Nucleic acids: theory and computer simulation, Y2K

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Molecular dynamics simulations on DNA and RNA that include solvent are now being performed under realistic environmental conditions of water activity and salt. Improvements to force-fields and treatments of long-range interactions have significantly increased the reliability of simulations. New studies of sequence effects, axis bending, solvation and conformational transitions have appeared.

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Abbreviations

FDPB	finite difference Poisson Boltzmann
GB	generalized Born
MD	molecular dynamics
NDB	Nucleic Acid Database
NOESY	nuclear Overhauser enhancement spectroscopy
PB	Poisson Boltzmann
PME	particle mesh Ewald
QM	quantum mechanics
rmsd	root mean square deviation
SA	solvent accessibility

Introduction

The biological functions of nucleic acids — information storage, replication and transcription, as well as novel catalytic events — are ultimately linked to the chemical elements of the base pair sequence and the biophysical elements of energetics, structure and molecular motion. X-ray diffraction, NMR spectroscopy, fluorescence depolarization, gel electrophoresis, microcalorimetry and other techniques are used in experiments to probe the biophysics of nucleic acids. The next step, interpreting the experiments, requires the formulation of molecular models consistent with observed results. The first and most instinctive source of molecular models is chemical intuition; however, competing forces determine almost all phenomena and one usually cannot reliably intuit the resultant of opposing terms as the magnitudes are not well known. Computational modeling via molecular simulation, in which some quantification of the relative contributions of various terms is provided using methods derived from theoretical physical chemistry [1], has thus come to assume an increasingly important role. The most rigorous method for modeling macromolecules in solution with an all-atom representation of solute and solvent molecules is molecular dynamics (MD) computer simulation. In principle, MD provides a complete microscopic description of the atomic structure and motions of macromolecules and solvent. In practice, MD modeling is a work in progress, with results dependent upon judicious approximations of the underlying empirical force-field, simulation protocols and system preparation. Run lengths of even nanoseconds, a considerable recent technical accomplishment, are still not long enough to access many nucleic acid phenomena of interest; however, much can and has been done. In this review, we describe the recent literature on all-atom MD applied to uncomplexed DNA and RNA systems, with an emphasis on studies published during 1998–2000, that serve to document simulation protocols and provide validations of the current level of accuracy of the simulations.

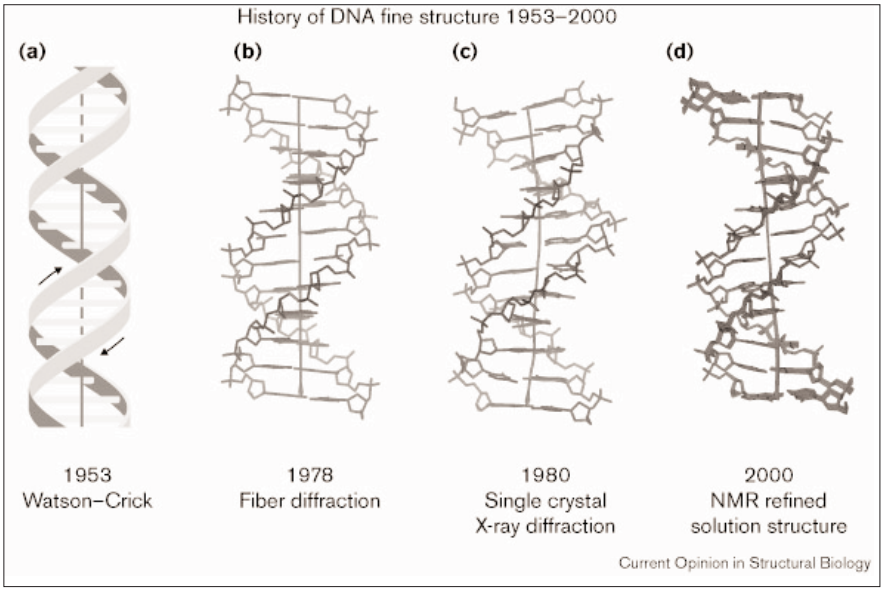
This review builds, in particular, on the most recent *Current Opinion in Structural Biology* review on this subject, that by Auffinger and Westhof [2], as well as a number of others [3–6]. A multi-author treatise on nucleic acid structure edited by Neidle [7•] contains a number of valuable reviews, particularly one on MD by Miller *et al.* [8] and a current overview by Lavery and Zakrzewska [9••] on the defining parameters of nucleic acid structure, in which conflicted issues surrounding the definition of global and local axis frames are resolved [10•]. Methods extending MD to longer time frames are of considerable importance to the field, but are beyond the scope of this review (for information on this topic, see [11–13]). Schlick *et al.* [14] have recently reviewed related algorithmic challenges.

Methodology and validation studies

Nucleic acids are an especially challenging problem to treat with MD simulation. DNA and RNA sequences are polyelectrolytes at neutral and physiological pH, with a multiplicity of backbone phosphate groups fully ionized. Thus, electrostatic charge–charge interactions can be expected to be a major component of the forces determining the structures and driving the MD modeling. Following Coulomb's law, charge–charge interactions fall off slowly with distance and are most susceptible to error when numerical truncation is applied. In addition, nucleic acids have a generally larger surface to volume ratio than proteins, making solvation effects generally more significant and, in some instances, a dominant effect. Thus, an accurate theoretical treatment of both long-range electrostatic interactions and solvent effects is necessary for proper MD modeling of nucleic acids.

Structures proposed, from 1953 to the present, for B-form DNA, as derived from experimental data, are shown in Figure 1. A number of characterization studies of MD modeling of nucleic acids have focused on a DNA oligonucleotide duplex with the sequence d(CGCGAATTCGCG). Dickerson and co-workers [15] reported a crystal structure for this sequence in 1980, the first single crystal structure of a Watson–Crick double helix (Figure 1c). This sequence contains the recognition site for the *Eco*RI restriction

Figure 1



Fifty years of DNA structures. **(a)** Watson and Crick's proposed structure, 1953 [159]. **(b)** Structure of canonical B-form DNA obtained using fiber diffraction in 1976 [160]. **(c)** X-ray crystal structure of the *Eco*RI DNA dodecamer determined in 1980 [15]. **(d)** Structure of the *Eco*RI DNA dodecamer obtained from NMR refinement, 2000 (PH Bolton *et al*., unpublished data).

endonuclease and is henceforth referred to in this review as the '*Eco*RI dodecamer'. Structures of many other DNA and RNA sequences have now been reported from crystallography [16] and serve as a basis for comparison with results from MD modeling. An ongoing issue is the extent to which these structures are influenced by crystal packing forces [17,18] and, thus, may not be good indicators of the structure of DNA or RNA in solution. Also, nucleic acid structural indices from experiment tend to be broadly distributed [19,20] and issues not only of static, but also of dynamical structure must be taken into consideration.

