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Metal Ion-Mediated Assembly of DNA Nanostructures for Cascade Fluorescence Resonance Energy Transfer-Based Fingerprint Analysis

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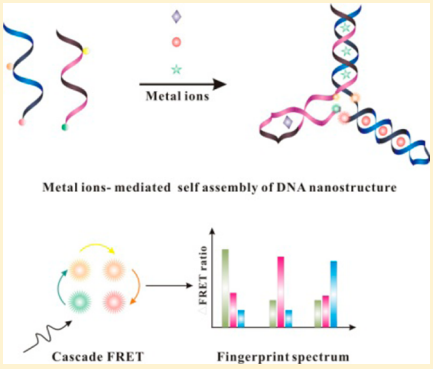
ABSTRACT: Contamination of heavy metal ions in an aquatic environment poses a serious threat to human health. More seriously, heavy metal ions are usually present in the environment in a mixture, and the synergetic toxicity of multiple heavy metal ions is revealed (Aragay et al. *Chem. Rev.* **2011**, *111*, 3433; Chu et al. *Aquat. Toxicol.* **2002**, *61*, 53). Unfortunately, most of the existing methods based on DNA sequences are focusing on the detection of one type of metal ions. Simple and multiplexed detection of multiple metal ions has been poorly investigated and remains challenging. Here, we re-engineered the DNA sequences for Pb^{2+}, Hg^{2+}, and Ag^{+}, through which the binding of multiple metal ions initiated the self-assembly of these DNA sequences. On the basis of our rationally designed multicolor fluorescent labeling of the DNA sequences, cascade fluorescence resonance energy transfer (FRET) occurred. As a result, a fingerprint fluorescent spectrum was produced to indicate the presence of a single type of metal ions or multiple metal ions. The major advantages of our cascade FRET fingerprint technology include the following: (1) the "mix and read" detection mode in homogeneous solution is simple without the need of complicated instruments; (2) only single excitation is required to provide the cascade FRET fingerprint spectrum; (3) multiplexed detection capability can be realized intuitively and sensitively.



Contamination of heavy metal ions in the aquatic environment poses a serious threat to human health. Accumulated evidence demonstrated that heavy metal ions cause serious diseases such as damaged or reduced mental ability and lower energy levels. Long time exposure may cause slowly progressing physical or neurological degenerative processes.[1] More seriously, heavy metal ions are usually present in the environment in a mixture, and the synergetic toxicity of multiple heavy metal ions is revealed.[1,2] Hence, the multiplexed detection of multiple metal ions is urgent to meet the requirements of the current severe situation of contamination.[3]

Unfortunately, sophisticated equipment such as ICP-AES (inductively coupled plasma−atomic emission spectrometry) is usually employed for the detection of metal ions.[3−6] However, the time-consuming, high cost, and large dimensions of the instruments make them not suitable for rapid on-site detection and limit their wide applications in resource-limited settings.[3]

Recently, emerging technologies such as aptamers and DNAzyme are widely employed for the detection of metal ions.[7−23] By coupling to the fluorescent, enzymatic, or colorimetric techniques, some simple detection platforms have been developed for Pb^{2+}, Hg^{2+}, and other metal ions.[7−23] However, most of the existing methods are focusing on the detection of one type of metal ions. Simple and multiplexed detection of multiple metal ions still remains challenging. In some existing methods for multiplexed detection,[9,10] masking agents are usually required, which increases the complexity and inconvenience of the detection.

Here, by using the specific binding between metal ions and DNA sequences, we re-engineered the DNA sequences and then assembled them into a DNA nanoassembly. The binding mediated DNA nanoassembly was employed to detect multiple heavy metal ions (Hg^{2+}, Pb^{2+}, and Ag^{+}), simultaneously. In our design, we engineered the specific DNA sequences for Hg^{2+}, Pb^{2+}, and Ag^{+} and integrated them in two DNA strands. Without the binding of heavy metal ions, the two DNA strands separated well from each other and remained as single stranded DNAs in the homogeneous solution. Upon the binding of multiple metal ions (Hg^{2+}, Pb^{2+}, and Ag^{+}), the two strands folded together and formed a three-way junction structure. By labeling the strands with multicolor fluorophores, we realized a cascade fluorescence resonance energy transfer (FRET), which requires only one excitation to produce a fingerprint-like spectrum to detect the multiple heavy metal ions (Figure 1).

