New tricks for modelers from the crystallography toolkit: the particle mesh Ewald algorithm and its use in nucleic acid simulations

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Structure March 1999, 7:R55–R60
http://biomednet.com/elecref/09692126007R0055

Introduction

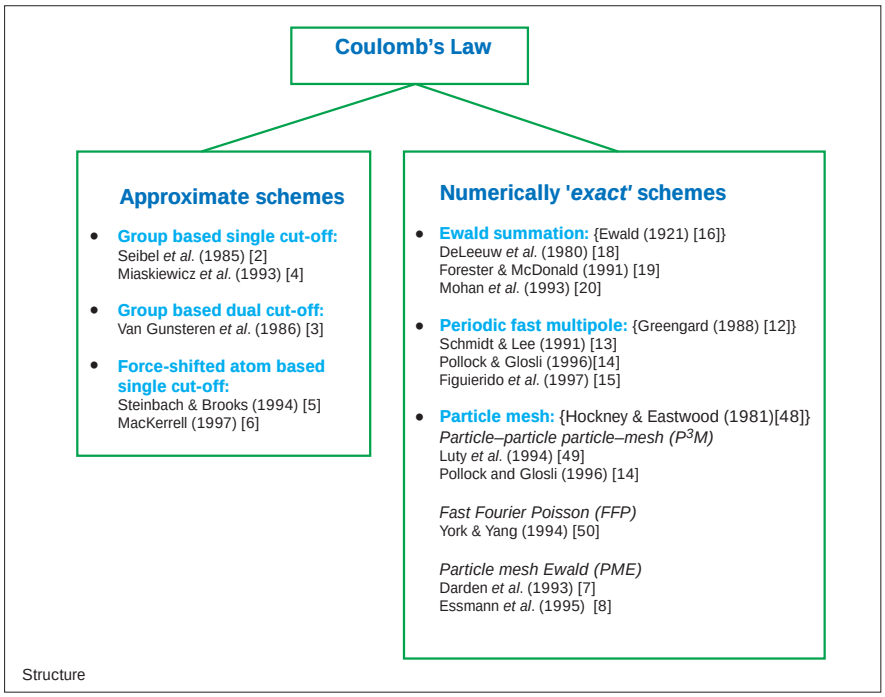
The accurate simulation of DNA and RNA has been a goal of theoretical biophysicists for a number of years. That is, given a high-resolution experimental structure, or perhaps a structure modeled from diverse experimental data, either of which provide a static view, we seek an accurate account (the simulation) of the relative motion of all of the atoms as time advances, sometimes under conditions that may differ significantly from any available experiment. How to treat long-range electrostatic interactions in simulations has been a major problem: DNA and RNA have charged phosphate backbones and, of course, realistic simulation systems will have a charge-neutralizing (or excess) complement of counter-ions. Early simulators treated electrostatics by essentially ignoring them [1], or by truncating the interactions at short range, 10 Å cut-offs [2] being common (computational cost scales as the cube of the cut-off). Larger cut-offs (e.g. 15 Å) were made possible through the use of methods such as 'twin-range' cut-off [3]. When long simulations began to be possible due to computer advances, substantial distortions were seen in DNA at the longer times [4], even when counter-ions and explicit solvent were present. Thus, nucleic acid simulations were seen to be particularly sensitive to the treatment of electrostatics. Improvements in DNA stability resulted with the introduction of more sophisticated cut-off methods [5,6], but it is now widely accepted that a rigorous treatment, such as the computationally expensive Ewald summation, is required for such highly charged systems. Thus, a significant step forward for DNA simulation work has been the implementation of fast algorithms for Ewald summation, such as the particle mesh Ewald (PME) algorithm [7,8]. The current state of biomolecular modeling is given in several recent reviews [9–11]. A description of the problem, the algorithm, applications (such as structure refinement) and current issues, in the context of DNA and RNA, follows here.

