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Molecular dynamics simulations with constrained roto-translational motions: Theoretical basis and statistical mechanical consistency

A. Amadei,[a] G. Chillemi,[b] M. A. Ceruso, A. Grottesi, and A. Di Nola
Department of Chemistry, University of Rome ''La Sapienza,'' P.le A. Moro 5, 00185, Rome, Italy

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From a specific definition of the roto-translational (external) and intramolecular (internal) coordinates, a constrained dynamics algorithm is derived for removing the roto-translational motions during molecular dynamics simulations, within the leap-frog integration scheme. In the paper the theoretical basis of this new method and its statistical mechanical consistency are reported, together with two applications. © *2000 American Institute of Physics.* [S0021-9606(00)50201-3]

I. INTRODUCTION

Often in molecular simulations the interest is focused on the structural properties of a molecule with internal degrees of freedom. For simulations of a single molecule in vacuum (ideal gas condition) as well as for simulations of a solute molecule in its solvent (infinite dilution condition), the roto-translational motions are in general uninteresting, while the behavior of the internal coordinates can be very important for many different studies. In particular with large organic molecules, and especially biomacromolecules, simulations are usually performed to obtain a detailed investigation of the conformational fluctuations, which can be studied only after removing the overall translation and rotation of the molecule. In these cases the ensemble of molecular configurations obtained from the simulation is normally manipulated to remove these roto-translational motions. In general the roto-translation is eliminated by over-imposing the center of mass of the actual configuration with that of a reference one, and then least square fitting the atomic displacements between the two structures rotating the actual structure around its center of mass.[1] This procedure, although usually efficient and widely used, has one disadvantage. Its implicit definition of external and internal coordinates is rather complicated, especially for the definition of conjugated momenta, and hence it is difficult to use this approach for theoretical mechanics or rigorous statistical mechanical studies, as well as to derive ideal constraint forces to stop the molecular roto-translational motions directly during the simulation. It should be considered that a rigorous method to constrain the roto-translational motions during a simulation can be advantageous in the following cases:

1. For large and flexible molecules the removal of the dynamical coupling between the internal motions and molecular roto-translations can shorten the system's relaxation time and hence provide a better configurational sampling for the internal coordinates in simulations of usual time lengths.
2. Simulations of a molecule in vacuum, with either a usual force field or using a mean field, are usually performed at zero angular momentum and this constraint can alter the statistical mechanical consistency of the simulation. On the contrary when ideal holonomic constraints are used to stop the roto-translational motions, and the angular momentum is not fixed anymore, the simulation can provide the exact statistical mechanics of the system.
3. For simulations of large nonspherical molecules (e.g., proteins) in water, with the presence of the roto-translational constraints we could use a simulation box shaped on molecular geometry, reducing significantly the number of necessary water molecules. The effect of large solute rotations is negligible for short length simulations (hundreds of picoseconds) but becomes relevant for longer time simulations. In fact, from the nanoseconds range the solute has enough time to rotate significantly and hence, without using a cubic simulation box, to interact directly with its periodic images.
4. For the calculation of free energy differences due to changes of the roto-translational configuration for interacting molecules or for a molecule interacting with an external field (e.g., molecular docking) the simulations with the roto-translational constraints could be extremely efficient.

In this paper we show that it is possible to use a definition of internal and external coordinates which is very suited for theoretical derivations and that allows direct simulation of only the molecular internal degrees of freedom. In fact, this definition of the molecular coordinates (standard in analytical mechanics) allows the use of ideal constraint forces to stop instantaneously the roto-translational motions during the simulation. It is well known from theoretical mechanics[2,3] that the use of ideal holonomic constraints in a Hamiltonian system still provides Hamiltonian dynamics in the constrained phase space (constraint subspace), and so a constrained Hamiltonian system can still be described by the microcanonical ensemble. We will show that more often in general the holonomic constraints do not alter the basic type of dynamics in the case of the usual molecular dynamics (MD) equations of motion involving a frictional term, and

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[a] Author to whom correspondence should be addressed; electronic mail: amadei@seurat.chem.uniroma1.it

[b] Inter-University Computing Consortium (CASPUR), University of Rome ''La Sapienza,'' P.le A. Moro 5, 00185, Rome, Italy.

hence the statistical mechanical ensemble which describes such MD systems in full phase space also exactly describes these systems in the constrained phase space. This means that a rigorous constrained dynamics algorithm can be used for theoretical studies in molecular simulations, and in general for molecular computational methods. It must be noted that a constrained dynamics algorithm, although statistical mechanically consistent, always alters the dynamics in the simulation as the equations of motion are not the same as those for the unconstrained system. Such a dynamical difference, which is usually assumed not very relevant, should be considered when the main interest in a simulation concerns the dynamics of a system.

The paper is organized as follows: in the first two theory sections we describe the definition used for the internal and external molecular coordinates and the derivation of the constrained dynamics. In the third theory section we show that for the typical MD equations of motion, which can provide exact statistical mechanical properties (the Hamiltonian or Lagrangian,[2,3] the isothermal Gaussian[4,5] and the Nosé-Hoover dynamics[6,7]), the application of ideal holonomic constraints does not prevent the system's behavior to converge in time to that of the correct statistical mechanical ensemble, although in a restricted phase space. In the fourth theory section we show that the statistical mechanics of a molecule in ideal gas or infinite dilution conditions can be obtained exactly from a simulation with roto-translational constraints. Finally we present as numerical examples two simulations of a large organic molecule in the microcanonical and canonical ensembles using the roto-translational constraints and some conclusions are given.

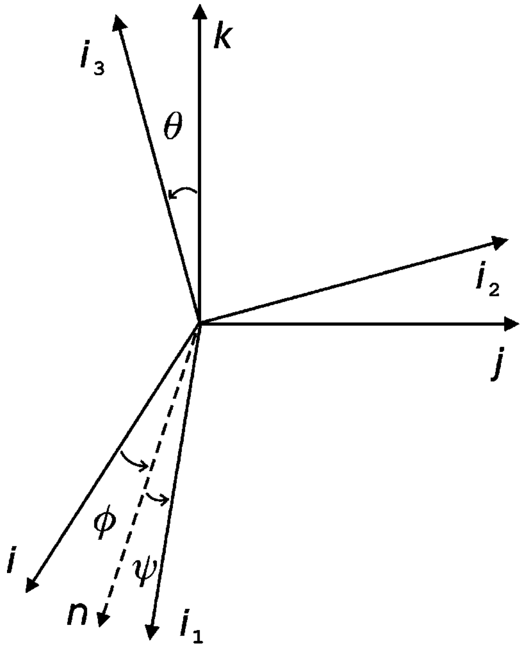

FIG. 1. The local orthonormal frame $(G,\mathbf{i}_1,\mathbf{i}_2,\mathbf{i}_3)$ is defined by the fixed orthonormal frame, moved in the center of mass $(G,\mathbf{i},\mathbf{j},\mathbf{k})$, and the three Eulerian angles (θ,ϕ,ψ). The Eulerian angles are defined as $\theta=\widehat{\mathbf{i}_3\mathbf{k}}$, $\phi=\widehat{\mathbf{i}\mathbf{n}}$, $\psi=\widehat{\mathbf{i}_1\mathbf{n}}$ where $\mathbf{n}$ is given by the intersection of the $\mathbf{i}$, $\mathbf{j}$ plane with the $\mathbf{i}_1$, $\mathbf{i}_2$ plane. It is worth noting that the unit vectors $\mathbf{n}$, $\mathbf{k}$, and $\mathbf{i}_3$ expressed in the $(G,\mathbf{i}_1,\mathbf{i}_2,\mathbf{i}_3)$ frame are:

$$\mathbf{k}^T=(\sin\theta\sin\psi \quad \sin\theta\cos\psi \quad \cos\theta),$$

$$\mathbf{n}^T=(\cos\psi \quad -\sin\psi \quad 0), \quad \mathbf{i}_3^T=(0 \quad 0 \quad 1).$$

II. THEORY

A. Definition of the translational, rotational and internal coordinates

Let's consider a flexible molecule made of N particles (atoms), where we define the coordinates of the ith particle $\mathbf{r}_i$ and the coordinates of the center of mass position of the molecule $\mathbf{r}_G$, both expressed in the fixed orthonormal frame $(O,\mathbf{i},\mathbf{j},\mathbf{k})$ (laboratory frame). We can define a local orthonormal molecular frame $(G,\mathbf{i}_1,\mathbf{i}_2,\mathbf{i}_3)$, with origin in the center of mass, where we have[2]

$$\sum_{i=1}^{N} m_i\mathbf{q}_i=\mathbf{0}, \tag{1}$$

$$\sum_{i=1}^{N} \mathbf{q}_i^0\times m_i\mathbf{q}_i=\mathbf{0}, \tag{2}$$

with

$$\mathbf{q}_i^0=\sum_{l=1}^{3} q_{il}^0\mathbf{i}_l, \tag{3}$$

$$\mathbf{q}_i=\sum_{l=1}^{3} q_{il}\mathbf{i}_l, \tag{4}$$

the position of the ith particle with respect to the center of mass, at an arbitrary reference structure with fixed coordinates q_{il}^0 in $(G,\mathbf{i}_1,\mathbf{i}_2,\mathbf{i}_3)$, and the actual position of the ith particle with respect to the center of mass. Equation (1) simply states that the center of mass always coincides with the origin of $(G,\mathbf{i}_1,\mathbf{i}_2,\mathbf{i}_3)$, and Eq. (2) is the usual way in analytical mechanics to define the rotation of an object with respect to a reference one. In fact Eq. (2) defines at every time the actual orientation of $(G,\mathbf{i}_1,\mathbf{i}_2,\mathbf{i}_3)$ with respect to the fixed frame moved in the center of mass of the molecule $(G,\mathbf{i},\mathbf{j},\mathbf{k})$, where the unit vectors $\mathbf{i}_1$, $\mathbf{i}_2$, $\mathbf{i}_3$ are expressed in $(G,\mathbf{i},\mathbf{j},\mathbf{k})$ via the Eulerian angles[2] (see Fig. 1). Hence for each configuration in $(O,\mathbf{i},\mathbf{j},\mathbf{k})$ we can obtain the local molecular frame $(G,\mathbf{i}_1,\mathbf{i}_2,\mathbf{i}_3)$ using the center of mass as origin and rotating an orthonormal frame around the center of mass until Eq. (2) is fulfilled. Note that Eq. (2) differs from the expression used by Eckart[8] to define the molecular frame, as in the latter the masses are not involved. Equations (1)–(2) provide a set of six linear algebraic equations which can be used to express six coordinates q_{il} in $(G,\mathbf{i}_1,\mathbf{i}_2,\mathbf{i}_3)$ as linear combinations of the other $3N-6$, and we chose to express q_{11}, q_{12}, q_{21}, q_{22}, q_{23}, q_{31} in terms of the others. Note that it is not possible to use Eqs. (1)–(2) to express six coordinates which define two particles as in this case the system of linear equations cannot be solved for all the six q_{il} (the six×six matrix of coefficients is singular). Hence we can replace the original set of $3N$ particle coordinates with a new one defined by the center of mass position $\mathbf{r}_G$, the Eulerian angles, θ, ϕ, ψ and the left $3N-6$ q_{il} coordinates. We do not treat explicitly the case of linear molecules where two Eulerian angles must be used and there are $N-1$ internal coordinates, or the case of two-dimensional molecules where there are $2N-3$ internal coordinates,[9] as they are special cases of our general derivations. It is clear that in the new set of coordinates the center of mass coordinates describe the overall translation of the molecule, the Eulerian angles describe the rotation and the $3N-6$ coordinates in $(G,\mathbf{i}_1,\mathbf{i}_2,\mathbf{i}_3)$ are the internal (intramolecular) degrees of freedom $\mathbf{x}_{\text{in}}$. The ith particle position can then be expressed as

