

SUPPLEMENTARY INFORMATION

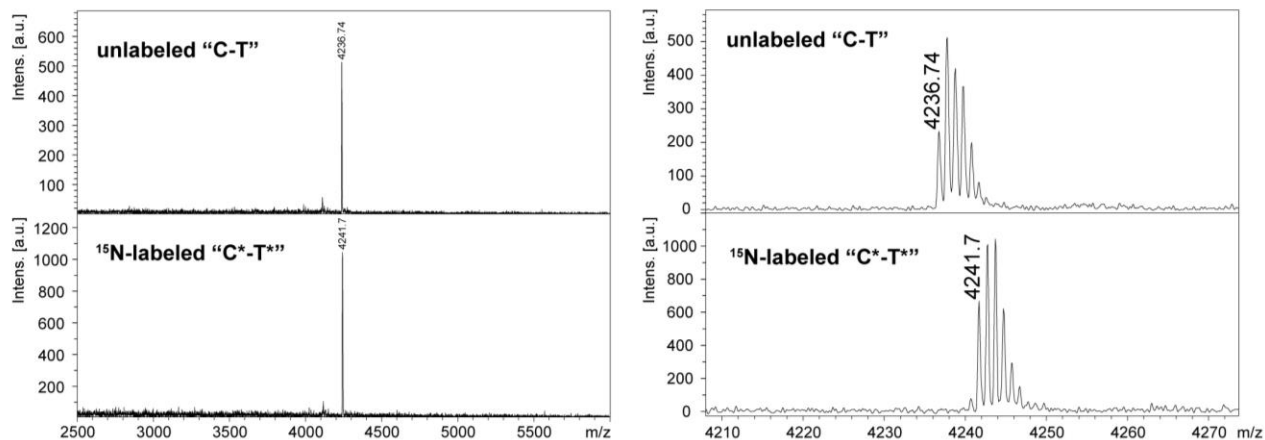
Concerted dynamics of metallo-base pairs in an
A/B-form helical transition

Schmidt et al.

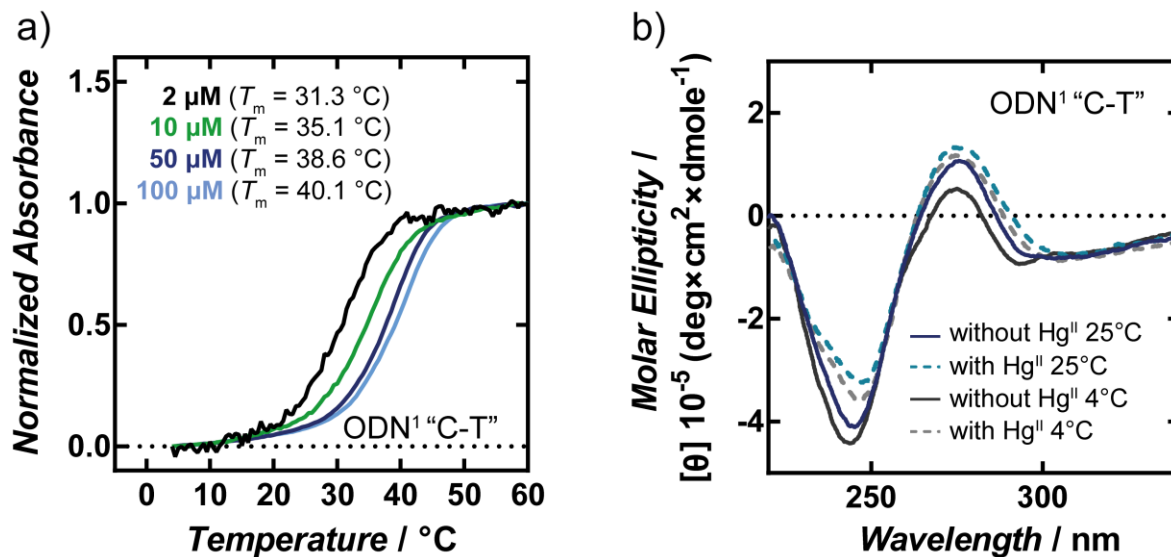
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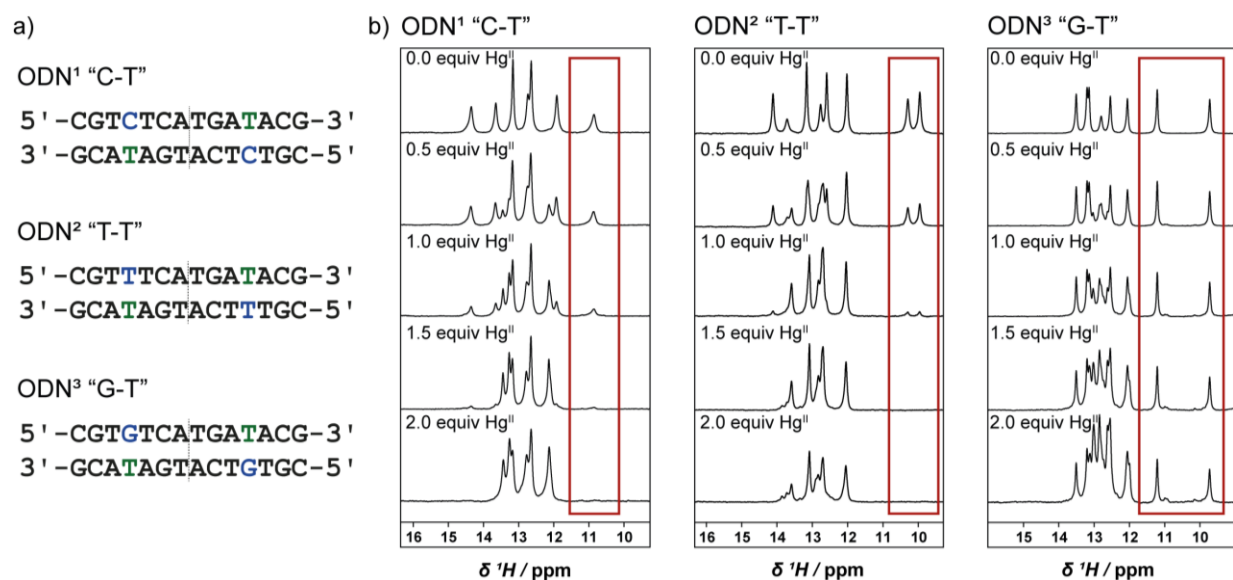
Supplementary Figures



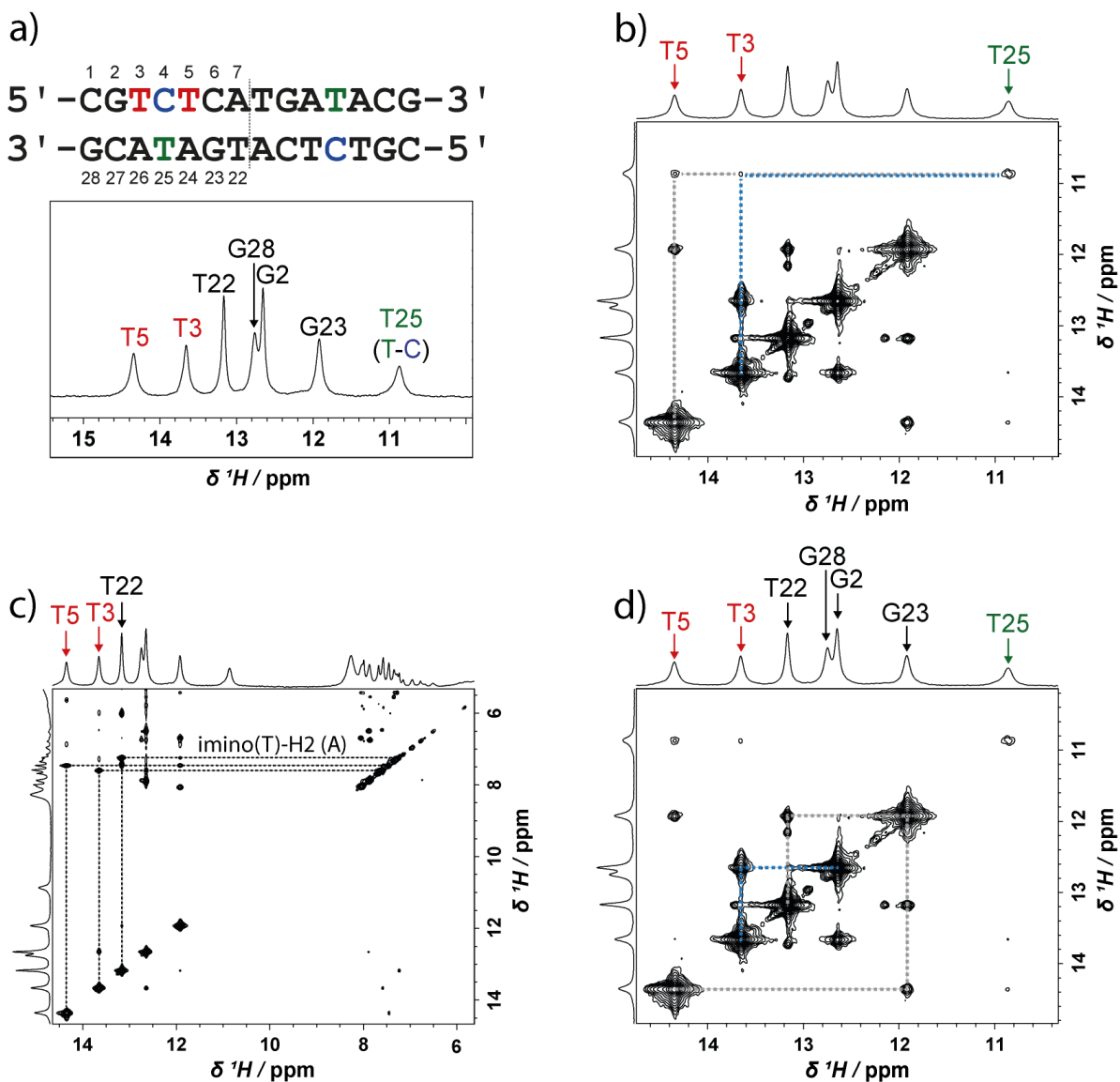
Supplementary Figure 1 | MALDI-MS analysis of unlabeled ODN¹ "C-T" and ¹⁵N-labeled ODN^{1*} "C*-T*". ¹⁵N-labeled "C*-T*" showed a mass elevation of 5 dalton.



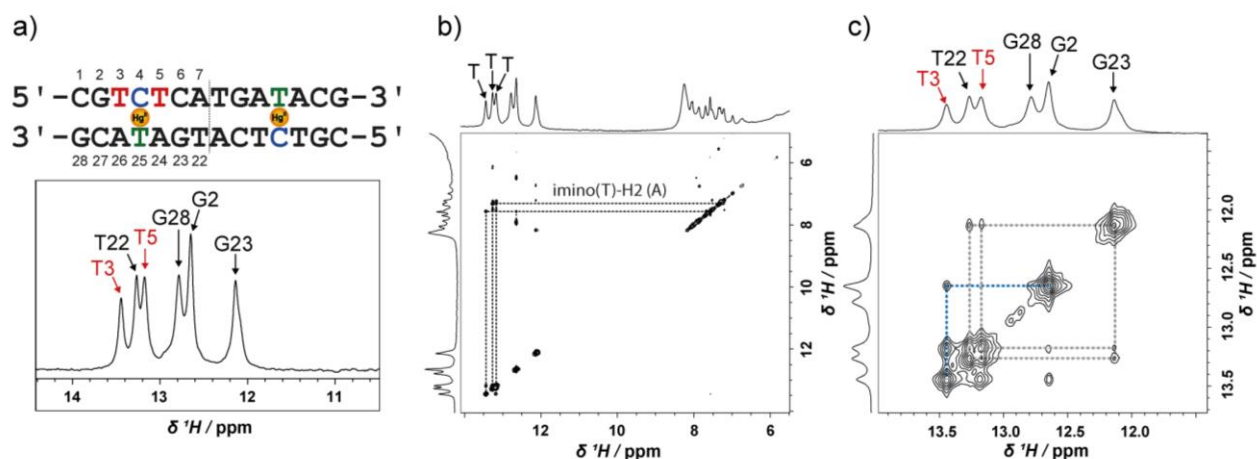
Supplementary Figure 2 | Melting temperature analysis (T_m) and circular dichroism spectroscopy (CD). a) Thermal melting of ODN¹ "C-T" at variable DNA concentrations.¹ b) CD spectra of ODN¹ "C-T" in the presence and absence of Hg^{II} at 4 °C and 25 °C.¹ Parts of the figure were taken from ref 1. For duplex sequence see Supplementary Table 1.



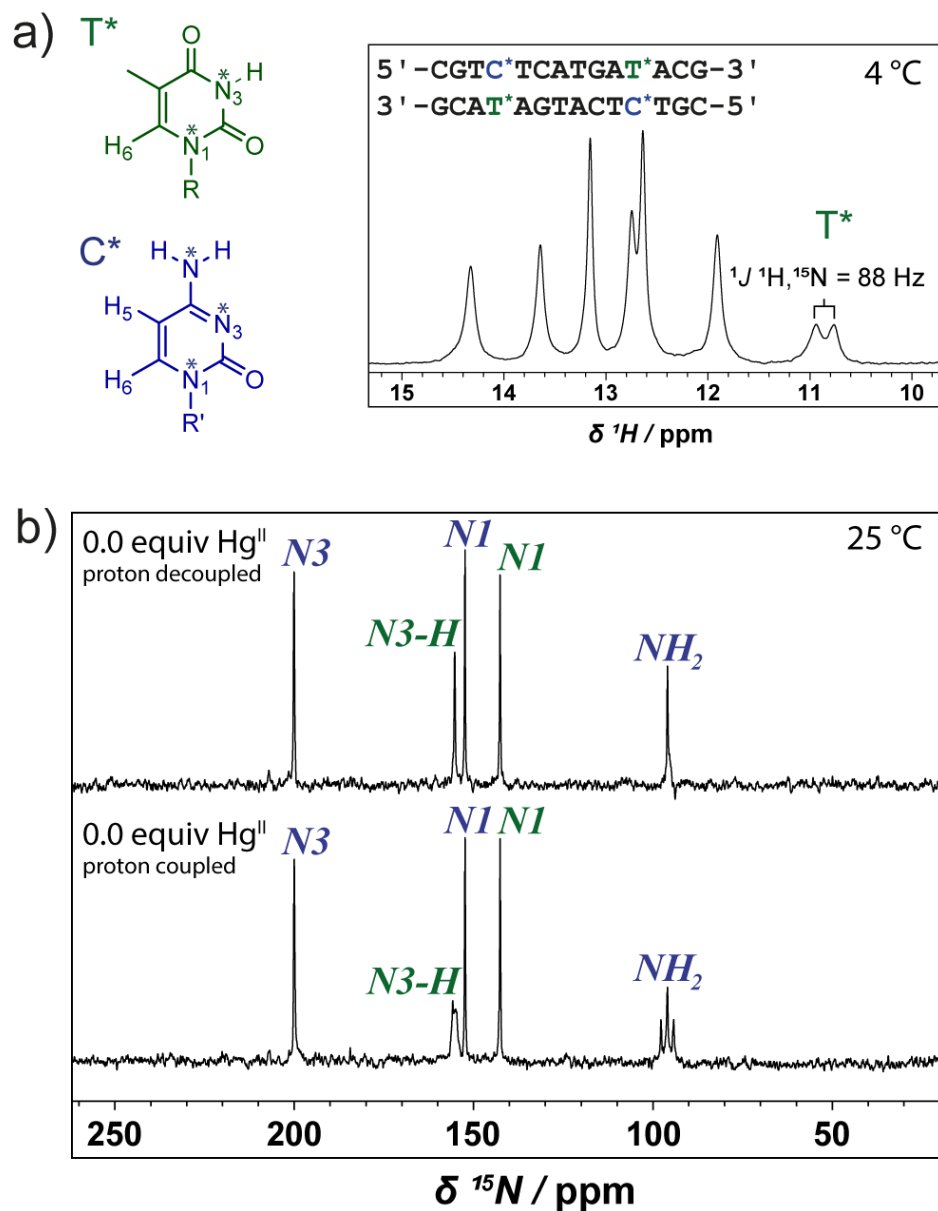
Supplementary Figure 3 | C-Hg^{II}-T base pair formation according to changes in ¹H NMR spectra upon addition of Hg^{II}. a) Names and sequences of palindromic, 14-mer duplex DNA "C-T" and "T-T", and "G-T". b) Disappearance of mismatched thymidine imino proton resonances upon addition of Hg^{II} to C-T- and T-T mismatch containing duplex DNA revealed stoichiometric binding of Hg^{II} to C-T- and T-T-mismatches. Addition of Hg^{II} to an analogous duplex containing G-T wobble base pairs caused no such deprotonation. All samples contained 0.5 mM of duplex DNA in aqueous buffer (200 mM NaClO₄, 50 mM cacodylic acid in H₂O / D₂O (9:1) at pH = 7.0). DNA samples were incubated with variable concentrations of Hg(ClO₄)₂ at r.t. for 15 min, followed by cooling at 4 °C for 15 min. Equiv of Hg^{II} given relative to mismatch.



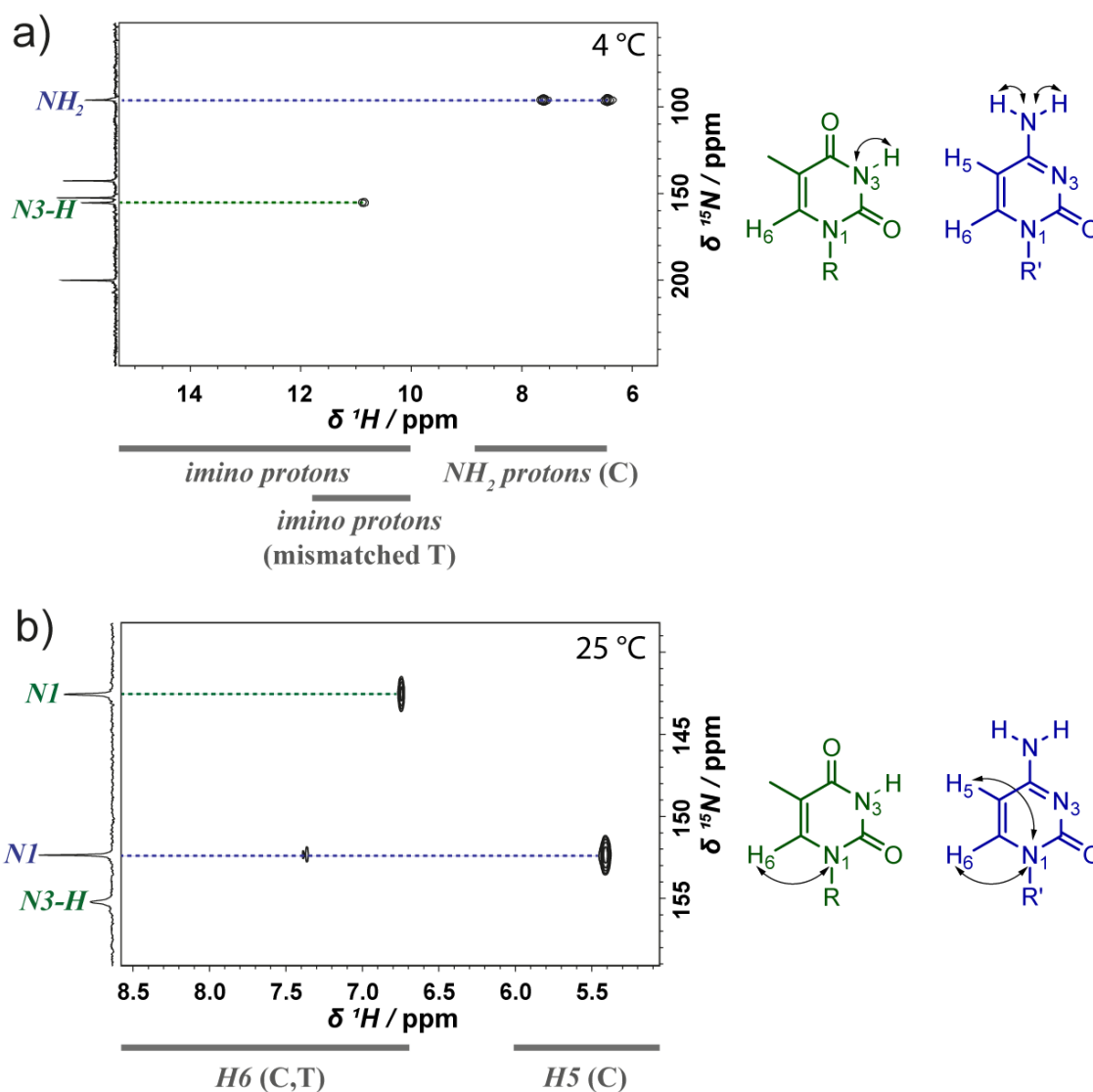
Supplementary Figure 4 | Assignment of *NH* imino protons of the duplex ODN¹ “C-T” in the absence of Hg^{II}. a) “C-T” sequence and imino region of ¹H NMR spectrum. Blue and green bases indicate C-T mismatches and red bases indicate residues flanking C-T mismatches. b) Assignment of thymidine residues flanking (red) the C-T mismatch (blue and green) according to NOE cross peaks between *NH* imino protons of thymidine. c) Assignment of thymidine imino protons according to NOE cross peaks between *NH* imino signals of thymidine residues and H2 of adenine residues. d) Assignment of guanosine residues of “C-T” according to NOE cross peaks between *NH* imino protons. The DNA sample contained 0.5 mM duplex DNA in aqueous buffer (200 mM NaClO₄, 50 mM cacodylic acid in H₂O / D₂O (9:1) at pH = 7.0). Spectra were recorded at 4 °C.



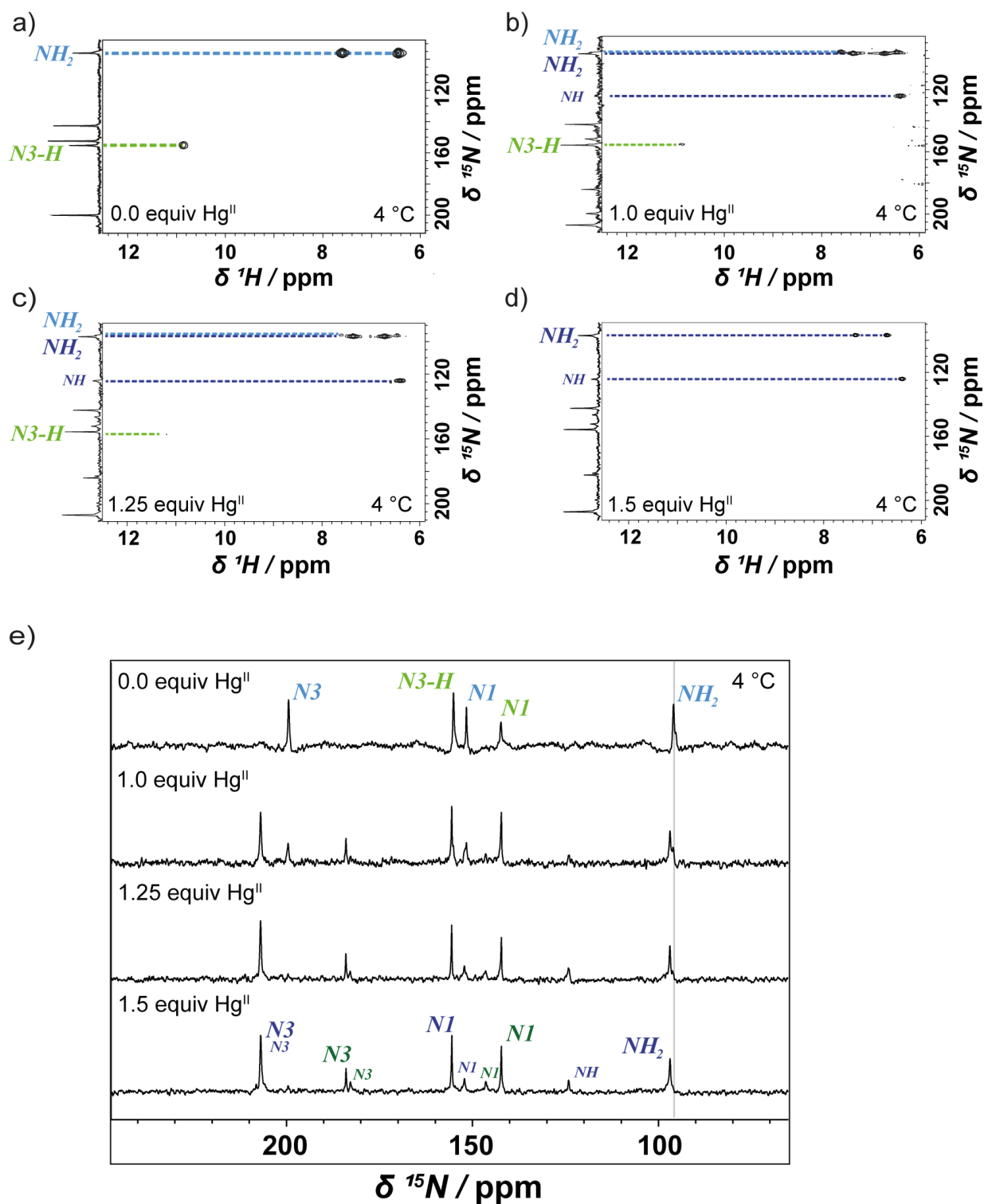
Supplementary Figure 5 | Assignment of *NH* imino protons of ODN¹ “C-T” in the presence of 1.5 equiv Hg^{II}. a) “C-T” sequence and imino region of ¹H NMR spectrum. Blue and green bases indicate C-T mismatches and red bases indicate residues flanking C-T mismatches. b) Assignment of thymidine imino protons according to NOE cross peaks between *NH* imino signals of thymidine residues and H2 of adenine residues. c) Assignment of thymidine and guanosine imino proton signals in the presence of Hg^{II} according to NOE cross peaks between *NH* imino protons. The DNA sample contained 0.5 mM duplex DNA and 1.5 mM Hg^{II} (1.5 equiv relative to mismatch) in aqueous buffer (200 mM NaClO₄, 50 mM cacodylic acid in H₂O / D₂O (9:1) at pH = 7.0). Spectra were recorded at 4 °C. Equiv of Hg^{II} given relative to mismatch.



