Journal of Biomolecular Structure & Dynamics, ISSN 0739-1102
Volume 15, Issue Number 6, (1998)

Molecular Dynamics Simulation of a 13-mer Duplex DNA: A PvuII Substrate

http://www.albany.edu/chemistry/sarma/jbsd.html

A.S. Kolaskar[1]* and R.R. Joshi[2]

[1]Bioinformatics Centre,
University of Pune,
Ganeshkhind,
Pune-411007, India

[2]Centre for Development of Advanced Computing,
University of Pune Campus,
Pune-411007, India

Abstract

Parallel version of AMBER 4.1 was ported and optimised on the Indian parallel supercomputer PARAM OpenFrame built around Sun Ultra Sparc processors. This version of AMBER program was then used to carry out molecular dynamics (MD) simulations on 5′-TGACCAGCTGGTC-3′, a substrate for PvuII enzyme. MD simulations in water are carried out under following conditions: (i) unconstrained at 300 K (230 ps); (ii) unconstrained at 283 K (500 ps); (iii) Watson-Crick basepair constrained at 283 K (1 ns); and (iv) Watson-Crick basepair constrained with ions at 283 K (1.2 ns). In all these simulation studies, the molecule was observed to be bending and maximum distortions in the double helix around was seen around the G7:C7′ basepair, which is the phosphodiester bond that is cleaved by PvuII. Analysis of MD simulation with ions carried out for 1.2 ns also pointed out that the conformation of double helix alternates between a conformation close to B-form and close to A-form. It is argued that a bent non-standard conformation is recognised by the PvuII enzyme. The maximum bend occurs at the G7:C7′ region, weakening the phosphodiester bond and allows His48 to get placed in such a fashion to permit the scission through a general base mechanism. The bending and distortion observed is a property of the sequence which acts as a substrate for PvuII enzyme. This is confirmed by carrying out MD studies on the Dickerson's sequence d(CGCGAATTCGCG)$_2$ as a reference molecule, which practically does not bend or get deformed.

Introduction

Complete genomes of more than fifteen different small organisms will be available in next few months. To assign the function to coding regions and to understand the information stored in them is a major challenge to computational biologists. Analysis of patterns and particularly those in coding regions has been making major strides and helping the biologists (1-3). However, deciphering the information in genomic sequences by taking into consideration the 3-D structure of the DNA is yet a virgin area and will lead to some interesting results. One of the reason for slow progress in the above area is, too little experimental data on 3-D structures of DNA and computationally it is even today a hard problem to carry out simulation studies on long double helical DNA. Unconstrained molecular dynamics studies in nanosecond time range, will give insight in this problem. Such long time MD simulations on double helical DNA molecules are only now beginning to be reported. Last few years have seen sudden interest in this area. The simulation studies on oligonucleotides in the nanosecond time range reported to date are on molecules having 10-12 residues (4-18). These studies also deal with the effect of ion concentration using periodic boundary conditions. The computational time of MD studies increases many fold with the increase of the size of the molecule as well as simulation time. One way to overcome this difficulty, without any approximation, will be to parallelize the molecular dynamics program such as AMBER and then use such a version to simulate molecules of biological interest. We have used this strategy. The parallel version of AMBER 4.1, obtained from University of California San Francisco, USA, was ported on Indian made parallel supercomput-

*Author to whom correspondence should be addressed. Phone: +91 212 350195, +91 212 355039; Fax: +91 212 350087; E-mail: kolaskar@bioinfo.ernet.in

er PARAM OpenFrame. This version of AMBER was used to study the oligomer 5′-TGACCAGCTGGTC-3′. This oligonucleotide was chosen as it contains the cleavage site for PvuII, a restriction endonuclease type II enzyme. The crystal structure of the PvuII enzyme and its complex with the oligomer mentioned above is also available (19). Thus, the results obtained from the MD studies could be compared with the snapshot available from X-ray diffraction data. In fact, 3-D structures of PvuII, BamHI, EcorI and EcorV, each of which is a endonuclease of type II, are now available (19-22). On the basis of the structures of these enzymes and their complexes, they are categorised in two separate classes (23). The crystal structures of the enzyme-DNA complex of EcoRI and EcoRV show the DNA molecule with a kink in its double helical conformation. On the other hand, the enzyme-DNA complexes of PvuII and BamHI which fall in the second category does not show the DNA to be kinked. Thus, it is still an open question, as to how the enzymes PvuII and BamHI specifically recognise only 5′-CAGCTG-3′ and 5′-GGATCC-3′ sequences respectively. The cleavage of the phosphodiester bond after enzyme is bound to the DNA can be explained with little difficulty in EcoRI and EcoRV, but one still does not have the complete idea about the exact mechanism of enzyme action in PvuII and BamHI. MD studies have proven to be very useful in understanding such problems (24-26) and is probably the only approach to find out the molecular behaviour at very early stages of recognition and action, with the exception of recent nanosecond spectroscopic method (27). Results as well as difficulties in such simulation studies are discussed. The method used to parallelize the AMBER and to optimise it on the PARAM OpenFrame is also briefly given. In fact, MD simulations are carried out at two different temperatures with water as a solvent. Further, ions are added to neutralize negative charges on the phosphate group and MD simulations are carried out upto 1.2 nanosecond. Not only the oligonucleotide chosen is one of the largest oligonucleotide on which unconstrained MD studies are carried out but studies at two different temperatures adds an additional dimension namely the effect on stability of molecular conformation by amplitude of motion. It has been observed that bending of DNA duplex, considered in the present study, is a property of its sequence dependent conformation and is observed under various conditions of simulations used in this study.

