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Protecting Heterochiral DNA Nanostructures against Exonuclease-Mediated Degradation

Tracy L. Mallette and Matthew R. Lakin*

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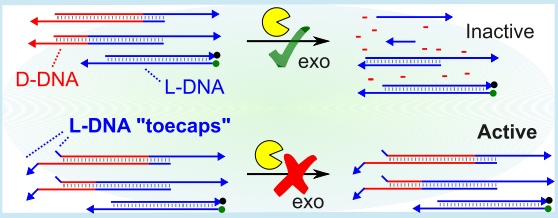
ABSTRACT: Heterochiral DNA nanotechnology employs nucleic acids of both chiralities to construct nanoscale devices for applications in the intracellular environment. Interacting directly with cellular nucleic acids can be done most easily using D-DNA of the naturally occurring right-handed chirality; however, D-DNA is more vulnerable to degradation than enantiometric left-handed L-DNA. Here we report a novel combination of D-DNA and L-DNA nucleotides in triblock heterochiral copolymers, where the L-DNA domains act as protective caps on D-DNA domains. We demonstrate that the D-DNA components of strand displacement-based molecular circuits constructed using this technique resist exonuclease-mediated degradation during extended incubations in serum-supplemented media more readily than similar devices without the L-DNA caps. We show that this protection can be applied to both double-stranded and single-stranded circuit components. Our work enhances the state of the art for robust heterochiral circuit design and could lead to practical applications such as *in vivo* biomedical diagnostics.



KEYWORDS: *heterochiral DNA, DNA strand displacement, molecular computing, DNA nanotechnology, exonucleases, synthetic biology*

INTRODUCTION

The goal of dynamic DNA nanotechnology is to engineer precise control of biomolecular systems at the nanoscale, exploiting the sequence-specific nature of DNA chemistry to program reaction pathways via sequence and structure. DNA strand displacement has emerged as a powerful technique for implementing such systems as enzyme-free competitive hybridization reactions nucleated by short, overhanging, single-stranded toehold domains.[1,2] Previous work has used this approach to implement a wide range of computational frameworks including digital logic circuits,[3,4] abstract chemical reaction networks,[5,6] and artificial neural networks.[7,8] DNA-based systems can directly interact with cellular biomolecules, in particular nucleic acids, making this technology potentially well suited for *in vivo* biomedical applications,[9−11] including diagnosing disease states based on observed cellular biomarkers and autonomously activating a therapeutic payload in response.

However, a practical difficulty for intracellular molecular computing is that cells have evolved defense mechanisms to detect and degrade foreign nucleic acids. Previous work on carrying out molecular logic reactions in mammalian cells was limited by this issue, even when chemical modifications, such as phosphorothioate backbone linkages, were included in the DNA strands that make up the system components.[10] The degradation of circuit components often causes "leak", that is, the undesired release of output signals in the absence of the corresponding inputs.[12] This problem has severely limited the scale-up of molecular computing in cells compared to that *in vitro*.[11] An additional problem is reduced levels of circuit activation due to degraded input recognition components.[13] Improving the resistance of molecular computing components to degradation in biological environments will greatly enhance the practical applicability of these systems for use in living cells.

Exploiting DNA chirality as a design parameter can produce components that are more resistant to degradation in biochemical environments. Naturally occurring DNA ("D-DNA") has a double helix that twists to the right, whereas enantiomeric "L-DNA" twists in the opposite direction: it is the chiral mirror-image of D-DNA (Figure 1a). This means that L-DNA is not recognized by cellular nucleases which have evolved to recognize D-DNA only; thus, L-DNA is degraded far less efficiently than D-DNA in the intracellular environment (Figure 1c). However, L-DNA does not interact with D-DNA according to the usual rules of nucleic acid sequence complementarity, which makes it challenging to interface pure L-DNA devices with cellular nucleic acids. Recent work by ourselves and others has produced a novel solution to this problem: using hybrid chiral, or "heterochiral", DNA strand displacement systems that combine both L- and D-DNA into single components.[13,14] Thus, D-nucleic acid targets can be

