Photon-Regulated DNA-Enzymatic Nanostructures by Molecular Assembly

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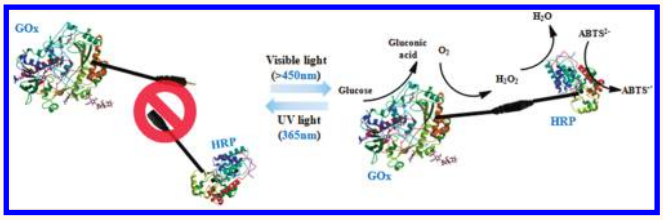
ABSTRACT



Future smart nanostructures will have to rely on molecular assembly for unique or advanced desired functions. For example, the evolved ribosome in nature is one example of functional self-assembly of nucleic acids and proteins employed in nature to perform specific tasks. Artificial self-assembled nanodevices have also been developed to mimic key biofunctions, and various nucleic acid- and protein-based functional nanoassemblies have been reported. However, functionally regulating these nanostructures is still a major challenge. Here we report a general approach to fine-tune the catalytic function of DNA-enzymatic nanosized assemblies by taking advantage of the *trans*–*cis* isomerization of azobenzene molecules. To the best of our knowledge, this is the first study to precisely modulate the structures and functions of an enzymatic assembly based on light-induced DNA scaffold switching. Via photocontrolled DNA conformational switching, the proximity of multiple enzyme catalytic centers can be adjusted, as well as the catalytic efficiency of cofactor-mediated DNAzymes. We expect that this approach will lead to the advancement of DNA-enzymatic functional nanostructures in future biomedical and analytical applications.

KEYWORDS: azobenzene · DNA–enzyme conjugation · enzyme cascade · photocontrollable

Molecular assembly enables the development of smart nanostructures designed to perform a desired function. Perfecting such assembled nanostructures depends on the availability of molecules that can serve as linkers or as both linkers and functional units for the assembly. In addition to its primary role as a carrier of genetic information, DNA has recently gained considerable attention as one of the most promising building blocks for the design and assembly of nanostructures.[1−3] Owing to its high sequence specificity and addressability, DNA molecules can also be used to direct the assembly of other functional molecules. Examples include DNA-templated organic synthesis,[4] identification of ligands for protein targets,[5] and DNA-guided nanoparticle[6,7] and protein[8−14] arrays. Because DNA molecules are both readily accessible and easily modified by chemical synthesis, decorating DNA with various functional moieties for analytical and biomedical applications is feasible. In particular, incorporating enzymatic functionality into DNA nanostructures could increase the utility of both types of macromolecules. In the present report, we demonstrate a general method for precisely controlling the catalytic activity of a DNA–enzyme assembly. To the best of our knowledge, this study is the first to report precise modulation of the structures and functions of an enzymatic assembly based on light-induced DNA scaffold switching.

As efficient and clean external triggers, light-regulated processes provide precise temporal and spatial control over various biological and analytical systems.[15] Among various photoresponsive molecular tools, the photoinduced isomerization of azobenzene molecules has been broadly studied and used to induce significant conformational and biochemical changes in nucleic acids,[16−18] peptides, and proteins.[19,20] The light-driven *cis/trans* isomerization of the azobenzene moiety is wavelength-dependent: UV light at 365 nm drives the *trans*-to-*cis* conversion, while visible light at around 465 nm corresponds to the *cis*-to-*trans* isomerization. As a consequence of spatial structure alternation during *cis/trans* isomerization, geometry-dependent biological activity changes occur, forming the foundation of the azobenzene-based photomodulation of biological processes.

In biology, different enzymatic reactions often work together in a cascade fashion.

