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Enzyme-Operated DNA-Based Nanodevices

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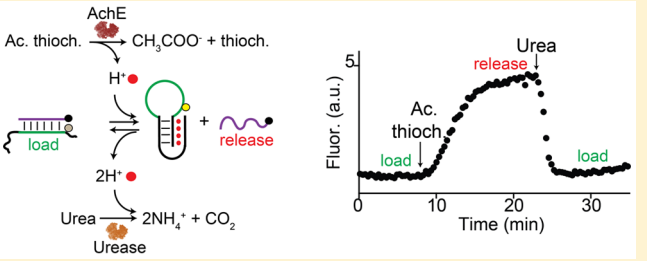
Supporting Information

ABSTRACT: Functional molecular nanodevices and nanomachines have attracted a growing interest for their potential use in life science and nanomedicine. In particular, due to their versatility and modularity DNA-based nanodevices appear extremely promising. However, a limitation of such devices is represented by the limited number of molecular stimuli and cues that can be used to control and regulate their function. Here we demonstrate the possibility to rationally control and regulate DNA-based nanodevices using biocatalytic reactions catalyzed by different enzymes. To demonstrate the versatility of our approach, we have employed three model DNA-based systems and three different enzymes (belonging to several classes, i.e., transferases and hydrolases). The possibility to use enzymes and enzymatic substrates as possible cues to operate DNA-based molecular nanodevices will expand the available toolbox of molecular stimuli to be used in the field of DNA nanotechnology and could open the door to many applications including enzyme-induced drug delivery and enzyme-triggered nanostructures assembly.



KEYWORDS: *DNA nanotechnology, molecular devices, enzymes, DNA nanostructures*

DNA nanotechnology takes advantage of the simple base-pairing code and the nanoscale dimension of DNA to rationally engineer stimuli-responsive nanodevices or nanomachines[1−8] that can be employed for sensing, drug-delivery, and imaging purposes.[1−19] In general, such nanodevices are based on input-induced conformational changes, that is, the binding of a specific target leads to a structural change[5,20−28] that can, for example, give a signal or release a ligand.[29,30] Alternatively, DNA nanomachines make use of specific and highly controlled DNA-based reactions. In this context, the best example is represented by the toehold-mediated DNA strand-displacement reaction, a process through which a DNA strand displaces another prehybridized strand in a highly controlled manner.[31−34]

Despite their potentialities and impressive performances, DNA nanodevices are generally activated by a restricted class of molecular stimuli. These include nucleic-acids (i.e., single- or double-stranded DNA or RNA strands)[3,27,28] and small molecules or proteins recognized by specific DNA/RNA sequences (i.e., aptamers).[35,36] The use of environmental changes such as temperature, light, or pH has been also recently demonstrated as a way to control the functionality of DNA-based nanodevices.[20−26] The constraint associated with the limited number of available stimuli ultimately slows further advancements in the field of DNA-based nanotechnology. In order to expand the possibilities of these molecular devices, it is thus crucial to be able to control their functions through a wider range of molecular cues.

Nature makes use of a large number of molecular inputs to control in a specific and selective manner different biological pathways and reactions. The majority of such processes rely on enzymes, highly evolved molecular machines that catalyze a wide range of chemical reactions within cells by recognizing in a very specific way a wide range of molecular substrates.[37] In addition to their high specificity toward their substrate (i.e., the molecular input), enzymes also display a high turnover rate of product formation that makes them particularly advantageous as input−output devices to transmit and amplify chemical information. For the above reasons, enzymes represent an excellent opportunity to expand the range of possible molecular inputs to be used in DNA nanotechnology. To date, several groups have reported the possibility to use enzymes to control DNA nanodevices. While these examples represent an important proof of the utility of controlling DNA nanodevices with enzymatic reactions, we note that they are based on the use of DNA-recognizing enzymes (enzymes that use nucleic acids as their substrate such as nuclease, ligase, polymerase, and nicking enzymes)[38−41] that represent only a small portion of the myriad of enzymes that Nature has evolved to catalyze chemical reactions in living systems.[12,37]

Motivated by the above arguments, here we propose to control a range of DNA-based nanodevices using enzymatic reactions. Of note, the enzymes we employ in this work do not belong to the restricted class of DNA-recognizing enzymes.[38−41] We used, instead, enzymes belonging to different

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classes that, by recognizing a specific molecular substrate can activate or inhibit a DNA-based nanodevice. More specifically we employed here different proton-consuming or proton-producing enzymes that can be used to finely tune and regulate the activity of different pH-dependent DNA reactions and nanodevices (Figure 1).

