DNA nanotechnology in the undergraduate laboratory: Toehold-less strand displacement in switchback DNA

Bharath Raj Madhanagopal and Arun Richard Chandrasekaran*

The RNA Institute, University at Albany, State University of New York, Albany 12222, NY, USA.

Email: arun@albany.edu

ABSTRACT

Dynamic DNA nanostructures that reconfigure into different shapes are used in several applications in biosensing, drug delivery and data storage. One of the ways to produce such structural transformations is by a process called strand displacement. This laboratory experiment demonstrates a strand displacement reaction in a two-stranded DNA nanostructure called switchback DNA by the addition of a third strand. In this process, the difference in the affinity between the component DNA strands is used to convert switchback DNA into conventional duplex DNA. Students are introduced to the concept through gel electrophoresis and quantitative analysis of DNA nanostructure reconfiguration. The experiment presented here follows a series of DNA nanotechnology-based exercises in an undergraduate setting and is tailored for adaptation in a chemistry, biology, or biochemistry laboratory with minimal costs.

KEYWORDS

Upper-division undergraduate, Biochemistry, Interdisciplinary/multidisciplinary, Hands-on learning/manipulatives, Electrophoresis, Molecular properties/structure, Nanotechnology, Nucleic acids/DNA/RNA, Undergraduate research, DNA nanotechnology

INTRODUCTION

DNA self-assembly has been used in the construction of a variety of nanostructures, such as polyhedral objects and multidimensional arrays.[1,2] Assembly of these structures involves different types of DNA motifs, with examples including the DNA double crossover (DX) motif,[3] triple crossover (TX) motif,[4] paranemic crossover (PX),[5] and multi-arm DNA stars.[6,7] Further, DNA nanostructures can be made to reconfigure based on external stimuli such as pH, ions, or other nucleic acids.[8] While various strategies have been used to produce structural reconfiguration in nucleic acid nanostructures, toehold-mediated strand displacement is a widely used concept that has been used in biosensing, molecular computation, drug delivery and materials science applications of dynamic DNA nanostructures.[9,10] As newer DNA motifs and nanostructures are being designed, modifications to displacement strategies have also been developed. For example, light-responsive linkers have been used to create caged toeholds,[11] photocleavable linkers to control displacement reactions,[12] and aptamers that respond to nucleic acids or proteins have been used for different functions.[13] In contrast to toehold-mediated strand displacement, reconfiguration of DNA nanostructures can also be based on the affinity of component DNA strands to form different types of DNA motifs.[14] In this Laboratory Experiment, we discuss the switchback DNA motif and the concept of toehold-less strand displacement process from switchback DNA to conventional duplex DNA, with the results visualized by gel electrophoresis. The pedagogical value of this experiment is to introduce undergraduate students to a new type of DNA motif, combined with practical skill development in gel electrophoresis, an often-used technique in biophysics, molecular biology, and biochemistry for a range of investigations such as analyzing photoinduced oxidative DNA damage,[15] quantifying DNAzyme activity,[16] determining protein concentration,[17] characterizing DNA-cleaving metal complexes,[18] biostability of DNA motifs[19] and analyzing the molecular topology of DNA nanoswitches,[20] all of which have been described in this *Journal*.

DNA nanostructures are typically characterized by methods such as atomic force microscopy, cryo-electron microscopy, transmission electron microscopy, scanning electron microscopy, dynamic light scattering, and size exclusion chromatography.[21,22] While such advanced techniques allow high-resolution analyses of assembled structures, gel electrophoresis remains an easy-to-adapt and routine method for DNA nanostructure characterization. Recently, the analysis of DNA origami structures by gel electrophoresis has been developed for middle school, high school, and undergraduate laboratories.[23] Our own undergraduate laboratory experiment protocols in this *Journal* have used gel electrophoresis for analyzing the molecular topology of DNA nanoswitches[20] and for evaluating the biostability of DNA motifs.[19] Several other articles and laboratory protocols published in this *Journal* have introduced various concepts of DNA nanotechnology to students.[16,24–27] The current laboratory experiment adds to this series of demonstrations on DNA nanotechnology concepts in an undergraduate laboratory setting.

HAZARDS

Acrylamide and tetramethylethylenediamine (TEMED) are hazardous chemicals. Acrylamide is a carcinogen, and exposure can occur via inhalation (if aerosolized), ingestion, and skin absorption. Care should be taken when using acrylamide to prepare gels. TEMED is a flammable liquid and must be handled with care and used only under a chemical fume hood. Avoid contact with skin or clothing and wear personal protective equipment/face protection while handling the chemicals to prevent accidental exposure. The electrophoresis apparatus and the power supply must be handled cautiously as they pose electrical hazards. To avoid electric shocks, students should use care when plugging the gel boxes into the power supply. Exercise caution while using the gel imager by wearing appropriate skin and protection to avoid potential exposure to UV irradiation.