Methods

The SANDER module in AMBER is the most compute intensive and therefore this module is parallellized (28,29). The parallel version of AMBER was obtained from University of California San Francisco. AMBER 4.1 was ported onto the Indian supercomputer PARAM OpenFrame with Sun Ultra Sparc processors and was optimised for the SUN Ultra Sparc processors. The Master-Slave topology was chosen and the load balancing is carried out dynamically to utilise each processor optimally with little idle time. In Master-Slave topology, the Master communicates with the slaves, but within the slaves there is no communication. Thus, the processor which acts as a master, sends the packets of work to various processors and after calculations, the results are sent back to the master processor. New packet of work is then sent to the slave processors. In PARAM, at present the inter-processor communication as well as the data transfer is done using fast-ethernet with only 100 Mbps bandwidth. The PVM message passing is layered over the TCP/IP layer in the PARAM OpenFrame system rather than at the microkernel level. Thus the present implementation of AMBER on PARAM is similar to a loosely coupled cluster of workstations. The essential correctness in porting of AMBER 4.1 on PARAM OpenFrame was checked by carrying out a test run on Plastocyanin in a water box (11585 atoms) for 100 steps. These results were identical with the output of the test programs that are available with the source code and this installation was used for further studies.

MD simulations, using the above parallel AMBER version 4.1, were carried out on the 13-mer duplex oligonucleotide sequence 5′-TGACCAGCTGGTC-3′ that con-

tains the cleavage site of PvuII restriction enzyme. The initial double helical conformation of the 13-mer duplex was built using the parameters of Hunter (30). A rectangular box was constructed with explicit solvent water using the EDIT module. The box size namely $60 \times 48 \times 54 Å^3$, was chosen on the basis of the size of oligonucleotide duplex helix. Periodic boundary conditions were used during these studies. Simulations were carried out using the SANDER module and the SHAKE algorithm. The protocol followed for simulation, described below briefly, is similar to that followed by Cheatham and Kollman (5). An integration time step of 1 fs each was used in these simulations at temperatures of 283 K and 300 K with Berendsen temperature coupling. The 12.0Å cutoff was applied to the Lennard-Jones interaction term. The non-bond pairlist was updated at every 50 steps. Initial equilibration was performed, by holding the DNA fixed (25 kcal/mol constraint) and allowing only the water molecules to relax for 10000 steps at 100 K. This was followed by removing the constraints on the DNA molecule and 2000 steps of MD where the temperature was raised from 100 K to 283/300 K. Final equilibration was achieved by carrying out 25 ps of free MD on the whole system and only then production runs were initiated (5). Two unconstrained simulation runs were carried out for 230 ps at 300 K and 500 ps at 283 K. The third run was carried out for one nanosecond at 283 K by imposing distance constraints and flat angle constraints of 10Kcal/mol on Watson-Crick base pairings. During the production runs the structures were saved at interval of one picosecond and thereby generating trajectories of 230, 500 and 1000 structures.

Simulations with Ions: In this simulation 24 Na^+ ions were added in the box of water to neutralize the phosphate charges on the DNA molecule. The ions were added at the phosphate groups at the bifurcating positions of the ∠O-P-O angle at a distance of 5.0Å from the phosphorus atom using the EDIT module of AMBER 4.1. This procedure is identical to the one described by Cheatham and Kollman (5). Other conditions for simulation are identical to those mentioned above without ions except that the final equilibration is achieved by free MD upto 100 ps instead of only 25 ps. The production runs for the DNA with ions and water was carried out for 1.2 nanoseconds. During these simulations hydrogen bond constraints of 10 Kcal/mol were applied to maintain WC base pairing and double helical conformation. A reference molecule having Dickerson's sequence $d(CGCGAATTCGCG)_2$ was chosen. Molecular dynamics simulations with water and ions on the Dickerson's sequence was also carried out for 250 ps. The trajectories were generated as in other production runs at the interval of every one picosecond.

The trajectories were animated and viewed using the MOIL-VIEW package and were analysed using the CURVES package (31-33). For each trajectory and also for the average structure, the helical parameters namely, number of base pairs per turn (n), the rise per residue (h), the helical twist (t), roll (ρ), twist (Ω), tilt (τ) and slide (Dy) *etc.* parameters were calculated to study changes in the conformation with respect to the initial conformation of the molecule. The shortening in the helix of the molecules was calculated by taking the difference between the path length and the end-to-end distance. Offset values, that are the perpendicular distances of successive reference points from a line drawn between the first and last reference points, were also calculated using the CURVES program. The shortening of DNA helix and offset values are used to measure the bending and curvature of the bend DNA. The various calculated helical parameters like the shift, slide, rise, tilt, roll and twist were plotted in a windows format and the torsion angles were plotted in the dial format, using a program which was developed in-house at the centre.

Results and Discussion

Unconstrained Simulations of PvuII Substrate

The simulations carried out in water without ions and without Watson-Crick base-pair constraints, points out that the initial straight B-helix conformation bends sig-

Table I

Simulation	Shortening		Offset
	Time	% shortening	
1. 300 K (230 ps)	42 ps	10%	A6, G7, C8
	157 ps	53%	
2. 283 K (500 ps)	361 ps	37%	A6, G7, C8
3. 283 K (1 ns) + WC contr.	363 ps	37%	A6, G7, C8
	670 ps	16%	
	781 ps	40%	
4. 283 K (1.3 ns) + WC contr. + ions	409 ps	45%	G7
	741 ps	18%	
	890 ps	58%	
	1028 ps	17%	
	1116 ps	57%	

nificantly within the short simulation time of 10-15 ps. As the simulation time increases the bending and the distortion continues to increase. As shown in Table I the DNA molecule bends maximally by 53% at 157 ps and 37% at 361 ps for simulations carried out at 300 K and 283 K respectively. In both these simulations the strands fall apart around 230 ps and 500 ps respectively. This bending of double helical DNA is understandable as the electrostatic interactions due to the negative on the phosphate will force the molecule to bend. Similarly, the two strands of the double helix will fall apart as the hydrogen bonds between the bases becomes weak and water molecule enters in the centre of the helix. The base pair rise (h) of G7:C7′ and C8:G6′ observed in these simulations is highly unrealistic. It is the phosphodiester bond between these nucleotides that is cleaved by the PvuII enzyme. The roll values (ρ) are seen to decrease for the basepair step of G7/C8 when the rise is maximum. As expected, the molecular dynamics simulations at 283 K (lower temperature), points out that the molecule is somewhat more rigid and the amplitude of motion of the groups of atoms decreases.

