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Mini-Review

Photoresponsive Small Molecule Enzyme Mimics

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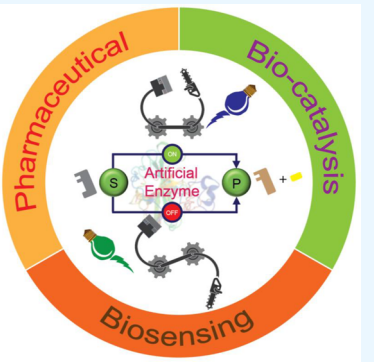
ABSTRACT: Enzyme mimics emulate the catalytic activities of their natural counterparts. Light-responsive enzyme mimics are an emerging branch of biomimetic chemistry where the catalytic activities can be controlled reversibly by light. These light-responsive systems are constructed by incorporating a suitable photoswitchable unit around the active-site mimic. As these systems are addressable by light, they do not leave back any undesired side products, and their activation−deactivation can be easily controlled. Naturally, these systems have enormous potential in the field of on-demand catalysis. The synthetic light-responsive enzyme mimics are robust and stable under harsh conditions. They do not require special handling protocols like those for real enzymes and can be tailor-made for improved solubility in a variety of solvents. How the introduction of the light-responsive systems has offered a new-edge to the field of small-molecule enzyme mimic has been elaborated in this Mini-review. Recent breakthroughs in light-responsive enzyme-like systems have been highlighted. Finally, the current obstacles and future prospects of this field have been discussed.



INTRODUCTION

Enzymes are biomacromolecules that serve as catalysts in cellular processes. They are primarily proteins with dynamic three-dimensional structures. These "nature's catalysts" outperform any synthetically developed catalyst in terms of their catalytic efficiency, selectivity, stereocontrol, and rate acceleration, which could be as high as 10^{17}-fold faster than the uncatalyzed reactions.[1] The high proficiency of the enzymes has been attributed to various factors, including the proximity effects between the reactant and the stabilization of the transition states for the reactions.[1]

Although the naturally occurring enzymes play a pivotal role in various biochemical reactions in living organisms, displaying excellent substrate selectivity and enormous rate enhancement, there are certain intrinsic pitfalls owing to their poor thermal stability, less versatility toward substrate choice, lack of stability under environmental conditions, and expensive extraction and purification.[2] Thermostable enzymes are produced by protein engineering for performing chemical reactions and processes at higher temperatures in industries including food and beverage, textiles, and paper industries.[3] An alternate way for carrying out difficult reactions for industrial processes may include the use of artificial enzymes or enzyme mimics. Therefore, even though there are no alternatives for enzymes in the living systems, there have been demands for the development of robust, cost-effective enzyme-like systems. Thus, the field of "artificial enzymes" has emerged. For the last half a century, there have been several pioneering works aiming to use cleverly designed synthetic analogues to simulate biocatalytic processes. These artificial enzymes consisting mainly of synthetic organic molecules are often known as the synzymes (synthetic enzymes).[4]

The metabolism processes in the cells are directed and regulated by a cascade of enzymes, and the physiological processes are controlled reversibly by chemical inputs, namely pH, cofactors, ions, and signaling molecules. The enzyme actions are turned "on" and "off" by means of feedback mechanisms. Certain biological processes are also known to be controlled by light. The photoisomerization of retinal in rhodopsin is the most common example of a protein function being regulated by light. In artificial enzymes, the activities can be tuned using light as a stimulus. There are a number of advantages to employing light as a stimulant because it does not produce any byproducts. A light stimulus is also important, as it ensures easy spatiotemporal control and can be used without requiring any physical contact. Moreover, utilizing a light stimulus to modulate catalytic activity can be rapid and reversible.

Some of the factors to be considered while designing these enzyme mimics include the incorporation of appropriately designed binding sites that selectively bind to the chosen substrates and lead them into the proper reactive conformations. A number of research studies using natural and modified supramolecular hosts, including macrocycles such as cyclo-

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dextrins (CDs),[5,6] calixarenes,[7] cyclophanes,[8] polymers, dendrimers,[9] and biomolecules (such as nucleic acids, proteins), as enzyme models have been reported. Thus, by emulating natural enzymes and incorporating a suitable photoactive unit, it is possible to rationally build light-responsive catalysts.

Attempts have been made to replicate conventional enzymatic reactions using structurally simpler photoresponsive molecules having light-triggered functional moieties. With the inclusion of photoresponsive molecular switches such as azobenzenes, a few photoswitchable enzyme mimics have been constructed. Photoisomerization of molecular photoswitches causes reversible shape changes, generating light-induced molecular motions that can lead to altered proximity as well as electronic structures of the system, which in turn leads to a change in the polarity, hydrophobicity, and a whole host of supramolecular interactions.[10] Researchers have recently focused on optically tunable artificial enzymes and discovered a range of photoresponsive enzymes with light-triggered photoactivity.

Light-controlled enzyme mimics are synthetically designed and can be customized for specific needs, such as on-demand activation for organic synthesis. It is to be noted that enzyme mimics have diversified into numerous other forms, such as nanozymes. In addition, light-induced enzyme activities have also been explored with photoregulated inhibitors. However, this review will focus solely on the small-molecule-based light-controlled enzyme mimics with photoswitchable moieties that emulate enzyme activities.

PHOTOREGULATED ENZYME MIMICS

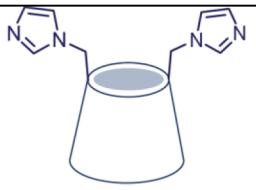
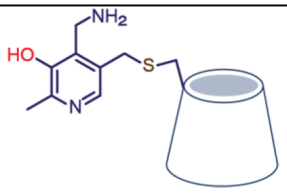
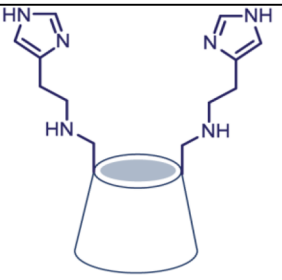
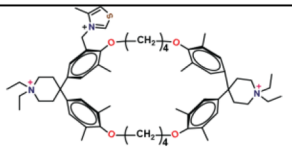
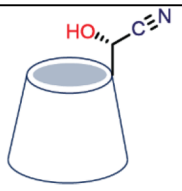
Inspired by natural enzymes, chemists have demonstrated that synthetic molecules can perform tasks similar to those of the natural enzymes. Some of the pioneers of biomimetic chemistry have employed synthetic molecules as artificial enzymes. Breslow, Cram, Kirby, and Shinkai among others are some of the early contributors to the field. Some of the prominent landmarks of the enzyme mimic are presented in Table 1.

