Nuclease Degradation Analysis of DNA Nanostructures Using Gel Electrophoresis

Arun Richard Chandrasekaran[1,2] and Ken Halvorsen[1]

[1]The RNA Institute, University at Albany, State University of New York, Albany, New York
[2]Corresponding author: *arun@albany.edu*

Custom-built DNA nanostructures are now used in applications such as biosensing, molecular computation, biomolecular analysis, and drug delivery. While the functionality and biocompatibility of DNA makes DNA nanostructures useful in such applications, the field faces a challenge in making biostable DNA nanostructures. Being a natural material, DNA is most suited for biological applications, but is also easily degraded by nucleases. Several methods have been employed to study the nuclease degradation rates and enhancement of nuclease resistance. This protocol describes the use of gel electrophoresis to analyze the extent of nuclease degradation of DNA nanostructures and to report degradation times, kinetics of nuclease digestion, and evaluation of biostability enhancement factors. © 2020 Wiley Periodicals LLC.

Basic Protocol: Timed analysis of nuclease degradation of DNA nanostructures
Support Protocol: Calculating biostability enhancement factors

Keywords: biostability • DNA motifs • DNA nanotechnology • drug delivery • gel electrophoresis • nucleases

How to cite this article:
Chandrasekaran, A. R., & Halvorsen, K. (2020). Nuclease degradation analysis of DNA nanostructures using gel electrophoresis. *Current Protocols in Nucleic Acid Chemistry*, *82*, e115. doi: 10.1002/cpnc.115

INTRODUCTION

DNA nanotechnology involves the construction of nanoscale shapes and structures using DNA (Chandrasekaran, Anderson, Kizer, Halvorsen, & Wang, 2016; Seeman, 2003; Xavier & Chandrasekaran, 2018). Over the past few decades, scientists have created many types of DNA nanostructures by cooperative self-assembly of DNA strands, hierarchical assembly of DNA motifs into larger structures, and using the DNA origami technique (Chandrasekaran & Levchenko, 2016; Hong, Zhang, Liu, & Yan, 2017; Seeman & Sleiman, 2018). Recently, the field has moved from proof-of-concept demonstrations of structural design to more application-oriented research. Custom-built DNA nanostructures are now used in applications such as biosensing, molecular computation, biomolecular analysis, enzyme cascades, material organization and drug delivery (Kizer, Linhardt, Chandrasekaran, & Wang, 2019; Mathur & Medintz, 2019; Wang et al., 2018; Xiao et al., 2019). While the functionality and biocompatibility of DNA makes DNA nanostructures useful in such applications, biostability is still a major challenge in the field. Biostability is an important factor especially when these structures are used for biological applications where DNA nanostructures face attack by nucleases in *in vivo* or physiological conditions. DNA nanostructures need to resist degradation by nucleases

CURRENT PROTOCOLS
A Wiley Brand
Current Protocols in Nucleic Acid Chemistry e115, Volume 82
Published in Wiley Online Library (wileyonlinelibrary.com).
doi: 10.1002/cpnc.115
© 2020 Wiley Periodicals LLC

present in the body to perform their functions. In biosensing applications, for example, most strategies involve a conformational change and separation of fluorophore-quencher pairs. In a real-world setting, detection is ideally from bodily fluids such as blood and urine and requires the DNA biosensors to be stable in these solutions. Similarly, DNA nanostructures used as carriers for drug delivery purposes need to withstand the harsh *in vivo* conditions to protect the encapsulated drugs (Madhanagopal, Zhang, Demirel, Wady, & Chandrasekaran, 2018).

Recently, several strategies have been developed to improve nuclease resistance of DNA nanostructures. Some of these include chemical ligation (Keum & Bermudez, 2009) and crosslinking of component strands within DNA nanostructures (Cassinelli et al., 2015), use of chemically modified DNA for construction (Conway, McLaughlin, Castor, & Sleiman, 2013), and protective coatings such as polymers (Kiviaho et al., 2016), proteins (Auvinen et al., 2017), and lipids (Perrault & Shih, 2014). In these studies, researchers analyze the degradation (and enhanced stability) of DNA nanostructures to nucleases using different methods such as gel-based analysis, fluorescence, real-time atomic force microscopy (AFM), and transmission electron microscopy (TEM). Among these, gel electrophoresis has been one of the most commonly used technique to evaluate nuclease degradation.

In our recent research, we studied the biostability of a DNA motif called paranemic crossover (PX) DNA and explored the crossover-dependent biostability of DNA motifs by comparing PX DNA with similar motifs that contained lesser number of crossovers (Chandrasekaran et al., 2020). PX DNA is a four-stranded DNA structure that consists of two adjacent and connected double helical DNA domains (Shen et al., 2004; Wang et al., 2019) (Fig. 1). The motif is formed by creating crossovers between strands of the same polarity at every possible point between two side-by-side helices (Shen et al., 2004). Each duplex domain of PX DNA contains alternating major (wide) groove (denoted by W) or a 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. In our study, we used a PX DNA motif with major/minor groove separations (W:N) of 6 and 5 nucleotides, respectively (Shen et al., 2004; Wang et al., 2019). 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 (Chandrasekaran et al., 2020). In this protocol, we provide a step-by-step description of nuclease degradation analysis of the paranemic crossover DNA motif. This protocol can be adapted for any DNA nanostructure and for any type of nuclease. The Basic Protocol describes gel-based analysis of timed nuclease degradation that can be used to measure the extent of degradation of a DNA nanostructure at various time points. The Support Protocol provides instructions for comparing nuclease resistance of two different DNA nanostructures and calculating biostability enhancement factors.

