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Laboratory Experiment

# DNA Nanotechnology in the Undergraduate Laboratory: Electrophoretic Analysis of DNA Nanostructure Biostability

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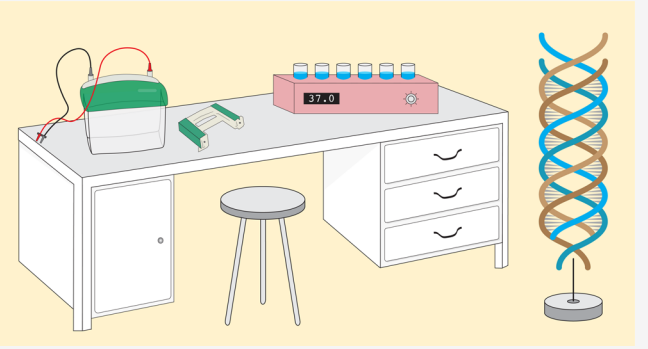
**ABSTRACT:** The field of DNA nanotechnology has grown rapidly in the past decade and has expanded to multiple laboratories. While lectures in DNA nanotechnology have been introduced in some institutions, laboratory components at the undergraduate level are still lacking. Undergraduate students predominantly learn about DNA nanotechnology through their involvement as interns in research laboratories. The DNA nanostructure biostability analysis experiment presented here can be used as a hands-on introductory laboratory exercise for discussing concepts in DNA nanotechnology in an undergraduate setting. This experiment discusses biostability, gel electrophoresis and quantitative analysis of nuclease degradation of a model DNA nanostructure, the paranemic crossover (PX) DNA motif. The experiment can be performed in a chemistry, biology, or biochemistry laboratory with minimal costs and can be adapted in undergraduate institutions using the instructor and student manuals provided here. Laboratory courses based on cutting edge research not only provide students a direct hands-on approach to the subject, but also can increase undergraduate student participation in research. Moreover, laboratory courses that reflect the increasingly multidisciplinary nature of research add value to undergraduate education.




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



## INTRODUCTION

DNA nanotechnology involves the construction of nanoscale shapes and structures using DNA.[1,2] DNA nanostructures have been used in biosensing, in molecular computation, in biomolecular analysis, as structural scaffolds, and in drug delivery.[3−5] For use in biological applications, one important factor is the stability of DNA nanostructures in physiological conditions. Several recent works have addressed the biostability and nuclease resistance of DNA nanostructures to make them better suited for in vivo applications.[6] Some of the methods to enhance the biostability of DNA nanostructures include design of multiple crossovers within the structures,[7,8] chemical ligation[9] and cross-linking[10] of component strands within DNA nanostructures, use of chemically modified DNA for construction,[11] and protective coatings such as polymers,[12] proteins[13] and lipids.[14] For analyzing nuclease degradation profiles of DNA nanostructures, different methods such as gel electrophoresis, fluorescence, real time atomic force microscopy (AFM), and transmission electron microscopy (TEM) have been used.[6] Among these, gel electrophoresis has been one of the most commonly used techniques to evaluate nuclease degradation. Here, I describe a laboratory experiment for undergraduate students to analyze the nuclease resistance of synthetic DNA motifs.

This laboratory experiment uses the paranemic crossover (PX) DNA motif[15] as a model nanostructure and DNase I enzyme to test the nuclease resistance levels. Nuclease activity on the DNA is evaluated using nondenaturing polyacrylamide gel electrophoresis (PAGE). An often-used laboratory method, gel electrophoresis-based experiments have been previously described in this *Journal*, including those for analyzing photoinduced oxidative DNA damage,[16] quantifying DNAzyme activity,[17] determining protein concentration,[18] characterizing DNA-cleaving metal complexes,[19] and analyzing molecular topology of DNA nanoswitches.[20] Beyond exposing students to a new field of research, this experiment is a key addition to the list of teaching opportunities that improve students' hands-on