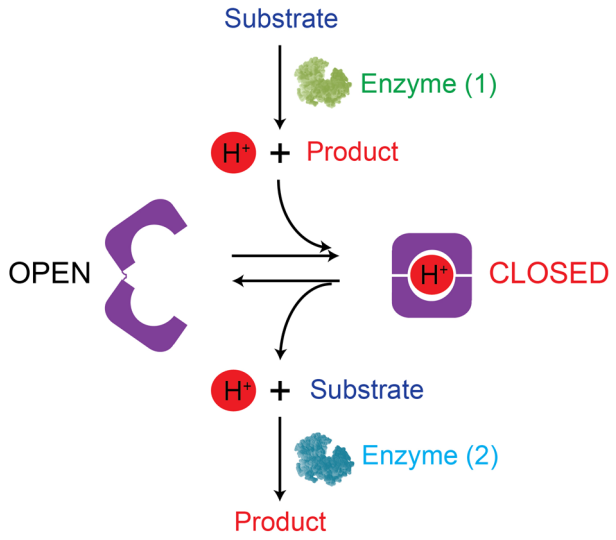

Figure 1. DNA nanodevices or nanomachines use configuration-switching DNA structures or DNA-based reactions that convert a mechanical motion induced by a molecular input into a signal or a useful action (e.g., the release of a ligand). Here we expand the toolbox of available molecular inputs to operate such DNA nanomachines to those produced by enzymatic reactions. In this work, we demonstrate three proof-of-principle applications of this strategy. More specifically, we used different proton-producing (top) or proton-consuming (bottom) enzymes to control a DNA-based nanoswitch, a strand-displacement reaction, and a DNA nanomachine for the controlled release of a ligand.

As a first proof-of-principle of our strategy, we demonstrate that we can trigger the opening and closing of a DNA-based molecular switch through an enzymatic reaction. To do this we have selected a recently reported pH-dependent optically labeled nanoswitch whose folding/unfolding can be triggered at specific pH values (Supporting Information Figure S1).[42] To first demonstrate the enzyme-induced closing of the nanoswitch, we employed Glutathione Transferase (GST), a detoxifying enzyme presents in all aerobic organisms that in the presence of its natural substrate GSH and CDNB leads to the production of a strong acid (i.e., HCl).[43] Such enzymatic reaction results in the nanoswitch's protonation thus ultimately triggering triplex-formation and nanoswitch's closing (see Figure 2A). By varying the concentration of GSH (from 0.05 to 1.0 mM) added to a solution containing a fixed concentration of GST and the cosubstrate CDNB we were able to finely modulate the nanoswitch's closing (Figure 2A) thus ultimately controlling the fraction of closed nanoswitches (Supporting Information Figure S2). We were also able to control the rate at which the nanoswitch closes (half-times, $t_{1/2}$ from 14 to 27 min) by using different concentrations of GST at a fixed level of substrates (Supporting Information Figure S3).

We also demonstrate the enzyme-induced switch's opening. To do so we used urease, an enzyme belonging to the class of hydrolases, that converts its specific substrate (i.e., urea) into ammonia and CO_2 (Figure 2B).[44] Under these conditions, the