BASIC PROTOCOL

TIMED ANALYSIS OF NUCLEASE DEGRADATION OF DNA NANOSTRUCTURES

Different research groups have developed various design strategies and annealing protocols to assemble DNA nanostructures (Bhatia et al., 2009; He et al., 2008; Rothemund, 2006; Rusling et al., 2014). Once the DNA nanostructure is assembled and characterized, the following protocol can be used to estimate the percent or fraction of DNA nanostructure digested by nucleases over time. In our case, we characterized assembly of PX DNA using polyacrylamide gel electrophoresis (PAGE), thermal melting profiles and circular dichroism (Fig. 1B-F). The gel electrophoresis protocol established for characterizing DNA nanostructures can be used for the nuclease degradation analysis. As an example, this protocol uses digestion of PX DNA by DNase I enzyme and includes a step-by-step

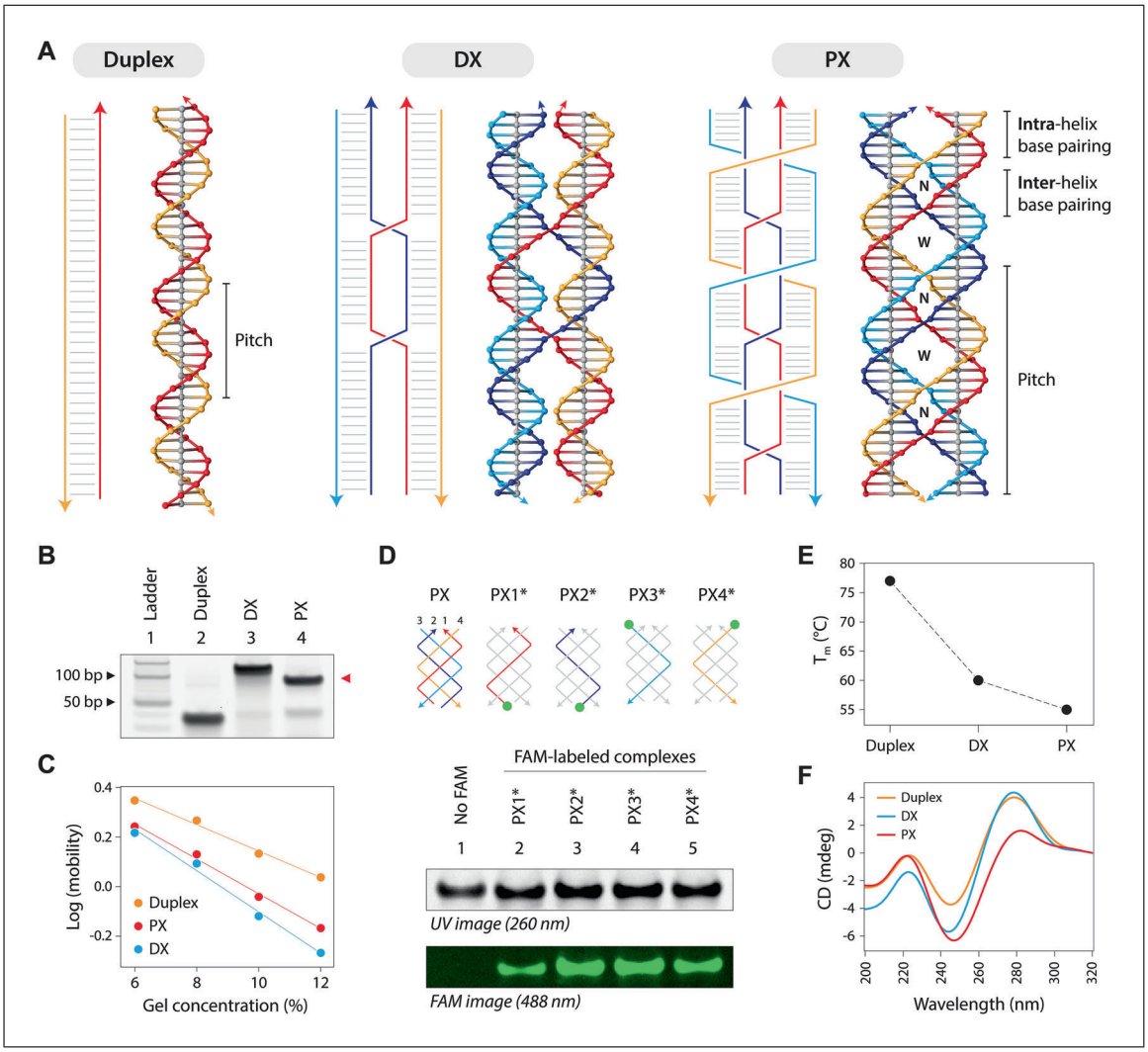

Figure 1 Design and validation of paranemic crossover (PX) DNA. (**A**) Schematic and molecular models of a B-DNA duplex, a double crossover (DX) motif and paranemic crossover (PX) DNA. (**B**) Non-denaturing PAGE showing formation of structures as predominant products in each lane, with PX migrating slightly faster than its DX counterpart. (**C**) Ferguson plot showing gel mobility characteristics of the control and PX structures as a function of gel concentration. (**D**) Validating incorporation of all four strands in the PX by making four structures each with a single FAM-labeled strand. Gel image under UV is shown for reference, with a control PX lane omitting FAM labels. (**E**) Melting temperatures determined from UV melting experiment show a decrease in thermal stability from duplex to DX to PX. (**F**) Circular dichroism spectra of the tested structures show that characteristics of PX are similar to those previously reported. Figure reproduced from (Chandrasekaran et al., 2020) with permission. Copyright 2020, American Chemical Society.

procedure to analyze degradation at different time points using PAGE. Further, we also describe analysis of a DNA duplex as a control structure (Fig. 1A). Sequences of duplex controls in such comparative studies are typically derived from part of the nanostructure under analysis (in this case, the duplex sequences form one half of the PX DNA). Full sequences of the duplex and PX DNA used in this protocol is provided in Table 1. This protocol can be used for any type of DNA nanostructure and nuclease and can also be performed using agarose gel electrophoresis depending on the size of the DNA nanostructure (for e.g., DNA origami structures). Additional background information on PAGE analysis of synthetic nucleic acids is available elsewhere (Andrus & Kuimelis, 2000).