The "turn-off" of natural enzymes are controlled by various factors collectively known as feedback regulation. The flexibility of enzyme active sites allows for effective catalytic activity modulation via allosteric effects and feedback loops. To gain similar control with artificial enzymes, novel methods had to be devised. Chemical stimuli such as pH or ions can provide such controls. However, the use of light as the stimulus has its own added advantages. Photoinduced transformation ideally happens almost quantitatively, that is, with high ratios of the isomers in the photostationary state (PSS), and it is maintained in multiple isomerization cycles. To accomplish green, clean, fast, and easy spatiotemporal control of the catalytic activities of artificial enzymes, light-sensitive compounds for mimicking natural enzymes have flourished as an emerging field.

AZOBENZENE-BASED PHOTORESPONSIVE SMALL MOLECULE ENZYME MIMICS

Despite the availability of a large variety of photochromic systems such as azobenzenes, stilbenes, diarylethenes (DTEs), spiropyrans, and indigoids, chemists have commonly used the azobenzene scaffold, as it offers certain benefits of convenient synthesis, well-established reversible cis → trans photo-

Table 1. A Few Representative Examples of Conventional Artificial Enzymes

Enzyme	Enzyme mimic (synthetic molecules)	Ref.
Ribonuclease A		[11]
Transaminase		[12]
Carbonic anhydrase		[13]
Pyruvate oxidase		[14]
Glycosidase		[15]

isomerization with a significant change in its molecular geometry upon irradiation with light. Our group has been extensively working on constructing photoregulated enzyme mimics. We have developed a strategy for light-controlled enzyme mimics. The use of a photochromic system in conjunction with a synthetic active-site mimic can trigger a turn-on or a turn-off response of the activity of the system. The change in the proximity between the catalytic residues can also modulate the pK_a of a molecule for general acid−general base catalysis.

The hydrolysis reaction is one of the most prevalent biological reactions. Hydrolases are a class of enzymes that facilitate the hydrolysis of a specific bond using water as a nucleophile. Hydrolases include phosphatases or phosphodiesterases for cleaving phosphate ester bonds, glycosidases for cleaving the ether bonds of polysaccharides, lipases for cleaving ester bonds, and proteases or peptidases for cleaving peptide bonds. Because hydrolases are an important class of enzymes, their mimics were attractive targets. Over the last few decades, several groups have attempted to mimic the activity of

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hydrolases. Breslow,[6] Cram,[5] and others contributed significantly to the development of enzyme-like active sites for the hydrolysis of esters using supramolecular host−guest complexes. Several other groups have also developed small synthetic molecules acting as hydrolytic enzyme mimics. Arriba et al. have developed an amide-based hydrolase mimic cleaving vinyl esters in a short span of time.[6] Although a significant number of hydrolase-like small molecules have been identified earlier in the literature, a stimuli-responsive hydrolase mimic added an additional controllable feature to the catalyst.[−16]

Access to the active-site of a supramolecular host as an enzyme mimic can be a strategy for light-controlled enzyme mimics. One of the earliest examples of a photoswitchable hydrolase was reported by Ueno and co-workers in 1981 with the photoswitchable hydrolysis of *p*-nitrophenyl benzoate catalyzed by azobenzene-capped *β*-cyclodextrin (*β*-CD) **1** (Figure 1). A small molecule, 4,4′-bis(carboxy)azobenzene,

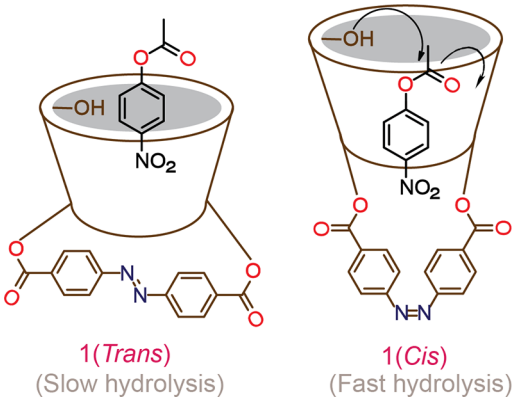

Figure 1. Reversible photocontrol of azobenzene-capped *β*-cyclodextrin **1**-catalyzed ester hydrolysis.

was employed as a capping unit and was covalently attached to the smaller opening of *β*-CD. The considerable alteration in molecular geometry upon irradiation with light translated to a change in the depth of the *β*-cyclodextrin's hydrophobic pocket, where the substrate was bound before being hydrolyzed. Thus, the *E*-isomer efficiently blocked the ester substrate from fitting in the cavity and impeded its hydrolysis. The production of *Z*-isomer on the other hand, in response to light irradiation, caused an increase in hydrolytic rate by up to five times.

The substrate could fit inside the larger hydrophobic cavity of *β*-CD in the case of the cis-isomer, resulting in an improved binding, whereas the cavity associated with the trans-isomer was too shallow to form a stable complex with the substrate. In this way, by modifying the depth of the hydrophobic pocket by photoinduced geometric changes of the azobenzene moiety, the substrate's binding to the *β*-CD cavity and consequent hydrolytic activity can be photoregulated.

Ueno's group has also published a more efficient system for hydrolase mimic.[17] They created photoresponsive catalyst **2** by linking an azobenzene unit to *β*-CD via a histidine spacer as a pendant group to control the ester hydrolysis. Three different model substrates (*p*-nitrophenyl acetate, Boc-L-alanine-*p*-nitrophenyl ester, and Boc-D-alanine-*p*-nitrophenyl ester) were studied in the presence of the trans- or cis-isomer of **2**. In the case of the cis-isomer, the cyclodextrin cavity served as the binding site for the substrate during imidazole-catalyzed ester hydrolysis. This was not achievable in the instance of the trans-isomer because of the inclusion of the *trans*-azobenzene moiety in the cyclodextrin cavity.

