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Controlled dehydration of a ruthenium complex-DNA crystal induces reversible DNA kinking

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Abstract

Hydration-dependent DNA deformation has been known since Rosalind Franklin recognised that the relative humidity of the sample had to be maintained to observe a single conformation in DNA fibre diffraction. We now report for the first time the crystal structure, at the atomic level, of a dehydrated form of a DNA duplex and demonstrate the reversible interconversion to the hydrated form at room temperature. This system, containing d(TCGGCGCCGA) in the presence of Λ -[Ru(TAP)₂(dppz)]²⁺ (TAP = 1,4,5,8-tetraazaphenanthrene, dppz = dipyridophenazine), undergoes a partial transition from an A/B hybrid to the A-DNA conformation, at 84-79% relative humidity. This is accompanied by an increase in kink at the central step from 22° to 51°, with a large movement of the terminal bases forming the intercalation site. This transition is reversible on rehydration. Seven datasets, collected from one crystal at room temperature, show the consequences of dehydration at near-atomic resolution. This result highlights that crystals, traditionally thought of as static systems, are still dynamic and therefore can be the subject of further experimentation.

Introduction

It has been known, since the original DNA fibre diffraction experiments conducted by Franklin, with model building conducted by Watson and Crick, that relative humidity can induce changes in DNA structure¹. In fact, when Franklin collected her fibre diffraction data, she controlled the relative humidity inside the sample chamber using saturated salt solutions. This vital step maintained the humidity of the fibre, resulting in a single conformation rather than a mixture.

Since her original work, changes which induce a deformation, or change in conformation, of the DNA duplex have been studied intensively. These structural changes can be induced in a number of ways including chemical modification,² covalent and non-covalent interactions with a ligand^{3,4} or protein⁵ and dehydration.⁶ A change in conformation, from the B to the A form, is observed in low humidity conditions and has been characterised by a number of methods including circular dichroism^{7,8} and other spectroscopic techniques.⁹

In contrast to the distortions induced by many small molecules, DNA kinking was first proposed as a necessary step required to enable the storage of DNA within the nucleus of a cell,¹⁰ making it more compact. The crystal structure of a nucleosome core particle¹¹ showed how DNA can be bent into a superhelix around histone proteins. In the superhelix the single highest roll angle, for an AA/TT pair, is 27° which is comparable to the 26° roll angle induced by the binding of cis-platin to a GpG step in a DNA duplex.¹² This is by no means the highest single-step kink which DNA can support whilst maintaining a Watson-Crick base pair. The structure of the TFAM-DNA (TFAM = mitochondrial transcription factor A) complex shows a 90° bend in the DNA helix, across three steps, with the highest roll angle at a single step of 60°.

Previously, we reported the crystal structure of the DNA duplex of sequence d(TCGGCGCCGA) crystallized in the presence of Λ -[Ru(TAP)₂(dppz)]²⁺,¹³ with the complex and structure illustrated in figure 1 and 2a respectively. The complex is a part of a family of dppz-containing ruthenium compounds which have been the subject of intense research due to their interesting photophysical properties.¹⁴⁻¹⁷ The complex was found to intercalate, through the dppz group, into the T₁C₂:G₉A₁₀ step at both ends of the duplex. One TAP ligand (TAP1) however, semi-intercalated into the G₃G₄:C₇C₈ steps inducing a 51° kink at each site, stabilised by a Ba²⁺ cation which is bound to the N7 position of G₃ and bifurcated to both O6 and N7 on G₄. The central unbound C₅G₆:C₅G₆ step possessed a 22° bend. Residue A₁₀ flipped out and formed a reverse Watson-Crick base pair with T₁ from a neighbouring duplex, thus cross linking all of the duplexes within the crystal.

Here we present the consequences of controlled dehydration on this system, as illustrated in figure 2b, using the HC1b humidity control device¹⁸ which replaces the cryojet on a macromolecular crystallography beamline and enables humidity control in real time. A reversible transition occurs at 84-79% relative humidity, where a striking reversal in terminal base conformation occurs, with T₁ now flipped out forming the reverse Watson-Crick base pair with A₁₀ from a neighbouring duplex, which requires the A₁₀ to flip in. A bend of 51° has been induced at the central C₅G₆:C₅G₆ step and the unit cell volume has been reduced to 76% of its original volume. Additionally, the duplex also shows a partial

transition from an A/B-hybrid to A-DNA conformation, which is the first such transition to be observed in the crystalline state. Unexpectedly, this transition is reversible on rehydration, to greater than 84% relative humidity, and seven crystal structures, charting this reversibility, were obtained from one crystal at room temperature, with one structure at near-atomic resolution. To our knowledge there is no record of such a reversible process in a nucleic acid crystal.