Watson-Crick Basepair Constrained Simulation of PvuII Substrate (1ns)

During this simulation a 10 Kcal/mole constraint was applied to the Watson-Crick hydrogen bonds in the DNA molecule. Such constraints thus make sure that the two

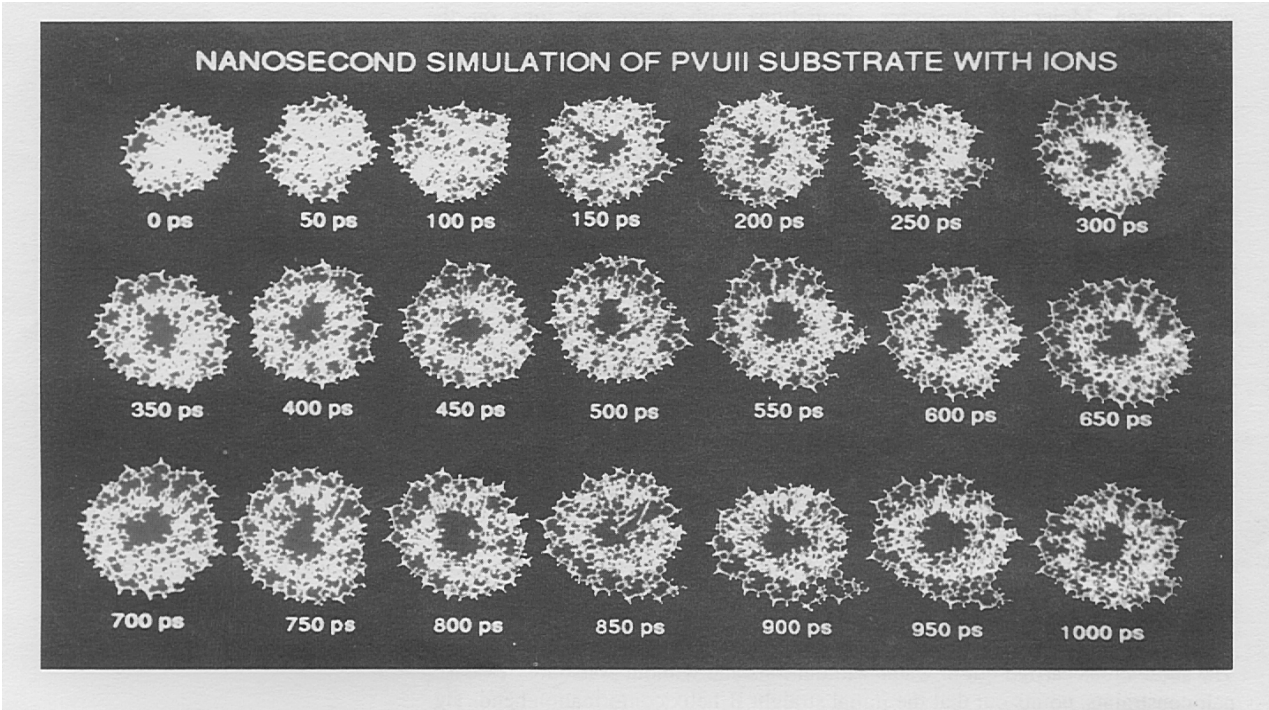


Figure 1: Snapshots giving the top view of the structures of the 1.2 ns simulation in presence of water and ions captured at 50 ps interval. The molecule is seen to move close to the A-DNA like structure.

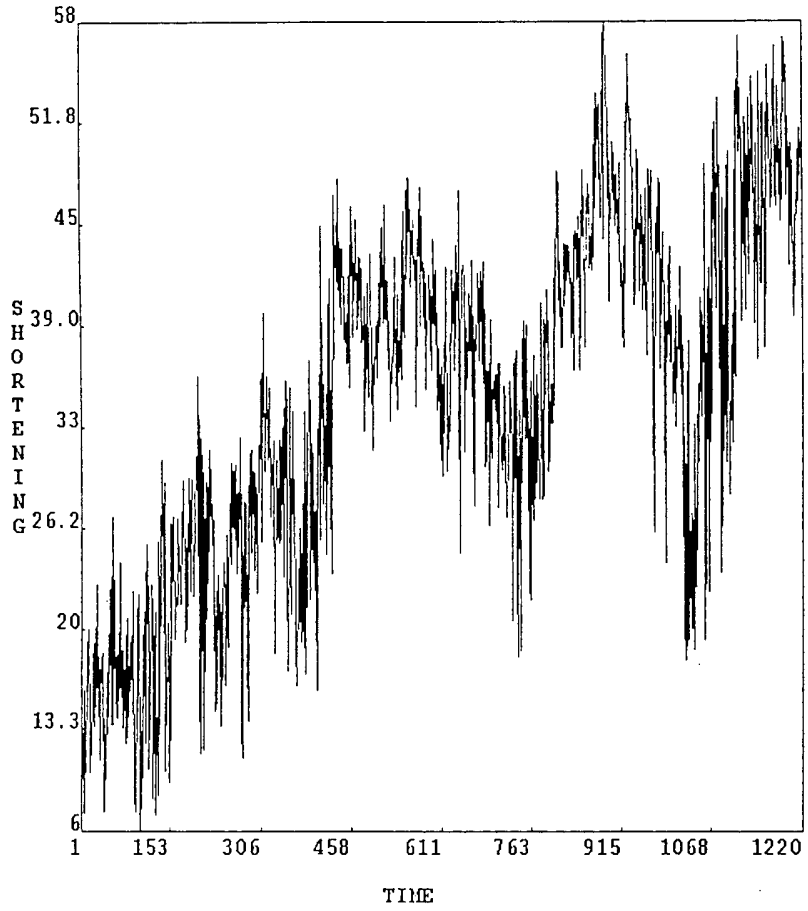


Figure 2: Plot of shortening versus time for the 1.2 ns molecular dynamics simulation in presence of water and ions.