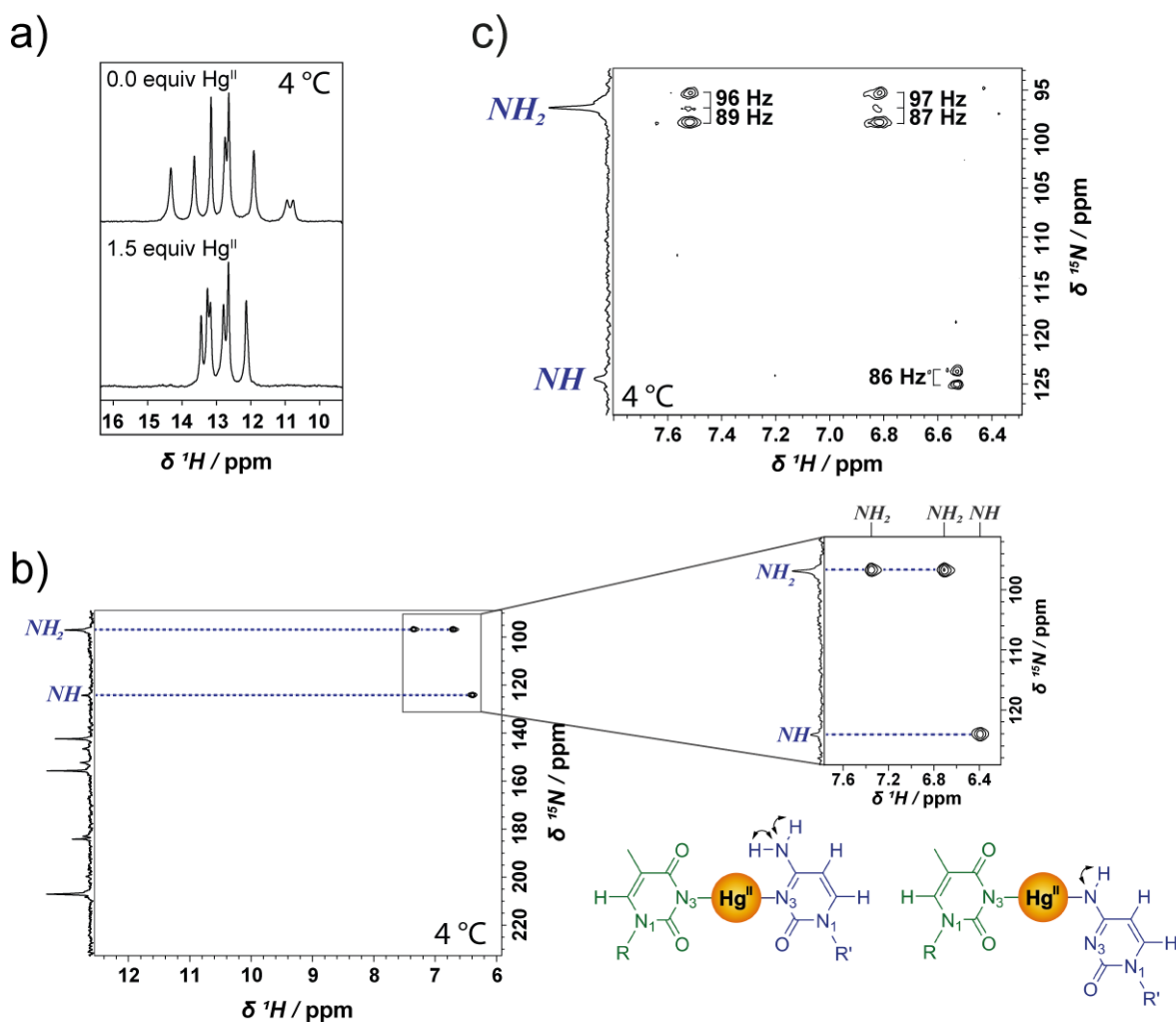
Supplementary Figure 6 | ^1H - and ^{15}N NMR spectra of ODN¹ “C*-T*” in the absence of Hg^{II} . a) $^1J \text{ } ^1\text{H}, ^{15}\text{N}$ coupling ($^1J = 88 \text{ Hz}$) of N3-H imino proton of mismatched thymidine according to ^1H NMR spectrum of ODN¹* “C*-T*” containing a site-selective, ^{15}N -labeled C-T mismatch at 4 °C ($\text{N}^* = ^{15}\text{N}$). b) Assigned proton decoupled- and proton coupled ^{15}N NMR spectra of ODN¹* “C*-T*” at 25 °C. Blue indicates ^{15}N resonances of C* and green indicates ^{15}N resonances of T*. DNA samples contained 0.5 mM (^1H) and 1 mM (^{15}N) duplex DNA in aqueous buffer (200 mM NaClO_4 , 50 mM cacodylic acid in $\text{H}_2\text{O} / \text{D}_2\text{O}$ (9:1) at pH = 7.8).



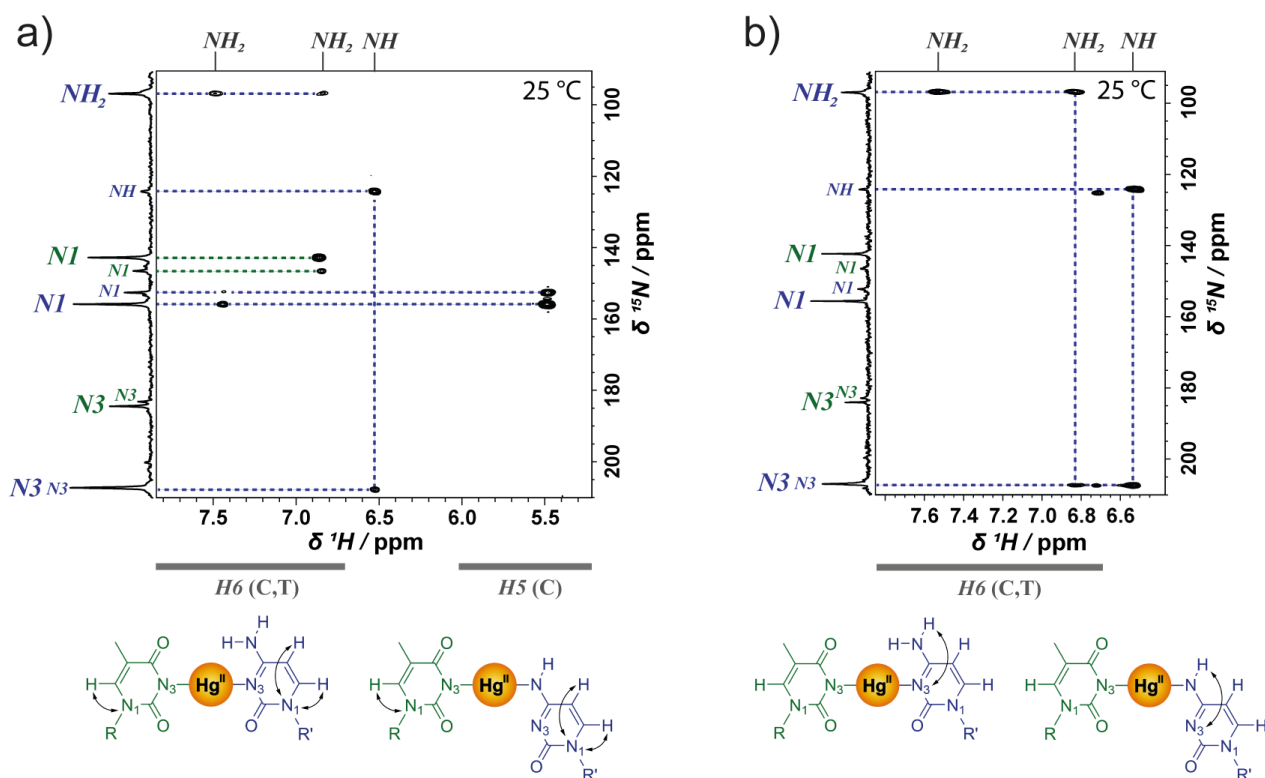
Supplementary Figure 7 | ^{15}N resonance assignment of ODN¹ “C*-T*” in the absence of Hg^{II} . a) $^1J\text{ }^1\text{H}, ^{15}\text{N}$ coupling of thymidine N3-H (green) and $^1J\text{ }^1\text{H}, ^{15}\text{N}$ coupling of cytosine exocyclic amine (blue) according to $[^{15}\text{N}, ^1\text{H}]$ -HSQC spectrum at $4\text{ }^{\circ}\text{C}$. b) $^2J\text{ }^1\text{H}, ^{15}\text{N}$ coupling between thymidine N1 and H6 (green) and 3J - and $^2J\text{ }^1\text{H}, ^{15}\text{N}$ coupling between cytosine N1 and H5 and H6 (blue) observed in the long-range $[^{15}\text{N}, ^1\text{H}]$ -HSQC spectrum at $25\text{ }^{\circ}\text{C}$. DNA samples contained 1.0 mM duplex DNA (ODN¹* “C*-T*” in aqueous buffer (200 mM NaClO_4 , 50 mM cacodylic acid in $\text{H}_2\text{O} / \text{D}_2\text{O}$ (9:1) at pH = 7.8) and were measured at the indicated temperature.



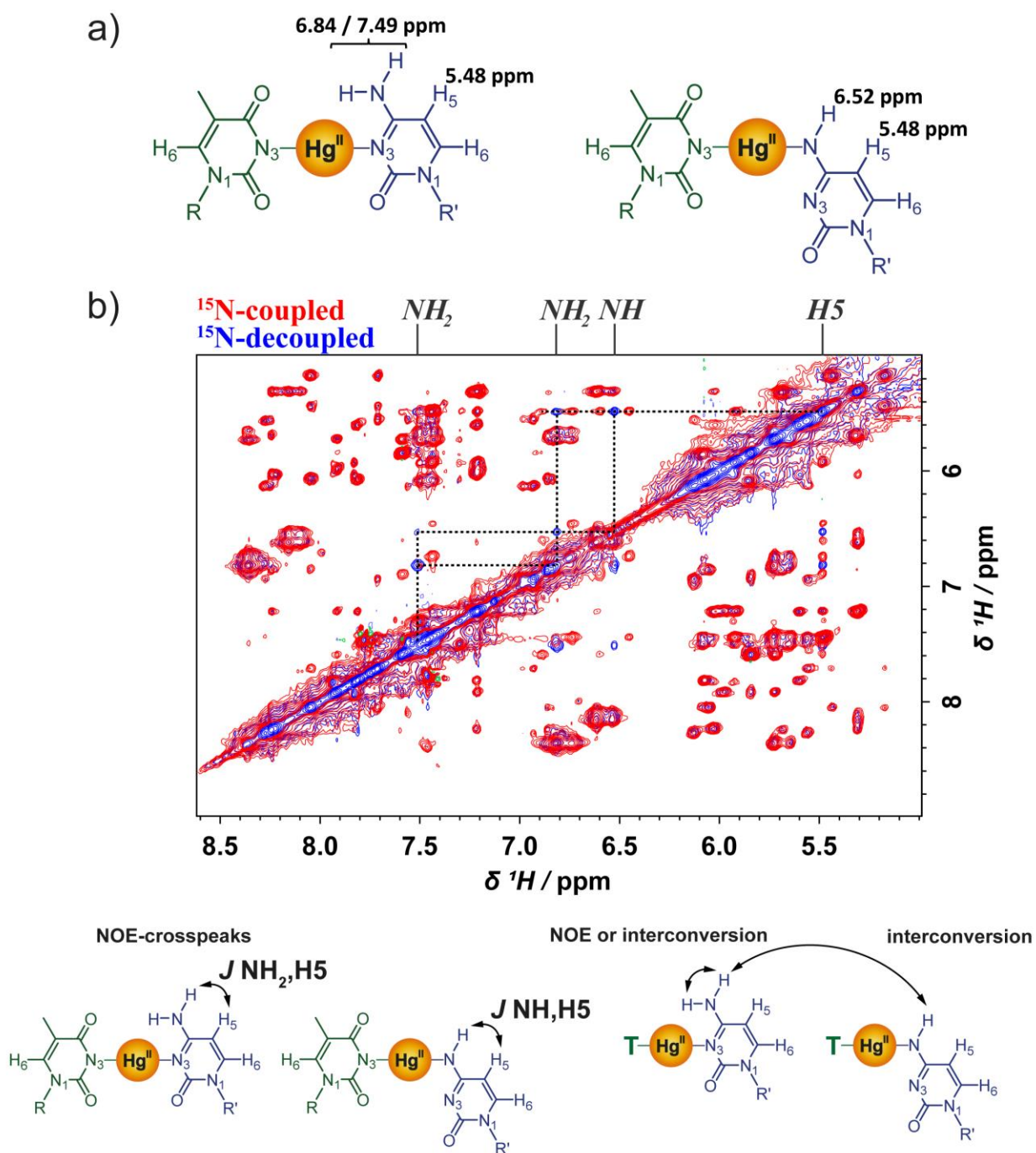
Supplementary Figure 8 | Changes in ^{15}N , ^1H -HSQC spectra and 1D ^{15}N spectra upon addition of Hg^{II} to ODN^{1*} "C*-T*". ^{15}N , ^1H -HSQC spectra (a – d) and e) 1D ^{15}N spectra. 1.5 equiv of Hg^{II} were needed to saturate C-T mismatch-containing duplex DNA and induce complete disappearance of ^{15}N signals of unbound duplex (Hg^{II} -unbound signals: light blue and light green; Hg^{II} -bound signals: dark blue, dark green). DNA samples contained 0.5 mM duplex DNA (ODN^{1*} "C*-T*") in aqueous buffer (200 mM NaClO_4 , 50 mM cacodylic acid in H_2O / D_2O (9:1) at pH = 7.8). Spectra were recorded at 4 °C. Equiv of Hg^{II} given relative to mismatch.



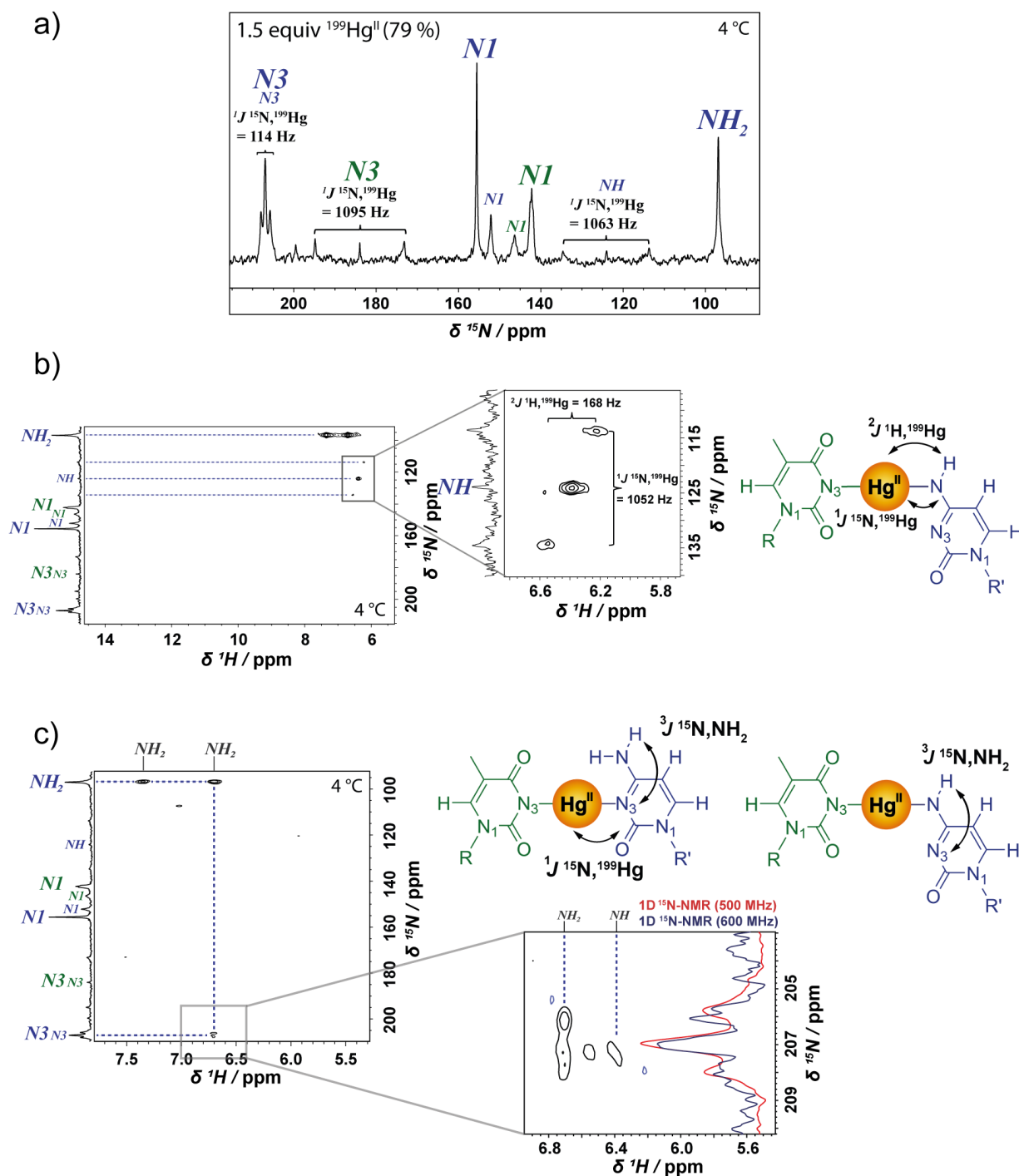
Supplementary Figure 9 | ^1H - and ^{15}N , ^1H -HSQC spectrum of ODN^{1*} “C*-T*” in the presence of Hg^{II} . a) Deprotonation of mismatched thymidine N3-H upon addition of 1.5 equiv of Hg^{II} . b) $^1J \text{ } ^1\text{H}$, ^{15}N coupling of thymidine (N4) $\text{NH}_2^{\text{major}}$ and (N4) NH^{minor} of cytosine according to ^{15}N , ^1H -HSQC spectrum. c) Doublet (124 ppm, $^1J = 86 \text{ Hz}$) observed in the proton-coupled ^{15}N , ^1H -HSQC spectrum confirmed deprotonation of exocyclic amine in the minor binding mode. Blue indicates ^{15}N resonances of C*. DNA samples contained 0.5 mM duplex DNA (ODN^{1*} “C*-T*”) and 1.5 mM Hg^{II} (1.5 equiv relative to mismatch) in aqueous buffer (200 mM NaClO_4 , 50 mM cacodylic acid in $\text{H}_2\text{O} / \text{D}_2\text{O}$ (9:1) at pH = 7.8) and were measured at the indicated temperature.



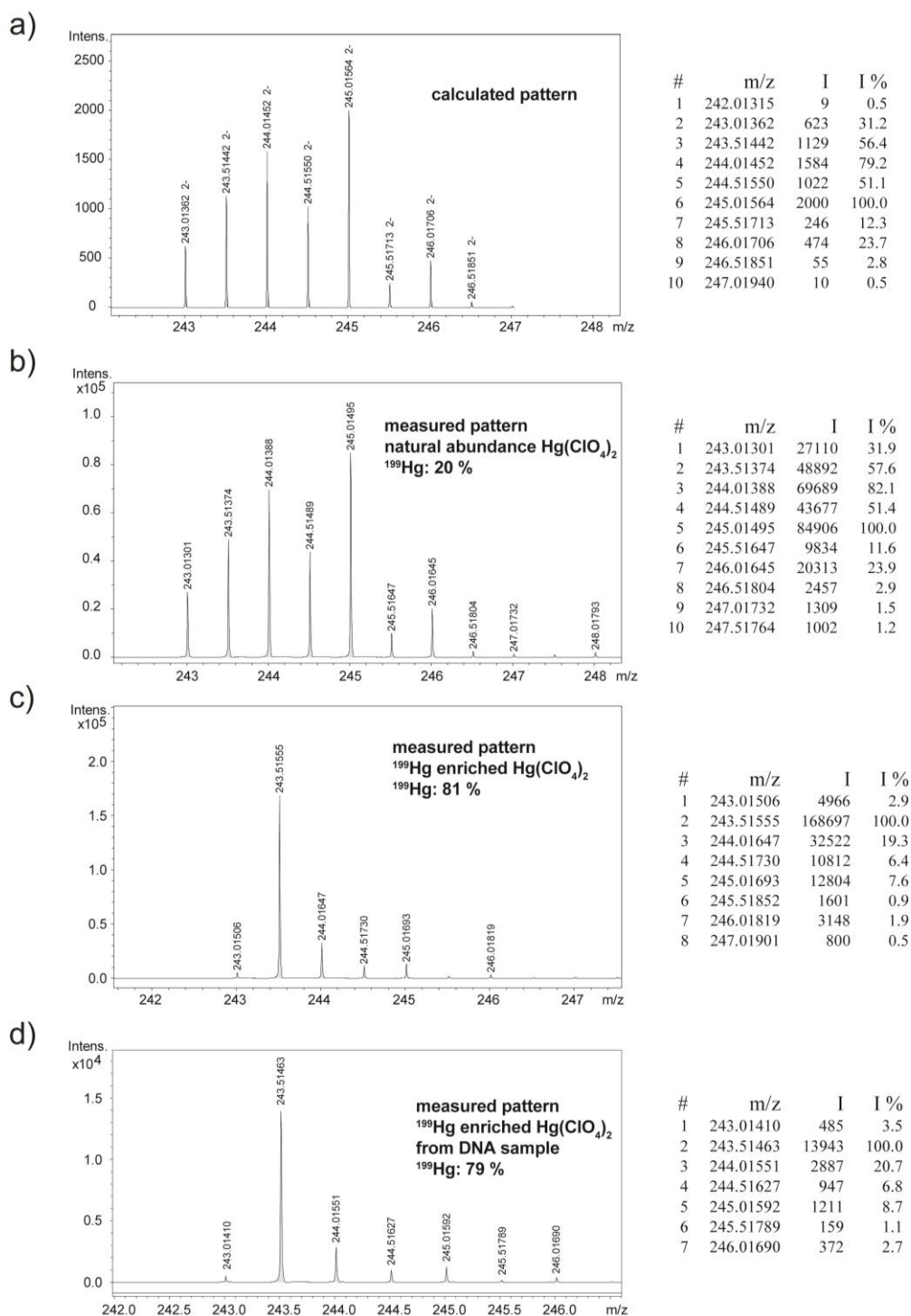
Supplementary Figure 10 | ^{15}N resonance assignment of major- and minor C- Hg^{II} -T coordination mode. a) Assignment of N1^{major} and N1^{minor} of thymidine and cytosine by 3J - and 2J ^1H , ^{15}N coupling between N1 and H5 and/or H6 in the long-range ^{15}N , ^1H -HSQC spectrum. b) Assignment of N3^{major} and N3^{minor} of cytosine according to 3J ^1H , ^{15}N coupling to $(\text{N4})\text{NH}_2^{\text{major}}$ and $(\text{N4})\text{NH}^{\text{minor}}$ in the band-selective, long-range ^{15}N , ^1H -HSQC spectrum at 25 °C. Blue indicates ^{15}N resonances of C* and green indicates ^{15}N resonances of T*. DNA samples contained 0.5 mM (a) and 1 mM (b) duplex DNA (ODN 1* “C*-T*”) and 1.5 equiv of $\text{Hg}(\text{ClO}_4)_2$ in aqueous buffer (200 mM NaClO_4 , 50 mM cacodylic acid in H_2O / D_2O (9:1) at pH = 7.8). Equiv of Hg^{II} are given relative to mismatch.



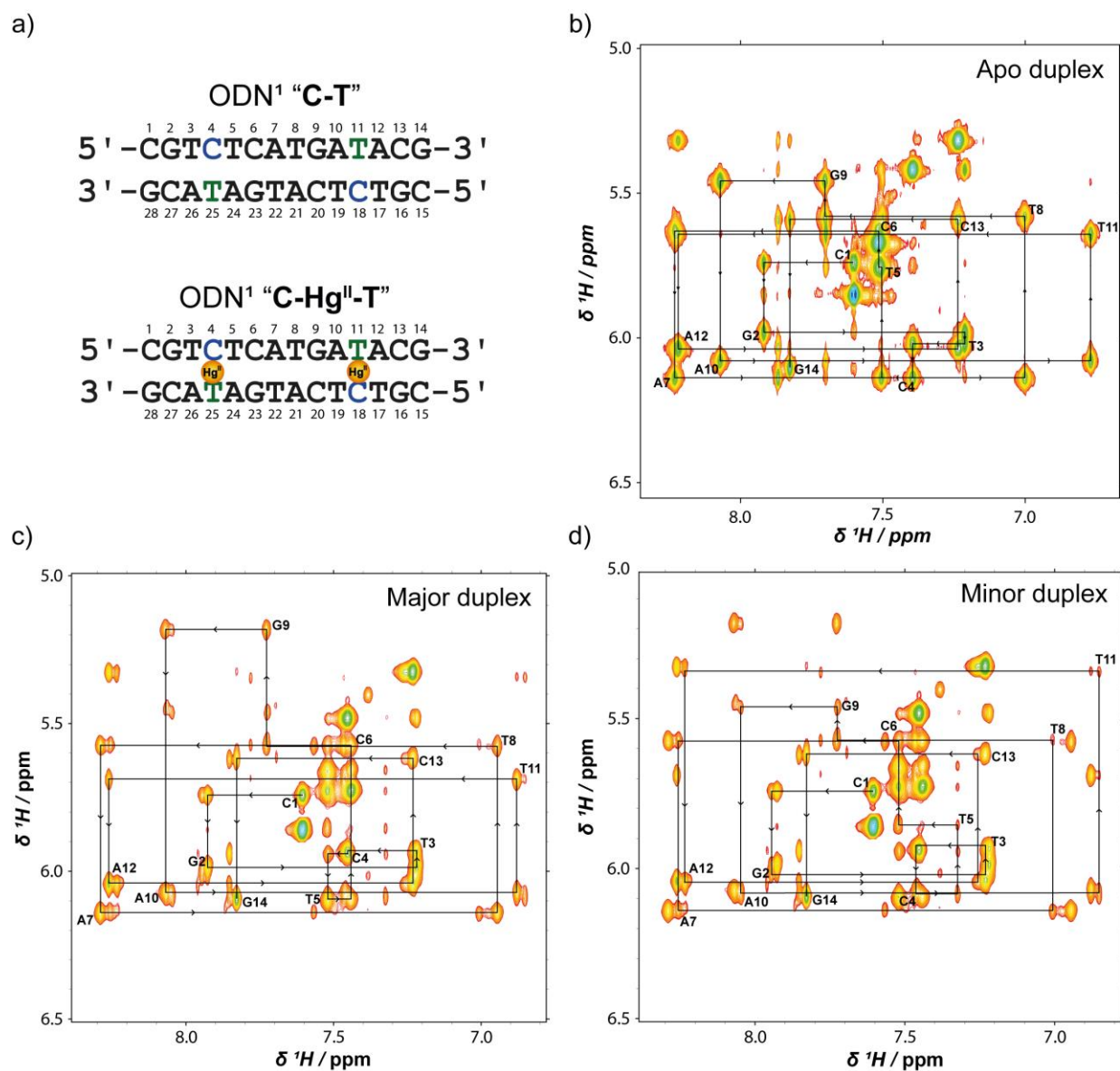
Supplementary Figure 11 | ^{15}N -coupled and ^{15}N -decoupled $[\text{}^1\text{H}, \text{H}]$ -NOESY spectra of duplex ODN^{1*} “ $\text{C}^* \cdot \text{Hg}^{\text{II}} \cdot \text{T}^*$ ”. NOE-cross peak between $(\text{N4})\text{NH}^{\text{minor}}$ and cytosine H5 confirms ^{15}N -resonance assignment as being the deprotonated exocyclic amine of cytosine. a) Chemical shift values of cytosine H5, $(\text{N4})\text{NH}_2^{\text{major}}$ and $(\text{N4})\text{NH}^{\text{minor}}$ derived from $[\text{}^{15}\text{N}, \text{H}]$ -HSQC and band-selective, long-range $[\text{}^{15}\text{N}, \text{H}]$ -HSQC spectra (Supplementary Figures 9 and 10). b) $\text{NH}_2, \text{H5}$ and $\text{NH}, \text{H5}$ NOE cross peaks and NH_2, NH exchange cross peaks observed in the ^{15}N -coupled (red) and ^{15}N -decoupled (blue) $[\text{H}, \text{H}]$ -NOESY spectra at 25 °C. The DNA sample contained 1 mM duplex DNA (ODN^{1*} “ $\text{C}^* \cdot \text{T}^*$ ”) and 3 mM of $\text{Hg}(\text{ClO}_4)_2$ (1.5 equiv Hg^{II} relative to mismatch) in aqueous buffer (200 mM NaClO_4 , 50 mM cacodylic acid in $\text{H}_2\text{O} / \text{D}_2\text{O}$ (9:1) at pH = 7.8).



