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DNA Polymerase Inhibition by High Kinetic Stability of T-Hg^{II}-T Base Pairs#

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§SCS-Metrohm Award for the best oral presentation in Medicinal Chemistry & Chemical Biology

Abstract: A fluorescent surrogate of thymidine called ^{DMA}**T** was used for the first fluorescence-based study of Hg^{II} binding to discrete T-T sites in duplex DNA. The fluorescent properties of ^{DMA}**T**-A base pairs were highly sensitive to wild-type T-Hg^{II}-T base pair formation at an adjacent site, allowing for a determination of the precise thermodynamic and kinetic parameters of these metal binding reactions. T-Hg^{II}-T complexes exhibited equilibrium dissociation constants of $K_d \approx$ 8–50 nM. These high-affinity binding interactions are characterized by very slow association and dissociation kinetics ($k_{on} \approx 10^4 - 10^5\ M^{-1}s^{-1}$, $k_{off} \approx 10^{-4} - 10^{-3}\ s^{-1}$), revealing exceptionally high kinetic stabilities of T-Hg^{II}-T base pairs (half-lives = 0.3–1.3 h). Duplex DNA containing ^{DMA}**T**-A and no T-T mismatch exhibited nonspecific Hg^{II} binding affinities of $K_d \approx$ 2.0 μM. The high kinetic stabilities of T-Hg^{II}-T resulted in the inhibition of dynamic processes such as DNA strand invasion and strand displacement during enzymatic DNA synthesis, which led to premature chain termination. These results demonstrated that T-Hg^{II}-T base pairs are kinetically distinct from T-A base pairs and therefore are likely to disrupt DNA metabolism *in vivo*.

Keywords: Base pair · DNA · Enzymatic catalysis · Fluorescence · Transition metal

Olivia P. Schmidt *was born and raised in Zurich (Switzerland). She studied at the University of Zurich, obtaining her Bachelor's degree in Chemistry with a minor in Biochemistry in 2011, and her Master's degree in Chemistry in 2013. Olivia is currently conducting her PhD studies in the group of Prof. Dr. Nathan W. Luedtke at the University of Zurich, where she is working on the biophysical evaluation of DNA-metal ion interactions using fluorescent nucleobase analogs.*

The interaction of Hg^{II} and DNA has been of great interest due to the infamous cytotoxic and mutagenic activities of Hg^{II}.[1] In addition to glutathione depletion and oxidative stress,[2] Hg^{II} also causes DNA point mutations,[3] DNA strand breaks,[4,5] inhibition of DNA synthesis and DNA repair in live cells,[5,6] possibly as a result of mercury–DNA binding interactions.[7] High-affinity mercury binding sites occur in cellular DNA with a frequency of about 0.3% of base pairs upon exposure of 5 μM Hg^{II} to live cells.[7] However, little is known about their exact composition or structure. The study of Hg^{II}–DNA binding *in vitro* was pioneered in the 1950s by Katz.[8] Studies by several research groups revealed that Hg^{II} preferentially binds to *N1* or *N7* of purines and to *N3* of thymidine residues.[9] Hg^{II} stoichiometrically binds to T-T mismatches in double-stranded DNA *in vitro* to give duplexes with thermal stabilities comparable to duplexes containing T-A base pairs.[10] Utilizing ^{1}H-NMR and ^{15}N-NMR spectroscopy, the preferred binding site for Hg^{II} was found to be composed of the *N3* positions of two deprotonated thymidine residues to give a 'T-Hg^{II}-T' base pair (Fig. 1).[11] The structural similarity between T-Hg^{II}-T and the canonical T-A base pair was revealed in a crystal structure of a duplex containing two consecutive T-Hg^{II}-T base pairs, which showed minimal distortion of the B-form duplex DNA.[12] As such, T-Hg^{II}-T can serve as a functional surrogate of T-A base pairs in primer hybridization[13] and by causing misincorporation of dTTP across from thymidine during enzymatic DNA primer extension to give T-Hg^{II}-T base pairs *in vitro* and possibly *in vivo*.[14]

A wide variety of spectroscopic methods have been utilized to detect and characterize T-Hg^{II}-T base pairs in duplex DNA. These include UV,[10,15] Raman,[16] CD,[17] ITC,[18] NMR,[19] EPR,[19c] and fluorescence.[20] With the exception of high resolution structural analysis, these studies provided information regarding changes in the global properties of each system and therefore mostly qualitative information regarding Hg^{II} binding. Despite broad attention and notable progress, there are no reports of the exact thermodynamic and

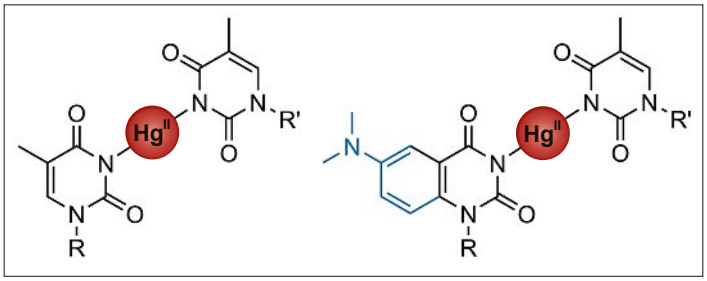

Fig. 1. T-Hg^{II}-T base pair (left) and ^{DMA}**T**-Hg^{II}-T base pair (right).