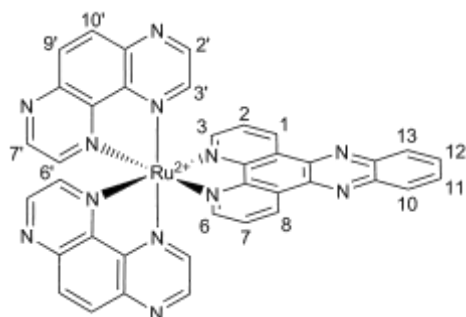


Figure 1: Diagram of Λ -[Ru(TAP)₂(dppz)]²⁺

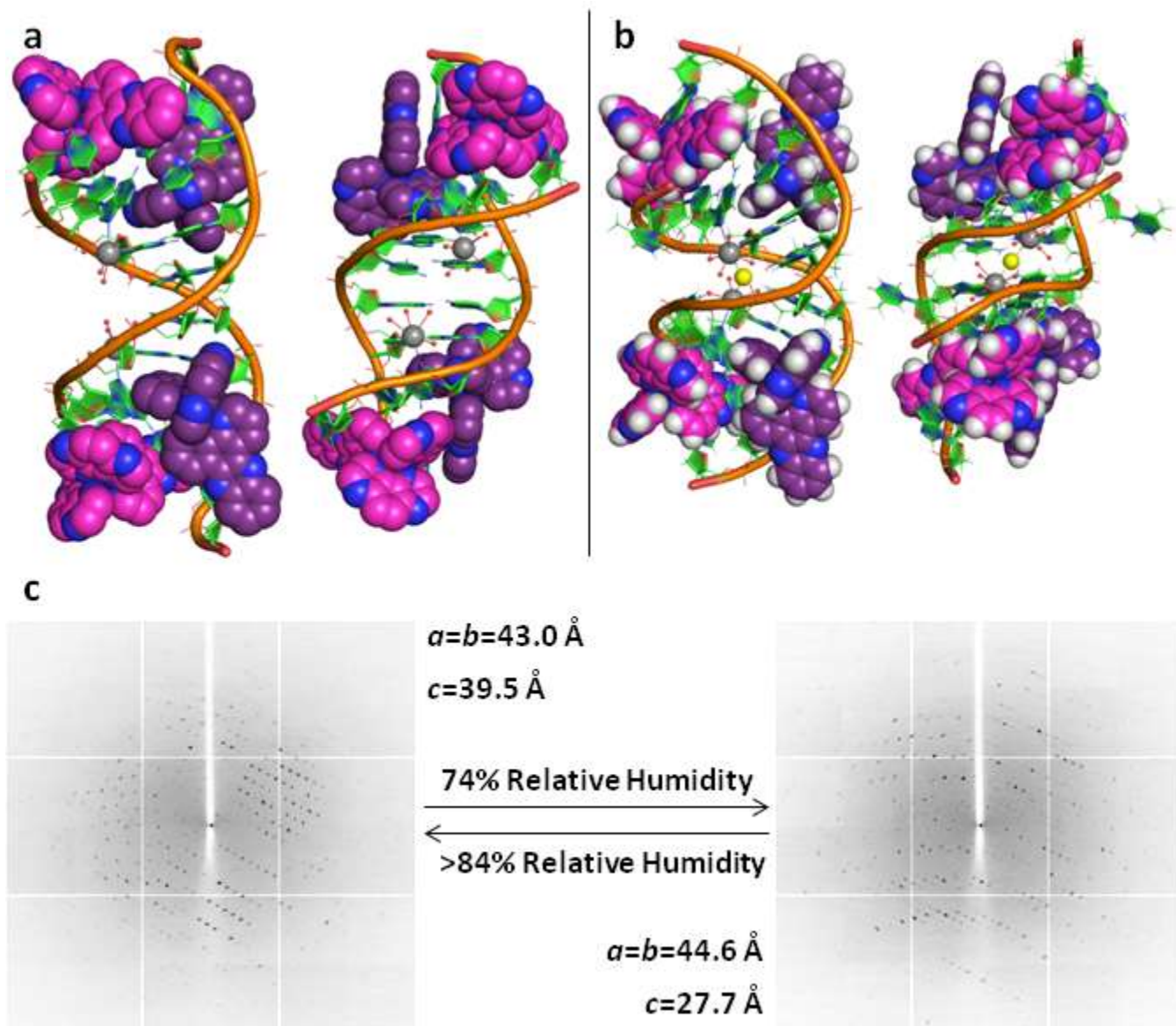


Figure 2: (a) Two views of the biological unit of structure **1** (99% relative humidity, left) and **2** (74% relative humidity, right). The left view in each panel is from the side of the duplex with the right view looking into the major groove. Note the narrowing of the major groove in the centre of the duplex and the chloride anion, which is bound to the Ba^{2+} in **2** but not in **1**. Intercalating complexes have their carbon atoms coloured magenta with the carbons of semi-intercalating complexes in purple. Other atoms are coloured according to type with nitrogen in blue, oxygen in red, DNA carbon atoms in green, barium cation in silver, bound water molecules as red spheres and the chloride anion in yellow. The DNA backbone is drawn as a tube. (b) Two diffraction images collected from a crystal, at the same orientation, at 89% (left) and 74% (right) relative humidity. As the structure changes, so does the diffraction pattern. Note that the reflections extend to a higher resolution in the image collected from the dehydrated crystal.

Results

Seven crystal structures were obtained from one crystal by varying both the beam position and relative humidity for each data collection. The structures are numbered **1-7** with key crystallographic statistics in table 1. Structures **1, 3, 5** and **7** are of the hydrated form with **2, 4** and **6** as the dehydrated. For a structural comparison, only structures **1** and **2**, the initial hydrated form and highest resolution dehydrated structure respectively, will be discussed. Subsequent structures in the same hydration state are isostructural apart from the number of ordered water molecules, as shown by the rmsd values in table 1, performed using LSQKAB, comparing the DNA and ligand atoms in structures of the same hydration state. The crystals, when dehydrated, give remarkably different diffraction (figure 2c) even though the spacegroup, $P4_32_12$, is maintained. The solvent content is reduced from 66% in the hydrated form to 55% in the dehydrated. Initially it was difficult to collect data from a crystal which had undergone dehydration and rehydration multiple times, because of radiation damage occurring at room temperature. However, by taking a large crystal and changing the beam position for each dataset, the transition was repeated both on multiple crystals and multiple times on the same crystal.