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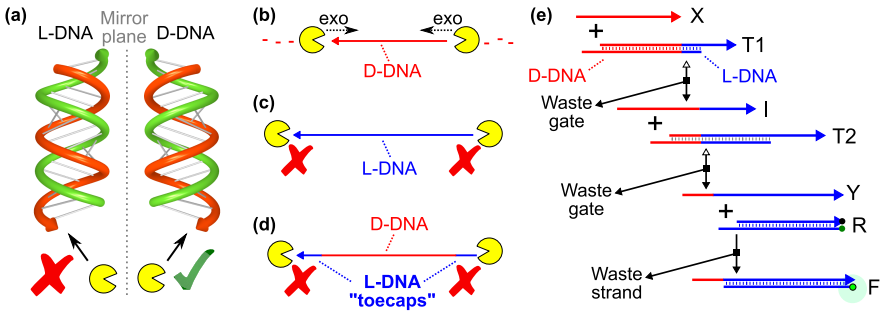

Figure 1. Heterochiral DNA nanotechnology for robust molecular computing devices. (a) L-DNA is the chiral mirror image of naturally occurring D-DNA. (b) D-DNA domains are expected to be degraded by exonucleases present in model biological fluids. (c) Since the chirality of the backbone sugars is flipped, exonucleases that have evolved to recognize D-nucleic acid strand termini do not readily recognize L-nucleic acids, enabling L-DNA to survive for extended periods of time in biological fluids. (d) Here we exploit this property to protect robust heterochiral DNA nanostructures by adding L-DNA "toecaps" to the strand termini of D-DNA domains, thereby protecting them against recognition by exonucleases. (e) Example system under study in this paper, based on our previous work on heterochiral DNA nanotechnology.[13] This cascade accepts a D-DNA input (X) and converts it into an L-DNA output (Y) via two leakless translator gates (T1 and T2).[12] The output is detected via an L-DNA strand displacement reporter probe (R).

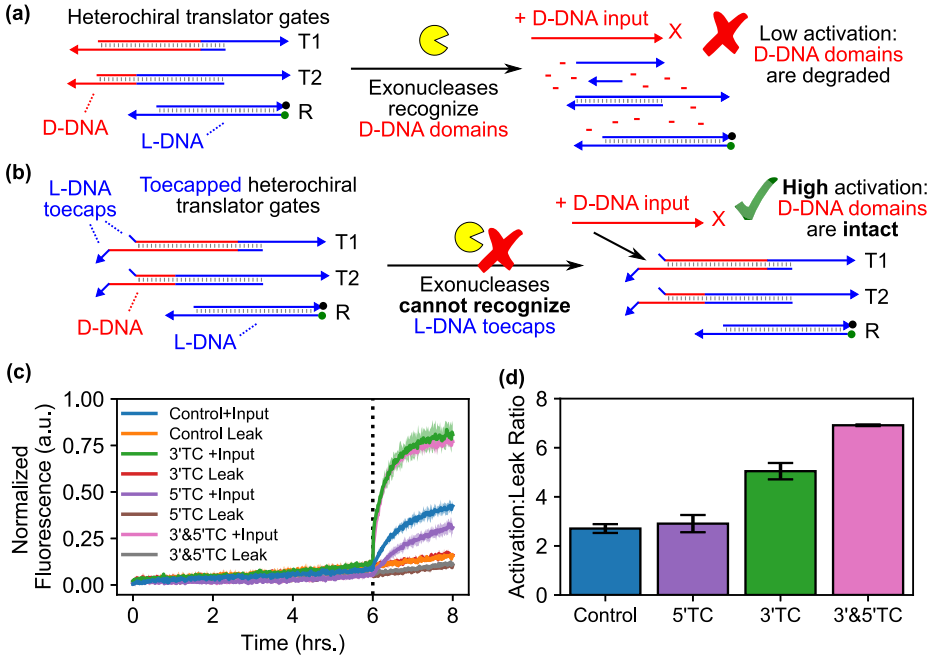

Figure 2. Protection of strand displacement gate complexes from exonuclease-mediated degradation. (a) Degradation of the D-DNA domains in a heterochiral strand displacement translator gate[13] can lead to a weakened response to the cognate D-DNA input strand. (b) Here we use short L-DNA "toecaps" to protect the D-DNA strand termini of gate complexes T1 and T2 from recognition by exonucleases, thereby preventing degradation of these domains and leading to an enhanced response when the gate is activated by the cognate D-DNA input strand. (c) Demonstration of protection afforded to D>L leakless translators by poly-T TTTTT toecap ("TC=5T") protection on some or all D-DNA strand termini. Translator gates and reporters were incubated at a 1× concentration of 300 nM concentration for 6 h in DMEM with 10% fetal bovine serum, at which point the circuits were triggered by the addition of a 10× concentration of D-DNA input strands. The control is the nontoecapped D>L translator system and nontriggered leak traces are also shown for comparison. Data have been normalized with respect to the end point fluorescence each system reached in a equivalent nondegradation test (see Figure S1) and background-subtracted as outlined in the Supporting Information. Line shows the mean of three technical replicates; error bars show the standard deviation. (d) Ratio of the end point fluorescence value upon activation with 10× input to the leak end point fluorescence value for each system from part (c), which shows the signal-to-noise ratio observed for each design variant.