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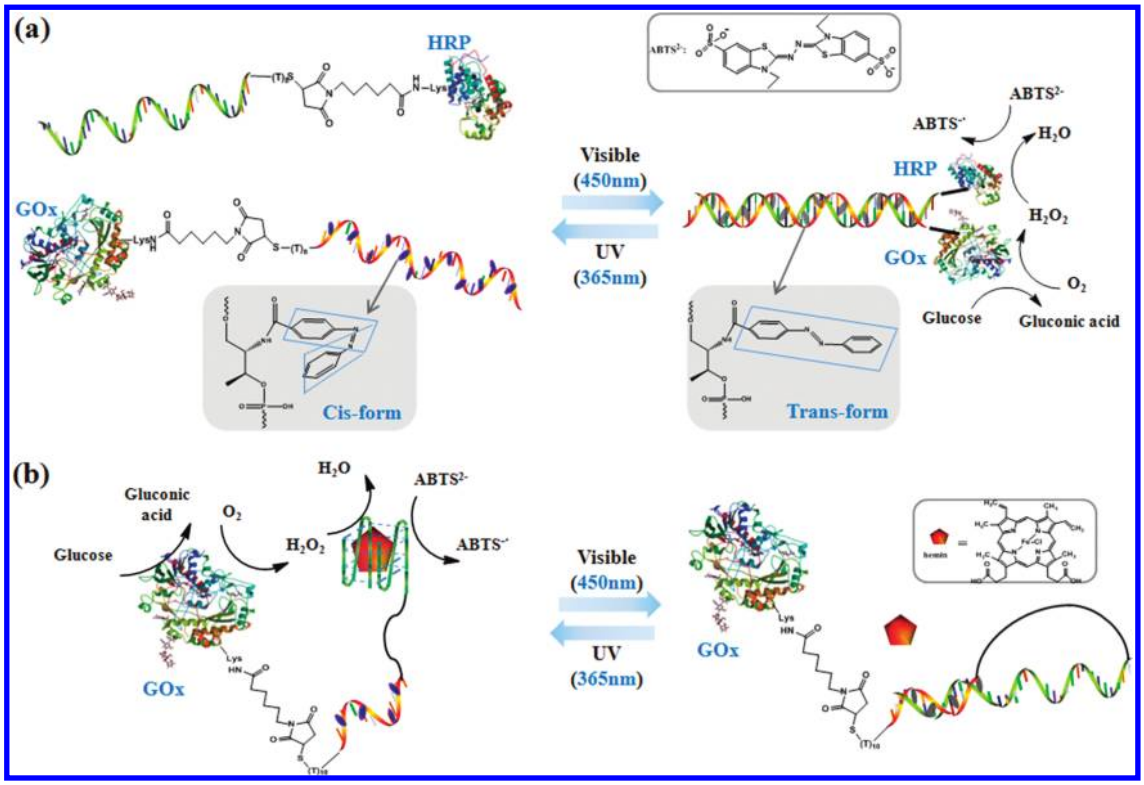

Figure 1. Working scheme for photoregulation of DNA−enzyme nanostructure. (a) Light-responsive azobenzene-integrated DNA duplex controlling glucose oxidase (GOx)/ horseradish peroxidase (HRP) protein enzyme cascade activity. (b) DNA switch probe for regulating the function of GOx/HRP DNAzyme hybrid enzyme nanodevice.

Such concatenated catalytic transformations are important for controlling cellular signaling, and they have also found applications in biotechnology,[21−27] *e.g.*, biosynthesis. The cellular response to a stimulus usually depends on when and how a specific enzyme is activated.[28−30] Thus, for example, the ability to control enzymatic activity in the context of physiological cell signaling would be very useful in clinical applications.

RESULTS AND DISCUSSION

Figure 1a illustrates the principle of using photoresponsive DNA to scaffold the glucose oxidase (GOx)/ horseradish peroxidase (HRP) multienzyme system. Hybridization/dehybridization of DNA nanoarchitectures modulates the proximity of the catalytic centers (*i.e.*, functional domains within GOx and HRP enzymes), which determines the efficiency of enzyme cascade reactions.[21,23] When in close proximity, the high local concentration of the intermediate product allows cascade reactions, which would otherwise be limited by the substrate diffusion rate, to occur. UV light induces the *trans*-to-*cis* conversion of azobenzene, which provides low binding affinity of the DNA duplex and keeps the enzymes separated. In contrast, visible light irradiation reverses the isomerization, thus enabling DNA duplex binding and direct proximity of the catalytic domains within the two enzymes.

