Concerted dynamics of metallo-base pairs in an A/B-form helical transition

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Metal-mediated base pairs expand the repertoire of nucleic acid structures and dynamics. Here we report solution structures and dynamics of duplex DNA containing two all-natural C-HgII-T metallo base pairs separated by six canonical base pairs. NMR experiments reveal a 3:1 ratio of well-resolved structures in dynamic equilibrium. The major species contains two (N3)T-HgII-(N3)C base pairs in a predominantly B-form helix. The minor species contains (N3)T-HgII-(N4)C base pairs and greater A-form characteristics. Ten-fold different 1J coupling constants (15N,199Hg) are observed for (N3)C-HgII (114 Hz) versus (N4)C-HgII (1052 Hz) connectivities, reflecting differences in cytosine ionization and metal-bonding strengths. Dynamic interconversion between the two types of C-HgII-T base pairs are coupled to a global conformational exchange between the helices. These observations inspired the design of a repetitive DNA sequence capable of undergoing a global B-to-A-form helical transition upon adding HgII, demonstrating that C-HgII-T has unique switching potential in DNA-based materials and devices.

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Transitions between A- and B-form duplexes were discovered by Franklin and Gosling when conducting X-ray fibre diffraction analyses under various humidities. In solution, protein binding reactions can also partially dehydrate duplex DNA, giving global A-form viral genomes, as well as local A-form perturbations at specific binding sites. Small molecules such as polyamines, aminoglycosides, hexamminecobalt (III), and cisplatin can induce local B → A transitions via mechanisms independent of global changes in hydration and water activity. The binding of metal ions to discrete coordination sites in nucleic acids can be coupled to the (re)folding of DNA and RNA molecules that activate DNAzymes, ribozymes, riboswitches, and DNA-based materials. Previous studies mostly focused on characterizing the changes in structure and function of metal-bound versus metal-free (apo) nucleic acids. Here we report the structures and dynamics of two interconverting structures of the same metallo duplex, where local changes in metal-nucleobase ionization and isomerization are directly coupled to a global conformational exchange. This observation, together with the structural differences between metal-bound and apo duplex structures, enabled our design and identification of a duplex DNA containing 15 C-T mismatches that exhibits a global B → A helical transition upon adding HgII.

Transition metal ions participate in reversible binding interactions between opposing nucleobases. T-HgII-T provided the first such example of an all-natural metal-mediated base pair composed of a pyrimidine-pyrimidine mismatch and a transition metal ion. T-HgII-T base pairs exhibit very high kinetic and thermodynamic stabilities and can serve as functional mimics of T-A by causing enzymatic misincorporation of dTTP across thymidine to give T-HgII-T in vitro and possibly in vivo. NMR studies confirmed early predictions that HgII binds to T-T mismatches via N3 coordination of two deprotonated thymidine residues. Structurally analogous C-AgII-C base pairs have also been reported, and in both these cases, little or no impact on the global structure of the B-form duplex was reported.

C-HgII-T is a newly discovered, all-natural metallo base pair for which relatively little information is available. Using fluorescent nucleobase analogues and 1H NMR spectroscopy, we recently reported stoichiometric, high affinity binding of HgII to DNA duplexes containing C-T mismatches. Conducted in parallel, crystal screening of various oligonucleotides and metal ions produced an X-ray structure of a short (8-mer), A-form parallel, crystal structure of the B-form duplex was reported. Therapeutic targets include DNA binding drugs and pharmacological agents that can bind directly to a global conformational exchange. This changes in metal-nucleobase ionization and isomerization are interconverting structures of the same metallo duplex, where local nucleic acids. Here we report the structures and dynamics of two ribozymes, riboswitches, and DNA-based materials. 

**Fig. 1** Proposed C-HgII-T binding modes. a, (N3)T-HgII-(N3)C coordination based on structural homology with T-HgII-T and small increases in thermal stabilities of duplexes containing C-T mismatches after adding HgII (Fig. 1). The global A-form structure observed in the crystal structure was inconsistent with circular dichroism (CD) data of slightly longer, 14-21-mer duplexes containing one or two C-HgII-T base pairs. The CD spectra suggested B-form helices, and little-to-no changes in their global conformation upon adding HgII. The metal binding mode(s) and global structural characteristics of duplex DNA containing C-HgII-T base pairs in solution were therefore unclear.

