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Leak-resistant Design of DNA Strand Displacement Systems*

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Abstract. Although a number of dynamically-controlled nanostructures and programmable DNA Strand Displacement (DSD) systems have been designed using DNA strand displacement, predictability and scalability of these DNA-based systems remain limited due to leakages introduced by spuriously triggered displacement events. We present a systematic design method for implementing leak-resistant DNA strand displacement systems in which each legitimate displacement event requires signal species to bind cooperatively at the two designated toehold binding sites in the protected fuel complexes, and thus inhibits spurious displacement events. To demonstrate the potential of the leak-resistant design approach for the construction of arbitrary complex digital circuits and systems with analog behaviors, we present domain-level designs and displacement pathways of the basic building blocks of the DNA strand displacement cascades, e.g. OR, AND gates, and an elementary bimolecular reaction.

Keywords: DNA Strand Displacement, Leakages, Leak-resistant, Leakless, Dynamic DNA Nanotechnology

1 Introduction

One of the goals of DNA nanotechnology is to rationally design robust DNA-based systems with programmable dynamic behaviors [26]. Toehold-mediated DNA Strand Displacement (TMSD) [23, 28] provides a versatile building block for designing dynamic DNA systems. Over two decades, a number of dynamic DNA systems, such as molecular motors [24, 1], walkers [15], DNA logic circuits [14, 11], and enzyme-free catalytic systems [27, 22] have been constructed. However, due to signal leakages that are mainly introduced by either defective sequences or spuriously triggered displacement events, the experimental performance degrades severely when these systems are constructed at larger scales [3].

Although the leakage caused by poorly optimized or defectively synthesized sequences can be minimized by improving the sequence design, reducing spuriously triggered leakages needs a systematic consideration of leakage sources at

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the design level. For example, a typical DNA strand displacement cascade comprises of partially double-stranded DNA complexes as fuels, having their one end as a designated *toehold* binding site and the other blunt end is ideally non-reactive. In practice, however, an eventual fraying [12] in the blunt ends of DNA complexes can trigger a TMSD, which may ultimately end up producing output signal even in the absence of input, and thus causing leakages [14, 11]. The other possible sources of leakages in DSD systems include: nicks, junctions [17], bulges and hairpins [4, 7].

There are a number of methods proposed to reduce the leakages introduced by spurious strand displacement events in the DSD systems. For example, a low concentration of reactants slows the kinetics of undesired strand displacement reactions between fuel complexes and mitigates leakages [11], but this also slows down the overall speed of the DSD system. Further, leak reduction methods have been proposed to inhibit fraying at the blunt ends of fuel complexes: 1) by designing stronger base-pair (C-G) bonds in the sequences that form blunt ends [27], 2) by adding a short sequence termed 'clamp' [22, 11] to protect the blunt end against fraying. Thachuk et al. [18] presents a systematic approach for designing leakless DSD systems by adding 'redundant' domains in the fuel complexes. The redundancy modifies the leak pathway by making it energetically less favorable due to an intermediate four-way branch migration [10] step. The method can potentially reduce leak to arbitrary low levels even at high concentration, as recently experimentally demonstrated in [19]. Kotani and Hughes [9] give another leak reduction approach based on multi-stranded fuel complexes in which the leakage pathway involves a four-way branch migration, however, a legitimate strand displacement is still a 3-way branch migration. Although multi-stranded approach reduces leakages without a significant decrease in the kinetics of the system, it can not be easily adopted for designing larger systems and DSD cascades due to lack of modularity.

We are particularly motivated by leakage resistant mechanisms in which leakage pathways are designed to become energetically less favorable, such as redundancy-based approach [19] and multi-stranded fuel complex design [9]. We present a systematic leakless design method using especially designed two-toehold multi-stranded DNA complexes. The central idea behind the design of multi-stranded DNA complexes is to keep them fully protected, except two single-stranded binding sites that act as toeholds. The design of DNA complexes enforces proximity of toeholds and raises their effective local concentration, triggering a cooperative binding event as *invader* signal engages with the toeholds. Following the cooperative binding event, a subsequent migration of respective branches ultimately releases the pre-assembled signal strand, which can then be used for downstream strand displacement process. There are two ways by which the proposed design inhibits DSD leakages: 1) the absence of blunt ends in the protected DNA complexes prohibits spurious displacement events caused by eventual fraying, and 2) a legitimate displacement event involves a "proof-reading" reinforced by a cooperative binding at the two toeholds and subsequent migration of branches.

In the following, Section 2 discusses the basic concepts and terminology used in this paper and introduces the problem of spuriously triggered leak in DSD cascades. Section 3 presents the design of two-toehold DNA complexes as fuels, a phenomenological description of cooperative two-toehold mediated strand displacement, and leakage modeling using an example of translator system design. Section 4 illustrates several examples of leak-resistant designs using domain-level representation and reaction pathways. Section 5 concludes with some general observations and further challenges.

2 DNA Strand Displacement Systems and Leakages

We start our discussion by briefly describing the terminology and conventions used in this paper. In the domain-level design of Figure 1 and other designs presented in the paper, each DNA strand is denoted by a line, where its 3' end is marked by a half-arrowhead. Each double-stranded DNA is represented by two anti-parallel lines. We use a small letter followed by subscripts to represent each domain, and its complementary domain is labeled by an asterisk, where subscripts are used to denote different domains within a strand. For example, x_t and x_b in Figure 1a represent toehold domain and branch migration domain [28] of the DNA strand 'x', respectively. The reversible and irreversible transitions between reactants and products species are shown by double-arrow and single-arrow lines, respectively.

