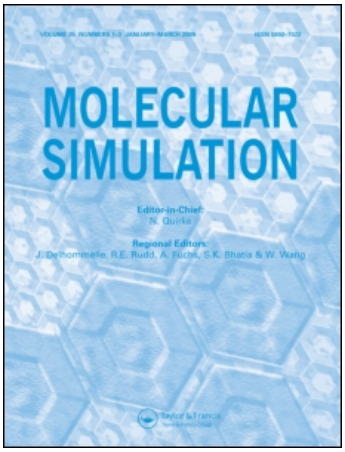

Molecular Simulation

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713644482

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To cite this Article Gallo, Marco T. , Grant, Barry J. , Teodoro, Miguel L. , Melton, Julia , Cieplak, Piotr , Phillips Jr., George N. and Stec, Boguslaw(2009) 'Novel procedure for thermal equilibration in molecular dynamics simulation', Molecular Simulation, 35: 5, 349 — 357

To link to this Article: DOI: 10.1080/08927020802647272

URL: http://dx.doi.org/10.1080/08927020802647272

Molecular Simulation
Vol. 35, No. 5, April 2009, 349–357

Taylor & Francis
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Novel procedure for thermal equilibration in molecular dynamics simulation

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(*Received 24 September 2008; final version received 20 November 2008*)

We describe a simple novel procedure for achieving thermal equilibration between a protein and a surrounding solvent during molecular dynamics (MD) simulation. The method uniquely defines the length of simulation time required to achieve thermal equilibrium over a broad range of parameters, thus removing ambiguities associated with the traditional heuristic approaches. The proposed protocol saves simulation time and avoids bias introduced by the inclusion of non-equilibrium events. The key element of the procedure involves coupling only the solvent atoms to a standard heat bath. Measuring progress towards thermal equilibration involves simply monitoring the difference in temperature between the solvent and the protein. Here, we report that the results of MD simulations using the above procedure are measurably improved relative to the traditional approaches in terms of root-mean-square deviations and principal components analysis both indicating significantly less undesirable divergence.

Keywords: molecular dynamics; thermal equilibrium; solvent coupling

1. Introduction

Molecular dynamics (MD) simulations have become a widespread tool for investigating the dynamical properties of biomolecules [1–3]. The initial stages of most MD simulations include a thermal equilibration period, where the temperature of the system is brought into the range of interest [4]. This process typically entails coupling all atoms of the system to a thermal bath at a given temperature by a prescribed computational procedure [5]. This action ensures that all atoms of the system will show a ensemble behaviour and decrease the probability that localised fluctuations in energy will persist throughout the simulation.

As thermal equilibration is an essential step in the majority of MD simulations, several different equilibration techniques have been proposed including variations in coupling schemes and gradual heating and quenching of the system [6]. Despite the expenditure of substantial effort there is no definitive algorithmic recipe for achieving thermal equilibration for a particular set of parameters used in a given simulation. The general practice is based on the heuristic approaches with no standard method available to estimate the length of time required to reach thermal equilibrium. Notwithstanding, it is commonly assumed that the initial equilibration (kinetic temperature) leads to a well-sampled (or dynamically equilibrated) system, to which the ergodic assumption is applicable. However, ergodicity is rarely proven and in many cases is practically unprovable. Therefore, the relationship between a single dynamic simulation and the system's thermodynamic behaviour remains tenuous in contradiction to the popular belief [7].

The subject of this paper is the attainment and assessment of thermal equilibration as defined by the classical kinetic theory of gases [8]. This theory states that the thermal equilibrium is reached when the average kinetic energy of two gases is equal, or equivalently, the temperatures of two systems in contact are equal (where kinetic energy is equivalent to temperature through the formula $\langle E_K \rangle = (1/2)nkT$, for a system with '$n$' degrees of freedom).

The distinction between thermal–kinetic and dynamic-long time equilibration can be illustrated with the example of a glass particle (non-ergodic phase that is never dynamically equilibrated at finite time) thermally equilibrated in water. The above example provides a clear distinction between the thermal and dynamic equilibrium and highlights that one can achieve short-term thermal equilibration without reaching the equilibration in a dynamical sense. In this work, we are only concerned with thermal equilibration in the kinetic sense. However, it should be noted that thermal equilibration is a necessary prerequisite for the attainment of dynamical equilibration.