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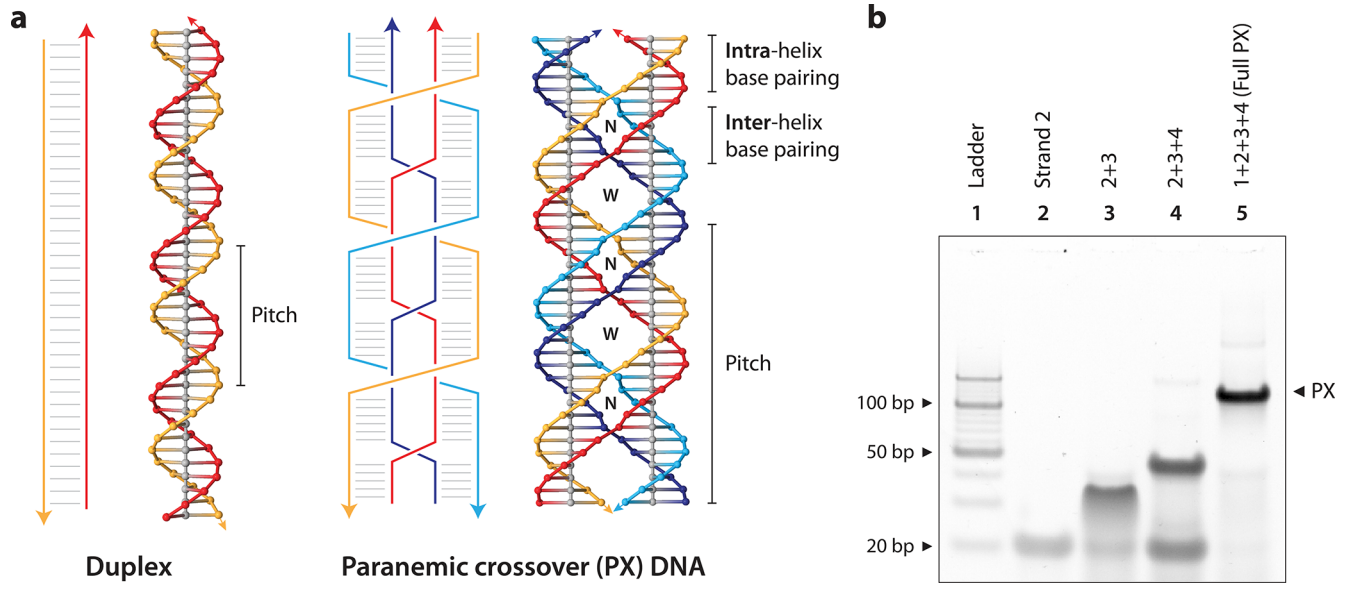


**Figure 1.** Design and assembly of paranemic crossover (PX) DNA. (a) Schematic and molecular models of a B-DNA duplex and paranemic crossover (PX) DNA. (b) Nondenaturing PAGE showing formation of PX DNA structure as the predominant product when all four component DNA strands are annealed together. Adapted with permission from ref 7. Copyright 2020 American Chemical Society.

