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Working the kinks out of nucleosomal DNA

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Abstract

Condensation of DNA in the nucleosome takes advantage of its double-helical architecture. The DNA deforms at sites where the base pairs face the histone octamer. The largest so-called kink-and-slide deformations occur in the vicinity of arginines that penetrate the minor groove. Nucleosome structures formed from the 601 positioning sequence differ subtly from those incorporating an AT-rich human α -satellite DNA. Restraints imposed by the histone arginines on the displacement of base pairs can modulate the sequence-dependent deformability of DNA and potentially contribute to the unique features of the different nucleosomes. Steric barriers mimicking constraints found in the nucleosome induce the simulated large-scale rearrangement of canonical B-DNA to kink-and-slide states. The pathway to these states shows non-harmonic behavior consistent with bending profiles inferred from AFM measurements.

Introduction

The exquisite management of long, double-helical DNA in the tight confines of the eukaryotic nucleus —a highly condensed chromatin structure that is remarkably accessible to the genetic machinery —takes advantage of structural and energetic signals encoded in the nucleotide sequence. For example, the eight histone proteins that make up the core of the nucleosome —the fundamental packaging unit of ~150 bp of eukaryotic DNA in chromatin —bind preferentially to so-called ‘positioning’ sequences with unique nucleotide repeating patterns. The proteins, however, make few, if any, direct contacts with the DNA bases in known high-resolution structures [1–4], seemingly recognizing the intrinsic conformation and/or the deformability of the DNA wrapped on their surfaces [5,6]. The sequence-neutral aspects of this association allow for the binding of nucleosomes on arbitrary genomic sequences and the concomitant compaction of DNA as a whole. The weak sequence-specificity of nucleosome positioning underlies the precise fold of the nucleosomal array and the access of proteins and other ligands to the encoded genetic information. The latter molecules can take advantage of the exposed nucleotides on the outer surfaces of the nucleosomes or, alternatively, compete with the histones for access to the nucleotides at the DNA-protein interface [7,8].

Until recently much of what was known about the contribution of nucleotide sequence to nucleosome structure and positioning derived from the superhelical pathway of the

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palindromic, A+T-rich human α -satellite DNA sequence featured in almost every solved crystal complex. The DNAs in these assemblies follow staircase-like routes with fragments of naturally straight, B-like helices interrupted by sharp turns/dislocations in three-dimensional structure [5], i.e., ~5 bp 'treads' of B DNA joined by dimeric 'risers' generated by concomitant bending and lateral displacement of successive base pairs (Figure 1). Moreover, these 'kink-and-slide' deformations occur almost exclusively at regularly spaced CA·TG steps along the DNA, i.e., paired dinucleotides made up of successive C·G and A·T base pairs, and act in concert to set the global curvature and superhelical pitch of the wrapped duplex [5,9,10]. The CA·TG steps are thought to be naturally deformable, not only taking up the major distortions of DNA in many nucleosome core-particle structures but also adopting a wide range of conformational states in other protein-bound complexes [6,11] and converting to highly distorted states at low energetic cost in molecular simulations [4,12].

Compared to a decade ago when the 'rules' of nucleotide deformability were first extracted from the configurations of DNA in high-resolution crystal structures [11] and years earlier when the concept of DNA sequence-dependent helical structure was formulated from the primary sequences of naturally occurring DNAs [13] and the sequence-dependent flexibility of DNA was deduced from the cyclization propensities and gel mobility profiles of short polymeric chains [14,15], there are now more than 3,000 publicly available DNA-containing high-resolution (X-ray and NMR) structures and even larger numbers of low-resolution images of DNA obtained from electron microscopy (EM) and atomic force microscopy (AFM) studies [16,17]. In other words, instead of interpreting an average property of a DNA sequence, such as its gel retardation, in terms of a simplified physical (harmonic) model, one can decipher the sequence-dependent behavior of a specific DNA from large numbers of configurations of the molecule. In addition, computational advances now make it possible to examine the influence of local sequence-induced deformations of DNA helical structure on the spatial configurations and properties of increasingly longer chains [18,19] and to reconsider the precise mechanics of nucleotide-dependent deformations of DNA [4,20]. For example, naturally flexible sequences, such as those containing CA·TG and other pyrimidine-purine, or YR, dimers, do not necessarily follow the ideal harmonic behavior expected from classical physical models [4]. Understanding the precise details of extreme helical distortions helps make sense of the observed positioning of nucleosomes and the binding of other proteins on DNA.