The glucose oxidase and horseradish peroxidase system was utilized for "proof-of-concept". Specifically, the primary enzyme GOx catalyzes the oxidation of glucose to gluconic acid, with the concomitant formation of H_2O_2, and the latter product acts as the substrate for HRP, mediating the oxidation of $ABTS^{2-}$ to the colored radical product $ABTS^{-}\cdot$. In this way, the efficiency of the cascade reactions can be directly monitored from the absorbance of the $ABTS^{-}\cdot$ radical at 415 nm.

First, the photoinduced conformational change of the DNA duplex structure was verified on the basis of distance-dependent fluorescence resonance energy transfer (FRET) between fluorescein and dabcyl dyes. The azo-modified DNA was synthesized with an azobenzene moiety every two bases and was labeled with dabcyl at the 3′ end. The cDNA sequence without azo modification was labeled at the 5′ end with fluorescein, the donor dye for the dabcyl quencher. The hybridization or dehybridization of the DNA strands led to quenched or highly fluorescent signals, respectively. By alternating UV and visible light irradiation, the reversible conformational change of the DNA duplex was demonstrated (Supporting Information, Figure S1).

Instead of direct chemical coupling, the conjugation of DNA and enzyme was facilitated by a commercial cross-linker, sulfo-EMCS. Following the standard protocol, the alkylthiol-modified DNA oligomers were conjugated at the active lysine site of GOx or HRP enzymes, as verified by polyacrylamide gel electrophoresis and absorption spectra (Supporting Information, Figure S3).

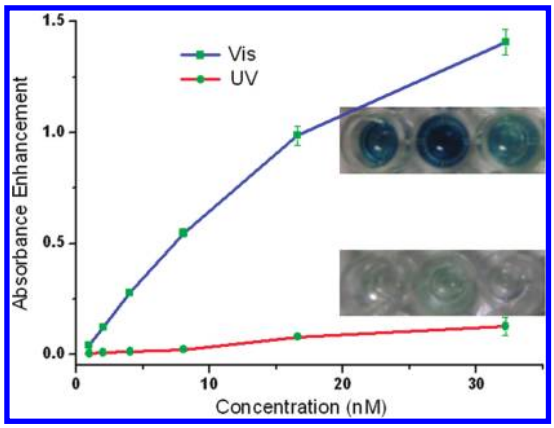

Figure 2. Photoregulation effect on various concentrations of GOx/HRP enzyme conjugates. The absorbance data were obtained 9 min after initiating the reactions. The image was taken for the 16, 32, and 8 nM enzyme samples, left to right, respectively.

The GOx enzyme was conjugated to the azo-modified DNA, while the HRP enzyme was linked to the cDNA. After conjugation, their activities were tested and found to correspond to *ca.* 85% of those of the native enzymes. The reactivity of the GOx/HRP cascade system was monitored by continuous measurement of the absorbance of the cascade product $ABTS^{-}\cdot$ at 415 nm. After 10 min of incubation at 450 nm (azobenzene *trans*; DNA hybridized), generation of $ABTS^{-}\cdot$ was observed immediately after adding the initial reactant (glucose). However, after 10 min of UV (365 nm) irradiation (azobenzene *cis*; DNA unhybridized), the production of $ABTS^{-}\cdot$ was blocked (Figure 2). The enhancement in cascade reactivity between UV and visible irradiation was calculated to be in the range of 10- to 25-fold, depending on the concentration of each enzyme moiety. The difference between *cis* and *trans* states could be observed by the colorless to yellow-green color change during the turnover of $ABTS^{2-}$ to $ABTS^{-}\cdot$. When UV irradiation was used as a control, the enzymatic activities of GOx and HRP were only marginally affected (3−18%) under these experimental conditions (Supporting Information, Figure S2), indicating that hybridization/dehybridization is the major reason for the observed photoregulation. The high local concentration of generated H_2O_2 in the vicinity of the secondary HRP catalytic center accelerates the cascade reaction, as noted previously, and guarantees a high turnover of $ABTS^{2-}$ to $ABTS^{-}\cdot$.

