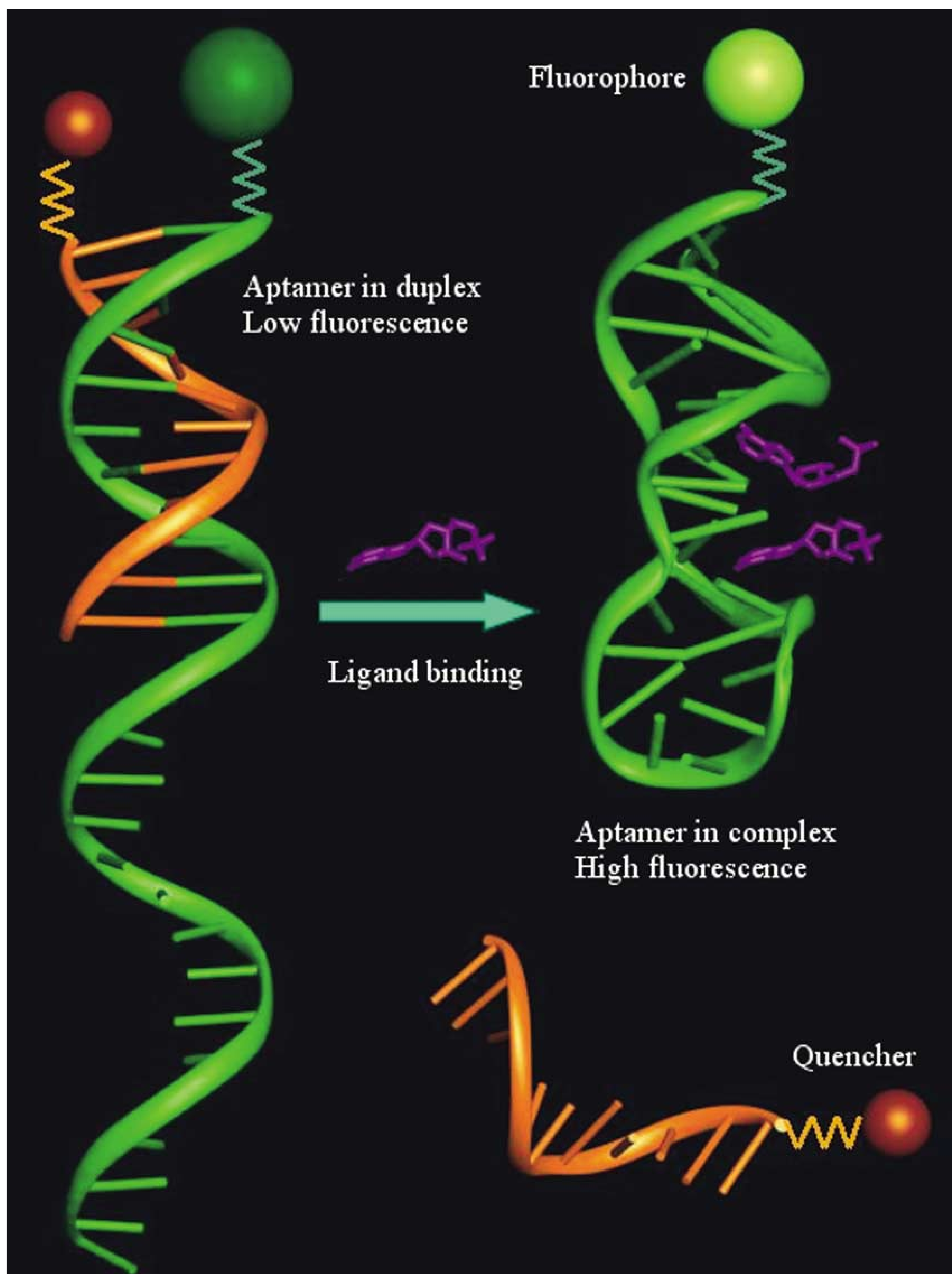

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Structure-Switching Signaling Aptamers: Transducing Molecular Recognition into Fluorescence Signaling

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Abstract: The development of aptamer technology considerably broadens the utility of nucleic acids as molecular recognition elements, because it allows the creation of DNA or RNA molecules for binding a wide variety of analytes (targets) with high affinity and specificity. Several recent studies have focused on developing rational design strategies for transducing aptamer–target recognition events into easily detectable signals, so that aptamers can be widely exploited for detection directed applications. We have devised a generalizable strategy for designing nonfluorescent aptamers that can be turned into fluorescence-signaling reporters. The resultant signaling probes are denoted "structure-switching signaling aptamers" as they report target binding by switching structures from DNA/DNA duplex to DNA/target complex. The duplex is formed between a fluorophore-labeled DNA aptamer and an antisense DNA oligonucleotide modified with a quencher (denoted QDNA). In the absence of the target, the aptamer hybridizes with QDNA, bringing the fluorophore into close proximity of the quencher for efficient fluorescence quenching. When this system is exposed to the target, the aptamer switches its binding partner from QDNA to the target. This structure-switching event is coupled to the generation of a fluorescent signal through the departure of QDNA, permitting the real-time monitoring of the aptamer–target recognition. In this article, we discuss the conceptual framework of the structure-switching approach, the essential features of structure-switching signaling aptamers as well as remaining challenges and possible solutions associated with this new methodology.

