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Molecular dynamics simulation using weak-coupling NOE distance restraining

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Summary

Application of the weak-coupling scheme to restrain the configurations of a molecular system to a set of NOE distance restraints is investigated using two test systems: (i) a 15-atom chain molecule with one distance restraint; and (ii) a protein molecule with hundreds of NOE distance restraints. Atom–atom distance restraining by the weak-coupling technique is possible, but this method does not produce as good results as the penalty function method normally used to maintain NOE distance restraints.

troduction

Restrained molecular dynamics (MD) simulations now ve a place as a standard tool for the refinement of ma-)molecular structures based on NMR data. At the heart the method is a means for persuading Q, some property the system, to reproduce a reference value Q^0, the quan- corresponding to the experimental measurement. Typi-ly, this is a distance derived from an NOE measurement dihedral angle derived from a ^3J coupling constant.
The standard method for enforcing distance restraints MD simulations is the addition of a restraining or ıalty function term V_{restr} to the physical potential ener-function V_{phys} (van Gunsteren et al., 1984; Kaptein et 1985). Usually this is done by defining V_{restr} so that artificial term added to the normal force field grows ıdratically as Q moves away from Q^0.
Although this has almost reached the level of dogma, gnores a wealth of experience in the broader field of) simulations. There is, in fact, a wide range of methods d to restrain various properties in the calculations (van nsteren et al., 1996). From this point of view, it might n that one should be able to take a method used to ple a thermodynamic quantity such as temperature apply it to guide a system to agree with some experi-ıtally measured quantity. In this vein, one might consider using a first-order relaxation equation to couple Q to a reference value Q^0. By another name, this approach is known as the weak-coupling method (Berendsen et al., 1984). It is routinely used to couple thermodynamic quantities such as temperature, pressure or chemical potential in MD simulations (Berendsen et al., 1984; Beutler and van Gunsteren, 1994) and is implemented in most simulation packages. It provides a simple but efficient way to maintain a property in a simulation.

The aim of this work was to see if the weak-coupling approach could be used in the context of structural refinement. That is, could one bypass a normal penalty function and simply use the first-order relaxation equation approach. We describe the introduction of the method into the equations of motion and its testing on a simple model system and a real polypeptide.

In parallel, the method was tested with experimental distances enforced as time averages. This reflects the fact that the spectroscopic measurements do not represent snapshots of a structure, but are time-averaged quantities (Torda et al., 1989,1990,1993). This was of interest, since it has been argued that time-averaged restraints yield a more realistic representation of the simulated molecule than instantaneous restraints (Pearlman and Kollman, 1991; Schmitz et al., 1992,1993; Pearlman, 1994a,b; Nanzer et al., 1994,1995). At the same time, it has been shown

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eviations: NOE, nuclear Overhauser effect; MD, molecular dynamics; PDB, protein data bank.

that time-averaged restraints can artificially increase mobility and cause local heating of the system (Pearlman, 1994a,b; Nanzer et al., 1995).

The two test systems were chosen so as to have very different properties. Firstly, we used a completely artificial system, a linear chain of 15 covalently bound carbon atoms (C-15) with one (end-to-end) distance restraint. This might be seen as a pentadecane chain in the united-atom representation. Secondly, as a more realistic case, we used a polypeptide, the 64-residue 627-atom N-terminal domain of chymotrypsin inhibitor 2 (CI-2) with 961 experimental NOE distance restraints. This second test molecule allowed an easy comparison with the penalty function method for distance restraining, since the latter method had been applied to the same molecule to analyse time-averaged distance-restrained MD simulations (Nanzer et al., 1995).

In particular, we wanted to address several questions. Firstly, could the weak-coupling approach be used to restrain a set of distances $r_{ij}(t)$ between atoms i and j to the reference value r_{ij}^0? Secondly, in the case of time-averaged restraints, would the disturbance of the dynamics be increased or decreased using the weak-coupling approach? Finally, would the method perform better or worse than conventional restraints?