$$\mathbf{r}_i=\mathbf{r}_G+\sum_{l=1}^{3} q_{il}\mathbf{i}_l,$$

$$\mathbf{i}_l=\mathbf{i}_l(\theta,\phi,\psi),$$

where in the case of the first three atoms q_{11}, q_{12}, q_{21}, q_{22}, q_{23}, q_{31} are clearly linear combinations of the internal coordinates $\mathbf{x}_{\text{in}}$ with time independent coefficients. It should be noted that it is not possible to use a linear reference configuration because in this case one of the Eulerian angles cannot be defined (the six×six matrix of the coefficients to solve the six q_{il} would be singular). It is also very interesting to express the kinetic energy in the new coordinates. In fact, from the last equation we can express the velocity of the ith particle in $(O,\mathbf{i},\mathbf{j},\mathbf{k})$ as[2]

$$\dot{\mathbf{r}}_i=\dot{\mathbf{r}}_G+\sum_{l=1}^{3}\dot{q}_{il}\mathbf{i}_l+\sum_{l=1}^{3} q_{il}\frac{d\mathbf{i}_l}{dt}=\dot{\mathbf{r}}_G+\sum_{l=1}^{3}\dot{q}_{il}\mathbf{i}_l+\boldsymbol{\omega}\times\sum_{l=1}^{3} q_{il}\mathbf{i}_l, \tag{5}$$

where we used

$$\frac{d\mathbf{i}_l}{dt}=\boldsymbol{\omega}\times\mathbf{i}_l \tag{6}$$

with the angular velocity $\boldsymbol{\omega}$ expressed in terms of the Eulerian angles time derivatives[2]

$$\boldsymbol{\omega}=\dot{\theta}\mathbf{n}+\dot{\phi}\mathbf{k}+\dot{\psi}\mathbf{i}_3 \tag{7}$$

(for the definition of the unit vector $\mathbf{n}$ see Fig. 1).

Hence from Eq. (5), using Eqs. (1)–(2) we obtain after some algebra, the kinetic energy[2]

$$K=\frac{1}{2}\sum_{i=1}^{N} m_i(\dot{\mathbf{r}}_i\cdot\dot{\mathbf{r}}_i)=K_G+K_{\text{rot}}+K_{\text{in}}+K_c, \tag{8}$$

where

$$K_G=\tfrac{1}{2}M(\dot{\mathbf{r}}_G\cdot\dot{\mathbf{r}}_G), \tag{9}$$

$$K_{\text{rot}}=\tfrac{1}{2}(\boldsymbol{\omega}\cdot\tilde{I}\boldsymbol{\omega}), \tag{10}$$

$$\tilde{I}\boldsymbol{\omega}=\sum_{i=1}^{N} m_i\{\mathbf{q}_i\times(\boldsymbol{\omega}\times\mathbf{q}_i)\}, \tag{11}$$

$$K_{\text{in}}=\frac{1}{2}\sum_{i=1}^{N} m_i(\dot{\mathbf{q}}_i'\cdot\dot{\mathbf{q}}_i'), \tag{12}$$

$$K_c=\boldsymbol{\omega}\cdot\left\{\sum_{i=1}^{N} m_i(\mathbf{q}_i-\mathbf{q}_i^0)\times\dot{\mathbf{q}}_i'\right\} \tag{13}$$

with

$$M=\sum_{i=1}^{N} m_i, \tag{14}$$

$$\dot{\mathbf{q}}_i'=\sum_{l=1}^{3}\dot{q}_{il}\mathbf{i}_l \tag{15}$$

and $\tilde{I}$ the instantaneous inertia tensor in $(G,\mathbf{i}_1,\mathbf{i}_2,\mathbf{i}_3)$, if the vector $\boldsymbol{\omega}$ is also expressed in the $(\mathbf{i}_1,\mathbf{i}_2,\mathbf{i}_3)$ basis set.

Hence using Eq. (7) and remembering that from Eqs. (1)–(2) we can express six coordinates as linear combination of the other $3N-6$ in $(G,\mathbf{i}_1,\mathbf{i}_2,\mathbf{i}_3)$, it is clear from Eqs. (8)–(13) that the kinetic energy is a quadratic form of the velocities of the new set of coordinates and when $\mathbf{q}_i\cong\mathbf{q}_i^0$ the kinetic energy reduces to the sum of independent quadratic terms with no cross products between the translational $(\dot{r}_{Gx},\dot{r}_{Gy},\dot{r}_{Gz})$, the rotational $(\dot{\theta},\dot{\phi},\dot{\psi})$ and the $3N-6$ internal velocities $\dot{\mathbf{x}}_{\text{in}}^T=(\dot{q}_{13}\dot{q}_{32}\dot{q}_{33}\cdots\ \dot{q}_{N1}\dot{q}_{N2}\dot{q}_{N3})$. This kinetic energy form in the new coordinates is very useful in statistical mechanics in order to evaluate the partition function and in general allows analytical derivations in statistical and theoretical mechanics.

B. Derivation of the constrained dynamics

We can use Eqs. (1)–(2) to define a set of six linear holonomic constraints in order to fix the $(G,\mathbf{i}_1,\mathbf{i}_2,\mathbf{i}_3)$ frame of the molecule. In fact, if we start our dynamics from a configuration which belongs to the constraint surface defined by Eqs. (1)–(2), we can derive ideal constraint forces, via the gradients of the constraints, to correct the motions of the system in order to keep the molecule on this constraint surface, stopping the translation and rotation of $(G,\mathbf{i}_1,\mathbf{i}_2,\mathbf{i}_3)$. Note that the left hand side expression of Eq. (2) is a function only of the Eulerian angles and hence the fulfillment of the three holonomic constraints defined by Eq. (2) in a simulation means to keep the Eulerian angles fixed at zero. Note also that the gradients of these three holonomic constraints are linear combinations of the gradients of the Eulerian angles.

If at the initial time $(G,\mathbf{i}_1,\mathbf{i}_2,\mathbf{i}_3)$ coincides with the fixed reference of frame moved in the center of mass of the molecule $(G,\mathbf{i},\mathbf{j},\mathbf{k})$, the two frames will coincide at every other time because of the application of the constraint forces. Hence defining with

$$\mathbf{q}_i=\mathbf{i}x_i+\mathbf{j}y_i+\mathbf{k}z_i, \tag{16}$$

the ith particle position in $(G,\mathbf{i},\mathbf{j},\mathbf{k})\equiv(G,\mathbf{i}_1,\mathbf{i}_2,\mathbf{i}_3)$ and with

$$\mathbf{q}_i^0=\mathbf{i}X_i+\mathbf{j}Y_i+\mathbf{k}Z_i, \tag{17}$$

the ith particle position of the reference configuration in the same frame, we can rewrite Eqs. (1) and (2)

$$\sum_{i=1}^{N} m_i\mathbf{q}_i=\mathbf{0}, \tag{18}$$

$$\sum_{i=1}^{N} m_i(\mathbf{q}_i^0\times\mathbf{q}_i)=\mathbf{0} \tag{19}$$

using the explicit components expressions in $(G,\mathbf{i}_1,\mathbf{i}_2,\mathbf{i}_3)$

$$g_1 = \sum_{i=1}^{N} m_i x_i = 0, \tag{20}$$

$$g_2 = \sum_{i=1}^{N} m_i y_i = 0, \tag{21}$$

$$g_3 = \sum_{i=1}^{N} m_i z_i = 0, \tag{22}$$

$$g_4 = \sum_{i=1}^{N} m_i (Y_i z_i - Z_i y_i) = 0, \tag{23}$$

$$g_5 = \sum_{i=1}^{N} m_i (Z_i x_i - X_i z_i) = 0, \tag{24}$$

$$g_6 = \sum_{i=1}^{N} m_i (X_i y_i - Y_i x_i) = 0. \tag{25}$$

Equations (20)–(25) define the six holonomic constraints and can be used to obtain the ideal constraint forces as usual via the gradients of the g functions. In fact, from their time derivatives we have

$$\dot g_k = \sum_{i=1}^{N} \left(\frac{\partial g_k}{\partial x_i} \dot x_i + \frac{\partial g_k}{\partial y_i} \dot y_i + \frac{\partial g_k}{\partial z_i} \dot z_i \right) = 0, \tag{26}$$

implying that in configurational space these gradients are always orthogonal to the velocities vector $(\dot x_1 \dot y_1 \dot z_1 \cdots \dot x_N \dot y_N \dot z_N)$, and then to the constraint surface. Therefore constraint forces defined as linear combinations of the g gradients will be also orthogonal to the velocities vector and so will not produce any work on the system, as required for ideal constraint forces. Hence using a leap-frog algorithm, we can express the change of the coordinates during one time step in the presence of a set of n_g holonomic constraints as

$$\Delta x_i = \dot x_i \left(t - \frac{\tau}{2} \right) \tau + \frac{f_{ix}(t)}{m_i} \tau^2 + \tau^2 \sum_{k=1}^{n_g} \frac{\lambda_k(t)}{m_i} \frac{\partial g_k}{\partial x_i}(t), \tag{27}$$

$$\Delta y_i = \dot y_i \left(t - \frac{\tau}{2} \right) \tau + \frac{f_{iy}(t)}{m_i} \tau^2 + \tau^2 \sum_{k=1}^{n_g} \frac{\lambda_k(t)}{m_i} \frac{\partial g_k}{\partial y_i}(t), \tag{28}$$

$$\Delta z_i = \dot z_i \left(t - \frac{\tau}{2} \right) \tau + \frac{f_{iz}(t)}{m_i} \tau^2 + \tau^2 \sum_{k=1}^{n_g} \frac{\lambda_k(t)}{m_i} \frac{\partial g_k}{\partial z_i}(t), \tag{29}$$

where f_{ix}, f_{iy}, f_{iz} are the x, y and z components of the systematic force on particle i (the sum of the forces due to the potential energy and, if present, the frictional force, but excluding the constraint force) and τ is the time step used. We can obtain the set of λ_k at one time step from the fact that $dg_k = 0$ at any time. In fact, using the leap-frog scheme we have

$$\Delta g_k = \dot g_k \left(t + \frac{\tau}{2} \right) \tau = \dot g_k \left(t - \frac{\tau}{2} \right) \tau + \ddot g_k(t) \tau^2, \tag{30}$$

and hence

$$\Delta g_k = \sum_{j=1}^{3N} \frac{\partial g_k}{\partial x_j} \left(t - \frac{\tau}{2} \right) \dot x_j \left(t - \frac{\tau}{2} \right) \tau + \sum_{j=1}^{3N} \frac{\partial g_k}{\partial x_j}(t) \ddot x_j(t) \tau^2 + h_k(t) \tau^2, \tag{31}$$

where x_j is now the generic coordinate, and

$$h_k = \sum_{j=1}^{3N} \sum_{j'=1}^{3N} \frac{\partial^2 g_k}{\partial x_j \, \partial x_{j'}} \dot x_j \dot x_{j'},$$

$$\ddot x_j = \frac{f_j}{m_j} + \sum_{k=1}^{n_g} \frac{\lambda_k}{m_j} \frac{\partial g_k}{\partial x_j}.$$

