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Laboratory Experiment

DNA Nanotechnology in the Undergraduate Laboratory: Analysis of Molecular Topology Using DNA Nanoswitches

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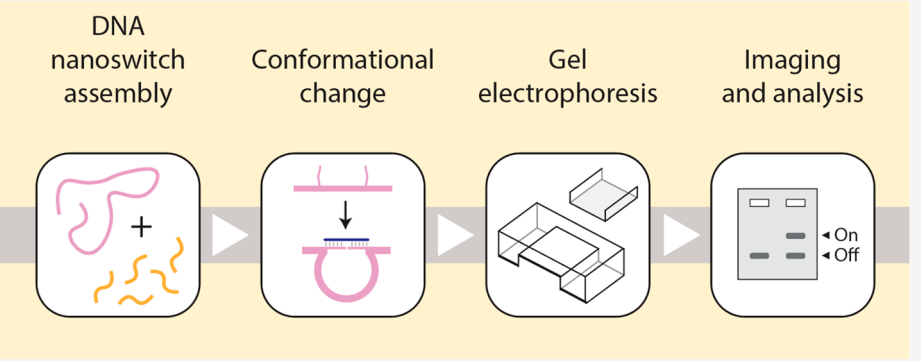

ABSTRACT: There is a disconnect between the cutting-edge research done in academic laboratories, such as nanotechnology, and what is taught in undergraduate laboratories. In the current undergraduate curriculum, very few students get a chance to do hands-on experiments in nanotechnology-related fields, most of which are through selective undergraduate research programs. In most cases, complicated synthesis procedures, expensive reagents, and the requirement for specific instrumentation prevent broad adaptation of nanotechnology-based experiments to laboratory courses. DNA, being a nanoscale molecule, has recently been used in bottom-up nanotechnology with applications in sensing, nanorobotics, and computing. In this article, we propose a simple experiment involving the synthesis of a DNA nanoswitch that can change its shape from a linear "off" state to a looped "on" state in the presence of a target DNA molecule. The experiment also demonstrates the programmable topology of the looped state of the nanoswitch and its effect on gel migration. The experiment is easy to adapt in an undergraduate laboratory, requires only agarose gel electrophoresis, has a minimal setup cost for materials, and can be completed in a 3 hour time frame.

KEYWORDS: *Upper-Division Undergraduate, Biochemistry, Interdisciplinary/Multidisciplinary, Hands-On Learning/Manipulatives, Electrophoresis, Molecular Properties/Structure, Nanotechnology, Nucleic Acids/DNA/RNA, Undergraduate Research, Biophysical Chemistry*

INTRODUCTION

As part of the undergraduate education program, laboratory courses have found as much importance as lectures and presentations.[1,2] Laboratory courses complement existing lectures and enhance students' education beyond just theoretical knowledge. Emphasis on laboratory courses has seen a steady rise over the past few decades,[2] with most basic courses in chemistry, biology, and physics accompanied by a lab course. In some cases, already existing fields (e.g., forensic science)[3,4] have been updated with newly proposed laboratory experiments for students.[5] This emphasis has only recently been devoted to newer fields such as nanotechnology, which are more research-based compared with standard curricula. Undergraduate students can benefit from engagement in research laboratories, and some of these skills can be developed by introducing new laboratory exercises to be part of existing courses.[6] According to the National Academies and the American Association for the Advancement of Science, early student engagement in project-based laboratory exercises can improve the level of student participation in research.[7,8] New laboratory exercises that mirror current developments in research are also needed because of the increasingly interdisciplinary nature of research within the chemistry community and other communities.[9]

The field of DNA nanotechnology involves the construction of nanoscale shapes and devices using DNA.[10] While structural DNA nanotechnology deals with the construction of different types of architectures, dynamic DNA nanotechnology

