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Structural changes in DNA upon binding in a 2:1 motif to a polyamide minor groove binder: f -IPP in 5'-TAGCTA-3'

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Abstract

Polyamide minor groove binders are known to disrupt cellular function when bound to DNA. In this project, the structural changes of the sequence 5'-TAGCTA-3', an important binding site for regulatory proteins in the EGFR pathway, were investigated when bound to f-IPP using molecular dynamics simulations. Changes in base-pair and base-pair-step parameters of slide, twist, and roll varied when f-IPP was added to the 5'-TAGCTA-3' system, causing the DNA backbone to stretch and increase in flexibility. Additionally, changes in dihedral angles of the DNA phosphate backbone upon binding impacted global features of the DNA in that the minor groove narrowed following f-IPP addition.

Introduction

Polyamide Minor Groove Binders and their Cognate DNA Sequences

The DNA segment 5'-TAGCTA-3' is a cis-acting element or binding site for regulatory proteins in the Epidermal Growth Factor Receptor (EGFR) pathway.[1] Specifically, this segment is recognized by the EGFR transcription factor complex. If the pathway is not properly regulated, increased cell growth and proliferation characteristic of cancer may occur via STAT3 phosphorylation.[2] Targeting this DNA segment allows for control of the EGFR complex so as to encourage a decrease in undesired cell growth and proliferation, and is therefore a prevalent area of study where new methods of chemoprevention and chemotherapy are concerned.[3,4] Minor groove binders will be utilized to achieve this targeting of 5'-TAGCTA-3'.

Analogues of minor groove binders have been synthesized and bound to segments like 5'-TAGCTA-3' to study the relationship between biologically relevant binding sites and their influence on controlling aberrant gene expression. Minor groove binders are molecules constructed with high specificity and affinity for the pre-determined DNA sequences they are targeting, mimicking the behaviors of DNA-binding proteins.[5] The drugs netropsin and distamycin A serve as models for this type of minor groove binder design.[5] The binder for this experiment, f-IPP (Figure 1), generally binds in the minor groove of the DNA by way of a 2:1 anti-parallel drug/DNA binding motif (Figure 2).[2]

Minor groove binders like f-IPP consist of repeating pyrrole and imidazole units that may be modified to successfully bind to a pre-determined sequence of DNA. Selective binding will be achieved via intentional selection of pyrrole and imidazole units in the f-IPP ligand (Figure 2c).

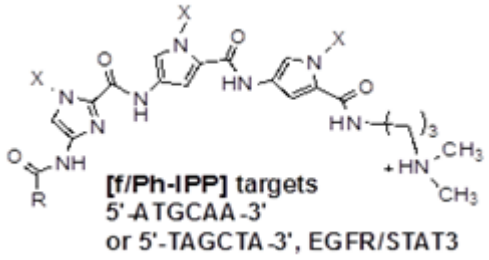


Figure 1[7]: Polyamide chain structures and sequences for promoter EGFR/STAT3.

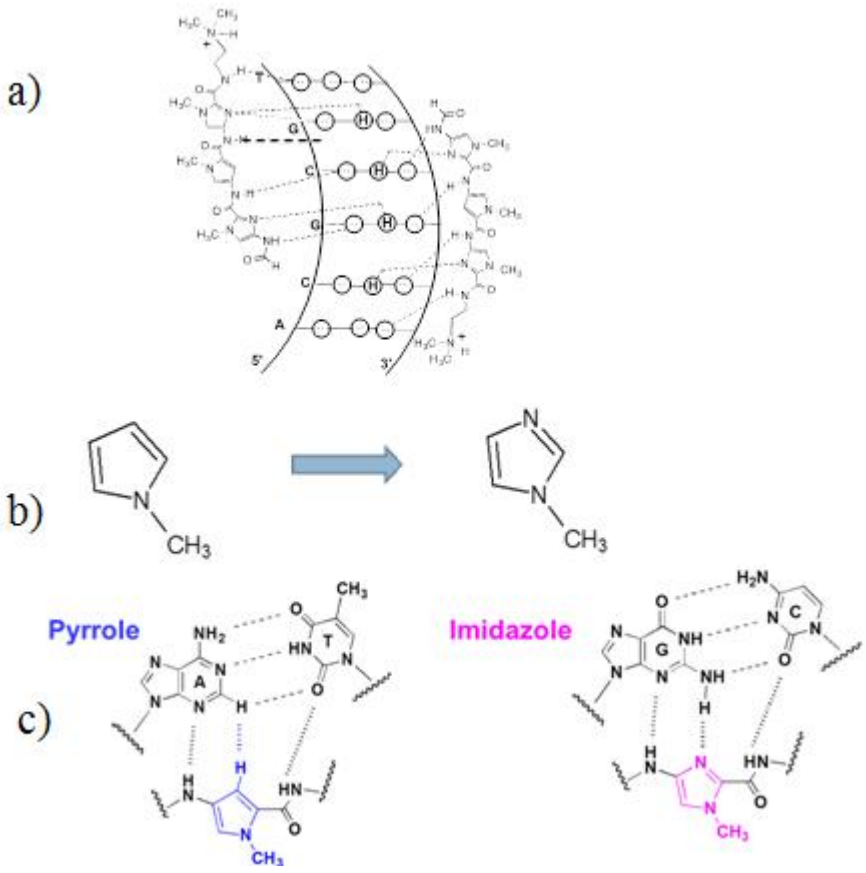


Figure 2[7]: a) Example molecular recognition pattern, b) structural modifications to change DNA sequence recognition and c) recognition units for sequence identification. Note that the sequence studied in this work is a modification of the one shown in Figure 2a where an imidazole has been substituted for a pyrrole, and thus the sequence it recognizes differs from the one shown in 2a.