Keywords: aptamers • biosensors • fluorescence • nucleic acids

Introduction

Nucleic acids are traditionally exploited as molecular recognition elements to detect DNA and RNA targets through Watson–Crick interactions. However, several landmark discoveries over the past two decades, including the demonstration of certain natural RNA molecules as enzymes (ribozymes) in the 1980s, followed by the revelation that artificial DNA or RNA molecules can function as man-made catalysts (ribozymes and deoxyribozymes) or as binding elements for non-nucleic acid targets (DNA or RNA aptamers) in the 1990s,[1–6] have led to the astounding exhibition of nucleic acids as a multitalented class of macromolecules.[7–10] Due to these advances, nucleic acids have become increasingly popular as biorecognition elements in a wide range of analytical applications.

Aptamers are single-stranded DNA, RNA, or modified nucleic acids that have the ability to form defined tertiary structures for specific target binding.[7,8] They are isolated from random-sequence DNA or RNA libraries by "in vitro selection" or SELEX (systematic evolution of ligands by exponential enrichment).[5,6] The development of aptamer technology significantly widens the utility of nucleic acids as molecular recognition elements, because DNA and RNA can now be engineered not only to detect nucleic acid targets, but also to recognize a broad scope of non-nucleic acid ligands, including proteins and metabolites.[7,8] Several strategies for transducing aptamer–target interactions into electrochemical, mechanical, piezoelectric, or fluorescent signals have been reported.[11–14] Among these methods, fluorescence signaling is most desirable, because of the convenience of detection, diverse measurement methods, and avail-

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ability of a large selection of fluorophores and quenchers for nucleic acid modification.[15] Therefore, designing fluorescence-signaling aptamers have become the focal point of several recent studies.[16–22]

Standard DNA and RNA aptamers do not have fluorescence-signaling capabilities when they are isolated from random-sequence libraries and can only be engineered into fluorescent reporters by rational design based post-selection modifications. An ideal rational design strategy should have the following three key features. First, the method should be easy to apply to any given aptamer regardless of its size and structural properties. This is important as aptamers have variable sizes and vastly different secondary and tertiary structures. Some aptamers do not have an easily determinable secondary structure, and the tertiary structures of most aptamers are not readily available. Second, the method should be capable of designing signaling aptamers with a large signaling magnitude and a fast, ideally real-time, reporting capability. Signaling aptamers that exhibit large fluorescence enhancements upon target binding increase the sensitivity and accuracy of detection assays. The real-time reporting capability allows rapid sample measurements and permits demanding applications such as high-throughput screening. Third, the method should lead to the design of a signaling aptamer from an existing one without significantly altering the affinity and specificity of the original aptamer.

In this article, we begin with a brief review of existing signaling aptamer design methods. We then discuss a rational design strategy we have recently developed for engineering a special class of aptamer reporters denoted "structure-switching signaling aptamers". Finally, we address some remaining challenges and possible solutions associated with our new methodology.

It is noteworthy that many RNA or DNA aptamers have been utilized by nucleic acid enzyme engineers in the design of allosteric ribozymes or deoxyribozymes.[10] These catalytic reporters are designed to contain both an aptamer domain and an enzymatic motif within a single molecule. The aptamer domain changes its conformation upon target binding and this conformational switch then turns on (or shuts off) the catalytic activity of the ribozyme or deoxyribozyme motif. Strategies for allosteric nucleic acid enzyme engineering have been reviewed elsewhere[10] and will not be discussed herein.

Known Rational Design Strategies

The existing rational design strategies can be divided into two groups: 1) monochromophoric approach, which involves labeling aptamers only with a single fluorophore, and 2) bischromophoric approach, in which signaling aptamers are doubly labeled with a fluorophore and a quencher.

Monochromophoric approach: The two key assumptions of this approach are: 1) the binding of a target to the aptamer will trigger a significant structural re-organization and 2) the induced conformational change can substantially alter spectroscopic properties of the attached fluorophore. Jhaveri et al. reported this approach in 2000 (Figure 1A).[16] They used two anti-ATP aptamers (one RNA and one DNA) with known tertiary structures as model examples.[23–26] Seven fluorophore-labeled aptamers, each with a fluorophore attached at a location away from the target-binding

