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The role of water in mediating DNA structures with epigenetic modifications, higher-order conformations and drug–DNA interactions

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Water is arguably one of the most important chemicals essential for the functioning of biological molecules. In the context of DNA, it plays a crucial role in stabilizing and modulating its structure and function. The discovery of water-bound motifs in crystal structures has greatly improved our understanding of the interactions between structured water molecules and DNA. In this manuscript, we review the role of water in mediating biologically relevant DNA structures, in particular those arising from epigenetic modifications and higher-order structures such as G-quadruplexes and i-motifs. We also examine water-mediated interactions between DNA and various small molecules, including groove binders and intercalators, and emphasize their importance for DNA function and therapeutic development. Finally, we discuss recent advances in tools and techniques for predicting water interactions in nucleic acid structures. By offering a fresh perspective on the role of water, this review underscores its importance as a molecular modulator of DNA structure and function.

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1. Introduction

Water exhibits unique properties that are essential for biological processes. Its polarity and ability to form hydrogen bonds make it a critical solvent. Despite its simple molecular structure,

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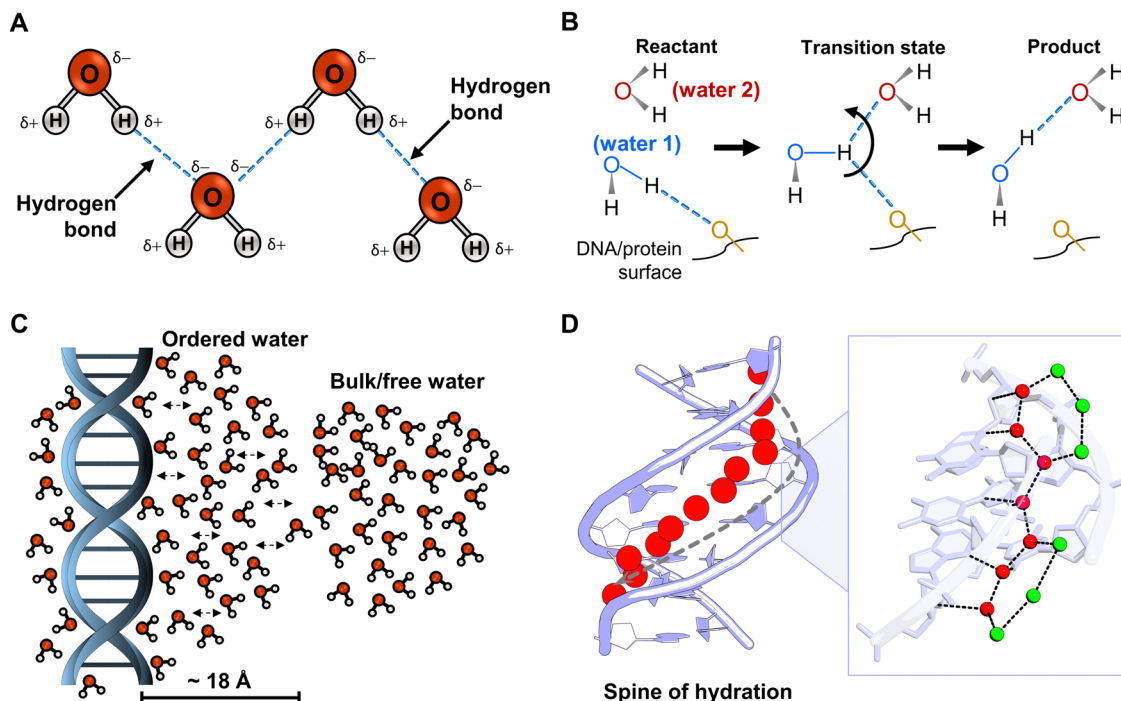
Fig. 1 (A) Schematic representation of hydrogen bonding between water molecules. (B) Illustration of the jump model depicting the reorientation of a water molecule (water 1) that forms a hydrogen bond with a nearby water molecule (water 2) and simultaneously breaks the hydrogen bonds with other molecules, such as DNA and proteins. The figure is adapted with permission from *J. Am. Chem. Soc.*, 2016, **138**(24), 7610–7620. (C) Diagram showing ordered water molecules in the hydration shell interacting with DNA. A range of ordered water extending up to 18 Å is depicted. (D) The linear spine of hydration (red spheres) observed in the AT-rich region of a B-DNA (PDB ID: 4C64). Enlarged view shows an extensive interconnected network of hydrogen bonds formed by water molecules in the first hydration shell (red spheres) interacting with DNA bases, the backbone, and water molecules in the second hydration shell (green spheres).

water's remarkable ability to form hydrogen bonds with other molecules is crucial to its life-supporting characteristics.[1,2] In water, hydrogen bonds form when each partially positively charged hydrogen atom is covalently bonded to a partially negative oxygen atom in one water molecule while also being attracted to the oxygen atom of a neighboring water molecule (Fig. 1A).[3] The hydrogen bonds in water molecules undergo continuous breaking and reformation. This dynamic behavior has been explained by the "jump model" mechanism.[4,5] When a water hydroxyl group switches its hydrogen bond acceptor, it undergoes large-amplitude angular jumps (Fig. 1B). These jumps regulate the reorientation dynamics of water molecules, whether they are free or interacting with other molecules, such as ions, DNA, and proteins.[6–8] This remarkable ability of water to form dynamic intermolecular hydrogen bonds, both within itself and with other molecules, is a key factor in enabling various biological functions.

Of particular interest, water plays a crucial role in influencing the stability and functionality of biomolecules, including proteins and nucleic acids. At protein interfaces, water forms a solvation layer that modulates intermolecular interactions and functions.[7,9,10] In the nucleosome, for example, water promotes interactions between histones and DNA, thereby stabilizing the entire structure.[11] During DNA–protein recognition, water-mediated polar contacts contribute to binding specificity.[12,13] Water at protein interfaces also assists in the binding of small molecules.[14] Conserved water molecules in the vicinity of

proteins have been shown to play a guiding role in the structure-based design of novel protease inhibitors, a promising approach in drug discovery.[15] Moreover, water can act as a channel for proton transport and plays a crucial role in the catalytic functions of proteins.[16] In addition to the more dynamic water molecules on the protein surface, Takano *et al.* demonstrated that water molecules within the interior cavities of proteins play a crucial role in enhancing structural stability by examining the crystal structures and conducting thermodynamic studies on a series of human lysozyme mutants.[17] This internal water provides stability and ensures the correct folding and unfolding of proteins. The dynamic role of water in maintaining protein structures and functions has been the subject of numerous reviews.[10,18–23]

Similar to proteins, the role of water in DNA structures has long been the subject of research.[24–28] Since DNA lacks a distinct interior hydrophobic surface like proteins, the majority of its atoms tend to interact with surrounding water molecules. DNA can transition between its B- and A-form conformations under varying hydration conditions.[29–31] This reversible conformational shift alters the DNA backbone and the groove widths, exposing buried residues to form contacts with proteins and small molecule ligands. The formation of A-DNA is also important for cellular defense mechanisms under harsh conditions.[32] Thus, solvation and desolvation in DNA not only maintain its structural integrity but also drive biochemical functions.

Moreover, water plays an essential role in mediating interactions between DNA and small molecule ligands. Numerous studies highlighted the importance of water interactions in drug–DNA complexes.[33–35] Unlike DNA, RNA typically exists as a single strand and can fold into complex three-dimensional structures necessary for its biological functions.[36,37] Water is critical to this folding process.[38] Water also mediates interactions between RNA and other molecules, improving the specificity and stability of RNA–protein and RNA–drug complexes.[39–42] In catalytic RNA, water plays a key role by participating in enzymatic reactions as a proton donor or acceptor.[43] Thus, water acts as an integral part of the molecular architecture in biological systems, essential for driving molecular interactions and biochemical functions.

Advances in structural biology have provided significant insights into the fundamental interactions between water and DNA. The increasing number of high-resolution structural motifs has yielded extensive data emphasizing the essential role of water in DNA. A detailed analysis of literature on water-mediated interactions within nucleic acids deepens our understanding on the role of water and its possible implications in nucleic acid functions and therapeutic development. In this review, we provide an overview of the importance of water interacting with nucleic acids, with a particular focus on its role in mediating biologically relevant DNA structures. Subsequently, we discuss water-mediated interactions between small molecules and DNA. We review advancements in new tools and techniques for predicting water interactions within nucleic acid structures. This review offers a new perspective on the critical role of water in molecular and chemical biology of DNA.

2. Ordered water molecules around DNA are essential for its structural stability and function

Water maintains the structural integrity of DNA by forming a robust, interconnected network of immobilized, ordered molecules known as the "hydration shell." Hydration shells are essential for stabilizing double-helical structures and facilitating the formation of the grooves. The hydration shell consists of multiple layers of water molecules (Fig. 1C). Water molecules directly interacting with DNA up to ${\sim}3$ Å typically form the first hydration shell, establishing direct hydrogen bonds with phosphate groups, sugar moieties, and nitrogenous bases. Water molecules located ${\sim}3$–8 Å from the DNA surface constitute the second hydration shell; these are less tightly bound and act as a bridge between the first hydration shell and bulk water. Beyond the second hydration shell, water molecules gradually transition to bulk-like behavior while still experiencing some influence from DNA's electrostatic field.