The method

The fundamental problem is that of computing the electrostatic energy (and first derivatives) of N particles, each with a unique charge, to arbitrary accuracy. Expressions for the energy and its derivatives are necessary to solve Newton's equations of motion. Figure 1 summarizes approaches that have been taken to date for simulations under periodic boundary conditions. Truncation-based methods [1–6] constitute approximations that are perhaps unnecessary with the high-speed high-storage computers of today (in fact the more sophisticated cut-off methods are less efficient than current fast Ewald methods). Of the 'exact' methods, the fast multipole method [12–15], which distributes the charges into subcells and then computes the interactions between the subcells by multipole expansions in a hierarchical fashion, shows promise, but to date applications to realistic molecular systems have been limited. The Ewald method, first described in 1921 [16], however, has seen a burst of recent activity with several existing formulations. The basic task is to compute the electrostatic energy and derivatives for an infinitely replicated unit cell. Each cell might be an actual crystallographic unit cell or might contain a single macromolecule or cluster surrounded by solvent. Steps 1 and 2 (Figure 2) take us to Ewald.

In his pioneering theoretical studies of X-ray diffraction in crystals (1912) Ewald needed to extract the contribution of an individual dipole oscillator within an ideal periodic array of such oscillators excited by a plane wave traveling through the lattice. The difficulty concerned the interconversion between spherical and plane wave representations. Ewald credits [17] Debye with introducing him to Riemann's theta transformation method, which was used to solve the problem. Later (1921) Ewald attacked the problem of calculating the electrostatic or Madelung energy of a crystal, which is precisely our problem. The unit-cell energy is written as an infinite sum $E(r_1,\ldots r_N)$ (Figure 2), that is only conditionally convergent (the value of the energy depends on the order of summation). Ewald [16] used the same theta transformation technique as before to convert $E(r_1,\ldots r_N)$ into a sum of absolutely (and rapidly) convergent sums in direct and reciprocal space. The remaining part of the electrostatic energy $E(r_1,\ldots r_N)$, a macroscopic reaction field term that captures the nature of the conditional convergence and that was missing in Ewald's treatment, was finally clarified 60 years later by DeLeeuw, Perram and Smith [18] (again Riemann's technique played an essential role).

Figure 1



Schemes for the evaluation of electrostatic interactions in periodic boundary conditions (see text for details).

