Nanoscale

A reversible metal ions fueled DNA three-way junction molecular device for "turn-on and -off" fluorescence detection of mercury ions (II) and biothiols respectively with high selectivity and sensitivity

Journal:	*Nanoscale*
Manuscript ID	NR-COM-07-2015-004688.R1
Article Type:	Communication
Date Submitted by the Author:	03-Sep-2015
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Journal Name

COMMUNICATION

A reversible metal ions fueled DNA three-way junction molecular device for "turn-on and -off" fluorescence detection of mercury ions (II) and biothiols respectively with high selectivity and sensitivity

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

www.rsc.org/

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We constructed a reversible molecule device in nanoscale based on a DNA three-way junction (3WJ) fueled by Hg^{2+} binding and sequestration. It is highly responsive to external stimulus, which brings about optically detectable global structural changes. Such a DNA device can serve as a novel "turn-on and -off" fluorescent sensor for Hg^{2+} and biothiols detection with high selectivity and sensitivity.

Mercury is believed as one of the most impactful heavy metal pollutants, it has varied forms (organic, inorganic, metallic or part of some other complexes) and accumulates in human body through food chain. Its overdose is causative of adverse neurological, renal, respiratory, immune, and reproductive disorders. Microbial biomethylation of solvated Hg^{2+} in aquatic ecosystems is highly harzardous, as it ends up with neurotoxic and genotoxic methyl mercury. The threat of Hg^{2+} creates urgent need for routine and facile monitoring it in aqueous solutions and aquatically derived food supplies.[1]

Since Ono group[2] initiated the idea that formation of thymine-Hg^{2+}-thymine complex can be applied for Hg^{2+} sensing, a number of DNA (DNA-dye interaction, DNAzyme, G-quadruplex, molecular beacon or strand displacement reaction)[3] or DNA-conjugates (with nanoparticle, nanorod or nanocluster)[4] based biosensors have been established. Compared with other methods, especially the ones requiring expensive instruments as inductively coupled plasma mass spectrometry (ICP-MS), atomic absorption spectrometry (AAS), and cold vapor atomic fluorescence (FL) spectrometry, they bear a few advantages including low cost, easy handling, satisfactory sensitivity and selectivity as to accomplish on-site detection purposes.[5]

It is dispensable to improve the feasibility of Hg^{2+} detection by introducing novelty of DNA structure design. We tried to construct a DNA three-way junction (3WJ) supramolecular system fueled by Hg^{2+} as depicted in Fig. 1. The 3WJ molecular device is composed of three single-stranded DNA (ssDNA), termed as signal strand (SS), quencher strand (QS) and assistant strand (AS). Each two of those have some hybridisation to form a dsDNA region termed as helical arm. SS and QS has a 3' BHQ1 (black hold quencher 1) and a 5' HEX (hexachlororfluorescein) labels in the end respectively. The close vicinity of HEX to BHQ1 give strongly repressed fluorescence in that HEX is highly quenched by BHQ1. DNA 3WJ motif as one type of forked oligonucleotides, it is found both naturally and synthetically. It usually adopts a Y-shaped structure, and not necessarily being planar. There are also the some nucleobases in the branch point staying unpaired even if for a fully base paired 3WJ. This results in a nanoscale cavity.[6] We assume that in the absence of Hg^{2+}, this 3WJ adopts a reasonably well defined global structure with three helical arms; while in the presence of Hg^{2+}, the metal ions can diffuse into the cavity and mediate the T-Hg^{2+}-T base pairing. This propensity leads to the reformation of the global structure at desirable temperature, in which SS and AS are double-stranded and QS is hence "pushed out", Consequently, the HEX dye is free from quenching, and a "turn-on" fluorescence can be obtained. Furthermore, due to the thiophilic nature of Hg^{2+},[7] it is reasonable to use biothiols including cysteine (Cys) and glutathione (GSH) as models to chelate Hg^{2+} and reset this molecular device to its original state as if a reversible device.