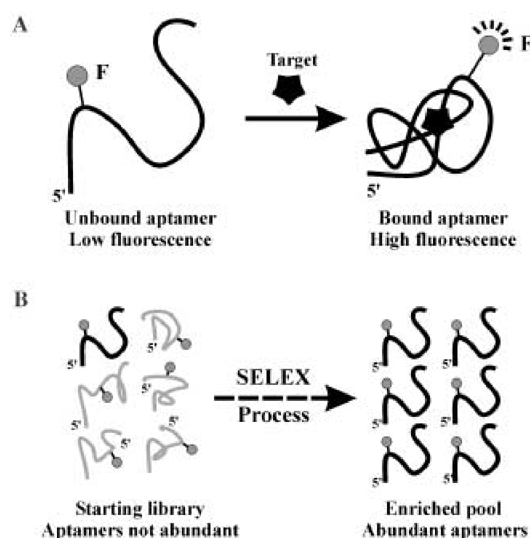

Figure 1. Designing signaling aptamers by monochromophoric approach. A) Rational design concept. Labeling an aptamer with a fluorophore at a location where, upon target binding, a substantial structural re-organization induces a change in the fluorescence property of the attached fluorophore. B) In vitro selection concept. Signaling aptamers are generated de novo by SELEX using a random-sequence DNA or RNA library in which each DNA or RNA molecule is labeled with one or a few fluorophores (for more information on SELEX, see references [5–9]). F dots denote fluorophores. Black curves represent aptamers, while the gray curves symbolize nucleic acid molecules without target binding capabilities. The star denotes the target for an aptamer.

site, were examined. Five modified aptamers did not show fluorescence changes in response to the addition of ATP and the remaining two registered 25–45% increase in fluorescence intensity upon ATP addition. The failed constructs might have been caused by disruption of the correct structural folding of the aptamer as a result of fluorophore attachment or by the insubstantial alteration of the fluorescence property of the attached fluorophore induced by target binding.

Jhaveri et al. then investigated the possibility of acquiring signaling aptamers by an "irrational" means, that is, by directly selecting signaling aptamers, through SELEX approach,[5–9] from a pool of random-sequence RNAs each containing one or a few fluoresceinated uridines (Figure 1B).[17] After several rounds of selective amplification with ATP as the binding target, a few aptamers that can act as real-time reporters for ATP sensing were obtained. The best signaling aptamer exhibited an approximate twofold increase in the fluorescence intensity at the saturating ATP concentration (1 mM ATP). Interestingly, it was found that the fluorescein label on the aptamer could be substituted for other fluorophores without affecting the aptamer's target-binding affinity and specificity. However, their selection also generated some aptamers that failed to register significant

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fluorescence enhancement upon target binding, suggesting that even the SELEX approach cannot guarantee the generation of single-fluorophore-labeled aptamers that can act as fluorescent reporters.

Bischromophoric approach: The bischromophoric approach is analogous to the molecular beacon approach for nucleic acid detection. A standard molecular beacon is a hairpin-shaped oligonucleotide labeled with a fluorophore on one end and a quencher at the other end (Figure 2A).[27] A molecular beacon can adopt two conformational states depending on the availability of the target. In the absence of the target, molecular beacons form an intramolecular stem that brings the fluorophore and the quencher into close proximity of each other for fluorescence quenching. When a complementary strand of DNA or RNA is present, molecular beacons adopt the more stable intermolecular duplex with the target, resulting in a fluorescence signal due to the separation of the fluorophore–quencher pair.

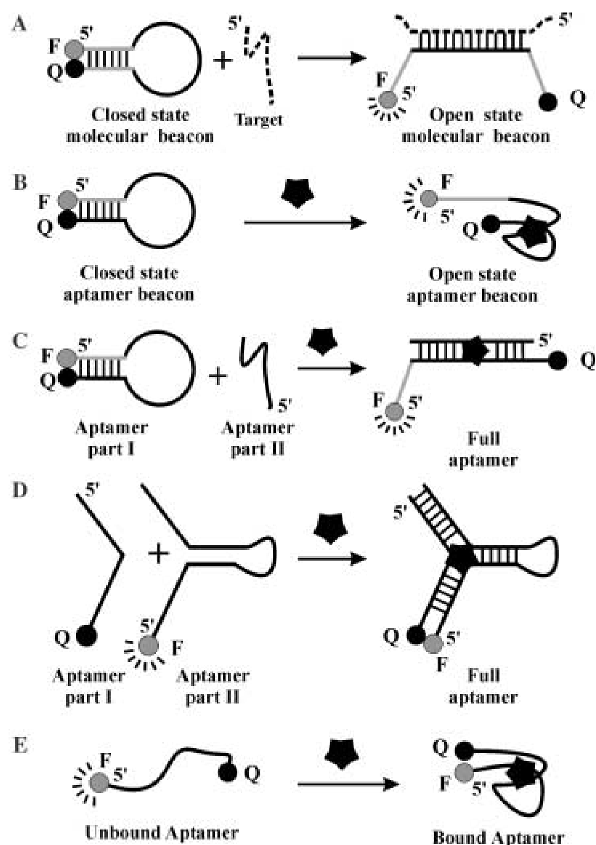

Figure 2. Designing signaling aptamers by bischromophoric approach. A) Standard molecular beacon and its signaling mechanism. B–E) Different ways to design aptamer beacons. Each design is discussed in the main text. F and Q dots signify fluorophore and quencher, respectively. The black lines or curves represent target-binding sequences and the gray lines denote nucleotides that are not involved in target binding. Nucleic acid and non-nucleic acid targets are shown in dashed curves and black stars, respectively.