While the hydration shell around biomolecules has been studied for a long time,[44,45] recent experimental and computational advances have uncovered new insights into the dynamics and structures of ordered water, which play a critical role in DNA conformation and functionality. For example, using a combination of dielectric relaxation, terahertz spectroscopy and all-atom molecular dynamics simulations, Singh *et al.* comprehensively analyzed the collective motions of hydrated DNA molecules.[46] Their results demonstrated that the hydration structure extends as far as ${\sim}18$ Å from the DNA surface, with the intensity of water molecule interactions determined by their proximity to DNA; *i.e.*, the water molecules exhibit different relaxation behavior depending on their interactions with the DNA. For example, water molecules closer to the minor grooves exhibit the slowest dynamics, followed by those interacting directly with the major grooves and phosphate groups. Meanwhile, water molecules farther from the DNA surface are more loosely bound and have faster dynamics.[46] These observations imply that DNA influences water molecules beyond just its immediate surroundings, *i.e.*, the first and second hydration shells. Interestingly, even such distant waters can interact with Watson–Crick base pairs but not with mismatched base pairs in DNA.[47] These studies suggest that ordered water interactions with Watson–Crick base pairing are important for the long-distance recognition of DNA hybridization processes and that these interactions could be affected by environmental conditions, such as pH, salt concentration, and temperature.

The properties of water interacting with biological molecules in hydration shells differ significantly from those of free bulk water. Laage *et al.* compared and summarized these dynamic properties across various biomolecules, including DNA, proteins, and phospholipids.[48] Briefly, unlike free bulk water, the hydration shell surrounding DNA consists of strongly bound water, an increased number of hydrogen bonds, and a higher degree of molecular ordering. These water molecules often act as bridges, mediating both intermolecular and intramolecular interactions within DNA or between DNA and other biomolecules. One of the most prominent features of water–DNA interactions is the "spine of hydration" in the minor groove (Fig. 1D), first reported in the crystal structure of the B-DNA dodecamer, d(CGCGAATTCGCG).[49] The water molecules in the hydration spine form a complex, interconnected network involving the first- and second-shell water molecules and their interactions with the base pairs lining the groove. These water sites are interdependent, exhibit cooperative behavior, and are less affected by modifications in DNA bases.[50,51] Notably, the hydration spine can also form in solution under ambient conditions.[52,53] These water interactions are crucial for maintaining integrity, stability, and functionality of DNA. In the following sections, we will explore the interactions between water and both canonical and non-canonical DNA structures, including those associated with epigenetic modifications, gene regulation, and disease-related conformations. Using examples primarily from X-ray crystallography studies, we will highlight these interactions and their critical roles in regulating DNA structure and function.

3. Water interactions in DNA structures

3.1. Water-mediated interactions in canonical DNA structures with epigenetic modifications

The B-form of the DNA duplex is the most biologically relevant structure within the genome, featuring a well-characterized

network of water molecules. In canonical structures including B-DNA, water molecules tend to form hydrogen-bonded interactions with base pairs, backbone, and the grooves.[26,54–56] The spine of hydration is a well-studied hydration aspect in B-DNA. Particularly in A–T rich regions, water in the minor groove creates the spine of hydration due to the narrow width of the groove, which facilitates close water-mediated interactions.[57] On the other hand, in the A-DNA conformation, water molecules exhibit more interactions with the interstrand phosphate oxygen atoms. Phosphate groups in A-DNA are positioned close enough for water molecules to bridge adjacent phosphates *via* their free oxygen atoms—a configuration not possible in B-DNA due to the larger spacing between phosphates.[26] Using *in silico* sampling methods and an in-house-developed thermodynamic parameter analysis, Ghosh *et al.* studied the dynamic and thermodynamic responses of local hydration in a variety of base pair sequences. They demonstrated that the structure, sequence, and composition of the DNA groove influence local hydration characteristics during the B-to-A transition, a process crucial for biological functions.[58]

In addition to interacting with DNA grooves and the backbone, water-mediated interactions with base pairs play a crucial role in maintaining DNA structure. The guanine base can form hydrogen bonds with water molecules using the 2-amino and N3 atoms in the minor groove and the 6-keto group and N7 atoms in the major groove.[59] Cytosine can form hydrogen-bonded interactions with water using the 2-keto group from the minor groove and the 4-amino group from the major groove. Similar to guanine, adenine can use the 6-amino group from the major groove and the free ring nitrogen atoms N3 and N7 in the minor groove to form water-mediated hydrogen bonds. Thymine forms hydrogen-bonded interactions with water using the 2-keto and 4-keto groups from the minor and major grooves, respectively. Interestingly, crystallographic analysis of the hydration sites around DNA bases showed that these hydration sites in purine bases are planar to the plane of the nucleobases and exhibit a consistent pattern, whereas those around pyrimidine bases are non-planar.[60] While the positions of hydration sites around DNA bases are generally consistent, local deformations in DNA can alter the distribution of ordered water molecules surrounding the base pairs. This redistribution of water is crucial for DNA repair proteins to distinguish canonical Watson–Crick base pairs from mismatched ones. For example, the formation of a semi-open conformation of the U:G mismatch, resulted in the reordering of water molecules around uracil in such a way that water acts as a hydrogen bond donor.[61] This contrasts with Watson–Crick C:G base pairing, which usually adopts a closed conformation, where water acts as a hydrogen bond acceptor to the cytosine and guanine bases. These differences in water interactions between mismatched and Watson–Crick base pairs create differences in electrostatic fields, which are likely to affect the base pair discrimination ability of DNA repair proteins.

DNA methylation is essential for regulating gene expression and maintaining genome stability.[62] In an all-atom molecular dynamics simulation study, Teng *et al.* found that cytosine methylation reorganizes the arrangement of water molecules around DNA bases

and strengthens their interactions with water.[63] Recently, Lin *et al.* reported the crystal structures of several native and ligand-bound methylated DNA sequences using X-ray crystallography.[64] Analysis of these structures revealed that cytosine methylation at non-CpG sites in native DNA can promote the formation of water-mediated Hoogsteen base pairs in methylated bases (Fig. 2A). The propensity for such water-mediated base pairing increases upon ligand binding. These findings were further corroborated in solution using NMR spectroscopy. This suggests that the positioning of water and its interactions in methylated base pairs are critical for recognition by external ligands. Thus, these types of water-mediated interactions might play an important role in the recognition of methylated bases by regulatory proteins.

Using X-ray diffraction, Renciuk *et al.* solved a series of crystal structures of B-DNA containing the epigenetic modifications 5-hydroxymethylcytosine or 5-methylcytosine.[65] Based on the analysis of these structures, they proposed that replacing a hydrophobic methyl group with a hydrophilic hydroxymethyl group in epigenetically modified cytosine creates a conserved network of water molecules that interact with the O5 atom of 5-hydroxymethylcytosine bases. The O5 atom of hydroxymethyl groups forms bifurcated hydrogen bonds with water molecules in the vicinity of approximately 3 Å. Similar water-mediated interactions were also observed in an independent study on 5-hydroxymethylation in B-DNA.[66] In another X-ray crystallography study, Wang *et al.* found that, regardless of DNA conformations, 5-hydroxymethylcytosine forms strong water-bridged hydrogen bonds with neighboring bases that stabilizes overall modified structures (Fig. 2B).[67]

Given the fact that hydroxymethylation of cytosine is an intermediate step in the methylation and demethylation processes, and it also plays a significant role in gene regulation, pluripotency, and development,[68,69] the occurrence of a conserved hydration network is supposed to have an active role in epigenetic regulation. Water-bridging interactions have also been observed in another cytosine modification, formylcytosine. In the crystal structure of DNA containing 5-formylcytosine, a water network linking the formyl group of modified cytosine to the phosphate backbone and adjacent nucleobases is identified (Fig. 2C).[70] This resulted in alteration in Watson–Crick base pairing, creating an unusual geometry in which two water molecules bridge the formyl group of cytosine with its complementary guanine. In addition, the extensive hydration of modified bases also plays a crucial role in their interactions with DNA-processing enzymes. For example, in the crystal structures of ten-eleven translocation (TET) proteins complexed with hydroxymethylcytosine- and formylcytosine-containing DNA, Hu *et al.* reported water-mediated hydrogen bonded interactions between the exocyclic amino (N4) nitrogen of the modified bases and threonine (T1393) residue of TET2 proteins suggesting a regulatory role of water in epigenetically modified DNA and its functions.[71]