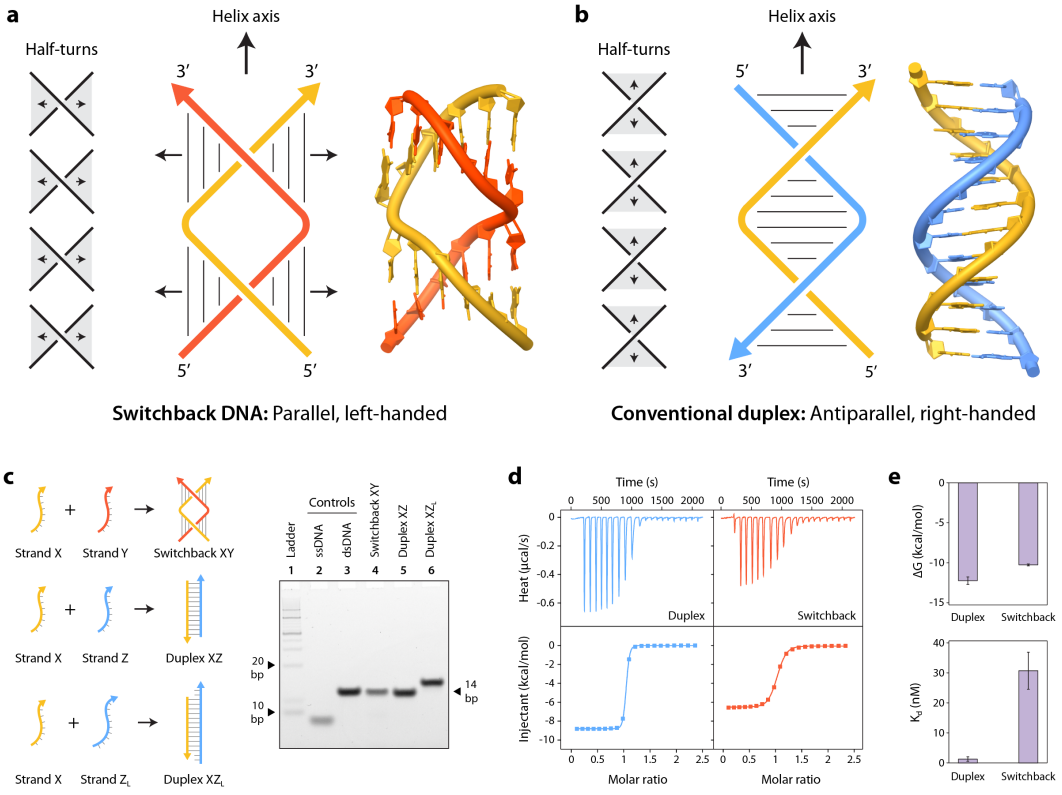

Fig. 1. ***Switchback DNA and conventional duplex.*** Schematic and model of (a) switchback DNA and (b) conventional duplex. The models are adapted from PDB ID: 8EPF[28] and PDB ID: 1BNA[29], respectively. Arrows denote 3′ ends of DNA strands. The structure contains two half-turn domains, with each half-turn domain consisting of six base pairs. The switchback DNA structure has a global left-handed twist, with the helical axis of the full structure being perpendicular to the helical axis of the half-turn domains. The yellow and red strands are complementary in the switchback sense. (c) Non-denaturing polyacrylamide gel electrophoresis (PAGE) analysis of switchback DNA. (d) Isothermal titration calorimetry (ITC) thermograms of conventional duplex and switchback DNA. (e) Thermodynamic parameters of conventional duplex and switchback DNA. Figure adapted from ref 14 with permission. The original article is open access and is a publication by the current authors.

