ACS SyntheticBiology

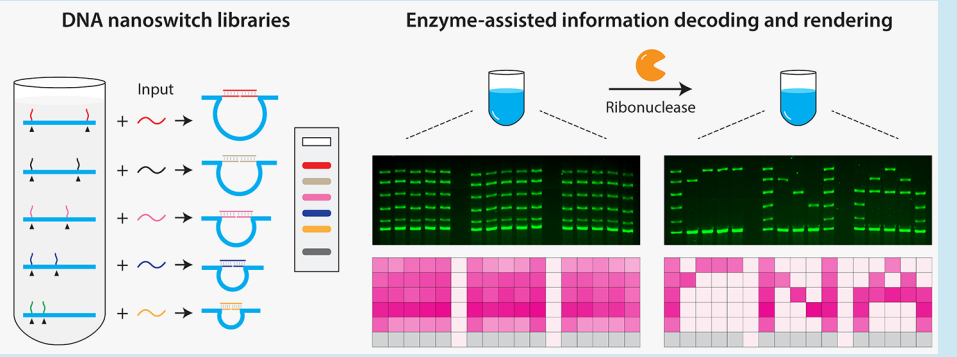
pubs.acs.org/synthbio Letter

Encoding, Decoding, and Rendering Information in DNA Nanoswitch Libraries

Hannah Talbot, Ken Halvorsen, and Arun Richard Chandrasekaran*

Cite This: https://doi.org/10.1021/acssynbio.2c00649 Read Online

ACCESS | Metrics & More | Article Recommendations | Supporting Information



ABSTRACT: DNA-based construction allows the creation of molecular devices that are useful in information storage and processing. Here, we combine the programmability of DNA nanoswitches and stimuli-responsive conformational changes to demonstrate information encoding and graphical readout using gel electrophoresis. We encoded information as 5-bit binary codes for alphanumeric characters using a combination of DNA and RNA inputs that can be decoded using molecular stimuli such as a ribonuclease. We also show that a similar strategy can be used for graphical visual readout of alphabets on an agarose gel, information that is encoded by nucleic acids and decoded by a ribonuclease. Our method of information encoding and processing could be combined with DNA actuation for molecular computation and diagnostics that require a nonarbitrary visual readout.

Nucleic acids are known for their ability to carry biological information, predominantly dictated by their sequence identities. With the development of nucleic acid nanostructures and nucleic acid copolymers, DNA structures can also code for information beyond just their sequences.[1,2] These features have made DNA useful in molecular computation, where the molecule has been used to perform logic operations,[3] mathematical calculations,[4] and to store and process data.[5] DNA is highly stable as a material, making it suitable for long-term (archival) storage of information.[6] This process typically involves information encoding in DNA sequences and uses a DNA sequencing readout to retrieve information.[7] Recent works have looked at random access of data stored in DNA where individual files can be recovered without having to sequence the entire library of molecules.[8] Further, different encoding methods[9] for DNA computing have aided in enhancing the density of data storage[10] with low error in data processing,[11] and new synthesis techniques have reduced the costs involved in DNA data storage.[12] Some recent strategies for DNA-based information encoding and data processing include the incorporation of artificial nucleotides to achieve higher coding efficiency,[13] DNA-silver nanoparticle composites for write-once read-many-times memory,[14] DNA and DNA-mimicking brush polymers as low-power-consuming memory devices,[15] temperature-controlled DNA hairpins for molecular memory[16] and one-dimensional DNA arrays for rewritable memory.[17]

We have previously used reconfigurable DNA nanoswitches for short-term storage of information encoded as 5-bit characters.[18] In our prior work and work by many others, the DNA devices are typically operated using other nucleic acids as inputs. The programmability of DNA allows construction of nanostructures that can reconfigure in response to different environmental stimuli (pH[19]), physical stimuli (temperature[20] or light[21]), and biological stimuli (nucleic acids,[22] antibodies[23] and antigens[24]). Thus, programming DNA nanostructures to respond to multiple types of stimuli expands the routes with which information can be encoded or decoded, and in some cases with graphical readouts.[25] In this context, here we present DNA nanoswitch libraries that can respond to a ribonuclease to provide a visual readout on an electrophoretic platform, either as segmented displays or as binary codes. Such graphical readouts

Received: December 1, 2022

of molecular events could be useful in diagnostics and computation.[26,27] Further, we also demonstrate the use of such nanoswitch libraries in information encryption using a combination of RNA and DNA inputs, with the information decoded by an end user using a ribonuclease.

