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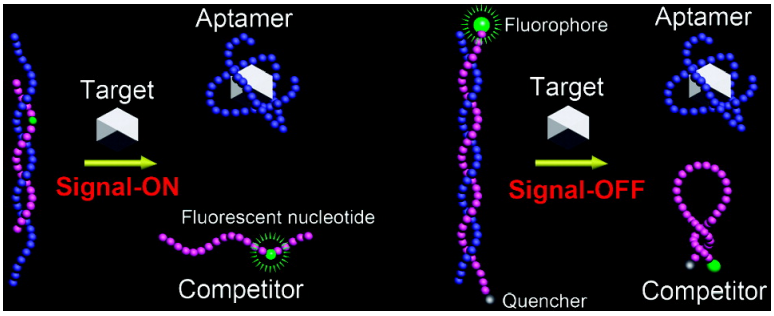

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Aptamer-Based Optical Probes with Separated Molecular Recognition and Signal Transduction Modules

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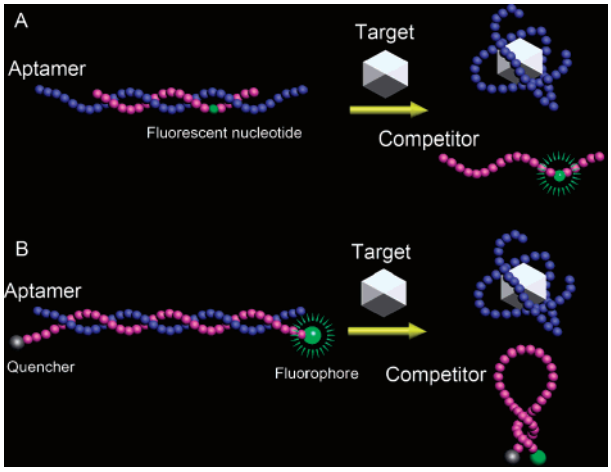
Aptamers are nucleic acid ligands isolated from combinatorial oligonucleotide libraries by in vitro selection.[1,2] Aptamers can bind to a great variety of molecular targets[3,4] with an affinity and a specificity comparable to that of antibodies, making them promising candidates for bio-detection applications.[5−7] Extensive efforts have been spent on developing aptamer-based optical sensors. Because even slight modifications on aptamers may lead to significant loss in their affinity and specificity,[8−10] lengthy optimization is often involved in strategies requiring labeling[11−18] or sequence alteration[19−22] of aptamers. Although recently a few label-free aptamer biosensors have been reported,[23−27] these methods used either photoactive polymer[23] or DNA-intercalating dyes[24−26] to report conformational change of aptamers upon recognition of given targets, therefore limiting their applicability for multiplex or in situ detection.

We present a generic design strategy for aptamer-based optical bio-sensors, in which an unmodified aptamer specific to the target serves as the molecular recognition element and a competitor oligo serves as the signal transduction element. The signaling competitor oligo is completely or partially complementary to the aptamer. In the absence of targets, competitor oligos bind to aptamers. However, in the presence of targets, the competitor oligos are partially displaced from aptamers by targets, which is accompanied by a change in the optical readout. The strategy of separating the recognition and signaling modules greatly simplifies the probe optimization procedures.

The signaling competitor oligo can be constructed in different ways to achieve either a signal-on (Scheme 1A) or a signal-off (Scheme 1B) detection. In the signal-on detection scheme, a fluorescent nucleotide analogue,[28] such as 2-aminopurine and pyrrolo-dC, is incorporated in the competitor oligo. The fluorescence quantum yield of these nucleotide analogues is dependent on the stacking interaction with their neighbor bases. The strongly quenched intrinsic fluorescence in the double-stranded competitor/aptamer complex is recovered when a competitor/aptamer duplex is dissociated upon addition of the target. In the signal-off detection scheme, the molecular beacon,[29] a hairpin oligo with a fluorophore attached on one end and a quencher attached on the other end, is used as a competitor oligo. When displaced by targets, the competitor oligos change from the "open" configuration to the "closed" configuration that brings fluorophore and quencher in close proximity, thus leading to a decrease in fluorescence. The signal-on detection is usually more sensitive than the corresponding signal-off detection.[30] The caveat is that for low-affinity aptamers, the signal-on detection has to be performed at low temperature to achieve the high sensitivity because the signal-on competitor/aptamer duplex is not stable at room temperature. In contrast, the signal-off competitor can be designed by tuning the length of the stems so that it forms a stable hybrid with the low-affinity aptamer without sacrificing the detection sensitivity. Detection of the target molecules with low-affinity aptamers tends to be more challenging than that with high-affinity aptamers. Therefore, to demonstrate the generality of the strategy, low-affinity aptamers and the signal-off competitors were used as model systems in this paper.

Scheme 1. General Strategy for Aptamer-Based Optical Detection: (A) Signal-on; (B) Signal-off.



