss of CO_2 and phthalate to yield the alkyl radical.[63] The use of alkynyl phenylsulfone proved to perform best in the coupling. After α-addition to the alkynyl sulfone and the sulfonyl radical elimination, the alkynylated product was obtained. The Ru(I) was obtained from the reduction of the Ru(II)* with Hünig's base, Hantsch ester or ascorbate. The reaction was performed in the presence of carbonic anhydrase (1.6 mol%) with only marginal reduction of enzymatic activity after the reaction, clearly establishing that the reductive cycle of ruthenium photocatalysis does not inhibit the function of this enzyme. The compatibility of photocatalyst with enzyme catalysis is attracting attention as a means to achieve non-natural biocatalytic transformations. A recent example using xanthene-based photocatalysts (rose bengal) enabled a double-bond reductase to catalyze an enantioselective deacetoxylation.[64] Another recent example made use of an iridium photocatalyst in conjunction with a monoamine oxidase to access enantioenriched amines in excellent yield and purity.[65] These results clearly highlight an excitant potential of photocatalysis with biocatalysis.

Turn-on of ruthenium catalysis: The previous sections discussed the vast opportunities of ruthenium-photocatalyzed reactions in chemical biology. Several studies have also demonstrated the possibility to engineer a turn on of this catalytic activity in response to biological stimuli. The Plaxco group has made extensive use of binding-induced conformational changes as a means to sense biomolecule interactions.[66] Amongst the different strategies pursued, they investigated the use of a peptide beacon functionalized on one side with a $[Ru(bpy)_2phen)]^{2+}$ complex and on the other side with viologen (Figure 10A), an excellent electron acceptor for the photoexcited ruthenium complex $[Ru(bpy)_2phen]]^{2+*}$.[67] The two ends of the unstructured peptide will collide on a time scale of 100 ns, however, when bound to its target antibody, the peptide adopts a rigid conformation that extend the two ends and dramatically reduces interactions of the conjugates protruding from the ends of the peptide (EKIRLR: epitope of HIV's p17 matrix protein). Based on the luminescence life time of the ruthenium complex (800 ns), electron transfer to viologen is faster than photon emission in the unbound state; however, once bound to the antibody, the emission increased 6-fold suggesting that the SET is largely inhibited. This example clearly illustrates the potential to tune the course of reaction of a ruthenium complex in response to a receptor-ligand interaction. An alternative strategy based on FRET rather than SET was reported by the Xing group (Figure 10B).[68]