The DNA nanoswitch[28,29] we use in this work is constructed from a commercially available single-stranded M13 viral genome (7249 nucleotides) and short complementary backbone oligos (Figure 1a and Figure S1). Two of the backbone oligonucleotides are modified to contain single stranded extensions (called latches) that can recognize specific nucleic acid inputs. On recognizing the input DNA or RNA, the nanoswitch reconfigures from a linear "open" state to a looped "locked" state. The two states of the nanoswitch provide a digital on/off signal when they become separated by differences in mobility using an agarose gel readout (Figure 1b). This mobility difference arises from structural reconfiguration of the nanoswitch from the linear to the looped state rather than differences in molecular weight that gels are typically associated with. We have previously used DNA inputs to loop nanoswitches and encode information using binary codes (locked and unlocked nanoswitches as 1s and 0s, respectively),[18] and in a separate work showed that nanoswitches can be looped by RNA and unlooped using a ribonuclease.[30] Here, we build on those works to reconfigure specific sets of DNA nanoswitches in a library using ribonuclease to encode, encrypt, and render specific patterns such as alphanumeric characters or 5-bit binary codes.

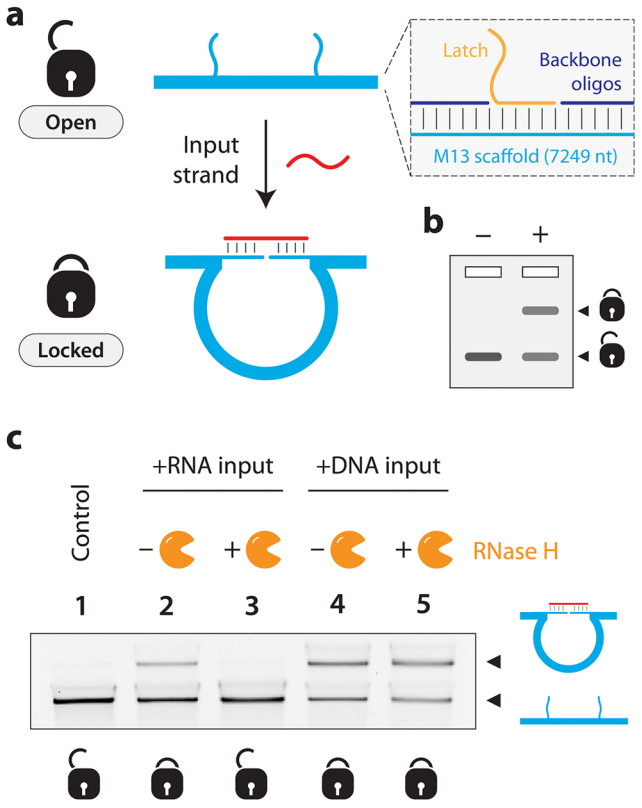


Figure 1. DNA nanoswitch design and operation. (a) The open state of the DNA nanoswitch is a linear structure with two single stranded DNA latches. Addition of an input strand reconfigures the nanoswitch to the looped locked state. Inset shows the components of the DNA nanoswitch. (b) Gel-based readout of the open and locked states. (c) Ribonuclease-triggered reconfiguration of the DNA nanoswitch from the locked to the open state achieved through RNase H triggered cleavage of an RNA input strand (lanes 2 and 3). The same nanoswitch can be erase-protected by using a DNA input strand (lanes 4 and 5).

To demonstrate the response of the nanoswitches to ribonuclease H (RNase H), we created a DNA nanoswitch that can bind to a specific RNA input sequence. On binding the RNA input, the DNA nanoswitch is reconfigured from the linear to the looped state, indicated by the appearance of the locked band (Figure 1c, lanes 1 and 2). On the addition of RNase H, the RNA input in the RNA-DNA hybrid of the input/latch duplex is cleaved, thus unlooping the nanoswitch and leading to the disappearance of the locked band (Figure 1c, lane 3). For example, a prewritten code (a locked nanoswitch corresponding to "1") can be "erased" using RNase H to reconfigure it to an open nanoswitch ("0"). Further, the specificity of RNase H to only cleave the RNA in a DNA−RNA hybrid duplex provides an "erase protection" feature. To demonstrate this, we locked the nanoswitch using a DNA strand of the same sequence as the RNA input so that this locked nanoswitch does not react to the addition of RNase H, thus remaining in the locked conformation (Figure 1c, lanes 4 and 5).

