Integrating DNA Strand Displacement with RNA Transcriptional Regulation

1   Background

RNA/DNA hybrids are an underexplored area with significant implications for RNA regulatory systems and strand displacement mechanisms [7]. Strand displacement is a technique where one strand replaces another, with the thermodynamics and kinetics of these mechanisms being well understood [9], [8].

DNA/RNA hybrid circuits, such as those based on the T7 transcriptional unit, showcase an innovative approach to molecular design. The genelet toolbox highlights this concept, where strand displacement directly regulates T7 transcription, allowing the newly synthesized transcripts to further participate in strand displacement activities [3]. It has led to the development of circuits like switches and oscillators [3], [4], [6].

Shifting from the genelet's experimental framework, our focus turns to RNA strand displacement mechanisms in vivo, aiming to bridge artificial networks like DNA strand displacement with in vivo RNA mechanisms of regulation. One example is the control of transcription termination by short termination activator RNAs (STARs) [1]. The use of short termination activator RNA (STAR) based on intrinsic termination [2] demonstrates the versatility of these systems in regulatory mechanisms, including E. coli transcription. This opens up possibilities for connecting DNA strand displacements with RNA regulatory mechanisms [5].

2   Goal

This project aims to integrate a strand displacement circuit with a transcriptional regulatory part involving the STAR system. We will focus on designing and implementing simple RNA/DNA hybrid strand displacement. This includes one part dedicated to strand displacement and another to transcription activation, both involving oligonucleotides that can be either RNA or DNA, and both resulting in the release of a DNA or RNA molecule. We intend to connect two independent units, varying their positions in a cascade. We will explore more complex circuits if time permits.

3   Requirements

Applicants should have:

- A grasp of fundamental molecular biology techniques such as PCR, PCR cleanup, agarose, and PAGE gels.
- Hands-on experience with enzymatic reactions and a solid understanding of their principles.
- Proficiency in English for effective communication.
- Skills in data processing, which will be advantageous for analyzing experimental results.
- Prior experience with in vitro systems, beneficial for understanding project requirements.

4   What You'll Learn

Through participation in this project, you will acquire knowledge in:

- The design of molecular circuits and understanding their operational principles.
- The potential and limitations of in vitro systems for simulating biological processes.
- Utilizing NUPACK for the prediction and design of strand displacement reactions, enhancing the accuracy of experimental designs.
- Applying simple models for fitting experimental data, a critical skill for interpreting results.

References

[1] James Chappell, Melissa K Takahashi, and Julius B Lucks. Creating small transcription activating RNAs. *Nature Chemical Biology*, 11(3):214–220, March 2015.

[2] Ivan Gusarov and Evgeny Nudler. The Mechanism of Intrinsic Transcription Termination. *Molecular Cell*, 3(4):495–504, April 1999.

[3] Jongmin Kim, Kristin S White, and Erik Winfree. Construction of an *in vitro* bistable circuit from synthetic transcriptional switches. *Molecular Systems Biology*, 2(1):68, January 2006.

[4] Jongmin Kim and Erik Winfree. Synthetic *in vitro* transcriptional oscillators. *Molecular Systems Biology*, 7(1):465, January 2011.

[5] Ádám Kun, András Szilágyi, Balázs Könnyű, Gergely Boza, István Zachar, and Eörs Szathmáry. The dynamics of the RNA world: insights and challenges: The dynamics of the RNA world. *Annals of the New York Academy of Sciences*, 1341(1):75–95, April 2015.

[6] Samuel W. Schaffter, Kuan-Lin Chen, Jackson O'Brien, Madeline Noble, Arvind Murugan, and Rebecca Schulman. Standardized excitable elements

for scalable engineering of far-from-equilibrium chemical networks. *Nature Chemistry*, 14(11):1224–1232, November 2022.

[7] Georg Seelig, David Soloveichik, David Yu Zhang, and Erik Winfree. Enzyme-Free Nucleic Acid Logic Circuits. *Science*, 314(5805):1585–1588, December 2006.

[8] David Soloveichik, Georg Seelig, and Erik Winfree. DNA as a universal substrate for chemical kinetics. *Proceedings of the National Academy of Sciences*, 107(12):5393–5398, March 2010.

[9] David Yu Zhang and Erik Winfree. Control of DNA Strand Displacement Kinetics Using Toehold Exchange. *Journal of the American Chemical Society*, 131(47):17303–17314, December 2009.