To study the structure of the minor-groove bound DNA system, Molecular Dynamics Simulations (MD) are performed. Once complete, the resulting analysis will be compared to that of the DNA alone under the same procedural conditions, serving as a foundation for comparison.

Molecular Dynamics (MD) Simulations and Structural Parameters

MD uses a classical Newtonian technique to define a force field (Figure 3) to mathematically model the dynamic behavior of the system studied.[1] This behavior is time dependent, where runs do not exceed 50 ns. The atoms, their connectivity, positions, and interparticle interactions are defined, and Newtonian equations of motion describe their time-dependent behavior. MD simulations have been instrumental in DNA characterization for awhile, as seen with the Ascona B-DNA Consortium.[2] The results of these simulations allow for a detailed visualization of the system's structural parameters.

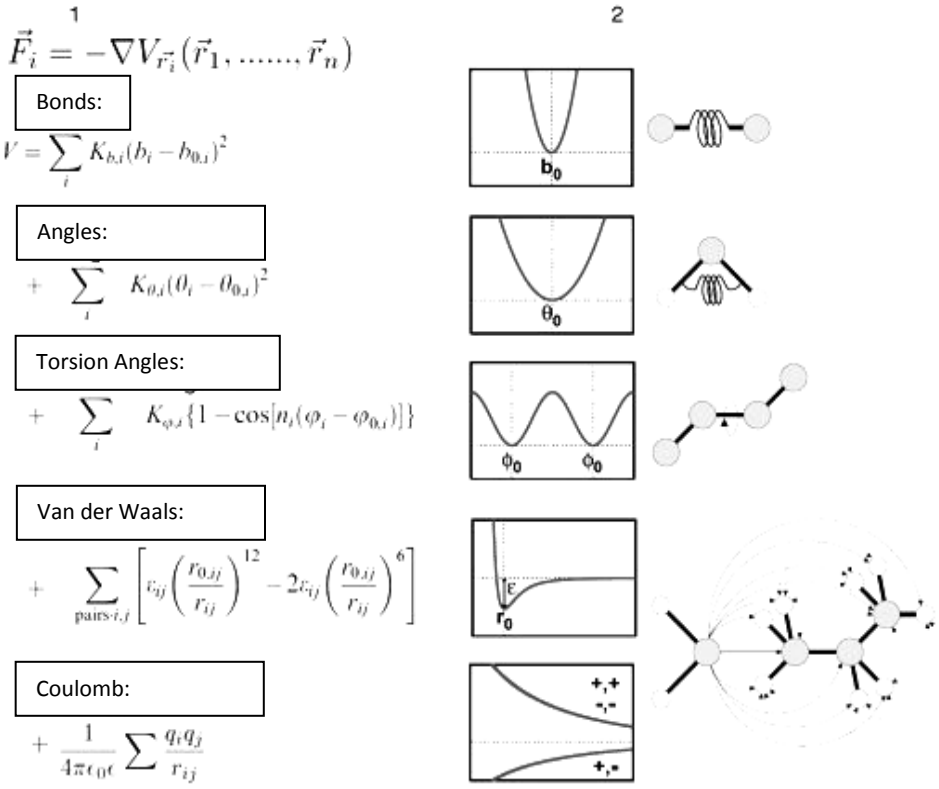


Figure 3:[6] An example of classical force field parameters. The potential energy of the system's molecular conformation is obtained from the summations of the energy terms found in the force field's functional form.

Several structural parameters are used to characterize a segment of DNA alone and when it is bound to a minor groove binder. Such parameters include zeta-epsilon backbone dihedral angles, slide, twist, and roll measurements for groove depth and width, and alpha/gamma helical measurements.

Torsion angles are closely examined to see how bonds between atoms of each nucleotide bend and stretch in order to arrive at its conformation as a nucleotide sequence. The zeta (ζ) and epsilon (ε) angles, specifically, were studied (Figure 3). These torsion angles have two

conformations: a lower energy BI state, ζ - ε less than -90°, and a higher energy BII state with the same difference greater than +90° (Figure 4).[8] The ratio of BI:BII is representative of a global structure. The BI state is roughly symmetrical in terms of its position with respect to the minor and major groove. The BII state, however, is not as symmetrical, where the phosphate faces towards the minor groove.[9] Changes in the magnitude of the BII state show how the minor groove accommodates the incoming minor groove binder like f-IPP.