New surprises in the crystal structures of positioned nucleosomes

The past year has witnessed the elucidation of five new nucleosome structures incorporating DNAs other than the popular α -satellite sequence [21-23], and thus provided the opportunity to learn more about the conformational responses of DNA on the nucleosome and the implications of these findings on the positioning of nucleosomes in chromatin. Significantly three of the new structures [21,22] incorporate the synthetic '601' sequence found to bind the histone octamer with very high affinity and widely used to set the positions of nucleosomes in experimental studies of chromatin [24]. Although the ends of the crystallized sequences terminate with different bases, the central 139 nucleotides include the known key repeating patterns found in solution —namely, five TA base-pair steps, i.e., TA·TA dimers, which recur at increments of ~10 bp, i.e., roughly complete double-helical turns, and the alternation, at half-helical turns, of A+T- and G+C-rich motifs long known to characterize native nucleosomal sequences [25] and recently detected in nucleosomes on the yeast genome [26]. Moreover, these sequence preferences play a central role in the organization of nucleosomes *in vivo*, such as positioning nucleosomes in the vicinity of elements that regulate transcription [27].

The crystallized 601 sequences occupy the same position on the histone core as that mapped to single-nucleotide resolution by site-directed hydroxyl-radical cleavage [28], i.e., the expected base pair lies on the structural dyad. One of the 601-containing structures (PDB_ID 3mvd) includes the *Drosophila* RCC1 (regulator of chromosome condensation) protein implicated in disparate cellular functions [21]. The other structures (PDB_IDs 3lz0, 3lz1) focus on the subtle differences between the two different asymmetric settings of the non-palindromic 601 sequence on the histone assembly [22].

The deformable nature of the TA dinucleotide steps in many observed and simulated structures [4,6,11,29] has long hinted of a conformational role for the periodically spaced TA steps in the 601 sequence much like that taken by the CA·TG steps in the many nucleosomes incorporating α -satellite DNA. Surprisingly, the crystallized 601 sequences make different use of the naturally deformable, periodically phased TA steps in binding the histone core. Although the phased TA steps lie in the expected rotational settings, i.e., with minor-groove edges facing the histone core, they constitute only a small proportion of the kink-and-slide steps in the structures. Most of the phased TA steps deform more ‘smoothly’, with the histone-induced bends into the minor groove and/or lateral displacement (Slide) of base pairs occurring in adjacent base-pair steps. This deformational ‘smoothing’ leads to greater conformational variability of DNA in the 601-containing nucleosomes compared to that found in nucleosomes containing the α -satellite sequence (Figure 2).

Some of the kink-and-slide steps in the 601 sequences entail CA·TG dimers and several involve less naturally deformable RR·YY dimers (see the overlap/non-overlap of kink-and-slide vs. YR steps in the mosaic in Figure 3). In contrast to the isolated CA·TG kink-and-slide steps found in nucleosomes containing α -satellite DNA, some of the strongly distorted CA·TG dimers in the 601 nucleosomes about a comparably distorted base-pair step (noted by ‘double’ bars in Figure 3). The crystallized 601-containing nucleosomes also include a higher than random proportion of CG dimers (~10%), other easily distorted YR steps [11] which are completely missing in the many of the nucleosomes incorporating α -satellite DNA. Most of the CG steps in the 601 nucleosomes occur at sites where the DNA bends into the major groove. Only one CG step (that at step 56 in the 3lz1 structure [22]) adopts a kink-and-slide conformation that narrows the DNA minor groove. As is clear from Figure 3, some of the kink-and-slide steps in the 601 nucleosomes coincide with the corresponding deformations in the 145-bp α -satellite DNA, specifically at the ends of the chain binding the H2A·H2B dimer. The deformations of 601 vs. α -satellite DNA, however, differ substantially in center of the nucleosome, where the DNA is in contact with the (H3·H4)₂ tetramer (Figure 3). The binding of the RCC1 chromosomal protein has only a small effect on the kink-and-slide pattern of the 601 sequence (compare entries for 3lz0 and 3mvd in Figure 3). In general, the 601 sequence is more strongly deformed around the histone core than the α -satellite DNA, e.g., more extreme bending between successive base pairs in the nucleosomal 601 sequences compared to the DNA found in the best-resolved core-particle structure (PDB_ID 1kx5) [30] incorporating a 147-bp α -satellite sequence. The areas of the ellipses constructed in Figure 2 from the values of Roll and Tilt in the 601- and α -satellite-containing nucleosomes illustrate these differences.