A typical TMSD process, as illustrated by domain-level designs in Figure 1, has three main components: (1) toehold assembly/binding, (2) branch migration, and 3) driving forces for the displacement reaction. In the TMSD process, a single-stranded DNA domain termed *toehold* serves as a binding site within a pre-assembled partially double-stranded DNA complex, also referred as *fuel*. The *toehold* in the *fuel* co-localizes another single-stranded DNA molecule termed *invader* or *signal*, as shown in Figure 1a. Although a toehold in the fuel complex strengthens binding of the signal strand and provides a strong driving force for the TMSD process, a double-stranded DNA molecule with blunt ends can potentially initiate a strand displacement by an eventual *fraying* of a few terminal base-pairs [8], marked by dotted-line rectangles in Figure 1d and e . The frayed base-pairs create a nick in the blunt end of the double-stranded molecule, enabling a short toehold binding site for the signal to trigger a strand displacement. Such blunt-end triggered strand displacements form the source of leakages that are studied in this paper.

The second step following the toehold assembly is the branch migration. The toehold assembly facilitates a 3-way branch migration [28] process in the *fuel* molecule, releasing the previously attached single-stranded species, as illustrated in Figure 1b. Another class of branch migration, known as 4-way branch migration [10], occurs when two double-stranded DNA molecules, having mutually complementary strands, exchange their pre-assembled strands (Figure 1e). The TMSD process is driven by a decrease in the free-energy, which is derived from a net gain in enthalpy of toehold assembly /binding and/or configuration

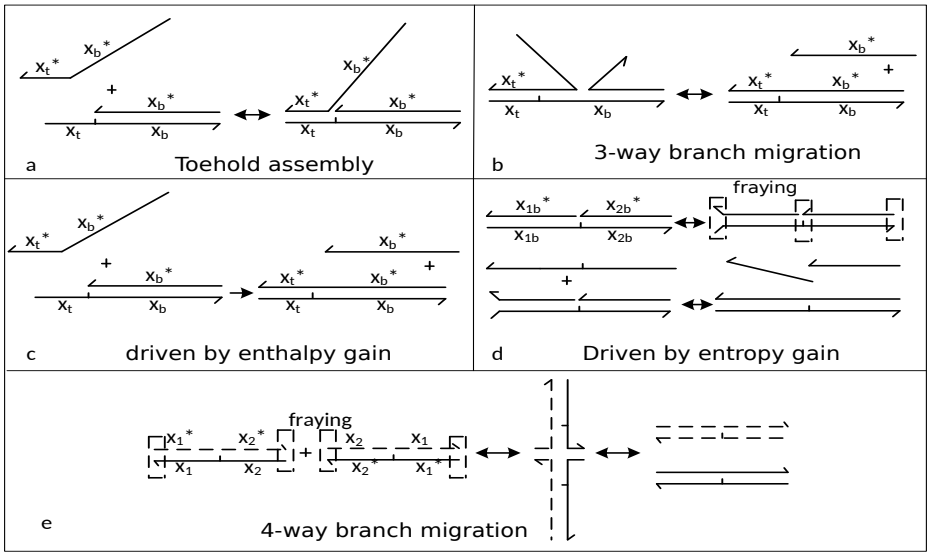

Fig. 1: The basic mechanisms in the TMSD toolbox. (a) Toehold assembly: the toehold domain x_t within the double-stranded complex serves as an active binding site for the complementary domain x_t^* within the invader signal strand $x_b^* x_t^*$. The bimolecular reaction of toehold assembly is driven by a net gain in enthalpy due to base-pairing of the two toeholds. (b) 3-way branch migration: following the toehold assembly, the domain x_b^* within the invader strand reconfigures the double-stranded DNA complex by dislodging its pre-assembled strand x_b^* via a competitive back-and-forth process within the transient DNA complex consisting of three DNA strands. (c) The displacement reaction is powered by a net gain in the enthalpy due to base-pairing of the toeholds. The configuration entropy does not change much, as the number of reactant and product species remains the same. (d) The multi-stranded DNA complex does not have a toehold for the binding of invader strand, but an eventual fraying on its blunt ends or at the nick creates a short toehold binding. In the absence of a toehold, the displacement reaction is powered by a net gain in the configuration entropy of the system. (e) 4-way branch migration: the two double-stranded DNA complexes with mutually complementary domains exchange the strands in a slow process, as there is no effective gain in either of enthalpy or entropy of the system.

entropy due to increase in the number of product species, as shown by Figure 1c and d.

The *toehold* plays a major role in the design of programmable DNA strand displacement systems. First, its length and sequence composition have a significant influence over the kinetic rate of strand displacement [28] – kinetic rate varies a million-fold over a toehold length six bases or less, and saturates for longer toeholds. Second, the toehold also serves as a recognition domain for

the input signal [28]. While the first feature provides a design mechanism for programmable kinetic control based on competing DNA strand displacement reactions [26], the second feature enables the design of DNA strand displacement cascades using DNA complexes with inactivated toeholds that are conditionally activated as the reaction proceeds sequentially [14]. In principle, any mechanism that sequesters the toehold domain and inhibits its hybridization can be used for the inactivation. For example, toeholds can be buried within double-stranded regions [14] or inside hairpin loops [4, 22] to make them inactive.

Here, we discuss the design principles of DNA strand displacement cascades using an example of signal translator design, as illustrated in Figure 2. There are three reactant species in the translator system: input signal x, and fuel complexes F_1 and F_2 (Figure 2a). Assuming that there are no spurious events that open up a set of potential toehold binding sites in the fuel complexes, the leakage causing reaction is inhibited in the absence of input signal x (Figure 2b). However, in the presence of input signal x, the translator DSD system produces an output signal y in a two-step DSD process, as illustrated by schematic diagram in Figure 2c. In the first step, the input strand x displaces an intermediate sequestered strand I_{xy} from the fuel F_1, which in turn displaces the output signal y from the fuel F_2 in the second step. The two complexes, W_1 and W_2, are also produced as nonreactive waste products.