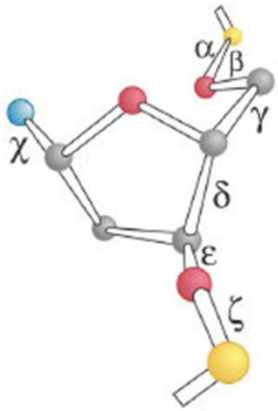


Figure 4:[10] Demonstration of the backbone torsion angles such as α, β, γ, σ, ε, and ζ, in addition to the glycosidic torsion angles like χ.

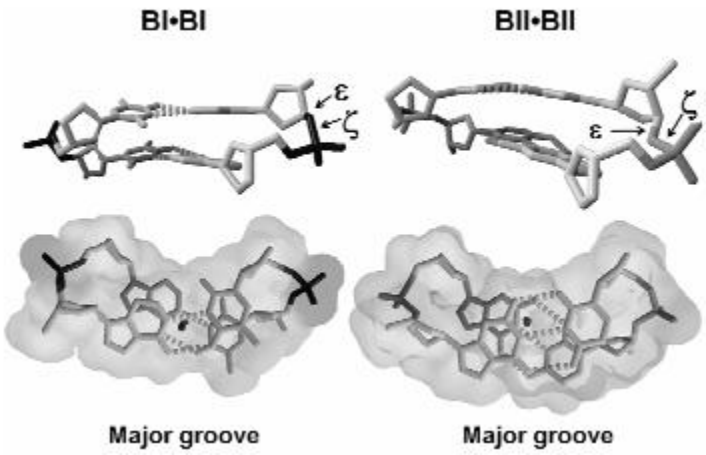


Figure 5:[11] Illustrates the BI (left) and BII (right) energy states and their positions in relation to the major groove of DNA.

Other structural parameter that may be studied are slide, twist, and roll (Figure 5[3]). These help to analyze the molecular structure of the DNA and demonstrate how the bases move. These movements are good indications of the movement of the whole DNA segment. Typically, the averages and standard deviations of the time steps for each simulation are reported for comparison between the DNA alone and in the presence of a minor groove binder. Slide, twist, and roll parameters also affect the previously mentioned torsion angle parameters, especially where the BII energy state is concerned. Occurrence of the BII state can cause twist values to

increase and roll values to become more negative.[8] Looking at the roll diagram in Figure 6, the BII state would lead to less of an opening motion of the base pairs while a large displacement would result from the stacked bases in the twist configuration.[8]

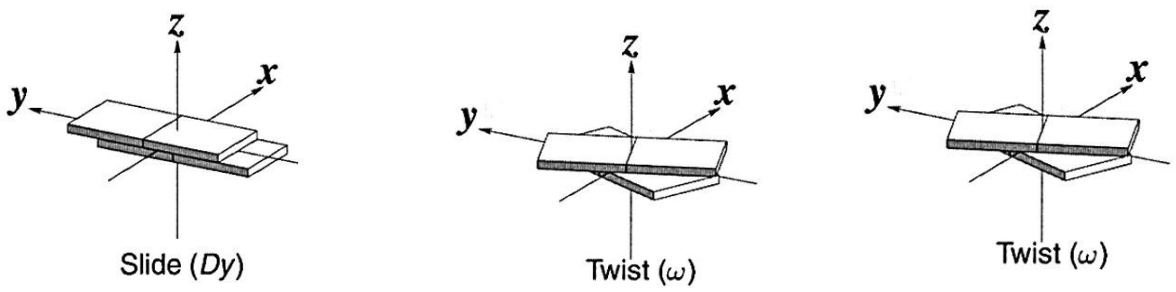


Figure 6:[3] The slide, twist, and roll step movements are shown. Measurements of these movements are usually expressed as a relative deviation from the standard positions pictured.

The final structural parameters to be studied are the alpha and gamma (α and γ) torsion angles. Since conformational changes in the B-DNA form affect DNA recognition by proteins, the study of alpha/gamma torsion angles in addition to the zeta-epsilon torsion angles are important in characterizing DNA strands.[8] Primarily, the alpha and gamma torsion angles describe the low twist conformations taking place during protein recognition of DNA, where the magnitude of each angle corresponds to a certain conformation (Figure 7).[8,10] The g^-/g^+ ground state is the most heavily populated for alpha and gamma angles of B-DNA.[7,9] Although, the regions that correspond to the g^-/t and g^+/t states are accessible.[8] The addition of a minor groove binder should lock the system into the g^-/g^+ ground state.

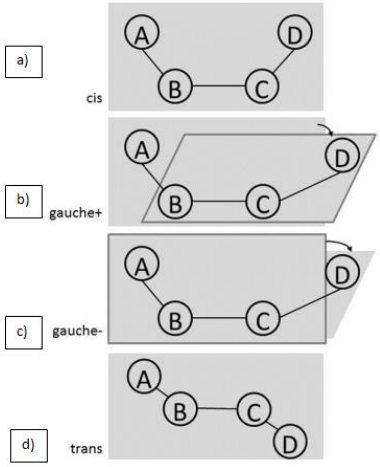


Figure 7[11]: The atoms A, B, C, and D are used to establish the torsion angles around the central B-C bond. Figure 7a refers to a cis configuration where the torsion angle is equal to 0◦, meaning that the four atoms lie in the same plane. Figure 7b shows how a positive angle results from rotating atom D towards the viewer, away from the plane. Rotating atom D away from the viewer as well as away from the plane causes a negative angle as seen in Figure 7c. Finally, if atom D were to be rotated in either direction so as to (away or towards the viewer) return to the plane where the other atoms are situated, an 180◦ angle would result, or a trans configuration.