Theory

The weak coupling (Berendsen et al., 1984) of distance restraints was implemented into the equations of motion for the atoms i= 1,2,...,N of the molecular system according to Eq. 1 (van Gunsteren et al., 1996):

$$\frac{d\mathbf{r}_i(t)}{dt} = \mathbf{v}_i(t) - \frac{1}{2\tau_{dr\text{-}wc}}\left[\langle r_{ij}^{-p}\rangle^{-1/p} - r_{ij}^0\right]\frac{\mathbf{r}_{ij}(t)}{r_{ij}(t)} \qquad (1)$$

where the value of p is 3 or 6 depending on the time period over which the average indicated by $\langle ... \rangle$ is taken. The position of atom i at time t is indicated by $\mathbf{r}_i(t)$ and its velocity by $\mathbf{v}_i(t)$, $r_{ij}=|\mathbf{r}_i-\mathbf{r}_j|$ is the scalar distance between atoms i and j, r_{ij}^0 the experimentally derived distance constraint between atoms i and j, and $\tau_{dr\text{-}wc}$ is the time constant for coupling. An additional factor of 2, when compared to the original weak-coupling equations (Berendsen et al., 1984) comes from the fact that Eq. 1 is applied to both atoms i and j involved in the distance restraint.

The quantity $\langle r_{ij}^{-p}\rangle^{-1/p}$ can be calculated instantaneously or as a time average. For time-averaged coupling we have:

$$\langle r_{ij}^{-p}\rangle = \overline{r_{ij}^{-p}(t;\tau_{dr})} \equiv \left(\frac{1}{\tau_{dr}\left(1-e^{-t/\tau_{dr}}\right)}\int_0^t e^{-t'/\tau_{dr}}\left[r_{ij}(t-t')\right]^{-p}\,dt'\right) \qquad (2)$$

where τ_{dr} is the characteristic time for the exponentia decay used to calculate the running average (Torda et al. 1989,1990,1993). In the limit of τ_{dr} approaching zero, Ec 2 reduces to $r_{ij}^{-p}(t)$ and $\langle r_{ij}^{-p}\rangle^{-1/p}$ in Eq. 1 to $r_{ij}(t)$. As in ou previous work, we assume that $t \gg \tau_{dr}$, so:

$$1 - e^{-t/\tau_{dr}} \approx 1 \qquad (3$$

and this factor was dropped from Eq. 2. Simulations witl time-averaged distance restraints require the choice of a initial value for:

$$\left[\overline{r_{ij}^{-p}(t=0;\tau_{dr})}\right]^{-1/p} \qquad (4$$

In all the runs using weak-coupling time-averaged dis tance restraints, for each distance restraint Eq. 4 was se to 0.2 Å less than r_{ij}^0.

The simulations using a penalty function V_{restr} to re strain the atom–atom distances r_{ij} were carried out as i earlier studies (Kaptein et al., 1985; Torda et al., 1989 1990; Nanzer et al., 1994,1995) using:

$$V_{restr} = \begin{cases} 0 & \text{when } 0 < \langle r_{ij}^{-p}\rangle^{-1/p} < r_{ij}^0 \\ \frac{1}{2}K_{dr}\left[\langle r_{ij}^{-p}\rangle^{-1/p} - r_{ij}^0\right]^2 & \text{when } r_{ij}^0 < \langle r_{ij}^{-p}\rangle^{-1/p} < r_{ij}^0 + \Delta r^0 \\ K_{dr}\left[\langle r_{ij}^{-p}\rangle^{-1/p} - r_{ij}^0 - \frac{1}{2}\Delta r^0\right]\Delta r^0 & \text{when } r_{ij}^0 + \Delta r^0 < \langle r_{ij}^{-p}\rangle^{-1/p} \end{cases} \qquad (5$$