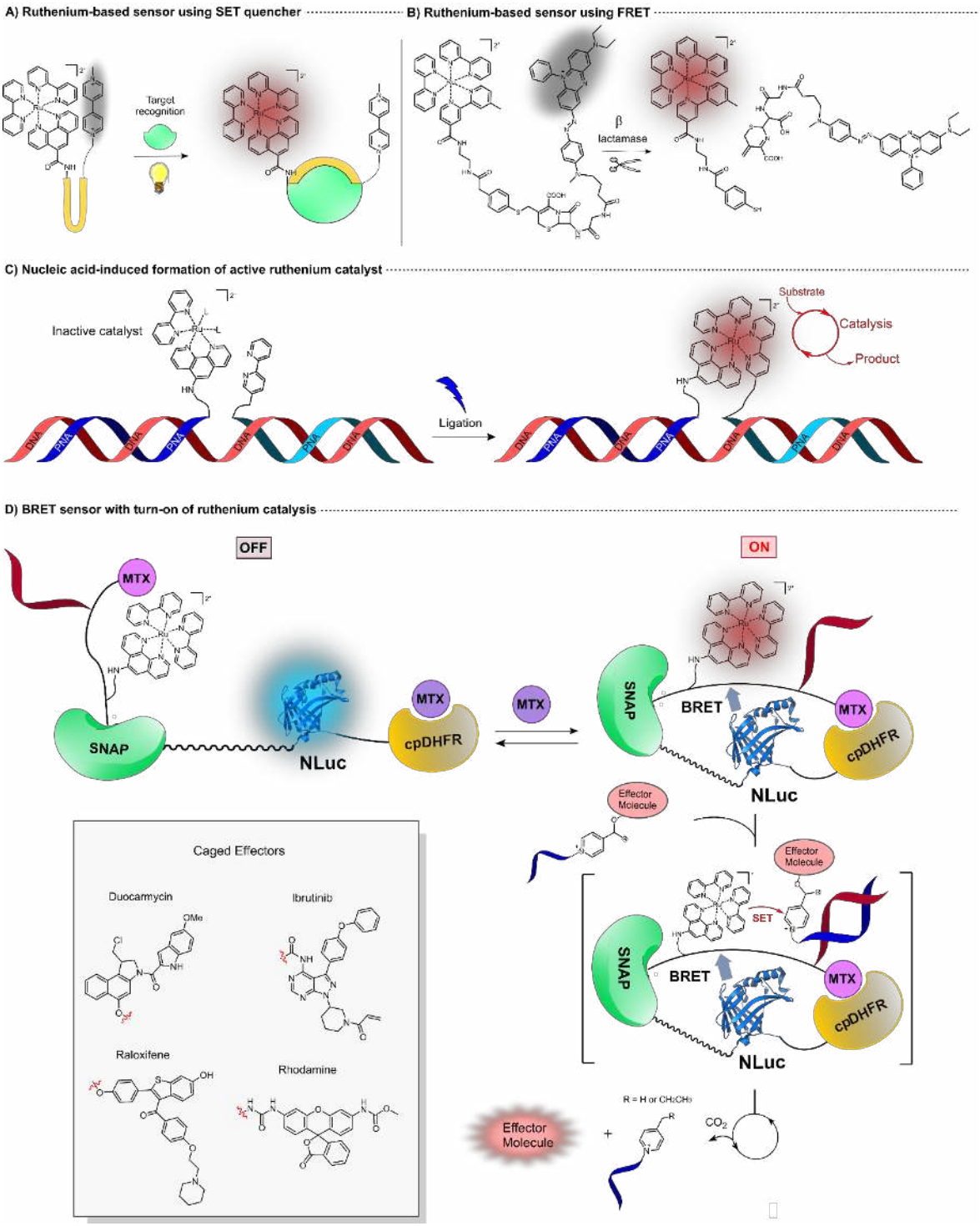

Figure 10. Turn-on of ruthenium photocatalytic activity. SNAP: soluble NSF attachment protein; MTX: methotrexate; DHFR: dihydrofolate reductase.

In this case, cephalosporin, a β-lactam antibiotic, was conjugated with a $[Ru(bpy)_3]^{2+}$ complex on one side and a black quencher (BHQ3: 550-700 nm) on the other side. In the presence of β-lactamase, an 8-fold increase in luminescence was observed. The BHQ3 quencher was also effective in suppressing the photogeneration of $^1O_2$. This quencher had been shown to be highly effective in suppressing $^1O_2$ formation in DNA-strand displacement studies of donor-quencher.[69] Comparison of the luminescence of the probe in bacteria strain that are sensitive to β-lactams (*S. aureus*) *vs* resistant strains (MRSA) that express β-lactamase showed a clear difference in luminescence. Importantly, an 8-fold gain in lethality was observed following irradiation, which is consistent with the production of toxic doses of singlet oxygen.

Our group made use of a DNA- or RNA-templated reaction to "turn-on" ruthenium photocatalysis via a ligation reaction between a catalytically inactive ruthenium complex and a bipyridine ligand (Figure 10C). As previously discussed, monodentate ligands on a complex such as $Ru(bpy)_2L_2$ will exchange upon photoexcitation. We showed that this exchange reaction can be harnessed to convert a catalytically inactive ruthenium complex into a catalytically active complex.[70] *In vitro*, the active catalyst was shown to be capable of tremendous signal amplification with >15 000 turnovers. The templated ligation was shown to proceed in cells. The sequence-specific ligation was detected using ruthenium photocatalysis (uncaging of a precipitating dye by reduction of the pyridinium immolative linker). Model studies on streptavidin beads showed that detection of < 100 attomoles of DNA was possible. The same reaction was also used to detect miR in cells.
In parallel work, we have shown that a bioluminescence resonance energy transfer (BRET) can be used to photoexcite the ruthenium and enable catalysis (Figure 10D).[71] BRET from luciferase to the ruthenium photocatalyst was used to uncage different effector molecules (drugs or a fluorophore) with up to 64 turnovers of the catalyst, achieving concentrations >0.6 μM effector with 10 nM luciferase construct. Using a BRET sensor, we further demonstrated that this catalysis could be modulated in response to an analyte, analogous to an allosterically controlled enzymes. While light is very appealing to control a turn-on of activity with temporal resolution, light penetration can be an issue. The use of BRET circumvents this problem by utilizing bioluminescence as the intrinsic light source and enables the conversion of BRET-based sensors to catalysts.