Materials

40% Polyacrylamide solution (19:1 or 29:1 acrylamide/bisacrylamide; National Diagnostics, cat. no. EC-850 or EC-852, respectively)

Table 1 Sequences of PX DNA and Duplex Control Used in this Protocol

Name	Sequence
Duplex1	5′-GTGGTGTCGCAACAGATATGTGTAGGCACCGAATCACT-3′
Duplex2	5′-AGTGATTCGGTGCCTACACATATCTGTTGCGACACCAC-3′
PX1	5′-GTGGTATCATCAATGCTATGTGTAGGCTTAGACCTGAG-3′
PX2	5′-ACTAGGTCGCAACAGACACAATACTTGACCGAATCACT-3′
PX3	5′-AGTGAGTCTAACAAGTCACATATCTGTGATGATCTAGT-3′
PX4	5′-CTCAGTTCGGTGCCTAATTGTGGCATTTGCGACACCAC-3′

10× TAE-Mg (Tris-acetate-EDTA-Mg^{2+}) buffer (see recipe)
10% ammonium persulfate (APS; see recipe)
Tetramethylethylenediamine (TEMED; Thermo Scientific, cat. no. PI17919)
1× TAE-Mg running buffer
Assembled DNA nanostructure (example shown here is PX DNA and a duplex as reported in Chandrasekaran et al., 2020)
DNase I reaction buffer (10× , New England Biolabs, cat. no. B0303S; included with DNase I enzyme purchase)
DNase I (2000 U/ml; New England Biolabs, cat. no. M0303S)
Nuclease-free water (Invitrogen, cat. no. 10977-015)
Ice
Gel Loading Dye, Blue (6×; New England Biolabs, cat. no. B7021S)
GelRed nucleic acid stain (10,000 × in water, Biotium cat. no. 41003)
10-bp ladder (ThermoFisher Scientific, cat. no. SM1313; see recipe), optional

Gel electrophoresis plates, chamber and comb (Mini-PROTEAN Tetra Vertical Electrophoresis Cell or similar)
Power supply for gel electrophoresis (BioRad PowerPac™ Basic Power Supply or similar)
Heating block (at 37°C or other nuclease-dependent temperature) *or* thermal cycler (e.g., BioRad T100 Thermal Cycler)
Timer
Gel loading tips
GelDoc imaging station or similar (Bio-Rad Gel Doc XR+ imager)
Gel analysis software such as Image Lab, Image J or similar
Graphing software (Origin Lab or similar)
PCR tubes or 0.5- or 1.5-ml tubes
15-ml falcon tubes

NOTE: We have provided specific vendors for the reagents but similar products from other vendors may also be used.

Prepare non-denaturing polyacrylamide gel (15 min)

1. Mix the following in a 15-ml falcon tube to make a 10% polyacrylamide gel. Make two similar solutions for 2 gels (one for PX DNA and one for duplex).

   2.5 ml of 40% polyacrylamide
   1 ml 10× TAE-Mg buffer
   6.5 ml de-ionized water
   80 µl of 10% APS
   4 µl TEMED

   NOTE: *This total volume of 10 ml is for one gel run on a Mini-PROTEAN Tetra Vertical Electrophoresis system. Volumes can be scaled up as needed for the number of gels to be run.*

2. Set up the gel plates and chamber according to the manufacturer's instructions.
3. Pour the gel solution into the glass plate set up and allow the gel to polymerize for 30-40 min.
4. Set up the gel chamber and pour 1× TAE-Mg running buffer for gel running.
5. Keep the entire set up in the cold room so that the gels are ready to be loaded when nuclease reaction with sample is completed.

   NOTE: *Addition of 10% APS and TEMED can be modified based on what is used in your respective laboratories. Higher amounts will cause quicker polymerization.*

DNase I time series (75 min)

6. Decide on the time points for the experiment. This protocol describes a time series experiment for reacting assembled DNA structure and control with DNase I at time points 0, 1, 2, 4, 8, 16, 32, and 64 min (i.e., 8 samples). Follow this protocol for both PX DNA and the duplex control.
7. Mix the assembled DNA nanostructure (PX DNA in this example) with DNase I reaction buffer to a final of 1× . Mix gently by pipetting.

   Assembled PX DNA or duplex (at 1 μM) (or any DNA nanostructure): 90 μl
   10× DNase I buffer: 10 μl

   NOTE: *If doing experiment in triplicates, prepare sample volume accordingly.*
8. Split the above mixture into 8 tubes with 10 μl in each tube.

   NOTE: *Use appropriate tubes depending on the type of heating block used. We use PCR tubes for incubation in a thermal cycler. 0.5-ml tubes or 1.5-ml tubes can be used for heating blocks.*
9. Dilute the DNase I enzyme to the desired amount.

   Stock concentration: 2000 U/ml (i.e., 2 U/μl)
   For 0.1 U/μl enzyme, mix 2 μl stock DNase I + 38 μl nuclease-free water.

   NOTE: *Keep the enzyme and enzyme dilutions on ice at all times.*
10. Set a heating block or thermal cycler to be at 37°C, the temperature at which DNase I has optimal activity. Choose the desired temperature depending on the nuclease used in the experiment.
11. Keep the tubes containing the nanostructure/buffer mix (from step 7) on the heating block once the set temperature has been reached (Fig. 2A).