sensed via hybridization to D-DNA domains and the signal transferred to a L-DNA downstream circuit via strand displacement across the chiral interface. However, the presence of D-DNA in the system means that there is still the possibility of degradation of those portions of the circuit by cellular nucleases, which would reduce the capability of the system to sense its programmed targets. Recent work has also used PNA as an achiral intermediary between D- and L-DNA,[14,15] though this can impact circuit kinetics.[16]

In this work, we address the challenge of degradation of the D-DNA components of heterochiral molecular circuits by using L-DNA to protect D-DNA domains from degradation. Specifically, we aim to reduce recognition of D-DNA strand termini by exonucleases, which can degrade D-DNA from

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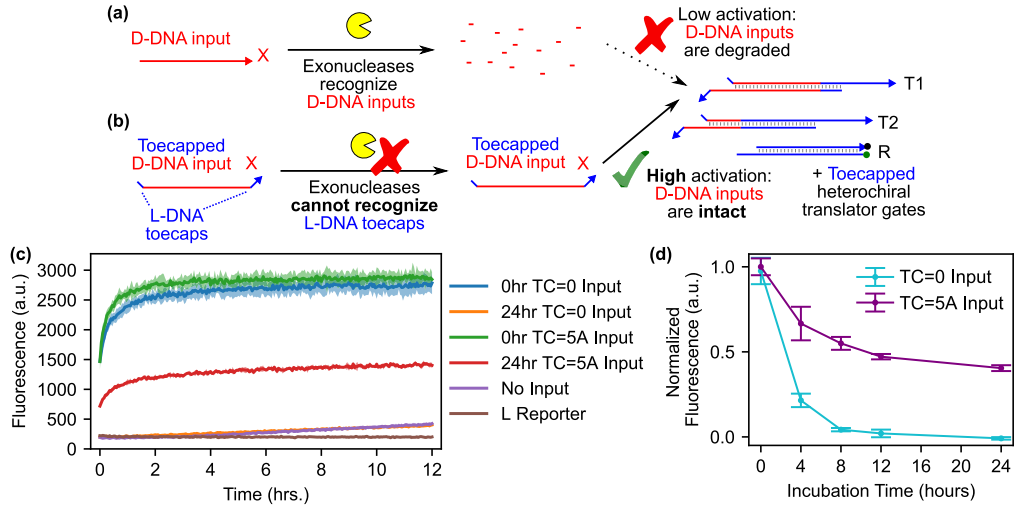

Figure 3. Protection of single-stranded D-DNA oligonucleotides from exonuclease-mediated degradation. (a) Unprotected D-DNA input strands are degraded by exonucleases on incubation in serum-supplemented media, which results in low activation when the incubated strands are used to activate a toecapped heterochiral translator system. (b) Adding L-DNA toecaps reduces the rate of recognition by exonucleases, enabling higher levels of activation of the strand displacement gates upon addition. (c) Kinetic traces of selected input incubations comparing the effectiveness of a L-DNA toecapped input (TC=5A Input) to a fully D-DNA input (0TC Input). Gates for all reactions had 5 base-pair poly-A L-DNA toecaps on all D-DNA strand termini. Standard concentrations of 300 nM were used throughout. Line shows the mean of three technical replicates; error bars show the standard deviation. (d) Normalized end point fluorescence of five different input incubation durations ranging from 0 to 24 h incubation. Plotted data points show the mean of normalized data from three technical replicates; error bars show the standard deviation. Fluorescence values were normalized and background-subtracted as outlined in the Supporting Information.

either terminus (Figure 1b), by "capping" those strand termini with short L-DNA sequences (Figure 1d). These domains, which we refer to as "toecaps", are intended to mask the strand termini from exonucleases that have evolved specifically to recognize D-DNA. We hypothesize that this approach would reduce recognition, and thus degradation, of D-DNA components by exonucleases in model biological fluids such as serum-supplemented media (Figure 1d). Specifically, we test this hypothesis in the context of our previously reported heterochiral translator system,[13] whose mechanism of operation is illustrated in Figure 1e, for an unprotected version.

