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Lattice- and Sequence-Dependent Binding of Mg^{2+} in the Crystal Structure of a B-DNA Dodecamer

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Crystal structures of nucleic acids often reveal multiple metal ions. Whether a certain ion is specific to the structural motif or the result of the crystal lattice and/or crystallization conditions is usually not obvious. Five Mg^{2+} ions per asymmetric unit were observed in the crystal structure of the B-form DNA duplex with sequence CGCGAATTCGCG. One of them binds at a GpC step, adjacent to a kink into the major groove near one end of the duplex. Two others link phosphate groups across the minor groove, resulting in a marked narrowing at one border of the AATT-tract. However, none of the ions binds to only a single duplex. Instead they mediate contacts between symmetry-related DNA dodecamers. Moreover, all Mg^{2+} ions exhibit either inner- or outer-sphere coordination to phosphate groups. Although some of the Mg^{2+} ions accentuate conformational features of the DNA duplex in the lattice, their particular locations and coordination modes indicate that ion binding to the dodecamer is primarily a consequence of the crystal packing and, to a lesser degree, of the sequence.

Metal ions play important roles in the stability of DNA (*1*) and RNA (*2, 3*), DNA conformational transitions (*4*) and phosphodiester cleavage and ligation reactions catalyzed by nucleic acids (*5*). The binding modes of ions can be grouped broadly into two categories: Diffuse and site-binding (*6*). Both can serve specific functions in the structural organization of nucleic acids and RNA catalysis. However, only site-bound or localized metal ions can be observed in X-ray crystal structures.

Many among the recently determined RNA crystal structures have revealed bound metal ions [(*7-11*), reviewed in (*12*)]. Similarly, improvements in the resolution of DNA oligonucleotide crystal structures have provided insight into the localizations of divalent (*13-17*) and monovalent metal cations (*18*, *19*) [reviewed in (*20*)]. Which of these ions have to be considered an integral part of a certain RNA folding motif or a DNA duplex may often be difficult to determine. For example, analysis of the effects of monovalent cations on the stability of a 58-nucleotide fragment from *E. coli* 23S rRNA showed that K^+ was most effective in terms of stabilizing the RNA tertiary structure and that the increased stability was due to a single K^+ ion (*3*). A subsequent crystal structure of the fragment led to the identification of this ion and the precise mode of tertiary structure stabilization (*10*). The structure of the internal loop E fragment of 5S rRNA is known to be highly sensitive to the concentration of divalent metal ions and in the high-resolution crystal structure five Mg^{2+} ions were found to coordinate to the loop E major groove (*7*). However, it appears that only one of them may be specific to the loop E and hence of biological importance (*21*).

It has been correctly pointed out that the more complex tertiary and quaternary structure of RNA compared with (duplex) DNA leads to a more versatile role of metal ions, particularly monovalent ions, in the stabilization of RNA folding (*17*). Oligodeoxynucleotide duplexes constitute a relatively simple class of structural motifs despite the existence of right-handed and left-handed geometries. Because phosphate groups lie on the outside of the double helix a large portion of the helical surface displays strong electronegative potential. Most of the localized metal ions in crystal structures of nucleic acid duplexes simply relieve electrostatic repulsion between phosphates from adjacent strands and thus provide the mortar between the DNA bricks. When analyzing the effects of site-bound or localized metal ions on DNA duplex structure, it is obviously important to differentiate between sites that are occupied as a result of packing interactions and those that may constitute intrinsic binding pockets, potentially resulting in specific ion-mediated conformational properties of DNA. Another variable that has to be taken into account when judging the specificity of metal ion binding is the often high concentrations of cations used for crystallizing nucleic acid compared with their levels *in vivo*.

The B-form DNA oligonucleotide with sequence CGCGAATTCGCG, the so-called Dickerson-Drew dodecamer (*22*) (DDD), provides an excellent example for analyzing the potential effects of a particular class of divalent metal ions (Mg^{2+}) on the conformation of a double helical fragment. The structure of the Mg form has been determined at atomic resolution [max. 0.95 Å; ref (*23*)]

and five Mg^{2+} ions could be located per crystallographic asymmetric unit (*13*, *14*). The dodecamer duplex exhibits an asymmetric kink into the major groove that is associated with a bound Mg^{2+} hexahydrate and two additional Mg^{2+} ions were observed to coordinate to the phosphate backbone outside the groove adjacent to a further hallmark of the DDD, the narrow AATT portion of the minor groove. In the present chapter, we review the coordination modes of Mg^{2+} to the DDD and examine their possible influence on its structure in the orthorhombic crystal lattice. As the reader will see, a further analysis of the best studied DNA duplex structure from the point of view of DNA-cation interactions demonstrates that, although bound Mg^{2+} ions can modulate the conformation of the DDD, metal ion coordination is primarily determined by crystal packing and sequence.

The Ionic Environment of the DDD in the Mg Form

The crystal structure of the DDD at 1.1 Å resolution furnished the locations of five Mg^{2+} ions in the asymmetric unit (*13*). In the crystal lattice, each duplex is surrounded by 13 divalent ions as a result of the packing interactions in the orthorhombic space group (*14*). The Mg^{2+} environment for an individual dodecamer with the associated packing contacts is depicted in Plate 1. The five crystallographically independent ions are termed Mg1 to Mg5 and DNA residues in the first strand are numbered 1 to 12 and those in the second are numbered 13 to 24. Mg1, Mg3 and Mg5 are hexahydrates and Mg2 and Mg4 are pentahydrates. The detailed coordination modes and geometries were described in reference (*14*). Mg1 is bound inside the major groove adjacent to one end of the duplex (Plates 1A, 2). It uses three of its water ligands to contact O6 and N7 and O6 of residues G2 and G22, respectively. The remaining water ligands are involved in hydrogen bonds to phosphate oxygens of residues A6 (O1P and O2P) and T7 (O2P) from a symmetry related duplex. Mg2 is engaged in contacts to three different DDDs in the crystal lattice. The inner-sphere contact is made to an O1P oxygen from a first duplex (residue T19). Two additional water-mediated contacts are made to two symmetry related DDDs and involve O1P oxygens from residues G12 and G24, respectively. Mg3 forms outer-sphere contacts to two neighboring duplexes. The ion interacts with both the O1P and O2P oxygen of residue G10 from the first duplex and with O1P of residue A18 in the second. Mg4 links three neighboring duplexes and is engaged in an inner-sphere interaction to O1P of A17 from a first duplex and outer-sphere interactions to both phosphate oxygens of C9 from a second duplex as well as to O2P of G24 from a third. By comparison Mg5 is exclusively involved in outer-sphere coordinations. It bridges two duplexes by forming contacts to O2P of residue C21 from one duplex and to O1P and O2P of residues A5 and A6, respectively, from a second. An additional outer-sphere contact is established to the 5'-hydroxyl group of residue C13.

