Molecular Dynamics Simulations on Solvated Biomolecular Systems: The Particle Mesh Ewald Method Leads to Stable Trajectories of DNA, RNA, and Proteins

T. E. Cheatham, III,† J. L. Miller,† T. Fox,†
T. A. Darden,‡ and P. A. Kollman*,†

Department of Pharmaceutical Chemistry
University of California
San Francisco, California 94143-0446
National Institute of Environmental Health Sciences
Research Triangle Park, North Carolina 27709

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This communication presents results from molecular dynamics (MD) simulations with AMBER 4.1[1] and the Cornell *et al.*[2] force field of three different, fully solvated, fully charged, macromolecular structures: X-ray-derived structures of d(CCAACGTTGG)$_2$ DNA[3] and ubiquitin[4] and an NMR-derived r(UUCG) RNA hairpin loop and stem structure.[5] We compare the use of the particle mesh Ewald (PME) method[6] for the treatment of long-range electrostatic interactions to standard charge group based truncation cutoff (CUT) methods used in simulations with periodic boundary conditions.

An accurate representation of long-range electrostatic interactions in MD simulations is extremely important in order to properly represent the structure, dynamics, and energetics of biomolecular systems.[7−9] This is particularly true for highly charged systems, such as DNA and RNA, where it has been difficult to obtain stable trajectories for a fully solvated system without imposing added restraints or artificially modifying the charges on the phosphate.[10] Stable trajectories, ideally without the addition of restraining forces, are necessary for applications such as accurately predicting the binding free energy of ligand−macromolecule interactions.[11]

The results on these three solvated biomolecular systems clearly demonstrate that, with a modest computational burden, the PME method is not only generally applicable but superior to standard cutoff methods. This is demonstrated by less rms deviation from the experimentally observed structures by the PME method while reasonable atomic positional fluctuations are maintained. In the case of the DNA and RNA simulations, both CUT simulations led to distortion and breakup of the structures. The PME results presented on RNA, to our knowledge, are the first demonstration of stable solvated RNA MD trajectories.

A number of studies to date have applied MD simulations to study macromolecular crystals.[12,13] In these simulations, and more generally, with standard solvated periodic boundary systems (e.g., not true experimentally derived crystal unit cells), it has been found that it is critical to properly treat the electrostatics, preferably through the use of methods which account for the periodicity of the unit cell, such as PME, but minimally through the use of large cutoffs and/or methods which smooth out the potential and forces resulting from a truncated cutoff.[8] To our knowledge, the Ewald[14] method has not been directly applied in standard solvated periodic boundary simulations of bimolecular systems, except for in the work of Smith *et al.*[15] studying a small zwitterionic peptide and Schreiber *et al.*[8] studying helix-forming peptides. Both found that better treatment of the electrostatics was obtained using the Ewald method, in contrast to standard cutoff or switching function techniques, even though the Ewald method is known to introduce long-range correlation of fluctuations.[16]

In each of the macromolecular systems investigated, MD simulations were run[17] using the PME method with tin foil boundary conditions, a charge group based truncation cutoff (CUT), and a group based truncation cutoff coupled with complete evaluation of all the solute−solute interactions (CUTSS). Over the course of the dynamics in each of these cases, the PME structures remained strictly closer to the experimentally observed structures (Figure 1), yet demonstrated significant positional fluctuations.

In the case of a 1 ns PME DNA simulation, the structure remained in a strictly B-DNA form with an average rms deviation 3.2 Å away from the crystal structure (2.9 Å away from canonical B-DNA[18]). This simulation, run with a fully charged DNA and explicit counterions, converged after roughly 200 ps to a structure which deviated from the average structure over 200 ps to 1 ns by an average rms deviation of 1.4 Å (0.2

† University of California.
‡ National Institute of Environmental Health Sciences.

