Aptamer Regulated Transcription for in-vitro detection and signal processing of Interferon Gamma

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Abstract

Aptamers, which are single-stranded DNA or RNA molecules, possess the capability to strongly and selectively bind to specific targets. However, the effectiveness of most aptamer-based biosensors relies on distinct structural changes that arise during interactions with proteins. Our objective was to devise a biosensor framework known as Aptamer Regulated Transcription for In-vitro Sensing and Transduction (ARTIST). By integrating the aptamer into a transcriptional circuit, aptamer functions as a switch to govern circuit output through the presence of target protein on the aptamer to stop polymerase transcription which product detectable output. Our findings exhibited that the ARTIST sensor designed for IFN-γ can precisely modulate transcriptional rates in correspondence with varying ligand concentrations. This modulation was influenced by distinct levels of ARTIST concentration. Moreover, we showcased the downstream processivity of ARTIST by incorporating it into an inverter and amplifier circuit.

ARTIST

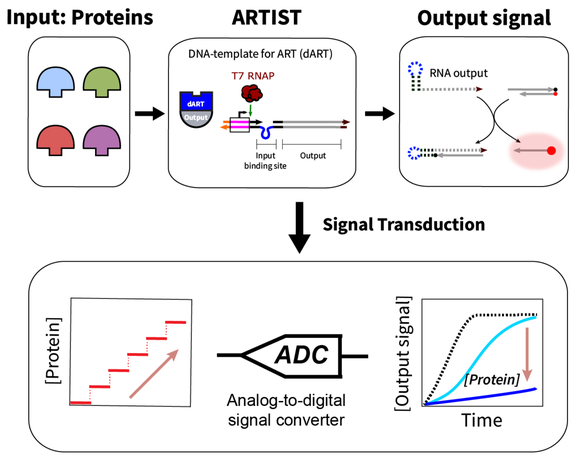


Figure from Lee et al.

We can produce an RNA-based output signal through the DNA Aptamer Regulated Template (dART). The input of the system would be proteins such as IFN-γ and the output from all ARTIST systems would be RNA that can be coupled to various downstream nucleic acid reactions.

Designs and Results

Result from the dART using IFN aptamer

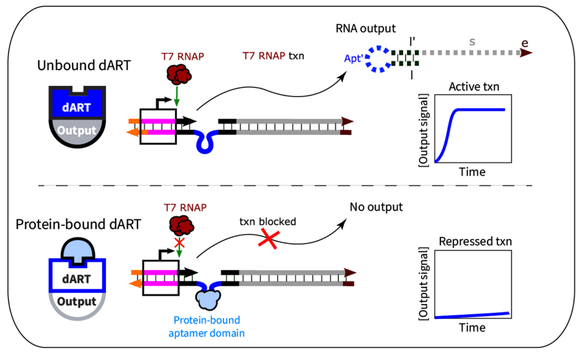
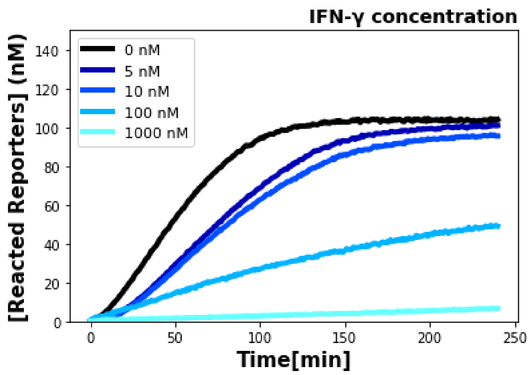


Figure from Lee et al.

dART works by protein-aptamer complex which interfere with transcription. Protein-aptamer complex could hinder the T7 polymerase transcription activities and suppress the production of the output RNA.



The dART can detect protein concentration in the solution by having different transcription rate. Higher protein concentjration yield a low transcription rate.

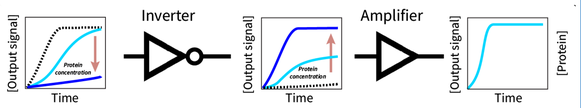


Figure from Lee et al.

