Molecular Motors

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A DNA–Protein Nanoengine for "On-Demand" Release and Precise Delivery of Molecules**

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We demonstrate that artificial DNA probes can be merged with natural biological processes to create highly active nanomechanical devices by developing a DNA–enzyme hybrid nanoengine that is triggered by a small molecule as the input to cyclically dispatch and retrieve a DNA strand as the output. Moreover, the movement of the output can be accurately directed between two specific DNA destinations. Joint exploration of small molecules, nucleic acids, and enzymes may eventually lead to the engineering of bionanodevices that can both detect a biological malfunction and send out a messenger to initiate a repair mechanism.

Nucleic acids encode "smart" molecular recognition properties that commend their exploitation in bionanotechnology.[1,2] It has been proven that DNA or RNA can be used as structural material for building complex self-assembled objects,[3–7] computational devices,[8–13] or active machines and motors.[14–22] In the latter case, DNA also represents the "fuel" that triggers the function of the DNA machine. However, the

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need of artificial DNA molecules for the manipulation of DNA-based devices limits their potential for biological applications. Herein we show that an artificial DNA-aptamer nanoswitch can be coupled to an enzymatic reaction to create a hybrid DNA–enzyme nanoengine capable of transporting an antisense DNA molecule between two specific destinations under the control of a small-molecule input.

DNA aptamers are highly structured single strands of DNA that are capable of binding a ligand with great affinity and specificity. The nature of these aptamers makes them inherently capable of recognizing two different molecules: the cognate non-nucleic acid target for which the aptamer is created and an antisense DNA molecule through Watson–Crick base pairing. It has been shown that the addition of a target to a solution containing the properly engineered aptamer/antisense duplex can trigger rapid structure switching from the aptamer/antisense duplex to the aptamer/target complex.[23] The dual binding capability of DNA aptamers and the reversibility of the duplex–complex switching process are the two key elements exploited in the design of the DNA nanoswitch presented herein.

The molecular model of the DNA nanoswitch is illustrated in Figure 1 A. The nanoswitch has three components: a modified version of a previously described adenosine-binding DNA aptamer (black line),[24] a short complimentary DNA strand as the output (gray line), and adenosine as the input (red star). In the absence of adenosine, the system is in the "off" state, as the output molecule is docked onto the DNA aptamer through Watson–Crick base pairing. Upon addition of adenosine, the system is switched to the "on" state, as adenosine displaces the output from the aptamer. Therefore, the presence of one biological entity is transduced into the release of another.

The evidence for the output displacement by the input was obtained by using agarose gel electrophoresis (Figure 1 B). For this experiment, the output DNA strand was labeled with fluorescein at the 3′ end (green circle in Figure 1 A). DNA strands of 11 and 12 nucleotides in length (named "output 11" and "output 12") were studied as the output molecules at two concentrations. Figure 1 C plots the percentage release of the output versus the concentration of the input for each gel. In each case, a clear dependence of the output release on the input concentration was observed. The experiments with 20 nM output 12 and 100 nM output 11 gave higher release percentages at all tested concentrations than those with 100 nM output 12 and 500 nM output 11. It is important to note that, by using different concentrations of the nanoswitch, the absolute amount of the output to be released can be controlled independently of the concentration of the input. For example, 1 mM adenosine can cause the release of the output in four different amounts: 10.4 nM (52% of 20 nM output 12), 16 nM (16% of 100 nM output 12), 50 nM (50% of 100 nM output 11), or 80 nM (16% of 500 nM output 11). This suggests the possibility that a certain amount of input can be accurately transduced into a desired amount of output by utilizing different sizes of the DNA output along with different concentrations of the DNA nanoswitch.