RESULTS

Figure 2 outlines this approach as applied to the gate complexes from our heterochiral translator system. This system consumes a D-DNA input strand and releases an L-DNA output strand via a multistep strand displacement cascade consisting of two toehold-mediated strand displacement reactions involving gate complexes T1 and T2, the second of which traverses the chiral interface between D-DNA and L-DNA. A third L-DNA strand displacement reaction activates a FRET reporter probe by displacing its quencher from the reporter complex R. We call this a D>L translator. In our previous work, the chiral interface in the translator gates was shown to have a minimal effect on the stability of the gate complex, as measured by melting temperature.[13] The D-DNA domains are responsible for sensing D-DNA input in this system, and we hypothesized that protecting these strand termini with L-DNA toecaps would enable this system to remain responsive to its input even after extended incubation in serum-supplemented media. This should be the case even without any additional modifications to the D-DNA regions such as locked nucleic acids (LNA),[17] phosphorothioate backbone linkages,[10] or 2′*O*-methyl ribonucleotides.[10]

Experimental data validating this system design as applied to heterochiral DNA strand displacement gate complexes are shown in Figure 2c,d. Several variants of the T1 and T2 gates from the D>L heterochiral translator were incubated in DMEM media supplemented with 10% fetal bovine serum for 6 h along with the L-DNA reporter gate R, at which point the circuit was activated via addition of 10× of the corresponding D-DNA input strand X. Fluorescence was then measured for an additional 2 h. We tested versions with poly-T (TTTTT) L-DNA toecaps on 3′ D-DNA strand termini only in the T1 and T2 translator gates (labeled 3′TC in Figure 2), 5′ D-DNA strand termini only (labeled 5′TC), and on all D-DNA strand termini (labeled 3′&5′TC). As in our previous work,[13] the leak was low in all cases.

The 5′TC variant and nontoecapped control both show the weakest response to input, which is not surprising given that most (but not all) exonucleases operate in the 3′ to 5′ direction and thus recognize their targets at 3′ strand termini. This hypothesis is also supported by the fact that the systems which have the 3′ strand termini protected (3′TC and 3′&5′TC) show stronger responses (Figure 2c), which also indicates that the 3′ toehold is more intact and can thus more readily consume the incoming signal strands. Importantly, the 3′&5′TC variant exhibited the strongest activation to leak ratio (Figure 2d), indicating that both 5′ and 3′ exonuclease-mediated degradation can be mitigated using our approach and that the most robust system is the one in which both termini are protected by toecaps; we therefore used these gate variants for all subsequent experiments. We note that, in order to separate this effect from potentially confounding factors such as the relative signal transduction efficiencies of the gate variants, the data in Figure 2c was normalized to the end point fluorescence value from the corresponding nondegradation control for each toecap variant (Figure S1; see also

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"Normalization of raw fluorescence data" in the Supporting Information).

We used 10× input in these experiments so that this was not the limiting factor in the observed signal, allowing us to correlate fluorescence obtained with the integrity of the D-DNA portions of the T1 and T2 translator gates. Similar results were obtained using just 2× of the D-DNA input (see Figure S2), thereby confirming that gate degradation was the limiting factor in these results. We additionally investigated the impact of toecaps on kinetics and found that the toecaps on the gates appear to slightly increase the observed reaction rate (see Figure S3). We hypothesize that the toecap may be acting as a weak extension to the toehold, as longer toeholds are known to increase reaction rates.[18] This effect is somewhat surprising given that hybridization between D- and L-nucleic acids does not follow the rules of Watson−Crick complementarity and we hypothesize that electrostatic effects or transient interactions between individual base-pairs may be responsible.

To investigate whether the toecapping effect is sequence-specific, we constructed a similar system using poly-A (AAAAA) L-DNA toecaps, which behaved almost identically to the poly-T version (see Figure S4). This suggests that toecap protection is *not* sequence-specific. It also suggests that L-DNA toecap sequences can be tailored as required for a particular system to prevent cross-hybridization with other L-DNA circuit components without affecting the conferred protection against exonuclease-mediated degradation. Taken together, these results suggest a design rule for enhancing the robustness of heterochiral DNA devices in biological fluids: add appropriate short L-DNA domains to all D-DNA strand termini on gate complexes, to serve as protective toecaps.