Here we report a detailed NMR study using 15N-labelled DNA and HgII enriched mercury salts to determine the solution structures and dynamics of C-HgII-T base pairs in duplex DNA. Unlike previous examples of metal-mediated base pairs, C-HgII-T exhibits two types of covalent connectivities that are dynamically coupled via a global conformational change in helical structure. A palindromic, 14-mer duplex with two C-HgII-T sites separated by six canonical base pairs (ODN1, Fig. 2a, Supplementary Table 1, and Supplementary Figs. 1, 2) exists as a 3:1 mixture of well-defined duplexes in dynamic equilibrium. Both structures exhibit groove and rise dimensions intermediate between ideal A- and B-form helices. The most abundant duplex contains (N3)T-HgII-(N3)C connectivity and mostly B-form helical characteristics, whereas the minor species contains (N3)T-HgII-(N4)C base pairs and more A-form characteristics. No indication of a third duplex containing one of each type of metallo base pair is evident, consistent with long-range conformational coupling between the two metal centres. Furthermore, the rate constants for nucleobase-metal-nucleobase isomerization measured using 1H NMR experiments are nearly identical to those of the global conformational exchange of duplex structures measured using 1H NMR experiments measured using 1H NMR experiments. These results therefore support the coupling of metal-ligand isomerization reactions over long distances (> 20 Å) via a global conformational change of the double helix. Taken together with the greater A-form characteristics upon metal binding, these results suggest that placing numerous C-T mismatches throughout a repetitive duplex sequence can facilitate a global B → A helical transition upon adding HgII. To test this possibility, we prepare and analyse a small library of hairpin duplex DNAs (n = 10) and identify a duplex sequence that adopts a global A-form structure upon adding stoichiometric HgII. This helical transition is rapid (< 30 s) and fully reversible upon addition of N-acetylcyesteine in a cycle that can be repeated more than 10 times on...
the same DNA. In addition to its broad implications in structural biology and biochemistry, such demand control of B → A and A → B helical switching may be utilized in the future development of advanced DNA-based materials and devices.

Results
Solution structure of HgII-free (apo) duplex ODN1 “C-T”

Our preliminary 1H NMR and CD studies utilized a 14-mer C2 symmetric, self-complementary sequence containing two C-T mismatches (ODN1 “C-T”, Fig. 2a)51. This sequence exhibits a thermodynamically predictable, two-state transition between single strand and duplex (Supplementary Fig. 2), as well as a 1H NMR exchangeable imino region that is well resolved at pH = 7 (Supplementary Fig. 3). Here the full assignment of proton resonances was conducted by sequential walking along H1′ and aromatic protons (H1′ → H6/H8n+1 ↔ H1′ Hn+1) (Supplementary Fig. 14). As cross references for our assignments, H2′/aromatic (H2′/H2n → H6/H8n+1 ↔ H2′/H2n+1), and aromatic/aromatic (H6/H8n → H6/H8n+1 → H6/H8n+2) regions in the [1H,1H]-NOESY, [1H,1H]-TOCSY (H1′ → H2′/H2′) and [13C,1H]-HSQC spectra (aliphatic and aromatic regions) were used. All signals of the duplex were well resolved and the sequential walking could be followed through the entire sequence. Models for the duplex were constructed based on 958 conformationally restrictive nuclear Overhauser effect (NOE) distance restraints (Table 1). The models did not contain any artificial constraints of co-planarity or hydrogen bonding for the C-T mismatches. Superimposition of the 20 lowest energy structures from 200 computed structures gave an overall root mean square deviation (r.m.s.d) of all heavy atoms of 0.74 ± 0.26 Å, and 0.54 ± 0.21 Å for the C-T mismatch (Table 1, Fig. 2b). The central region forms a canonical B-form duplex, yet local perturbations about the non-coplanar C-T mismatches cause a 19 ± 3° bend in the helical axis at each mismatch. Given the high dynamics of this system and limitations of the modelling used, it is difficult to ascertain the exact pattern(s) of hydrogen bonding present in C-T mismatches. Consistent with an early NMR model, the mismatched pyrimidines are stacked inside the duplex in a co-facial orientation (Fig. 2c)53. Our structure suggests C-T mismatches containing only one or two very weak hydrogen bonds. Evidence for weak hydrogen bonding is observed in the C-T imino proton resonances at 10.9 ppm that are broader than the imino resonances of an analogous duplex ODN2 “T-T” containing T-T mismatches, and much broader than those of ODN3 “G-T” containing G-T wobble base pairs (Supplementary Fig. 3). These observations correlated very well with the thermal stabilities of these duplexes (Tm ODN1 “C-T” = 35 °C, (Tm ODN2 “T-T” = 38 °C), and (Tm ODN3 “G-T” = 47 °C)54, as well as other reported duplexes containing C-T, T-T, and G-T52,53,55. A weak interaction between C and T is further supported by the 10-fold faster HgII binding of C-T versus T-T mismatches51. Taken together with the axial bending in our structure, these results are consistent with the fact that C-T mismatches are among the most thermodynamically destabilizing mismatches known in duplex DNA52,53,55.

Table 1 NMR restraints and statistics.a

| Restraint Type                  | Apo duplex | HgII duplex (major form) | HgII duplex (minor form) |
|--------------------------------|------------|--------------------------|--------------------------|
| NOE-derived distance restraintsb | 958        | 646                      | 640                      |
| Intra-nucleotide               | 302        | 266                      | 264                      |
| Inter-nucleotide (i → j = 1)   | 566        | 306                      | 302                      |
| Long-range (i → j ≥ 2)         | 90         | 74                       | 74                       |
| C-T, C-HgII-T                  | 250        | 158                      | 148                      |
| Repulsive                      | 0          | 0                        | 0                        |
| NOE restraints per residue      | 34.21      | 23.07                    | 22.86                    |
| NOE violation >0.2 Å            | 0          | 0                        | 0                        |
| Dihedral restraintsb,c         | 168        | 168                      | 168                      |
| Dihedral violations >5.0°       | 0          | 0                        | 0                        |
| Hydrogen-bond restraintsb,c     | 60         | 62                       | 62                       |
| Planarityc                      | 24         | 24                       | 24                       |
| r.m.s.d (all heavy atoms vs. best structure) | 0.74 ± 0.26 Å | 1.21 ± 0.44 Å | 1.09 ± 0.36 Å |
| Helix                          | 0.83 ± 0.29 Å | 1.37 ± 0.48 Å | 1.21 ± 0.40 Å |
| C-T, C-HgII-T base pairs       | 0.54 ± 0.21 Å | 0.76 ± 0.28 Å | 0.75 ± 0.29 Å |