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kinetic parameters of T-Hg^{II}-T base pair formation at discrete sites in duplex DNA. These kinetic parameters could govern a wide variety of biochemical processes involving DNA metabolism and are important for understanding the potential impact on dynamic processes involving DNA.

With excellent spatial and temporal resolution, fluorescent nucleobase analogs constitute an important family of fluorescent probes and can facilitate highly sensitive biophysical measurements.[21] There are only a small handful of previous studies that have utilized fluorescent nucleobase analogs to probe transition metal binding of DNA,[22] and there are no examples of their use to characterize wild-type, site-specific metal-nucleobase binding interactions. To determine the kinetic and thermodynamic properties of native, site-specific T-Hg^{II}-T binding interactions we synthesized a new fluorescent thymidine analog '*N,N*-dimethylaniline-2'-deoxythymidine' (^{DMA}**T**) (Fig. 1).[23]

^{DMA}**T** exhibits fluorescence properties that are highly sensitive to the local environment, and it has the same pK_a and Watson-Crick base pairing face as native thymidine. DNA oligonucleotides containing a single ^{DMA}**T** at variable positions '**X**' were synthesized using standard phosphoramidite chemistry by automated DNA synthesis (Fig. 2).[23] Circular dichroism (CD) and thermal denaturation experiments revealed that ^{DMA}**T**-A and ^{DMA}**T**-Hg^{II}-T-containing duplexes exhibited the same global structures and thermal stabilities as wild-type sequences containing T-A or T-Hg^{II}-T base pairs.[23] ^{DMA}**T** fluorescence quenching was used to track Hg^{II} binding of duplexes containing a single ^{DMA}**T**-T mismatch at position **X13** or **X15** (Fig. 2). Our results revealed that ^{DMA}**T**-T mismatches have the same metal binding properties as native T-T mismatches thereby providing the first example of a fluorescent nucleobase analog used as a probe for site-specific binding between DNA and Hg^{II}.[23]

To determine the thermodynamic parameters of T-Hg^{II}-T formation, equilibrium titration experiments were performed using dilute solutions of duplex DNA (25 nM) containing a ^{DMA}**T**-T mismatch at position **X13** (Fig. 2). Alternatively, Hg^{II} was titrated into a solution of duplex DNA containing a ^{DMA}**T**-A base pair at position **X13** to determine the non-specific DNA binding affinity of Hg^{II}. The DNA solutions were equilibrated with variable concentrations of Hg^{II} for 1 h at 25 °C and the fluorescence intensity was measured (Fig. 3a). The decreases in fluorescence intensity were plotted against Hg^{II} concentration and fit to a monoexponential equation from which the binding affinity (K_d) was determined (Fig. 3b). Surprisingly, ^{DMA}**T**-T mismatch-containing duplex DNA exhibited a very high Hg^{II} binding affinity with a K_d = 77 ± 4 nM (Fig. 3c).[24] In contrast, duplex DNA containing a ^{DMA}**T**-A base pair and no T-T mismatch exhibited a 26-fold lower, apparent affinity (K_d = 1.97 ± 0.08 μM), reflecting non-specific binding. To evaluate whether ^{DMA}**T** can be utilized as a probe for formation of a wild-type T-Hg^{II}-T base pair, duplex DNA was prepared containing a T-T mismatch at a neighboring site to a ^{DMA}**T**-A base pair ('**X15** ^{DMA}**T**-A, X16 T-T', Fig. 2). Upon addition of Hg^{II} the same concentration-dependent fluorescence quenching (apparent K_d = 57 ± 7 nM) was observed as for duplex '**X13** ^{DMA}**T**-T' (Fig. 3c), suggesting that a ^{DMA}**T**-A base pair can serve as a reporter for the formation of a neighboring, wild-type T-Hg^{II}-T base pair with little to no impact on the affinity of the reaction. These results showed that ^{DMA}**T** can be utilized to derive T-T mismatch specific and non-specific binding affinities of Hg^{II} to DNA.

To determine Hg^{II} association rates to duplexes containing a single ^{DMA}**T**-T mismatch at position **X13** or **X15**, time-dependent fluorescence quenching of ^{DMA}**T** was measured upon addition of Hg^{II}. Alternatively, a wild-type T-T mismatch was placed adjacent to a ^{DMA}**T**-A base pair in '**X15** ^{DMA}**T**-A, **X16** T-T' (Fig. 2). Control experiments using duplexes containing a ^{DMA}**T**-A base pair but lacking a T-T mismatch revealed that non-specific fluorescence quenching had little or no impact on the reported reaction rates. Using pseudo-first-order approximations, association rate constants (k_{on}) were determined by addition of 2, 4, and 6 equiv. of Hg^{II} (Fig. 4a). For '**X13** ^{DMA}**T**-T' and '**X15** ^{DMA}**T**-T' similar k_{on} values of 0.8 x 10^4 M^{-1}s^{-1} and 1.9 x 10^4 M^{-1}s^{-1} were obtained (Table 1), which were about 10^5-fold lower than those reported for outer-sphere binding of divalent ions to polynucleotides.[25] The probe itself had little or no impact on k_{on}, because '**X15** ^{DMA}**T**-A, **X16** T-T' gave a similar k_{on} of 9.0 x 10^4 M^{-1}s^{-1} (Table 1).