Having demonstrated the validity of our approach to protect the D-DNA domains of a multistranded heterochiral gate complex, we next tested a similar approach to protect single-stranded D-DNA oligomers from degradation under similar conditions. Given that many multistep DNA strand displacement circuit designs require auxiliary single-stranded fuels to drive the reaction forward,[5] developing such a capability is important to enable our approach to be scaled up to larger circuits. We therefore created a toecapped version of the D-DNA oligonucleotide X that serves as the input to our D>L heterochiral translator system using the poly-A L-DNA toecap, as shown in Figure 3a,b. In a variation of our previous experiment, we incubated toecapped and nontoecapped versions of this input (X) for various lengths of time before transferring them into a reaction volume containing the heterochiral gates (T1 and T2) with 3′&5′ poly-A L-DNA toecaps and the L-DNA reporter probe (R), to assess the intactness of the single strands via their ability to activate the reaction cascade.

The toecap-protected input continues to exhibit over 50% activation of the circuit after 24 h of incubation (Figure 3c). This is a significant improvement compared to the non-toecapped input, which degrades below 50% activation in less than 4 h (Figure 3d). The trendline for the system with the TC=5A input is still dropping at the 24 h time point, albeit slowly, which implies that still longer incubations could produce further drops in the signal. However, it is also possible that the TC=5A input might never degrade completely, which we hypothesize could occur due to denaturation of the nucleases and/or depletion of required metal ion cofactors during the extended incubation. In summary, these results demonstrate that our toecapping approach can be applied not just to D-DNA domains of strand displacement gate complexes but also to single-stranded nucleic acids, which are critical components of many DNA-based molecular computing devices.

Finally, we sought to determine if the toecap protection of the D-DNA could be conveyed with fewer L-DNA base pairs (Figure 4a). The five base-pair length of the toecap for the initial designs was chosen to match the toehold length; however, a shorter sequence of L-DNA would be preferable to simplify the sequence design process and to reduce the possibility of undesired cross-hybridization with other L-DNA circuit components. Additionally, shorter overhangs may enhance stability of the base-pairs at the end of any neighboring duplexes, which should also help to reduce leaking via blunt-end strand invasion. Therefore, we created variants of the D-DNA input strand X with L-DNA poly-A toecaps consisting of five, three, or one base on both the 5′ and 3′ ends, and a control input with no toecaps. Here we referred to these as TC=5A, TC=3A, TC=1A, and TC=0 variants, respectively. In an expansion of the experiment presented in Figure 3d, these input variants were incubated in serum supplemented media, and their ability to activate the reaction cascade was tested. We observed the expected trends with the level of activation broadly increasing with the length of the toecap domain and decreasing with extended incubation times. The control 0TC input was almost completely inactive after a 4 h incubation, whereas it took 24 h for the input with the shortest L-DNA toecap (TC=1A) to become similarly degraded. The TC=5A and TC=3A variants were the most

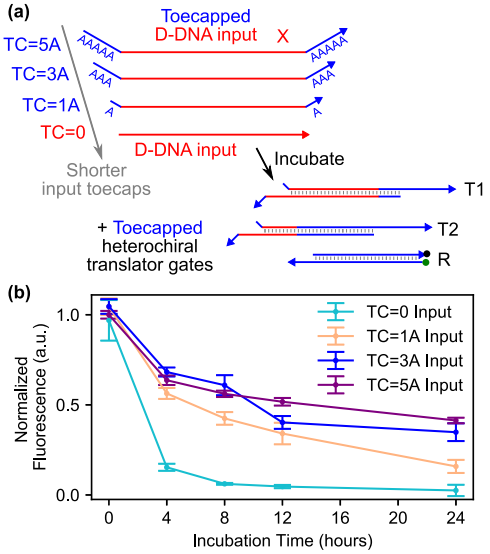

Figure 4. Effect of decreasing toecap length on protection of single-stranded input oligonucleotides. (a) Schematic of X input strands with 5, 3, 1, and 0-length L-DNA poly-A toecaps. After incubation, toecapped heterochiral translator gates are added to measure integrity of input strands via their ability to initiate the programmed strand displacement cascade. (b) Normalized fluorescence of reaction end points after 8 h. All circuit components were added at a concentration of 300 nM. Plotted data points show the mean of normalized data from three technical replicates; error bars show the standard deviation. Fluorescence values were normalized as outlined in the Supporting Information.