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predominantly involves reconfigurable devices that can change shapes upon sensing a biological (e.g., nucleic acid) or chemical (e.g., pH) stimulus.[11,12] Many academic laboratories are currently doing research in this field, but there are very few courses designed on the topic for undergraduate students. The importance of discussing self-assembly and nanotechnology to students has been presented in this *Journal* before.[13] Nanotechnology-based experiments have previously been proposed for the general chemistry lab courses.[14] These proposals are typically materials-science-oriented, with heavy focus on inorganic nanomaterials. Here we propose a lab experiment based on a reconfigurable DNA nanoswitch for third-year biology or chemistry undergraduate students, specifically those in biochemistry and genetics lab courses where students already run gels (as in our own institution). This experiment will address two teaching concepts: (1) experimental demonstration of the dynamic DNA nanotechnology concept used for biosensing and (2) simple validation of topological changes in DNA using gel electrophoresis. Laboratory exercises designed to mimic research experiences often fall under the "expository instruction" category, where the outcome is predetermined or known to both the instructor and the students.[15] However, these experiments not only help students understand what they have learned in theory[16] but also improve their hands-on skill set in the laboratory (e.g., pipetting and electrophoresis).[17,18] Some related experiments described in this *Journal* include DNA topology analysis based on supercoiled DNA and the enzyme topoisomerase I.[19] Lab experiments based on gel electrophoresis have also been reported.[20,21] Other DNA-related experiments previously discussed in this *Journal* include observation of DNA molecules using fluorescence microscopy and atomic force microscopy (AFM).[22] However, these are complicated techniques to adapt for a larger chemistry or biology class, especially with instruments like AFM that are sensitive to noise and vibrations in the room.[23] In this context, the experiment we propose will be an introductory peek into DNA nanotechnology that will help students understand characteristics of DNA devices. In addition, this exercise will also help them understand gel electrophoretic patterns based on structure or topology, in contrast to the typical migration based on molecular weight.[24] With some investment in component DNA strands, this experiment can be executed using equipment already available in a typical biology or biochemistry laboratory and does not require expensive instrumentation or microscopy to read out and analyze results.

DNA NANOSWITCHES: DESIGN AND WORKING PRINCIPLES

Apart from being well-known as the genetic material, DNA is also an inherently nanoscale molecule that is used for bottom-up fabrication of nanostructures, where molecules are assembled together to form nanometer-scale objects.[10] DNA-based nanostructures can be planar or three-dimensional objects, multidimensional arrays, and programmable devices that can respond to external stimuli.[25] DNA nano-objects have found applications in drug delivery, while periodic lattices and frameworks made from DNA are useful as scaffolds to align guest molecules such as proteins and nanoparticles.[10] Devices made from DNA are useful in biosensing applications, where the presence of a target molecule or biomarker causes a conformational change in the DNA device, leading to a signal readout.[26] One such example is the DNA nanoswitch,[27] constructed on the basis of the principles of DNA origami, where a long strand of DNA scaffold is folded into any desired shape using short complementary strands.[28] The DNA nanoswitch is a linear double-stranded DNA consisting of a single-stranded M13 scaffold (7249 nucleotides) and short complementary strands (49−60 nucleotides) called staples or, in this case, "backbone oligonucleotides" (Figure 1). Although

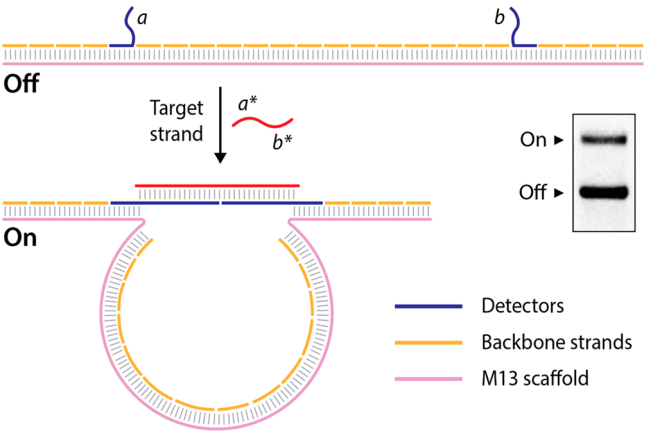

Figure 1. Design and working principle of the DNA nanoswitch. The "off" state of the nanoswitch is a duplex with two single-stranded extensions (detectors) that are partially complementary to parts of a target DNA strand. Target recognition and binding cause the formation of the looped "on" state. The off and on states of the nanoswitch can be easily identified on an agarose gel (inset).