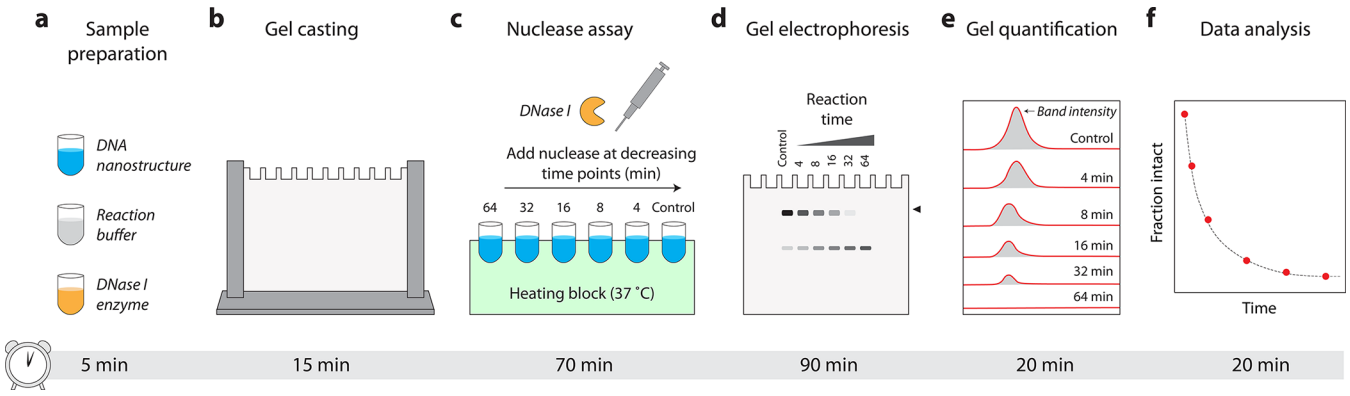


**Figure 2.** Nuclease degradation assay and gel analysis. (a) Sample preparation. (b) Casting nondenaturing polyacrylamide gel. (c) The assembled PX DNA is incubated at the optimal temperature for the nuclease (for example, 37 °C for DNase I), and the nuclease is added at different times starting with the longest time point. (d) DNase I-treated samples are loaded on a nondenaturing polyacrylamide gel and run for the desired time at specific voltages. (e) Bands corresponding to PX DNA in the gel image are quantified to yield the fraction of the intact structure at various time points tested. (f) Quantified band intensities are plotted to obtain degradation time constants. Estimated time durations for each step are shown below the illustration.

skill set in the laboratory (for example, pipetting and electrophoresis).

## HAZARDS

Acrylamide is a hazardous chemical and carcinogen, and exposure can occur via inhalation (if aerosolized), ingestion, and skin absorption. While toxicity and potential for exposure substantially decrease after polymerization, care should be taken when using polyacrylamide to prepare gels. Electrocution is also a potential hazard as typical voltages of 100 V can be applied across gels. To avoid electric shocks, students should use care when plugging the gel boxes into the power supply.

## RESULTS

This undergraduate laboratory experiment is designed based on our recent study of the biostability of paranemic crossover (PX) DNA motif.[7] PX DNA is a four-stranded DNA structure that consists of two adjacent and connected double helical DNA domains (Figure 1a, Figure S1 and Table S1).[15,21] The motif is formed by creating crossovers between strands of the same polarity at every possible point between two side-by-side helices.[15] Each duplex domain of PX DNA contains alternating major (wide) groove (denoted by W) or minor (narrow) groove separation (denoted by N) flanking the central dyad axis of the structure, with one helical repeat containing a mixture of four half turns (Figure 1a). In that study,[7] we annealed the PX DNA in tris-acetate-EDTA-$Mg^{2+}$ (TAE-$Mg^{2+}$) buffer and checked their formation using nondenaturing PAGE (Figure 1b). Using gel-based analysis, we reported degradation times, kinetics of nuclease digestion, enzyme-specific concentration gradients on DNA motifs, and evaluation of biostability enhancement factors.[7] Compared to duplexes and other control structures with a lesser number of crossovers, PX DNA showed exceptional biostability when tested against DNase I and in biofluids such as fetal bovine serum, human serum, and human urine.


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In this laboratory experiment, students tested the degradation profile of PX DNA when incubated with DNase I, using PAGE. In previous work, we annealed PX DNA to 4 °C and performed nondenaturing PAGE in the cold room (4 °C). To be easily adaptable for an undergraduate laboratory, here, we modified the protocol for the gels to be run at room temperature (~20 °C) and confirmed that the assembled PX DNA was intact using this new protocol (Figure S2). For testing PX DNA biostability, students used DNase I, one of the most widely used endonucleases that nonspecifically cleaves both strands of double-stranded DNA. To obtain degradation profiles, we chose 0.5 U/$\mu$L DNase I concentration which showed a gradual degradation rate in our previous work.[7] We probed enzymatic degradation by incubating the PX DNA with DNase I enzyme for different times at 37 °C and quantifying the reduction of the band representing the structure on nondenaturing gels (Figure 2).

To evaluate the nuclease degradation of PX DNA in an undergraduate laboratory setting, I provide an experimental workflow that can be performed in under 3 h (Figure 2a–d). The suggested skill level for students is basic pipetting techniques and familiarity with gel electrophoresis. To start the laboratory experiment with nuclease degradation analysis, the DNA nanostructure (PX DNA in this case) can be prepared ahead of the laboratory time and provided to students. I've provided a separate document for lab instructors to assemble the PX DNA and prepare reagents for this laboratory experiment (Supporting Information, Table S2 and Supplementary Note 1: Reagent preparation for instructor). After the experimental portion of the laboratory is completed, gel quantification and data analysis can be performed by the students as postlab exercises (Figure 2e,f). Instructors can also perform additional experiments using control structures to provide students with data for comparing their analysis and for discussion of results (Figure S3 and Table S3).[22]