Next, we showed that the ribonuclease activity can be used on specific nanoswitches in a library. To demonstrate this, we designed five nanoswitches, each containing single stranded latches separated by different distances on the scaffold strand (600, 1200, 1800, 2400, and 3000 bp) (Figure 2a). We then created a nanoswitch mixture containing the five nanoswitches. Addition of specific input strands results in nanoswitches of different loop sizes which in turn have specific migration patterns on an agarose gel (Figure 2b). We chose these latch placements based on our earlier multiplexed biosensing work,[31] so that the reconfigured nanoswitches yield loop sizes that are easily resolvable on a gel (Figure 2b, gel inset). It is to be noted that the migration of nanoswitches is dependent on the different loop sizes and not the molecular weight. We first confirmed that RNase H can act on a specific nanoswitch in this mixture by locking one nanoswitch with an RNA input and the other four nanoswitches with DNA inputs (Figure 2c). We tested different cases where we locked a nanoswitch of a different loop size using an RNA input. On the addition of RNase H, only the specific nanoswitch is unlocked, while all the other nanoswitches in the mixture remain locked (Figure 2d and Figure S2).

We then used the gel-based readout of DNA nanoswitch reconfiguration as a graphical readout strategy, similar to seven-segmented displays where a series of bits display a particular pattern (for example, a number or a letter). To create an alphanumeric display, we created DNA nanoswitch libraries that consisted of the 5-nanoswitch mixture incubated with specific combinations of RNA or DNA inputs that are predetermined for a specific character. Once all the inputs are added, the nanoswitches in each library are reconfigured, resulting in 5 loops per lane (Figure 2e and Figure S3). We started with a set of 5 × 5 matrix displayed across five gel lanes where each looped band provides a "pixel". To read the encoded message, we added RNase H to the library and incubated the nanoswitches for 1 h. On the addition of RNase H, the enzyme cleaves only the RNA inputs bound to the specific set of nanoswitches and unlooping them, yielding a graphical display of the letters "rNA" on the gel formed by the remaining nanoswitches locked by DNA inputs (Figure 2e).

The DNA nanoswitches can also be used to encrypt information as binary codes, with each looped state corresponding to a "bit". Specific DNA or RNA inputs can encode specific