Although standard molecular beacons are only useful for nucleic acid detection, several groups have attempted to adapt the same principle to design signaling aptamers (denoted aptamer beacons).[18–22] Hamaguchi et al. made the most straightforward adaptation (Figure 2B) by adding a few nucleotides (shown in gray) onto the 5′-end of a small, anti-thrombin DNA aptamer[28] (shown in black) to engage the 3′-end of the aptamer into a hairpin structure.[18] When the ligand is absent, the aptamer beacon forms the closed-state structure in which fluorescence is quenched. In the presence of thrombin, the aptamer beacon forms the complex structure in which the fluorophore and the quencher are separated in distance, resulting in an increased fluorescence signal. Yamamoto et al. reported a two-chain aptamer beacon approach by splitting an HIV Tat protein binding RNA aptamer[29] into two molecules, one of which was formulated into a molecular beacon (Figure 2C).[19] In the absence of Tat, the two RNA molecules exist independently and the solution has a low fluorescence intensity. When Tat is introduced, the molecular beacon and the other half of the aptamer assemble into a single unit for Tat binding. The dissociation of the hairpin structure is accompanied by an increase of fluorescence intensity.

Since signal generation comes from effective fluorescence dequenching, the signaling magnitude exhibited by aptamer beacons is usually much higher than signaling aptamers designed by single fluorophore approach. For example, the aforementioned Tat aptamer beacon has been shown to generate a 14-fold fluorescence enhancement when incubated with Tat at saturating concentrations.[19]

Other groups explored fluorescence quenching as an alternate way to track target–aptamer recognition.[20–22] Stojanovic et al. used a two-chain assembly approach in the design of two aptamer reporters,[20] one that binds cocaine and the other for ATP recognition (Figure 2D). In the absence of the target, the two half chains of the aptamer (one labeled with a quencher and the other with a fluorophore) do not associate strongly and, therefore, the fluorescence intensity of the solution is high. Introduction of the target promotes the assembly of the two half chains for target binding, resulting in fluorescence quenching. Moreover, it was shown that the above two reporters when labeled with different fluorophores could report both ligands in the same solution, suggesting that it is possible to use labeled aptamers for multiplex detection. The anti-cocaine aptamer has also been shown to work in a dually labeled single-chain format.[21] Recently, a fluorescence-quenching anti-thrombin signaling aptamer, as illustrated in Figure 2E, was described.[22] It is known that the two ends of the anti-thrombin DNA aptamer are located next to each other in the folded, guanine-quartet-based complex structure.[30,31] Li et al. exploited this folding property and designed a signaling aptamer by appending a pair of fluorophore and quencher to the two ends of the aptamer sequence. The resulting aptamer was able to perform real-time reporting of thrombin by fluorescence quenching.

Structure-Switching Signaling Aptamers

All the signaling aptamers discussed above function by the principle that target-induced folding and resultant conformational change of the aptamer alter fluorescent properties

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of the attached fluorophore either directly (in monochromophoric approach) or through fluorophore-quencher separation as well as association (in bischromophoric approach). One feature common to all the above rational design strategies is the reliance on the availability of information on the concerned aptamers' key secondary or tertiary structures, which could lead to difficulties in generalizing each strategy for any given aptamer. Inspired by the above approaches and by our success on the design of tripartite molecular beacons,[32] we sought to develop a new, bischromophoric method that could be used to rationally design real-time signaling aptamer reporters from any given DNA or RNA aptamer without prior knowledge of its secondary or tertiary structure.[33]

Conceptual framework: We reasoned that a general strategy should be possible if we could take advantage of the unique dual structure-forming capabilities of DNA (or RNA) aptamers. Because an aptamer is created for engaging a non-nucleic acid target for binding, it has the ability to form a defined tertiary structure (denoted complex structure) in the presence of its cognate target. Since an aptamer is a nucleic acid molecule, it also has the natural ability to form a duplex structure with an oligonucleotide that has a complementary sequence. If the aptamer is labeled with a fluorophore and the complementary oligonucleotide with a quencher, a mixture of the two will produce a low fluorescent state due to the formation of the duplex structure and associated fluorescence quenching. If the aptamer–target complex is more stable than the aptamer–oligonucleotide duplex, adding the target into the above mixture will trigger the release of the quencher-labeled oligonucleotide, which should result in a fluorescent signal.