The cost of ‘threading’ DNA [5,6] on the pathways reported in the 601-containing nucleosome structures is higher than that for the DNA found in nucleosomes incorporating α -satellite sequences [9], i.e., there are more 601 base-pair steps far from their minimum-energy rest states. The deformation scores of the 601-bound DNAs, based on sequence-dependent potentials derived from the structures of other protein-bound DNA molecules [6], are over threefold greater than that for the 147-bp α -satellite DNA and twofold higher than that for the ‘stretched’ 145-bp α -satellite DNA with two G·C pairs missing on either side (± 3 bps) of the dyad (PDB_ID 2nzd) [31]. In other words, the nucleotide steps along the

histone-bound 601 sequences exhibit conformational responses more drastic than those found in other nucleosome structures. The deformation scores of the 601 sequences also exceed those of the DNA in nucleosomes of comparable resolution, e.g., scores of 1624 and 2118 for the two settings of the 601 sequence crystallized at 2.5-Å resolution on *Xenopus* histones [21] vs. scores of 1086 and 750 for the α -satellite DNA wrapped respectively around chicken [32] and human [33] histone assemblies (PDB_IDs 1eqz and 2cv5). (Note that these scores take no account of the sizable distortions of the Watson-Crick base pairs in the 601 structures; see below.) Whether the greater scores of the 601 DNA reflect real differences in macromolecular sequence and structure or differences in data collection and refinement methods are open questions.

There is yet another difference between the two sets of structures, which is hidden within the double-stranded (base-pair step) parameters described above but visible in the rigid-body parameters describing the spatial arrangements of complementary and/or successive bases, i.e., base-pair and single-stranded base-step parameters. Specifically, the base pairs comprising the histone-bound 601 sequence show signs of ‘melting’ not found in earlier structures of nucleosomes containing α -satellite DNA. The Watson-Crick geometry, particularly that of A·T pairs, is highly distorted. For example, the planes of A and T are systematically staggered (vertically displaced) in the 3′-direction by ~0.3 Å on average from the ‘natural’ 0 Å rest state found in other nucleosomal DNAs and by as much as 2.5 Å at some locations. Moreover, the angle between base planes, measured in terms of Buckle and/or Propeller, sometimes exceeds 50°. Whether these structural signals mirror the measured sequence-dependent thermal stabilities of DNA [34], and in particular the extremely low stability of TA steps, is an open question. Further analysis of the stacking of bases along the DNA strands, i.e., single-stranded base-step parameters, and the interactions of histones with the individual strands may shed light on this question.