Whilst gross structural features such as intercalation and semi-intercalation into the minor groove and barium cation coordination remain the same, the overall conformation of the DNA chain showed a transition further towards the A-DNA form. Full data collection and refinement statistics can be found in table S1 in the supplementary information. It should also be noted that when the crystal was dehydrated for the first time, to give structure **2**, the data were of sufficient resolution to include hydrogen atoms, at calculated positions, within the model. This was not the case with the other six datasets.

Intercalation Site

Intercalation by the dppz of the complex into the $T_1C_2:G_9A_{10}$ step is observed in both forms. In **1** A_{10} is flipped out, as illustrated in figure 3a, and forms a reverse Watson-Crick base pair with T_1 from a neighbouring symmetry equivalent duplex. Remarkably, in **2** this base flipping is reversed with T_1 flipped out (figure 3a), forming the reverse Watson-Crick base pair with A_{10} from a neighbouring duplex, with A_{10} flipped back in. In **1** the long axis of the dppz group is offset from the G_9-C_2 hydrogen bonds by 55° which is increased to 65° in **2**. As a consequence of this reorientation, there is an increase in distance between the complex and DNA bases. The distance between $C7'$, on the TAP which is not semi-intercalating, and $G_9(NH_2)$ is increased from 3.48 \AA to 4.08 \AA in structures **1** and **2** respectively, displayed in figure S1 in the supplementary information. Additionally, the distance between $C7'$ and $G_3(NH_2)$ is also increased from 3.82 \AA to 4.40 \AA , along with a similar increase to the Ru^{2+} which is given in table S4 in the supplementary information. This increase in distance in structure **2** is combined with slight

pyramidalization of the Ru²⁺ coordinated dppz nitrogens which is not present in structure **1**. As the complex is known to become strongly oxidising upon photoexcitation^{19–21}, these structural changes might be expected to affect the rate of the forward electron transfer process. Equally the back reaction, which will determine the extent of permanent DNA damage, is expected to depend on the distance between the reduced TAP ligand in the metal complex and the transiently oxidised guanine.

Table 1 – Selected Data Collection and Refinement Statistics for **1-7**

Structure	1	2	3	4	5	6	7
Cell dimensions							
<i>a</i> , <i>c</i> (Å)	42.9, 39.5	44.3, 27.5	42.9, 39.4	44.1, 27.4	43.1, 39.4	44.1, 27.4	43.1, 39.3
Resolution (Å)	29.09-1.50 (1.54-1.50)*	31.32-1.18 (1.21-1.18)	30.39-1.60 (1.64-1.60)	31.25-1.41 (1.45-1.41)	30.48-1.80 (1.85-1.80)	27.49-1.45 (1.19-1.45)	30.5-2.07 (2.12-2.07)
Relative Humidity (%)	97	74	97	74	97	74	97
Structural Form	Hydrated	Dehydrated	Hydrated	Dehydrated	Hydrated	Dehydrated	Hydrated
Unit Cell Volume (Å ³)	72696	53968	72512	53287	73189	53287	73004
*Outer shell statistics given in parentheses							
Refinement							
No. Reflections	5301	8525	4341	4994	3575	4913	2376
R _{work} /R _{free}	0.13/0.15	0.08/0.11	0.14/0.17	0.13/0.16	0.14/0.18	0.13/0.15	0.15/0.19
No. Atoms							
DNA	202	315	202	202	202	202	202
Complex	51	73	51	51	51	51	51
Chloride	-	1	-	1	-	1	-
Barium cation	1	1	1	1	1	1	1
Water	37	43	32	37	28	31	17
Average B factors							
DNA	29.20	20.02	29.63	20.18	32.59	20.85	39.91
Complex	24.02	14.19	25.13	16.51	26.72	16.51	31.74
Chloride	-	13.24	-	15.45	-	17.12	-
Barium cation	24.12	12.24	24.85	16.56	27.61	15.69	34.40
Water	45.14	37.17	42.04	33.03	41.25	31.55	42.80
RMSD of all non-water atoms, with respect to 1 or 2 (Å)	NA	NA	0.058	0.173	0.072	0.074	0.134
PDB ID	4LTF	4LTG	4LTH	4LTI	4LTJ	4LTK	4LTL