https://doi.org/10.1021/acssynbio.2c00649
ACS Synth. Biol. XXXX, XXX, XXX−XXX

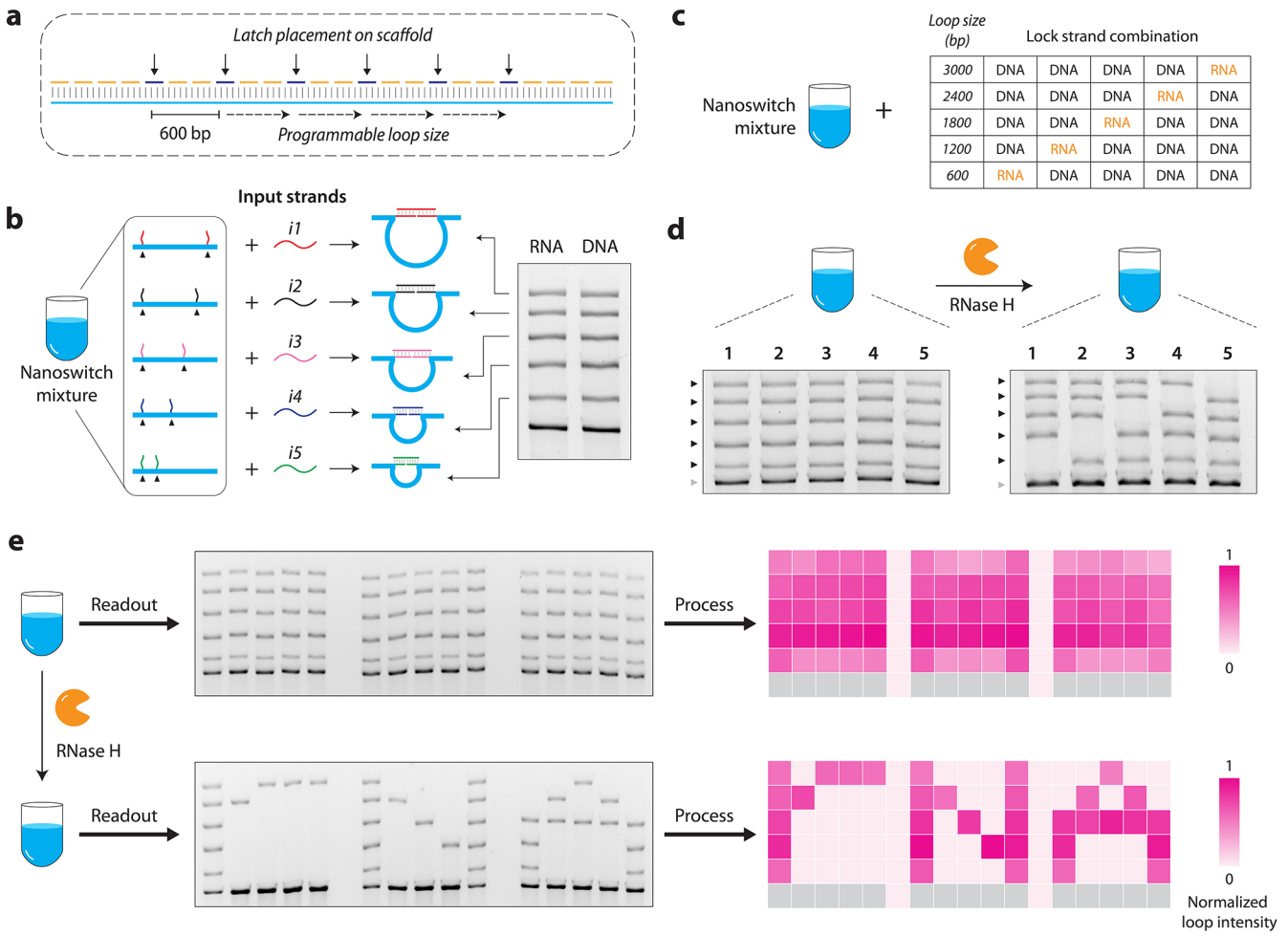


Figure 2. Graphical readout using ribonuclease-triggered DNA nanoswitches. (a) Placement of latches separated by different distances. (b) Design of nanoswitch library consisting of nanoswitches of different loop sizes with a unique readout signature on a gel. Gel shows bands corresponding to each nanoswitch locked by RNA or DNA inputs. (c) Combination of DNA or RNA inputs with a nanoswitch library containing five nanoswitches. (d) Nanoswitches within a library can be specifically reconfigured using RNase H, leading to erasing of a single pixel within a group. (e) A segmented display pattern using DNA nanoswitch libraries, reading out "rNA" on treatment with RNase H. Heatmap of signal intensity from gels for the displayed information is shown on the right. Band intensities in the heat map are normalized to the highest intensity band in the gel.

combinations of bits and RNase H can be used to erase specific bits, resulting in the readout of the encrypted message. Using the nanoswitch mixture, we first encoded 5 bits using a combination of DNA or RNA inputs. We used a library of input strands that, when treated with RNase H, will yield a specific 5-bit code corresponding to an alphabet according to the Baudot code (Figure 3a and Table S1). Nanoswitches locked by DNA input strands remain looped while those looped by RNA inputs are unlocked. Using this strategy, we also demonstrate encryption of a specific message using a set of 5-bit codes (Figure 3b). As an example, we encrypted a 7-letter message that displays the binary code "11111" for all the letters when read out on an agarose gel (Figure 3c and Figure S4). The receiver of the message can add RNase H to the tube, and the resulting gel readout now displays binary codes that translate the encrypted message "NEWYORK" (Figure 3d and Figure S4).