The majority of contacts by Mg^{2+} ions in the lattice of the DDD are formed to phosphate groups. In fact Mg1 and Mg5 are the only ions that exhibit interactions to base (O6 and N7 of G) and sugar atoms (5'-OH), respectively, besides interactions to phosphates. This clearly demonstrates the importance of Mg^{2+}-phosphate interactions for lattice formation and stabilization. Our own work has yielded evidence that the Mg^{2+} concentration in crystallizations of the DDD correlates with the packing density and the resolution of X-ray diffraction data (*13*). Increased Mg^{2+} concentrations improve the diffraction limit, in turn making possible observation of divalent ions previously not visible in electron density maps based on lower resolution data or observation of partially occupied ions. Because Mg1 was also present in structures of the DDD at medium resolution (*24*), independent of the concentration of spermine used for growing crystals, we may assume that this particular site is occupied first in the Mg-from lattice of the DDD. Mg2 and Mg3 coordinate in close vicinity from each other and together bridge phosphate groups across the minor groove. The two ions were not observed in earlier structures at lower resolution and their occupancies may depend on the relative concentrations of Mg^{2+} and spermine in crystallizations of the DDD. Mg4 and Mg5 exhibit only partial occupancies even in the structure of the DDD at atomic resolution, based on crystals grown in the presence of relatively high concentrations of Mg^{2+} (typically > 25 mM). Therefore, it is reasonable to conclude that these sites may only become occupied as the Mg1, Mg2 and Mg3 ions are fully bound.

The Major Groove Mg^{2+} Ion

Mg1 bridges G2 and G22 from opposite strands in the major groove, adjacent to a distinct kink into that groove (Plates 1 to 3). In the 1.1 Å crystal structure of the DDD the kink amounted to ca. 11° (*13*). This asymmetric compression of the major groove near one end of the duplex is a long noted feature of the DDD (*22*) and has been referred to as a 'facultative bend' (*25*) or 'annealed kinking' (*26*) [briefly reviewed in ref. (*27*)]. The latter term referred to the smooth and continuous nature of the bending in the DDD, whereby an increase in roll appeared to lead to a cascade of alterations in propeller twisting that was propagated halfway down the helix (*26*). The issue of bending was later revisited and it was concluded that GC/AT junctions are inherently bendable and can adopt straight or bent conformations under the influence of crystal packing forces (*28*). The bend itself is produced by rolling one base pair over the next along their long axes such that the major groove gets compressed.

Structures of DDD duplexes studied under different conditions or in crystals grown from a range of conditions display various degrees of kinking. For example, the absence of a kink in the crystal structure of d(CGCGAATT^{Br}CGCG) at high concentrations of 2-methyl-pentane-2,4-diol (MPD) was attributed to the specific steric limitations as result of the additional bromine located at the edge of the major groove (*26*) (Plate 2B). Although the

exact origins of the asymmetric kink remained unclear, the tendency to roll and bend must be consequence of the particular sequence context (*28*). Both packing forces (*22*) and kinetics of crystallization (*26*) were invoked as explanations for the asymmetric kink. The observation that the $[d(CGCGAATT^{Br}CGCG)]_2$ duplex was straight suggests that a single bromine can overcome the local packing forces (intermolecular hydrogen bonds) in DDD crystals. It is noteworthy that both straight and bent DDDs can apparently be accommodated in the orthorhombic crystal lattice (*28*).

In the original structures of the DDD with resolutions between 2.3 and 3.0 Å Mg1 had not been observed (*22*, *25*, *26*). In some reports, a spermine molecule was described to cross the major groove at the site of the greatest bending (*26*, *29*), but such a location of the polyamine was never confirmed and in the structures at high resolutions, spermine was not found in that region either. However, the presence of Mg1 near the site of the kink in recent crystal structures of the DDD provided new support to the idea that a cation could somehow promote the kink (*20*). Mg1 was initially observed in crystal structures of the DDD with base pair mismatches or drugs bound [i.e. (*30*)], perhaps as a result of the improved quality of electron density maps due to the advent of low-temperature data collection. This Mg^{2+} ion was also present in DDD structures at medium resolution (*24*, *31*) and high occupancy of the site is apparently independent of the specific ratios between Mg^{2+} and spermine used for growing crystals. However, other divalent metal cations such as Ca^{2+} do not enter the site – Ca^{2+} actually coordinates in the minor groove of the DDD (*14*) – and alkali metal cations may not be able to replace Mg^{2+} unlike at a site inside the major groove of an A-form decamer duplex (*19*). In terms of the role of Mg^{2+} in inducing or stabilizing bending, a helix with an asymmetric kink and Mg^{2+} bound nearby may crystallize easily, in any case more easily than a symmetrically kinked duplex with ions bound near both ends or a straight duplex (*26*). However, one should not discount a potentially crucial role of packing forces in either inducing or enhancing the kink. As described above, Mg1 does not just engage in contacts to a singe duplex, but is inserted between the major groove and phosphates of the C1-G12 strand from a second duplex (Plates 1, 2). Mg5 is bound in close vicinity but only forms a hydrogen bond to a phosphate oxygen of C21 in an outer-sphere fashion and none of the ligand waters are shared between the two ions. This close approach between phosphates and a major groove involving two duplexes is not observed at the other end of the DDD (Plate 4). Because the environments of the duplex ends are different in the orthorhombic crystal and the asymmetric kink occurs near the end that is involved in tight Mg^{2+}-stabilized inter-duplex contacts, packing forces could be enhancing and stabilizing the particular conformation of the DDD in the Mg form.

