Azobenzene Functionalized DNA for Light-Induced DNA Stringency

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Abstract and Introduction:

Azobenzene functionalized deoxyribonucleic acid (DNA) and DNA modified gold nanoparticles (AuNP) are used to study light-induced DNA stringency, a method to distinguish perfectly matched DNA from partially matched DNA using light. To do this, AuNP are functionalized with DNA [1], and azobenzene modified DNA is attached to glass slides to use chip-based assays. The photoisomerization quantum yield of azobenzene, chemically attached with DNA molecules, is also measured in order to study the temperature's effect on azobenzene isomerization efficiency.

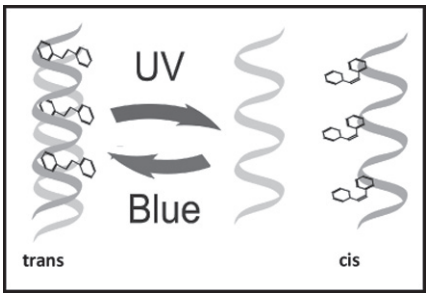

Figure 1: Azobenzene modified DNA switching between dsDNA and ssDNA with light. (See full-color version on inside cover.)

Azobenzene is an organic molecule that can photoisomerize between the *trans*- and *cis*-form. *Trans*-azobenzene can isomerize into the *cis*-form under UV light, and the reverse will happen under blue light. Azobenzene can be chemically attached to DNA [2], which will permit control over whether DNA is a double or single strand using light (Figure 1).

Complementary single-stranded DNA (ssDNA) will form double-stranded DNA (dsDNA) when azobenzene is in the *trans*-form. Under exposure to UV light, a properly formed dsDNA incorporating azobenzene modifications will denature into ssDNA, since the formation of *cis*-azobenzene will induce steric hindrance. Previous research has shown that azobenzene resists photoisomerization into the *cis*-form when it is attached to a perfectly matched dsDNA, as compared with when it is attached to a partially matched dsDNA [3]. This result explains the phenomenon that with the same amount of UV energy, perfectly matched dsDNA stays bound while partially matched dsDNA denatures. This difference in behavior allows light to be used to perform DNA hybridization stringency.

While previous studies have shown photo-controlled DNA stringency in solution, the focus of this summer's research was to attach DNA to a glass substrate and study its photoswitching properties.

Experimental Procedure:

To achieve chip-based DNA stringency, a glass substrate was functionalized with azobenzene modified DNA, 5′-/5Thio MC6-D/AAA AAA AAA TG/ iAzoBenz/ AA/iAzoBenz/ CT/iAzoBenz/ AA/iAzoBenz/ CG-3′. A silanized glass slide was covered with a hybridization chamber that was filled with succinimidyl 4-(p-maleimidophenyl)butyrate, SMPB, overnight. Azobenzene was added for six hours and bonded to the SMPB. Unbound SMPB was pacified with 6-mercapto-hexanol for thirty minutes.

A target strand of DNA was attached to the azobenzene DNA for two hours. The glass was exposed to UV light for two hours. Then DNA modified AuNP were inserted into the chamber for one hour. A silver enhancement solution was added for ten minutes so that the silver ions ionized around the AuNP creating a spot where the AuNP remained.

Quantum yield is a calculation based on how much *trans*-azobenzene has isomerized into *cis*-azobenzene divided by the number of photons [4]. To measure quantum yield, a solution of ssDNA containing azobenzene was prepared. The experimental apparatus for calculations consisted of an Agilent 8453 UV-Vis spectrometer, UV LEDS, a temperature controller, and a stirring plate. A photodiode was used to measure the UV light intensity before photoswitching. The DNA solution was placed in a cuvette and allowed to equilibrate in the apparatus without UV exposure for an hour. After the time elapsed, the solution was exposed to the UV light, and UV-Vis spectra were taken over the next hour at specific times. The data from the spectra was collected focusing on the 330 nm wavelength. This data was then analyzed with a curve-fitting program which created a numerical analysis of the quantum yield as the azobenzene transformed from *trans* to *cis*.

Result and Conclusions:

When looking at the glass slides that had been silver enhanced, a solid grey square was expected when a perfectly matched target strand of DNA was present. However, when it was a partial match, the intensity of the grey was expected to be less. This research focused on perfecting these steps to make the process more effective. To do this, the different steps were analyzed. From this, the importance of attaching the DNA in high quantities to the glass was understood. Otherwise, the AuNP could directly attach to the glass via non-specific interactions. This would create a false positive when the silver enhancer was used.

Another key factor was the age of the DNA attached to the glass (Figure 2 and Figure 3). From the figures, it is shown that the newer DNA allowed for a greater difference for observation of perfectly matched DNA compared to completely mismatched DNA. This shows that the older DNA began to break down and allowed the AuNP to directly attach to the glass.

The quantum yield was studied for A-azo, 5′-AAA AAA AAA /iAzoBenz/ AAA AAA AAA-3′, and T-azo, 5′-TTT TTT TTT/ iAzoBenz/TTT TTT TTT -3′. Research has shown that A-azo is more rigid than T-azo [5]. Because of the differing rigidity, the effect of temperature on the DNA strands was studied. A-azo at 29°C had a quantum yield of 0.044 ± 0.001, while at 37°C the quantum yield was 0.053 ± 0.00005. T-azo at 29°C had a quantum yield of 0.067 ± 0.014, while at 37°C the quantum yield was 0.064 ± 0.016. A-azo had a 19.5% increase in quantum yield at higher temperatures, and T-azo had a 5.1% decrease in quantum yield at higher temperatures.

As a result, it can be concluded that the higher temperatures have a greater effect on the more rigid DNA.

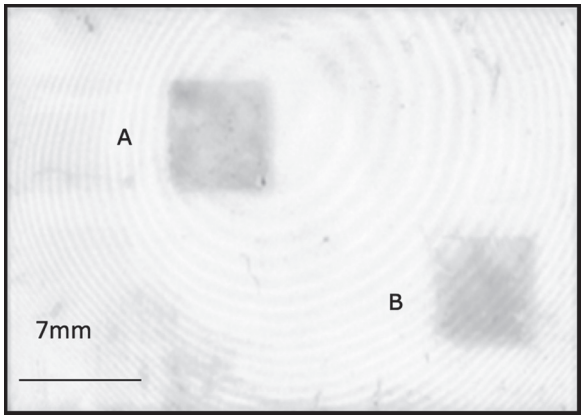

Figure 2: Old DNA; A) Perfectly matched DNA, and B) Mismatched DNA.

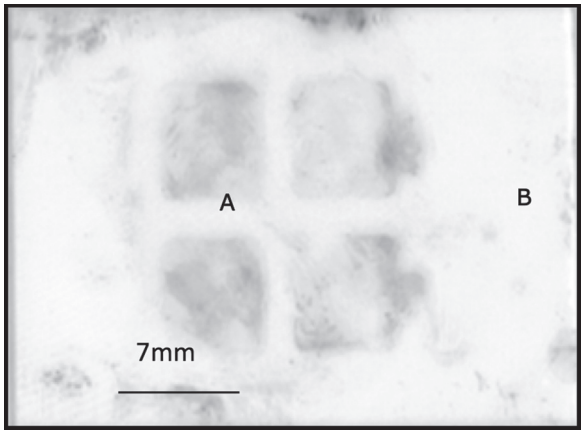

Figure 3: New DNA; A) Perfectly matched DNA, and B) Mismatched DNA.

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References:

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