Note that the systematic acceleration in the presence of a frictional force is

$$\frac{f_j}{m_j} = -\frac{1}{m_j} \frac{\partial \Phi}{\partial x_j} + \gamma \dot x_j, \tag{32}$$

where Φ is the potential energy of the system and the frictional constant γ is the same for all the coordinates. Hence from Eq. (26) we can rewrite Eq. (31) as

$$\Delta g_k = \sum_{j=1}^{3N} \frac{\partial g_k}{\partial x_j}(t) a_j(t) \tau^2 + \sum_{k'=1}^{n_g} \lambda_{k'} \sum_{j=1}^{3N} \frac{\partial g_k}{\partial x_j}(t) \frac{\partial g_{k'}}{\partial x_j}(t) \frac{\tau^2}{m_j} + h_k(t) \tau^2 \tag{33}$$

with a_j the acceleration of the j coordinate due only to the conservative force $(-\partial \Phi / \partial x_j)$, and keeping only the terms up to τ^2

$$h_k(t) = \sum_{j=1}^{3N} \sum_{j'=1}^{3N} \frac{\partial^2 g_k}{\partial x_j \, \partial x_{j'}}(t) \dot x_j \left(t - \frac{\tau}{2} \right) \dot x_{j'} \left(t - \frac{\tau}{2} \right).$$

So using the fact that $\Delta g_k = 0$ we can solve the $\boldsymbol{\lambda}$ set via

$$\boldsymbol{\lambda} = -\tilde{\Theta}^{-1} \mathbf{c}, \tag{34}$$

where the column vector $\mathbf{c}$ and the matrix $\tilde{\Theta}$ are given by

$$c_k = \sum_{j=1}^{3N} \frac{\partial g_k}{\partial x_j}(t) a_j(t) \tau^2 + h_k(t) \tau^2, \tag{35}$$

$$\Theta_{k,k'} = \sum_{j=1}^{3N} \frac{\partial g_k}{\partial x_j}(t) \frac{\partial g_{k'}}{\partial x_j}(t) \frac{\tau^2}{m_j}. \tag{36}$$

Equations (34)–(36) provide a completely general solution for $\boldsymbol{\lambda}$ associated with any possible set of holonomic constraints. For the constraints defined by Eqs. (20)–(25) the symmetric matrix $\tilde{\Theta}$ is a block diagonal matrix

$$\tilde{\Theta} = \begin{pmatrix} \Theta_{1,1} & 0 & 0 & 0 & 0 & 0 \\ 0 & \Theta_{2,2} & 0 & 0 & 0 & 0 \\ 0 & 0 & \Theta_{3,3} & 0 & 0 & 0 \\ 0 & 0 & 0 & \Theta_{4,4} & \Theta_{4,5} & \Theta_{4,6} \\ 0 & 0 & 0 & \Theta_{5,4} & \Theta_{5,5} & \Theta_{5,6} \\ 0 & 0 & 0 & \Theta_{6,4} & \Theta_{6,5} & \Theta_{6,6} \end{pmatrix}, \tag{37}$$

with

$$\Theta_{1,1}=\Theta_{2,2}=\Theta_{3,3}=\sum_{i=1}^{N} m_i, \tag{38}$$

$$\Theta_{4,4}=\sum_{i=1}^{N} m_i(Z_i^2+Y_i^2), \tag{39}$$

$$\Theta_{4,5}=\sum_{i=1}^{N} m_i(-Y_iX_i), \tag{40}$$

$$\Theta_{4,6}=\sum_{i=1}^{N} m_i(-X_iZ_i), \tag{41}$$

$$\Theta_{5,4}=\sum_{i=1}^{N} m_i(-X_iY_i), \tag{42}$$

$$\Theta_{5,5}=\sum_{i=1}^{N} m_i(X_i^2+Z_i^2), \tag{43}$$

$$\Theta_{5,6}=\sum_{i=1}^{N} m_i(-Y_iZ_i), \tag{44}$$

$$\Theta_{6,4}=\sum_{i=1}^{N} m_i(-X_iZ_i), \tag{45}$$

$$\Theta_{6,5}=\sum_{i=1}^{N} m_i(-Y_iZ_i), \tag{46}$$

$$\Theta_{6,6}=\sum_{i=1}^{N} m_i(X_i^2+Y_i^2). \tag{47}$$

Note that the matrix $\tilde{\Theta}$ given by Eqs. (38)–(47) is singular only in the case that the reference configuration is linear.

Realizing that in our case the g functions are linear in the coordinates, we have

$$\frac{\partial g_k}{\partial x_j}\left(t-\frac{\tau}{2}\right)=\frac{\partial g_k}{\partial x_j}(t)=\text{constant}, \tag{48}$$

$$h_k=0, \tag{49}$$

and so using again Eq. (26) we obtain

$$c_k=\sum_{j=1}^{3N}\frac{\partial g_k}{\partial x_j}\Delta x_j', \tag{50}$$

where

$$\Delta x_j'=\dot{x}_j\left(t-\frac{\tau}{2}\right)\tau+a_j(t)\tau^2 \tag{51}$$

is the displacement of the coordinate x_j in one time step due only to the conservative force. Hence we can solve the translational $(\lambda_1,\lambda_2,\lambda_3)$ and rotational $(\lambda_4,\lambda_5,\lambda_6)$ coefficients independently. It must be noted that in the presence of extra holonomic constraints involving particle distances like the bond length constraints, the translational constraints (given by g_1,g_2,g_3) are still independent, with matrix elements always zero apart from the diagonal ones $(\Theta_{11},\Theta_{22},\Theta_{33})$. On the contrary, the rotational constraints (given by g_4,g_5,g_6) can in general interact with these new constraints having nonzero elements between the rotational and the bond constraints blocks of the $\tilde{\Theta}$ matrix. In this case we should invert each step a large $\tilde{\Theta}$ matrix (involving all the constraints in the system) to solve instantaneously the $\boldsymbol{\lambda}$ set [Eq. (34)]. However, this might be computationally very expensive and an iterative procedure could be used instead. In test simulations where we used fixed bond lengths, we solved consecutively the bond constraints with the SHAKE algorithm[10] and the roto-translational ones with $\tilde{\Theta}$ given by Eqs. (38)–(47). This procedure can be iteratively applied until the proper accuracy is reached. For the systems we studied a single application of the roto-translational constraints at every time step, after applying SHAKE, was always enough to obtain a proper constraints fulfilment. However, for accurate statistical mechanical calculations it is preferable not to use such an approximate procedure, but either the exact $\tilde{\Theta}$ matrix solution, involving the bond constraints, or simply not to use any bond constraints (this latter choice is in general better as the simulation physical consistency is higher[11]). Note that when we want to stop the roto-translational motions of a solute molecule surrounded by solvent molecules, the described procedure will apply the ideal constraint forces only to the atoms of the solute.

A schematic description of the implementation of the algorithm in a usual MD code is:

(1) evaluate (unconstrained) conservative accelerations due to the conservative forces $\mathbf{F}(t)$ on all atoms

$$\ddot{\mathbf{r}}_i'(t)=\mathbf{F}_i(t)/m_i; \tag{52}$$

(2) compute velocities

$$\dot{\mathbf{r}}_i'\left(t+\frac{\tau}{2}\right)=\dot{\mathbf{r}}_i'\left(t-\frac{\tau}{2}\right)+\ddot{\mathbf{r}}_i'(t)\tau; \tag{53}$$

(3) compute new (unconstrained) positions

$$\mathbf{r}_i'(t+\tau)=\mathbf{r}_i(t)+\dot{\mathbf{r}}_i'\left(t+\frac{\tau}{2}\right)\tau; \tag{54}$$

(4) if required, apply SHAKE constraints to coordinates;

(5) apply roto-translational constraints to coordinates;

(6) compute constrained velocities

$$\dot{\mathbf{r}}_i\left(t+\frac{\tau}{2}\right)=\frac{\mathbf{r}_i(t+\tau)-\mathbf{r}_i(t)}{\tau}; \tag{55}$$

(7) if required, apply temperature and/or pressure coupling.

Note that in the case the bond constraints are used steps 4 and 5 could be reduced to a single step implementing the roto-translational constraints directly in the SHAKE routine, using either the exact solution or the iterative procedure. It must be considered that to initialize the MD trajectory we have to start from a configuration which is already on the constraint surface, and use initial constrained velocities. Consider also that the reference configuration should be an energy minimized structure as this provides a more stable algorithm. Note that Eqs. (1)–(2) can be used also to remove the roto-translational motions from a trajectory obtained by a usual simulation. In that case, after the trivial removal of the center of mass motion, we should solve Eq. (2) for every configuration obtained by MD in the form

$$\sum_{i=1}^{N} \mathbf{R}_i \times m_i \widetilde{T}(\mathbf{r}_i - \mathbf{r}_G) = \mathbf{0},$$

$$\mathbf{R}_i^T = (q_{i1}^0 q_{i2}^0 q_{i3}^0), \tag{56}$$

where $\widetilde{T}$ is the orthogonal rotational matrix which overimposes $(G,\mathbf{i},\mathbf{j},\mathbf{k})$ to $(G,\mathbf{i}_1,\mathbf{i}_2,\mathbf{i}_3)$. Hence for a given reference configuration, Eq. (56) provides for every MD time frame a set of three equations. These equations can be solved obtaining directly the three rotational angles which define the matrix $\widetilde{T}$, and in this way also the rotation can be removed.