CONCEPTS AND RESULTS

This undergraduate laboratory experiment is based on our recent study of switchback DNA, a motif assembled from two strands of DNA.[14] In switchback DNA, units of six base pairs that constitute a B-DNA half-turn are aligned laterally in contrast to the co-axial arrangement of half-turns in a conventional double helical structure of DNA (**Figure 1a**).[14,28,30] That is, the helical axis of the half-turns is perpendicular to the axis of the global helix. Because the strands switch back after each half-turn, the overall helix is left-handed with the strands running parallel to each other. In our previous study on switchback DNA, we assembled a heterodimeric switchback DNA using two strands X and Y (**Table S1**). To compare the properties of switchback DNA with that of a conventional duplex, we designed a strand (Z) that can pair with strand X to form a conventional duplex DNA. In this context, strands X and Y are complementary in the switchback sense, while strands X and Z are complementary in a duplex sense. We validated the assembly of the switchback XY using non-denaturing polyacrylamide gel electrophoresis (PAGE) (**Figure 1c**). The formation of switchback DNA was confirmed by the fact that it migrated similar to the conventional duplex of the same length and the absence of bands corresponding to single strands. We also studied the thermodynamics of switchback DNA and conventional duplex formation using isothermal titration calorimetry (ITC) in that work, reporting a ΔG of −12.23 kcal/mol for the conventional duplex (with a Kd of 1.3 nM) and a ΔG of −10.26 kcal/mol and a Kd of 30.7 nM for switchback DNA (**Figure 1d,e**). These results indicated that the conventional duplex was thermodynamically more stable than its switchback counterpart. The difference in thermodynamic stability of the two complexes manifested in the preference of strand X to bind to strand Z over strand Y. This led us to test whether a duplex complement (Z) can displace a switchback complement (Y) from an assembled switchback DNA, a process that would be toehold-less strand displacement.

In a typical toehold-mediated strand displacement reaction, an incoming single stranded DNA with a full sequence complementarity displaces a partially complementary strand from a pre-hybridized duplex (**Figure 2a**).[31] The invading strand initially binds to a single-stranded region called a toehold, and this binding event triggers a branch migration process that results in the removal of the previously partially bound strand to produce a duplex with more base pairs than the original duplex.[9,10] In the context of switchback DNA, a duplex complement displaces a switchback complement from a pre-assembled switchback DNA, resulting in the formation of a conventional duplex (**Figure 2b**). Unlike the toehold containing duplex in the toehold-mediated strand displacement strategy, no bases in the switchback DNA are unpaired, and there is no single-stranded region in the structure. Despite that, the displacement of the switchback complement strand is favored due to the higher thermodynamic stability of the conventional duplex (formed by the invading strand Z with the common strand X) than the initial switchback DNA XY.

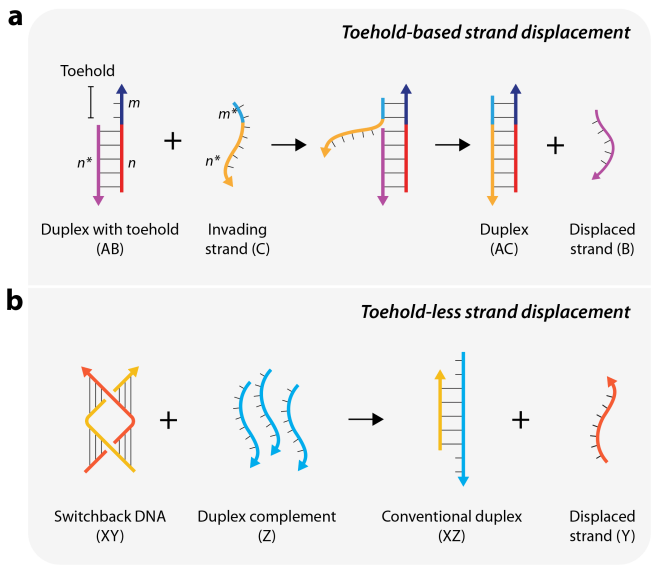

Figure 2. ***Strand displacement reactions.*** (a) In toehold-mediated strand displacement, strand C binds to the toehold region *m* in strand A and proceeds to displace strand B from duplex AB producing AC with a higher number of base pairs. (b) In toehold-less strand displacement, strand Z displaces strand Y from switchback DNA (XY) producing a conventional duplex (XZ).