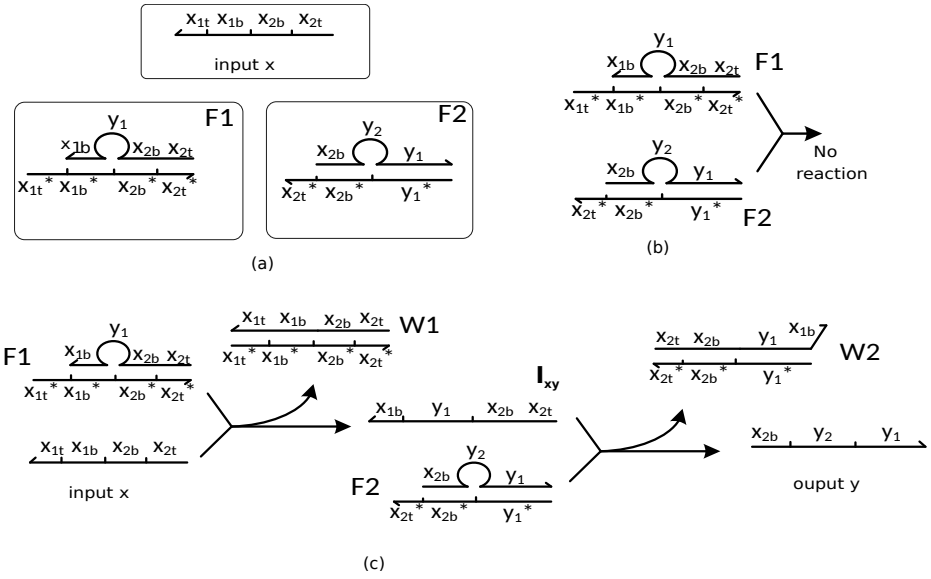

Fig. 2: Design of a signal translator using DSD cascade and toehold inactivation mechanism. (a) Initial reactant species in the translator DSD system: input signal x, and fuel complexes F_1 and F_2. (b) Translator reaction in the absence of input signal. (c) Translator reaction pathway in the presence of the input signal x.

The main design concept of our translator is adopted from [14], but the implementation approach has several distinctions. First, to comply with the conventions used in the leak-resistant design method discussed in Section 3, we use signal strands with four domains. Note that the domain lengths are critical here. For example, if x_{1b} is too short, it will spontaneously fluctuate between open and closed states, like a hairpin. The toehold domains are shorter than the branch migration domains. For example, x_{1t} is shorter than x_{1b}, and the same applies to the other combinations of toehold and branch migration domains in this design. Although one might need to play around with these domain lengths for experimental implementations, we can imagining that "t" domains are length 5nt while "b" domains are length 15nt. Second, reactive domains ($y_1 = y_{1b}y_{1t}$ and $y_2 = y_{2b}y_{2t}$ in 5' to 3' orientation) of intermediate signal species stay protected inside the bulge loops of fuel complexes. The toehold domain x_{2t} is inactivated by making it double-stranded inside the fuel F_1. Therefore, a direct strand displacement reaction between the two fuel molecules can not occur. However, in the presence of input x, the fuel F_1 co-localizes x and displaces its intermediate signal strand ($x_{2t}x_{2b}y_1x_{1b}$) in which the toehold x_{2t} is now activated. The intermediate signal with activated toehold further displaces the output y from the fuel F_2. The signal translator cascade also produces two unreactive waste products, W_1 and W_2.

3 Two-toehold DNA Strand Displacement

The centerpiece of the proposed leakless translator design is a four-stranded DNA molecular structure termed *Two-Toehold DNA Complex*, abbreviated as TTDC. The molecular structure of TTDC, as shown in Figure 3a, is derived from the Double Crossover (DX) DNA molecule [5]. Here we selected a DX structure of the type Double Crossover Antiparallel with Even spacing (DAE). The design of TTDC has several features, as shown in Figure 3a. First, the structure is fully protected, except the two toeholds (*Toehold1* and *Toehold2*), and therefore there are no blunt ends to initiate fraying events susceptible to leaks. Second, the toeholds are designed to be localized [1] so that they bind cooperatively with the toeholds of an incoming signal and initiate the branch migration process that ultimately displaces the strand for a downstream displacement event. A cartoonish view of the TTDC structure is shown in Figure 4a using cylinders to represent antiparallel helices and vertical dotted lines to show the two crossover junctions. The two crossover junctions would constrain the movement of two helices, and thus the two ends representing toeholds would stay in proximity. Third, the incumbent signal is wrapped around the two arms of the complex that makes its illegitimate displacement energetically less favorable. Fourth, the other two

[1] The antiparallel DX molecule provides a rigid structure [21], where its two helices are tightly held together (helical axes separated by ≈ 4.0 nm) by two crossovers. Note that, since we use only two ends of the helices to sequester the signal and create two toehold sticky ends, the second crossover is replaced by a half-crossover [20].

toehold domains in the incumbent signal (*Inactive Toehold1* and *Inactive Toehold2*), which are intended to participate in a downstream displacement process, are inactivated by burying them deep inside the double-stranded regions.

The second important component in a translator system is *signal* DNA strand that drives the DSD system by initiating a strand displacement process that passes through several downstream displacement stages mediated by intermediate signal species and releases the final output signal species. Therefore, the structure and composition of the *signal* strand should be carefully chosen so that an arbitrary large DSD system can be composed in a modular fashion, where the input trigger signal, intermediate signals and the output signal do not change significantly in composition. The most commonly used signal is composed of two domains: toehold domain and branch migration domain. The other types of signal structure include: (1) 3-domain [2], (2) 4-domain [16], and (3) multi-domain signals used in the redundant leakless design method [19]. Here we use a redundant 4-domain signal structure ($s_{12t}s_{12b}s_{11b}s_{11t}LLs_{21t}s_{21b}s_{22b}s_{22t}$), where the two arms of the signal strand are linked by a four bases long linker (LL), as shown in Figure 3b. The linker adds spacing between the two arms of the signal strand so that its toehold domains can successfully bind with the two arms of the DNA complex shown in Figure 3a.