The classical method for equilibration (i.e. discarding the initial portion of the simulation to provide stability of

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ISSN 0892-7022 print/ISSN 1029-0435 online

DOI: 10.1080/08927020802647272
http://www.informaworld.com

the averages of dynamical entities such as energy) has proved to be quite successful and is commonly used despite its heuristic foundation [9–11]. However, one can argue that an artificial computer model of the heat bath is replacing a physical bath that in macromolecular systems is comprised of solvent molecules. It can also be argued that a more physical thermal bath model is provided by a thermally equilibrated solvent. There are several motivations for investigating a solvent as a heat bath. Not only can it be considered a more realistic physical representation of the heat bath (solvent is in direct contact with the surroundings not the protein atoms), but also the speed of equilibration for solvent (typically water) is well determined by MD and Monte Carlo simulations. In numerical simulations, an isolated system of weakly interacting particles (like water molecules) achieves equilibration in around 50 ps of MD or ~5000 steps of Monte Carlo simulation [12]. In addition, equilibration through the solvent offers possibilities for investigating the energy transfer rates in off-equilibrium phenomena (for instance, rapid heating) [13].

In order to determine when a solvated macromolecule has reached thermal equilibrium we have designed a simple computational procedure. Guided by the definition taken from the kinetic theory of gases, we postulated that the surrounding solvent constitutes a more realistic heat bath. The process of reaching thermal equilibrium, while heating up only the solvent atoms, is monitored by comparing the separately calculated temperature (i.e. average kinetic energy) of the macromolecule with the temperature of the surrounding solvent. When the two temperatures reach the same value thermal equilibrium is reached (achieved?). The numerical implementation of the above protocol has the advantage of providing a unique measure of time required for the equilibration to be completed (for a given set of parameters). The results of the simulations implementing this procedure showed important differences in the behaviour of the system compared with more traditional methods that suggests a number of additional advantages. These include low divergence from the original structure suggesting a much more stable simulation.

2. Materials and methods

2.1 The models

All simulations were carried out using the NAMD program [14]. The proposed equilibration method was incorporated into the NAMD submission scripts and the results were evaluated with XPLOR [15] and bio3d [16] programs. The design of the method makes implementation simple in any major MD package. In this report, we present the results of testing of our method in several simulations carried out on two protein models representing different architectures. Namely, the simulations have been performed for the $\alpha + \beta$ protein crambin [17] and the mostly random coil kingle-4 domain from the human plasminogen [18]. The crystallographic structures of both molecules were determined and refined to a resolution of 0.83 Å and 1.67 Å with an R factor of 0.09 and 0.143, respectively [17,18]. The structures were selected for their high quality in which they accurately represent the physical reality including robust disorder. Such structures were expected to be good probes for our methods because unmodelled disorder must inevitably lead to more structural divergence in MD simulations.

2.2 The MD procedures

Prior to simulation, the atomic structures were modified in order to represent a single dominant conformer, with lower occupancy disordered atoms and crystallographic waters deleted. Each structure was solvated along with neutralising counter ions using the standard procedures. Initially, protein atoms were fixed and the system energy minimised followed by 50 ps of MD simulation at 300 K. The heat bath was coupled to the solvent atoms as described by Berendsen [4] and the pressure was kept at 1 atm. Finally, the protein atom restraints were removed and a quenched energy minimisation was performed. Subsequent production-phase simulations were carried out for 1 ns with a 0.5 fs time step with resulting position and velocity information written every 0.5 ps.

During the main simulations we used the following protocol. The temperature was gradually raised from 0 to 293 K every 50 steps of the simulation and after reaching the desired temperature the simulation were continued for a further 1 ns. Two basic protocols of temperature control were evaluated: (i) coupling all atoms to the heat bath and (ii) coupling only water molecules to the heat bath. Both protocols were implemented with the Langevin (explicit solvent) and Berendsen methods of temperature control. Additionally, we evaluated the influence of using the SHAKE algorithm on the results obtained in our simulations.

2.3 Test for equilibration

To evaluate the equilibration progress, the average kinetic energy and corresponding temperatures were calculated for the protein and the solvent atoms separately with the XPLOR program [15]. As the final test of equilibration, we calculated the time average of the kinetic energy for the centre of mass of the protein. This average kinetic energy converted to temperature should be equal to that of solvent and that of the protein (i.e. also equilibrated). The instantaneous average temperature for the protein and the solvent were plotted together with the instantaneous temperature of the centre of mass of the protein. Finally, the time averages were calculated for the instantaneous temperatures of the protein

and the solvent including their standard deviations (SDs). The SDs in a well-equilibrated system should obey the fluctuation–dissipation theorem [8]. Indeed, comparison of the instantaneous temperatures (Figure 1) for various components of the system shows that the lowest variation is for the solvent, is larger for the protein (fewer atoms) and is largest for the centre of mass (a single variable).