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Table 1. Survey of Some Related Work[10,14,24,26] on Various Mechanisms for Protection of DNA Oligonucleotides in Various Model Biological Fluids[a]

Paper	Modification(s)	Model Fluid	Result(s)
Groves et al.[10]	(i) PS backbone	Transfected CHO K1 cells	After 6 h: (i) Marginal improvement
	(ii) 2′*O*Me-RNA		(ii) >4× reporter activation
	(iii) PS backbone and 2′*O*Me-RNA		(iii) Similar to PS only
Fern and Schulman[24]	(i) 5′ vs 3′ toeholds on reporter gate complex	Nuclease-screened media (actin and decoy ssDNA/dsDNA in DMEM) with 10% FBS	(i) After 70 h: 25% loss in 5′ toehold reporter activity, 85% loss in 3′ toehold reporter activity
	(ii) 3′ hairpin on ssDNA input		(ii) After 7 h: 4× greater reporter activation
Wahlested et al.[26]	(i) Intermixed LNA and DNA bases	Rat blood serum	(i) Stable to 20 h
	(ii) LNA-DNA-LNA triblock sequence		(ii) Partially stable to 5 h
	(iii) PS backbone		(iii) Partially stable to 20 h
Young and Sczepanski[14]	Combined L-DNA and D-2′*O*Me-RNA	DMEM with 10% FBS	After 6 h: 3.5× less leak than full D-DNA system

[a]Abbrevations: PS, phosphorothioate; 2′*O*Me-RNA, 2′*O*-methyl ribonucleotides; CHO, Chinese hamster ovary; LNA, locked nucleic acid; ssDNA, single-stranded DNA; dsDNA, double-stranded DNA; DMEM, Dulbecco's Modified Eagle Medium; FBS, fetal bovine serum.

effective; interestingly, these two variants were similarly effective at reducing degradation of the input for the first 8 h of incubation, though a small improvement is seen with the 5-base-pair system in longer incubation times (Figure 4b). This suggests that the TC=3A variant might be a good compromise between protection and compactness of sequence. However, even a single L-DNA base pair toecap showed significant improvement over nonprotected DNA. Taken together, these results show that protection against exonucleases is a function of toecap length and incubation time, suggesting that future systems could be tuned to add only the protection needed for the time the system needs to resist exonuclease-mediated degradation to perform its programmed function.

DISCUSSION

To summarize, we have shown that adding L-DNA domains to "cap" D-DNA strand termini can enhance the robustness of heterochiral DNA devices to degradation in serum-supplemented media, a model biological fluid. Survival in such conditions would be of practical importance for the delivery of therapeutic DNA circuit components to targeted cells and tissues via the bloodstream. This is therefore a potentially relevant model system for the detection of circulating biomarkers such as microRNAs, which could be used to diagnose a range of conditions.[19−21] Here, however, we mainly use activation of the circuit in large part as a means of measuring the intactness and functionality of the circuit components after incubation.

Our L-DNA modifications were shown to be effective at protecting both double- and single-stranded D-DNA and could reasonably be expected to be less immunogenic than other chemical modifications.[22] The protection conferred does not appear to be dependent on the sequence of the L-DNA toecap, allowing for flexibility in applying this technique to future system designs. While in this work we have focused exclusively on the D>L heterochiral translator system, similar protective techniques could be applied to other molecular computing circuit architectures, including fully D-DNA circuits of various kinds,[4,12] which would likely experience even greater relative protection compared to the heterochiral system studied here, or to the inverse L>D translator from our previous work.[13]

Other approaches to cross-chiral interaction, such as raising L-RNA aptamers against targets of interest,[23] are promising but require selection to be done on a case-by-case basis, whereas the heterochiral approach allows hardened D-nucleic acid components to be straightforwardly designed to interact directly with complementary D-nucleic acids via hybridization. Related work[24] on the degradation of DNA strand displacement circuit components in serum-supplemented media focused on D-DNA gates only. In that work, actin and mimic DNA were used as decoys to titrate nucleases away from the circuit components. Our heterochiral approach does not require such additions. In addition, small hairpin loops were added to some strand termini to reduce degradation; this approach could potentially be combined with our system to further reduce degradation of circuit components. Furthermore, that work studied the effects of situating gate toeholds on the 5′ or 3′ end of the strand and found that gates with 5′ toeholds were better able to operate after incubation. Table 1 presents a summary of some additional results from that and some other previous work on the stability of various forms of modified DNA in various model biological systems, with the goal of providing a broad overview of approaches adopted. We refer the reader to published reviews[22] for a more detailed treatment of this field. The practical advantage of our approach is that the protection demonstrated here relies solely on the presence of the L-DNA toecap and not on any other additional chemical modifications to the DNA.

For historical reasons drawing on our previous work,[13] and based on our desire to test our system in the most challenging scenario, our study used gates with toeholds on the 3′ end of the corresponding gate strand. This suggests that redesigning this aspect of our gates could also further enhance protection against degradation. Other previous work on DNA nanomachines in serum[25] found additional topological effects which could be leveraged to further enhance the protection of our heterochiral nanostructures in similar conditions.