3.2. Water-mediated interactions in non-canonical higher-order DNA structures

In non-canonical DNA structures, such as G-quadruplexes and i-motifs, the role of water becomes even more pronounced. These alternative conformations are essential not only for
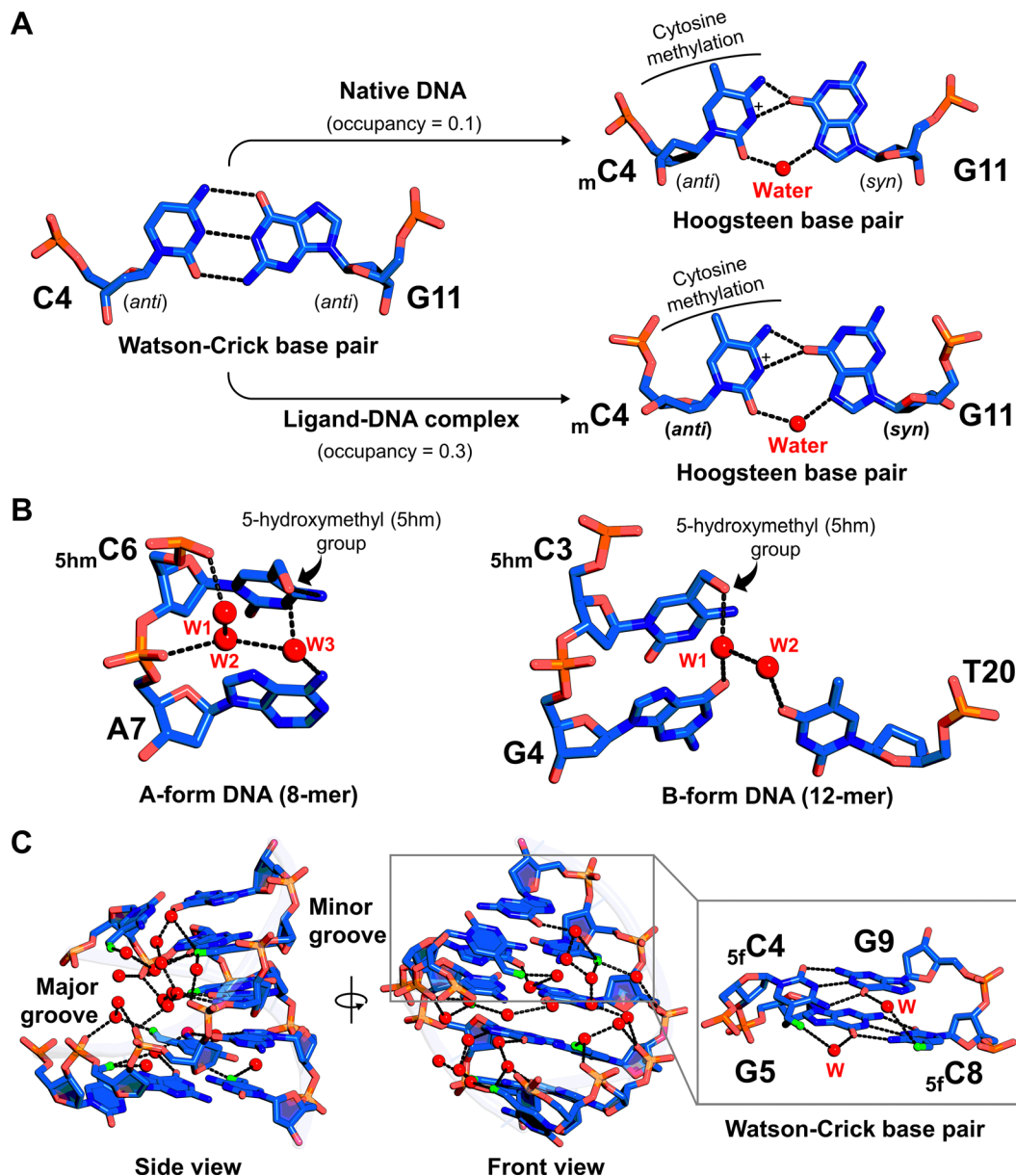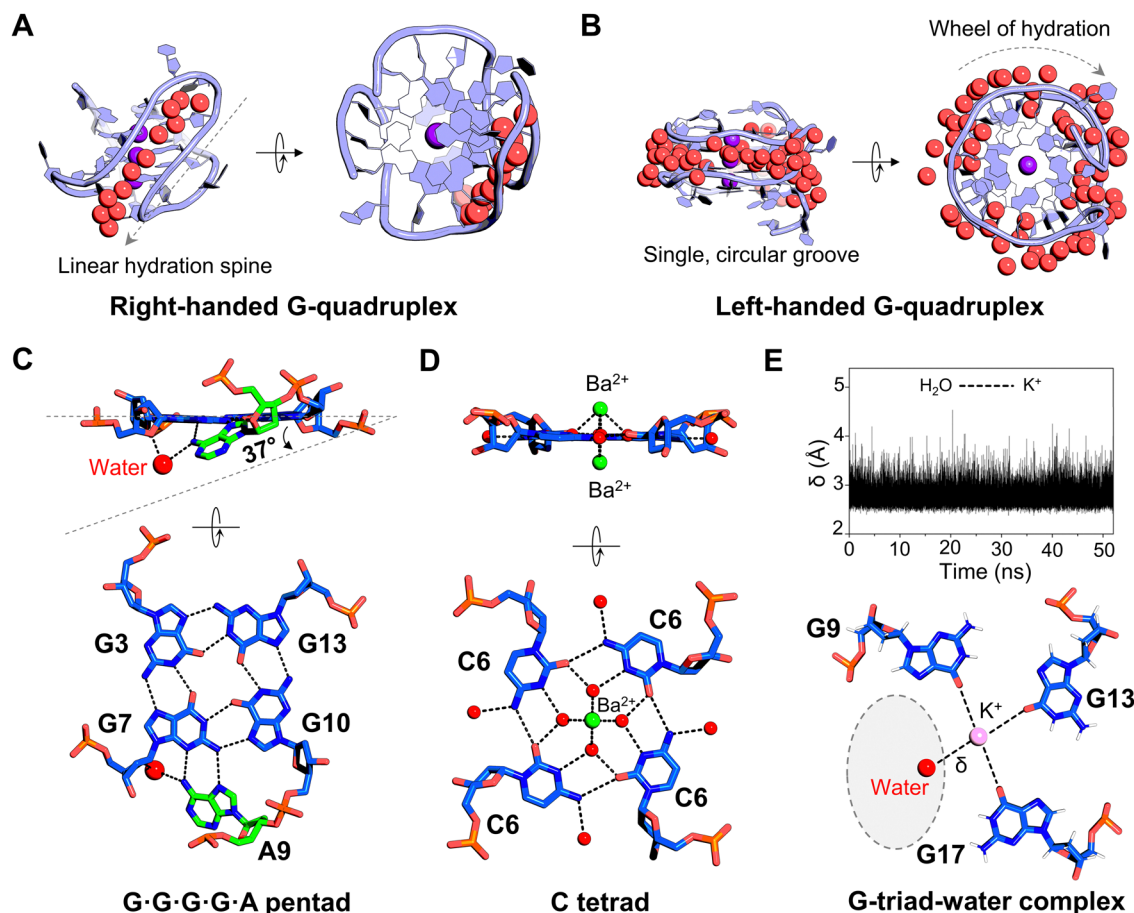
Fig. 2 Water-mediated interactions in epigenetically modified DNA structures. (A) Induction of water-mediated Hoogsteen base pairing upon cytosine methylation in unliganded native DNA (PDB ID: 8XPA) and upon ligand binding (PDB ID: 8XP8). (B) Water-mediated interactions in 5-hydroxymethylcytosine ($_{5hm}$C)-modified DNA in the A-form conformation (PDB ID: 5CH0) and in the B-form conformation (PDB ID: 5CJY). (C) Views of the major and minor groove of DNA with 5-formylcytosine ($_{5f}$C) modification (PDB ID: 4QKK). A network of water in the major groove is shown, with the water molecules shown as red spheres and the modified 5-formyl group at the cytosine bases highlighted in green. On the right, an enlarged view of unusual Watson–Crick base pairing is shown.

regulating gene expression processes, but also have significant potential as drug targets.[72–74] In conventional G-quadruplexes, water often forms a clustered network around the four grooves, which results in an increased complexity of the grooves and the overall quadruplex diversity.[75–77] The presence of water molecules around different G-quadruplex topologies is important for the stabilization of overall quadruplex arrangements by small molecule ligands.[78–80] In recent years, a novel topology of G-quadruplexes has been identified, called left-handed G-quadruplexes.[81–83] These left-handed structures are characterized by a single, circular groove. The arrangement of water molecules around both right- and left-

handed G-quadruplex structures has recently been surveyed using crystallographic analysis (Fig. 3A and B).[84] Interestingly, there is significant distinction between the hydration pattern of these two conformations. In right-handed helical G-quadruplexes, the hydration tends to follow a linear pattern, similar to that found in right-handed DNA duplexes. In contrast, the left-handed G-quadruplexes display a unique, circular hydration arrangement within a single groove, termed the "wheel of hydration". Although biological implications of left-handed G-quadruplex structures are still unclear, the distinct and consistent hydration pattern associated with these structures can be

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Fig. 3 Water interactions in DNA G-quadruplexes. (A) Representative view of a narrow groove in a right-handed G-quadruplex structure, illustrating a linear arrangement of water molecules (PDB ID: 6JKN). (B) View of a single circular groove with water molecules in a left-handed G-quadruplex structure (PDB ID: 4U5M). (C) Formation of a water-mediated pentad base pair arrangement in the G-quadruplex structure of an HIV-1 integrase aptamer (PDB ID: 7XDH). (D) Water-mediated cytosine tetrad (C-tetrad) surrounding the central Ba^{2+} ion channel (green sphere) in the G-quadruplex structure (PDB ID: 4U92). (E) Formation of a G-triad–water complex in the G-quadruplex structure. Top: The distance (δ) between water molecules and K^+ ions (pink sphere) during the simulation. Bottom: The orientation of three guanine bases and their interactions with water molecules. A dashed oval highlights a potential region for the binding of an external ligand. The figure is adapted with permission from *Nucleic Acids Res.*, 2016, **44**(2), 910–916. In all panels, the water molecules are shown as red spheres and the hydrogen bonds as dashed black lines.

considered as their integral component which may play a crucial role in their functions.

In addition to the grooves, water molecules also mediate planar arrangements of the bases, which is essential for the integrity and structural framework of G-quadruplex structures. For example, in an interlocked G-quadruplex structure, a conserved water-mediated pentad base-pair arrangement forms between four planar guanines and *ca.* 37° tilted adenine (Fig. 3C).[85] This water-mediated pairing is crucial for interactions and functions of this G-quadruplex as an HIV-1 integrase inhibitor. Zhang *et al.* studied a biologically relevant G-quadruplex structure induced by the incorporation of a nucleoside analog, cyanovinylcarbazole, into DNA.[86] They solved the 1.50 Å crystal structure of the DNA decamer containing cyanovinylcarbazole modification. This G-quadruplex structure formed a planar arrangement of four cytosines (called a C-tetrad) with water molecules mediating contacts between a divalent cation and the C-tetrad (Fig. 3D). These water molecules can donate protons to nearby cytosine bases, which in turn increases

the electron density of the lone pair on water oxygen atoms. Consequently, the water oxygen can shield the charges between adjacent divalent metal ions. Thus, the presence of water has a favourable effect on stabilizing the unusual C-tetrad pairing and in forming continuous stacking interactions of the other guanine tetrads, thereby maintaining the overall integrity of the G-quadruplex structure.[86] Water-mediated tetrad base pairs are also found in G-quadruplex RNA structures.[87] Using NMR spectroscopy and molecular dynamics simulations, Heddi *et al.* demonstrated that in parallel G-quadruplexes, if one of the guanines is missing from guanine tetrads, water molecules can mimic the position of the missing guanine and form a G-triad–water complex at that site (Fig. 3E).[88] These water-mediated sites can serve as binding sites for various metabolites, with implications in metabolite sensing, regulation and drug development. In an antiparallel human-telomeric G-quadruplex structure, water-mediated intra-strand interactions between two thymine bases were found to enhance stacking on the G-tetrad, further contributing to the stability of the quadruplex.[89] Thus, in

addition to stabilizing the grooves, water also aids in interactions with base-pair arrangements in quadruplexes and facilitates the formation of unique structural motifs, which can further influence ligand binding by stabilizing DNA conformations, enhancing specificity, and modulating binding affinities.