Procedure

The DNA sequence 5'-TAGCTA-3' was manually built and f-IPP was docked using Sybyl (Figure 8).

Figure 8[13]: Visual representation of 5'-TAGCTA-3' alone (left) and fIPP bound to 5'-TAGCTA-3' via a 2:1 binding motif using Sybyl (right).

Then, the complex was loaded into AMBER12 where TIP3P water molecules were added, followed by sodium and chloride ions. This created an approximately 0.15 M NaCl environment. This was done to match experimental conditions. The system was then restricted to a truncated octahedron box. This was accomplished under periodic conditions. The AMBER 12 software suite with the parmbsc0 force field15 modifications was utilized in preparing the DNA-minor groove binder system and in executing the MD simulations. Equilibration was achieved via the Shields technique.[3] For a thorough analysis of the system, the total amount of time dedicated to the MD simulations was divided into smaller time frames. These time frames were referred to as production runs. Typically, the total time for this type of MD simulation is 50 ns. Longer time frames were not necessary since previous research has shown that MD simulations longer than 50 ns do not provide additional information about the conformational parameters of the DNA.[3]

Following the MD simulations, *Canal* and *Curves*$^{+}$ were used for trajectory analysis. The data were transferred to Excel for further study and observation.[12]

Results and Discussion

The base pair step movements and torsion angles of the MD simulations performed with the f-IPP bound 5'-TAGCTA-3' were compared to those of MD simulations performed with 5'-TAGCTA-3' alone.

Slide, Twist, and Roll

Slide, twist, and roll base step pair movements were graphed and reported as time-averaged values with standard deviations for 5'-TAGCTA-3' with and without f-IPP bound (Figures 8).