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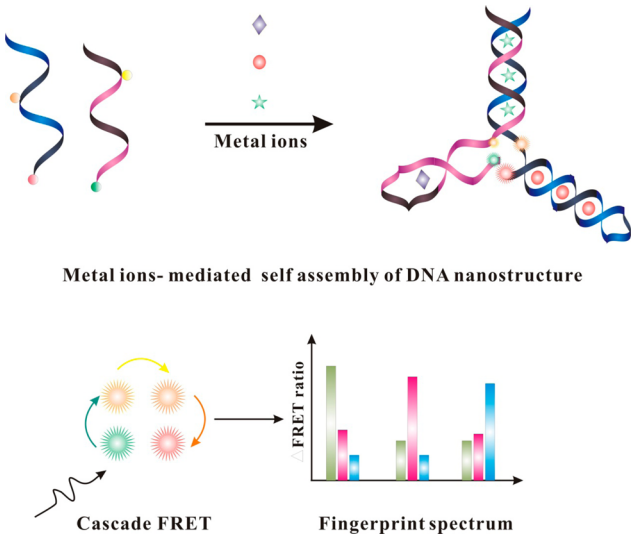

Figure 1. We re-engineered the DNA sequences that specifically bind to Pb^{2+}, Hg^{2+}, and Ag^{+}, respectively, to produce two single stranded fragments (fragment 1 and fragment 2). Without the binding of metal ions, these two fragments remained single stranded and separated well in homogeneous solution. Upon the binding of multiple metal ions, these two fragments folded and self-assembled into a three-way junction like nanoassembly. By labeling the fragments with appropriate fluorescent dyes, cascade FRET occurred to produce a fingerprint like spectrum, which can be employed to detect multiple metal ions simultaneously.

The major advantages of our cascade FRET fingerprint technology include the following: (1) the "mix and read" detection mode in homogeneous solution is simple without the need of complicated instruments; (2) only a single excitation is required to present the cascade FRET fingerprint spectrum; (3) multiplexed detection capability can be realized intuitively and sensitively.

EXPERIMENTAL SECTION

Materials. Oligonucleotides were synthesized and purified by Takara Biotechnology Co. (Dalian, China). 3-Morpholinopropanesulfonic acid (MOPS), lead(II) nitrate, silver(I) nitrate, and mercury(II) perchloride monohydrate were purchased from Sigma-Aldrich. Manganese(II) sulfate monohydrate, copper(II) sulfate pentahydrate, zinic (II) sulfate heptahydrate, nickle sulfate heptahydrate, magnesium(II) nitrate hexahydrate, iron(II) sulfate heptahydrate, calcium(II) nitrate tetrahydrate, cobalt(II) nitrate hexahydrate, and sodium(I) nitrate was obtained from Aladdin (Shanghai, China). All reagents were used as received without further purification. Milli-Q water (18.2 MΩ cm^{-1}) was used for all experiments.

Fragment 1: 5′-FAM-AAAAGGTTGGTGTGGTTGGAT-(Cy3)TTCTTTCTTCC-3′

Fragment 2: 5′-CCTTGTTTGTTTCAT(TexRed)ACTCT-CTTCTCTTCATTTTTCAACACAACACACA-Cy5-3′

Fluorescence Spectrum Measurement. All fluorescence measurements were carried out with a F900 Fluorescence spectrophotometer (Edinburgh, United Kingdom), and slits for both the excitation and emission were at 1 nm. Ten mM of MOPS buffer (pH 7.0) containing 50 mM $NaNO_3$ was used as working buffer. The stock solutions of oligonucleotides were prepared in MOPS buffer and stored at −20 °C. The heavy metal ions were prepared with 0.5% HNO_3 to avoid hydrolyzation.

For the Pb^{2+} analysis, fragment 1 (10 μL, 5.0 μM) was first added to MOPS buffer (10 mM MOPS, 50 mM $NaNO_3$, pH 7.0) with the appropriate volume, and then, different concentrations of Pb^{2+} ions were introduced into the above solution; the total volume of the final solution was fixed at 100 μL. After incubation for 20 min at room temperature, the spectrum of FAM and Cy3 from the fragment 1 probe was recorded with the excitation wavelength of 492 nm. The ratio of the fluorescence intensity at the emission wavelength of 564 and 515 nm was used for quantitative analysis of Pb^{2+}.

