Conserving Energy during Molecular Dynamics Simulations of Water, Proteins, and Proteins in Water

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Molecular dynamics simulations have been carried out for a series of systems of increasing complexity including: pure water, a model polypeptide (α-helical decaglycine) in vacuo, a protein (Pancreatic Trypsin Inhibitor, PTI) in vacuo, and a fully solvated protein (PTI in water). The equations of motion were integrated using Andersen's velocity version of the Verlet algorithm with internal contraints (the RATTLE algorithm). The accuracy with which the equations of motion are integrated has been analyzed for several different simulation conditions. The effects of various nonbonded interaction truncation schemes on the conservation of energy have been examined, including the use of atomic cutoffs, and (neutral group) residue cutoffs. The use of a smoothing function to eliminate the discontinuities in the potential at the cutoff leads to a significant improvement in the accuracy of the integration for each of the systems studied. The accuracy with which the equations of motion are integrated using the RATTLE algorithm for pure water and for the solvated protein are found to be comparable when the nonbonded interactions are tapered with a smoothing function at the cutoff.

INTRODUCTION

Molecular dynamics simulations of proteins provide the most detailed theoretical method available for studying their structural, dynamical, and thermodynamic properties. With this method, Newton's equations of motion are solved step by step for all the atoms of the system. Due to the large number of atoms in a protein these calculations are computationally intensive, and for this reason the earliest molecular dynamics simulations of proteins were carried out in vacuo. With the vastly increased speed of supercomputers, it has become possible to include the solvent environment explicitly in the simulations. The current generation of simulations can handle proteins of molecular weight up to ca. 20,000 solvated by several thousand water molecules. The technology has progressed to the point where there are now commercial software packages available to perform these calculations. However, there are still fundamental questions regarding the accuracy that can be achieved under diverse simulation conditions. Many comparisons between the results of simulations and experimental probes of protein structure and dynamics have been reported.[1-3]. Differences between calculated and experimental results are most often attributed to the approximate nature of the potential functions, and there are ongoing major efforts in many laboratories to improve the empirical potentials. An additional source of error in simulations of these very large systems can arise from inaccurate integration of the equations of motion. Such errors can result from the integration algorithm employed, the time step used or, very commonly, from the way in which long range interactions are treated. The analysis of these effects has most frequently been reported in the context of liquid state simulations of uncharged homogeneous systems.[4,5] The evaluation of the relative accuracies of different integration schemes as applied to proteins in vacuo has also been reported.[6-8] Calculating trajectories accurately for solvated proteins presents additional problems beyond those for pure liquids or proteins in vacuo. This analysis is beginning to receive attention in the literature.[9] In this article we review the problem of the accurate integration of the equations of motion taking as examples a series of systems of increasing complexity including: pure water, a model polypeptide (α-helical decaglycine) in vacuo, a protein (Pancreatic Trypsin Inhibitor,

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PTI) in vacuo, and finally a solvated protein (PTI in water).

Errors in the integration of the equations of motion for these complex systems can be manifest in several ways: for example large fluctuations in the total energy and/or large temperature increases over the course of the trajectory. If, in the microcanonical ensemble, the equations of motion were integrated with no error, the total energy would be conserved and no fluctuation of the total energy would be observed. In practice, the total energy does fluctuate during simulations within the (N, V, E) ensemble. One standard measure of the accuracy of a simulation is given by the ratio of the fluctuations in the total energy, $\langle \Delta E^2 \rangle^{1/2}$ to the average of the total energy $\langle E \rangle$.[5] For liquid state simulations, a rule of thumb often employed is that $\langle \Delta E^2 \rangle^{1/2}/\langle E \rangle$ should be less than 0.01%[5] (note: all ratios in this paper are expressed as %; 0.01% = 0.0001). Unfortunately, there has been no systematic investigation of the relationship between the magnitude of $\langle \Delta E^2 \rangle^{1/2}/\langle E \rangle$ and variations in average structural or thermodynamic quantities calculated from a simulation; we return to this point in the discussion. Another criterion sometimes applied to assess the accuracy of the integration is given by the ratio of $\langle \Delta E^2 \rangle^{1/2}/\langle \Delta KE^2 \rangle^{1/2}$ where the denominator is the square root of the fluctuations in the kinetic energy of the system.[6] It should be noted that the two criteria will scale differently with the size of the simulated system as the total energy is proportional to N, the total number of atoms in the system, while the square root of the fluctuations in the kinetic energy increases as $N^{1/2}$.[10] The fluctuation in the kinetic energy (and therefore in the temperature) is proportional to the heat capacity of the system. Therefore when the error criteria is expressed as $\langle \Delta E^2 \rangle^{1/2}/\langle \Delta KE^2 \rangle^{1/2}$, the ratio ties the error in the integration to an experimental quantity since this value is directly related to the error in the estimate of the heat capacity of the system.[10] Different authors have suggested acceptable values for this quantity for protein simulations ranging from 1–10%. Berendsen et al. and Levitt have compared different integration algorithms.[6–8] Higher order integration methods provide increased accuracy for small time steps at a cost of slightly longer cpu time per iteration and larger memory requirements. Berendsen et al. point out however, that higher order methods may actually be more sensitive to spurious effects which arise from the truncation of long range forces.[6]

