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Orthogonal Control of DNA Nanoswitches with Mixed Physical and Biochemical Cues

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ABSTRACT: Nanoscale devices that can respond to external stimuli have potential applications in drug delivery, biosensing, and molecular computation. Construction using DNA has provided many such devices that can respond to cues such as nucleic acids, proteins, pH, light, or temperature. However, simultaneous control of molecular devices is still limited. Here, we present orthogonal control of DNA nanoswitches using physical (light) and biochemical (enzyme and nucleic acid) triggers. Each one of these triggers controls the reconfiguration of specific nanoswitches from locked to open states within a mixture and can be used in parallel to control a combination of nanoswitches. Such dynamic control over nanoscale devices allows the incorporation of tunable portions within larger structures as well as spatiotemporal control of DNA nanostructures.

From branched DNA junctions to origami, DNA nanotechnology has evolved in the past three decades to include a variety of nanoscale structures.[1] While structural DNA nanotechnology has resulted in the construction of different types of DNA architectures using many strategies, dynamic DNA nanotechnology has shown the versatility of DNA nanostructures to respond to cues.[2] The applications of such dynamic DNA nanostructures hinge on their reconfigurable states. Once assembled, DNA nanostructures can be designed to undergo different types of conformational changes that can be read out using a variety of techniques.[3] Some of the well-known applications of such dynamic DNA nanostructures are in biosensing, where recognition and binding of a specific biomarker (DNA,[4] RNA,[5] proteins,[6] or antibodies[7]) or environmental conditions (temperature,[8] pH,[9] ions[10]) cause the starting nanostructure to change its shape. In drug delivery, the response to stimuli (temperature,[11] light,[12] cellular fragments[13]) is beneficial in the triggered release of an encapsulated drug. While many of these structures respond to a single trigger, activation by simultaneous triggers can provide multiple functionalities. For example, parallel stimuli of different types (RNA and protein or different wavelengths of light) could allow independent processing of DNA devices with spatial and temporal control over the response. In this work, we demonstrate orthogonal control of DNA nanoswitches using three types of triggers: nucleic acids (specific DNA strands), enzymes (a ribonuclease), and light (ultraviolet radiation).

To demonstrate dynamic control of DNA nanostructures using external triggers, we use DNA nanoswitches[14] as a model system here. The "unlocked" state of the nanoswitch is a linear duplex assembled using the M13 scaffold strand (7249 nt) and short complementary backbone oligonucleotides. Two of the backbone oligonucleotides are modified to contain single-stranded extensions (latches) that are partly complementary to a target nucleic acid (Figure 1 and Figure S1). When the target binds to both the latches, it forms a loop between them, reconfiguring the nanoswitch to a looped "locked" state. The two configurations of the DNA nanoswitch can be read out on an agarose gel (Figure 1, inset). This binary reconfiguration allows only the two states of the nanoswitch to exist (locked and open) without any intermediate states. The only possible intermediate would be a looped-to-unlooped transition during the gel running time, which can be avoided with high-affinity locking strands.

To be able to operate the nanoswitch from the locked to the unlocked states, we designed three different types of locking strands and tested them on specific nanoswitches. For the first mechanism, we used a DNA locking strand that contained a photocleavable linker in the middle of the sequence (PC-DNA lock); once locked, the nanoswitch can be unlocked by shining UV (Figure 2a). For the second mechanism, we used an RNA lock to loop the nanoswitch, which can be unlocked using a ribonuclease (RNase H) that cleaves the RNA in a DNA/RNA hybrid (Figure 2b). For the third, we used a locking strand containing a toehold (TH-DNA lock) to reconfigure the nanoswitch. In this case, the addition of a strand that is fully complementary to the locking strand can remove the TH-DNA lock, thus unlocking the nanoswitch (Figure 2c). These three triggers are representative of biochemical and physical triggers, demonstrating control of DNA devices using nucleic acids, enzymes, and light.

The programmable nature of the DNA nanoswitch allows the creation of different loop sizes (i.e., different locked states)

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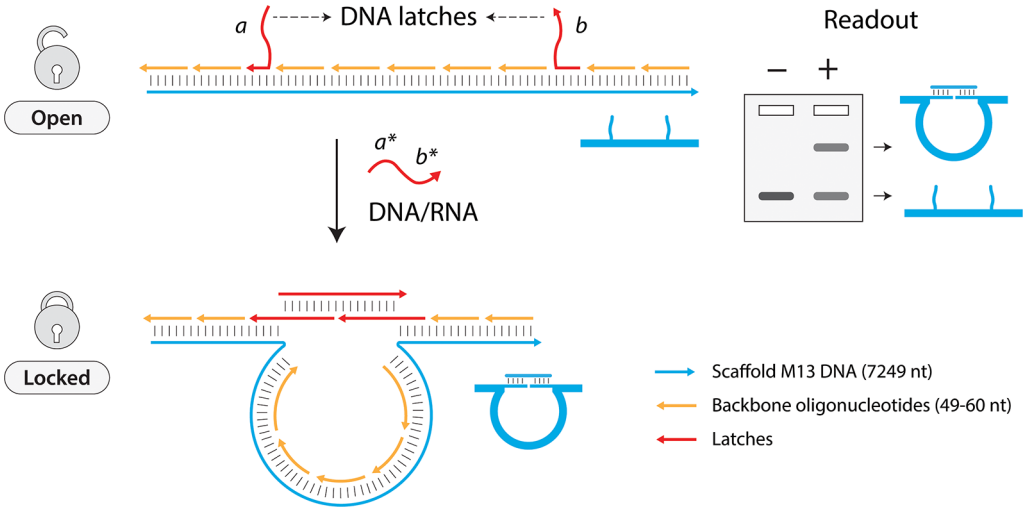