Methods

All MD simulations were carried out using software from the GROMOS suite of programs (van Gunsteren and Berendsen, 1987). The simulations were performed using the standard GROMOS 37D4 united-atom force field for in vacuo simulation (van Gunsteren and Berendsen, 1987). The temperature was held constant by weak coupling (τ_T=0.1, 0.05 or 0.01 ps) to an external bath of 300 K (Berendsen et al., 1984). The SHAKE algorithm was used to constrain all bond lengths with a relative precision of 10^{-4} (Ryckaert et al., 1977) allowing an integrator time step of 0.002 ps. Nonbonded interactions were truncated at 8 Å.

The first test molecule was a linear chain of 15 aliphatic CH_2 and CH_3 united atoms with a single distance restraint of 6 Å between the first and the last CH_3 atoms. The starting structure was the extended conformation. The second test molecule was the 64-residue structured domain of chymotrypsin inhibitor 2 (CI-2, PDB acquisition code 3ci2) for which a set of 961 distance restraints and refined solution structures have been published (Clore

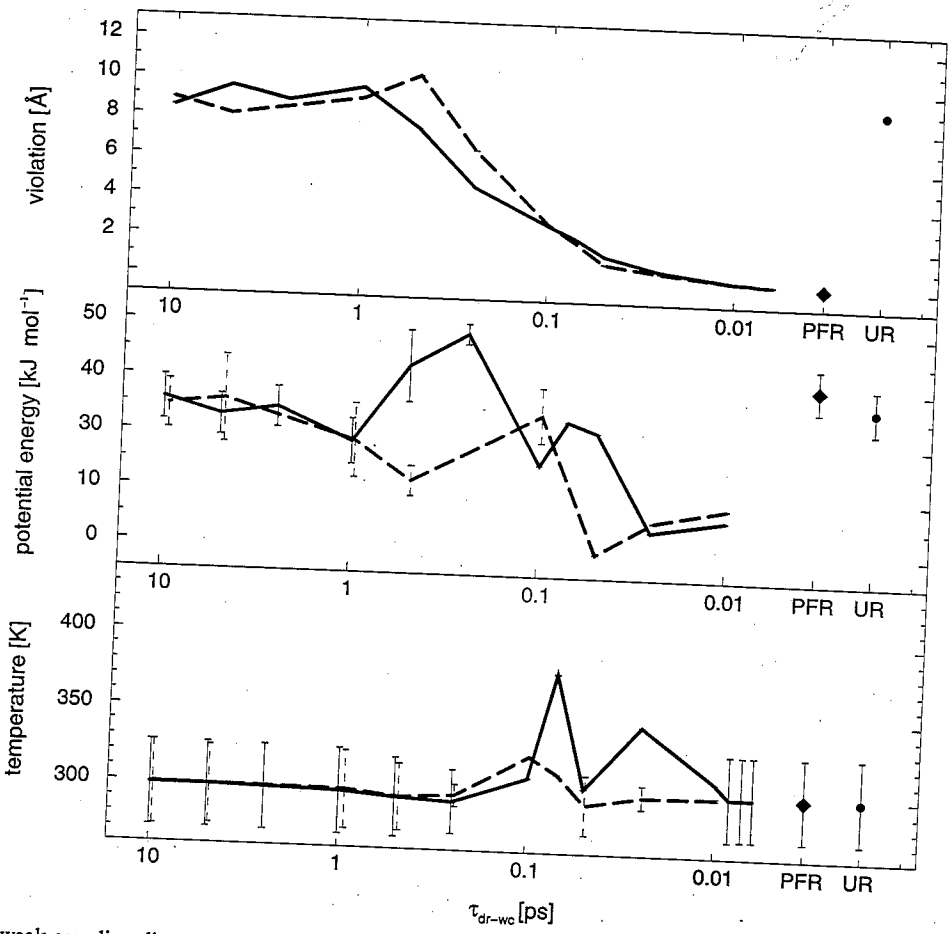

Fig. 1. Instantaneous weak-coupling distance-restraining MD simulation of the C-15 molecule with one distance restraint. The top graph shows the violation of the distance restraint, the middle the physical potential energy V_{phys} ± rms fluctuation, and the bottom the temperature ± rms fluctuation plotted as a function of weak-coupling times $\tau_{dr\text{-}wc}$. The values for the penalty function restraining (PFR, ◆) and the unrestrained (UR, ●) MD simulations are indicated on the right. The solid line corresponds to a temperature coupling time τ_T of 0.1 ps and the dashed line to 0.05 ps.

et al., 1987; Ludvigsen et al., 1991a,b; Nanzer et al., 1994). The single starting structure was the same as the one used by Nanzer et al. (1994,1995). Pseudo- and virtual atoms were defined as in Wüthrich et al. (1983), van Gunsteren et al. (1985) and van Gunsteren and Berendsen (1987). A value of p = 3 was used in Eqs. 1–5.

