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Highly Sensitive Aptasensor for Trace Arsenic(III) Detection Using DNAzyme as the Biocatalytic Amplifier

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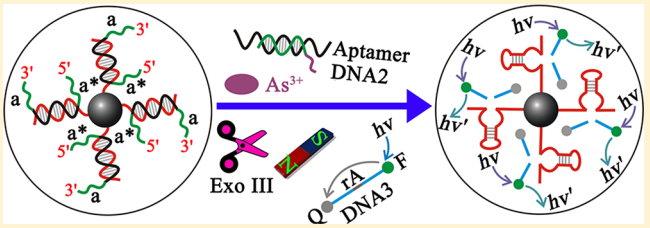
Supporting Information

ABSTRACT: A highly sensitive fluorescence biosensing system was designed for the detection of trace amounts of arsenic(III) (As^{3+}) based on target-triggered successive signal amplification strategy. The specific recognition between the target As^{3+} and the aptamer sequence results in the release of the blocking DNA to trigger the subsequent signal amplification steps. Exonuclease III (Exo III)-mediated DNA recycling digest process is introduced into the sensing system to generate numerous Mg^{2+}-dependent DNAzymes. After magnetic separation, the active DNAzyme with multiple turnovers could catalyze the continuous cleavage of the fluorophore-quencher-functionalized substrate strands, thus yielding a significantly amplified fluorescence signal for target detection. Due to the synergetic signal amplification of Exo III and DNAzyme, the fluorescent biosensor exhibits ultrasensitivity for As^{3+} monitoring, with a detection limit of 2 pM. Our established biosensor also displays excellent selectivity toward the target As^{3+} and has been successfully applied to the determination of As^{3+} in water samples with satisfactory accuracy. This sensing platform can be developed as a universal approach for the fast, sensitive, and accurate detection of aptamer-binding molecules.



As one of the most toxic heavy metals, arsenic (As) is becoming a severe worldwide threat to ecosystems and human health.[1] Long-term exposure to As can cause skin lesions, various circulatory and respiratory diseases, and many types of cancers.[2] Arsenite (As^{3+} as H_3AsO_3) and arsenate (As^{5+} as H_3AsO_4) are the two predominant forms of arsenic species in aqueous environments.[3] As^{3+} has been identified as the most harmful heavy metal, and it is 60 times more toxic than As^{5+}.[4] Considering its high toxicity, the U.S. Environmental Protection Agency (EPA) and the World Health Organizations (WHO) have set the maximum contaminant level (MCL) for arsenic in drinking water to be 10 ppb.[5]

To date, conventional techniques including atomic absorption spectroscopy (AAS),[6] atomic fluorescence spectrometry (AFS),[7] and inductively coupled plasma mass spectrometry (ICPMS)[8,9] are commonly used for As detection. Although sensitive and accurate quantification of As can be realized, these methods require cumbersome equipment, complex sample pretreatment, and highly trained personnel, which limit their wide applications for routine measurement. Very recently, as an alternative to conventional techniques, several elegant and efficient sensing protocols have been proposed for As^{3+} determination using As^{3+}-aptamer as the molecular recognition element, such as electrochemical,[10,11] colorimetric,[12−16] and fluorescent assays.[17−21] Among these approaches, fluorescent biosensors have received increasing interest for the rapid and reliable quantification of trace heavy metal ions with the remarkable properties of flexible design, simple operation, and low cost.[18,21] However, the fluorescent biosensors without a signal amplification procedure often suffer from relatively poor detection limits.[19,20]

To improve the detection sensitivity, various sensing strategies have been employed to amplify the response signals, in which DNAzyme-based signal amplification technology has gained remarkable attention in recent years.[22,23] DNAzymes are catalytic nucleic acids that can cleave the specific substrates using metal ions as the cofactors to activate the cleavage reactions.[24,25] The hydrolytic cleavage of DNAzymes can undergo multiple turnovers without losing the catalytic activities toward substrates, thus generating an amplified detection signal.[26,27] Due to their good stability, high catalytic efficiency, and easy functionalization, DNAzymes can be chosen as the ideal biocatalytic amplifiers to achieve significant signal enhancement for sensing events.[28,29] For example, the Mg^{2+}-dependent DNAzyme has been adopted as the catalytic cleavage units to construct biosensors for DNA,[30] protein,[31,32]