The azobenzene moiety changed from the trans- to the cis-isomer under UV irradiation, causing the catalytic mechanism to be active ("turn-on") (Figure 2). However, upon visible light irradiation, the azobenzene was isomerized to the trans-form, causing catalytic activity to "turn-off".

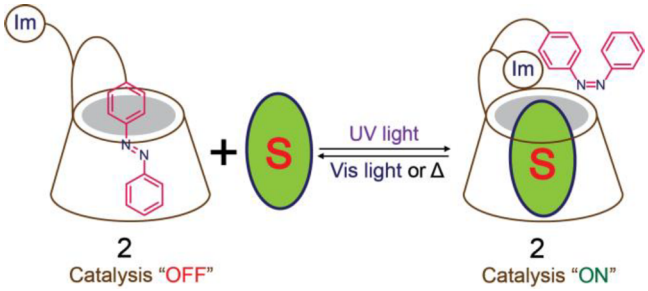

Figure 2. Schematic representation of Ueno's hydrolase mimic (Im: imidazole, S: ester substrate).

Ribonucleases (RNases) are a wide class of hydrolytic enzymes that break down ribonucleic acid (RNA) molecules. The reversible photoregulation of RNA hydrolysis was a remarkable work of ingenuity by Komiyama and co-workers.[18] The mimic was composed of a Zn(II) complex tethered to a cationic bulky moiety (regulator) via an azobenzene-based photoresponsive linker **3** (Figure 3). The shape of the linker

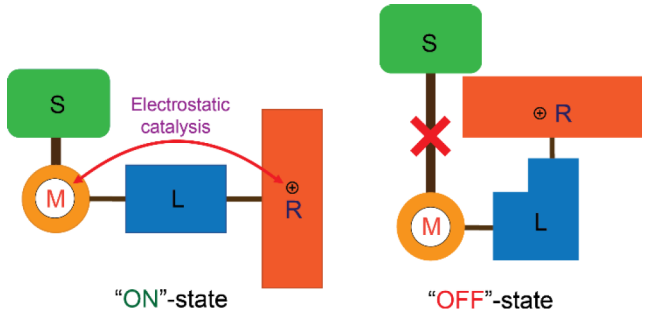

Figure 3. Komiyama's photoregulated RNase mimic with an external stimulus; M = metal center, R = regulator, S = substrate. In response to the stimuli, linker L modifies the structure.

can be altered by light irradiation to toggle between the two states. In the "off" state, the regulator is too close to the catalytic center, encountering steric hindrance. In the "on" state, the regulator is at an optimum distance from the catalytic metal center, resulting in a photocontrolled hydrolytic activity of the RNA. Thus, ligand **3** (Figure 4) displays light-controlled catalytic activity of the Zn(II) complex.

The reaction mixtures included fractions of the 2:1 Zn(II)/**3** complex's cis- or trans-isomers. The trans-isomer of the complex was capable to hydrolyze RNA, while the cis-isomer was essentially inactive. When the azobenzene in the complex assumed the trans-form, the two Zn(II) ions were dispersed and behaved independently. One of these served as the adenylyl-(3′-5′)-adenosine (APA) hydrolysis catalytic center, and the other accelerated the reaction as an electrostatic catalyst. The two Zn(II) ions came in close proximity when the azobenzene was isomerized to the cis-isomer. The bulky

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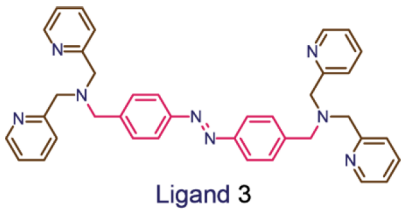

Figure 4. Molecular structure of ligand **3**, which involves photoresponsive azobenzene in the linker.

moieties coordinating with the other Zn(II) ion sterically prevented the substrate from coordinating with one of the two Zn(II) ions, which resulted in almost no catalytic activity.

Liu et al. have published another remarkable work demonstrating for the first time a reversible photoregulated activity of an artificial glutathione peroxidase (GPx) with a model system.[19] Cytosolic enzyme glutathione peroxidase (GPx) is responsible for catalyzing the reduction of hydrogen peroxide to water and oxygen, as well as the reduction of peroxide radicals to alcohols and oxygen. Its active site contains a catalytic triad of tryptophan and glutamine, which triggers a selenium moiety to reduce peroxides effectively. Ebselen, an organoselenium compound with comparable peroxidase activity, was used initially to mimic this enzyme.[21] Later on, several other research groups attempted to mimic GPx either by structural modification of ebselen or by including some structural characteristics of enzymes in their synthetically developed molecules. Recently, several nanozymes have been developed, portraying GPx-like activity more efficient than that of the above-mentioned enzyme mimics.[20] But the number of light-responsive mimics of this enzyme having photoregulated control over their activity is rare.

Using a photocontrolled inclusion−exclusion reaction of azobenzene, Liu et al. created two conventional GPx mimics and photoregulated the activity of artificial glutathione peroxidase (GPx). Azobenzene-based telluride β-cyclodextrin (β-CD) dimer **4a** and ditelluride β-CD dimer **4b** were designed to mimic the function of GPx (Figure 5).

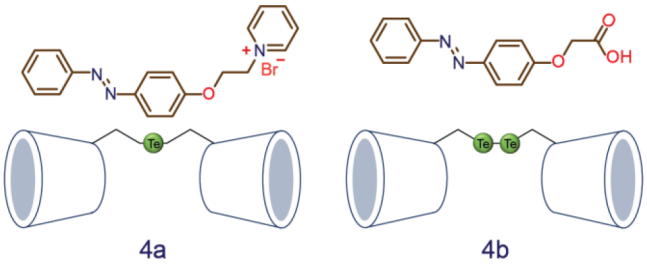

Figure 5. Molecular structures of the designed azobenzene derivatives and GPx mimics.

In the trans form, charged azobenzene was inside the cavity of cyclodextrin, keeping no active site available for substrate, and thus the enzymatic activity was inhibited. However, upon UV light irradiation, when the trans form was converted to the cis form (Figure 6), the azobenzene was forced out of the cavity, leaving the active site available for catalysis.