The DDD duplex does not exhibit a kink at the opposite end (C12:G13; Plate 4). The helical axes of the $[d(CGCG)]_2$ and the central $[d(GAATTC)]_2$ duplex portions are virtually parallel (Plate 3). The drawings of the two halves of the DDD duplex and the surrounding duplexes depicted in Plates 2 and 4

illustrate that the packing around the C1:G24 end of the duplex is tighter than that around the G12:C13 end. The superposition of DDD duplexes from different structures reveals that C13 enjoys a certain degree of freedom (Plate 4B), clearly supporting the notion that packing there is somewhat less tight. Although certain features of the environment of the two DDD duplex ends are quite similar, such as for example the way they overlap with the terminal base pairs from duplexes above and below in the crystallographic *z*-direction of the lattice (*25*), the packing deviate to a certain extent. In some structures of the DDD, fragments of spermine resided near the major groove at a site that is equivalent to the location of Mg1 at the opposite end of the duplex (*32*, *33*). However, the presence of the cation there appears to have no conformational consequences (Plate 4B). In all DDD structures reported to date this end of the molecule is straight. Therefore, it is reasonable to suspect that alternative influences by the packing might play a role in bringing about the particular geometry of the DDD duplex observed in virtually all of these crystals.

The Minor Groove Mg^{2+} Ions

Mg2 and Mg3 are located at the periphery of the narrow central minor groove of the DDD duplex (*13*). Thus, they link phosphates from residues T19 and G10 across the groove (Plate 5). A symmetry-related Mg^{2+} pair, M2*a* and M3*a* (Plate 5), also gets to lie near the minor groove, interacting with phosphate oxygens of residues G12 and A18, respectively. None of these ions establishes any interaction to a base atom at the floor of the groove and the coordination spheres are only involving phosphate groups. Interestingly, the groove at the site of the Mg^{2+} bridge between P10 and P19 is markedly narrower relative to DDD duplexes in structures that did not contain these ions (*14*) (Plate 6). In fact, the groove at that site is more compressed than in any other structure of the DDD, the actual reduction in width amounting to around 1 Å relative to the duplexes included in the comparison shown in Plate 6.

The crystallographic data clearly demonstrated that divalent metal cations, even in cases where they are located at the periphery of a groove, can modulate the groove width by relieving electrostatic repulsion between closely spaced phosphate groups from opposite strands. Apparently, this can occur in sections of the minor groove that already exhibit sequence-dependent narrowing, as is the case for the AATT stretch in the DDD (Plate 6). By contrast, coordination of alkali metal cations inside the central portion of the DDD minor groove has not resulted in any observable changes of groove width according to all available crystallographic data (*18*, *20*, *31*, *32*). However, in a recent crystal structure of a stilbenediether-capped DNA hexamer duplex with an A_4:T_4 tract, a Mg^{2+} hexahydrate complex was located inside the A-tract minor groove (*34*). The Mg^{2+} ion interacts with O2 and N3 atoms of a thymine and two adenines, respectively, and forms further contacts to 4'-oxygen atoms of two residues. It is noteworthy that in this structure, unlike in the case of Mg1 in the DDD major

groove or Mg2 and Mg3 in the DDD minor groove, the Mg^{2+} ion binds to only one duplex. As a result, the A-tract minor groove is narrower by almost 1 Å compared with a second molecule in the asymmetric unit which does not display Mg^{2+} coordination at that site.

Stabilization of Short Intermolecular P···P Contacts by Mg^{2+}

What determines the locations of Mg^{2+} ions in the orthorhombic lattice of the DDD? As pointed out in the introduction, X-ray crystallography even at high resolution can only shed light on the whereabouts of site-bound ions whereas delocalized ones remain unaccounted for. So an answer to the question regarding factors that affect the preference for particular sites is necessarily incomplete as only a subset of cations is commonly included in such an analysis. With the exception of a handful of cases (*35-38*), the cations retrieved in crystal structures of oligonucleotides normally do not account for a complete neutralization of the negatively charged phosphate groups.

To answer the above question in the case of the five Mg^{2+} ions per asymmetric unit in DDD crystals, let us first look at the coordination spheres of these ions. All of them feature at least one interaction to a phosphate group. For two among the five, this interaction is of the inner sphere mode. Moreover, none of the Mg^{2+} ions binds only to a single duplex; they all establish contacts to at least two adjacent DDD molecules. Next, let us analyze the inter- and intra-duplex (inter-strand) distances between phosphorus atoms. These are graphically summarized in Plate 6. The graphs at the top and at the bottom show distances between phosphorus atoms in strands 1 and 2, respectively, and phosphorus atoms from symmetry-related duplexes. In each case, the two closest P···P contacts in Å are depicted. The graph in the middle shows distances between phosphorus atoms from opposite strands across the minor groove. Basically, these distances provide a measure for the minor groove width.

There are eleven inter-duplex P···P distances below 7 Å (Plate 6, top and bottom). If we account for symmetry only seven unique interactions remain. The shortest intra-duplex P···P distance is 7.8 Å (between P10 and P19; Plate 6, middle) and corresponds to the narrowest site of the minor groove. Except for the close contact between P2 and P14# from two different DDD molecules (# indicates a symmetry-related duplex), each of these eight close P···P contacts in the lattice is stabilized by a Mg^{2+} ion (P10 and P19 are bridged by two ions across the minor groove as described in the previous section). The P2···P14# contact is visible in Plate 2A (upper left), although P14# is partly obscured in the drawing. Inspection of the surroundings of the P2···P14# pair reveals that they face a solvent channel and are bridged by a single water molecule. Interestingly, Mg1 and Mg5*c* bracket the two phosphates, although neither ion establishes a direct (inner- or outer-sphere) coordination to either of the two

phosphates. The distance between Mg1 and P2 is 9.4 Å (Plate 2A) and Mg5*c* is equidistant from P2 and P14# (8.9 Å; Plate 4A).

From this analysis it appears that the basic role of ordered Mg^{2+} ions in DDD crystals is the alleviation of potentially destabilizing electrostatic repulsions between closely spaced phosphate groups, residing either in two different duplexes or within a duplex. Therefore, the particular relative orientation of DDD duplexes in the three-dimensional lattice emerges as a major determinant of the arrangement of Mg^{2+} ions around an individual oligonucleotide duplex. In turn, ions trapped by the lattice can affect the local geometry of the DDD duplex, as illustrated by the asymmetric kink into the major groove (Plates 2, 3) and the local contraction of the minor groove (Plate 5).

