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主　論　文　の　要　旨

論文題目　Studies on the Reversible Photo-regulation of Strand Displacement with Azobenzene-tethered DNA
(アゾベンゼン導入 DNA による鎖交換反応の可逆的な光制御に関する研究)

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論　文　内　容　の　要　旨

Recent decades of years, DNA has drawn much attention for its possibility as an outstanding engineering molecule in nanotechnology. By programming sequence of DNA, precise control of DNA binding behavior is accessible. Various nanodevices, nanostructures as well as new materials have been developed by manipulating DNA sequences and these achievements provide alternative solutions for such as cell imaging, drug development and clinical diagnosis.

In DNA nanotechnology, toehold strand displacement has been successfully employed to realize controllable or spontaneous motions. An overhang tile called toehold in the DNA duplex triggers a strand migration process, giving the result that one of the DNA stand in duplex is replaced by another strand that has longer binding portion. This method achieved first reversibly controllable DNA nano-devices, a DNA tweezer. The "open" and "close" of the DNA tweezer can be simply manipulated by alternatingly adding two DNA strands. However, the efficiency of this DNA tweezer declines after several cycles due to the increased waste duplex produced every cycle. The excess pollutions contaminate the nano-environment by breaking the dynamic equilibrium of strand displacement, resulting in the decrease of efficiency. This is one of the common problems in designing a controllable DNA nano-machine utilizing toehold strand displacement reaction.

As a solution for above drawbacks, a light-driven DNA strand displacement system was established and thoroughly studied in this study. It contains two main part:

1) Study on photo-driven DNA strand displacement

2) Study on photo-regulatable DNA amplification reaction

The first chapter includes general introductions to nucleic acids and DNA nanotechnology. History of DNA nanotechnology as well as basic concept of DNA binding behavior are described to provide reader a background knowledge of this field. A brief introduction to photochemistry of DNA was also reviewed to build the basis for further discussion of azobenzene-tethered DNA in the following chapters.

The second chapter contains the strategy and validation of photo-driven DNA strand displacement reaction. Azobenzene-incorporated oligonucleotides are employed as modulators to driven the system. When UV (~365 nm) is irradiated, azobenzene isomerizes to *cis*-form that dissociates DNA duplex due to significantly increased steric hindrance, while visible light (400 nm~) irradiation converts azobenzene moieties to *cis*-form thus duplex is able to hybridize and further stabilized by hydrophobic stacking effect (Fig. 1). This photo-regulatable DNA binding behavior raised the idea of developing a photo-driven strand displacement, as shown in Fig. 2. There were three strands used in this method: a template (**Tem**), a product (**Prod**), and a modulator (**Mod**). **Tem** is a 22-mer DNA and **Prod** is fully complementary to **Tem**; together, they form a blunt-ended duplex. **Mod** is fully complementary to **Tem** and contains several azobenzene moieties.

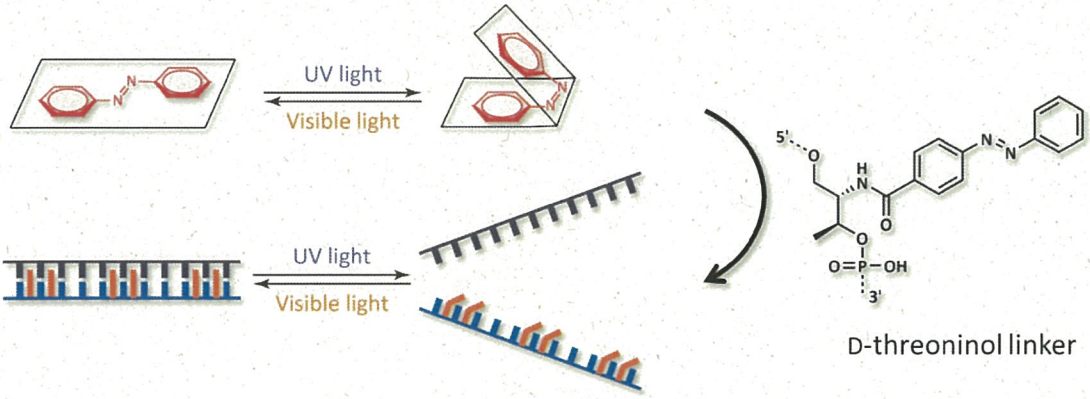

Figure 1. Photo-regualtion of DNA

In the initial state, **Mod** binds to the **Tem** and **Prod** is single stranded. Upon irradiation with UV light, *trans*-to-*cis* isomerization induces dissociation of the **Tem/Mod** duplex, which allows the duplex formation of **Tem** and **Prod**. Next, the sample is irradiated with visible light. This induces reversion of the azobenzenes to *trans*-form, thereby **Mod** again binds to **Tem** to release **Prod** and initializes the system. Through