For the weak-coupling distance-restrained MD simulations, coupling times between $\tau_{dr\text{-}wc} = 0.002$ and 10 ps were tested. The length of the memory decay time τ_{dr} was 10 ps. For comparison, MD simulations with the penalty function restraining (PFR) method were performed using instantaneous and time-averaged restraining. The parameters used in Eq. 5 were $K_{dr} = 20$ kJ mol^{-1} Å^{-2} and $\Delta r^0 =$ 1 Å. Unrestrained (UR) MD simulations were also performed for comparison. In these simulations a value of $\tau_T = 0.1$ ps was used.

Four series of MD simulations using weak-coupling distance restraining were performed. The C-15 molecule and the CI-2 macromolecule were both tested with the instantaneous and the time-averaged restrained weak-coupling method. All systems were first simulated for 100 ps to equilibrate and then for another 100 ps for analysis. Results are averages over these 100 ps trajectories. When calculating a time average as in Eq. 2 from an MD trajectory, normal averaging, i.e. Eq. 2 with infinite τ_{dr}, is used.

Results

MD simulations with a C-15 molecule.

First, the instantaneously restrained weak-coupling method was tested with the artificial system (15 carbon atoms with a single distance restraint). The restrained MD simulation should easily fulfill the distance restraint. Weak-coupling distance restraining MD simulations with different coupling times $\tau_{dr\text{-}wc}$ to distance restraints and coupling times τ_T to the temperature bath were performed. Figure 1 gives an overview of this first series of calculations. The three graphs show the distance-restraint violation, the total potential energy V_{phys} (± root-mean-square (rms) fluctuation) and the temperature (± rms fluctuation) as a function of $\tau_{dr\text{-}wc}$. This is repeated for temperature