Comprehensive reviews of the literature on MD modeling of DNA and RNA pre-1995 are available [21]. This earlier work is now of primary interest as examples of the difficulties in making a proper MD model of nucleic acids using 'first generation' force-fields, distance-dependent dielectric screening models of solvent and the truncation of long-range interactions. The development of the particle mesh Ewald (PME) method [22] for long-range forces rendered the classic Ewald treatment of periodic boundary conditions tractable for general purpose MD. In 1995 came the publication of three new complete all-atom force-fields [23–25], including a full set of parameters for nucleic acids, designed particularly for simulations including explicit consideration of solvent — termed 'second generation' force-fields [23]. Here, we provide an update on developments in each of these areas and on progress towards a simplified treatment of solvent on the basis of continuum electrostatics.

Particle mesh Ewald treatment of long-range interactions

Advances in the Ewald and PME methodology, including consideration of the improved performance of the particle–particle particle mesh (PPPM or P3M), have been reviewed by Sagui and Darden [26]. An independent confirmation of the performance of PME versus P3M is provided by Deserno and Holm [27]. At this point, most MD simulations utilize the PME or force-shifted method for treating long-range interactions, rather than using truncated potentials. The latter is known to produce artifacts at the cut-offs [28], although it has also been demonstrated that feathering the potentials can minimize the problem [25,29,30•,31,32••]. The Ewald model is strictly applicable to treatments of crystalline solids, but is used to model solutions by making the simulation cell large and mostly solvent, net neutral and positioning the solute at the center.

The first MD calculations on DNA, as well as proteins, using PME showed significantly improved dynamical stability; however, solution models developed on the basis of PME could be subject to spurious long-range correlations caused by the quasi-crystalline boundary conditions. Darden *et al.* [33] studied possible artifacts in the solvation free energy of small ions using PME compared with using cluster models with spherical cut-offs. Significant differences were found and attributed to the effect of the vacuum/solvent interface in cluster models. Hunenberger and McCammon [34,35••] described potential artifacts in conformational preferences using PME if the system is not neutral or exhibits large effective charge separation. Examples of potential PME problems concerning the solvation free energy of a spherical ion and the potential of mean force between two spherical ions were provided, both examples having potential implications for MD on DNA and RNA using PME. Bogusz *et al.* [36•] have described a method to remove artifacts of energy and pressure caused by the use of the PME method for systems with a net charge using net charge correction term. The proposed correction term is general and is applicable to a

number of cell sizes, shapes and content. The 'flying ice cube' problem [37•] is an artifact of periodic velocity rescaling and can be treated either by periodically removing the translational and rotation motion with respect to the center of mass or by using velocity reassignments.

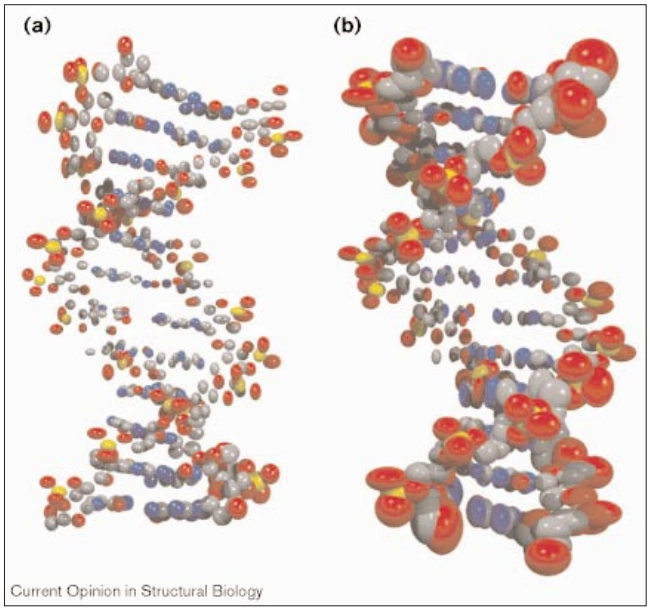
Energy functions and force-fields

Several independent studies documented that the AMBER 5.0 suite of programs [38], the force-field derived by Cornell *et al.* [23] and PME [22] produce stable MD trajectories for DNA on the nanosecond time frame and provide a reasonable description of the B-form double helix in the crystalline state and in solution. The first applications of the Cornell *et al.* force-field [23] to DNA and RNA sequences were reported by Kollman and coworkers [39]; this and other subsequent calculations were reviewed previously [40–42]. Cheatham *et al.* [43•], concerned about the distributions of sugar pucker and helical repeat, subsequently explored modifications and improvements to the Cornell *et al.* force-field. The new modifications better reproduce B-DNA crystal distributions. Using the new force-field, however, simulations of both A-DNA in ethanol and A-DNA with bound hexaammine cobalt(III) ions show a gradual transition away from A-DNA, contrary to the behavior observed using the Cornell *et al.* force-field.

The validation of results from MD simulations is an essential step in the application of theory to understanding biological problems. A recent paper by van Gunsteren and Mark [44••] surveys issues concerning the validation of MD simulations. For DNA simulations, issues of validation are especially acute because of the particular sensitivity of highly charged systems to parameters and protocols. Using AMBER, Young *et al.* [45] reported an MD model of the *Eco*RI dodecamer in solution, including counterions and water, that was recently extended to 14 ns [46] with no essential changes observed in the dynamical structure of the DNA (Figure 2b). Detailed comparisons of the distributions of key helicoidals with the corresponding distributions found for all current examples of B-form DNA crystal structures confirmed that this MD model describes B-form DNA reasonably well for this sequence. Duan *et al.* [47] showed, for the same sequence, that, in 1 ns of MD, the protein-bound conformation of the DNA moved to within 1 Å rmsd of the crystal structure of the unbound form. Sprous *et al.* [48] have characterized an AMBER MD model for an A-form structure of the *Eco*RI dodecamer, obtained from a simulation carried out in 85% ethanol/water mixed solvent.

