Chapter 1

Introduction

Nucleic acids, deoxyribonucleic acid (DNA) and ribonucleic acid (RNA), are essential macromolecules for known forms of life. DNA stores the genetic information of a cell while RNA molecules participate in transmitting, expressing and controlling this information. Fortunately, one can do more with nucleic acids than carry genetic information. Using the base-pairing properties of nucleic acids, one can use nucleic acids to design molecules that self assemble into specified structures. This field is called nucleic acid nanotechnology or nucleic acid engineering.

The field emerged three decades ago with the seminal work of Nadrian C. Seeman who devised that it is possible to generate nucleic acid sequences that form 3- and 4-arm junctions rather than linear double helix duplex [1, 2]. Since then, many other complex structures have been engineered from DNA; 2-D structures such as lattices, tubes and DNA origami as well as 3-D structures [3–7]. Compared to DNA, RNA can form non-canonical base-pairs and is less hydrolitically stable. However, it has a rich conformational and catalytic space and combined with its genetic encodability make it a powerful medium for engineering nucleic acids for biological applications [8, 9]. As with DNA, a wide variety of 2-D as well as 3-D structures have been composed out of RNA [9–12].

The goal of nucleic acid engineering is to design structures for real-life applications. Structures can be made functional by using them as scaffolds for precise spatial positioning

of other molecules. For example, metals can be positioned to serve as nano-wires or proteins can potentially be arranged for X-ray crystallography or to bring together enzymatic activities [13–16]. Three-dimensional structures such as molecular cages, can potentially be used in medical applications as a drug delivery vehicle where the cargo (drug) is released in a programmable way [17–20]. Self-assembled DNA nanoparticles functionalized with a targeting ligand and fused to siRNAs can be used for targeted drug delivery into tumor cells [21]. Furthermore, the fact that nucleic acids are used as the engineering material opens up the opportunity to interface with biological samples for controlling gene expression. For example, a biomolecular computer can be designed to analyze the levels of messenger RNA species and in response produces a molecule that affects the level of gene expression [22].

A majority of nucleic acid structures described in the literature are static structures formed by thermal annealing (a process in which the reactants are mixed together, heated and then gradually cooled until they reach the desired structure). The target structure is designed to have the lowest free energy compared to all other possible structures and is therefore thermodynamically favorable to form. Dynamically formed nucleic acids structures can also be designed utilizing an isothermal process of strand displacement [23]. In this process, two nucleic acid strands hybridize to each other while displacing a strand that was previously hybridized. The newly revealed sequence can also initiate the reveal of another sequence, resulting in a cascade. Strand displacement reactions are facilitated by single-stranded regions called 'toeholds' that mediate a branch migration. Reactions are driven forward by the gain of base-pairs in each step of the cascade and entropy gain from disassembly reactions. Strand displacement cascades have been used to create logic circuits [24, 25], walkers [26–29] and catalytic self-assembly [28].

In this field, our lab has introduced the concept of kinetically controlling strand displacement cascades based on hairpin motifs (a type of small conditional RNA or DNA (scRNA and scDNA, respectively)). The hairpin motif is composed of three features: a toehold, a double-stranded stem and a loop (Figure 1.1(a)). Strand displacement reac-

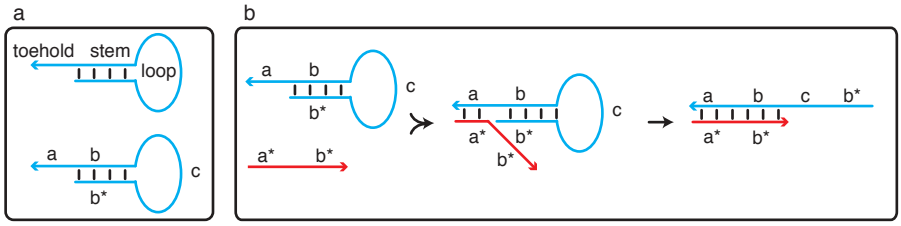

Figure 1.1: **Hairpin and toehold-mediated branch migration nomenclature and schematics.** 5′ and 3′ polarity are differentiated by an arrow representation of the 3′ end. Letters marked with * are complementary to the corresponding unmarked letter (e.g., 'a' is complementary to 'a*'). Base-pairs are represented by a black line. (a) Hairpin schematic. A hairpin consists of three domains: a single-stranded toehold, a double-stranded stem and an unpaired loop. Letters represent domains in the structure ('a' is a toehold, 'b' and 'b*' compose the stem and 'c' is the loop). (b) Toehold-mediated strand displacement schematic. The red strand (termed initiator) displaces the stem of the hairpin. Domain 'a*' of the initiator binds to toehold 'a' of the hairpin; next, branch migration occurs and domain 'b*' of the initiator displaces domain 'b*' of the hairpin. The binding of the initiator to the hairpin results in a new structure, initiator·hairpin, with an exposed new toehold 'c'. Either 'c' or both 'c -b*' can serve to initiate a downstream reaction mediated by another component (not shown).

tions cause the hairpin to change conformation and open up, resulting in a new initiator that instead of being released is still bound to the initial initiator (Figure 1.1(b)). Using hairpin-based cascades we have demonstrated autonomous locomotion [30], self-assembly of branched dendrimers and catalytic self-assembly [28] and formation of a long dsDNA or dsRNA polymer (hybridization chain reaction, HCR) [31, 32]. By functionalizing scRNAs with fluorophores, a fluorescent HCR polymer can be grown. This strategy was used for multiplexed detection of fluorescent in situ hybridization (FISH) with improved signal-to-background compared to traditional FISH [32].

The focus of the work presented here is to expand the repertoire of applications available from engineered nucleic acids using toehold-mediated strand displacement. Engineered systems have the capacity to sense molecular cues (e.g., mRNA) and to produce an output that leads to a change in the cell. Chapters 2, 3 and 4 present mechanisms with the aim

of conditionally down-regulating gene expression via RNA interference (RNAi) in response to detection of an input signal of an unrelated mRNA molecule. Chapter 2 presents a catalytic system based on scRNAs that generates an RNAi effector molecule in response to an mRNA target. In Chapter 3 we examine some possible explanations as to why conditional RNAi is not obtained in tissue culture. Chapter 4 presents a system based on scDNAs that conditionally transcribes an RNAi effector molecule. Finally, Chapter 5 expands upon the work presented by Choi et al. [32] to use fluorescent scRNAs as an output signal for a sensitive, multiplexed northern blot detection of mRNAs and miRNAs. The presented method has the potential to improve the study of transfected or expressed scRNAs described in Chapter 3.

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