Similar to G-quadruplexes, i-motifs, another type of four-stranded non-canonical structure, can form in cytosine-rich sequences in the regulatory regions of the human genome.[90–92] In the formation of i-motif structures, water plays an important role. It has been shown that variations in hydration conditions can regulate the folding and unfolding of i-motif structures.[93] Although fewer i-motif structures have been reported compared to G-quadruplexes, the available structures provide valuable insights into the potential role of water in stabilizing and influencing the i-motif structures. The i-motifs are characterized by two wide grooves and two narrow grooves, with the majority of water molecules concentrated in the wider grooves, as the narrow grooves are too restricted to accommodate water molecules.[94–96] In i-motif structures, water-mediated bridge interactions typically occur between phosphate oxygens and the N4 amino group of cytosine, playing a crucial role in stabilizing these structures. In a recently reported i-motif structure derived from the HRAS oncogene sequence, a robust network of water molecules is observed within the wider groove (Fig. 4A).[97] This water network forms hydrogen bonds with the phosphate backbone, with N4 atoms in cytosine bases, or with other water molecules, similar to previous i-motif structures. In addition to these interactions, the current structure displays two high-electron density water positions: the first coordinated with two extrahelical guanine bases and another water molecule, while the second is located in the loop region of the i-motif. A similar water position is present in the loop of another HRAS i-motif solved in the same study, highlighting the

possibility that water in these regions is conserved in HRAS i-motifs which might have important functional roles. Another biologically relevant intramolecular i-motif structure from the promoter region of the insulin gene has been recently reported.[98] In this structure, water molecules formed bridging hydrogen-bonded interactions with the N4 atoms of cytosine bases and phosphate oxygens. Several water molecules were observed in the loop region of the i-motif, indicating potential sites for hydrogen-bonding. Such water-bound structure motifs can also provide crucial information for designing gene-selective ligands.

The structures of i-motifs associated with specific tandem repeat sequences have been studied, revealing several important water-mediated interactions. Chen *et al.* reported the crystal structure of an i-motif formed from the CCG repeat sequence, which is linked to fragile X syndrome.[99] This structure features a network of bridging water molecules mediating inter-strand interactions within the i-motif. In this structure, water molecules bridge interactions between two strands of the asymmetric units and stabilize the i-motif core (Fig. 4B). A similar water interaction network is found in another CCG-repeat-related i-motif, suggesting its importance in maintaining the i-motif conformation.[100] This conserved network of water further plays an important role in stabilizing the base-extruded duplex induced by a small-molecule ligand.[100] Sato *et al.* reported a higher-order structure formed by d(GCGA[A]₁AGC), which is associated with d(GAAA)ₙ tandem repeats in the non-coding region near telomeres.[101] This structure adopts an octaplex conformation, incorporating an i-motif with water-mediated adenine quartets (A-quartets). Based on the analysis of this structure and the other octaplex structure formed by the d(GCGA[G]₁AGC) repeat sequence,[102] it is clear that the water-mediated hydrogen bonding network is critical in stabilizing the octaplex conformation.
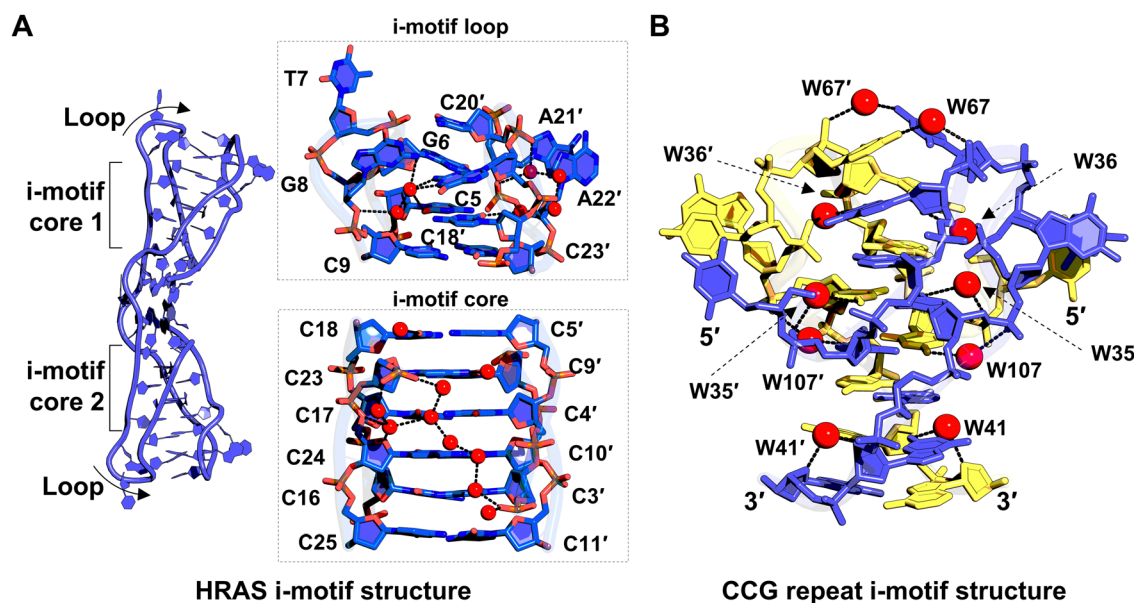
Fig. 4 Water interactions in DNA i-motifs. (A) Overall structure of the HRAS oncogene i-motif (PDB ID: 8CXF). On the right, detailed views of water-mediated interactions in the loop and core regions are shown. (B) Inter-strand coordination of water molecules in the i-motif structure of CCG repeats (PDB ID: 4PZQ). In the figure, the two strands are colored yellow and blue. In all panels, water molecules are shown as red spheres.

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They further hypothesized a potential mechanism for sequence slippage during DNA replication in d(GAAA)$_n$ tandem repeats. The presence of water-mediated A-quartet structures likely stabilizes transient misaligned base-pairing events and facilitates structural rearrangements during slippage. Understanding these intricate structural details could facilitate therapeutic intervention. Since d(GAAA)$_n$ repeat expansion has been linked to approximately 34% of renal cell carcinomas,[103,104] small molecules that selectively stabilize or destabilize this structure could modulate replication-associated instability, potentially offering a novel strategy for cancer treatment. Targeting the water-mediated hydrogen bonding network could allow for the rational design of drugs that either prevent pathological repeat expansion or induce structural changes that disrupt oncogenic pathways.

4. Water interactions in small molecule–DNA complexes

Water is crucial for the formation of complexes between small molecules and DNA. Its ability to form hydrogen bonds and modulate the electrostatic and structural environment of DNA binding sites has established water molecules as a key focus in drug–DNA binding mechanisms.[105–107] In this section, we will examine the role of water in mediating small molecule–DNA interactions and their significance in influencing DNA structure and function.

4.1. Water-mediated interactions between DNA and groove-binding agents

DNA targeting molecules typically bind DNA through two major modes: minor groove binding and intercalation.[108,109] Structured water molecules are critical in mediating interactions between small molecule ligands and the DNA minor groove, improving binding affinity and biological activity.[35] DNA groove-binding agents like netropsin and distamycin interact with the minor groove by displacing ordered water molecules to achieve strong binding.[110] However, these molecules exhibit different binding affinities due to their distinct interactions with water.[111] Distamycin, being more hydrophobic, interacts with DNA-associated water to a lesser extent than netropsin. This resulted into a more pronounced loss of distamycin mobility upon DNA binding which led to its more unfavourable entropy to the binding free energy and consequently to lower affinity.[111] These examples explain how water-mediated interactions modulate the binding specificity and affinity of small molecule compounds to DNA, with implications for their biological activity.

Moreover, groove-binding agents usually exhibit either shape-specific or sequence-specific interactions with DNA. In shape-specific binding, the molecules often achieve strong interactions by being isohelical, *i.e.* they complement the shape of the minor groove. However, even non-isohelical molecules can achieve strong minor groove binding through water-mediated interactions. For example, the non-isohelical linear heterocyclic compounds DB921, DB1055, and CGP 40215A utilize water molecules to establish hydrogen bonds that compensate for their lack of curved shape (Fig. 5A).[112–115] In a X-ray crystallography-based study, Wei *et al.* showed that in addition to the well-reported spine of hydration in the minor groove, a conserved network of 11 ordered water molecules is present within the minor groove (Fig. 5B).[116] This water-network mediates non-covalent interactions with linear hetero-cyclic groove-binding agents. The water-mediated interactions complete the molecular curvature required for high-affinity binding to DNA. These findings emphasize the significance of water in stabilizing the linear geometry containing small molecules in interacting with the DNA minor groove.

