ChemPubSoc Europe
DOI: 10.1002/cbic.201800057
CHEMBIOCHEM Communications

Reconfigurable DNA Nanoswitches for Graphical Readout of Molecular Signals

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This study reports a DNA nanoswitch that can be used for visual display of outputs resulting from hybridization events of specific molecular inputs. Conformational changes of the DNA nanoswitch triggered by input DNA strands yield different "pixels" that collectively produce a graphical output on an agarose gel. This system has potential in molecular computation and biosensing approaches in which individual binding results can be translated into macromolecular visual readouts.

DNA is used as a building block for the construction of nanoscale materials with applications ranging from macromolecular scaffolding and biosensing to drug delivery.[1] One other application is in molecular computation, in which DNA nanostructures and devices have been used to compute mathematical tasks,[2] to perform logic operations,[3] and to store and process data.[4] In these processes, the DNA-based structures generate specific outputs from distinct inputs, and in some cases are designed to display the output as a visual readout of a computational result[5] or a biosensing event[6,7] (Figure 1). This work presents a DNA-nanoswitch-based system with the capability to store and to read out specific information by using molecular signals. The method is based on the sequence specificity of DNA and provides a molecular platform with distinct outputs and a simple gel-electrophoresis-based visual readout.

The DNA nanoswitch is constructed on the basis of the principles of DNA origami[8] and contains specific addressable sites (Figure 2). The "*off*" state of the nanoswitch is a long duplex formed by the single-stranded M13 scaffold (7249 nucleotides)

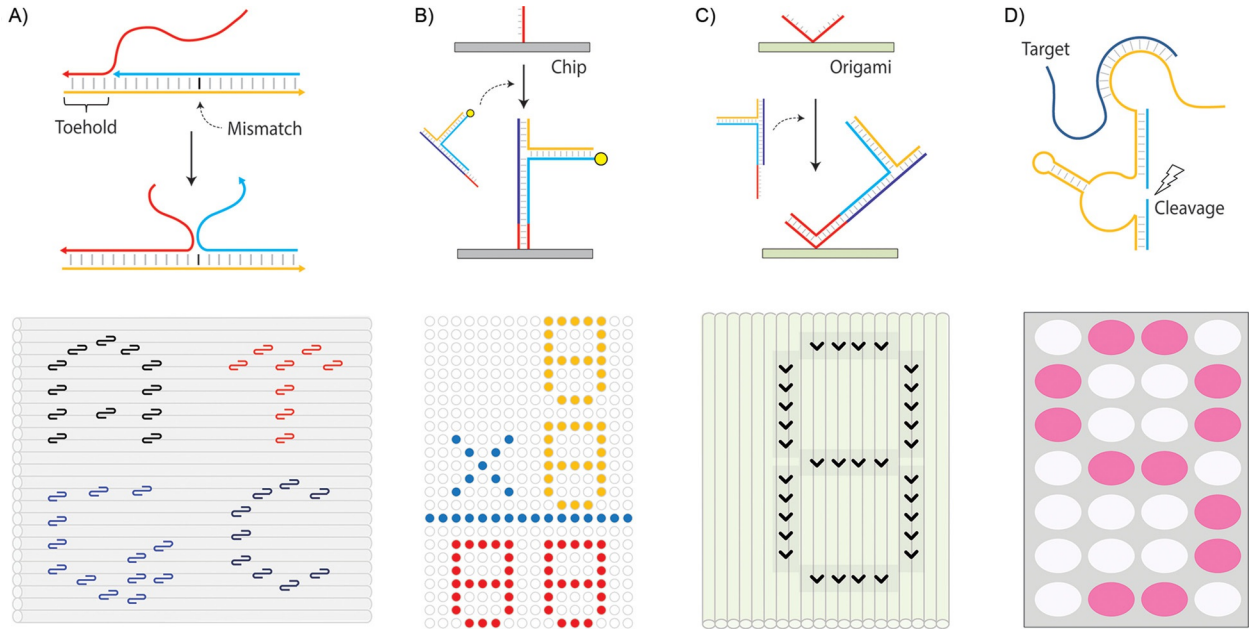

Figure 1. Examples of visual molecular readout strategies. A) An origami-based system for direct visual readout of single-nucleotide polymorphisms.[6] B) A DNA chip that can display the result of multiplication of two input numbers denoted by DNA strands.[5] C) The strategy shown in (B) can also be used to display the end result of multiplication on a DNA origami platform.[5] D) A deoxyribozyme-based graphics processing unit for nucleic acid detection with alphanumerical read-outs through a fluorescent display.[7]