When simulating systems containing thousands of atoms, it is necessary to introduce a cutoff in the interaction pair potential at some distance R_c, typical choices of R_c vary between 7 Å and 12 Å. The simplest way to implement such a cutoff is to abruptly truncate both the potential and the force at the cutoff distance. However, the truncated force is not the negative gradient of the truncated potential, because it does not include the impulsive forces introduced by the discontinuity of the potential. The truncated force is in general not the negative gradient of any function, and in the resulting dynamics there is no conserved quantity analogous to a total energy. The net effect is a steady increase in the temperature of the system over time. This effect can be understood in a simple sense by considering the fact that molecular groups within the cutoff but moving beyond it are, on average, more correlated in their orientation and thus have lower energy than molecular pairs which cross the boundary in the opposite direction. To compensate for this effect in simulations which employ an abrupt spherical cutoff, the temperature of the system must be rescaled during the simulation. Several different schemes for rescaling the temperature (kinetic energy) have been proposed, including those designed to approximate the simulation of a canonical ensemble.[11,12] Two drawbacks of temperature scaling are: (1) it can mask errors resulting from inaccurate integration of the equations of motion and (2) for heterogeneous solute in solvent systems, uniform temperature scaling of the entire system leads to a disparity between solute and solvent temperatures (with the solvent hotter than the solute). With respect to masking errors, a prudent practice is to carry out trial simulations of the system under study—without temperature scaling—in order to assess the relative accuracy of the trajectory under the particular simulation conditions to be used for production runs, which are performed with temperature scaling. The problem of disparate temperatures between solute and solvent is not completely solved at present; a common practice is to scale the temperature of the solvent and solute separately.

An alternative approach for dealing with truncation effects is to alter the form of the potential function in a zone at the cutoff boundary so that the potential and its first derivative (the force) go to zero smoothly at the cutoff. The application of a smoothing function leads to much more stable trajectories in simulations of liquids.[4,13] The smoothing procedure does not affect the short range liquid structure and has a very small effect on the internal energy, but it does have a large effect on the long range orientational order. As the long range orientational order is related to the dielectric response of the liquid, the use of a smoothing function will, in general, alter the dielectric properties of the liquid.[14] For pure liquid simulations, formulas have been derived which account for the effect of smoothing on the dielectric response,[15] but analogous treatments

are not available for heterogeneous systems such as simulations of proteins in water. Thus it must be kept in mind that the use of smoothing functions in simulations of solvated proteins may affect the dielectric properties in these simulations in ways that are not well understood at present.

In this article we analyze the effect of various simulation conditions on the accuracy with which the equations of motion are integrated. We have studied the effect of such parameters as step size, frequency with which the nonbonded list is updated, and methods used to truncate the long range interactions, on the conservation of energy during the simulation. Several properties of the truncation scheme have been evaluated including the cutoff distance, the use of a smoothing function, and the use of atomic versus neutral group truncation.

METHODS

All of the simulation results reported in this article were carried out using the simulation package IMPACT (Integrated Modeling Program/Applied Chemical Theory) developed in our laboratory over several years.[16–18] IMPACT is a program for simulations of condensed phase systems, including macromolecular solutes in solution. Integration of the equations of motion is accomplished within IMPACT using the velocity version of the Verlet algorithm with constraints (the RATTLE algorithm) of Andersen:[19]

$$r(t + h) = r(t) + hv(t) + \frac{h^2[f(r(t)) + g_{RR}(t)]}{2m}$$

$$v(t + h) = v(t) + \frac{h[f(r(t)) + g_{RR}(t) + f(r(t + h)) + g_{RV}(t)]}{2m} \quad (1)$$

where $r(t)$, $v(t)$ are the atomic positions and velocities at time t, $f(r(t))$ is the force on an atom at time t, m is the atomic mass and h is the time step. The constraint forces due to the RATTLE algorithm are contained in g_{rr} and g_{rv}, respectively.[19] This form of the Verlet algorithm has two advantages over the conventional Verlet algorithm. First, the velocities are calculated explicitly at each step which is necessary for simulations in which temperature scaling is used. Temperature scaling can then be used to simulate the canonical ensemble while at the same time controlling the drift in the total energy during the course of a simulation. Also there is higher numerical precision associated with the way terms in powers of (h) are combined. To our knowledge, the RATTLE algorithm has not previously been applied to protein simulations. As reported in the following section, our experience has been that the algorithm performs well for simulations of solvated macromolecules, including charged systems.

Prior to carrying out simulations at constant total energy for analysis, the systems were first equilibrated during simulations lasting ca. 5 ps at a temperature close to 300 K using temperature scaling. The temperature scaling procedure employed during the equilibration was Berendsen's "coupling to a heat bath" method.[12] At each step the velocities are scaled by a factor, λ:

$$\lambda = \left[1 + \frac{h}{\tau_T}\left(\frac{T_0}{T} - 1\right)\right]^{1/2} \quad (2)$$

where τ_T is the (approximately) exponential relaxation time to the target temperature T_0. Both temperature and pressure bath coupling have been implemented within IMPACT. In the presence of constraints, the equations coupling the system to an external bath and the constraint equations must be iterated in a particular order. The iterative procedure for determining positions and velocities using RATTLE with temperature and pressure bath coupling is listed below:

Given Velocities $V(t)$, Positions $X(t)$, and Forces $F(t)$ at $t = 0$

1. Rescale velocities (if coupling to heat bath turned on)
2. Compute pressure and rescale positions (if coupling to pressure bath turned on)
3. Compute new positions, solving iteratively constraint equations for positions (if internal constraints turned on)
4. Compute new velocities, solving iteratively constraint equations for velocities (if internal constraints turned on)
5. Go to 1

None of the simulations reported here have used pressure scaling; temperature scaling was used during the equilibration phase of the simulations.

Simulations were carried out on four different systems: (1) pure water, (2) an α-helical polypeptide (decaglycine) in vacuum, (3) a protein (Pancreatic Trypsin Inhibitor, PTI) in vacuum, (4) a protein (PTI) in water. The amino and carboxyl terminii of decaglycine and PTI were capped with charged groups (zwitterionic form). In the protein simulations, all the side chains which are ionized at neutral pH were fixed in their ionic form. For the simulations involving protein in water, a box of water with dimensions 43.2 Å × 46.9 Å × 47.3 Å was used with cubic periodic boundary conditions. Six chloride atoms were added to the system to maintain an electrically neutral system. The protein was placed in the box of waters by removing all waters whose distance to any protein atom was within the sum of the overlap distances of the water oxygen and the protein atom (the overlap

distance being defined as the σ parameters from the Lennard–Jones energy terms). Chloride ions were used to replace sequentially the six water molecules with the greatest electrostatic potential due to the protein. The potential parameters used were the SPC values[20] for water; for the protein simulations the molecular mechanics force field of Kollman and coworkers was employed.[21] In this force field, the electrostatic properties of sulfur atoms on e.g., methionine and cysteine residues are modeled using pseudo atomic groups with unit mass to represent the lone pair electrons, placed at a distance of 0.67 Å from the sulfur nucleus. We found that the very short bond length and light mass of this pseudo atomic group leads to unstable behavior in the integration of the equations of motion, and constraints were therefore always applied to bonds involving these groups during the molecular dynamics simulations. Additionally, in some of the simulations reported in the following section, constraints were applied to all the bonds of the protein.