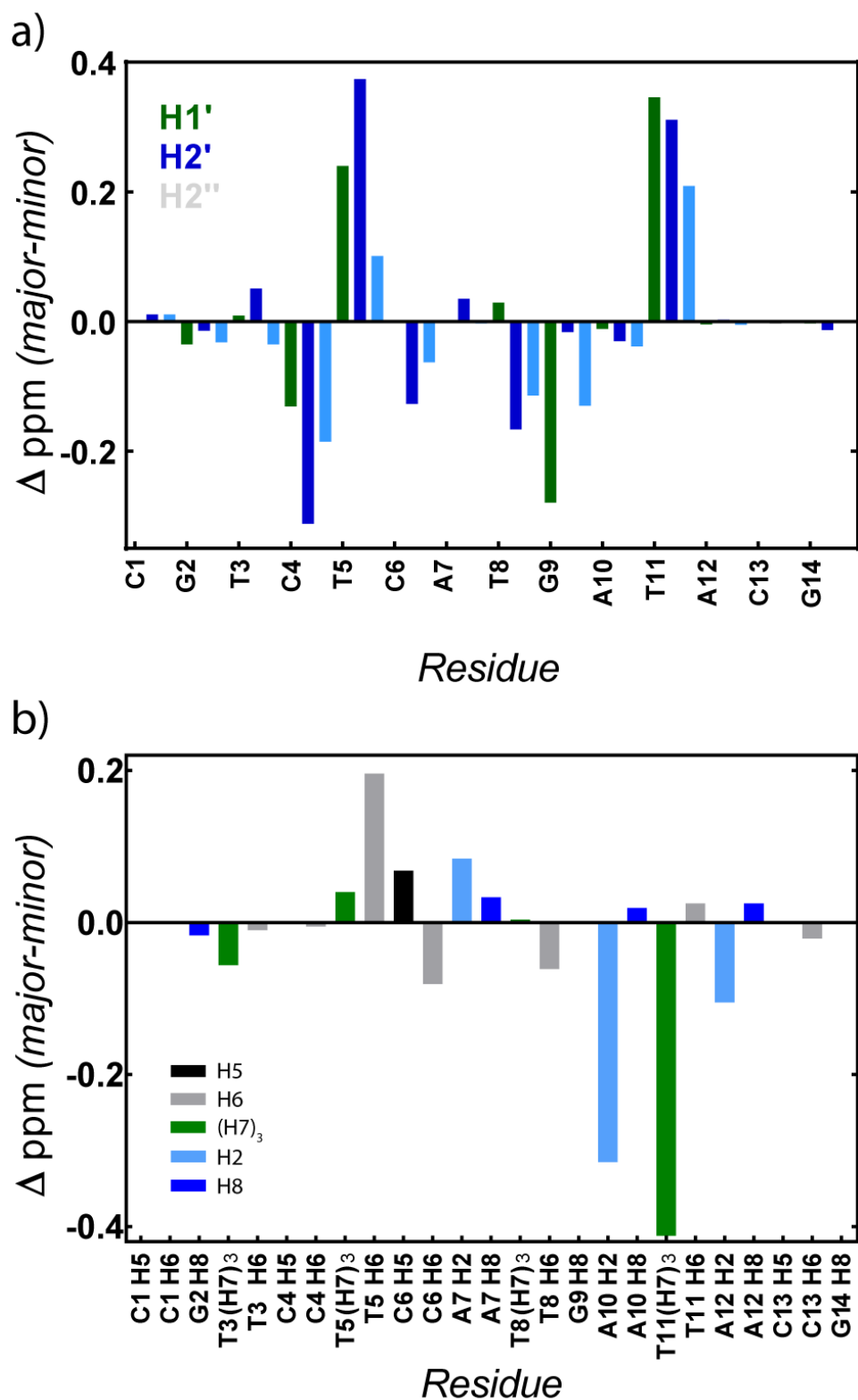
Supplementary Figure 12 | Unambiguous determination of metal-nucleobase connectivities. Changes in ^{15}N - and $^{15}\text{N}, ^1\text{H}$ -HSQC spectra of ODN1* "C*-T*" upon addition of ^{199}Hg -isotopically enriched $\text{Hg}(\text{ClO}_4)_2$ provided direct evidence for Hg^{II} -nucleobase coordination sites. a) $^1J\ ^{15}\text{N}, ^{199}\text{Hg}$ coupling according to ^{15}N NMR spectrum of ODN1* "C*-T*" with 1.5 equiv of ^{199}Hg -enriched $\text{Hg}(\text{ClO}_4)_2$ (79 % enriched, Supplementary Figure 13). b) $^1J\ ^{15}\text{N}, ^{199}\text{Hg}$ and $^2J\ ^1\text{H}, ^{199}\text{Hg}$ coupling according to $^{15}\text{N}, ^1\text{H}$ -HSQC. c) $^3J\ ^1\text{H}, ^{15}\text{N}$ coupling between N3^{major} and $(\text{N4})\text{NH}_2^{\text{major}}$ of cytosine and $^3J\ ^1\text{H}, ^{15}\text{N}$ coupling between N3^{minor} and deprotonated $(\text{N4})\text{NH}^{\text{minor}}$ of cytosine observed in the band-selective, long-range $^{15}\text{N}, ^1\text{H}$ -HSQC spectrum. Blue indicates ^{15}N resonances of C* and green indicates ^{15}N resonances of T*. The DNA sample contained 1 mM duplex DNA and 3 mM of ^{199}Hg -enriched $\text{Hg}(\text{ClO}_4)_2$ (1.5 equiv Hg^{II} relative to mismatch) in aqueous buffer (200 mM NaClO_4 , 50 mM cacodylic acid in $\text{H}_2\text{O} / \text{D}_2\text{O}$ (9:1) at pH = 7.8), and the spectra were measured at 4 °C.



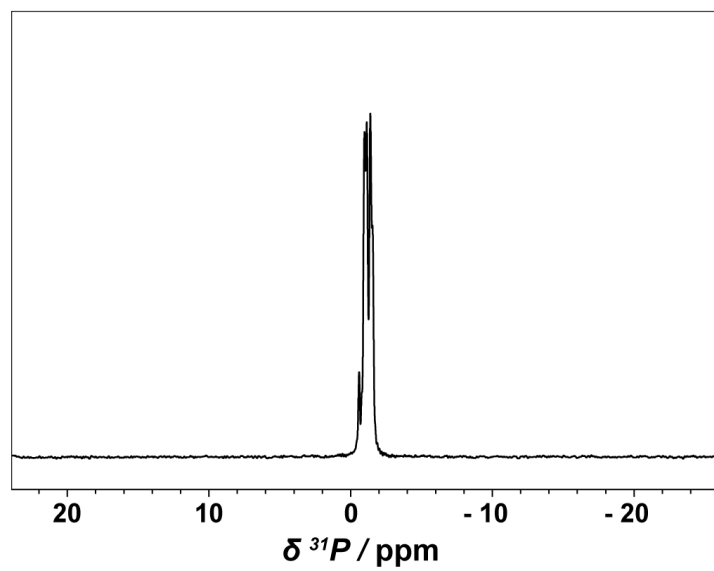
Supplementary Figure 13 | HR-ESI-MS analysis of Hg^{II} -EDTA $^{2-}$ complex. a) Calculated isotopic pattern of Hg^{II} -EDTA $^{2-}$. b) Measured isotopic pattern of natural abundance Hg^{II} -EDTA $^{2-}$. c) Measured isotopic pattern of ^{199}Hg -enriched Hg^{II} -EDTA $^{2-}$. d) Measured isotopic pattern of ^{199}Hg -enriched Hg^{II} -EDTA $^{2-}$ from “C*-T*” duplex DNA.



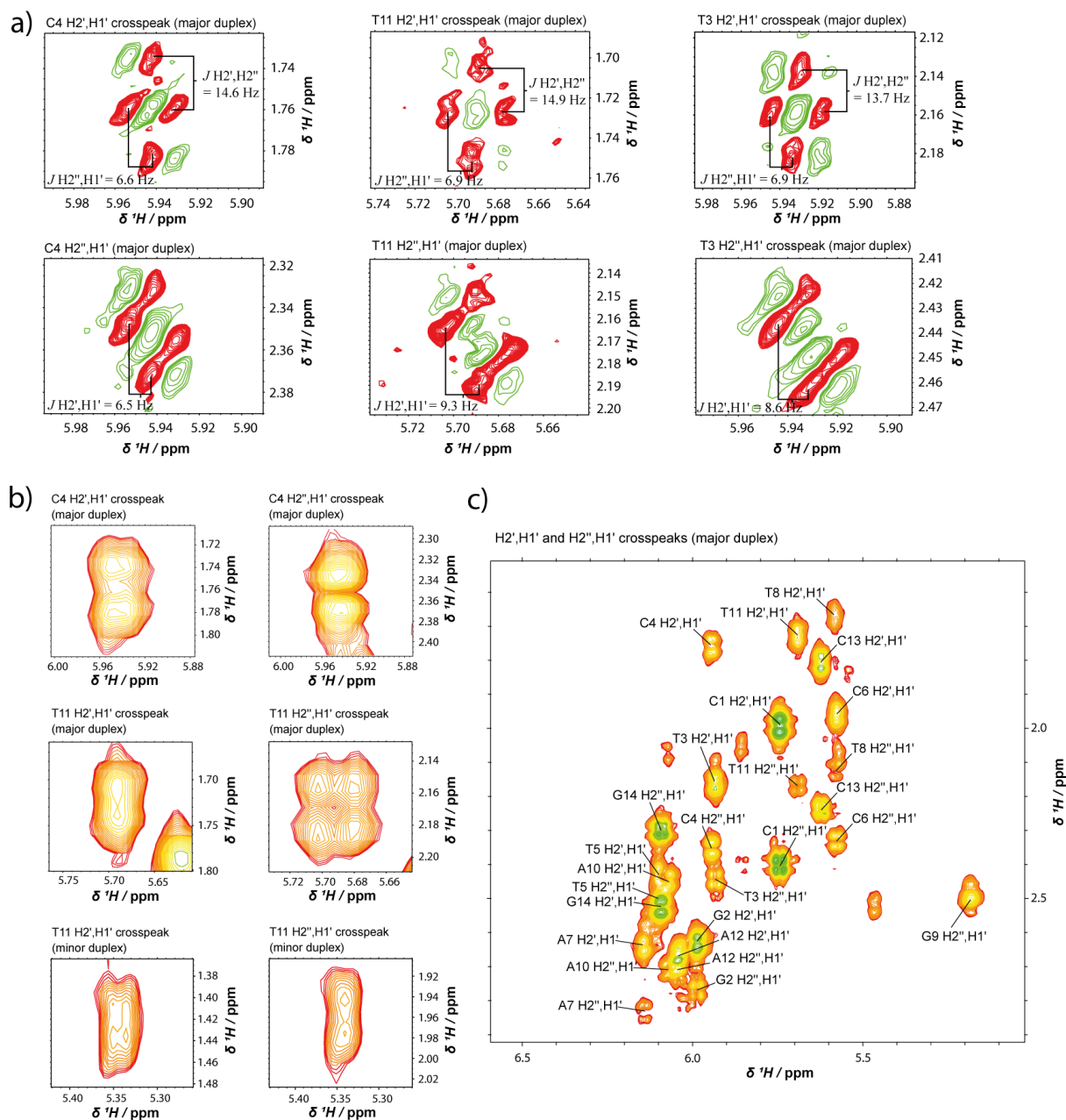
Supplementary Figure 14 | Sequential walking along H1' and aromatic protons in $[^1\text{H}, ^1\text{H}]$ -NOESY spectra. a) ODN¹ “C-T” and ODN¹ “C-Hg^{II}-T” sequence. Blue and green bases indicate C-T mismatches. Sequential walking along H1' and aromatic protons in the $[^1\text{H}, ^1\text{H}]$ -NOESY spectra through the entire sequence from residues C1 to G14 for apo- (b), major- (c) and minor (d) duplex DNA. The DNA sample of the apo duplex (ODN¹ “C-T”) contained 0.3 mM duplex DNA in an aqueous solution of NaClO₄ (50 mM, D₂O, pD = 7.75) and was measured at 25 °C. The DNA sample of the “C-Hg^{II}-T” contained 0.4 mM duplex DNA (ODN¹ “C-T”) and 1.2 mM Hg^{II} (1.5 equiv Hg^{II} relative to mismatch) in an aqueous solution of NaClO₄ (50 mM, D₂O, pD = 7.75) and was measured at 25 °C.



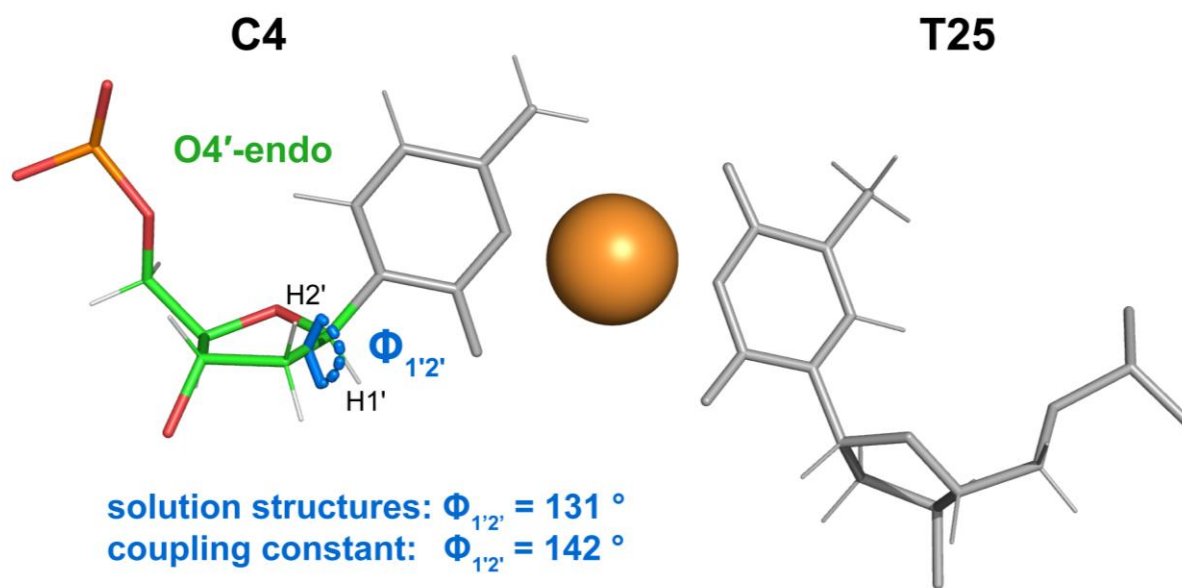
Supplementary Figure 15 | Chemical shift differences between major and minor duplexes. a) Chemical shift difference of H1' (green), H2' (blue), and H2'' (grey) sugar proton signals, and b) of aromatic nucleobase signals H5 (black), H6 (grey), (H7)₃ (green), H2 (light blue), and H8 (dark blue). Δ ppm calculated as ppm(major) - ppm(minor). The DNA sample contained 0.4 mM duplex DNA (ODN¹ "C-T") and 1.2 mM Hg^{II} (1.5 equiv Hg^{II} relative to mismatch) in an aqueous solution of NaClO₄ (50 mM, D₂O, pD = 7.75) and was measured at 25 °C. Source data are provided as a Source Data file.



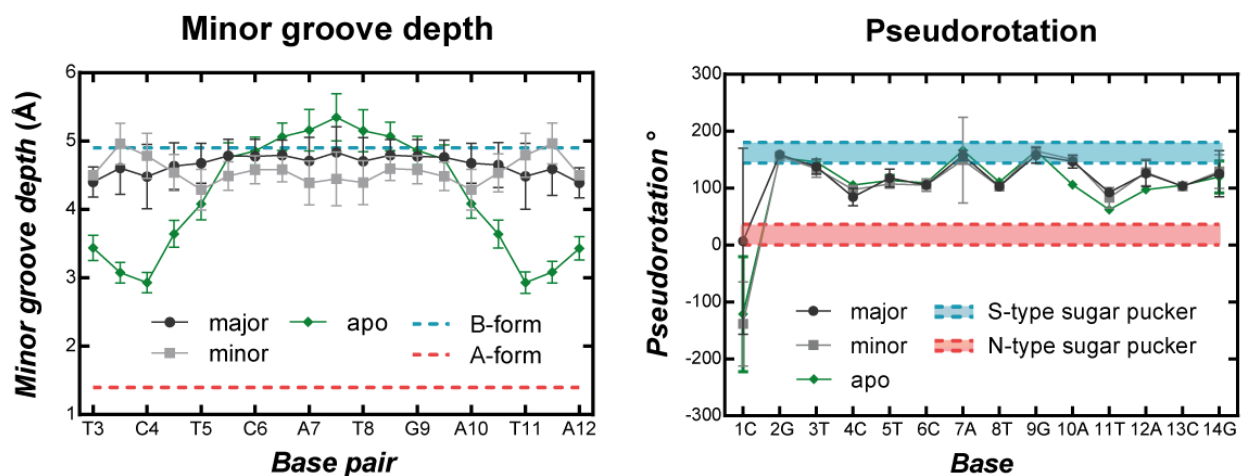
Supplementary Figure 16 | ^{31}P NMR of duplex ODN¹ “C-T”. The DNA sample contained 0.4 mM duplex DNA and 1.2 mM Hg^{II} (1.5 equiv Hg^{II} relative to mismatch) in an aqueous solution of NaClO_4 (50 mM, D_2O , $\text{pD} = 7.75$) at 25 °C.



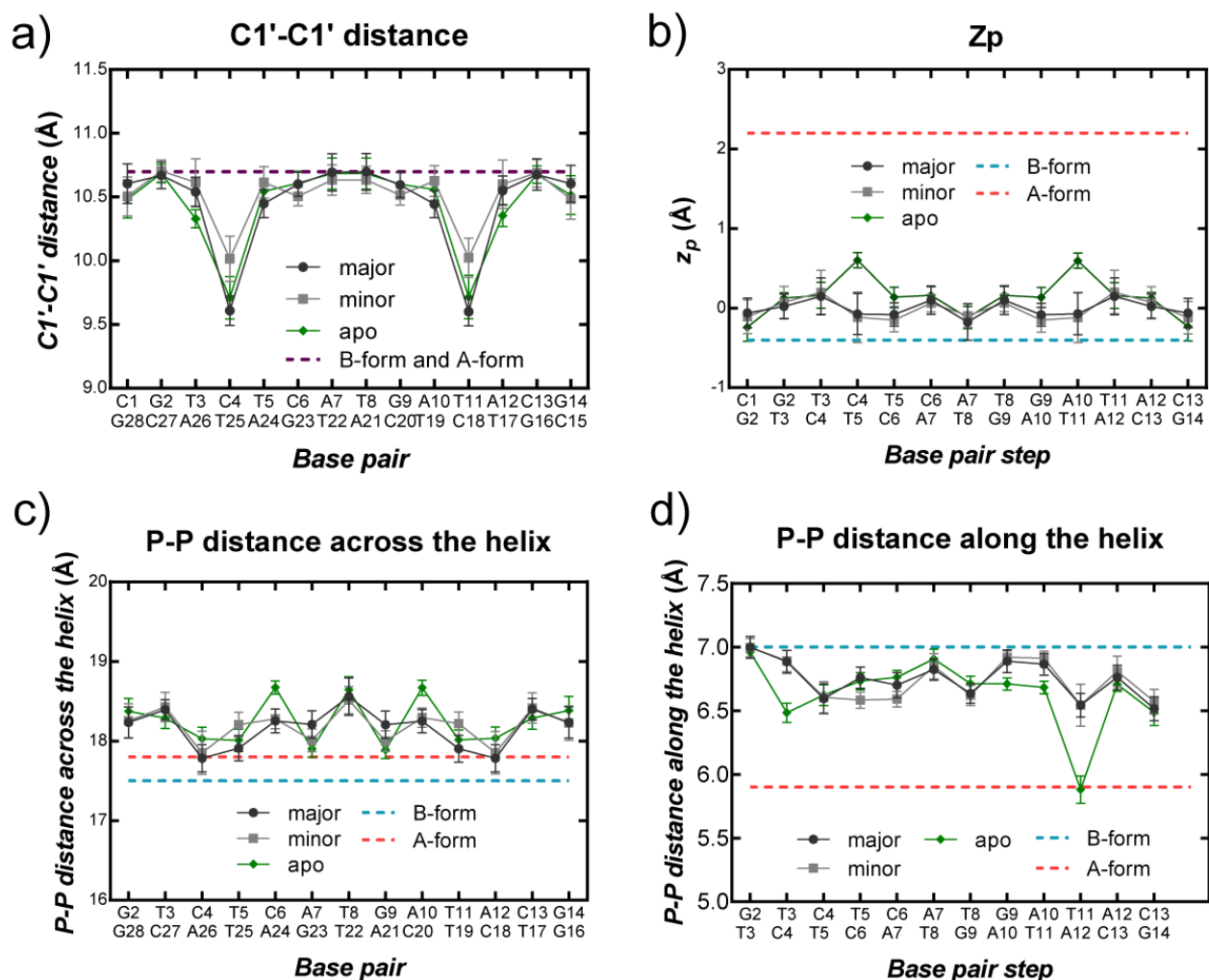
Supplementary Figure 17 | [$^1\text{H}, ^1\text{H}$]-E.COSY and magnitude-processed [$^1\text{H}, ^1\text{H}$]-COSY. a) Selected H_1', H_2' and $\text{H}_1', \text{H}_2''$ cross peaks in the [$^1\text{H}, ^1\text{H}$]-E.COSY spectrum used to determine coupling constants for Hg^{II} -bound residues C4 and T11 and coupling constants of T3 as a selected example for a Hg^{II} unbound residue. b) H_1', H_2' and $\text{H}_1', \text{H}_2''$ cross peaks in the magnitude processed [$^1\text{H}, ^1\text{H}$]-COSY spectrum for Hg^{II} -coordinated residues. c) H_1', H_2' and $\text{H}_1', \text{H}_2''$ cross peaks of the major duplex in the magnitude processed [$^1\text{H}, ^1\text{H}$]-COSY spectrum illustrating similar shape of T11 cross peaks of the minor duplex species and C4 cross peaks of the major duplex species. The DNA sample contained 0.4 mM duplex DNA (ODN¹ “C-T”) and 1.2 mM Hg^{II} (1.5 equiv Hg^{II} relative to mismatch) in an aqueous solution of NaClO_4 (50 mM, D_2O , pD = 7.75) and was measured at 25 °C.