To measure the rate constants of Hg^{II} dissociation (k_{off}), '**X13** ^{DMA}**T**-T', '**X15** ^{DMA}**T**-T', and '**X15** ^{DMA}**T**-A, **X16** T-T' were incubated with 2 equiv. of Hg^{II} for 3 h to generate duplexes containing a ^{DMA}**T**-Hg^{II}-T or T-Hg^{II}-T base pair. A large excess

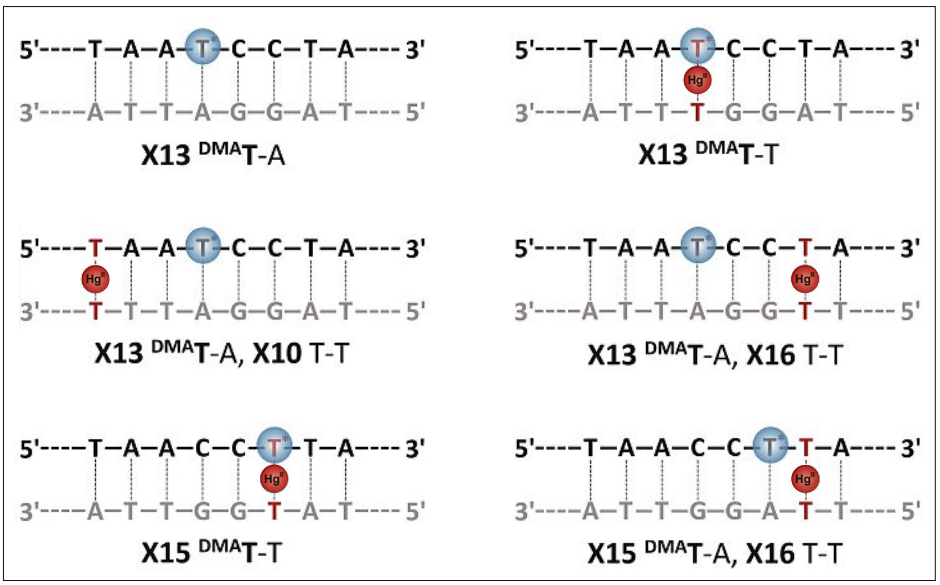

Fig. 2. Variable regions (underlined) and names of ^{DMA}**T**-containing duplex DNA used in these studies: **X13**: 5'-CCC-TAA-CCC-TAA-**X**CC-TAA-CCC-3'; **X15**: 5'-CCC-TAA-CCC-TAA-CC**X**-TAA-CCC-3'; where **X** = T or T* (^{DMA}**T**).

Table 1. Rate constants of association (k_{on}), dissociation (k_{off}), and calculated dissociation constants (K_d) of ^{DMA}**T**-Hg^{II}-T or T-Hg^{II}-T base pairs in duplex DNA.[a]

sequence	k_{on} [M^{-1}s^{-1}]	k_{off} [s^{-1}][b]	K_d [nM][c]
X13 ^{DMA}**T**-T	0.8 ± 0.2 x 10^4	4.0 ± 0.5 x 10^{-4}	50 ± 14
X15 ^{DMA}**T**-T	1.9 ± 0.1 x 10^4	1.5 ± 0.2 x 10^{-4}	8.0 ± 1.1
X15 ^{DMA}**T**-A, **X16** T-T	9.0 ± 2.0 x 10^4	9.0 ± 4.0 x 10^{-4}	10 ± 5.0

[a]Each value represents an average of three independent measurements ± standard deviation. All measurements were performed in aqueous buffer (200 mM Na_2HPO_4, 100 mM citric acid, and 100 mM $NaNO_3$ (pH = 7.35)). [b]50 equiv. of scavenger DNA containing a T-T mismatch were added to determine dissociation rate constants. [c]The K_d values were calculated as $K_d = k_{off} / k_{on}$.