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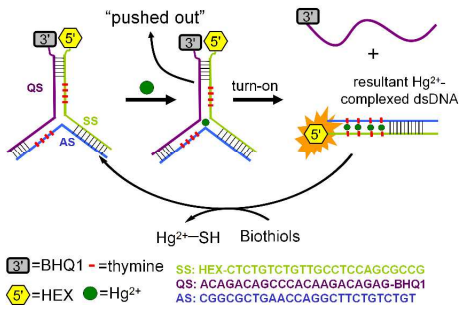

Fig. 1. Working principle and DNA sequences of the Hg^{2+} fueled DNA device. SS and AS are designed to have multiple thymine bases.

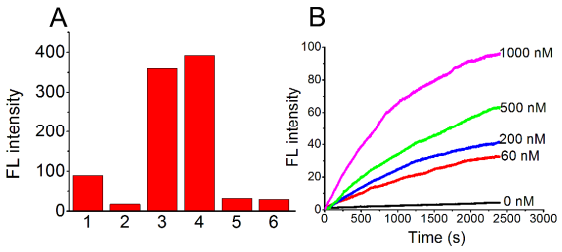

Fig. 2. HEX fluorescence analysis for characterising the DNA 3WJ based molecular device. A depicts the HEX fluorescence in different states. Column 1=SS:QS:AS (1:1:1) without annealing; 2= annealed 3WJ; 3=3WJ+Hg^{2+}; 4=SS strand only; 5=3WJ+Hg^{2+}glutathione; 6=3WJ+Hg^{2+}+cysteine. Please note that the 3WJ used was 10 nM, Hg^{2+}, glutathione and cysteine used were all 500 µM. B shows the real time fluorescence when different concentrations of Hg^{2+} (denoted next to the curves) were added into the 10 nM 3WJ molecular device.

Fig. 2A depicts the analysis of HEX fluorescence in different DNA structures. Simply, compared with physically mixed three ssDNA sample without annealing (column 1), fully annealed 3WJ has a weaker fluorescence as shown in column 2, on account of significant contact quenching effect of BHQ1 to HEX. The observation of column 3 proves that the **"**turn-on**"** effect of Hg^{2+}. In contrast to column 2, it has several-fold enhancement in fluorescence. We also note that the fluorescence intensity for column 3 is merely lower than column 4 (SS strand only, theoretically the one gives the strongest fluorescence, as it is free from quenching). This is indicative of a major structural disturbance upon Hg^{2+} addition. The column 5 and 6 explicate that cysteine and glutathione can effectively restore the fluorescence, indicative of a reformation of 3WJ. As shown in Fig. 2B, this global dynamic change of 3WJ after addition of Hg^{2+} can be reflected in real time *via* the fluorescence of HEX at 555 nm upon excitation of 535 nm (fluorescence spectra are shown in Fig. S1). In the absence of Hg^{2+}, the fluorescence of 3WJ is rather steady and aligns well with baseline; while with the addition of increasing amounts of Hg^{2+}, it can be noticed that the initial velocity of this global structure change mirrored by real time fluorescence mounts accordingly. It is well illustrated that the **"**turn-on**"** process is triggered by Hg^{2+}, showing a concentration dependent manner.

This striking structural change in DNA can be deduced by CD spectra. CD spectroscopy is arguably a reliable approach for diagnosing the conformational polymorphism of nucleic acids. CD measures the interactions of chiral molecules with circularly polarised electromagnetic beams. Spectral CD investigation of DNA is sensitive in the UV region from 180 to 300 nm and it uses ellipticity (expressed in degrees) as output.[8] Though not being able to provide structural information of molecules at atomic level, CD spectroscopy is considered to be used empirically in study of DNA. To be detailed, The CD spectra for a piece of canonial B-form DNA shows a positive and a negative band centered around 275 nm (due to base stacking) and 245 nm (due to right-handed helicity) respectively and meets the baseline around 260 nm. These two bands are also sensitive to the mode of DNA