Utilization of a photochromic system as an enzyme mimic was achieved by our group in a glycosidase mimic with a different approach.[21] Natural glycosidases have two carboxylates in their active sites. Thus, a lysozyme has an Asp and a Glu in its active site, serving as a proton donor and a

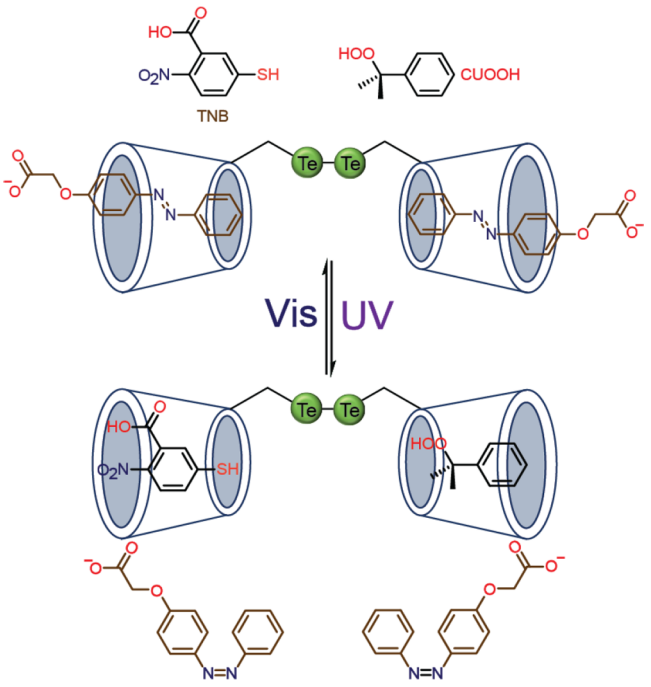

Figure 6. Reversible photoregulation of the artificial GPx.

nucleophile, respectively. This was mimicked efficiently by a rather simple small molecule, azobenzene-3,3′-dicarboxylic acid **5** (Figure 7). The concept of photomodulation of pK_a in the 3,3′-azobenzenedicarboxylic acid was used to emulate the mimetic activity.

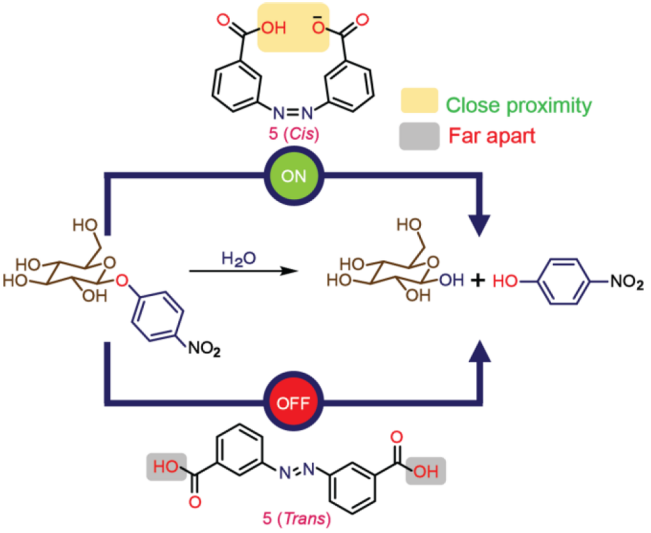

Figure 7. (*Z*)-Diacid **5** catalyzed hydrolysis of 4-nitrophenyl-β-D-glucopyranoside, whereas the (*E*)-isomer remains inactive in terms of hydrolytic activity.

In the pH range of 4.7 to 6.5, employing the concept of a general acid−general base mechanism, the cis-form enhanced the hydrolysis of the glycosidic bond in 4-nitrophenyl-β-D-glucopyranoside (as the model substrate) by 6 orders of magnitude compared to the background reaction. The deprotonation of the carboxylic acid groups in the (*E*)-form occurs instantly, whereas it proceeds in a stepwise manner in the (*Z*)-form. The (*Z*)-isomer's monoanionic form functions

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as a glycosidase mimic, with a conventional general acid–general base catalytic mechanism (Figure 8).

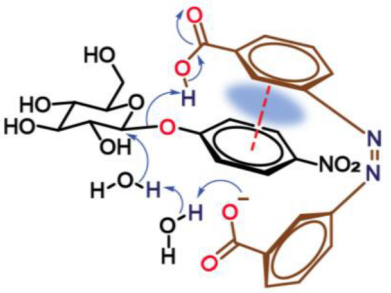

Figure 8. Proposed catalytic mechanism of the (*Z*)-diacid **5** using 4-nitrophenyl-β-D-glucopyranoside as a model substrate.

Another innovative way of modulating the catalytic activity with light was accomplished by Chen et al.[22] Heparin is an anticoagulant, and this property makes it incredibly vital in the human body. For nearly a century, unfractionated heparin (UFH), one of its variants, was used as an anticoagulant drug. However, it was highly dangerous due to its detrimental effects on the human body, such as the occurrence of hemorrhage and heparin-induced thrombocytopenia. Later, low molecular weight heparin (LMWH) having a higher anticoagulant effect and devoid of the other adverse effects emerged. Consequently, the large-scale production of LMWH is necessary, which requires extreme control of the enzymatic depolymerization of heparin. Because of the difficulties of the procedure and the consequences, achieving a controlled depolymerization of heparin became a major concern. Incorporating a light-responsive moiety into the degradation system could offer on-demand control of binding with such systems.

The enzyme heparinase III can selectively depolymerize heparin and is involved in the enzymatic production of LMWH. A phototriggered biocatalyst K130C–DMAA for heparinase III was rationally designed by employing a photoresponsive polymeric azobenzene moiety site-specifically coupled with the mutant K130C of PhHepIII (heparinase III from *Pedobacter heparinus*). *N*,*N*-Dimethylacrylamide-*co*-4-phenyl azophenyl acrylate (DMAA) was employed as an azobenzene–polymer, **6** (Figure 9), which was linked with a cysteine residue in the heparinase III mutant, that offered a greater control of the substrate degradation.