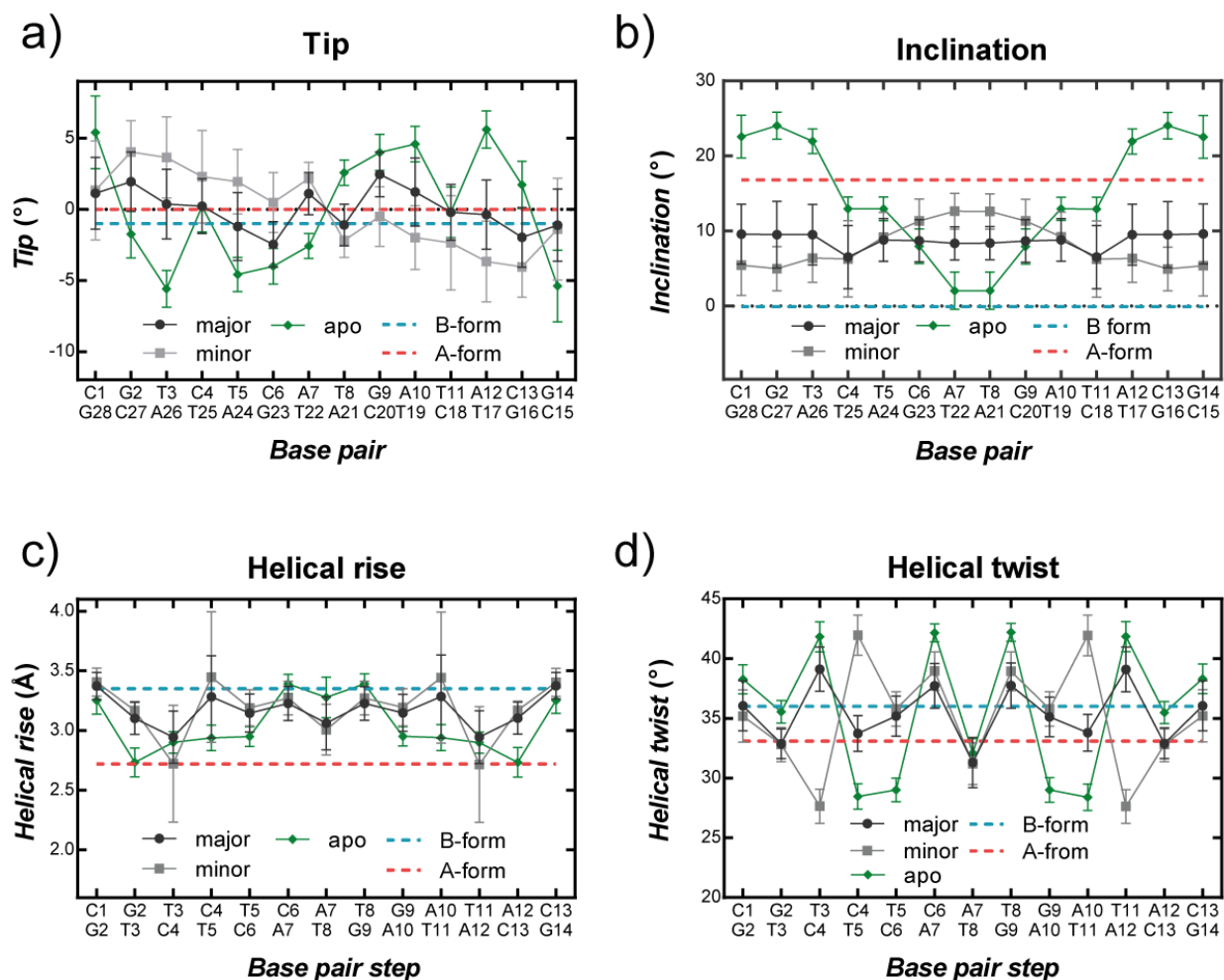
Supplementary Figure 18 | O4'-endo sugar pucker at the Hg^{II}-bound C4 residue of the major duplex species. The dihedral angle ($\Phi_{1'2'} = 131^\circ \pm 5^\circ$) determined from solution structures was in excellent agreement with the dihedral angle calculated from the experimentally determined coupling constant ($\Phi_{1'2'} = 142^\circ$) according to the Karplus relationship (Supplementary Equation 1).^{2,3} This value is inconsistent with both C2'-endo ($^3J_{H1',H2'} = 9.5 \text{ Hz}$)⁴ and C3'-endo ($^3J_{H1',H2'} = 1.5 \text{ Hz}$)⁴ sugar conformations (Supplementary Table 5). According to the Karplus relationship a pseudorotation phase angle (P)² of 85° and 202° was calculated for the Hg^{II}-coordinated cytosine residue of the major duplex species. A pseudorotation phase angle of 85° corresponds to an O4'-endo sugar pucker.^{2,5} $\Phi_{1'2'}$ determined from the solution structures represents the mean value and standard deviation of the 20 lowest energy conformations. Figure was generated with PyMol.⁶



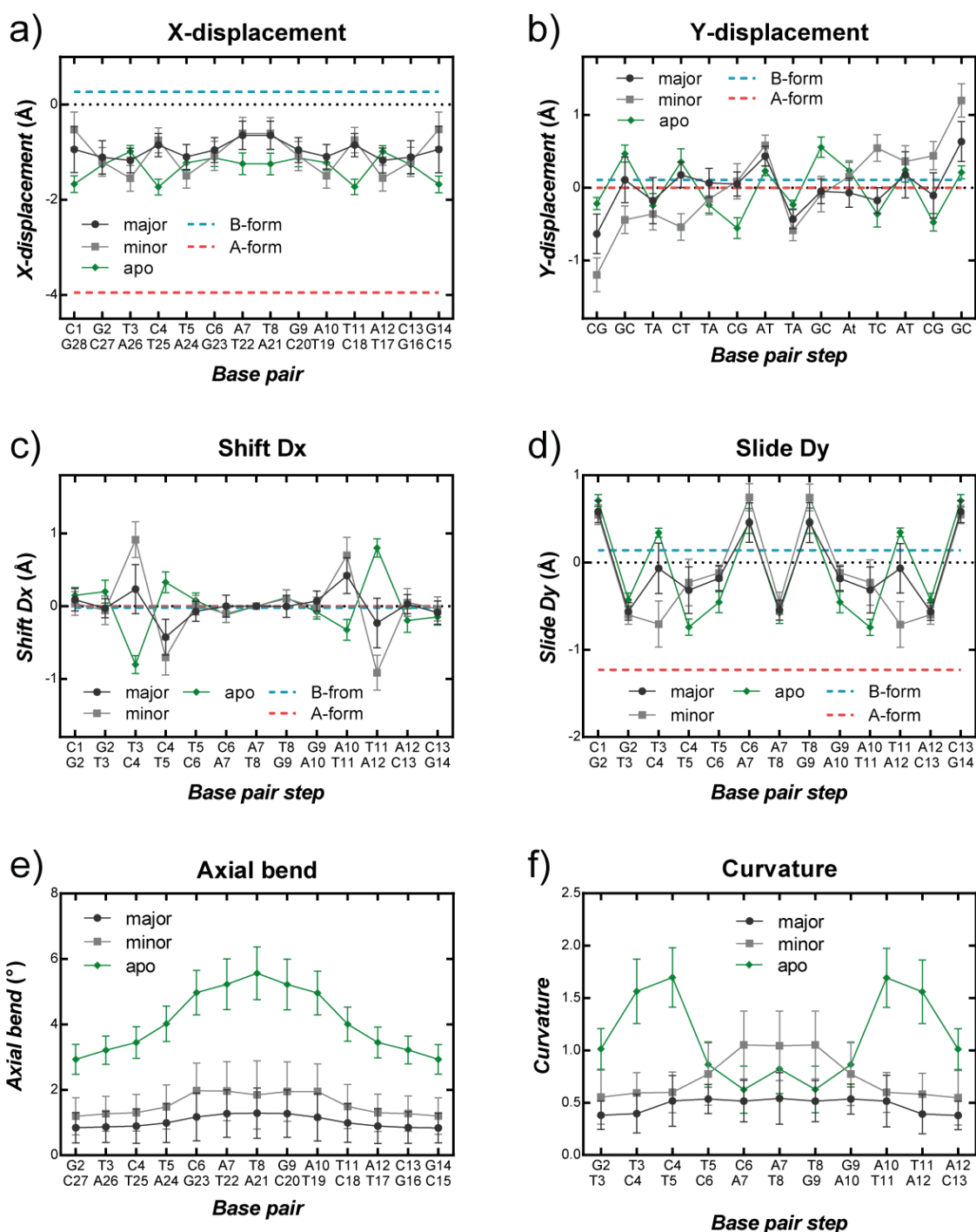
Supplementary Figure 19 | Minor groove depth and pseudorotation phase angles. Parameters were analyzed using Curves⁺ and represent mean and standard deviation of the 20 lowest energy conformations of major- (black), minor- (grey) and apo (green) duplex species. Reference A-form (red) and B-form (blue) values for pseudorotation were taken from (ref 8) and for minor groove depth from (ref 7) which analyzed crystal structures of A-DNA (PDB ID 1d13⁹) and B-DNA (PDB ID 1bna¹⁰) dodecamers. Source data are provided as a Source Data file.



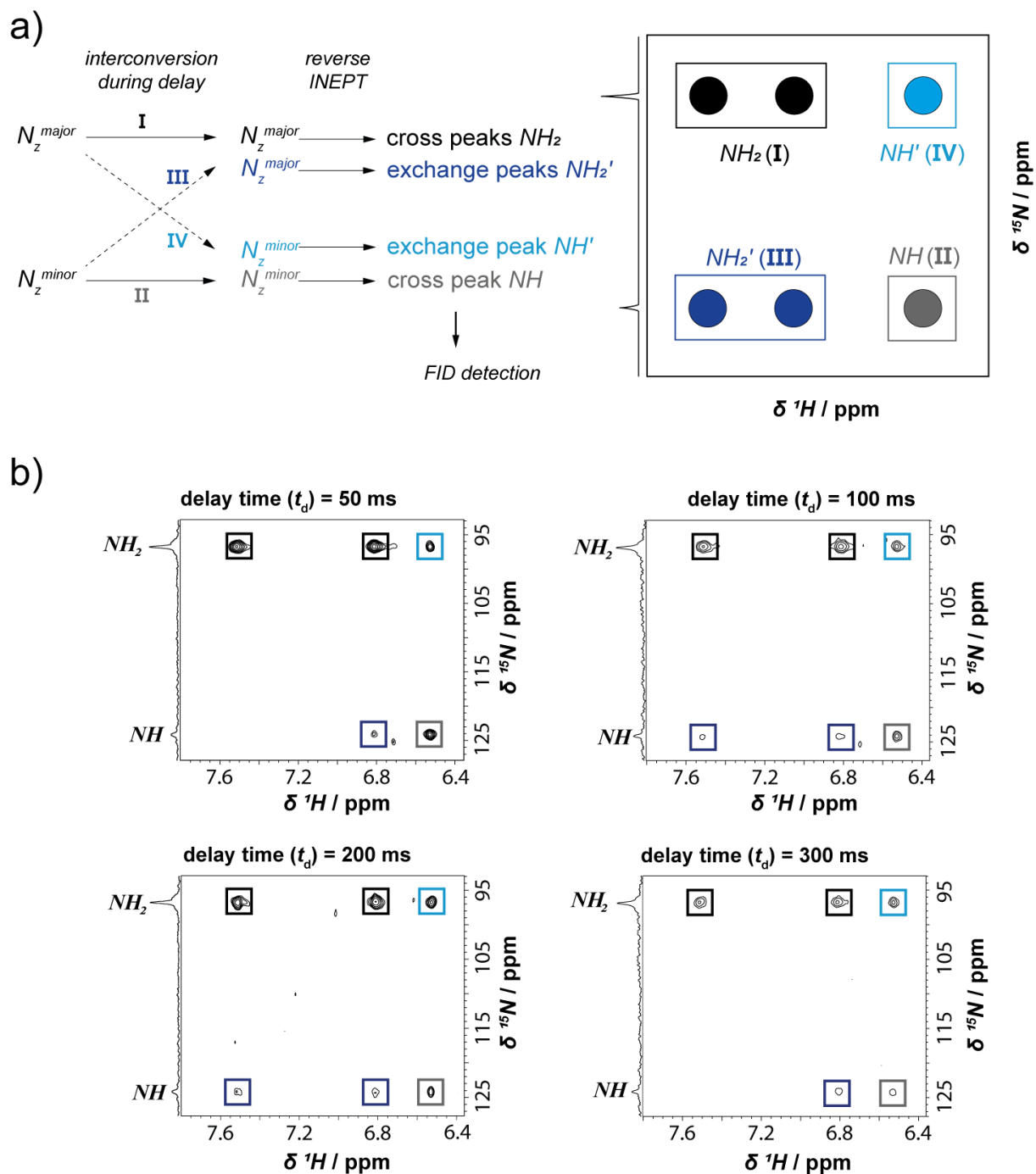
Supplementary Figure 20 | Helical parameters. a) C1'- C1' distances in each base pair. b) Displacement of phosphate group from the middle of a base pair step (Z_p). c) P-P distance across the helix. d) P-P distance along the helix. C1'- C1' distance, phosphate displacement, and P-P distance along the helix were calculated using 3DNA (a-b)^{11,12} and P-P distance across the helix were determined using PyMOL.⁶ Standard A-form (red) and B-form (blue) values for C1'- C1' distance and phosphate displacement were taken from (ref 13) and (ref 14), respectively. Reference A-form and B-form values for P-P distance across the helix were determined by generating an ideal A-form and B-form using scfbio-iitd software¹⁵ and for P-P distance along the helix from (ref 11,12). Reported values are mean and standard deviation of the 20 lowest energy conformations of major- (black), minor- (grey) and apo (green) duplex species. Source data are provided as a Source Data file.



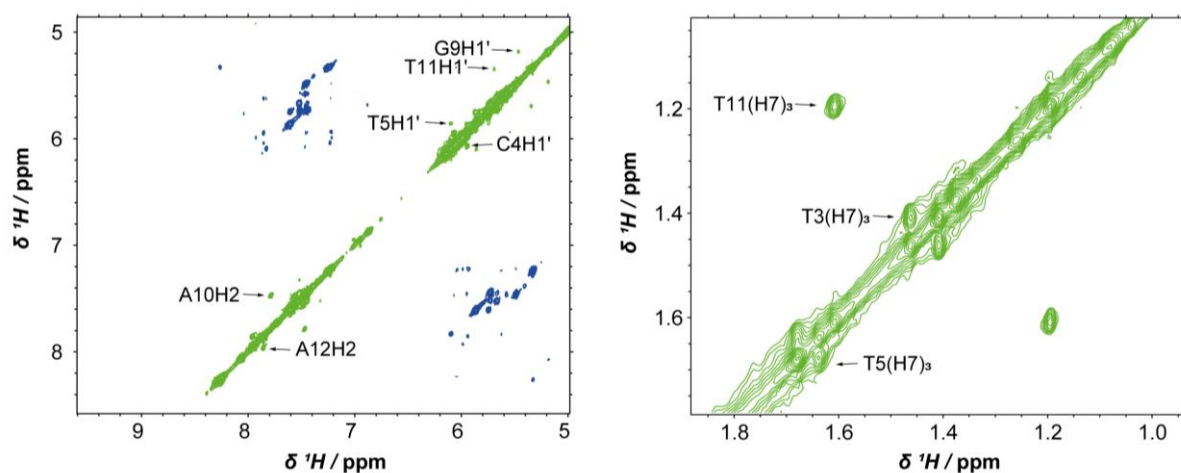
Supplementary Figure 21 | Base pair-axis parameters tip and inclination and helical parameters helical rise and helical twist. a) tip (rotation around the long axis of the base pair) and b) inclination of base pairs with respect to the helical axis (rotation around the short axis of the base pair). c) Helical rise and d) helical twist angles. All parameters were analyzed using Curves⁺⁷ and represent mean and standard deviation of the 20 lowest energy conformations of major- (black), minor- (grey) and apo (green) duplex species. Reference A-form (red) and B-form (blue) values were taken from (ref 7) which analyzed crystal structures of A-DNA (PDB ID 1d13⁹) and B-DNA (PDB ID 1bna¹⁰) dodecamers. Source data are provided as a Source Data file.



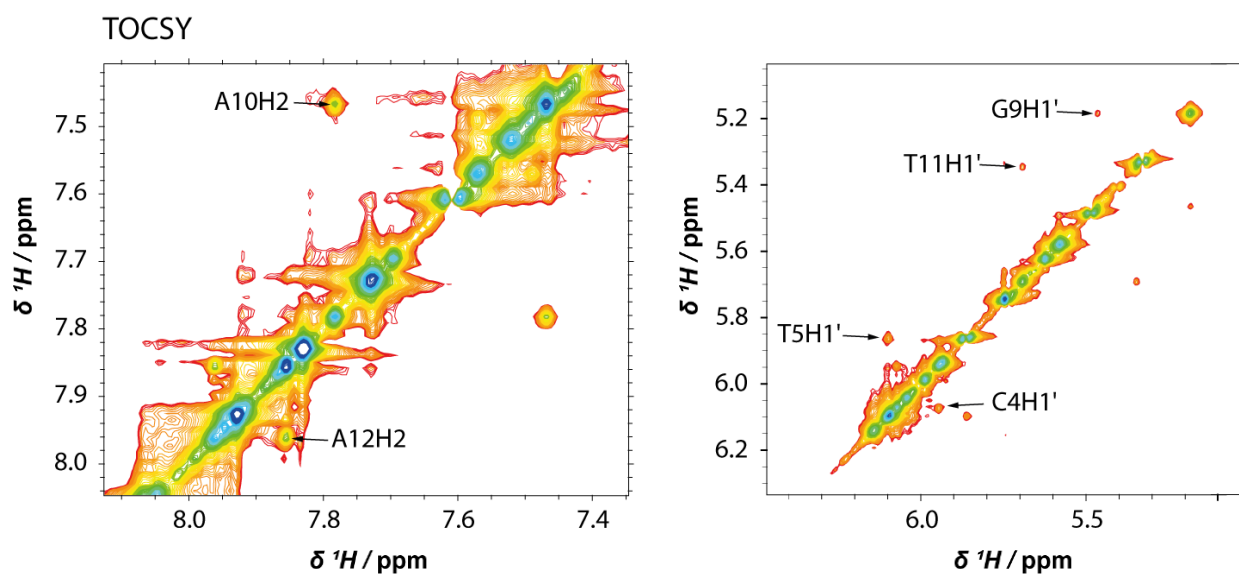
Supplementary Figure 22 | Base pair parameters X- and Y-displacement, shift and slide and curvature analysis. a) Translation of base pair towards grooves (a, x-displacement) and perpendicular to the grooves (b, y-displacement). Local dimer step parameters shift (c, Dx) and slide (d, Dy) describing relative positioning of successive base pairs with respect to the x- and y-axis of the relative base pair reference frame. e) axial bend and f) curvature analysis of major-, minor-, and apo duplexes. Curvature is a dimensionless quantity normalizing the curvature to DNA on the nucleosome (curvature ~ 1).¹⁶ All parameters were analyzed using Curves⁺ and represent mean and standard deviation of the 20 lowest energy conformations of major- (black), minor- (grey) and apo (green) duplex species. Reference A-form (red) and B-form (blue) values were taken from (ref 7) which analyzed crystal structures of A-DNA (PDB ID 1d13⁹) and B-DNA (PDB ID 1bna¹⁰) dodecamers. Source data are provided as a Source Data file.



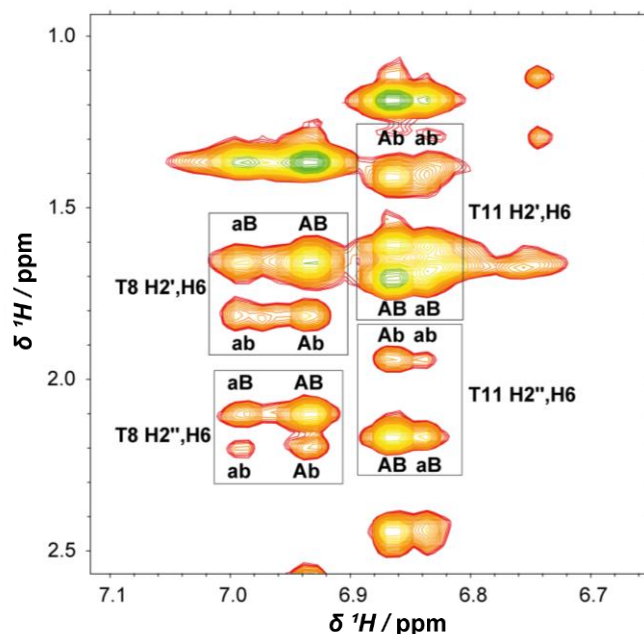
Supplementary Figure 23 | Dynamic changes in local metal ion coordination. a) Schematic description of a $[^{15}\text{N},^1\text{H}]$ -HSQC experiment with a delay introduced before the reverse INEPT showing all possible ^{15}N z-z exchange during delay (I-IV), followed by INEPT transfer. Exchange I and II give cross peaks NH_2 (black) and NH (grey), corresponding to those of $[^{15}\text{N},^1\text{H}]$ -HSQC experiments without delay. Interconversion of major- and minor structures during the delay results in exchange III and IV to give exchange cross peaks NH_2' (dark blue) and NH' (light blue). b) Interconversion of major- and minor structures according to exchange crosspeaks in $[^{15}\text{N},^1\text{H}]$ -HSQC spectra with various delay times before reverse INEPT at 25 °C. The DNA sample contained 0.5 mM duplex DNA (ODN¹ “C-T”) and 1.5 mM of $\text{Hg}(\text{ClO}_4)_2$ (1.5 equiv Hg^{II} relative to mismatch) in aqueous buffer (200 mM NaClO_4 , 50 mM cacodylic acid in $\text{H}_2\text{O} / \text{D}_2\text{O}$ (9:1) at pH = 7.8).



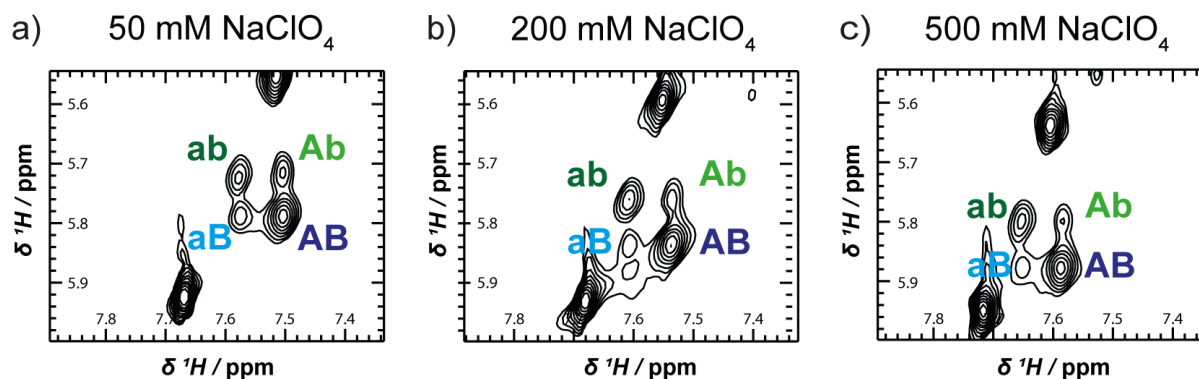
Supplementary Figure 24 | Sections of the $[^1\text{H}, ^1\text{H}]$ -ROESY spectrum of ODN¹ “C-T”. Exchange signals can be distinguished from ROE cross peaks by the phase. ROE cross peaks are opposite in phase to the diagonal and exchange cross peaks in phase to the diagonal. The DNA sample contained 0.4 mM duplex DNA and 1.2 mM Hg^{II} (1.5 equiv Hg^{II} relative to mismatch) in an aqueous solution of NaClO_4 (50 mM, D_2O , $\text{pD} = 7.75$) and was measured at 25 °C.



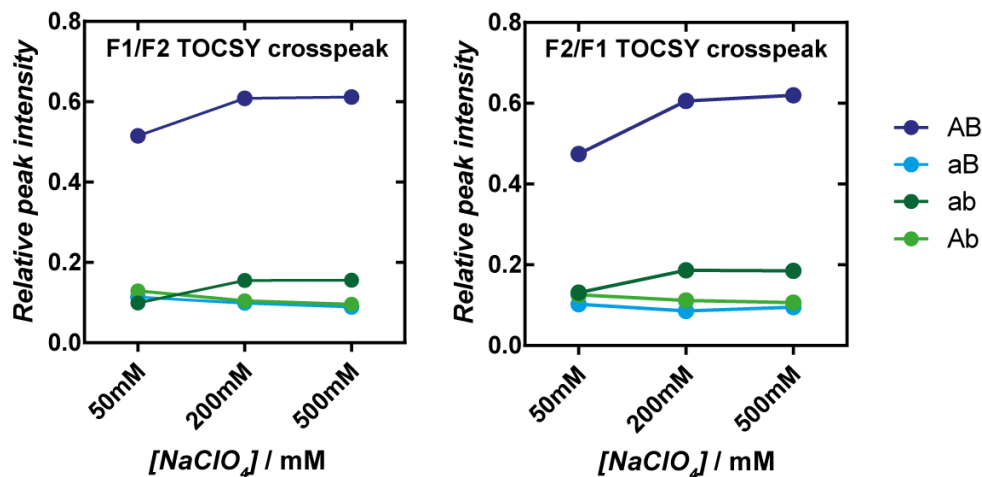
Supplementary Figure 25 | Exchange cross peaks in $[^1\text{H}, ^1\text{H}]$ -TOCSY spectrum. The DNA sample contained 0.4 mM duplex DNA (ODN¹ “C-T”) and 1.2 mM $\text{Hg}(\text{ClO}_4)_2$ (1.5 equiv Hg^{II} relative to mismatch) in an aqueous solution of NaClO_4 (50 mM, D_2O , $\text{pD} = 7.75$) and was measured at 25 °C.



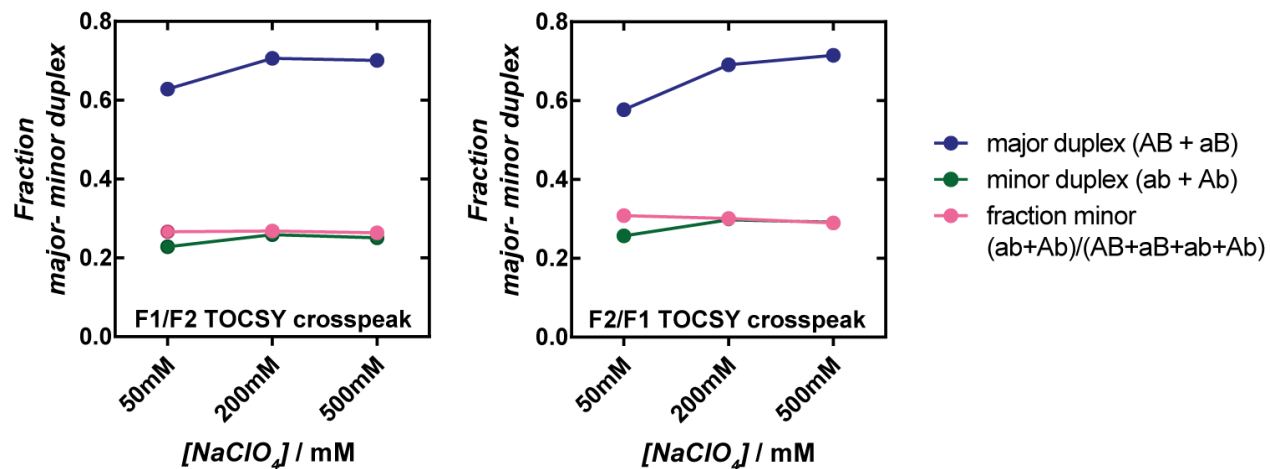
Supplementary Figure 26 | Exchange-mediated NOE cross peaks in the [^1H , ^1H]-NOESY spectrum. ‘AB’ and ‘ab’ are defined as NOE cross peaks of major- and minor duplex species, respectively, and ‘Ab’ and ‘aB’ exchange-mediated NOE cross peaks (Supplementary Figure 36). The DNA sample contained 1 mM duplex DNA (ODN¹ “C-T”) and 3 mM of ^{199}Hg -enriched $\text{Hg}(\text{ClO}_4)_2$ (1.5 equiv $^{199}\text{Hg}^{\text{II}}$ relative to mismatch) in aqueous buffer (200 mM NaClO_4 , 50 mM cacodylic acid in H_2O / D_2O (9:1) at pH = 7.8) and was measured at 25 °C.



