

## Loop-Ligation of DNA Nanoswitch for Sensitivity Increase

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### Abstract

DNA nanoswitches are tools to detect nucleic acids such as microRNAs and viral RNAs. The DNA nanoswitch is a linear duplex structure created from single-stranded circular viral M13 DNA using a DNA origami approach. Each nanoswitch can be designed to have explicit detector strands that bind to a specific DNA sequence. In the presence of a particular target sequence, the DNA nanoswitch will undergo a conformational change in which it switches from a linear “off” state to a looped “on” state. The shape of the DNA nanoswitch can then be detected using standard gel electrophoresis, a cheap and simple test. In this project, we aim to improve the sensitivity of the DNA nanoswitches in detecting target strands using DNA loop-ligation. This strategy will allow the target strand to be “recycled” in solution after a more permanent looped nanoswitch is formed and continue to convert more linear nanoswitches into looped nanoswitches through target recycling. We were able to confirm permanently looped nanoswitches after ligation using T4 DNA Ligase enzyme. We will optimize the reaction conditions to show stability and signal amplification of the nanoswitch. Ultimately, we hope to amplify detection signal at least 10-100 fold using this approach, bringing our sensitivity to the aM ( $10^{-18}$ M) range.

### Introduction

DNA nanotechnology has become an important influencer on multiple disciplines due to the unusual physical and chemical characteristics of nanostructures. On the nanometer scale, arrangements of particles can be designed to function in highly specific conditions and with greater accuracy. The development of programmable DNA nanoswitches has provided scientists with a tool to detect nucleic acid sequences, which can be applicable to many issues present specifically in the medical and forensic fields. They have potential use for drug delivery, medical diagnostics, and information storage.

Our lab focuses on creating inexpensive and highly specific sensors for detection of molecular interactions. The DNA nanoswitch will undergo a conformational change and become looped in

the presence of specific inducers, such as a target strand, in which will bind to the complementary detector strands on the scaffold to loop a part of the DNA. The loop size of the nanoswitch can be programmed by incorporating detector strands in desired location along the M13 backbone and the functionality can be modified by varying the sequence of the detector strand. The conformation change of the nanoswitch from the linear “off state” to the looped “on state” can be detected using gel shift assay. The looped nanoswitch migrates slower in a fixed electric field compared to linear nanoswitch. A single target strand can only create one looped nanoswitch in solution and the detection is therefore limited to the amount of target in solution. To improve the efficiency of nanoswitch looping, a target recycling approach can be implemented. To achieve this goal the loop portions of the nanoswitch can be ligated to create a more stable structure that is no longer dependent on a target strand to keep it in the looped state. The nanoswitch can be heat annealed to kick off the target strand and recycled back into solution for the next looping-ligation-reannealing cycle (Figure 2-A). This research shows our preliminary data for the confirmation and optimization of nanoswitch ligation.

## **Methods**

### Linearization of M13 circular DNA

The process of designing the phosphorylated nanoswitch begins with linearizing circular single-stranded M13 DNA. To a 5 $\mu$ l solution containing circular M13 (250 ng/ $\mu$ l), 2.5 $\mu$ l of 10x Cut Smart buffer, 1 $\mu$ l of BtsCI restriction-site complimentary oligonucleotide(100uM) and 16.5 $\mu$ l nuclease free water was added. The mixture was heated to 95°C and cooled down to 50°C to heat anneal the BtsCI restriction-site oligo with the circular M13 DNA and create the BtsCI restriction site. 1 $\mu$ l of BtsCI enzyme is added and incubated at 50°C for 15 minutes to linearize the M13 DNA strand, then heated up to 95 °C to deactivate the enzyme.

### Phosphorylation of Oligonucleotides

The two specific detector strands 45 nucleotides in length, positioned at the 40<sup>th</sup> and 80<sup>th</sup> position from 3' site on scaffold are mixed in 1:1 ratio and diluted to a final concentration of (x M). T4 DNA Ligase buffer is added to ensure optimal activity of the enzyme, and T4 Polynucleotide Kinase (PNK) is used to phosphorylate the 5' end of the DNA. This will prepare the detector

strands for ligation as it adds the phosphate group on the 5' site of detector oligonucleotide. After incubating the mixture at 37°C for 5 hour the enzyme is deactivated.