aStatistics are given for the 20 lowest energy structures from 200 calculated structures. bExperimentally derived constraints (Supplementary Figs. 14, 16). cIntroduced constraints. The two additional hydrogen-bond restraints in the HgII-containing duplexes re

Nucleobase-metal-nucleobase connectivity of C-HgII-T

Adding three equivalents of HgII (1.5 : 1.0 with respect to the number of mismatches) to duplex ODN1 “C-T” caused disappearance of the mismatched imino resonance at 10.9 ppm (Fig. 3a and Supplementary Fig. 3)51. Similar results were obtained for the duplex ODN2 “T-T” containing two T-T mismatches (Supplementary Fig. 3). These results reflect specific binding reactions, since the addition of HgII to an analogous duplex containing two G-T wobble base pairs (ODN3 “G-T”) caused no such deprotonation (Supplementary Fig. 3). [1H,1H]-NOESY cross peaks between NH of thymidine and guanine residues, and between thymidine NH and H2 of adenine residues enabled assignment of all imino proton signals of ODN1 “C-T” in both the presence and absence of HgII (Supplementary Figs. 4, 5). The imino proton resonances of the thymidine residues flanking the C-T mismatch exhibited the largest changes in chemical shifts upon HgII addition, giving a final spectrum similar to that of ODN2 “T-T” containing the widely studied T-HgII-T base pairs (Supplementary Fig. 3, Supplementary Table 2)51,52,54,66,69.

To characterize the structure(s) of C-HgII-T base pairs, we synthesized a 15N-labelled ODN1 “C-T” by synthetic incorporation of 15N-labelled C and T residues at positions 4 and 11 in an otherwise unlabelled duplex. The splitting of the 1H resonance at 10.9 ppm by 15N (88 Hz), and its disappearance upon adding HgII (Fig. 3a) further confirmed its assignment as the mismatched thymidine NH-resonance. The five 15N-resonances in the absence of mercury were assigned by proton-coupled and proton-decoupled 15N NMR spectra, 1H,15N coupling of thymidine N3-H and cytosine NH2 by heteronuclear single quantum coherence (HSQC), and 1H,15N and 1H,15N coupling...
between N1 and H5 and/or H6 by long-range $^{15}$N-$^1$H-HSQC (Supplementary Figs. 6, 7). After adding three equiv of HgII (1.5:1.0 with respect to the number of mismatches), two sets of $^{15}$N-resonances were observed, corresponding to a “major” and a “minor” species in a 3:1 ratio (Fig. 3b). When adding only two equiv of HgII, these same signals were observed, in addition to those of unbound DNA, confirming that the minor species was not a result of any excess of HgII (Supplementary Fig. 8). $^{15}$N-$^1$H-HSQC spectra allowed assignment of all $^{15}$N signals for both the major and minor complexes (Fig. 3c, d, Supplementary Figs. 9, 10). Disappearance of the N3-H cross peak in the $^1$H-$^{15}$N-HSQC spectrum confirmed deprotonation of thymidine N3 upon addition of 1.5 equiv of HgII (Supplementary Figs. 7, 8). The downfield shift of thymidine N3major ($\Delta$ppm = +29) and N3minor ($\Delta$ppm = +28) suggested that direct (N3)T-HgII coordination was present in both binding modes (Fig. 3b, Supplementary Tables 3, 4). $^3$J$^1$H,${^{15}}$N and $^2$J$^1$H,${^{15}}$N coupling of N1 to H5 and/or H6 observed in long-range $^{15}$N-$^1$H-HSQC spectra allowed for assignment of N1major and N1minor resonances, as well as H5 and H6 protons for cytosine and H6 of thymidine (Fig. 3d and Supplementary Fig. 10). The appearance of a single cross peak in the direct $^{15}$N-$^1$H-HSQC spectrum at 124/6.39 ppm indicated deprotonation of cytosine (N4)NHminor in the minor binding mode (Fig. 3e). The large downfield shift of cytosine (N4)NHminor ($\Delta$ppm = +28) (Fig. 3b, Supplementary Tables 3, 4) and a doublet observed in the $^{15}$N-$^1$H-proton-coupled HSQC ($J = 86$ Hz, Supplementary Fig. 9c) further suggested displacement of one (N4)NH2 proton by HgII and direct HgII coordination to N4 in the minor species. An NOE-cross peak between (N4)NHminor to cytosine H5 (6.52/5.48 ppm at 25 °C) confirmed this $^{15}$N-resonance assignment (124 ppm) as being the deprotonated exocyclic amine of cytosine (Supplementary Fig. 11). $^3$J$^1$H,${^{15}}$N coupling of the $^{15}$N-signal at 207 ppm to (N4)NHminor, as well as to (N4)NHmajor was observed in a band-selective, long-range $^{15}$N-$^1$H-HSQC (Supplementary Fig. 10b). This allowed assignment of the overlapping $^{15}$N-resonances of the N3major and N3minor of cytosine. It was still unclear, however, if HgII was directly coordinated to cytosine N3major.

