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Single-molecule photon-fueled DNA nanoscissors for DNA cleavage based on the regulation of substrate binding affinity by azobenzene†

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A pair of single-molecule photo-responsive DNA nanoscissors for DNA cleavage based on the regulation of substrate binding affinity was designed and fabricated. Compared with other DNA nanomachines, our DNA nanoscissors have the advantages of a clean switching mechanism, as well as robust and highly reversible operation.

DNA is an extremely powerful and versatile building block for nanoscience and nanotechnology, due to its highly sequence-specific hybridization to form a predictable double-helix with suitable flexibility.[1] Many exquisite 2D or 3D DNA nanostructures with different shapes and geometries,[2] such as tiles, sheets, nanoflasks, boxes, cubes, and tetrahedra, have been reported recently.[3] Integrated with controllable elements, these building blocks have led to a variety of functional DNA nanodevices,[4] including walkers, tweezers, rotaxanes and gates, which can perform elegant functions such as movement, scission, rolling and computing. Most traditional DNA nanodevices have been powered by single-stranded DNA (ssDNA).[5] Such ssDNA fueled nanomotors accumulate double stranded DNA (dsDNA) segments as waste products that quickly deteriorate device performance and ultimately bring it to a halt.[6] Recently, various DNA nanodevices driven by other external fuels,[7] such as protons, photons, and magnetism, have been reported. Among them, light is an ideal source for exogenous control of biological systems as it possesses several advantages, especially the ability to control light irradiation in both spatial and temporal fashion.[8] Moreover, photon radiation is a clean, non-invasive and non-contact source without waste accumulation.[7b,9]

Recently, azobenzene has been proven to be a photo-responsive molecule that can be introduced into DNA to regulate the hybridization–dehybridization process.[10] The reversible stereo isomerization of azobenzene from the *trans* to *cis* at 300–380 nm and from *cis* to *trans* at wavelengths over 400 nm[11] allows the photo-manipulation of DNA structures.[7b] Based on this technique, Asanuma *et al.* designed DNA tweezers powered by light irradiation[7b] and Tan *et al.* constructed a light-driven single molecular DNA nanomotor.[7d] More recently, a machine-like DNAzyme for RNA digestion by photo-regulating the topological structure of the enzyme active site was reported.[10b] Compared to regulating topological structure of active site, modulating substrate binding affinity is a simpler, more straightforward, and much easier-to-execute strategy to manipulate enzyme activity. In this study, single-molecule photo-responsive DNA nanoscissors for DNA cleavage based on the regulation of substrate binding affinity are proposed. The photon-fueled DNA nanoscissors were constructed by attaching complementary azobenzene-modified sequences to one end of the DNAzyme as the regulatory domain for substrate binding modulation. This unimolecular DNA nanodevice can be easily and precisely regulated *via* the change of irradiation wavelength. Compared with other DNA nanomachines, our DNA nanoscissors have the advantages of a clean switching mechanism (without DNA waste), as well as robust and highly reversible operation. More importantly, the proposed strategy of substrate binding regulation is also applicable to other DNAzymes, allowing the construction of other photo-responsive nanodevices for DNA nanotechnology.

To construct the photo-fueled DNA nanoscissors, a DNAzyme for DNA cleavage (Enz35) is used as the model system.[12] As shown in Scheme 1, the DNA nanoscissors consist of two hairpin structures **H1** and **H2**. **H1** originally exists as the functional portion of DNAzyme and readily forms a triplex structure with the substrate. **H2** is made from DNAzyme and a complementary azobenzene-modified sequence as the regulatory domain extended at the 5′-end of DNAzyme. Since the conformational change of the photoisomerizable azobenzene group from *trans* in visible light to *cis* in UV light could strongly affect the DNA hybridization, the formation of **H2** is light-dependent. When visible light is applied, the azobenzene groups (X) are in the *trans* form, and the regulatory domain assumes a closed (OFF) state to form very stable **H2**. This strong intramolecular hybridization prohibits the intermolecular hybridization between DNAzyme and the substrate, thus suppressing the DNA-cleavage activity. On the other hand, with UV irradiation, the azobenzene groups convert to the *cis* form, and the regulatory

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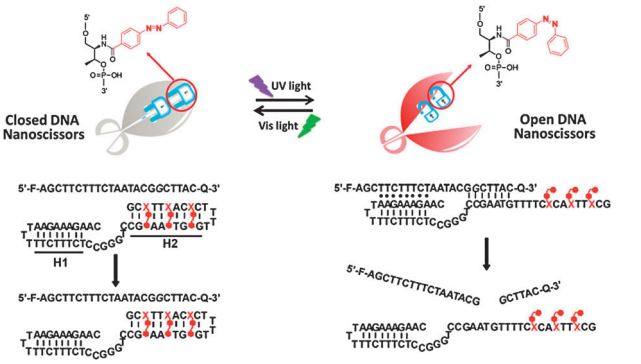