As only physical interactions are involved in the function of the nanoswitch, the release of the output is a reversible

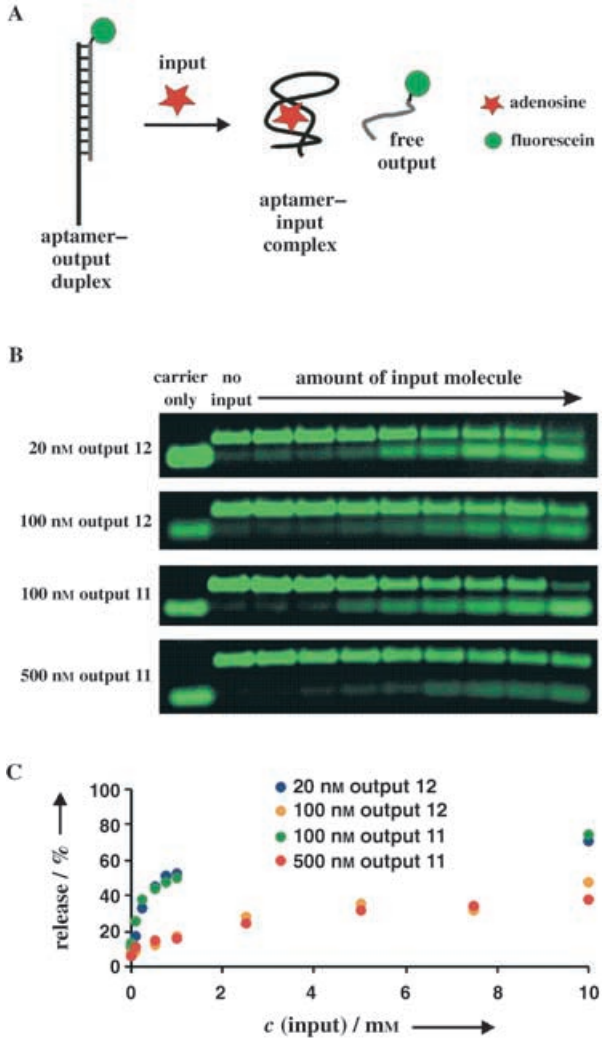

Figure 1. A DNA nanoswitch (see text). A) The working principle. B) Gel evidence for output release. Lanes 3–10 show the response of the nanoswitch to gradually increasing concentrations of the input (10^{-3}–10 mM). C) The dependence of output release on input concentration and overall concentration of the nanoswitch.

process. Therefore, the nanoswitch can be programmed to work cyclically when it is coupled to adenosine deaminase (ADA, blue oval in Figure 2 A)—an enzyme that converts adenosine (red star) into inosine (red pentagon), for which the aptamer has no affinity.[24] ADA is expected to convert free adenosine into inosine and this process should cause more and more aptamer–adenosine complexes to dissociate. Thus, freed aptamer molecules will then engage the output DNA molecules to reform the aptamer–output duplex. The inclusion of ADA in the system therefore converts the DNA nanoswitch into a two-stroke DNA nanoengine. The first stroke, triggered by the addition of adenosine, is represented by the release of the output; the second stroke, driven by the transformation of adenosine into inosine, is the return of the output to the aptamer.

A fluorescence quenching/dequenching experiment was performed to provide a physical readout of the cyclic function of the DNA nanoengine (Figure 2 B). First, the aptamer was