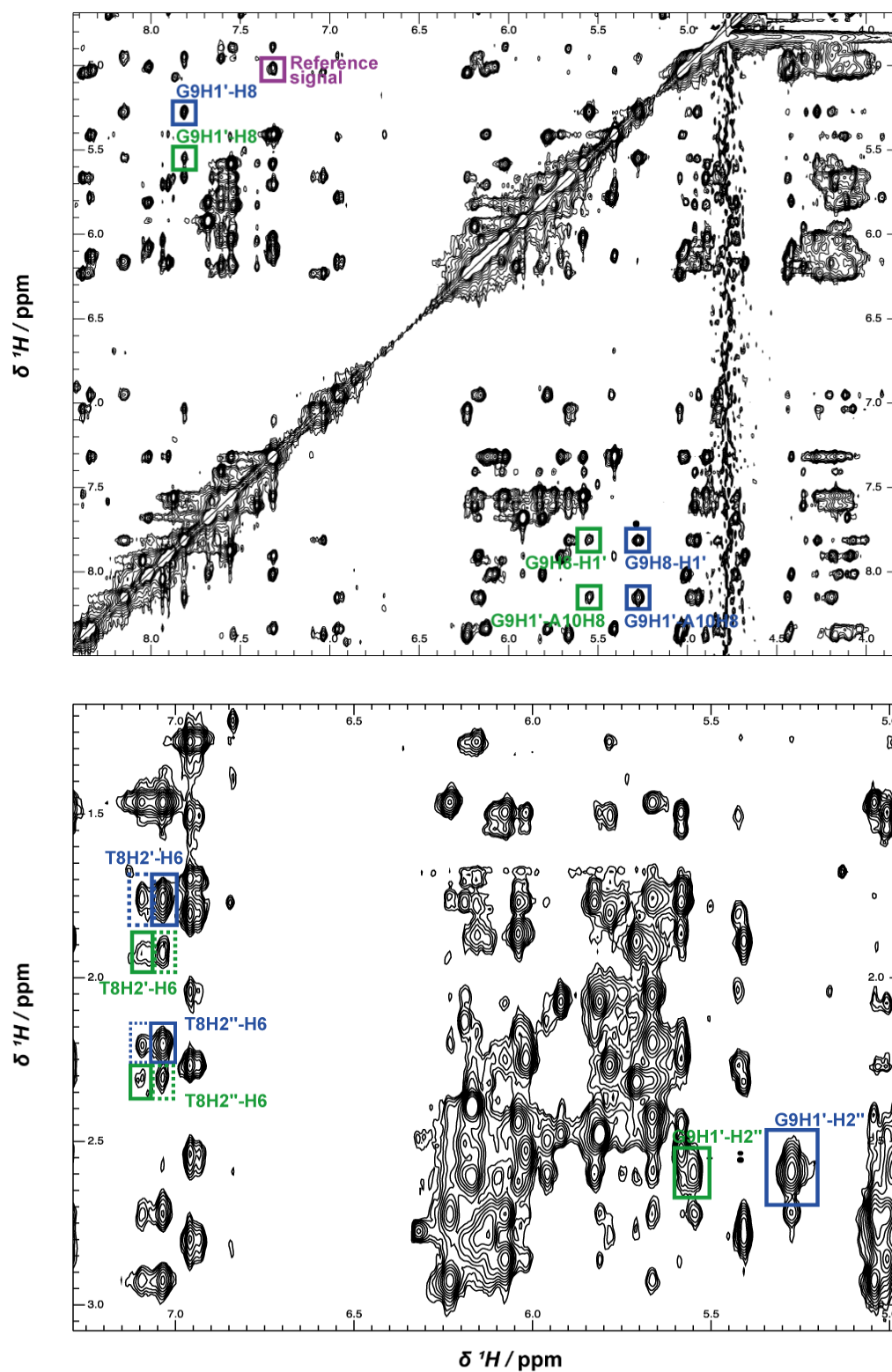
Supplementary Figure 27 | Decrease in overall exchange rate of major- and minor duplexes with increasing ionic strength. Cytosine C4 H5/H6 [^1H , ^1H]-TOCSY resonances of major- (AB, dark blue) and minor (ab, dark green) and their exchange-mediated cross peaks (aB, light blue) and (Ab, light green) in the presence of a) 50 mM NaClO_4 , b) 200 mM NaClO_4 , and c) 500 mM NaClO_4 . Decreasing signal intensities of the exchange-mediated cross peaks (aB and Ab) compared to [^1H , ^1H]-TOCSY cross peaks (AB and ab) revealed a slower overall interconversion rate of the helices with increasing ionic strength. The DNA samples contained 0.96 mM duplex DNA and 2.88 mM of $\text{Hg}(\text{ClO}_4)_2$ (1.5 equiv Hg^{II} relative to mismatch) in aqueous buffer (50 mM, 200 mM or 500 mM NaClO_4 , 50 mM cacodylic acid in H_2O / D_2O (9:1) at pH = 7).



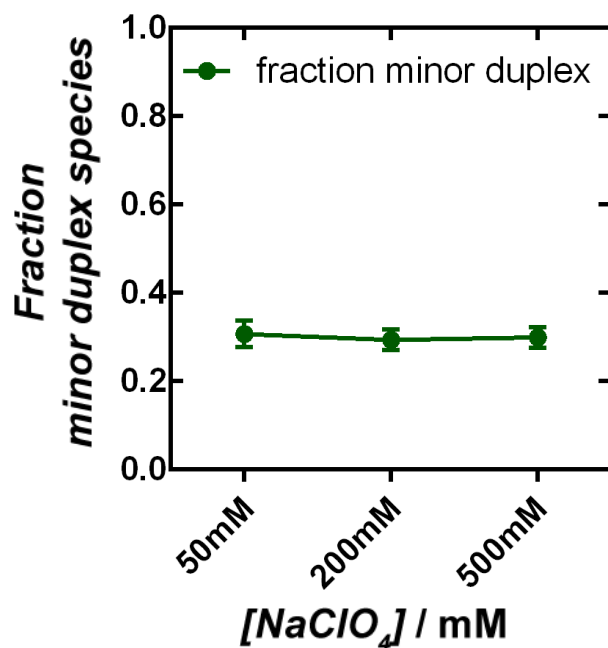
Supplementary Figure 28 | Relative peak intensities of F1/F2- and F2/F1 cross peaks at variable ionic strength. For definitions of labels see Supplementary Figure 27. Source data are provided as a Source Data file.



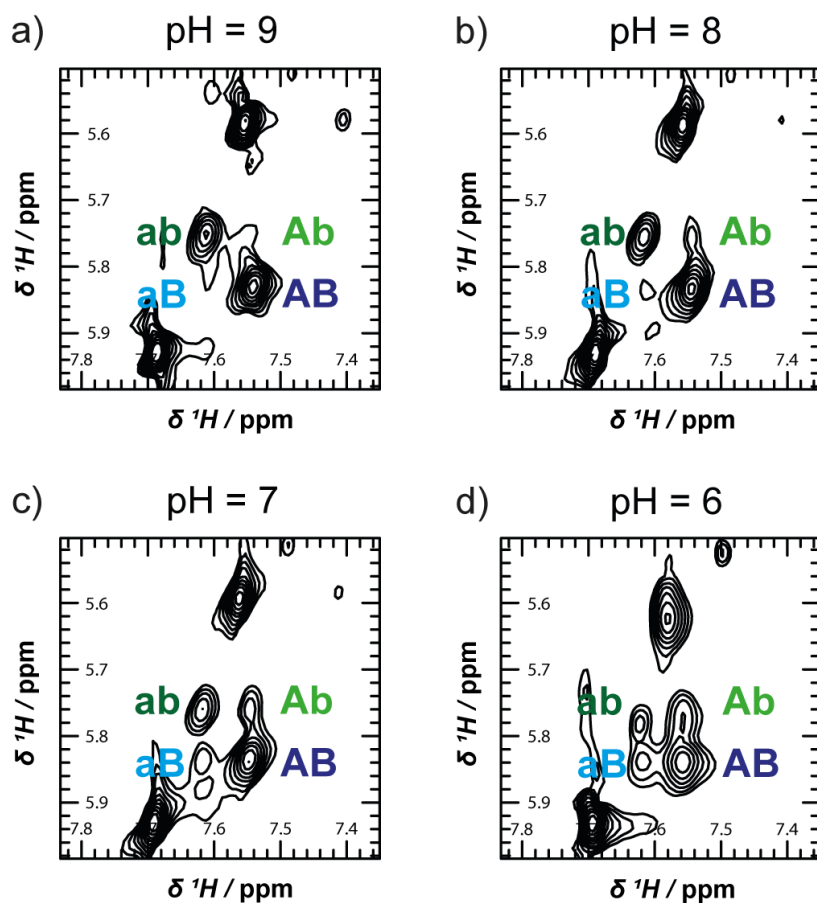
Supplementary Figure 29 | Fraction minor duplex species at variable ionic strength. Sum of integrals of the major- (AB + aB, blue) and minor species (ab + Ab, green), and the fraction minor species ((ab + Ab)/(AB + aB + ab + Ab), pink) of F1/F2 and F2/F1 cross peaks at variable ionic strength. Source data are provided as a Source Data file.



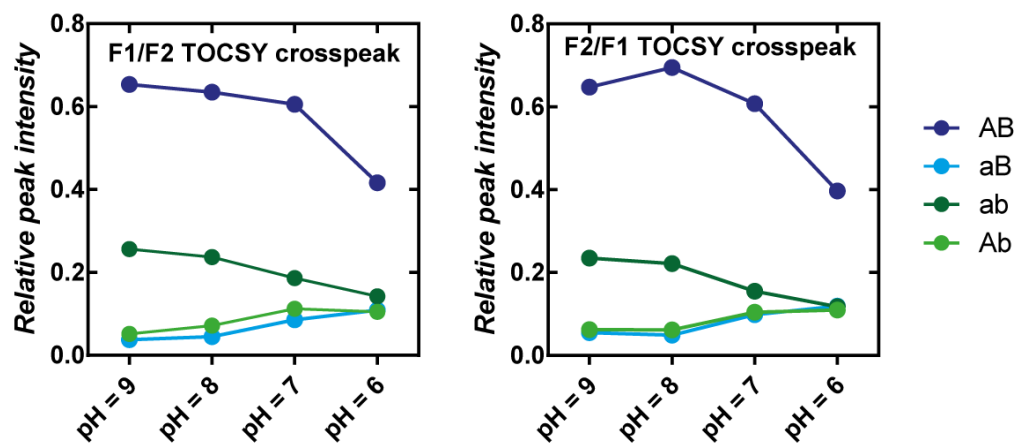
Supplementary Figure 30 | NOE cross peaks used to determine the fraction of minor duplex species at variable ionic strength. NOE cross peaks of the major duplex species are depicted in blue and of the minor duplex species in green. NOE cross peak of the selected reference signal (A12H3' – C13H6) is depicted in purple. The DNA samples contained 0.96 mM duplex DNA and 2.88 mM of $\text{Hg}(\text{ClO}_4)_2$ (1.5 equiv Hg^{II} relative to mismatch) in aqueous buffer (50 mM, 200 mM or 500 mM NaClO_4 , 50 mM cacodylic acid in $\text{H}_2\text{O} / \text{D}_2\text{O}$ (9:1) at pH = 7).



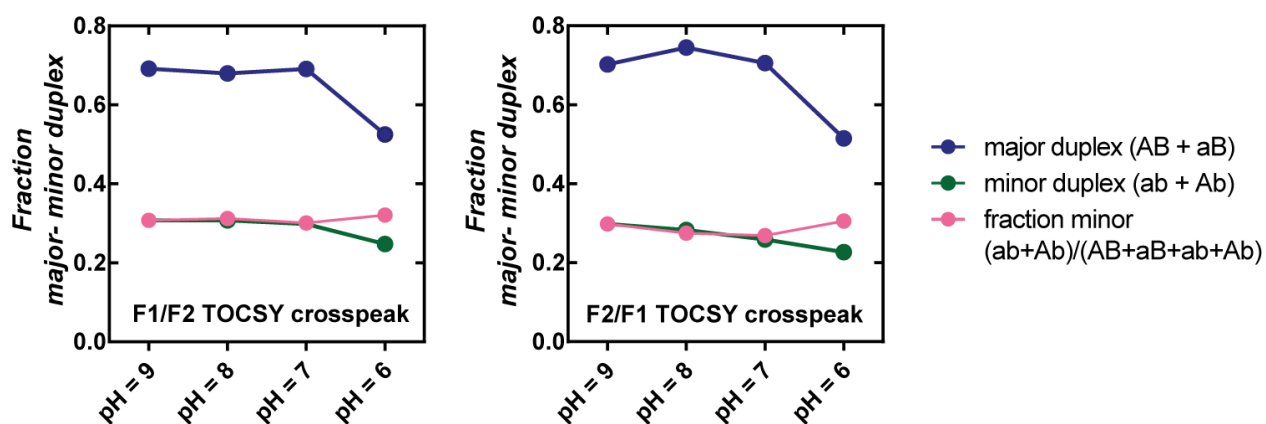
Supplementary Figure 31 | Fraction minor duplex species at variable ionic strength. Six well resolved NOE signals in the [¹H,¹H]-NOESY spectra of metallo duplex samples were used to determine fraction minor duplex species (Supplementary Figure 30). The DNA samples contained 0.96 mM duplex DNA and 2.88 mM of Hg(ClO₄)₂ (1.5 equiv Hg^{II} relative to mismatch) in aqueous buffer (50 mM, 200 mM or 500 mM NaClO₄, 50 mM cacodylic acid in H₂O / D₂O (9:1) at pH = 7). The data represent the mean value from six NOE signals and standard deviation is indicated by error bars. Source data are provided as a Source Data file.



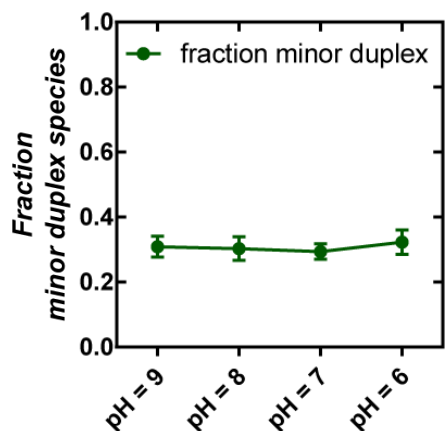
Supplementary Figure 32 | Decrease in the overall exchange rate of major- and minor duplexes with increasing pH. Cytosine C4 H5/H6 [^1H , ^1H]-TOCSY resonances of major- (AB, dark blue) and minor (ab, dark green) and their exchange-mediated cross peaks (aB, light blue) and (Ab, light green) at a) pH = 9, b) pH = 8, c) pH = 7, and d) pH = 6. Decreasing signal intensities of the exchange-mediated cross peaks (aB and Ab) compared to [^1H , ^1H]-TOCSY cross peaks (AB and ab) revealed a slower overall interconversion rate of the helices with increasing pH. DNA samples contained 0.76 mM duplex DNA and 2.28 mM of $\text{Hg}(\text{ClO}_4)_2$ (1.5 equiv Hg^{II} relative to mismatch) in aqueous buffer (200 mM NaClO_4 , 50 mM cacodylic acid in $\text{H}_2\text{O} / \text{D}_2\text{O}$ (9:1) at pH = 6 – 9).



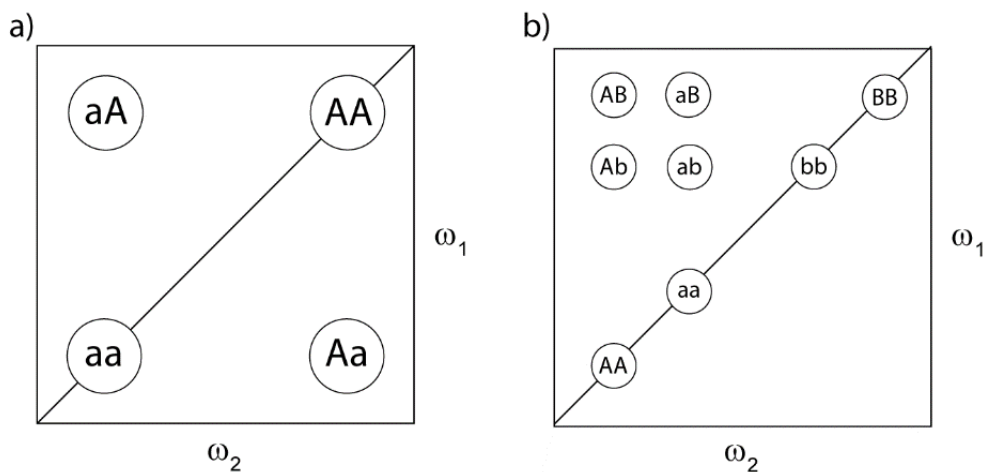
Supplementary Figure 33 | Relative peak intensities of F1/F2- and F2/F1 cross peaks at variable pH. For definitions of labels see Supplementary Figure 32. Source data are provided as a Source Data file.



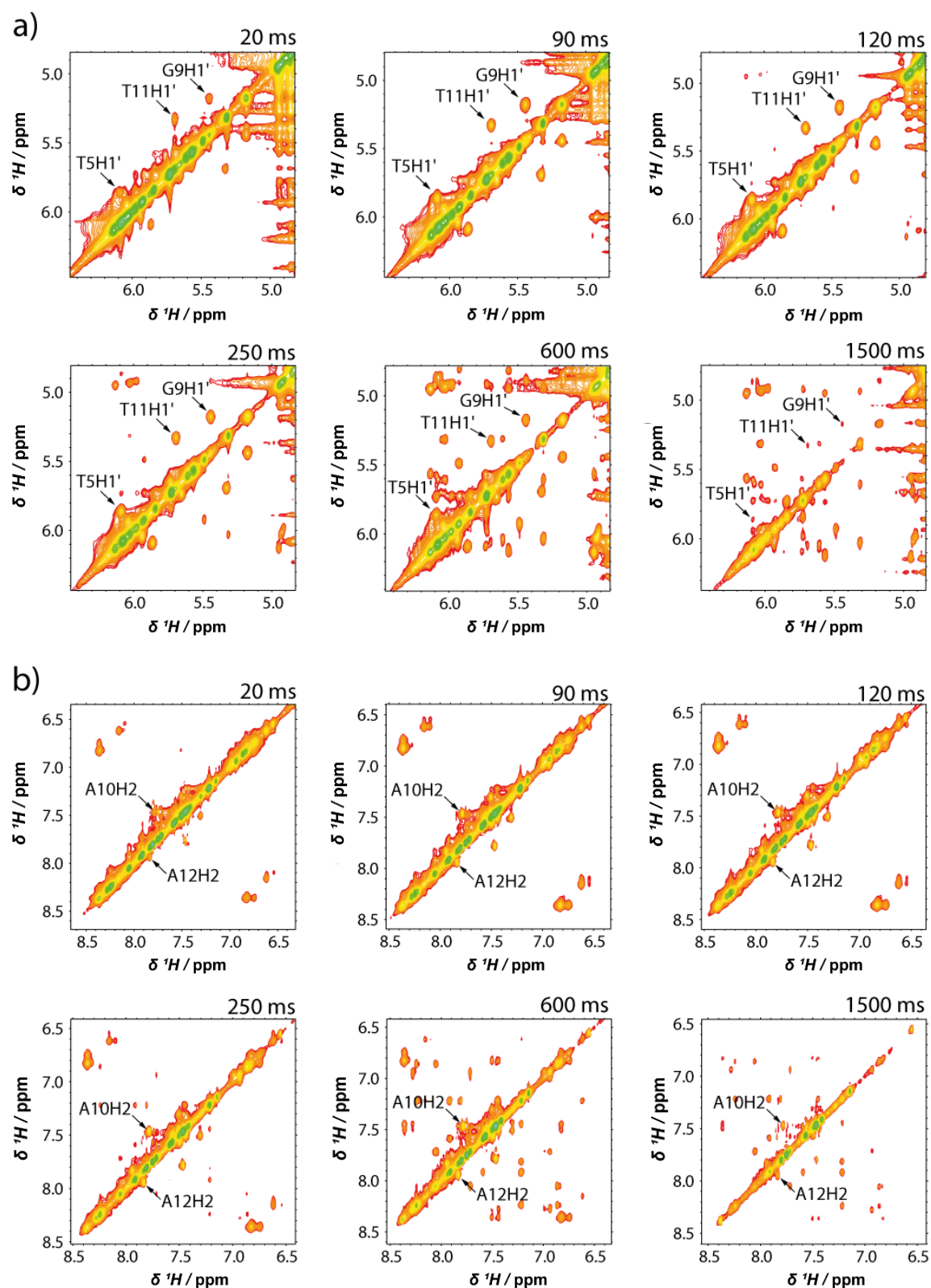
Supplementary Figure 34 | Fraction minor duplex species at variable pH. Sum of integrals of the major- (AB + aB, blue) and minor species (ab + Ab, green), and the fraction minor species $((ab + Ab)/(AB + aB + ab + Ab))$, pink of the F1/F2- and F2/F1 cross peaks at variable pH. Source data are provided as a Source Data file.



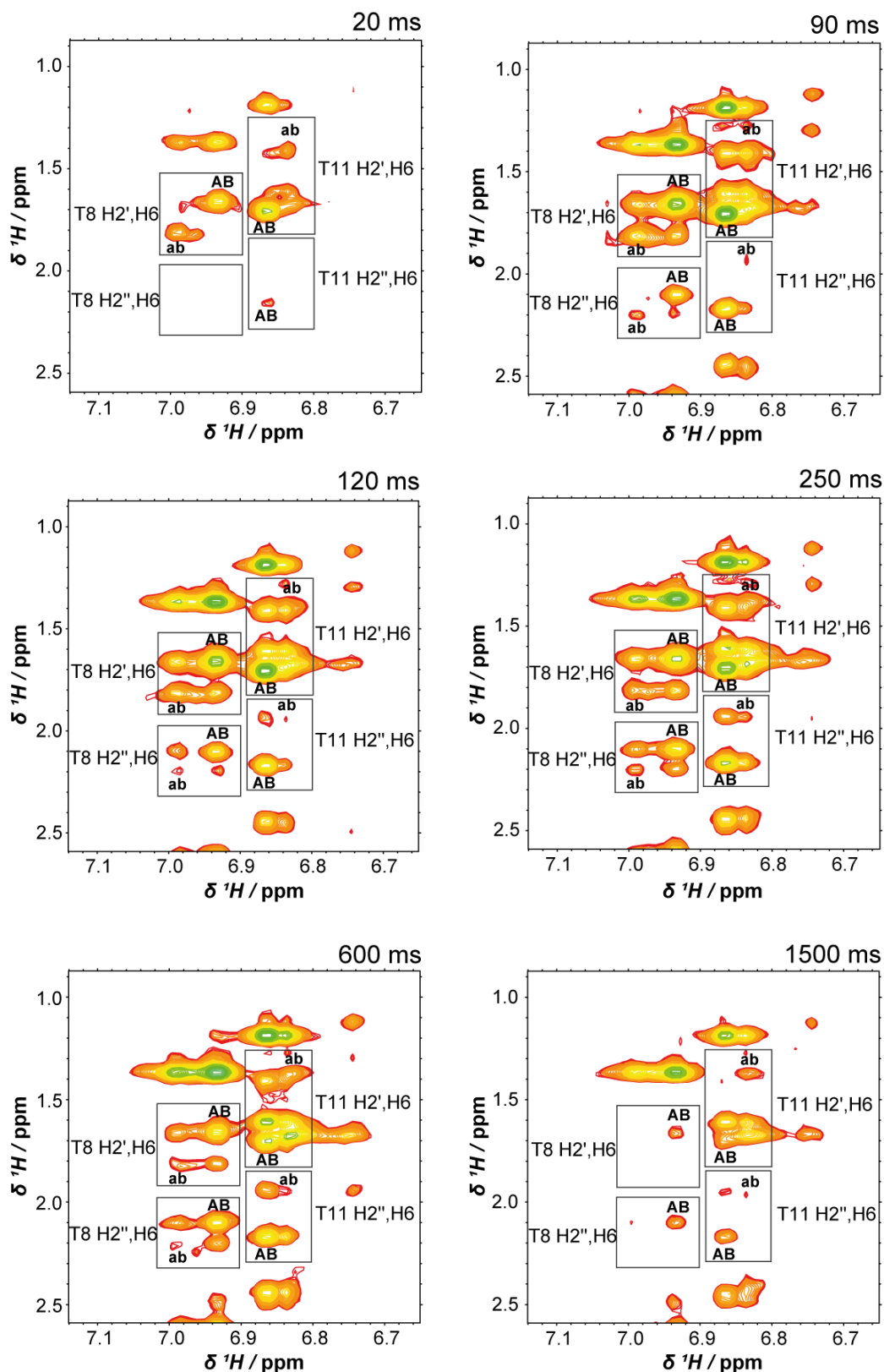
Supplementary Figure 35 | Fraction minor duplex species at variable pH. Six well resolved NOE signals in the [^1H , ^1H]-NOESY spectra of metallo duplex samples were used to determine fraction minor duplex species (Supplementary Figure 30). DNA samples contained 0.76 mM duplex DNA and 2.28 mM of $\text{Hg}(\text{ClO}_4)_2$ (1.5 equiv Hg^{II} relative to mismatch) in aqueous buffer (200 mM NaClO_4 , 50 mM cacodylic acid in H_2O / D_2O (9:1) at pH = 6 – 9). The data represent the mean value from six NOE signals and standard deviation is indicated by error bars. Source data are provided as a Source Data file.