Conclusions

The organization of divalent metal ions around the DDD duplex in the crystal structure of the Mg form is chiefly determined by packing interactions. There are two main reasons for the importance of the crystal lattice, or, in other words, the relative orientations of DNA duplexes, in controlling Mg^{2+} ion localization. One is the absence of complex tertiary and quaternary structural elements in double helical DNA (*17*). Mg^{2+} ions can interact with the backbones or the grooves or both. However, because the negatively charged phosphate groups are exposed on the surface of the DNA duplex, they dominate the coordination spheres of Mg^{2+} ions. Thus, none of the five ions in the DDD structure exhibits binding to base atoms only; each Mg^{2+} ion forms at least one contact to a phosphate group, either via the inner- or outer sphere mode. The second reason is related to the relatively high enthalpy of hydration of the magnesium hexahydrate ion. Two of the Mg^{2+} ions in the crystal structure of the Mg form of the DDD show a single inner-sphere interaction to a DNA phosphate group (Mg2 and Mg4). Both are engaged in at least one additional water-mediated interaction to a phosphate group from a symmetry-related DDD. They are representative of a more general observation with regards to the coordination preferences of Mg^{2+}: There is not a single case of a DNA crystal structure where Mg^{2+} was observed to form a direct (inner-sphere) contact to just one DNA molecule. The water ligands of such ions are always hydrogen bonded to one or more phosphate groups from adjacent DNAs. Apparently, a single phosphate group cannot compensate for the loss of a water or a binding site located within a single DNA molecule is no match for those that involve two or three neighboring molecules offering geometrically more optimally oriented phosphate groups that fit the rigid octahedral coordination geometry of Mg^{2+}. In principle, one could imagine a Mg^{2+} ion being bound between two adjacent phosphate groups from the same backbone. However, in the structure of the DDD such a coordination mode is not present. It is likely that the spacing of duplexes in DNA crystal structures is such that an ion bound to phosphates from

one backbone would get to lie in close vicinity of a backbone from a symmetry-related duplex. An alternative explanation for the absence of the above coordination mode is that, perhaps, an ion bound only to adjacent phosphate groups of one duplex and facing an open solvent-filled region in the crystal exhibits high mobility and may then be 'invisible' in electron density maps even at high resolution.

There are two instances in the crystal structure of the DDD where Mg^{2+} coordination is associated with a change in DNA conformation. The first is the kink into the major groove adjacent to Mg1 (Plates 2 and 3) and the second is the further narrowing of the minor groove as a result of the Mg2-Mg3 bridge in the AATT portion of the DDD (Plate 5). These cases demonstrate the influence of sequence in directing Mg^{2+} binding in conjunction with that of the packing described above. Thus, sequence-dependent narrowing of the minor groove in the A-tract of the DDD allows bridging by the Mg^{2+} tandem in the first place; ion binding then brings about a further contraction. Regarding the coordination of Mg1 at the G2pC3 step, it is certainly possible that the conservation of the kink in most crystal structures of the DDD is a result of the kinetics of crystallization. However, this still leaves the DNA sequence as the basic impetus for the preferred binding of Mg^{2+} at the GpC step. Again, packing plays an important role as well because Mg1 links the DDD duplex at the site of the kink to the backbone of an adjacent duplex (Plate 2). At the chemically equivalent G14pC15 step near the other end of the duplex no ion is found (Plate 4). The absence of Mg^{2+} at that site is likely related to the looser packing in the DDD crystal there, with the major groove being farther removed from the backbones of neighboring DDDs.

Regarding the order of events that result in sequence-dependent conformational changes associated with binding of divalent metal ions, the recent analysis of Mn^{2+} coordination in the crystal structure of the nucleosome core particle (NCP) has been very instructive (*39*). The overall bend and topology of the 147 base pair DNA duplex in the NCP are a consequence of the histone proteins in its core. Packing forces are necessarily less important in directing ion binding in the case of the NCP. Moreover, the softer Mn^{2+} ion compared with Mg^{2+} exhibits more inner-sphere coordination to N7 of guanine and is therefore more commonly associated with a single DNA molecule rather than being trapped between neighboring molecules. In the structure of the NCP, GpG and GpC steps harboring Mn^{2+} ions display characteristic roll, slide and shift parameters (*39*), demonstrating the importance of the duplex DNA conformation imposed by the nucleosome core on divalent metal ion binding. Accordingly, DNA conformation dictates metal ion binding and not *vice versa*.

In summary, DNA sequence (determines DNA conformation and hence) directs metal ion binding and, in turn, bound cations may modulate DNA conformation. In crystal structures of oligonucleotide DNA duplexes, such as the DDD discussed here, the lattice assumes a role in governing Mg^{2+} coordination that is more important than sequence.

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Plate Caption List

Plate 1. Mg^{2+} environment and packing contacts of the DDD duplex in the Mg form lattice. The dodecamer duplex viewed (**A**) into the minor groove, (**B**) into the major groove and (**C**) roughly along the z-direction of the orthorhombic unit cell. All molecules and metal cations are shown in van der Waals mode. Atoms of the DNA backbone are colored yellow, red and orange for carbon, oxygen and phosphorus, respectively. Nucleobase atoms are colored gray, pink and cyan for carbon, oxygen and nitrogen, respectively. Phosphate groups from neighboring duplexes are highlighted in magenta. Mg^{2+} ions are colored green and are labeled M1 through M5 with small letters in italic font designating individual symmetry mates.

Plate 2. Close-up views of the top half of the DDD duplex (base pairs C1:G24 to A6:T19). (**A**) The duplex viewed across the major and minor grooves, illustrating the kink into the major groove and Mg1 bound at the G2pC3 (C22pG23) step. The color scheme for atoms is identical to that in plate 1 and Mg^{2+} ions and selected residues as well as phosphate groups from symmetry related duplexes are labeled. (**B**) Superposition of the top halfs of DDD duplexes in selected structures: Nucleic acid database [NDB (*40*)] code BD0007 [(*13*), green], BD0005 [(*32*), cyan], BDL001 [(*22*), gray] and BDLB04 [(*26*), pink], demonstrating the similar degrees of kinking into the major groove in the first three structures and the absence of a kink in the structure of the brominated DDD $[d(CGCGAATT^{Br}CGCG)]_2$.