any long single-stranded DNA can be used, M13 DNA is a popular choice as a scaffold in the field of DNA origami since it is commercially available and cheap. Two of the backbone oligonucleotides can be modified to have single-stranded extensions ("detectors") that are partly complementary to a target oligonucleotide. Once the target is added, it binds to both detectors of the nanoswitch and reconfigures it to form a loop. We call this linear form the "off" state and the looped configuration the "on" state of the DNA nanoswitch. The presence of the target can be identified by running the samples on an agarose gel, as the linear (off) and looped (on) states of the nanoswitch migrate differently (Figure 1 inset). These DNA nanoswitches have previously been used for single-molecule studies[29] and biomolecular analysis[30] and in rewritable memory[31,32] and biosensing.[27,33] Since the readout of the nanoswitch is topology-based, we use the programmable nature of the nanoswitch to create a laboratory experiment to analyze molecular topology and gel migration.

HAZARDS

To reduce the risk of burns, students should wear personal protective equipment and use heat-proof mitts or tongs while handling the hot flask of melted agarose. To avoid fire hazards, students should turn off and unplug the hot plate after use. To avoid electric shocks, students should use care when plugging the gel boxes into the power supply.

RESULTS AND DISCUSSION

The visual readout of the two states of the DNA nanoswitch is a simple system to study topological changes of DNA-based structures and the effect of topology on gel migration (instead of the "usual" molecular-weight-based separation). The topology of the looped state can be further programmed by

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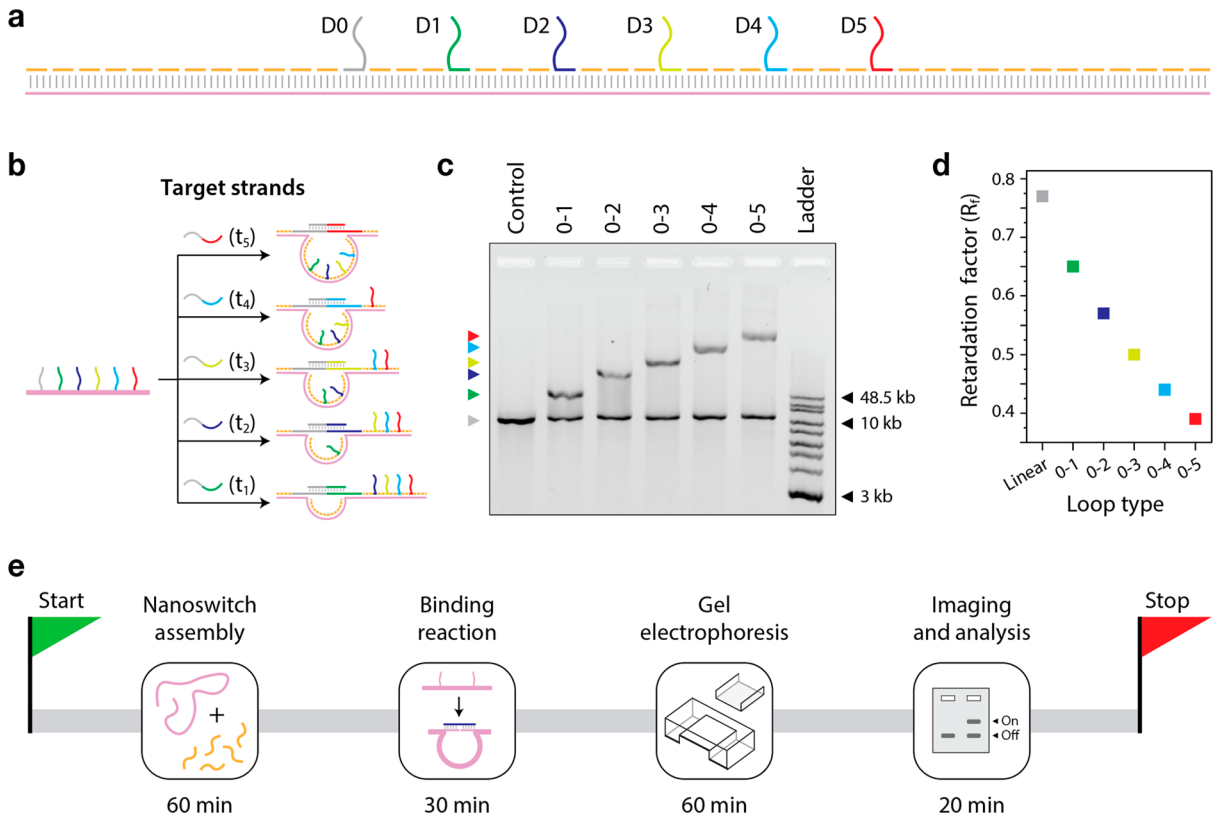