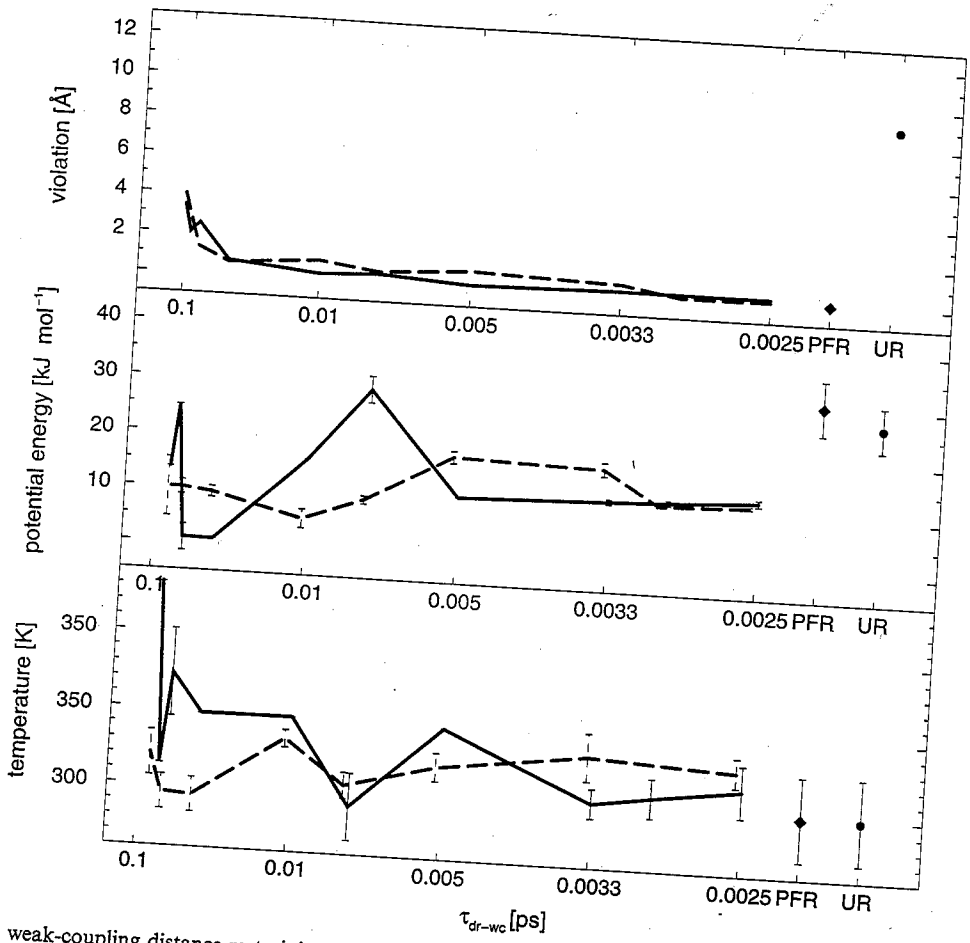

Fig. 2. Time-averaged weak-coupling distance-restraining MD simulation of the C-15 molecule. The graphs show the same quantities as Fig. 1. The solid line corresponds to a temperature coupling time τ_T of 0.1 ps and the dashed line to 0.05 ps.

coupling times τ_T of 0.1 and 0.05 ps. The corresponding values obtained with penalty function restraining (PFR, ◆) and in unrestrained (UR, ●) MD simulations are indicated on the right by additional symbols. The figure shows that tight coupling ($\tau_{dr\text{-}wc}$ below 0.01 ps) is required to fulfill the distance restraint. The potential energy is generally reduced for tighter coupling and its fluctuation is almost reduced to zero beyond $\tau_{dr\text{-}wc} = 0.1$ ps. Tight coupling immobilises the system. At the same time, the velocities of restrained atoms increase due to the resetting of the atomic positions based on Eq. 1, and the system is heated up. Only shorter coupling times τ_T can keep the system near the given reference temperature of 300 K. Since the temperature coupling is a global one, nonrestrained atoms will cool off, when restrained atoms heat up, leading to a complete immobilisation of the system: restrained atoms are immobilised by Eq. 1 and the nonrestrained atoms by the coupling induced by the temperature bath. Coupling times $\tau_{dr\text{-}wc}$ longer than 1 ps have little restraining effect and result in more-or-less unrestrained simulations, whereas coupling times $\tau_{dr\text{-}wc}$ below 0.005 ps cause shifts in the atom positions which lead to SHAKE errors, because the bond lengths cannot be maintained anymore. To conclude, instantaneous-restrained weak-coupling MD simulations are able to restrain one distance in an artificial C-15 molecule to a reference value r^0, but the molecule becomes immobilised without any fluctuation of the potential energy. This means the energy surface is not properly sampled.