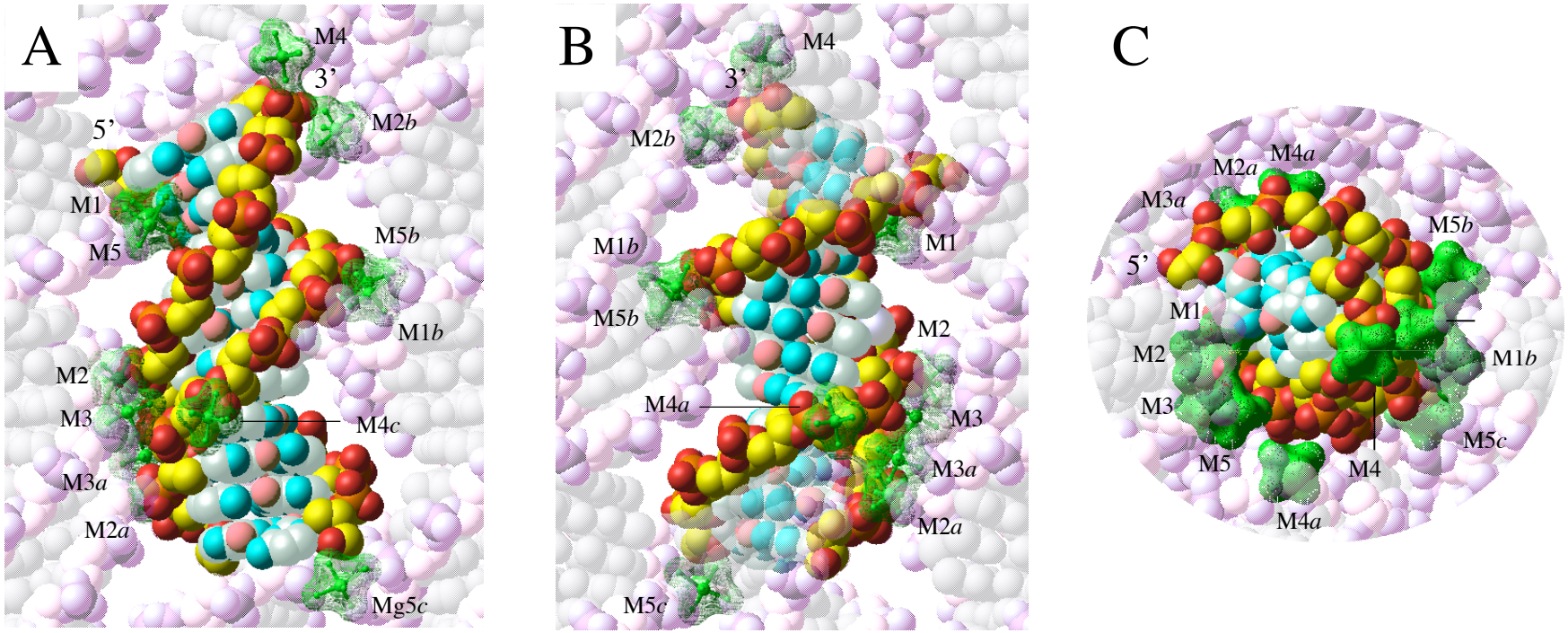
Plate 3. Helical geometry of the DDD duplex in the Mg form [BD0007 (*13*)]. (**A**) The duplex viewed into the minor groove, roughly along the molecular dyad. Helical axes for the CGCG tetramers at both ends and the central hexamer GAATTC were calculated with the program CURVES (*41*) and are depicted in blue. The color scheme for atoms is identical to that in plate 1 and residues of strand 1 are labeled. The drawing illustrates the asymmetric kink of the DDD duplex into the major groove, based in part on a positive roll at the C3pG4 (C21pG22) step. (**B**) Superposition of the top (pink) and bottom (cyan) halves of the DDD duplex. Accordingly, the central hexamer displays almost perfect twofold rotational symmetry, while the kink near one end compresses the major groove and results in deviating geometries of the two 'G-tracts'. The DDD duplex in the orthorhombic lattice can be thought of as composed of a trimer and a nonamer duplex that are themselves straight but exhibit a ca. 11° kink at their interface.

Plate 4. Close-up views of the bottom half of the DDD duplex (base pairs C13:G12 to A18:T7). (**A**) The duplex viewed across the major and minor grooves, illustrating the absence of a kink at the site equivalent to that in the top half of the duplex (plate 2) and the absence of Mg^{2+} in that portion of the major groove. The color scheme for atoms is identical to that in plate 1 and Mg^{2+} ions and selected residues as well as phosphate groups from symmetry related duplexes are labeled. (**B**) Superposition of the bottom halves of DDD duplexes in selected structures: Nucleic acid database [NDB (*40*)] code BD0007 [(*13*), green], BD0005 [(*32*), cyan], BDL001 [(*22*), gray] and BDLB04 [(*26*), pink], demonstrating that all four duplexes are straight and that binding of a spermine molecule in one case [only 6 atoms were observed (*32*)] does not affect the helical geometry. The drawing also illustrates the conformational flexibility of the terminal base pair C13:G12, resulting in effective unstacking of C13 in some duplexes. This provides an indication that the constraints due to packing are considerably different for the two DDD duplex ends, the packing around the C13:G12 base pair apparently being less tight.

Plate 5. Mg^{2+} coordination in the minor groove of the DDD. (**A**) Superposition of DDD duplexes based on structures BD0007 [(*13*), green], BD0005 [(*32*), cyan], BDL001 [(*22*), gray] and BDLB04 [(*26*), pink], indicating the slight local narrowing of the minor groove at the site of Mg^{2+} coordination in the BD0007 duplex. (**B**) In the structure of the DDD duplex at 1.1 Å resolution, a tandem of Mg^{2+} ions (M2 and M3) crosses the minor groove at the periphery, linking phosphate groups from opposite strands (*13*). Two symmetry-related Mg^{2+} ions (M2a, M3a) get to lie in the vicinity of the minor groove near the G12:C13 end of the same duplex. The color scheme for atoms is identical to that in plate 1 and selected DNA residues are labeled.

Plate 6. Intra- (defining minor groove width) and inter-duplex P···P distances in Å in the high-resolution crystal structure of the DDD (*13*) and Mg^{2+} ions stabilizing closely spaced phosphate pairs in the orthorhombic lattice. The graphs at the top and at the bottom depict inter-duplex distances between phosphorus atoms in strands C1-G12 and C13-G24, respectively, and phosphorus atoms from neighboring duplexes. The closest contacts for Ps in each strand are connected by a solid line and the second closest contacts are connected with a dashed line. The graph in the middle depicts minor groove widths in four DDDs: BD0007 [(*13*), green], BD0005 [(*32*), cyan], BDL001 [(*22*), gray] and BDLB04 [(*26*), pink] and illustrates the narrower minor groove of the DDD as a result of the Mg2-Mg3 bridge across the groove in the BD0007 structure. The coordination sites of Mg^{2+} ions are indicated.