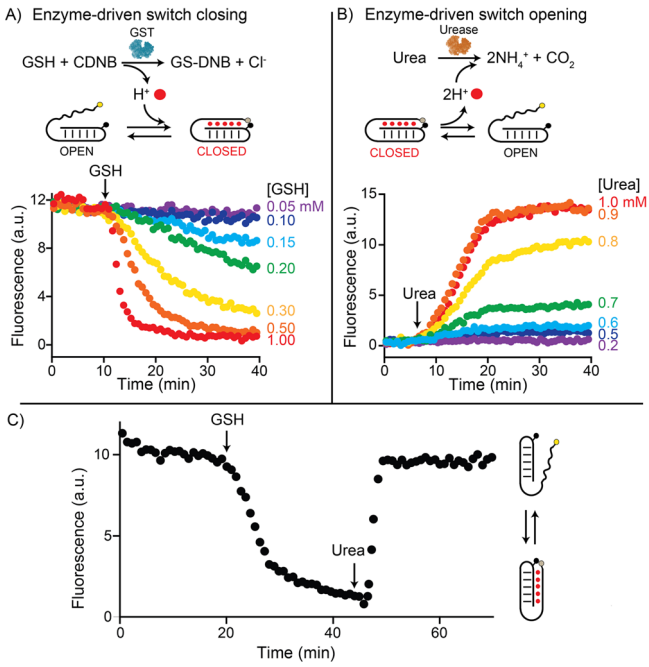

Figure 2. Enzyme-operated opening/closing of a DNA nanoswitch. (A) We control the nanoswitch's closing by using the enzyme GST. By varying the GST's substrate (GSH) concentration, we can finely control the enzyme-induced closing of the pH-sensitive DNA nanoswitch.[42] (B) We have also demonstrated the enzyme-induced opening of the nanoswitch by using urease. Again, at different concentrations of urea we can control the opening of the switch. (C) Finally we show that we can reversibly close and open the same nanoswitch in the presence of both enzymes by sequentially adding the two substrates. Fluorescence measurements were performed at 25 °C in a citrate/phosphate/borate buffer +2 mM $MgCl_2$ + 0.050 M NaCl at a pH of 7.8 or 5.0 for GST and Urease experiments, respectively. The DNA nanoswitch concentration used was 10 nM. Urease and GST were used at a concentration of 0.15 mg/mL and 2 μg/mL, respectively.

nanoswitch behaves as a Bronsted–Lowry acid and releases protons to the enzymatically produced ammonia thus destabilizing the triplex structure and leading to the nanoswitch's opening. We demonstrate that we can finely control enzyme-driven nanoswitch opening by varying the concentration of enzymatic substrate (from 0.2 to 1.0 mM) in the presence of a fixed concentration of urease (Figure 2B). By doing this, we demonstrate that we can rationally modulate the fraction of opened nanoswitches over a quite narrow range of substrate's concentration (Supporting Information Figure S4). Also in this case, by varying the concentration of urease at a fixed level of urea we are able to control the nanoswitch's opening kinetic achieving half-times ($t_{1/2}$) of nanoswitch's opening from 13 to 43 min (Supporting Information Figure S5). Finally, as a further proof of the versatility of such approach, we have demonstrated the possibility to reversibly open and close the nanoswitch by alternatively adding the two substrates in the presence of both enzymes (Figure 2C).

The nanoswitches used above represent only a specific example of a much larger family of DNA-based nanodevices that include molecular motors,[5,10] tweezers,[12] autonomous nanomachines,[13,14] circuits,[17,18] walkers,[15,16] and catalytic amplifiers.[19] Interestingly, the majority of these DNA nanodevices rely on a simple highly controllable fundamental DNA-based reaction named toehold-mediated strand-displacement, a

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process through which two DNA strands hybridize with each other displacing one (or more) prehybridized strands.[31−34] Despite the advantages represented by the strand-displacement reaction to build and engineer functional DNA nanodevices in a controlled fashion, it would be important to find new ways to control this process using a wide range of molecular cues. In fact, only few examples have been reported to date that allow to activate strand-displacement reactions with non-nucleic acids inputs.[45−47]