A concept-demonstrating scheme is shown in Figure 3A.[33] In this particular design, three short single-stranded DNA molecules are used: FDNA (modified with a fluorophore at the 5′-end), QDNA (labeled with a quencher at the 3′-end) and MAP (made of an aptamer element, shown in black, and an FDNA-binding motif, shown in gray). The QDNA is designed to have a sequence that is complementary to the aptamer element near the FDNA-binding motif. Without the target, the three DNA molecules naturally assemble into a tripartite duplex structure to bring the fluorophore and the quencher into close proximity of each other, leading to efficient fluorescence quenching. Because the aptamer is selected on the basis of target binding, the target addition should cause the switch of the duplex structure to the complex structure, resulting in fluorescence enhancement.

We designed the first structure-switching reporter based on a 27-nt (nt = nucleotide)) DNA aptamer originally created by Huizenga and Szostak for ATP binding.[24] Evidence of structure-switching was obtained by using the simple temperature-changing procedure illustrated in Figure 3B:[33] both ATP-containing (data series shown in circles) and ATP-lacking (diamonds) signaling aptamer solutions were incubated for a total of 90 min at different temperature settings, the first 10 min at 15 °C, the next 50 min at 37 °C, and final 30 min at 22 °C. Rapid structure switching did not occur at 15 °C (indicated by steady fluorescence observed for both solutions), because the pre-formed FDNA–QDNA–MAP duplex assembly was stable and ATP was not able to displace QDNA. Structure transition occurred at 37 °C (indicated by the progressive fluorescence intensity increase in the ATP-containing sample), because the duplex assembly was weakened, allowing ATP to gradually replace QDNA molecules in the assembly. When the temperature was lowered to 22 °C, while more free QDNA molecules naturally reassembled back onto MAP in the ATP-lacking solution, the formation of the ATP–aptamer complex in the ATP-containing solution prevented the QDNA from reassembling.

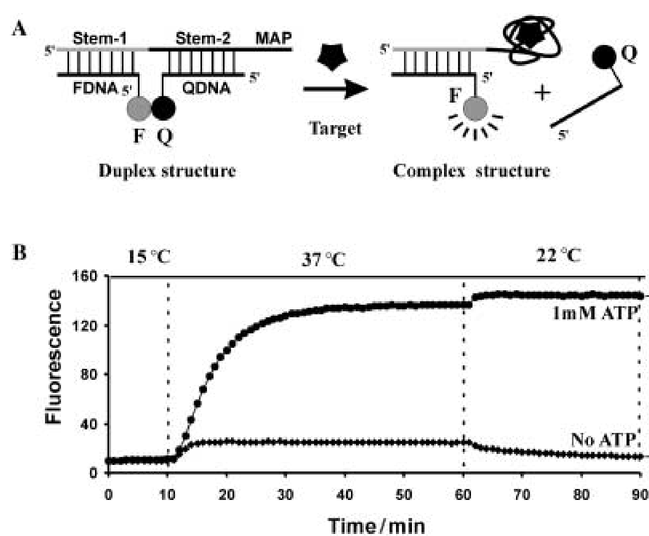

Figure 3. Designing structure-switching signaling aptamers: A) Structure switching concept. A modified aptamer (MAP) is configured into a low-fluorescence, two-stem duplex structure state with the fluorophore-containing FDNA and quencher-bearing QDNA. In the presence of the target (star), QDNA is released, leading to a highly fluorescent complex structure state. The aptamer element is shown in black, the FDNA-binding element in gray. B) Confirmation of structure switching by a temperature-changing experiment. The FDNA/QDNA/MAP duplex is relatively stable at 15 °C; however, raising temperature to 37 °C promotes the structure switching from the FDNA/QDNA/MAP duplex to the FDNA/MAP/target complex in the ATP-containing solution (circles). Lowering temperature to 22 °C results in the reassembly of more free DNA molecules into FDNA/QDNA/MAP duplex in ATP-lacking solution (diamonds), while the presence of ATP prevents such reassembly in the ATP-containing solution.

Key features of structure-switching aptamers

Adaptability of structure-switching concept: Our structure-switching design strategy exploits the common dual structure-forming capabilities of all aptamers and should, in principle, be adaptable for all aptamers. In reality, however, a single modification scheme (such as the one shown in Figure 3A, re-shown in Figure 4A as the first design option) may not be perfectly suited for any given aptamer, particularly considering that aptamers have vastly different sizes. Fortunately, the structure-switching concept can be easily expanded to cover many simple variations, including the three illustrated in Figure 4B–D. In the second option (Fig-