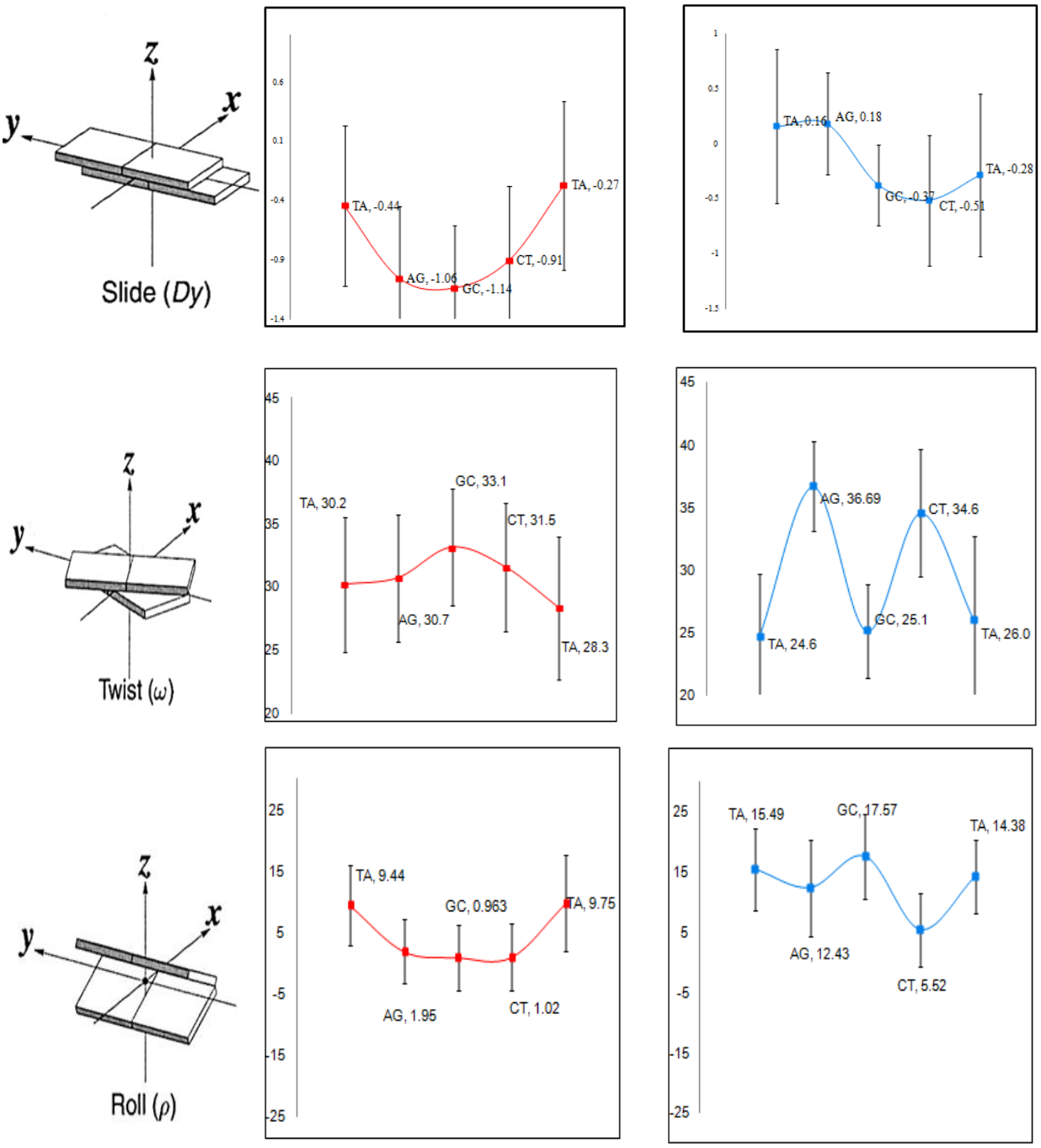


Figure 8: The charts show the time-dependent values for each base pair step over the 50 ns production run. The charts on the left corresponds to slide, twist and roll parameters for 5'-TAGCTA-3' without f-IPP bound while the chart on the right exhibits the resulting parameters for the same DNA sequence bound to f-IPP.

Slide: The slide values for 5-TAGCTA-3' alone are more negative than those of the DNA segment bound to f-IPP. The increase in the slide values is the result of f-IPP forcing the bases comprising the minor groove apart, contributing to more flexibility within the backbone, allowing it to stretch at the ends.[14]

Twist: More variation is observed between the twist values for the 5'-TAGCTA-3' system alone versus that bound to f-IPP. As with the slide values, higher twist values occur due to the minor groove binder forcing the bases apart in the minor groove.

Roll: Negative roll values are significant for this research since they indicate the presence of the BII state.[15] Even though the roll values were higher for the DNA/binder system versus the DNA alone, there were no negative values to report. Still, this shows an overall increase in the bending of the DNA to accommodate the incoming minor groove binder.[14]

For this experiment, significant changes in the slide, twist, and roll parameters were observed when comparing the DNA alone to the DNA/binder system. For example, the slide averages were higher overall, indicating that the bases in the minor groove were being forced apart once the binder was added. However, as seen with the error bars in Figure 8, there was significant variation in the slide values than is observed with other systems such as f-IPI bound DNA segments. Again, this is the result of the stretching of the ends of the DNA backbone and the narrowing of the minor groove following f-IPP addition. The twist values demonstrated the same trends but with some variation. The bases in the minor groove were not interacting as closely with one another in the presence of f-IPP. Finally, the roll values were higher for the DNA/binder system versus that of the DNA alone, corresponding to the increase in BII described above by the zeta and epsilon torsion angle parameters, and also because of the increased curvature of the DNA backbone causing narrowing of the minor groove and an overall stretching of the DNA. The variations in this experiment are confirmed by the slide, twist, and roll values found in literature, primarily for simulations involving f-IPI. These values for f-IPI are displayed in Figure 13.

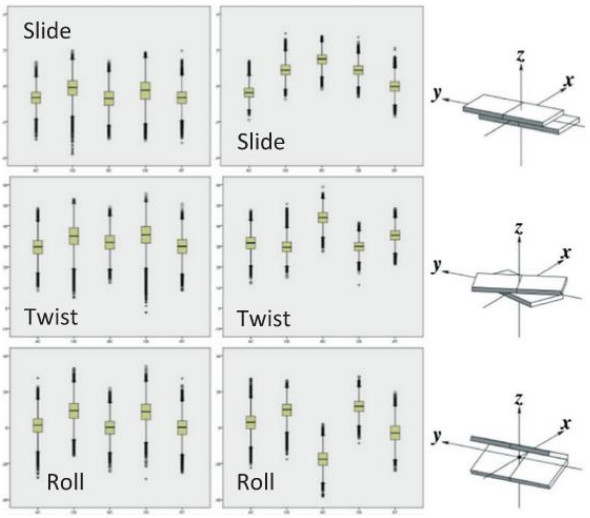


Figure 13:[14] Box and whisker plots for slide, twist, and roll parameters comparing the DNA alone (left) to the DNA bound to the minor groove binder (right). Slide and Twist averages were higher for the DNA/binder system than for the DNA alone while the roll averages were more negative for the DNA/binder system.

It is important to note that no negative roll values were observed for this experiment, but the increase in roll values for the DNA/binder system do show how the DNA backbone becomes more curved when a minor groove binder is added.

Zeta-Epsilon

The zeta-epsilon torsion angles were analyzed in terms of percent BII present for 5'-TAGCTA-3' with and without f-IPP bound (Figure 10). The BII state was studied as opposed to the BI state due to its relevancy to this experiment. Increases in the percent BII indicate a widening of the minor groove, allowing for enough room in the DNA backbone to accommodate the incoming minor groove binder.