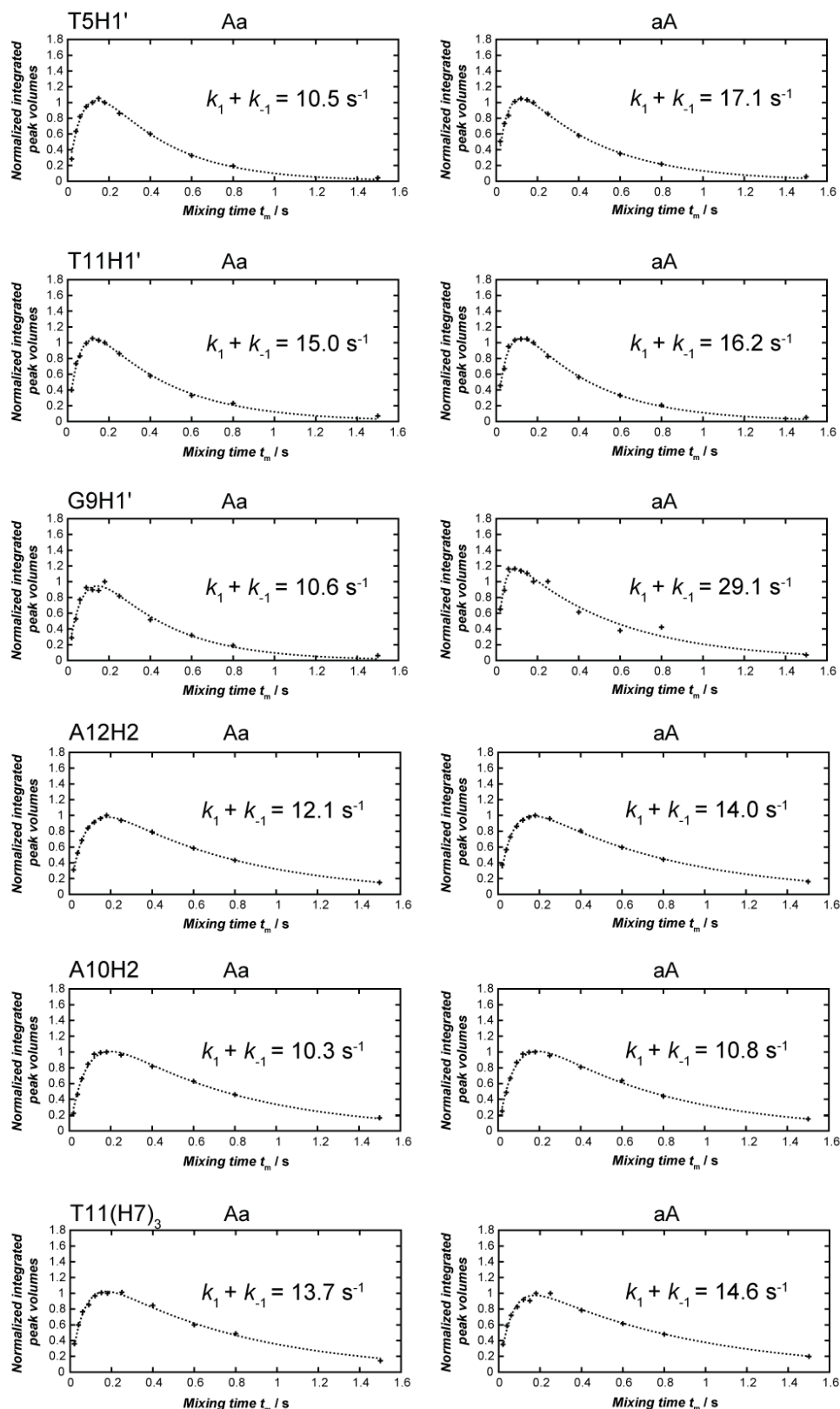
Supplementary Figure 36 | Definition of diagonal peaks, exchange cross peaks, and exchange-mediated NOE cross peaks. a) diagonal peaks ('AA' and 'aa') undergoing conformational interconversion to give exchange cross peaks ('aA' and 'Aa') and b) 'AB' and 'ab' NOE cross peaks undergoing conformational interconversion to give exchange-mediated NOE cross peaks 'Ab' and 'aB'.¹⁷ Figure adapted from ref 17.



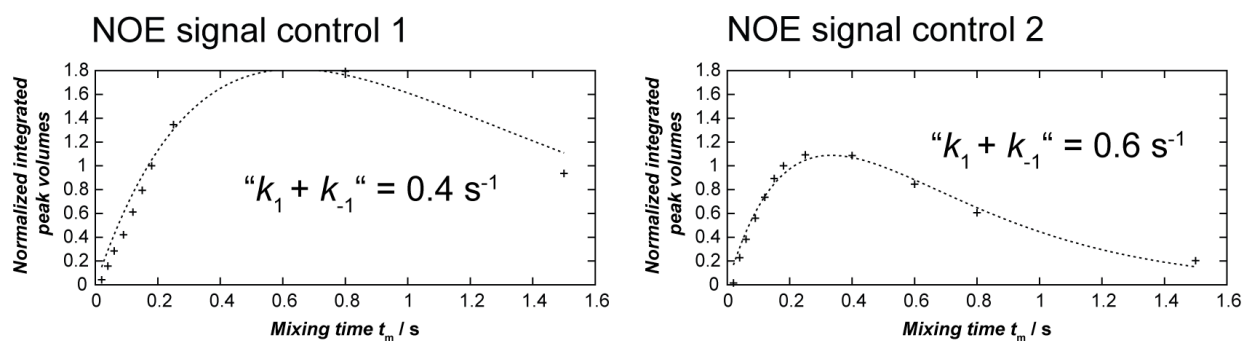
Supplementary Figure 37 | ^1H , ^1H -NOESY spectra with selected mixing times ranging from 20 ms to 1.5 s. Change of exchange cross peak intensities as a function of mixing time for a) H1' protons and b) H2 protons. The DNA sample contained 1 mM duplex DNA and 3 mM of ^{199}Hg -enriched $\text{Hg}(\text{ClO}_4)_2$ (1.5 equiv $^{199}\text{Hg}^{\text{II}}$ relative to mismatch) in aqueous buffer (200 mM NaClO_4 , 50 mM cacodylic acid in $\text{H}_2\text{O} / \text{D}_2\text{O}$ (9:1) at pH = 7.8).



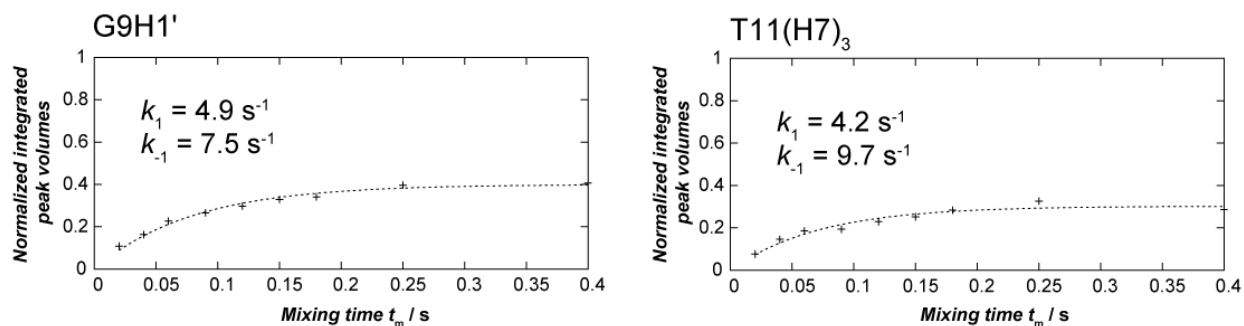
Supplementary Figure 38 | Changes of exchange-mediated NOE cross peak intensities as a function of mixing time. Exchange-mediated NOE cross peaks in the aromatic $\rightarrow$ H2'/H2'' region of the [^1H , ^1H]-NOESY spectrum with selected mixing times ranging from 20 ms to 1.5 s. The DNA sample contained 1 mM duplex DNA and 3 mM of ^{199}Hg -enriched $\text{Hg}(\text{ClO}_4)_2$ (1.5 equiv $^{199}\text{Hg}^{\text{II}}$ relative to mismatch) in aqueous buffer (200 mM NaClO_4 , 50 mM cacodylic acid in $\text{H}_2\text{O} / \text{D}_2\text{O}$ (9:1) at pH = 7.8).



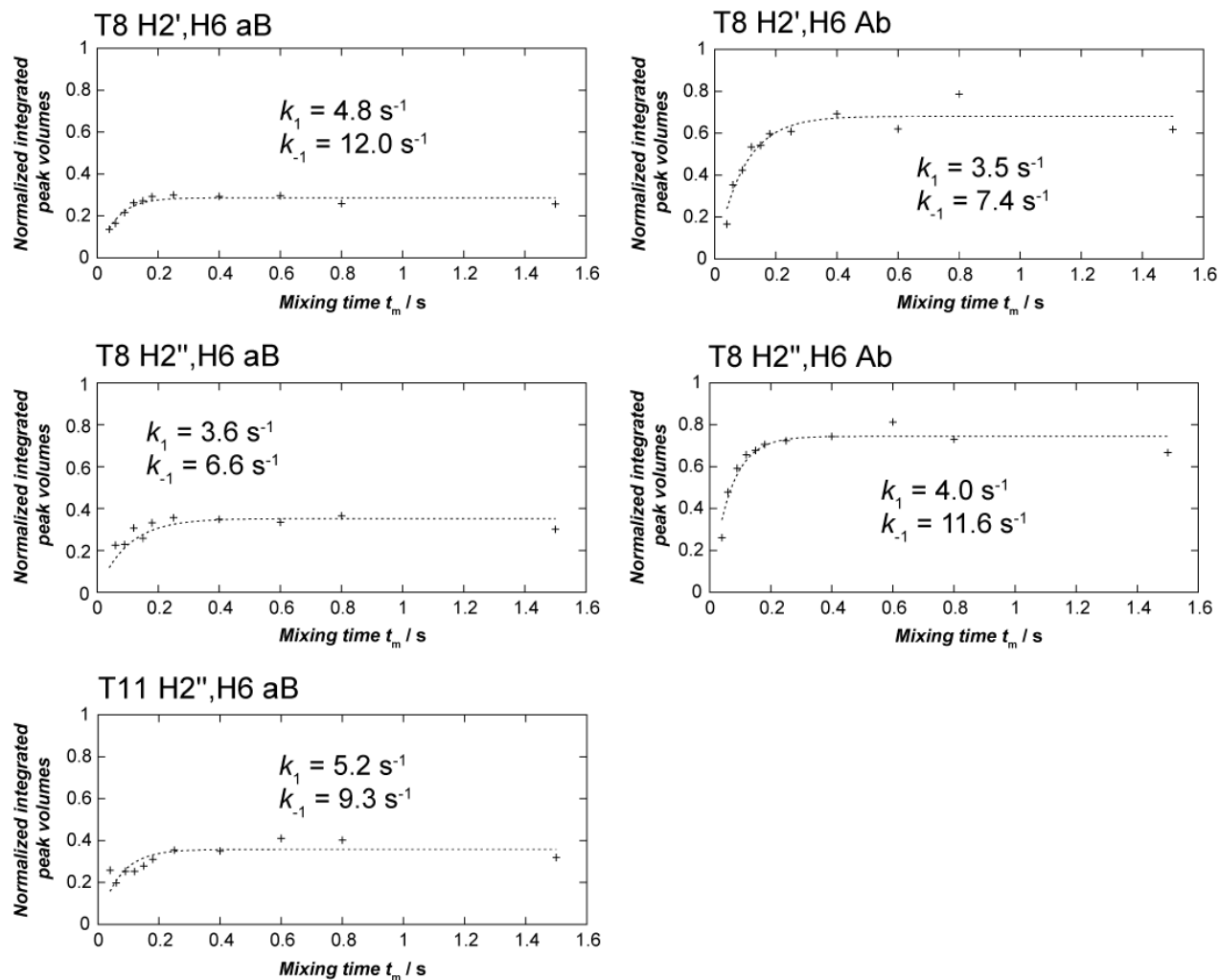
Supplementary Figure 39 | Sum of interconversion rate constants (k_1+k_{-1}). Sum of rate constants were determined by fit (Supplementary Equation 12) of change in cross peak intensities vs mixing time (t_m). The DNA sample contained 1 mM duplex DNA and 3 mM of ^{199}Hg -enriched $\text{Hg}(\text{ClO}_4)_2$ (1.5 equiv $^{199}\text{Hg}^{\text{II}}$ relative to mismatch) in aqueous buffer (200 mM NaClO_4 , 50 mM cacodylic acid in $\text{H}_2\text{O} / \text{D}_2\text{O}$ (9:1) at pH = 7.8). Source data are provided as a Source Data file.



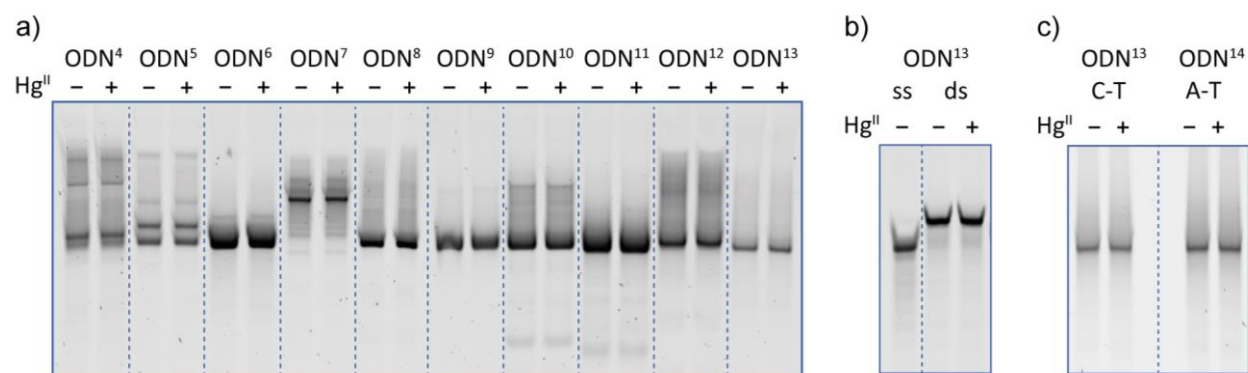
Supplementary Figure 40 | NOE signals A7H3',H1' processed equivalent to exchange signals as a control experiment. Both examples showed large deviations from the values obtained for exchange cross peaks. The DNA sample contained 1 mM duplex DNA and 3 mM of ^{199}Hg -enriched $\text{Hg}(\text{ClO}_4)_2$ (1.5 equiv $^{199}\text{Hg}^{\text{II}}$ relative to mismatch) in aqueous buffer (200 mM NaClO_4 , 50 mM cacodylic acid in H_2O / D_2O (9:1) at pH = 7.8). Source data are provided as a Source Data file.



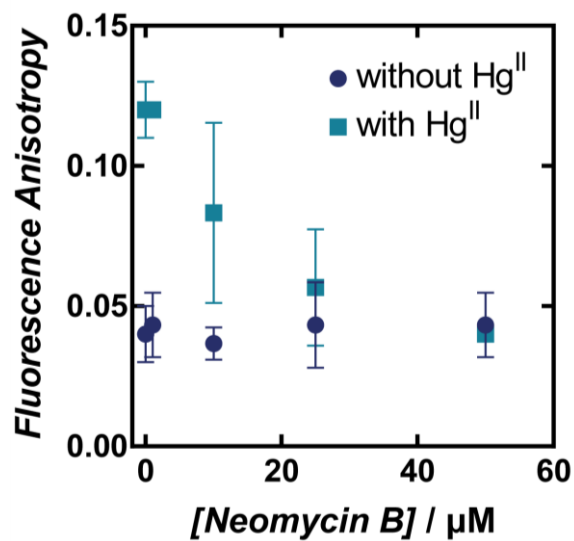
Supplementary Figure 41 | Exchange cross peak intensities normalized to the intensities of diagonal peaks. $aA(t_m) / (aA(t_m) + AA(t_m))$ is plotted as a function of mixing time (t_m). Rate constants of interconversion were determined by fit with Supplementary Equation 14. The DNA sample contained 1 mM duplex DNA and 3 mM of ^{199}Hg -enriched $\text{Hg}(\text{ClO}_4)_2$ (1.5 equiv $^{199}\text{Hg}^{\text{II}}$ relative to mismatch) in aqueous buffer (200 mM NaClO_4 , 50 mM cacodylic acid in H_2O / D_2O (9:1) at pH = 7.8). Source data are provided as a Source Data file.



Supplementary Figure 42 | Normalized areas of exchange-mediated NOE cross peaks as a function of mixing time (t_m). Rate constants of interconversion were determined by fit with Supplementary Equation 18 ('aB') or Supplementary Equation 19 ('Ab'). The DNA sample contained 1 mM duplex DNA and 3 mM of ^{199}Hg -enriched $\text{Hg}(\text{ClO}_4)_2$ (1.5 equiv $^{199}\text{Hg}^{\text{II}}$ relative to mismatch) in aqueous buffer (200 mM NaClO_4 , 50 mM cacodylic acid in H_2O / D_2O (9:1) at pH = 7.8). Source data are provided as a Source Data file.

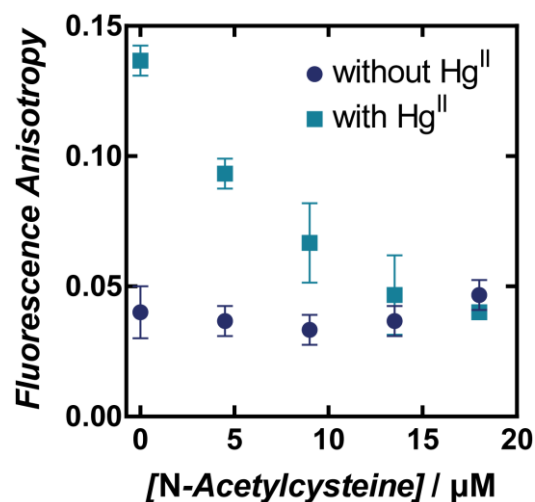


Supplementary Figure 43 | Native gel analysis of DNA repeat sequences in the presence and absence of Hg^{II} . a) intramolecular hairpin formation, b) ODN¹³ duplex DNA formation, and c) intramolecular hairpin formation of ODN¹³ containing C-T mismatches and ODN¹⁴ containing A-T base pairs in the absence or presence of Hg^{II} . All samples were prepared in aqueous buffer containing 200 mM NaClO_4 and 50 mM cacodylic acid (pH = 7.8). Sucrose (40 %, 5 μl) was given to pre-annealed hairpin or duplex DNA (5 μl , 5 pmol) prior to loading on gel. DNA samples measured in the presence of Hg^{II} were incubated with $\text{Hg}(\text{ClO}_4)_2$ (1.5 equiv relative to mismatch present) for 3 h prior to use. For oligonucleotide sequences see Supplementary Table 8. Source data are provided as a Source Data file.

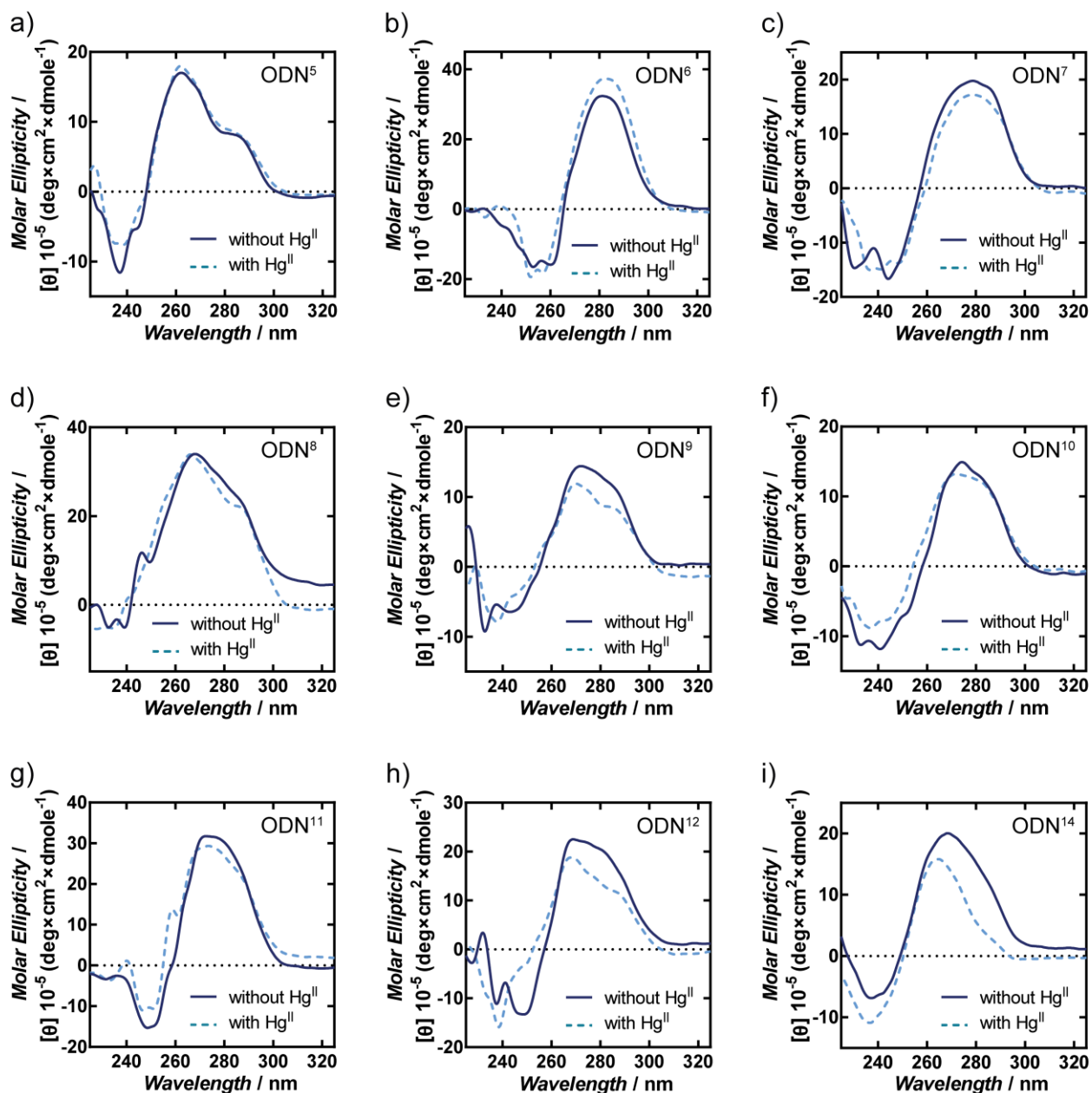


Supplementary Figure 44 | Changes in fluorescence anisotropy of Neo-BODIPY upon addition of unlabeled neomycin B.

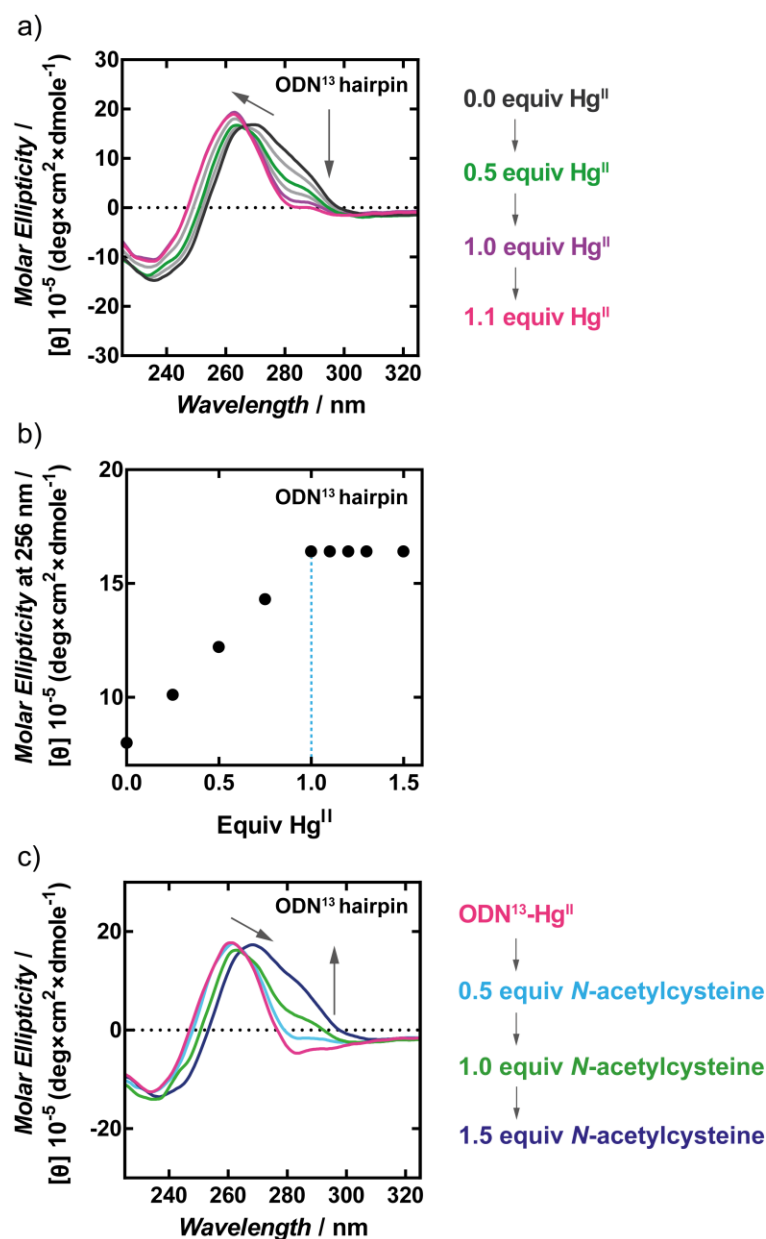
Unlabeled neomycin B was added to samples of ODN¹³ preincubated with Neo-BODIPY in the absence or presence of Hg^{II}. Samples in the absence of Hg^{II} (dark blue circles) contained 40 nM Neo-BODIPY, 0.6 μM ODN¹³ hairpin, and 0 – 50 μM unlabeled neomycin B. Samples in the presence of Hg^{II} (light blue squares) contained 40 nM Neo-BODIPY, 0.6 μM ODN¹³ hairpin, 13.5 μM Hg(ClO₄)₂ (1.5 equiv relative to mismatch present), and 0 – 50 μM unlabeled neomycin B and were incubated with Hg^{II} for 3 h prior to addition of Neo-BODIPY and unlabeled neomycin B. Samples were excited at $\lambda_{\text{ex}} = 480$ nm and fluorescence anisotropy was monitored at $\lambda_{\text{em}} = 515$ nm. All samples were prepared in aqueous buffer containing 200 mM NaClO₄ and 50 mM cacodylic acid (pH = 7.8). The data represent mean values and error bars represent standard deviation of three independent measurements. Source data are provided as a Source Data file.



Supplementary Figure 45 | Changes in fluorescence anisotropy of Neo-BODIPY upon addition of *N*-acetylcysteine. *N*-acetylcysteine was added to samples of ODN¹³ preincubated with Neo-BODIPY in the absence or presence of Hg^{II} . Samples in the absence of Hg^{II} (dark blue circles) contained 40 nM Neo-BODIPY, 0.6 μM ODN¹³ hairpin, and 0 – 18 μM *N*-acetylcysteine. Samples in the presence of Hg^{II} (light blue squares) contained 40 nM Neo-BODIPY, 0.6 μM ODN¹³ hairpin, 13.5 μM $\text{Hg}(\text{ClO}_4)_2$ (1.5 equiv relative to mismatch present), and 0 – 18 μM *N*-acetylcysteine and were incubated with Hg^{II} for 3 h prior to addition of Neo-BODIPY and *N*-acetylcysteine. Samples were excited at $\lambda_{\text{ex}} = 480$ nm and fluorescence anisotropy was monitored at $\lambda_{\text{em}} = 515$ nm. All samples were prepared in aqueous buffer containing 200 mM NaClO_4 and 50 mM cacodylic acid (pH = 7.8). The data represent mean values and error bars represent standard deviation of three independent measurements. Source data are provided as a Source Data file.