    NOTE: It is important to keep all the sample tubes at the reaction temperature regardless of the time the enzyme is added; this eliminates any temperature variation effect on the results.
12. Start a timer immediately with 64 min and counting down to 0.
13. Starting with the 64-min time point, add 1 μl of 0.1 U/μl enzyme (from step 9) to the tubes corresponding to each time point (at 32-, 16-, 8-, 4-, 2-, and 1-min mark) (Fig. 2A).
14. For the 0 min tube, add 1 μl nuclease-free water instead of DNase I enzyme.
15. When the time points are completed, keep all the tubes on ice to quench the reaction (read next step).

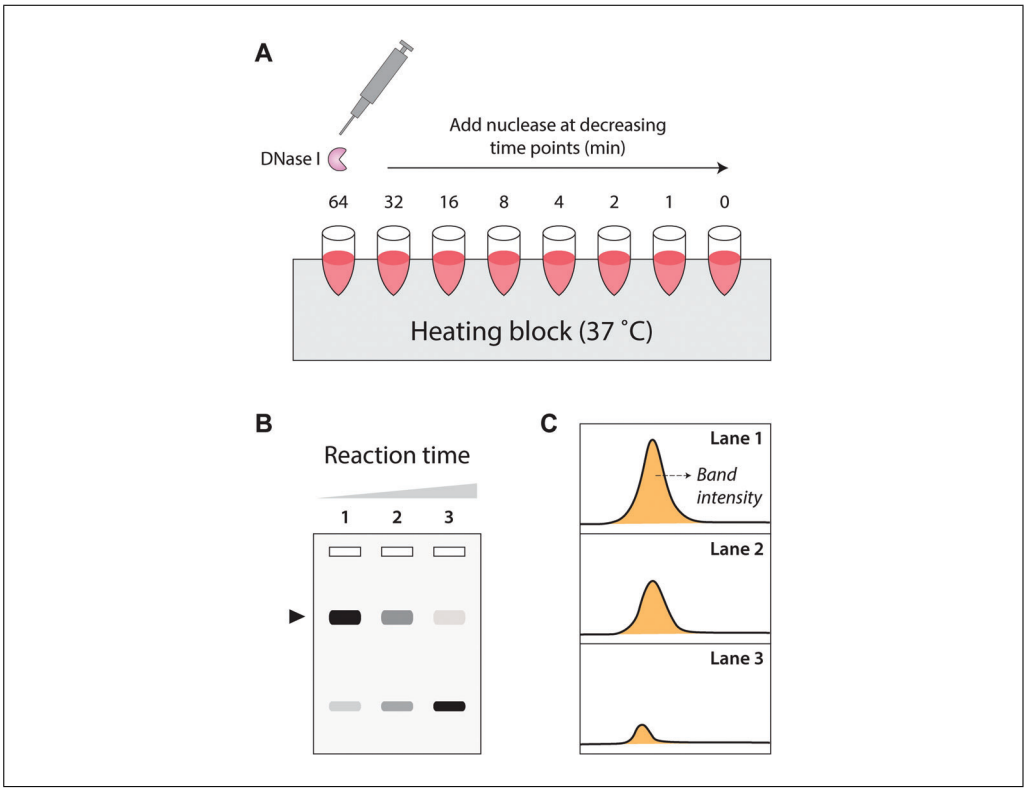

Figure 2 Pictorial representation of nuclease time series and gel analysis. (**A**) The assembled DNA nanostructure is kept at the optimal temperature for the nuclease (e.g., 37°C for DNase I) and the nuclease is added at different times starting with the longest time point. (**B**) The nuclease-treated samples are loaded on a polyacrylamide or agarose gel and run for the desired time at specific voltages. (**C**) The bands corresponding to the structure in the gel image are quantified to yield the fraction of the intact structure at various time points tested.

Gel electrophoresis of nuclease-treated samples (60 min)

The prepared samples will be run on a non-denaturing PAGE gel to analyze decrease in intensity of the band corresponding to the DNA nanostructure.

16. Quickly add 1 µl loading dye to the tubes (from step 14) while keeping track of the time until the gel is loaded. Any lag in loading time much be kept consistent for all the samples.

17. Starting with time 0, load 10 µl of each sample into the gel (from steps 13-14) in order of time (from 0 to 64 min), totaling 8 lanes.

18. Optionally, a 10-bp ladder can be loaded for reference.

19. Run the gel in the cold room (4°C) by applying 100 V for 1 hr. For structures that are stable at higher temperatures, gels can be run at room temperature.

    NOTE: *We use the Mini-PROTEAN Tetra cell gel box (Bio-Rad) and optimized gel running conditions to be typically 100 V for 60 min. The voltage and running time should be optimized based on DNA nanostructure used and will typically be similar to gel conditions used to characterize the structure.*

Gel staining (30 min)

20. Prepare gel staining trays with 50 ml of 1× TAE-Mg.

21. Add 2.5 µl of GelRed stock solution to the staining buffer and mix well (this yields 0.5 × GelRed in the staining solution).