For the above reasons, we propose here to rationally control a toehold-mediated DNA strand-displacement process using enzymatic reactions. As a proof of principle, we have employed here a previously reported pH-controlled strand-displacement system that is activated only under basic conditions.[34] In this system, the target duplex is designed to contain a triplex forming tail that under acidic pHs forms a stable triplex complex that acts as a molecular padlock preventing strand-displacement (Figure 3A and Supporting Information Figure S6). By coupling this system with an enzyme (i.e., urease) that produces a base, we demonstrated that we can finely trigger the displacement process through the catalyzed enzymatic reaction. For example, at pH 5.0 at which strand-displacement in our system is inhibited, the addition of the invader strand does not result in any significant signal change (Figure 3B), suggesting that no displacement occurs. Conversely, under the same conditions we observe a signal increase after the addition of urea (5.0 mM) thus suggesting that the enzymatically produced ammonia, triggers the strand-displacement process by causing the padlock opening (Figure 3B). Also, in this case the enzymatic-driven activation of the strand-displacement system can be finely controlled by changing the substrate concentration (from 0.2 to 5.0 mM) (Figure 3C and Supporting Information Figure S7).

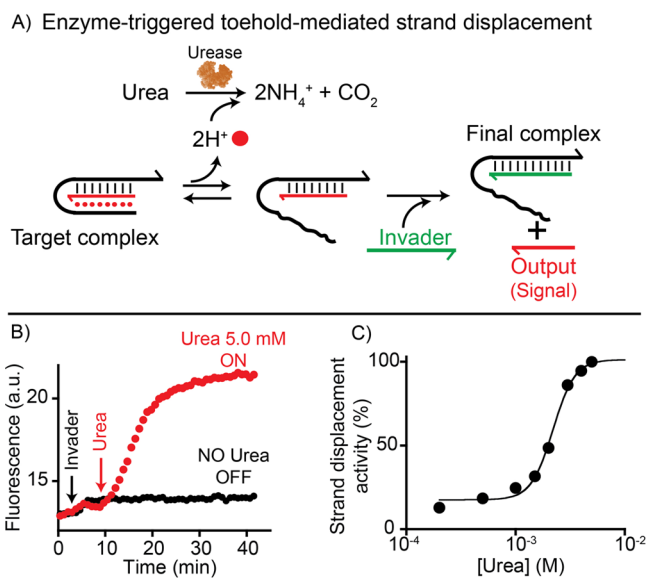

Figure 3. Enzyme-triggered toehold-mediated DNA-based strand-displacement reaction. (A) We control a pH-dependent DNA strand-displacement system with urease. (B) In the presence of the invader strand, we observe optimal strand-displacement only after the addition of the enzyme's substrate (urea). (C) By varying the concentration of urea (from 0.2 to 5.0 mM) we can finely control the strand-displacement efficiency (%). [target] = 10 nM, [invader] = 30 nM, [urease] = 0.15 mg/mL in a 0.01 M Tris buffer +0.01 M $MgCl_2$, pH 5.0, at 25 °C. The release of the output strand (see cartoon) results in a fluorescence signal increase due to the reaction with a fluorescent-labeled reporter strand[31−34] (see Supporting Information Figure S6 for details).

As a further demonstration of how enzymatic reactions can improve the current toolkit of possible molecular inputs in the field of DNA-based nanotechnology, we also propose here the use of enzymatic reactions as a way to regulate DNA-based nanomachines for the controlled load and release of a ligand. To do this, we employed a recently reported DNA-based receptor that allows the load/release of a specific ligand through pH changes.[30] More specifically, we used a molecular beacon re-engineered to contain a pH-sensitive stem that by folding/unfolding at different pHs releases and loads a specific ligand (here a DNA strand complementary to the loop) (Figure 4 and Supporting Information Figure S8). We first demonstrate the possibility to enzymatically trigger the release of the ligand using acetyl-cholinesterase (AchE), a hydrolase enzyme that, by catalyzing the hydrolysis of the substrate acetylthiocholine, leads to the production of acetic acid and thiocholine.[48,49] Under these conditions, the cytosines present in the triplex-forming stem behaves as Bronsted−Lowry bases and accept protons from the enzymatically produced acetic acid ($pK_a \approx 4.7$). This leads to the formation of the triplex structure in the stem of the molecular beacon with the subsequent ligand's release (Figure 4A,B). Of note, we can finely modulate the release of the ligand strand by varying the concentration of acetylthiocholine in the presence of a fixed amount of AchE (Figure 4C and Supporting Information Figure S9). We also demonstrate that we can control the loading of the ligand to the molecular beacon by using another enzyme (i.e., urease) (Figure 4D). The enzymatically produced ammonia (in the presence of urea) leads to the unfolding of the triplex stem in the molecular beacon thus ultimately favoring the binding of the ligand strand to the complementary domain (Figure 4E). Also in this case, by adding different concentrations of urea we rationally controlled the amount of ligand bound to the