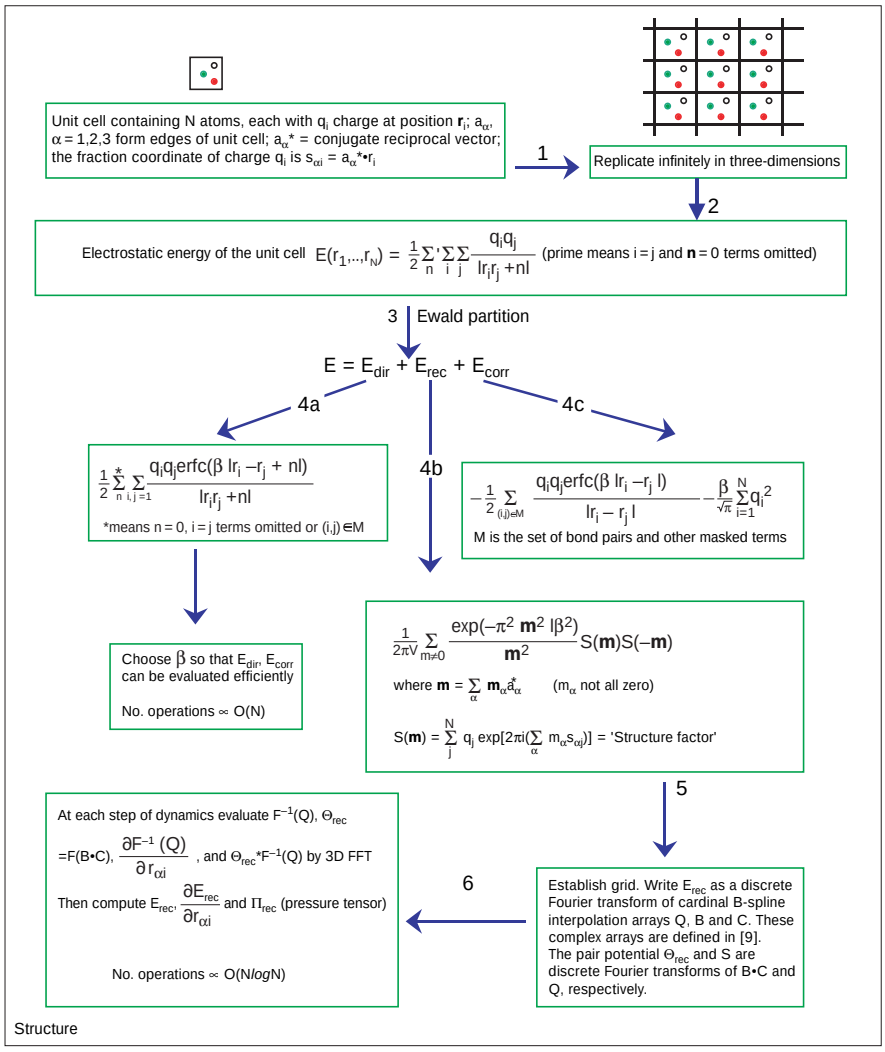
Algorithms that employ Ewald's formulation have been used in DNA simulations [19,20], but the time requirements scale as $O(N^2)$ and hence the application is limited to small systems. If, however, the Ewald formulation is written as in Figure 2 — direct space sums (steps 4a,c) and a reciprocal or Fourier space sum (step 4b) — a more time efficient algorithm is possible. The total energy is invariant to the choice of the Ewald convergence coefficient β, which determines the fraction of the total sum that is in Fourier versus direct space. For example, β can be chosen so that, regardless of system size, the direct sum can be truncated at 10 Å without loss of accuracy. This is possible because, unlike the original sum $E(r_1,\ldots r_N)$, the individual terms decay exponentially with distance. In this case the sums that result from steps 4a and 4c can be computed in O(N) steps. Unfortunately, this forces the Fourier space term to have an $O(N^2)$ dependence on system size. The number of structure factors $S(\mathbf{m})$ needed for an accurate calculation grows linearly with system size (note that the reciprocal vector $\mathbf{m}$ and thus the exponent in step 4b varies inversely with the unit-cell size) and each structure factor requires O(N) operations to calculate. However, this is very like a problem faced earlier by macromolecular crystallographers. The structure factors, $S(\mathbf{m})$, are functions of the particle positions. As in fast Fourier transform (FFT) based crystallographic refinement methods [21], the essential step (step 5) is to create a mesh on which the structure factors, and therefore the energies, the forces and the pressure tensor, can be smoothly approximated. The PME method effects this by approximating the trigonometric functions appearing in the structure factors using the Euler spline, a smooth function that expresses the value of the trigonometric function at the actual charge coordinates in terms of its values at neighboring mesh points. The resulting trigonometric sums over regular meshes can be efficiently evaluated using the FFT. All of the summations for step 4b can be evaluated in $O(N\mathit{log}(N))$ steps. The PME method was originally implemented in the AMBER simulation package [22], but is now available in many other simulation codes. A public domain version is available from the authors. The method has been recently analyzed and compared to other methods [23,24].

Validation and applications

Studies of the dynamics of highly charged proteins and nucleic acids in the early 1990s using conventional cut-off schemes, were dogged by the fact that artifactual behavior resulted at long simulation times. Papers began to appear that suggested that Ewald methods should be used [25]. By 1993 progress had been made [7], and by 1995 studies showed that DNA [26–28] and RNA [26,29] simulations were much improved by employment of the PME method. DNA and RNA were stable for nanoseconds without restraints or charge reduction. Particularly exciting was the discovery by Cheatham and Kollman [30] that non-trivial structural refinement (i.e. convergence to a force-field-dependent free energy minimum) occurred spontaneously

Figure 2

A flow chart of the steps involved in computing the electrostatic energy, force and pressure tensor by the PME scheme [9]. E_{dir}, E_{rec} and E_{corr} denote the direct and reciprocal summations, and the correction terms, respectively.