Figure 2. Analysis of nanoswitch reconfiguration and loop size vs gel migration. (a) Design of the multidetector nanoswitch that can respond to five different targets. (b) Different target DNA strands activate the formation of different loops. (c) Nanoswitches forming different loops migrate differently on an agarose gel, providing unique signals. (d) Measured retardation factors (R_f) for different loop sizes. (e) Workflow for a lab-based experiment for an undergraduate course.

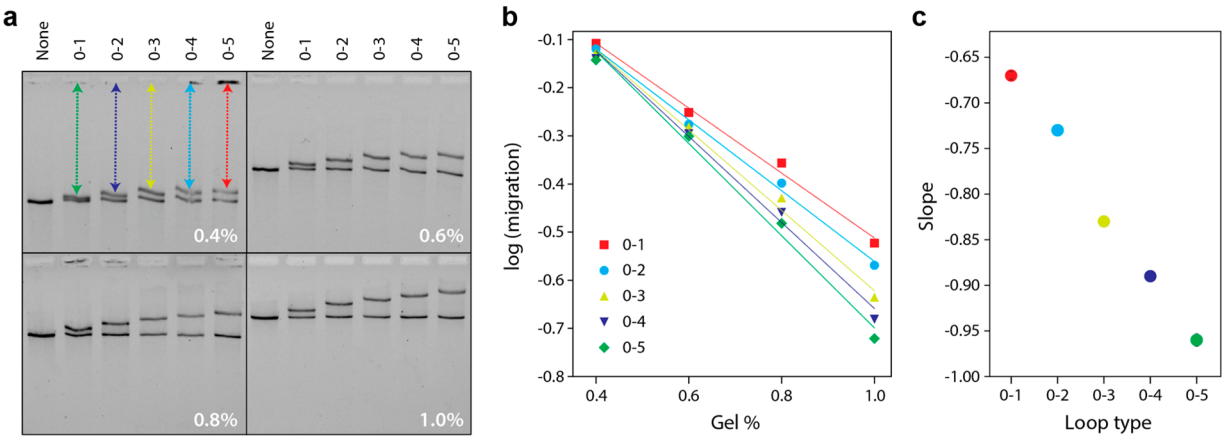

Figure 3. Ferguson plot analysis of looped DNA nanoswitches. (a) Migration of different loop sizes in various agarose gel percentages. (b) Ferguson plot of the migration vs gel percentage. (c) Slope of the Ferguson plot provides the frictional coefficient for each looped species.

changing the detector positions on the scaffold DNA, which in turn changes the loop size (Figure 2a). To systematically study the effect of loop size on gel migration, we redesigned the nanoswitch to form five different loops of various sizes. In the design shown in Figure 2a, the nanoswitch has six detector strands that can be triggered by different target DNA strands. The detectors are spaced ~600 bp apart and form five different loop sizes upon binding specific target strands (Figure 2b). The first detector (D0) is complementary to one half of all five target DNA strands. The other half of each target strand is complementary to one of the other five detectors (D1−D5). Each loop has a unique migration pattern on the gel based on the loop size (Figure 2c). This topology-based gel migration can also be a discussion point when taught in an undergraduate laboratory. In routine gel shift experiments, band separation is based on molecular weight (as shown by the ladder on the right lane). The migration of the DNA ladder can be compared to migration of the different nanoswitches, which have the same molecular weight but different loop sizes. Bigger loops migrate slower in the gel because of the larger molecular radius. The mobility of each looped species is characterized by its retardation factor (R_f) (Figure 2d). This experiment