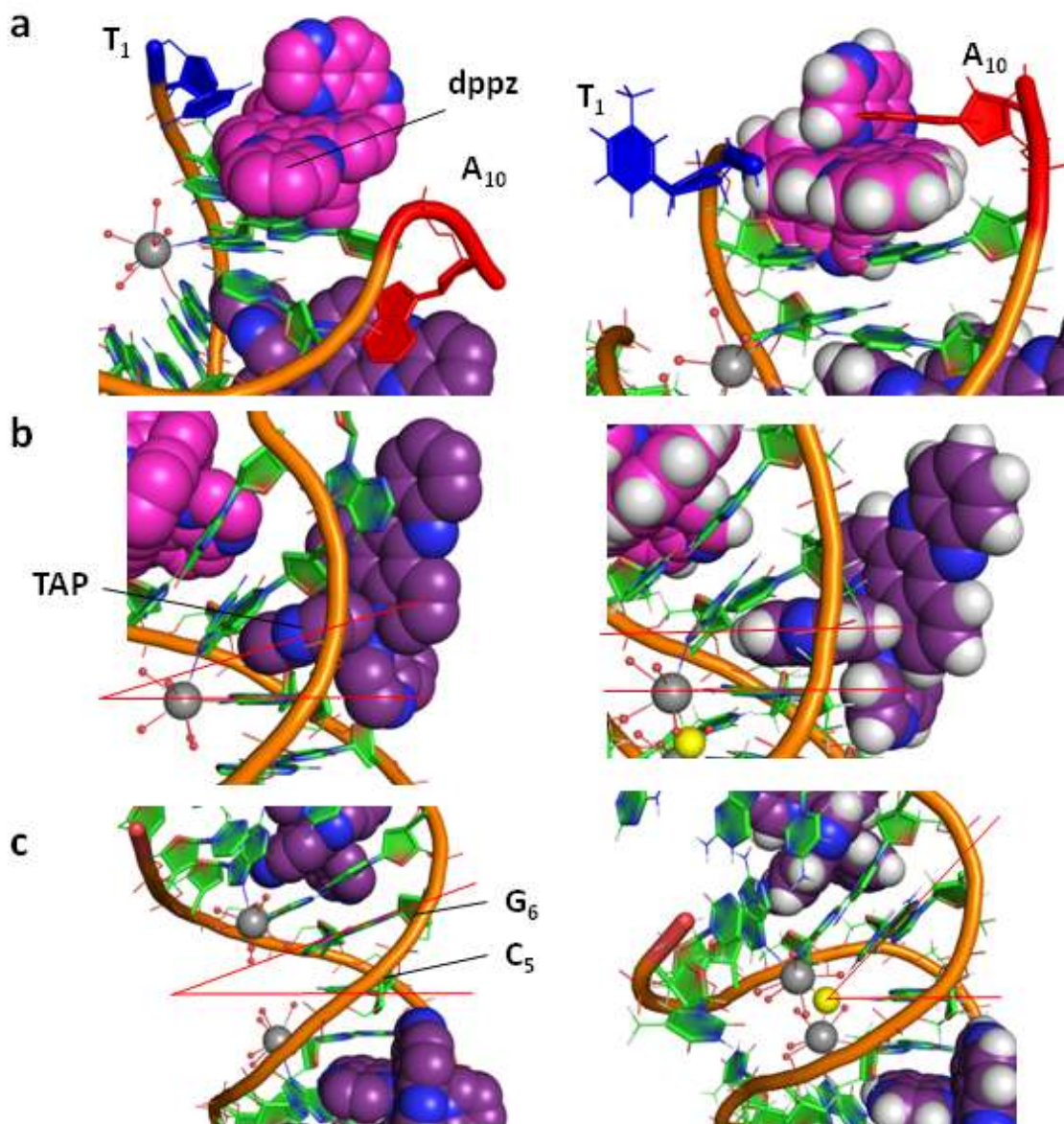


Figure 3: (a) In **1** (hydrated) the T₁ (blue) is stacked on top of the dppz with A₁₀ (red) flipped out (left). However, in the dehydrated form this is reversed so that T₁ is flipped out and A₁₀ is flipped back in. (b) In **1** a TAP lies between the G₃C₇ and G₄C₆ base pairs. The angle between the plane of the TAP and G₄C₆ base pair, indicated by red lines, is approximately 20°. This is reduced to <10° in structure **2** and the TAP no longer stacks onto the G₃C₇ base pair. (c) The roll angle at the central C₅G₆:C₅G₆ base pair is 23° but, when dehydrated to give **2**, is increased to 51°, as indicated by red lines. Also note the barium cations, drawn in silver, have moved closer together in **2** and are bridged by a chloride anion (yellow). Unless otherwise specified carbons from the intercalating complexes are in magenta with those from the semi-intercalating compound in purple. All other atoms are coloured according to element with carbon in green, nitrogen in blue, oxygen in red, phosphorus in orange, hydrogen in white and bound water molecules as red spheres.

Semi-intercalation Site and Barium Coordination Environment

One TAP group (TAP1) from the complex semi-intercalates into the G₃G₄:C₇C₈ base pairs, inducing a kink in the duplex. The size of this kink is 51° in **1** but is reduced to 48° in **2**. The kink is stabilised by a Ba²⁺ cation which is bound to G₃(N7) and bifurcated to G₄. In **1** the TAP is almost equidistant from G₃:C₈ and G₄:C₇ but, when dehydrated to give **2**, the TAP group stacks fully onto G₄:C₇, as illustrated in figure 3b. The barium cations at these steps are 9.3 Å apart in **1**, with a full coordination shell of six water molecules, but move closer to 5.9 Å when dehydrated. This is attributed to the increased kink at the central step, which will be discussed later. As a consequence of this movement there is no longer space to accommodate a full coordination shell of water molecules around the cations, and one water molecule from each barium coordination sphere has been replaced by a bridging chloride anion (figure 3c). The assignment of a chloride anion was based on a typical Ba-Cl bond distance of 3.2 Å²² and a 7σ peak in the F_o-F_c difference map when the peak is assigned as water. The Cl⁻ comes from the solvent channels, which would contain NaCl from the crystallization solution, and are able to move into the structure as the data are collected at room temperature.