For the Hg^{2+} analysis, fragment 1 (10 μL, 5.0 μM) and fragment 2 (11.0 μL, 4.52 μM) were mixed. The mixture was first added to MOPS buffer (10 mM MOPS, 50 mM $NaNO_3$, pH 7.0) with the appropriate volume, and then, different concentrations of Hg^{2+} ions were introduced into the above solution; the total volume of the final solution was fixed at 100 μL. After incubation for 20 min at room temperature, the spectrum of TexRed from fragment 2 and Cy3 from the fragment 1 probe was recorded with the excitation wavelength of 555 nm. The ratio of the fluorescence intensity at the emission wavelength of 612 and 564 nm was used for quantitative analysis of Hg^{2+}.

For the Ag^{+} analysis, fragment 2 (11.10 μL, 4.52 μM) was first added to MOPS buffer (10 mM MOPS, 50 mM $NaNO_3$, pH 7.0) with the appropriate volume, and then, different concentrations of Ag^{+} ions were introduced into the above solution; the total volume of the final solution was fixed at 100 μL. After incubation for 20 min at room temperature, the spectrum of TexRed and Cy5 from the fragment 2 probe was recorded with the excitation wavelength of 595 nm. The ratio of the fluorescence intensity at the emission wavelength of 663 and 612 nm was used for quantitative analysis of Ag^{+}. For the multiplexed detection of metal ions, the operation steps were the same with the detection of Hg^{2+}.

RESULTS AND DISCUSSION

Here, we employed the DNA sequences that can bind to Pb^{2+}, Hg^{2+}, and Ag^{+}, respectively. For the binding of Pb^{2+}, we employed a DNA sequence that folds into a G-quartet structure through the coordination of Pb^{2+} to the eight surrounding guanine O6 atoms.[15] For the binding of Hg^{2+}, we employed the DNA sequence with enriched thymines,[8] which formed a hairpin structure in the T-Hg^{2+}-T configuration upon binding of Hg^{2+}. For the binding of Ag^{+}, we employed the specific interaction of Ag^{+} with cytosine−cytosine mismatches to design the cytosine rich DNA sequence.[16]

In order to realize the multiplexed detection, we designed a DNA fragment (fragment 1) that combined the specific sequence for Pb^{2+} and a part of the DNA sequence for the specific binding of Hg^{2+}. Then, we modified the DNA with a fluorophore (FAM) at the 5′ end and another fluorophore (Cy3) at an internal position (Figure 1). In the same pattern, we designed another DNA fragment (fragment 2) that integrated the specific sequence for Ag^{+} and another part of the DNA sequence for the specific binding of Hg^{2+}. Fragment 2 was labeled with two fluorophores (TexRed and Cy5) as well. We estimated that fragment 1 and fragment 2 can self-assembled into a DNA three-way junction like nanoassembly in the presence of all three types of metal ions (Pb^{2+}, Hg^{2+}, and Ag^{+}) (Figure 1). With our rational designed fluorophore modification, the formation of the DNA nanoassembly initiated the cascade FRET with a single excitation to produce a

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fingerprint-like fluorescent spectrum, with which the multiplexed detection of metal ions can be realized intuitively.

To testify the conformational change of our re-engineered DNA sequences upon ion binding, we employed the FRET technique that is well established and used to characterize the structure change of DNA.[24,25] At first, we incubated the Pb^{2+} (2000 nM) with fragment 1 for 20 min and collected the fluorescent spectrum. We observed an obvious decrease in the emission of FAM dye and an increase in the emission of Cy3 dye (Figure 2). This increased FRET efficiency indicated that