In this work, we demonstrate the use of DNA nanoswitch libraries that can be reconfigured using specific nucleic acid inputs, and further respond to a ribonuclease to yield graphical readouts of alphanumeric characters. Our strategy is also useful in encoding information using binary codes, allowing the encryption of hidden messages that can be decrypted using RNase H. In the current work, we've shown a segmented display type graphical readout that includes a 5 × 5 matrix, and a 5-bit binary code. The number of bits can be expanded by increasing the number of nanoswitches in the mixture, with additional latch placements corresponding to different loop sizes. The ∼7 kb long scaffold is an advantage in this case, allowing the placement of latches at different locations. Our typical design has the latches separated by 600 bp, but more latch combinations can be placed at shorter distances for multiplexing with a higher number of nanoswitches within a library. We've demonstrated up to 8 bits using nanoswitches in our prior work,[18] and the number is mainly limited by the resolution of the gel to distinguish the different looped states. The readout efficiency can also be expanded using high-throughput gel electrophoresis systems[32] and capillary gel electrophoresis.[33] While we use a gel-based readout here, reconfiguration of the nanoswitch can also be monitored using FRET or fluorescence. However, our present work using a gel-based readout is low cost, and uniquely does not require any labeling and only requires typical gel stains. The gel-based readout also has high sensitivity due to the intercalation of thousands of dye molecules (DNA gel stains) in the ∼7 kbp nanoswitch.[29] If combined with fluorescent readout,