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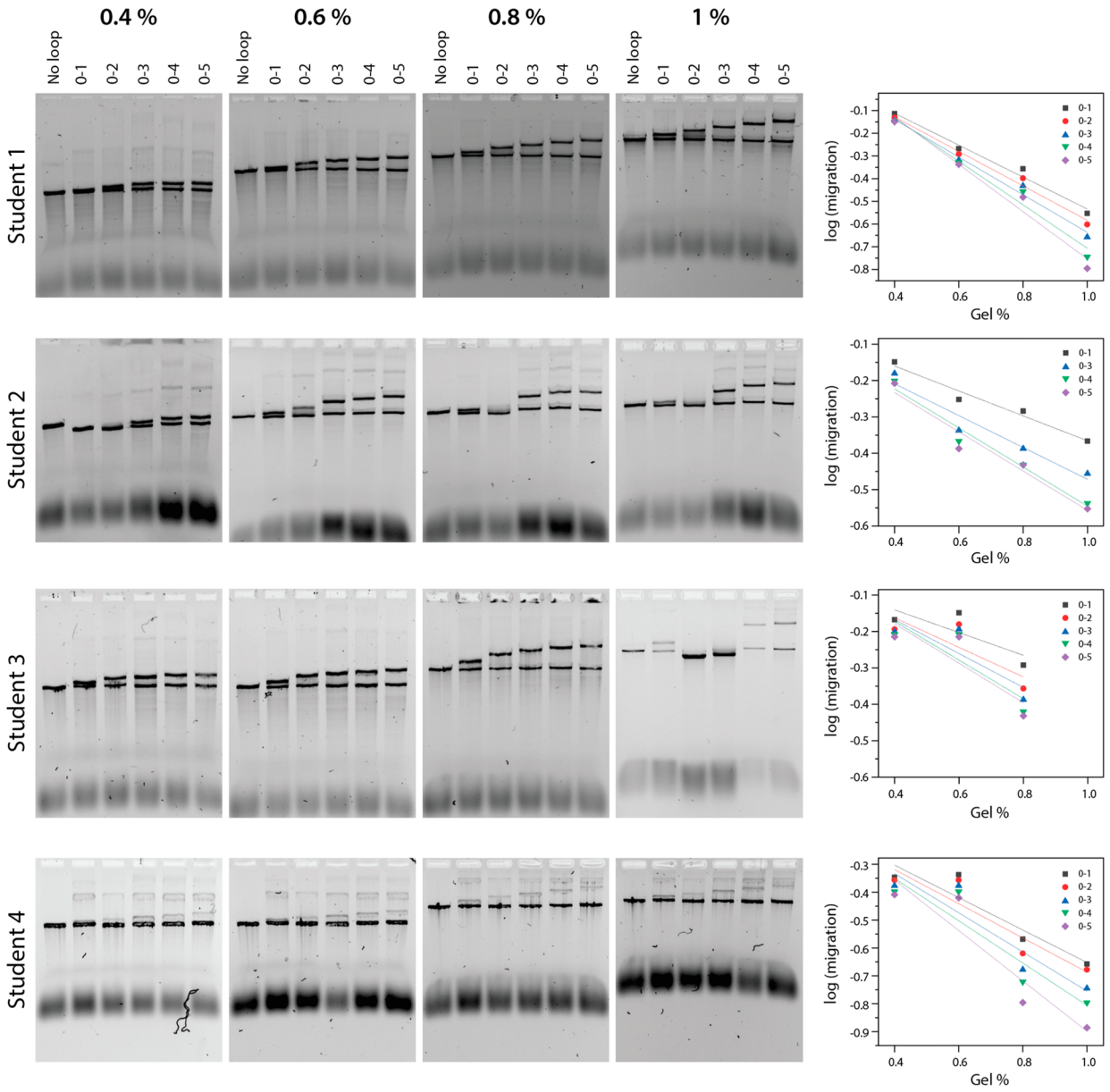

Figure 4. Representative results from experiments performed by undergraduate students. Students were given the manual in Supplementary Note 2. All of the students finished the lab work within 3 h as proposed in our experiment. Gel analysis was done later using ImageJ, and trends were plotted using Origin. Students can use other software such as Microsoft Excel to plot the results. If a gel by a student is inconsistent, student groups can analyze the other three gels to obtain the Ferguson plot (e.g., results from Student 3).

involves only a mixing step and incubation of the nanoswitch with the target strands, followed by agarose gel electrophoresis to read out the results (Figure 2e).