Central Step

In both forms the central C₅G₆:C₅:G₆ step is unbound. In **1** the roll angle for this step is 23° but, when dehydrated, this increases to 51° (figure 3c) which is almost identical to the bend induced by semi-intercalation of the TAP1 group, although there is no ligand binding at this step. This kink is believed to be stabilised by interstrand water mediated hydrogen bonds, which are discussed later.

Conformational Analysis

The sugar pucker analysis shows that structure **1** possesses a conformation which is a hybrid between the B and A-DNA form. Five nucleotides possess a B-DNA pucker with the other five adopting an A-DNA conformation. However, after dehydration, the helix has adopted a form closer to that of A-DNA, with eight out of ten bases possessing the typical A-DNA C3'-*endo*/C4'-*exo* pucker, and two guanines, G₄ and G₆, retaining the C2'-*endo* pucker of B-DNA. This could be because by preserving this pucker the stacking between G₄ and the complex is maximised. However, for G₆, the retention of the sugar as C2'-*endo* maximises the stacking onto C₇, as a transition to C3'-*endo* could result in a translation of the base towards the major groove. Details of the sugar pucker analysis can be found in table 2. All calculations were performed using W3DNA²³.

The highly detailed study, reported by Shing Ho *et al*, reports a transition from B to A-DNA observed in 13 different crystal structures of d(GGCGCC)²⁴. Whilst this sequence is the same as the core

of the DNA used in this study, d(T₁C₂G₃G₄C₅G₆C₇C₈G₉A₁₀) with the core highlighted in red, comparison with this work is difficult. The reason is that at the G₃G₄:C₇C₈ and C₇C₈:G₃G₄ steps a TAP group is semi-intercalated into the DNA. In addition to this, in the dehydrated form, the DNA is kinked at the central C₅G₆:C₅G₆ site. A comparison of the distribution of χ and δ torsion angles for this core is displayed in figure S2 and table S2 in the supplementary information, and shows that both **1** and **2** display an approximately equal set of these two torsion angles. The rise distance and twist angle at each step have also been calculated and can be found in table S3 in the supplementary information. These show that at semi-intercalated sites the rise distance is increased to approximately 5 Å, compared to the 3.4 Å rise for B-DNA, but this is combined with a reduction at non-interacting steps, from 3.24 Å in **1** to 2.97 Å in **2**. However, there is also an increase in rise distance at the central C₅G₆:C₅G₆ step, from 3.56 Å in **1** to 4.78 Å in **2**, which is attributed to the formation of a kink. The DNA is unwound at the semi-intercalated sites, to 15°, but is more tightly wound at the G₄C₅ and G₆C₇ steps to 38°. The twist at the central step is lower in **2** (16°) than in **1** (26°) which is again attributed to the kink.

Therefore, for this work, the sugar pucker has been chosen as the most appropriate criterion as both intercalation and semi-intercalation of a ligand will limit, or bias, the conformational flexibility of the bases around the interaction site. For example, intercalation by a planar ligand will limit the flexibility of a base to rotate around χ as if the base rotates it would clash with the intercalating group. In addition to this the rise distance at the intercalation site would be increased and, depending on the identity of the group, the twist could either be increased²⁵ or decreased²⁶ compared to B-DNA.

Water Structure

In structure **1** (hydrated form) the water structure is largely unremarkable. A majority of the molecules are located in the major groove, as the minor groove is largely blocked by the four interacting Ruthenium complexes. Hydrogen bonding between water molecules and the phosphate backbone is common in the structure. The Ba²⁺ cations possess a coordination shell with six water molecules. A hydrogen bond is present between HOH 225, which is coordinated to the Ba²⁺ cation, and G₄(O6) which is illustrated in figure 4a. This is the only example of an interstrand cross-link in the structure, and is mediated by a single water molecule.

However, in the transformation to structure **2**, five water mediated interstrand crosslinks are formed. These occur either by (1) forming a hydrogen bonded bridge through a single water molecule between two phosphate groups on opposing strands or (2) via a water molecule coordinated to the Ba²⁺ cation on one strand and a phosphate group on the other, as illustrated in figure 4b. This hydrogen bonding network could explain why the dehydrated form diffracts to a higher resolution, as an increase in

intermolecular bonding would increase the order of the system. Whilst a majority of the water molecules are found in the major groove, or major groove side of the duplex, there is a slight increase in the number found in the minor groove. This could be because the kink at the central step opens up the minor groove side of the DNA allowing hydrogen bond formation with groups on the floor of the groove.

The formation of the bridging network is only possible because the phosphate backbone of the two strands approach so closely to each other. The separation between $G_3(P)$ on one strand and $G_4(P)$ on the other is 7.76 \AA but the $G_3(P)$ - $G_3(P)$ distance is even shorter at 6.09 \AA , much smaller than the major groove separation observed, in multiple A-DNA crystal structures, of approximately 10 \AA ²⁷. This is attributed to the combination of the A-DNA conformation and the inducement of an increased kink, bending the helix towards the major groove, at the central step.