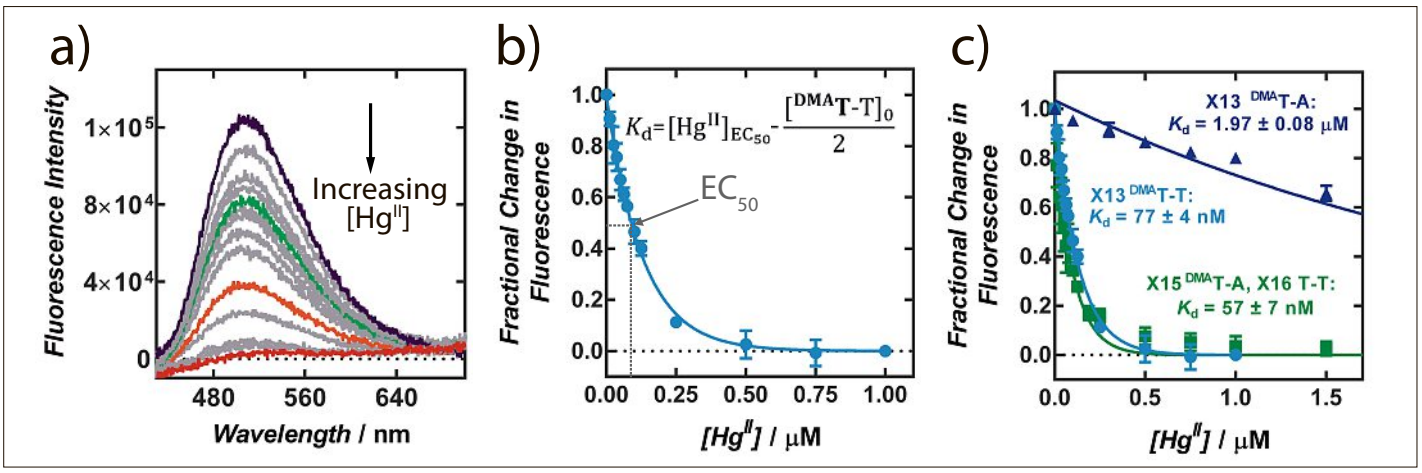

Fig. 3. a) Fluorescence quenching (λ_{ex} = 370 nm) of '**X13** ^{DMA}**T**-T' upon addition of variable equiv. of Hg^{II}. b) Plot of normalized fluorescence intensity (λ_{em} = 500 nm) versus concentration of Hg^{II}, from which the EC_{50} and K_d values were determined. c) Normalized changes in fluorescence of '**X13** ^{DMA}**T**-A' (triangles), '**X13** ^{DMA}**T**-T' (circles), and '**X15** ^{DMA}**T**-A, **X16** T-T' (squares) upon addition of Hg^{II}. All DNA samples contained 25 nM of DNA in aqueous buffer (200 mM Na_2HPO_4, 100 mM citric acid and 100 mM $NaNO_3$ (pH = 7.35)) and were incubated with $Hg(ClO_4)_2$ for 1 h at 25 °C prior to reading.

of nonfluorescent duplex DNA containing a T-T mismatch was added as a passive Hg^{II} scavenger, and the resulting increase in fluorescence intensity was measured as a function of time (Fig. 4b). To obtain a concentration-independent, first order dissociation curve, 40 equiv. of the scavenger DNA were needed. From the monophasic fit of the data, half-lives of dissociation ($t_{1/2}$) were obtained, from which the k_{off} could be calculated (Fig. 4b). For all three duplexes similar $t_{1/2}$ values of 0.3–1.3 h were obtained giving k_{off} values ranging from 1.5–9.0 x 10^{-4} s^{-1} (Table 1). The combination of slow on-rates with extremely slow off-rates resulted in high binding affinities of K_d = 8–50 nM, which are in excellent agreement with the K_d values determined by the equilibrium titrations.

Most biochemical processes occur at rates of microseconds to seconds. To evaluate whether the exceptionally high kinetic stabilities of T-Hg^{II}-T base pairs ($t_{1/2}$ = 0.3–1.3 h) could thereby pose significant barriers to dynamic processes involving DNA, strand displacement was used as a model reaction. Duplexes containing a short, single-stranded overhang (red, Fig. 5a) and a single ^{DMA}**T**-Hg^{II}-T were prepared. Alternatively, a wildtype T-Hg^{II}-T was placed three base pairs upstream or downstream from a ^{DMA}**T**-A base pair. Addition of excess unlabeled invading strand 'I' initiated displacement of the ^{DMA}**T**-containing strand to give a longer duplex DNA as the product, which is thermodynamically more stable. ^{DMA}**T** exhibits a two-fold higher quantum yield in duplex DNA as compared to single-stranded DNA.[23] As such, rates of strand displacement were determined by monitoring changes in ^{DMA}**T** fluorescence in real time. Using four different concentrations of invading strand 'I', rate constants of strand displacement (k) were determined using pseudo-first-order