Plates 1 to 6



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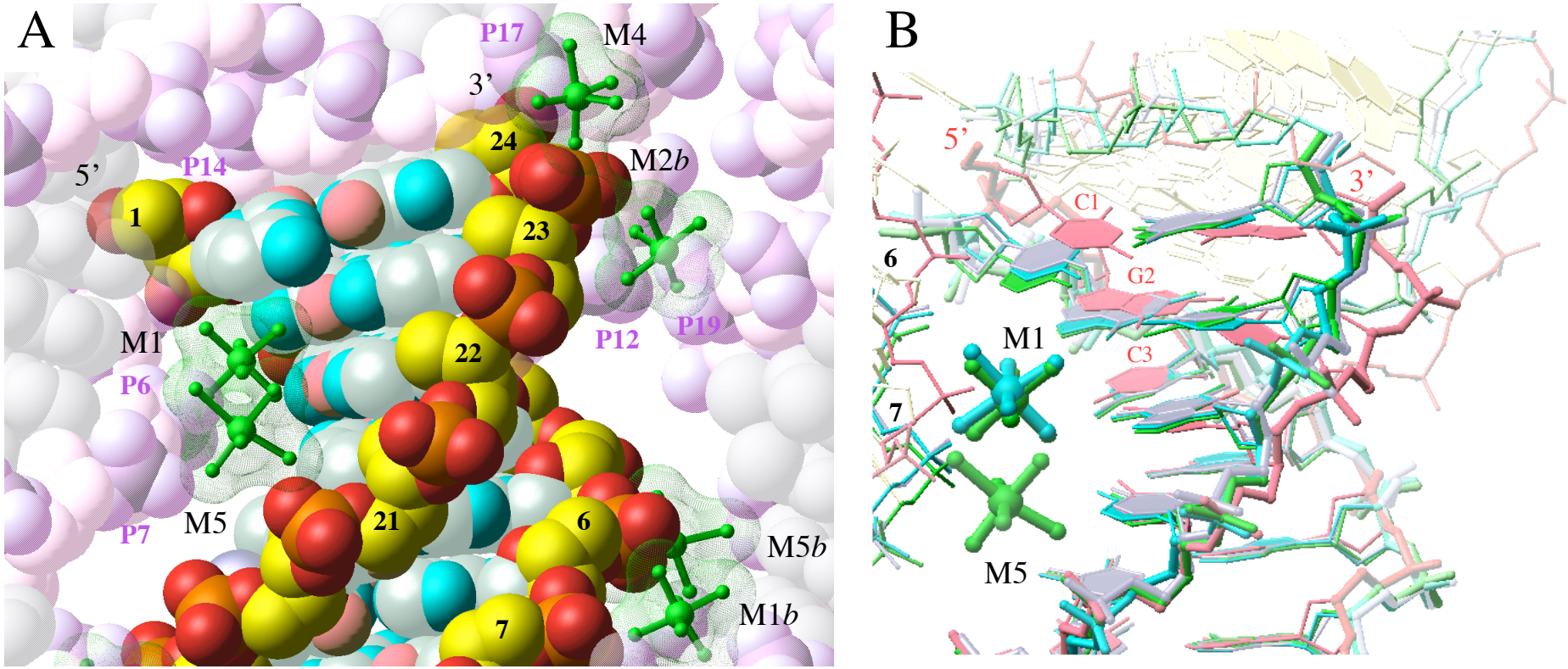

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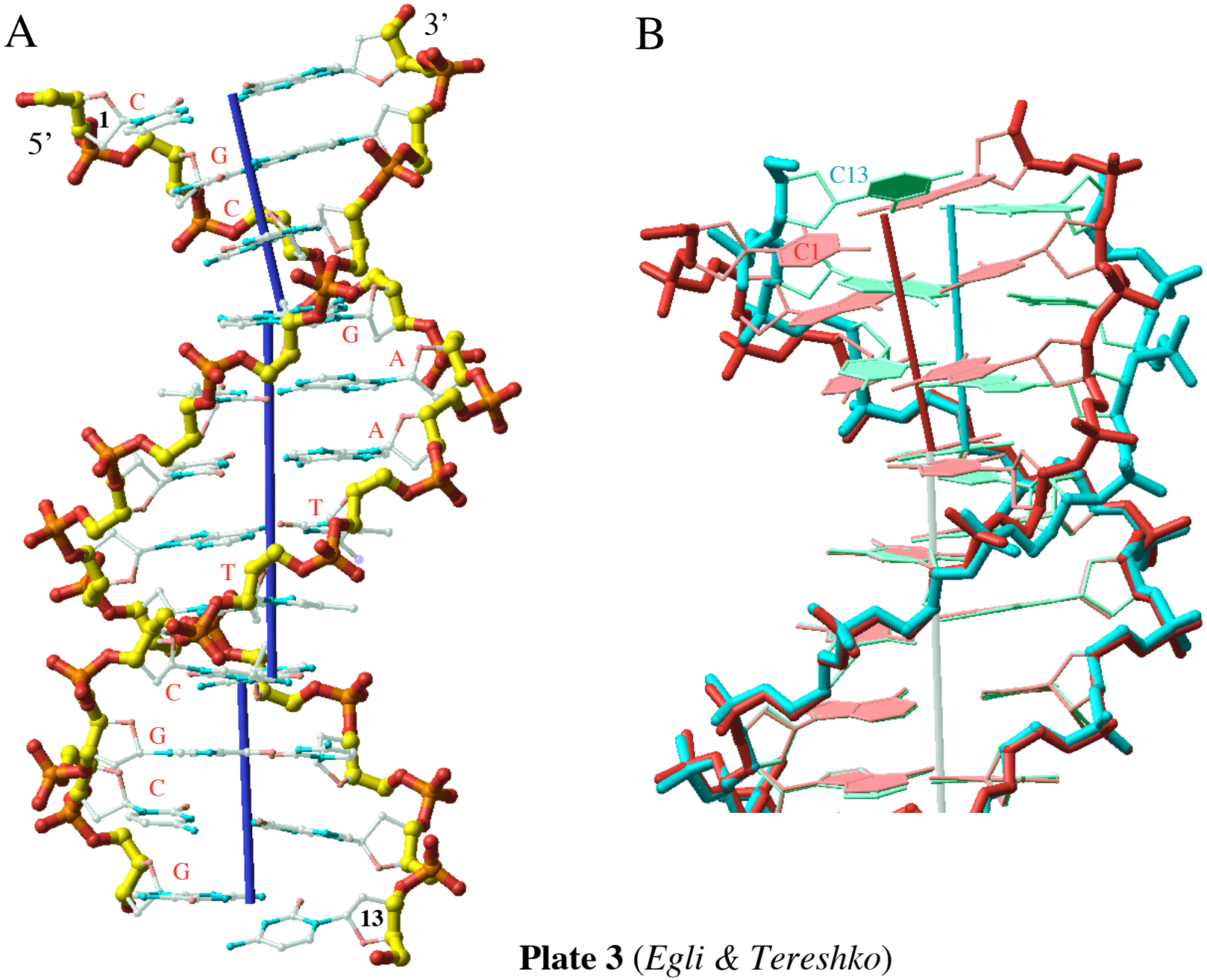