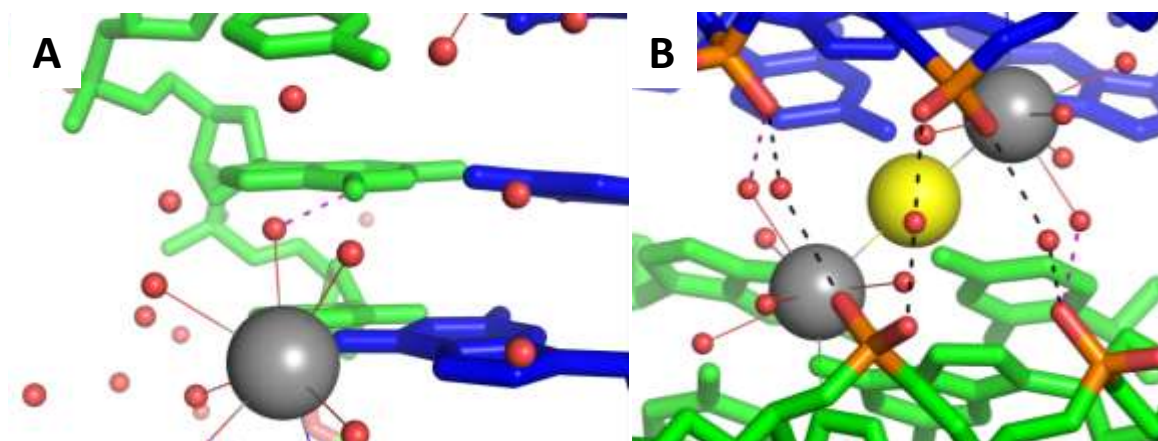


Figure 4: Water mediated interstrand crosslinking, in the major groove, in structure **1** (left) and **2** (right). The only example of this in structure **1** is where a water coordinated to the Ba^{2+} forms a hydrogen bond with $G_6(O6)$ from the opposing strand. However in structure **2** there are three water molecules which form bridging hydrogen bonds between $G_3(OP1)$, $G_3(OP2)$ and $G_4(OP2)$ on opposing strands. Additionally two hydrogen bonds are formed between waters coordinated to the Ba^{2+} and $G_4(OP2)$. The two opposing DNA strands are illustrated in green and blue. Hydrogen-bonded phosphate groups are coloured according to type with oxygen in red and phosphorus in orange. Other atoms are coloured according to type with the Ba^{2+} cation displayed in grey, chloride anion in yellow and water molecules as red spheres. Interstrand hydrogen bonds are indicated by dashed lines, in black to indicate (P)O-HOH-O(P) links and magenta to highlight Ba^{2+} -HOH-O interactions.

Table 2: Conformational analysis for structures **1** and **2**. In all cases the parameter before the forward slash is for structure **1** with structure **2** given after.

Base	Phase angle of pseudorotation	Assigned Sugar Pucker	Closest DNA Conformation
T	34.7/30.1	C3'-endo/C3'-endo	A/A
C	36.1/17.2	C3'-endo/C3'-endo	A/A
G	18.2/15.8	C3'-endo/C3'-endo	A/A
G	161/177.1	C2'-endo/C2'-endo	B/B
C	27.7/34.5	C3'-endo/C3'-endo	A/A
G	155.7/173	C2'-endo/C2'-endo	B/B
C	53.6/47.2	C4'-exo/C4'-exo	A/A
C	88.7/30.3	O4'-endo/C3'-endo	B/A
G	156.9/8.0	C2'-endo/C3'-endo	B/A
A	162.7/14.1	C2'-endo/C3'-endo	B/A

Reversibility

What is striking, and quite unforeseen, about this study is that the transition, between the hydrated and dehydrated forms, is reversible when the relative humidity around the sample is increased. This is despite a large difference in the length of the *c* axis, which is 39 Å in the hydrated form but is reduced to 27 Å when dehydrated. The unit cell volume for the hydrated form is 72,696 Å³ and the volume of the dehydrated form is 53,968 Å³ so, after dehydration, the unit cell is 74% of its original volume. The solvent content of the hydrated and dehydrated form is 66% and 55% respectively, calculated using UCSF Chimera,²⁸ showing a drop in solvent content within the crystal. When water is removed the solvent channels contract in size, as illustrated in figure 5 and a 51° kink is induced in the central step, as discussed above. In addition to this the dehydrated form, in all cases, possesses lower average B-factors for the DNA and exhibits higher resolution diffraction than the preceding or subsequent hydrated form, as illustrated in figure S3 in the supplementary information. In addition to this there are more ordered water molecules in the dehydrated structures compared to the hydrated, despite the latter possessing a higher solvent content.