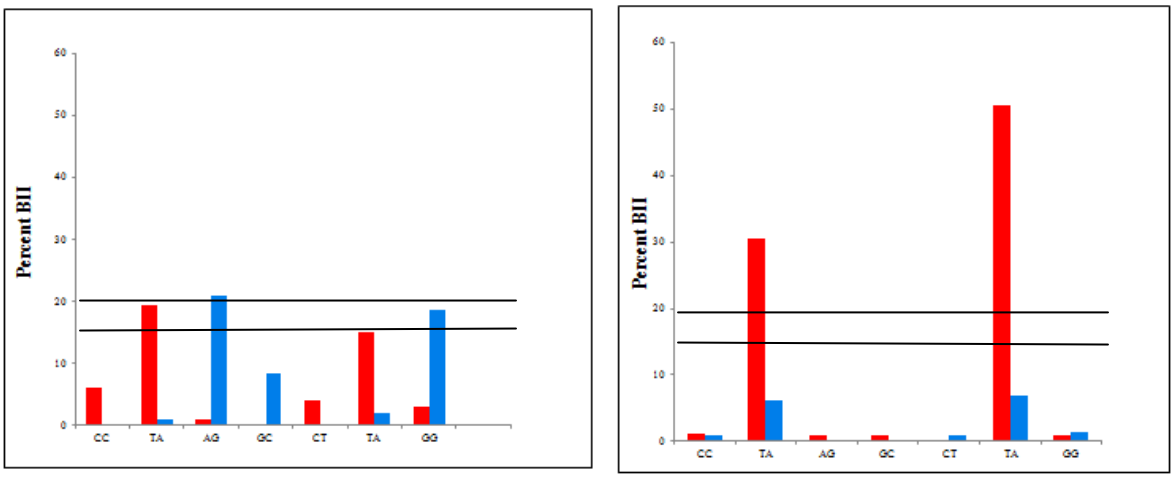
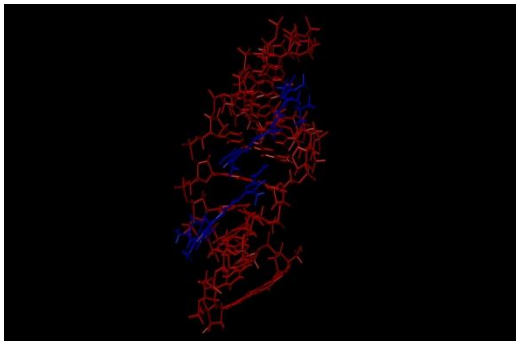
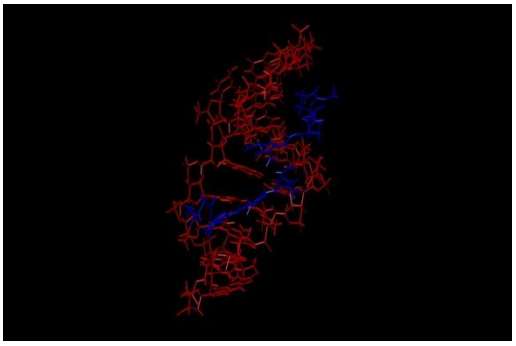


Figure 10: Shown is the percent BII present for each base on 5'-TAGCTA-3' alone (left) and the f-IPP bound 5'-TAGCTA-3' system (right). The blue color refers to the Watson strand, while the red color refers to the Crick strand. Experiments indicate and average of 15 to 20% BII present in most sequences.

The increase in percent BII for the Watson and Crick Strands of the DNA/binder system indicate a widening of the ends of the DNA along with a narrowing in the middle. The change in conformation of the DNA is visualized in Figure 11.

a)  b) 

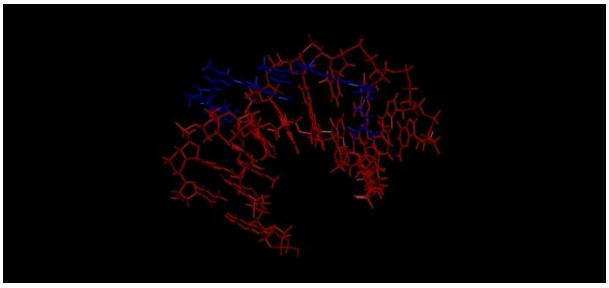
c) 

Figure 11[13]: Although the ends of the DNA have widened and the curvature has become more pronounced (11a and 11c), the minor groove binder is still attached to the backbone as seen in the narrowing of the middle of the system (11b).

In addition to these trends, it should be noted that not all percentages of BII increased for each base step for the DNA/binder system, especially AG, GC, and CT. This may be attributed to narrowing of the minor groove, showing a significant deviation in movement from the original system as seen in Figure 11.

The fraction of bases in the BII state as defined by the zeta and epsilon torsion angles agreed somewhat with trends found in literature comparing the DNA alone to the effects of adding a minor groove binder. In general, the increase in BII indicated a narrowing of the minor groove upon the addition of the minor groove binder, which was not expected. Previous experiments show how the percent BII increases for both the Watson and Crick strands when a minor groove binder, in this case, f-IPI, is added to a DNA backbone system, correlating to the widening of a minor groove binder (Table 1). Table 2 illustrates the same comparison for this experiment.