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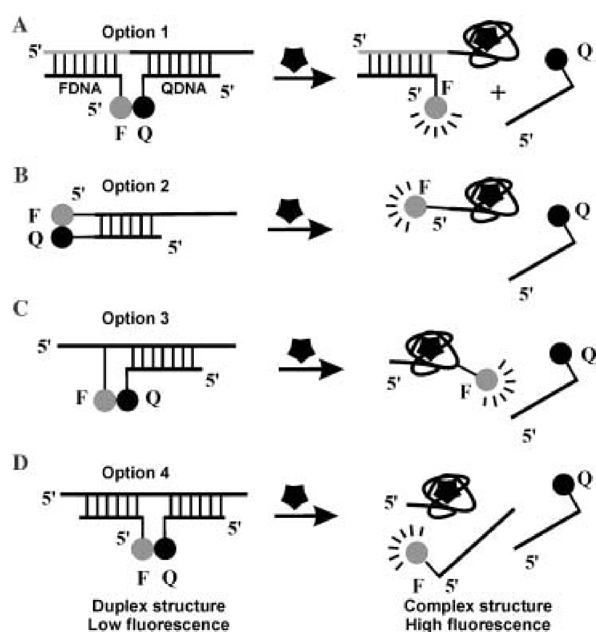

Figure 4. Variations of structure-switching aptamer design schemes. Each option is discussed in the main text. F and Q dots symbolize a fluorophore and quencher, respectively. Aptamer elements are shown in black line and targets as stars.

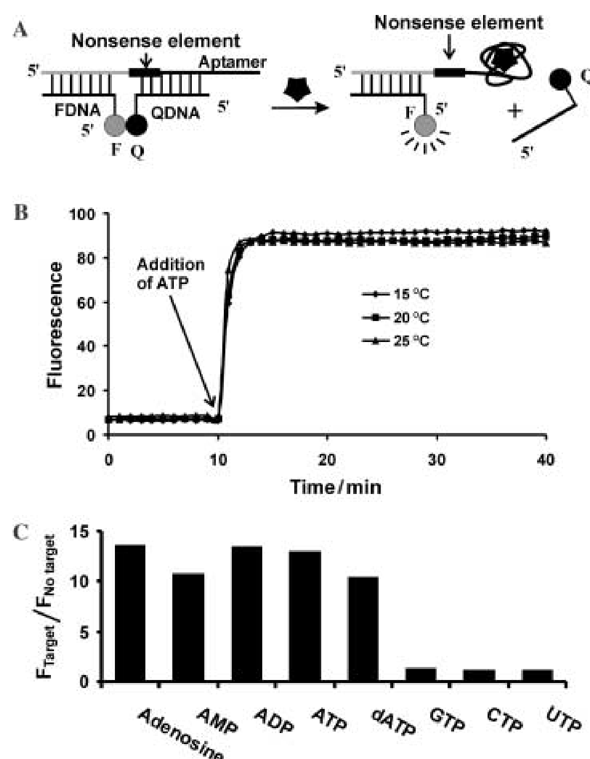

Figure 5. Designing structure-switching aptamers with real-time signaling capability at low temperatures. A) Design strategy. The symbols are identical to the one used in Figure 4 except that a thick line is introduced to indicate the nonsense sequence (see the main text). B) Signaling profile. The signaling DNA aptamer mixture was incubated at 15 (diamonds), 20 (squares), 25 °C (triangles) for 10 min before the addition of 1 mM ATP. Each target-aptamer mixture was further incubated for 30 more minutes after the ATP addition. C) Reporting specificity. Signaling profiles similar to 5B were obtained for a series of signaling aptamer solutions each containing an indicated target at 1 mM concentration. The ratio of fluorescence intensity at 40th min (denoted F_{Target}) and the 10th minute (denoted $F_{No\ Target}$) is plotted as the *y* axis.

ure 4B), the FDNA binding domain is excluded and the fluorophore is directly attached onto one end of the aptamer. In the third option (Figure 4C), FDNA is also eliminated and the fluorophore is covalently placed somewhere in the middle of the aptamer sequence. In the fourth option (Figure 4D), FDNA and QDNA are used to block two adjacent stretches of an aptamer sequence. Each of these three variations was tested with the same anti-ATP aptamer and was found equally effective as the first option.[33] Hypothetically, for short aptamers, both options 1 and 2 can be good choices. For long aptamers, option 4 should be more suitable. For aptamers with the key target-binding nucleotides located near the middle of the sequence, option 3 should be the best choice. Furthermore, the location of the fluorophore and the quencher can be interchanged. With many variations available, the structure-switching concept should be applicable to any given aptamer.

Desirable signaling properties: The signaling aptamer shown in Figure 3 exhibited an approximate sevenfold fluorescence enhancement, but required more than 30 minutes to reach maximal fluorescence intensity at 37 °C. At lower temperatures, the signaling magnitude increased, but much more time was required to reach the maximum fluorescence.[33] The inability of the signaling aptamer to switch structures at low temperatures was attributed to blockage of a long stretch of the aptamer binding site by QDNA and was rectified by a subtle adjustment to the design strategy, as illustrated in Figure 5 A. An arbitrary 5-nt sequence (denoted nonsense element, shown in thick line) was introduced between the aptamer segment and the FDNA-binding motif and a 12-nt QDNA was designed to form base pairs with both the inserted five nucleotides and the first seven nucleotides in the aptamer sequence. Upon addition of 1 mM ATP, the re-designed anti-ATP signaling aptamer was able to switch very quickly even at 15 °C and displayed a fluorescence enhancement of 14.1-, 13.0-, and 10.4-fold at 15, 20, and 25 °C, respectively (Figure 5B).[33] These experiments clearly demonstrated that structure-switching signaling aptamers could be designed to have very attractive real-time signaling properties.

