

COMMUNICATION

Highly sensitive and selective mercury sensor based on mismatched base pairing with ^{diox}T

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A highly selective and sensitive sensor for mercury was designed based on a new fluorescent nucleobase, ^{diox}T. Its metal-sensing ability was investigated using mismatched ^{diox}T-T and ^{diox}T-C base pairing. The sensor exhibited a high sensitivity (quenching efficiency, 80%, 1:1 binding mode) and selectivity upon the addition of mercury ions.

Heavy metal ions are well known for their involvement in important environmental issues, because of their severe effects on human health.¹ In particular, the highly toxic mercury(II) ions are widespread pollutants that arise from industrial waste materials and a variety of natural sources.^{2–4} Although the mechanisms underlying the cytotoxicity and metabolic damage induced by mercury remain unclear, mercury(II) ions mainly cause several severe health problems, such as kidney failure, brain damage, and heart damage.^{1–4} Since the first report of T-Hg(II)-T binding by Katz and the seminal studies of Ono and coworkers,^{5,6} the knowledge of the binding of mercury ions to T-T mismatched base pairs has led to the rapid development of metal sensors based on DNA. DNA is a very stable and highly programmable biomolecule that can provide a specific metal coordination site in a unique secondary structure. These features make DNA a very attractive material for the generation of metal-sensing devices. In this context, several mercury sensors have been developed based on single-nucleotide polymorphisms,⁷ nanomaterials,^{8,9} DNAzymes,^{10,11} and fluorescent nucleobases.^{12–15} Fluorescent nucleobase analogues are powerful and important tools in the chemical biology of nucleic acids, and their applications have enabled a variety of investigations,^{16–18} such as single-molecule polymorphism,¹⁹ monitoring of structural changes,^{20,21} elucidating enzyme activities,^{22,23} and the development of the orientation-

sensitive FRET system.^{24,25} Another advantage of fluorescent nucleobase analogues compared with conventional dyes, such as Cy3, Alexa dyes, FAM, and Texas Red, is that they are particularly useful to investigate the properties of the local structures of nucleic acids. Despite these advantages, the use of fluorescent nucleobase analogues and of their specific metal-binding interactions in the generation of metal sensors is relatively uncommon. A sensing system based on pyrrolo-dC (PdC) was reported in 2012; it was shown that the mismatched PdC-C base pair can serve as a detector of silver ions.²⁶ A few years later, a thymine-modified fluorescent nucleobase, ^{DMAT}, was reported as a fluorescent mercury detector, as ^{DMAT}-T was able to bind to mercury ions.^{27,28} Recently, it was also reported that the mismatched ^{DMAT}-C base pair selectively binds to mercury and silver ions.²⁹ Although these fluorescent nucleobase analogues exert a quenching effect in the presence of certain metal ions, their quenching efficiencies are not very high in the presence of 1 eq of a specific metal ion. Therefore, the development of a more effective metal-sensing system is warranted.

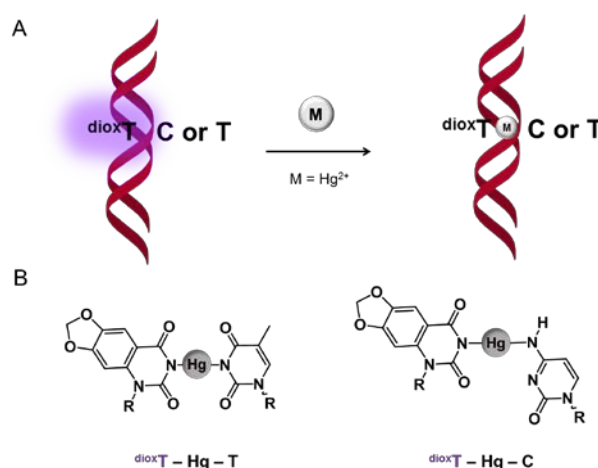


Figure 1. (A) Schematic illustration of the metal sensors based on ^{diox}T-C and ^{diox}T-T mismatched base pairing that are used to detect mercury ions. (B) Metal-mediated base pairing of ^{diox}T-Hg-T and ^{diox}T-Hg-C.