https://doi.org/10.1021/acssynbio.2c00649
ACS Synth. Biol. XXXX, XXX, XXX−XXX

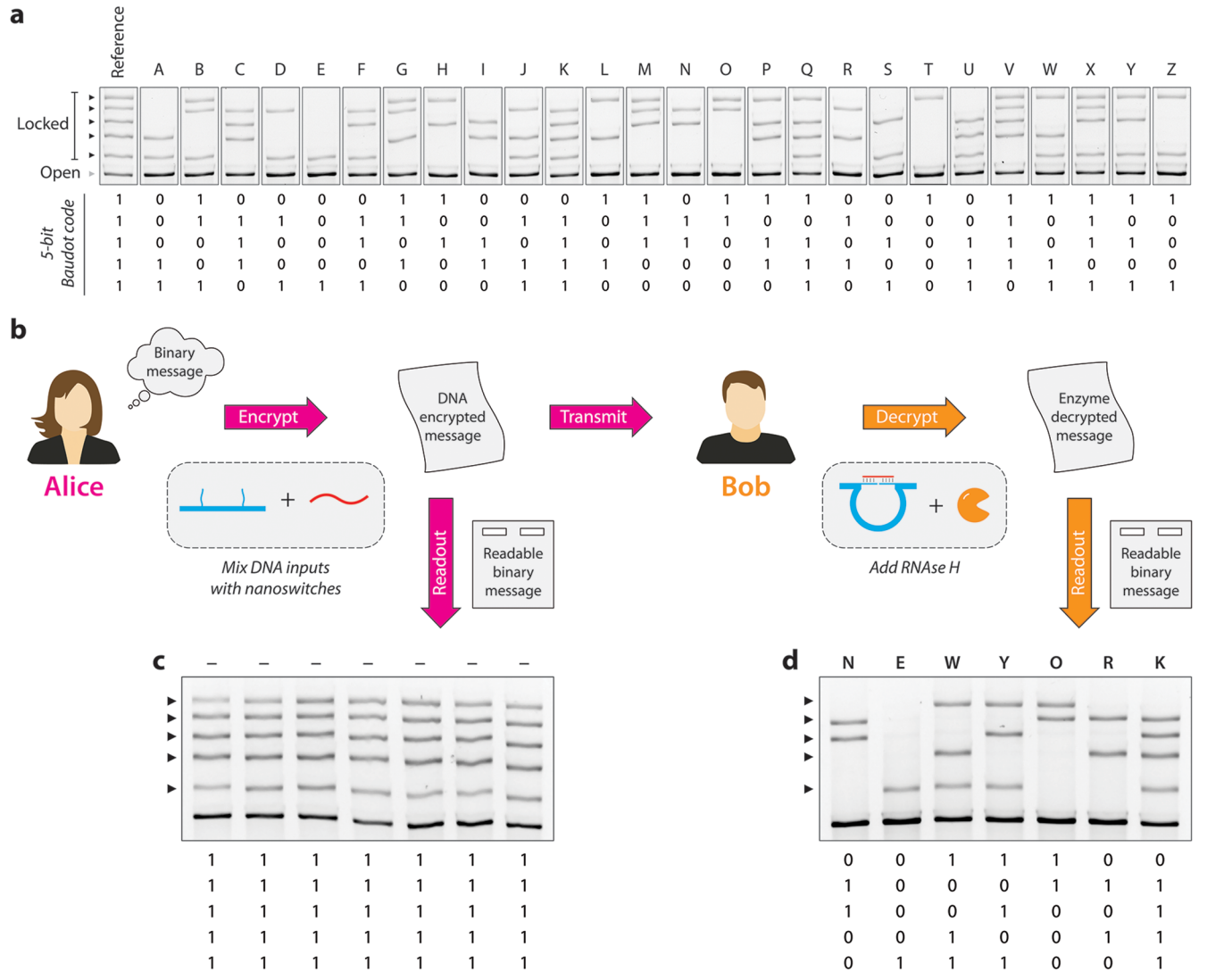


Figure 3. Data encryption using DNA nanoswitches. (a) Encoding 5-bit binary codes for the English alphabet characters using ribonuclease-responsive DNA nanoswitches and corresponding gel readout. (b) Encryption of the message "NEWYORK" using nanoswitches that can be translated by treatment with RNase H and a gel based readout. (c) Gel readout of encrypted message. (d) Gel readout of RNase-treated sample that yields the decrypted message.

it is possible that such a system will allow macroscopic readouts on plates[34] or paper-based[27] platforms similar to other reported biomolecular computational studies. The work described here uses nucleic acid inputs (DNA and RNA), and the system is intended for short-term memory. Based on our other works that use DNA nanoswitches for detecting proteins and antibodies,[31] one can also expand this information processing using a larger variety of biomolecules as inputs, and other stimuli such as light[35] to decrypt information. Such a molecular readout system could also be useful in biosensing (for display of biomarker presence) or in molecular computation (for readout of results) where molecular events can lead to macroscopic outputs.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acssynbio.2c00649.

Methods, additional experimental results, and oligonucleotide sequences used (PDF)

■ AUTHOR INFORMATION

Corresponding Author

Arun Richard Chandrasekaran − *The RNA Institute, University at Albany, State University of New York, Albany, New York 12203, United States;* orcid.org/0000-0001-6757-5464; Email: arun@albany.edu

Authors

Hannah Talbot − *The RNA Institute, University at Albany, State University of New York, Albany, New York 12203, United States*

Ken Halvorsen − *The RNA Institute, University at Albany, State University of New York, Albany, New York 12203, United States;* orcid.org/0000-0002-2578-1339

Complete contact information is available at: https://pubs.acs.org/10.1021/acssynbio.2c00649

https://doi.org/10.1021/acssynbio.2c00649
ACS Synth. Biol. XXXX, XXX, XXX−XXX

Author Contributions

H.T. performed experiments; K.H. aided design of experiments and edited the manuscript; A.R.C. conceived the project, designed and performed experiments, analyzed and visualized data, supervised the project, and wrote the manuscript.

Notes

The authors declare the following competing financial interest(s): A.R.C. and K.H. are inventors on patents and patent applications related to DNA nanoswitches.

■ ACKNOWLEDGMENTS

Research reported in this publication was supported by the NIH through NIGMS under Award R35GM124720 to K.H. and through The RNA Institute and University at Albany startup funds to A.R.C.

