Biological Assays

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Amplified Aptamer-Based Assay through Catalytic Recycling of the Analyte**

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Interest in nanomaterials has increased rapidly in recent years as a result of their size- and shape-dependent properties, which make them extremely useful in bioanalytical and biomedical applications.[1] A useful property of nanomaterials is that they can protect DNA from nuclease cleavage owing to the steric-hindrance effect, which prevents nucleases from binding to the DNA adsorbed on the surface of nanomaterials.[2] This feature has encouraged the use of nanomaterials, such as gold nanoparticles[3] and carbon nanotubes,[4] in therapeutic DNA delivery.[5]

Aptamers are single-stranded nucleic acids isolated from random-sequence DNA or RNA libraries by an in vitro selection process termed the systematic evolution of ligands by exponential enrichment (SELEX).[6] The ability of aptamers to bind to a great variety of targets with high affinity and with specificity comparable to that of antibodies makes them promising molecular receptors for bioanalytical applications.[7] Extensive effort has been devoted to the development of aptamer-based homogeneous optical assays.[8] However, in these homogeneous assays, each aptamer binds to only a single target molecule (Figure 1, top). This 1:1 binding ratio limits signal enhancement and thus the sensitivity of the assay.

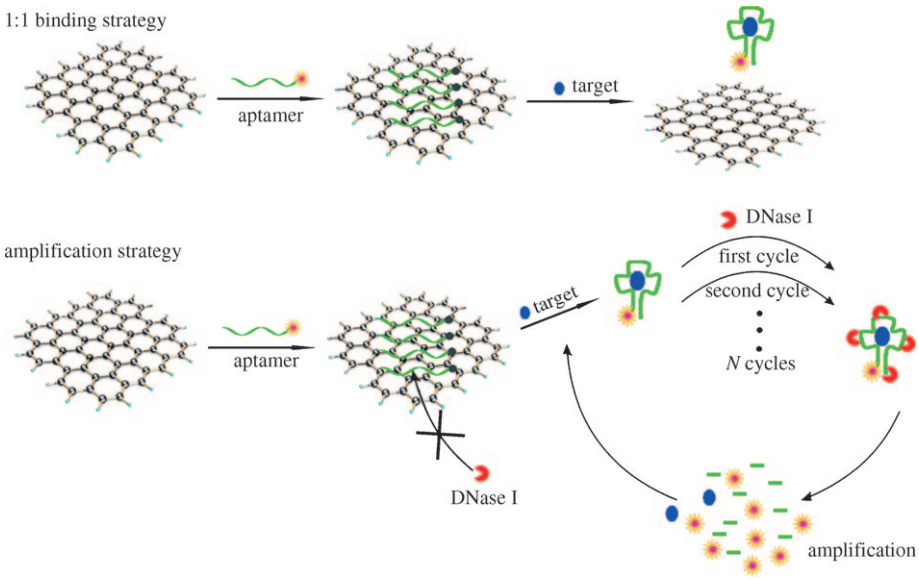

Figure 1. Top: A limitation of the 1:1 binding strategy is that each aptamer binds to only a single target molecule. Bottom: Amplification strategy based on a DNA-protective nanomaterial.

To overcome this problem, Willner and co-workers designed an ingenious aptamer-based machine for the amplified analysis of a target.[9] However, this machine is relatively complex and requires a polymerase replication process. Herein, we describe the development of a convenient amplified aptamer-based assay which relies on the DNA-protection properties of nanomaterials (graphene sheets and single-wall carbon nanotubes in this case; Figure 1, bottom). Graphene, a new kind of carbon nanostructure, is a single-atom-thick, two-dimensional carbon material.[10] It has been proven that graphene sheets can strongly bind single-stranded DNA, such as aptamers, as a result of hydrophobic and π-stacking interactions between the nucleobases and graphene. These interactions protect aptamers from nuclease cleavage.[11] Furthermore, graphene sheets can quench the fluorescence of fluorophores conjugated with aptamers as a result of the excellent electronic transference of graphene. When challenged with a target, the aptamer forms a stable, rigid structure and is released from the graphene substrate,[11a] whereupon the nuclease can cleave the free aptamer, thereby liberating the fluorophore and ultimately releasing the target. The released target then binds another aptamer, and the cycle starts anew, which leads to significant amplification of the signal. By monitoring the increase in fluorescence intensity, we could detect the target with very high sensitivity.