Secondly, the weak-coupling distance restraining method was tested with the C-15 molecule and time-averaged distance restraining. Figure 2 shows the same quantities as Fig. 1, but for simulations using time-averaged restraining. In contrast to the case of instantaneous restraints, the system has to fulfill the restraint as an average over a time window of about $\tau_{dr} = 10$ ps. Not surprisingly, the atoms can be coupled much tighter to the distance restraint without producing SHAKE errors. The restraint violation approaches the ideal value of zero with coupling times $\tau_{dr\text{-}wc}$ below 0.05 ps. The potential energy is lower than in the unrestrained simulation and the penalty function restrained MD simulations. Again, for coupling times $\tau_{dr\text{-}wc}$ below 0.005 ps the fluctuation of the potential energy is very small and the system becomes immobilised. Additionally the restrained atoms, and with them the system, are artificially heated for most of the coupling

times $\tau_{dr\text{-}wc}$ for both values of τ_T. In summary, the restraints can be satisfied by applying time-averaged weak-coupling distance restraints, but the system heats up and the sampling is reduced for shorter coupling times.

MD simulations with a macromolecule

In order to test weak-coupling distance restraining MD simulation under more realistic conditions, a second series of calculations with chymotrypsin inhibitor 2 (CI-2) and real NOE distance restraints was performed. Again both instantaneous and time-averaged weak-coupling restraining were tested.

Instantaneous weak-coupling distance restraints with CI-2 quickly showed the limits of this approach. All tested coupling times $\tau_{dr\text{-}wc}$ below 5.0 ps resulted in considerable heating of the molecule and SHAKE errors. The tight coupling of the restrained distances caused such large corrections of atom positions, that SHAKE could not maintain the bond lengths. When less tight coupling was applied, the NOE restraints were not satisfied. Therefore, instantaneous weak-coupling restraining is not a practical method to incorporate distance restraints in MD simulations.

Finally, time-averaged weak-coupling restrained MD simulations were performed with CI-2. Again, several coupling times to the distance restraints ($\tau_{dr\text{-}wc}$ between 1.0 and 0.167 ps) and to the temperature bath ($\tau_T = 0.1$, 0.05 and 0.01 ps) were tested. The results are summarised in Fig. 3. As in the instantaneous restraining case, the weak-coupling technique was not able to fulfill the restraints without heavily disturbing the dynamics of the molecule. Applying a normal coupling time τ_T of 0.1 ps to the temperature bath, the simulation produced SHAKE errors for all distance restraining coupling times $\tau_{dr\text{-}wc}$ below 0.5 ps, and the sum of violations is of the same size as for an unrestrained MD simulation. A similar picture is obtained for the temperature coupling times $\tau_T = 0.05$ and 0.01 ps. The simulations could not fulfill the distance restraints and the temperatures are increased compared to the penalty function restraining method. Compared to the instantaneous weak-coupling restraining MD simulation, the system could be coupled much tighter to the restraints. This is no surprise, because the averaging window for the restrained distances ($\tau_{dr} = 10$ ps) is much larger than that for instantaneous restraints (time step = 0.002 ps).