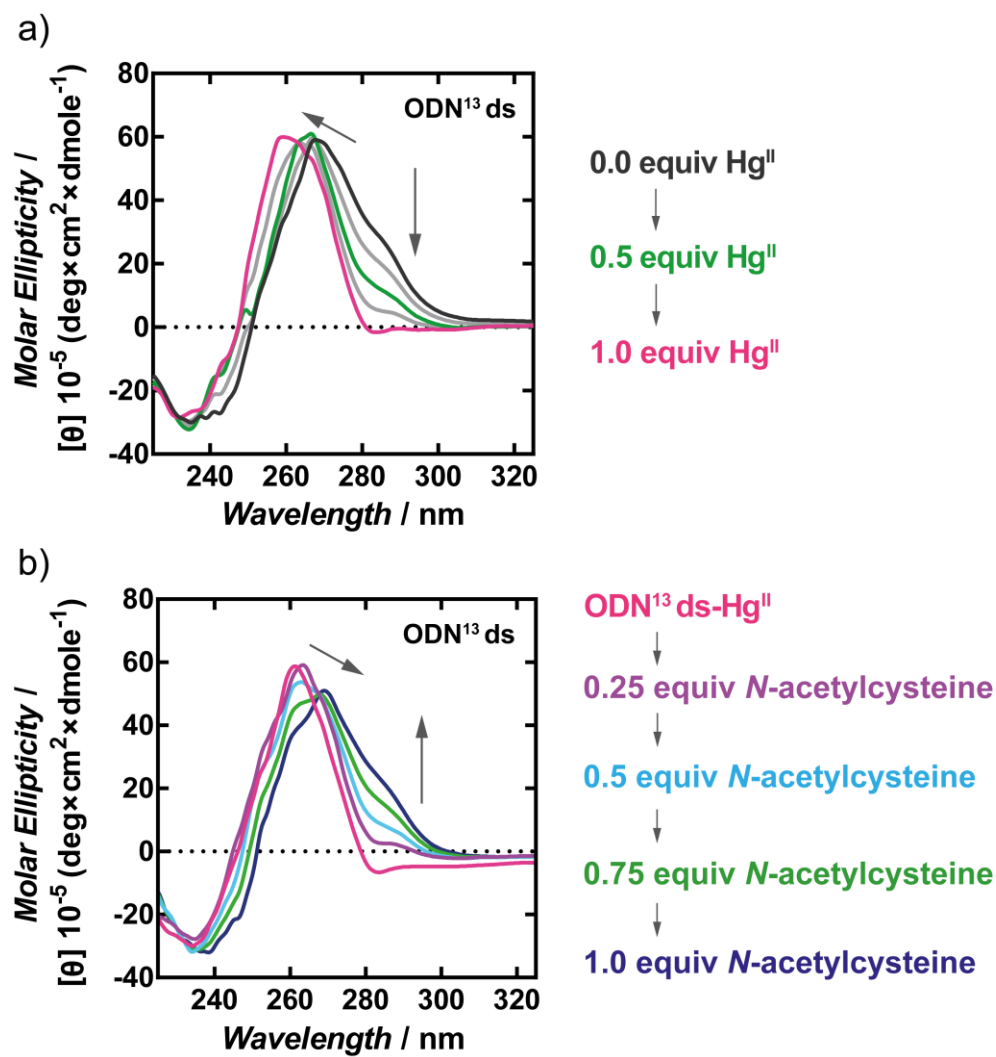


Supplementary Figure 46 | CD spectra of DNA hairpin repeat sequences in the presence and absence of Hg^{II}. CD spectra of a) ODN⁵, b) ODN⁶, c) ODN⁷, d) ODN⁸, e) ODN⁹, f) ODN¹⁰, g) ODN¹¹, h) ODN¹², and i) ODN¹⁴ in the presence (dashed, light blue) and absence (solid, dark blue) of 1.5 equiv Hg^{II}. Samples in the absence of Hg^{II} contained 1 μM hairpin DNA. Samples in the presence of Hg^{II} contained 1 μM hairpin DNA and were incubated with 1.5 equiv Hg(ClO₄)₂ (equiv relative to mismatch present) for 3 h prior to use. All samples were prepared in an aqueous buffer containing 200 mM NaClO₄ and 50 mM cacodylic acid (pH = 7.8).



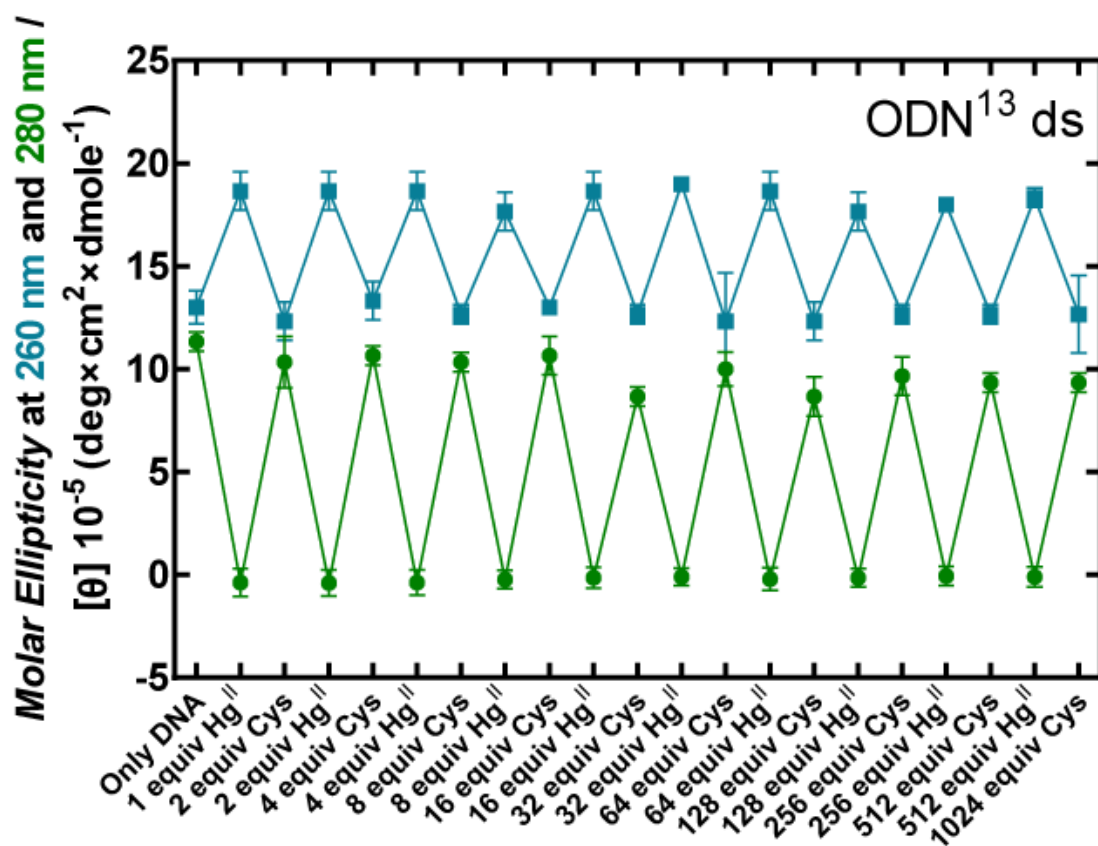
Supplementary Figure 47 | Stoichiometric Hg^{II} binding to ODN¹³ and reversibility of B-A transition by *N*-acetylcysteine.

a) Binding of Hg^{II} to C-T mismatches induces B- to A-form helical transition of ODN¹³ hairpin according to changes in CD spectra. b) 1:1 stoichiometric binding of Hg^{II} to the number of C-T mismatches present in ODN¹³ hairpin according to changes in molar ellipticity at 256 nm. Equiv of Hg^{II} are given relative to mismatch present. c) Reverse A- to B-form transition upon addition of *N*-acetylcysteine. ODN¹³ hairpin (1 μM) was preincubated with $\text{Hg}(\text{ClO}_4)_2$ (22.5 μM , 1.5 equiv relative to mismatch) for 10 min prior to addition of *N*-acetylcysteine. All samples contained ODN¹³ hairpin (1 μM) in aqueous buffer (200 mM NaClO_4 and 50 mM cacodylic acid (pH = 7.8)). CD spectra were recorded after 10 min incubation with aliquots of $\text{Hg}(\text{ClO}_4)_2$ (a) or *N*-acetylcysteine (c). Source data are provided as a Source Data file.



Supplementary Figure 48 | Stoichiometric Hg^{II} binding to ODN¹³ ds and reversibility of B-A transition by *N*-acetylcysteine.

a) Stoichiometric binding of Hg^{II} to C-T mismatches induces B- to A-form helical transition of ODN¹³ ds according to changes in CD spectra. b) Reverse A- to B-form transition upon addition of *N*-acetylcysteine. ODN¹³ duplex (3 μM) was preincubated with Hg(ClO₄)₂ (45 μM , 1 equiv relative to mismatch present) for 10 min prior to addition of *N*-acetylcysteine. All samples contained ODN¹³ ds (3 μM) in aqueous buffer (200 mM NaClO₄ and 50 mM cacodylic acid (pH = 7.8)). CD spectra were recorded after 10 min incubation with aliquots of Hg(ClO₄)₂ (a) or *N*-acetylcysteine (b).



Supplementary Figure 49 | Helical switching cycle from B- to A-form and A- to B-form. Reversibility of A/B-form helical switching by the addition of Hg^{II} and *N*-acetylcysteine in alternating order according to changes in molar ellipticity at 260 nm (blue) and 280 nm (green). Samples contained ODN¹³ ds (1 μM) in aqueous buffer (200 mM NaClO₄ and 50 mM cacodylic acid (pH = 7.8)). Samples were incubated with Hg(ClO₄)₂ or *N*-acetylcysteine for 30 sec prior to measuring. Equiv of Hg^{II} and *N*-acetylcysteine are given relative to mismatch present. The data represent mean values and error bars represent standard deviation of three independent measurements. Source data are provided as a Source Data file.

Supplementary Tables

Supplementary Table 1: Names and Sequences of Duplex Oligonucleotides Used For T_m measurements and NMR spectroscopy.^[a]

name		duplex sequence	ϵ_{260}
ODN ¹	“C-T”	5'-CG TCT CAT GAT ACG-3' 3'-GC ATA GTA CTC TGC-5'	133000 M ⁻¹ cm ⁻¹
ODN ^{1*}	“C*-T*”	5'-CG TC*T CAT GAT* ACG-3' 3'-GC AT*A GTA CTC* TGC-5'	133000 M ⁻¹ cm ⁻¹
ODN ²	“T-T”	5'-CG TTT CAT GAT ACG-3' 3'-GC ATA GTA CTT TGC-5'	133900 M ⁻¹ cm ⁻¹
ODN ³	“G-T”	5'-CG TGT CAT GAT ACG-3' 3'-GC ATA GTA CTG TGC-5'	136500 M ⁻¹ cm ⁻¹

^[a] Bold bases indicate C-T mismatches. Stars indicate ¹⁵N-labeled residues (C* = ¹⁵N-labeled C, T* = ¹⁵N-labeled T).

Supplementary Table 2: Imino-Proton Chemical Shift Values (ppm) of ODN^{1*} “C*-T*” with and without Hg^{II} at 4 °C.^[a]

Imino-proton signal	ppm in the absence of Hg ^{II}	ppm in the presence of Hg ^{II}
G28	12.75	12.79 (+0.04)
G2	12.64	12.65 (+0.01)
T3	13.64	13.45 (-0.19)
T25	10.86 (<i>d</i> , ¹ <i>J</i> ¹ H, ¹⁵ N = 88 Hz)	-
T5	14.33	13.26 (-1.07)
G23	11.91	12.13 (+0.22)
T22	13.15	13.17 (+0.02)

^[a] Values in brackets represent chemical shift changes upon addition of Hg^{II}. The DNA sample contained 0.5 mM duplex DNA in aqueous buffer (200 mM NaClO₄, 50 mM cacodylic acid in H₂O / D₂O (9:1) at pH = 7.8) in the absence and the presence of 1.5 mM of Hg(ClO₄)₂ (1.5 equiv relative to mismatch).

Supplementary Table 3: ^{15}N -Chemical Shift Values (ppm) of ODN^{1*} “C*-T*” with and without Hg^{II} at 25 °C.^[a]

^{15}N signal	ppm in the absence of Hg^{II}	ppm in the presence of Hg^{II}
N1 (T)	142.6	N1 ^{major} : 142.7 (+0.1) N1 ^{minor} : 146.4 (+3.8)
N3 (T)	155.2	N3 ^{major} : 184.3 (+29.1) N3 ^{minor} : 183.1 (+27.9)
N1 (C)	152.3	N1 ^{major} : 155.7 (+3.4) N1 ^{minor} : 152.5 (+0.2)
N3 (C)	200.0	N3 ^{major} : 207.2 (+7.2) N3 ^{minor} : 207.2 (+7.2)
NH_2 (C)	95.9	(N4) NH_2 ^{major} : 96.7 (+0.8) (N4) NH ^{minor} : 124.1 (+28.2)

^[a] Values in brackets represent chemical shift changes upon addition of Hg^{II} . The DNA sample contained 1 mM duplex DNA and 3 mM of $\text{Hg}(\text{ClO}_4)_2$ (1.5 equiv Hg^{II} relative to mismatch) in aqueous buffer (200 mM NaClO_4 , 50 mM cacodylic acid in H_2O / D_2O (9:1) at pH = 7.8).

Supplementary Table 4: ^{15}N -Chemical Shift Values (ppm) and $^1J^{15}\text{N},^{199}\text{Hg}$ coupling constants of ODN^{1*} “C*-T*” with and without Hg^{II} at 4 °C.^[a]

^{15}N - signal	ppm in the absence of Hg^{II}	ppm in the presence of Hg^{II}	$^1J^{15}\text{N},^{199}\text{Hg}$
N1 (T)	142.3	N1 ^{major} : 142.2 (-0.1) N1 ^{minor} : 146.4 (+4.1)	- -
N3 (T)	155.1	N3 ^{major} : 184.0 (+28.9) N3 ^{minor} : 182.8 (+27.7)	1095 Hz n. d.
N1 (C)	151.6	N1 ^{major} : 155.5 (+3.9) N1 ^{minor} : 152.1 (+0.5)	- -
N3 (C)	199.4	N3 ^{major} : 206.9 (+7.5) N3 ^{minor} : 206.9 (+7.5)	114 Hz -
NH_2 (C)	95.9	(N4) NH_2 ^{major} : 96.9 (+1.0) (N4) NH ^{minor} : 124.2 (+28.3)	- 1063 Hz

^[a] Values in brackets represent chemical shift changes upon addition of Hg^{II} . The DNA sample contained 1 mM duplex DNA and 3 mM of ^{199}Hg -enriched $\text{Hg}(\text{ClO}_4)_2$ (1.5 equiv Hg^{II} relative to mismatch) in aqueous buffer (200 mM NaClO_4 , 50 mM cacodylic acid in H_2O / D_2O (9:1) at pH = 7.8).

Supplementary Table 5: $^3J\ ^1\text{H},^1\text{H}$ Coupling Constants of the major duplex species.^[a]

Residue	H1',H2'	H1',H2''	H2',H2''
C1	7.8	6.9	13.8
G2	9.2	6.6	13.2
T3	8.6	6.9	13.7
C4	6.5	6.6	14.6
T5	n. d.	n. d.	n. d.
C6	8.4	7.1	13.5
A7	9	6.6	14.1
T8	n. d.	n. d.	n. d.
G9	n. d.	n. d.	n. d.
A10	n. d.	n. d.	n. d.
T11	9.3	6.9	14.9
A12	n. d.	n. d.	n. d.
C13	8.3	6.6	13.8
G14	8.4	6.6	13.9
S-type (C2'-endo) ⁴	9.5	5.8	14.1
N-type (C3'-endo) ⁴	1.5	7.7	14.1

^[a] The DNA sample contained 0.4 mM duplex DNA (ODN¹ “C-T”) and 1.2 mM Hg^{II} (1.5 equiv Hg^{II} relative to mismatch) in an aqueous solution of NaClO₄ (50 mM, D₂O, pD = 7.75) and was measured at 25 °C. n.d. = not determined.

Supplementary Table 6: Frequency of C2'-Endo, O4'-Endo, and C1'-Exo Sugar Pucker Conformation.^[a]

Residue	C2'-endo (%)			O4'-endo (%)			C1'-exo (%)		
	major	minor	apo	major	minor	apo	major	minor	apo
C1	50	5	10	0	0	0	0	0	0
G2	100	100	100	0	0	0	0	0	0
T3	50	35	60	0	0	0	50	65	40
C4	0	0	0	65	100	80	10	0	20
T5	10	0	0	40	55	25	50	45	75
C6	0	0	0	75	80	45	25	20	55
A7	95	95	100	0	0	0	5	0	0
T8	0	0	0	100	90	10	0	10	90
G9	95	100	100	5	0	0	0	0	0
A10	75	90	0	5	0	70	20	10	30
T11	0	0	0	100	65	5	0	5	0
A12	25	45	0	15	30	95	55	25	5
C13	0	0	0	70	100	75	30	0	25
G14	45	40	25	35	35	60	10	25	15

^[a] % represent percentage of sugar pucker in the 20 lowest energy conformations of major-, minor-, and apo duplex species. Sugar pucker conformations were determined using Curves+.⁷ A single C3'-endo sugar pucker was observed in one of the 20 models of the major duplex. Otherwise no C3'-endo sugar pucker were observed.

Supplementary Table 7: Rate Constants of Nucleobase-Metal-Nucleobase Isomerization and of Duplex Interconversion.^[a]

	$k_1 + k_{-1}$ (s ⁻¹)	k_1 (s ⁻¹)	k_{-1} (s ⁻¹)
[¹⁵N,¹H]-HSQC exchange peaks	11.2	3.5	7.7
exchange cross peaks			
T5H1'	10.5	n.d.	n.d.
T11H1'	15.0	n.d.	n.d.
G9H1'	10.6	4.9	7.5
A12H2	12.1	n.d.	n.d.
A10H2	10.3	n.d.	n.d.
T11(H7)₃	13.7	4.2	9.7
mean ± standard deviation	12.0 ± 1.8	4.6 ± 0.4	8.6 ± 1.1
exchange-mediated NOE cross peaks			
T8 H2',H6	n.d.	4.2	9.7
T8 H2'',H6	n.d.	3.8	9.1
T11 H2'',H6	n.d.	5.2	9.3
mean ± standard deviation	n. d.	4.4 ± 0.6	9.4 ± 0.2

^[a] Rate constants of nucleobase-metal-nucleobase isomerization were determined by global fitting of integrated peak volumes versus delay time (t_d) using Supplementary Equations 6-9, SI. Sums of rate constants (k_1+k_{-1}) were determined by fitting exchange cross peak vs mixing time (t_m) with Supplementary Equation 12. Rate constants k_1 and k_{-1} were determined from normalized exchange cross peak areas vs mixing time (t_m) by fitting with Supplementary Equation 14, or from plot of exchange-mediated NOE cross peaks by fit with Supplementary Equation 18 or Supplementary Equation 19. Values for T8 H2',H6 and T8 H2'',H6 were calculated as the mean of both exchange mediated NOE cross peaks. The DNA sample contained 0.5 mM duplex DNA and 1.5 equiv of Hg(ClO₄)₂ (nucleobase-metal-nucleobase isomerization) or 1 mM duplex DNA and 1.5 equiv of ¹⁹⁹Hg-enriched Hg(ClO₄)₂ (global helix interconversion) in aqueous buffer (200 mM NaClO₄, 50 mM cacodylic acid in H₂O / D₂O (9:1) at pH = 7.8) and spectra were measured at 25 °C. For spectra see Supplementary Figures 23 and 37-38. For fits see Figure 5a and Supplementary Figures 39-42. Equiv of Hg^{II} are given relative to mismatch.

Supplementary Table 8: Names and Sequences of 120-mer DNA Hairpin Small Library.^[a]

[illegible]

[^a] Bold bases indicate C-T mismatches (ODN⁴ – ODN¹³) or T-A base pairs (ODN¹⁴). Bold italic bases indicate loop regions.

Supplementary Methods

Synthesis and Folding of Duplex DNA

Unmodified oligonucleotides were purchased from Sigma-Aldrich as HPLC-purified sequences. ^{15}N -labeled cytosine and thymidine phosphoramidites were purchased from Cambridge Isotope Laboratories, Inc. ^{15}N -containing sequences were synthesized on a 1 μmol scale using a Bioautomation Mermade 4 DNA synthesizer using standard trityl-on procedure, except that 5 % dichloroacetic acid in CH_2Cl_2 was used for the deprotection step. For incorporation of ^{15}N -labeled nucleosides three coupling reactions were performed with prolonged coupling times (400 s). ^{15}N -containing oligonucleotides were purified using Glen-Pak cartridges purchased from Glen Research according to their procedure. Purified oligonucleotides were analyzed with MALDI-MS spectroscopy using THAP as matrix in a reflector-ion mode on a Bruker Ultraflextreme MALDI mass spectrometer. Unlabeled oligonucleotide was measured in parallel. In comparison to the unlabeled oligonucleotide, the ^{15}N -labeled oligonucleotide showed a mass elevation of 5 dalton (Supplementary Figure 1). Oligonucleotide stock solutions were prepared in deionized water and their concentrations were determined by absorbance at 260 nm using a molar extinction coefficient (ϵ_{260}) calculated using a nearest-neighbor model.^{18,19} For calculated extinction coefficients see Supplementary Table 1. Double stranded oligonucleotides were prepared by diluting the self-complementary sequences in the indicated aqueous buffer and heating to 95 °C for 5 min, followed by slow cooling to room temperature over 4 h.

Melting Temperature Analysis (T_m) and CD Spectroscopy

Thermal denaturation temperatures of duplex DNA were determined by measuring the absorbance at 260 nm as a function of temperature in a 1 mm path length thermo-controlled strain-free quartz cuvette on a JASCO J-715 spectrometer equipped with a temperature control system. Solutions of pre-folded duplex DNA in aqueous buffer (200 mM NaClO_4 , 50 mM cacodylic acid at pH = 7.0) were equilibrated at 4 °C for a minimum of 20 min and slowly ramped to 90 °C with 0.5 °C steps at a rate of 25 °C h^{-1} . The melting temperatures were determined from the obtained maximum after differentiation of the received curves on Spectra Manager. T_m values were calculated as the average from the heating and cooling curves and were measured in triplicate.

Circular dichroism spectra of pre-annealed duplex DNA (10 μM) were measured from 200 nm to 350 nm at 4 °C and 25 °C with a 2 nm band width with 0.1 nm steps at a scanning rate of 20 nm min^{-1} in 1 mm

path length thermo-controlled strain-free quartz cuvette on a JASCO J-715 spectrometer. Duplex DNA (10 μ M) was formed by dissolving the self-complementary sequences in aqueous buffer (200 mM NaClO₄, 50 mM cacodylic acid at pH = 7.0), heating to 95 °C for 5 min, and slowly cooling to room temperature over 4 h. The pH was then adjusted to 7.9 by addition of a solution of NaOH. CD spectra of duplex DNA measured in the presence of Hg^{II} were incubated with 2.0 equiv of Hg(ClO₄)₂ for 3 h prior to use.¹

Sample Preparation of Duplex DNA used for NMR studies

Duplex DNA (0.5 mM or 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. For Hg^{II} titration experiments, aliquots of Hg(ClO₄)₂ were added, and NMR spectra were recorded after incubating each sample for 15 min at r.t. followed by 15 min at 4 °C. ¹⁵N-labeled duplex DNA (0.5 mM or 1.0 mM) was prepared by dissolving 1.0 mM or 2.0 mM of the self-complementary sequence in an aqueous buffer (200 mM NaClO₄, 50 mM cacodylic acid in H₂O / D₂O (9:1) at pH = 7.0) and annealed as described above. The pH was adjusted to approximately pH = 7.8 by addition of an aqueous solution of NaOH. For solution structure determination, oligonucleotides were desalted, and 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 7.75 by addition of an aqueous solution of NaOH. The samples were annealed as described above and if necessary the pH was readjusted to pH = 7.75. Samples measured in D₂O were prepared in an aqueous solution of NaClO₄ (50 mM) and the pH was adjusted to 7.75 by addition of an aqueous solution of NaOH and annealed as described above. The samples were lyophilized, dissolved in 99.9% D₂O and the pD was adjusted to 7.35 by addition of a solution of NaOD in 99.9 % D₂O. DNA samples used for structure determination measurements and containing Hg^{II} were treated with Chelex-100 (BIO-RAD) for 10 min after addition of Hg^{II} (1.5 equiv with respect to mismatch) to remove unspecific Hg^{II} coordination.²⁰ 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 at the given temperature. The spectra were processed with a line broadening factor of 10 Hz. [¹H, ¹H]-NOESY spectra for unlabeled oligonucleotides in 200 mM NaClO₄, 50

mM cacodylic acid in H₂O / D₂O (9:1) at pH = 7.0 and for ¹⁵N-labeled oligonucleotides (200 mM NaClO₄, 50 mM cacodylic acid in H₂O / D₂O (9:1) at pH = 7.8) were recorded on a Bruker Avance 600 MHz spectrometer equipped with a TCI z-axis gradient CryoProbe at 4 °C with mixing times of 100 ms and 120 ms, respectively. Proton chemical shifts were referenced to the water line at 4.70 ppm at the given temperature. 1D ¹⁵N 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. An inter-scan delay of 1 s was used with a nominal 30° pulse for excitation and the pulse sequence employed a gradient spin echo of 2.5 ms duration prior to acquisition in order to get rid of the strong background signal. The total experiment time was typically on the order of 72 h. ¹⁵N chemical shifts were indirectly referenced against ¹H using $\Xi = 0.101329118$.²¹ The spectra were processed with a line-broadening factor of 10 Hz. ¹J [¹⁵N, ¹H]-HSQC spectra were recorded on a Bruker Avance II 500 MHz spectrometer equipped with a BBO z-axis gradient CryoProbe at 4 °C. Proton chemical shifts were referenced to the water line at 4.70 ppm at 4 °C and ¹⁵N chemical shifts were indirectly referenced against ¹H using $\Xi = 0.101329118$.²¹ The INEPT time in the ¹J [¹⁵N, ¹H]-HSQC's was set to select for a 90 Hz coupling. z-z exchange ¹J [¹⁵N, ¹H]-HSQC spectra containing a delay time before reverse INEPT were recorded on a Bruker Avance 600 MHz spectrometer equipped with TCI z-axis gradient CryoProbe at 25° C. Long-range [¹⁵N, ¹H]-HSQC spectra were recorded on a Bruker Avance 600 MHz spectrometer equipped with TCI z-axis gradient CryoProbe at 4 °C or on a Bruker Avance 700 MHz spectrometer equipped with TXI z-axis gradient CryoProbe at 25 °C. The INEPT time was set to select for a 20 Hz coupling. In order to enhance sensitivity, band-selective long-range [¹⁵N, ¹H]-HSQCs were measured in some instances in which the 180° ¹⁵N pulses in both INEPT blocks were replaced by band-selective pulses with a minimal excitation to the ¹⁵N nuclei of the NH₂ and NH resonances. The INEPT time was set to 25 Hz coupling. Water flip-back pulses together with the WATERGATE method were used for water suppression. For solution structure determination non-exchangeable resonances were assigned from [¹H, ¹H]-NOESY spectra (4 °C and 25 °C and mixing times of 60 ms and 250 ms), [¹H, ¹H]-total correlation spectra (TOCSY) (4 °C, 50 ms mixing time), and [¹H, ¹H]-correlation spectra (COSY) in D₂O, recorded either 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. Exchangeable protons were assigned from [¹H, ¹H]-NOESY spectra (4 °C, 150 ms mixing time) recorded on a Bruker Avance 600 MHz spectrometer equipped with a TCI z-axis gradient CryoProbe in H₂O / D₂O (9:1). ³¹P spectra were recorded on a Bruker Avance II 500 MHz spectrometer equipped with a BBO z-axis gradient CryoProbe at 25 °C. [¹H, ¹H]-Exclusive correlation spectra (E.COSY)²² were recorded on a Bruker Avance 600 MHz spectrometer equipped with a TCI z-axis gradient CryoProbe.