To perform the strand displacement reaction with switchback DNA in an undergraduate laboratory setting, here we provide an experimental workflow that can be completed in under 3 hours (**Figure 3a, Table S2**). In this laboratory experiment, students analyze the reconfiguration of switchback DNA to conventional duplex DNA structures using PAGE. To identify the product and to discern it from the initial switchback structure, we used a longer duplex complement Z_L (same sequence as strand Z but with 2 additional Ts at each terminus). The strand displacement can be observed on a gel by the different migration of the start and end structures (**Figure 1c, lane 6**). The use of longer complement Z_L obviates the need to use more sophisticated techniques or expensive fluorophore-labeled strands and makes the experiment suitable for an undergraduate laboratory. In previous work, we annealed switchback DNA using a thermal annealing protocol that required a thermal cycler and performed non-denaturing PAGE in the cold room (4 °C) (**Figure 3b**). For adaptation in an undergraduate setting, we tested the assembly of switchback DNA using a hot water bath and modified the gel electrophoresis protocols to be performed at room temperature on the bench (~20 °C) instead of a cold room. We confirmed that these changes did not affect our original reported assembly and analysis of the displacement process (**Figure 3c**). The bands corresponding to the product duplex appeared with progressive intensities as the concentration of strand Z_L increased. The product band was well resolved from the substrate switchback band under the modified protocol as well as the original electrophoresis protocol. The intensity of

the duplex band increased steadily reaching the maximum intensity at [strand X]:[strand Z_L] molar ratio of 1:1.75, similar to our previous report.[14]

The structures can be assembled by the instructor using the protocol in **Supplementary Note 1** and provided to students when they start the laboratory experiment. The in-lab experiment involves mixing the duplex complement at different ratios to the switchback DNA, incubation at room temperature, gel electrophoresis, and gel imaging (a separate step-by-step student hand-out is provided in the SI: **Supplementary Note 2**). Our experimental design suggests gel quantification and data analysis as post-lab exercises but can be performed in the lab if timings are modified. In our own laboratory, our workflow included a demonstration of gel analysis and plotting to all the students together, followed by each student performing the analysis independently. A control experiment could be performed by the instructors beforehand for students to compare their results. As an alternative, we have provided the data set from our previous research experiments (the data shown in Figure 3b) that can be used as a control data set to validate results (**Table S3**).

