Supporting Material

Comment on using a too large integration time step in molecular dynamics simulations of coarse-grained molecular models

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This Supporting Material contains a detailed description of how the instantaneous and reported temperatures are calculated for the GROMOS, GROMACS, and CHARMM packages, and additional simulations (both coarse grained and atomistic) showing that the observed temperature shifts are only apparent.

Reported temperature at full time step is defined differently in GROMACS and GROMOS:

Both GROMOS[1,2] and GROMACS[3] codes integrate Newton's equations of motion using a Leap-frog algorithm with positions known at full times and velocities at half times. In the Leap-frog scheme, Newton's equations of motion are integrated numerically in an expansion up to second order in time, alternately updating the positions at full times $t = n\Delta t$, and velocities v_i at half times $t - \Delta t/2 = (n - 1/2)\Delta t$; n is the number of steps taken in the integration and Δt is the time-step.

In constant temperature simulations, both GROMOS and GROMACS codes implement the same weak coupling scheme due to Berendsen *et al.*[4]. Velocities at half times are scaled by a factor computed from the instantaneous temperatures at half times earlier[5].

$$v_i'(t + \Delta t/2) = \lambda(t - \Delta t/2)v_i(t + \Delta t/2); \quad \lambda(t - \Delta t/2) = \left[1 + \frac{\Delta t}{\tau_T}\left\{\frac{T_{ref}}{T(t - \Delta t/2)} - 1\right\}\right]^{1/2} \quad \text{(Eq. S1)}$$

Here, λ is the temperature scaling factor, τ_T is a parameter determining the strength of the coupling (relaxation time toward the target temperature) and T_{ref} is the target temperature. Thus, GROMOS and GROMACS codes should be able to maintain the same temperature, all other things being equal.

However, the reported temperature in both GROMACS and GROMOS codes is $T(t)$, the instantaneous temperature at time t. In general, the instantaneous temperature in MD simulations is defined from the kinetic energy.

$$T(t) = \frac{2E_{kin}(t)}{k_B N_{dof}} \quad \text{(Eq. S2)}$$

Here, $E_{kin}(t)$ is the kinetic energy at time t, k_B is Boltzmann's constant, and N_{dof} is the number of degrees of freedom for which the kinetic energy is computed. This formula derives from the equipartition theorem for energy of motion. The kinetic energy is computed from the masses *m* and velocities *v* of the particles defining the degrees of freedom.

$$E_{kin}(t) = \sum_{i=1}^{N_{part}} \frac{1}{2} m_i v_i^2(t) \qquad \text{(Eq. S3)}$$

Temperatures at full times *t* may now be calculated in two manners. We found that the main reason for the differences observed between reported temperatures using GROMACS and GROMOS codes, respectively, is due to the implementation of two different calculations of the temperature at full times.

GROMACS codes (from version 3.3.1)[3] implement the calculation of the temperature at full times from the average of the kinetic energies at half times, which uses the known velocities at half times.

$$\begin{aligned} T_{GROMACS}(t) &= \frac{2\left(\frac{E_{kin}(t+\Delta t/2)+E_{kin}(t-\Delta t/2)}{2}\right)}{k_B N_{dof}} \\ &= \frac{2}{k_B N_{dof}} \sum_{i=1}^{N_{part}} \frac{1}{2} m_i \left(\frac{v_i^2(t+\Delta t/2)+v_i^2(t-\Delta t/2)}{2}\right) \end{aligned} \qquad \text{(Eq. S4)}$$

GROMOS codes[1,2] implement the calculation of the temperature at full times from the kinetic energy at full times, which uses the average velocities at half times.

$$T_{GROMOS}(t) = \frac{2E_{kin}(t)}{k_B N_{dof}} = \frac{2}{k_B N_{dof}} \sum_{i=1}^{N_{part}} \frac{1}{2} m_i \left(\frac{v_i(t+\Delta t/2)+v_i(t-\Delta t/2)}{2}\right)^2 \qquad \text{(Eq. S5)}$$