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Very recently, we developed new fluorescent thymine analogues based on the dioxoloquinazoline core, **dioxT**. **dioxT** has desirable biophysical properties such as high brightness ($\Phi_e = 3602 \text{ M}^{-1}\text{cm}^{-1}$) and quantum yield (0.36) as a T surrogate, including complementary base pairing with A. Moreover, **dioxT**-containing DNA displays an excellent quantum yield (~ 0.2) and a remarkable brightness ($\Phi_e = \text{average } 622 \text{ M}^{-1}\text{cm}^{-1}$ in double stranded DNA).³⁰ The notable biophysical and photophysical features of **dioxT** instigated us to develop a fluorescent nucleic-acid-based metal sensor. Herein, we report the development of a highly sensitive and selective mercury sensor using a new fluorescent nucleobase analogue, **dioxT**.

dioxT was incorporated into the center of an 18-mer oligonucleotide (ODN1: 5'-CGTCCGTAATACGCACGC-3', where X = **dioxT**) via automatic solid-phase synthesis. Complementary strands containing matched or mismatched counter bases in the center (ODN1'(Y): 5'-GCGTGCCTATACGGACG-3', where Y = A, T, G, C, and **dioxT**) were also prepared. We envisioned that mismatched base pairs containing **dioxT** can form the metallo base pairs **dioxT**-Hg-C and **dioxT**-Hg-T (Figure 1A).^{5,31-33} The binding of **dioxT**-Hg-T could be characterized based on previous studies; the N3 nitrogen atoms of the **dioxT**-T mismatched base pair coordinate with mercury ions. In the case of **dioxT**-Hg-C, mercury ions coordinate with the N3 of **dioxT** and the N4 of cytosine (Figure 1B).^{27,31}

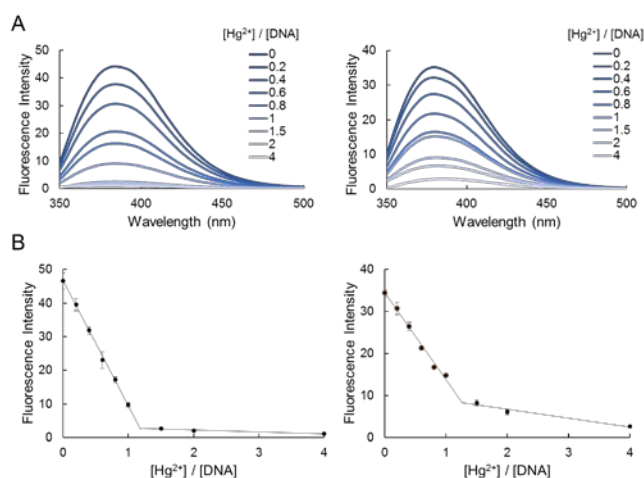


Figure 2. (A) Fluorescence spectra of the DNA-containing mismatched base pairs **dioxT**-T (left) and **dioxT**-C (right) upon titration of HgCl_2 . (B) Plot of fluorescence intensity based on the ratio of $[\text{Hg}^{2+}]$ to $[\text{DNA}]$. ODN1 hybridized with ODN1'(T) or ODN1'(C). All samples contained $2 \mu\text{M}$ DNA and 100 mM NaNO_3 in 10 mM MOPS buffer (pH 7.0). After the metal ions were added, the samples were incubated for 3 h.

To confirm the effect of the mercury ion, we performed steady-state fluorescence measurements. Upon the addition of mercury ions, **dioxT** mismatched with C and T showed high quenching efficiencies ($\sim 70\%$). In particular, a ratio of 1:1 binding between mercury ions and **dioxT**-T yielded a significant quenching efficiency

($\sim 80\%$). This is the highest quenching efficiency to date compared with a previous system with DMAT-Hg-T that showed quenching efficiency 65% .²⁷ Remarkable brightness and great quantum yield³⁰ as well as the highest quenching efficiency indicate the usefulness of **dioxT** as a mercury sensor. Furthermore, the **dioxT**-**dioxT** mismatched base pair also showed a high quenching efficiency (60%), which was not greater than that of the mismatched **dioxT**-T base pair. The **dioxT**-A and **dioxT**-G mismatched base pairs afforded a low quenching efficiency, which was considered as nonspecific binding of mercury to DNA. By gradually increasing the concentration of the Hg^{2+} ion, the fluorescence intensity of both the **dioxT**-T and **dioxT**-C mismatched base pairs decreased significantly between a $[\text{Hg}^{2+}]/[\text{DNA}]$ ratio of 0 and 1. After further addition of 1 eq of Hg^{2+} ion, we observed a low quenching effect, which was caused by nonspecific binding of mercury ions to DNA (Figure S1).

Table 1. Melting temperature (T_m) of unmodified and modified duplexes in the absence and presence of 1 eq Hg^{2+} ions. ODN1 hybridized with ODN1'(Y) (where Y = A, T, G, and C). All sample contained $2 \mu\text{M}$ DNA and 100 mM NaClO_4 in 10 mM MOPS buffer (pH 7.0). The sample was incubated for 3 h after the addition of the metal ions.