The three 601 sequences form 145-bp core particles spanning the same number of double-helical turns as both the 147-bp α -satellite DNA in the best-resolved crystal structure [30] and the related 145-bp nucleosomal sequence [31]. All five structures are slightly (2-4%) overstretched compared to ideal B DNA—a known numerical consequence [35] of the nonplanar buckling of Watson-Crick geometry that accompanies the insertion of arginines into the narrow DNA minor groove at locations where the minor groove faces the histones. The alteration of sequence, however, has subtle, previously unreported effects on the helical twist and the apparent setting of specific base pairs on the histone core. Although the average twist of the 601 sequences is identical to that of the nucleosomal 145-bp α -satellite DNA (10.4 bp/turn, based on the so-called twist of supercoiling [36]), the local repeating patterns differ appreciably over two stretches centered around superhelical positions ± 2.5 , i.e., 20-30 bp from the dyad in the vicinity of the dimerization interface between histones H3-H4 and H2A-H2B, and over two stretches at either end of the DNA (Figure 3). Differences such as these, which can be detected with chemical footprinting techniques, may account for the apparent discrepancies between early solution measurements of the helical twist of nucleosomal DNA [37] compared to that of the DNA captured in high-resolution structures.

The 601 and α -satellite sequences share limited identity. Alignment of the crystallized 145-bp sequences with respect to the base pair on the dyad of each structure shows that only 33 bases lie in the same register and that only six base-pair steps are identical, i.e., correspondence at the level of two randomly occurring sequences. The spacing of YR dimers also differs in the two sequences, with a greater concentration of such steps at the ends of the α -satellite DNA and in the middle of 601 DNA (Figure 3). The regularly spaced TA dimers in the 601-nucleosome structure drape over the surface of the (H3·H4)₂ tetramer rather than the H2A·H2B dimers. Many of the kink-and-slide deformations of the 601 DNA

found at H2A·H2B binding sites entail RR·YY or RY steps. Other sequences with YR steps optimized to fit all the arginine binding sites may thus fit on nucleosomes more tightly than the 601 sequence. Clearly, many more new high-resolution structures with a greater variety of bound DNA sequences are needed to understand the physical basis of DNA deformability and nucleosome positioning.

Insights into sequence-dependent DNA deformations from atomic simulations

Despite the many examples of extreme and complex deformations of DNA in the structures of nucleosomes and other protein-DNA complexes, most of the theories used to interpret the sequence-dependent properties of DNA still rely on oversimplified physical models of the double helix, e.g., DNA treated as an ideal wormlike chain subject to isotropic bending without consideration of either the coupling of bending to twisting and lateral displacement or the effects of localized sequence-dependent deformability [38]. Clear evidence of the breakdown of the classic DNA models comes from the unexpected prevalence of large bends in DNA chains of a few thousand base pairs in AFM images [16]. Interestingly, the cost of duplex bending extracted from the observed deflections of DNA does not follow simple quadratic behavior.

Although the tightly clustered, Gaussian distributions of the rigid-body parameters used to characterize the spatial arrangements of DNA base pairs in high-resolution structures are consistent with a quadratic increase in energy for small thermal fluctuations, e.g., dimeric bend angles of 10-20° (directed toward the grooves), the conformational dependence of the deformation energy is not clear for the isolated, severely distorted states captured in the structures of numerous protein-DNA complexes [6,11] and in specific crystallographic lattices [39]. The increase in energy with sharp bending, when the backbone conformation is altered and neighboring base-stacking interactions are lost, becomes less than quadratic. That is, the second derivative of the energy with respect to bending deflection decreases for large angles compared to small angles. One (out of many possibilities) is that the energy function becomes quasi-linear for angles greater than some threshold, e.g., 40-50° [16].

Indeed, this quasi-linear behavior has surfaced in recent computer simulations of DNA subject to strong bending. The uptake of extreme deformations depends upon the sequence and the assumed force field. In particular, the Lavery group [20] has found that implementation of the updated parmbsc0 force field [40] in molecular dynamics simulations of oligonucleotide duplexes yields different types of kinks in GC-rich versus AT-rich DNA. Whereas the kinks of the GC-rich oligomers are directed toward the major groove, those at the 3'-end of an A-tract, in the AAC trimer, are directed toward the minor groove. These structural distortions, termed kinks II, span three consecutive base pairs as opposed to a 'classical' kink I, which unstacks a single base-pair step. By contrast, use of the older parm94 force field [41] generates both type I and II kinks, which enhance the writhing of small DNA minicircles [18].