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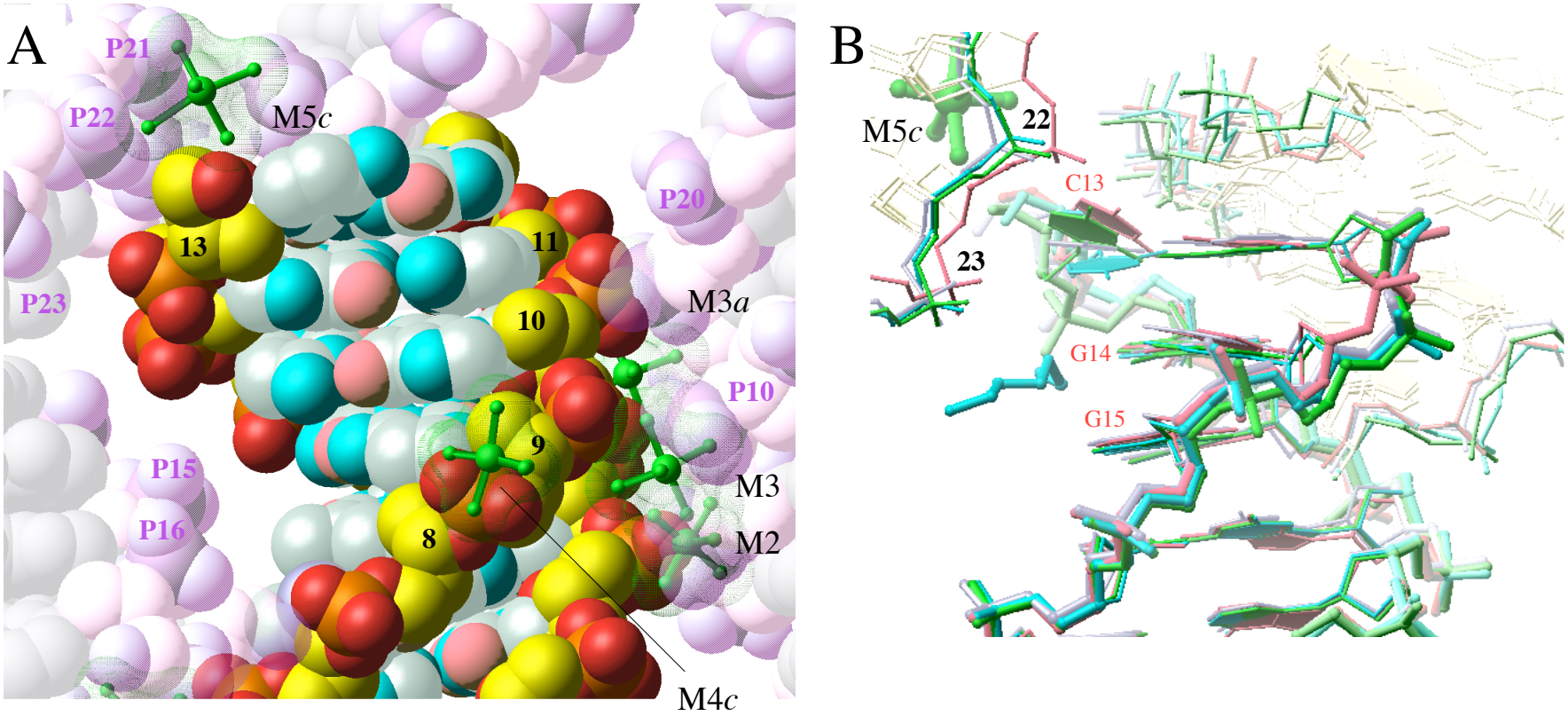

