Decreasing Leaks of DNA Strand Displacement Circuits

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Leaks of Nucleic Acid Circuits

Nucleic acid circuits are prone to leaks, which result from unintended displacement interactions between nucleic acid strands. Such leaks can grow combinatorially with circuit size, are challenging to mitigate, and can significantly compromise circuit behavior.

<table>
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<tr>
<th>approach</th>
<th>advantages</th>
<th>challenges</th>
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<tr>
<td>G–C bonds at ends of complexes</td>
<td>Effective leak reduction by reducing fraying; Maintains circuit design at the domain level.</td>
<td>Used in specific contexts, may not eliminate all leaks.</td>
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<tr>
<td>Base pair mismatches</td>
<td>Maintains circuit design at the domain level; Substantial leak reduction with modestly decreasing rates in catalytic hairpin assembly; Identification of target areas for leak reduction and quantification of secondary structure effects, allowing a 4-fold leak reduction.</td>
<td>May slow down circuit behavior; Requires careful tuning of circuit; Used in specific contexts.</td>
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<td>Clamps</td>
<td>Successfully implemented in complex DSD circuits; Simple experimental implementation.</td>
<td>Used in specific contexts, may not eliminate all leaks; May require modification of circuit design.</td>
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<td>Molecular cascades with additional long domains</td>
<td>Additional domains can be introduced to further reduce leaks; Capable of reducing leaks to arbitrarily low levels at the cost of increased complexity.</td>
<td>Validated for translator circuits, further work required for AND circuits with two inputs.</td>
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<td>Interdomain bridging</td>
<td>Helps to eliminate toehold-independent leakages while enhancing strand displacement kinetics across a three-way junction.</td>
<td>Used in specific contexts, may not eliminate all leaks.</td>
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<td>Multicistron junctions with four-way branch migration</td>
<td>Increases leakage energy barrier through four-way branch migration; Potential to design complex, robust, and efficient molecular systems.</td>
<td>Limitations for implementing some circuit types such as AND gates.</td>
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<td>Shadow cancellation</td>
<td>Does not require modification of existing circuit; Agnostic to leak mechanism; Works in conjunction with other leak mitigation strategies.</td>
<td>Requires a shadow copy of the circuit and additional cancellation complexes; Requires a sufficiently good match between the primary and shadow circuits; Slows down circuit behavior.</td>
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<td>Thresholds</td>
<td>Mitigates the effects of initial leakage; Works well for digital logic circuits.</td>
<td>Limited application to continuous leakage in dynamic circuits; Requires sufficiently strong binding to threshold, may not eliminate all leaks.</td>
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<td>Spatial localization</td>
<td>Uses spatial separation to reduce interference; Allows for faster circuit dynamics and reduced leakage between separated components.</td>
<td>Higher experimental complexity required to achieve localization; Barriers to communication between locations may affect circuit behavior.</td>
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<td>Enzymatic DNA synthesis</td>
<td>Reduces leakage due to malformed complexes caused by synthesis errors. Enzymatically prepared DNA hairpins exhibit substantially reduced leakage compared to chemically synthesized hairpins.</td>
<td>Specific to certain architectures amenable to enzymatic synthesis; Increased complexity of experimental preparation.</td>
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<tr>
<td>Hybrid DNA/Locked Nucleic Acid (LNA) systems</td>
<td>High leakage rate reduction (over 50-fold) while maintaining high circuit performance; Maintains original circuit architecture.</td>
<td>Requires synthesis of hybrid oligonucleotides; Requires care when incorporating LNA due to their strong binding affinity.</td>
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Table 2. Overview of Existing Approaches for Mitigating Leaks, Together with Some Advantages and Challenges

Automated Leak Analysis of Nucleic Acid Circuits (Zarubileva & Philips, ACS Synth. Biol. 2022)
Techniques & Software for Automated Leak Analysis of Nucleic Acid Circuits

- Microsoft’s Visual DSD language was developed for the design and analysis of nucleic acid circuits.

- Developed additional techniques & software for the automated leak analysis of nucleic acid circuits, referred to as DSD Leaks.

- Extended the logic programming functionality of the Visual DSD language, with:
  - predicates for leak generation,
  - a leak reaction enumeration algorithm, and
  - predicates to exclude low probability leak reactions.

- Used Software to identify the critical leak reactions affecting the performance of control circuits, and to analyze leak mitigation strategies by automatically generating leak reactions.