The next stage of validation involves comparing MD results on a crystalline unit cell with corresponding crystal structure data, and MD models of solution structures with data taken directly for the solution state. Following the report by York *et al.* [49] on the use of AMBER 3.0 and the force-field derived by Weiner *et al.* [50], Young [51] carried out 3 ns of MD on an *Eco*RI crystalline unit

Figure 2



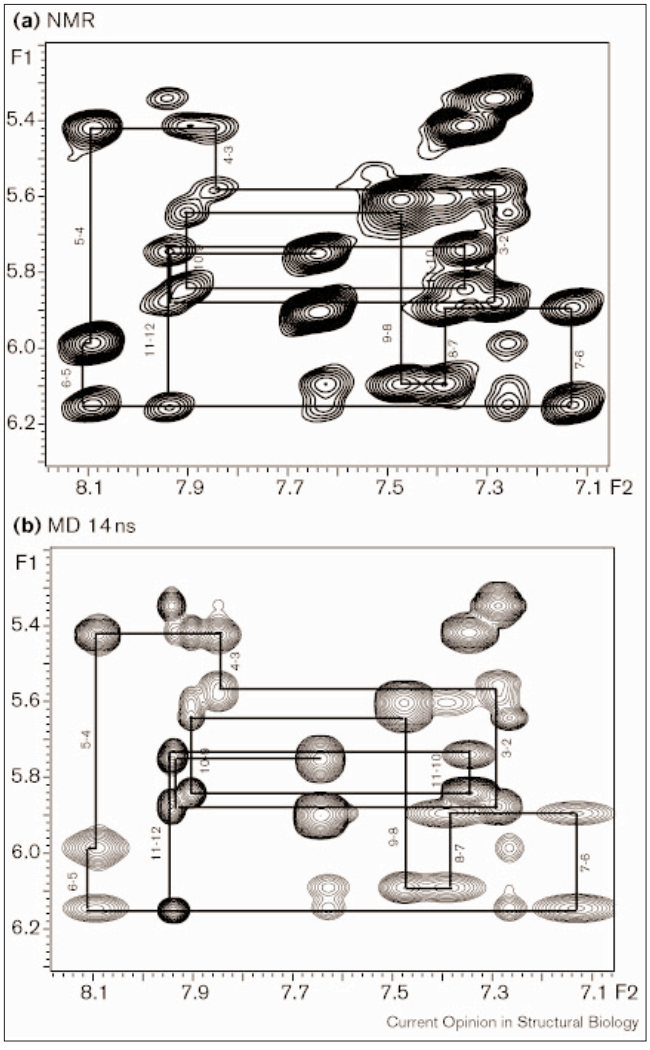
Computational models of the dynamical structure of the *Eco*RI DNA dodecamer obtained from MD simulations, with atomic motions presented as thermal ellipsoids. **(a)** Prediction of the crystal structure (KJ McConnell *et al.*, unpublished data). **(b)** Prediction of the solution structure [45].

cell comprising four dodecamers, neutralizing sodium counterions and water using AMBER and the Cornell *et al.* force-field [23]. The results show approximately 1 Å rmsd between the MD time-averaged structure and the crystal structure, and confirm this MD model of the crystal to be an improvement. Comparing the MD results on a B-DNA sequence in the crystal with those for the same sequence in solution provides a basis for a purely theoretical study of crystal packing effects. The results (c.f. Figure 2a,b) show that the dynamic range of motions for the MD models of DNA is significantly less in the crystal than in solution, but reveal good agreement of the average structures (2.1 Å rmsd) for the two phases. Differences between the crystal and solution model MD structures show packing effects at the 3′ end of the sequence to be prominent. A similar study by Bevan *et al.* [52••] of the $d(CCAACGTTGG)_2$ decamer also shows differences between solution and crystalline MD models.

NMR spectroscopy is the primary source of structures for DNA in solution and a number of studies of the *Eco*RI dodecamer have been reported. In particular, Lane *et al.* [53] have shown that the NMR results are quite consistent with a good B-form of DNA in solution for the *Eco*RI sequence. McConnell *et al.* (KJ McConnell *et al.*, unpublished data) address the question of how well the AMBER MD model described above for DNA in solution compares with NMR results. To pursue this, new 2D NOESY (nuclear Overhauser enhancement spectroscopy) spectra were obtained using NMR and the 14 ns MD trajectory of

Young *et al.* [46] was used as the basis for the calculation of a corresponding theoretical spectrum. A comparison of the observed and calculated results is shown in Figure 3, revealing close accord and supporting the accuracy of the MD solution structure. This study has been extended to deal with some current problematic issues concerning NMR structure refinement of DNA in solution; the NMR structure shown in Figure 1d was obtained using our improved procedure. Recent studies by Konerding *et al.* [54] describe the use of the Cornell *et al.* force-field with PME in restrained MD based on NMR-derived distance and torsional restraints for two previously determined DNA structures [55] and compare the resulting conformations with free MD simulations. They found agreement for some structural aspects of the NMR-derived structure and the free MD simulation, but comment on significant differences in helical twist.

Figure 3



(a) Observed versus **(b)** calculated 2D NOESY NMR spectra for the aromatic and sugar region of the *Eco*RI dodecamer (PH Bolton *et al.*, unpublished data).

The 1995 version of the CHARMM force-field (CHARMM *22*) was described in detail by Mackerell *et al.* [24] and applied to several nucleic acid demonstration cases. Subsequent studies by Feig and Pettitt [56,57•] and, independently, by Langley [30•] showed, for sequences expected to be B-form DNA, that this version of CHARMM resulted in a tendency for the model DNA to assume structures intermediate between A- and B-form. Langley [30•] subsequently explored this issue and suggested a modified set of CHARMM-type parameters coupled with AMBER charges, known as the Bristol-Myers-Squibb (BMS) force-field. Torsional angle values, initially derived from quantum mechanics (QM) calculations on model compounds, were subjected to iterative refinement against values obtained from A- and B-form DNA crystal structures. Validation was based on 41 ns of MD on 12 different A-, B- and Z-DNA sequences under various environmental conditions.

Refinement of the CHARMM nucleic acid parameters was subsequently undertaken. Yin and Mackerell [58•] used *ab initio* QM and empirical data for rare gas atoms and model compounds to optimize CHARMM Lennard–Jones parameters and tested them on free energies of solvation. QM was applied to the systematic refinement of parameters affecting the conformational properties of the deoxyribose and ribose moieties [59] and the phosphodiester backbone and glycosyl linkages [60•]. It was found that the coupling of nucleotide bases and furanose accounts for the sugar pucker conformations of the purines versus the pyrimidines in the Nucleic Acid Database (NDB). The energy barriers for the backbone conformational parameters are all found to be larger than the thermal kinetic energy (kT) at 298K and, thus, may have significant effects on DNA conformation. A study of the intrinsic conformational properties of deoxyribonucleosides provides leading evidence for a special role for cytosine in the equilibrium between the A-, B- and Z-forms of DNA ([61•] and see also [43•]).