The sequence-dependent deformability of DNA also depends on the imposed constraints that mimic the presence of bound protein (Figure 4). For example, modeled, 'protein-free' hexameric duplexes [4] bend more easily in the direction of the minor groove at RY steps and in the direction of the major groove at YR steps, a finding obtained many years ago in simulations of DNA tetramers [29] and consistent with the stereochemical rules introduced by Calladine to account for subtle, sequence-dependent conformational effects seen in the earliest DNA crystal structures [42]. That is, major-groove bending relieves purine-purine clashes in the minor groove of YR steps, and minor-groove bending diminishes unfavorable clashes in the major groove of RY steps. If, however, the base pairs are laterally displaced,

along their long axes, as at the kink-and-slide sites in the nucleosome core particle [30], the computed conformational preferences switch, with the YR steps easily bent into the minor groove. Moreover, the constraint imposed on Slide [4] also reproduces the large increase in twist and the BI→BII rearrangement of the DNA backbone usually found in combination with the kink-and-slide deformations in high-resolution structures and responsible for the transformation of the canonical B-DNA double helix with 10 bp per helical turn to the C-like form with 9 bp/turn [43]. The coupling of these and other conformational variables may account for the unexpectedly large kink-and-slide deformations of several RR· YY steps and the differences in twist found in the 601 vs. α -satellite nucleosome structures.

Interestingly, the predicted uptake of energy depends on the direction of bending. Whereas the cost of deformations into the major groove shows a quadratic increase with (Roll) bend angles up to 20-25°, that for deformations into the minor groove is more linear [4]. The different responses may reflect some kind of ‘asymmetry’ associated with minor- vs. major-groove bends, such as the ‘exposure’ of the glycosidic bonds in the minor groove, but the precise reasons for this behavior are unknown. The bending of TA into the minor groove shows a significant non-harmonicity in the Roll angle and Slide displacement not found in the case of AT (Figure 4). In addition to the correlation between Roll and Slide, there is significant ‘widening’ of the energy ‘ravine’ between positive to negative Roll values (major-groove vs. minor-groove bending). This novel effect may ‘shift’ the Boltzmann equilibrium toward negative Roll values for TA dimeric steps.

Concluding remarks

The positions of nucleosomes on DNA determine the global organization of chromatin and the accessibility of the genomic sequence to the cellular machinery. High-resolution structures of nucleosome-binding sequences offer the unique opportunity to deduce the ‘rules’ that govern nucleosome positioning, i.e., the sites on DNA most likely to take up the severe kinks and lateral displacements of base pairs that give rise to the compact, superhelical fold of the double helix on the surface of the histone octamer. Currently, there is no consensus on the precise ‘rules’ of nucleosome positioning —see, for example, the significant difference of opinions expressed in the recent review by Trifonov [44] and the seven accompanying commentaries. Understanding the different uptake of the 601 sequence on the nucleosome in recent high-resolution structures [21,22] compared to the wrapping of α -satellite DNA found in earlier crystallographic studies is therefore critical for elucidating these ‘rules’ and drawing meaningful conclusions regarding the placement of nucleosomes relative to key elements on genomes.

The unexpected conformational response of the 601 positioning sequence to histone-core binding merits serious investigation. Why RR· YY base-pair steps heretofore characterized as naturally stiff undergo the large deformations expected of the regularly spaced, flexible TA dimers in the 601 sequence and why TA steps behave differently from CA· TG steps, i.e., DNA deformations are sometimes spread over 2-3 steps rather than localized at the TA dimer, are not clear. Re-refinement of the data with different software may lead to different solutions in better agreement with structural expectations much like the independent refinements decades ago of the DNA dodecamer structure [45-47]. For example, some of the CA· TG and CG dimers abutting the currently deformed RR· YY steps may take up the kink-and-slide states, much like the shuffled YR steps found in different α -satellite sequences (where the kink-and-slide steps move to neighboring positions [6]). On the other hand, histone-DNA complexes may not fit the general conformational trends deduced from other protein-DNA structures. That is, the arginines that penetrate the minor groove of nucleosomal DNA may present steric barriers that induce unexpected structural responses in what otherwise appear to be naturally stiff base-pair steps. Computational studies of

appropriately constrained model systems along the lines of recent simulations of highly kinked DNA duplexes may shed light on this question.