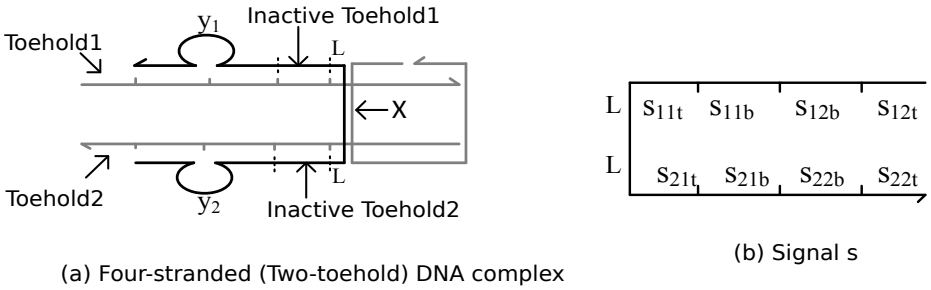

Fig. 3: Fuel and signal designs for two-toehold DNA strand displacement. (a) The design of four-stranded DNA complex used as a fuel molecule for the implementation leak-resistant DSD systems. (b) design of redundant four-domain signal strand *s*.

Having described the designs of two-toehold complex and signal species, we present a schematic diagram to phenomenologically explain the process of strand displacement involving the two-toehold complex, as shown in Figures 4b–d. The basic design idea is that the two short toeholds of signal strand cooperatively bind with their respective complementary toehold binding sites of a TTDC structure. The cooperative binding is reinforced by structural stiffness provided by one and a half crossovers of the TTDC molecule, which helps in holding its two toehold ends localised in a small volume. If a signal binds by only one of its toeholds, the toehold-mediated strand displacement is least likely to succeed as the

toehold binding is weaker due to short length of the toehold and the DNA strand is tightly held in the other arm of the TTDC structure. However, if both toeholds have matching toehold binding sites in the TTDC structure, they would swiftly bind cooperatively, and the effective toehold binding would be strong enough to ultimately succeed in displacing the associated DNA strand.

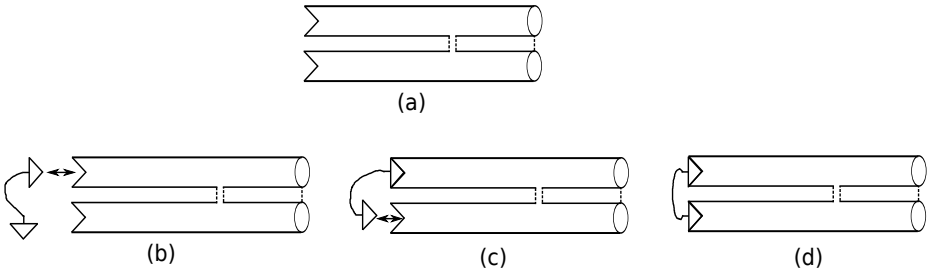

Fig. 4: A sketch to explain the cooperative binding phenomena involving two localised toeholds of the TTDC structure. (a) A schematic representation of the TTDC molecule using two cylinders as the two helices of the molecular structure. The cylinders are aligned and tightly held together by two junctions (dotted vertical lines) representing the full crossover in the mid and the half crossover on the right end. (b)–(d) illustrate the cooperative binding phenomenon using a key-lock representation.

3.1 Leak-resistant Signal Translator Design

We use the TTDC structure to design a leak-resistant translator ($x \rightarrow y$), where x and y are DNA strands, representing the translator's input and output, respectively. The translator system consists of three reactant species: input x and two fuel complexes (F_1 and F_2), as shown in Figure 5a.

In the following discussion, we propose two reaction pathways of the translator system: (1) the intended leakless pathway involving fuel species F_1 and F_2 in the presence of input x, and (2) a leaky pathway in the absence of input x. The intended pathway (see Figure 5b) of the translator has two displacement stages: (1) $x + F_1 \rightarrow W_1 + I_{xy}$; (2) $I_{xy} + F_2 \rightarrow W_2 + y$, where I_{xy} represents the intermediate signal released from the first stage, and W_1 and W_2 are the waste products. In the first stage, the two toeholds (x_{11t} and x_{21t}) of the input signal x cooperatively bind with their respective complement domains (x^*_{11t} and x^*_{21t}) in the fuel F_1. The subsequent domains ($x_{11b}x_{12b}x_{12t}$ and $x_{21b}x_{22b}x_{22t}$) in the two arms of the assembled signal x start migrating the respective branches ($x_{11b}y_{11}x_{12b}x_{12t}$ and $x_{21b}y_{21}x_{22b}x_{22t}$) of the intermediate signal that is sequestered with the fuel F_1. Note that the domains, y_{11} and y_{22}, respectively consist of $y_{11b}y_{11t}$ and $y_{22b}y_{22t}$ in 5' to 3' orientation. At the end of this branch migration process, the intermediate signal, which is now attached just by a few linker (LL) bases,