The difference between these definitions becomes apparent if the difference between the velocities at consecutive half times is written as δ, and the GROMOS and GROMACS expressions are written out explicitly.

$$v_i(t+\Delta t/2) = v_i(t-\Delta t/2) + \delta_i \qquad \text{(Eq. S6)}$$

$$T_{GROMACS}(t) \propto \sum_i \frac{(v_i+\delta_i)^2 + v_i^2}{2} = \sum_i v_i^2 + v_i\delta_i + \frac{1}{2}\delta_i^2 \qquad \text{(Eq. S7)}$$

$$T_{GROMOS}(t) \propto \sum_i \left(\frac{v_i+\delta_i+v_i}{2}\right)^2 = \sum_i v_i^2 + v_i\delta_i + \frac{1}{4}\delta_i^2 \qquad \text{(Eq. S8)}$$

In the latter two equations, $v_i = v_i(t-\Delta t/2)$. Assuming the δ_i are normally distributed, it is clear that the reported temperature at full times in GROMOS will always be lower than the reported temperature at full times in GROMACS. Also, in general δ_i will be larger as the time-step increases, and therefore reported temperatures will differ more between GROMOS and GROMACS codes with increasing time-step. The issue of the calculation of velocity-dependent properties in the Leap-frog scheme is also discussed by Cuendet and van Gunsteren[6], with the suggestion to use (Eq. S4) rather than (Eq. S5) in MD simulation. Fully consistent velocity-dependent properties at full and half times can only be obtained by accounting for higher order terms in the Taylor expansion of the equations of motion; it is however possible to compute the velocity to higher order using the forces[7].

Testing reported and actual temperature for a hexadecane system at atomic resolution:

Testing for a system of liquid hexadecane using the atomistically detailed united-atom GROMOS 53A6 force field, for which GROMOS and GROMACS codes allow the same force field terms (straight van der Waals cut-off, internal bond, angle, and dihedral potentials, without using constraints; note that the electrostatic interaction is absent) showed that both codes (GROMOS96 cq. GROMACS 3.3.3) were able to maintain the temperature at half times close to the reference temperature. Making the codes print the instantaneous kinetic energies at half times, in both cases, the instantaneous temperatures at larger time-steps were seen to be slightly higher than the reference temperature consistent with the heating commonly observed due to integration and cut-off errors. Simulation results are collected in Table S1.

The simulations were run using weak temperature coupling due to Berendsen (Eq. S1), with a fixed ratio of the time-step to the coupling parameter, the value of the coupling parameter being 50 times the value of the time-step. The system consisted of 320 hexadecane molecules described by the GROMOS 53A6 force field as implemented in GROMOS and GROMACS, respectively, without using any constraints. Note that a time-step of 0.5 fs is recommended in the GROMOS manual (Table 2.7.3.1)[1] in case of the absence of bond constraints to ensure proper energy conservation. (In a control run using bond constraints and a time-step of 2 fs, the instantaneous and reported temperatures were found to be much closer than in the unconstrained run with the same time-step). A single cut-off of 1.4 nm was used in all simulations, and the neighbor list was updated every time-step. Simulations were performed with and without weak pressure coupling. In case of weak pressure coupling, the coupling time constant for the pressure scaling was chosen to be 5 times that of the coupling constant for the temperature scaling. Center of mass motion was removed every time-step. The simulations were run for 10,000 steps, and were all started from the same conformation. The instantaneous temperatures at half times, $T(t-\Delta t/2)$, reported temperatures[8] at full times, T_{rep}, and temperature coupling parameters, λ, collected in Table S1 are averages over the last 9,000 steps of the simulation, except where noted.