strands of the molecule do not fall apart as observed in earlier unconstrained studies. Even under these constraints, an increase in rise at the G7/C8 basepair step is seen. Analysis of the nanosecond simulation run also points out that the molecule has a considerable bending potential. The molecule can be seen to bend even at the 50th picosecond. The extent of the curvature increases till 363 ps (37%) and then seems to be reducing and is least around 670 ps (16%), later again the bending increases in the same direction as seen previously reaching maximum around 781 ps. Peak shortening of 37% and 40% was observed at around 370 ps and 781 ps respectively. It was seen that the phenomena of bending of the molecule is reoccurring. It should be noted that phosphodiester bond between the bases G7 and C8 is cleaved by the PvuII enzyme. Interesting changes are seen at and around the 7th base G which is the cleavage site of the PvuII enzyme. There is large rise (h) at around 495 ps at the G7/C8 basepair step and this is accompanied by a large negative value of roll (data not shown). The tilt value was also found to be significantly different than observed for a standard B-DNA. The increase in the rise and change in tilt occur slowly with the increase of the bending of the DNA. The analysis of windows plot of the global basepair axis parameters namely xdisp, ydisp, inclination and tip for each basepair calculated using CURVES indicates fluctuations around the normal values.

Watson-Crick Basepair Constrained Simulation of PvuII Substrate with Ions (1.2 ns)

Analysis of the nanosecond simulation run with ions, points out that the molecule has considerable bending potential. The Figure 1, which shows the top view of the snapshots indicates that the starting molecule, which was in B conformation, seems to be moving more towards the A-DNA conformation. The molecule can be seen to bend even at the 50th picosecond. The extent of the curvature increases till 409 ps (45 %) (Figure 2) and then seems to be reducing and is least around 741 ps (18 %).

Later, the bending again increases in the same direction as seen previously reaching maximum around 890 ps (58%) followed by a decrease at 1028 ps (17%) and again a maxima at 1116 ps (57%). It was seen that the phenomena of bending of the molecule is reoccurring and confirming that the simulation time of 1.2 nanosec-