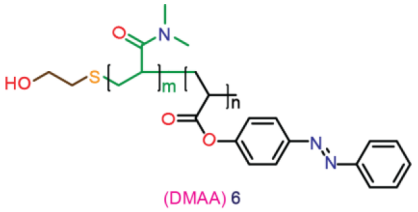

Figure 9. Molecular structures of the photoresponsive azobenzene–polymer **6**.

To control the enzymatic activity precisely, the allosteric azobenzene fragment was triggered by UV/vis light. The *trans*-azobenzene copolymer species was inactive toward degradation, whereas upon UV irradiation the trans-isomer was converted to the cis-isomer and the degradation activity was restored, probably due to the close proximity and enhanced accessibility of the substrate to the active site for the cis-isomeric species. Upon heating or exposure to blue light, the inactive trans-form predominated and the degradation activity was diminished. As a result, upon photoswitching, the enzymatic breakdown of heparin could be regulated artificially to produce LMWH with a more uniform molecular weight and increased anticoagulant action (Figure 10). Thus, using light to fine-tune biocatalytic activity was successful in this work. In addition, the polymer was reusable.

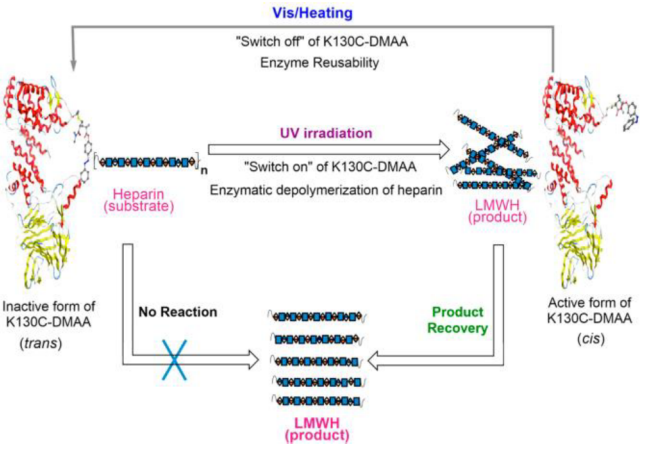

Figure 10. For the enzymatic synthesis of LMWH, a photoswitchable K130C–DMAA conjugate is shown schematically. The photoswitchable mutant K130C of PhHepIII is represented by K130C–DMAA. *N*,*N*-Dimethylacrylamide-*co*-4-phenyl azophenyl acrylate copolymer is abbreviated as DMAA. (Adapted with permission from ref 22. Copyright 2018, American Chemical Society).

Proton transfer reactions are the most biocatalytic reactions. Proton-coupled steps are present in almost all enzymatic reactions where hydrogen-bonding interactions frequently help to stabilize the transition state. In a recent example, Wang and co-workers demonstrated another remarkable photoregulated activity of a peptide-based artificial hydrolase enzyme.[23] They designed a peptide-based enzyme mimic with a photoresponsive azobenzene moiety incorporated into the peptide chain to tune the catalytic activity and provide hydrophobic interaction in a self-assembled system. When photoirradiated, peptide Azo-Gly-Phe-Gly-His **7** (Azo-GFGH **7**) (Figure 11) undergoes a structural transition from an antiparallel β-sheet to a random coil, leading to the peptide fibril's assembly and disassembly, respectively. The hydrogen bonding between the peptides and the $\pi-\pi$ stacking of *trans*-azobenzene helped to form an antiparallel β-sheet structure that supported the assembly of the Azo-GFGH **7** into nanofibers. The *trans*-azobenzene was converted into the cis-form when exposed to UV light, causing the β-sheet structure to be destroyed and the supramolecular assemblies to be disassembled.

The inclusion of phenylalanine provided an extra hydrophobic interaction to aid the assembly, while the two glycines stabilized the rigidity of the $\pi-\pi$ stacking, making the entire peptide skeleton flexible. Because most natural hydrolases have an imidazole group in the active site, the imidazole moiety of histidine at the C-terminal chain was cleverly grafted in the peptide system. Imidazole plays a crucial role in the hydrolytic activity (Figure 12). The common model substrate, *p*-nitrophenyl acetate (*p*-NPA), was employed to assess subsequent catalytic behavior (Figure 12).

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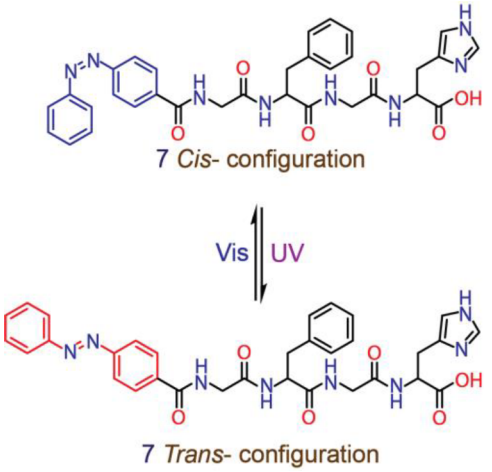

Figure 11. Molecular structure of the azobenzene-derived tetrapeptide Azo-GFGH **7**.

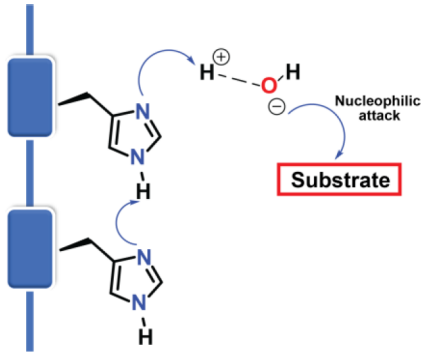

Figure 12. Proposed mechanism for the increased hydrolytic activity of the Azo-GFGH assemblies.

In a conceptually related study, Luo et al. effectively constructed a model photoresponsive hydrolase (V-$H_{10}R_1$) based on the assembly and disassembly of a supramolecular structure.[24] The designed principle was created on the spatial arrangement of histidine/arginine peptide-based supra-amphiphiles **10** that were held together by cucurbit[8]uril (CB[8]), methyl viologen (MV **8a** and **8b**) (Figure 13), and azobenzene-linked hydrophobic alkyl chains (Azo-TBA **9**) ternary complexation (1:1:1).