In all simulations, the average values of the coupling parameter λ were consistent with heating for the largest time-steps investigated: the average λs were below 1 indicating the bath removing heat from the system. For the smallest time-steps, the average λs were above 1 indicating heat was pumped into the system. Both codes managed to maintain the instantaneous temperatures quite close to the target temperature, except when the simulations were numerically unstable, which occurred for GROMOS with a time step of 6 fs, and for GROMACS with time step of 5 fs for NpT runs and with a time step of 8 fs for both codes for NVT runs. The reported temperatures at full times, did, however, differ strongly between the codes, with the reported temperature of GROMOS being consistently lower than that of GROMACS and more so with increasing time-step. In fact, the reported averages of λ and T of GROMOS started to show an inconsistency: the average λ is below 1 indicating the thermostat is cooling the system whereas the average temperature is below the target temperature. Of course, the inconsistency is only an apparent one, because the average value of λ reflects on the deviation of the instantaneous temperature from the reference temperature, with which it is fully consistent.

Thus, the apparent strong cooling reported by Winger et al.[9] does not reflect the instantaneous temperature of the system but is a consequence of the way in which GROMOS codes calculate the temperature at full times. Closer inspection of Figure 3 of Winger et al.[9] reveals that the values of λ reflect the stability of the results with increasing time-step. If the system was really cooling as dramatically as shown from the temperature, the λ should have followed suit, which it clearly does not.

Testing the effect of large time steps with the CHARMM code:

The issue of apparent versus instantaneous temperatures may also be important in implementations of the MARTINI model with other codes. We tested the CHARMM code[10], results are shown below. As an independent check, we also used the CHARMM code to probe different manners to use the weak-coupling algorithm together with the Leap-frog algorithm to integrate the equations of motion. The different schemes for weak coupling are summarized in Table S2. The names of the schemes are based on the program using it (i.e. CHARMM, GROMOS, GROMACS), but they were all implemented in the CHARMM code. All modifications were performed in the source/dynamc/dynamc.src file in the subroutine DYNAMC.

CHARMM couples to the temperature as calculated by (Eq. S5), which shall be denoted as $T^{VEL}(t)$, i.e. from the kinetic energy calculated from the velocities at full times. The coupling parameter is calculated from

$$\lambda\left(t+\Delta t/2\right)=\left[1+\frac{\Delta t}{\tau_T}\left\{\frac{T_{ref}-T^{VEL}\left(t\right)}{T\left(t+\Delta t/2\right)}\right\}\right]^{1/2} \qquad \text{(Eq. S9)}$$

It also reports $T^{VEL}(t)$, and therefore, the reported and internal temperatures at full time steps are always close together. In contrast, as explained before, both GROMOS and GROMACS schemes couple to the instantaneous temperature at half times, (Eq.

S1), but report different temperatures. The temperature reported by GROMACS, (Eq. S4), shall be denoted T^{KE}.

In Fig. S1 we report the effect of the integration time-step used in the CHARMM scheme on a number of properties related to the velocities. The time-step used ranges from 2 to 50 fs. The system simulated contained 400 MARTINI water beads in a cubic box. The van der Waals interactions were calculated using the "force switch" shifting function implemented in CHARMM with CTONNB=9.0, CTOFNB=12.0 and CUTNB=14.0 Å. This is similar to the parameters used in the original MARTINI model and the properties of CG water were reproduced. The temperature is controlled with the original CHARMM weak-coupling scheme (see Table S2), at a value of 300 K. In Fig. S1a, we report the average values of the difference in temperatures at full times, as calculated from Eqs. S4 and S5, denoted as $T^{COR}=T^{KE}-T^{VEL}$. It is clear that the value of T^{COR} increases with increasing time-step. The deviation becomes significant (>5 K) with a time-step above 30 fs and is ~15 K with a time-step of 50 fs. Note that the instantaneous velocities themselves are not affected by the time-step, as shown by their average and standard deviation shown in Fig. S1b. It was also observed that the distributions of the instantaneous velocities are virtually identical at each time-step, with time-steps in the range from 2 to 50 fs (data not shown). On the other hand, the differences between consecutive instantaneous atomic velocities increase linearly with the time step. This is shown in Fig. S1c. These observations are consistent with a linear loss of correlation between consecutive instantaneous atomic velocities as a function of the time-step resulting in a quadratic effect on T^{COR}. Again it becomes clear that the calculation of the temperature on-step from the average velocities at half-time-step, T^{VEL}, instead of from the average kinetic energies at half-time-step, T^{KE}, will give an apparent temperature of the system systematically lower by T^{COR}.