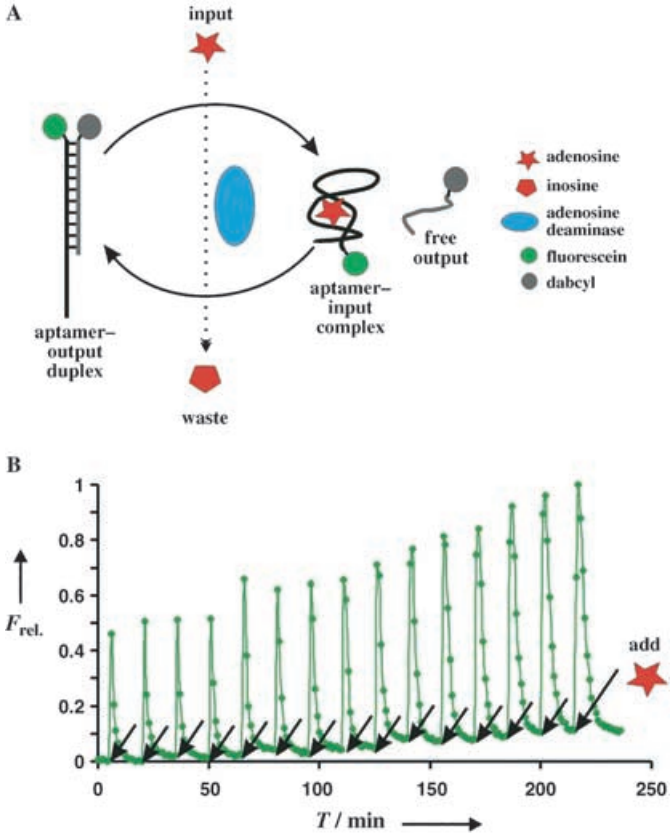

Figure 2. Transformation of the nanoswitch into a two-stroke DNA–enzyme nanoengine (see text). A) Cycling the nanoengine. B) A fluorescence quenching/dequenching experiment illustrates the working of the nanoengine. The figure presents the first 15 cycles of the nanoengine, where every cycle is initiated by the addition of fresh adenosine (black arrows).

labeled with fluorescein (green circle in Figure 2A) at its 5′ end and the output was labeled with 4-(4-(dimethylamino)-phenylazo)benzoic acid (dabcyl, a universal fluorescence quencher, gray circle) at its 3′ end. The fluorophore-labeled aptamer and the quencher-labeled output were then mixed with ADA and the fluorescence of the system was recorded over time. Prior to adenosine addition, the output was docked onto the aptamer, thereby bringing the quencher into close proximity with the fluorophore for efficient fluorescence quenching; therefore the system had a low fluorescence reading. The addition of adenosine after 5 min (the first black arrow in Figure 2B) elicited a rapid increase of the fluorescence intensity of the system, a result reflecting the fast release of the output (the first stroke of the engine). Slowly but gradually, ADA converted adenosine into inosine, thereby prompting the reannealing of the output onto the aptamer (the second stroke), which was indicated by the progressive decrease of the fluorescence intensity of the system.

A full cycle of the engine, under our experimental conditions, took about 15 min to complete. However, this interval could be easily altered by increasing or decreasing the amount of ADA in the system. Although only 15 cycles are shown in Figure 2B, each one triggered by the addition of fresh adenosine (black arrows), in theory the nanoengine should work for an indefinite period of time. However, in practice, the lack of an exhaust system will result in an accumulation of inosine and ammonia that will inhibit ADA[25] and will eventually arrest the engine. This may explain the gradual increase of the fluorescence intensity of the system as well as the steady peak widening observed in Figure 2B.

The above nanoengine could also be designed to include a defined destination for the output molecules upon their release. Two extra strands of DNA were introduced into the system to create two different destinations for the output: a mutagenized version of the aptamer as the desired destination and a control DNA strand as the unwanted destination. The mutant aptamer contained three mutations: A7→T7, G15→T15, and G27→T27. The first mutation, introduced into the output-binding site, caused the mutant aptamer to have a decreased affinity for the output, whereas the other two mutations, introduced into the adenosine-binding site, abolished the adenosine-binding ability. The control DNA had a sequence completely unrelated to that of the original aptamer. The active aptamer, the mutant aptamer, and the control DNA were labeled with Cy3, fluorescein, and Cy3.5, respectively, while the output was still labeled with the dabcyl quencher. Therefore, the arrival of the output at a given destination would be transduced into a decrease in the fluorescence intensity of the corresponding fluorophore.

When all the DNAs were mixed with ADA in the absence of adenosine, the high level of fluorescence of the mutant aptamer and the control DNA as well as the low level of fluorescence of the aptamer (Figure 3B) indicated that the output was docked onto the aptamer as planned. However, when adenosine was introduced, the rapid fluorescence increase of the active aptamer, coupled with the rapid fluorescence decrease of the mutant aptamer, confirmed the prearranged direction of movement of the output (Figure 3A). Importantly, the fluorescence of the control DNA remained unchanged, thereby demonstrating that the movement was indeed driven by specific molecular recognition. The action of ADA promoted the reverse movement of the output from the mutant aptamer to the active aptamer, reflected in the decrease of the fluorescence of the active aptamer and the increase of the fluorescence of the mutant aptamer. The dependence of the pattern of the fluorescence signal on the DNA sequence was verified by permutating the three fluorophores on the three DNA sequences (data not shown).