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Supporting information and the ORCID identification number for the author of this article can be found under https://doi.org/10.1002/cbic.201800057: experimental procedures, a note on nanoswitch library schemes for segmented display, detailed nanoswitch designs, results of nanoswitch characterization and optimization and the DNA sequences used.

and short complementary backbone oligonucleotides (49–60 nucleotides). Two of these backbone oligonucleotides can be modified to contain single-stranded extensions (address strands), each of which can bind to parts of the input strand through sequence complementarity (details in Figure S1 in the Supporting Information). Hybridization of the input strand to the address sites reconfigures the switch to form a loop, thus changing it to the "*on*" state. The *off* and *on* states of the DNA

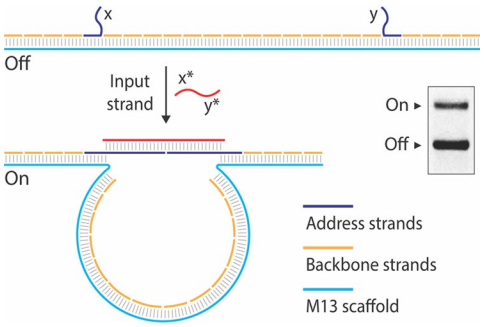

Figure 2. Design and operation of the DNA nanoswitch. The linear "*off*" state of the nanoswitch is formed from a long single-stranded scaffold strand and short complementary backbone oligonucleotides. Single-stranded extensions from two of the backbone oligonucleotides serve as address sites and, on binding to a complementary input strand, form a looped "*on*" state. The on and off states of the nanoswitch are easily identifiable on a gel (inset).

nanoswitches migrate differently on an agarose gel (containing routine DNA gel stains), thus providing a binary readout in the absence or in the presence of the input strand (Figure 2, inset). The DNA nanoswitch has been used previously in the context of single molecule studies,[9] as a biomolecular analysis platform,[10] and for the detection of nucleic acids[11] and antigens.[12]

The programmable nature of the nanoswitch allows multiple address sites to be positioned at desired intervals along the scaffold, thereby resulting in loops of different sizes on binding the input strand (Figure 3A). As an example, five nanoswitches were designed to contain address sites separated by different distances on the scaffold (600, 1200, 1800, 3000, and 5400 bp; Figures 3B and S2). On the addition of specific input strands, the nanoswitches reconfigure to form loops of different sizes corresponding to the distances between address sites. Figure 3C shows the gel results: lane 0 shows the switch by itself and lanes 1–5 show different looped states of the five different nanoswitches that are formed by five different input strands. The separations of these states were chosen in such a way that all five nanoswitches (or a subset) can be run on the same lane and still be distinctly visible on a gel (lane 6). The output intensity of the looped bands is presented as a heat map in Figure 3D. I would like to point out here that all of the looped nanoswitches have the same molecular weight, and that the migration of each *on* state depends on the loop size. The electrophoretic mobility for each species was analyzed by using a Ferguson plot (Figures 3E and S3), a means to estimate the retardation (frictional) coefficient of the looped species.[13] It can be seen that the frictional coefficient increases with loop size (increase in effective molecular radius) thus causing retardation in the gel (Figure 3F and Table S1). The efficiency of loop formation (short vs. long loops) was characterized by using a nanoswitch that can form multiple loops from a single input strand; looping efficiency was higher for address sites separat-