    NOTE: *Other DNA stains such as ethidium bromide or SYBR gold can also be used.*

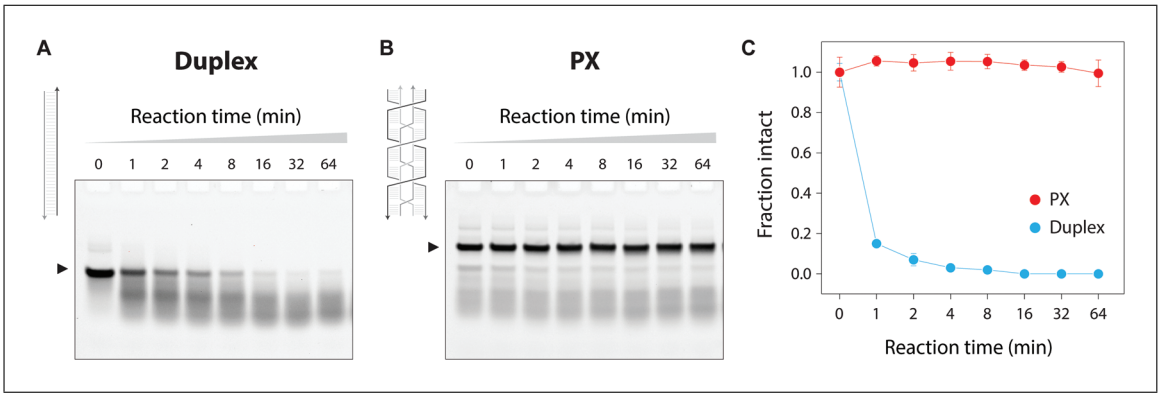

Figure 3 Sample data set for nuclease degradation analysis of a DNA duplex and PX DNA. (**A and B**) show polyacrylamide gels with structures treated with DNase I for different times (0 to 64 min). (**C**) Analysis showing fraction of the structures intact at each time point. Figure adapted from Chandrasekaran et al. (2020) with permission. Copyright 2020, American Chemical Society.

22. Open the gel plates and place the gel carefully in the staining solution.

    NOTE: *For convenience, gel can remain on one of the plates so that it is easier to move the gel from staining to de-staining solutions. Extra care has to be taken for low percent gels that can be fragile.*

23. Place the gel-containing trays on a rotator and stain for 20 min.

24. After staining, move the gel to a tray with 50 ml water. De-stain for 10 min.

    NOTE: *Staining and de-staining times can be optimized to be shorter or longer.*

Gel imaging (5 min)

25. Remove the gel from the de-staining tray, carefully take gel off the glass plate and place on the imaging plate on a Bio-Rad Gel Doc XR+ gel imager (or any gel imager available). Image using the gel analysis tool in the Image Lab software package available with Bio-Rad Gel Doc XR+ (or the software available with your gel imager) (Fig. 2B).

    NOTE: *Make sure to take gel images at multiple exposure times to ensure that the band corresponding to the structure is not saturated. If saturated, the intensity values will not be accurate.*

Gel image analysis (15 min)

26. Use ImageJ or the image analysis software provided with the gel imaging station to analyze the gel (Gallagher, 2014). Measure the intensity of the band corresponding to the structure in the lane for each time point (Fig. 2C). Plot the values to obtain the degradation pattern. A sample data set of DNase I degradation of duplex and PX DNA, as well as the analyzed band intensities is shown in Figure 3.

CALCULATING BIOSTABILITY ENHANCEMENT FACTORS

SUPPORT PROTOCOL

There are several studies by different groups on the nuclease resistance of DNA nanostructures (Anastassacos, Zhao, Zeng, & Shih, 2020; Castro et al., 2011; Kim & Yin, 2020; Wang et al., 2020). In these studies, researchers typically compare the nuclease degradation pattern of DNA nanostructures to a duplex DNA or other nanostructures. To establish a parameter for enhanced nuclease resistance of a DNA nanostructure, we created a metric called the biostability enhancement factor (BioEF values). To calculate this, we first choose an optimal nuclease concentration at which the structures show a trend of degradation with the nuclease (reducing structural intactness) and complete degradation (0% intact) at the end point. For example, a nuclease concentration at which the nanostructure is immediately degraded will not provide useful data for analysis and will thus require

a lower nuclease concentration. At this chosen nuclease concentration, one can analyze the nuclease degradation of the two structures, obtain decay kinetics, and use the BioEF values to compare the fold increase in nuclease resistance. Data obtained using Basic Protocol for duplex and PX DNA with 0.1 U/μl DNase I are used as examples here.

Materials

Band intensity data obtained using Basic Protocol for two different structures (duplex and PX DNA)
Origin Lab or other similar software

Data analysis and BioEF calculation (45 min)

1. Repeat the degradation analysis using Basic Protocol for another structure that you want to compare with. Here, we show PX and duplex structures as examples.

   NOTE: *Characterize nuclease degradation of the structures before-hand and pick enzyme concentrations at which the structures are completely degraded at some defined time point.*

2. Plot the band intensities for the structures in separate plots using Origin Lab or other similar software (Fig. 4A and 4B).

3. Using the tools provided within the graphing software, fit an exponential decay to the plotted values. This fit will yield the time constant (τ) for degradation of the structures (Fig. 4A and 4B).