We have demonstrated above that a small molecule, an aptamer, an antisense DNA, and an enzyme can be configured into a two-stroke DNA nanoengine that can function in a cyclic fashion. The input-removing enzyme ADA is an essential component of our nanoengine. However, ADA functions independently of the output. A more desirable feature, particularly for real-world applications, that has yet to be demonstrated is the intricate linking of the enzyme activity with the freed output. Such a system can first detect a specific problem (input) and then dispatch a signal (output) for activating a rescuing protein or pathway (equivalent to ADA) for the correction of the problem. The completion of the final

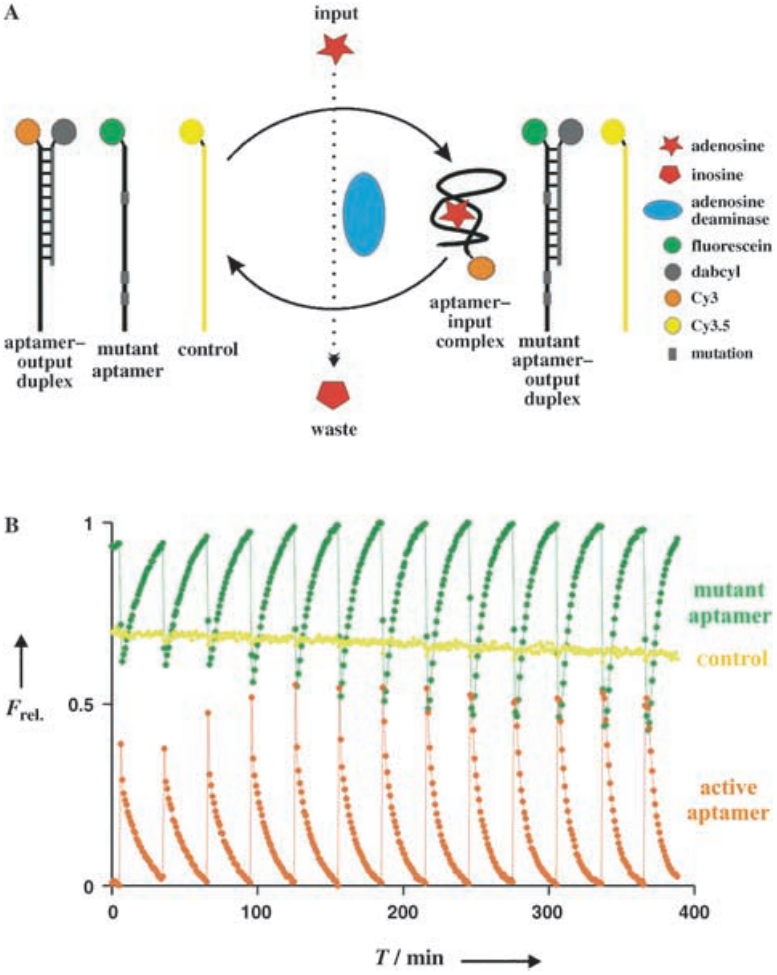

Figure 3. Precise delivery of the output (see text). A) Schematic representation. One cycle of the nanoengine is represented by the travel of the output from the aptamer to the mutant and back. B) A three-color fluorescence quenching/dequenching experiment.

step will deliver the output back to the aptamer, which resets the nanoengine for a new round of action, if necessary. Our nanoengine prototype does not offer a solution to this practical problem and can only dispatch a DNA strand as the output in response to a relatively high concentration of the small-molecule input. However, on the basis of the functional versatility of natural enzymes and the ease of in vitro selection with which diverse aptamers can be identified for virtually any target,[26,27] we speculate that highly practical nanoengines of a similar nature with a better input sensitivity and the ability to dispatch a desirable output will be created in the future for different input/output systems and that these nanoengines will offer new ways for the delivery of a drug or the regulation of a disease-causing biological process.

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