A full description of the new CHARMM *27* force-field has just appeared [62••], including details of the dynamical properties of A-, B-, and Z-DNA sequences under a variety of conditions [32••]. The results show the new force-field to provide an improved account of the distributions of conformational properties as observed in crystals, as well as selected dynamical properties from solution state experiments. Some limitations in reproducing dynamic properties of Z-form DNA and uncertainties in describing the B to A form transition of DNA in mixed solvent conditions are noted. Applications of CHARMM *27* have been reported for DNA located between two phospholipids [63], as well as for free energy component analyses on nucleic acid prototype systems [64].

The GROMOS simulation program has been recently discussed by van Gunsteren and colleagues [65] and several recent papers have reported results for MD on DNA using the GROMOS force-field. Bonvin *et al.* [66] report

GROMOS96 MD on DNA at 277K, describing the hydration of DNA and comparing the results to NMR residence times and crystal structures. Tapia *et al.* [67] applied GROMOS to a DNA decamer that remained stable in the B-form, but required a modification of certain Lennard–Jones terms to make the aliphatic carbons more hydrophobic and to constrain the counterions around the protein–DNA complex to keep them from collapsing on the DNA. A fuller discussion of the parameterization of the Lennard–Jones parameters for aliphatic united-atom carbons has been provided [68]. At this point, the GROMOS force-field has not received as extensive critical validation for studies on nucleic acid systems as AMBER and CHARMM, but work in this direction is in progress.

Levitt's ENCAD potential has not been extensively applied to nucleic acids, but preliminary results are promising [25]. Note that this approach uses a cut-off based force-shifted methodology, in contrast to PME. The parameterization of a new flexible three-centered water (F3C) model by Levitt *et al.* [69], developed for the ENCAD macromolecular potential [25], shows good results over a wide range of temperatures and pressures.

An assessment of the performance of various empirical potentials compared with results from *ab initio* QM calculations has been provided by Hobza and Sponer [70•], who note the possible importance of polarization effects on nucleic acid base pairs. Cybulski and co-workers [71] report systematic studies of electron correlation effects in nucleic acid bases.

Generalized Born/solvent accessibility solvation methods

Ironically, with the advent of second generation force-fields designed for explicit inclusion of solvent comes a very promising new development in implicit solvent models. The 'generalized Born' (GB) method results in rapid estimation of the electrostatic free energy of hydration. Originally suggested by Still and co-workers [72], the GB method can be combined with well-established estimates of the nonelectrostatic contributions to solvation that are derived from solvent accessibility (SA) calculations to provide a description of the total free energy of hydration. It can be augmented with a Debye–Huckel term to approximate the effects of added salt [73,74•]. A comparison of the results of the GB/SA method with experiment shows that, appropriately parameterized, GB/SA provides quite accurate estimates of hydration free energy [75•,76•]. An appraisal of the performance of the GB/SA model in estimating the solvation energies of small molecules and the pK_a shifts of dicarboxylic acids was carried out by Jayaram *et al.* [77]. The development of GB parameters compatible with the AMBER force-field was reported [75•] and a similar initiative is underway with respect to CHARMM [76•].

Two applications of this idea relevant to nucleic acid studies are currently being pursued: *post hoc* estimates of the hydration free energy of MD snapshots from a simulation [78•,79•]; and performing MD in a GB/SA model solvent [80,81]. The use of GB/SA as a post-MD calculation of the relative free energy difference of conformational states has been independently applied to the A to B transition of DNA by Srinivasan *et al.* [78•] and Jayaram *et al.* [79•] (for a discussion of results, see below). Srinivasan *et al.* [78•] examined the stability of A- and B-forms of DNA, RNA and phosphoramidate DNA helices by both finite difference Poisson Boltzmann (FDPB) and GB/SA methods. Further studies of the conformational stability comparing the sequence-dependent preferences of $dA_{10}{\cdot}dT_{10}$ and $dG_{10}{\cdot}dC_{10}$ show that the GC base pairs have a greater preference for A-form DNA than AT base pairs, as is seen in the van der Waals interaction energies [82]. Srinivasan *et al.* [74•] provide a detailed comparison of the difference between the FDPB and GB/SA methods for a peptide, three proteins and four nucleic acid structures. They show that both the GB/SA and FDPB methods reproduce the overall energies, but GB/SA can underestimate the contribution of buried residues, which is canceled out in the free energy cycle. They conclude that GB/SA can be a good substitution for the FDPB method, but add a strong note of caution for systems with extensive buried dipoles and charges. They also note larger differences between FDPB and GB/SA at low salt concentrations. A comprehensive review of continuum models of solvent for molecular modeling has been provided by Cramer and Truhlar [83]. Vlachy [84] discusses ion effects beyond the PB theory.

DNA oligonucleotides

Sequence effects

A notable sequence effect on DNA structure, biologically significant in nucleosome structure and in regulatory processes, is concerted axis bending in sequences with successive A-tracts in phase with a full turn of the B-form helix. At issue in the interpretation is whether axis bending occurs by deformation at ApA steps within A-tracts (the wedge model [85–87]), between A-tracts and flanking sequences (the junction model [88]) or at non-A-tract regions (the general sequence bending model [89,90]) (for reviews, see Sundaralingam and Sekharudu [91] and Olson and Zhurkin [92]). Six crystal structures of DNA oligonucleotides with A-tracts show little evidence of significant wedge-like deformations at ApA steps [93,94], a result apparently at odds with the wedge model. MD simulations have been performed for solution models of oligonucleotide sequences with A-tracts for which crystal structures are known, first with GROMOS [95] and subsequently with AMBER force-fields [96•]. These results, as well as MD simulations of decamers of all 10 base pair steps flanked by CGCG, show that ApA steps exhibit the smallest standard deviation in twist, roll and tilt, and support relatively rigid ApA steps, exhibiting little deformation from canonical B-form structural parameters.