The promising photoregulation demonstrated for the GOx/HRP system led us to extend the design to nucleic acid enzymes. In addition to naturally occurring protein-based enzymes and ribozymes, so-called DNAzymes have been developed to catalyze reactions, such as RNA/DNA cleavage, ligation, phosphorylation, and branching.[31,32] We further demonstrate that a general method for photoregulation of DNA-based enzymatic nanostructures can also be developed on the basis of azobenzene-modified DNA structures.

TABLE 1. Regulation of the Activities of HRP-DNAzyme Probes by UV or Visible Light

probes[a]	ΔA_{415}(vis)[b]	ΔA_{415}(UV)	$\Delta A_{UV}/\Delta A_{VIS}$
7c-3azo	0.047 ± 0.003	0.123 ± 0.009	2.6
7c-6azo	0.056 ± 0.002	0.129 ± 0.002	2.3
8c-4azo	0.038 ± 0.003	0.156 ± 0.001	4.1
9c-4azo	0.028 ± 0.003	0.123 ± 0.006	4.4
9c-8azo	0.059 ± 0.001	0.135 ± 0.007	2.3
10c-5azo	0.020 ± 0.001	0.126 ± 0.003	6.3
10c-9azo	0.045 ± 0.002	0.137 ± 0.004	3.0
11c-5azo	0.019 ± 0.002	0.121 ± 0.005	6.4
11c-10azo	0.043 ± 0.006	0.144 ± 0.011	3.3
12c-6azo	0.019 ± 0.005	0.129 ± 0.012	6.8
14c-7azo	0.010 ± 0.001	0.123 ± 0.006	12.3

[a] The sequences of different probes are shown in the Supporting Information. [b] The absorbance of $ABTS^{-}\cdot$ was measured at 415 nm, 3 min after initiating the cascade.

The HRP-DNAzyme, perhaps the most widely used biocatalytic DNAzyme for amplified biosensing,[33−36] consists of a hemin cofactor-intercalated G-quadruplex structure. This DNAzyme was chosen for concatenation to GOx, as shown in Figure 1b. The azobenzene moieties were positioned within a DNA oligomer complementary to the HRP-DNAzyme sequence. The basis for photomodulation is the competition between the formation of the DNA duplex and the G-quadruplex. The cascade reaction is deactivated under visible irradiation because the DNAzyme hybridizes with cDNA in the *trans*-azobenzene configuration. However, when UV is applied, the *trans*-to-*cis* conversion induces dissociation of the DNA duplex, freeing the DNAzyme to bind hemin and catalyze the cascade reaction.

First, the photoregulation efficiency of the DNA switch probe was optimized. Since even minor modification to the DNAzyme sequence could result in significant disruption of enzymatic activity, we decided to incorporate the azobenzene moieties into the complementary part, not the DNAzyme sequence itself. Optimization of the probe was based on maximizing the efficacy of photoisomerization by adjusting the number and positions of azobenzene moieties and the resulting DNA duplex binding strength. One merit of employing DNAs as scaffolds is their flexibility in synthesizing sequences with various binding strengths and functional groups. A series of probes was prepared with varying base pair number, azobenzene content, and position (Supporting Information, Table S1). The probes were named "Xc-Yazo", where X is the number of complementary base pairs, and Y is the number of azobenzene moieties in the regulatory domain. Similar to the protocol followed in assessing the GOx/HRP cascade, the DNA probe and H_2O_2 were added to determine HRP-DNAzyme activity, and $ABTS^{-}\cdot$ absorbance was monitored (415 nm) after applying either UV or visible light irradiation (Supporting Information, Figure S5). A 2- to 12-fold enhanced catalytic efficiency after 10 min of UV light irradiation was observed for the