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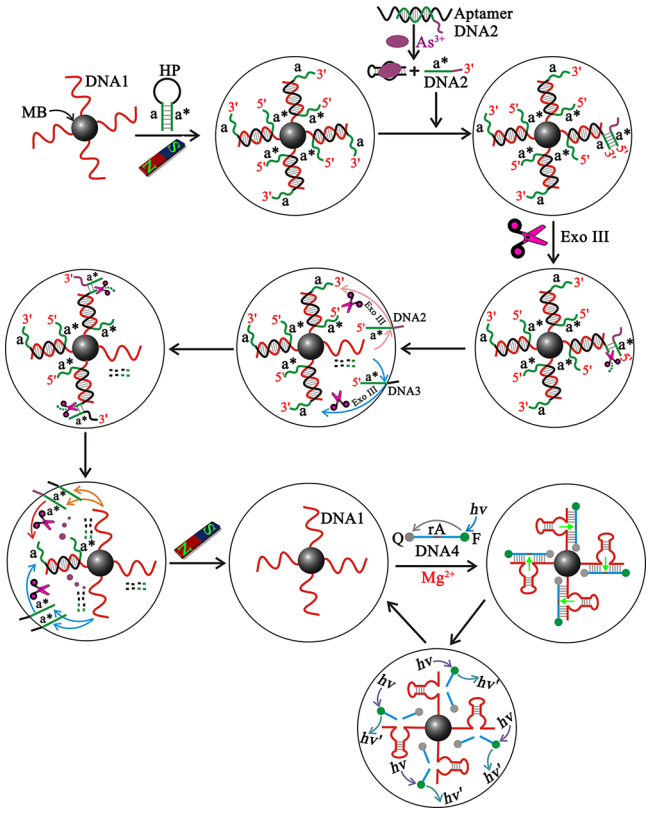
and small molecule[33−35] detection with high sensitivity. Herein, we designed a ultrasensitive sensing platform for As^{3+} detection using Mg^{2+}-dependent DNAzyme as the amplifier. Protein enzyme Exo III[36−38] is integrated with the DNAzyme technology to achieve the successive signal amplification.

The principle of the designed successive signal amplification strategy for As^{3+} assay is illustrated in Scheme 1. A hairpin probe (HP) is first hybridized with DNA1 immobilized on magnetic beads (MB). With the aid of magnetic separation, excess HP is then removed. The obtained partial duplex DNA (DNA1-HP) containing the protruding 3′ terminus cannot be digested by Exo III. The addition of target As^{3+} into the sensing system would cause the aptamer-DNA2 complex to unwind and lead to the release of DNA2, which could subsequently trigger the enzymatic cycling signal amplification process in the presence of Exo III. The recognition of domain a* in DNA2 with the dangling 3′ end of HP (domain a) makes the DNA-HP probe have a 3′ blunt terminus. Exo III thus can catalyze the stepwise cleavage of mononucleotides in the 3′ direction, liberating DNA2 to initiate a new cleavage reaction. At the same time, Exo III continues to work down DNA1, cleaving the HP DNA, and another DNA fragment containing domain a* in HP (DNA3) is also liberated, which can serve as the new activator sequence to trigger the continuous digestion of HP. Due to the successive cleavage process, only DNA1 is left on MB after magnetic separation to remove DNA2, DNA3, and Exo III. It is worth noting that the sequence of DNA1 is the Mg^{2+}-dependent DNAzyme. Incubation with the cofactor Mg^{2+}, DNAzyme will be activated to cleave substrate strand DNA4, which is modified with fluorophore (FAM) and quencher (TAMRA). The resulting fluorophore-functionalized DNA fragment can generate the fluorescence signal for As^{3+} detection. Importantly, each active DNAzyme can undergo many cycles to catalyze the cleavage of numerous DNA4 substrates, achieving a further increase in the output signal for the sensing event. In the absence of As^{3+}, DNA1 is occluded by hybridization with HP, which prevents DNA1 to form an active DNAzyme.

Scheme 1. Schematic Illustration of the Assay Principle for As^{3+} Detection Based on Target-Triggered Successive Signal Amplification Strategy[a]



[a]Domain a is complementary to domain a*. The sequence of DNA1 corresponds to the Mg^{2+}-dependent DNAzyme. MB, magnetic beads; HP, hairpin probe; Exo III, exonuclease III; F, fluorophore (FAM); Q, quencher (TAMRA).

To verify the feasibility of the proposed signal amplification strategy for As^{3+} detection, fluorescence emission spectra of the solution are recorded at different control experiments. As shown in Figure 1, in the absence of target As^{3+}, only background fluorescence signal can be observed (curve a), which indicated that the sensing probes (DNA1-HP) are kept intact on MB and fail to be triggered for the signal amplification process. Upon the addition of As^{3+} into the sensing system without Exo III, the fluorescence response is almost negligible (curve b). That is because no cleavage reaction toward HP could occur in the absence of Exo III. In the absence of Mg^{2+}, the cleavage activity of the DNAzyme is suppressed and only small fluorescence signal can be obtained (curve c). In the presence of As^{3+}, Exo III, and Mg^{2+}, the fluorescence intensity of the solution is sharply increased (curve d). Such an increase can be contributed to the recycling digest process by Exo III and the multiple turnover capability of the DNAzyme. Those results demonstrate that the developed fluorescent sensor can be used to detect As^{3+}.