C. Constrained dynamics simulations and statistical mechanics

In the previous sections we described the molecular coordinates used to define the molecular internal and external degrees of freedom, and how to obtain molecular dynamics simulations with the roto-translational motions constrained. In this section we show that the usual MD equations of motion, that for unconstrained systems can provide an exact statistical mechanical behavior (the Hamiltonian or Lagrangian,[2,3] the isothermal Gaussian,[4,5] and the Nosé–Hoover dynamics[6,7]), provide in the presence of holonomic constraints a dynamics which is still consistent with the correct statistical mechanical ensemble, although in a restricted phase space.

Consider a system with Hamiltonian $H(\mathbf{x},\mathbf{p})$ and a set of (ideal) holonomic constraints. Its equations of motion are

$$\dot{p}_i = -\left(\frac{\partial H}{\partial x_i}\right) + \gamma p_i + f_i^c,$$

$$\dot{x}_i = \left(\frac{\partial H}{\partial p_i}\right), \tag{57}$$

or

$$\dot{p}_i = \left(\frac{\partial L}{\partial x_i}\right) + \gamma p_i + f_i^c, \tag{58}$$

$$p_i = \left(\frac{\partial L}{\partial \dot{x}_i}\right), \tag{59}$$

where

$$L(\mathbf{x},\dot{\mathbf{x}}) = 2K - H = K - \Phi \tag{60}$$

is the Lagrangian of the system, with K and Φ the kinetic and potential energies, respectively, $\mathbf{x}$ are the coordinates and $\mathbf{p}$ their conjugated momenta, $\mathbf{f}^{\mathbf{c}}$ are the ideal constraint forces and γ is the frictional coefficient necessary for the temperature coupling of the system (which is clearly zero for the pure Lagrangian or Hamiltonian dynamics). Note that $\mathbf{x}$, $\dot{\mathbf{x}}$, $\mathbf{p}$ and $\mathbf{f}^{\mathbf{c}}$ are multidimensional vectors, and the partial derivatives in the coordinates are always at fixed momenta in the Hamiltonian representation and at fixed velocities in the Lagrangian one. If we define with $\boldsymbol{\xi}$, $\boldsymbol{\beta}$ a set of generalized coordinates where $\boldsymbol{\beta}=\mathbf{0}$ defines the positions on the constraint surface, $\boldsymbol{\pi}$ the conjugated momenta of the $\boldsymbol{\xi}$ coordinates

$$\pi_i = \left(\frac{\partial L}{\partial \dot{\xi}_i}\right), \tag{61}$$

and $\boldsymbol{\nu}$ the conjugated momenta of the $\boldsymbol{\beta}$ coordinates

$$\nu_i = \left(\frac{\partial L}{\partial \dot{\beta}_i}\right), \tag{62}$$

we can express the Lagrangian or Hamiltonian of the system as a function only of the $\boldsymbol{\xi}$ coordinates and, respectively, their velocities $\dot{\boldsymbol{\xi}}$ or conjugated momenta $\boldsymbol{\pi}$. In fact in the presence of the holonomic constraints, expressed by the ideal constraint forces, the potential energy of the system is $\Phi(\boldsymbol{\xi},\boldsymbol{\beta}=\mathbf{0})$ and hence a function only of the $\boldsymbol{\xi}$ coordinates. Moreover in such a constrained system from the conjugated momenta definition we have

$$\begin{pmatrix} \boldsymbol{\pi} \\ \boldsymbol{\nu} \end{pmatrix} = \begin{pmatrix} \widetilde{G}_{\xi,\xi} & \widetilde{G}_{\xi,\beta} \\ \widetilde{G}_{\beta,\xi} & \widetilde{G}_{\beta,\beta} \end{pmatrix} \begin{pmatrix} \dot{\boldsymbol{\xi}} \\ \dot{\boldsymbol{\beta}} \end{pmatrix} = \begin{pmatrix} \widetilde{G}_{\xi,\xi} & \widetilde{G}_{\xi,\beta} \\ \widetilde{G}_{\beta,\xi} & \widetilde{G}_{\beta,\beta} \end{pmatrix} \begin{pmatrix} \dot{\boldsymbol{\xi}} \\ \mathbf{0} \end{pmatrix}, \tag{63}$$

and so

$$\boldsymbol{\pi} = \widetilde{G}_{\xi,\xi}\dot{\boldsymbol{\xi}}, \tag{64}$$

$$\boldsymbol{\nu} = \widetilde{G}_{\beta,\xi}\dot{\boldsymbol{\xi}} = \widetilde{G}_{\beta,\xi}\widetilde{G}_{\xi,\xi}^{-1}\boldsymbol{\pi}, \tag{65}$$

where

$$\widetilde{M} = \begin{pmatrix} \widetilde{G}_{\xi,\xi} & \widetilde{G}_{\xi,\beta} \\ \widetilde{G}_{\beta,\xi} & \widetilde{G}_{\beta,\beta} \end{pmatrix} \tag{66}$$

with $\widetilde{G}_{\beta,\xi} = \widetilde{G}_{\xi,\beta}^T$ is the mass tensor expressed for the velocities and conjugated momenta of the $\boldsymbol{\xi}$, $\boldsymbol{\beta}$ coordinates. From these last equations we can express the kinetic energy as

$$\begin{aligned} 2K &= (\dot{\boldsymbol{\xi}}^{\tau} \quad \dot{\boldsymbol{\beta}}^{\tau}) \begin{pmatrix} \widetilde{G}_{\xi,\xi} & \widetilde{G}_{\xi,\beta} \\ \widetilde{G}_{\beta,\xi} & \widetilde{G}_{\beta,\beta} \end{pmatrix} \begin{pmatrix} \dot{\boldsymbol{\xi}} \\ \dot{\boldsymbol{\beta}} \end{pmatrix} \\ &= (\dot{\boldsymbol{\xi}}^{\tau} \quad \mathbf{0}^{\tau}) \begin{pmatrix} \widetilde{G}_{\xi,\xi} & \widetilde{G}_{\xi,\beta} \\ \widetilde{G}_{\beta,\xi} & \widetilde{G}_{\beta,\beta} \end{pmatrix} \begin{pmatrix} \dot{\boldsymbol{\xi}} \\ \mathbf{0} \end{pmatrix} \\ &= \dot{\boldsymbol{\xi}}^T \widetilde{G}_{\xi,\xi}\dot{\boldsymbol{\xi}} = \boldsymbol{\pi}^T \widetilde{G}_{\xi,\xi}^{-1}\boldsymbol{\pi}. \end{aligned} \tag{67}$$

Hence, considering that the mass tensor $\widetilde{M}$ as the potential energy is also a function only of the $\boldsymbol{\xi}$ coordinates, we have that in the presence of holonomic constraints the Hamiltonian or Lagrangian can be expressed as functions only of the $\boldsymbol{\xi}$ coordinates and, respectively, their conjugated momenta $\boldsymbol{\pi}$ or velocities $\dot{\boldsymbol{\xi}}$ as the $\boldsymbol{\beta}$ coordinates are fixed and so $\dot{\boldsymbol{\beta}}=\mathbf{0}$. This clearly means that the equations of motion for the full system reduce to the equations of motion only in the $\boldsymbol{\xi}$, $\boldsymbol{\pi}$ phase space which can be obtained directly by the Lagrangian and Hamiltonian functions that can be expressed now as $L(\boldsymbol{\xi},\dot{\boldsymbol{\xi}})$ and $H(\boldsymbol{\xi},\boldsymbol{\pi})$. Note that the time dependence of the momenta $\boldsymbol{\nu}$ is fully determined by the equations of motion in the $\boldsymbol{\xi}$, $\boldsymbol{\pi}$ phase space as they are linear combinations of the $\boldsymbol{\pi}$ momenta; see Eq. (65).

The equations of motion in the $\boldsymbol{\xi}$, $\boldsymbol{\pi}$ phase space are then

$$\dot{\pi}_i=\frac{d}{dt}\left(\frac{\partial L}{\partial \dot{\xi}_i}\right)=\frac{d}{dt}\left[\sum_j \left(\frac{\partial L}{\partial \dot{x}_j}\right)\left(\frac{\partial \dot{x}_j}{\partial \dot{\xi}_i}\right)\right]$$
$$=\sum_j \dot{p}_j\left(\frac{\partial \dot{x}_j}{\partial \dot{\xi}_i}\right)+\sum_j \left(\frac{\partial L}{\partial \dot{x}_j}\right)\sum_k \frac{\partial}{\partial \xi_k}\left(\frac{\partial x_j}{\partial \xi_i}\right)\dot{\xi}_k, \tag{68}$$

where we used

$$\left(\frac{\partial \dot{x}_j}{\partial \dot{\xi}_i}\right)=\left(\frac{\partial x_j}{\partial \xi_i}\right). \tag{69}$$

Substituting in Eq. (68) the expression for $\dot{\mathbf{p}}$, and using again Eq. (69) we obtain

$$\dot{\pi}_i=\sum_j \left(\frac{\partial L}{\partial x_j}\right)\left(\frac{\partial x_j}{\partial \xi_i}\right)+\gamma\sum_j \left(\frac{\partial L}{\partial \dot{x}_j}\right)\left(\frac{\partial \dot{x}_j}{\partial \dot{\xi}_i}\right)$$
$$+\sum_j f_j^c\left(\frac{\partial x_j}{\partial \xi_i}\right)+\sum_j \left(\frac{\partial L}{\partial \dot{x}_j}\right)\frac{\partial}{\partial \xi_i}\sum_k \left(\frac{\partial x_j}{\partial \xi_k}\right)\dot{\xi}_k$$
$$=\left(\frac{\partial L}{\partial \xi_i}\right)+\gamma\pi_i \tag{70}$$

or from

$$\left(\frac{\partial L}{\partial \xi_i}\right)=-\left(\frac{\partial H}{\partial \xi_i}\right) \tag{71}$$

in the Hamiltonian representation

$$\dot{\pi}_i=-\left(\frac{\partial H}{\partial \xi_i}\right)+\gamma\pi_i, \tag{72}$$

$$\dot{\xi}_i=\left(\frac{\partial H}{\partial \pi_i}\right). \tag{73}$$

Note that in Eq. (70) we used the fact that the multidimensional vector of the ideal constraint forces is by definition a linear combination of the gradients of the constraints, and hence

$$\sum_j f_j^c\left(\frac{\partial x_j}{\partial \xi_i}\right)=0 \tag{74}$$

for all the ξ_i coordinates. Equations (70) and (72) show that the constrained dynamics expressed in terms of $\boldsymbol{\xi}$, $\boldsymbol{\pi}$ is equivalent to the dynamics of an unconstrained system in a reduced dimensional phase space. This fact implies that if for the unconstrained system this type of equations of motion provides a density distribution of the trajectory which converges in time to a given statistical mechanical ensemble distribution, the same is true for the constrained system in its constrained phase space (constraint surface). We will explicitly show this in the case of the isothermal Gaussian dynamics since it is a prototypical example: similar derivations provide the same result for the Lagrangian or Hamiltonian dynamics (as is well known from theoretical mechanics[2,3]) and for Nosé–Hoover dynamics.