Y	X = T		X = dioxT	
	Without Hg^{2+}	With Hg^{2+} (ΔT_m)	Without Hg^{2+}	With Hg^{2+} (ΔT_m)
T	58.4	66.2 (+7.8)	59.8	65.0 (+5.2)
C	56.6	60.1 (+3.5)	58.1	59.7 (+1.6)
A	65.9	66.3 (+0.4)	64.5	64.4 (-0.1)
G	59.2	60.0 (+0.8)	58.8	59.1 (+0.3)

Based on quenching experiment with assumption of 1:1 binding stoichiometry, we calculated equilibrium binding affinities (K_d) (Figure S6). At 25°C , K_d values were obtained as 45 nM for **dioxT**-T, and 106 nM for **dioxT**-C, respectively. In previous report by Luedtke, K_d values of DMAT-T and DMAT-C were 77 nM and 152 nM , respectively.²⁹ The results suggest that higher quenching efficiency of **dioxT**-Hg-T might be attributed to higher binding affinity of **dioxT**.

As decreasing temperature at 5°C , K_d of **dioxT**-T to mercury ion is changed to 127 nM and that of **dioxT**-C is varied to 59 nM . By plotting of ΔG versus temperature from K_d values at 5°C and 25°C , ΔS of **dioxT**-T and **dioxT**-C show 260 Jmol^{-1} and 66 Jmol^{-1} . These results were consistent with previous report on DMAT by Luedtke,²⁹ indicating that **dioxT**-C is less water released from mercury binding to mismatched base pair rather than **dioxT**-T mismatched base pair. We also measured the time-resolved fluorescence of duplexes with **dioxT**-T and **dioxT**-C mismatched base pairs. In absence of mercury ion, both of **dioxT**-C and **dioxT**-T mismatched base pair show biexponential fluorescence decay curves (Figure S8 and Table S1). The short lifetime is attributed by stacking interaction between **dioxT** and nearest neighboring bases and long lifetime comes from unstacked **dioxT**. Upon addition of 1 eq mercury ion, the average lifetime of **dioxT**-T mismatched base pair decrease from 3.47 ns to 2.92 ns , while that of **dioxT**-Hg-C mismatched base pair did not change (2.33 ns). Upon addition of 2 eq mercury ion, both **dioxT**-T and **dioxT**-C mismatched

base pair resulted in decreased average lifetimes (τ_1 and τ_2) and the extended lifetime (τ_3) that can be explained by the high spin-orbit coupling associated with the heavy metal ion.³⁵ These results suggest that dioxT-C mismatched base pair has different metal binding behavior from that of dioxT-T base pair. Further investigation is underway to understand the interaction between mercury ion and dioxT at the molecular level.³⁹

To investigate the stabilization of mismatched base pairs containing dioxT in the presence of mercury ions, we performed melting temperature measurements (Table 1 and Figure S9). The thermal stabilities of duplexes with the A-dioxT or G-dioxT pair were slightly lower than those of unmodified duplexes with A-T or G-T. Regarding the latter, the addition of mercury ions did not affect much the thermal stabilities of the duplexes. Consistent with previous studies, the melting temperature of duplexes containing mismatched base pairs with dioxT (dioxT-C and dioxT-T) was higher than that of unmodified duplexes with a mismatched pair (T-C and T-T) without metal ions. In particular, the T_m values of the dioxT-dioxT mismatched base pair was significantly higher ($T_m = 63.6^\circ\text{C}$) than that of the T-T mismatched base pair ($T_m = 58.4^\circ\text{C}$). This may have been caused by the high stacking property of dioxT . After the addition of mercury ions, the thermal stability of duplexes containing T-T or dioxT-T increased significantly (ΔT_m of T-T = $+7.8^\circ\text{C}$ and ΔT_m of dioxT-T = $+5.2^\circ\text{C}$), as expected. Interestingly, the T_m values of duplexes containing dioxT-dioxT and dioxT-T mismatched base pairs were similar to that of the native T-T mismatched base pair upon the addition of mercury ions. This may be because the coordination of the mercury ion with the N3 of the thymine moiety rearranges the location of dioxT between the nearest neighboring bases, resulting in a thermal stability that is similar to that of native T. Duplexes with T-C or dioxT-C mismatched base pairs also showed a slightly increased thermal stability (ΔT_m of T-C = $+3.5^\circ\text{C}$ and ΔT_m of dioxT-C = $+1.6^\circ\text{C}$) in the presence of mercury ions.

Our T_m results are consistent with those of previous reports that showed that the T-T and T-C mismatched base pairs are highly selective for Hg^{2+} ions.^{32–34} We also performed CD measurements (Figure S10). Upon addition of 1 eq of Hg^{2+} ion, dioxT-T and dioxT-C did not change the global structures of duplexes. This indicates that this sensing system based on a fluorescent nucleobase analogue, dioxT , can be a powerful tool in the investigation of local structural changes without deformation of the global structure.