2.4 Trajectory analysis

In order to gain a deeper insight into the dynamical behaviour of the systems equilibrated by different methods we investigated the spatio-temporal behaviour of individual simulations by calculating the root-mean-square deviations (RMSD) from the initial structure and performing principal components analysis (PCA) on the combined trajectories for each system. As expected, from the significant differences in the architectural features of the two proteins studied we observed larger positional divergence of the space trajectory of kringle than for tightly folded crambin. In order to provide the computational base for principal components (PC) calculation we extracted coordinates for the common core of the protein by analysing the available Protein Data Bank (PDB) structures for kringle domains, as well as for crambin by iterative RMSD analysis [16]. The residues of the common

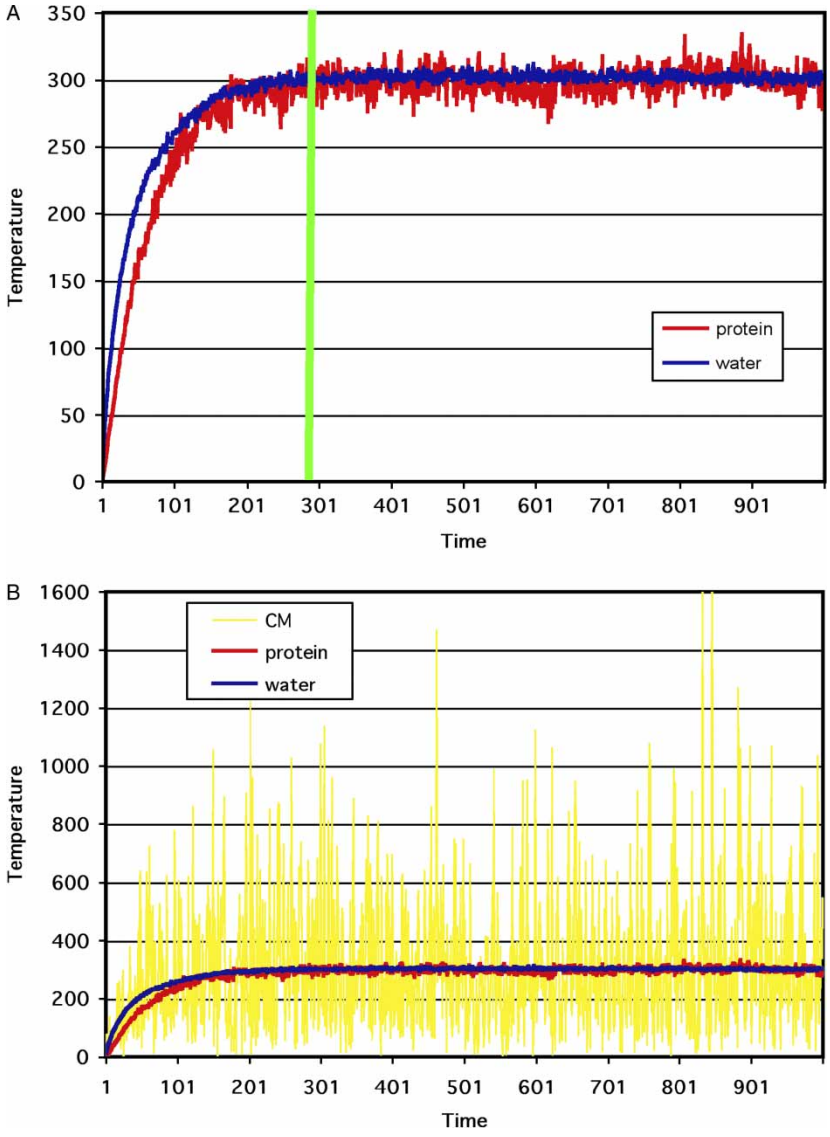

Figure 1. The time dependence of instantaneous average temperatures obtained during simulation of the kringle-4 domain of human plasminogen using Langevin coupling to water atoms. The protein atoms are in red, water atoms in blue and an instantaneous temperature for centre of mass in yellow. (a) The plot represents the water and protein atoms. The levelling of plots after an initial 250 ps of simulation indicated by the green vertical line defines the equilibration time. (b) All three temperatures (water, protein and centre of mass) are plotted.