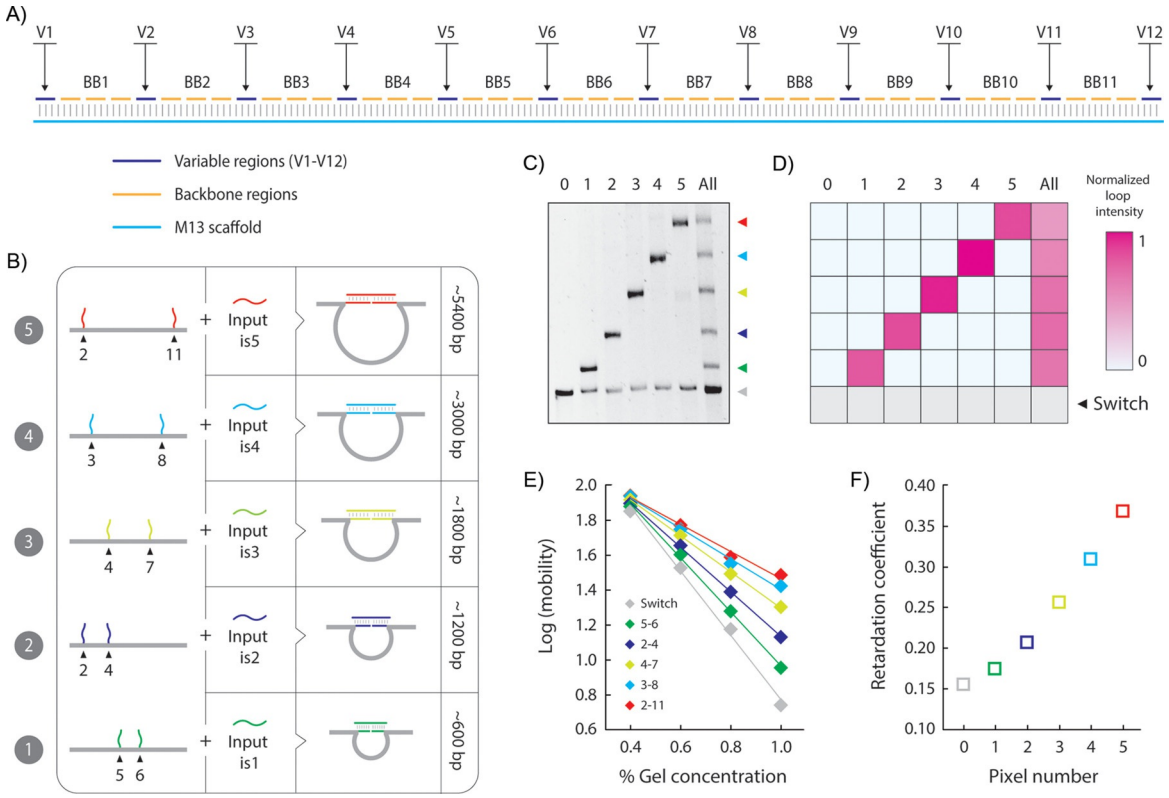

Figure 3. Characterization of looped DNA pixels. A) Strand arrangement of the nanoswitch, showing the variable regions where address sites can be inserted. B) The five different nanoswitches with address sites placed at different distances. Numbers indicate the locations of the address sites: for example, the 5–6 nanoswitch has address sites on variable regions 5 and 6, thus producing a 600-base-pair loop. C) Gel results of the loops resulting from the five nanoswitches shown in (B). Lane 6 has a mixture of all five nanoswitches, showing five distinct pixels from the five loops. D) A heat map showing the looped intensities from the gel in (C). E) A Ferguson plot showing the gel mobility characteristics of the five pixels across different gel percentages. F) The gel retardation coefficients for different pixels (loop sizes) calculated from the Ferguson plot.

ed by shorter distances (Figure S4). Moreover, shorter loops also formed more rapidly than longer loops (Figure S5).

Using this nanoswitch-based system I have demonstrated a "DNA pixel" strategy by choosing a set of address sites to store and to display alphanumeric characters. The readout of the stored information is inspired by digital display circuits in which a series of bits display a particular pattern (e.g., a number or a letter). For this purpose, I designed a multi-input nanoswitch[14] (Figure 4A) that allows different loops to be triggered on the same nanoswitch (instead of using a collection of different nanoswitches as described in the previous paragraph). This nanoswitch design has six address strands (spaced ≈600 base pairs apart) that can form five unique loops on binding five different input strands (Figure 4B). The first address site (A0) is common to all five loops; the input strands are all partly complementary to this address strand and partly complementary to any of the other five address strands. If the readout is considered to be a segmented display board with a 5×5 matrix, each looped band provides a DNA pixel on the matrix. As an example, specific input strands were used to trigger and to display three alphabet characters: "r", "N", and "A" (Figure 4C). The number of pixels in the vertical direction is five, corresponding to the five inducible looped states of the nanoswitch. This number can potentially be increased by including additional address sites on the nanoswitch, but will be limited by the resolution of the gel to display each looped band as a unique pixel. Any number of gel lanes can be used as the horizontal matrix length. On thinking of the resolution in the context of an image, the *on* and *off* states of the nanoswitch only provide a modest level of contrast for a multi-pixel graphical display (e.g., to create a pixelated image).[15] However, the main use of this system lies in a simple digital output that works like a segmented display. A library of such nanoswitches with distinct address strand pairs can potentially be used as a seven-segmented display, similar to those used to display the output results of multiplication of numbers[5] and to diagnose specific viral sequences.[7] A proposed design for use of nanoswitch libraries for segmented display is discussed in Supplementary Note 1 (see Figures S6–S7 for illustration).