Pyrrole-imidazole (Py-Im) polyamides are a class of synthetic compounds designed to target the DNA minor groove.[117,118] These compounds are excellent examples of shape complementarity, as they can recognize specific DNA sequences by matching the minor groove curvature. Chenoweth and Dervan elucidated the crystal structure of an eight-ring cyclic Py-Im polyamide bound to DNA, showing the compound bridge interactions between adenine and guanine base pairs *via* water-mediated hydrogen bonds.[119] In another X-ray crystallographic study, Oh *et al.* demonstrated how hairpin Py-Im polyamides block transcription by trapping DNA and RNA polymerase II.[120] In this structure, two ordered water molecules were found to mediate hydrogen bonding interactions between the polyamide and the DNA minor groove, underscoring the role of water in driving the biological activity of Py-Im minor groove binding ligands.

Structured water molecules also play a critical role enabling the sequence specificity to the DNA minor groove binders. Many X-ray crystallographic studies have been reported on sequence-specific groove binders, highlighting how water molecules bridge the interactions between DNA and groove-binding agents. For example, the antitrypanosomal agent berenil has been shown to bind asymmetrically to AT-rich DNA sequences (–AATT– sequence) through water-mediated bridging interactions (Fig. 5C).[121] In the asymmetric binding mode, one of the amidinium groups of the berenil forms a hydrogen-bonded interaction with the N3 atom of the adenine base, while the other amidinium group does not directly interact with the complementary strand adenine in DNA. Instead, the amidinium nitrogen atom interacts with the 5′ end of the adenine base through a water-mediated interaction. Interestingly, another independent crystal structure showed that when berenil binds symmetrically to another AT-rich sequence (–AAATTT–), the water mediation is not required.[122] In the symmetric binding mode, the two amidinium groups of berenil interact directly with two adenine bases on each strand through hydrogen bonding, without requiring a bridging water molecule. These studies demonstrate that water-mediated interactions can vary depending on the DNA sequence and the drug binding orientation. Similarly, propamidine, another minor groove binder with a longer linker than berenil, relies on water-mediated interactions to recognize AT-rich sequences.[123,124] Recent studies have shed light on the water-mediated interactions between the minor groove binders and biologically important DNA sequences. For example, the X-ray diffraction structure of diamidinobenzimidazole compounds shows that they selectively bind to AT-rich DNA sequences from the promoter regions of
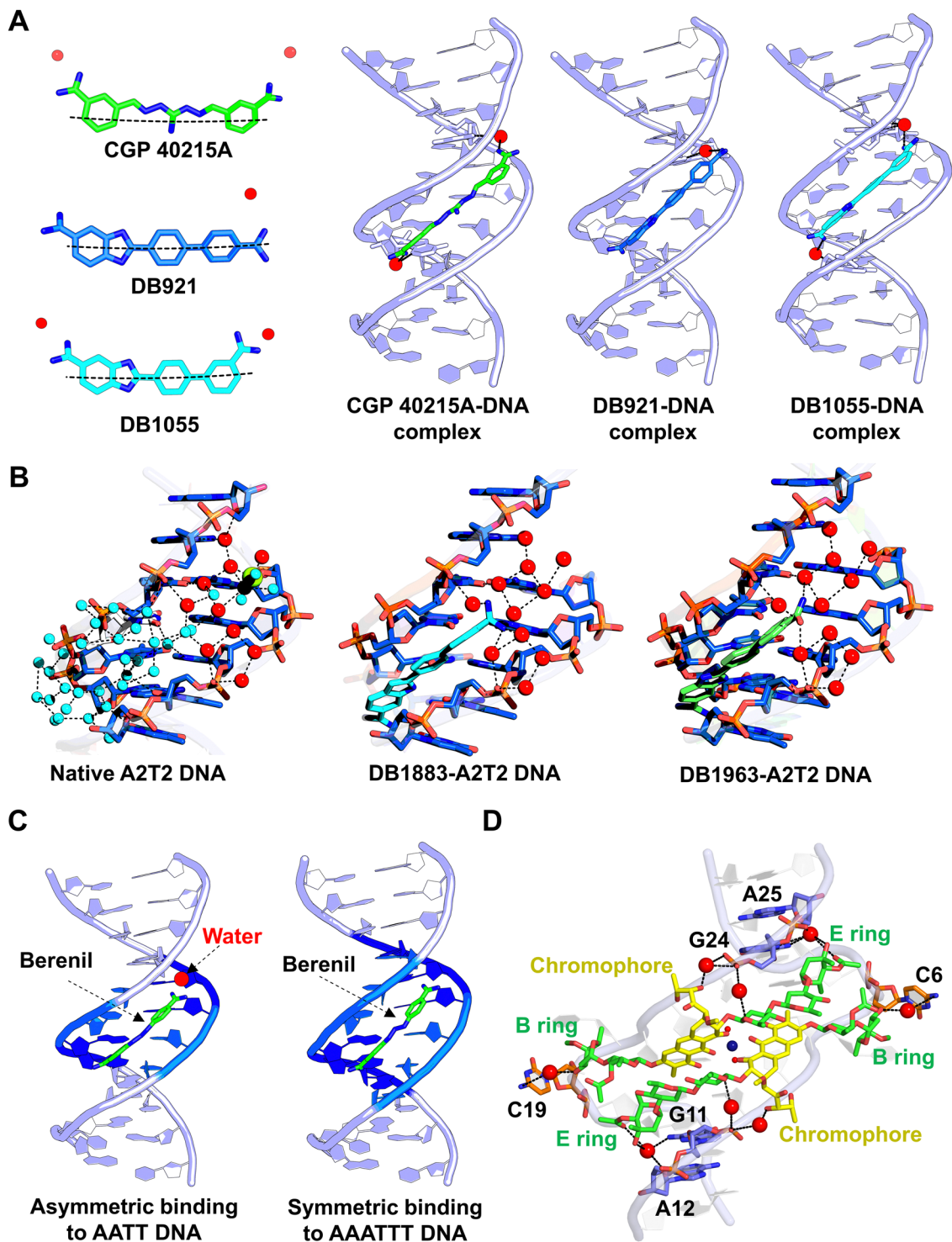
Fig. 5 Water interactions in DNA groove binders. (A) Water-mediated binding of the linear-shaped groove binders CGP 4-215A (PDB ID: 1M6F), DB921 (PDB ID: 2B0K), and DB1055 (PDB ID: 2I5A) to DNA. The water molecules are shown as red spheres. (B) Water cluster with water molecules of the first shell (cyan spheres) and a network of 11 conserved water molecules (red spheres) in native DNA (PDB ID: 3U2N). After binding of the ligands DB1883 (PDB ID: 3U0U) and DB1963 (PDB ID: 3U08), the conserved water cluster contributes to the stabilization of the ligand–DNA complex. (C) Binding of berenil to DNA in the asymmetric mode (PDB ID: 2DBE) involves water-mediated interactions, whereas water-mediated stabilization is not required for symmetric binding (PDB ID: 1D63). (D) Water-mediated bridging interactions stabilize the flipped-out residues in chromomycin-induced CCG repeat DNA (PDB ID: 5XEW). The water molecules are shown as red spheres.

human genes.[125] These compounds adopt two possible binding orientations, with a preference for water-assisted hydrogen bonding at their cationic ends.[125] Using long-timescale molecular dynamics simulations, Carter *et al.* demonstrated the structural

basis of a heterocyclic diamidine, DB2277, which exhibits a preference for binding to GC sites in DNA.[126] This minor-groove binder displaces the ordered water molecules from the groove and forms several water-mediated contacts with the bases and the backbone of the DNA. These studies suggest the potential for designing a new class of minor groove binders that incorporate bound water molecules into their DNA complexes.