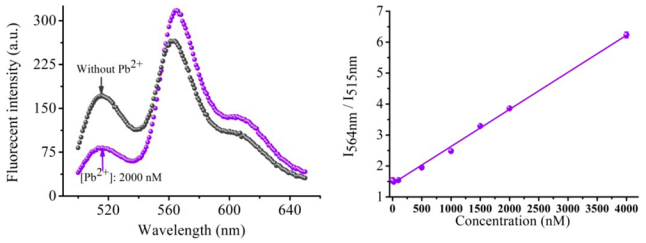

Figure 2. (Left) The fluorescent spectrum of fragment 1 with and without Pb^{2+}. The change of FRET efficiency indicated the successful binding of Pb^{2+} and conformational change of fragment 1. (Right) The fluorescent intensity ratio between 564 and 515 nm increased along with the increase of the concentration of Pb^{2+}.

the FAM and Cy3 were forced into close proximity via the target induced folding of fragment 1, which is consistent with the previous studies.[11,15] As a further step, we interrogated the detection capability of fragment 1 to Pb^{2+}. We can detect as low as 20 nM of Pb^{2+}. Above this concentration, we obtained a monotonic increase in the ratio of fluorescent intensity at 564 and 515 nm. The detection limit of our technique is sensitive enough to fulfill the requirement of the US Environmental Protection Agency (US EPA), which is 75 nM (Figure 2).

We then evaluated the conformational change of fragment 2 and detection capability of fragment 2 to Ag^{+}. We observed an obvious increase of FRET efficiency upon Ag^{+} binding (Figure 3), which indicated the folding of fragment 2. The dynamic range of Ag^{+} detection spans from 100 nM to 2 μM.

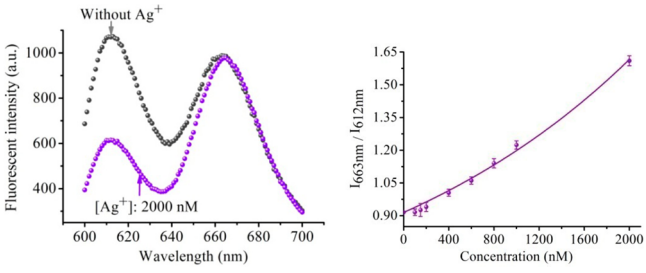

Figure 3. (Left) The fluorescent spectrum demonstrated the increase in FRET efficiency after the binding of Ag^{+}. (Right) We can detect as low as 100 nM Ag^{+}. The fluorescent intensity ratio increased with the increase of the concentration of Ag^{+}.

Next, by using the DNA fragments with enriched thymines that were embedded in fragment 1 and fragment 2, we assembled these two fragments and realized the sensitive detection of Hg^{2+}. As demonstrated (Figure 4), the binding of Hg^{2+} led to an obvious increase in FRET efficiency, which indicated that the fragment 1 and fragment 2 were assembled successfully. The assembly of these two fragments can be

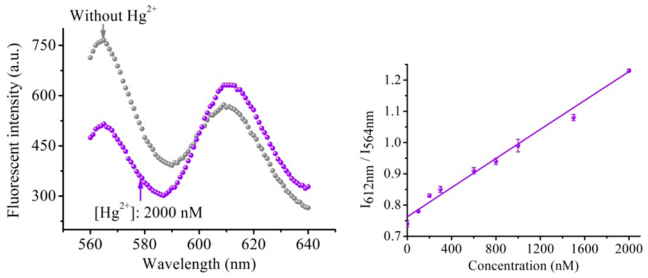

Figure 4. (Left) In the presence of Hg^{2+} (2000 nM), we observed an increase in FRET efficiency, which was originated from the assembly of fragment 1 and fragment 2 upon the binding of Hg^{2+}. (Right) The relationship between fluorescent intensity ratio and the concentration of Hg^{2+} indicated that we can detect Hg^{2+} with a dynamic range from 100 nM to 2 μM.

employed to realize the sensitive detection of Hg^{2+}. We obtained detection sensitivity of 100 nM of Hg^{2+}.

