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Technical Note

Robust Heterochiral Strand Displacement Using Leakless Translators

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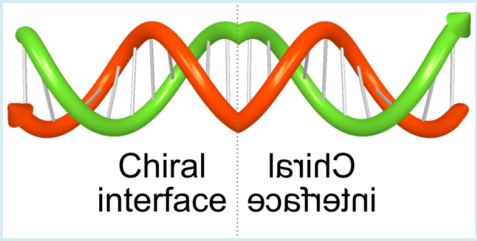
ABSTRACT: Molecular computing offers a powerful framework for *in situ* biosensing and signal processing at the nanoscale. However, for *in vivo* applications, the use of conventional DNA components can lead to false positive signals being generated due to degradation of circuit components by nuclease enzymes. Here, we use hybrid chiral molecules, consisting of both L- and D-nucleic acid domains, to implement leakless signal translators that enable D-nucleic acid signals to be detected by hybridization and then translated into a robust L-DNA signal for further analysis. We show that our system is robust to false positive signals even if the D-DNA components are degraded by nucleases, thanks to circuit-level robustness. This work thus broadens the scope and applicability of DNA-based molecular computers for practical, *in vivo* applications.



KEYWORDS: *chirality, DNA strand displacement, biosensing, leak, molecular computing*

DNA strand displacement is a powerful chemical framework for implementing enzyme-free molecular circuits.[1] Previous work has highlighted its potential to sense biomarkers in cells and make diagnostic or therapeutic decisions *in vivo*.[2,3] However, a key issue preventing the application of these systems in cells is the degradation of the circuit components by nucleases.[4] Our solution is to use L-nucleic acids, the chiral mirror images of naturally occurring D-nucleic acids, as alternative materials for constructing nucleic acid circuits. L-nucleic acids are not recognized by naturally occurring nuclease enzymes, which have evolved to target D-nucleic acids only,[5] and therefore should resist degradation in cells. However, detecting D-nucleic acids using L-nucleic acids is challenging because the two helices have opposing twists and thus the usual Watson−Crick rules of base-pairing no longer apply.[6]

One possible approach is to raise L-DNA aptamers against D-nucleic acid targets on a case-by-case basis.[7] A more general approach is to use hybrid L-DNA/D-DNA systems, in which the D-DNA component is used for sensing and the sensed signal is translated, *e.g.*, *via* toehold-mediated strand displacement (TMSD), into an L-DNA signal, which could be processed further by a robust L-DNA backend circuit. That circuit could draw on the wide variety of existing TMSD architectures, including logic circuits[8] and artificial neural networks.[9] Previous work has shown that translation between chiralities can be achieved using PNA as an achiral intermediary[10] or by using chimeric L-DNA/D-DNA strands to construct the components.[11] However, these approaches are prone to undesired signal release if the D-DNA components of the circuit are degraded, necessitating further chemical modifications to harden the D-DNA component.[11]

Here, we report an alternative design for chimeric L-DNA/D-DNA signal translators with enhanced robustness. We use a heterochiral variant of previously reported "leakless" strand displacement gates,[12] whose structure means they are intrinsically more robust to nonspecific generation of output signal ("leak") in the absence of inputs: even if the D-DNA components of our circuit are degraded, the output signal remains low. Our system is thus more robust in the sense that it will "fail safe" when the D-DNA components are degraded. We also demonstrate, for the first time, that a TMSD reaction will actually proceed across the interface between the L-DNA and D-DNA duplexes in a hybrid chimeric molecule (Figure 1a). Thus, our work not only demonstrates a new design paradigm in heterochiral molecular computing but also offers enhanced robustness guarantees that make our circuits more applicable for practical biomedical applications.

RESULTS AND DISCUSSION

Our goal was to develop a heterochiral signal translation system that is robust even to degradation of the D-DNA domains by nucleases. We developed a variant of a previously reported leakless translator system,[12] in which the input signal (X) is an ssDNA of one chirality but the output is an ssDNA of the opposite chirality, as shown in Figure 1b. The leakless translator is a two-step design consisting of two TMSD gates