Table 1.[14] Previous experimentation depicting an increase in the BII percentage backbone dihedrals for the DNA strands accompanied by the minor groove binder f-IPI as opposed to those of the DNA alone. Both systems underwent molecular dynamics simulations. Strands A and B correspond to the Watson and Crick strands, respectively.

Base Step	A/C	C/G	G/C	C/G	G/T
DNA Alone –Strand A	11.3	17.5	12.6	6.0	3.3
DNA Alone –Strand B	3.2	3.5	2.9	12.2	12.2
Complex –Strand A	13.6	11.4	87.8	0	0
Complex –Strand B	0	0	0	99.8	0.1

Table 2. BII percentage backbone dihedrals for the Watson (A) and Crick (B) strands of the DNA alone and for the DNA attached to the minor groove binder f-IPP for this experiment.

Base Step	T/A	A/G	G/C	C/T	T/A
DNA Alone –Strand A	30.5	0.1	0.1	0	50.5
DNA Alone –Strand B	6.2	0	0	0.7	7
Complex –Strand A	19.4	0.8	0	4	15.1
Complex –Strand B	1.1	21	8.4	0	1.9

Even though, in general, the percent BII values in Table 1 increase for the complex versus the DNA alone, there is some variation observed that also characterized the percent BII values for this experiment, particularly for the central base pair steps- CG and GT for f-IPI bound DNA, and TA and GC for the f-IPP bound DNA. In Table 2, the variation in the percentage of BII states emphasizes different effects following the addition of a minor groove binder. Typically, a broadening of the minor groove binder would be expected. However, as seen through the base pair steps TA and GC, the BII percentages decrease once f-IPP is added to the complex for the Crick Strand (Strand A), corresponding to a narrowing of the minor groove rather than its broadening.

Alpha/Gamma

The final structural parameters studied were the alpha and gamma torsion angles for the DNA alone and the DNA/binder system. To observe the relationship between the two torsion angles, plots were made of the gamma angles in degrees versus the alpha angles in degrees, along with different boxes featuring the g^-/g^+ energy state, the g^-/t energy state, the t/t energy state and the g^+/t energy state. For the majority of the trajectories studied, the bases of the two systems remained in the ground state, g^-/g^+ once f-IPP was added. This is most likely due to the narrowing of the minor groove and the stretching of the backbone ends. This is observed for the sixth base of the 5'-TAGCTA-3' central DNA sequence for the Crick strand (Figure 12a-b) and the sixth base of the same DNA sequence for the Watson Strand (Figure 12c-d).