Ruthenium catalysis (not photocatalysis) in chemical biology: The demand for novel bioorthogonal reactions has led several groups to consider transition metal catalysis as a means to uncage or to conjugate molecules *in cellulo*.[5, 72] Notable achievements include ruthenium complex that accumulates in specific organelles and remains capable of catalytic transformations such as a ruthenium complex competent in π-allyl transfer chemistry (Alloc deprotection).[73]

CONCLUSION

A number of examples have now validated the use of ruthenium photocatalysis in chemical biology. Ruthenium complexes have been used either as reagents or as conjugates. These complexes have been harnessed for their photocatalytic oxidative and reductive properties. In the oxidative cycle, SET from electron rich aromatics (such as tyrosine) and further radical coupling reaction have been leveraged to crosslink proteins, interrogate protein-protein interactions and ligand-protein interactions. Ruthenium complexes have also been used as a singlet oxygen sensitizer in order to degrade a target protein of interest in live cells. Furthermore, ruthenium-photocatalyzed cross-coupling reactions between boronic acids and benziodoxole-alkynes have been demonstrated in the presence of biomolecules.
The reductive cycle has been shown to provide chemoselective transformations in live cells and live vertebrates. Reduction of azides or pyridiniums have been shown to proceed at high rate in the presence of biomolecules. Azides stand out as an important functionality in chemical biology considering its stability and unique reactivity. Immolative linkers leveraged on azide- or pyridinium reductions have been used to uncage fluorophores and drugs in responsive systems. This chemistry lends itself particularly well for templated processes were reagents are brought in close proximity though a binding to a biomolecule of interest (proteins or nucleic acids). The compatibility of these reactions with living systems open up new avenues in the engineering of abiotic metabolic and signal transduction pathways.
A unique feature of this chemistry is the fact that it is catalyzed by visible light, empowering it with temporal control. The complexes most prominently used have an excitation wavelength of 450 nm and low intensity LED lights are sufficient to promote the chemistry. This is important because high intensity lasers at this wavelength would result in significant phototoxicity in cells or organisms.
While ruthenium complexes have been used to target DNA through intercalation and charge association,[74] the efficiency of this targeting highly depends on the nature of the ligands. The examples presented in this review with $[Ru(bpy_3)]^{2+}$ and closely related analogs clearly illustrate that it is possible to achieve ruthenium photocatalysis in cells outside of the nucleus and it is possible to confine this reaction to a target protein.
While certain ruthenium(II) arene complexes have been shown to have anticancer activity with a profile that is different in comparison with cis-platin anticancer complexes,[75] the stability of $Ru(bpy)_3$-type complex and the fact that their coordination sphere is saturated means that they do not engage in this cytotoxic activity.

ACKNOWLEDGMENTS

We thank our collaborators who have contributed to our work in the area of ruthenium photocatalysis as well as the funding agencies (NCCR Chemical Biology, Swiss National Science Foundation, ERC). We thank Eric Lindberg for helpful discussions. This review was not intended to be comprehensive but aimed to highlight recent studies, and we apologize for arbitrary omissions.

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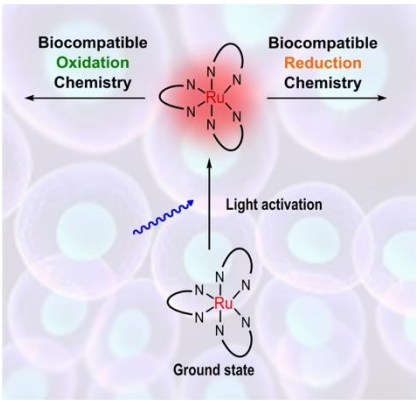
TOC