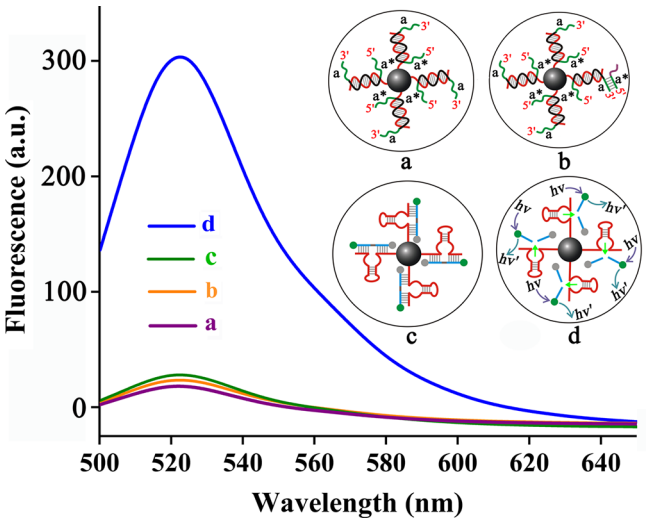


Figure 1. Fluorescence signals of the sensing system at different experimental conditions: (a) no As^{3+}, (b) no Exo III, (c) no Mg^{2+}, and (d) 10 nM As^{3+}, 2 unit/μL Exo III, and 100 mM Mg^{2+}.

Under optimal experimental conditions (Figures S1−S4, Supporting Information), the developed biosensor was applied to detect different concentrations of target As^{3+}. As shown in Figure 2A, upon increasing the target As^{3+} concentrations from 0 to 10 μM, a consistent increase of the fluorescence intensity can be observed, indicating that more FAM-labeled DNA fragments are liberated with increasing As^{3+} concentrations.

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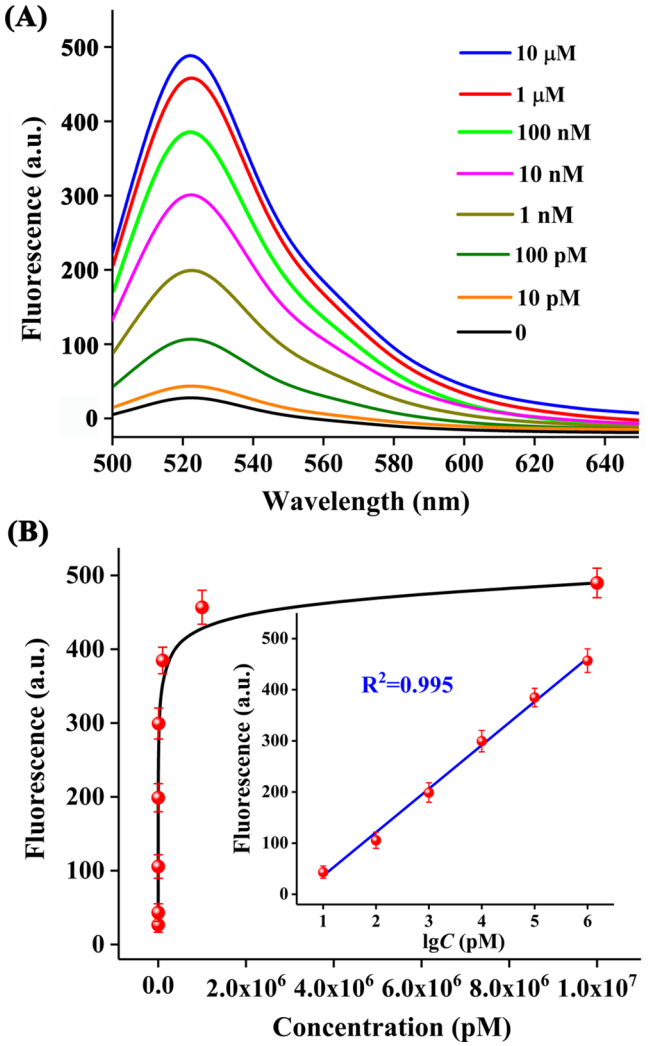

Figure 2. (A) Fluorescence spectra of the sensing system in the presence of different As^{3+} concentrations. (B) Correlation of the fluorescence intensity at 520 nm with the As^{3+} concentrations. The error bars are standard derivation obtained from three independent experiments.

