Advances in Light-based Transformations in Biological Systems

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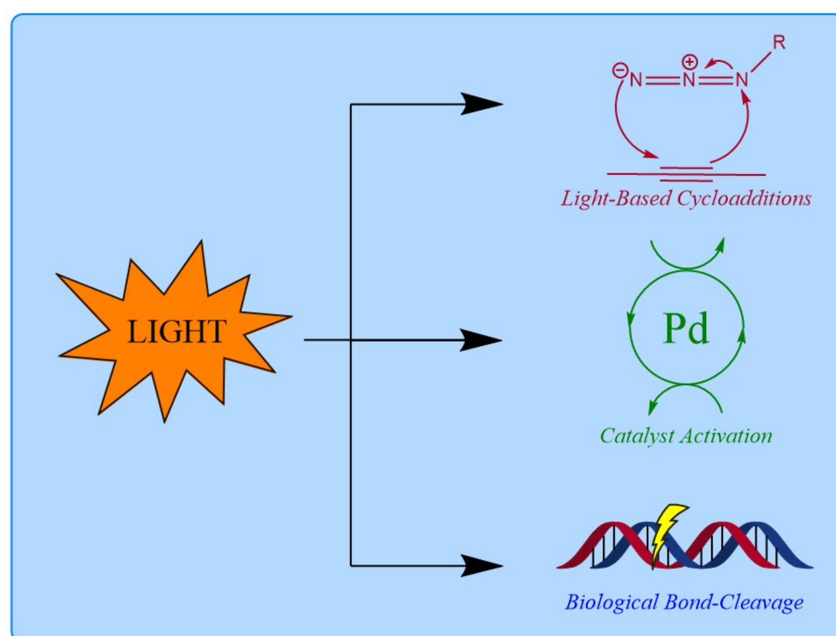
Abstract

Light is commonly known as the ultimate biorthogonal reagent, due to its biologically and chemically tolerant nature, as well as its ease of usage and high selectivity. It can effect cycloadditions, activate catalysts, break covalent bonds, or otherwise cause significant structural changes in biological systems. In this review we will look at the scope of light-promoted reactions within biological systems and their applications in chemical biology.



1. Introduction

Over the last two decades, bioorthogonal methods have become widespread within chemical biology. These methods involve reactions which have high chemoselectivity and kinetic rates, that are also tolerant enough of biological conditions such that they can be performed within living cells [1]. Processes such as biopolymer labelling, enzyme activity profiling, and drug target identification can all be studied with bioorthogonal techniques [1]. Many such reactions have hence been developed, including some based on transition metal catalysts, ultimately creating a 'toolkit' for chemical biologists to probe cellular processes with [1] [2].

One of the most prominent bioorthogonal methods is with light-induced reactions. With the advent of light-promoted transition-metal catalysis, cycloadditions, and bond cleavage, light itself has become a 'reagent' of choice. Further, light is often highly tolerant of normal

living conditions, and can offer highly selective spatiotemporal control [3] [4]. This review will hence offer an overview of the ways that light itself can act as a 'reagent,' that give us more insight into biological processes.

2. Cycloadditions

Cycloadditions are a common way to link two separate entities together in bioorthogonal chemistry. After the advent of the click reaction by Sharpless, many variations of bioorthogonal cycloaddition have been developed. Specifically, cycloadditions based on alkyne ring-strain, nitrones, metal complexation, etc have all seen use [1] [5].