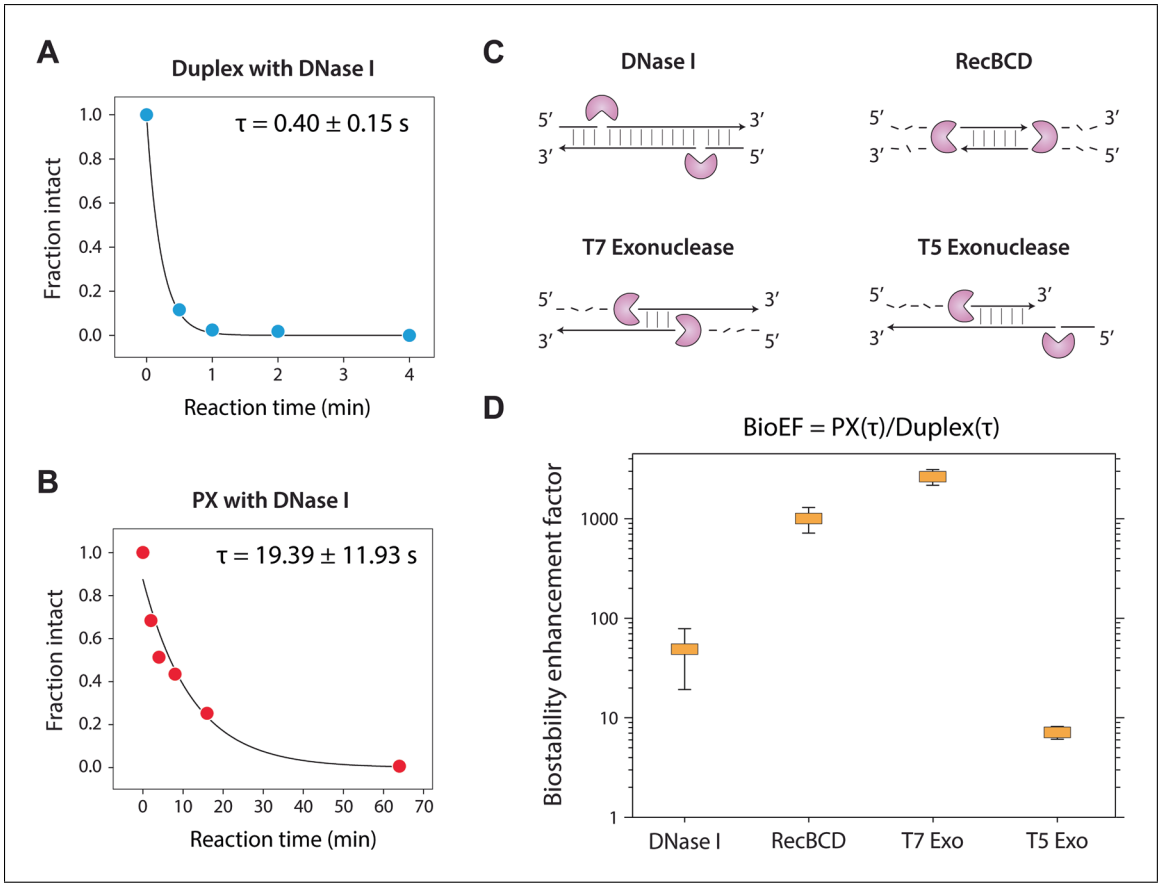

Figure 4 Sample data set of biostability enhancement factor analysis. (**A and B**) show nuclease degradation analysis of duplex and PX DNA with exponential decay fits (black lines). The slope of this fit is the time constant (τ values). (**C**) Similar analysis can be carried out for nucleases with different activities; cartoon shows the known activity of four different nucleases on single and double stranded DNA. (**D**) The biostability enhancement factor for PX in comparison to duplex for enzymes DNAse I, RecBCD, T7 exonuclease and T5 exonuclease. Figure adapted from Chandrasekaran et al. (2020). Copyright 2020, American Chemical Society.

NOTE: *This analysis can be performed for any type of nuclease to obtain degradation kinetics. Different enzymes may have different activities on the DNA nanostructures (Fig. 4C).*

4. The enhancement factor of PX over duplex is calculated by dividing the values PX(τ)/Duplex(τ) (Fig. 4D).

REAGENTS AND SOLUTIONS

10-bp ladder (preparation for 1 lane)

0.5 µl of 10-bp ladder
1 µl of 10× TAE-Mg
8.5 µl deionized water
Add loading dye as mentioned in the protocol for other samples and load 10 µl per gel lane.

Ammonium persulfate (APS), 10%

0.5 g ammonium persulfate in 5 ml of deionized water

Tris-Acetate-EDTA with magnesium (TAE-Mg), 10×

48.5 g Tris base (VWR, cat. no. 97061-794)
200 ml acetic acid (1 M solution, Fisher Scientific, cat. no. S25840A)
40 ml EDTA (0.5 M solution, pH 8; VWR cat. no. BDH7830-1)
26.8 g Magnesium acetate (Fisher Scientific, cat. no. BP215-500)
Make up to 1 L using deionized water
Store up to 1 year at room temperature

Contains 400 mM Tris base (pH 8.0), 200 mM acetic acid, 20 mM EDTA, and 125 mM magnesium acetate.

Tris-Acetate-EDTA with magnesium (TAE-Mg), 1×

100 ml 10× TAE-Mg (see recipe)
900 ml deionized water
Store up to 1 year at room temperature

COMMENTARY

Background Information

DNA has been used as a material for the construction of nanoscale structures. Over the years, many different DNA motifs have been created that serve as building blocks for bottom-up construction (Seeman & Sleiman, 2018). These (usually) multi-stranded structures typically contain double helical domains that are connected together by strand crossovers. A wide variety of structures have been made using bottom-up DNA construction, ranging from small objects and devices to larger, trigger-responsive cages that have emerging applications in drug delivery. To be successfully used in biological applications such as biosensing and drug delivery, DNA objects must overcome a major challenge of surviving harsh physiological environments such as blood and cellular matrices (Madhanagopal et al., 2018). Strategies to improve the biostability of DNA structures include polymer (Agarwal, Matthies, Gür, Osada, & Schmidt, 2017) and protein-based coatings (Anastassacos et al., 2020; Auvinen et al., 2017; Ponnuswamy et al., 2017), viral capsid encapsulation (Perrault & Shih, 2014), dendritic oligonucleotides (Kim & Yin, 2020), modified nucleotides (Kim et al., 2016; Liu et al., 2017), crosslinking (Anastassacos et al., 2020; Cassinelli et al., 2015), and increased crossovers (Chandrasekaran et al., 2020). Analysis of the stability of these structures requires a widely accessible and cost-effective technique. Gel electrophoresis has been the choice of method in most of the nuclease resistance studies. The protocol we described here could be used as a standard reference for analyzing any type of DNA nanostructure, either using polyacrylamide or agarose gel electrophoresis. These papers also report different metrics for nuclease resistance and biostability such as lifetimes, half-lives or percent structure intact after a certain time with nucleases or body fluids. The biostability

enhancement factor (BioEF value) described here could be a new metric that can attribute a particular structure's enhanced biostability compared to a standard structure such as a DNA duplex.