■ REFERENCES

(1) Ceze, L.; Nivala, J.; Strauss, K. Molecular Digital Data Storage Using DNA. *Nat. Rev. Genet.* **2019**, *20*, 456.
(2) Rutten, M. G. T. A.; Vaandrager, F. W.; Elemans, J. A. A. W.; Nolte, R. J. M. Encoding Information into Polymers. *Nat. Rev. Chem.* **2018**, *2* (11), 365−381.
(3) Chatterjee, G.; Dalchau, N.; Muscat, R. A.; Phillips, A.; Seelig, G. A Spatially Localized Architecture for Fast and Modular DNA Computing. *Nat. Nanotechnol.* **2017**, *12* (9), 920−927.
(4) Qian, L.; Winfree, E. Scaling Up Digital Circuit Computation with DNA Strand Displacement Cascades. *Science* **2011**, *332* (6034), 1196−1201.
(5) Erlich, Y.; Zielinski, D. DNA Fountain Enables a Robust and Efficient Storage Architecture. *Science* **2017**, *355* (6328), 950−954.
(6) Cox, J. P. L. Long-Term Data Storage in DNA. *Trends Biotechnol.* **2001**, *19* (7), 247−250.
(7) Zhirnov, V.; Zadegan, R. M.; Sandhu, G. S.; Church, G. M.; Hughes, W. L. Nucleic Acid Memory. *Nat. Mater.* **2016**, *15* (4), 366−370.
(8) Organick, L.; Ang, S. D.; Chen, Y.-J.; Lopez, R.; Yekhanin, S.; Makarychev, K.; Racz, M. Z.; Kamath, G.; Gopalan, P.; Nguyen, B.; Takahashi, C. N.; Newman, S.; Parker, H.-Y.; Rashtchian, C.; Stewart, K.; Gupta, G.; Carlson, R.; Mulligan, J.; Carmean, D.; Seelig, G.; Ceze, L.; Strauss, K. Random Access in Large-Scale DNA Data Storage. *Nat. Biotechnol.* **2018**, *36* (3), 242−248.
(9) Garzon, M. H.; Bobba, K. V.; Hyde, B. P. Digital Information Encoding on DNA. In *Aspects of Molecular Computing: Essays Dedicated to Tom Head, on the Occasion of His 70th Birthday*; Jonoska, N., Păun, G., Rozenberg, G., Eds.; Lecture Notes in Computer Science; Springer: Berlin, Heidelberg, 2004; pp 152−166. DOI: 10.1007/978-3-540-24635-0_11.
(10) Goldman, N.; Bertone, P.; Chen, S.; Dessimoz, C.; LeProust, E. M.; Sipos, B.; Birney, E. Towards Practical, High-Capacity, Low-Maintenance Information Storage in Synthesized DNA. *Nature* **2013**, *494* (7435), 77−80.
(11) Yazdi, S. M. H. T.; Gabrys, R.; Milenkovic, O. Portable and Error-Free DNA-Based Data Storage. *Sci. Rep* **2017**, *7* (1), 5011.
(12) Antkowiak, P. L.; Lietard, J.; Darestani, M. Z.; Somoza, M. M.; Stark, W. J.; Heckel, R.; Grass, R. N. Low Cost DNA Data Storage Using Photolithographic Synthesis and Advanced Information Reconstruction and Error Correction. *Nat. Commun.* **2020**, *11* (1), 5345.
(13) Ren, Y.; Zhang, Y.; Liu, Y.; Wu, Q.; Su, J.; Wang, F.; Chen, D.; Fan, C.; Liu, K.; Zhang, H. DNA-Based Concatenated Encoding System for High-Reliability and High-Density Data Storage. *Small Methods* **2022**, *6* (4), 2101335.
(14) Hung, Y.-C.; Hsu, W.-T.; Lin, T.-Y.; Fruk, L. Photoinduced Write-Once Read-Many-Times Memory Device Based on DNA Biopolymer Nanocomposite. *Appl. Phys. Lett.* **2011**, *99* (25), 253301.
(15) Lee, J.; Kim, Y.; Kim, C.; Ree, M. New High Performance Digital Memory Devices Fabricated with DNA and DNA-Mimics. *Mater. Horiz.* **2017**, *4* (3), 423−430.
(16) Takinoue, M.; Suyama, A. Hairpin-DNA Memory Using Molecular Addressing. *Small* **2006**, *2* (11), 1244−1247.
(17) Shin, J.-S.; Pierce, N. A. Rewritable Memory by Controllable Nanopatterning of DNA. *Nano Lett.* **2004**, *4* (5), 905−909.
(18) 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.
(19) Ji, W.; Li, D.; Lai, W.; Yao, X.; Alam, Md. F.; Zhang, W.; Pei, H.; Li, L.; Chandrasekaran, A. R. PH-Operated Triplex DNA Device on MoS2 Nanosheets. *Langmuir* **2019**, *35* (14), 5050−5053.