Future studies to probe the potential applicability of L-DNA in such a protective role could combine L-DNA with other forms of chemical modification such as phosphorothioate backbone linkages and 2′*O*-methyl ribonucleotides, which are typically applied within the D-chirality. This should provide even stronger protection than for either modification alone. Testing our systems in the presence of specific exo- or endonucleases could help to elucidate which pathways and enzymes are most responsible for any DNA circuit degradation

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that occurs. Integrating structural motifs such as L-DNA terminal hairpins[24] could also be a promising direction. To demonstrate biomedical relevance it would be necessary to test our system in more realistic model systems such as transfected human cell lines. Given that complementary DNA and RNA of the same chirality can interact directly via hybridization, and indeed undergo toehold-mediated strand displacement reactions,[27] it should also be straightforward to use D-RNA inputs for an L-DNA toecap-protected strand displacement circuit. In conclusion, our approach can extend the useful operational life of DNA nanodevices in harsh biological environments, paving the way for future biomedical applications such as cellular imaging[28] or *in vivo* detection of disease biomarkers.[29]

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acssynbio.2c00105.

Detailed information on materials and methods and sequences of all oligonucleotides used in this work, as well as supplementary data figures (PDF)

■ AUTHOR INFORMATION

Corresponding Author

Matthew R. Lakin − *Department of Computer Science and Department of Chemical & Biological Engineering, University of New Mexico, Albuquerque, New Mexico 87131, United States; Center for Biomedical Engineering, University of New Mexico, Albuquerque, New Mexico 87131, United States*; Phone: +1 (505) 277-3351; Email: mlakin@cs.unm.edu; Fax: +1 (505) 277-6927

Author

Tracy L. Mallette − *Center for Biomedical Engineering, University of New Mexico, Albuquerque, New Mexico 87131, United States;* orcid.org/0000-0002-6197-4197

Complete contact information is available at: https://pubs.acs.org/10.1021/acssynbio.2c00105

Author Contributions

T.L.M. and M.R.L. conceived research and designed experiments. T.L.M. carried out experiments and analyzed data. T.L.M. and M.R.L. interpreted data and wrote the manuscript.

Notes

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

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■ REFERENCES

(1) Zhang, D. Y.; Seelig, G. Dynamic DNA nanotechnology using strand-displacement reactions. *Nat. Chem.* **2011**, *3*, 103−113.

(2) Simmel, F. C.; Yurke, B.; Singh, H. R. Principles and Applications of Nucleic Acid Strand Displacement Reactions. *Chem. Rev.* **2019**, *119*, 6326−6369.

(3) Seelig, G.; Yurke, B.; Winfree, E. Catalyzed Relaxation of a Metastable DNA Fuel. *J. Am. Chem. Soc.* **2006**, *128*, 12211−12220.

(4) Qian, L.; Winfree, E. Scaling up digital circuit computation with DNA strand displacement cascades. *Science* **2011**, *332*, 1196−1201.

(5) Soloveichik, D.; Seelig, G.; Winfree, E. DNA as a universal substrate for chemical kinetics. *Proc. Natl. Acad. Sci. U.S.A.* **2010**, *107*, 5393−5398.

(6) Chen, Y.-J.; Dalchau, N.; Srinivas, N.; Phillips, A.; Cardelli, L.; Soloveichik, D.; Seelig, G. Programmable chemical controllers made from DNA. *Nat. Nanotechnol.* **2013**, *8*, 755−762.

(7) Qian, L.; Winfree, E.; Bruck, J. Neural network computation with DNA strand displacement cascades. *Nature* **2011**, *475*, 368−372.

(8) Cherry, K. M.; Qian, L. Scaling up molecular pattern recognition with DNA-based winner-take-all neural networks. *Nature* **2018**, *559*, 370−376.

(9) Hemphill, J.; Deiters, A. DNA Computation in Mammalian Cells: MicroRNA Logic Operations. *J. Am. Chem. Soc.* **2013**, *135*, 10512−10518.

(10) Groves, B.; Chen, Y.-J.; Zurla, C.; Pochekailov, S.; Kirschman, J. L.; Santangelo, P. J.; Seelig, G. Computing in mammalian cells with nucleic acid strand exchange. *Nat. Nanotechnol.* **2016**, *11*, 287−294.

(11) Chen, Y.-J.; Groves, B.; Muscat, R. A.; Seelig, G. DNA nanotechnology from the test tube to the cell. *Nat. Nanotechnol.* **2015**, *10*, 748−760.