Critical Parameters

This protocol details analysis of degradation of DNA nanostructures using gel electrophoresis. One main aspect to this is prior characterization of the DNA nanostructure on a gel (either PAGE or agarose). The gel percentage used for this experiment is based on the gels run for routine characterization of the structure. It is also important to test different amounts of the nuclease to determine degradation levels. To obtain kinetics of cleavage, we recommend using an enzyme concentration at which there is gradual decrease of band intensity on reaction with the nuclease. It is also important to load and start the gel right after the last time point to avoid large errors in time points. One can also keep track of the lag in loading the gel, especially at shorter time points so errors can be calculated uniformly for all lanes.

Understanding Results

The sample data set for PAGE analysis of nuclease degradation of duplex and PX DNA is shown in Figure 3. It is important to characterize the DNA nanostructures of interest on PAGE before performing nuclease degradation analysis so as to identify the band that corresponds to the structure. To obtain the fraction or percent degradation (Fig. 3C), the untreated sample (DNA nanostructure without the enzyme) is used as the control band (lane corresponding to 0 time in Fig. 3A and 3B). For kinetics analysis, the enzyme concentration also plays a role. To fit a decay model to degradation data, one might have to test different enzyme concentrations to decide which concentration of the enzyme provides a few data points before complete degradation of the nanostructure. In the duplex and PX DNA data shown in Figure 4A and 4B, 0.1 U/μl DNase I was used. At higher enzyme concentrations, the duplex was completely degraded at the first time point (within 1 min), preventing the fitting of a decay model to the data (Chandrasekaran et al., 2020). These pre-characterization steps also apply to any other nuclease that is tested.

Time Considerations

The typical time taken in our laboratory to complete the different experiments and analyses in this protocol are provided at each step. Some of these steps include wait times–for example, while waiting for the gel to polymerize and in between longer time points. Researchers can plan the experiment in such a way that a few steps overlap and are set up during wait times of an earlier step.

Acknowledgment

We acknowledge support by the NIH through NIGMS under award R35GM124720 to K.H.

Author Contributions

Arun Richard Chandrasekaran: Conceptualization; data curation; formal analysis; investigation; methodology; project administration; supervision; validation; visualization; writing-original draft; writing-review & editing. **Ken Halvorsen:** Conceptualization; funding acquisition; project administration; resources; supervision; writing-review & editing.

Literature Cited

Agarwal, N. P., Matthies, M., Gür, F. N., Osada, K., & Schmidt, T. L. (2017). Block copolymer micellization as a protection strategy for DNA origami. *Angewandte Chemie International Edition*, *56*, 5460–5464. doi: 10.1002/anie.201608873.

Anastassacos, F. M., Zhao, Z., Zeng, Y., & Shih, W. M. (2020). Glutaraldehyde cross-linking of oligolysines coating DNA origami greatly reduces susceptibility to nuclease degradation. *Journal of the American Chemical Society*, *142*, 3311–3315. doi: 10.1021/jacs.9b11698.

Andrus, A., & Kuimelis, R. G. (2000). Polyacrylamide gel electrophoresis (PAGE) of synthetic nucleic acids. *Current Protocols in Nucleic Acid Chemistry*, *1*, 10.4.1–10.4.10. doi: 10.1002/0471142700.nc1004s01.

Auvinen, H., Zhang, H., Nonappa, Kopilow, A., Niemelä, E. H., Nummelin, S., … Kostiainen, M. A. (2017). Protein coating of DNA nanostructures for enhanced stability and immunocompatibility. *Advanced Healthcare Materials*, *6*, 1700692. doi: 10.1002/adhm.201700692.

Bhatia, D., Mehtab, S., Krishnan, R., Indi, S. S., Basu, A., & Krishnan, Y. (2009). Icosahedral DNA nanocapsules by modular assembly. *Angewandte Chemie International Edition*, *48*, 4134–4137. doi: 10.1002/anie.200806000.

Cassinelli, V., Oberleitner, B., Sobotta, J., Nickels, P., Grossi, G., Kempter, S., … Manetto, A. (2015). One-step formation of "Chain-Armor"-stabilized DNA nanostructures. *Angewandte Chemie International Edition*, *54*, 7795–7798. doi: 10.1002/anie.201500561.

Castro, C. E., Kilchherr, F., Kim, D.-N., Shiao, E. L., Wauer, T., Wortmann, P., … Dietz, H. (2011). A primer to scaffolded DNA origami.

Nature Methods, *8*, 221–229. doi: 10.1038/nmeth.1570.

Chandrasekaran, A. R., Anderson, N., Kizer, M., Halvorsen, K., & Wang, X. (2016). Beyond the fold: Emerging biological applications of DNA origami. *ChemBioChem*, *17*, 1081–1089. doi: 10.1002/cbic.201600038.

Chandrasekaran, A. R., & Levchenko, O. (2016). DNA nanocages. *Chemistry of Materials*, *28*, 5569–5581. doi: 10.1021/acs.chemmater.6b02546.

Chandrasekaran, A. R., Vilcapoma, J., Dey, P., Wong-Deyrup, S. W., Dey, B. K., & Halvorsen, K. (2020). Exceptional nuclease resistance of paranemic crossover (PX) DNA and crossover-dependent biostability of DNA motifs. *Journal of the American Chemical Society*, *142*, 6814–6821. doi: 10.1021/jacs.0c02211.

Conway, J. W., McLaughlin, C. K., Castor, K. J., & Sleiman, H. (2013). DNA nanostructure serum stability: Greater than the sum of its parts. *Chemical Communications*, *49*, 1172–1174. doi: 10.1039/c2cc37556g.

Gallagher, S. R. (2014). Digital image processing and analysis with ImageJ. *Current Protocols Essential Laboratory Techniques*, *9*, A.3C.1–A.3C.29. doi: 10.1002/9780470089941.eta03cs9.