In the following we present two applications in which the choice of the coupling scheme reveals the importance of T^{COR}. The system simulated was a box of 2,000 MARTINI water beads under NpT conditions (p=1 atm, τ_P=5.0 ps). A 50 fs time-step was used to magnify T^{COR}. Three temperature-coupling schemes were used: CHARMM, GROMOS and GROMACS. GROMOS and GROMACS differ only by the reported temperature, T^{VEL} and T^{KE}, respectively. The instantaneous ($T(t-\Delta t/2)$) and reported temperatures are shown in Fig. S2a. With both CHARMM and GROMOS schemes the reported temperature is not consistent with the instantaneous temperature, i.e. the actual temperature of the system. In both cases the difference is equal to the value of T^{COR} as reported in Fig. S1a: ~15 K. Since CHARMM couples to and reports T^{VEL} (see Table S2), the actual temperature of the system (instantaneous temperature: T^{KE}) is 15 K higher than the reported one. Alternatively, in GROMOS, although the dynamics runs at the correct temperature, T^{KE}, the reported temperature, T^{VEL}, is ~15 K lower than the instantaneous one and could be erroneously interpreted as a cooling of the system (see main manuscript). With the GROMACS scheme the instantaneous and reported temperatures are consistent.

In a second application we have calculated the freezing temperature of the MARTINI water model by simulating a box of 2,000 water beads using a time-step of 50 fs. Starting from a conformation in which about half of the system was already frozen, the water was considered in the liquid/ice phase if complete melting/freezing was observed within 5 ns. Note that the system equilibrated to either phase within a few hundred ps. This operation was performed at several temperatures and the freezing temperature was determined. In Fig. S2a, the resulting phase diagrams are shown for two different coupling schemes: CHARMM and GROMACS. The freezing temperature is 270-275 K based on the reported temperature for the CHARMM scheme. This about 15-20 K lower than the value obtained with the GROMACS scheme, 290-295 K, and consistent with the difference T^{COR} between T^{VEL} and T^{KE}, ~15 K, using a time step of 50 fs.

Table S1: Instantaneous internal temperatures (printed from inside the code) and reported temperatures (from energy file) using GROMOS96 [1] and GROMACS 3.3.3 [3] codes for a system of hexadecane molecules under NpT and NVT, respectively. For more details, see text. In case of GROMOS, the temperature was calculated from the kinetic energy after averaging over respective kinetic energies[8].

	GROMOS			GROMACS		
Δt (fs)	$T\ (t\text{-}\Delta t/2)$	T_{rep}	λ	$T\ (t\text{-}\Delta t/2)$	T_{rep}	λ
NpT						
2	323.96 (7)	318.24 (5)	1.000002	323.94 (11)	323.95 (11)	1.000002
4	323.87 (6)	299.4 (7)	1.000005	323.98 (6)	323.98 (6)	1.000001
5	323.81 (5)	283 (1)	1.000007	328 (2)[b]	324.7 (2)	0.999979
6	324.56 (6)[a]	252 (1)	0.999984	Not stable[c]		
NVT						
0.5	324.0 (1)	323.7 (1)	1.000001			
2	323.93 (7)	318.25 (7)	1.000003	324.02 (7)	324.02 (8)	1.0000000
4	323.87 (6)	299.21 (9)	1.000004	324.01 (6)	324.01 (6)	1.0000000
5	323.81 (5)	283 (1)	1.000006	324.02 (5)	324.02 (5)	1.0000000
6	324.6 (3)[d]	253.3 (5)	0.999982	324.03 (4)	324.03 (5)	0.9999997
8	Not stable[c]			Not stable[c]		

[a] Numerically instable after 6,038 steps; averages over 5,000 steps.