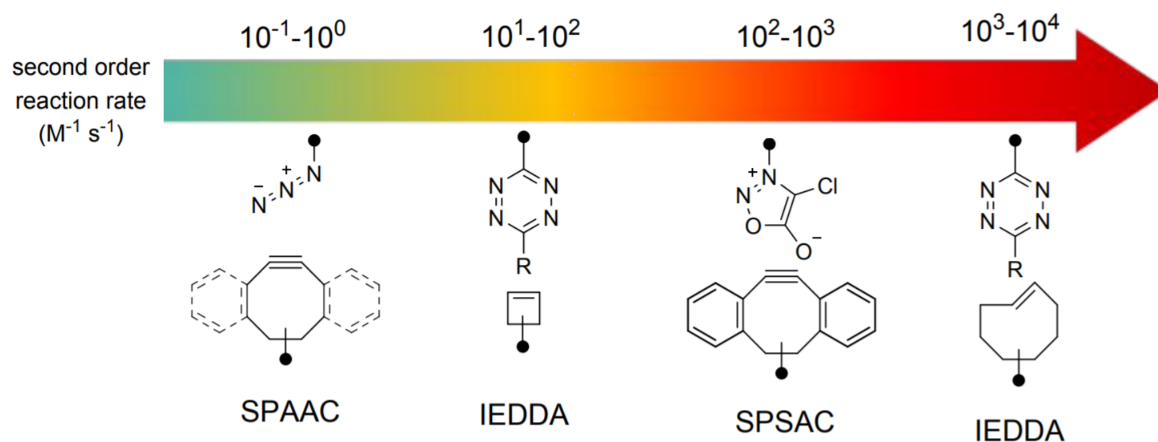


Figure 1: Some cycloaddition methodologies and their rates. Adapted from [6].

Accordingly, light-based cycloadditions have also been developed. These methods offer another way to impart selectivity and orthogonal labelling. Like their predecessors, these reactions often use reactive species that can undergo cycloaddition reactions, which can be generated by light.

One common method of creating a light-activated cycloaddition is simply to have a functional group that results in a reactive species when cleaved with light. A direct application of this methodology is in the transformation of cyclopropenone-caged bicyclononynes into cyclooctynes, which can then react with a tetrazine moiety through an inverse-electron demand Diels-Alder reaction [7].

Unhindered cyclopropenes can also react directly with tetrazine moieties. Thus, in order to have light-based control, a blocking group can be put into place to prevent such reaction. For example, the use of a sterically-demanding light-cleavable carbamate moiety has been demonstrated to block the cyclopropene-tetrazine cycloaddition [8].

Other methods to generate reactive species is through cleavage of heterocycles. Specifically, chlorosydnones have been discovered to generate a reactive species, most likely a nitrene, after light-promoted loss of carbon dioxide. This reaction has been used to incorporate radioactive flourine units within biological systems [6]. Similarly, tetrazoles have been shown to generate a reactive nitrile imine species after loss of nitrogen gas [9]. Both of these species can then go on to react with alkynes, creating pyrazole units. A tetrazole-containing RNA monomer has also been synthesized [10], which can be incorporated into

standard RNA-synthesizing machines. Exposure to light then generates the nitrile imine species which can be used to bioconjugate RNA.

Methods not based on bond cleavage have also been developed. Isomerization of cyclic azo groups or cycloheptenes within rings can lead to significant ring strain that helps promote cycloadditions with azide or nitrile imine moieties [11]. Systems based solely on photochemical excitation also have been developed and are highly valuable, as they bring increased orthogonality. One such system is the excitation of 9,10-phenanthrenequinone, which is able to undergo cycloaddition with electron-rich vinyl ethers after photoexcitation [12].

With each of these light-promoted cycloadditions, different mechanisms ultimately reveal different reactive species, which selectively react with some species over others. Though tetrazine-based systems often react the fastest, each of these cycloaddition methodologies react on a reasonable timescale and can individually exploited or combined to quickly link molecules together in a spatiotemporally controlled manner.

3. Catalyst Activation

Another method of inducing reactions is with transition metals that are incorporated into biological systems. The scope of reactions that transition metals can catalyze are much more versatile than even enzymatic reactions, and transition metals can be incorporated into biological molecules of interest in many ways [1].

Further, these transition metal catalysts can sometimes be controlled with light itself. Specifically, light energy is able to excite certain molecules, from which photoredox-active catalysts are able to produce the desired chemical transformation, often through single-electron transfer processes [13] [14].