Moreover, water-mediated interactions are crucial for achieving optimal binding of some groove-binding antibiotics. For example, antibiotics such as chromomycin and mithramycin often adopt an octahedrally coordinated, metal-chelated dimer conformation to bind DNA.[127,128] In these complexes, water molecules serve as the fifth and sixth ligands to complete the coordination. In addition, the other bridging water molecules play a key role in mediating the interactions between the drugs and disease-associated DNA structures. For example, the binding of metal-chromomycin A3 complexes to the CCG repeat DNA associated with fragile X syndrome induces large-scale DNA deformations, including the extrusion of cytosine bases and distortions in the backbone.[100,129] In this unusual DNA conformation, structured water molecules stabilize the interactions between the extruded bases and drug moieties (Fig. 5D). The N4 amine atom on the extruded cytosines forms water-mediated hydrogen bonds with the O3 oxygen atoms of the disaccharide B ring of chromomycin, while the N2 atoms of the terminal guanines form water-mediated hydrogen bonds with the O3 oxygen atoms of the trisaccharide E ring. Two bridging water molecules further mediate interactions between the O1 oxygen atoms of the trisaccharide C ring of chromomycin and guanine bases. These water-mediated interactions contribute to the specific recognition of $(CCG)_n$ repeats by chromomycin complexes. Furthermore, in a G-G mismatched DNA structure stabilized by chromomycin A3, water molecules are shown to stabilize an unusual *anti–syn* geometry.[130] The interaction of water with mismatched bases pushes the guanines toward the exterior of the helix, creating a single hydrogen bond between them. The stability of this more distant *anti–syn* pairing is compensated by water-mediated interactions between the mismatched bases. The study further demonstrated that this unusual water-mediated mismatch geometry serves as a hotspot for G–G mismatch recognition by another DNA-intercalating drug. Thus, water-mediated interactions are essential for stabilizing antibiotic–DNA complexes by completing metal coordination, bridging drug and DNA interactions, and facilitating the specific recognition of disease-associated DNA structures, such as tandem repeats and mismatches, through hydrogen bonding networks that induce large-scale conformational changes and structural stabilization.
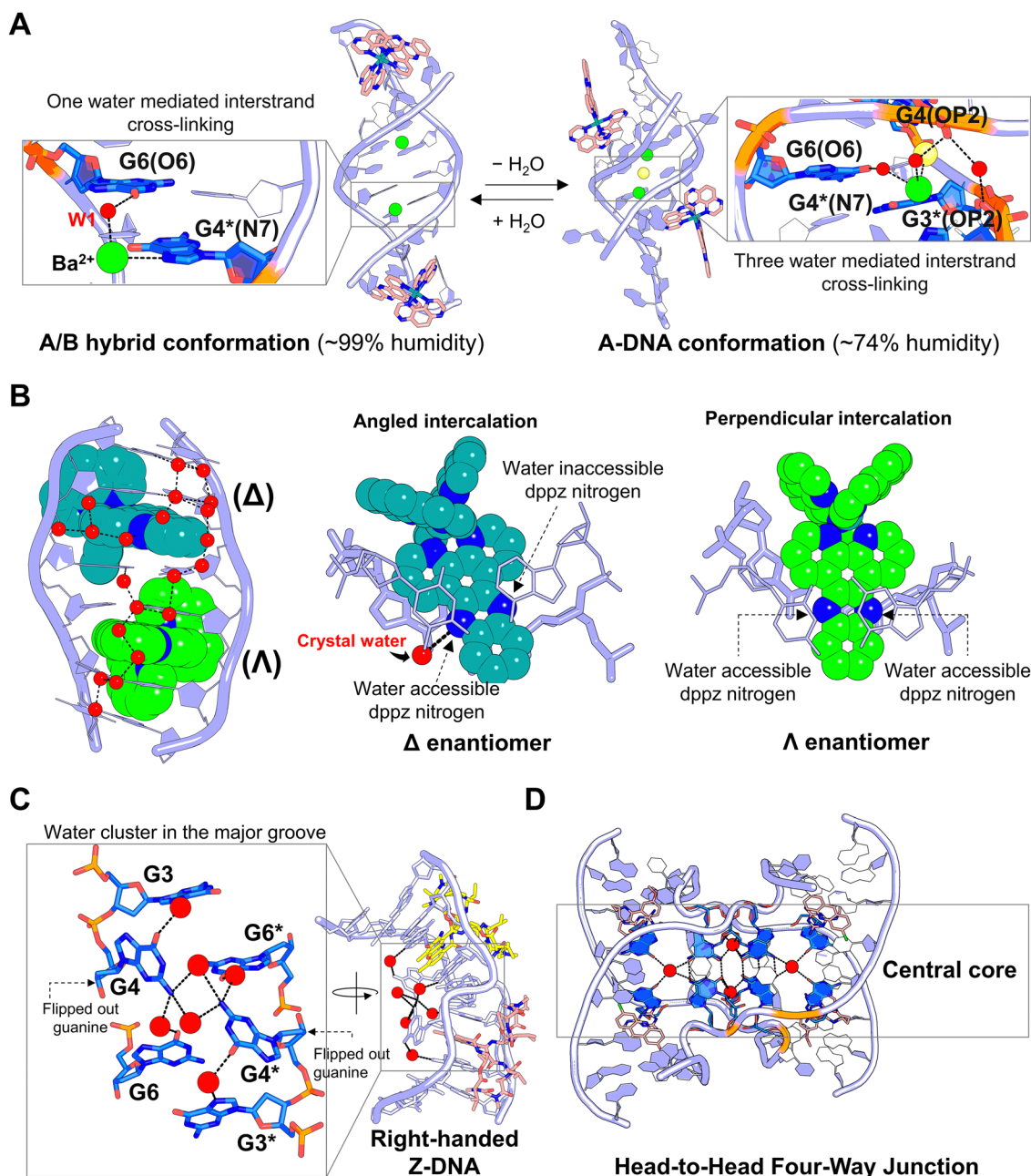
4.2. Water-mediated interactions between DNA and intercalating agents

DNA intercalation is another important binding mode for many DNA-targeting agents, in which intercalators insert their planar aromatic ring structures between DNA base pairs.[131] Unlike groove binders, the role of water in DNA intercalation remains less well-defined, possibly because intercalators primarily rely on stacking interactions rather than direct hydrogen bonding with DNA. However, studies have revealed a significant relationship between water and intercalating agents, emphasizing their unique roles in the functionality and structural stability of DNA. For example, ruthenium-based compounds exhibit photophysical properties upon DNA intercalation.[132–134] These compounds interact with DNA through the photooxidation of guanine bases and also display luminescence properties.[135,136] Hall *et al.* collected seven independent X-ray data sets from a single DNA crystal at room temperature under varying relative humidity conditions to understand the effects of dehydration on DNA structures.[33] Their results demonstrated that the controlled dehydration and rehydration of a complex containing the ruthenium compound $[Ru(TAP)_2(dppz)]^{2+}$ (TAP = 1,4,5,8-tetraazaphenanthrene, dppz = dipyrido[3,2-*a*:2′,3′-*c*]phenazine), intercalated in DNA, induces reversible DNA kinking and a B-to-A transition (Fig. 6A). In its hydrated form, the complex structure shows a single water-mediated bridging interaction. Upon dehydration, however, three water-mediated interstrand cross-links form per Ba^{2+} ion. This enhanced water network arises from the adoption of an A-DNA-like conformation and a kink induced by the ligand intercalation. These findings show promise for the application of water-controlled ruthenium intercalators in DNA self-assembly and molecular engineering. Moreover, the chemical properties of ruthenium compounds are influenced by the orientation of their dppz moiety and its interactions with surrounding water molecules.[137–139] When the dppz ligand is intercalated deeply within DNA base pairs, its nitrogen atoms become inaccessible for interaction with water. This phenomenon can be exemplified by the crystal structure of $[Ru(phen)_2(dppz)]^{2+}$ (phen = phenanthroline) in complex with the $d(ATGCAT)_2$ DNA duplex.[139] In this structure, the two enantiomers, Δ and Λ, of $[Ru(phen)_2(dppz)]^{2+}$ exhibit different orientations when simultaneously intercalated into DNA. The distinct binding modes of the two enantiomers result in the formation of differently structured water monolayers. Upon comparing these two structural halves, it was found that the Δ enantiomer intercalates at an angled orientation, exposing only one dppz nitrogen atom to mobile water molecules (Fig. 6B). In contrast, the perpendicular intercalation of the Λ enantiomer allows mobile water to access both phenazine nitrogen atoms. Since water access to the dppz phenazine nitrogen determines the photochemical lifetime of ruthenium complexes, luminescence measurements show that the Λ enantiomer exhibits lower emission intensity upon DNA interaction. This highlights the critical role of water in modulating the function of ruthenium-based DNA intercalating agents.

Intercalators often induce stronger structural changes in DNA compared to groove binders.[140–142] These changes result in the formation of unusual structural motifs, such as a kinked backbone or base pair flipping. Base pair flipping occurs when an intercalator inserts its planar rings between DNA base pairs, causing the nucleobases to rotate about their glycosidic bonds and protrude out of the helical axis.[138] This disruption in base stacking and hydrogen bonding interactions exposes the flipped-out bases to the external environment, including proteins or solvent molecules. Water-mediated interactions play a

Fig. 6 Water-mediated interactions in DNA intercalators. (A) Controlled hydration and dehydration of the d(TCGGCGCCGA) duplex in the presence of Λ-[Ru(TAP)$_2$(dppz)]$^{2+}$ (TAP = 1,4,5,8-tetraazaphenanthrene; dppz = dipyrido[3,2-a:2′,3′-c]phenazine) induce conformational changes in DNA (PDB IDs: 4LTF and 4LTG). The enlarged view shows the water-mediated cross-linking between the strands in the major groove. (B) A water network formed by ordered water molecules in the major groove of the d(ATGCAT)$_2$ DNA duplex containing Δ- and Λ-enantiomers of *rac*-[Ru(phen)$_2$dppz]$^{2+}$ (phen = phenanthroline; dppz = dipyridophenazine) (PDB ID: 4JD8). (C) Water-mediated hydrogen bonding stabilizes flipped-out guanine residues and thus contributes to the overall stability of actinomycin D bound to the DNA duplex (PDB ID: 6J0H). (D) Water-mediated stabilization of central core residues in the DNA structure induced by the triamino–triazine–acridine conjugate Z1 (PDB ID: 6M4T). In all panels, the water molecules are shown as red spheres and the hydrogen bonds as dashed black lines.

crucial role in stabilizing such unusual structures. For example, the binding of actinomycin D to G–G mismatch in DNA induces a kinked right-handed Z–DNA conformation, in which the guanine bases are flipped out of the duplex.[130] In this structure, a cluster of water molecules plays a key structural role in stabilizing the kinked structure with the flipped-out guanines (Fig. 6C). This water cluster holds and stabilizes the entire kinked DNA backbone structure toward the major groove side. These water-mediated stabilizing interactions of flipped bases are also observed in other drug–DNA complexes and protein–DNA complexes.[129,143] Moreover, in an X-ray crystallography structure, Chien *et al.* reported the formation of a U-shaped head-to-head four-way junction structure upon intercalation by the triaminotriazine–acridine conjugate Z1 (Fig. 6D).[144] In this

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highly hydrated complex structure, water molecules mediate significant interactions to stabilize the residues at the central crossover site of the junction conformation. In addition, three conserved water molecules further stabilize the Z1–DNA complex by directly mediating interactions between the ligand Z1 and DNA bases. In a recent structure of a DNA side-by-side junction formed due to the intercalation of diaminoacridine bis-intercalator DA5, a specific water-mediated interaction at the inter-duplex contact has been observed.[145] In addition to bridging direct interactions between intercalators and DNA, water often supports intercalator–DNA complexes through several indirect interactions. For example, in the structure of echinomycin bound to T–T mismatched DNA, Wu *et al.* showed water-mediated stabilization of a drug–drug interaction and the mismatch.[146] Water-mediated mismatch stabilization has also been observed in other intercalators and DNA structures.[147] In general, intercalating agents induce significant structural changes in DNA, often leading to unusual motifs stabilized by water-mediated interactions, such as kinked Z–DNA conformations, four-way junctions, and inter-duplex contacts. The water interactions not only support ligand–DNA interactions but also facilitate cooperative drug binding, enhancing structural integrity and biological activity.