[b] Numerically instable after 2,000 steps; averages over 1,000 steps.

[c] Numerically instable after a few steps.

[d] Numerically instable after 7,389 steps; averages over 6,000 steps.

Table S2: Details of the temperature schemes tested in this study. Importantly, the CHARMM scheme couples to and reports T^{VEL}. The GROMACS and GROMOS schemes couple to T^{KE} and while GROMACS reports T^{KE}, GROMOS reports T^{VEL}.

scheme	CHARMM	GROMOS	GROMACS
λ	$\lambda = \left[1 + \frac{\Delta t}{\tau_T}\left\{\frac{T_{REF} - T^{VEL}(t)}{T(t+\Delta t/2)}\right\}\right]^{1/2}$	$\lambda = \left[1 + \frac{\Delta t}{\tau_T}\left\{\frac{T_{REF}}{T(t-\Delta t/2)} - 1\right\}\right]^{1/2}$	
T coupled to	$T^{VEL}(t)$	$T(t-\Delta t/2)$	
$T(t)$ reported	$T^{VEL}(t)$	$T^{VEL}(t)$	$T^{KE}(t)$

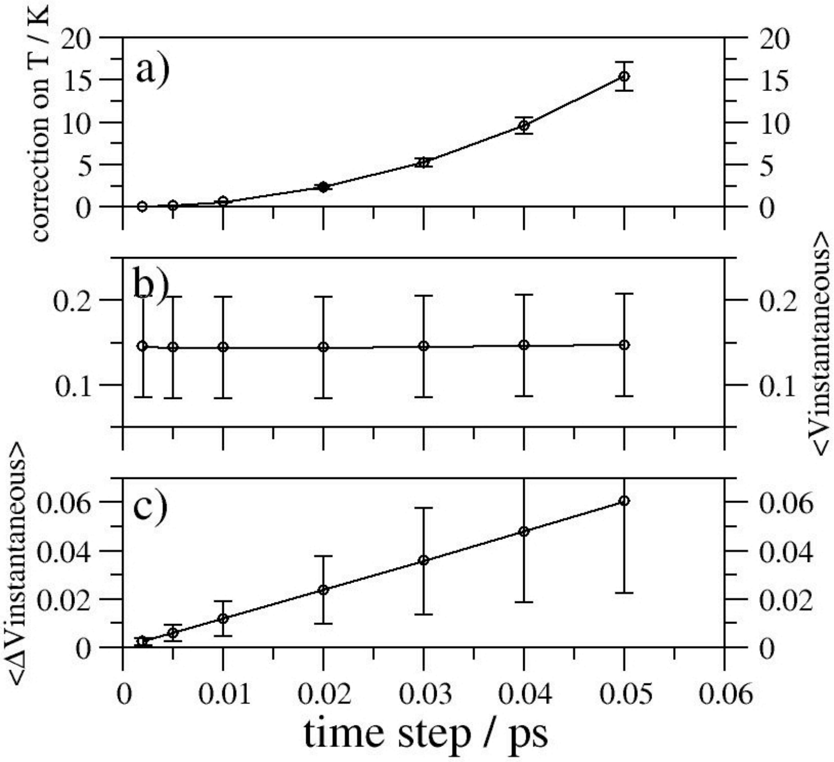

Figure S1: Quantities affected by the time step. a) Difference between temperatures calculated according to Eqs. S4 and S5, T^{COR}; b) Average instantaneous velocity at half time, <v(t-Δt/2)>; c) Average absolute difference in velocities at minus and plus half time step, < |v(t-Δt/2)-v(t+Δt/2)| .