Treatment of Nonbonded Interactions

There are several methods available for constructing nonbonded interaction lists. The simplest is to construct the list based on a strict interatomic cutoff distance criteria. A major difficulty with this method is that electrostatic interactions are treated in an inconsistent way when dipolar units located on pairs or groups of atoms are split during the generation of the list. In order to circumvent this problem, list generation can be based on the evaluation of the interactions between neutral groups rather than individual atoms. For proteins, a logical choice for the grouping of atoms into "neutral" groups is the amino acid residue; for charged residues, the group has a unit charge. In the following section, we refer to the neutral group method of generating the nonbonded list as the residue cutoff method. When using a neutral group cutoff criteria, a decision must be made regarding how to calculate a cutoff distance between the groups as well. For pure water simulations, the choice of a cutoff distance between a pair of water molecules can be based either on the oxygen-oxygen distance as is usually the case, or on the distance between the center of mass of the two molecules, a more general method useful for simulating nonaqeuous polyatomic solvents. For proteins, a criterion based on the distance between the centers of mass of the two residues is not a good choice because the spatial extent of the residues may lead to the neglect of significant interatomic interactions. In the program IMPACT, we have implemented a residue cutoff criteria based on a minimum distance between any two atoms of the residue pair; that is if any atom i on residue α is less than R_{cut} away from any atom j on residue β, then the interactions between all the atoms in residue α and all the atoms in residue β are included in the nonbonded interaction list.

There is a significant computational advantage to the use of a residue-based nonbonded list as compared with an atomic nonbonded list associated with storage requirements. For simulations of proteins in water, the number of nonbonded interactions that must be included with a cutoff of ca. 8–10 Å is very large. Consequently, when using an atom based method for constructing nonbonded pair lists, the (two) arrays which store the partner information must be very large; whereas when the list is constructed using residue criteria, the partner lists are much smaller. For example, the size of an atom-based list in the PTI/water simulation using a 9 Å cutoff is on the order of 1.5 million interactions, all of which would be stored explicitly. A residue-based list would store only 200,000 pairs to represent roughly the same number of atomic interactions. An additional advantage in the use of a residue-based list is that the number of pairwise interactions which must be calculated when generating the list is greatly reduced. Thus for water, looping over molecular (centers of mass) pairs rather than atom pairs saves a factor of nine in the generation of the nonbonded list. In IMPACT, the cutoff criterion used for the solvent (water) is always a molecular (residue) criteria, while for the protein various options are permitted.

Multiplication of the nonbonded interactions close to the cutoff by a smoothing function leads to more stable trajectories. The smoothing function used in IMPACT for the simulations presented in the following section is given by the fifth-order polynomial[4]

$$S(R) = 1 - (C_3\,\delta^3 + C_4\,\delta^4 + C_5\,\delta^5) \qquad (3)$$

$$C_3 = \frac{10}{(R_H^2 - R_L^2)^3} \qquad C_4 = \frac{-15}{(R_H^2 - R_L^2)^4}$$

$$C_5 = \frac{6}{(R_H^2 - R_L^2)^5} \qquad \delta = (R^2 - R_L^2)$$

This function and its first and second derivatives are continuous. The smoothing function tapers continuously from the value 1 at R_L to the value 0 at R_U; the derivative of the smoothing function is zero at the lower and upper cutoff. The distance R can be chosen with IMPACT to be either the distance between any two atoms, the distance between any two (neutral) groups of atoms specified by the centers of mass of the groups, or the distance between an atom (on the protein) and a neutral group (a solvent water molecule). We have evaluated the effects of a smoothing function on the conservation of energy in simulations of pure water (using a neutral group or molecular cutoff), simulations of protein

in vacuum (using an atomic cutoff), and simulations of protein in water (using a neutral group cutoff criterion for the water, and an atomic criterion for the protein). The nonbonded interaction energy between atom or group i and atom or group j is given by:

$$E'_{IJ} = S_{IJ}E_{IJ} \tag{4}$$

A shifting function may be obtained from this smoothing function by setting the lower cutoff (R_L) to zero as shown below,

$$S(R) = 1 - \left(\frac{10R^3}{(R_H^2)^3} - \frac{15R^4}{(R_H^2)^4} + \frac{6R^5}{(R_H^2)^5}\right) \tag{5}$$

The fact that S_{IJ} may be a function of a molecule's center of mass complicates the derivatives of Eq. (5). Equation 6 shows the first derivative of (4) with respect to an arbitrary atom-atom distance ($R_{i'j'}$) when center of mass coordinates are used to calculate the smoothing function value.

$$\frac{\partial E'_{IJ}}{\partial R_{i'j'}} = S_{IJ}\frac{\partial E_{i'j'}}{\partial R_{i'j'}} + \frac{\partial S_{IJ}}{\partial R_{i'j'}}E_{IJ} \tag{6a}$$

$$\frac{\partial R_{IJ}^2}{\partial x_{i'}} = \frac{m_{i'}}{m_T}(2)\left(\frac{\Delta x_{i'j'}}{R_{IJ}}\right) \tag{6b}$$

$$\frac{\partial S_{IJ}}{\partial R_{i'j'}} = \frac{\partial S_{IJ}}{\partial R_{IJ}}\frac{\partial R_{IJ}}{\partial R_{i'j'}} \tag{6c}$$

where R_{IJ} is the distance between the center of mass of group I and group J and $\Delta x_{i'j'}$ is the difference between the x coordinates of atom i' in group I and atom j' in group J. Center of mass coordinates are often used to calculate the smoothing function distance; in this case the second term in (6a) must also be used in the calculation of the derivatives. This additional term complicates the programming of the derivative since E_{IJ} is the total group-to-group potential energy and therefore the nonbonded list must be looped over twice in order to calculate the E_{IJ} first, and then to calculate the forces using the E_{IJ}.