A full explanation behind the observed geometry of ApA steps in an A-tract has not yet been unequivocally determined. A steric clash argument has been proffered,

suggested by the higher propeller twist of AT base pairs [97,98]. The possibility of a novel 'bifurcated' hydrogen bond at ApA steps, involving adenine N6 on one strand and thymine 04 on the other, has been proposed on the basis of crystal structure evidence [99] and has received considerable attention [94,100•,101]. MD simulations have been used to compare the structure and dynamics of DNA dodecamers [96•], comparing the sequences AAA and AIA (where I is the inosine mutant), in which there is structurally no opportunity for a bifurcated hydrogen bond to form. The results indicate that the inosine substitution does more to affect the dynamical structure of the oligonucleotides than might be expected from just eliminating a bifurcated hydrogen bond across the major groove and point to the importance of DNA flexibility, as much as its static structure, in determining macroscopic behavior. It was suggested that the foreshortened N6H–TO4 distance associated with the bifurcated hydrogen bond across the major groove could arise as a *de facto* consequence of characteristic high propeller twist in A-tracts, rather than being the driving force behind it. Earlier MD on A-tract oligonucleotides [102], as well as more recent calculations [96•,100•], indicates that structures with possible bifurcated hydrogen bonding comprise only a small fraction of the trajectory, whereas other features of A-tracts, such as minor groove narrowing, remain intact. The examination of AAA steps in 17 different oligonucleotide crystal structures reveals that only 13% of the possible examples show structures with interatomic N6H–TO4 distances of 2.8 Å or less [103]. However, NMR and resonance Raman studies support the occurrence of an interaction [101,104] and the issue reduces to whether free energy of stabilization is contributed or not. The net contribution from each instance would be expected to be small at best, but could, of course, be cumulative. A QM perspective is available from Sponer and Hobza [105], who stress the nonplanarity of the NH_2 group.

Pastor *et al.* [100•] have investigated this issue using the CHARMM *22* force-field in simulations of the TATA-box sequence [d(CTATAAAAGGGC)] and a similar sequence substituting the adenine with inosine. They also found that inosine substitution introduces more flexibility and independently conclude that the bifurcated hydrogen bond in the major groove does not contribute to the stability of the A-tract over the I-tract. Interestingly, the authors describe very different hydration patterns in the minor grooves of the two sequences, despite the identical chemical nature of the two sequences, and suggest that this accounts for the difference in the binding of the TATA-box-binding protein of some three orders of magnitude. Flatters *et al.* report TATA-box MD studies [106] and explore the effect of making a double mutation [107•]. They found that the wild-type sequence tends towards the A-form protein-bound conformation, whereas the mutant lies closer to B-form, and conclude that the MD model is able to predict sequence-dependent effects consistent with experiment. In the case of the double mutant, the DNA structure is not as close to the 'adapted' conformation as observed in the crystal structure of the bound form. Contrary results on the roll of YpR steps (where R represents a purine and Y a pyrimidine) compared with the MD simulations of Pastor *et al.* [108] were noted.

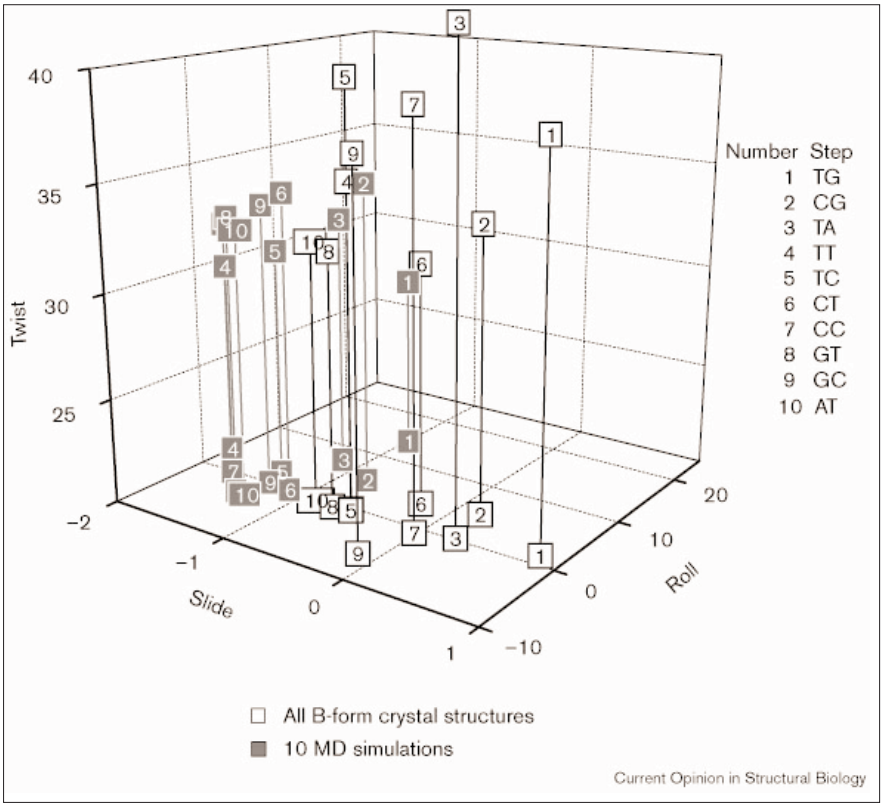
Bevan *et al.* [52••] report MD simulations on the DNA decamer $d(CCAACGTTGG)_2$ in crystalline and solution conditions. From this study, they found that the majority of helicoidal parameters from the crystal and solution MD are nearly identical, with the notable exception of the backbone torsion angles and helical twist. They also found that almost all of the base step twists center around 30° and suggest that sequence-dependent information in twist may be lost.

MD calculated sequence effects for the 10 unique base pair steps in DNA oligonucleotides are being used as a basis for a more detailed comparison of theoretical and observed values, and provide a higher resolution characterization of force-fields. Results from *27* B-DNA crystal structures [16] are shown as a function of the helicoidal parameters roll, twist and slide in Figure 4. The YR, RR(=YY) and RY steps over all B-form DNA crystal structures are found to fall into fairly distinct clusters. The corresponding results from MD simulations on DNA sequences, including water and counterions, using AMBER and the Cornell *et al.* force-field are shown as dark squares in Figure 4. Clearly, the results show a systematic displacement in slide and twist for the calculated values compared with the observed, indicating a previously unnoticed possible systematic force-field error for slide or (less likely) a packing effect from the crystals. The implications of this are not clear as yet, but slide makes a critical differentiation between A-form versus B-form DNA for helicoidal parameters derived with respect to a local base-pair centered frame of reference [9••,10•]. The results in Figure 4 confirm the AMBER MD model with parm94 to be undertwisted. A similar effect, though not as pronounced, is evident for CHARMM *27* [32••] and modified parm98 AMBER [43•] force-fields.