Following that, they self-assemble into gigantic vesicles in water with a high degree of uniformity. The photoisomerization of azobenzene causes a photoirradiation-induced reversible morphological transformation of vesicular structures to nonassembled peptide fragments. This allowed a drastic change in hydrolytic activity due to the assembly/disassembly of its enzyme-like active site.

The imidazolyl moiety of His activated a water molecule, resulting in the formation of a hydroxide ion that attacked the carbonyl of the substrate *p*-nitrophenyl acetate (*p*-NPA), forming a tetrahedral transition state that was stabilized by the guanidyl group of Arg for C−O bond cleavage. Furthermore, UV irradiation led to the trans to cis isomerization of the azobenzene unit, resulting in the dissociation of peptide-based supra-amphiphiles. The His/Arg ratio-mediated coassembly of two short peptide derivatives on the surface of supra-amphiphilic vesicles was tuned to form an enzyme-like active site.

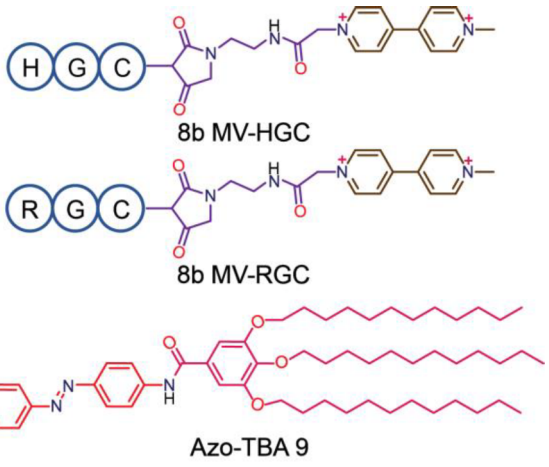

Figure 13. Molecular structure of the methyl viologen derivatives (MV **8a** and **8b**) and azobenzene-linked hydrophobic alkyl chains, Azo-TBA **9**.

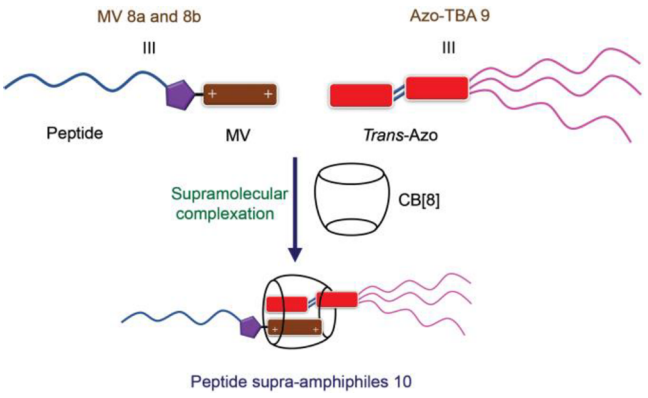

Figure 14. Schematic illustration of the design of the photoresponsive hydrolase model containing peptide supra-amphiphiles **10**.

Carbonic anhydrase (CA) is a natural enzyme that reversibly converts carbon dioxide to a bicarbonate ion. It aids in the maintenance of acid−base balance, pH regulation, and fluid balance. The human carbonic anhydrase II enzyme (hCAII) regulates a wide range of physiological functions in the human body, from neurological functioning to cancer. Until now, a number of ingenious designs of CA mimics have been reported. Relying on those essential features, we have designed a light-controlled dynamic catalyst to control the bioinspired activity of carbonic anhydrase.

The hCAII enzyme has a Zn ion coordinated to three histidine residues of the enzyme and a molecule of water in its active site. Tabushi et al. mimicked this in the 1980s using a bis(histamino)-β-cyclodextrin−Zn−imidazole complex.[13] However, the molecule could not be used for on-demand catalysis. Our group has reported the first photoregulated CA mimic.[25] The coordination motif was photoregulated by employing two imidazole appendages at the two terminals of a photoresponsive azobenzene moiety, i.e., imidazole-appended azobenzene **11** (Figure 15), resulting in a light-gated activity modulation of this enzyme mimic.

To stabilize the tetrahedral oxyanionic transition state, a cationic amphiphilic copolymer having a quaternary ammonium linkage was exploited. Indeed, a significant rate increase was seen in the presence of the cationic polymer, which supported our strategy. Owing to the close proximity of two

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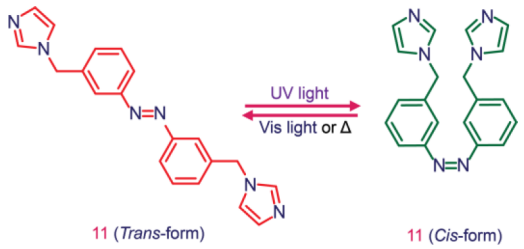

Figure 15. Molecular structures of the imidazole-appended azobenzene **11**.

attached imidazole moieties in the cis-form of azobenzene, the zinc ion produced a persistent and active tetrahedral catalytic site which enhanced the hydrolysis of the model substrate (Figure 16).

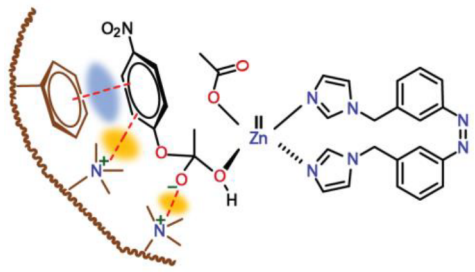

Figure 16. Probable catalytic mechanism for the hydrolysis of the *p*-NPA model substrate in the presence of the cationic polymer.

Thus, the [Zn^{2+}.cis-**11**] performed efficiently as an active site mimic of the CA enzyme. Gaseous CO_2 could also be converted efficiently to carbonic acid using the cis-catalyst.

Racemase enzymes convert L-amino acids to their D-isomer. The unusual D-amino acid residues are found in our brains and have a regulatory role. It is also found in peptidoglycans, a primary structural polymer on the bacterial cell surface. The D-Ala isomers are generated biosynthetically when the naturally abundant L-alanine (L-Ala) is racemized by employing the bacterial cellular machinery of the alanine racemases, a pyridoxal 5′-phosphate (PLP)-dependent enzyme. Using this approach, a well-designed PLP−photoswitch−imidazole triad **12** (Figure 17) was recently reported by our group,[26] in which azobenzene functions as a molecular photoswitch. Thus, the molecule acted as a photoregulated mimic of the racemase enzyme.