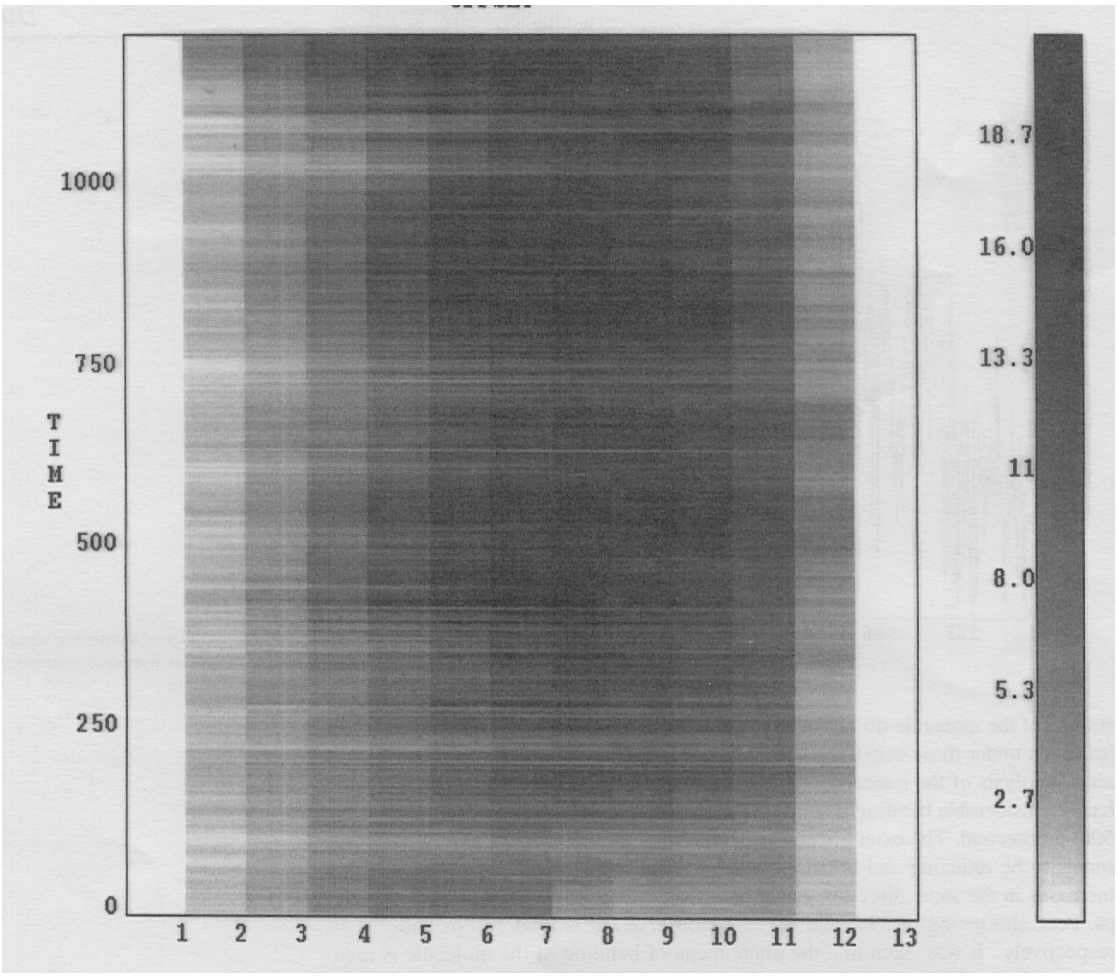


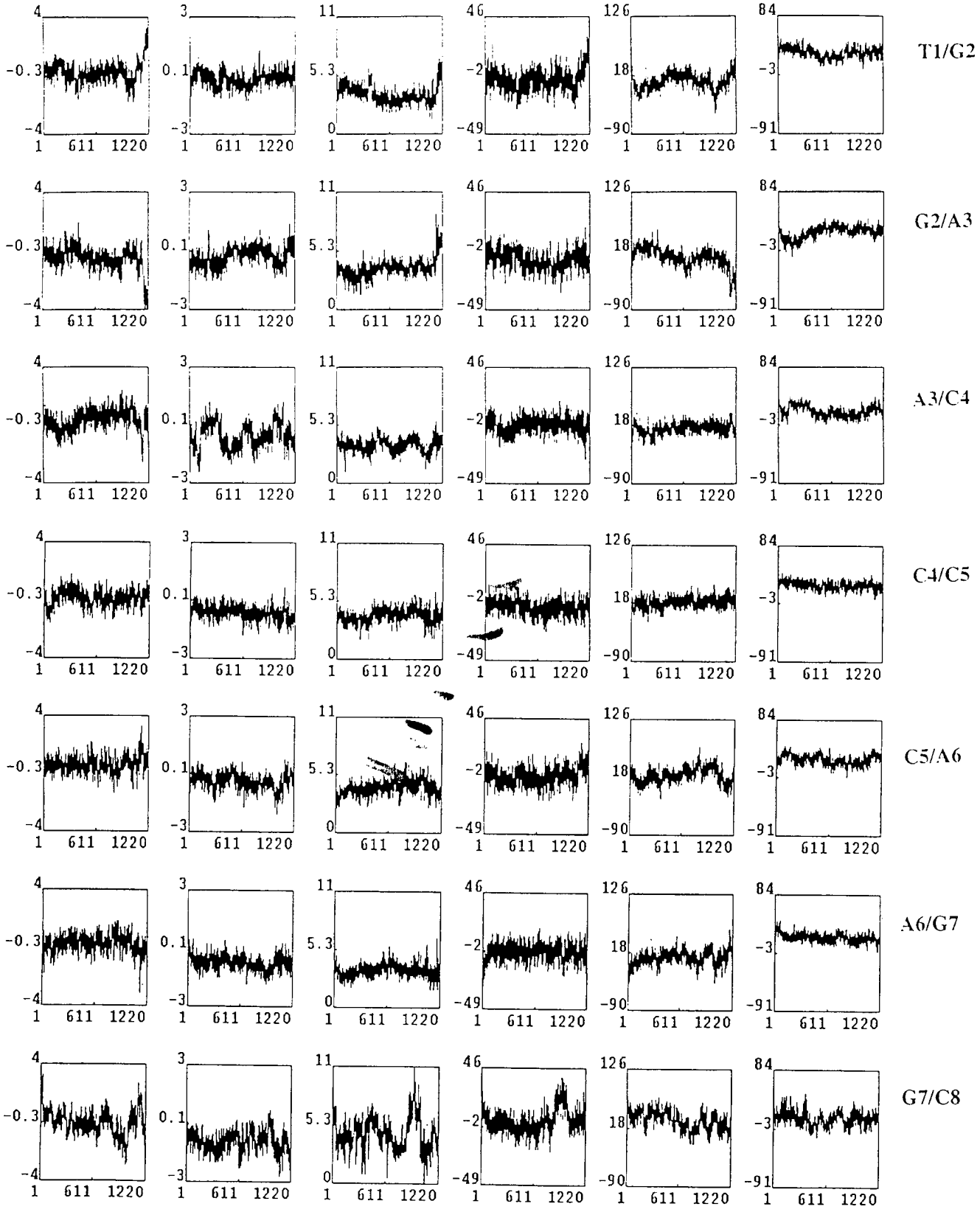
Figure 3: Plot of the offset values of each base versus time for the 1.2 ns simulation. The intensity of the offset is shown using a gray scale.

ond is sufficient to study the dynamic aspects of conformation of this molecule. The analysis of the offset values of each of the 13 nucleotides in the molecule considered, show that the offset value is maximum at the nucleotide 7 (Figure 3). At G7, it is observed that the phosphodiester bond is cleaved by the PvuII enzyme. Other helical parameters have different values compared to either the ideal A or B-DNA conformation. Figure 4 shows the plots of the shift, slide, rise, tilt, roll and twist parameters for the each base pair in the DNA molecule. The increase in the rise and change in tilt occur slowly with the increase of the bending of the DNA. In order to confirm that the structural changes seen in the simulation are not artifacts, simulations were also carried out under identical conditions with water and Na^+ ions, on the Dickerson's dodecamer $d(CGCGAATTCGCG)_2$ as mentioned in the methods section. Analysis of the simulation results clearly showed that this molecule, did not show any significant bending or distortion and essentially remained in a B-DNA conformational state. Thus, the results of studies on PvuII substrate, namely bending of double helix and partial conversion from B-DNA to A-DNA is a sequence effect.

The average global basepair axis parameters, namely the x-disp and inclination

were calculated using CURVES. The major geometric parameters that can distinguish A-DNA from B-DNA are the x-displacement and inclination. X-disp represents the location of the nucleic acid bases relative to the helix axis. Figure 5 shows the plot of the x-disp versus the simulation time. When the x-disp value is near zero,

Figure 4: Windows plot of the global inter base-pair parameters, namely shift, slide, rise, tilt, roll and twist, for the 1.2 ns simulation. Figure 4 continued on following page.