- Designed new control circuits with substantially reduced leakage including a sophisticated proportional-integral controller circuit, which can in turn serve as building blocks for future circuits.
Microsoft Visual DSD
Software for Designing and Simulating a DNA Strand Displacement Reaction Networks

Microsoft Visual DSD:
https://dsd.azurewebsites.net/

Visual DSD User Manual:

Read pp.4 – 14 from User Manual.
Then try out examples: (1) DSD/Code/Join (2) Join–Inference (3) Join–CTMC

Video link: Microsoft Research's DNA Strand Displacement tool (DSD)
https://www.youtube.com/watch?v=_cEklwMYypo
Improved Microsoft Visual DSD
To model Leak Reactions
in DNA Strand Displacement Reaction Networks
Improved Microsoft Visual DSD To model Leak Reactions: Example Catalysis Circuit: A signal $X$ to catalyzes the production of a signal $Y$, where built-in predicates of the Logic DSD language are highlighted in blue.

(A) Visual DSD code for the species of the Catalysis circuit (left column) and their corresponding graphical representations (right column). The full Visual DSD program is shown.

(B) Chemical Reaction Network (CRN) generated from the species in (A) according to the logic predicates defined in (C–G).

(C) Logic predicates for automated CRN generation, defined using a special reaction predicate, which takes as arguments a list of reactant species, a reaction rate, and a product process.

- A reaction is generated by merging a single elementary slow reaction with a maximal sequence of elementary fast reactions, defined using the merge predicate, which also ensures that the same process is not visited more than once.
- The slow and fast predicates define elementary slow and fast reactions, respectively.
- Separate reaction rules are defined for unimolecular and bimolecular reactions.

(D) The bind predicate states that two processes $P_1$ and $P_2$ can bind if they contain complementary domains $D$ and $D'$. The product is a process $Q$ in which a fresh bond $i$ is shared between the two domains.

(E) The displace predicate states that an unbound domain $D$ can displace a bound domain $D_{li}$ in the 5’ to 3’ direction if the unbound domain directly overhangs the bound domain. A symmetric displaced predicate defines strand displacement in the 3’ to 5’ direction.

(F) A symmetric unbind predicate states that complementary bound domains $D$ and $D'$ can unbind if $D$ is a toehold and if there are no adjacent bound domains on either side of the toehold, as defined by the adjacent predicate.
**DSD Leaks methodology, illustrated using the Catalysis circuit:** A signal $X$ to catalyzes the production of a signal $Y$, where built-in predicates of the Logic DSD language are highlighted in blue.

(A) **Reactions of the Catalysis circuit**, which are the same as previously given, but with a slower binding rate $c$ for reaction (1).

(B) **The leak predicate** states that an unbound domain from one species can displace a bound domain from another species, provided the bound domain does not itself contain adjacent bound domains on both sides, as defined by the clamped predicate.

(C) When applied to the Catalysis circuit, this allows the fuel strand to displace the output in the absence of the catalyst.

(D) **The migrate predicate** states that two opposing pairs of bound domains can swap complementary domains if they are held next to each other. When combined with the leak predicate and applied to the Catalysis circuit, this allows two fuel complexes to leak, resulting in the displacement of the output in the absence of the catalyst.

(E) The number of leak reactions can be reduced by considering only first order leaks, defined as leaks in which both species are generated from nonleak reactions only, as defined by the nonLeaked predicate. A limit on the size of polymers can also be enforced, by requiring that the combined number $N$ of strands in the reactants is less than a given threshold, where tolist and length are built-in predicates that, respectively, turn a complex into a list of strands and compute the length of a list.

(F) To improve the efficiency of leak enumeration we encoded a generalized method for merging fast reactions.

(G) **This method using the Catalysis circuit.** A slow elementary bind reaction is followed by fast elementary displace and unbinding reactions, represented as a reactions graph enclosed in a dashed outline. Strongly connected components (SCCs) of fast reactions are enclosed in solid boxes, with the terminal SCC shown in red. (I) The merging results in a single reaction whose products are given by the terminal SCC.

(H) **Simulation results of the Catalysis circuit with four different leak hypotheses:** full leaks (FL), first order leaks (FoL), no leaks (NL) and classic leaks (CL).

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**Automated Leak Analysis of Nucleic Acid Circuits (Zarubiieva&Philips, ACS Synth. Biol. 2022)**
Improving the Performance of DNA Strand Displacement Circuits by Shadow Cancellation

Song & Reif, ACSNano2018

DNA strand displacement circuits are powerful tools that can be rationally engineered to implement molecular computing tasks because they are programmable, cheap, robust, and predictable.