To verify whether metal-binding selectivity is a useful sensing system, we performed a metal screening using a mismatched base pair containing dioxT-T . The fluorescence of the dioxT-T mismatched base pair indicates the presence of a high quenching efficiency exclusively for the mercury ions; in contrast, an additional nine metal ions, i.e., Ag^+ , Co^{2+} , Ca^{2+} , Cu^{2+} , Mg^{2+} , Ni^{2+} , Zn^{2+} , Cd^{2+} , and Pb^{2+} , displayed no quenching effect. The dioxT-C mismatched base pair also exhibited a high quenching efficiency with mercury ions. Regarding the dioxT-C mismatched base pair, fluorescence quenching was observed in the presence of silver ions, albeit at a lower level ($\sim 30\%$) than that of mercury ions.⁴⁰ Other metal ions, with the exception of mercury and silver ions, did not afford fluorescence quenching for the dioxT-C mismatched base pair. These results indicate that dioxT is very sensitive for the detection of specific metal ions. Furthermore,

the limit of detection (LOD) ($3\sigma/\text{slope}$) was estimated to be ca. 8.4 nM for dioxT-Hg-T and ca. 17.1 nM for dioxT-Hg-C , respectively (Figure S11). These values are comparable to previous mercury sensors based on DNA functionalized chemosensors.^{36–38} It is noteworthy that LOD of dioxT-Hg-T is lower than 10 nM, the allowable value of Hg^{2+} in drinking water by Environmental Protection Agency (EPA) regulation.

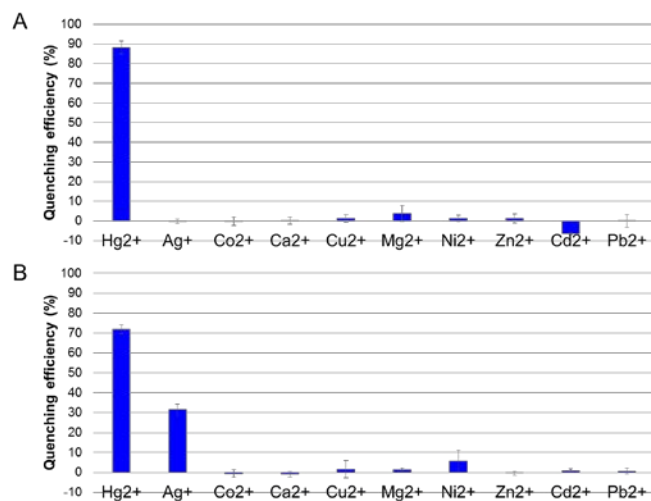


Figure 3. (A) Fluorescence selectivity of the DNA-containing mismatched dioxT-T base pair or (B) mismatched dioxT-C base pair after the addition of various metal ions (2 μM). All samples contained 2 μM DNA and 100 mM NaNO_3 in 10 mM MOPS buffer (pH 7.0). The sample was incubated for 3 h after the addition of the metal ions.

In summary, we report a mercury sensor that was generated using a new size expanded fluorescent nucleobase, dioxT . The dioxT-T and dioxT-C mismatched base pairs induced a strong fluorescence-quenching effect after binding to mercury ions. The results presented here clearly indicate that the dioxT-T and dioxT-C mismatched base pairs in duplexes can serve as a nucleic-acid-based mercury sensor. Moreover, the dioxT-C base pair may be used as a detector of silver ions. To our knowledge, the dioxT -based system has the highest fluorescence-quenching efficiency for mercury ions compared with existing metal sensors using fluorescent nucleobases reported to date. Furthermore, we believe that this system can provide deeper insights for the study of the kinetics and dynamics of nucleic acids that are affected by specific metal ions, such as the Hg^{2+} ion.

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- 40 Unlike mercury ion, fluorescence intensity decreased significantly after addition of over 2 eq of silver ion (Figure S3). The fluorescence of duplexes with ^{diox}T-A matched base pair did not change by silver ion (Figure S4), suggesting that non-specific binding of silver ion is probably not main cause of fluorescence quenching in the presence of greater than 1 eq of Ag(I) ion. CD spectra of ^{diox}T-C mismatched base pair upon the titration of silver ion showed the decreased magnitude at 255 nm with red-shifted the highest positive band from 280 nm to 285 nm (Figure S5). These results suggest that excess amount of silver ion induce local structure change of DNA and this can affect fluorescence intensity of ^{diox}T-C. Further investigation is underway to understand the different quenching efficiency between mercury ion and silver ion.