Given the close spacing of the negatively charged phosphate groups across the DNA minor groove, bending deformations that further narrow the groove are generally considered unfavorable. The neutralization of the DNA phosphates (and the constraint imposed on Slide) by the histone arginines facilitates DNA bending into the minor groove. The highly distorted kink-and-slide states that effect this bending differ significantly from canonical B DNA. The sugar-phosphate backbone typically undergoes a large conformational rearrangement as opposed to randomly fluctuating within the conformation space of the B-DNA energy minimum. The likelihood of the change of DNA helical state, a BI→BII transition, should not necessarily follow the classical mechanics of ideal elastic rods or the sequence-dependent rules of DNA deformability found in ‘intact’ double-helical structures. The complex interplay of nucleotide sequence, DNA backbone architecture, base-pair geometry, groove structure, and histone-DNA interactions along the kink-and-slide pathway is only beginning to be understood [4].

Finally, given that the twist of simulated DNA duplexes increases significantly only if constraints are placed on the lateral displacement (Slide) of adjacent base pairs, the extreme bending of DNA detected in AFM and EM images likely differs in character from the kinked base-pair steps found in nucleosomes, in terms of both the direction of bending (major- vs. minor-groove) and the accompanying rearrangement of the sugar-phosphate backbone. Whether these bends entail BI→BII transitions or other large backbone rearrangements, e.g., sugar repuckering, is an open question. Intriguingly, CG and CA·TG are among the base-pair steps most likely to adopt the BII form in NMR solution studies of protein-free B-DNA duplexes [48]. The transition propensity, which is inferred from P³¹ NMR chemical shifts, is substantially lower for TA steps. The greater propensity of GC-rich sequences for the BII form may be one of the reasons why the TA steps in the three recent 601 nucleosome structures behave differently from the CA·TG steps in the many nucleosomes formed with α -satellite DNA.

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Figure 1.

(a) Simplified representation, adapted from [5], of the staircase-like superhelical pathway taken by DNA in high-resolution nucleosome crystal structures. The 'steps', depicted by yellow rods, span 5-bp stretches of an ideal B-DNA double helix and run in the direction of the local helical axes. Red and blue arrows at the 'risers' highlight the lateral displacements associated with the histone-induced deformations that alternately convert DNA base-pair steps to A- and C-like helical states. The localized bending of adjacent base pairs into the major and minor grooves (red and blue arrows, respectively) occurs in concert with the lateral displacements and twisting needed to effect the B→A and B→C conformational transformations. The dyad passing through the central base pair is depicted by the diamond and the global axis of the nucleosome by the dotted black line. (b) Atomic-level view of the two helical turns of DNA centered about the dyad of the nucleosomal model. Note the dislocations of the helical axes (yellow lines joining the centers of base pairs) at the sites of DNA deformation and the uniform direction of helical-axis displacement in the detailed and simplified images, *i.e.*, along the global axis of the nucleosome.

Figure 2.

Scatter plots in the Slide-Roll and Roll-Tilt planes of the observed parameters and derived 'energy' contours of base-pair steps from nucleosomes incorporating α -satellite [30,31] and 601 [21,22] DNA (upper and lower images, respectively). Parameters in individual nucleosome structures are denoted by the symbols specified in the inset. Ellipses are projections of the six-dimensional 'equipotential' surfaces obtained from covariance analysis [11]. The contours correspond to 'energies' of 4.5, *i.e.*, ellipses where parameters deviate from their mean values by no more than $3\Delta\theta$, three times the root-mean-square deviation $\Delta\theta$. Note (i) the broader ranges of Roll and Slide and the more isotropic bending in the 601 dimers compared to the α -satellite steps, (ii) the more extreme deformations in the nucleosome containing 145 vs. 147-bp α -satellite DNA (2nzd vs. 1kx5), and (iii) the more limited deformations in the 601 nucleosome binding RCC1 versus those crystallized in the absence of the chromosomal protein (3mvd vs. 3lz0 and 3lz1). The kink-and-slide steps discussed in the text are those where Roll. -7° , Slide. $1.4 \text{ \AA}$ (C-like steps) and Roll. $+7^\circ$, Slide. $-1 \text{ \AA}$ (A-like steps). Block images on the right illustrate positive values of the three parameters. The lightly shaded edges of the blocks correspond to the minor-groove edges of the base pairs. Step parameters and images computed with the 3DNA software [49,50].