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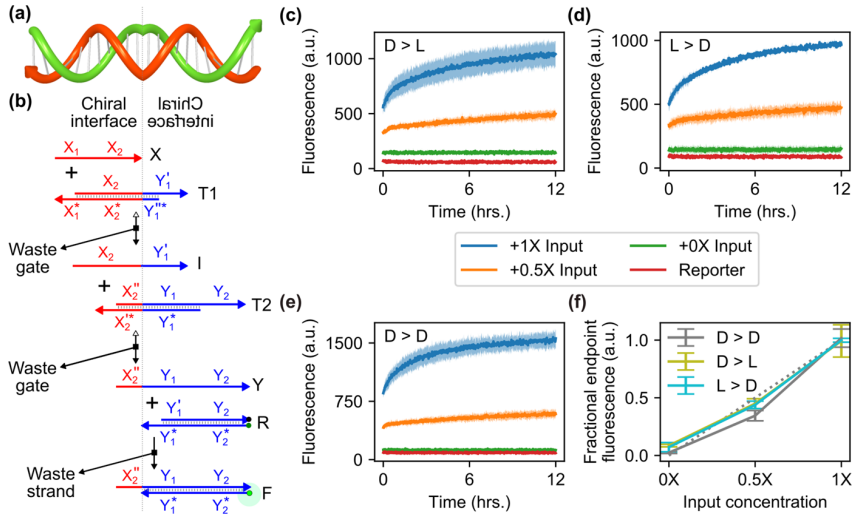

Figure 1. Leakless heterochiral translator architecture and operation. (a) Cartoon of a hybrid chiral duplex. (b) The leakless translator architecture involves a two-step translation process in which an input oligonucleotide (X) is converted to an output oligonucleotide (Y) of the opposite chirality, either D-DNA to L-DNA or vice versa. The translation is mediated by translator complexes (T1 and T2), and the output is sensed by a strand displacement reporter probe (R) of the same chirality as Y. Domain lengths: X_1, 5 nt; X_2, 25 nt; X_2', 15 nt; X_2'', 10 nt; Y_1, 20 nt; Y_1', 15 nt; Y_1'', 5 nt; Y_2, 27 nt. (c–e) Fluorescence time courses of signal translation in heterochiral translators from (c) D-DNA input to L-DNA output (D > L) and (d) from L-DNA input to D-DNA output (L > D). (e) A pure D-DNA translator (D > D) is included as a control. Reporter and gates are at concentrations of 300 nM, and 1× input (300 nM) is supplied. The plot shows mean and standard deviation of three replicates. (f) Fractional end point fluorescence of kinetic assay for 0×, 0.5×, and 1× signal demonstrating the linearity of signal translation relative to the maximum signal in each case. The plot shows the mean and standard deviation of three replicates. The dotted line indicates a 1:1 input/output ratio. Reporter and gates are at concentrations of 300 nM which are also the 1× input concentration.

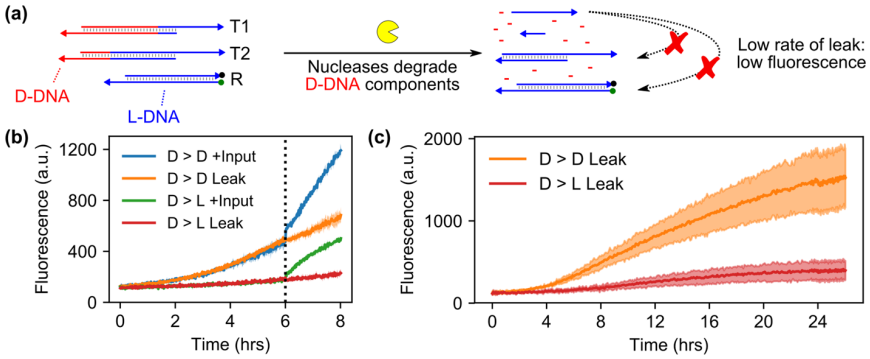

Figure 2. Robustness of leakless heterochiral translators in serum. (a) Cartoon of the degradation of D > L leakless heterochiral translator in serum. The translator design means that, even if the upstream D-DNA domains are fully degraded, the only reaction that can proceed is a blunt end strand displacement reaction which has a low rate constant; therefore, a low rate of leak should be observed. (b) Incubation of D > D and D > L leakless translators in 10% serum for 6 h, at which point circuits are triggered with 10× input. Even without any protection of the D-DNA domains *via* chemical modifications, a far lower leak is observed in the D > L circuit than the control D > D system. Plot shows mean and standard deviation of three replicates. (c) Demonstration of longer-term stability of leakless D > L translator in 10% serum over 26 h. Even after this long incubation, a minimal leak is observed. Plot shows mean and standard deviation of three replicates. If triggered with input, circuit response in the D > L circuit is low because the D-DNA domains that sense the input have likely been degraded (data not shown). Standard concentrations of 300 nM were used throughout.

(T1 and T2), in which the first gate (T1) translates the input strand to an intermediate (I) whose displacement domain is partly of one chirality and partly of the opposite chirality. The translation process is completed by the second gate (T2). The resulting output signal, (Y), whose displacement domain is of the opposite chirality to that of X, is sensed by a FRET-based TMSD probe (R).