The calculation of the nonbonded interactions is by far the most time consuming part of the computation. On vector processors the efficiency is determined by the ability of the compilers to vectorize the code. The main roadblock to vectorization is conditional execution of code. In the case of protein/water simulations this becomes the most difficult problem to overcome. The nonbonded interactions between atom (or group) I and atom (or group) J are calculated using partner lists which are updated at a frequency determined at run time. There are several decisions which must be made regarding the form of the nonbonded energy calculation function: (1) should periodic boundary conditions be applied on the distance, (2) should distances be calculated based on center of mass or on atomic positions for condition (1), (3) what type of dielectric approximation should be made (e.g., constant, distance dependent, exponential), (4) what type of cutoff should be used (atomic or neutral group)? The most efficient approach is to set up different subroutines for each method of calculating the nonbonded interactions so that the nonbonded pair loops have no conditional statements inside of them. Unfortunately, this leads to a large amount of redundance in the computer code. We have chosen to structure the code so that group versus atomic cutoffs decisions are made outside of all loops but each time through the atom loops decisions are made regarding (1) through (3) above. We are continuing to investigate new ways to store this information such that these decisions will be made outside of the nonbonded energy loops.

The relative timings of the various cutoff approximations differ depending on the system which is being modeled. Table I compares three methods which were used in these studies: atomic cutoff/no smoothing, atomic cutoff/smoothing and residue cutoff. For PTI in water, the relative timings are approximately equal for these three methods. This is due to the fact that nearly all interactions are of the water-water type (Table VI). For vacuum protein simulations the differences between methods are much more substantial. Residue cutoffs include more interactions and therefore take much more time than atomic cutoffs. Smoothing increases the calculation time because the smoothing function must be recalculated for each nonbonded interaction. With an atomic cutoff, the smoothing function must be calculated for each atom pair in the boundary zone; with a residue cutoff (i.e., for water-water interactions), the smoothing function must be recalculated for each residue pair (i.e., approximately 1/9th as often for water-water interactions). Actual timings on several different machines for the pure water and PTI in water simulations are also listed in Table I. The nonbonded energy and nonbonded list generation subroutines have been fully vectorized. The simulations run approximately 55 times faster on a Convex C2 and 350 times faster on a Cray Y/MP as compared with a VAX 11/780.

RESULTS AND DISCUSSION

Pure Water

The conservation of energy in a series of pure water simulations as measured by the fluctuations in the total energy relative to the average total energy and to the fluctuations in the kinetic energy are shown in Table II. These results provide a useful reference with which the simulations of solvated protein may be compared. We first comment on the accuracy with which the equations of

Table I. Comparative timings.

System	Cutoff distance	Atomic no smoothing	Atomic smoothing	Residue
Relative timings[a]				
PTI				
	9.0	1.0	1.40	2.42
PTI/Water				
	6.0		0.69	
	7.0		0.81	
	8.0	0.81	0.97	0.87
	9.0	1.0	1.20	1.05
	10.0		1.55	1.27
Actual timings (CPU-time in seconds)				
		Water[b]	PTI/Water[c]	
Cutoff distance		7.5	8.0	9.0
Machine				
Vax 11/780		4500.		
Silicon Graphics Iris 240		235.	7400.	8945.
Convex C2		81.	2898.	3245.
Cray Y/MP		13.	465.	514.

[a]In the two sets of protein simulations (in vacuum and in water), the cpu times are listed relative to the simulations for that system with a 9 Å atomic cutoff without smoothing.

[b]CPU times for 100 steps of molecular dynamics of 216 water molecules in an 18.6206 Å cubic periodic box. The nonbonded list was updated every 10 steps and the number of nonbonded interactions calculated per MD step was approximately 55000. Rattle constraints were applied. The cutoff was molecular with no smoothing.

[c]CPU times for 100 steps of molecular dynamics of PTI in water (912 protein atoms and 2943 water molecules) as described in the text. The nonbonded list was updated every 5 steps and the number of nonbonded interactions calculated per MD step was approximately 1,000,000 with the 8 Å cutoff. No smoothing was used for these two runs.

Table II. Pure water simulations.[a]

Step size (fs)	List update (fs)	Cutoff radius (Å)	Truncation scheme	Shake/rattle	$\langle \Delta E^2 \rangle^{1/2} / \langle E \rangle$ (%)	$(\langle \Delta E^2 \rangle / \langle \Delta KE^2 \rangle)^{1/2}$ (%)	Temperature (K)
H_2O							
1	1	8.0	no smoothing	y	0.123	20.2	299.64
1	1	8.0	smoothing	y	0.0065	1.03	296.83
1	1	8.0	shifting	y	0.0023	3.23	300.59
1	5	8.0	no smoothing	y	0.173	27.2	310.30
1	5	8.0	smoothing	y	0.0098	1.67	295.09
1	5	8.0	shifting	y	0.0068	1.05	302.16
1	1	9.0	no smoothing	y	0.0988	15.4	309.35
1	1	9.0	smoothing	y	0.0049	0.74	296.11
1	1	9.0	no smoothing	n	0.173	27.2	310.30
1	1	9.0	smoothing	n	0.0098	1.67	295.09
2	2	8.0	no smoothing	y	0.120	18.9	296.29
2	2	8.0	smoothing	y	0.0071	1.29	306.72
4	4	8.0	no smoothing	y	0.147	19.3	304.21
4	4	8.0	smoothing	y	0.028	4.2	296.89

[a]Smoothing parameters: with 8 Å cutoff smoothing is between 7.5 Å and 8.0 Å; with 9 Å cutoff smoothing is between 8.0 Å and 9.0 Å.