We used gel electrophoresis to investigate the viability of our strategy. Adenosine triphosphate (ATP) and an ATP aptamer were used as our models. The free ATP aptamer was

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digested completely upon incubation with the nuclease (DNase I in this case) for 1 hour (Figure 2, lane 3). DNase I also digested the ATP aptamer in the presence of ATP (Figure 2, lane 4). However, there was no evident enzymatic

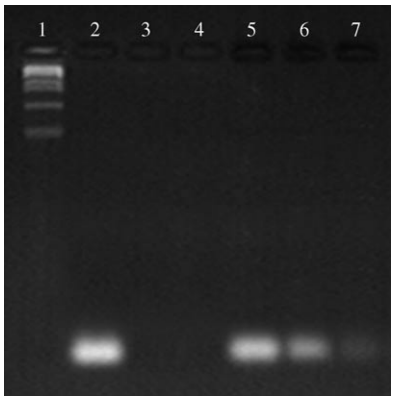

Figure 2. Gel electrophoresis of an ATP aptamer. Lane 1: DNA marker; lane 2: ATP aptamer only; lane 3: ATP aptamer treated with DNase I for 1 h; lane 4: ATP–ATP aptamer complex treated with DNase I for 1 h; lane 5: ATP aptamer–graphene complex treated with DNase I for 1 h; lanes 6 and 7: ATP aptamer–graphene complex treated with DNase I and ATP for 1 and 2 h, respectively.

hydrolysis of the ATP aptamer in the presence of graphene (Figure 2, lane 5). When DNase I and ATP were added to the aptamer–graphene complex simultaneously, the ATP aptamer was digested slowly during the incubation time (Figure 2, lanes 6 and 7). These results demonstrated that the aptamer was protected from DNase I cleavage after adsorption on the graphene surface. However, in the presence of the target molecule, aptamers were released from graphene one by one and thus digested by the nuclease.

We prepared the amplified assay based on the use of an aptamer and the DNA-protection property of graphene by first incubating the carboxyfluorescein-labeled ATP aptamer (50 nM) with graphene for 10 minutes to form an aptamer–graphene complex. We then added ATP and DNase I simultaneously, incubated the mixture for another 2 hours (see Figure S1 in the Supporting Information), and measured the fluorescence intensity. The amplification strategy based on the DNA-protection property of graphene led to a dramatic increase in the final fluorescence intensity upon the addition of the ATP target (Figure 3). The cleavage of some of the aptamers adsorbed on graphene by DNase I led to an increase in the background fluorescence in concert with the observed increase in the signal. Although the background fluorescence could be lowered by decreasing the reaction temperature, a longer reaction time was then required. However, the net signal produced by this biological assay was significantly enhanced. By using our amplification strategy, we observed a $330 \pm 9\%$ signal increase. In contrast, for the 1:1 binding strategy, only a $62 \pm 5\%$ increase in the signal was observed (in both cases, an ATP concentration of 50 μM was used).

The amplified aptamer-based assay is sensitive and specific. Figure 4 shows the fluorescence-emission spectra of the FAM-labeled ATP aptamer–graphene complex upon the