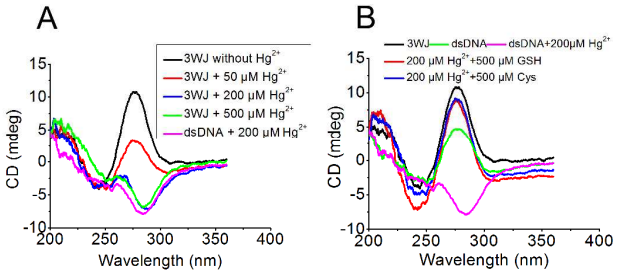

Fig. 3. CD analysis for monitoring the structural changes of the Hg^{2+} fueled DNA device.

interactions with small molecules.[9] As shown in Fig. S2, the three ssDNA components of 3WJ show a good agreement with aforesaid features of DNA. It is also perceptible that 3WJ has a greater positive value in the wavelength around 275 nm in comparation with all three ssDNA, since the helical arm region of 3WJ has stronger base stacking mediated by base pairing than three ssDNA. Taking further, as shown in Fig. 3A, adding ascending amounts of Hg^{2+} from 50 µM to 500 µM (red, blue and green respectively), it has a distinct influence on DNA dynamics as the the positive band around 275 nm declines sharply and even ends up with a negative band peaking at ~280 nm, which indicates a pronounced structural transition. The CD curves of 3WJ with excessive 200 and 500 µM Hg^{2+} (blue and green lines respectively) fairly resemble the resultant 200 µM Hg^{2+}-complexed dsDNA (pink line, sequence is shown in Fig. 1). This implies the disruption of the 3WJ structure and meanwhile the reformation of Hg^{2+} mediated dsDNA. Fig. 3B demonstrates that adding excessive 500 µM glutathione or cysteine into a 200 µM Hg^{2+}-treated 3WJ (red and blue lines respectively) can restore the 3WJ structure (black line) as the CD signals of the formers quite approximate the later.

We next thought to test the utility of this molecular device for Hg^{2+} detection. In order to do so, some factors, which are in correlation with the performance of the fluorescence

measurements, have been investigated in details. The dependence of the fluorescence intensity on the incubation time was studied. A discontinuous assay was used, , as shown in Fig. S3, the initial addition of Hg^{2+} into 3WJ causes rise in fluorescence emission intensity as expected. With increase in incubation time, the intensity enhances gradually. This increase remains until about 20 min and reaches a plateau afterward. Further incubation of the mixture does not cause evident change in fluorescence intensity. While in the absence of Hg^{2+}, when the interaction time (incubation time) is varied from 10 to 90 min, the background signal almost stays unchanged (data not shown). Hence, for the following experiments, the incubation time has been determined to be 30 min. The dependence of the fluorescence intensity on the incubation temperature was also investigated, as shown in Fig. S4. The incubation temperature has a moderate affect on the fluorescence response. The strongest fluorescence intensity of Hg^{2+}- treated 3WJ appears when the temperature is 37°C.

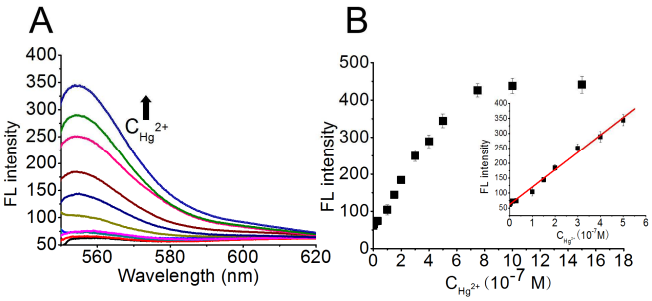

Fig. 4. The detection utility of DNA device for sensing Hg^{2+}. (A) Fluorescence spectra for different concentrations of Hg^{2+}. (B) The dependence of fluorescence on concentration of Hg^{2+} and the linear relationship between the fluorescence intensity and the concentration of Hg^{2+} (inset).