All runs: 2 psec of equilibration with temperature scaling; 1 psec data collection.

motion are integrated without the application of a smoothing function. The first row in Table II lists the results for a standard set of parameters frequently used in simulations of pure water systems: an integration step of 1 femtosecond, update of the nonbonded interaction list every step, and a nonbonded cutoff radius R_{cut} = 8 Å. Under these conditions and without smoothing the nonbonded interactions at the cutoff, the relative fluctuation in the total energy is 0.123% and the fluctuation relative to the kinetic energy is 20.2%. These values are considered to be rather large for pure water simulations. The results with a smoothing function show substantial improvement over the

results with abrupt truncation. Using the standard set of parameters, the relative fluctuation in the total energy (0.0063%) and fluctuation in kinetic energy (1.03%) are both decreased by a factor of ca. 20 when a smoothing function is applied at the cutoff boundary. It is of interest to note that there is only a small increase in the error when the list update is decreased by a factor of 5 to once every 5 femtoseconds (step size 1 femtosecond). The frequency with which the nonbonded list is updated is determined by the average atomic displacement per step; for water, an update frequency of 5 femtoseconds appears to be a reasonable choice.

We have investigated the effects of a shifting function on the fluctuations in the total energy during the pure water simulations. There is a modest decrease in the fluctuations in the total energy as compared with the smoothing function results. The increased stability is obtained at the expense of introducing a significant change in the pair potential energy of the model. Since the use of a shifting function necessitates reparameterization of the pair potentials, and because it results in only a small improvement over a smoothing function in the integration of the equations of motion, the use of a shifting function appears to be unwarranted.

Results are also listed in the table for simulations with a flexible water model developed by Levitt[9] basd on the SPC and TIPS models.[20,22] This model includes explicit bond stretching and bending potentials and uses partial charges on the atomic centers which are slightly reduced. For this model the relative fluctuations in the total energy and kinetic energy were 0.0098% and 1.67% respectively, with a 9 Å nonbonded cutoff. These values are comparable to those obtained with the rigid SPC water potential.

α-Helical Decaglycine in Vacuum

α-helical decaglycine is a simple model which has been used previously in molecular dynamics studies of the properties of α helices.[23,24] The model has 76 atoms. The results of several 1 ps simulations under varying conditions are listed in Table III. As for the pure water simulations, the use of a smoothing function has a large effect on the conservation of energy in α helix simulations. With smoothing, the relative fluctuations in the total energy and kinetic energy are about 20 times smaller than the fluctuations without smoothing. When a smoothing function is used, the ratio of the fluctuations in total energy to kinetic energy varies between ca. 1–4% for the un-

Table III. Decaglycine vacuum simulations.[a]

Step size (fs)	List update (fs)	Cutoff radius (Å)	Truncation scheme	Shake/rattle	$\langle \Delta E^2 \rangle^{1/2} / \langle E \rangle$ (%)	$\langle \langle \Delta E^2 \rangle / \langle \Delta KE^2 \rangle \rangle^{1/2}$ (%)	Temperature (K)
$(Gly)_{10}$ Zwitterionic end groups							
1	10	8.0	no smoothing	n	20.3	109.1	304.1
1	10	8.0	smoothing	n	0.81	4.1	296.4
1	10	8.5	extended list	n	4.88	2.43	297.8
1	—	∞	—	n	0.34	2.7	318.4
2	20	8.0	no smoothing	n	22.5	110.9	264.5
2	20	8.0	smoothing	n	2.84	18.0	311.7
2	20	8.0	extended list	n	6.70	11.4	308.6
2	—	∞	—	n	1.87	13.41	310.6
1	10	8.0	no smoothing	y	16.8	115.5	324.4
1	10	8.0	smoothing	y	0.93	4.5	303.5
1	10	8.5	extended list	y	0.006	0.57	286.5
1	—	∞	—	y	0.533	0.74	300.5
2	20	8.0	no smoothing	y	18.3	115.4	277.0
2	20	8.0	smoothing	y	3.9	16.1	354.7
2	20	8.0	extended list	y	0.32	2.8	306.2
2	—	∞	—	y	1.7	3.0	321.3
$(Gly)_{10}$ Neutral end groups							
1	10	8.0	no smoothing	n	23.2	74.4	292.0
1	10	8.0	smoothing	n	1.10	3.5	318.8
1	—	∞	—	n	0.36	2.3	305.8
1	5	8.0	no smoothing	n	19.8	65.9	274.4
1	5	8.0	smoothing	n	1.3	2.7	302.9
1	1	8.0	no smoothing	n	14.0	64.7	301.1
1	1	8.0	smoothing	n	0.4	2.2	331.7

[a]Smoothing parameters: Smoothing between 7.5 and 8.0 Å for 8.0 Å cutoff; extended list: Smoothing between 7.5 and 8.0 Å with the list generated at 8.5 Å; infinite: All nonbonded interactions included (no cutoff) All simulations: Equilibration of the structure was provided by 1000 steps of conjugate gradient minimization followed by 2 psec of equilibration with molecular dynamics using temperature scaling (relaxation time was 10 times the time step); 1 psec data collection.

charged decaglycine simulations, which is similar to the values calculated for the pure water simulations. The ratio of the fluctuations in the total energy to the average total energy however is on the order of 1%, which is considerably larger than the corresponding values obtained in the pure water simulations using a smoothing function. This effect is in part due to the fact that the average total energy for the decaglycine simulations (20 Kcal/mol) is considerably smaller than the average total energy in the pure water simulations, and that fluctuations in total energy do not scale simply with the total energy.