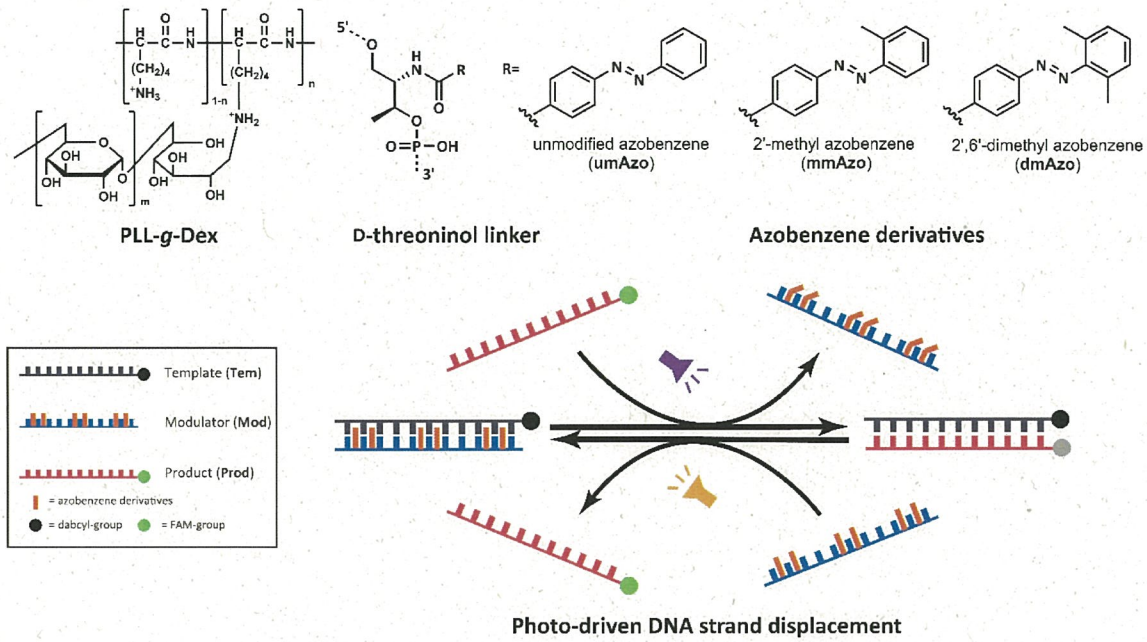

Figure 2. Photo-driven DNA strand displacement

alternating UV/visible light irradiation, the strand displacement occurs reversibly. PLL-*g*-Dex is expected to facilitate strand displacement in the absence of a toehold.

The kinetic and thermodynamic studies suggested that both forward and backward strand displacements are feasible, after which light irradiation experiments showed the reversibility and controllability of this photo-driven strand displacement method. We further demonstrated that chemical modification (*ortho*-methylation) of azobenzene and appropriate incorporating quantity of azobenzene moieties could significantly enhance the efficiency of our method. We also discussed some other factors that influenced the performance of photo-driven DNA strand displacement reaction, and finally showed a non-toehold but reversibly controllable behavior with constant efficiency 65% (Fig. 3).

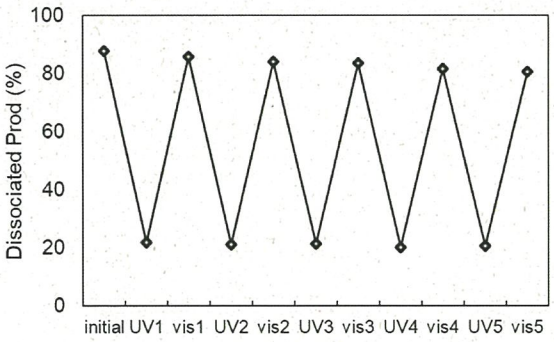

Figure 3. Reversible and constant photo-regulation of DNA strand displacement.