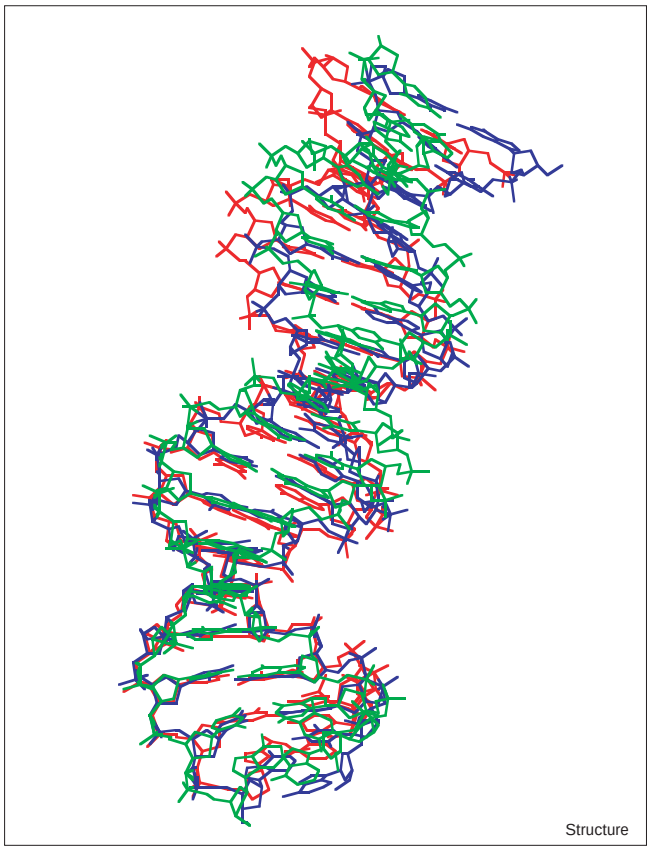
in DNA simulations on this time-scale (reviewed in [11]). This observation demonstrated that the enhanced stability seen with the new methods was not artifactual, and hinted that a host of interesting biophysical questions about DNA were within reach of current simulation methodology.

The PME method has been used to study counter-ion distributions around B-DNA [31]. In these simulations, the minor groove was found to narrow around tracts rich in A–T base pairs due in part to localization of Na^+ ions [32]; this predicted effect was recently directly observed in high-resolution crystallographic studies [33]. The latter also interestingly provided a caution for the study of sequence-specific structure effects in DNA based on medium-resolution structures. The bending of long 25 base pair DNA with and without A–T-rich tracts under high-salt conditions (60 mM KCl, 10 mM $MgCl_2$, neutralizing cations) was studied by simulation and showed remarkable concurrence with experimental data [34]. Modification of the buffer (using Na^+ ions only or by removal of Mg^{2+}) decreased the agreement with experimental results.

An interesting question is the extent to which one can hope to predict correct structures from incorrect analogs. An encouraging study [35] that tested the novel locally enhanced sampling (LES) method showed that when employing PME, an incorrectly folded RNA tetraloop moved to the correct structure in approximately 200 ps. A similar simulation repeated with a cut-off led to unfolded RNA. This study clearly points to the possibility that molecular dynamics utilizing PME coupled with new sampling methods, such as LES, may lead to improved structures from low-resolution structural determinations.

New methods have recently emerged to estimate conformational free-energy differences [36,37]. In contrast to

Figure 3



Comparison of a DNA model for the template–primer–HIV-1 reverse transcriptase complex (L Li, unpublished results, green) with two recent X-ray crystal structures ([51], red; [52], blue). The model complex comprises 19 base pairs of template and 18 base pairs of primer – the HIV-1 reverse transcriptase is not shown. Base pairs 2–8 (from top) were used for the fit. The model was developed prior to the availability of the X-ray structures by merging PME-MD-refined fragments based on earlier crystal structures [53,54]. The all-atom 2 ns simulation used to prepare the model contained 180,000 atoms, including the HIV-1 reverse transcriptase, DNA, solvent and counter-ions.

potential of mean force calculations, which require that the reaction paths for conformational transitions be determined, these methods estimate the absolute free energy of nucleic acid conformations using continuum solvation free-energy calculations in combination with gas phase enthalpy and solute entropy approximations. These estimates are then averaged over a set of neighboring conformers taken from 'snapshots' of a PME molecular dynamics (PME-MD) trajectory using explicit solvent. This averaging greatly alleviates the sensitivity of the estimates to the fine details of a particular conformation. The sensitivity of the B-DNA↔A-DNA equilibrium to environmental factors, such as salt and ethanol–water composition, has been studied by these methods; the key factor appears to be that the relative free energy associated with explicit organization of the mobile counter-ions and solvation in the major groove favors the A form in alcohol-rich solvent. Other applications of this technique are rapidly appearing.