Scheme 1 Working principle of photon-fueled DNA nanoscissors. With visible light irradiation, hairpin **H2** with azobenzenes (in red) maintains its hairpin structure to prevent substrate binding, thus shutting down enzymatic activity. With UV irradiation, **H2** can be opened due to azobenzene isomerization from the planar *trans* to the nonplanar *cis* form, thereby preventing duplex formation and restoring strong binding affinity towards the DNA substrate and activating enzyme function.

domain is converted to an open (ON) state due to the steric hindrance of the nonplanar *cis* structure, thereby dehybridizing **H2** and liberating the binding site of DNAzyme for hybridization with the substrate. In this case, enzymatic activity is restored, and the substrate is cleaved by the active DNAzyme. The ON–OFF transition leads to a dramatic change in substrate binding affinity, and a sharp difference in the enzymatic activity. This photon-fueled DNA nanomachine can be regarded as DNA nanoscissors[13] for DNA cleavage.

In order to inhibit the cleavage activity of DNAzyme, we first optimized the length of the regulatory domain. Since the cleavage starts by the hybridization of DNAzyme and the substrate, the cleavage activity is strongly affected by the affinity of the DNAzyme–substrate complex, which, in our case, has to compete with the affinity of the regulatory domain/DNAzyme. We designed different regulatory domain lengths from 4 to 8 bases, and the cleavage efficiencies were investigated (Fig. S1, ESI†). As expected, the cleavage efficiency of the DNAzyme decreased with the increase in length of the regulatory domain. The relative enzymatic activity of R4Enz35 (4 bases in the regulatory domain) was as high as that of Enz35 without the regulatory domain. After the length of the regulatory domain reached 7 bases (R7Enz35), the relative cleavage activity decreased to 5%, and addition of another base did not further decrease the enzymatic activity. As a result, we chose the R7Enz35 as the prototype for our DNA nanoscissors.

It has been reported that insertion of azobenzenes between at least 2 nucleotides can increase the duplex stability by enhanced π–π stacking interaction, but continued insertion of azobenzenes causes significant reduction in stability.[14] Therefore, with one azobenzene per two nucleotides, three azobenzene molecules were inserted into the 7-base regulatory domain of R7Enz35, named A3R7Enz35. The light-controlled closing and opening of **H2** were confirmed *via* analyzing the T_m of nanoscissors upon visible and UV light treatments, respectively. The T_m of **H2** under visible light (closed state) was measured to be 85 °C (Fig. S2, ESI†). However, after UV irradiation, no clear melting transition was found, suggesting an open linear structure of **H2**. These results confirmed that the regulatory domain of the nanoscissors could be maintained in the closed hairpin state under visible light and switched to the open linear state *via* UV irradiation at ambient temperature. The photo-controlled isomerization efficiency was further studied by reversed phase HPLC analysis of the nanoscissors after different light treatments, and the data showed that almost 100% of A3R7Enz35 was in the closed hairpin state with visible light irradiation and 85% of A3R7Enz35 transformed to the open state after UV activation (Fig. S3, ESI†).

To characterize the active and inactive states of A3R7Enz35, a DNA substrate was designed as a TaqMan probe with a fluorophore (FITC) and a quencher (DABCYL) covalently attached to the 5′- and 3′-end, respectively, to investigate the photo-responsive kinetics. In Fig. 1A, the cleavage activity of the nanoscissors (indicated by an increase in FITC fluorescence) increased with increasing UV irradiation time. Without UV irradiation, the fluorescence was very low (Fig. S4A, ESI†), indicating little cleavage activity because the nanoscissors were in the default inactive state. In contrast, after 15 s UV irradiation the enzymatic activity increased dramatically to 55%, due to the *trans*-to-*cis* conversion. After 120 s UV irradiation, the enzymatic activity reached the maximum, about 8-fold higher than that prior to UV irradiation. The ON–OFF regulation of the nanoscissors was further confirmed by UV/Vis spectroscopy. The UV/Vis absorption spectra were recorded after UV irradiation (from 0 to 180 s), and the results are shown in Fig. 1B and Fig. S5 (ESI†). When the azobenzene molecules were in the thermally stable *trans* configuration, the spectra showed a local maximum at 330 nm, assigned to an intense π–π* transition of the *trans* azobenzene chromophore.[11] However, with the increase in UV irradiation time, the absorbance at 330 nm decreases with a corresponding increase at 430 nm, assigned to the n–π* transition of the *cis* azobenzene. The absorption spectra also confirmed the opening of the nanoscissors under UV irradiation. The results were consistent with fluorescence results, thereby demonstrating rapid photoisomerization kinetics, especially during the first 30 s.