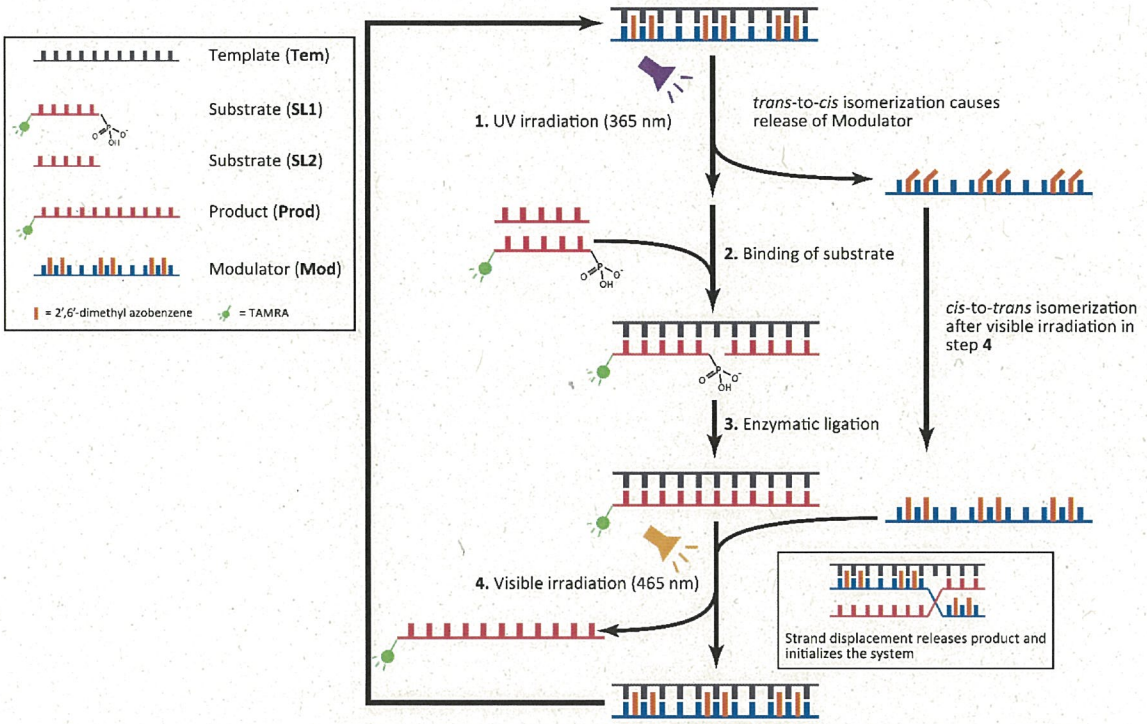

Figure 4. Photo-regulatable isothermal DNA amplification

In Chapter 3, based on the successful development of photo-driven DNA strand displacement, I then proposed a photo-regulatable DNA amplification method, as shown in Fig. 4. The system consists of a template strand (**Tem**), an azobenzene-tethered modulator (**Mod**) that is complementary to template strand, and two substrate strands (**SL1** and **SL2**), each of which is complementary to half of the **Tem**. **SL1** is labelled with a TAMRA-group at the 3' end and has a phosphate group at the 5' end.

Initially, the azobenzene moieties in the **Mod** strand are in *trans*-form, and this allows its hybridization onto **Tem**. Once irradiated with UV light, the azobenzene moieties isomerize to the *cis*-form, inducing dissociation of the duplex **Tem/Mod** thereby permitting **Tem** to hybridize with two substrate strands, **SL1** and **SL2**. Enzymatic ligation then occurs to produce a product strand (**Prod**). The system is then irradiated with visible light (465 nm) so that the azobenzene moieties revert to the *trans*-form. This induces the backward strand displacement that **Mod** binds to **Tem** releasing the **Prod** and the system is initialized. In this way, by alternating irradiation with UV and visible light, amplification of **Prod** can be precisely, quantitatively, and repetitively controlled.

As a result, we achieved at least 14 cycles of successful linear amplification of **Prod**, which is quantified by electrophoresis, as shown in Fig. 5. A series of control experiments verified the amplification was obediently regulated by alternating light irradiation, and the average yield of 60% is coincident with the efficiency of photo-driven strand

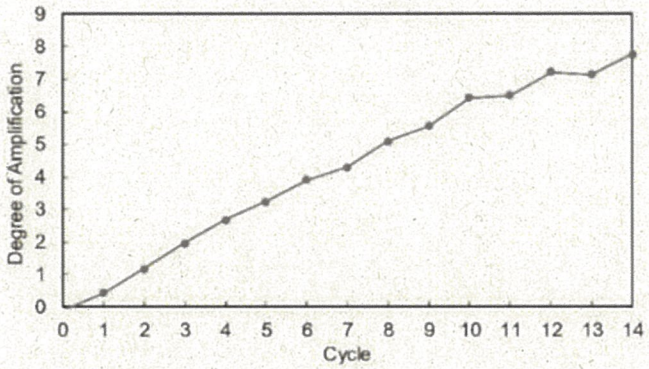

Figure 5. Photo-regulatable isothermal amplification of DNA

displacement in the previous study. We also demonstrated the predominant discriminability of mismatches in template strand, which provides an alternative solution for designing new diagnostic tool of such as genetic diseases.