### Synthesis of DNA nanoswitch

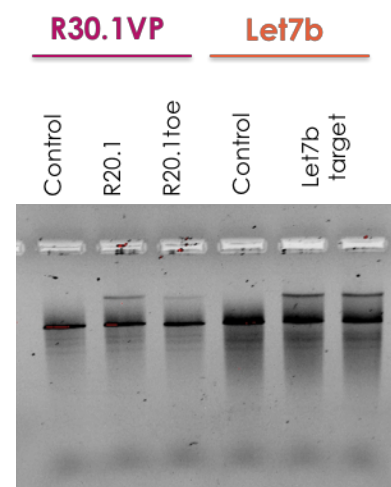
The DNA nanoswitch is constructed by adding 123 staple strands including two detector strands to the linearized M13 DNA. The mixture of M13 and staple strands were heat annealed in the thermal cycler. The constructed nanoswitch is then purified using liquid chromatography to remove excess oligonucleotides and is ready to be used.

### Gel Electrophoresis

All experiments were done in 0.8% agarose gel made by dissolving agarose in 0.5x TBE (Tris-borate EDTA) buffer by heating the mixture in a microwave. The mixture was then solidified in a casting tray with combs to form the indentations for the nanoswitch mixtures. All gel electrophoresis experiments were run at room temperature under constant voltage (75V) in 0.5X TBE buffer for 45 minutes. Each sample was pre stained with GelRed™ (Biotium) and loading dye and all images were taken using a Biorad gel imager.

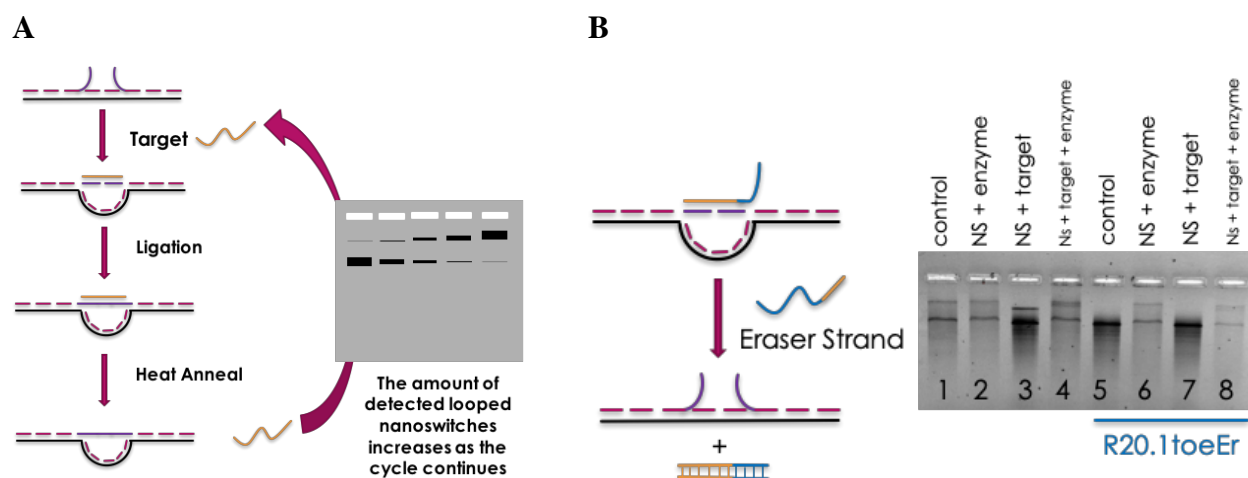
## Results and Discussion

We began to study the abilities of newly phosphorylated nanoswitches with varying target strands and temperature cycling. In order to ensure the reliability of this new nanoswitch, we compared the looping activity against a functional and commonly used nanoswitch Let7b (Figure 1). The phosphorylated nanoswitch was successful in forming loops that were congruent to the known looped nanoswitch when incubated with two different target strands, R20.1 and R20.1 with a toe-hold strand at 20nM concentrations. The toe-hold region on the data strand will allow the target to be removed in the presence of a complementary eraser strand and confirm that ligation has occurred (Figure 2-B).



**Figure 1:** Gel image comparing the looping of the phosphorylated nanoswitch with two target strands, with and without a toe-hold region, to a known Let7B nanoswitch.