To assess the sensitivity and specificity of the strategy, a signal-off detection system was developed for ATP based on its DNA aptamer.[31] In the design, the most important consideration is the relative stability among aptamer/competitor duplex, aptamer/target complex and the self-structure of competitor. Thermodynamic prediction was used to help select the appropriate sequence for the competitors. Because of the weak affinity between ATP and its DNA aptamer (6 ± 3 μM),[31] along with the loop part one stem in the competitor is also complementary to the aptamer, which ensures the separation of fluorophore and quencher when competitors bind to aptamers. On the basis of thermodynamic prediction, a 22 bp competitor oligo with 6-carboxyfluorescein at 3′ and Iowa Black FQ quencher (Integrated DNA Technologies, USA) at 5′ was designed. In the experiment, 0.2 μM competitor/aptamer (300 mM NaCl, 5 mM $MgCl_2$, 0.1 mM EDTA, 10 mM Tris, pH 8.0) was incubated at 65 °C for 5 min, followed by the addition of ATP, followed by an incubation of 30 min at room temperature before fluorescence measurements. Figure 1A shows the fluorescence intensity observed upon adding different concentrations of ATP or GTP. With the increase of ATP concentration, the resulting fluorescence intensity decreases owing to the increasing of aptamer/ATP complexes, releasing the free competitor oligos to self-quench in hairpin configuration. The reduction of fluorescence signal can be normalized as Figure 1B. Without extensive optimization, a detection sensitivity of 10 μM, which is comparable to other ATP DNA aptamer-based detection methods,[11,20] has been achieved with the current design of the competitor oligo. The sensitivity could

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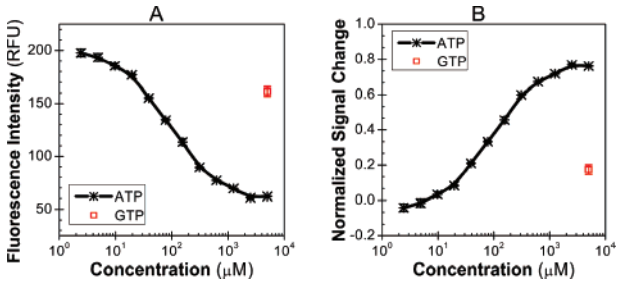

Figure 1. ATP sensor based on ATP DNA aptamer: (A) fluorescence intensity; (B) signal decrease normalized with the difference between competitor only and competitor/aptamer only.

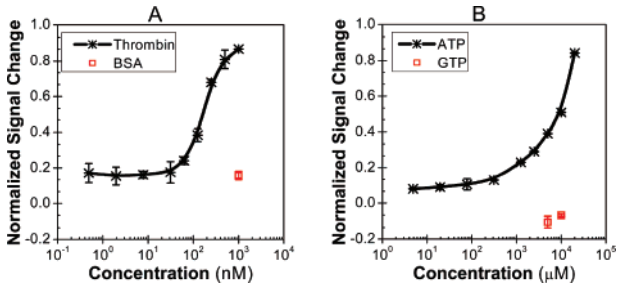

Figure 2. General applicability of the detection strategy: (A) thrombin sensor based on thrombin DNA aptamer (BSA, Bovine Serum Albumin); (B) ATP sensor based on ATP RNA aptamer.

be further improved by redesigning the competitor sequence and using optimal dye (organic dyes and quantum dots) and quencher pairs. The specificity of our detection system is tested by introduction of nonspecific target GTP. The result is nominal signal change compared to that of the specific target ATP with same concentration, thus demonstrating a high degree of specificity.

To illustrate the generality of our design strategy, we applied this strategy to another two systems. The first one was to detect the human thrombin protein based on its DNA aptamer.[32] The second one was for detection of ATP, but distinct from the previous design since an RNA aptamer[33] was used. In both systems, the competitors designed as the above were able to detect the corresponding targets with both high sensitivity and specificity (Figure 2).

In conclusion, we developed a generic strategy to convert the aptamer-target recognition event into an optical signal. By separating the molecular recognition element and signal transduction element, there is no need to make any modification on the original aptamer. This eliminates the laborious aptamer-specific optimization process, which is required in many of aptamer-based detections to preserve the affinity and specificity of the original aptamer. The generality of this strategy has been demonstrated not only with different types of targets (small molecules and proteins), but also with different types of aptamers (DNA and RNA). Moreover, the strategy is extremely versatile in that both signal-off and signal-on detections can be easily realized and easily adapted for other detection methods including electrochemical as well as optical detection. Finally, in the practical application, this strategy could be further improved. For example, the sensitivity could be increased by using an aptamer with a higher affinity to the target and the detection time could be shortened with appropriate competitor design and experimental conditions such as temperature and buffer composition. Therefore, with its simplicity, sensitivity, and specificity, this strategy holds great promise in applications such as high-throughput drug screening and intracellular studies.

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Supporting Information Available: Sequence of aptamers and competitors and experimental details. This material is available free of charge via the Internet at http://pubs.acs.org.

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