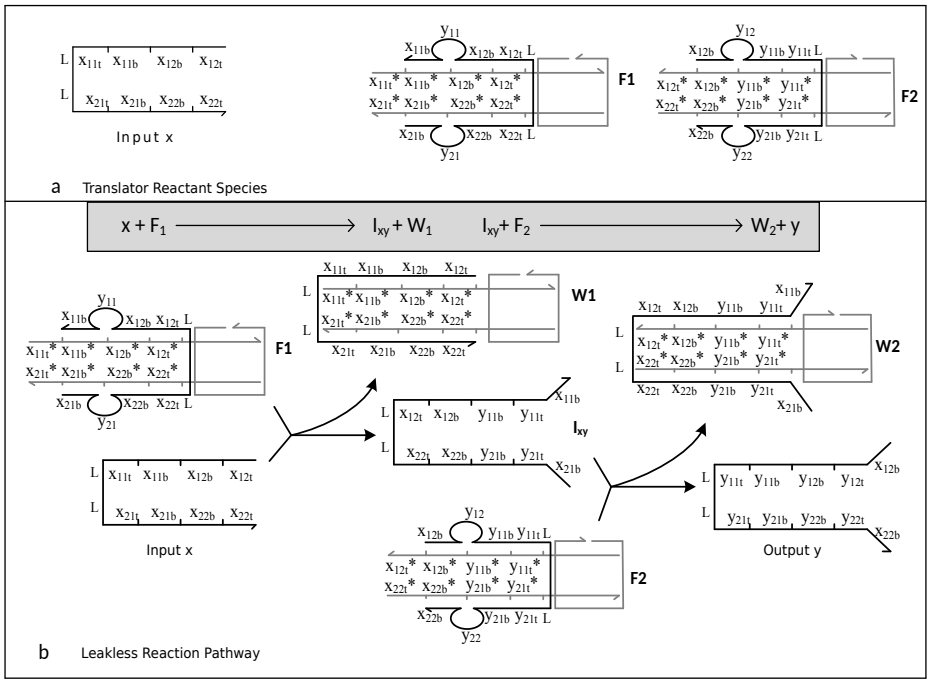

Fig. 5: The leak-resistant translator system. (a) The translator reactant species: input x, fuel F_1 and F_2. Intermediate signal I_{xy} and output signal y (black lines) are sequestered within fuel complexes F_1 and F_2, respectively. (b) The intended leakless translator reaction pathway. From left to right the two stages of the leakless reaction pathway are: $x + F_1 \rightarrow W_1 + I_{xy}$; $I_{xy} + F_2 \rightarrow W_2 + y$.

dissociates due to thermal instability and produces the intermediate signal I_{xy} with activated toeholds (x_{12t} and x_{22t}). In the presence of activated intermediate signal, the fuel F_2 similarly initiates the second displacement event that ultimately produces the output signal y and the waste product W_2.

The two fuel species F_1 and F_2 do not have any mutually complementary active binding domains that can initiate a displacement event in the absence of input signal x. Moreover, the fully protected fuel structures have no blunt ends susceptible to fraying that can eventually create an ad-hoc toehold binding site and initiate a leak-prone displacement event. Nonetheless, we hypothesize a possible leak pathway initiated by opening up of a few bases enclosing the bulge loops [2] in the sequestered signals of the fuel species.

The proposed leaky pathway of the translator in the absence of the input strand x is illustrated in Figure 6 using domain-level representations and reac-

[2] The stability of the base-pairs flanking a bulge loop within the DNA duplex depends on the types of flanking bases and other structural aspects [13]. The destabilizing effect can be mitigated by using stronger G-C pairs on each side of the bulge loop.

tions *a*–*i*. The first two reactions *a* ($F_1 \leftrightarrow FI_1$) and *b* ($F_1 \leftrightarrow FI_1$) represent a possible opening up of a few bases (labeled by dotted rectangles) enclosing the bulge loops in fuel F_1 and F_2, respectively. Although it would be sterically unfavorable for the two transient fuel species FI_1 and FI_2 to bind using the recently revealed bulge enclosing bases (see reaction *c*), there remain possibility of short toeholds binding between the complementary domains (reaction *d*: x_{12b} and x^*_{12b}, and reaction *e*: x_{22b} and x^*_{22b}), as the two molecules stack on one another. For clarity in the the combined transient state molecule $FI_1 : FI_2$, the domains within the FI_1 and FI_2 molecules are shown in thin and thick lines, respectively. These toehold binding events initiate 4-way branch migration reactions (see reaction *f*) involving the subsequent complementary domains from the sequestered signals ($x_{12b}x_{12t} \leftrightarrow x_{12b}$ and $x_{22b}x_{22t} \leftrightarrow x_{22b}$ following reactions *d* and *e*, respectively). Note that the output sequestered signal y is still completely bound within the transient molecular complex $FI_1 - FI_2$.

Although the bulge loop domains y_{11} and y_{21} of the intermediate sequestered signal I_{xy} within the molecular complex can potentially displace the domains $y_{11b}y_{11t}$ and $y_{21b}y_{21t}$ of the sequestered signal y, the bulge loops not open for binding, as the I_{xy} is still fully bound. To consider a further possibility of progress in the leak reaction towards the end, we introduce a hypothetical reactant species hs_1 with domains $x_{11t}x_{11b}$ and $x_{11t}x_{11b}$. This reactant could be considered as a possible motif that is part of the reaction system, for example within the reporter complex. Now the domains x_{11t} and x_{21t} of the hypothetical signal h_{s1} have their respective complementary binding domains active within the molecular complex to initiate a toehold-mediated strand displacement of the domains x_{11b} and x_{21b} of the intermediate sequestered signal I_{xy} (see reaction *g*). At this stage the bulge loops y_{11} and y_{21} become free, which can eventually displace the domains $y_{11b}y_{11t}$ and $y_{21b}y_{21t}$ of the signal y (see reaction *h*). Note that the reactive domains of the output signal y are now available, however it is still attached by domains x_{12b} and x_{22b} with the transient complex. A further hypothetical signal hs_2 can ultimately release the leaky output signal y in a subsequent displacement reaction *i*.

4 Leak-resistant Design Examples

In this Section we apply the leak-resistant design method to demonstrate its potential for designing more complex DSD systems. We present the leak-resistant designs and intended leakless pathways of the basic building blocks of the DSD systems, i.e. logic gates *OR*, *AND*, and a bimolecular elementary chemical reaction ($x + y \rightarrow z + w$).