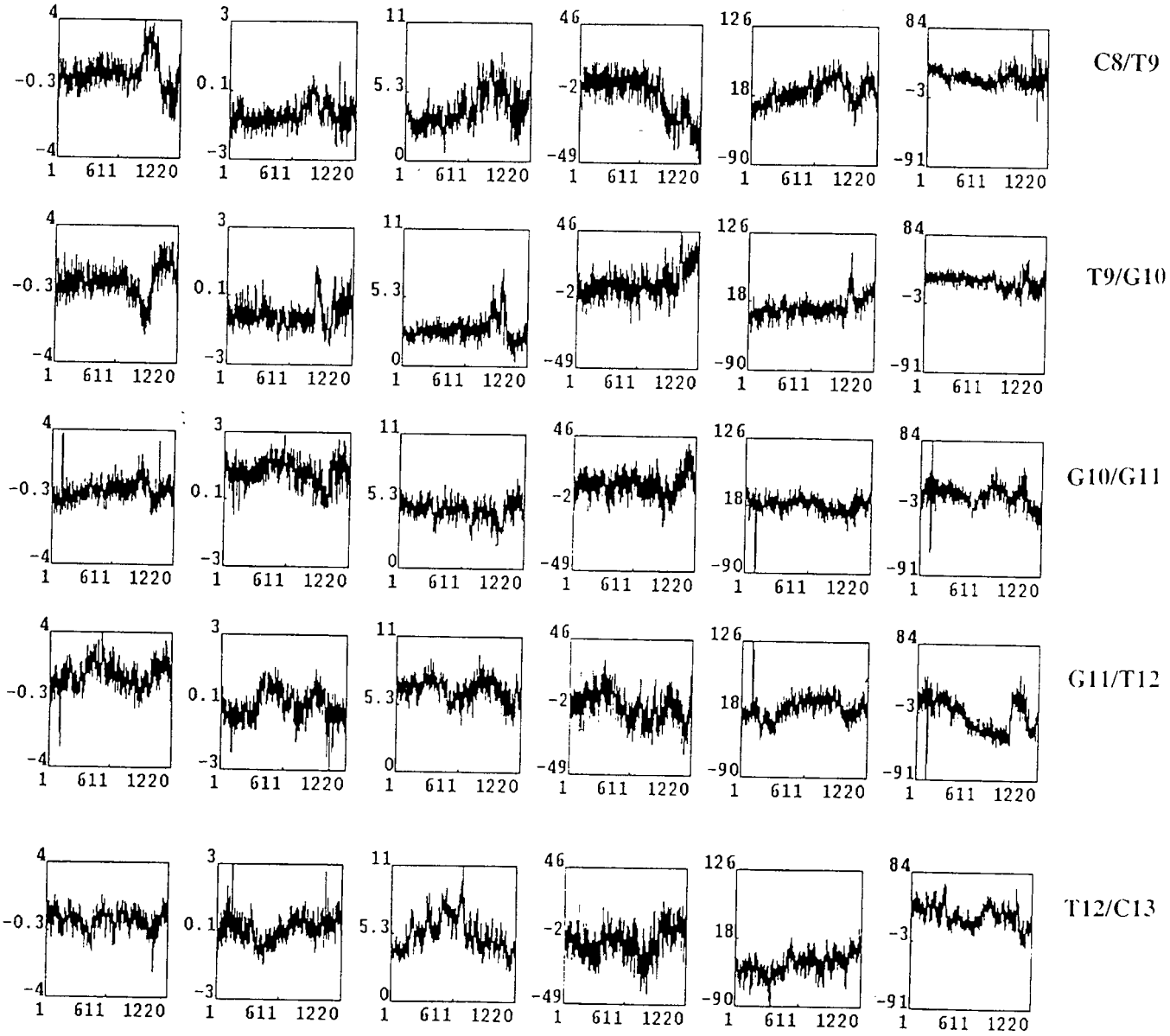


Figure 4 continued.

it corresponds to structures having no hole in the middle, while those having a hole have significantly negative x-disp values (-4 to -5Å). As can be seen from plot the molecule is seen to be in a conformation close to A-DNA conformation from 100-400 ps and later goes back to B-DNA at around 500 ps. Another significant transition is seen around 1000 ps where it is seen that the DNA conformation from 1000-1220 ps is closer to an ideal A-DNA conformation. Figure 6 shows the plot of inclination versus simulation time. The inclination for canonical A-DNA conformation is around 19.2 degrees and for canonical B-DNA conformation is around -6.2 degrees. As can be seen, Figure 6 further confirms that the molecular conformation alternates from B-DNA conformation towards A-DNA conformation and vice versa. The transition points observed from analysis of x-disp (see Figure 5) and inclination values (see Figure 6) match with each other. The molecule is seen to be close to A-DNA from 100-400 ps and close to B-DNA around 400-600 ps simulation time. Again as seen in case of the x-disp the molecule moves close to A-form around 1000-1220 ps. During these transitions the hole in the centre of the molecule is clearly seen (see Figure 1). The calculated x-disp and inclination values points out that the molecule is neither in the ideal A-form nor in the ideal B-form.

It is usually argued that only Particle Mesh Ewald method can take into considera-

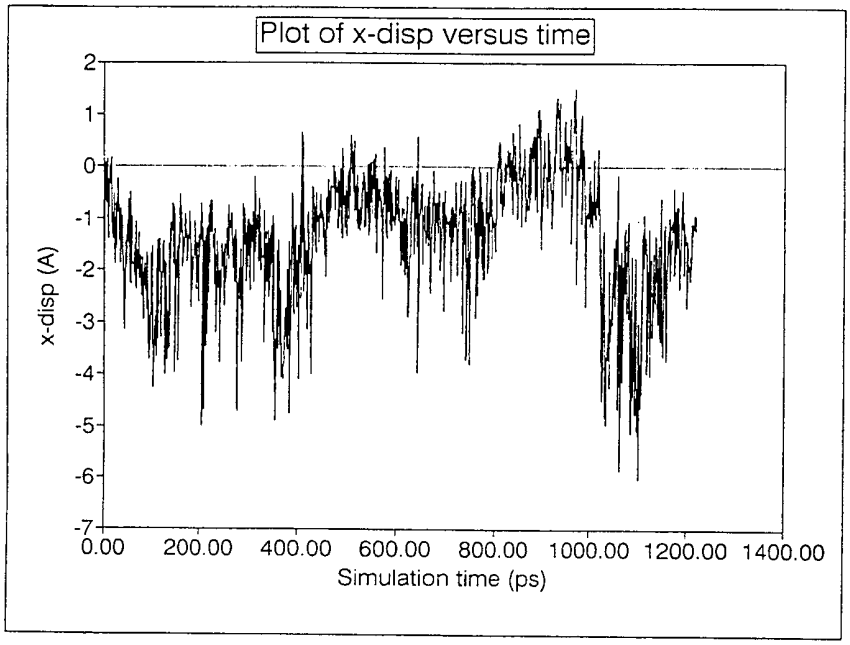


Figure 5: Plot of x-displacement versus simulation time, indicating that conformation alternates from close to B-DNA to close to A-DNA structure.

tion properly the electrostatic interactions. Even though this is true and a few artifacts do arise in MD simulation studies even after addition of ions, water and large cutoff values, the overall qualitative picture does not seem to be different. In fact as argued out by Crowley (34), the 12Å cutoff used in the non-PME run with the periodic boundary conditions and a function to smoothen the cutoff, gives similar electrostatic energy term as with the PME method and 9Å cutoff. Thus the results obtained in the present study where 12Å cutoff has been used should be reliable. Further, the DNA double helix was found to be in the bent conformation in all the runs which includes with and without constraints and in presence of ions and water. Morever the bending is maximum at the G7:C7′ basepair , which is the position at which the DNA is cleaved by the PvuII enzyme. Such large bending as mentioned above did not occur for the Dickerson's sequence d(CGCGAATTCGCG)$_2$ suggesting utility of these studies.