Retention of binding specificity: All anti-ATP signaling aptamers retained the binding specificity of the original DNA aptamer. For example, the ATP reporter shown in Figure 5A exhibited more than tenfold fluorescence enhancement in the presence of 1 mM ATP, ADP, AMP, dATP, and adenosine, but the addition of 1 mM CTP, UTP, or GTP was not able to induce a fluorescence signal (Figure 5C).[33] The observed specificity pattern is consistent with that reported for the original aptamer.[24] The specificity consistency was also observed for an anti-thrombin signaling aptamer designed from a thrombin-binding DNA aptamer[28] with the same design strategy.[33] These observations indicate that struc-

ture-switching signaling aptamers are able to retain the same binding specificity of their parental aptamers.

Alteration of binding affinity: Blockage of an aptamer's target-binding site by QDNA may hinder the binding of the target to the aptamer. Therefore, we expected that the structure-switching approach might lead to a drop in the aptamer's target-binding affinity. Indeed, we observed a 60-fold affinity reduction with the ATP reporter and a twofold drop with the thrombin reporter. Since the K_d values of the two original aptamers are ~10 μM and ~200 nM, respectively, the above observation seems to suggest that the structure-switching-based modification strategy may cause more affinity reduction to low-affinity aptamers than to high-affinity ones. Two possible solutions to minimize this effect are discussed below.

Future Directions

Further validation of general adaptability of structure-switching concept: Thus far, we have successfully designed real-time reporters from two DNA aptamers with variable sizes (27 nt in anti-ATP aptamer and 15 nt in thrombin-binding aptamer), different structural features, and dissimilar affinities (10 μM and 200 nM, respectively).[24,26,28,30,31] Although our initial experiments have produced encouraging results, more aptamers (including RNA and modified nucleic acid aptamers) need to be studied to fully validate the generality of this simple design strategy.

Lessening affinity reduction: It is apparent that the structure-switching design strategy causes a certain degree of affinity reduction to the modified aptamers. Although this may not significantly diminish the utility of the aptamer reporter, it is certainly preferred that the affinity reduction be kept at a minimum. Since all QDNAs tested so far are DNA molecules that are completely complementary to the modified aptamers, it is conceivable that one potential way to minimize the affinity decrease is to use QDNAs with sequences that form less perfect duplexes with the aptamer. The other solution is to incorporate the structure-switching concept into an in vitro selection scheme and use it to directly derive structure-switching signaling aptamers from a random-sequence DNA library. Such strategies have been used by Jhaveri et al.[17] to directly derive single-fluorophore-modified, signaling aptamers from a random-sequence nucleic acid library and by Rajendran and Ellington for the in vitro selection of molecular beacons.[34]

Minimizing false signaling possibilities: Reporting molecular recognition simply by fluorescence intensity measurements can be complicated by false-positive possibilities imposed by many unexpected factors. A potential false-positive opportunity relevant to the structure-switching approach is the dissociation of duplex structures promoted by some unfavorable conditions (such as the presence of a DNA denaturing reagent, reduced metal-ion concentrations or temperature variations) or digestion of DNA molecules by a contaminating nuclease in the testing sample. Such a false-positive signal can, in principle, be conveniently revealed by the use of a control reporter, as illustrated in Figure 6. The control

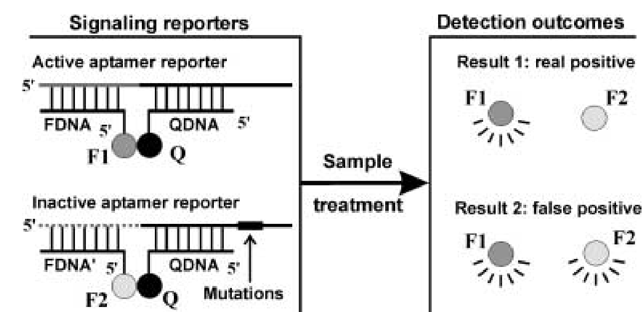

Figure 6. Strategies for designing control reporters to distinguish real-positive signals from false-positive ones. The inactive aptamer reporter contains a different FDNA-binding sequence (dashed gray line) and mutated nucleotides (shown in thick line) within the aptamer element (shown in black), but outside of the QDNA binding domain. A signal from only the bone fide reporter indicates the presence of the target of interest; signals from both reporters suggest a potential false positive signal.

reporter can be designed to have a different FDNA-binding sequence (dashed line in gray) to engage a new FDNA, FDNA′ (labeled with a different fluorophore) and a mutant aptamer with one or more crucial nucleotide mutations (shown in thick line) within the target-binding site. It is desirable to place the mutations outside of the QDNA binding domain so that the same QDNA can be used for both bona fide and control reporters. The base mutations are introduced to render the mutant aptamer inactive in target binding. When the signaling aptamer mixture is treated with a sample to be tested, a signal from the active reporter, but not from the control reporter, indicates the presence of the target of interest, and a signal from both reporters suggests that the sample may contain denaturants or other interfering factors and needs further testing.