The cleavage efficiencies of the nanoscissors A3R7Enz35 in active and inactive states were then investigated with two control

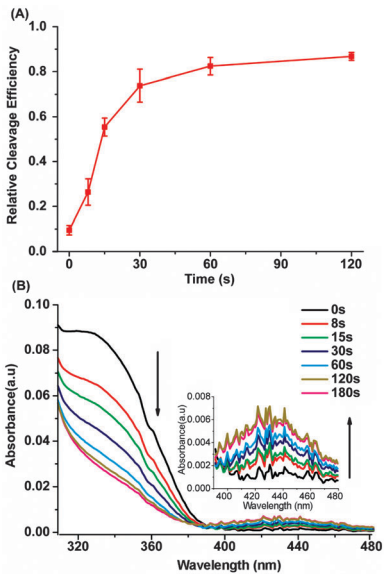

Fig. 1 Photo-switching kinetics study of nanoscissors by fluorescence (A) and absorbance (B) at different lengths of UV light irradiation time. The arrows indicate increasing irradiation time.

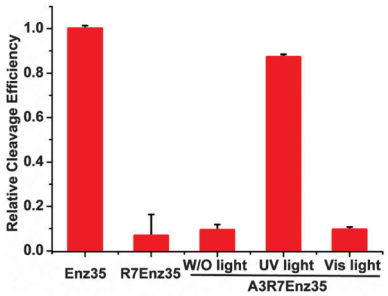

Fig. 2 Investigation of DNA cleavage efficiency of the photo-responsive DNA nanoscissors A3R7Enz35 after different light activation (UV light: 2 min; visible light: 5 min). Enz35 (DNAzyme only) and R7Enz35 (DNAzyme with the regulatory domain lacking azobenzene) were used as positive and negative controls, respectively.

DNAzyme sequences, positive control Enz35 (always-opened DNAzyme without the regulatory domain) and negative control R7Enz35 (always-closed DNAzyme with the regulatory domain lacking azobenzene). In order to compare the results, the cleavage efficiency of Enz35 was defined to be 100%. As shown in Fig. 2, in the initial state, the photo-responsive DNA nanoscissors were in the OFF state (Fig. S4A, ESI†), and less than 10% DNA substrate was cleaved even after 60 min reaction, and the result was the same as that of always-closed R7Enz35. When UV light was applied, the regulatory domain was opened, activating the nanoscissors to cleave the substrate (Fig. S4B, ESI†). About 85% substrate was cleaved by the active nanoscissors after 2 min, an 8-fold enhancement compared to the inactive ones. With subsequent visible irradiation for 5 min, the nanoscissors closed again *via* the formation of **H2**, and the cleavage efficiency decreased to less than 10%. Overall, the relative cleavage efficiency of the nanoscissors is consistent with the structure transformation efficiency, indicating that the formation of the secondary structure favoring substrate binding is essential for the cleavage function of DNAzyme. Thus, efficient photo-regulation of DNA cleavage was successfully achieved by introducing a regulatory domain modified with azobenzene moieties.

Our photon-fueled design provides a simple, clean, and long-lived nanodevice that can reversibly control the enzymatic activity by using light of different wavelengths. Multiple cycling of the machine is demonstrated in Fig. 3A. The cyclic cleavage efficiency changes were associated with the controlled opening and closing of the nanoscissors by successive irradiation with UV and visible light, demonstrating that UV light turns on cleavage while visible light turns it off. More importantly, the efficiency of photoregulation does not change with the number of operation cycles. The UV/Vis absorption spectra also confirmed that the azobenzene moieties could be transformed reversibly by UV and visible light (Fig. 3B), further verifying that the photo-regulated DNA nanomachine is stable and robust.

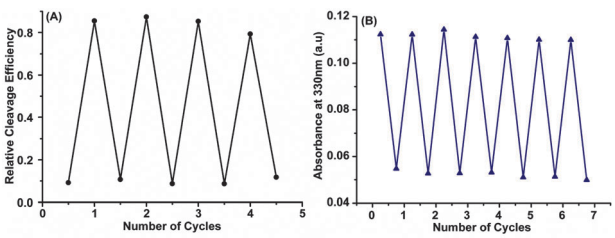

Fig. 3 Reversible cleavage efficiency of the nanoscissors (A) and *trans*-to-*cis* structural transition of the azobenzene group (B) by alternate 2 min UV and 5 min Vis irradiation were recorded.

In conclusion, photon-fueled DNA nanoscissors for DNA cleavage were constructed by attaching complementary azobenzene-modified sequences to one end of the DNAzyme as the regulatory domain for substrate binding modulation. This unimolecular DNA nanodevice can be easily and precisely regulated *via* the change of irradiation wavelength. Compared with other DNA nanomachines, our DNA nanoscissors have the advantages of a clean switching mechanism (without DNA waste), as well as robust and highly reversible operation. More importantly, a simple function for DNA cleavage has been realized based on DNAzyme and photo-responsive azobenzene groups. Our proposed strategy of substrate binding regulation is also applicable to other DNAzymes for construction of many other photo-responsive nanodevices, which provides a new method for practical use of DNA nanotechnology.

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