Additionally as shown in Fig. S5, at 37°C the ($F-F_0$) seems to reach maximum value (F and F_0 denote 3WJ solution in the presence and absence of Hg^{2+} respectively), thus we have decided to use 37°C as the incubation temperature. We also find that the linearity between fluorescence intensity and concentration of Hg^{2+} is very sensitive to pH value and have worked out pH 7.4 works well (data not shown).

Upon the completion of the experimental conditions optimisation, the relationship of fluorescence intensity and added Hg^{2+} was tested as shown in Fig. 4, with the incremental amounts of Hg^{2+}, more T-Hg^{2+}-T structures form, which results in more 3WJ changing the global structure. This leads to the gradual enhancement in fluorescence intensity (Fig. 4A). There is a linear relationship (R=0.997) between fluorescence intensity and Hg^{2+} concentration over the range from 5 nM to 500 nM, and a 3.0 nM limit of detection (LOD) for Hg^{2+} analysis is achieved, based on 3σ/slope equation. The Hg^{2+} fueled molecular device can be rescued by biothiols, which gives rise to reduced **"turn-off"** fluorescence. As shown in Fig. 5, a linear relationship of between fluorescence intensity and glutathione (R=0.999) or cysteine (R=0.996) can be seen. This demonstrates that this Hg^{2+} fueled device can also be applied for glutathione and cysteine analysis. Fluorescence intensity *vs*. concentration of glutathione and cysteine in the full range (20-1000 nM) are listed in Fig. S6 and 7 respectively. Besides, millimole level of glutathione has been tested using our system, as shown in Fig. S8.

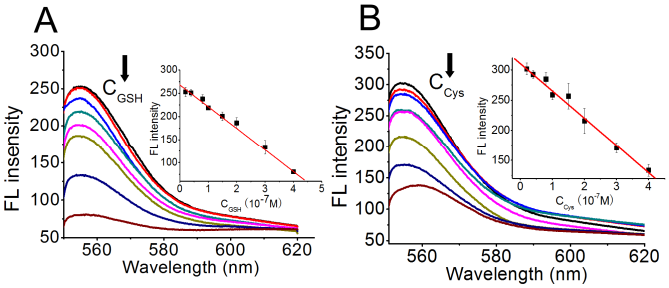

Fig. 5. The detection utility of DNA device for sensing cysteine and glutathione. Fluorescence spectra for sensing different glutathione (A) and cysteine (B), which range from 20 to 400 nM. The linear relationship between the fluorescence and the concentrations of glutathione and cysteine respectively (inset).

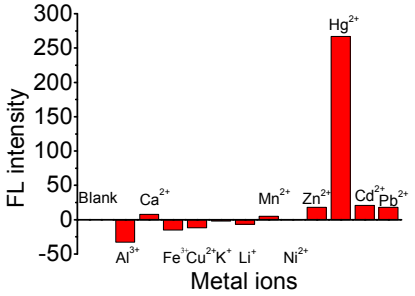

Fig. 6. The selectivity of the DNA device for Hg^{2+} over other common metal ions.

The selectivity of this DNA device is crucial and has been tested. The selectivity of Hg^{2+} was evaluated over some other common metal ions. Fig. 6 presents the histograms of fluorescence intensity in solutions with Hg^{2+} (100 μM) and other metal ions (500 μM). It can be observed that, compared with Hg^{2+}, even if having a five-fold concentration in place, the rest of the metal ions do not interfere the system as the fluorescence intensity is almost undisturbed. It can be concluded that these competing metal ions have only negligible effects on our detection system. This approach exhibits high selectivity for Hg^{2+}, which enables it scope for futuristic use.