Ruthenium-based catalysts have been reported to effect oxidative crosslinking, oxidative receptor labelling, protein photo-inactivation, cross-coupling reactions, and bond cleavage [13]. Recently, methodologies involving ruthenium-based catalysts have been further developed. For example, ruthenium photocatalysts have been shown to selectively react with tyrosine residues in proteins, with residues closer in proximity to the ruthenium being selectively labeled upon exposure to light [15]. A ruthenium photocatalyst which selectively reacts with tryptophan residues has also been developed [16]. This catalyst has the additional advantage that multiple proximal tryptophan residues can bind, allowing for cross-linking of polypeptides.

Proximal-labelling photocatalysts have also been developed further, with Nickel and Iron complexes. These photocatalysts often use reactive oxygen species in order to affect the desired oxidative transformation. Each of these complexes has a 'labelling radius,' which can be fine-tuned to the biological system of interest. Further, these methods can also lead to protein-selective labelling [17].

Transition metal catalysts can also be 'trapped' and released with light, as demonstrated in a silica - Pd^0 azobenzene/cyclodextrin system, which causes a structural change upon irradiation by light [18]. The light causes isomerization of the azobenzene unit, which then releases cyclodextrin-attached palladium nanoparticles that can go on and catalyze certain cross-coupling reactions, including ones that make fluorescent molecules from non-fluorescent starting materials.

Further, a light-based redox reaction of the metal itself can also be realized in cells. Specifically, some metal complexes (such as cisplatin) are biologically active, and a reduction from the Pt^{IV} state to the active Pt^{II} state is possible through light activation. Instead of the metal, a organic flavin photoredox catalyst and NADH is used instead to reduce the metal itself, ultimately creating a light-activated prodrug to drug transition [19].

4. Bond Cleavage and Applications

Through the development of these light-based techniques, many applications in probing, manipulating, and tracking biological systems have been realized. These often involve unmasking photolabile protecting groups, which activates the molecule of interest and allows for biological application [20].

One such application is through the light-controlled incorporation of a photo-caged azido amino acid into proteins. Once the amino acid is exposed to light, the cage is removed and the azido amino acid is able to be recognized by native cellular ribosomes. Due to the azide functionality, the synthesized protein can be further modified as needed, through either normal or light-based azide cycloadditions [21].

A site-specific protein modification methodology has also been reported [22]. The authors use a transglutaminase enzyme in order to modify select glutamine residues with wide scope, from which selective light-based cleavage results in the native protein.

When light-activatable units are incorporated into proteins, they can also be used to directly control function. Wang. et al. has reported a proximal decaging system from which a photocaging group is added to an 'anchor site,' thus preventing the functional site from being reached [23]. Cleavage with light allows for the functional site to maintain full activity. This anchor site can actually be found using computational techniques, from which experimental verification is necessary in order to verify inhibition.

Specific proteins can also be directly activated with high precision through light based techniques. For example, PROTACs rely on a ubiquitin-proteasome system to degrade proteins, and the PROTAC itself can be inhibited with the use of a photolabile protecting group, which allows for light-activated protein degradation with good control [24]. A similar system allows for high-precision control of CRISPR-Cas9, using a photoactivatable Cas9 activator in order to do so [25]. These two approaches allow for significant applications in light-based control.

5. Conclusion

In recent years, light itself has become one of the more promising tools of control within biological systems. It is able to efficiently catalyze cycloadditions of various types to link molecules together, activate photoredox catalysts to make unviable transformations happen, and cleave bonds that activate proteins, all with high spatiotemporal control and biological inertness. Many light-based transformations have been developed with demonstrated applications in controlling many proteins of interest. Facile caging/uncaging and linking of specific proteins of interest are now all possible with light-based techniques. Overall, the development of these techniques have significantly improved the scope of bioconjugation and have created a foundation for a wide variety of further applications in biological control.

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