To unambiguously determine metal-nucleobase connectivities, we monitored changes in the $^{15}$N NMR spectrum of ODN1* “C*-T*” upon addition of $^{199}$Hg-isotopically enriched (79 %) Hg(ClO4)$_2$ (Fig. 4a). In the major binding mode, the N3-resonances for both cytosine and thymidine appeared as doublets, thereby revealing their direct coordination to HgII (Fig. 4a). The large $^1$J$^1$N,$^1$H$^{199}$Hg coupling constant of 1095 Hz for thymidine HgII-N3major is consistent with T(N3)-HgII binding reported for a T-HgII-T dinucleoside complex measured in d6-DMSO. Cytosine N3major-$^1$H-$^{199}$Hg exhibited a much smaller coupling constant $^1$J$^1$N,$^1$H$^{199}$Hg = 114 Hz (Fig. 4a), consistent with a longer, weaker bond. A doublet with a coupling constant $^1$J$^1$N,$^1$H$^{199}$Hg = 1052–1063 Hz confirmed direct HgII coordination to cytosine (N4)NHminor (Fig. 4a, b). This larger coupling constant is consistent with a stronger, shorter bond for HgII-C (N4) versus HgII-C(N3). Further support of our assignments was observed in $^1$H,$^{199}$Hg through-bond coupling with $^2$J$^1$H,$^{199}$Hg...
The metal binds to cytosine via two distinct coordination modes in solution. The major species contained two identical and unambiguous (N3)T-HgII-(N3)C base pairs, and the minor species two identical and unambiguous (N3)T-HgII-(N3)C base pairs. No evidence for a lower symmetry duplex containing one of each type of base pair was observed in any NMR experiment.

Global structures of duplexes containing C-HgII-T. Consistent with 15N NMR spectra (Figs. 3, 4), 3H NMR experiments confirmed the presence of two C2 symmetrical species (Supplementary Fig. 14). Proton resonance assignments and modelling for each duplex were conducted exactly the same as for the metal-free duplex. Aside from the first and last residues of the duplexes (C1 and G14), all signals of the major and minor metallo duplexes were well resolved and the sequential walk proceeded through the entire sequence (Supplementary Figs. 14, 15). Models for major and minor duplex were constructed based on 646 and 640 NOE-derived conformationally restrictive distance restraints, respectively (Table 1). Superimposition of the 20 lowest energy structures of 200 computed structures gave an overall root mean square deviation (r.m.s.d) of all heavy atoms of 1.21 ± 0.44 Å for the major duplex and 1.09 ± 0.36 Å for the minor duplex (Fig. 5a, Table 1).

As compared to the major duplex, the minor duplex exhibits more axial bending and a deeper, narrower major groove (Fig. 5b). The differences between the metal-bound and metal-free structures are also the greatest for the minor duplex (Fig. 5c). Inspection of the 20 lowest energy models for all three structures (apo, major metallo, and minor metallo) revealed a high frequency (65–100 %) of an unusual, O4′-endo sugar pucker at the cytosine residue of the C-T and C-HgII-T base pairs (Supplementary Table 6). Direct support for this was observed in the 3J H1′,H2′ coupling constant for the HgII-coordinated cytosine residue (3J H1′,H2′ = 6.5 Hz) in the major structure (Supplementary Table 5, Supplementary Fig. 17). Using the Karplus equation (Supplementary Equation 1)61 a dihedral bond-angle of Φ,2 = 142° was calculated, which is in excellent agreement with dihedral angles observed in the solution structure models (Φ,2 = 131°) (Supplementary Fig. 18, Supplementary Equation 1)61,62. However, the apparent O4′-endo sugar puckers present in all three structures likely reflect the averaged conformations of rapidly interconverting C2′- and C3′-endo sugar puckers that occur much faster than the time scale of these NMR measurements63–67.
Support for this conclusion can be found in the analyses of global parameters, where the sugar pucker amplitudes throughout all three duplexes were much smaller than both A-form and B-form duplexes (Fig. 6a). In contrast, nearly all other structural parameters including groove dimensions, twist and rise gave values intermediate between A- and B-form duplexes (Fig. 6b–f, and Supplementary Figs. 19–22).

**Dynamics of major—minor metallo duplex interconversion.**

The presence of two individual sets of NMR signals, together with exchange cross peaks and exchange-mediated cross peaks between them (Supplementary Figs. 23–26 and Supplementary Figs. 36–38) indicated the presence of conformational changes with rates suitable for determination by standard NMR methods. To investigate the dynamic changes in local metal ion coordination, z-z exchange $[^{15}\text{N},^{1}\text{H}]$-HSQC spectra were measured. With increasing delay time ($t_m$), new exchange signals appeared (Fig. 7a, Supplementary Fig. 23). Global fitting of integrated peak volumes versus exchange delay times (Supplementary Equations 6–9) furnished rate constants of $k_1 = 3.5 \text{ s}^{-1}$ and $k_{-1} = 7.7 \text{ s}^{-1}$ for the forward and reverse reactions of nucleobase-metal-nucleobase isomerization, respectively (Fig. 7a).