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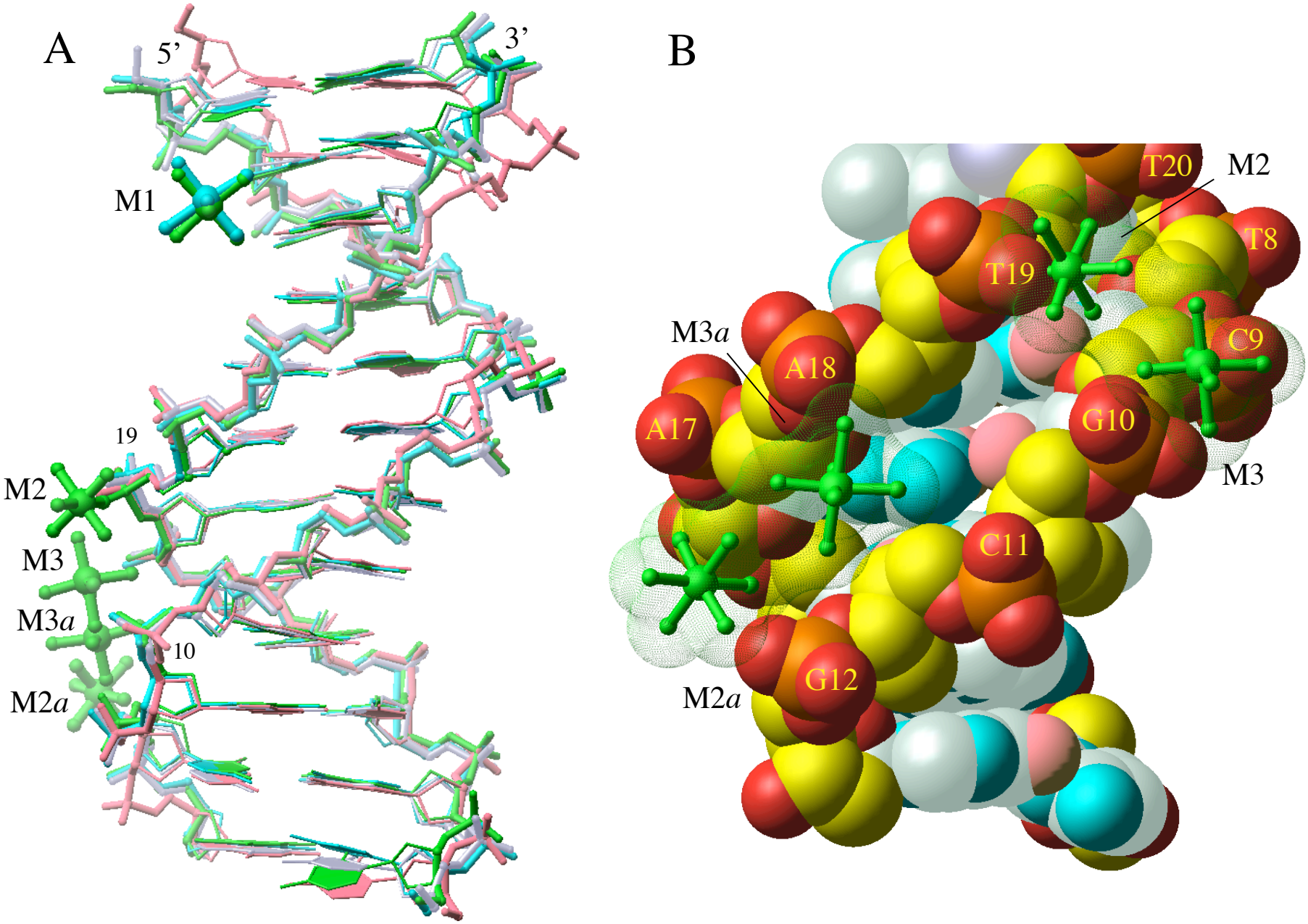

Plate 5 (*Egli & Tereshko*)

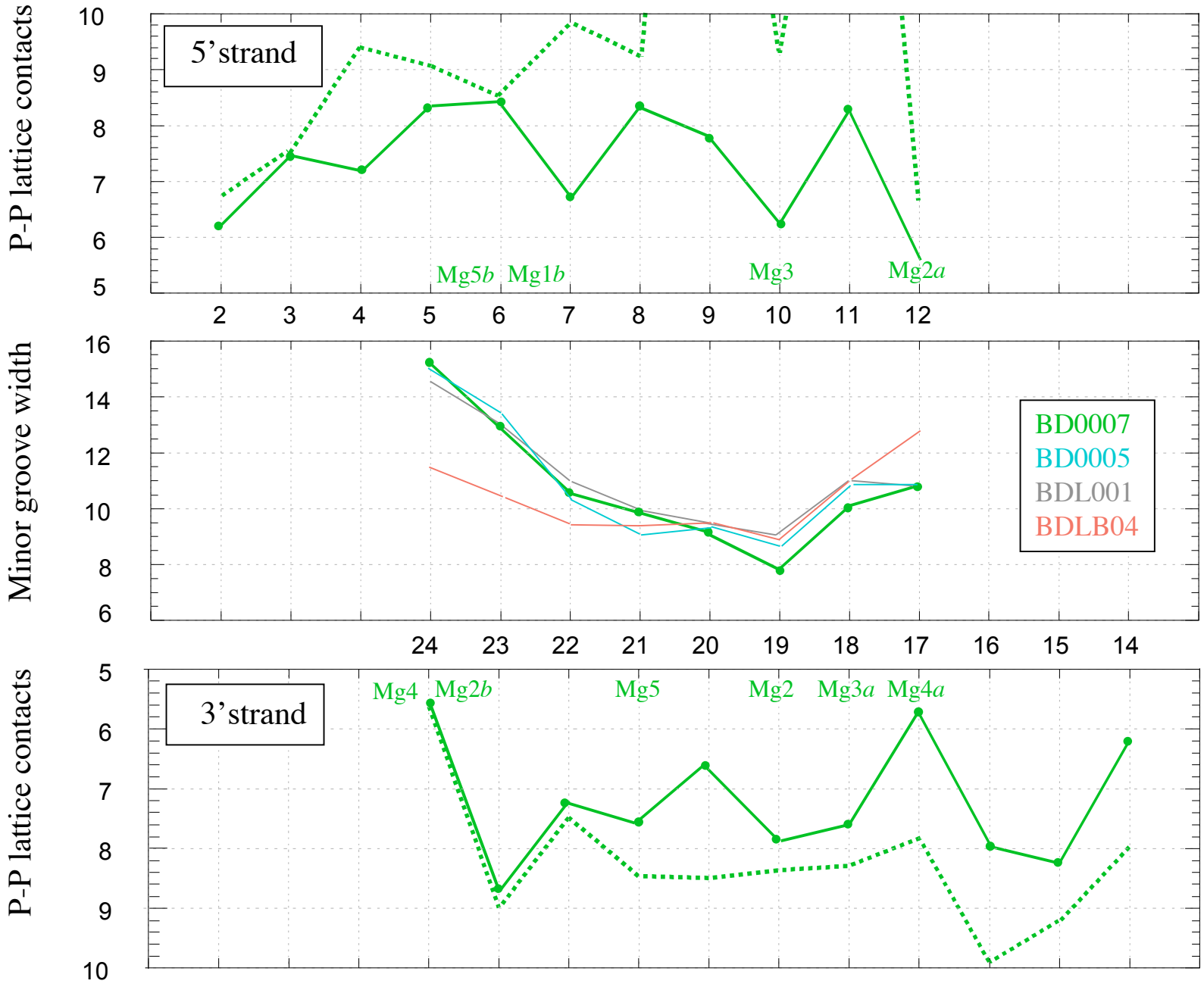

Plate 6 (*Egli & Tereshko*)