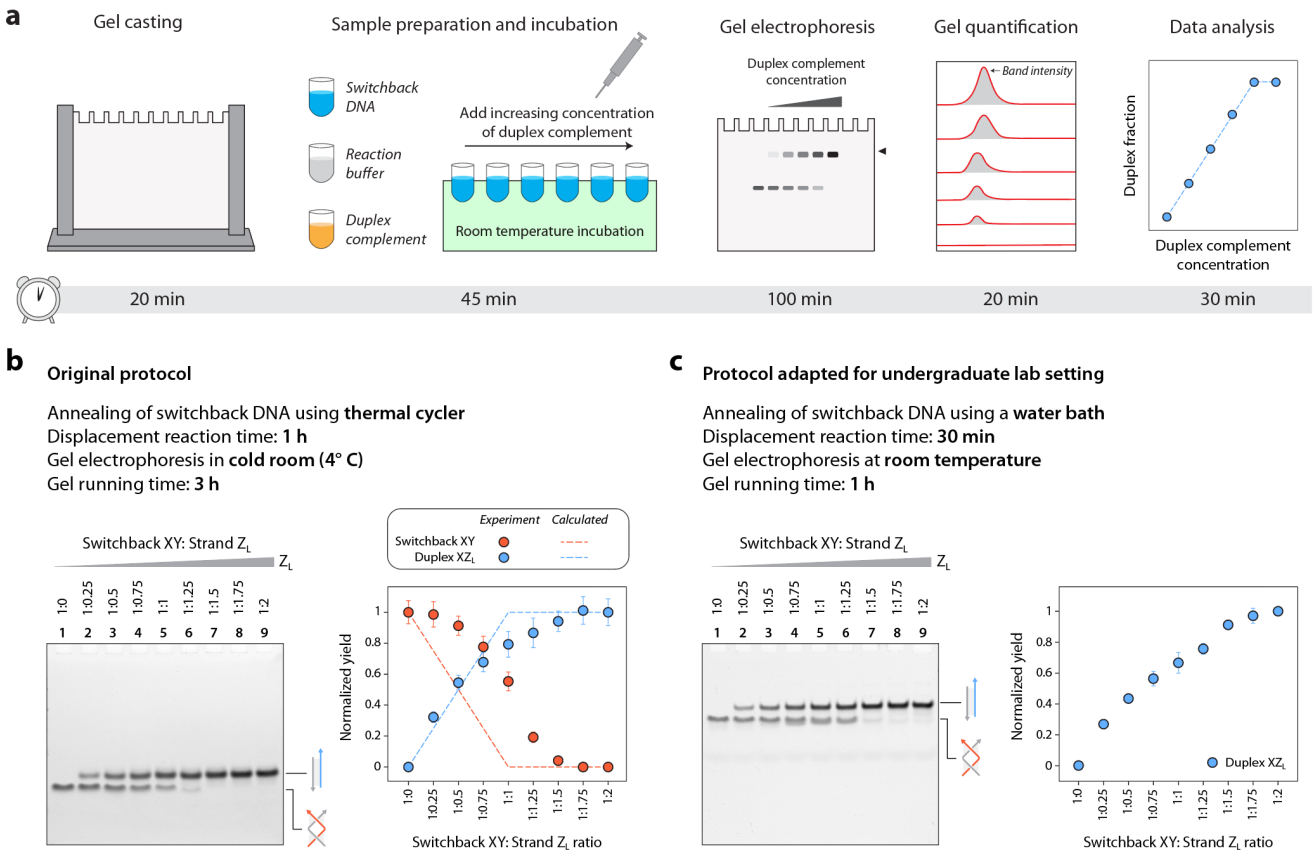

Fig. 3. Protocol and expected results. (a) Non-denaturing polyacrylamide gel casting, sample preparation, loading incubated samples on a non-denaturing polyacrylamide gel and running the gel for specific time, quantification of bands corresponding to duplex DNA in the gel image to estimate the yield of duplex product at various concentrations tested.

Estimated time durations for each step is shown below the illustration. (b) Addition of duplex complement to a pre-assembled switchback DNA causes displacement of one of the strands in the switchback DNA, resulting in duplex formation. Data reproduced from ref 14 with permission. (c) Experiment adapted for an undergraduate laboratory setting yielded results similar to previously published data shown in (b).

Representative results of experiments performed by undergraduate students in our laboratory are provided in **Figure 4**. Students were provided with the assembled switchback DNA motif, duplex complement DNA, and tris-acetate-EDTA buffer containing Mg^{2+} (10×), the buffer in which the structures are assembled (1×). Students performed the displacement assay at room temperature (on the bench), loaded the samples and ran them on an 18% non-denaturing polyacrylamide gel, stained the gels using GelRed, imaged the gels, quantified the bands, and analyzed the data. The plots created using data from the gels showed expected trends and were consistent between different students. Results obtained by the students were also similar to our previously published results.

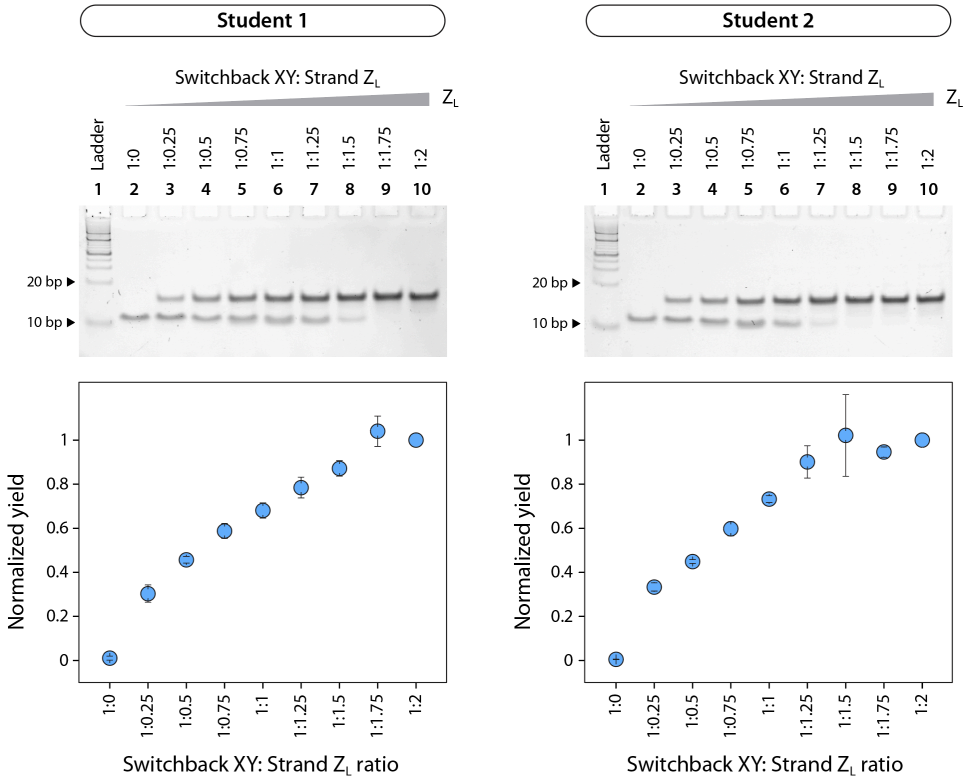

Figure 4. ***Representative results from experiments performed by undergraduate students.*** Students were given the manual in Supplementary Note 2 and were provided with annealed DNA complexes and reagents. Gel analysis was done using ImageLab and data plotted using Origin. Error bars are standard deviations from experiments performed in triplicates by each student.