In the case we use the isothermal Gaussian dynamics we have[4,5]

$$\gamma=\frac{\dot{\Phi}}{2K_0}=\frac{\nabla_{\xi}\Phi\cdot\dot{\boldsymbol{\xi}}}{\boldsymbol{\pi}\cdot\dot{\boldsymbol{\xi}}}, \tag{75}$$

which provides a constant kinetic energy K_0 during the simulation. From the conservation of the probability density ρ in phase space, applying the divergence theorem, we have

$$-\left(\frac{\partial \rho}{\partial t}\right)=\sum_i \frac{\partial}{\partial \xi_i}(\rho\dot{\xi}_i)+\sum_i \frac{\partial}{\partial \pi_i}(\rho\dot{\pi}_i)$$
$$=\sum_i \left(\frac{\partial \rho}{\partial \xi_i}\right)\dot{\xi}_i+\sum_i \left(\frac{\partial \rho}{\partial \pi_i}\right)\dot{\pi}_i+\rho\sum_i \left(\frac{\partial \dot{\xi}_i}{\partial \xi_i}\right)$$
$$+\rho\sum_i \left(\frac{\partial \dot{\pi}_i}{\partial \pi_i}\right)$$
$$=\sum_i \left(\frac{\partial \rho}{\partial \xi_i}\right)\dot{\xi}_i+\sum_i \left(\frac{\partial \rho}{\partial \pi_i}\right)\dot{\pi}_i+\rho\sum_i \left(\frac{\partial^2 H}{\partial \xi_i\,\partial \pi_i}\right.$$
$$\left.-\frac{\partial^2 H}{\partial \pi_i\,\partial \xi_i}+\frac{\partial}{\partial \pi_i}(\gamma\pi_i)\right), \tag{76}$$

and hence

$$\dot{\rho}=-\rho\sum_i \frac{\partial}{\partial \pi_i}(\gamma\pi_i)=-D\gamma\rho-\rho\sum_i \pi_i\left(\frac{\partial \gamma}{\partial \pi_i}\right), \tag{77}$$

where

$$\dot{\rho}=\left(\frac{\partial \rho}{\partial t}\right)+\sum_i \left(\frac{\partial \rho}{\partial \xi_i}\right)\dot{\xi}_i+\sum_i \left(\frac{\partial \rho}{\partial \pi_i}\right)\dot{\pi}_i \tag{78}$$

and D is the total number of coordinates ξ_i. From Eq. (75) we have

$$\left(\frac{\partial \gamma}{\partial \pi_i}\right)=\frac{1}{\boldsymbol{\pi}\cdot\dot{\boldsymbol{\xi}}}\sum_j \left(\frac{\partial \Phi}{\partial \xi_j}\right)\left(\frac{\partial \dot{\xi}_j}{\partial \pi_i}\right)$$
$$-\frac{\dot{\Phi}}{(\boldsymbol{\pi}\cdot\dot{\boldsymbol{\xi}})^2}\left[\sum_j \pi_j\left(\frac{\partial \dot{\xi}_j}{\partial \pi_i}\right)+\dot{\xi}_i\right]$$
$$=\frac{1}{\boldsymbol{\pi}\cdot\dot{\boldsymbol{\xi}}}\sum_j \left(\frac{\partial \Phi}{\partial \xi_j}\right)\left(\frac{\partial \dot{\xi}_j}{\partial \pi_i}\right)-2\dot{\Phi}\frac{\dot{\xi}_i}{(\boldsymbol{\pi}\cdot\dot{\boldsymbol{\xi}})^2}, \tag{79}$$

where we used

$$\sum_j \pi_j\left(\frac{\partial \dot{\xi}_i}{\partial \pi_j}\right)=\sum_j \pi_j\left(\frac{\partial \dot{\xi}_j}{\partial \pi_i}\right)=\dot{\xi}_i. \tag{80}$$

Finally, inserting Eq. (79) into Eq. (77) and using again Eq. (80), we obtain

$$\begin{aligned}\dot{\rho}&=-D\gamma\rho-\frac{\rho}{\boldsymbol{\pi}\cdot\dot{\boldsymbol{\xi}}}\sum_i \pi_i\sum_j \left(\frac{\partial\Phi}{\partial\xi_j}\right)\left(\frac{\partial\dot{\xi}_j}{\partial\pi_i}\right)\\&\quad+\frac{2\dot{\Phi}\rho}{(\boldsymbol{\pi}\cdot\dot{\boldsymbol{\xi}})^2}\sum_i \pi_i\dot{\xi}_i\\&=-D\gamma\rho-\frac{\rho}{2K_0}\sum_j \left(\frac{\partial\Phi}{\partial\xi_j}\right)\sum_i \pi_i\left(\frac{\partial\dot{\xi}_j}{\partial\pi_i}\right)+2\frac{\dot{\Phi}\rho}{2K_0}\\&=-\frac{\dot{\Phi}(D-1)}{2K_0}\rho.\end{aligned}\tag{81}$$

The last equation means that if $2K_0=(D-1)kT$, when the stationary condition $\partial\rho/\partial t=0$ is reached, the probability density for an ensemble of isothermal Gaussian dynamics trajectories (with no constants of motion other than the kinetic energy in the $\boldsymbol{\xi}$, $\boldsymbol{\pi}$ phase space[12]) is

$$\rho(\boldsymbol{\xi},\boldsymbol{\pi})=\frac{e^{-\beta\Phi}\delta(K-K_0)}{\int e^{-\beta\Phi}\delta(K-K_0)d\boldsymbol{\xi}\,d\boldsymbol{\pi}}\tag{82}$$

with $\beta^{-1}=kT$, where k is the Boltzmann constant and T the reference temperature. Note that Φ is evaluated at each $\boldsymbol{\xi}$ configuration on the constraint surface $\boldsymbol{\beta}=\mathbf{0}$.

In order to obtain the configurational probability density we have to integrate the probability density in phase space over the momenta $\boldsymbol{\pi}$ at each configuration

$$\rho(\boldsymbol{\xi})=\int\rho(\boldsymbol{\xi},\boldsymbol{\pi})d\boldsymbol{\pi}=\frac{e^{-\beta\Phi}}{\int e^{-\beta\Phi}\delta(K-K_0)d\boldsymbol{\xi}\,d\boldsymbol{\pi}}\times\int\delta(K-K_0)d\boldsymbol{\pi}.\tag{83}$$

Note that in Eq. (83) we can obtain the integral over the momenta via subsequent transformations. First we can express the kinetic energy in terms of the $\boldsymbol{\pi}$ momenta

$$K=\tfrac{1}{2}\boldsymbol{\pi}\cdot\dot{\boldsymbol{\xi}}=\tfrac{1}{2}\boldsymbol{\pi}^T\tilde{G}_{\xi,\xi}^{-1}\boldsymbol{\pi}.\tag{84}$$

Second using the orthogonal transformation of the momenta which diagonalizes $\tilde{G}_{\xi,\xi}^{-1}$, the kinetic energy in these ''eigenvectors'' momenta $\boldsymbol{\pi}_1$ transforms into

$$K=\frac{1}{2}\sum_{i=1}^{D}\lambda_i^{-1}\pi_{1i}^2,\tag{85}$$

where clearly λ_i are the eigenvalues of $\tilde{G}_{\xi,\xi}$. Third, applying to the $\boldsymbol{\pi}_1$ momenta a rescaling transformation

$$\pi_{1i}=(2\lambda_i)^{1/2}\pi_{2i}\tag{86}$$

the kinetic energy in the new momenta $\boldsymbol{\pi}_2$ is

$$K=\sum_{i=1}^{D}\pi_{2i}^2\tag{87}$$

with the Jacobean for this last transformation equal to $2^{D/2}(\det\tilde{G}_{\xi,\xi})^{1/2}$. Finally, expressing the integral in multidimensional polar coordinates, where the square root of the kinetic energy is the radius length, we have

$$\begin{aligned}\int\delta(K-K_0)d\boldsymbol{\pi}&=2^{D/2}(\det\tilde{G}_{\xi,\xi})^{1/2}\\&\quad\times\int A_0K^{(D-1)/2}\delta(K-K_0)d(K^{1/2})\\&=2^{D/2}(\det\tilde{G}_{\xi,\xi})^{1/2}A_0\int\frac{K^{(D-1)/2}}{2K^{1/2}}\delta(K-K_0)dK\\&=2^{D/2}(\det\tilde{G}_{\xi,\xi})^{1/2}A_0\frac{K_0^{D/2-1}}{2},\end{aligned}\tag{88}$$

with A_0 a pure constant depending only on the dimension D of the system. Hence the probability density in the configurational space is

$$\rho(\boldsymbol{\xi})=\frac{e^{-\beta\Phi}(\det\tilde{G}_{\xi,\xi})^{1/2}}{\int e^{-\beta\Phi}(\det\tilde{G}_{\xi,\xi})^{1/2}d\boldsymbol{\xi}}.\tag{89}$$

Note that $\det\tilde{G}_{\xi,\xi}$ is in general a function of the coordinates. This last equation provides exactly the same expression for the probability density in configurational space which is obtained from statistical mechanics. In fact, for a system in the canonical ensemble with the same holonomic constraints we have

$$\begin{aligned}\rho(\boldsymbol{\xi})&=\frac{\int e^{-\beta(K+\Phi)}\delta(\boldsymbol{\beta})\,\delta(\boldsymbol{\nu}-\boldsymbol{\nu}(\boldsymbol{\pi}))d\boldsymbol{\beta}\,d\boldsymbol{\nu}\,d\boldsymbol{\pi}}{\int e^{-\beta(K+\Phi)}\delta(\boldsymbol{\beta})\,\delta(\boldsymbol{\nu}-\boldsymbol{\nu}(\boldsymbol{\pi}))d\boldsymbol{\xi}\,d\boldsymbol{\beta}\,d\boldsymbol{\pi}\,d\boldsymbol{\nu}}\\&=\frac{\int e^{-\beta(K+\Phi)}\,d\boldsymbol{\pi}}{\int e^{-\beta(K+\Phi)}d\boldsymbol{\xi}\,d\boldsymbol{\pi}},\end{aligned}\tag{90}$$

where

$$\boldsymbol{\nu}(\boldsymbol{\pi})=\tilde{G}_{\beta,\xi}\tilde{G}_{\xi,\xi}^{-1}\boldsymbol{\pi}$$

and in the last integral of Eq. (90)

$$K=\tfrac{1}{2}\boldsymbol{\pi}^T\tilde{G}_{\xi,\xi}^{-1}\boldsymbol{\pi}\tag{91}$$

and Φ is obtained for each $\boldsymbol{\xi}$ configuration at $\boldsymbol{\beta}=\mathbf{0}$. Using again the orthogonal transformation for the momenta that diagonalizes $\tilde{G}_{\xi,\xi}^{-1}$ we obtain

$$\rho(\boldsymbol{\xi})=\frac{e^{-\beta\Phi}(\det\tilde{G}_{\xi,\xi})^{1/2}}{\int e^{-\beta\Phi}(\det\tilde{G}_{\xi,\xi})^{1/2}d\boldsymbol{\xi}}.\tag{92}$$

From this last equation follows that assuming ergodicity the probability density in configurational space obtained from the MD trajectory in time is statistically mechanically completely correct even in the presence of a set of (ideal) holonomic constraints.