He, Y., Ye, T., Su, M., Zhang, C., Ribbe, A. E., Jiang, W., & Mao, C. (2008). Hierarchical self-assembly of DNA into symmetric supramolecular polyhedra. *Nature*, *452*, 198–201. doi: 10.1038/nature06597.

Hong, F., Zhang, F., Liu, Y., & Yan, H. (2017). DNA origami: Scaffolds for creating higher order structures. *Chemical Reviews*, *117*, 12584–12640. doi: 10.1021/acs.chemrev.6b00825.

Keum, J.-W., & Bermudez, H. (2009). Enhanced resistance of DNA nanostructures to enzymatic digestion. *Chemical Communications*, 7036–7038. doi: 10.1039/b917661f.

Kim, K.-R., Kim, H. Y., Lee, Y.-D., Ha, J. S., Kang, J. H., Jeong, H., … Ahn, D. R. (2016). Self-assembled mirror DNA nanostructures for tumor-specific delivery of anticancer drugs. *Journal of Controlled Release*, *243*, 121–131. doi: 10.1016/j.jconrel.2016.10.015.

Kim, Y., & Yin, P. (2020). Enhancing biocompatible stability of DNA nanostructures using dendritic oligonucleotides and brick motifs. *Angewandte Chemie International Edition*, *59*, 700–703. doi: 10.1002/anie.201911664.

Kiviaho, J. K., Linko, V., Ora, A., Tiainen, T., Järvihaavisto, E., Mikkilä, J., … Kostiainen, M. A. (2016). Cationic polymers for DNA origami coating – examining their binding efficiency and tuning the enzymatic reaction rates. *Nanoscale*, *8*, 11674–11680. doi: 10.1039/C5NR08355A.

Kizer, M. E., Linhardt, R. J., Chandrasekaran, A. R., & Wang, X. (2019). A molecular hero suit for in vitro and in vivo DNA nanostructures. *Small*, *15*, 1805386. doi: 10.1002/smll.201805386.

Liu, Q., Liu, G., Wang, T., Fu, J., Li, R., Song, L., … Chen, F. (2017). Enhanced stability of DNA nanostructures by incorporation of unnatural base pairs. *ChemPhysChem*, *18*, 2977–2980. doi: 10.1002/cphc.201700809.

Madhanagopal, B. R., Zhang, S., Demirel, E., Wady, H., & Chandrasekaran, A. R. (2018). DNA nanocarriers: Programmed to deliver. *Trends in Biochemical Sciences*, *43*, 997–1013. doi: 10.1016/j.tibs.2018.09.010.

Mathur, D., & Medintz, I. L. (2019). The growing development of DNA nanostructures for potential healthcare-related applications. *Advanced Healthcare Materials*, *8*, 1801546. doi: 10.1002/adhm.201801546.

Perrault, S. D., & Shih, W. M. (2014). Virus-inspired membrane encapsulation of DNA nanostructures to achieve in vivo stability. *ACS Nano*, *8*, 5132–5140. doi: 10.1021/nn5011914.

Ponnuswamy, N., Bastings, M. M. C., Nathwani, B., Ryu, J. H., Chou, L. Y. T., Vinther, M., … Shih, W. M. (2017). Oligolysine-based coating protects DNA nanostructures from low-salt denaturation and nuclease degradation. *Nature Communications*, *8*, 1–9. doi: 10.1038/ncomms15654.

Rothemund, P. W. K. (2006). Folding DNA to create nanoscale shapes and patterns. *Nature*, *440*, 297–302. doi: 10.1038/nature04586.

Rusling, D. A., Chandrasekaran, A. R., Ohayon, Y. P., Brown, T., Fox, K. R., Sha, R., … Seeman, N. C. (2014). Functionalizing designer DNA crystals with a triple-helical veneer. *Angewandte Chemie International Edition*, *53*, 3979–3982. doi: 10.1002/anie.201309914.

Seeman, N. C. (2003). DNA in a material world. *Nature*, *421*, 427–431. doi: 10.1038/nature01406.

Seeman, N. C., & Sleiman, H. F. (2018). DNA nanotechnology. *Nature Reviews Materials*, *3*, 17068. doi: 10.1038/natrevmats.2017.68.

Shen, Z., Yan, H., Wang, T., & Seeman, N. C. (2004). Paranemic crossover DNA: A generalized holliday structure with applications in nanotechnology. *Journal of the American Chemical Society*, *126*, 1666–1674. doi: 10.1021/ja038381e.

Wang, S.-T., Gray, M. A., Xuan, S., Lin, Y., Byrnes, J., Nguyen, A. I., … Gang, O. (2020). DNA origami protection and molecular interfacing through engineered sequence-defined peptoids. *Proceedings of the National Academy of Sciences*, *117*, 6339–6348. doi: 10.1073/pnas.1919749117.

Wang, X., Chandrasekaran, A. R., Shen, Z., Ohayon, Y. P., Wang, T., Kizer, M. E., … Seeman, N. C. (2019). Paranemic crossover DNA: There and back again. *Chemical Reviews*, *119*, 6273–6289.

Wang, X., Lai, W., Man, T., Qu, X., Li, L., Chandrasekaran, A. R., & Pei, H. (2018). Biosurface engineering with DNA scaffolds for

theranostic applications. *Nanofabrication*, *4*, 1–16. doi: 10.1515/nanofab-2018-0001.

Xavier, P. L., & Chandrasekaran, A. R. (2018). DNA-based construction at the nanoscale: Emerging trends and applications. *Nanotechnology*, *29*, 062001. doi: 10.1088/1361-6528/aaa120.

Xiao, M., Lai, W., Man, T., Chang, B., Li, L., Chandrasekaran, A. R., & Pei, H. (2019). Rationally engineered nucleic acid architectures for biosensing applications. *Chemical Reviews*, *119*, 11631–11717. doi: 10.1021/acs.chemrev.9b00121.