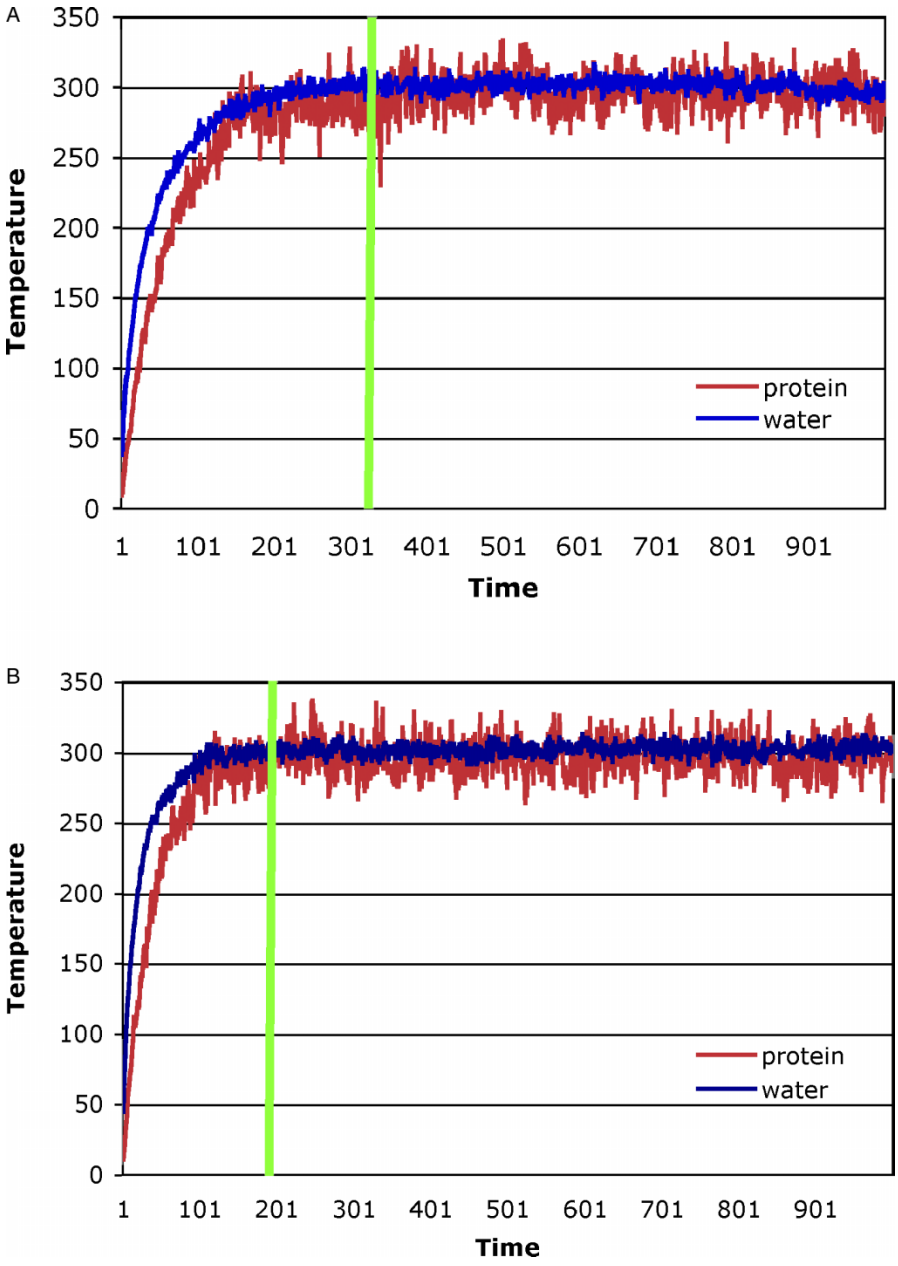

Figure 2. The time dependence of instantaneous average temperatures obtained during the simulation of crambin using Langevin coupling to water atoms. The protein atoms are in red and water atoms in blue. (a) The plot representing the water and protein atoms obtained in simulation with coupling constant 0.05. (b) The same as in (a) obtained in simulation with coupling constant 0.1. The equilibration time is marked by the green vertical line.

core were used to superpose all the frames of the MD trajectories and the full positional covariance matrix was calculated. The dominant eigenvectors corresponding to the largest eigenvalues were extracted, the trajectory was then projected onto the two-dimensional space spanned by the components and plotted (Figures 3 and 4). The PCA is based on the assumption that only a few dominating modes account for most of the trajectory variation. This expectation is well supported by the vast body of previous results.

3. Results and discussion

3.1 Comparison of equilibration protocols

Thermal equilibration is an important element of most MD simulations. It is designed not only to remove residual experimental errors but also to create a representative dynamical state of the molecule suitable for the initiation of a simulation at the given thermodynamic parameters (T, p). The kinetic or thermal equilibration is frequently confused with thermodynamic equilibration