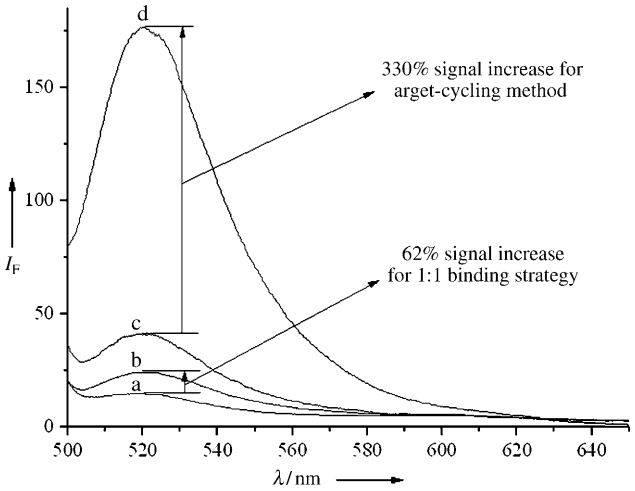

Figure 3. Fluorescence-emission spectra of the FAM-labeled ATP aptamer (50 nM) under different conditions: a) ATP aptamer + graphene; b) ATP aptamer + graphene + ATP (50 μM); c) ATP aptamer + graphene + DNase I; d) ATP aptamer + graphene + DNase I + ATP (50 μM).

addition of DNase I and ATP at different concentrations. A dramatic increase in the FAM fluorescence intensity was observed as the ATP concentration increased from 0.1 to 1000 μM. The detection limit (3σ/S, in which σ is the standard deviation for the blank solution, $n = 8$, and S is the slope of the calibration curve) was 40 nM. In contrast, the detection limit was 10 μM when we used the 1:1 binding strategy without amplification (see Figure S2 in the Supporting Information). Although the system has not yet been optimized for maximum efficacy, the sensitivity of this amplified aptamer-based assay for the detection of ATP was more than two orders of magnitude higher than that of reported traditional unamplified aptamer-based homogeneous assays.[12] The amplified aptamer-based assay is also specific. To evaluate this property, we challenged the system with several ATP analogues: guanidine triphosphate (GTP), cytosine triphosphate (CTP), and uridine triphosphate (UTP). Significantly higher fluorescence was observed with the target ATP than with its analogues (Figure 5). These results clearly demonstrate the high specificity of our amplified aptamer-based assay.

To illustrate the generality of our design, we applied this strategy to the detection of cocaine by using an appropriate DNA aptamer. As shown in Figure 6, the amplified assay based on the use of an aptamer and a DNA-protective nanomaterial detected cocaine with a detection limit of 100 nM, which is 200-fold lower than that of the 1:1 binding strategy (see Figure S3 in the Supporting Information).

Other nanomaterials, such as single-wall carbon nanotubes (SWNTs), can also be used in the amplified aptamer-based assay. It has been reported that single-stranded DNA can wrap around carbon nanotubes and is thus protected from nuclease cleavage.[4c] An amplified aptamer-based assay with SWNTs also showed high sensitivity: we observed a detection limit for ATP of 50 nM.

In conclusion, we have developed a simple and highly sensitive amplified aptamer-based assay which relies on the