The weak-coupling distance restraining method was

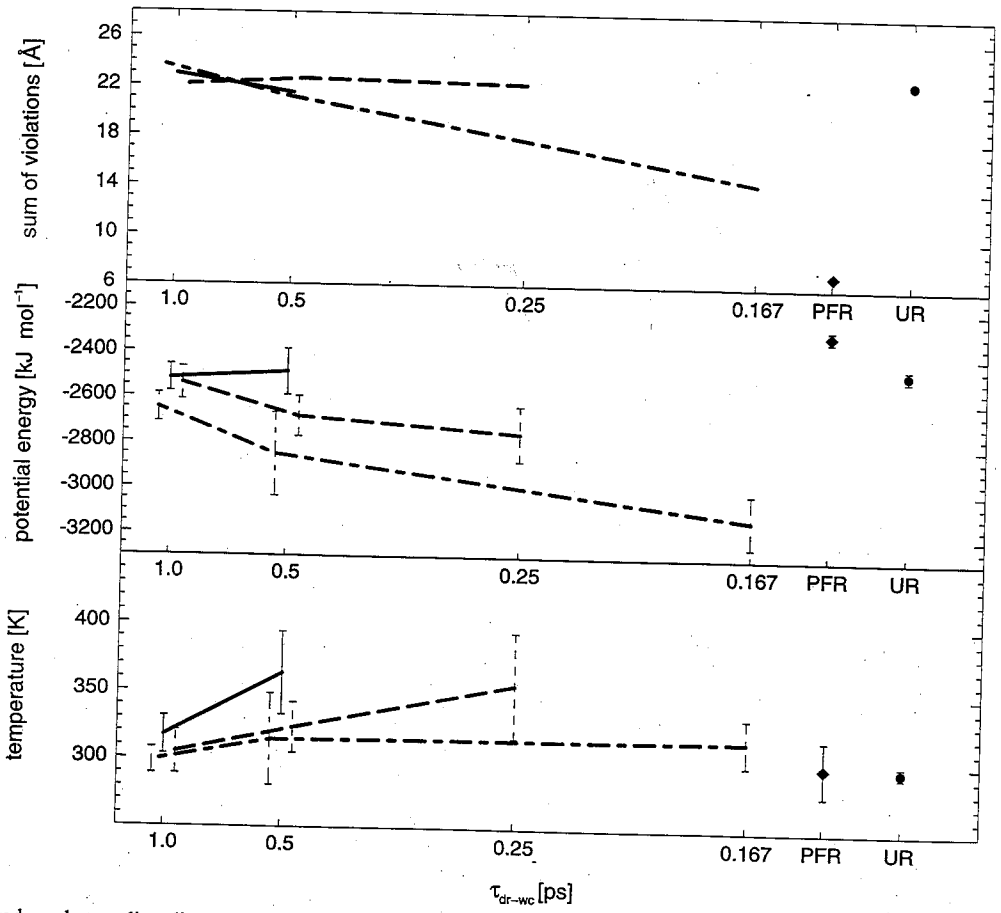

Fig. 3. Time-averaged weak-coupling distance-restraining MD simulation of the CI-2 macromolecule with 961 NOE distance restraints. The graphs show the same quantities as Fig. 1. The solid line corresponds to a temperature coupling time τ_T of 0.1 ps, the dashed line to 0.05 ps and the dot-dashed line to 0.001 ps.

also tested without applying the SHAKE routine by reducing the integration time step to 0.0005 ps and applying harmonic forces to maintain the bond lengths. As in the calculations using SHAKE, tighter coupling to distance restraints resulted in a temperature increase with coupling times below 0.1 ps, the equations of motion could only be properly integrated when the integration time step was further reduced beyond 0.0005 ps.

Discussion and Conclusions

The MD simulations presented here showed mainly two things: (i) when restraining a macromolecule to a set of NOE distance restraints with the weak-coupling method, both restrained and nonrestrained atoms are immobilised, and the restrained atoms are heated up. With atom–atom distance restraining by the weak-coupling scheme, a set of distance restraints can be fulfilled, but at the expense of considerable heating of the system and with a reduction of the integration time step to avoid failure of the procedure SHAKE that is used to maintain bond-length constraints; (ii) the weak-coupling method does not perform better than the conventional penalty function distance-restraining method. The weak-coupling technique is therefore not a practical method to maintain distance restraints. Major reasons for investigating the weak-coupling technique are the simple formulation of the weak-coupling equation and the easy implementation of the scheme in the MD algorithm. The result is not an obvious one, since weak coupling is typically used with great success to maintain thermodynamic quantities such as temperature or pressure. The reason is that when considering extensive properties, the correction to the atomic velocities or positions is averaged over the whole system, normally containing several hundred atoms and the perturbation of the atomic motion is small. Consequently, small coupling times ($\tau_T = 0.1$ ps, for temperature or pressure coupling) are sufficient to keep the system at a certain reference value of the temperature or pressure restraint. In contrast, the correction due to NOE distance restraints is applied for each distance restraint, that is, for each pair of atoms. The correction factor is not averaged over the whole system and can even be applied more than once for the same atom in different directions. Furthermore, the correction factor increases rapidly for tighter coupling times and large coordinate corrections heat the restrained atoms and thereby the system and cause SHAKE errors. In cases where several NOEs to one atom are present (often the case for macromolecules) these problems are aggravated.