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(17) DNA/RNA simulations: A rectangular box was constructed with counterions placed by the AMBER EDIT module. Simulations were run with SHAKE on hydrogens, a 2 fs time step, a temperature of 300 K with Berendsen temperature coupling, a 9 Å cutoff (also applied to Lennard-Jones interactions in PME), and constant pressure. The nonbonded pairlist was updated every 10 steps. No extra restraints were placed on the DNA/RNA systems following the equilibration period. Results were analyzed with the CARNAL module of AMBER 4.1. The PME charge grid spacing was approximately 1.0 Å, and the charge grid was interpolated on a cubic grid. The DNA box size was 64.0 × 45.0 × 45.0 Å^3, and the RNA box size was 47.0 × 44.0 × 39.0 Å^3. Equilibration was performed by placing 25 kcal/mol restraints on all solute atoms, minimizing the water for 1000 steps, followed by 3 ps of MD, which allowed the water to relax around the solute. This was followed by five rounds of 600 step minimizations, reducing the solute restraints by 5 kcal/mol during each round. Finally, the system was heated to 300 K over 10 ps of MD and production runs were initiated. Ubiquitin simulation: A rectangular box of 4385 TIP3P waters was constructed. Simulations were run with SHAKE on hydrogens, a 1.5 fs time step, a temperature of 300 K with Berendsen coupling, an 8 Å cutoff, and constant pressure. The nonbonded pairlist was updated every 10 steps. The system was equilibrated as follows: after initial minimization of the sovlent box down to an rms gradient of 0.5 kcal mol^{-1} Å^{-1}, 12 ps of dynamics on the solvent only was performed. This was followed by six minimizations of the whole system with the backbone atoms of the proteins restrained to the crystal positions by force constants of 1000, 100, 50, 15, 2, and 0 kcal/mol. Then two 3 ps MD at 100 and 200 K were performed, followed by production runs.
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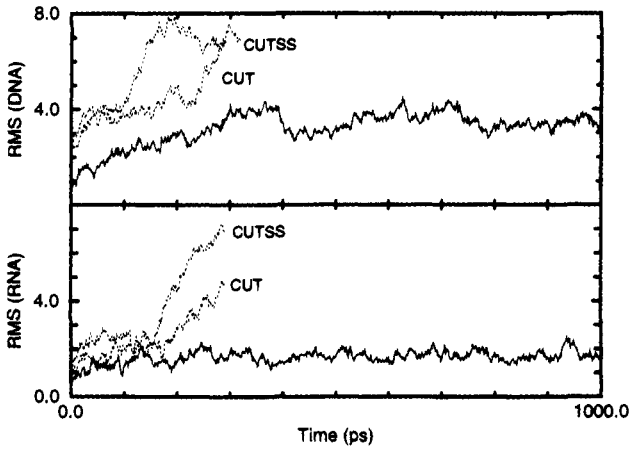

Figure 1. Time evolution of the all-atom rms deviation from the initial structure for (top) d(CCAACGTTGG)$_2$ and (bottom) 5'GGAC(UUCG)-GUCC. The solid line represents the PME, and the labeled dotted lines represent the CUT and the CUTSS simulations.

Å standard deviation) and did not demonstrate any persistent substates.[19] In contrast, 300 ps CUT and 300 ps CUTSS simulations demonstrated significant bending and structural distortion of the DNA, leading to a rms deviation from the crystal structure of over 6 Å (Figure 1, top). The distortion was particularly apparent in the CUTSS simulations (in both rectangular and cubic box simulations) where the initial bending was so severe it led to breaking of the three terminal base pairs.

MD on the RNA system was performed using the CUT and CUTSS methods for 300 ps each, while the PME simulation was run for 1 ns. The two non-PME simulations diverged significantly (>4 Å) from the solution structure within 300 ps (Figure 1, bottom). This deviation is primarily due to disruptions of the stem structure. As was seen in the DNA simulations, the CUTSS method demonstrated significantly worse behavior than the CUT method. The PME simulation stayed close to the solution structure throughout the entire 1 ns simulation (~1.5 Å rms deviation). More importantly, the PME method correctly maintained the A-form stem structure. The largest deviations seen in the stem are in the closing base pair of the loop (C4:G9), where significant buckling occurred. Analysis of the loop structure showed that the major structural features, as reported by Cheong *et al.*,[5] were also maintained. The stability and accuracy of the PME method gives us confidence that these simulations can be used to examine some of the unresolved questions concerning the unique stability of this tetraloop.