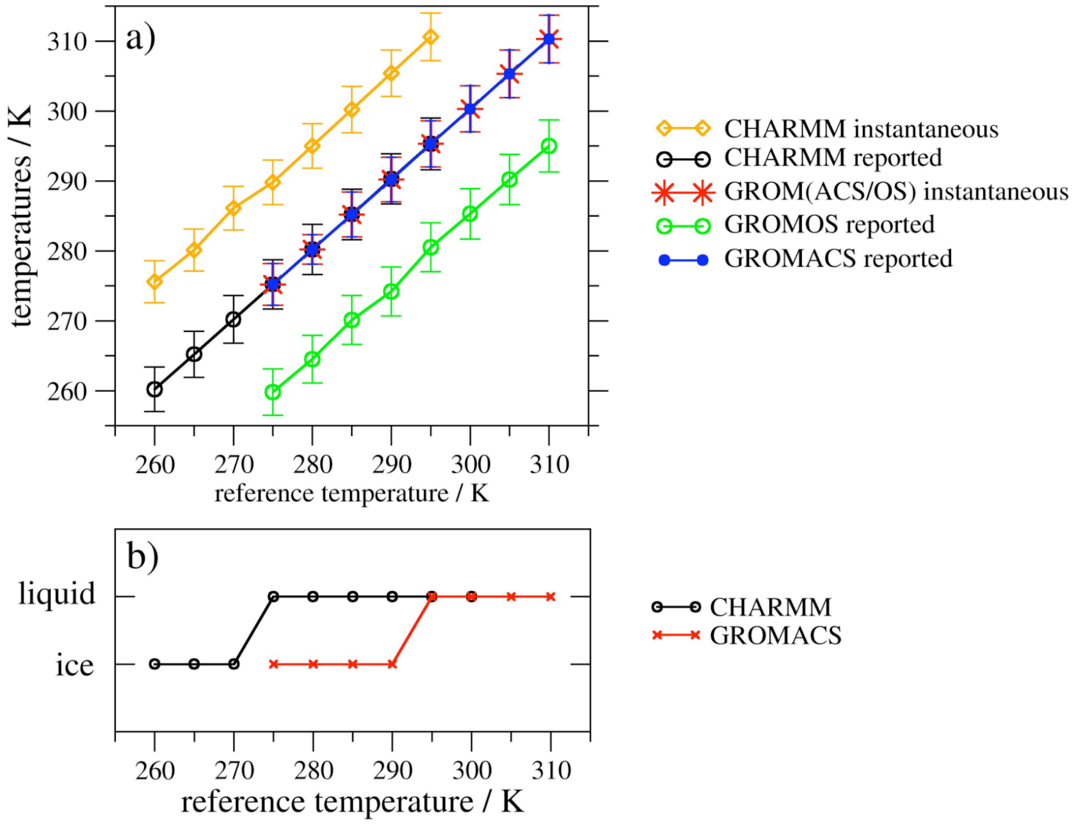

Figure S2: Effect of the reference temperature for the different schemes used: CHARMM, GROMOS and GROMACS. a) Instantaneous and reported temperatures. b) Freezing temperature of CG water (see text for details).

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[2] M. Christen *et al.* The GROMOS software for biomolecular simulation: GROMOS05. *J. Comput. Chem.,* 205, **26**, 1719-1751.

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[5] The GROMOS96 code actually uses the ratio of the target kinetic energy and the instantaneous kinetic energy instead of the corresponding temperatures, which is equivalent.

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[7] H.J.C. Berendsen and W.F. van Gunsteren. Practical algorithms for dynamic simulations. In 'Molecular Dynamics Simulation of Statistical Mechanical Systems' ed. G. Ciccotti and W. Hoover, Amsterdam, North Holland.

[8] It should be noted that GROMOS does not output the temperature to the energy trajectory file, nor does it use the temperature internally. Instead, the kinetic energy at full time steps is output to the energy trajectory file. The reported temperature is calculated from the kinetic energy at full time steps by (Eq. S2), where $N_{dof} = 15{,}357$ for the system under study. The average temperature calculated this way is consistent with the average temperature over the whole simulation as printed in the standard output file of GROMOS.

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