In cases where the target itself is either a fluorophore or a quencher, false-negative possibilities could exist; that is, the target binding could inherently result in some degree of fluorescence quenching, partially negating fluorescence dequenching produced by the QDNA departure. One or several simple solutions can be applied to deal with these possibilities depending on the exact nature of the target. For example, one can choose a particular fluorophore, whose fluorescence cannot be quenched by the target, to label the aptamer (either directly or through FDNA strategy). One could also exchange the position of FDNA and QDNA: QDNA is used to label the aptamer and FDNA is designed to block part of the target-binding site. In this design, FDNA dissociates from the aptamer into the solution; therefore, target binding still leads to fluorescence generation. Other options include: 1) performing FRET (fluorescence resonance energy transfer) analysis between the target and the fluorescent aptamer instead of measuring fluorescence intensity (if the target is a fluorophore that can engage the fluorophore on the aptamer for FRET), and 2) eliminating QDNA and directly measuring fluorescence quenching induced by the target binding.

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Increasing detection dynamic range: Although not discussed in this article, we found that the signaling aptamers can be used as quantitative reporters with a detection dynamic range of 2–3 orders of magnitude.[33] In order to explore signaling aptamers as quantifying tools, a larger dynamic range is highly desirable. One possible solution for expanding the detection dynamic range is to simultaneously use two or more QDNAs of different sizes in the same mixture. At low target concentrations, only small-size QDNAs will be displaced. As the concentration of the target increases, longer QDNAs will be progressively replaced. We expect that by choosing an adequate group of QDNA molecules and by setting each QDNA to the appropriate concentration, a signaling aptamer mixture can be generated that can render a significantly increased detection dynamic range.

Designing multiplexed assays: The versatility of the structure-switching approach makes it convenient to create multiplexed assays. For instance, to design a 4-plexed assay by using four existing aptamers, one could simply use the strategy illustrated in Figure 7, whereby a common QDNA and four FDNAs (each labeled with a different fluorophore with a distinct emission maximum) are used. Each FDNA is designed to partially block the target-binding site of a particular aptamer, therefore only a matching target for a particular signaling aptamer could release the coding FDNA. The mixture of these reporters should allow the reporting of multiple targets in the same solution.

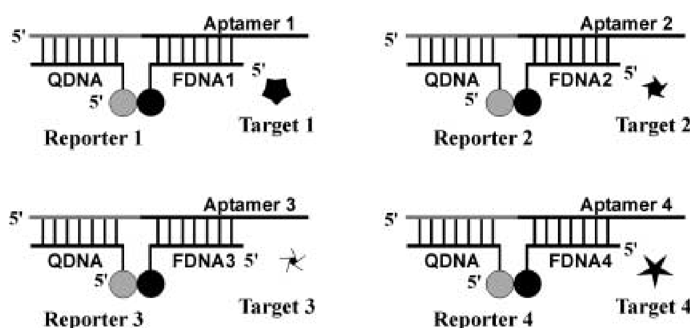

Figure 7. Designing structure-switching reporters for multiplex assays. In this particular example, four FDNAs (FDNA1–4) are designed to block the target binding sites of the four aptamer elements. The existence of one or more matching targets (Target 1–4, different stars) will release corresponding FDNAs, resulting in fluorescence signals indicative of coding targets.

Investigating surface immobilization: So far, we have only demonstrated the utility of structure-switching signaling aptamers as solution-based reporters. It is uncertain whether the same signaling aptamers can be used as surface-bound reporters. The immobilization of short oligonucleotides similar to the aptamers in our study is feasible,[35–37] and surface-bound aptamers or allosteric ribozymes (aptamer–ribozyme conjugates) have been shown to retain full function.[14,38] However, it remains to be determined whether hybridization between FDNA, QDNA, and surface-bound aptamers, as well as the release of QDNA by target addition will be as effective as observed in solutions.

Concluding Remarks

DNA is easy to prepare and has exceptional chemical stability. DNA aptamers can be readily derived from random-sequence DNA libraries by in vitro selection for binding a diverse range of targets with both high binding affinity and specificity. The simplicity and potential generality of the structure-switching design strategy, and the effectiveness of resultant fluorescence-signaling reporters, may significantly facilitate the exploration of DNA aptamers as molecular tools for many detection directed applications, including real-time monitoring of metabolites and proteins, in vitro diagnostics, and drug screening.

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