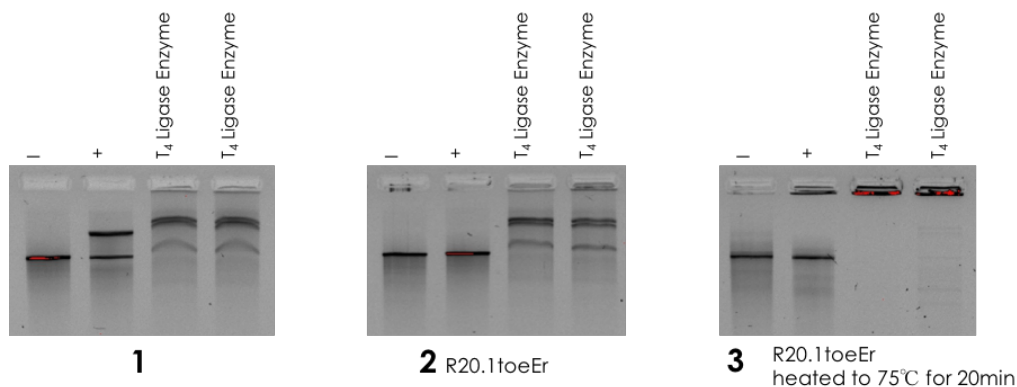
We continued to use the R20.1toe strands in the proceeding experiments in addition to deionized water, 10x BSA, and T4 DNA Ligase buffer to optimize activity. The phosphorylated detector strands were incubated overnight with R20.1toe strands and 40k/mL T4 DNA Ligase enzyme. Although the enzyme caused other structures to be formed like dimers shown in lanes 4 and 8, there was still detection of looping activity (Figure 2-B). To understand the stability of the new phosphodiester linkage between the oligonucleotides on the looped nanoswitch, the complementary eraser strand was used to revert back to the original linear structure. We were able to conclude that not all of the looped nanoswitch was destroyed after the bonds were attacked by the eraser strand (comparing lanes 4 and 6 Figure 2-B). This indicated that T4 DNA Ligase enzyme was successful in ligating the target region of the nanoswitch and created a more permanent loop.



**Figure 2:** (A) Illustration of the formation of a looped nanoswitch when induced by a target strand that is then ligated. With each cycle of ligation and heat annealing to recycle the target strand in solution, the detection of looped nanoswitches will be amplified. (B) Shows how the target strand with a toe-hold region will unloop the nanoswitch when a complementary eraser strand is introduced. Gel image compares the loop-ligated nanoswitch detection with and without an eraser strand.

We next wanted to observe the thermostability of the loop-ligated nanoswitch and the nanoswitch solution was heated in a thermocycler to 75°C for 20 minutes. The solution was subsequently pre-stained and run under normal gel electrophoresis conditions (Figure 3-3). As predicted the nanoswitch and all other structures were not completely degraded in this temperature cycle (last

lane in figure 3). Although the detector loop was ligated and more stable, it was not enough to hold the entire nanoswitch together or the nanostructures were trapped in the wells of the gel. From this we were able to conclude the need to ligate the entire nanoswitch in order for the target strand to be kicked off without degrading the structure. If the entirely ligated nanoswitch remains unaffected by the temperature cycle, the signal of looped nanoswitches will dramatically increase due to the detector strands ability to be recycled in solution.



**Figure 3:** Shows looping of the nanoswitch with T4 DNA Ligase enzyme (1), after incubation with R20.1 toe-hold target eraser strand (2), and degradation of the nanoswitch after temperature cycle (3).

## Conclusion

The focus of our preliminary testing was to create a nanoswitch that could undergo ligation, which began with ligating only a small region of the nanoswitch where the loop was formed. A protocol for developing the phosphorylated DNA nanoswitch was designed and we learned what conditions are necessary for the ligation to occur with T4 DNA Ligase enzyme. Additionally, we were able to determine that the loop-ligated nanoswitch is more permanent and remains looped in the presence of a complementary eraser strand that is meant to unloop and linearize the nanoswitch. Although signal amplification was not yet achieved by recycling the target into solution, future experiments will focus on developing an entirely ligated nanoswitch that can undergo the temperature cycle to remove the target strand from the looped nanoswitch without degrading the entire structure. Future experiments are planned to optimize the conditions for this nanostructure, help improve the efficiency of the target detection, and increase the overall sensitivity of the nanoswitch to respond to concentrations in the aM ( $10^{-18}$ M) range.

## References

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