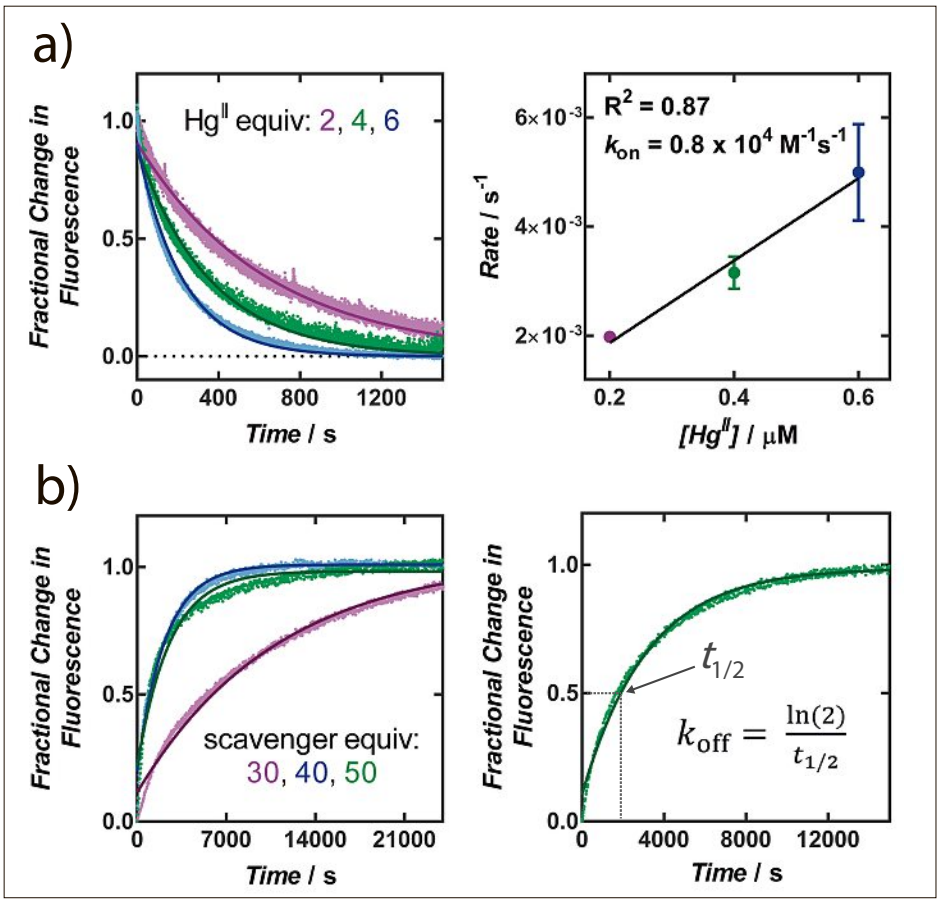

Fig. 4. a) Association rates determined by fluorescence quenching of '**X13** ^{DMA}**T**-T' upon addition of Hg^{II} (left). Association rate constants (k_{on}) were calculated from the slope of the association rate versus Hg^{II} concentration (right). b) Dissociation rate constants according to fluorescence changes upon addition of excess of scavenger DNA containing a T-T mismatch. Duplexes were incubated with 2 equiv. of $Hg(ClO_4)_2$ for 3 h at 25 °C to generate ^{DMA}**T**-Hg^{II}-T or T-Hg^{II}-T base pairs. Rate constant of dissociation was calculated as $k_{off} = \ln(2)/t_{1/2}$. Samples contained 0.1 μM of DNA for k_{on} and 4 μM of DNA for k_{off} measurements.

approximations. The rate constants for duplexes obtained in the absence of Hg^{II} were all in a similar range of k = 29–247 $M^{-1}s^{-1}$ with half-lives of 1.5–33 min (Table 2, Fig. 5b). Duplexes containing a single ^{DMA}**T**-Hg^{II}-T or T-Hg^{II}-T base pair, however, exhibited 100- to 2000-fold lower rate constants, ranging from k = 0.05–0.47 $M^{-1}s^{-1}$ with half-lives of 10–77 h (Table 2, Fig. 5b). Unspecific Hg^{II}-DNA binding did not influence the strand-displacement kinetics as control experiments with duplexes lack-

ing a ^{DMA}**T**-T or T-T mismatch had the same rate constants in the presence and absence of Hg^{II} (Table 2, Fig. 5b). These results demonstrated that the exceptionally high kinetic stabilities of T-Hg^{II}-T base pairs can pose a large barrier to passive DNA–DNA strand-displacement reactions.

To evaluate the ability of T-Hg^{II}-T base pairs to inhibit energy-dependent DNA metabolism their impact on enzymatic DNA synthesis was investigated using primer extension assays. The activity of the DNA polymerase 'Klenow Fragment' was investigated. This enzyme possesses a DNA polymerase domain, but lacks any exonuclease activity (*exo*-). Two nicked duplex DNA constructs were used, either containing a T-T or T-A base pair at the first position (ODN1) or the seventh position (ODN2) of the non-template strand (Fig. 6a). The Klenow Fragment binds to the nick site and displaces the non-template (displaced) strand during DNA synthesis (arrow, Fig. 6a). ODN1 and ODN2 were incubated with variable concentrations of Hg^{II} for 3 h, prior to addition of dNTP's and Klenow Fragment. At different time points, aliquots of the reaction mixture were removed and quenched by addition of EDTA. The progress of enzymatic DNA synthesis was analyzed by denaturing polyacrylamide gel electrophoreses (Fig. 6b). Comparing rates of enzymatic DNA synthesis of duplexes containing T-T versus T-A revealed that Hg^{II} caused both specific and nonspecific inhibition of primer extension. In the absence of Hg^{II}, DNA synthesis for ODN1 containing T-T mismatch was 2.7-fold faster than the duplex containing T-A (Table 3). Upon addition of Hg^{II} (5–10 μM) little to no change in the rate of DNA synthesis (k_{obs}) was observed for the duplex containing a T-A base pair. In contrast, a 7 to 13-fold decrease in the k_{obs} was observed for the duplex containing a T-Hg^{II}-T base pair (Table 3). For ODN2 a specific inhibition of DNA synthesis was observed and a pronounced stalling of the Klenow Fragment occurred at the site of the T-Hg^{II}-T base pair (Table 3, Fig. 6b). These results demonstrated that the high kinetic stabilities of T-Hg^{II}-T base pairs pose a large barrier to enzymatic DNA synthesis *in vitro*. Interestingly, this occurred over the same Hg^{II} concentration range as reported previously to inhibit DNA synthesis *in vivo*.[5]

Here we reported the use of a novel fluorescence-based assay to study site-specific T-Hg^{II}-T binding reactions by incorporating a non-perturbing fluorescent ^{DMA}**T**-T mismatch or a ^{DMA}**T**-A adjacent to a T-T mismatch in duplex DNA. Quenching of ^{DMA}**T** fluorescence by Hg^{II} provided a highly sensitive, site-specific probe of T-Hg^{II}-T formation in real time that was used to determine the kinetic and thermodynamic parameters of site-specific T-Hg^{II}-T binding in duplex DNA. Previous studies have demonstrated that T-Hg^{II}-T can serve as a functional surrogate of T-A by stabilizing T-T during primer extension and by the enzymatic misincorporation of dTTP across from thymidine in the presence of Hg^{II} to give T-Hg^{II}-T in the new duplex. In contrast, our results demonstrated that T-Hg^{II}-T base pairs are kinetically distinct from T-A base pairs. Consistent with the formation and breakage of partially-covalent bonds, T-Hg^{II}-T base pairs exhibited slow on- and off-rates. Equilibrium dissociation constants obtained by kinetic and thermodynamic analyses were in excellent agreement giving K_d values ranging between 8–77 nM. The exceptionally high kinetic stabilities of T-Hg^{II}-T might impact a wide variety of metabolic processes. Here we demonstrated the inhibitory effects of T-Hg^{II}-T on DNA strand displacement and enzymatic synthesis reactions. The inhibition and premature termination of DNA synthesis offers a potential mechanism for DNA strand breaks that are reported to occur in living cells treated with Hg^{II}.[4,5] Other dynamic processes including transcription, translation and DNA repair could also be impacted by T-Hg^{II}-T base pairs in DNA and/or RNA. These effects could explain some of the cytotoxic and mutagenic activities associated with Hg^{II} exposure.