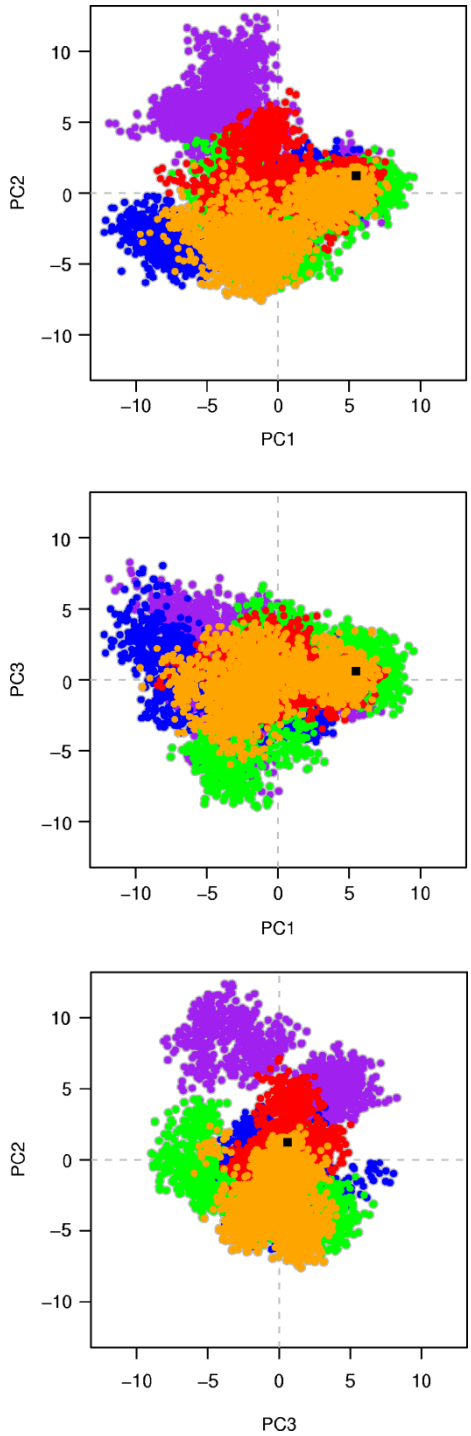

Figure 3. The plot of the 1 ns trajectory of the same simulation as in Figure 1 (kringle) plotted in the space of three dominant PCs calculated for Cα atoms. The purple dots represent the simulation with heat bath coupling by the Berendsen method to all atoms, the blue dots represent the Langevin coupling to all atoms, the red dots represent the Berendsen coupling to the solvent atoms, and the orange dots the Langevin coupling to the solvent atoms only. The green dots represent the Berendsen coupling to the solvent atoms but with use of the SHAKE procedure. The black dot represents the position of the X-ray structure in common PC space.

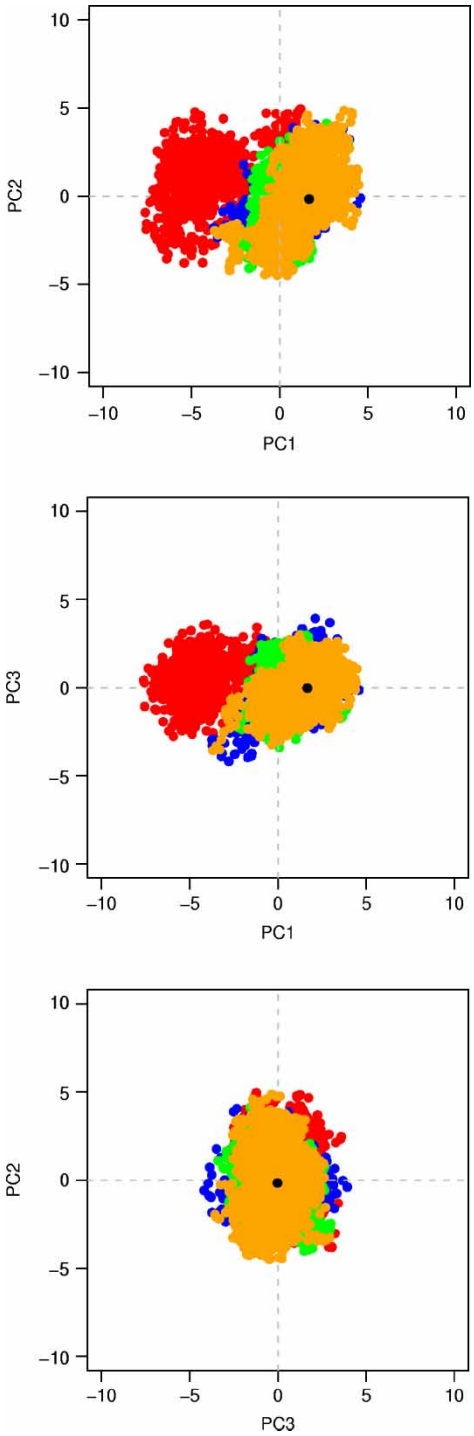

Figure 4. The plot of the 1 ns trajectory of the same simulation as in Figure 2(b) (crambin) plotted in the space of three dominant PCs calculated for Cα atoms. The red dots represent the simulation with heat bath coupling by the Berendsen method to all atoms, the blue dots represent the Berendsen coupling to the solvent atoms, the green dots represent the Langevin coupling to the solvent atoms with the use of SHAKE, and the orange dots represent the trajectory obtained in the simulation with the use of the Langevin coupling to the solvent atoms only. The black dot represents the X-ray structure positioned in PC space.

(here termed dynamic equilibration). Proteins are believed to be glassy systems; therefore, the only reasonable expectation is to achieve thermal equilibration at the beginning of the simulation and follow the time evolution of the system over subsequent iterations.