The lab experiment can be extended by the instructor to include a Ferguson plot for calculating friction coefficients of the looped structures. The target-bound nanoswitches, despite having the same molecular weight, show unique migration patterns on a gel due to the difference in topology (loop size). The electrophoretic mobility for each of these looped nanoswitches can be analyzed using a Ferguson plot[34] (Figure 3a,b), where the migration of looped DNA is examined on agarose gels of varying percentage (0.4% to 1%). This plot is used to estimate the frictional coefficient of the looped species as given by the slope of the line (Figure 3c and Table S1 in the Supporting Information). Bigger loop size indicates an increase in effective molecular radius, thus causing retardation in the gel. This trend is defined by the change in loop size between the different nanoswitches (roughly 600, 1200, 1800, 2400, and 3000 base pairs). Students learn about the effect of molecular topology on gel migration and compare it to sized-based gel separation. This experiment also indicates how DNA nanostructures and DNA structures in general (e.g., circular vs linear DNA) are characterized.

■ ADAPTATION TO THE UNDERGRADUATE LABORATORY

Exposure of students to DNA nanotechnology research predominantly occurs through their involvement as interns in research laboratories in the field. As part of their regular curriculum, undergraduates do not typically have a course in DNA nanotechnology, except in a few universities. The nanoswitch-based topological analysis we propose is easily

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adaptable to a chemistry, biology, or biochemistry laboratory. Similar to typical lab experiments, the nanoswitch materials can be prepared beforehand by the teaching instructor and provided to students (Supplementary Note 1 in the Supporting Information: Reagent Preparation for Instructor). Students can assemble the nanoswitch, add target strands, incubate, and execute gel electrophoresis for the samples. Students can analyze the gels and measure R_f values as a postlab exercise. We modified previously published experiments to fit a typical laboratory course timing (~3 hours) and provide a detailed step-by-step protocol (Supplementary Note 2: Student Instruction Manual). We have used this protocol as a training experiment for undergraduate students in our research laboratory (typically sophomores, juniors, and seniors). Students were provided with the protocol in Supplementary Note 2 and the materials, and all of the students completed the exercise within the proposed time. The Ferguson plots created using data from the gels showed the expected trends, and these were consistent among different students. We have included representative results of the experiments performed by undergraduate students in Figure 4. These results suggest that the DNA nanoswitch system is a feasible platform for adaptation to undergraduate laboratories, not just in a topological context but also in potential biosensing[33] and biophysical applications.[29] Instructors can adapt the nanoswitch for other nucleic acid targets or to explain different concepts using guidelines in our earlier work.[33,35]

From the outcome of these experiments, we list here a few considerations for adaptation to the undergraduate laboratory, as well as steps that might not be successful:

(1) The importance of the mixing step should be emphasized. In some cases, students may forget to add the target DNA, which will result in no loop formation in that lane. Instructors can run a control gel from the material prepared to keep as a reference.

(2) The experimental design allows data from different students to be combined. Thus, if one of the gels does not provide expected data (or other reasons), there will still be three data points that can be used to fit the trend.

(3) Overheating of agarose solutions and evaporation can result in a higher gel percentage than prescribed, resulting in altered migration.

One additional parameter for adaptation to undergraduate laboratories is the cost of the materials. The starting materials for the DNA nanoswitch are tabulated in Table S2. A startup investment to buy the DNA scaffold and backbone strands at the lowest synthesis scale is approximately $450 (purchased from IDT). Combined with other reagents such as agarose and enzymes, the total setup cost is approximately $1000. These materials, including the DNA, can be used for more than 5 years and thus have an estimated average cost of $200 per year (or $100 per semester). To offset the initial trial phase for community colleges that cannot afford this, our lab is also open to providing aliquots of the backbone oligonucleotide mix to programs or universities on reasonable request. These materials can be used for many years if kept frozen, and the cost for the experiments described here is only ~3 cents per lane, with the most expensive reagent being the agarose. Thus, the proposed experiment may suit the budget of most undergraduate laboratory courses. In addition, this experiment uses GelRed nucleic acid stain, which is nontoxic, as opposed to ethidium bromide staining. The experiment is safe for undergraduate students, with no requirements for specific waste disposal once the experiments are finished (gels can be discarded in regular trash and buffers can be discarded in sinks).