D. Statistical mechanics in the infinite dilution conditions

In the last section we showed that the use of ideal holonomic constraints in a simulation does not perturb the statistical mechanical consistency of the dynamics, and hence from such a constrained MD trajectory we can obtain the exact statistical mechanics of a constrained system. In this section we show that the exact statistical mechanics of a molecule in the infinite dilution conditions (including the special case corresponding to the ideal gas) can be obtained from that of a system with ideal holonomic constraints for the roto-translational degrees of freedom of the molecule, via a simple correction.

The partition function of a solute-solvent system with a classical Hamiltonian, where for the solute molecules we use the molecular coordinates described in the first theory section, is

$$Q=\int \frac{e^{-\beta K_s}e^{-\beta K}e^{-\beta\mathcal{U}}}{(1+\gamma_s)^{n_s}(1+\gamma)^n n_s!n!h^D}\,d\mathbf{p}_s\,d\mathbf{x}_s\prod_{i=1}^{n}\,d\mathbf{x}_i\,d\mathbf{p}_i$$

$$=\int \frac{e^{-\beta K_s}e^{-\beta K}e^{-\beta\mathcal{U}}}{(1+\gamma_s)^{n_s}(1+\gamma)^n n_s!n!h^D}\,d\mathbf{p}_s\,d\mathbf{x}_s$$

$$\times\prod_{i=1}^{n}\,|\sin\theta_i|\,d\mathbf{x}_i\,d\mathbf{p}_i', \tag{93}$$

$$\mathbf{p}_i'=\tilde{A}_i^T\left(\frac{\partial L}{\partial\dot{\mathbf{x}}_i}\right)=\tilde{A}_i^T\mathbf{p}_i=\tilde{M}_i'\dot{\mathbf{x}}_i', \tag{94}$$

$$\dot{\mathbf{x}}_i'=\tilde{A}_i^{-1}\dot{\mathbf{x}}_i, \tag{95}$$

$$\mathbf{x}_i=\begin{pmatrix}\mathbf{r}_{i,G}\\ \theta_i\\ \phi_i\\ \psi_i\\ \mathbf{x}_{i,\mathrm{in}}\end{pmatrix},\quad \dot{\mathbf{x}}_i=\begin{pmatrix}\dot{\mathbf{r}}_{i,G}\\ \dot{\theta}_i\\ \dot{\phi}_i\\ \dot{\psi}_i\\ \dot{\mathbf{x}}_{i,\mathrm{in}}\end{pmatrix},\quad \dot{\mathbf{x}}_i'=\begin{pmatrix}\dot{\mathbf{r}}_{i,G}\\ \omega_{i1}\\ \omega_{i2}\\ \omega_{i3}\\ \dot{\mathbf{x}}_{i,\mathrm{in}}\end{pmatrix},$$

where K_s is the kinetic energy of the n_s solvent molecules, $\mathbf{x}_s$ and $\mathbf{p}_s$ are the coordinates and conjugated momenta of the solvent, and the kinetic energy of the n solute molecules is

$$K=\frac{1}{2}\sum_{i=1}^{n}\,\mathbf{p}_i'^T(\tilde{M}_i')^{-1}\mathbf{p}_i'. \tag{96}$$

Furthermore, $\mathbf{x}_i$ and $\mathbf{p}_i$ are the coordinates and the conjugated momenta of the ith solute molecule, $\mathcal{U}$ is the potential energy and $1+\gamma$ and $1+\gamma_s$ are the symmetry coefficients per molecule for the solute and solvent molecules necessary to correct the partition function from the rotations and intramolecular atomic displacements which correspond to permutations of identical particles which do not change the physical state of the system.[13,14] For each ith solute molecule we used the corresponding transformation for the solute momenta given by $\mathbf{p}_i=(\tilde{A}_i^T)^{-1}\mathbf{p}_i'$ (with Jacobean $\det(\tilde{A}_i^T)^{-1}=|\sin\theta_i|$), associated with the transformation of the vector $\boldsymbol{\omega}_i$ from the $(\mathbf{n},\mathbf{k},\mathbf{i}_3)$ to the $(\mathbf{i}_1,\mathbf{i}_2,\mathbf{i}_3)$ basis set defined by

$$\omega_{i1}=\dot{\theta}_i\cos\psi_i+\dot{\phi}_i\sin\theta_i\sin\psi_i, \tag{97}$$

$$\omega_{i2}=-\dot{\theta}_i\sin\psi_i+\dot{\phi}_i\sin\theta_i\cos\psi_i, \tag{98}$$

$$\omega_{i3}=\dot{\phi}_i\cos\theta_i+\dot{\psi}_i. \tag{99}$$

Note that the mass tensor $\tilde{M}_i'$ is expressed in the form which is appropiate for using the $\mathbf{p}_i'$ momenta or $\dot{\mathbf{x}}_i'$ velocities, and it can be obtained from the mass tensor $\tilde{M}_i$ expressed for the $\mathbf{p}_i$ momenta and $\dot{\mathbf{x}}_i$ velocities via $\tilde{M}_i'=\tilde{A}_i^T\tilde{M}_i\tilde{A}_i$. Note also that $\tilde{M}_i'$ differs from $\tilde{M}_i$ only for the elements affected by the transformation of the angular velocity given by Eqs. (97)–(99), and hence the mass tensor blocks corresponding to the velocities of the center of mass and internal coordinates are identical in $\tilde{M}_i'$ and $\tilde{M}_i$. Finally, h is the Planck constant, D the total number of degrees of freedom and $\beta=1/kT$. For a given configuration we can solve the integral over the solutes momenta using the orthogonal transformations of these momenta which diagonalize each $(\tilde{M}_i')^{-1}$ obtaining

$$\int\; e^{-\beta\,\frac{1}{2}\mathbf{p}_i'^T(\tilde{M}_i')^{-1}\mathbf{p}_i'}d\mathbf{p}_i=((2\pi kT)^{3N}\det\tilde{M}_i')^{1/2}, \tag{100}$$

where N, from the first theory section, is the number of atoms in a solute molecule. Using this last equation in Eq. (93) we have

$$Q=\int \frac{e^{-\beta K_s}e^{-\beta\mathcal{U}}(2\pi kT)^{3Nn/2}}{(1+\gamma_s)^{n_s}(1+\gamma)^n n_s!n!h^D}\,d\mathbf{p}_s$$

$$\times d\mathbf{x}_s\prod_{i=1}^{n}\,(\det\tilde{M}_i')^{1/2}|\sin\theta_i|\,d\mathbf{x}_i. \tag{101}$$

If the n solute molecules are in the infinite dilution condition, we can simplify the integral in the previous equation considering that there is no interaction between the solute molecules. Hence for the great majority of the roto-translational configurations of the solute molecules the integral on the other coordinates and solvent momenta is a constant. Therefore

$$\int\; e^{-\beta(\mathcal{U}+K_s)}\,d\mathbf{p}_s\,d\mathbf{x}_s\prod_{i=1}^{n}\,(\det\tilde{M}_i')^{1/2}|\sin\theta_i|\,d\mathbf{x}_i$$

$$\cong(V8\pi^2)^n\int\; e^{-\beta(\mathcal{U}^0+K_s)}\,d\mathbf{p}_s\,d\mathbf{x}_s\prod_{i=1}^{n}\,(\det\tilde{M}_i')^{1/2}\,d\mathbf{x}_{i,\mathrm{in}}, \tag{102}$$

where in this last equation we used the fact that $|\sin\theta|d\theta\,d\phi\,d\psi$ corresponds to the product of the instantaneous rotation differential angles for the axes $\mathbf{i}_1$, $\mathbf{i}_2$ and $\mathbf{i}_3$, which is equivalent to the product of a solid angle differential with the differential of a third usual angle, and hence

$$\int\;|\sin\theta|\,d\theta\,d\phi\,d\psi=8\pi^2. \tag{103}$$

Note that in the special case we deal with a linear molecule with two Eulerian angles corresponding to the usual polar coordinates angles, the previous integral reduces to $2\pi^2$. Note also that $\mathcal{U}^0$ is the potential energy function evaluated at fixed roto-translational coordinates for the solute molecules where these are homogeneously distributed in the volume V of the full system, and $\tilde{M}'$ is now evaluated for each solute molecule at the fixed roto-translational configuration. Finally, considering again that n/n_s is almost zero we have

$$\int e^{-\beta(\mathcal{U}^0+K_s)}\,d\mathbf{p}_s\,d\mathbf{x}_s\prod_{i=1}^{n}(\det\tilde{M}_i')^{1/2}\,d\mathbf{x}_{i,\mathrm{in}} \cong \frac{n_s!}{(n^0!)^n}\left(\int e^{-\beta(u^0+k^0)}(\det\tilde{M}')^{1/2}d\mathbf{p}^0\,d\mathbf{x}^0\,d\mathbf{x}_{\mathrm{in}}\right)^n, \tag{104}$$

where u^0 is the potential energy of a subsystem defined by a single solute molecule with fixed roto-translational coordinates, in the center of the subsystem, n^0 is the number of solvent molecules in the subsystem given by the closest integer number to n_s/n, and k^0 is the corresponding solvent molecules kinetic energy. The right-hand integral in the last equation is taken over the volume V/n of the subsystem, and $\mathbf{x}^0$ and $\mathbf{p}^0$ are the coordinates and momenta of the n^0 solvent molecules of the subsystem. Clearly $\mathbf{x}_{\mathrm{in}}$ and $\tilde{M}'$ represent the $3N-6$ internal coordinates and the mass tensor of the single solute molecule. Note that the factor $n_s!/(n^0!)^n$ is a simple degeneration factor due to the number of ways we can distribute n^0 out of n_s solvent molecules in n subsystems. Hence we can rewrite the whole partition function as $Q \cong (Q_0V)^n/n!$ with Q_0 the partition function of a single subsystem given by

$$Q_0 = B_0\int e^{-\beta u^0}(\det\tilde{M}')^{1/2}\prod_{i=1}^{n^0}(\det\tilde{M}_i^0)^{1/2}\,d\mathbf{x}^0\,d\mathbf{x}_{\mathrm{in}}, \tag{105}$$

with

$$B_0 = \frac{(2\pi kT)^{3N/2}(8\pi^2)(2\pi kT)^{D^0/2}}{(1+\gamma)(1+\gamma_s)^{n^0}n^0!\,h^{(D^0+3N)}}.$$