DNA hydration

A recent *Current Opinion in Structural Biology* review of DNA hydration was provided by Pettitt *et al.* [109•], who draw the useful distinction between 'solvation site' and 'distribution function' approaches. Nucleic acid crystal structure articles reporting ordered water positions have been analyzed in depth by Schneider and Berman [110,111], who delineate characteristic transferable features of the hydrophilic hydration sites of nucleotide bases and the 'cones of hydration' around anionic phosphates. Crystallographically ordered water positions are, of course, only a fraction of the total hydration and correspond to sharply peaked regions of the overall nucleic acid/water distribution functions. MD calculated distribution functions presented via computer graphics as hydration densities provide a more complete (albeit theoretical) view

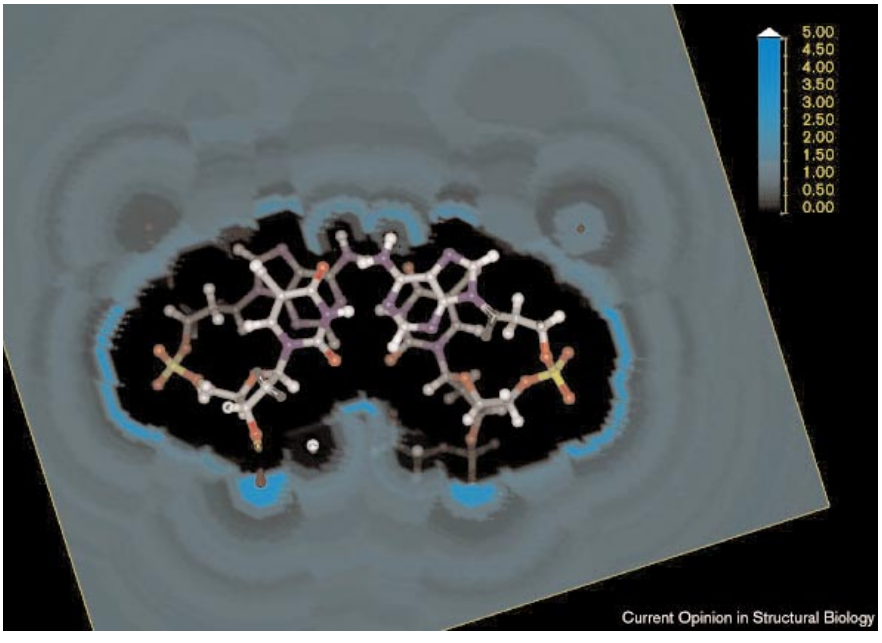
Figure 4



Distribution of average values for base pair roll, twist and slide for the 10 unique base pair steps in a DNA sequence, obtained from 23 B-form oligonucleotide crystal structures (white boxes) and from MD simulations using AMBER on 10 decamer oligonucleotides (dark boxes) (Y Lui, DL Beveridge, unpublished data).

of hydration (Figure 5) and can be decomposed using the proximity method [112] to arrive at useful interpretations, as illustrated and extended in a recent paper by Pettitt and co-workers [113]. A calculated solvent structure for the primary solvation shell of DNA sequences was compared with the location of ordered solvent positions in the corresponding crystal structure by Feig and Pettitt [114] with considerable success. Analysis of the results on the *Eco*RI

Figure 5



Calculated hydration density from MD simulations around the ApT step of the *Eco*RI DNA dodecamer. The number scale refers to the relative local water density.

dodecamer by Young *et al.* [45] permitted an estimate of the degree of order required for water molecules to be crystallographically ordered and indicated that ordered solvent positions in crystals are roughly twice as structured as in bulk water.

Bonvin *et al.* [66] studied the hydration patterns and structural properties of the *trp* operator DNA sequence on the basis of a 1.4 ns MD simulation using the GROMOS forcefield. The average MD structure was found to be in closer agreement to the bound form of the DNA than the unbound crystal form. Analysis of the mechanism of water exchange was carried out for the major and minor grooves. MD calculated and NMR determined water residence times and the percentage of hydrogen bonding are shown to be in good agreement. The sensitivity of DNA bases to hydration has recently been examined by Cubero *et al.* [115], who observed base pair opening and closing or 'breathing' events for a modified base sequence in a 14 ns MD simulation. The difference in observed dynamical properties between the modified and unmodified bases is a result of a difference in hydration and hydrogen-bonding interactions, whereas stacking appears to be similar in the two cases. Earlier studies by Mezei [116] have shown the differentiation of hydration in the grooves of DNA.

The local dielectric environment in MD simulations on DNA was analyzed by Jayaram and co-workers [46] on the basis of a variation of the Kirkwood–Grunwald theory. The computed dielectric profile near DNA increased rather rapidly with distance and displayed bulk behavior beyond 5 Å. Proximity analysis of the dielectric function revealed that the relative permittivities in the first shell of DNA obey the following trend: phosphate backbone > major groove > minor grove. Estimates of the local dielectric constants in the major groove are consistent with interpretations based on fluorescence measurements [117], indicating that MD models of solvent around DNA are providing a reasonably accurate account of the local solution environment of a complicated polyelectrolyte. The calculated dielectric profile was fit to a sigmoidal function, which could be used to estimate the strength of charge–charge interactions around DNA.

At this point, crystal structures are being obtained at a much higher level of resolution (approximately 1 Å versus 2.2 Å for the *Eco*RI dodecamer in 1980). As a consequence, much more of the electron density of the ordered solvent is 'seen' and more elaborate networks of water molecules (and possibly ions, see below) are derived from X-ray structure refinement. Water as an associated liquid is readily expected to form more ordered structures than simple liquids and it is truly impressive to be seeing more of this in crystallography. However, the link between water dimers, tridents, clusters, spines, filaments and other 2D and 3D networks observed and the corresponding free energies of stabilization are not firmly established. Thus, the structural biology and possible functional implications of hydration structures around nucleic acids remains to be fully clarified and is an important agenda item for the field (*vide infra* for progress on this issue in DNA conformational stability). Grand canonical Monte Carlo simulations of the dCpG–proflavin complex by Resat and Mezei [118] show that empirical potentials can accurately reproduce the water structure as observed in crystals. So far, DNA sequences have been treated far more so than RNA sequences; a useful review of salient issues related specifically to RNA hydration is provided by Egli *et al.* [119].