Figure 3.

Color-coded depictions of the kink-and-slide deformations, YR base-pair steps, and the variation in helical twist in nucleosome core-particle structures incorporating fragments of α -satellite [31] and 601 [21,22] DNA. Nucleotide positions are reported with respect to the central base pairs of the crystallized 145-bp sequences at position 0. The numerals below the graph denote the base-pair position, and the numerals above the mosaic show the superhelical position [1], *i.e.*, the number of helical turns of a given base pair from the dyad. The Protein Data Bank identifiers (PDB_IDs) of individual structures are noted on the left of the mosaic and in the plot inset. The sites of C-like kink-and-slide deformations in individual structures, where Roll. -7° and Slide. $1.4 \text{ \AA}$, are denoted by the black lines in the upper half of each structural 'bar code'. The identities and locations of YR steps are depicted by the differently colored lines in the lower half of each entry —CA·TG (blue), CG (green), TA (red). Note (i) the very different sequence composition and distribution of kink-and-slide steps in the two nucleosomal sequences (601 in 3lz0 and 3mvd vs. α -satellite in 2nzd), (ii) the coincidence of most kink-and-slide states with sites where the DNA is overtwisted, with close to the 9 bp/turn helical repeat characteristic of C DNA, and (iii) the differences in twist at the ends vs. middle of the 601 sequence in rough agreement with the average values of 10.0 and 10.5 bp/turn detected experimentally on the 5S RNA positioning sequence [37]. Helical twist is computed from the twist of supercoiling Tw^{sc} [36] and expressed in terms of the number of residues per turn, *i.e.*, $360 / \langle \text{Tw}^{\text{sc}} \rangle$, where $\langle \text{Tw}^{\text{sc}} \rangle$ is the average value, in degrees, over five successive base-pair steps.

Figure 4.

(Top) Composite stick/space-filled atomic representations, adapted from [4], illustrating the relative overlap/repulsions of the purine bases on opposing strands of (left) TA and (right) AT base-pair steps constrained to kink-and-slide arrangements (Roll = -20° , Slide = $2.5 \text{ \AA}$) like those found in high-resolution nucleosome core-particle structures. The magenta and blue arrows denote the respective directions of the bending deformation (into the minor groove via negative Roll) and the lateral displacement of base pairs (along their long axes via positive Slide). Note the weaker purine-purine repulsions (lesser overlap between pink and green surfaces) in the TA dimer compared to the AT dimer.

(Bottom) Two-dimensional (Roll vs. Slide) energy contour plots illustrating the greater deformability of the central TA base-pair step of the $d(\text{CTTAAG})_2$ hexamer duplex compared to the corresponding AT dimer in the $d(\text{GAATTC})_2$ structure with the reverse sequence [4]. DNA structures and conformational energies simulated with DNAMiniCarlo [51]. Conformational energies are the values minimized over all hexamer arrangements with the specified (Roll, Slide) values. The contour line separation is 2 kcal/mol. Cyan circles: the optimal structures. Note that for TA, the optimal Roll > 0 , while for AT, the optimal Roll < 0 . Pink circles: the kink-and-slide arrangements illustrated above. White thick lines: boundary between BI and BII forms, *i.e.* structures with backbone dihedral angles ϵ and ζ switched from *trans*, *gauche*⁻ (BI) conformational states to *gauche*⁻, *trans* (BII) arrangements.