- A key feature of these circuits is the use of catalytic gates to amplify signal.

Catalytic gates tend to leak:

- that is, they generate output signal even in the absence of intended input.
- Leaks are harmful to the performance and correct operation of DNA strand displacement circuits.

We present “shadow cancellation”, a general-purpose technique to mitigate leak in catalytic DNA strand displacement circuits.

- Shadow cancellation involves constructing a parallel shadow circuit that mimics the primary circuit and has the same leak characteristics.
- It is situated in the same test tube as the primary circuit and produces “anti-background” DNA strands that cancel “background” DNA strands produced by leak.

We demonstrate the feasibility and strength of the shadow leak cancellation approach through a challenging test case, a cross-catalytic feedback DNA amplifier circuit that leaks prodigiously.

- Shadow cancellation dramatically reduced the leak of this circuit and improved the signal-to-background difference by several fold. Unlike existing techniques, it makes no modifications to the underlying amplifier circuit and is agnostic to its leak mechanism.
- Shadow cancellation also showed good robustness to concentration errors in multiple scenarios.
- This work introduces a direction in leak reduction techniques for DNA strand displacement amplifier circuits and can potentially be extended to other molecular amplifiers.
Strategy of shadow cancellation:

• The shadow circuit mimics the primary circuit and has the same leak profile.
• The two leaks cancel each other by designated reactions to stop the leak from propagating in the primary circuit.
A cross-catalytic seesaw amplifier:

consists of:

• two DNA seesaw gate complexes, GA1 and GA2, and
• two DNA fuel strands, FA1 and FA2.
• Strands OA1 and OA2 are the outputs of gates GA1 and GA2, respectively.
• The output of a gate feeds into the other gate and acts as a catalyst.
• Reporter complex RC is used to monitor the performance of the amplifier and is triggered by one of the output strands, OA1.

The shadow circuit: has an identical architecture to the primary amplifier and consists of two DNA seesaw gate complexes, GB1 and GB2, and two DNA fuel strands, FB1 and FB2. Middle: The DNA complexes C1 and C2 are cooperative hybridization gates that implement the leak cancellation reactions. C1 sequesters strands OA1 and OB1, and C2 sequesters strands OA2 and OB2.
DNA strand displacement reactions of the cross-catalytic amplifier:

(a) The intended reactions:
- DNA reactions 1 and 2 implement reaction 1a.
- DNA reactions 3 and 4 implement reaction 1b.

The reactants of a reaction are indicated by triangular solid arrows if the reaction is reversible, and the products of a reaction are indicated by regular plain arrows.
DNA strand displacement reactions of the cross-catalytic amplifier:
(a) The intended reactions:
- DNA reactions 1 and 2 implement reaction 1a.
- DNA reactions 3 and 4 implement reaction 1b.
(b) The leak reactions:
- DNA reaction 5 implements reaction 1c.
- DNA reaction 6 implements reaction 1d.
DNA strand displacement reactions of the cross-catalytic amplifier:

(c) The reactions between OA1 and RC to generate fluorescence:

The red triangle represents fluorophore (6-FAM) and the black circle represents quencher (Iowa Black).
After the reactions, the fluorophore leaves the quencher and emits fluorescence.
Improving the Performance of DNA Strand Displacement Circuits by Shadow Cancellation (SongACSNano2018)

Performance of shadow cancellation:
- Amplifier was tested:
  - alone,
  - with cancellation complexes, and
  - with shadow cancellation,
  and the SBDavs were 0.076, 0.084, and 0.631, respectively.

In each subfigure, the "signal" curve represents the case where 5 nM of target strand OA2 was added, and the "leak" curve represents the case where no OA2 was added. Each experiment was repeated three times.

Improving the Performance of DNA Strand Displacement Circuits by Shadow Cancellation (SongACSNano2018)
Utility of shadow cancellation with shadow circuit with various concentrations

- Utility of shadow cancellation with shadow circuit that was 10% lower, the same, and 10% higher in concentration than the amplifier.

- When the shadow circuit had a lower concentration than the amplifier, the $SBD_{av}$ was smaller than when the two circuits were balanced.

- When the shadow circuit had a higher concentration than the amplifier, the $SBD_{av}$ was comparable to when the two circuits were balanced.

- For $\alpha$% shadow, $[GB1] = [GB2] = 100 \times \alpha$% nM; $[FB1] = [FB2] = 200 \times \alpha$% nM.

- The cancellation complexes are at 75 nM in all three scenarios.