Single exchange signals in ROESY spectra were observed for the Hg$^{II}$-bound nucleosides as well as for sugar- and aromatic proton signals of various residues throughout the duplex (Supplementary Fig. 24). The same exchange signals were found in the $[^{1}\text{H},^{1}\text{H}]$-TOCSY spectrum in the H1'/H5 region ($\sim$5.0–6.2 ppm, Supplementary Fig. 25). Within that region, no protons belonged to the same spin system. The observed cross peaks therefore occurred by exchange rather than through-bond coupling. Exchange-mediated cross peaks were observed in the aromatic $\rightarrow$ H2'/H2* region of the $[^{1}\text{H},^{1}\text{H}]$-NOESY spectrum (Supplementary Fig. 26), confirming the global nature of the conformational change. To evaluate the potential impact of variable ionic strength on the exchange rates, samples of the metallo duplex were prepared in the presence of 50 mM, 200 mM and 500 mM NaClO$_4$. The overall exchange rates of interconversion between the two structures decreased with increasing ionic strength, but the ratio of the two structures remained the same (Supplementary Figs. 27–31). Likewise, pH-dependent measurements revealed slower overall exchange rates with increasing pH from pH 6–9 while maintaining the same ratio (Supplementary Figs. 32–35). The lower rates of exchange correlate with the increasing persistence length and therefore rigidity of the duplex with increasing pH and ionic strength.

To determine rate constants of global interconversion of the two duplexes, we measured $[^{1}\text{H},^{1}\text{H}]$-NOESY spectra with various mixing times ($t_m$) using samples prepared in the presence of 200 mM NaClO$_4$ at pH = 7.8. Selected exchange cross peaks (‘Aa’ and ‘Aa’, Supplementary Fig. 37) and exchange-mediated NOE cross peaks (‘Ab’ and ‘aB’, Supplementary Fig. 38) at residues throughout the duplex were integrated, normalized to signal intensity at mixing time $t_m = 200$ ms, and plotted as a function of mixing time ($t_m$) (Supplementary Table 7, Supplementary Figs. 39–42). For definitions of peak labels, see Supplementary Fig. 36. Exchange cross peaks were fit to Supplementary Equation 12 to determine the sum of rate constants $k_1 + k_{-1}$ (Fig. 7b, Supplementary Fig. 39 and Supplementary Table 7). For signals having sufficient resolution of the diagonal peaks (‘Aa’ and ‘aa’) for integration purposes, exchange cross peaks were normalized by AAA ($t_m$) / (AAA ($t_m$) + Aa ($t_m$)) and fit to Supplementary Equation 14 to determine individual rate constants $k_1$ and $k_{-1}$ (Fig. 7b and Supplementary Fig. 41). Dividing exchange-mediated cross peaks (‘Ab’ and ‘aB’) by the sum with their corresponding NOE cross peak (‘AB’ and ‘ab’) allowed the determination of rate constants $k_1$ and $k_{-1}$ by fitting to Supplementary Equations 18 and 19 (Supplementary Fig. 42). The sum of rate constants $k_1 + k_{-1}$ determined for the global conformational change according to exchange cross peaks ($10.5–15.0 \text{ s}^{-1}$), as well as the individual rate constants $k_1$ (3.8–5.2 $\text{ s}^{-1}$) and $k_{-1}$ (7.5–9.7 $\text{ s}^{-1}$) independently determined...
from exchange cross peaks and exchange-mediated cross peaks, were all in excellent agreement with rate constants determined for changes in metallo base pair structure ($k_1 = 3.5 \text{s}^{-1}$, $k_{-1} = 7.7 \text{s}^{-1}$, Fig. 7a, Supplementary Table 7). Together with the absence of a third, lower symmetry duplex containing one of each type of metallo-base pair, these results revealed that dynamic changes in local metal-ligand isomerization were coupled to the global interconversion of the two duplex structures (Fig. 7c).