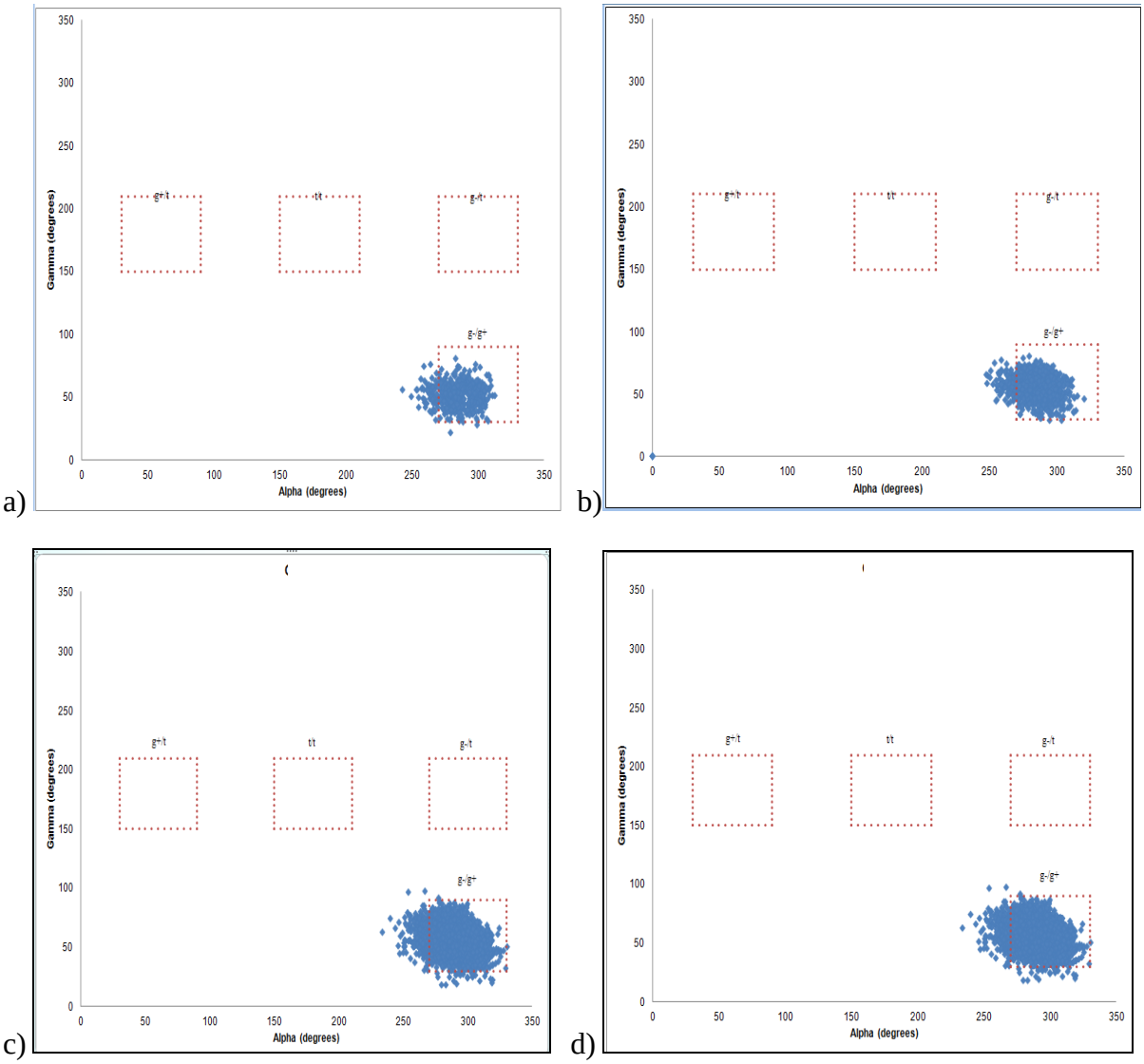


Figure 12: Top Row: Sixth base of 5'-TAGCTA-3' for the Crick Strand for the DNA alone (12a) and the DNA/binder complex (12b). Bottom Row: Sixth base of of 5'-TAGCTA-3' for the Watson Strand for the DNA alone (l2c) and the DNA/binder complex (12d).

All charts show how that the minor groove binder does not further encourage occupation of a higher energy state. Rather, it maintains this low-energy conformation.

Finally, the alpha and gamma torsion angle parameters for this experiment share similar patterns with those found in other experiments when comparing a DNA sequence with it complexed to a minor groove binder. As shown in the results section, the alpha and gamma torsion angles remained in the low energy, or ground state, conformation of g^-/g^+. This ground state was further locked in with the addition of the minor groove binder. Previous studies have shown agreement with this trend, especially in extreme cases where less than 100% of the angles were in the ground state, instead migrating to other energy states like g^+/t, t/t and g^-/t for bases in

the DNA sequence alone. The incorporation of the minor groove binder kept the DNA to the ground state (Figure 14).

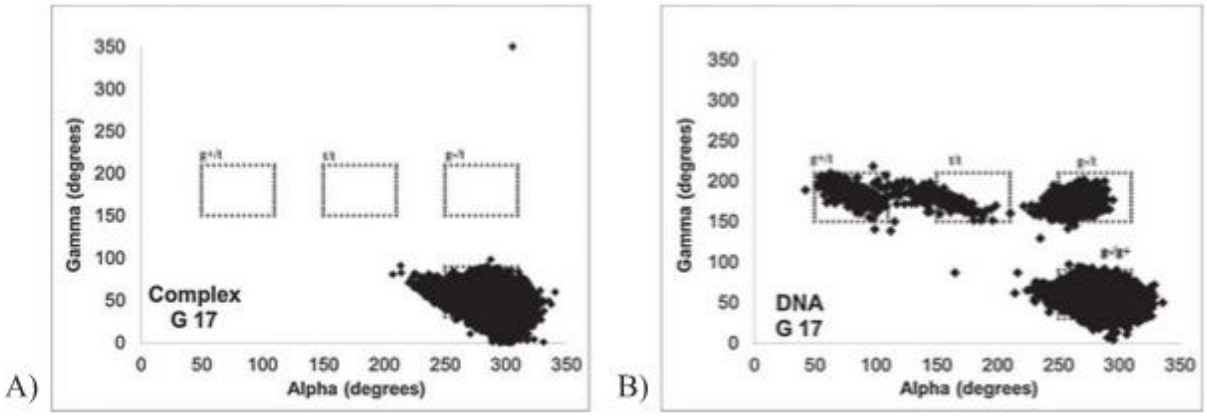


Figure 14:[14] Alpha and gamma angles for 5′-ACGCGT-3′ with (a) and without (b) a f-IPI.

These trends highlight the flexibility free DNA has so to be able to access other conformational energy states. Adding a minor groove binder restricts this movement, which is why the system is restricted to the ground energy state.[14]

Conclusions

The ratio of BI to BII states and the time-averaged values of Slide/Twist/Roll in the 5'-TAGCTA-3' are vastly different with the addition of the minor groove binder. This is as expected, because the binder modifies both the local and global structure of the DNA. The increase in BII states indicates a widening of the minor groove. Changes in Slide/Twist/Roll indicate more flexibility in the motion of the stretched DNA backbone in the presence of the f-IPP binder than might be otherwise expected. The alpha and gamma torsion angles did not demonstrate significant changes in the conformation state inhabited by the two systems studied. However, adding the minor groove binder increased curvature of the DNA and narrowed the minor groove binder, allowing the system to continue occupying the g^-/g^+ ground state conformation.

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