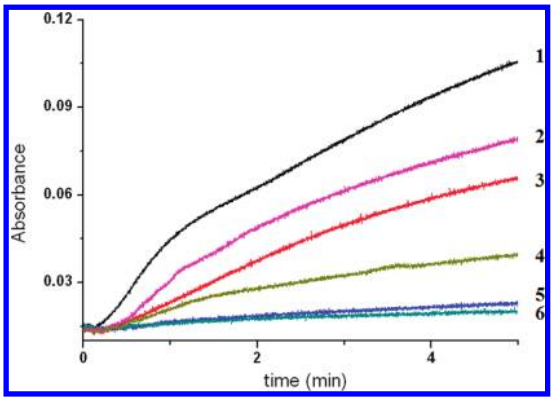

Figure 3. Photoregulation of GOx/DNAzyme cascade activity: (1, 3) covalently linked GOx−DNA after 10 min under UV or visible light irradiation; (2, 4) freely mixed GOx and DNA after UV or visible light irradiation; (5, 6) GOx after UV or visible light irradiation.

DNA switch probes investigated (Table 1). DNA probes with a single azobenzene moiety after every 2 bases showed higher regulation efficiency than probes possessing the maximum azobenzene number, consistent with the previous report.[37,38]

For probes with similar azo/cDNA base number ratio, the enhanced hybridization strength, achieved by elongating the DNA duplex, resulted in an attenuated catalytic activity of DNA switch probes under visible light: *e.g.*, 14c-7azo < 11c-5azo < 9c-4azo < 7c-3azo. However, this trend was not obvious after UV irradiation, where different probes gave almost the same signal enhancement (Supporting Information, Figure S6). These results are attributed to the efficient light-regulated DNA duplex dehybridization. That is, in the *cis* form (ON state), different azo-DNAs all have very low binding affinities with the complementary oligomer, but in the *trans* form (OFF state), the difference in cascade efficiency can be distinguished at room temperature. The almost fully recovered catalytic efficiency after UV irradiation was also confirmed by a readout similar to that of the original HRP-DNAzyme ($\Delta A = 0.15 \pm 0.01$ under the same experimental conditions). When the cDNA length exceeded 10 base pairs, relatively similar photoregulation efficiency was observed. Therefore, the 10c-5azo probe was chosen for subsequent experiments, considering that the hybridization/dehybridization rate would probably be faster compared to that of longer DNA probes.[39]

To prove the feasibility of modulating DNA−enzyme conjugation, molecular assembly of the protein-based enzyme GOx and nucleic acid-based HRP-DNAzyme cascade system was further studied. The azobenzene-modified cDNA was linked on one end to glucose oxidase, with the other end linked to the HRP-DNAzyme by a polyethylene glycol (PEG) spacer. Photoregulation was demonstrated in both covalently linked and freely mixed GOx/HRP-DNAzyme cascade systems (Figure 3). In both cases, the gentle UV light irradiation before initiating the reactions activated the formation of HRP-DNAzyme and enhanced the reaction efficiency. The covalently linked enzymatic constructions displayed higher cascade ability, consistent with the immediate proximity of the catalytic centers, accelerating the reactions, which would otherwise be limited by substrate diffusion. Moreover, the reversible ON/OFF regulation of the enzymatic activity for several rounds was demonstrated by alternated UV and visible light irradiation (Supporting Information, Figure S7).