Table 2. Rate constants k ($M^{-1}s^{-1}$) of DNA-DNA strand-displacement reactions in the absence or presence of Hg^{II}.[a]

initial duplex	k [$M^{-1}s^{-1}$], no Hg^{II}	k [$M^{-1}s^{-1}$], with Hg^{II}
X13 ^{DMA}**T**-A	29 ± 3	22 ± 3[b]
X13 ^{DMA}**T**-T	97 ± 12	0.05 ± 0.01
X13 ^{DMA}**T**-A, **X10** T-T	55 ± 15	0.47 ± 0.03
X13 ^{DMA}**T**-A, **X16** T-T	247 ± 16	0.21 ± 0.06

[a]Each value represents an average of three independent measurements ± standard deviation. All samples contained 4 μM of DNA. [b]Estimated from a single concentration of added invading strand I.

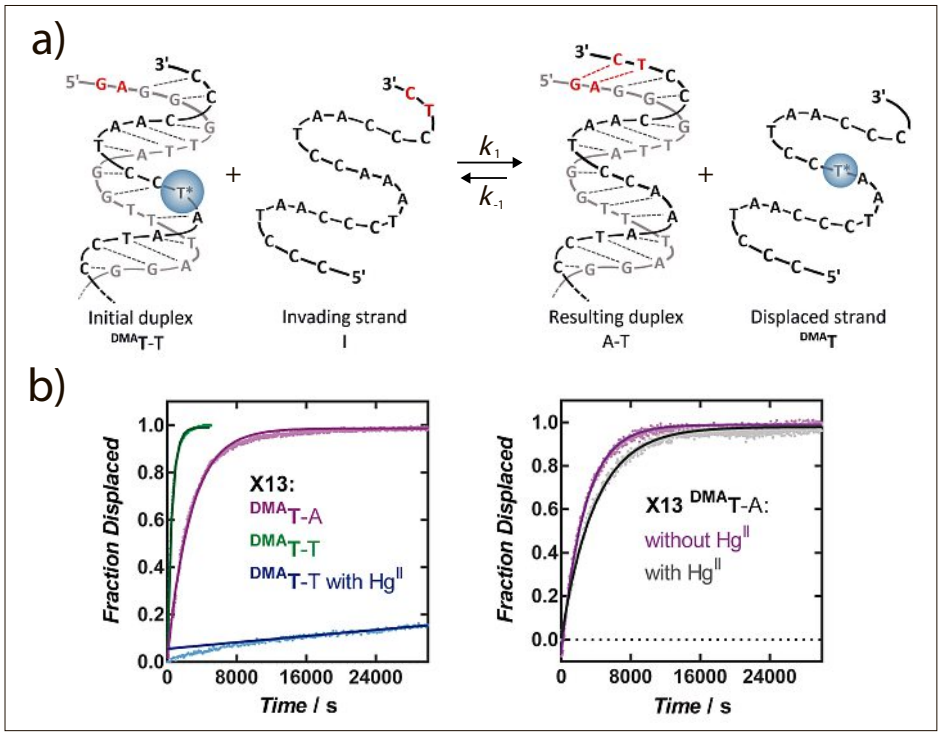

Fig. 5. a) Representative DNA–DNA strand-displacement reaction with **X13** ^{DMA}**T**-T, where T* = ^{DMA}**T**. b) Strand displacement of **X13** containing a ^{DMA}**T**-A base pair (purple) or a ^{DMA}**T**-T mismatch in the absence (green) or presence (blue) of Hg^{II} (left). Strand displacement of **X13** ^{DMA}**T**-A in the absence (purple) or presence (grey) of Hg^{II} (right).

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Table 3. Observed rates (k_{obs}) of primer extension by Klenow Fragment (*exo*-) of ODN1 or ODN2.[a]

Hg^{II} [μM]	'Y'	ODN1 k_{obs} [min^{-1}]	ODN1 k_{obs} rel	ODN2 k_{obs} [min^{-1}]	ODN2 k_{obs} rel
0	A	0.18 ± 0.05	1.0	0.28 ± 0.04	1.0
	T	0.48 ± 0.12	1.0	0.25 ± 0.03	1.0
5	A	0.23 ± 0.07	1.3	0.29 ± 0.04	1.04
	T	0.07 ± 0.01	0.15	0.17 ± 0.03	0.68
10	A	0.14 ± 0.05	0.8	0.18 ± 0.03	0.64
	T	0.038 ± 0.002	0.08	0.066 ± 0.009	0.26