Representative results of experiments performed by undergraduate students in our laboratory are provided in Figure 3. Students were provided with the assembled PX DNA motif, DNase I enzyme at 0.5 U/$\mu$L concentration and DNase I reaction buffer (10×). Students performed the timed nuclease assay at 37 °C, loaded and ran the samples on an 8% nondenaturing polyacrylamide gel, imaged and quantified the gel, and analyzed quantified data. In the set of experiments described here, the longer time points were started first, followed by shorter time points, and the gels were loaded and run at time zero. The protocol can also be adapted to use a stop solution (typically 50 mM EDTA) to inactivate the DNase I after incubation with PX DNA for specific time points. The nuclease degradation plots created using data from the gels showed expected trends and were consistent between different students. Students also analyzed the plots to obtain time constants of the PX DNA degradation (Table S4), which were within the error of previously published results (Figure S4). In our prior work, we compared the nuclease degradation profiles of PX DNA and to those of DNA duplexes that degrade within a few minutes when treated with the same concentration of DNase I (0.5 U/$\mu$L) (Figure S3). Instructors can discuss these results with the students to compare the difference in nuclease resistance of the PX DNA and duplex DNA and to verify their own results based on previously published data.

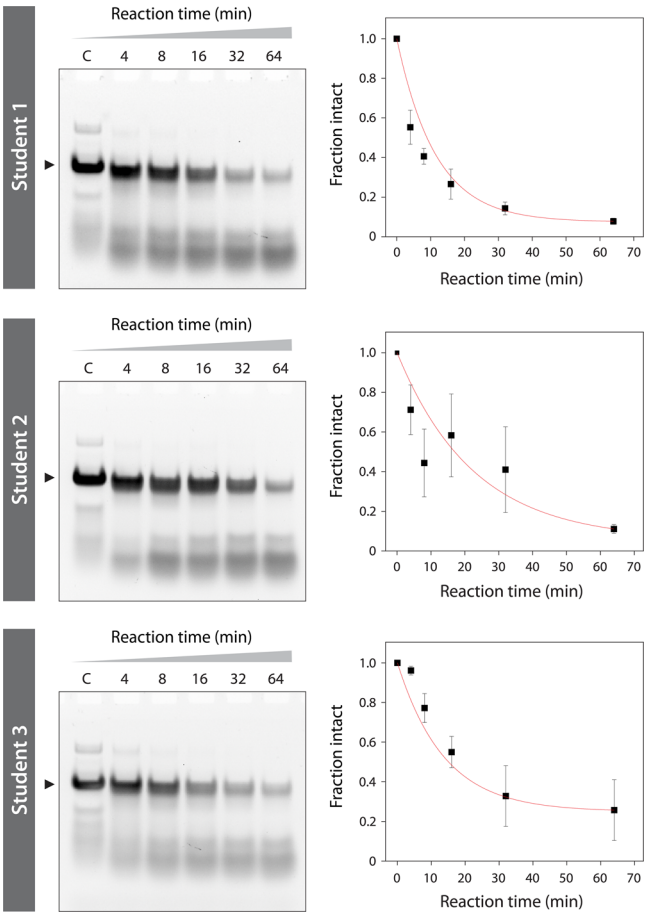


**Figure 3.** Representative results from experiments performed by undergraduate students. Students were given the manual in Supplementary Note 2 and were provided with annealed DNA complexes and reagents. Gel analysis was done using ImageLab and data plotted using Origin (C = control). Error bars are standard deviations from experiments performed in triplicates by each student.

## EXPERIMENTAL CONSIDERATIONS

The following considerations can be useful to achieve consistent results:

(1) Always keep enzymes on ice to avoid denaturation.
(2) Using the same enzyme dilutions across replicates and different students will provide consistent results to avoid any differences in diluting the enzymes.
(3) The assembled PX DNA (or other DNA nanostructure) should not be vortexed or heated up, which will result in disassembly or denaturation of the DNA nanostructure.
(4) After adding the enzyme to the DNA sample, it is important to mix the solution gently but thoroughly to make sure the enzyme is uniformly mixed with the DNA nanostructure. Uneven mixing may lead to skewed results.
(5) There might be a lag in time when loading the gels right after the timed nuclease assay is finished; students can keep track of the delay in loading and running the gels when discussing results. Alternatively, a stop solution can also be used.
(6) Instructors can perform a set of the experiments and have a demonstration data set for students to compare their results to.