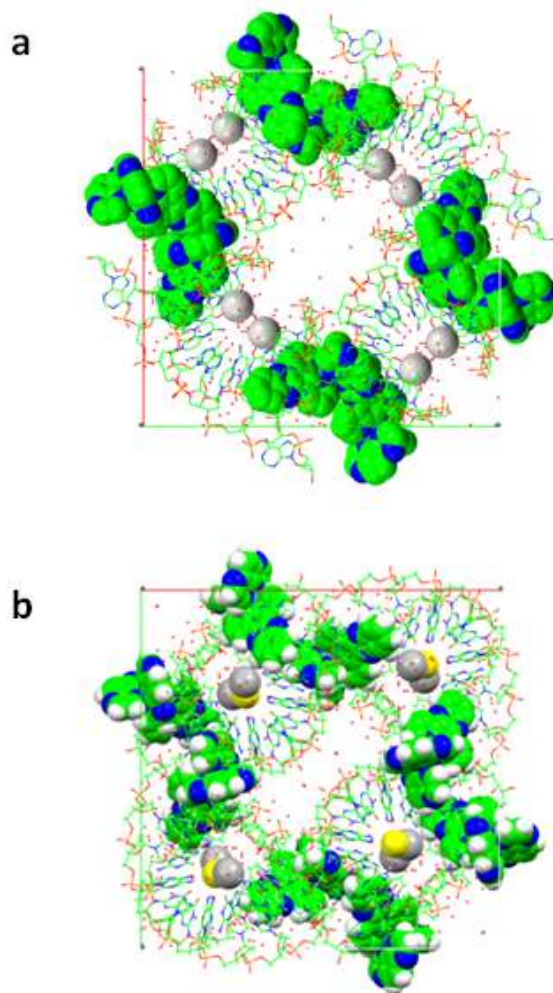


Figure 5: Packing diagrams, in space group $P4_32_12$, viewed down the c axis, for (a) structure **1** and (b) **2**. Note the contraction in the central solvent channel in structure **2** (dehydrated) compared to **1** (hydrated). Atoms are coloured according to type with carbon in green, oxygen in red, nitrogen in blue, phosphorus in orange, hydrogen in white, Ba^{2+} in silver and chloride in yellow. The Λ -[Ru(TAP)₂(dppz)]²⁺, Ba^{2+} and Cl^- are displayed as a space filling model.

Conclusions

Previously it has been found that at a low relative humidity it is possible to obtain multiple DNA conformations, including the A²⁹ and Z-DNA³⁰ forms, using a variety of techniques. Yet, whilst the A and B-DNA forms have been crystallized individually,^{31,32} there have been no reports of inducing a full, or partial, transition between the two in a single crystal. Dehydration of DNA crystals has previously been reported but only resulted in an increase in diffraction resolution and not accompanied by a transition between DNA forms,³³ possibly due to the presence of a minor groove binding agent which would restrict conformational changes.

Whilst the transition between **1** and **2** is not a full transformation, it does represent a partial conformational shift, from an A/B hybrid to an 80% A-DNA conformation. This was an unexpected consequence of the dehydration, which is traditionally used to increase diffraction quality in poorly diffracting crystals.³⁴ In fact, an increase in order in the system when performing the dehydration systematically was observed. The ability to induce the transition between the hydrated and dehydrated form, and then reverse it again on demand, was not expected and is made even more unusual as it was accompanied by a large structural change.

The induction of a kink is particularly interesting as it highlights the inherent flexibility of the DNA duplex, even in the crystalline state. This flexibility is exploited by DNA-mismatch repair proteins, which travel along the helical axis of the duplex searching for regions of increased flexibility, compared to well matched base pairs, which indicates the presence of a mismatch.³⁵ The question of why a single kink is induced instead of a bend is an interesting one. Instead of completely destacking the central C₅G₆:C₅G₆ base pair, the G₄C₅:G₆C₇ and G₆C₇:G₄C₅ steps could have been partially destacked, along with the central step, forming a less sharp bend across the three base pairs. A GC base pair stack is more stable than a CG stack, stabilising the duplex by -2.70 kcal/mol⁻¹ and -1.44 kcal/mol⁻¹ respectively,³⁶ and therefore it is more favourable to destack the central step rather than partially destack all three pairs.

In terms of biological relevance, the hydrated form could be said to be more representative as the dehydrated structure can only be formed when the crystal is dehydrated to a relative humidity which is comparable to that of a 6.3M NaCl solution.³⁷ This is difficult to compare to the solvent environment *in vivo* as information about the solvent content of organelles, in a particular cell type, is limited. One study has reported that the water content of organelles within HeLa cells is 65-80%³⁸ but, as the solvent content of crystals is a direct consequence of crystal packing, this does not enable comparison with known crystal structures. Relative humidity may be a more suitable metric as it is demonstrated here, and with previously reported systems, that structural transitions can be induced by dehydration.³⁹

The relative humidity at which a structure exists should be reported to ensure that it is possible for this form to exist *in vivo*. What should be highlighted is that, in our case, an analysis of the dehydrated form would have led to the conclusion that intercalation at the central step of the decamer was impossible, due to the large kink. Instead, we have previously reported the symmetrical intercalation of an additional ruthenium complex at this central step, using a slightly different sequence.²⁵

This study reports the consequences to DNA structure which are observed when the DNA is both dehydrated and kinked simultaneously and reveals an interesting new hydrogen-bonding motif. When DNA is kinked, for example by *cis*-platin, the interstrand phosphate distance in the major groove can be reduced to approximately 10 Å^{3,12}, similar to the 9 Å separation observed in A-DNA²⁷. However, in both cases, individual water molecules are unable to form a P(O)-HOH-(O)P bridge as the distance is too great. In our study we observe an intermolecular P-P distance as low as 6.1 Å, enabling single water molecules to form H-bonded bridges between the phosphate oxygen atoms. The extra hydrogen bonds from both the bridging water and Ba²⁺ coordinated water would stabilise the new form, and is presumably one reason why this dehydrated conformation is stable in the crystal and is formed uniformly.

This new binding motif has implications for both molecular engineering and DNA self assembly. Water content has been found to affect the formation of twists in a cationic amphiphile consisting of pyridinium and a long chain glutamide (PULG)⁴⁰. It was reported that altering the amount of water changed the magnitude of the uniform twist observed in a single PULG strand. In addition to this, gelation was only able to occur in the presence of water, and not organic solvents alone, suggesting that one key factor in the formation of the gel was the occurrence of water mediated intermolecular hydrogen bonds between the polar groups. Our system also changes structure as a function of relative humidity and therefore is an example of a hydration sensitive supramolecular switch. It has also been shown that DNA intercalators, such as ethidium, can promote the formation of error-free self-assembled DNA nanostructures⁴¹ and therefore one must consider whether the introduction of a semi-intercalator could promote a kinked structure. If assembly were performed in a low-humidity environment, in the presence of a semi-intercalator, then it may be possible that water mediated interstrand crosslinks could form, in a similar manner to those observed here, affecting the final structure. It should also be noted the crystals reduce in size, on the micron scale, when dehydrated and therefore could be used to activate a mechanical switch at a particular relative humidity.