DISCUSSION

The field of DNA nanotechnology has recently expanded to several new concepts, necessitating the development of new curricula to introduce the field to students at early educational levels. The laboratory experiment presented here is one such example that introduces a new DNA motif and demonstrates strand displacement, a key tool used in DNA nanotechnology. The experiment is most suited for undergraduate students who have learnt basic pipetting and gel electrophoresis techniques, such as third-year undergraduate students who have taken chemistry, biology, biochemistry, molecular biology, or genetics laboratory courses and have performed gel electrophoresis. We would like to note that we sometimes have first year undergraduate students who get trained in these skill sets and are able to perform gel electrophoresis on their own in a research lab setting. In the student manual provided with this article (**Supplementary Note 2**), each student performs the strand displacement experiment in triplicates (so did undergraduates in our lab, data provided in **Figure 4**). Depending on the resources available and any time constraints, the experiment can be performed in groups of three, with each student performing a replicate. The experiment uses equipment typically available in an undergraduate lab, and the DNA cost is minimal. The DNA strands required for this experiment can be ordered from companies such as Integrated DNA Technologies (IDT), and costs a total of ~$19 for all three strands when ordered at the 100 nmol scale. For the replicate experiment described here, we only use 0.33 nmoles for each strand of DNA per student. The remaining DNA strands can be stored frozen for many years and be used for several courses. Typical gel electrophoresis setups can accommodate up to four gels at a time, which allows this experiment to be performed by 20-30 students using 6-8 gel setups if done in parallel. Our method already uses the non-toxic intercalating dye GelRed for gel staining, making this experiment student-safe and does not require special waste disposal procedures. An alternative to reducing the overall time of the experiment is the use of commercially available pre-cast polyacrylamide gels (eg: from Millipore Sigma or ThermoFisher) but that option will increase the overall cost of the experiment.

The usefulness of this laboratory experiment extends beyond improving the undergraduate curriculum. It provides students an opportunity to understand scientific concepts in a new and emerging field of study and may motivate them to pursue research in related areas. Introducing the original research work related to the experiments performed in the undergraduate laboratory will also help in enhancing student learning. The gel analysis and post-lab exercises allow students to compare the two DNA structures, and can also help assess student learning in large undergraduate laboratories. In a research setting for undergraduate students such as ours, student evaluation is achieved through their presentations in weekly group meetings with constant feedback for improvement of laboratory skills, data analysis and interpretation, and accurate representation of data. Some key skills that this laboratory experiment provides students are pipetting skills, nucleic acid gel

electrophoresis and imaging, gel image quantification, and replicate data analysis to calculate average and error values. As part of a series of laboratory experiments on DNA nanotechnology for undergraduate students,[19,20] this experiment takes us a step closer to developing a collection of laboratory protocols for a DNA nanotechnology undergraduate laboratory course.

SUPPORTING INFORMATION

Materials and methods, notes for instructors, student instruction manual, worksheet for students.

CONFLICT OF INTEREST

The author declares no conflict of interest.

ACKNOWLEDGMENTS

Research reported in this publication was supported by the National Institutes of Health (NIH) through National Institute of General Medical Sciences (NIGMS) under award number R35GM150672 to A.R.C. We thank undergraduate students Leah Punnoose, Abigail Tsuji and Antoinette Mastrangelo for the experiments done in the lab following this protocol.

REFERENCES

(1) Xavier, P. L.; Chandrasekaran, A. R. DNA-Based Construction at the Nanoscale: Emerging Trends and Applications. *Nanotechnology* **2018**, *29* (6), 062001. https://doi.org/10.1088/1361-6528/aaa120.

(2) Chandrasekaran, A. R.; Anderson, N.; Kizer, M.; Halvorsen, K.; Wang, X. Beyond the Fold: Emerging Biological Applications of DNA Origami. *ChemBioChem* **2016**, *17* (12), 1081–1089. https://doi.org/10.1002/cbic.201600038.

(3) Fu, T. J.; Seeman, N. C. DNA Double-Crossover Molecules. *Biochemistry* **1993**, *32* (13), 3211–3220. https://doi.org/10.1021/bi00064a003.