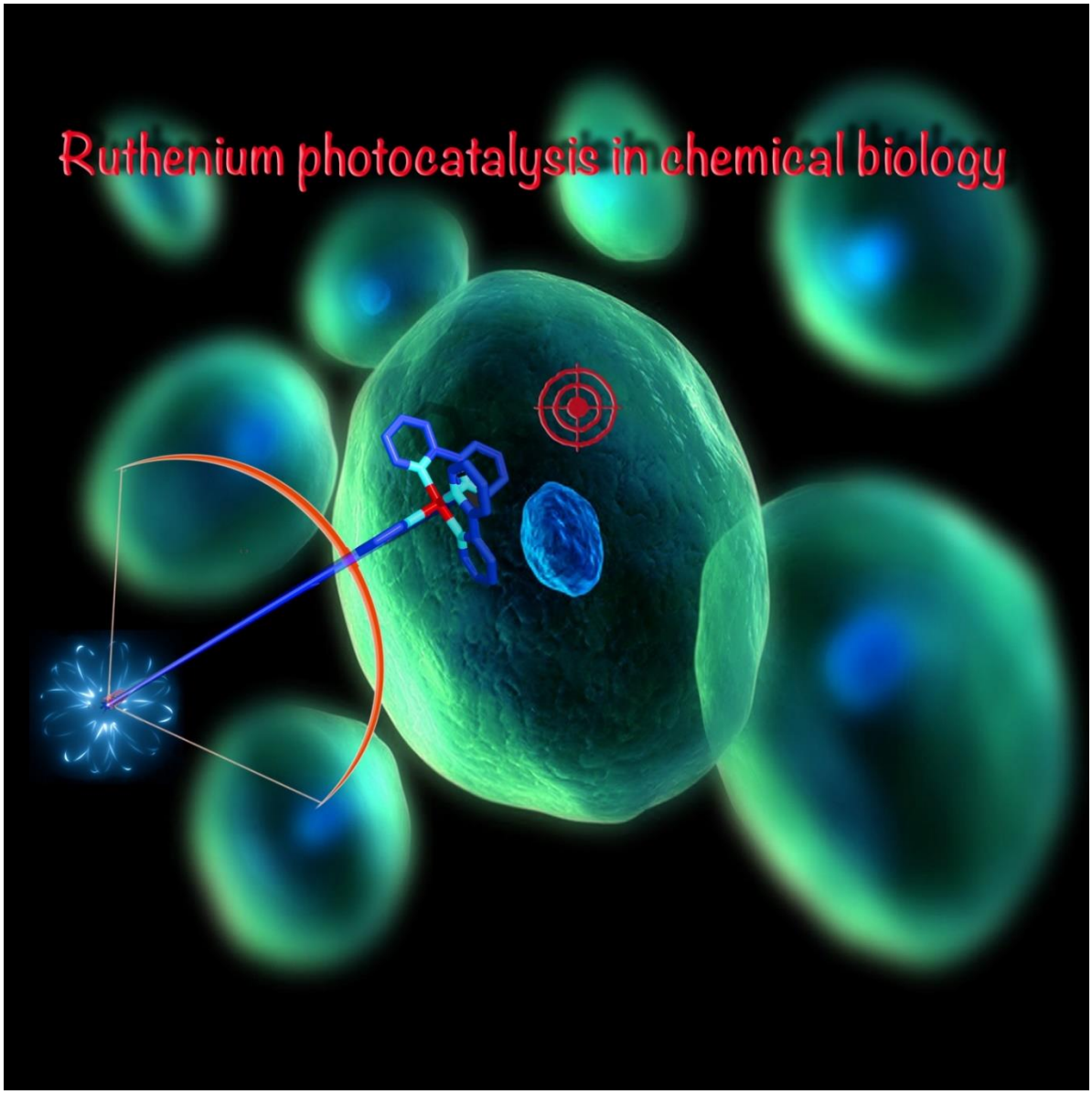
Let there be light. The use of photoredox catalysis with ruthenium complexes in chemical biology is reviewed. A unique and attractive property of ruthenium photocatalysts is their ground-state stability coupled to their photoexcited state reactivity. The fact that the catalyst is activated by light provides control of the reactivity in space and time.

Biographies

Simona Angerani studied chemistry at the University of Milan where she obtained her Bachelor's and Master's degree. Since 2016, she is a Ph.D. student in the group of Prof. Nicolas Winssinger where she works on ruthenium photocatalyzed release of effector molecules.

Nicolas Winssinger's research interests lie in bioorganic chemistry, developing enabling methods in chemistry to further our understanding of complex biological systems. An important theme throughout his research is the use of oligonucleotides as supramolecular tags to encode molecules, program spatial organization of ligands and biorthogonal reactions. In parallel, he is interested in natural products as a privileged starting points in the development of chemical biology probes and tool compounds. He received his BS from Tufts University before joining K. C. Nicolaou at the Scripps Research Institute for his PhD. He remained at Scripps as a postdoctoral fellow in the group of P. G. Schultz. In 2002, he began his independent career at the Institut de Science et Ingénierie Supramoléculaires, University of Strasbourg. In 2012, he moved to the University of Geneva where he is professor in the organic chemistry department and a member of the NCCR chemical biology.

Front piece graphic



The image illustrates the fact that selective transformations catalyzed by ruthenium complexes can be achieved in complex biological settings. A rather unique property of ruthenium photocatalysts is their ground-state stability coupled to their photoexcited state reactivity. The fact that the catalyst is activated by light provides control of the reactivity in space and time.