This result highlights that crystals, traditionally thought of as static systems, can still be dynamic at room temperature and can therefore be the subject of further experimentation after growth. Now that this crystal system has been established as robust, the possibility of exploring the reactivity of the

complex within the crystalline state will be explored because, as commented on above, the hydration-induced changes in the interaction of the metal complex might have a significant effect on the photophysical properties of the complex.

Methods

Crystallization

The oligonucleotide d(TCGGCGCCGA) was purchased from ATDBio as a solid purified by double-HPLC. Λ -[Ru(TAP)₂(dppz)].2Cl was synthesised according to literature methods.^{13,19} Crystals containing the nucleotide and the complex Λ -[Ru(TAP)₂(dppz)].2Cl were grown using vapour diffusion from sitting drops at 18°C. The drops contained 1 μ l of 2mM oligonucleotide, 1 μ l of 4mM *rac*-[Ru(TAP)₂(dppz)]Cl₂ and 6 μ l of the following solution; 10% (vol/vol) MPD, 40 mM sodium cacodylate (pH 6.0), 12mM spermine tetra-HCl, 80mM sodium chloride and 20 mM barium chloride. The drop was equilibrated against 1ml of 35% (vol/vol) MPD and reddish-yellow rhombohedra appeared after approximately one week. It should be noted that individual crystals possessing the hydrated or dehydrated unit cells were found in the same crystallization drop.

Data Collection and Refinement

All seven data sets were collected from a single crystal, of approximate dimensions 150 x 180 x 100 μ m with a 0.3s exposure time on beam line I02 at Diamond Light Source Ltd with radiation of wavelength 0.8266 Å. Data were collected at 295 K. A total of 90° of data were collected in 90 images with a 1° oscillation angle. Each dataset was collected from a separate volume of the crystal to minimise the effects of radiation damage. The data were integrated with xia2⁴² with XDS⁴³ and processed using SCALA⁴⁴. The structures were solved by molecular replacement using Phaser⁴⁵ with **3QRN** as the starting model for the hydrated data sets and **3S80**, a low resolution structure solved from data collected on the Gemini-S-Ultra single crystal diffractometer in the Chemical Analysis Facility at the University of Reading, for the dehydrated data sets. The models were refined using REFMAC5⁴⁶ after being updated with Coot⁴⁷. The CCP4 suite was used throughout⁴⁸. Five percent of reflections were reserved for the R_{free} set. All models were deposited in the Protein Data Bank with the following ID's: **4LTF**, **4LTG**, **4LTH**, **4LTI**, **4LTJ**, **4LTK** and **4LTL**. Selected statistics can be found in table 1 with full data collection and refinement statistics in table S1 in the Supplementary Information.

Dehydration

A drop (<1 μ l) of the crystal mother liquor was mounted on a Micromesh from MiteGen and placed on the goniometer of beamline I02 at Diamond Light Source Ltd. The initial relative humidity (RH) of the mother liquor was 99% and was found according to literature methods.³⁹ The crystal was then mounted, on the same Micromesh, and two minutes allowed for the crystal humidity to equilibrate. Next, three diffraction frames 45° apart were taken to establish a baseline for both the unit cell dimensions and diffraction quality. Automated processing available on the beamline using EDNA⁴⁹ was used throughout to index the diffraction frames.

Next, a crystal was dehydrated at a rate of 2% RH per minute, with three frames of data collected after each two percent change. A drop in diffraction quality was observed at 84% relative humidity and the diffraction spots became poorly defined. Diffraction quality then increased at 74% relative humidity, associated with a change in unit cell dimensions. The relative humidity was then increased to 99%, at 2% per minute, and three frames of data, 45° apart, were taken. This gave an increase in unit cell dimensions

Another crystal was mounted on a Micromesh from MiTeGen and was equilibrated to 97% relative humidity, with the mother liquor humidity found using the same method. The crystal was repeatedly dehydrated and rehydrated to give seven datasets which in turn yielded seven structures (**4LTF**, **4LTG**, **4LTH**, **4LTI**, **4LTJ**, **4LTK** and **4LTL** in the Protein Data Bank). The HC1b humidity control device was used throughout to control the relative humidity at the sample position. This device replaces the cryojet and allows for monitoring of the crystal by diffraction whilst changing relative humidity. Figure S4, showing the appearance of the crystal before and after dehydration, is given in the supplementary information.

Associated Information

Full data collection and refinement statistics, along with figure S5, showing **1** and **2** superimposed, are available in the supplementary information.

Author Contributions

J.P.H wrote the paper with input from all other authors. J.P.H and J.S-W carried out the experiments, using materials synthesised by C.A, C.H.Q, K.O'S, J.M.K and D.J.C. J.P.H, C.J.C and J.S-W designed the experiment. J.A.B, T.S and G.W assisted with analysis of the results and useful discussions.

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Competing Financial Interests

The authors declare no competing financial interest

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