CONCLUSION

In this article, we have described how a simple DNA nanostructure can be used to analyze molecular topology and gel migration in an undergraduate laboratory setting. The nanostructure is easy to assemble, and the mixtures can be frozen for long-term use. Concepts discussed in this laboratory experiment can be extended to other DNA nanostructures as well as complement any existing courses or lectures on DNA nanotechnology.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available at https://pubs.acs.org/doi/10.1021/acs.jchemed.9b01185.

Reagent list; detailed experimental procedures; notes for instructors (figures in instructor notes are labeled I#); student instruction manual (figures in students' manual are labeled S#); worksheet for students (PDF, DOCX)

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Notes

The authors declare the following competing financial interest(s): A.R.C. and K.H. are inventors on patents and patent applications covering aspects of the DNA nanoswitch design and applications.

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REFERENCES

(1) Round, J.; Lom, B. In Situ Teaching: Fusing Labs & Lectures in Undergraduate Science Courses to Enhance Immersion in Scientific Research. *J. Undergrad. Neurosci. Educ.* **2015**, *13* (3), A206−A214.

(2) Feisel, L. D.; Rosa, A. J. The Role of the Laboratory in Undergraduate Engineering Education. *Journal of Engineering Education* **2005**, *94* (1), 121−130.

(3) Kaplan, L. J. Forensic Science: Crime in the Chemistry Curriculum. *J. Chem. Educ.* **1993**, *70* (7), 574.
(4) Clark, M. J.; Keegel, J. F. Chemistry in the Crime Lab. A Forensic Science Course. *J. Chem. Educ.* **1977**, *54* (1), 38.
(5) Cresswell, S. L.; Loughlin, W. A. An Interdisciplinary Guided Inquiry Laboratory for First Year Undergraduate Forensic Science Students. *J. Chem. Educ.* **2015**, *92* (10), 1730−1735.
(6) Kloser, M. J.; Brownell, S. E.; Chiariello, N. R.; Fukami, T. Integrating Teaching and Research in Undergraduate Biology Laboratory Education. *PLoS Biol.* **2011**, *9* (11), No. e1001174.
(7) National Research Council. *BIO2010: Transforming Undergraduate Education for Future Research Biologists*; The National Academies Press: Washington, DC, 2003. DOI: 10.17226/10497
(8) Woodin, T.; Carter, V. C.; Fletcher, L. Vision and Change in Biology Undergraduate Education, A Call for Action—Initial Responses. *LSE* **2010**, *9* (2), 71−73.
(9) Porter, L. A. Chemical Nanotechnology: A Liberal Arts Approach to a Basic Course in Emerging Interdisciplinary Science and Technology. *J. Chem. Educ.* **2007**, *84* (2), 259.
(10) Seeman, N. C. DNA in a Material World. *Nature* **2003**, *421* (6921), 427−431.
(11) DeLuca, M.; Shi, Z.; Castro, C. E.; Arya, G. Dynamic DNA Nanotechnology: Toward Functional Nanoscale Devices. *Nanoscale Horiz.* **2020**, *5*, 182−201.
(12) Xavier, P. L.; Chandrasekaran, A. R. DNA-Based Construction at the Nanoscale: Emerging Trends and Applications. *Nanotechnology* **2018**, *29* (6), 062001.
(13) Bagaria, H. G.; Dean, M. R.; Nichol, C. A.; Wong, M. S. Self-Assembly and Nanotechnology: Real-Time, Hands-On, and Safe Experiments for K-12 Students. *J. Chem. Educ.* **2011**, *88* (5), 609−614.
(14) Winkelmann, K. Nanotechnology for Freshmen. *ACS Symp. Ser.* **2010**, *1010*, 135−154.
(15) Domin, D. S. A Review of Laboratory Instruction Styles. *J. Chem. Educ.* **1999**, *76* (4), 543.
(16) Grisham, J. Students Rate Science, Hands-on Learning High. *Chem. Eng. News* **1997**, *75* (18), 62.