Below, we compare the effectiveness of the proposed protocol with traditional methods for thermal equilibration on two systems, kringle and crambin. Specifically, we compare the simulations carried out with a heat bath coupled to (i) all atoms and (ii) the solvent atoms only, using the standard Berendsen or Langevin temperature control. The results of analyses of trajectories obtained with and without SHAKE are also discussed.

In Figures 1 and 2 (kringle and crambin, respectively), we present a time plot of the average temperature for the protein and for the solvent atoms separately during which only the solvent atoms were thermally coupled to the heat bath using the Berendsen method. The average temperature of the protein increases with the raising temperature of the heat bath with the protein lagging behind the solvent. Because, the energy is transferred to the protein through the solvent, the protein temperature is significantly lower during the equilibration stage. The moment of equilibration is defined precisely, for any set of parameters, as the time needed for both data curves to reach the same level. The time of equilibration for kringle with the coupling parameter 0.1 is approximately 300 ps (Figure 1). The plot for traditional method with coupling to all atoms would be uninformative because both curves would overlap.

In order to obtain an independent measure of the time it takes (required?) to achieve equilibration we extracted the data for the centre of mass. As stated in Section 2, this additional degree of freedom must also be equilibrated. In Figure 1(b), we show the time behaviour of the temperature of the centre of mass in comparison to that shown in Figure 1(a). This additional parameter varies widely when viewed as a single variable but its time average proved to be highly informative allowing us to follow the equilibration more precisely. Even when the temperatures of protein and solvent components were equal, as is the case of coupling to all atoms, the average temperature of the centre of mass departed from other averages significantly by tens of degrees. Only in the protocol that uses equilibration through water the average kinetic energies of all components were equal (Figure 1(b)).

Similar observations were made in simulations performed on crambin, which has a more compact heat resistant structure (this is reflected in the PC projections—with a more compact spread of points, Figures 3 and 4). The presence of the helical elements connected by a disulphide bridge changes the internal rigidity as well as elastic properties of the protein. The time needed to achieve thermal equilibration with a coupling constant of 0.1 was significantly shorter than that for kringle ($\sim$210 ps). Additionally, when the coupling constant was changed to 0.05 the time was longer ($\sim$310 ps) and more similar to kringle (Figure 2(a) and (b)). This example demonstrates the dependency of the time needed to thermally equilibrate the system on the number of protein atoms involved in the simulation, the architecture of the protein, as well as to the coupling constant applied in the particular simulation. These parameters are system dependent indicating that different proteins will require different equilibration periods. Nevertheless, the times obtained here are qualitatively within the range recommended by existing heuristic methods.

These results suggest that previously observed artifacts, such as collective motion or the flying ice cube [19,20] artifact, may have originated because of incorrect energy redistribution. The heat bath coupling to all atoms disregards the differences between atoms that are covalently bonded in a single large molecule and the solvent atoms that equilibrate faster. As a result, the procedure disproportionately redistributes the energy to the centre of mass. In support of this claim we observed that in our simulations the kinetic energy of the centre of mass for the macromolecule was larger than expected when the heat bath was coupled to all atoms. Further studies are warranted to investigate this observation.

3.2 Evaluation of the equilibration procedures by PC analysis

To further investigate the spatial behaviour of the system we performed PCA of trajectory conformers as described in Section 2. PCA (sometimes called essential dynamics) [21] is a popular dimension reduction method used in an analysis of nonlinear systems. Briefly, a covariance matrix is built from the positions of Cα atoms of the molecule sampled every half a picosecond, after the removal of net translational and rotational degrees of freedom. The matrix is then diagonalised to yield the eigenvectors corresponding to the largest eigenvalues given the largest contribution to the displacement values calculated according to the displacements along the eigenvectors. In all our simulations, the three dominant eigenvalues captured more than 50% of the observed variance or mean square fluctuations within the trajectories. The projections on the three most dominant eigenvectors correspond to the components that dominate the trajectory; therefore, they represent the direction of the largest conformational changes. One might expect that in the relatively short time of our simulation (1 ns) and quite stable proteins, the spatial divergence should be limited. Actually, even much longer simulations usually do not lead to an adequate sampling of remote conformational states.

Analysis results for individual trajectories obtained using different equilibration methods are shown in Figure 3.