In contrast to the highly charged RNA and DNA systems, all the simulations of ubiquitin stay close to the initial structure. Without PME, the rms deviation from the crystal structure rises for all non-hydrogen atoms to 1.8 Å and for the backbone atoms to 1.2 Å after 300 ps. With PME, the corresponding values are 1.2 and 0.9 Å. The low rms deviation observed in the PME simulation is not due to damped motion since the calculated

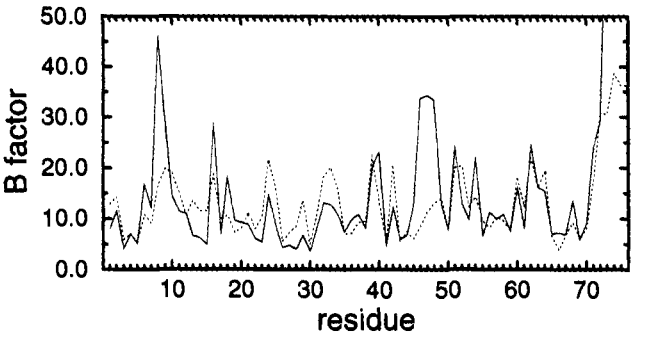

Figure 2. Experimental [4] (dotted line) and PME trajectory derived (solid line) thermal B factors for ubiquitin, averaged over residues. The calculated values from the PME trajectory were obtained from the atomic fluctuations $\langle \Delta r^2 \rangle^{1/2}$ by $B = \frac{8\pi^2}{3} \langle \Delta r^2 \rangle$.

thermal factors are of magnitude similar to the crystal thermal factors (Figure 2). In the solvent exposed surface residues the fluctuations are, not surprisingly, higher than that observed for the crystal[4].

In conclusion, it is demonstrated that PME is a powerful method to study the dynamics of macromolecules in solution, particularly for highly charged systems, and superior to standard cutoff schemes with a modest computational burden (40−50% additional cost). Other studies have analyzed various approaches to electrostatic cutoffs for proteins and have concluded that "shifted" potentials seem to be the most robust.[9,22] It is especially encouraging that the PME demonstrated stable, unrestrained nucleic acid trajectories. Although accurate agreement with a crystal structure is not expected, since we are not mimicking experimental crystal conditions, it may be significant that the NMR-derived RNA structure remains closer to the initial structure than does the X-ray-derived DNA structure. The low ubiquitin rms backbone deviation is consistent with an available NMR study[20] which suggests a hydrogen-bonding pattern in solution that is similar to that of the crystal structure. Furthermore, it can also be related to prior findings on BPTI, which show a 0.8 Å backbone deviation between the crystal structures and the NMR solution structure,[21] comparable to the 0.9 Å found here for the comparison between the MD and X-ray ubiquitin backbone structures. More in depth analysis of these simulation results will be published elsewhere.

Note Added in Proof: While this article was in press, the following article appeared which presents results on the application of the Ewald method to a DNS triple helix: Weerasinghe, S.; Smith, P. E.; Mohan, V.; Cheng, Y.-K.; Pettitt, B. M. *J. Am. Chem. Soc.* **1995**, *117*, 2147−2158.

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(19) This compares to the 1 ns switched cutoff simulations of McConnell *et al.*[10] of d(CGCGAATTCGCG)$_2$, run with no explicit counterions, a reduced (to −0.24 au) phosphate charge, and an extra hydrogen potential which converged to two structures approximately 4.5 and 7.5 Å away from canonical B-DNA.

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