It is clear from the results shown in Table III that there are errors in the integration unrelated to the truncation of long range interactions. This can be seen in the results of simulations for which all nonbonded interactions between all pairs of atoms in the molecule (2400 interactions) were calculated at every step. These simulations correspond to the use of an infinite cutoff radius. For the simulation without bond length constraints and using an infinite cutoff the error relative to the kinetic energy fluctuations is 2.7%; this value is reduced to 0.74% when bond length constraints are applied. The error relative to the total energy is ca. 0.5% with the infinite cutoff.

The conservation of energy in the neutral decaglycine simulations is similar to the results obtained with zwitterionic form. In all the α-helix simulations (and PTI in vacuum as well) a distance dependent dielectric has been employed. This large (but artificial) screening of the electrostatic interaction makes it much easier to integrate the equations of motion with a truncated electrostatic potential. For example, at 8 Å separation, the discontinuous change in the potential between two charged atoms which move across the boundary is 40 Kcal/mol when $\varepsilon = 1$, as compared with 5 Kcal/mol when $\varepsilon = R$. The actual discontinuous changes in the total energy will be even smaller because (1) the charge on a residue is partitioned among several atoms and (2) interactions between atoms of like charge tend to cancel those of opposite charge. Still, it is important to recognize that when solvent is included in protein simulations and therefore dielectric effects are being simulated explicitly, the coulomb interactions between charged groups at standard cutoff distances are very large.

As shown in Table III, the application of constraints to bond lengths does not have a significant effect on the conservation of energy with a choice of stepsize up to 2 femtoseconds. Presumably, this is due to the fact that the major portion of the observed error is associated with artifacts which arise from the use of a nonbonded list with cutoff. When the list update interval is varied between 1 and 10 femtoseconds, the error in the integration remains acceptably small ($\langle \Delta E^2 \rangle^{1/2} / \langle \Delta KE^2 \rangle^{1/2}$ less than 5%); when the list is updated less frequently than once every 10 femtoseconds, the error grows rapidly ($\langle \Delta E^2 \rangle^{1/2} / \langle \Delta KE^2 \rangle^{1/2}$ greater than 10%).

In summary, for α-helical decaglycine in vacuum, acceptably small errors in the integration of the equations of motion are obtained when an 8 Å cutoff for the nonbonded interactions is used, the nonbonded list is updated every 10 steps (stepsize 1 femtosecond) and the nonbonded interactions are tapered at the cutoff with a smoothing function.

Protein (Pancreatic Trypsin Inhibitor) in Vacuum

The results of the PTI in vacuum simulations are presented in Table IV. In these simulations PTI has a net charge (+6) and solvent screening effects are modeled using a distance dependent dielectric. In the equilibration stages of the simulations, we performed energy minimization of the X-ray structure and room temperature molecular dynamics with temperature scaling. The use of a smoothing function has a very small effect on the minimization; energy refined structures with and without smoothing remained close (rms deviation <0.9 Å) to the crystal structure. Also we note that in a trial simulation lasting 35 ps with temperature scaling, the rms deviation between the final structure and the crystal structure was 1.38 Å. The vacuum simulations for protein (with temperature scaling) sample conformations close to the crystal structure for many tens of picoseconds. Even though these vacuum simulations are inadequate for many kinds of studies (i.e., thermodynamic perturbation simulations), they provide a useful and cost effective approach for other kinds of modeling, such as the generation of macromolecular structures with experimentally derived distance constraints.

The magnitudes of the fluctuations in total energy relative to the average total energy in the simulations without temperature scaling are slightly larger than the values obtained in the decaglycine simulations. Again, the smoothing function has a very large effect on the fluctuations in the total energy. Without smoothing, the error relative to the total energy (ca. 25%) and to the fluctuations in kinetic energy (>100%) are unacceptably large. The smallest integration errors are obtained for simulation parameters which include a 9 Å atomic cutoff with smoothing, step size of 1 fs, and list update interval of 5 steps. In this simulation the error relative to the total energy is 0.59% and relative to the kinetic energy is 5.2%. We have completed one vacuum PTI simulation using a residue cutoff. In this

Table IV. PTI in vacuum simulations.[a]

Step size (fs)	List update (fs)	Cutoff radius (Å)	Truncation scheme	Shake/rattle	$\langle \Delta E^2 \rangle^{1/2} / \langle E \rangle$	$\langle \Delta E^2 \rangle / \langle \Delta KE^2 \rangle)^{1/2}$ (%)	Temperature (K) (%)
PTI (vacuum)							
1	5	9.0	smoothing	y	0.59	5.2	294.9
1	5	9.0	no smoothing	y	26.3	118.9	295.6
1	10	9.0	smoothing	y	4.51	19.2	305.1
1	10	9.0	no smoothing	y	24.5	115.4	298.4
1	10	8.0	residue[a]	y	1.81	13.8	301.1
1	5	8.0	smoothing	y	1.13	18.7	303.3
1	5	8.0	no smoothing	y	31.9	125.7	292.1
1	5	8.0	smoothing[b]	y	4.0	21.7	300.5
1	5	8.0	smoothing[c]	y	0.4	19.4	296.9

Equilibration process: 1. Minimization—2000 steps of Conjugate Gradient (CG) 2. Molecular Dynamics initial temperature 10 K, time step = 0.5 fs. 5 ps with temperature scaling to 298 K, 0.2 fsec relaxation time. 3. Equilibration with 1 fs time step for 5 ps, temperature scaling to 298 K with a 0.01 ps relaxation time 4. statistics gathered over 1 psec with no temperature scaling smoothing: 7.5–8 Å for 8 Å cutoff; 8–9 Å for 9 Å cutoff.

[a]stages i, ii, equilibration 10 psec, 1 fs time step and 0.01 relaxation time. Residue type cutoff: infinite cutoff for charged residues, 8 Å for other residues.

[b]weight of 0.75 on electrostatic terms (hydrogen bonds full weighting).