The corresponding calibration plots show that there is a good linear relationship between the fluorescence intensity and the logarithm of As^{3+} concentration in the range from 10 pM to 1 μM with a correlation coefficient (R^2) of 0.995 (Figure 2B). The linear equation is $F = 85.2 \lg C - 49.1$, where F is the fluorescence intensity and C is the As^{3+} concentration. The limit of detection (LOD) is calculated to be 2 pM (S/N = 3), which is much sensitive than those of aptamer-based sensors for As^{3+} detection without signal amplification.[16,20] The excellent sensitivity can be attributed to the cascade signal amplification process by the rational integration of Exo III and DNAzyme. Therefore, our proposed sensing system used here has quite a superior sensitivity, which is vital for the detection of trace level of As^{3+}.

To investigate the selectivity of this sensing platform toward As^{3+}, other competing heavy metal ions including As^{5+}, Cd^{2+}, Pb^{2+}, Hg^{2+}, Cu^{2+}, Cr^{3+}, Zn^{2+}, and Mn^{2+} were also tested. As shown in Figure 3, the presence of the potential interfering metal ions at 100 nM displayed negligible fluorescence responses compared with the blank solution. In contrast, the addition of the target As^{3+} (10 nM) into the sensing system

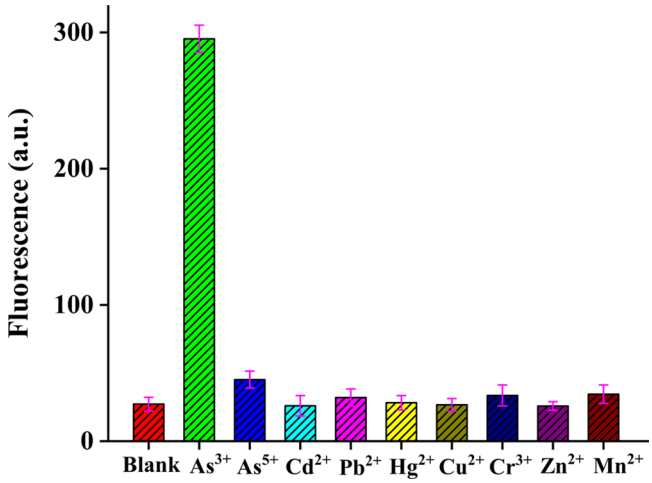

Figure 3. Selectivity investigation of the sensing platform for As^{3+} against other competing heavy metal ions. As^{3+}, 10 nM; control metal ions, 100 nM. The error bars are standard derivation obtained from three independent experiments.

generated a significantly enhanced fluorescence signal. The data revealed that our proposed sensor exhibited excellent specificity for the discrimination of As^{3+} from other heavy metal ions. Such high sensitivity could be attributed to the specific recognition between the aptamer and its ligand.

To validate the assay reliability of the proposed fluorescent biosensor in practical applications, this sensing system was applied to the detection of As^{3+} in real river water, tap water, and lake water samples. The concentrations of As^{3+} in the collected real water samples were certified by the ICPMS standard method. The testing data are summarized in Tables S2–S4 (Supporting Information). The concentrations of As^{3+} in real water samples determined by the biosensor are in good accordance with the ICPMS data, confirming the satisfactory accuracy of the proposed assay for As^{3+} detection in real samples.

In conclusion, we have successfully demonstrated a highly sensitive fluorescence sensing platform for As^{3+} detection based on target-triggered successive signal amplification strategy. As^{3+}-aptamer is used as the recognition unit. In the presence of As^{3+}, the blocking DNA is released to trigger the cascade signal amplification process. The integration of Exo III-assisted DNA recycling and DNAzyme-based catalytic cleavage with multiple turnovers results in the generation of significantly amplified fluorescence signals for highly sensitive quantification of trace levels of As^{3+}, with a detection limit of 2 pM. Meanwhile, the fluorescent biosensor can discriminate As^{3+} from other competing metal ions with high selectivity. This sensing platform is robust and has been successfully applied to the determination of As^{3+} in real water samples. In this design, nucleic acids were used to construct the sensing platform. The DNA probes can be stored at −20 °C for 1 year without losing their original conformations and can be used immediately to built the sensing system after dissolving in buffer solution. This successive signal amplification strategy based on Exo III and DNAzyme had the potential to become a powerful tool in fabricating an ultrasensitive biosensor with the advantages of simple operation, wide linear range, and excellent selectivity. Importantly, because different recognition units might be integrated into the sensing system, our proposed versatile fluorescence detection platform can be

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employed to detect various analytes by simply substituting the aptamer sequence.

■ ASSOCIATED CONTENT

Supporting Information

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Experimental details; DNA sequences employed in this work; effects of reaction temperature, molar ratio of DNA1 to DNA4, cleavage time of DNAzyme, and pH on the biosensor performance; and detected levels of As^{3+} in river water samples, tap water samples, and in lake water samples using the proposed biosensor and ICPMS (PDF)

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Author Contributions

The manuscript was written through contributions of all authors.

Notes

The authors declare no competing financial interest.

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