Applications of the PME-MD method to structural and dynamical questions have begun in earnest. The details of how large ions like $[Co(NH_3)_6]^{3+}$ stabilize A-DNA (by hydration and ion association in the major groove) and reduce its tendency to undergo the A-DNA→B-DNA transition have been elucidated by a PME-MD simulation [38]. Issues concerning the degree of cytosine protonation in DNA triplexes were investigated using PME-MD, with the conclusion that a certain fraction of the neutral form is present [39]. Finally, the PME-MD method was used to merge several X-ray structures, each of which was incomplete in some important detail, to construct an equilibrated all-atom model for HIV-1 reverse transcpriptase interacting with primer–template strands of DNA. The model has been useful in the analysis of vertical-scanning mutagenesis experiments [40] (Figure 3).

Recently, the PME-MD method has been employed in the nuclear magnetic resonance (NMR) structure refinement of nucleic acid structures and protein–DNA complexes [41–43]. In these studies, restrained molecular dynamics using full solvent and counter-ions was used as a final refinement step following the standard simulated annealing protocol *in vacuo*. Aspects of the structure that are not well defined by the nuclear Overhauser effect (NOE) intensities can be treated more precisely in this way. In particular, the local effects of solvent and counter-ions can be studied. The PME-MD method was used to refine high-resolution NMR data of hairpin dimer quadruplexes $d(G_3T_4G_3)$ in the presence of potassium. The full inclusion of electrostatics allowed a study of the effects of cation coordination on the structure and stability of telomeric DNA [41]. A similar application of this method was used to refine the Ca^{+2} binding γ-carboxy glutamic acid (Gla) domain of the coagulation protein factor IX [44]. In this study, part of the protein structure determined by the NMR refinement was estimated and the calcium ions, also invisible to NMR, were initially placed using a genetics algorithm and then annealed by PME-MD. As discussed by Konerding *et al.* [43], in the NMR refinement of nucleic acid structures the force field and simulation protocol have a non-negligible influence on the final results, even in well-determined systems. Future improvements in force fields will thus have a direct bearing on the quality of experimental structures.

The underlying suggestion in many of these studies is that the current force fields may be suitable for describing the dynamics of charged macromolecules for several nanoseconds (at least) if the electrostatic interactions are computed to high accuracy. In effect, the useful life of the force fields, which are also improving via parameterization advances, may have been extended.

The future

The PME-MD method has clearly been useful for numerous applications. Future applications in the area of improved refinement procedures for the determination of X-ray crystal, NMR and electron diffraction structures, are likely to follow. For example, the modeling of missing loops and other conformationally flexible features may improve. The range of applicability of molecular replacement methods may be extended. Other work will focus on theoretical and computational limitations of the method, and extensions to other simulation contexts. The 'flying ice cube', a difficulty experienced in some early PME-MD applications [45], resulted from energy drains due to parameter choices and was simply fixed. Simulation artifacts due to periodic boundary conditions have been assessed [46], the result (so far) being that systems in high dielectric solvents are largely unaffected [47]. There is current activity to extend the PME-MD method into free-energy perturbation methods, to improve the electrostatics (at least in principle) by implementing polarizability corrections, to improve sampling methodology through the use of LES [35], and to improve PME-MD performance on parallel systems.

Acknowledgements

We thank the North Carolina Supercomputing Center, the Pittsburgh Supercomputing Center and the National Cancer Institute Supercomputing Center for access to resources. LGP thanks the National Institutes of Health for HL-06350 and the National Institute of Environmental Health Sciences (NIEHS) for access to their facilities.

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