[a] 'k_{obs} rel' = k_{obs} (X μM Hg) / k_{obs} (0 μM Hg), where X = 5, 10

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[1] J.-D. Park, W. Zheng, *J. Prev. Med. Public Heal.* **2012**, *45*, 344.
[2] D. A. Monteiro, F. T. Rantin, A. L.Kalinin, *Ecotoxicology* **2010**, *19*, 105.
[3] a) J. C. Codina, C. Pérez-Torrente, A. Pérez-Garcia, F. M. Cazorla, A. deVicente, *Arch. Environ. Contam. Toxicol.* **1995**, *29*, 260; b) M. E. Ariza, M. V. Williams, *J. Biochem. Mol. Toxicol.* **1999**, *13*, 107; c) F. Schurz, M. Sabater-Vilar, J. Fink-Gremmels, *Mutagenesis* **2000**, *15*, 525
[4] O. Cantoni, R. M. Evans, M. Costa, *Biochem. Biophys. Res. Commun.* **1982**, *108*, 614.
[5] M. V. Williams, T. Winters, K. S. Waddell, *Mol. Pharmacol.* **1987**, *31*, 200.
[6] N. T. Christie, O. Cantoni, M. Sugiyama, F. Cattabeni, M. Costa, *Mol. Pharmacol.* **1986**, *29*, 173.
[7] O. Cantoni, N. T. Christie, A. Swann, D. B. Drath, M. Costa, *Mol. Pharmacol.* **1984**, *26*, 360.
[8] S. Katz, *J. Am. Chem. Soc.* **1952**, *9*, 2238.
[9] a) S. Katz, *Biochim. Biophys. Acta, Spec. Sect. Nucleic Acids Relat. Subj.* **1963**, *68*, 240; b) G. L. Eichhorn, P. Clark, *J. Am. Chem. Soc.* **1963**, *85*, 4020; c) R. B. Simpson, *J. Am. Chem. Soc.* **1964**, *86*, 2059; d) G. W. Buchanan, J. B. Stothers, *Can. J. Chem.* **1982**, *60*, 787; e) M. Polak, J. Plavec, *Eur. J. Inorg. Chem.* **1999**, *1999*, 547.
[10] Y. Miyake, H. Togashi, M. Tashiro, H. Yamaguchi, S. Oda, M. Kudo, Y. Tanaka, Y. Kondo, R. Sawa, T. Fujimoto, T. Machinami, A. Ono, *J. Am. Chem. Soc.* **2006**, *128*, 2172.
[11] Y. Tanaka, S. Oda, H. Yamaguchi, Y. Kondo, C. Kojima, A. Ono, *J. Am. Chem. Soc.* **2007**, *129*, 244.
[12] J. Kondo, T. Yamada, C. Hirose, I. Okamoto, Y. Tanaka, A. Ono, *Angew. Chem. Int. Ed.* **2014**, *53*, 2385.
[13] K. S. Park, C. Jung, H. G. Park, *Angew. Chem. Int. Ed.* **2010**, *49*, 9757.
[14] H. Urata, E. Yamaguchi, T. Funai, Y. Matsumura, S.-I. Wada, *Angew. Chem. Int. Ed.* **2010**, *49*, 6516.
[15] a) D. W. Gruenwedel, M. K. Cruikshank, G. M. Smith, *J. Inorg. Biochem.* **1993**, *52*, 251; b) D. W. Gruenwedel, *Biophys. Chem.* **1994**, *52*, 115; c) Y. Tanaka, H. Yamaguchi, S. Oda, Y. Kondo, M. Nomura, C. Kojima, A. Ono, *Nucleosides, Nucleotides Nucleic Acids* **2006**, *25*, 613.
[16] a) R. W. Chrisman, S. Mansy, H. J. Peresie, A. Ranade, T. A. Berg, R. S. Tobias, *Bioinorg. Chem.* **1977**, *7*, 245; b) T. Uchiyama, T. Miura, H. Takeuchi, T. Dairaku, T. Komuro, T. Kawamura, Y. Kondo, L. Benda, V. Sychrovsky, P. Bour, I. Okamoto, A. Ono, Y. Tanaka, *Nucleic Acids Res.* **2012**, *40*, 5766.
[17] a) D. W. Gruenwedel, *J. Inorg. Biochem.* **1994**, *56*, 201; b) Z. Kuklenyik, L. G. Marzilli, *Inorg. Chem.* **1996**, *35*, 5654.
[18] a) H. Torigoe, A. Ono, T. Kozasa, *Chem.-Eur. J.* **2010**, *16*, 13218; b) H. Torigoe, Y. Miyakawa, A. Ono, T. Kozasa, *Thermochim. Acta,* **2012**, *532*, 28.
[19] a) Y. Tanaka, A. Ono, *Dalton Trans.* **2008**, 4965; b) T. Dairaku, K. Furuita, H. Sato, J. Šebera, D. Yamanaka, H. Otaki, S. Kikkawa, Y. Kondo, R. Katahira, F. M. Bickelhaupt, C. F. Guerra, A. Ono, V. Sychrovský, C. Kojima, Y. Tanaka, *Chem. Commun.* **2015**, *51*, 8488; c) U. Jakobsen, S. A. Shelke, S. Vogel, S. T. Sigurdsson, *J. Am. Chem. Soc.* **2010**, *132*, 10424.
[20] a) A. Ono, H. Togashi, *Angew. Chem. Int. Ed.* **2004**, *43*, 4300; b) C.-W. Liu, Y.-T. Hsieh, C.-C. Huang, Z.-H. Lin, H.-T. Chang, *Chem. Commun.* **2008**, 2242; c) J. Wang, B. Liu, *Chem. Commun.* **2008**, 4759; d) X. Xue, F. Wang, X. Liu, *J. Am. Chem. Soc.* **2008**, *130*, 3244; e) R. Yang, J. Jin, L. Long, Y. Wang, H. Wang, W. Tan, *Chem. Commun.* **2009**, 322.
[21] For review articles see: A. Okamoto, Y. Saito, I. Saito, *J. Photochem. Photobiol., C* **2005**, *6*, 108; b) R. W. Sinkeldam, N. J. Greco, Y. Tor, *Chem. Rev.* **2010**, *110*, 2579; c) L. M. Wilhelmsson, *Q. Rev. Biophys.* **2010**, *43*, 159; d) A. A. Tanpure, M. G. Pawar, S. G. Srivatsan, *Isr. J. Chem.* **2013**, *53*, 366; e) A. C. Jones, R. K. Neely, *Q. Rev. Biophys.* **2015**, *48*, 244. f) A. Matarazzo, R. H. E. Hudson, *Tetrahedron* **2015**, *71*, 1627.
[22] a) S. J. Kim, E. T. Kool, *J. Am. Chem. Soc.* **2006**, *128*, 6164; b) A. Dumas, N. W. Luedtke, *Chem.-Eur. J.* **2012**, *18*, 245; c) A. Omumi, C. K. McLaughlin, D. Ben-Israel, R. A. Manderville, *J. Phys. Chem. B* **2012**, *116*, 6158; d) S. K. Jana, X. Guo, H. Mei, F. Seela, *Chem. Commun.* **2015**, *51*, 17301.
[23] G. Mata, O. P. Schmidt, N. W. Luedtke, *Chem. Commun.* **2016**, *52*, 4718.
[24] A previous ITC-based study reported a much lower affinity of T-Hg^{II}-T (K_d ~ 2 μM)[18], probably due to high DNA concentrations (40 μM), short equilibration times, and excess Hg^{II} used in their experiments.
[25] a) D. Pörschke, *Biophys. Chem.* **1976**, *4*, 383; b) J. Granot, J. Feigon, D. R. Kearns, *Biopolymers* **1982**, *21*, 181.