Ions and ion atmosphere

Sharp and Honig [120] and Jayaram and Beveridge [121] have provided recent reviews of this area. Analysis of the distribution of mobile counterions from MD has produced an independent description the DNA ion atmosphere [122,123,124•]. The results exhibit features in reasonable accord with the core concept of 'counterion condensation' of the Manning theory and provide an independent account of the Manning fraction for monovalent counterions. A surprising result came from the analysis of solvent around the *Eco*RI dodecamer — the presence of a Na^+ ion found to assume some fractional occupancy in the minor groove at the ApT step, indicating that counterions may intrude into the venerable spine of hydration [122]. This 'ApT pocket' had previously been noted to be of uniquely low negative electrostatic potential relative to other positions of the groove [125] and was supported by the location of a Na^+ ion in the crystal structure of the rApU miniduplex [126]. Support for the fractional occupation of ions in the minor groove of B-DNA has recently been obtained by NMR [127,128] and crystallography, particularly in the case of K^+, Rb^+ and Cs^+ salts [129,130]. Lyubartsev and Laaksonen [123] used MD to study the dynamics of Li^+, Na^+ and Cs^+ ions. The Li^+ ions showed a propensity for greater coordination to the phosphates than the others, consistent with the slower diffusion rate observed by NMR [131].

Williams and co-workers [132], using the idea of structural deformations arising from the modulation of phosphate repulsion [133], have argued the case for 'cations in charge' of DNA structure. This idea has been recently disputed by Dickerson and co-workers [134], who reported the crystal structure of a cross-linked dodecamer under environmental conditions without appreciable monovalent cations. The structure was not significantly altered compared with their 1980 result [15], indicating that either ions were not present in the spine or, if present, were not causing structural perturbations or 'electrostatic collapse.' Theory provides some guidance here — analysis of the MD structures with and without ions in the 14 ns MD trajectory of the *Eco*RI dodecamer shows that ions have little significant effect on the width of the major and minor grooves, and other structural indices. The MD results are, however, consistent with the Williams' group report of some 10% fractional occupancy of ions [129] in the 'hydrat-ion' spine [130] of the minor groove. Propensities for the fractional

occupation of ions at other steps in both the major and minor grooves have been calculated from MD simulations (KJ McConnell, DL Beveridge, unpublished data). There is leading evidence and a proposed model for the role of ions as bending loci in the grooves of DNA [135].

Axis bending and bendability

An MD model of a DNA oligonucleotide duplex featuring A-tracts phased by a full helix turn was reported [136•]. Specifically, a series of nanosecond level MDs was carried out for a 25 base pair phased A-tract duplex at various concentrations of saline solution. A 30 base pair duplex composed of three 10 base pair repeats of the *Bam*HI recognition sequence was simulated as a control. The MD results, for a concentration of 60 mM KCl and 10 mM $MgCl_2$ added salt plus minimal neutralizing cations, show concerted axis bending to the extent of 16.5° per A-tract, which compares favorably with the 17–21° bending per A-tract inferred from cyclization experiments. The MD model also exhibits a progressive 5′ to 3′ narrowing of the minor groove region of the A-tracts, a feature inferred DNA footprinting experiments. Evidence for a role for divalent ions at bending loci was found from the MD. Subsequent studies were aimed at investigating aspects of sequence polarity as an important parameter in determining DNA structure and curvature.

DNA sequences with the motif $d(CA_4T_4G)_n$ or $d(GA_4T_4C)_n$ ('Hagermers') show significant gel anomalies with respect to random sequence oligonucleotides, whereas sequences of the form $d(CT_4A_4G)_n$ or $d(GT_4A_4C)_n$ do not [137]. MD was performed on the DNA duplexes $d(G_5\text{-}\{GA_4T_4C\}_2\text{-}C_5)$ and $d(G_5\text{-}\{GT_4A_4C\}_2\text{-}C_5)$ to 3.0 ns and 2.5 ns, respectively [138•], at ionic concentrations roughly comparable to a ligase buffer. Analysis of the results shows that the $d(G_5\text{-}\{GA_4T_4C\}_2\text{-}C_5)$ simulation exhibits strong gross curvature, consistent with experiment. The most significant locus of curvature in the MD structure was found at the central C15–G16 step, with an average roll angle of $12.8 \pm 6.40°$. The A-tracts were themselves, on average, relatively straight. The dynamic structure of $d(G_5\text{-}\{GA_4T_4C\}_2\text{-}C_5)$ exhibited minor groove deformation comprising expansion at the 5′ end and progressive narrowing towards the 3′ end, supporting the interpretation of hydroxyl radical footprinting results on Hagamers set forth previously by Berkhoff and Tullius [139].

A benefit of all-atom MD is that a molecular model for axis bending in DNA emerges as a result, rather than as an *ad hoc* assumption. MD results from several laboratories [96•,100•,107•,136•,138•] support the idea of essentially straight, relatively rigid A-tracts, with axis deformations more likely to appear at the junctions, but extending into the general sequence region as well. It is likely that this question cannot be resolved from studies on short oligonucleotides and, as of writing, our opinion is that longer range compensatory patterns may be a critical element in the DNA bending story.

Conformational stability and transitions

Systems with solvent water, counterions and co-ions at experimental and, even, physiological ionic strengths can now be treated using MD and make interesting problems in the area of conformational stability and transitions accessible to more rigorous theoretical study. Earlier studies have been reviewed [2,41,42]. Cheatham and Kollman [140] reported MD on d(CCAACGTTGG) in which the expected A/B DNA conformational preferences were observed in water, including an A to B DNA transition. At low water activity, however, a simulation beginning in the B-form did not make the expected conformational transition to the A-form in a trajectory of 500 ps. In follow-up studies [82,141], minor modifications of a single dihedral force constant in the sugars enabled the MD structure to convert to A-form, but agreement on issues concerning the preferential stability of the B-form in water was noted. The role of sugar repuckering in the A to B DNA transition was also investigated by Soliva *et al.* [142], demonstrating that the driven change of one dihedral angle in the sugar ring leads to a fast complete change in the DNA conformation.