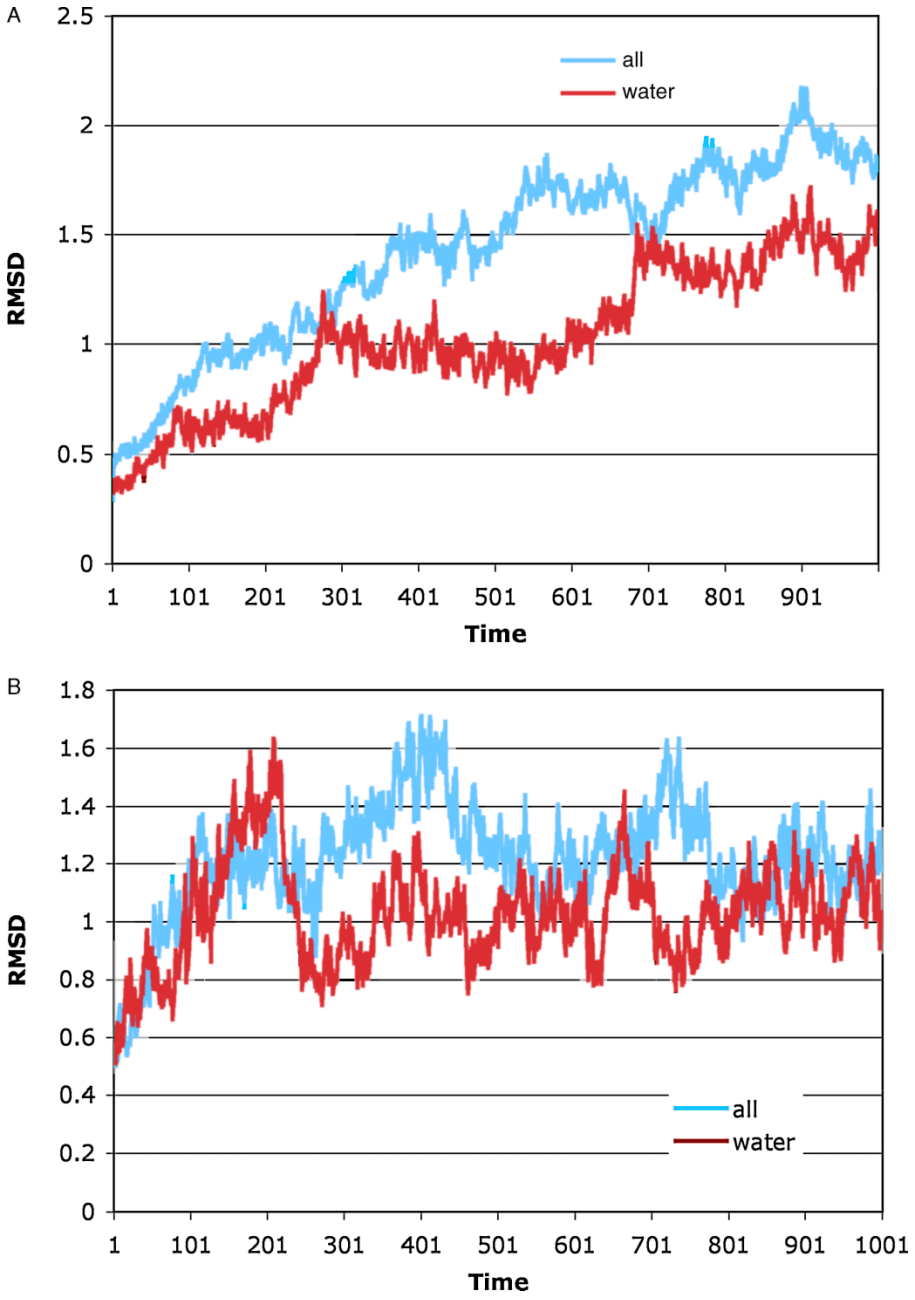

Figure 5. The time dependence of the RMSD calculated for all Cα during the 1 ns simulation carried out using the Berendsen coupling to all atoms (blue) and to the solvent atoms (red) for kringle (a) and crambin (b).

The figure represents (in different colours) five different trajectory files obtained from simulations, where the heat bath was coupled to (i) all atoms, (ii) the solvent atoms using a Berendsen approach and (iii) the solvent atoms applying a Langevin scheme. The last two coupling methods were also applied to analyse trajectories generated using SHAKE, cases (iv) and (v). For both kringle and crambin, the coupling of the heat bath to all atoms lead to larger divergence (almost 200% as measured by the spread of the trajectory in the dominant PC space) in the PC space regardless of the coupling method (Berendsen or Langevin). Additionally, the region swept was becoming disjointed with several sub-basins as can be seen by the purple and blue colour trajectories. The heat bath coupled to only solvent led to less overall trajectory divergence (Berendsen—red and Langevin—orange dots in Figure 3) in the space spanned by dominant PC when compared with trajectories obtained with a heat bath coupled to all atoms. This trend is also notable in the RMSD profiles in Figure 5. In particular, the trajectory space for the crambin

simulation is more uniform and centred around the X-ray structure (Figure 4). These features indicate a more uniform motion of the trajectory without obvious artifacts.