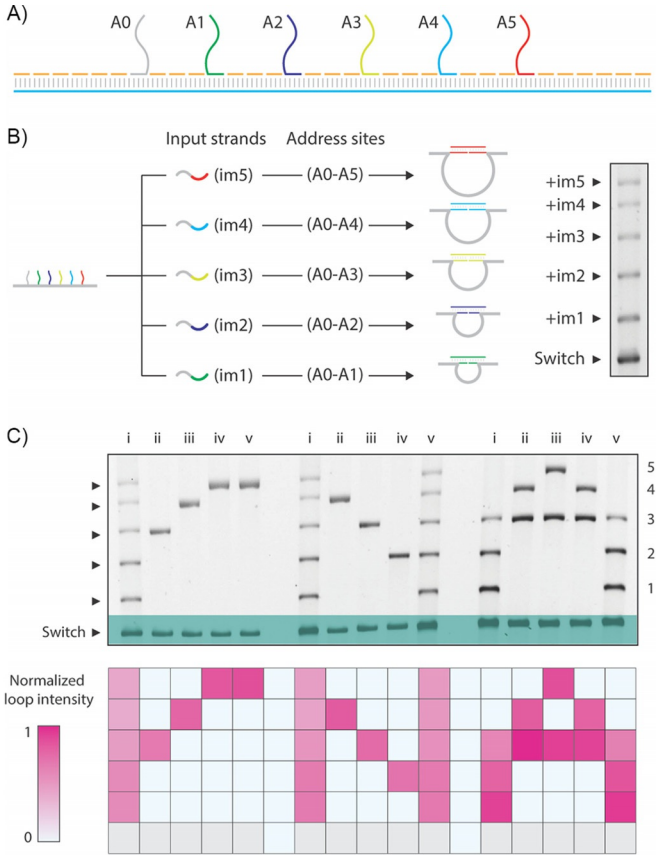

Figure 4. DNA pixel strategy for graphical readout. A) A multi-input nanoswitch with six address sites that can form five unique loops from five different input strands. B) Operation of the multi-input nanoswitch and gel result showing the five loops that can be triggered on addition of five inputs. C) A visual readout of a three-panel (5×5) matrix display showing the characters "r", "N", and "A" created by specific sets of input strands in each lane.

The system presented here converts a nanoscale molecular input directly into a macroscale output. Moreover, multi-input processing is feasible on the basis of design of address site locations, thus providing distinct output signals. This strategy does not involve complex, inconvenient processes such as separation by using magnetic beads or a solid substrate to obtain the readout and it also eliminates the need for use of fluorescent dyes or modified strands. Moreover, the nanoswitches are functional when stored dry,[14] and the purification process can be automated by using liquid chromatography techniques.[16] It should be noted that the mobility differences on the gel are induced by the conformational change of the nanoswitch from a linear to a looped state and that migration patterns remain unchanged for different types of inputs. The system discussed here uses DNA strands as inputs; however, it could easily be expanded to include proteins or antigens as inputs[12] by modifying the address strands to contain protein-specific antibodies (instead of single-stranded extensions). Given the sequence-specific response of this system, it is possible that this strategy can be coupled with biosensing devices, while providing a simple, amplification-free readout.

Acknowledgements

This work was done at The RNA Institute, University at Albany, State University of New York. I thank my postdoctoral advisor Dr. Ken Halvorsen for comments and discussion, for providing the lab space and resources for this project, and in general, for letting me "do stuff with DNA". I also thank Dr. Hari K. K. Subramanian for comments and suggestions on the manuscript. I would like to mention Reviewer 1's humor, which is a rarity in the current publishing scenario. It is a nice feeling to know that scientific review/criticism does not always have to be serious.

Conflict of Interest

A.R.C. is listed as an inventor on patent applications covering aspects of this work.

Keywords: DNA · DNA origami · nanodevices · nanoswitches · nanotechnology

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Manuscript received: January 28, 2018
Accepted manuscript online: March 24, 2018
Version of record online: April 23, 2018