NMR Solution Structure Calculations

The integrated peak volumes from the [^1H , ^1H]-NOESY spectrum measured at 25 °C with a mixing time of 250 ms were calibrated to distances using CALIBA macro in DYANA.²³ 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.^{24,25} For C-T mismatch- and C-Hg^{II}-T base pair residues no backbone dihedral angle-, backbone torsion angle-, or sugar pucker restraints were applied. For the other residues the following angle restraints were used: Based on ^{31}P NMR, α and ζ backbone dihedral angles were restrained to $\alpha = -70^\circ \pm 20^\circ$ and $\zeta = -85^\circ \pm 20^\circ$,²⁶ respectively, to exclude trans-conformation (Supplementary Figure 16).²⁷ Based on H1',H2', H1',H2'', and H1',H3' cross peaks in [^1H , ^1H]-NOESY spectra and cross peaks in [^1H , ^1H]-COSY and [^1H , ^1H]-TOCSY spectra, the torsion angles β ($180^\circ \pm 20^\circ$)²⁶, γ ($60^\circ \pm 20^\circ$)²⁸, δ ($140^\circ \pm 25^\circ$)²⁸, and ϵ ($-170^\circ \pm 20^\circ$)²⁹ were set to B-form values. Glycosidic angles χ were restrained to $-120 \pm 20^\circ$ (*anti*-conformation).²⁶ Planarity and H-bond distance restraints were used for all residues, except for C-T mismatch- and C-Hg^{II}-T base pair residues. Hg^{II} ions were included as a 2+ positive charge. For C-Hg^{II}-T base pairs in the major duplex species, the following constraints were included based on crystal structures of similar base pairs: (i) distance N3(C)–N3(T) = 4.1 ± 0.3 Å;³⁰ (ii) bond length N3(C)–Hg^{II} = 2.04 Å, bond length N3(T)–Hg^{II} = 2.04 Å;³⁰ (iii) angle N3(T)–Hg^{II}–N3(C) = 180° ,³¹ angle thymidine C2/C4–N3–Hg^{II} = 117° ,³² angle cytosine C2–N3–Hg^{II} = 113.4° ,³³ angle cytosine C4–N3–Hg^{II} = 126.1° .³³ For C-Hg^{II}-T base pairs in the minor duplex species, the following constraints were included based on a crystal structure of a C-Hg^{II}-T-containing duplex DNA:³⁴ (i) distance N4(C)–N3(T) = 4.3 ± 0.3 Å; (ii) bond length N4(C)–Hg^{II} = 2.13 Å, bond length N3(T)–Hg^{II} = 2.13 Å; (iii) angle N3(T)–Hg^{II}–N4(C) = 180° , angle thymidine C2/C4–N3–Hg^{II} = 116° , angle cytosine C4–N4–Hg^{II} = 118° , angle H–N4–Hg^{II} = 120° . H3', H4', H5', and H5'' sugar protons were only assigned for the major duplex species. For the minor duplex, same NOE-coupling patterns and NOE-cross peak intensities for the above mentioned sugar proton resonances were taken as for the major species for the structure calculation. Given the experimental C_2 symmetry observed in all NMR spectra, a non-crystallographic symmetry term was introduced for the calculations.

Starting from an extended structure 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. For the apo duplex species model #7 was replaced with #21 due to a bond angle outlier in model #7. The representative model (apo: model 1, major: model 1, minor: model 6) were chosen based on fewest outliers in the geometric quality criteria (standard geometry (bond lengths,

bond angles, chirality, and planarity)) and too-close contacts between non-bonded atoms with unfavorable steric overlap of van der Waals shells. Root mean square deviations (r.m.s.d.) were calculated using MOLMOL³⁵ and duplexes were visualized using PyMOL.⁶ Base-pair parameters were determined using Curves+⁷ or 3DNA.^{11,12} Structure coordinates and NMR chemical shift assignments are deposited in the Protein Data Bank as 6RLS (apo species), 6FY6 (major species), and 6FY7 (minor species).

HR-MS of Hg(ClO₄)₂

Ethylenediamine tetraacetic acid disodium salt (Na₂EDTA) (60 μM) was added to ODN^{1*} “C*-T*” (4 μM) containing 12 μM ¹⁹⁹Hg-enriched Hg(ClO₄)₂. After incubating for 20 min, high-resolution electrospray mass spectra (HR-ESI-MS) of the Hg^{II}-EDTA²⁻ complex were recorded on a Bruker maXis QTOF-MS. As control experiments the HR-ESI-MS of natural abundance Hg(ClO₄)₂ and ¹⁹⁹Hg-enriched Hg(ClO₄)₂ without DNA were recorded after incubation with EDTA. Samples were measured via continuous flow injection with a flow rate of 3 μl min⁻¹. The mass spectrometer was calibrated between *m/z* 118 and 2721 using an Agilent ESI-L low concentration tuning mix solution at a resolution of 20'000 and a mass accuracy below 2 ppm.

Calculation of Pseudorotation Phase Angle

The pseudorotational phase angle (P) was determined from a plot of ³*J* H1',H2' as a function of P.² The dihedral angle Φ_{12'} was calculated according to the Karplus relation.³ (Supplementary Equation 1)

$$J = 10.2 \cos^2 \Phi - 0.8 \cos \Phi$$

Supplementary Equation 1

Rate Constants of Nucleobase-Metal-Nucleobase Isomerization

To investigate dynamic changes in local metal ion coordination, we measured [$^{15}\text{N}, ^1\text{H}$]-HSQC with a delay before reverse INEPT. To determine rate constants of interconversion, the decreases of cross peaks and increases of exchange cross peaks were monitored as a function of delay time (t_d) at 25 °C (Supplementary Figure 23). The integrals of the cross peaks (NH_2 and NH) and exchange peaks (NH_2' and NH') were taken, normalized to one of the $\text{NH}_2^{\text{major}}$ signals (6.81/96.71 ppm) from the [$^{15}\text{N}, ^1\text{H}$]-HSQC spectrum with a delay time (t_d) = 100 ms, and plotted as a function of delay time (t_d). The rate constants of interconversion were determined by global fitting of integrated peak volumes versus delay times (t_d) using Supplementary Equations 6-9,³⁶ respectively, under the assumption that all proton- and nitrogen atoms have the same relaxation rate (R_1) and that the NH proton is converted equally to both NH_2 protons during the exchange.³⁶ A global fit was used, where R_1 , k_1 , and k_{-1} was constrained to be equal for all four curves.

The decay of $\text{NH}_2 \text{ } ^1J \text{ } ^1\text{H}, ^{15}\text{N}$ crosspeak can be expressed as

$$\frac{d(\text{NH}_2)}{d(t_d)} = -\text{NH}_2(t_d)(k_1 + R_1) + k_{-1}\text{NH}'(t_d) \quad \text{Supplementary Equation 2}$$

The decay of $\text{NH } ^1J \text{ } ^1\text{H}, ^{15}\text{N}$ crosspeak can be expressed as

$$\frac{d(\text{NH})}{d(t_d)} = -\text{NH}(t_d)(k_{-1} + R_1) + k_1\text{NH}_2'(t_d) \quad \text{Supplementary Equation 3}$$

The build-up of $\text{NH}' \text{ } ^1J \text{ } ^1\text{H}, ^{15}\text{N}$ crosspeak can be expressed as

$$\frac{d(\text{NH}')}{d(t_d)} = -\text{NH}'(t_d)(k_{-1} + R_1) + k_1\text{NH}_2(t_d) \quad \text{Supplementary Equation 4}$$

The build-up of $\text{NH}_2' \text{ } ^1J \text{ } ^1\text{H}, ^{15}\text{N}$ crosspeaks can be expressed as

$$\frac{d(\text{NH}_2')}{d(t_d)} = -\text{NH}_2'(t_d)(k_1 + R_1) + k_{-1}\text{NH}(t_d) \quad \text{Supplementary Equation 5}$$

with t_d = delay time, R_1 = autorelaxation rate and k_1 and k_{-1} = rate constants of interconversion.

Integration of Supplementary Equations 2 – 5 gives Supplementary Equations 6 – 9, respectively.

$$\text{NH}_2(t_d) = \frac{a_0 c e^{-R_1 t_d} (k_{-1} + k_1 e^{t_d(-k_1 - k_{-1})})}{k_1 + k_{-1}} \quad \text{Supplementary Equation 6}$$

$$\text{NH}(t_d) = \frac{q c_0 e^{-R_1 t_d} (k_1 + k_{-1} e^{t_d(-k_1 - k_{-1})})}{k_1 + k_{-1}} \quad \text{Supplementary Equation 7}$$

$$\text{NH}'(t_d) = - \frac{q a_0 e^{-R_1 t_d} k_1 (-1 + e^{t_d(-k_1 - k_{-1})})}{k_1 + k_{-1}} \quad \text{Supplementary Equation 8}$$

$$\text{NH}_2'(t_d) = - \frac{c c_0 k_{-1} e^{-R_1 t_d} (-1 + e^{t_d(-k_1 - k_{-1})})}{k_1 + k_{-1}} \quad \text{Supplementary Equation 9}$$

with t_d = delay time, R_1 = autorelaxation rate, k_1 and k_{-1} = rate constants of interconversion and a_0 , c , and c_0 , q = constants.

Variable Ionic Strength

To evaluate the impact of variable ionic strength ^1H , ^1H]-TOCSY and ^1H , ^1H]-NOESY spectra were recorded of metallo duplex samples in the presence of 50 mM, 200 mM, and 500 mM NaClO_4 . Comparing signal intensities of cytosine (C4) H5/H6 ^1H , ^1H]-TOCSY resonances of major- (AB) and minor species (ab) to their exchange-mediated cross peaks (aB) and (Ab) revealed a decrease in the overall exchange rate of the helices with increasing ionic strength (Supplementary Figure 27).

To investigate changes in the ratio of major- and minor duplex species, the areas of integration of AB, ab, aB, and Ab were determined, scaled to the intensity of the cytosine C1 H5/H6 ^1H , ^1H]-TOCSY correlation, and globally normalized to the spectrum at pH = 9 (Supplementary Figure 32a) to give a sum of integrals = 1. The symmetrical F1/F2 (Supplementary Figure 28) and F2/F1 cross peaks (Supplementary Figure 28) were analyzed separately. The sum of integrals of the major- (AB + aB) and minor species (ab + Ab), and the fraction minor species $((ab + Ab)/(AB + aB + ab + Ab))$ revealed minimal change in the ratio of the two species at different ionic strengths (Supplementary Figure 29).

Further evidence for this is found in the ^1H , ^1H]-NOESY spectra of metallo duplex samples in the presence of 50 mM, 200 mM, and 500 mM NaClO_4 (Supplementary Figure 30 – 31). Six well resolved NOE

signals of the major- (blue) and minor species (green) were integrated and the area of integration was normalized to a selected reference signal (A12H3' – C13H6, purple, Supplementary Figure 30). The fraction of the minor species was determined for each signal by dividing the area of integration by the sum of the integrals of major- and minor signals. If exchange-mediated signals were present they were included in the sum of integrals (Supplementary Figure 31).

Variable pH

To evaluate the impact of variable pH, [¹H,¹H]-TOCSY and [¹H,¹H]-NOESY spectra were recorded of metallo duplex samples at pH = 9, 8, 7, and 6. Comparing signal intensities of cytosine (C4) H5/H6 [¹H,¹H]-TOCSY resonances of major- (AB) and minor species (ab) to their exchange-mediated cross peaks (aB) and (Ab) revealed a decrease in the overall exchange rate of the helices with increasing pH (Supplementary Figure 32).

The pH dependence on the ratio of major- and minor duplex species was determined as described above for varying ionic strength. Analysis of the [¹H,¹H]-TOCSY and [¹H,¹H]-NOESY experiments revealed that the ratio of the two species remained the same from pH 6 – 9 (Supplementary Figure 33 – 35).

Rate Constants of Global Helix Interconversion

To determine rate constants of global interconversion of the duplexes, we measured [¹H,¹H]-NOESY spectra with various mixing times (t_m) at 25 °C (Supplementary Figures 37 and 38). Selected exchange cross peaks ('Aa' and 'aA', Supplementary Figures 36a and 37) and exchange-mediated NOE cross peaks ('Ab' and 'aB', Supplementary Figures 36b and 38) were integrated, normalized to signal intensity at mixing time = 200 ms, and plotted as a function of mixing time (t_m) (Supplementary Figures 39-42). Two NOE signals were also included to the analysis as controls (Supplementary Figure 40). At longer mixing times, a decrease in signal intensity was observed due to auto relaxation (R_1).

The area of integration of diagonal peaks ('AA' and 'aa') and exchange cross peaks ('Aa' and 'aA') were determined at various mixing times (t_m) (Supplementary Figure 37, for definition of labels see Supplementary Figure 36a). Under the assumption that auto relaxation $R_{1,A} = R_{1,a}$ and cross relaxation $\sigma = 0$, change in area of integration can be expressed by Supplementary Equations 10 – 12.^{17,37} Fit with Supplementary Equation 12 of change in area of integration of exchange cross peaks as a function of mixing time (t_m) furnishes the sum of rate constants $k_1 + k_{-1}$ (Supplementary Figure 39, Supplementary Table 7).

$$\text{diagonal peak 'AA' } (t_m) = \frac{k_{-1}}{(k_1 + k_{-1})^2} e^{-R_1 t_m} (k_{-1} + k_1 e^{-(k_1 + k_{-1}) t_m}) \quad \text{Supplementary Equation 10}$$

$$\text{diagonal peak 'aa' } (t_m) = \frac{k_1}{(k_1 + k_{-1})^2} e^{-R_1 t_m} (k_1 + k_{-1} e^{-(k_1 + k_{-1}) t_m}) \quad \text{Supplementary Equation 11}$$

$$\begin{aligned} \text{exchange cross peak 'Aa' } (t_m) &= \text{exchange cross peak 'aA' } (t_m) \\ &= \frac{k_1 k_{-1}}{(k_1 + k_{-1})^2} e^{-R_1 t_m} (1 - e^{-(k_1 + k_{-1}) t_m}) \end{aligned} \quad \text{Supplementary Equation 12}$$

For signals where the resolution of the diagonal peaks was sufficient for integration, Supplementary Equation 12 can be normalized to the intensity of diagonal peak by 'Aa' (t_m) / ('Aa' (t_m) + 'aa' (t_m)) and 'aA' (t_m) / ('aA' (t_m) + 'AA' (t_m)) to give the simplified Supplementary Equations 13 and 14, respectively, from which the rate constants k_1 and k_{-1} can be determined (Supplementary Figure 41).^{17,37}

normalized exchange cross peak =

$$\frac{'Aa'(t_m)}{'Aa'(t_m) + 'aa'(t_m)} = \frac{k_{-1}}{k_1 + k_{-1}} (1 - e^{-(k_1 + k_{-1}) t_m}) \quad \text{Supplementary Equation 13}$$

normalized exchange cross peak =

$$\frac{'aA'(t_m)}{'aA'(t_m) + 'AA'(t_m)} = \frac{k_1}{k_1 + k_{-1}} (1 - e^{-(k_1 + k_{-1}) t_m}) \quad \text{Supplementary Equation 14}$$

The area of integration of NOE cross peaks ('AB' and 'ab') and exchange-mediated NOE cross peaks ('Ab' and 'aB') (for definition of labels see Supplementary Figure 36b) were determined at various mixing times (t_m) (Supplementary Figure 38). Under the assumption that auto relaxation $R_{1,A} = R_{1,a}$, change in area of integration of NOE cross peaks as a function of mixing time (t_m) can be expressed by Supplementary Equation 15 and Supplementary Equation 16.^{17,37} Change in area of integration of exchange-mediated cross peaks as a function of mixing time (t_m) can be expressed by Supplementary Equation 17.^{17,37}

$$\text{NOE cross peak 'AB' } (t_m) = \frac{k_{-1} e^{-t_m(k_1 + k_{-1} + R_1 + \sigma)} (-1 + e^{2\sigma t_m}) (k_1 + k_{-1} e^{t_m(k_1 + k_{-1})})}{2(k_1 + k_{-1})^2} \quad \text{Supplementary Equation 15}$$

$$\text{NOE cross peak 'ab' } (t_m) = \frac{k_1 e^{-t_m(k_1 + k_{-1} + R_1 + \sigma)} (-1 + e^{2\sigma t_m}) (k_{-1} + k_1 e^{t_m(k_1 + k_{-1})})}{2(k_1 + k_{-1})^2} \quad \text{Supplementary Equation 16}$$

$$\begin{aligned} \text{exchange – mediated cross peak 'Ab' } (t_m) &= \text{exchange – mediated cross peak 'aB' } (t_m) \\ &= \frac{k_1 k_{-1} e^{-t_m(k_1 + k_{-1} + R_1 + \sigma)} (-1 + e^{2\sigma t_m}) (-1 + e^{t_m(k_1 + k_{-1})})}{2(k_1 + k_{-1})^2} \end{aligned} \quad \text{Supplementary Equation 17}$$

By dividing exchange-mediated cross peaks 'Ab' and 'aB' over the sum with their corresponding NOE cross peak gives the simplified Supplementary Equations 18 and 19, respectively, from which the rate constants k_1 and k_{-1} can be determined (Supplementary Figure 42, Supplementary Table 7).^{17,37}

$$\begin{aligned} \text{normalized exchange – mediated NOE cross peak} &= \\ \frac{'aB'(t_m)}{'AB'(t_m) + 'aB'(t_m)}} &= \frac{k_1 - k_{-1} e^{-t_m(k_1 + k_{-1})}}{k_1 + k_{-1}} \end{aligned} \quad \text{Supplementary Equation 18}$$

$$\begin{aligned} \text{normalized exchange – mediated NOE cross peak} &= \\ \frac{'Ab'(t_m)}{'Ab'(t_m) + 'ab'(t_m)}} &= \frac{k_{-1} - k_{-1} e^{-(k_1 + k_{-1})t_m}}{k_1 + k_{-1}} \end{aligned} \quad \text{Supplementary Equation 19}$$

with t_m = mixing time, R_1 = autorelaxation rate and k_1 and k_{-1} = rate constants of interconversion. For definition of labels 'AB', 'ab', 'Ab', and 'aB' see Supplementary Figure 36.

Hg^{II}-Induced Conformational Switching from B- to A-Form

Oligonucleotides were purchased from Sigma-Aldrich as HPLC- and PAGE purified sequences. Oligonucleotide stock solutions were prepared in deionized water and their concentrations were determined by absorbance at 260 nm using a molar extinction coefficient (ϵ_{260}) calculated using a nearest-neighbor model.^{18,19} Hairpins were prepared by diluting the sequences in aqueous buffer (200 mM NaClO₄, 50 mM cacodylic acid (pH = 7.8)) and heating at 95 °C for 5 min, followed by rapid cooling on ice at 0 °C. Duplex DNA ODN¹³ ds was prepared by dissolving the complementary sequences in aqueous buffer (200 mM NaClO₄, 50 mM cacodylic acid (pH = 7.8)) heating at 95 °C for 5 min, followed by slow cooling to room temperature over 4 h.

Gel Electrophoreses

Sucrose (40 %, 5 μ l) was given to pre-annealed hairpin or duplex DNA (5 μ l, 5 pmol) and the solution was loaded onto a 20 % native gel (1.6 V, 55 min). The gel was treated with SYBR gold staining and scanned on a Typhoon FLA 9500. DNA samples measured in the presence of Hg^{II} were incubated with Hg(ClO₄)₂ (1.5 equiv relative to mismatch present) for 3 h prior to use.

Neo-BODIPY Binding Assay

Pre-folded DNA hairpin (5 μ M) in aqueous buffer (200 mM NaClO₄ and 50 mM cacodylic acid (pH = 7.8)) was mixed with Hg(ClO₄)₂ (1.5 equiv relative to mismatch present) and incubated at r.t. for 3 h. Neo-BODIPY was added to give a final concentration of 40 nM Neo-BODIPY and 0 – 3 μ M DNA hairpin. The samples were incubated at r.t. for 30 min (ODN¹³, Figure 7a) or 1 h (ODN⁴ – ODN¹³, Table 2) prior to measurement. Fluorescence anisotropy values were determined by measuring fluorescence polarization at $\lambda_{em} = 515$ nm ($\lambda_{ex} = 480$ nm). Fluorescence polarization was measured in three independent trials at 25 °C using a Molecular Devices Spectra spectrofluorophotometer in 384-well plates.

A → B Helical Switching by Unlabeled Neomycin B

Pre-folded DNA hairpin (1 μM) in aqueous buffer (200 mM NaClO_4 and 50 mM cacodylic acid (pH 7.8)) was mixed with $\text{Hg}(\text{ClO}_4)_2$ (22.5 μM , 1.5 equiv relative to mismatch present) and incubated at r.t. for 3 h. Neo-BODIPY (66.7 nM) was added and incubated at r.t. for 60 min. Unlabeled neomycin B trisulfate was added to the samples to give a final concentration of 40 nM Neo-BODIPY, 0.6 μM ODN¹³, 13.5 μM Hg^{II} and 0 – 50 μM unlabeled neomycin B and the mixture was incubated at r.t. for 60 min. Anisotropy was recorded at 25 °C using a Molecular Devices Spectra spectrofluorophotometer in a 384-well plate. Fluorescence anisotropy was recorded in triplicate by fluorescence polarization measurement ($\lambda_{\text{ex}} = 480 \text{ nm}$, $\lambda_{\text{em}} = 515 \text{ nm}$).

A → B Helical Switching by *N*-Acetylcysteine

Pre-folded DNA hairpin (1 μM) in aqueous buffer (200 mM NaClO_4 and 50 mM cacodylic acid (pH 7.8)) was mixed with $\text{Hg}(\text{ClO}_4)_2$ (22.5 μM , 1.5 equiv relative to mismatch present) and incubated at r.t. for 3 h. Neo-BODIPY (66.7 nM) was added and incubated at r.t. for 60 min. *N*-acetylcysteine (0 – 2 equiv relative to mismatch present) was added to the sample to give a final concentration of 40 nM Neo-BODIPY, 0.6 μM ODN¹³, 13.5 μM Hg^{II} and 0 – 18 μM *N*-acetylcysteine and the mixture was incubated at r.t. for 60 min. Anisotropy was recorded at 25 °C using a Molecular Devices Spectra spectrofluorophotometer in a 384-well plate. Fluorescence anisotropy was recorded in triplicate by fluorescence polarization measurement ($\lambda_{\text{ex}} = 480 \text{ nm}$, $\lambda_{\text{em}} = 515 \text{ nm}$).

Circular Dichroism Spectroscopy of DNA Repeat Sequences

Circular dichroism spectra of pre-folded DNA hairpins (1 μM) were measured from 200 nm to 350 nm at 25 °C with a 2 nm band width with 0.1 nm steps at a scanning rate of 20 nm min⁻¹ in 1 mm path length thermo-controlled strain-free quartz cuvette on a JASCO J-715 spectrometer. CD spectra were recorded as an average of three measurements, each with three scans. CD spectra of hairpin DNA measured in the presence of Hg^{II} were incubated with 1.5 equiv (relative to mismatch present) of $\text{Hg}(\text{ClO}_4)_2$ for 3 h prior to use.

Hg^{II}–ODN¹³ Binding and Reversibility by *N*-Acetylcysteine

Aliquots of Hg(ClO₄)₂ (0 – 1.5 equiv relative to mismatch) were added to pre-folded ODN¹³ hairpin (1 μM) in aqueous buffer (200 mM NaClO₄ and 50 mM cacodylic acid (pH = 7.8). CD spectra were recorded as described above after 10 min incubation time. After complete B- to A-form helical transition (after addition of 1.5 equiv of Hg^{II} relative to mismatch) aliquots of *N*-acetylcysteine (0 – 1.5 equiv relative to mismatch) were added and CD spectra were recorded after 10 min of incubation.

Hg^{II} –ODN¹³ ds Binding and Reversibility by *N*-Acetylcysteine

Aliquots of Hg(ClO₄)₂ (0 – 1.0 equiv relative to mismatch) were added to pre-folded ODN¹³ ds (3 μM) in aqueous buffer (200 mM NaClO₄ and 50 mM cacodylic acid (pH = 7.8). CD spectra were recorded as described above after 10 min incubation time. After complete B- to A-form helical transition (after addition of 1.0 equiv of Hg^{II} relative to mismatch) aliquots of *N*-acetylcysteine (0 – 1.0 equiv relative to mismatch) were added and CD spectra were recorded after 10 min of incubation.

To investigate the reversibility of A/B-form helical switching, various equiv Hg^{II} (equiv relative to mismatch) and *N*-acetylcysteine (equiv relative to mismatch) were added in alternating order to pre-folded ODN¹³ ds (1 μM) in aqueous buffer (200 mM NaClO₄ and 50 mM cacodylic acid (pH = 7.8). CD spectra were recorded after 30 sec of incubation and changes in molar ellipticity were monitored at 260 nm and 280 nm.

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