Comparison of Crystal Data and MD Simulation Results

The crystal structure of the complex of PvuII enzyme with cognate DNA has point-

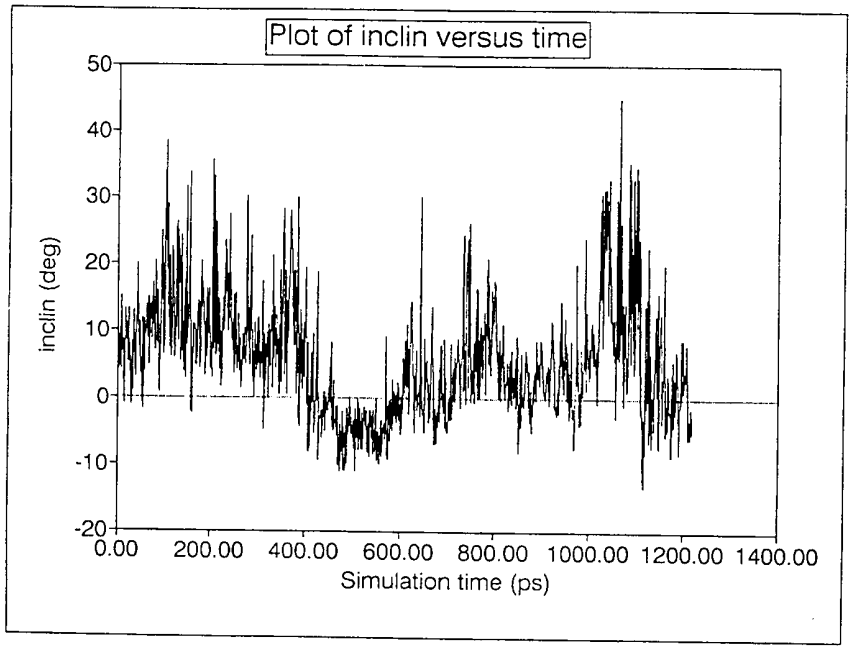


Figure 6: Plot of inclination versus simulation time, indicating that conformation alternates from close to B-DNA to close to A-DNA structure.

ed out that the DNA molecule has a small bend (19). Calculation of helical parameters using the CURVES program, for the DNA from the crystal structure suggest that the DNA has neither a standard B-DNA nor an A-DNA conformation. It has a low average twist value of 29° similar to A-DNA but a high average rise value of 3.7Å, higher than even that for B-DNA conformation. It should be noted that the rise at G7/C8 in the complex crystal structure is 4.13Å is higher than the normal B-DNA. During the course of our simulations, we too have noticed, higher value of rise at G7/C8 basepair step. It is known that phosphate-phosphate interactions play an important role in the bending of the DNA, but that does not seem to be the only factor. As can be seen from the Figure 1, though the transformation from B ⇌ A occurs, it is not reaching to ideal A or B-DNA conformation. The x-disp plot (Figure 5) and the inclination plot (Figure 6) clearly point out that the conformation of the double helix is distorted. The bending of the double helix is also observed in the crystal structure similar to that seen in the simulation studies. In fact, in our earlier studies on promoters, we have pointed out the ability of the DNA to bend and the flexibility of the molecule depends on the sequence (8). Thus these MD studies point out that the cognate DNA molecule is flexible around 5′-TGACCAGCTGGTC-3′ and because of this flexibility the DNA molecule bends and this bent conformation which is neither B-form nor A-form is recognized by the PvuII enzyme as discussed below. The snapshot observed in the crystal structure also points out that, the conformation of DNA bound to PvuII is neither an ideal B-form nor A-form and is also bent indicating the flexibility of cognate DNA. It must be noted that at the ionic concentration, as well as the pH at which the complex is crystallized, the substrate cleavage is inhibited and is not attacked by the enzyme (35). This clearly points out that the observed conformation of the DNA in the complex is not identical to the *in vivo* conformation of the DNA cleaved by the PvuII enzyme.

Model for PvuII Recognition of DNA and Cleavage

The crystal structure suggests that the active site of PvuII consists of Lys70, Glu68, Asp58 and Glu55 (19). The Lys70 is seen to interact with the phosphate between G7 and C8. The residues His84 and Asp34 also come close to the bases G7 and C8 and interact with them. The acidic residues Asp58 and Glu68 are well positioned to coordinate a Mg^{2+} at the active site with the reactive phosphate providing an additional ligand. The Mg^{2+} is thought to promote Sn2 nucleophilic attack at the phosphorus by chelating the phosphoryl oxygens and favouring transition of the phosphate from tetrahedral to trigonal bipyramidal geometry in the transition state (19). Later the Lys70 could act as a general base to deprotonate the attacking water molecule. Thus it has been proposed that scission may occur through a general base mechanism.

On the basis of crystal structure of the complex of PvuII and cognate DNA, as well as the present MD simulation studies we have tried to explain the steps involved in recognition and cleavage of the phosphodiester bond at G7/C8. In all our MD simulation studies we find that specific distortions occurs at the G7 and C8 bases of the recognition sequence. The bulky side chain of His will need more space to come in contact with the DNA. During the simulations the maximum bending was obtained at G7 and C8, which could actually favour the interaction of His84 and Asp34 with the bases. Our molecular dynamics studies throw more light on how the local structural deformations in the recognition sequence may lead to an active site geometry that will favour the interaction between the different side chains in the proteins and the bases in the DNA. We are aware that the DNA cleaved by the PvuII is usually much larger than the one considered in this study and thus the actual curvature of this region will be some what less than mentioned above, which may also reduce the rise value. Other changes from standard B-helical conformation, may also be small.