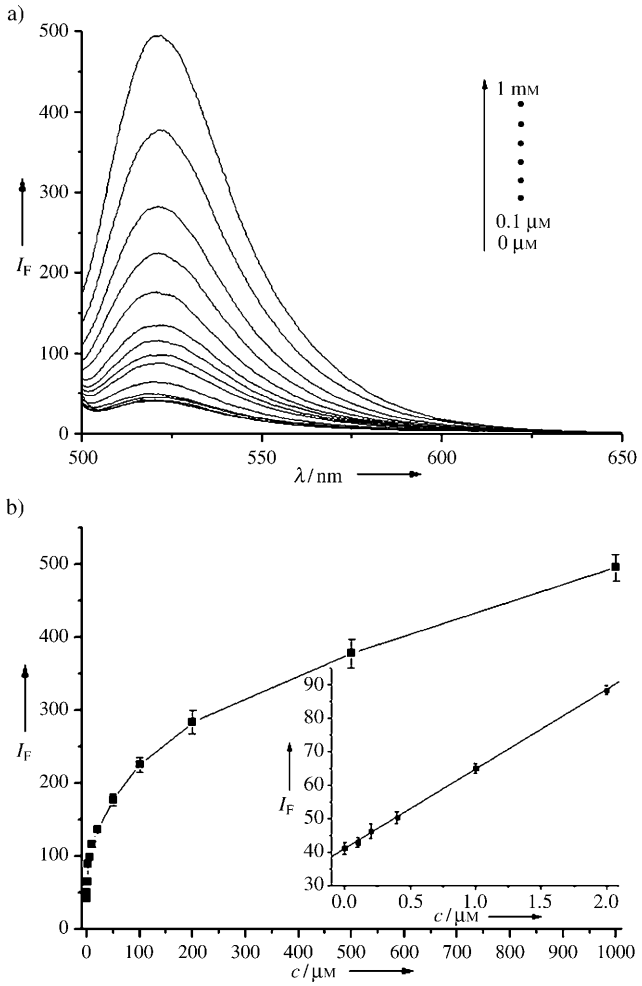

Figure 4. a) Fluorescence-emission spectra of the FAM-labeled ATP aptamer–graphene complex upon the addition of DNase I and ATP at different concentrations. b) Calibration curve for ATP detection. Inset: magnification of the plot in the range 0.0–2 μM. FAM = carboxyfluorescein.

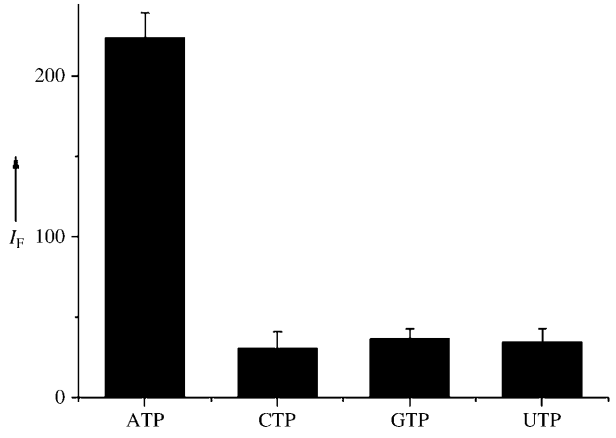

Figure 5. Selectivity of the amplified ATP-aptamer-based assay for ATP over CTP, GTP, and UTP (all at a concentration of 100 μM).

ability of nanomaterials to protect aptamers from nuclease cleavage. The assay can be prepared by simply mixing the aptamer, the nanomaterial, the nuclease, and the target molecule. The sensitivity of this new type of aptamer-based assay was more than two orders of magnitude higher than that of traditional unamplified aptamer-based homogeneous assays. Thus, the proposed amplified assay based on the use of a DNA-protective nanomaterial and an aptamer can be expected to provide a sensitive platform for the detection and subsequent analysis of target molecules.

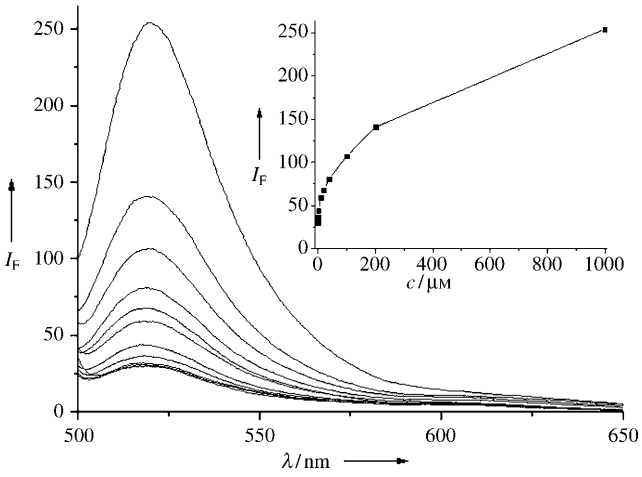

Figure 6. Fluorescence-emission spectra of the FAM-labeled cocaine aptamer–graphene complex upon the addition of DNase I and cocaine at different concentrations (0.2–1000 μM). Inset: calibration curve for cocaine detection.

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