CONCLUSIONS

This study has demonstrated the molecular assembly of nanostructures functionalized by light regulation. The azobenzene-modified DNA linkers can be used as "arms" to mediate signal transduction in an enzyme assembly. To the best of our knowledge, this is the first study to report the photomanipulation of DNA-enzymatic molecular assemblies. Taking advantage of the photoisomerization property of azobenzene moieties and highly specific self-assemblies of DNA oligomers, we were able to achieve rapid and precise translocation of either small molecules (cofactors like hemin) or macromolecules (protein enzymes) to activate a cascade reaction.

We believe that this approach can be applied to different types of protein enzyme or DNA enzyme structures. With the increased number of DNAzymes isolated by *in vitro* selection procedures,[31,32] more enzyme cascade reactions based on photocontrollable capture of cofactors (*e.g.*, hemin in HRP-DNAzyme) will be realized. Such assemblies will be useful in future biomedical and pharmaceutical applications, especially since ordered sequential cascade processes are central to many complex biological phenomena, such as the coagulation cascade for blood clotting and the apoptosis cascade for controlled cell deletion. The location, timing, and overall activity of any biochemical transformation inside the cell can have distinct biological consequences. While there may be initial concern that the UV light may harm biological systems, the speed of this light-driven procedure, as well as the recent development of azobenzene molecules responsive to visible, or even near-infrared light,[40,41] could help to overcome this issue.

MATERIALS AND METHODS

HRP-DNAzyme Activity Photomodulation Assays. The Xc-Yazo probe (300 nM) was first annealed 20 min in HEPES buffer (25 mM, pH 7.8, mixed with 30 mM KCl and 200 mM NaCl), followed by addition of 2 μM hemin (all concentrations refer to the final solution). After a 10 min incubation period at RT, 190 μL

of the above solution was either irradiated 10 min by a 6 W portable UV lamp (60 Hz with center wavelength at 365 nm and measured light source power around 0.2 mW) or kept under room light. Immediately after removing the sample from the light source, 2.5 mM $ABTS^{2-}$ and 5 mM H_2O_2 were added, in order (final volume = 200 μL). The time-dependent absorbance change was recorded at 415 nm, and the value was recorded 3 min after adding H_2O_2.

GOx/HRP-DNAzyme Activity Assays. As shown in Figure S3 in the Supporting Information, the conjugation between GOx and 10c-5azo probe reached an approximate 1:1 ratio, based on the absorbance result. An aliquot of 100 nM conjugated GOx/10c-5azo probe (or separately added 100 nM GOx and 10c-5azo probe) was incubated with 50 nM hemin in HEPES buffer (25 mM, pH 7.8, mixed with 30 mM KCl and 200 mM NaCl). Then, 190 μL of the above solution was either irradiated 10 min by the portable UV lamp or kept under room light. Immediately after removing the sample from the light source, 2.5 mM $ABTS^{2-}$ and 10 mM glucose were added, in order (final volume = 200 μL). The time-dependent absorbance change was recorded at 415 nm.

GOx/HRP Cascade Activity Assays. After spectrophotometric determination of concentrations, equal amounts of GOx-azo-cDNA and HRP-24mer DNA probe from 1 to 32 nM were incubated in HEPES buffer (25 mM, pH 7.8, mixed with 20 mM $MgCl_2$ and 200 mM NaCl). A 190 μL aliquot of the above solution was either irradiated 10 min by the portable UV lamp or kept under room light. Immediately after removing the sample from the light source, 2.5 mM $ABTS^{2-}$ and 5 mM glucose were added, in order (final volume = 200 μL). The time-dependent absorbance change was recorded at 415 nm 9 min after initiating the reactions.

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Supporting Information Available: Details of the synthesis of azobenzene phosphoramidite and DNA oligonucleotide sequences, preparation and characterization (gel electrophoresis and absorbance spectra) of DNA-conjugated HRP and DNA-conjugated GOx enzyme, effect of UV or visible irradiation on enzymatic activity, and FRET assay for demonstrating the hybridization/dehybridization cycle have been included. This material is available free of charge *via* the Internet at http://pubs.acs.org.

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