4.3. Water-mediated interactions between DNA G-quadruplexes and small molecule ligands

DNA G-quadruplexes are increasingly recognized as important molecular targets for small molecule compounds in drug development for treating cancer and neurological diseases.[148–150]

Studies on G-quadruplexes and their interactions with small molecules have identified several common features of quadruplex binders that are essential for defining their binding mechanisms. In general, G-quadruplex-binding ligands possess planar heteroaromatic groups which enabled them to stack effectively on G-quartet surfaces through π–π interactions. Additionally, these ligands often feature cationic side chains that interact with the DNA grooves and bases. Structural studies by Neidle and colleagues have highlighted the critical role of water-mediated interactions in G-quadruplex-ligand binding.[151–157] Water molecules usually occupy conserved sites near the ligand-binding region and the grooves in quadruplex structures. These water molecules form hydrogen bonds with both the ligand and the DNA. The crystal structure of an acridine-based ligand, BSU6039, in complex with a telomeric G-quadruplex, revealed that water molecules from the spine of hydration in the groove interact with the protonated nitrogen atom of the pyrrolidino group and the amide nitrogen of BSU6039.[151] Another well-studied quadruplex binder, BRACO-19, also employs extensive water-mediated hydrogen bonding interactions to stabilize the human G-quadruplex structure (Fig. 7A).[153] Naphthalene diimide derivatives show conserved water-mediated interactions, where water molecules bridge DNA phosphate groups and the nitrogen atom of the *N*-methyl-piperazine ring of the ligands.[155,156] Natural compounds such as berberine have shown potential for binding to G-quadruplex structures. In the crystal structure with a 2:1 berberine-to-G-quadruplex molar ratio, a water molecule occupies the central channel, mediating a conventional hydrogen bond with the
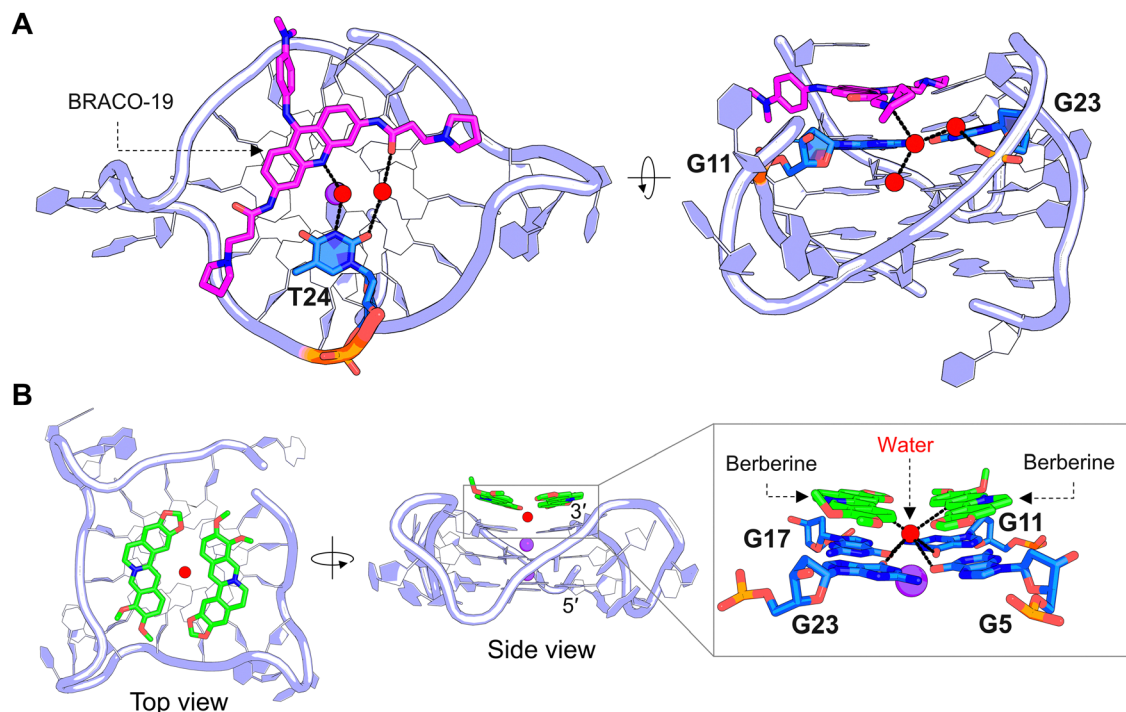
Fig. 7 Water-mediated interactions between small molecule ligands and G-quadruplexes. (A) Binding of the experimental anticancer drug BRACO-19 to human telomeric G-quadruplex DNA involves water-mediated interactions that stabilize the ligand-DNA complex (PDB ID: 3CE5). (B) In the berberine G-quadruplex complex, a water molecule at the 3'-end of the binding site forms a hydrogen bond that establishes a critical interaction between the drug and DNA (PDB ID: 3R6R).

carbonyl oxygen of guanine and a CH···O interaction with berberine (Fig. 7B).[158] This specific interaction stabilizes the orientation of berberine molecules within the G-quadruplex structure. Similarly, a ruthenium polypyridyl complex has been shown to bind to a unimolecular chair G-quadruplex conformation. In this, extensive structured water molecules form a network that stabilizes the compact globular ligand-G-quadruplex structure.[159]

In an analysis of X-ray crystal structures of G-quadruplexes in both native and ligand-bound states, Neidle observed clusters of conserved water molecules.[34] These water molecules serve as platforms for ligand binding and are crucial for establishing the structure–activity relationship of G-quadruplex-targeting ligands. This analysis led to an important conclusion about G-quadruplex binders: when identifying potential G-quadruplex binding ligands using high-throughput computational methods, it is imperative to account for solvent-mediated interactions in G-quadruplex binding. Neglecting water-mediated interactions increases the risk of inaccurate predictions of low-energy ligand-binding states and their interactions. Incorporating solvent-mediated considerations can not only enhances the binding affinity of these ligands but also improves their selectivity for G-quadruplexes over canonical duplex DNA structures.
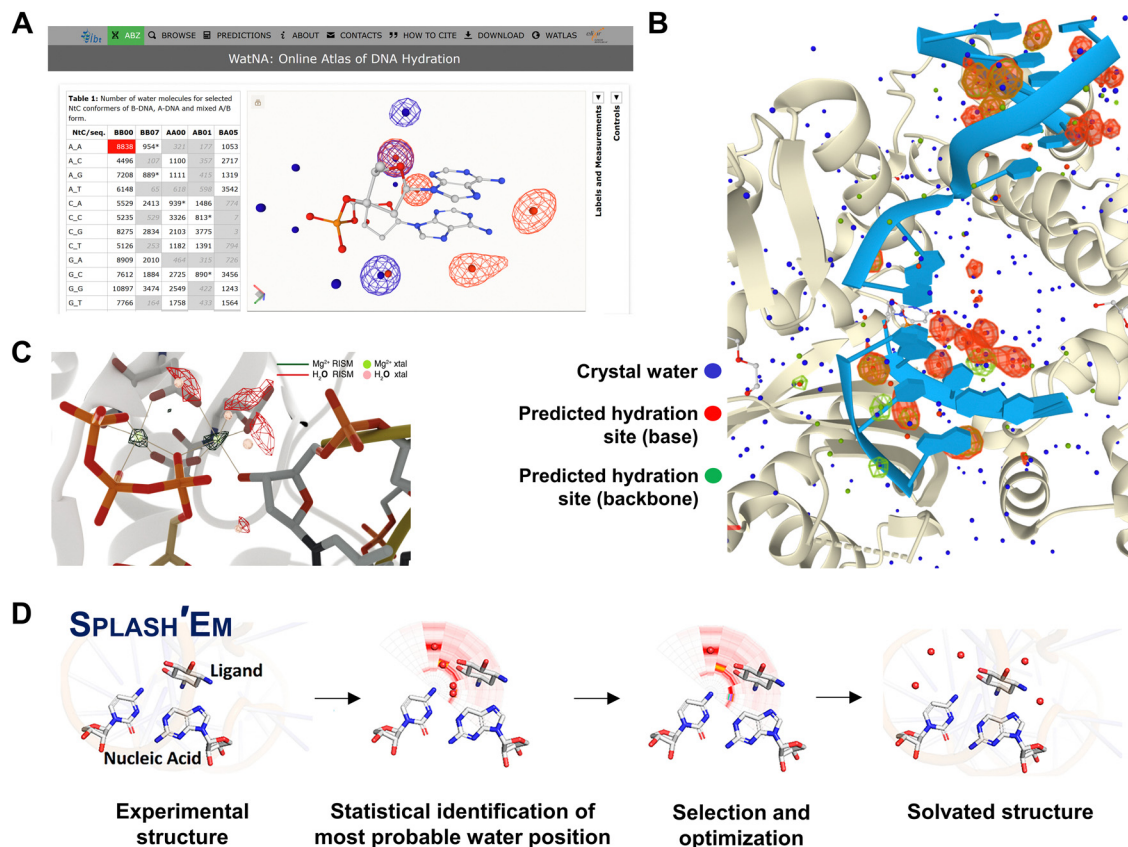
5. Emerging approaches for predicting water interactions with nucleic acids