4.1 Leak-resistant Design of *OR* Gate

We implement a DSD system for the *OR* logic gate (x *OR* $y \rightarrow z$) using the leak-resistant design method, where x and y are the input signal strands and z is the output signal, as shown in Figure 7.

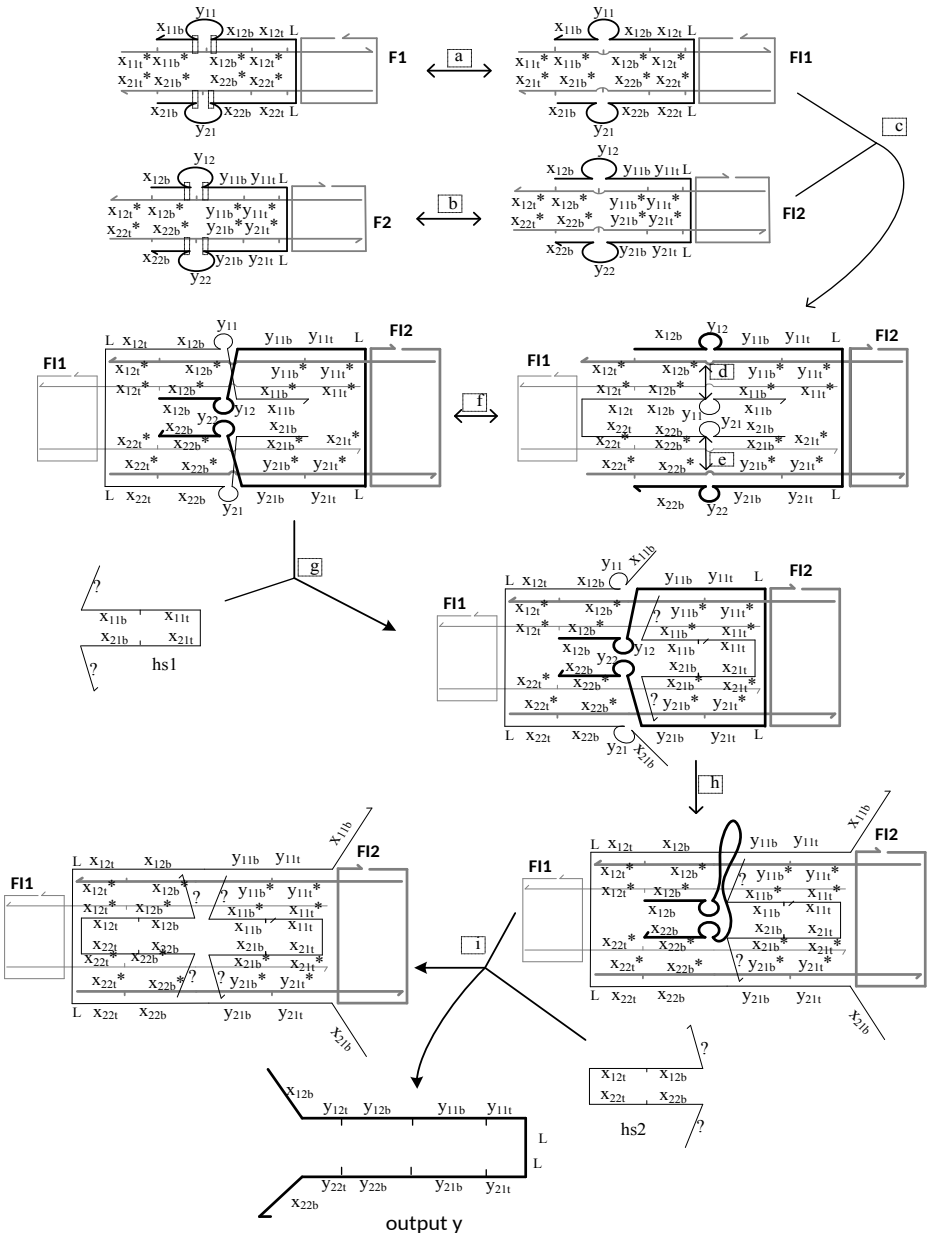

Fig. 6: A hypothesized leak pathway of the leak-resistant translator ($x \to y$) in the absence of the input x. The pathway is represented by reactions a–i, where reactions g–i occur in the presence of hypothetical signal species hs_1 and hs_2.

The two-input OR gate can be implemented by cascading three signal translators such that two parallel translators ($x \to w$ and $y \to w$) drive the third