The racemization method involves the formation of the alanine-PLP Schiff's base, which lowers the pK_a of the chiral α-proton. The acidic α-H is then abstracted by a basic amino acid residue. A histidine served as a general base in the compound **12**. In the trans-form of the azobenzene switch, it was distant from the chiral α-H of the PLP-conjugated alanine. However, in the cis-form, the histidine base and the α-H came in close proximity, causing an increase in the effective molarity of the basic residue. This resulted in an effective deprotonation. The reprotonation of the achiral quinoidal carbanion took place from both stereotopic faces, leading to a racemization reaction.

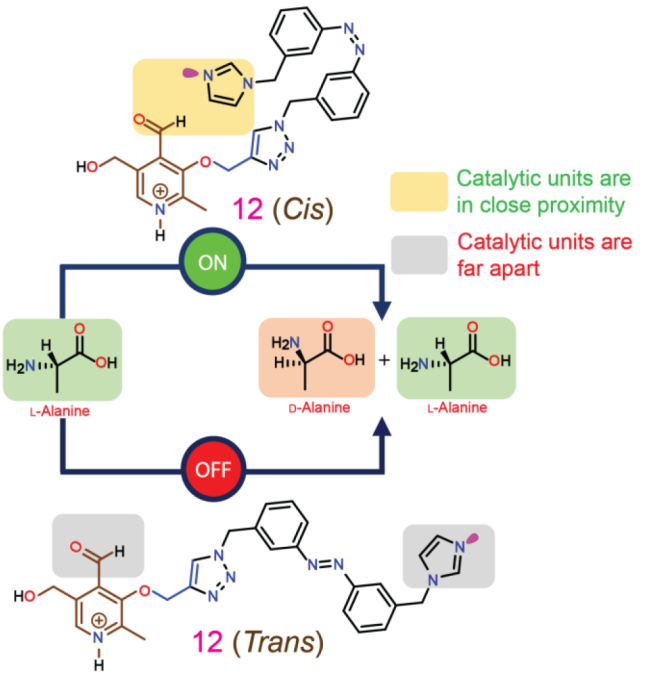

Figure 17. PLP−azobenzene−imidazole triad **12** photoregulates L-Ala to D-Ala transformation.

OTHER PHOTOSWITCH-BASED PHOTORESPONSIVE SMALL MOLECULE ENZYME MIMICS

Despite the advantages of azobenzene, chemists have also used alternative photochromic systems, such as diarylethenes (DTEs), as a photochromic switching unit to control the bioinspired activity of artificial enzymes. Diarylethenes have been employed by scientists on account of their fast photoresponsibility, excellent thermal stability, and fatigue resistance.[27] One such example of utilization of a dithienylethelene photochromic system (Figure 18) as an enzyme mimic

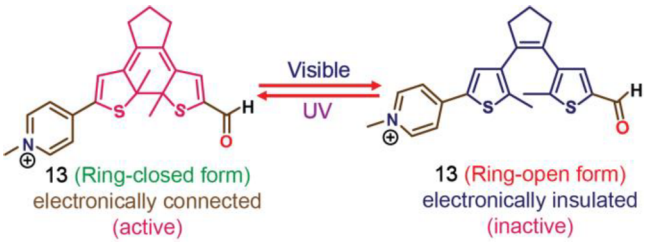

Figure 18. Molecular structure of the dithienylethene-based aldehyde **13**.

was achieved by Branda et al., in a pyridoxal 5′-phosphate mimic.[28] They devised for the first time a photomodulated mimic of this PLP cofactor employing a dithienylethene which toggles between closed form (active) and open form (inactive) upon photoirradiation of UV and visible light, respectively.

Broken electronic communication makes the open form of dithienylethene inactive, but upon ring closing, the restoration of electronic communication activates the photoswitch. Utilizing this concept, they developed the dithienylethene-based aldehyde, which generated the stabilized quinonoid intermediate (Figure 19) when the system was electronically "connected", but it inhibited the formation of quinonoid when

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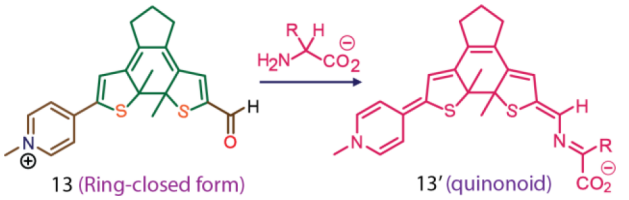


Figure 19. Structures of the quinonoid form (produced by deprotonating the α-H of an aldimine, formed by a reaction between a compound **13** and an amino acid).

the photoswitch was electronically "insulated". Thus, implementing a photocontrol over the catalytic activity of this system that showed a decent extent of racemization of the L-alanine molecule.

Oxidases belong to the oxidoreductase class of enzymes that employ oxygen for oxidizing various substrates without employing H_2O_2. In natural oxidases, flavin coenzyme is typically present. Examples of significant oxidases include cytochrome c oxidase, NADPH oxidase, glucose oxidase, xanthine oxidase, and so on. An earlier instance of the use of a nitrospiropyran photochromic system in a modified FAD cofactor was achieved by Willner et al.[29] An enzyme photoswitch was created by incorporating a photoisomerizable nitrospiropyran−FAD derivative **14** (Figure 20) in place of the

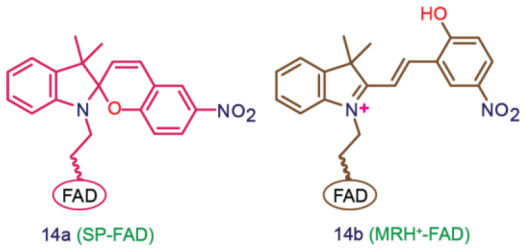


Figure 20. Molecular structure of the unisomerized form of the nitrospiropyran−FAD derivative **14a** and the photoisomerized form of the protonated nitromerocyanine−FAD derivative **14b**.

native FAD cofactor for glucose oxidase (GOx). Thus, a photoisomerizable GOx was created by reconstituting apo-glucose oxidase, a flavo-apoenzyme, with the semisynthetic nitrospiropyran−FAD cofactor.