A large number of techniques have been used to study the dynamic interactions between water and nucleic acids.[53,160–163] Structural techniques, including X-ray and neutron crystallography, remain the gold standards for studying these interactions, providing detailed spatial and dynamic insights into the water shell surrounding nucleic acid surfaces. While these methods yield the most valuable structural information, they have limitations in fully capturing the solvent and nucleic acid interactions. For example, resolution constraints or incomplete electron density in crystallographic structures can prevent the precise localization of water near biomolecules. Moreover, hydration networks and water positioning are significantly affected by artifacts introduced during the cryo-cooling process in biomolecular crystallography.[164,165] For instance, ultrahigh-resolution structures of the protein crambin have been reported at various temperatures ranging from 100 K to 293 K.[166] Teeter et al. observed that at temperatures above 200 K, the extensive hydrogen-bonding network of water molecules, identified as crystal packing artifacts, was disrupted.[166] To mitigate these artifacts and obtain more detailed insights into atomic motions and solvent interactions, room-temperature X-ray crystallography has been proposed.[167] However, despite its advantages, room-temperature data collection remains relatively uncommon, with cryogenically collected structures dominating the Protein Data Bank. These challenges can impede a comprehensive understanding of the role of water in nucleic acids. With the growing repository of high-resolution nucleic acid structural motifs, researchers are focusing on developing approaches and tools to accurately predict water positions and interactions with nucleic

acid. Biedermannová et al. proposed a Fourier averaging procedure that analyzes hydrated nucleic acid dinucleotide conformers, known as NtC blocks, as a method to predict DNA hydration structures.[168] Using data from approximately 41 000 NtC blocks associated with 316 265 water molecules, they developed a strategy to predict the hydration patterns within the DNA structures. This work led to the creation of a web server, the WatNA atlas (https://www.watlas.datmos.org/watna/), which provides insights into DNA hydration (Fig. 8A and B). This is a promising approach to creating fully automated prediction servers, which could aid in the interpretation of experimental data. Meisburger et al. introduced methods for decomposing X-ray scattering profiles of DNA, water, and metal ions using small-angle X-ray scattering and validated it through anomalous scattering measurements.[169] This approach provides an additional means of studying hydration patterns around nucleic acids. In addition, computational tools have become essential for studying nucleic acid dynamics and interactions. Giambaşu et al. introduced a toolset based on the three-dimensional reference interaction site model (3D-RISM) and molecular solvation theory to predict cation and water binding sites in G-quadruplex nucleic acids (Fig. 8C).[170] By refining force field parameters using structural and thermodynamic data, they successfully modeled cation and water interactions in G-quadruplexes with 40% accuracy in placing water molecules within 1 Å of crystallographic water positions in experimental structures. Wei et al. developed an improved method called Solvation Potential Laid around Statistical Hydration on Entire Macromolecules (SPLASH'EM), which accurately places water molecules in nucleic acid cavities (Fig. 8D).[171] By employing a particle waters force field with an angular contribution function and a hybrid scoring function based on a statistical analysis of crystal structures, their method enhances water placement predictions in nucleic acids with 62% accuracy. Such computational studies not only provide valuable insights into water-binding properties of nucleic acids, but also offer medicinal chemists enhanced tools for designing water-mediated nucleic acid-binding ligands with greater precision.

Spectroscopic techniques can also significantly contribute to advancing our understanding of the relationship between hydration and nucleic acids. For example, Franck et al. used the Overhauser effect dynamic nuclear polarization technique to demonstrate that water at DNA surfaces exhibits fast, bulk-like behavior, moving much more rapidly than water at protein or lipid surfaces.[172] Terahertz time-domain spectroscopy has been employed to illustrate how the hydration shells of nucleic acids differ from free bulk water.[173] McDermott et al., using second-order nonlinear optical spectroscopy, discovered that the DNA helical structure is surrounded by a network of chiral water molecules, particularly in the minor groove.[52] More recently, by combining chiral-selective sum frequency generation spectroscopy (chiral-SFG) with computational analysis, Perets et al. demonstrated that the chiral water structures around DNA are confined to the first hydration shell.[174] Notably, the population of chiral water molecules varies among the minor groove, major groove, and phosphate backbone. These findings contrast with earlier observations, which suggested

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Fig. 8 Tools and techniques for predicting water interactions in nucleic acid structures. (A) Screenshot of the WatNA online atlas of DNA hydration showing the predicted water positions in an AA dinucleotide step. (B) Screenshot of the predicted water positions represented by green and red spheres, together with the crystal waters shown as blue spheres in the PDB structure 6WID. (C) Predicted positions of metal ions and water densities (in dark green mesh) together with crystallographically resolved positions (in light green and pink mesh), illustrating the prediction accuracy of the three-dimensional reference interaction site model (3D-RISM) and molecular solvation theory. The figure is adapted with permission from *J. Am. Chem. Soc.*, 2019, **141**(6), 2435–2445. (D) The procedure for predicting water sites in nucleic acid–drug complex structures as proposed for the SPLASH'EM program. The figure is adapted with permission from *J. Chem. Inf. Model.*, 2019, **59**(6), 2941–2951.

that chiral water was confined solely to the minor groove. This updated understanding underscores a more intricate distribution of water across different regions of the DNA structure. Thus, the spectroscopy-based studies deepen our understanding of interactions of water with nucleic acids. Exploring the biological relevance of these findings could provide valuable insights into DNA function and aid in designing next-generation nucleic acid binding agents.

6. Conclusions and perspectives

Water plays a critical role in stabilizing biomolecular structures and supporting their functions.[48,175,176] In this review, we have emphasized the importance of water in DNA structures by examining structural studies focused on water interactions. Through various examples, we have highlighted how water is essential in maintaining biologically relevant DNA structures and how water–nucleic acid interactions are vital for their stability and functionality. We also explored how water-bound motifs in DNA can act as binding sites for small molecules,

including DNA-targeting drugs. These analyses reinforce the idea of water as a molecular modulator, not only for nucleic acid structures but also for their biological functions. Most of the water-mediated structures discussed in this review were determined through X-ray crystallography, which typically provides time-averaged, static representations of water interactions with DNA. This raises the question of whether the observations from crystal structures are consistent with in-cell conditions and accurately represent the real-time dynamic interactions between water and biomolecules. Lang *et al.* studied water structures within live cells and observed the hydration shells around soluble proteins.[177] Tros *et al.* studied the random orientational motion of water molecules inside living cells using ultrafast vibrational spectroscopy and dielectric-relaxation spectroscopy.[178] Interestingly, they found that slow water bound mostly to proteins and, to a lesser extent, to other biomolecules. The FTIR spectra of live cells have demonstrated DNA conformational changes upon hydration and dehydration, suggesting that the conformational changes observed in *in vitro* experimental conditions can also happen inside live cells.[179] Although there is no direct tool to investigate the atomic-level details of water interactions with

genomic DNA within cells, the current structural data on water-mediated DNA complexes are limited. However, the analysis of these structures has offered valuable insights into the broader role of water in biology and drug discovery.

Similar to DNA, water plays a crucial role in mediating different RNA structures. The structures of RNA containing repeat expansions have been extensively studied, revealing significant insights. For example, in CGG and CAG repeat RNA structures, the non-canonical geometries of G–G and A–A mismatches are heavily hydrated within the major groove.[180,181] These solvent-exposed base pair geometries attract charged species and bridge the interactions between mismatched pairs, suggesting a potential role for water in ligand–RNA interactions. Wang *et al.* reported the structures of myotonic dystrophy type 1-related CUG repeat RNA, where water molecules specifically bridge U–U mismatches through both the major and minor grooves.[182] Their study demonstrated that water enables the interchangeable conformations of U–U mismatches in these RNA structures, which may be critical for the recognition by external ligands. Moreover, water has been shown to play an important role in the formation of tertiary structures and in facilitating specific interactions between RNA and other molecules in functional nucleic acids, such as riboswitches and aptamers.[183–186] The formation of water-mediated interactions in RNA structures highlights the role of water, where it not only stabilizes non-canonical pairings but may also serve as a critical factor for the binding of RNA-binding proteins or small-molecule ligands. While a detailed discussion of water interactions in RNA structures is beyond the scope of this review, it is an important topic deserving a separate, dedicated analysis.

Meanwhile, advancing our understanding of water–nucleic acid interactions requires addressing the limitations of current methods. Structural techniques such as X-ray crystallography and NMR are instrumental in elucidating nucleic acid structures at the atomic level. However, these methods have limitations in capturing the dynamic and transient nature of water around nucleic acids.[187] For example, while X-ray crystallography is useful for identifying structured water molecules bound to DNA, it cannot reliably depict the behavior of bulk or dynamic water molecules. Similarly, NMR spectroscopy provides valuable information about hydration patterns but may not be able to capture fast-exchanging water molecules. To overcome these limitations, more sophisticated methods need to be developed by combining experimental and computational approaches.[188] Advanced techniques such as two-dimensional infrared spectroscopy and neutron scattering offer promising opportunities to study hydration shell dynamics, providing real-time, high-resolution data on water interactions.[189–192] On the computational front, MD simulations are powerful tools for modeling water behavior around nucleic acids. Enhancing these simulations with improved force fields and incorporating quantum mechanical calculations can significantly boost the accuracy of predictions related to water-mediated interactions. Emerging fields such as artificial intelligence and deep learning also hold great potential for predicting water–biomolecule interactions. For example, a deep learning-based model, HydraProt, has recently been developed to accurately predict water positions within protein structures.[193] Such approaches could pave the way for predictive models of water interactions with nucleic acids that would be faster and more accurate than traditional methods alone. The integration of new water-mediated structural motifs with experimental and computational methods has the potential to revolutionize our understanding of the effect of water on nucleic acid structures and functions. This, in turn, could open new opportunities in drug discovery by enabling the design of novel molecules that specifically target water-mediated nucleic acid structures.

Author contributions

Data availability

No primary research results have been included and no new data were generated as part of this review.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

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