For the multiplexed detection, we employed the cascade FRET to produce a fingerprint-like spectrum for each combination of metal ions. In the cascade FRET process, FAM was employed as a donor for the other three dyes[26] (Cy3, TexRed, and Cy5); Cy3 was employed as the acceptor for FAM but as the donor for TexRed and Cy5. TexRed was employed as the acceptor for FAM and Cy3 but as the donor for Cy5, and Cy5 was employed as acceptor for FAM, Cy3, and TexRed.[26] Then, we collected the fingerprint spectrum by using the excitation of 492 nm and analyzed the FRET ratio (Figure 5). We compared the fingerprint spectrum of blank solution to the

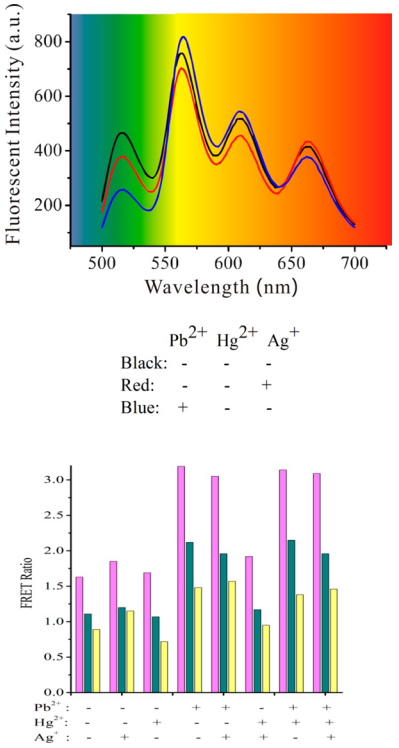

Figure 5. (Top) Representative fingerprint-like spectrum for the detection of metal ions. The characterized features of fluorescent intensity ratio can be employed to detect multiple metal ions. The background color indicates the approximation of spectral colors at different wavelength. (Bottom) The patterns of fluorescent intensity ratio for different combination of multiple metal ions.

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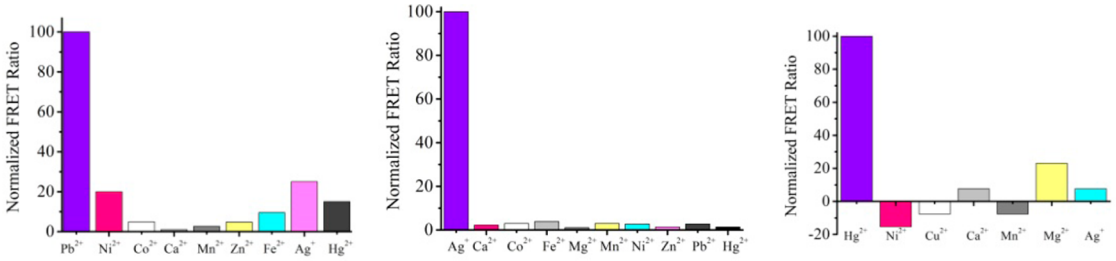

Figure 6. Our detection platform exhibited excellent specificity for the detection of Pb^{2+} (left), Ag^{+} (middle), and Hg^{2+} (right), respectively.

spectrum of solution with Ag^{+}, and we observed different features in the fingerprint patterns. For example, the emission in 612 nm decreased, while the emission in 663 nm increased. In the fingerprint spectrum of Pb^{2+}, we observed different characterized features, too (Figure 5).

At last, we collected a fingerprint like spectrum for various combinations of multiple metal ions. We obtained FRET ratio patterns with fingerprint features, which indicated that our detection platform can be employed to detect multiple heavy metal ions simultaneously (Figure 5).

Our re-engineered DNA fragments had excellent specificity. For example, we challenged our detection with various metal interferences when we detect Pb^{2+} using fragment 1 (Figure 6). We obtained an obvious increase in FRET efficiency in the presence of Pb^{2+}. Other metal ions can only introduce negligible signal change. Impressively, we obtained excellent specificity for Ag^{+} and Hg^{2+}, too (Figure 6).

CONCLUSION

Here, we have demonstrated a simple and fast detection platform for the multiplexed detection of heavy metal ions. To realize the detection, only a simple mixing step and a reading step are required, and the detection result can be presented in 20 min. In such a way, expensive instruments and trained expertise are not required, which makes it suitable for the point-of-care detection. The cascade FRET and fingerprint spectrum technique provide an effective way for the multiplexed detection with only one single excitation. Compared to some previous multiple detection platforms,[7,27] the preparation of nanoparticles and the conjugation of DNA to nanoparticles are not required. Therefore, the present metal-DNA mediated DNA nano assembly provides a rapid, simple, and sensitive detection for multiple heavy metal ions.

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Notes

The authors declare no competing financial interest.

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