(4) LaBean, T. H.; Yan, H.; Kopatsch, J.; Liu, F.; Winfree, E.; Reif, J. H.; Seeman, N. C. Construction, Analysis, Ligation, and Self-Assembly of DNA Triple Crossover Complexes. *J. Am. Chem. Soc.* **2000**, *122* (9), 1848–1860. https://doi.org/10.1021/ja993393e.

(5) Shen, Z.; Yan, H.; Wang, T.; Seeman, N. C. Paranemic Crossover DNA: A Generalized Holliday Structure with Applications in Nanotechnology. *J. Am. Chem. Soc.* **2004**, *126* (6), 1666–1674. https://doi.org/10.1021/ja038381e.

(6) He, Y.; Chen, Y.; Liu, H.; Ribbe, A. E.; Mao, C. Self-Assembly of Hexagonal DNA Two-Dimensional (2D) Arrays. *J. Am. Chem. Soc.* **2005**, *127* (35), 12202–12203. https://doi.org/10.1021/ja0541938.

(7) He, Y.; Tian, Y.; Ribbe, A. E.; Mao, C. Highly Connected Two-Dimensional Crystals of DNA Six-Point-Stars. *J. Am. Chem. Soc.* **2006**, *128* (50), 15978–15979. https://doi.org/10.1021/ja0665141.

(8) Chandrasekaran, A. R.; Wady, H.; Subramanian, H. K. K. Nucleic Acid Nanostructures for Chemical and Biological Sensing. *Small* **2016**, *12* (20), 2689–2700. https://doi.org/10.1002/smll.201503854.

(9) Simmel, F. C.; Yurke, B.; Singh, H. R. Principles and Applications of Nucleic Acid Strand Displacement Reactions. *Chem. Rev.* **2019**, *119* (10), 6326–6369. https://doi.org/10.1021/acs.chemrev.8b00580.

(10) Zhang, D. Y.; Seelig, G. Dynamic DNA Nanotechnology Using Strand-Displacement Reactions. *Nature Chem* **2011**, *3* (2), 103–113. https://doi.org/10.1038/nchem.957.

(11) Xing, Y.; Yang, Z.; Liu, D. A Responsive Hidden Toehold To Enable Controllable DNA Strand Displacement Reactions. *Angewandte Chemie International Edition* **2011**, *50* (50), 11934–11936. https://doi.org/10.1002/anie.201105923.

(12) Faheem, H.; Mathivanan, J.; Talbot, H.; Zeghal, H.; Vangaveti, S.; Sheng, J.; Chen, A. A.; Chandrasekaran, A. R. Toehold Clipping: A Mechanism for Remote Control of DNA Strand Displacement. *Nucleic Acids Research* **2023**, *51* (8), 4055–4063. https://doi.org/10.1093/nar/gkac1152.

(13) Wang, G. A.; Wu, X.; Chen, F.; Shen, C.; Yang, Q.; Li, F. Toehold-Exchange-Based Activation of Aptamer Switches Enables High Thermal Robustness and Programmability. *J. Am. Chem. Soc.* **2023**, *145* (5), 2750–2753. https://doi.org/10.1021/jacs.2c10928.

(14) Madhanagopal, B. R.; Talbot, H.; Rodriguez, A.; Louis, J. M.; Zeghal, H.; Vangaveti, S.; Reddy, K.; Chandrasekaran, A. R. The Unusual Structural Properties and Potential Biological Relevance of Switchback DNA. *Nat Commun* **2024**, *15* (1), 6636. https://doi.org/10.1038/s41467-024-50348-3.

(15) Shafirovich, V.; Singh, C.; Geacintov, N. E. Photoinduced Oxidative DNA Damage Revealed by an Agarose Gel Nicking Assay: A Biophysical Chemistry Laboratory Experiment. *J. Chem. Educ.* **2003**, *80* (11), 1297. https://doi.org/10.1021/ed080p1297.

(16) Flynn-Charlebois, A.; Burns, J.; Chapelliquen, S.; Sanmartino, H. An Undergraduate Investigation into the 10-23 DNA Enzyme That Cleaves RNA: DNA Can Cut It in the Biochemistry Laboratory. *J. Chem. Educ.* **2011**, *88* (2), 226–228. https://doi.org/10.1021/ed100607k.

(17) Carter, J.; Petersen, B. P.; Printz, S. A.; Sorey, T. L.; Kroll, T. T. Quantitative Application for SDS–PAGE in a Biochemistry Lab. *J. Chem. Educ.* **2013**, *90* (9), 1255–1256. https://doi.org/10.1021/ed300390j.