[c]weight of 0.75 on all electrostatic terms (including hydrogen bonds).

simulation two residues were included on the nonbonded pair list if any two atoms within the residues were closer than 8 Å apart. The use of a residue cutoff criterion leads to a signifcant decrease in the fluctuations in the total energy; the relative error in the integration is comparable to the values obtained with an atomic cutoff together with a smoothing function. Loncharich and Brooks have compared the effects of varying the long range cutoff on the structures and fluctuations in a series of molecular dynamics simulations of myoglobin in vacuum.[25] They report that the use of a neutral-group-based cutoff and long cutoff distance most closely reproduce structural and dynamical results of corresponding simulations of myoglobin in vacuum which included all nonbonded interactions explicitly.

Protein (Pancreatic Trypsin Inhibitor) in Water

The results of the PTI in water simulations are presented in Table V. In these simulations the net (+6) charge on PTI is neutralized by the addition of 6 Cl^- counterions and the dielectric constant is set to 1 in the electrostatic potential as water is

Table V. Simulations of PTI in water.

Step size (fs)	List update (fs)	Cutoff radius (Å)	Truncation scheme	Shake/rattle	Equilibration conditions	$\langle \Delta E^2 \rangle^{1/2} / \langle E \rangle$ (%)	$\langle \Delta E^2 \rangle / \langle \Delta KE^2 \rangle)^{1/2}$ (%)	Temperature (K)
PTI (in water)								
1	5	8.0	no smoothing	y	a	0.811	447.0	320.6
1	5	8.0	no smoothing	y	b	0.603	367.	320.4
1	5	9.0	no smoothing	y	d	0.2987	244.	302.1
1	5	8.0	smoothing	y	c	0.055	51.1	313.5
1	5	9.0	smoothing	y	c	0.0018	1.24	302.3
2	10	9.0	smoothing	y	c	0.0325	17.27	304.4
1	5	10.0	smoothing	y	c	0.00068	0.439	300.2
2	10	10.0	smoothing	y	c	0.0022	2.047	302.0
1	5	8.0	residue/nosm	y	d	0.0393	107.00	299.1
1	5	9.0	residue/nosm	y	d	0.034	105.21	301.6
1	5	10.0	residue/nosm	y	d	0.029	31.01	298.7
1	5	8.0	smoothing	y	e	0.0013	0.79	300.0
1	5	9.0	smoothing	y	e	0.00067	0.40	299.7

Equilibration process: 1. Minimization—500 steps of Conjugate Gradient (CG) 2. Molecular Dynamics initial temperature 10 K, time step = 0.5 fs. 1 ps with temperature scaling to 298 K, 0.02 fsec relaxation time. 3. Equilibration with 1 fs time step, temperature scaling to 298 K with 0.01 ps relaxation time 4. collection of statistics for 1 ps unless otherwise indicated Total equilibration times for stage 2 a–e.

[a]4 ps equilibration (1 fs time step).

[b]6 ps equilibration (1 fs time step).

[c]8 ps equilibration (1 fs time step).

[d]1 ps equilibration beginning with b (no smoothing) statistics over 200 fs in stage 4.

[e] Charge on the chloride ions was switched to −0.50, equilibration for 1 ps with temperature scaling.

included explicitly. In the solvated protein simulations, it was necessary to equilibrate the system with temperature scaling for times up to 8 ps before initiating the constant energy portion of the simulations. With insufficient equilibration, the temperature of the system rose by as much as 100 K when temperature scaling was turned off.

When an atom based cutoff criterion is used without smoothing, the ratio of the fluctuations in the total energy to the fluctuations in the kinetic energy is unacceptably large. For example, with an 8 Å cutoff, a stepsize of 1 fs and a nonbonded list update every 5 steps, the ratio of the root mean square fluctuations in the total energy to kinetic energy is greater than 400%. The very large fluctuations in the total energy are likely due to the effects of truncating the coulomb interactions at relatively short range in this ionic system. It is clearly not possible to calculate thermodynamic quantities from statistical mechanical fluctuation formulas with any accuracy in these simulations. Despite this, we note that the total energy is still conserved to better than 1% over the course of the simulation. In contrast, the PTI simulations in vacuum without smoothing exhibited much larger fluctuations in the total energy relative to the average total energy ($\langle \Delta E^2 \rangle^{1/2} / \langle E \rangle \cong 25\%$). These results reflect the fact that the total energy and the fluctuations in the kinetic energy scale differently with the number of atoms, so that when the fluctuations in the total energy are scaled by each of these quantities, the ratios will scale differently. It is instructive to decompose the total energy and other energy quantities in the solvated PTI simulations into contributions from the water-water, the protein-protein, and the protein-water interactions. These simulations included one protein molecule (912 atoms, including all hydrogens), and 2,943 water molecules (8,829 atoms). For this energy component analysis the six chloride ions were partitioned as solute (protein) atoms. The decomposition of the total energy into components for one such simulation (with smoothing) is displayed in Table VI. The average total energy is −30,173 Kcal/mol in this simulation, while the root mean square fluctuation in the total energy over 1,000 steps is 4.4 Kcal/mol. Partitioning the average potential energy into components, ca. 80% of the energy arises from the water-water interactions, ca. 14% comes from the intramolecular energy of the protein, while the interaction between the protein and solvent accounts for ca. 6% of the average potential energy in the system.