Hg$^{II}$-induced, conformational switching from B- to A-form. The long-range coupling (>20 Å) between the metal centres as well as the increased A-form characteristics in the centre region of the duplex upon metal binding, suggested that placing numerous C-T mismatches throughout a repetitive duplex sequence could facilitate a global B → A helical transition upon adding Hg$^{II}$. To test this possibility, we introduced C-T mismatches into (G$_n$C$_m$)$_R$ type DNA sequences that are known to exhibit partial A-form characteristics50,72–74. To suppress formation of intramolecular G-quadruplex structures that would otherwise interfere with intermolecular duplex formation of such repetitive sequences, we designed a small library of 120-mer DNA hairpins (Supplementary Table 8) containing the tetraloop sequence cGCTAg that is known to stabilize both RNA and DNA hairpins75. To fold the hairpins, dilute solutions of DNA (1 μM) were heated (95 °C, 5 min) and rapidly cooled on ice at 0 °C. Samples were then incubated with 0.0 or 1.5 equiv of Hg$^{II}$ (relative to number of C-T mismatches present) at 25 °C for 3 h prior to their analysis. Gel electrophoresis revealed clean, intramolecular hairpin formation for most sequences in both the presence and absence of Hg$^{II}$, including our hit ODN13 (Supplementary Fig. 43). To screen for the induction of A-form DNA, fluorescence anisotropy of a 40 nM solution of a Neomycin-BODIPY conjugate “Neo-BODIPY”76 were therefore measured in the presence and absence of each DNA (600 nM) pre-treated with Hg$^{II}$ (0.0 or 1.5 equiv per C-T mismatch). No changes in
anisotropy were observed upon addition of all hairpins in the absence of HgII; however, the pre-incubation of ODN13 with HgII caused a 3.5-fold increase in fluorescence anisotropy of Neo-BODIPY (Table 2). Titration of the ODN13-HgII complex into solutions of Neo-BODIPY revealed an apparent dissociation constant \( K_d = 1.4 \pm 0.7 \mu M \) (Fig. 8a). This value is similar to the values reported for binding of neomycin to A-form, duplex RNA\(^{77}\). The ternary complex formed between Neo-BODIPY and ODN13. HgII was disrupted by the addition of unlabelled neo- mycin B (Supplementary Fig. 44), as well as N-acetylcysteine that sequesters HgII (Supplementary Fig. 45). These results demonstrate the reversibility of Neo-BODIPY binding, a lack of significant impact by the BODIPY tag, as well as the switch-like (on/off) effect of HgII binding to ODN13. The analogous hairpin containing T-A base pairs (ODN14) in place of T-C mismatches exhibited no such behaviour (Table 2). These observations are confirmed using CD spectroscopy.

The (G\(_5\)C\(_3\))\(_n\)-containing hairpins ODN4 and ODN5 exhibited CD spectra consistent with previous publications\(^{72}\), having a double maximum at 260 nm and 280 nm (Fig. 8b and Supplementary Fig. 46) that are thought to reflect a mixture of A-form and B-form-like stacking of the guanine and cytosine nucleobases, respectively. The addition of HgII had little-to-no impact on the CD spectra of hairpins ODN4\(^{12}\) or ODN14 (Fig. 8b and Supplementary Fig. 46). However, addition of HgII to hairpin ODN13 caused changes in its CD spectrum indicative of a global B- to A-form helical transition (Fig. 8c and Supplementary Fig. 47a)\(^{78}\). This HgII-induced conformational change exhibited a 1:1 stoichiometry between HgII and the number of C-T mismatches present (Supplementary Fig. 47b), which was extremely rapid (<30 s to complete) and fully reversible upon addition of N-acetylcysteine (Supplementary Fig. 47c). Given the relatively low G-quadruplex propensity of sequence ODN13, we were successful in preparing the corresponding intermolecular duplex lacking a hairpin turn. This simple duplex "ODN13 d\(^{x}\)" also exhibited a reversible, HgII-induced switching between global B- and A-form helices (Supplementary Fig. 48). By alternating between the addition of HgII and N-acetylcysteine, the helical switching cycle from B- to A-form, and A-form to B-form could be repeated more than 10 times on the same DNA (Supplementary Fig. 49).

### Table 2 DNA hairpin repeat sequences and fluorescence anisotropy of Neo-BODIPY in the presence of each DNA with and without HgII.

| Name | DNA repeat sequence | no HgII | + HgII |
|------|---------------------|---------|--------|
| -    | (Neo-BODIPY only)   | 0.05 ± 0.01 | 0.05 ± 0.01 |
| ODN4 | [CGGGCCGGGGCC]\(_3\)\(_S\) | 0.05 ± 0.01 | 0.06 ± 0.01 |
| ODN5 | [CGGGCCGGGGCCCC]\(_3\)\(_S\) | 0.05 ± 0.01 | 0.06 ± 0.01 |
| ODN6 | [CGGG]\(_3\)\(_A\) | 0.05 ± 0.02 | 0.05 ± 0.01 |
| ODN7 | [TTCTCGGG]\(_3\)\(_B\) | 0.04 ± 0.01 | 0.05 ± 0.02 |
| ODN8 | [TTGGGGCTGGCG]\(_3\)\(_F\) | 0.05 ± 0.01 | 0.04 ± 0.01 |
| ODN9 | [TTGGGGCTGGCC]\(_3\)\(_J\) | 0.05 ± 0.01 | 0.05 ± 0.01 |
| ODN10| [CGGGGGCGGCG]\(_3\) | 0.04 ± 0.01 | 0.05 ± 0.01 |
| ODN11| [TTGGGGCTGGCC]\(_3\) | 0.05 ± 0.01 | 0.05 ± 0.01 |
| ODN12| [TTCTCGGG]\(_3\)\(_G\) | 0.04 ± 0.02 | 0.05 ± 0.01 |
| ODN13| [TTGGCCCTCCG]\(_3\) | 0.04 ± 0.01 | 0.14 ± 0.01 |
| ODN14| [TTGGCCCTCCG]\(_3\) | 0.04 ± 0.01 | 0.05 ± 0.01 |

*Bold bases indicate C-T mismatches. Italic bases in ODN14 indicate T-A base pairs.*

\(^{a}\)All samples contained 40 nM of Neo-BODIPY, 600 nM of DNA, and 0 or 1.5 equiv of HgII per C-T mismatch in an aqueous buffer containing 200 mM NaClO\(_4\) and 50 mM cacodylic acid (pH = 7.8). Averaged anisotropy values and standard deviations of three independent measurements are shown. Source data are provided as a Source Data file.