Figure 1. DNA nanoswitch design and operation. The open state of the nanoswitch is assembled from a single-stranded M13 scaffold and short complementary backbone oligonucleotides. Two single-stranded DNA latches are complementary to target nucleic acids. On binding the target DNA or RNA, the nanoswitch is reconfigured into the looped locked state. The open and locked states of the DNA nanoswitch can be identified using agarose gel electrophoresis (inset), due to different migration based on the topology.

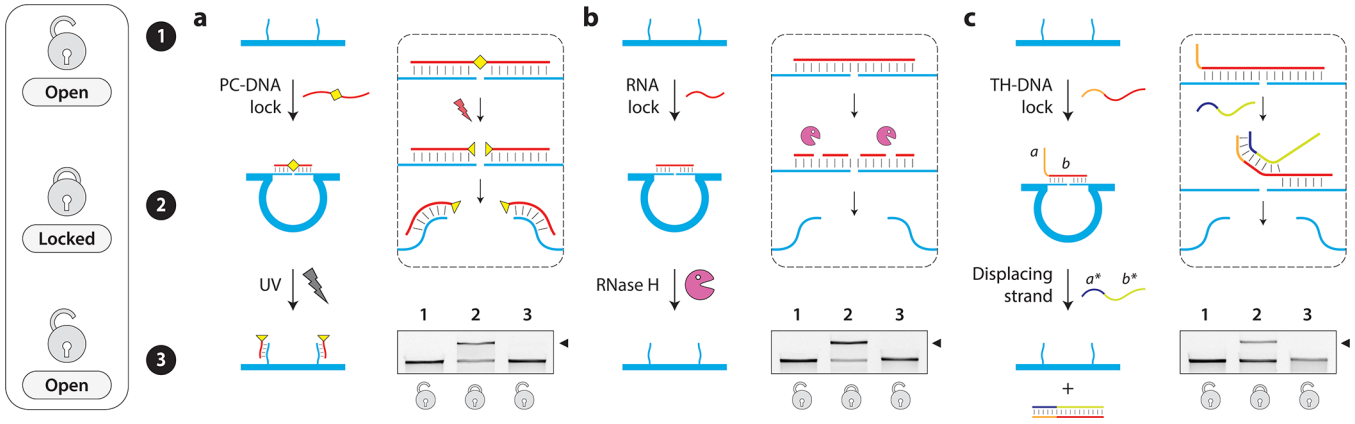

Figure 2. DNA nanoswitch reconfiguration using external triggers. (a) A photocleavable linker-containing DNA lock and ultraviolet (UV) light trigger, (b) RNA lock and ribonuclease trigger, and (c) toehold-containing DNA lock and a displacing strand as a trigger. For each case, the gel inset shows the open nanoswitch (lane 1) that is locked by a specific strand (lane 2) and unlocked by UV, RNase H, or a displacing strand (lane 3).

by placing the DNA latches at specific locations on the scaffold strand (Figure 3a and Figure S1). Latches placed further apart yield larger loops on recognizing the target, while smaller loops are formed by latches placed in closer proximity.[15] The different loop sizes of these locked nanoswitches yield unique bands on the gel, which we have previously used in multiplexed sensing assays.[5] The different gel migration patterns of the devices do not arise from a difference in molecular weight of the locked and unlocked states (the target nucleic acid is ∼20 nt and is thus negligible in comparison to a ∼7 kbp nanoswitch). Instead, it is because of a topological change from a linear to a looped state and the difference in the size and position of the loop. We characterized the electrophoretic mobility of different loop sizes using a Ferguson plot, a means to estimate the retardation (frictional) coefficient of the looped states (Figure S2).[16] Next, we designed a molecular mix that contained three nanoswitches with different loop sizes in which each of the nanoswitches recognizes only the PC-DNA, RNA, or TH-DNA locks (Figure 3b). We locked all three by adding the corresponding locking strands, confirmed by the presence of three unique bands on the gel (Figure 3c, lane 1). We used this mixture and added one trigger (lanes 2−4) or a subset (lanes 5−7) or all three triggers (lane 8), showing selective processing of each locked state into an unlocked state. This change can be visualized by the disappearance of the band corresponding to the particular locked nanoswitch (Figure 3c). In using multiple locking strands, a higher percentage of the open state nanoswitches are reconfigured into locked states, showing a diminished band corresponding to the open state. We then performed a time series for each of the triggers (UV irradiation, RNase H and strand displacement), showing unlocking of a single locked state, while the other two remained locked (Figure S3). In each case, depending on the trigger concentration, the unlocking event can be accomplished within an hour.