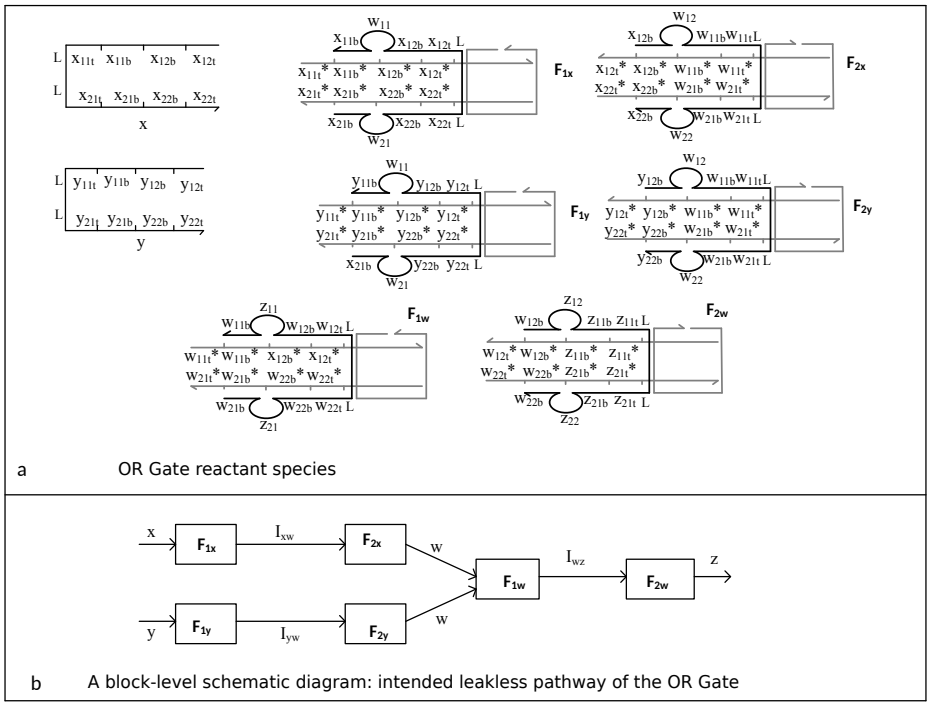

Fig. 7: The leak-resistant implementation the *OR* gate ($xORy \rightarrow z$). (a) Reactant species of the system: two input signals (x, y), and six fuel complexes (F_{1x},F_{2x}, F_{1y}, F_{2y}, F_{1w}, F_{2w}) with intermediate signals sequestered within them (black lines). (b) A block diagram of the intended leakless pathway of the *OR* gate DSD system has three connected translator modules: (1) $x \rightarrow w$ (2) $y \rightarrow w$, and (3) $w \rightarrow z$.

translator ($w \rightarrow z$) through a common intermediate signal w, as shown in Figure 7b. Therefore, we need six different fuel complexes with sequestered signals (F_{1x}, F_{2x}, F_{1y}, F_{2y}, F_{1w}, F_{2w}), as shown in Figure 7a. The *OR* gate implementation has four layers of strand displacement. First, input signals x and y displace the sequestered signals I_{xw} and I_{yw} from the fuels F_{1x} and F_{1y}, respectively. Second, both the displaced signals I_{xw} and I_{yw} further displace the intermediate signal w from the fuels F_{2x} and F_{2y}, respectively. Third, the signal w from either of the two preceding translators can displace the intermediate signal I_{wz} from the fuel F_{1w} in the third layer. Fourth, the intermediate signal I_{wz} finally displaces the signal z from the fuel F_{2w}. The modular implementation of two-input *OR* gate can easily be extended to construct an arbitrary circuit of *OR* gates. For example, any all-OR circuit of depth n can be implemented using $6 \times 2^{n-1} + 2^n - 2$ different fuel complexes.

4.2 Leak-resistant Design of *AND* Gate

Since *OR* gate operates in the presence of at least one of its input signals, it can easily be implemented using two translators connected in parallel to a third translator. The *AND* gate, however, needs both its inputs to be present simultaneously to operate. A typical scheme for the implementation of *AND* gate would be a cooperative binding [25] of the two inputs to displace the pre-assembled output signal from the fuel complex. However, the cooperative displacement is a trimolecular reaction, therefore the effective kinetics of the *AND* gate slows down severely in the low-concentration regime. Another mechanism of AND gate implementation that uses bimolecular reactions is based on a sequential rather than simultaneous presence of the the inputs [6].

Here, we present the leak-resistant implementation of a two-input *AND* gate (x *AND* $y \rightarrow z$) using the two inputs to sequentially execute displacement steps, as shown in Figure 8. We redesign the fuel complex structure to implement an integrator fuel (*IF*) structure, as shown in Figure 8a.
An additional signal is sequestered within the IF structure that is displaced by one of the input signals (x in this case), which in turn activates the toeholds (Δy_{11t} and Δy_{21t}) for the second concomitant displace by signal y. The intermediate signal, which is displaced from the *IF* complex, in turn displaces the output signal z from the fuel complex (F).

The intended leakless pathway of the designed AND gate is illustrated in Figure 8b using reactions *a*–*d*. First, the input signal x displaces the sequestered signal at the front of the *IF* complex, but it still remains attached with the complex by two toehold domains Δy_{11t} and Δy_{21t}, which eventually dissociates due to thermal instability and activates short toehold domains (see reactions a and b). Second, the activated short toehold domains withing the IF complex provide complementary toehold binding sites for the signal y, which initiates a concomitant displacement (reaction c). Finally, the displaced intermediate signal from the second displacement engages with the fuel F for the third displacement, producing the the output signal z. The pathway also produces three waste products, W_1, W_2 and W_3.

4.3 Leak-resistant Design of Bimolecular Reaction

Although increasingly larger circuits of logic gates have been implemented using DSD cascades, analog nature of the DSD reactions also opens up possibilities for designing DSD cascades for a variety of analog behaviors represented by chemical reaction networks. In general, an arbitrary chemical reaction network can be implemented using an DSD cascade of approximately equivalent behavior [16].

Here, we present the leak-resistant implementation of a elementary bimolecular reaction scheme ($x + y \rightarrow u + v$) that can easily be adopted for the implementation of a variety of other reactions, such as catalysis, amplification and oscillation. We use the previously discussed translator building blocks to design the fuel complexes for the implementation, as shown in Figure 9. The reactant species include (see Figure 9a): two input signals (x and y), an integrator type