(17) Hudson-Smith, N. V.; Cahill, M. S.; Klein, N. D.; Krause, M. O. P.; Haynes, C. L. A Macroscale Model for Hands-On Activities Demonstrating Transmission Electron Microscopy. *J. Chem. Educ.* **2019**, *96* (7), 1377−1382.
(18) Amaris, Z. N.; Freitas, D. N.; Mac, K.; Gerner, K. T.; Nameth, C.; Wheeler, K. E. Nanoparticle Synthesis, Characterization, and Ecotoxicity: A Research-Based Set of Laboratory Experiments for a General Chemistry Course. *J. Chem. Educ.* **2017**, *94* (12), 1939−1945.
(19) Keck, M. V. DNA Topology Analysis in the Undergraduate Biochemistry Laboratory. *J. Chem. Educ.* **2000**, *77* (11), 1471.
(20) 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.
(21) 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.
(22) Ito, T. Observation of DNA Molecules Using Fluorescence Microscopy and Atomic Force Microscopy. *J. Chem. Educ.* **2008**, *85* (5), 680.
(23) Blonder, R.; Joselevich, E.; Cohen, S. R. Atomic Force Microscopy: Opening the Teaching Laboratory to the Nanoworld. *J. Chem. Educ.* **2010**, *87* (12), 1290−1293.
(24) Michieletto, D. The Role of Topology in DNA Gel Electrophoresis. In *Topological Interactions in Ring Polymers*; Michieletto, D., Ed.; Springer: Cham, Switzerland, 2016; pp 95−120. DOI: 10.1007/978-3-319-41042-5_6
(25) Chen, Y.-J.; Groves, B.; Muscat, R. A.; Seelig, G. DNA Nanotechnology from the Test Tube to the Cell. *Nat. Nanotechnol.* **2015**, *10* (9), 748−760.
(26) Xiao, M.; Lai, W.; Man, T.; Chang, B.; Li, L.; Chandrasekaran, A. R.; Pei, H. Rationally Engineered Nucleic Acid Architectures for Biosensing Applications. *Chem. Rev.* **2019**, *119* (22), 11631−11717.
(27) Chandrasekaran, A. R.; Zavala, J.; Halvorsen, K. Programmable DNA Nanoswitches for Detection of Nucleic Acid Sequences. *ACS Sens.* **2016**, *1* (2), 120−123.
(28) Rothemund, P. W. K. Folding DNA to Create Nanoscale Shapes and Patterns. *Nature* **2006**, *440* (7082), 297−302.
(29) Halvorsen, K.; Schaak, D.; Wong, W. P. Nanoengineering a Single-Molecule Mechanical Switch Using DNA Self-Assembly. *Nanotechnology* **2011**, *22* (49), 494005.
(30) Koussa, M. A.; Halvorsen, K.; Ward, A.; Wong, W. P. DNA Nanoswitches: A Quantitative Platform for Gel-Based Biomolecular Interaction Analysis. *Nat. Methods* **2015**, *12* (2), 123−126.
(31) Chandrasekaran, A. R.; Levchenko, O.; Patel, D. S.; MacIsaac, M.; Halvorsen, K. Addressable Configurations of DNA Nanostructures for Rewritable Memory. *Nucleic Acids Res.* **2017**, *45* (19), 11459−11465.
(32) Chandrasekaran, A. R.; Abraham Punnoose, J.; Valsangkar, V.; Sheng, J.; Halvorsen, K. Integration of a Photocleavable Element into DNA Nanoswitches. *Chem. Commun.* **2019**, *55* (46), 6587−6590.
(33) Chandrasekaran, A. R.; MacIsaac, M.; Dey, P.; Levchenko, O.; Zhou, L.; Andres, M.; Dey, B. K.; Halvorsen, K. Cellular MicroRNA Detection with MiRacles: MicroRNA- Activated Conditional Looping of Engineered Switches. *Sci. Adv.* **2019**, *5* (3), No. eaau9443.
(34) Rodbard, D.; Chrambach, A. Estimation of Molecular Radius, Free Mobility, and Valence Using Polyacrylamide Gel Electrophoresis. *Anal. Biochem.* **1971**, *40* (1), 95−134.
(35) Chandrasekaran, A. R.; Dey, B. K.; Halvorsen, K. How to Perform MiRacles: A Step-by-Step MicroRNA Detection Protocol Using DNA Nanoswitches. *Curr. Protoc. Mol. Biol.* **2020**, *130* (1), No. e114.

https://dx.doi.org/10.1021/acs.jchemed.9b01185
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