$\tilde{M}_i^0$ and D^0 are the mass tensor of the ith solvent molecule and the total number of solvent molecules degrees of freedom in the subsystem. Note that Eq. (101) reduces to the usual expression, valid for typical small molecules, in the case $(\det\tilde{M}')^{1/2}$ as well as $\Pi_{i=1}^{n^0}(\det\tilde{M}_i^0)^{1/2}$ are constants over the configurations, and hence the integral over the momenta in Eq. (93) can be considered independent of the coordinates, although the integrand generally is not. In that case the partition function can be factorized into two independent integrals: one over the coordinates involving the potential energy and another over the momenta involving the kinetic energy.[13,15] However, in the presence of large and flexible molecules, especially in ideal gas or infinite dilution conditions where large structural fluctuations can occur because of the absence of the intermolecular potential or of the solute-solute interactions, such a factorization might be not accurate. Hence, in such cases one should use the completely general and always exact expression of the partition function, given by Eq. (101) or (105). Considering that for usual rigid or semi-rigid solvent molecules like water, $\Pi_{i=1}^{n^0}(\det M_i^0)^{1/2}$ is independent of the coordinates, the probability density for $\mathbf{x}^0$ and $\mathbf{x}_{\mathrm{in}}$ readily follows from Eq. (105)

$$\rho(\mathbf{x}^0,\mathbf{x}_{\mathrm{in}}) = \frac{(\det\tilde{M}'/\det\tilde{G}_{x_{\mathrm{in}},x_{\mathrm{in}}})^{1/2}}{\langle(\det\tilde{M}'/\det\tilde{G}_{x_{\mathrm{in}},x_{\mathrm{in}}})^{1/2}\rangle_c}\rho_c(\mathbf{x}^0,\mathbf{x}_{\mathrm{in}}), \tag{106}$$

$$\rho_c(\mathbf{x}^0,\mathbf{x}_{\mathrm{in}}) = \frac{e^{-\beta u^0}(\det\tilde{G}_{x_{\mathrm{in}},x_{\mathrm{in}}})^{1/2}}{\int e^{-\beta u^0}(\det\tilde{G}_{x_{\mathrm{in}},x_{\mathrm{in}}})^{1/2}\,d\mathbf{x}^0\,d\mathbf{x}_{\mathrm{in}}}, \tag{107}$$

$$\begin{aligned}\langle(\det\tilde{M}'/\det\tilde{G}_{x_{\mathrm{in}},x_{\mathrm{in}}})^{1/2}\rangle_c &= \int(\det\tilde{M}'/\det\tilde{G}_{x_{\mathrm{in}},x_{\mathrm{in}}})^{1/2}\rho_c\,d\mathbf{x}^0\,d\mathbf{x}_{\mathrm{in}}\\ &= \frac{\int e^{-\beta u^0}(\det\tilde{M}')^{1/2}\,d\mathbf{x}^0\,d\mathbf{x}_{\mathrm{in}}}{\int e^{-\beta u^0}(\det\tilde{G}_{x_{\mathrm{in}},x_{\mathrm{in}}})^{1/2}\,d\mathbf{x}^0\,d\mathbf{x}_{\mathrm{in}}},\end{aligned} \tag{108}$$

where, using the same notation as in the previous section, $\tilde{G}_{x_{\mathrm{in}},x_{\mathrm{in}}}$ is the block of the mass tensor of the solute molecule, corresponding to the velocities of the $3N-6$ internal coordinates $\mathbf{x}_{\mathrm{in}}$. Note that Eqs. (106)–(108) are in agreement with recent papers on related subjects,[11,16] and that $\det\tilde{G}_{x_{\mathrm{in}},x_{\mathrm{in}}}/\det\tilde{M}'$ is known as the Fixman determinant.[17] From the first theory section we also have that the elements of $\tilde{G}_{x_{\mathrm{in}},x_{\mathrm{in}}}$ are independent of the coordinates, as it follows from Eq. (12) considering that the six q_{il} which are expressed in terms of the others are linear combinations of the other $3N-6$ with coordinates independent coefficients; see also the Appendix. Hence the previous equations can be simplified to

$$\rho(\mathbf{x}^0,\mathbf{x}_{\mathrm{in}}) = \frac{(\det\tilde{M}')^{1/2}}{\langle(\det\tilde{M}')^{1/2}\rangle_c}\rho_c(\mathbf{x}^0,\mathbf{x}_{\mathrm{in}}), \tag{109}$$

$$\rho_c(\mathbf{x}^0,\mathbf{x}_{\mathrm{in}}) = \frac{e^{-\beta u^0}}{\int e^{-\beta u^0}\,d\mathbf{x}^0\,d\mathbf{x}_{\mathrm{in}}}, \tag{110}$$

$$\begin{aligned}\langle(\det\tilde{M}')^{1/2}\rangle_c &= \int(\det\tilde{M}')^{1/2}\rho_c\,d\mathbf{x}^0\,d\mathbf{x}_{\mathrm{in}}\\ &= \frac{\int e^{-\beta u^0}(\det\tilde{M}')^{1/2}\,d\mathbf{x}^0\,d\mathbf{x}_{\mathrm{in}}}{\int e^{-\beta u^0}\,d\mathbf{x}^0\,d\mathbf{x}_{\mathrm{in}}},\end{aligned} \tag{111}$$

where $\det\tilde{M}'$ is a function only of the internal coordinates $\mathbf{x}_{\mathrm{in}}$.[18] The ideal gas statistical mechanics is simply a special case of the infinite dilution one. In fact from the previous equations we have for the ideal gas case

$$\rho(\mathbf{x}_{\mathrm{in}}) = \frac{(\det\tilde{M}')^{1/2}}{\langle(\det\tilde{M}')^{1/2}\rangle_c}\rho_c(\mathbf{x}_{\mathrm{in}}), \tag{112}$$

$$\rho_c(\mathbf{x}_{\mathrm{in}}) = \frac{e^{-\beta u^0}}{\int e^{-\beta u^0}\,d\mathbf{x}_{\mathrm{in}}}, \tag{113}$$

$$\langle (\det \tilde{M}')^{1/2} \rangle_c = \int (\det\tilde{M}')^{1/2} \rho_c \, d\mathbf{x}_{\mathrm{in}} = \frac{\int e^{-\beta u^0} (\det \tilde{M}')^{1/2} \, d\mathbf{x}_{\mathrm{in}}}{\int e^{-\beta u^0} \, d\mathbf{x}_{\mathrm{in}}}, \tag{114}$$

where, obviously, u^0 now reduces to the intramolecular potential.

It is easy to show that ρ_c is exactly the configurational probability density for a system with a single solute molecule, n^0 solvent molecules and volume V/n in the presence of holonomic constraints for the solute roto-translational degrees of freedom expressed by the center of mass coordinates and Eulerian angles or equivalently, choosing G, $\mathbf{i}_1$, $\mathbf{i}_2$, $\mathbf{i}_3$ coinciding with G, $\mathbf{i}$, $\mathbf{j}$, $\mathbf{k}$, by the center of mass coordinates and the three generalized coordinates g_4, g_5, g_6 given by Eqs. (23)–(25). In fact, setting

$$\boldsymbol{\xi} = \begin{pmatrix} \mathbf{x}^0 \\ \mathbf{x}_{\mathrm{in}} \end{pmatrix}$$

and $\boldsymbol{\beta}$ as the solute roto-translational coordinates, from Eqs. (90)–(92) we have that in such a constrained system the configurational probability density is

$$\rho(\mathbf{x}^0, \mathbf{x}_{\mathrm{in}}) = \frac{e^{-\beta u^0} (\det \tilde{G}_{x_{\mathrm{in}}, x_{\mathrm{in}}})^{1/2} \Pi_{i=1}^{n^0} (\det M_i^0)^{1/2}}{\int e^{-\beta u^0} (\det \tilde{G}_{x_{\mathrm{in}}, x_{\mathrm{in}}})^{1/2} \Pi_{i=1}^{n^0} (\det M_i^0)^{1/2} \, d\mathbf{x}^0 \, d\mathbf{x}_{\mathrm{in}}} = \frac{e^{-\beta u^0}}{\int e^{-\beta u^0} \, d\mathbf{x}^0 \, d\mathbf{x}_{\mathrm{in}}}, \tag{115}$$

or in the ideal gas case, where $\boldsymbol{\xi} = \mathbf{x}_{\mathrm{in}}$

$$\rho(\mathbf{x}_{\mathrm{in}}) = \frac{e^{-\beta u^0} (\det \tilde{G}_{x_{\mathrm{in}}, x_{\mathrm{in}}})^{1/2}}{\int e^{-\beta u^0} (\det \tilde{G}_{x_{\mathrm{in}}, x_{\mathrm{in}}})^{1/2} \, d\mathbf{x}_{\mathrm{in}}} = \frac{e^{-\beta u^0}}{\int e^{-\beta u^0} \, d\mathbf{x}_{\mathrm{in}}}. \tag{116}$$

In Eq. (115) the potential energy u^0 is evaluated at a fixed solute roto-translational configuration, and in Eq. (116) u^0 is clearly independent of the roto-translational coordinates. Note that $\tilde{G}_{x_{\mathrm{in}}, x_{\mathrm{in}}}$ is identical using either the Eulerian angles or g_4, g_5, g_6 to express the rotational degrees of freedom, but the full mass tensors and the Fixman determinants are different.[19] This means that the statistical mechanical equivalence between the Eulerian angles and g_4, g_5, g_6 is valid only in the case the system is fully constrained and hence the partition function is evaluated constraining the roto-translational coordinates as well as their conjugated momenta; see Eqs. (90)–(92).[20] From Eqs. (106)–(116) it follows that a simulation with a canonical ensemble dynamics (e.g., the isothermal Gaussian dynamics), in the presence of (ideal) holonomic constraints for the center of mass coordinates and the Eulerian angles, can be used not only to obtain the exact statistical mechanics of the constrained system (as shown in the previous section) but also, if required, the exact statistical mechanics of the unconstrained system using a correction involving the mass tensor determinant (mass tensor correction) that can be evaluated from the constrained simulation. Such a mass tensor correction can be physically interpreted as the effect due to the centrifugal forces resulting in the coupling of the rotational and internal motions.