A substantial improvement in the accuracy with which the equations of motion are integrated is obtained using either an atomic cutoff with smoothing function or a residue cutoff. The results obtained with these boundary conditions are also listed in Table V. While the residue based cutoff scheme conserves energy better than the atom based method without smoothing, the fluctuations in the total energy are still much greater than those obtained with a smoothing function and atom cutoff. With a 9 Å smoothed, atom-based cutoff, the fluctuation in the total energy is 0.01% relative to the average total energy and 1.24% relative to the fluctuation in the kinetic energy. The fluctuation in the total energy is substantially reduced by using a 9 Å cutoff instead of an 8 Å or shorter cutoff. Using a 10 Å cutoff with smoothing, the fluctuations are reduced to one third of the 9 Å smoothing results. We suspected that the large decrease in the fluctuations in the total energy which occur when the cutoff is increased from 8 Å to 10 Å involved ionic interactions with the six chloride counterions which are included in these solvated PTI simulations and to test this we have completed two simulations in which the charge on the chloride ions was reduced to −0.50. The system was equilibrated for 1 ps following the reduction in the chloride charge and statistics were gathered over an additional 1 ps. The results of the two simulations with the reduced chloride charges using cutoffs of 8 Å and 9 Å are listed at the bottom of Table V. Reducing the charge on the chloride ions leads to a substantial reduction in the fluctuations in the total energy. Of course, reducing the charges on the counterions does not have a physical basis and will lead to artificial changes in the simulated thermodynamic properties. While it is possible to improve the mathematical stability of simulations by reducing the charges on ions or atoms, such ad hoc procedures reduce the utility of these simulations for exploring the properties of real biomolecules in solution.

We have examined the effect of increasing the timestep on the fluctuations in total energy;

Table VI. Averages of properties for a typical run. PTI/H_2O 9 Å cutoff with smoothing. Averages over 1 psec.

Temperature	302.04
Average total energy	−30173.
Average kinetic energy system	5842.2
Average potential energy	−36015.
Bond, angles, torsions	3691.7
Lennard—Jones	
PTI-PTI	−154.6
PTI-water	−50.8
water-water	5409.6
Hydrogen-bond (10–12)	
PTI-PTI	−8.7
Electrostatic	
PTI-PTI	−8516.5
PTI-water	−1988.4
water-water	−34397.

the results are listed in Table V. With a 9 Å cutoff, the fluctuation in the total energy increase from 0.002% to 0.03% relative to the total energy and from 1% to 17% relative to the fluctuations in the kinetic energy when the stepsize is doubled from 1 fs to 2 fs. These values are still considerably smaller than the corresponding values for simulations with a 1 fs timestep and an 8 Å cutoff. The increase in computation time going from an 8 Å cutoff to a 9 Å cutoff is ca. 20%, therefore our results suggest that to achieve a prescribed level of accuracy most efficiently, it is better to choose a longer time step coupled to a longer range cutoff of the nonbonded interactions. Finally, we note that with the largest cutoff studied (10 Å), the increase in the energy fluctuations with stepsize is close to the global error of the RATTLE algorithm, which is of the order of the square of the timestep.[19]

SUMMARY

We have performed simulations to investigate the effects of various simulation parameters and truncation schemes on the conservation of energy in molecular dynamics simulations. Studies were performed on four systems: pure water, α-helical decaglycine in vacuo, PTI in vacuo, and fully solvated PTI. The equations of motion were integrated using Andersen's velocity version of the Verlet algorithm with internal constraints (the RATTLE algorithm). The temperature of all simulations was approximately 300 K.

All of the pure water simulations employed a molecular (residue-based) cutoff criterion. For a cutoff radius of 9 Å or less, unacceptably low levels of energy conservation were seen when an abrupt truncation of the potential was used. Introduction of either a smoothing or shifting function to eliminate the abrupt truncation led to acceptable results. Neither increasing the nonbonded list update interval from 1 to 5 fs nor increasing the integration stepsize from 1 to 2 fs had a substantial effect on energy conservation; the choice of a rigid or flexible water model also had little effect (for a 1 fs integration step).

Most of the simulations of α-helical decaglycine in vacuo employed an atom-based cutoff criterion, and those that did not used no cutoff at all; a distance-dependent dielectric was used in all simulations. Again, abrupt truncation of the potential led to unacceptable results, while introduction of a smoothing function at the cutoff radius produced levels of energy conservation comparable to that seen in the pure water simulations. Some lack of energy conservation is unavoidable, and is seen even in simulations which use no truncation of the potential. Thus it was found that most of the error introduced by the (smoothed) potential truncation comes from not updating the nonbonded list frequently enough. By either updating the list at every step or using an extended nonbonded list, levels of energy conservation comparable to that found with no potential truncation were achieved. However, even without these measures, acceptable energy conservation is seen with list update intervals of as much as 10 fs. Introduction of bond length constraints enhances energy conservation, but only in those simulations where the potential truncation affected the energy conservation very little (i.e., those with no truncation or which used an extended nonbonded list).

The simulations of PTI—both in vacuo and solvated—indicate that for the protein a residue based cutoff without a smoothing function conserves energy much better than a nonsmoothed atom based cutoff, and can sometimes lead to levels of energy conservation comparable to those seen with a smoothed atom-based cutoff (as long as a molecular cutoff is used for the solvent water molecules, as was done in all the simulations here). We find the most favorable simulation parameters to choose for simulations of solvated proteins—from the standpoint of minimizing the error with which the equations of motion are integrated—is an atomic cutoff at relatively large distances (9 Å or greater) with the use of a smoothing function. The residue based cutoff scheme leads to a considerably larger ratio of the fluctuations in total energy to fluctuations in kinetic energy; although the relative error in the total energy is still small. The advantages of a residue based cutoff are twofold: (1) there is a saving in computer storage requirements associated with the much smaller size of a residue (as opposed to atomic) pair list and (2) the interaction potential is not distorted by the application of an artificial (smoothing) function. The residue based cutoff has the disadvantage that the cutoff boundary around any given atom is irregular. With respect to the cpu time required per cycle, both cutoff schemes are roughly comparable. Unfortunately, there has been little analysis done on the effects of different nonbonded cutoff methods on the average structural or dynamic properties of proteins in molecular dynamics simulations. Some results for molecular dynamics simulations of proteins in vacuo have been reported.[25] We are currently analyzing these effects in a series of solvated PTI simulations. The goal is to delineate conditions for simulating proteins in solution which maximize the accuracy of the simulations while minimizing the computational cost.

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