(20) Franch, O.; Iacovelli, F.; Falconi, M.; Juul, S.; Ottaviani, A.; Benvenuti, C.; Biocca, S.; Ho, Y.-P.; Knudsen, B. R.; Desideri, A. DNA Hairpins Promote Temperature Controlled Cargo Encapsulation in a Truncated Octahedral Nanocage Structure Family. *Nanoscale* **2016**, *8* (27), 13333−13341.
(21) Ryssy, J.; Natarajan, A. K.; Wang, J.; Lehtonen, A. J.; Nguyen, M.-K.; Klajn, R.; Kuzyk, A. Light-Responsive Dynamic DNA-Origami-Based Plasmonic Assemblies. *Angew. Chem., Int. Ed.* **2021**, *60* (11), 5859−5863.
(22) Chandrasekaran, A. R.; Halvorsen, K. Controlled Disassembly of a DNA Tetrahedron Using Strand Displacement. *Nanoscale Adv.* **2019**, *1* (3), 969−972.
(23) Ranallo, S.; Sorrentino, D.; Ricci, F. Orthogonal Regulation of DNA Nanostructure Self-Assembly and Disassembly Using Antibodies. *Nat. Commun.* **2019**, *10* (1), 5509.
(24) Engelen, W.; Sigl, C.; Kadletz, K.; Willner, E. M.; Dietz, H. Antigen-Triggered Logic-Gating of DNA Nanodevices. *J. Am. Chem. Soc.* **2021**, *143* (51), 21630−21636.
(25) Chandrasekaran, A. R. Processing DNA-Based Molecular Signals into Graphical Displays. *ACS Synth. Biol.* **2020**, *9* (7), 1490−1498.
(26) Connelly, R. P.; Morozkin, E. S.; Gerasimova, Y. V. Alphanumerical Visual Display Made of DNA Logic Gates for Drug Susceptibility Testing of Pathogens. *ChemBioChem.* **2018**, *19* (3), 203−206.
(27) Li, J.; Pollak, N. M.; Macdonald, J. Multiplex Detection of Nucleic Acids Using Recombinase Polymerase Amplification and a Molecular Colorimetric 7-Segment Display. *ACS Omega* **2019**, *4* (7), 11388−11396.
(28) 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.
(29) 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. *Science Advances* **2019**, *5* (3), No. eaau9443.
(30) Chandrasekaran, A. R.; Trivedi, R.; Halvorsen, K. Ribonuclease-Responsive DNA Nanoswitches. *Cell Rep. Phys. Sci.* **2020**, *1* (7), 100117.
(31) Chandrasekaran, A. R.; MacIsaac, M.; Vilcapoma, J.; Hansen, C. H.; Yang, D.; Wong, W. P.; Halvorsen, K. DNA Nanoswitch Barcodes for Multiplexed Biomarker Profiling. *Nano Lett.* **2021**, *21* (1), 469−475.
(32) Wang, D.; Shi, J.; Carlson, S. R.; Cregan, P. B.; Ward, R. W.; Diers, B. W. A Low-Cost, High-Throughput Polyacrylamide Gel Electrophoresis System for Genotyping with Microsatellite DNA Markers. *Crop Science* **2003**, *43* (5), 1828−1832.
(33) Greenough, L.; Schermerhorn, K. M.; Mazzola, L.; Bybee, J.; Rivizzigno, D.; Cantin, E.; Slatko, B. E.; Gardner, A. F. Adapting Capillary Gel Electrophoresis as a Sensitive, High-Throughput Method to Accelerate Characterization of Nucleic Acid Metabolic Enzymes. *Nucleic Acids Res.* **2016**, *44* (2), No. e15.
(34) Poje, J. E.; Kastratovic, T.; Macdonald, A. R.; Guillermo, A. C.; Troetti, S. E.; Jabado, O. J.; Fanning, M. L.; Stefanovic, D.; Macdonald, J. Visual Displays That Directly Interface and Provide Read-Outs of Molecular States via Molecular Graphics Processing Units. *Angew. Chem., Int. Ed.* **2014**, *53* (35), 9222−9225.

https://doi.org/10.1021/acssynbio.2c00649
ACS Synth. Biol. XXXX, XXX, XXX−XXX

(35) Chandrasekaran, A. R.; Punnoose, J. A.; Valsangkar, V.; Sheng, J.; Halvorsen, K. Integration of a Photocleavable Element into DNA Nanoswitches. *Chem. Commun.* **2019**, *55* (46), 6587−6590.

https://doi.org/10.1021/acssynbio.2c00649
ACS Synth. Biol. XXXX, XXX, XXX−XXX