Recent experiments as well as model building studies clearly point out that the DNA double helix is highly stretchable and extendable (36,37). Our studies reported here for 1.2 nanosecond MD simulations point out that DNA double helix can

bend considerably. The distortions in the helical conformation are local for fairly long duration of simulation. This is in agreement with the now accepted fact that DNA molecules are highly flexible and the flexibility is dependent on the sequence of the DNA (38). Realistic quantitative values of the flexibility or bending potential can be determined once the problem of treatment of counterions in DNA simulation is solved. MD simulations on large DNA using parallel computers seems to be the best solution for these compute intensive studies for the time being. As the network speeds are reaching to gigabits per second, computing in a distributed environment will emerge as the natural solution to carry out MD studies on the large DNA molecules which will give insight into the genomic data.

Acknowledgements

The authors thank Prof.R.Lavery, IBPC, France for giving permission to use the CURVES program developed by him. The authors would like to place on record their sincere thanks to referee-II who has not only critically evaluated the paper, but also made positive suggestions to improve the quality of the paper.

References and Footnotes

1. Schneider, R. and Sander, C., *Nucl. Acids. Res. 24*, 201-205 (1996).
2. Vandervies, S.M., *Trends Biochem. Sci. 20*, 14-15 (1995).
3. Lipman, D.J. and Pearson, W.R., *85*, 2444-8 (1988).
4. McConnel, K.J., Nirmala, R., Young, M.A., Ravishanker, G. and Beveridge, D.L., *J. Am. Chem. Soc. 116*, 4461-4462 (1994).
5. Cheatham III, T.E. and Kollman, P.A., *J. Mol. Biol. 259*, 434-444 (1996).
6. Yang, L. and Pettitt, B.M., *J. Phys. Chem.100*, 2564-2566 (1996).
7. Flatters, D., Young, M., Beveridge, D.L. and Lavery, R. J. Biomol. Struc. Dyn. 14, 757-765 (1997).
8. Kolaskar, A.S. and Joshi, R.R., *Res. Commun. Biochem. Cell. Mol. Biol.* (1997) (in press).
9. Norberto de Souza, O and Ornstein, R.L., *Biophysical Journal 72*, 2395-2397 (1997).
10. Cieplak, P., Cheatham III, T.E. and Kollman, P.A., *J. Am. Chem. Soc. 119*, 6722-6730 (1997).
11. Norberto de Souza, O. and Ornstein, R.L., *J. Biomol. Struct. Dyn. 14*, 607-611 (1997).
12. Feig, M. and Pettitt, B.M., *J. Phys. Chem. 101*, 7361-7363 (1997).
13. Cheatham III, T.E. and Kollman, P.A., *Structure 5*, 1297-1311 (1997).
14. Mackerell Jr., A.D., *J. Phys. Chem. 101*, 646-650 (1997).
15. Duan, Y., Wilkosz, P., Crowley, M. and Rosenberg, J.M., *272*, 553-572 (1997).
16. Cheatham III, T.E. and Kollman, P.A., *J. Am. Chem. Soc. 119*, 4805-4825 (1997).
17. Miller, J.L., and Kollman, P.A., *Biophys. J. 73*, 2702-2710 (1997).
18. Young, M.A., Ravishanker, G. and Beveridge, D.L., *Biophys J. 73*, 2313-2336 (1997).
19. Cheng, X., Balendiran, K., Schildkraut, I. and Anderson, J.E., *EMBO J. 13*, 3927-3935 (1994).
20. Newman, M., Strzelecka, T. Dorner, L.F., Schildkraut, I., Aggarwal, A.K., *Science 269*, 656-663 (1995).
21. McClarin, J.A., Fredrick, C.A. Wang, B.C., Greene, P., Boyer, H.W., Grable, I. and Rosenberg, J.M., *Science 234*, 1526-1541 (1986).
22. Winkler, F.K, Banner, D.W., Oefner, D., Tsernoglou, D., Brown, R.S., Heathman, S.P., Bryan, R.K., Martin, P.D., Petratos, K. and Wilson, K.S., *EMBO J 12*, 1781-1795 (1993).
23. Aggarwal, A.K., *Curr. Opinion Struct. Biol. 5*, 11-19 (1995).
24. Van Gunsteren, W.F. and Berendsen, H.J.C., *Angew. Chem. Int. Ed. Engl. 29*, 992-1023 (1990).
25. Bruccoleri, R.E. and Karplus, M., *Biopolymers 29*, 1847-62 (1990).
26. Bishop, T.C. and Schulten, K., *Proteins: Struct, Func & Genetics 24*, 115-133 (1996).
27. Jayaraman, V., Rodgers, K.R., Mukherji, I. and Spiro, T.G., *Science 269*, 1843-8 (1995).
28. Vincent, J.J. and Merz, K.M., *J. Comp. Chem. 16*, 1420-1427 (1995).
29. Swanson, E. and Lybrand, T.P., *J. Comp. Chem. 16*, 1131-1140 (1995).
30. Hunter, C.A., *J. Mol. Biol. 230*, 1025-1054 (1993).
31. Lavery, R. and Sklenar, H., *J. Biomol. Struct. Dyn. 6*, 63-91 (1988).
32. Lavery, R. and Sklenar, H., *J. Biomol. Struct. Dyn. 6*, 655-667 (1989).
33. Lavery, R., *Adv. Comp. Biol. 1*, 69-145 (1994).
34. Crowley, M., Personal Communication.
35. Balendiran, K., Bonventre, J., Knott, R., Jack, W., Benner, J., Schildkraut, I. and Anderson, J.E., *Proteins: Structure, Function Genet., 19*, 77-79 (1994).
36. Smith, S.B., Cui, Y. and Bustamante, C., *Science 271*, 795-798 (1996).
37. Cluzel, P., Lebrun, A., Heller, C., Lavery, R., Viovy, J.L., Caron, F., *Science 271*, 792-794 (1995).
38. Hagerman, P.J., *Annu. Rev. Biophys. Biophys. Chem. 17*, 265-86 (1988).

Date Received: October 13, 1997

Communicated by the Editor Rick L. Ornstein