The concept of orthogonal control of dynamic DNA nanoswitches presented here opens up opportunities to control nanoscale structures for a variety of applications. The type of triggers for different applications can also be chosen depending on their reaction rates.[15,17,18] For example, higher concentrations of the displacing strand or RNase H can be used to induce faster unlooping. Our previous work showed that UV-

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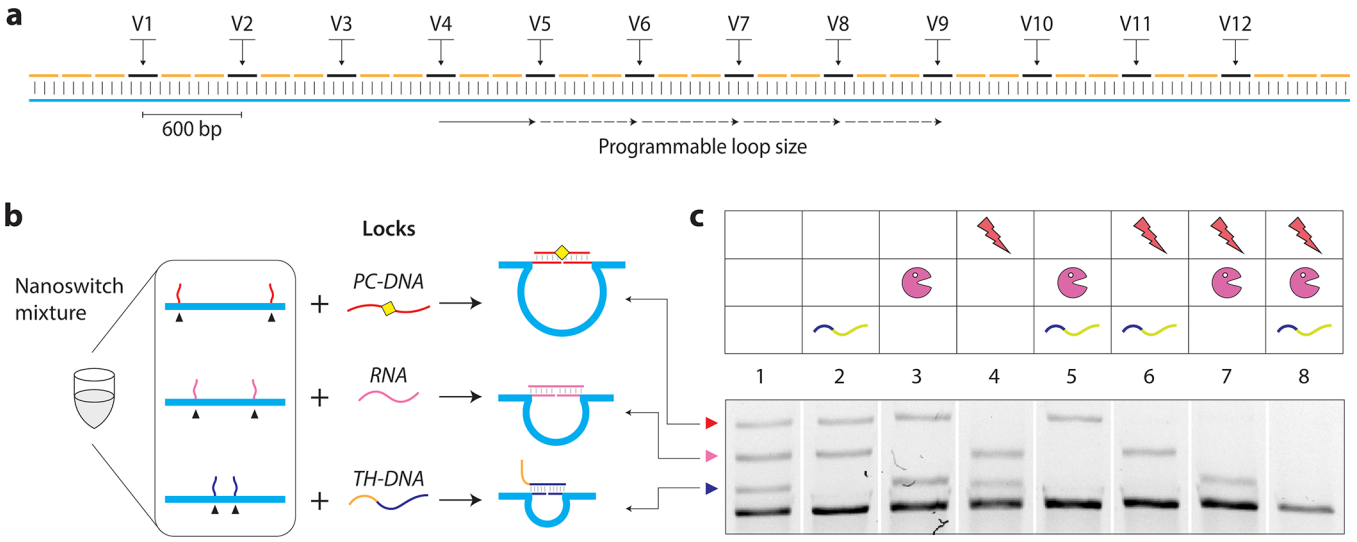

Figure 3. Orthogonal control of DNA nanoswitches using three different triggers. (a) DNA latches can be positioned on different variable regions (V1−V12) on the DNA nanoswitch. (b) A mixture containing DNA nanoswitches with latches programmed to yield different loop sizes. (c) The three loop sizes (locked states) are read out as unique bands on a gel (lane 1). Physical (UV light), enzymatic (RNase H), or DNA can be used to reconfigure individual nanoswitches (lanes 2−4), a subset (lanes 5−7), or all three nanoswitches (lane 8).

triggered conformational change was faster than DNA strand displacement even when an order of magnitude higher concentration of DNA was used.[17] It is also possible to control the kinetics of the formation of individual states by changing the concentration of specific triggers.[19] In this work, we focused on reconfiguring the nanoswitches from the locked to unlocked states; reversibility of such reconfiguration might also be useful in some applications. Each of the unlocked states described here can be reset to the locked conformation by the addition of excess locking strands after unlooping (Figure S4). Such dynamic control over nanoscale devices provides additional design capabilities for molecular circuits,[20] incorporation of tunable portions in a larger DNA origami structure,[21] as well as a selective response when spatiotemporal control[22] is needed in bioimaging, drug delivery, or molecular computation.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.biochem.0c00952.

Nanoswitch design, additional experimental results, and oligonucleotide sequences used (PDF)

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Author Contributions

N.F., J.V., and K.A. performed experiments and analyzed data. K.H. supervised the project and edited the manuscript. A.R.C. conceived the project, designed and performed experiments, analyzed and visualized data, and wrote the manuscript.

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Notes

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

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