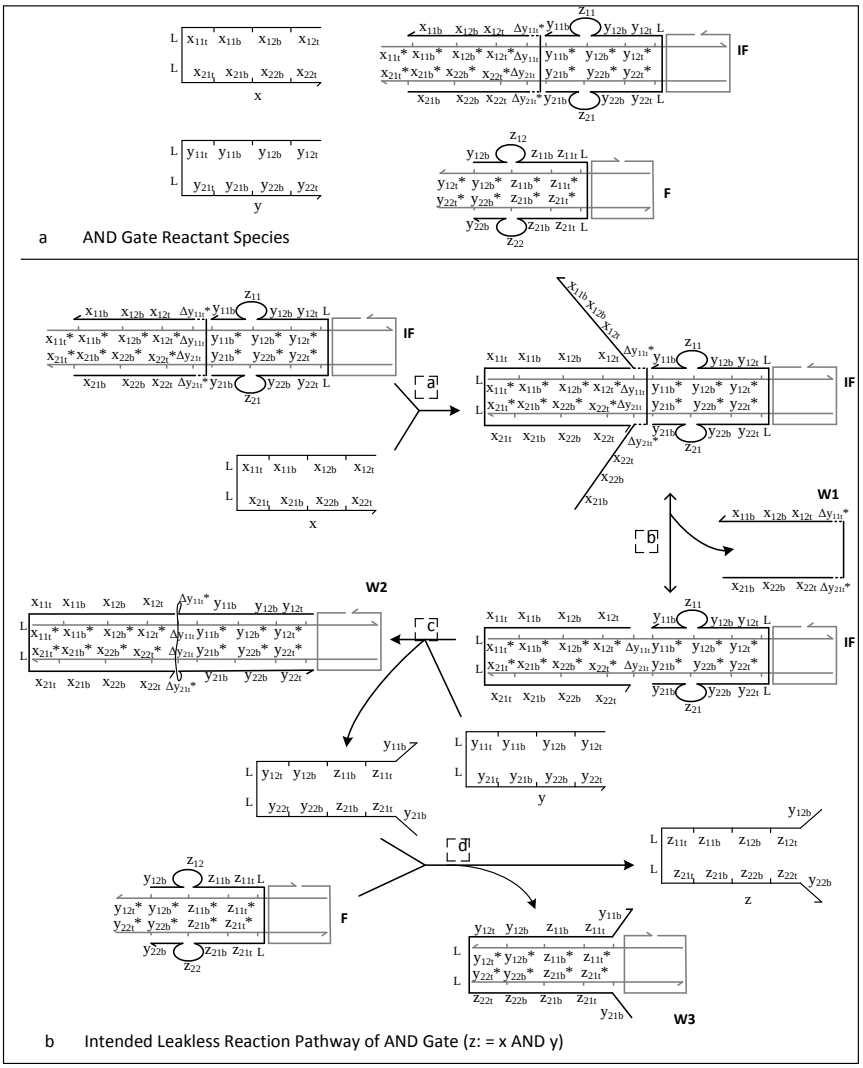

Fig. 8: The leak-resistant design of *AND* gate (x *AND* $y \rightarrow z$). (a) Reactant Species: input signals (x and y), and fuel complexes (F and IF). The integrator fuel complex (IF). (b) Intended leakless reaction pathway (reactions a–d).

fuel complex (IF_{xy}), and five different fuel complexes (F_z, F_{u1}, F_{u2}, F_{v1}, F_{v2}). The intended pathway of the DSD system, as shown in Figure 9b, can be represented by a four layer displacement system in which an AND gate translator (x *AND* $y \rightarrow v$) drives the two signal translators in parallel ($z \rightarrow u$).

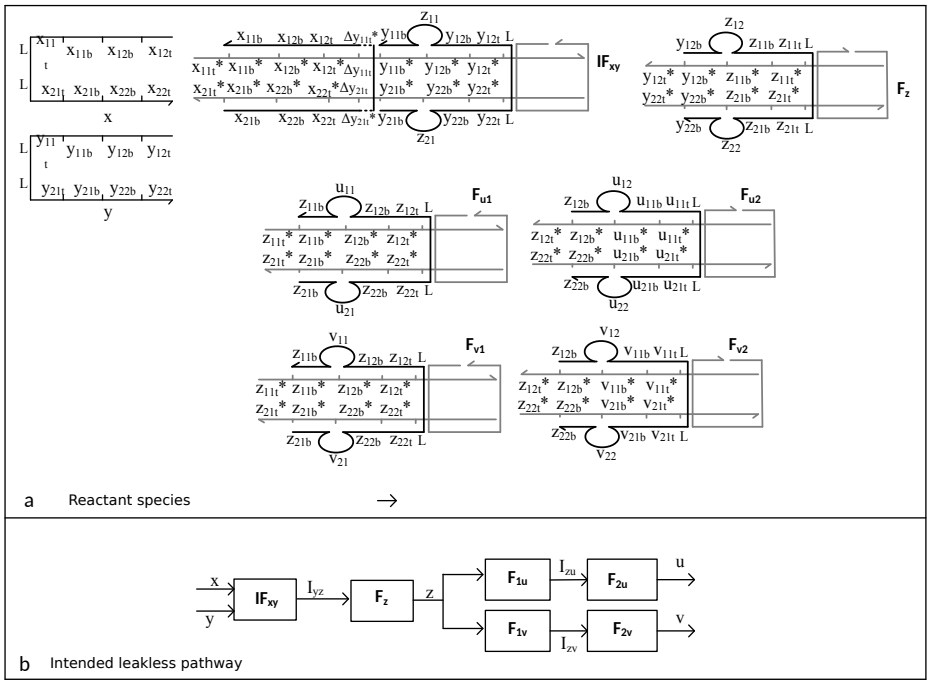

Fig. 9: The leak-resistant implementation of bimolecular reaction ($x+y \rightarrow u+v$). (a) reactant species: input signal strands (x and y), and the fuel complexes (F_z, F_{u1}, F_{u2}, F_{v1}, F_{v2}). (b) a modular representation of the proposed leakless pathway.

5 Conclusions and Future Work

In the context of DNA strand displacement systems, we discussed the problem of spuriously triggered leak, its sources, and presented a leak-resistant design method that can be applied for designing complex, modular systems. The proposed method and its potential for generalization are discussed theoretically using domain-level designs and DNA strand displacement reaction pathways. The design and analysis still needs to be extended to include reaction kinetics for a quantitative evaluation of leak resistance, but this involves several considerations, e.g. (2) enumeration of reactions, and (2) derivation of realistic kinetics for the cooperative toehold binding and branch migration reactions involving multi-stranded DNA complex, that we are currently investigating.

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