### Discussion

Here we report solution structures of C-HgII-T base pairs. In addition to providing fundamental insights into C-HgII-T binding and dynamics, these results provide \( J^{15N,199Hg} \) and \( J^{1H,199Hg} \) coupling constants. Prior to these studies, little or no such information was available for these coupling constants in water\(^{46,47,58,79}\). Large differences in \( J^{15N,199Hg} \) coupling constants for complexes containing (N3)C-HgII (114 Hz) versus (N4) C-HgII (1052 Hz) will provide a basis for future computational studies that address the relationships between coupling constants, ligand ionization and metal binding\(^{57}\).

Aside from the gain/loss of a proton from (N4)C, the major/minor duplexes of ODN1 bound to HgII are constitutional isomers with respect to each other. Isomerization-coupled conformational exchange between these structures was relatively slow on the chemical-shift NMR time scale, yet it was fast enough to allow direct characterization by monitoring changes in exchange cross peaks as a function of mixing time (\( t_m \)). The rate constants of nucleobase-metal-nucleobase isomerisation \( k_1 = 3.5 \pm 1 \) s\(^{-1}\) and \( k_{-1} = 7.7 \) s\(^{-1}\) were nearly identical to rate constants independently measured for the global conformational exchange of the two duplex structures \( k_1 = 4.3 \pm 0.6 s^{-1} \), \( k_{-1} = 8.8 \pm 0.9 s^{-1} \). No evidence for a third duplex containing one of each type of metal-base pair was observed, giving further support for coupling between the metal centres via global conformational exchange.

![Fig. 8](image-url)
The forward and reverse rate constants of this process were ~10^3-fold faster than dissociation of HgII from C-HgII-T base pairs. The HgII ions therefore remained bound to the DNA during multiple structural exchanges. Given the high quality of the structures and dynamics reported here, this system provides a highly attractive model for the development of molecular dynamics simulations aimed at elucidating the pathways of dynamic conformation exchange processes.

Consistent with the structure of our minor duplex (Figs. 4, 5), crystals of a short, A-form DNA sequence also contained two C-HgII-T base pairs having (N3)T-HgII-(N4)C connectivity. However, the global A-form structure observed in the crystal structure was inconsistent with our circular dichroism (CD) data collected in solution using five different, slightly longer (14 to 21-mer) duplexes containing one or two C-HgII-T base pairs. Together with the observation that (N3)T-HgII-(N3)C binding present in our major structure is associated with more B-form character, and the (N3)T-HgII-(N4)C present in the minor structure is associated with more A-form character, a general picture is suggested. (N3)T-HgII-(N3)C binding and B-form structure is likely dominant in most DNA sequences, in direct analogy with high resolution structures of T-HgII-T base pairs.

However, the 10-fold larger J coupling constants (15N,199Hg) observed for (N4)C-HgII (1052 Hz) connectivity in the minor duplex as compared to the (N3)C-HgII (114 Hz) in the major structure indicate that stronger nucleobase-metal-nucleobase binding interactions are present in the minor structure. The minor duplex, however, also exhibits a greater degree of structural perturbation away from a canonical B-form duplex that likely destabilizes the complex as compared to the major form. The lower bonding energy of (N3)T-HgII-(N3)C versus (N3)T-HgII-(N4)C is therefore compensated by the overall higher stability of B-form versus A-form in the major and minor structures, respectively. However, in situations that favour A-form (crystallization, dehybridation, G/C-rich DNA sequences, etc.) the stronger (N3)T-HgII-(N4)C base pairs suggested the combined effects of base pair geometry, neighbouring base effects, and a bridging water molecule in the minor groove were involved.

For both of our metal-bound structures in solution, the amount of A-form character was greater in the centre of each helix, suggesting a medium-range influence of C-HgII-T formation. In contrast, the opposite pattern was observed in the structure of the metal-free duplex, having the greatest B-form characteristics in the centre of the helix. Together with the long-range coupling (>20 Å) between the two metallo-base pairs via a global conformational exchange, these results suggested that placing numerous C-T mismatches throughout a repetitive duplex sequence could support a global B-form → A-form helical transition upon addition of stoichiometric HgII. Indeed, we were able to identify one such sequence exhibiting a fully-reversible switching cycle from B- to A-form, and A-form to B-form by tandem additions of HgII and N-acetylcyesteine (Supplementary Fig. 49). Both transitions were complete in <30 s, and could be repeated more than 10 times. While numerous examples of local A-form perturbations caused by DNA-protein and DNA-small molecule binding interactions have previously been reported, the previous examples of global B-form → A-form helical transitions involved global dehydration of the duplex. Here the global B- to A-form helical transition was a result of discrete, reversible metal binding. In addition to its broad implications in structural biology and biochemistry, this type of A/B-form helical switching can be potentially be utilized in the development of advanced DNA-based materials and devices.