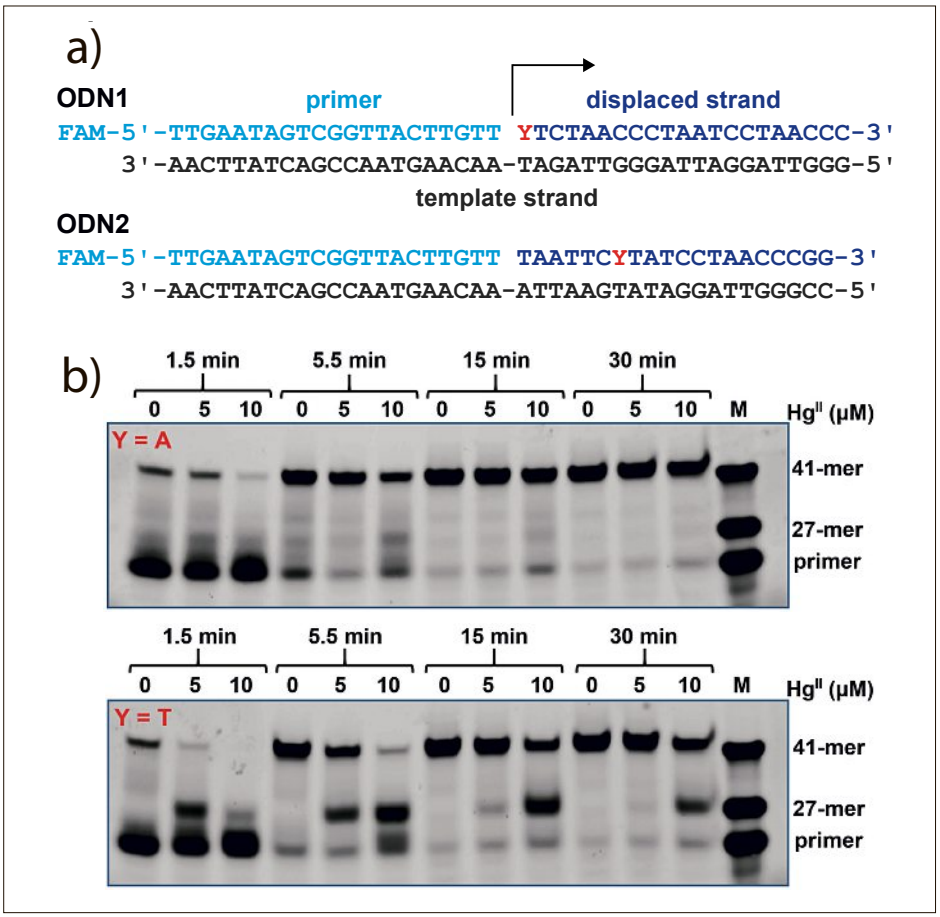

Fig. 6. a) Nicked duplex DNAs 'ODN1' and 'ODN2' containing a T-T mismatch (Y = T) or a Watson–Crick base pair (Y = A), where 'FAM' = fluorescein. PAGE analysis of 'ODN2' primer extension by Klenow Fragment at different time points and Hg^{II} concentrations. 'M' = marker for primer, stalled and full-length products.