The direct comparison of the results presented in Figures 3 and 4 indicates that kringle, with a looser architecture is less conformationally restricted than crambin. It also indicates that the trajectory diverges more from the X-ray-determined structure (marked by the black dots in both figures). While crambin is represented by a single conformational state in the PDB, kringle has been crystallised in at least three conformational states. Therefore, the simulation is expected to diverge more. However, as stated above, the divergence using coupling only to the solvent atoms is significantly smaller than when all atoms are coupled to the heat bath.

It is also interesting to note that trajectories calculated with the SHAKE procedure resulted in more divergence in PC and Cartesian space (green and orange trajectories in Figure 3). The use of SHAKE also introduced an additional equilibration artifact, namely that the centre of mass temperature as measured by the average kinetic energy was always lower by several tens of Kelvin (~ 20 K) than that of the heat bath (~ 300 K). It is possible that whilst SHAKE reassigns the positions of hydrogen atoms it never correctly estimated the kinetic energy content of the system's hydrogen atoms. This creates an energy sink that is responsible for the lack of equilibrium. Therefore, the use of procedures such as SHAKE, despite their obvious advantages, may not be advisable in circumstances where thermodynamic quantities are to be evaluated.

The majority of the thermostats employed today rely on calculating the temperature of the system from the mean kinetic energy of its particles. However, this is not the only approach to calculate the thermodynamic temperature of a system, which can be evaluated solely from the knowledge of the configuration of a system (instantaneous atomic coordinates 'static snapshots' and not momenta 'dynamics') known as configurational temperature [22–24]. The configurational temperature is computed from the first and second derivatives of the intermolecular potential energy. In non-equilibrium studies such as non-equilibrium MD of decane undergoing Couette flow [25], at high-shear rates isokinetic thermostats yield higher intermolecular potential energies as the system heats up. On the contrary, configurational temperature thermostats takes into account all degrees of freedom, and prevents the internal degrees of freedom from heating up, and maintains the intramolecular potential energy of the system under shear close to its equilibrium value, in accordance with real experiments. Delhommelle and Petravic [26] also studied the shear thickening behaviour of colloidal particles using MD simulations that take into account the solvent explicitly. In order for the system to reach steady state they introduce a configurational temperature thermostat coupled only to the solvent atoms. This configuration thermostat accounts correctly for the dissipation of heat in the system. The configurational thermostat was convenient since it does not fix the amplitude of the streaming velocity, in contrary to the majority of other thermostats.

A configurational thermostat has been also used by Rathore et al. [27] to calculate successfully the density states of a 16-residue β-hairpin from the C-terminal fragment of protein G facilitating its use to study the biological systems.

The configurational temperature has been calculated experimentally from the macroionic interactions (pair potentials as inputs) in monolayers of charged colloidal silica spheres dispersed in water and confined between parallel plates [28].

It would be very interesting to further study how fast thermal equilibrium is reached in the systems studied in this paper by the application of configurational temperature thermostat coupled only to the solvent atoms and compare it to our thermal equilibration approach based on the kinetic energy temperature.

4. Summary and recommendations

In this paper, we presented a simple computational procedure for reaching thermal equilibration in MD simulations. The procedure relies on coupling of only the solvent atoms to a heat bath. This procedure provides an estimate of the time needed to reach thermal equilibration for a particular system and a set of parameters, by observing the convergence of the average temperature of the protein and the solvent atoms.

This novel method for equilibrating the system prior to production-phase simulation is simple to implement in major simulation programs. It has clear thermodynamic advantages such as allowing for the natural fluctuation of atoms in the macromolecule not coupled directly to the heat bath. By changing the coupling constant of the solvent to the external heat bath we can control the heat transfer rates to the macromolecule. In several schemes tested in the framework of Langevin dynamics, it appears to be the best behaving when considering the averages of kinetic variables and the overall sweep of the trajectory. The systems tested in this work equilibrated in less than 300 ps which fully corresponds to the widely used heuristic recommendation that advocates a rejection of the first $\sim 100-200$ ps. The results also indicated that the SHAKE procedure creates an artifact that is not consistent with accurate equilibration (or energy transfer through degrees of freedom). This algorithm should not be used in most circumstances.

This result suggests that the method of temperature control through the coupling through the solvent atoms shows significant advantages over heuristic methods

of equilibration used for macromolecular systems in MD simulations. Such a method provides three major advantages: (i) it algorithmically determines the equilibration time for the particular protein and selected set of parameters thus avoiding an unnecessary loss of trajectory segments, (ii) it provides a more 'physical' method for heat bath and energy transfer through the intervening media, and (iii) it allows to study phenomena away from thermal equilibrium such as heat transfer rates. We believe that the implementation of our method will lead to improved reproducibility of MD simulation results.

Acknowledgements

This work was supported by research grants from the NIH to B. S. (GM64481) and partially supported by a grant to the W. M. Keck Center for Computational Biology in Houston (2T15LM07093); M. T. G. is a CONACyT Researcher.

Note

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