(18) Hormann, J.; Streller, S.; Kulak, N. Synthesis and Evaluation of Artificial DNA Scissors: An Interdisciplinary Undergraduate Experiment. *J. Chem. Educ.* **2018**, *95* (10), 1848–1855. https://doi.org/10.1021/acs.jchemed.7b00662.

(19) Chandrasekaran, A. R. DNA Nanotechnology in the Undergraduate Laboratory: Electrophoretic Analysis of DNA Nanostructure Biostability. *J. Chem. Educ.* **2023**, *100* (1), 316–320. https://doi.org/10.1021/acs.jchemed.2c00656.

(20) Abraham Punnoose, J.; Halvorsen, K.; Chandrasekaran, A. R. DNA Nanotechnology in the Undergraduate Laboratory: Analysis of Molecular Topology Using DNA Nanoswitches. *J. Chem. Educ.* **2020**. https://doi.org/10.1021/acs.jchemed.9b01185.

(21) Mathur, D.; Medintz, I. L. Analyzing DNA Nanotechnology: A Call to Arms For The Analytical Chemistry Community. *Anal. Chem.* **2017**, *89* (5), 2646–2663. https://doi.org/10.1021/acs.analchem.6b04033.

(22) Neyra, K.; Everson, H. R.; Mathur, D. Dominant Analytical Techniques in DNA Nanotechnology for Various Applications. *Anal. Chem.* **2024**, *96* (9), 3687–3697. https://doi.org/10.1021/acs.analchem.3c04176.

(23) Beshay, P. E.; Kucinic, A.; Wile, N.; Halley, P.; Rosiers, L. D.; Chowdhury, A.; Hall, J. L.; Castro, C. E.; Hudoba, M. W. Translating DNA Origami Nanotechnology to Middle School, High School, and Undergraduate Laboratories.

(24) He, Z.; Ding, S.; Wang, L.; Wang, G.; Liang, X.; Takarada, T.; Maeda, M. Introducing DNA Nanosensor to Undergraduate Students: Rapid Non-Cross-Linking Aggregation of DNA-Functionalized Gold Nanoparticles for Colorimetric DNA Assay. *J. Chem. Educ.* **2021**, *98* (11), 3553–3559. https://doi.org/10.1021/acs.jchemed.1c00605.

(25) Petrosko, S. H.; Coleman, B. D.; Drout, R. J.; Schultz, J. D.; Mirkin, C. A. Spherical Nucleic Acids: Integrating Nanotechnology Concepts into General Chemistry Curricula. *J. Chem. Educ.* **2021**, *98* (10), 3090–3099. https://doi.org/10.1021/acs.jchemed.1c00441.

(26) Loretan, C.; Müller, A. Nano, Bits, and Feynman's Dream: There's Plenty of Room at the (Molecular) Bottom. *J. Chem. Educ.* **2023**, *100* (3), 1366–1370. https://doi.org/10.1021/acs.jchemed.2c00941.

(27) Chandler, M.; Shevchenko, O.; Vivero-Escoto, J. L.; Striplin, C. D.; Afonin, K. A. DNA-Templated Synthesis of Fluorescent Silver Nanoclusters. *J. Chem. Educ.* **2020**, *97* (7), 1992–1996. https://doi.org/10.1021/acs.jchemed.0c00158.

(28) Zhang, C.; Zhao, J.; Lu, B.; Seeman, N. C.; Sha, R.; Noinaj, N.; Mao, C. Engineering DNA Crystals toward Studying DNA–Guest Molecule Interactions. *J. Am. Chem. Soc.* **2023**, *145* (8), 4853–4859. https://doi.org/10.1021/jacs.3c00081.

(29) Drew, H. R.; Wing, R. M.; Takano, T.; Broka, C.; Tanaka, S.; Itakura, K.; Dickerson, R. E. Structure of a B-DNA Dodecamer: Conformation and Dynamics. *Proceedings of the National Academy of Sciences* **1981**, *78* (4), 2179–2183. https://doi.org/10.1073/pnas.78.4.2179.

(30) Tian, C.; Zhang, C.; Li, X.; Li, Y.; Wang, G.; Mao, C. Artificial, Parallel, Left-Handed DNA Helices. *J. Am. Chem. Soc.* **2012**, *134* (50), 20273–20275. https://doi.org/10.1021/ja309908s.

(31) Yurke, B.; Turberfield, A. J.; Mills Jr, A. P.; Simmel, F. C.; Neumann, J. L. A DNA-Fuelled Molecular Machine Made of DNA. *Nature* **2000**, *406* (6796), 605–608. https://doi.org/10.1038/35020524.