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## DISCUSSION

The laboratory experiment presented here is designed for upper-class undergraduate students (third year chemistry or biology undergraduate students), specifically those in biochemistry, molecular biology, or genetics laboratory courses where students already run gels. In our laboratory, some first and second year undergraduates have also performed the experiment. To conduct the experiment in an undergraduate laboratory, groups of three students could be asked to perform one replicate of the timed assay each and then combine the data to obtain average values for data analysis. The defined timeline of the experiment, skill level, and characterization of results make this laboratory experiment easily adaptable for undergraduate laboratories and can be modified for other nucleic acid nanostructures with either polyacrylamide or agarose gel electrophoresis.[22] The cost of DNA strands for such an experiment is minimal, making it viable for adoption in undergraduate laboratories. The four DNA strands used to assemble the PX DNA motif cost ∼$30 at the 25 nmol synthesis scale (when purchased from Integrated DNA Technologies) and can be stored frozen for many years and used for multiple experiments. When ordered at the 25 nmol scale, we obtained ∼200 μL of each of the four strands at ∼100 μM concentration. If using 1 μM concentration of the PX DNA motif for the experiments (like in this publication), the 25 nmol scale of DNA order can be used for a total of ∼2000 gel lanes or ∼350 timed assays (with 6 time points per assay). Further, the experiment uses equipment and reagents typically available in a biochemistry or molecular biology laboratory. We used a thermocycler set to 37 °C in our laboratory, but a dry heating bath can be used instead. Our gel setup typically allows four gels to be run in one gel chamber; thus, the experimental design also allows for undergraduate laboratories of 20−30 students to accomplish the experiment using 5−6 workstations (more details of experimental setup is provided in Supplementary Note 2). While ethidium bromide is frequently used for gel staining, we used GelRed nucleic acid stain which is nontoxic, making the experiment safe for undergraduate students, and does not require specific waste disposal protocols. Articles in this *Journal* have described the exploration of alternatives to ethidium bromide for nucleic acid staining (however, that paper did not discuss the use of GelRed),[23] showing the importance of nontoxic dyes for undergraduate laboratory experiments. Precast polyacrylamide gels (e.g., from Millipore Sigma or ThermoFisher) can be used if there is a need to shorten the time frame of this laboratory experiment but will increase the cost associated with the experiment compared to gels made in the laboratory.

Similar to typical undergraduate laboratory experiments, the assembly of DNA motifs and reagent preparation can be performed beforehand by the teaching instructor and provided to students (Supporting Information, Supplementary Note 1: Reagent preparation for instructor). In the laboratory, students can incubate the PX DNA motif with DNase I, perform gel electrophoresis for the samples, and image the gels. As a postlab exercise, students can analyze the gels and measure band intensities corresponding to PX DNA motif at each time point. In our laboratory, students typically finished the gel part of the experiment on time, which is also aided by the timed nature of the assay. For the suggested postlab exercises, students can be given a demonstration of gel analysis during the introduction of the laboratory or in the lecture. The time taken by students for gel analysis and graphing varied between students, and thus it is suggested that this be given as a postlab exercise. Students can discuss their results in the postlab exercise by comparing the biostability of PX DNA to a duplex structure (Figure S3) and verify their results by comparing it with prior research (Figure S4). Student learning was assessed by their presentation of results in group meetings, specifically to address the pedagogical goals of experimental skill development, data analysis, and conceptual understanding of DNA nanostructure biostability. Students produced a report for an oral presentation that included labeled gel images of triplicate experiments, analyzed data with error bars (similar to Figure 3), and discussed any deviations from expected results. The experiment discussed here improved students' pipetting skills (some students repeated the exercise for better results), provided more efficient gel image analysis (not typically covered in laboratory courses), and introduced them to statistical analysis (of simple triplicate experiments). Based on this experience with our students, I've provided a detailed student instruction manual that is designed to fit this experiment in typical laboratory course timing (∼3 h) and a postlab worksheet (Supporting Information, Supplementary Note 2: Student instruction manual).

Utility of DNA as a material for constructing nanostructures is one of the rapidly advancing fields of multidisciplinary research. There is a vast body of literature that provides a collection of construction methods, history, and applications of DNA nanotechnology. The topic is being covered predominantly at the graduate level with some courses available at the undergraduate level. Introducing laboratory experiments that involve some aspects of DNA nanotechnology will expose students to this new field of research. 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.2c00656.

Materials and methods, notes for instructors, student instruction manual, and worksheet for students (PDF)

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### Notes

The author declares no competing financial interest.

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