This design requires that one of the TMSD reactions proceeds across the interface between the D-DNA and L-DNA duplexes in the T2 gate. Such a cross-chiral TMSD reaction has not been previously demonstrated. Therefore, we sought to test whether this reaction was possible and to study its behavior. We prepared three versions of the translator system: one in which an L-DNA input is translated into a D-DNA output (L > D), one in which a D-DNA input is translated into an L-DNA output (D > L) and a homochiral control in which a D-DNA input is translated into a D-DNA output (D>D). Raw fluorescence results of kinetic tests of this system are presented in Figure 1c–e. While the behavior is qualitatively similar, the absolute fluorescence values are different (see Figure S1 for results normalized for each reporter). These differences could be attributable to chemical differences between the reporters,

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stoichiometry errors in gate or circuit preparation, or inefficiency in the cross-chiral strand displacement reaction itself. However, the end point fluorescence in each case is linear in the input, as shown in Figure 1f. Altogether, these show that the two heterochiral systems perform similarly to the D > D system, and the time scale of the reaction is similar or slightly slower than previously published homochiral translator systems run at similar concentrations.[12]

We demonstrated the chiral specificity of our system by switching out various gates in the cascade for their D > D counterparts and observing that the resulting signal was not significantly above the background (Figures S2 and S3). Thus, our work shows that not only can individual signals be translated between L- and D-DNA *via* TMSD, but also that individual TMSD reactions can successfully cross the chiral interface. This is the first time that such TMSD reactions have been reported, and thus our work significantly broadens the design space for heterochiral TMSD systems compared with previous work.[11]

We hypothesized that the presence of a chiral interface part way along the duplex might destabilize the complex by disrupting base stacking. Therefore, we used UV−vis spectroscopy to determine the melt curves of the three T1 gates but did not detect a significant difference between the heterochiral and homochiral versions (Figure S4). This may be because the duplexes are relatively long (30 bp), so the local disruption at the chiral interface may have a smaller effect than those seen in previous work that studied shorter duplexes.[13] This result is encouraging as it removes a design limitation for heterochiral translators.

Finally, we sought to determine the protection against leak conferred by the leakless circuit architecture. Previous work has shown that D-DNA molecular circuits,[4] and the D-DNA components of heterochiral duplexes[11] are degraded in biological fluids unless further chemical modifications such as locked nucleic acids[14] or 2′ O-methyl D-ribonucleotides[2,11] are used. We hypothesized that the two-step nature of the D > L leakless translator should mitigate the leak because degradation of the D-DNA should not produce structures that can undergo TMSD. At worst, they may undergo the far slower blunt-end strand displacement process (Figure 2a, Figure S5). We tested this hypothesis by incubating the D > L translator system in serum-supplemented media (DMEM with 10% FBS) for 6 h and then triggering the translator by spiking in the D-DNA input. We observed a minimal leak over 6 h, followed by an increase in fluorescence as the input activated those gates with D-DNA domains still intact (Figure 2b). To demonstrate long-term survival, we also incubated D > D and D > L translators for 26 h and observed a far lower leak in the D > L system than the D > D system (Figure 2c). This demonstrated that leakless translators can detect D-nucleic acids in serum with minimal additional chemical modifications and that they "fail safe", *i.e.*, do not generate false positive signals even when their D-DNA domains are degraded. Results from similar experiments on L > D translators in serum are presented in Figures S6 and S7 and are similar to the D > D results, as expected.

CONCLUSIONS

In summary, we have demonstrated robust signal translation between D-DNA and L-DNA using a multistep leakless translator architecture. To our knowledge, this work reports the longest cascade to date in a heterochiral strand displacement system containing just L-DNA and D-DNA. It is also the first work to demonstrate that TMSD reactions can proceed across the chiral interface in an L-DNA/D-DNA hybrid duplex, both from D-DNA to L-DNA and vice versa. We have also studied the effect of the chiral interface on the stability of the gates themselves, which is minimal. Finally, we have shown that D > L translators are robust against the leak caused by degradation of the D-DNA components in biological fluids, by virtue of the circuit architecture itself. Future work to harden the D-DNA domains against degradation should produce an even more robust system. These advances will broaden the design space and future applications of heterochiral DNA strand displacement systems, including for *in vivo* deployment for tasks such as intracellular imaging[15] and detection of biomarkers such as microRNAs, which have been previously shown to be detectable *via* D-DNA strand displacement logic gates.[3]

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acssynbio.0c00131.

Materials and methods, oligonucleotide sequences, supplementary figures including additional data on reaction kinetics, reaction end point fluorescence, and results from UV−vis experiments (PDF)

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Notes

The authors declare the following competing financial interest(s): A provisional patent application has been filed on this research.

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