Free MD simulations on the sodium salt of the *Eco*RI dodecamer in water and in a mixed solvent composed of 85% (v/v) ethanol:water were reported by Sprous *et al.* [48]. This sequence is observed to assume a B-form structure in the solid state and in aqueous solution, and is expected to assume an A-form structure in the mixed solvent environment. This study built on the results of a previous 14 ns MD [42,45], which resulted in a well-stabilized B-form dynamical structure. Three additional simulations were reported: one simulation starts from the A-form in water; the second starts from the A-form in 85% (v/v) ethanol:water; and the third starts from the B-form in 85% (v/v) ethanol:water. The MD on the A-form structure in water undergoes an A to B DNA transition and stabilizes in the B-form. The corresponding 2.0 ns MD in ethanol:water remains an A-form structure, as expected; however, the B-form structure in the 85% (v/v) ethanol:water remains B-form, even after 2.0 ns of MD. Similar results have now been obtained with the new CHARMM 27 force-field [32••]. One thing lacking in this area of study is experimental data on shorter oligonucleotides under well-defined environmental conditions for comparison with MD results; whether or not the *Eco*RI dodecamer actually undergoes an A to B DNA transition has been cast in doubt [143].

The above set of MD simulations formed the basis for a subsequent free energy analysis of the conformational preferences of A- and B-forms of DNA in solution as a function of water activity [78•,79•]. A set of DNA–counterion snapshots was culled from the MD on A- and B-forms in various environmental circumstances and an A to B DNA transition, and was used as a basis for making *post facto* estimates of the conformational free energy using a version of the free energy component analysis procedure. The estimates of the intramolecular energetics were calculated from the empirical energy functions used in the MD,

with corresponding entropies calculated from the ensemble of MD structures using the quasiharmonic method [144], as applied by Duan *et al.* [145]. The free energies of solvation were evaluated from those same structures using modified GB/SA [75•,77]. The trends observed experimentally were well reproduced and the calculations provided a plausible, but unexpected, explanation of the results. Lower interphosphate repulsions favor the B-form, independent of solvent conditions. Counterion–DNA interactions favor the A-form as a consequence of its more compact structure and higher charge density. In approaching closer to the DNA, however, the counterions pay a heavier desolvation penalty. In water, solvation favors the B-form DNA–Na complex with a magnitude that wins out in the balance of terms. In 85% ethanol, the magnitudes of these terms are reduced and the balance is shifted. At lower water activity, the solvation free energy is greatly reduced and the free energy contribution originating in the more compact and energetically more favorable organization of counterions wins out and stabilizes the A-form structure. Srinivasan *et al.* [78•] came to similar conclusions using an ion continuum Debye–Huckel method. With respect to the dialog about "what's in charge?" [132], this theoretical analysis of the free energy of the A to B DNA transition presents a means other than electrostatic collapse by which counterions and the counterion atmosphere influence DNA structure.

Radiation damage and chemical modification produce conformational changes in DNA that are of considerable interest from the biological point of view. MD simulations investigating these systems are beyond the scope of this review and the reader is directed to several recent reviews on the subject for more detailed information. Particularly, carcinogen-containing DNA simulations and modeling methods have been recently reviewed by Broyde and Hingerty [146]. Chemically modified DNA has been reviewed by Ninaber and Goodfellow [147]. Ayadi *et al.* [148] develop the issue of the sequence dependence of abasic site mutations in DNA conformation and curvature by modeling CXC and GXG sequences with JUMNA [149].

RNA oligonucleotides

Auffinger *et al.* [150] reported a new 500 ps MD simulation of a solvated $tRNA^{Asp}$. The calculations led to a dynamically stable model of the tRNA with all secondary and tertiary base pairs maintained, although fluctuations in the base triples were noted. Tang and Nilsson [151] reported a 1.2 ns MD simulation of the free hairpin II of the U1 snRNA, a 21-base sequence. Conformational analysis showed that changes in the RNA occurred primarily in the loop region, whereas the stem maintained the A-form. The loop region is primarily involved in RNA protein-binding motifs. A series of seven 2.5 ns MD simulations explored the stability of the wild-type wobble base pair G:U 3:70 and six mutants of the RNA $microhelix^{Ala}$ [152•]. The base pair steps above and below the wobble base pair are observed to be underwound relative to a canonical A-form helix. Hydration analysis of these simulations shows a link between the strength of a bound water at the wobble site and experimentally determined aminoacylation activity.

MD has been used to investigate the relative stability of two NMR structures of an RNA hairpin loop [153]. Miller and Kollman report that both conformational forms are equally stable, but when the backbone is modified to deoxyribose, the MD showed a conversion from one form to the other. Simmerling *et al.* [154•] extended studies of the RNA hairpin in the two conformational forms using locally enhanced sampling (LES) [155] and observed the conversion of the incorrect hairpin loop to the correct form, with the best results obtained with five copies of the central six residues in 150–250 ps. Using the central four residues of the loop, the correct base pairing was observed, but the backbone torsion angles did not show a strong correlation to the values obtained from NMR. Srinivasan *et al.* [156] report MD on RNA hairpin loop that shows a transition from an incorrect form to the conformation observed by NMR.

Unrestrained stochastic dynamics simulations focusing on the UUCG tetraloop of the RNA hairpin in a GB/SA solvent have been reported by Williams and Hall [80]. Several alternatives were pursued, but the GB/SA simulations produced average structures with close agreement with corresponding NMR results and a conversion of an incorrect to correct loop geometry. This result is especially promising as the computer time for dynamics in implicit solvent models is much reduced.

Conclusions

The field of MD on DNA can be described as now being at 'second generation', with much improved results obtained over the past three years. Several groups have specialized in developing, with impressive coordinated efforts, suites of programs for MD modeling and have generously made computer codes and force-fields from their efforts readily available to the basic research community. Results to date from current MD studies on nucleic acids have provided the field with highly promising case studies. Agreement with experiment can never unequivocally prove a molecular model correct [44••,157], so pushing MD models to failure or, at least, to the point of revealing limitations is essential in advancing the science. A leap into biological applications without sufficient validation of whether a force-field can successfully deal with the particular problem at hand carries the concomitant risk of inadvertently studying what Auffinger and Westhof [2] termed 'force-field polymorphisms'. As nucleic acid force-fields from various sources evolve, it will be essential to be clear on whether the best force-fields are, in fact, providing similar results or whether each describes a significantly different 'model chemistry' with particularized realms of applicability. Is one force-field 'better' than another and, if so, on what objective criteria? With computational power just now to the point at which running

multiple simulations on large complex systems is feasible, a consensus set of well-chosen prototype systems, excluded from parameter training sets, might be reported in any new force-field development or substantive modifications. Although this field may not readily lend itself to blind prediction contests, such as the Critical assessment of methods of protein structure prediction (CASP) [158], variations on a community wide approach to this problem could ultimately avoid redundancies and are thus well worth considering.

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