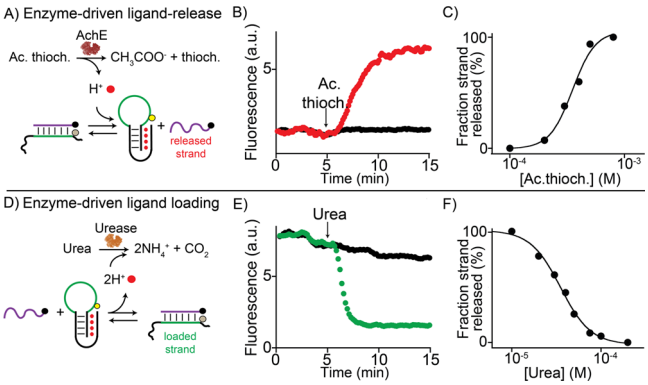

Figure 4. Enzyme-driven loading/release of a ligand. (A) Enzyme-driven ligand release: the enzymatic reaction catalyzed by AchE is used to release a ligand from a DNA receptor. (B,C) By using different concentrations of AchE's substrate, we can modulate the fraction of released ligand. (D) Enzyme-driven ligand loading: the reaction catalyzed by urease is used to induce ligand's binding to the DNA receptor. (E,F) Also in this case, by using different concentrations of urea we can tune the amount of ligand loaded to the receptor. Ligand load/release is followed by fluorescence measurements obtained at 25 °C in a phosphate buffer solution 0.1 mM + 0.01 M $MgCl_2$ at a pH of 8.0 or 5.0 for AchE (0.03 μg/mL) and urease (0.15 mg/mL) experiments, respectively.

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molecular beacon (Figure 4F and Supporting Information Figure S10). As a further demonstration of the versatility of such approach we have demonstrated the possibility to cyclically load and release a ligand by using as molecular inputs urea and acetylthiocholine in the presence of both enzymes (Supporting Information Figure S11).

Enzymes are the most important protein-based machines that operate in cells. They can recognize a large class of molecular substrates in a highly specific fashion and catalyze a wide range of chemical reactions.[37] Recently, several reports have demonstrated the advantages of controlling synthetic nanomotors or drug-releasing nanodevices using enzymes and enzymatic substrates.[50−57] For example, Sanchez et al. have used reactions catalyzed by three different enzymes to power the motion of nanomotors based on hollow mesoporous silica nanoparticles.[53] The possibility of using biocompatible fuels to operate such nanomotors makes the use of enzymes particularly advantageous.

Motivated by the above arguments here, we have demonstrated for the first time the possibility to use naturally occurring non-DNA-recognizing enzymes and enzymatic substrates as possible molecular cues to rationally control and regulate DNA-based nanodevices and reactions. The possibility to control DNA-based processes and reactions with the wide range of chemistries allowed by the variety of enzymatic reactions appears particularly promising to expand the available toolbox of molecular cues to be used in the field of DNA nanotechnology[58−60] and can open the future to new and exciting perspectives. For example, the possibility to regulate at specific concentrations of an enzymatic substrate the strand-displacement reaction (a process often used to build complex DNA nanostructures) can be used to introduce additional control over the formation and function of DNA nanostructures. Future promising research efforts might also be devoted to use enzymes whose activity relies on the presence of specific cofactors (ATP, GMP, and others) for which available DNA-based receptors are known. This will further expand the class of enzymes that can be used to control the function of DNA-based nanodevices.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.nanolett.5b04566.

Description of sequences, materials and methods, and additional experiments. (PDF)

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

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