**Methods**

**Sample preparation.** For Fig. 2, duplex DNA (0.3 mM) was prepared by dissolving a self-complementary sequence in aqueous NaClO4, (50 mM, 90:10 H2O/D2O) and the pH was adjusted to pH = 7.8 by addition of an aqueous solution of NaOH. The sample was annealed by heating to 95 °C for 5 min and slow cooling to room temperature over 4 h. For Figs. 3, 4, 7, duplex DNA (0.5–1.0 mM) was prepared by dissolving 1.0 mM or 2.0 mM of the self-complementary sequence in aqueous buffer (200 mM NaClO4, 50 mM cacodylic acid in H2O / D2O (9:1) at pH = 7.0), heating to 95 °C for 5 min, and slowly cooling to room temperature over 4 h. HgCl2 was added, and the pH adjusted to pH = 7.8 by addition of an aqueous solution of NaOH. For Figs. 5, 6, duplex DNA (0.4 mM) was prepared by dissolving 0.8 mM of the self-complementary sequence in an aqueous solution of NaClO4 (50 mM, 90:10 H2O/D2O) and the pH was adjusted to pH = 7.8 by addition of an aqueous solution of NaOH. The samples were annealed as described above, mixed with HgII (1.5 equiv with respect to mismatch), the pH re-adjusted to pH = 7.8, and the sample was treated with Chemex (BIO-RAD) for 10 min to remove excess HgII. Samples measured in D2O were prepared the same way and then lyophilized, dissolved in 99.9% D2O and the pH adjusted to ~7.4 by addition of a solution of NaOD in 99.9% D2O. Samples measured at 4 °C were equilibrated at 4 °C for 15 min prior to measuring.

**NMR spectra measurements.** 1H NMR spectra were recorded on a Bruker Avance II 500 MHz spectrometer equipped with a TXI z-axis gradient probe head using excitation sculpting for water suppression. Proton chemical shifts were referenced to the water line at 4.70 ppm. The spectra were processed with a line-broadening factor of 10 Hz. 1H NMR spectra were recorded on a Bruker Avance II 500 MHz spectrometer equipped with a BBO z-axis gradient CryoProbe at 4 °C or 25 °C using either inverse gated or no proton decoupling. The spectra were processed with a line-broadening factor of 10 Hz. [1H,NH]-HSQC spectra were recorded at 4 °C or 25 °C on a Bruker Avance II 500 MHz spectrometer equipped with a BBO z-axis gradient CryoProbe, Bruker Avance 600 MHz spectrometer equipped with TCI z-axis gradient CryoProbe or on a Bruker Avance 700 MHz spectrometer equipped with TXI z-axis gradient CryoProbe. The INEPT times were set to select for a 90 Hz coupling for [1H,NH]-HSQCs, 20 Hz coupling for [1H,1H]-HSQCs and [1H,NH]-QC spectra. 15N,199Hg double-selective NOESY spectra were measured in D2O (4 °C and 25 °C and mixing times of 60 ms and 250 ms). Exchangeable protons were assigned from [1H,1H]-NOESY spectra measured in D2O/D2O (90:10) (4 °C, 150 ms mixing time). Spectra were recorded on a Bruker Avance 600 MHz spectrometer equipped with a TCI z-axis gradient CryoProbe or on a Bruker Avance 700 MHz spectrometer equipped with TXI z-axis gradient CryoProbe. For additional information see supporting information.

**NMR solution structure calculations.** The integrated peak volumes from a representative [1H,1H]-NOESY spectrum (mixing time = 250 ms) measured at 25 °C were calibrated to distances using CALIBA macro in DYANA. The NOE signals were grouped into four categories: (i) strong (1.8–3.0 Å), (ii) medium (1.8–4.5 Å), (iii) weak (3.0–6.0 Å), and very weak (4.0–7.0 Å). Structure calculations were performed with XPLOR-NIH 2.46 using standard implemented force field parameters. For introduced restraints for the calculations see supporting information. Starting from a strand generated based on the sequence of nucleoside residues, 2000 structures were calculated based on NOE-, dihedral-, planarity- and H-bond distance restraints using simulated annealing. The 20 lowest energy structures were selected and used for further refinement using additional RAMA and ORIE database terms. 200 refined structures were calculated and the 20 lowest energy structures were visualized and analyzed. Root mean square deviation (r.m.s.d.) were calculated using MOLMOL and duplexes were visualized using PyMOL. Base-pair parameters were determined using Curves and JDNA as described above.

**Data availability**

Structures of the metal free (PDB 6RLS), HgII-bound major (PDB 6FY6) and HgII-bound minor (PDB 6FY7) duplex structures have been deposited in the Protein Data Bank (https://www.rcsb.org/). All other data generated and analyzed in this study are included in this article, supplementary information, source data file, and are also available from the authors upon reasonable request. Source data for Table 1, Table 2, Figs. 6, 7, 8, and Supplementary Figs. 15, 19–22, 28, 29, 31, 33–35, 39–44, 45, 47, 49 are provided in the Source Data file. Accepted: 16 October 2018; Accepted: 5 September 2019; Published online: 23 October 2019
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Author contributions
O.S. and N.L. conceived the project. O.S.; S.Ju.; S.Jo.; A.K.; R.S., and N.L. contributed to analysing the data. O.S. and N.L. wrote the manuscript.

Competing interests
The authors declare no competing interests.

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