Last, the applicability of this system was further explored by testing a real sample. A sample of tap water from Tianjin University of Science and Technology was acquired. The collected sample was filtered. Then the filtered water was spiked with a series of concentrations of Hg^{2+} (5, 10, 30, 300 and 400 nM as final concentration). The analytical results are listed in Tab. S1, the recovery values range from 97.5 to 103.0% and the triplicate measurements for all samples are less than 5.5%. These indicate that this proposed sensor is able to detect Hg^{2+} in aqueous-derived samples.

Conclusions

In summary, we have successfully carried out proof-in-principle experiments for fabricating a conceptually new Hg^{2+} fueled molecular device, which is based on the global conformational change of a forked DNA supramolecular system. This type of spatial change can be fine governed by addition of Hg^{2+} and biothiols, enabling it a useful biosensor for quantifying these agents. People have realised the secondary structure, as a signature for each DNA molecule can be rationally designed in response to a variety of external stimuli. These topologically distinct conformational states can be recorded, amplified and translated to discrete information even in a mutual manner, which could be highly useful to power nanoscale devices or sensing analystes.[10] A convincing example could be protein interfaced DNA have been extensively studied and used in this regard.[11] In diverse ways, DNA topology, likewise, influences molecular devices in certain aspects such as lifetime, recognition and activity.[12] In this work, we adapted this rational, to validate the notion the global structure changes of a forked DNA, irrespective of sequence context, can be harnessed for detection. Our work extend the reach of DNA molecular device fueled by metal ions, compared with previous work of this sort,[13] it couples DNA conformational dynamics with two-channel optical outputs. Its simplicity makes it avoid expensive organic dyes and and complex conjugations in order to give recordable readouts. Moreover, the nature of this molecular device is capable of being responsive to external stimulus, which not only highlighting the importance of metal ion-interacted DNA base pairs[14], but also with huge potential for setting up built-in sensors, DNA based nanomachines and "smart" bio-materials. Because structural controllability, tunability and responsiveness is the key features thereof.[15] A quintessential type of DNA template for building biosensor is molecular beacon which commonly has a stem-loop structural motif with a fluorescence donor and a corresponding acceptor in each end. Molecular beacons have been widely used and documented; however, one disadvantage is that the two ends of nucleic acid are both occupied, which obstructs further engineering by other labels. Also double labels in one ssDNA requires a sophisticated synthetic route and this might lead to loss affinity and specificity, for example, with cognate nucleic acid enzymes.[16] Researchers have always been seeking improved alternatives. DNA 3WJ, in these regards, circumvents the aforementioned drawbacks. Our work have afforded a successful proof-of-principle study of DNA 3WJ in application to bio-sensing, which implies that DNA 3WJ can be readily put into the toolkit for biosensor constructions. Last, Hg^{2+} is environmentally relevant. Biothiols (such as cysteine and glutathione) are biologically important for human; they play a crucial role in life, particularly for some diseases as heart diseases, cancers and *etc*. This device integrates high sensitivity and selectivity with good reversibility for sensing them. The LOD of this molecular device for Hg^{2+} is 3.0 nM, lower than the guideline value of Hg^{2+} (0.006 mg/L, equals to 30 nM) in drinking water recommended by World Health Organization (WHO).[17] Also this LOD and detection range for Hg^{2+} (up to 500 nM) is comparable or better than the most of reported methods, particularly for fluorescent methods (a comparison of this approach with others for Hg^{2+} sensing is listed in Tab. S2). Certainly the other functionality and applicability will be further explored.

Acknowledgements

This work is supported by National Natural Science Foundation of China (No. 81503086), a starting funding (No. 20140520) from Tianjin University of Science & Technology, a research funding of "1000 Talents Plan" of Tianjin (to LM) and Foundation of Key Laboratory of Industrial Fermentation Microbiology of Ministry of Education and Tianjin Key Lab of Industrial Microbiology (No. 2015IM106).

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