(12) Wang, B.; Thachuk, C.; Ellington, A. D.; Winfree, E.; Soloveichik, D. Effective design principles for leakless strand displacement systems. *Proc. Natl. Acad. Sci. U.S.A.* **2018**, *115*, E12182−E12191.

(13) Mallette, T. L.; Stojanovic, M. N.; Stefanovic, D.; Lakin, M. R. Robust heterochiral strand displacement using leakless translators. *ACS Synth. Biol.* **2020**, *9*, 1907−1910.

(14) Young, B. E.; Sczepanski, J. T. Heterochiral DNA Strand-Displacement Based on Chimeric D/L-Oligonucleotides. *ACS Synth. Biol.* **2019**, *8*, 2756−2759.

(15) Kabza, A. M.; Young, B. E.; Sczepanski, J. T. Heterochiral DNA Strand-Displacement Circuits. *J. Am. Chem. Soc.* **2017**, *139*, 17715−17718.

(16) Kundu, N.; Young, B. E.; Sczepanski, J. T. Kinetics of heterochiral strand displacement from PNA-DNA heteroduplexes. *Nucleic Acids Res.* **2021**, *49*, 6114−6127.

(17) Olson, X.; Kotani, S.; Yurke, B.; Graugnard, E.; Hughes, W. L. Kinetics of DNA Strand Displacement Systems with Locked Nucleic Acids. *J. Phys. Chem. B* **2017**, *121*, 2594−2602.

(18) Zhang, D. Y.; Winfree, E. Control of DNA strand displacement kinetics using toehold exchange. *J. Am. Chem. Soc.* **2009**, *131*, 17303−17314.

(19) Wang, H.; Peng, R.; Wang, J.; Qin, Z.; Xue, L. Circulating microRNAs as potential cancer biomarkers: the advantage and disadvantage. *Clinical Epigenetics* **2018**, *10*, 59.

(20) Roser, A. E.; Gomes, L. C.; Schünemann, J.; Maass, F.; Lingor, P. Circulating miRNAs as Diagnostic Biomarkers for Parkinson's Disease. *Frontiers in Neuroscience* **2018**, *12*, 625.

(21) Salloum-Asfar, S.; Satheesh, N. J.; Abdulla, S. A. Circulating miRNAs, Small but Promising Biomarkers for Autism Spectrum Disorder. *Frontiers in Molecular Neuroscience* **2019**, *12*, 253.

(22) Kabza, A. M.; Kundu, N.; Zhong, W.; Sczepanski, J. T. Integration of chemically modified nucleotides with DNA strand displacement reactions for applications in living systems. *WIREs Nanomedicine and Nanobiotechnology* **2022**, *14*, e1743.

(23) Kabza, A. M.; Sczepanski, J. An L-RNA Aptamer with Expanded Chemical Functionality that Inhibits MicroRNA Biogenesis. *ChemBioChem.* **2017**, *18*, 1824−1827.

(24) Fern, J.; Schulman, R. Design and Characterization of DNA Strand-Displacement Circuits in Serum-Supplemented Cell Medium. *ACS Synth. Biol.* **2017**, *6*, 1774−1783.

(25) Goltry, S.; Hallstrom, N.; Clark, T.; Kuang, W.; Lee, J.; Jorcyk, C.; Knowlton, W. B.; Yurke, B.; Hughes, W. L.; Graugnard, E. DNA topology influences molecular machine lifetime in human serum. *Nanoscale* **2015**, *7*, 10382−10390.

(26) Wahlestedt, C.; et al. Potent and nontoxic antisense oligonucleotides containing locked nucleic acids. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 5633−5638.

(27) Liu, H.; Hong, F.; Smith, F.; Goertz, J.; Ouldridge, T.; Stevens, M. M.; Yan, H.; Šulc, P. Kinetics of RNA and RNA:DNA Hybrid Strand Displacement. *ACS Synth. Biol.* **2021**, *10*, 3066−3073.
(28) Hao, Y.; Li, J.; Li, Q.; Zhang, L.; Shi, J.; Zhang, X.; Aldalbahi, A.; Wang, L.; Fan, C.; Wang, F. Programmable Live-Cell CRISPR Imaging with Toehold-Switch-Mediated Strand Displacement. *Angew. Chem., Int. Ed.* **2020**, *59*, 20612−20618.
(29) Jung, C.; Ellington, A. D. Diagnostic Applications of Nucleic Acid Circuits. *Acc. Chem. Res.* **2014**, *47*, 1825−1835.

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