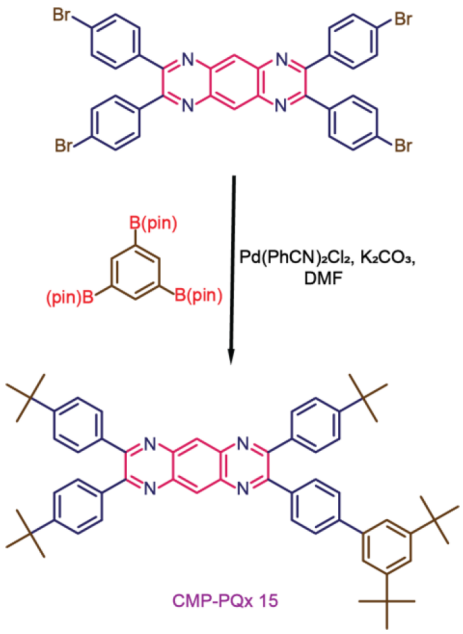
Amperometric transduction and amplification of captured optical signals were made possible by the photoswitchable activation and deactivation of redox proteins. A monolayer of the photoisomerizable enzyme with electrocatalytic characteristics was constructed on an Au electrode. This enzyme−FAD analogue complex underwent a reversible photoisomerization upon light irradiation to the photoisomerized protonated nitromerocyanine−FAD derivative form (MRH$^+$−FAD, **14b**). The unisomerized version of the enzyme (SP−FAD, **14a**) was active and involved in the bioelectrocatalytic oxidation of glucose utilizing the mediator l-(1-[dimethylamino]ethyl)-ferrocene but was inactive using ferrocenedicarboxylic acid mediator. However, in the aforementioned mediators, the photoisomerized form exhibited a reverse behavior. Thus, by fine-tuning the electrical connections between the electron mediator and the photoisomerizable enzyme, it was possible to alter the direction of "on" or "off" switching of the photoisomers (Figure 21). However, we note that the use of such a photoswitchable-FAD cofactor for on-demand catalysis cannot always be used as a general strategy, as the coenzyme/

Figure 21. Reconstitution of a flavoenzyme with a semisynthetic photoisomerizable FAD cofactor to create a photoswitchable biocatalyst.

cofactor binding site in some enzymes may be snug and therefore will have a limited space to accommodate a photoswitchable group. Hence, a new way to use proteins in optobioelectronic devices was revealed by the photoswitchable characteristics of the reconstituted enzymes and the increased amperometric transduction of the recorded optical signal by the electrobiocatalyzed transformation.

The following example illustrates a slightly different approach from the ones that were mentioned earlier. Use of photosensitized units rather than molecular photoswitches to regulate the bioinspired activity of synthetic enzymes are also known. One such example of the utilization of a photosensitized unit as an oxidase mimic was demonstrated in a recent study published by Su, Liang, and co-workers. They developed a visible light-driven oxidase mimic.[30] For the first time, a type of photosensitized conjugated microporous polymer (CMP) containing pyrazino[2,3-*g*]quinoxaline (CMP-PQx **15**) was effectively synthesized (Scheme 1) and

Scheme 1. Synthesis of a Conjugated Microporous Polymer Containing Pyrazino[2,3-*g*]quinoxaline (CMP-PQx 15)



found to successfully simulate oxidase activity. Following visible light irradiation, CMP-PQx used dissolved oxygen as an electron acceptor to create superoxide radicals that quickly oxidized a variety of the substrates.

Nanoparticle-based enzyme mimic "nanozymes" were developed in the late 1990s, possessing a number of advantages over the small molecule-based enzyme mimics. There are a number of reports where nanozymes have been constructed by incorporating photoswitchable moieties. However, the light-responsive functions of the nanozymes in this context are

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beyond the scope of this Mini-review. However, it is noteworthy to emphasize that nanozymes are also important players in enzyme mimics.

CONCLUSION AND FUTURE PROSPECTS

This critical Mini-review summarized the recent progress in photoresponsive artificial enzymes, ranging from design strategy, to regulating their activity, to catalysis. The emerging field of photoregulated biomimetic chemistry has attracted attention for its ability to reproduce biological processes by creating artificial model systems with light-responsive synthetic molecules. It is evident from the illustrative examples discussed herein that over the past two decades, a significant progress has been accomplished in this recently developed field of study. The careful selection of a photochromic unit and its precise use for the creation of artificial enzymes give unmatched opportunities to control the activity of the enzyme mimics. Embedding molecular photoswitchable entities into the catalyst structure has led to the effective development of a number of photoswitchable synthetic enzymes. Furthermore, the combination of light control techniques enabled accurate and dynamic control of artificial enzymes. The presence of various scaffolds and the capacity to modify them appropriately allows enzyme mimics to be used for a wide range of functions. Even though a variety of processes can be catalyzed by enzyme mimics, there still exist a number of major obstacles in the field. On the basis of the current scientific premise, there is still a lot of room for the development of photoresponsive artificial enzymes.

However, there are certain pitfalls of these synthetic systems that should be noted. In terms of enzymatic proficiency, artificial enzymes lag far behind the natural enzymes. This is also true for the photoswitchable enzyme mimics. In addition, photochromic systems such as azobenzenes display thermal reversibility. Thus, these systems may not be usable under elevated temperature conditions. Artificial enzyme mimics may also have biocompatibility issues. Not all enzyme mimics are thermally and chemically stable under physiologically relevant conditions, which precludes their usage in vivo. Also, the majority of the reactions that were monitored are on an analytical scale, so it makes sense to demonstrate their synthetic applicability in larger volumes and at higher concentrations. Most of the photoswitchable systems have traditionally needed UV light as a stimulus. Low tissue penetration by UV light and the dangers of biological samples under UV radiation prohibit these molecular photoswitches from reaching their full potential. Therefore, photoswitchable systems that operate in the biological window in the near-infrared (NIR) region of the spectra are highly coveted.

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All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

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