We can coupled dART output with the genelet system created by Schaffter et al. to create inverter and amplifier circuits downstream of the ARTIST sensor. Genelets are short transcriptional nodes that can be turned on and off to regulate transcription rate. We can build circuits with ARTIST using these transcriptional nodes to adjust ARTIST output to invert and amplify the signal coming from ARTIST.

Result from the Inverter and Amplifier

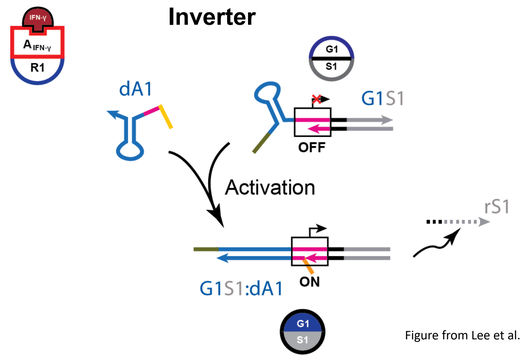


Figure from Lee et al.

The inverter system functions by IFN-γ repressing the production of a repressor RNA (R1). R1 inhibits G1S1 by preventing DNA activator (dA1) binding to G1S1 and produce fluorescent signal.

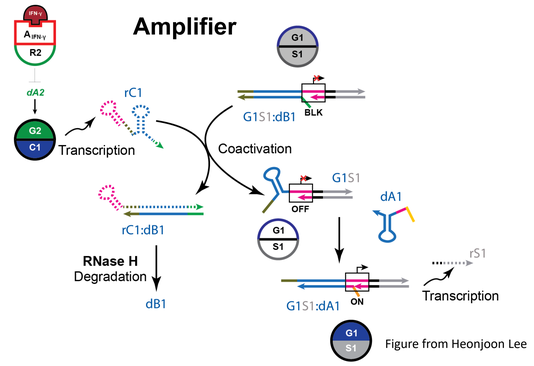
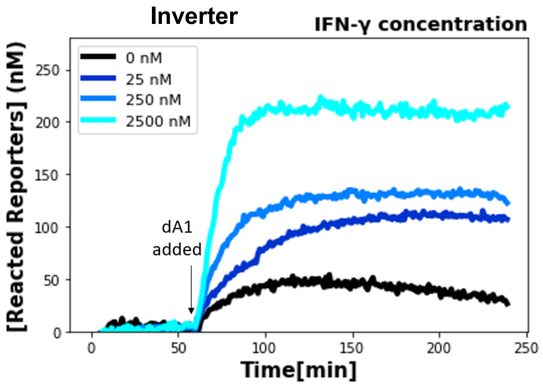
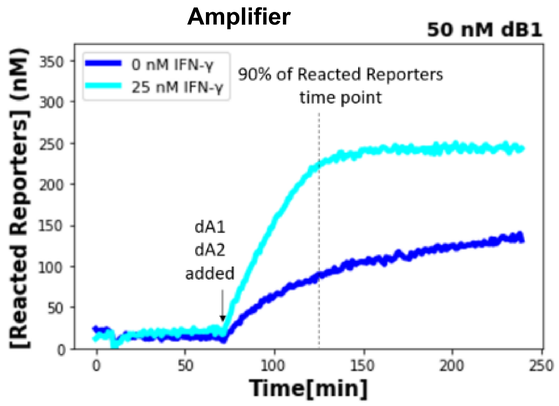


Figure from Heonjoon Lee

The amplifier system has an additional node compared to the inverter that increase output at low protein concentration which enhance the sensitivity of the ARTIST.





By coupling the dART with the inverter and amplifier, we were able to invert and amplify the repressive effect of the protein on the signal output. We demonstrated greater signal output in conditions with high protein presence with enhanced signal output for low protein concentration condition.

Conclusion

We have created the ARTIST system that utilize aptamers as biosensor which produce bio-relatable outputs. ARTIST would allow fast swapping of aptamers to perform multiplex protein detection and can be coupled to downstream nucleic acid reactions. Additional works are taking place to enhance the ARTIST's sensitivity.

Acknowledgement

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