Despite this negative result we think that a few constructive conclusions can be drawn. First, our study underlines the efficacy of the penalty function restraining method for restraining macromolecular structures to satisfy a set of NOE distance restraints. Second, it illustrates why the weak-coupling scheme is an effective method fo restraining thermodynamic quantities to reference values Third, the results suggest that investigation of the use o weak-coupling restraining for other atomic quantities e.g dihedral angle restraining or ^3J coupling constant restraining is not very useful. Fourth, it shows again that time-averaged restraints offer advantages compared to instantaneous restraints, specially if several restraints compete for the same atom. Finally, every theoretical method should at least be investigated once with respect to its practical advantages and limits.

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Appendix

An atom–atom distance restraint r_{ij}^0 may refer to virtual or pseudo-atoms, that is, to an atom that is not explicitly treated in the simulation or to a nonatomic site (Wüthrich et al., 1983; van Gunsteren et al., 1985; van Gunsteren and Berendsen, 1987). The position $\mathbf{r}_i$ of such a virtual or pseudo-atom is defined in terms of the positions $\mathbf{r}_{i_1}$, $\mathbf{r}_{i_2}$, ... of neighbouring real atoms. The weak-coupling distance-restraining Eq. 1 yields positional corrections:

$$\Delta\mathbf{r}_i = -\left(\frac{\Delta t}{2\tau_{dr\text{-}wc}}\right)\left[\left\langle r_{ij}^{-p}\right\rangle^{-1/p} - r_{ij}^0\right]\frac{\mathbf{r}_{ij}}{r_{ij}} \qquad (A1)$$

and

$$\Delta\mathbf{r}_j = -\Delta\mathbf{r}_i \qquad (A2)$$

for a time step Δt. When atom i (or atom j) is a virtual or pseudo-atom, the positional correction (Eq. A1) must be transformed to positional corrections $\Delta\mathbf{r}_{i_1}$, $\Delta\mathbf{r}_{i_2}$, ... for the real atoms defining the position of atom i. Using the 3×3 matrices $\underline{A}_1$, $\underline{A}_2$, ...,

$$\underline{A}_n = \begin{pmatrix} \frac{\partial x_i}{\partial x_{i_n}} & \frac{\partial y_i}{\partial x_{i_n}} & \frac{\partial z_i}{\partial x_{i_n}} \\ \frac{\partial x_i}{\partial y_{i_n}} & \frac{\partial y_i}{\partial y_{i_n}} & \frac{\partial z_i}{\partial y_{i_n}} \\ \frac{\partial x_i}{\partial z_{i_n}} & \frac{\partial y_i}{\partial z_{i_n}} & \frac{\partial z_i}{\partial z_{i_n}} \end{pmatrix} \qquad (A3)$$

containing the partial derivatives of $\mathbf{r}_i$ with respect to $\mathbf{r}_{i_n}$, we find

$$\Delta\mathbf{r}_{i_n} = (\underline{A}_n)^{-1}\,\Delta\mathbf{r}_i \qquad (A4)$$

The matrices $\underline{A}_n$ have been given by van Gunsteren et al. (1985) and van Gunsteren and Berendsen (1987).

The positional corrections (Eq. A1) resulting from the weak-coupling distance-restraining Eq. 1 have been implemented in the leap-frog algorithm used to integrate the equations of motion in a manner that is analogous to the incorporation of positional corrections resulting from weak-coupling to a pressure bath, as described by Berendsen et al. (1984).