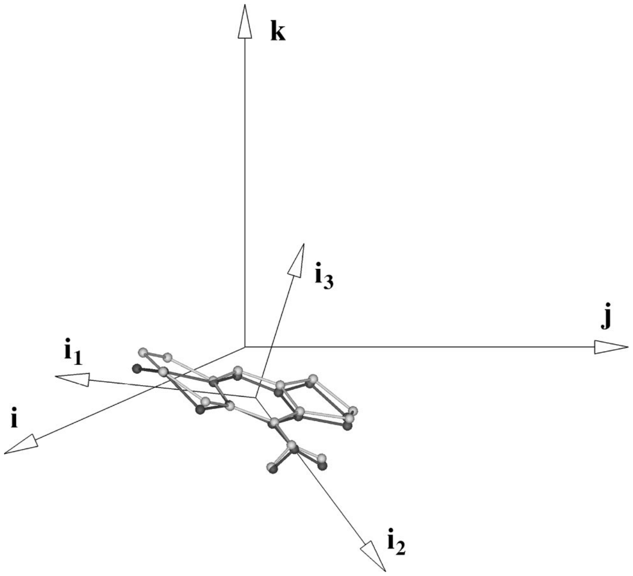

FIG. 2. Structures of the 4-aminopyridine derivative molecule obtained from molecular dynamics simulation with the roto-translational constraints applied. The fixed frame $(O,\mathbf{i},\mathbf{j},\mathbf{k})$ and the local frame $(G,\mathbf{i}_1,\mathbf{i}_2,\mathbf{i}_3)$ are shown. The light gray structure is the reference conformation, and the dark structure is taken after several thousand simulation steps.

In the Appendix a direct way to obtain the mass tensor $\tilde{M}'$, and a simple example for a biatomic molecule where a fully analytical derivation is possible are shown.

III. MOLECULAR DYNAMICS RESULTS

In this section we show the results obtained applying the roto-translational constraints in MD simulations performed with microcanonical and canonical ensemle dynamics. The algorithm has been implemented by us in the GROMACS simulation package,[21] and an aromatic 4-aminopyridine derivative molecule in vacuum was used as a test system. Molecular geometry was optimized at SCF level with the Dunning Huzinaga (D95) double zeta basis set, using the GAUSSIAN94 package.[22] The arbitrary reference configuration used to define the rotational degrees of freedom, and hence to constrain the molecule during the simulations, was an energy minimized structure, as this provides a more stable constrained dynamics. All the simulations started from such an energy minimized structure, and an initial run was used to thermally equilibrate the system. The simulations used to collect the data (productive simulations) were started from the final structures of the equilibration runs, using initial velocities obtained from Maxwellian distributions at the desired temperatures. Note that for the simulations with the roto-translational constraints the final structures of the equilibration runs needed to be stored at high precision (eight floating digits were enough) to start the productive runs. This is due to the fact that the constrained runs must always start from a constrained structure, and an initial constrained structure stored with low precision is equivalent to a slightly unconstrained configuration. Note also that especially at low temperature, it is important to start the productive simulation

TABLE I. Average energies and corresponding instantaneous energy standard deviations, obtained from the microcanonical simulations.

Constraints		Potential energy		Kinetic energy		Total energy	
rototr.	bonds	aver. (kJ/mol)	st. dev.	aver. (kJ/mol)	st. dev.	aver. (kJ/mol)	st. dev.
Yes	Yes	−401.5	4.34	22.3	4.34	−379.18	5.2742×10^{-4}
No	Yes	−401.4	4.83	22.3	4.83	−379.13	4.1548×10^{-4}
Yes	No	−401.4	4.28	24.4	4.28	−377.67	5.1216×10^{-4}
No	No	−402.0	4.04	24.4	4.04	−377.62	6.8820×10^{-4}

after equilibration, providing a new set of initial velocities from a Maxwellian distribution. In this way we ensure for the productive run a correct velocities distribution which could have been altered during the equilibration run. Finally to obtain very accurate and ''exact'' simulation data we always used a short time step in the range 0.15–0.20 fs. However, the algorithm described is numerically stable also with more usual time steps in the range 0.5–2.0 fs (data not shown).

A. Microcanonical simulations

Four simulations were carried out with the microcanonical ensemble (NVE) dynamics: two simulations with the roto-translational constraints, with and without the SHAKE algorithm,[10] and for comparison two usual simulations, with no roto-translational constraints also with and without the SHAKE algorithm. The SHAKE algorithm tolerance was chosen equal to 10^{-6}, the time step was 0.15 fs for all the simulations, and an ''infinite'' cutoff radius was used in order to guarantee that each atom could interact with any other in the molecule.

For each simulation, after equilibration, 20 000 steps were used to collect the data. In Fig. 2 we show the local molecular frame $(G,\mathbf{i}_1,\mathbf{i}_2,\mathbf{i}_3)$ and the fixed frame $(O,\mathbf{i},\mathbf{j},\mathbf{k})$, together with the reference structure and one structure taken after several thousand steps of simulation with the roto-translational constraints applied. In Table I we report the average total, potential and kinetic energy, with the standard deviations for the corresponding instantaneous energies, obtained from the four simulations. The table clearly shows that the application of the translational and rotational constraints during the simulation does not perturb the numerical accuracy of the trajectories. As expected the dynamics in the presence of the roto-translational constraints is conservative on the constraint surface, showing that the algorithm is numerically stable, even in the case in which the SHAKE constraints are also applied. Note that for an isolated molecule the translational constraints should not alter the trajectory with respect to a usual one where the velocity of the center of mass has been initially set to zero. Hence in this case the use of the translational constraints can be useful only to remove possible numerical errors. On the contrary the rotational constraints really change the dynamics of the system confining the molecular motions in a configurational subspace, the rotational constraint surface. Note also that with our definition of the rotational degrees of freedom, via Eq. (2), the angular momentum is not conserved during the constrained simulation but fluctuates around zero (see Fig. 3).

B. Canonical simulations

Three simulations were carried out with the canonical ensemble (NVT) dynamics using the isothermal Gaussian temperature coupling,[4,5] at 5300 and 700 K. In the three simulations no SHAKE algorithm was applied, the time step was 0.2 fs and an ''infinite'' cutoff radius was used in order

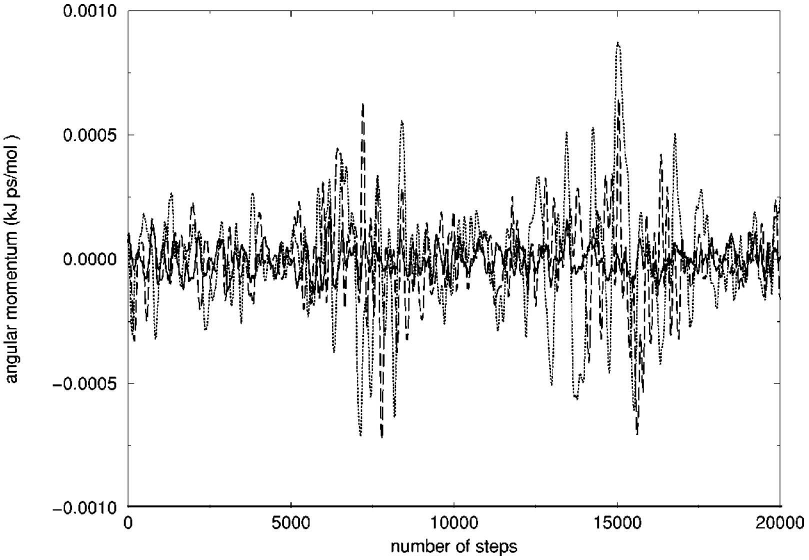

FIG. 3. Total angular momentum of the 4-amino-pyridine derivative molecule versus the number of steps, as obtained from the microcanonical simulation with no SHAKE algorithm and roto-translational constraints applied. Solid line: $\mathbf{i}_1$ component; dashed line: $\mathbf{i}_2$ component; dotted line: $\mathbf{i}_3$ component.

TABLE II. Average potential energies and corresponding isochoric heat capacities, together with their root mean square deviations, obtained from the canonical constrained dynamics. In the table we also show the differences between these average potential energies or heat capacities and the mass tensor corrected values.

Temp.	$\langle \mathcal{U}' \rangle$	$\sigma_{\langle u' \rangle}$	$\Delta\langle \mathcal{U}' \rangle$	C'_V	$\sigma_{C'_V}$	$\Delta C'_V$
K	kJ/mol		kJ/mol	J/(mol K)		J/(mol K)
5	-424.870	0.002	3.0×10^{-5}	181.8	3.2	2.0×10^{-3}
300	-374.5	0.10	3.3×10^{-2}	167.6	2.4	-2.66×10^{-1}
700	-309.1	0.25	2.0×10^{-1}	169.7	2.7	9.7×10^{-1}

to guarantee that each atom could interact with any other in the molecule. At each temperature after equilibration we used 5.0 million steps to collect the data, as we wanted to obtain well equilibrated properties. In Table II we show the values of the average potential energy with the corresponding isochoric heat capacity, obtained directly from the constrained simulations, together with the estimates of the corresponding root mean square deviations $\sigma_{\langle \mathcal{U}' \rangle}$ and $\sigma_{C'_V}$. In the table we also show the differences between these average potential energies or heat capacities and the values obtained using the mass tensor correction described in Sec. II D. From Table II it is evident that the mass tensor correction alters the values of the average energies within their errors (shifts always within a couple of $\sigma_{\langle \mathcal{U}' \rangle}$) and hence such a correction is not relevant. The same is true for the heat capacity calculated at each temperature via the second potential energy central moment

$$C'_V = \frac{M'_2}{kT^2},$$

$$M'_2 = \langle (\mathcal{U}' - \langle \mathcal{U}' \rangle)^2 \rangle,$$

where $\mathcal{U}'$ is the potential energy of the system. From the table it is also clear that the effect of the mass tensor correction is extremely small except at high temperature (700 K) where it becomes larger, although still not relevant. It should be noted that at 5 K, where the harmonic behavior is expected, the heat capacity for the potential energy matches almost the pure harmonic value for a molecule of 16 atoms given by

$$C'_V = \frac{(16\times 3 - 6)}{2} R = 174.6\ \mathrm{J/(mol\,K)}$$

with R the ideal gas constant. This clearly shows that the simulation procedure is extremely accurate, as it can properly reproduce the second momemt of the energy.

IV. CONCLUSIONS

In this paper we introduced a new constrained dynamics method to stop the roto-translational motions of a molecular system during MD simulations. Using the standard analytical mechanical definition of the roto-translational degrees of freedom, which is very suited for theoretical studies and alternative to the usual least square fitting one, we derived an exact algorithm for fixing the molecular frame without altering the statistical mechanical consistency of the simulation. We also showed that from such constrained simulations it is possible to obtain the exact statistical mechanics of the unconstrained systems in the ideal gas or infinite dilution conditions, via a simple correction (mass tensor correction) which provides the effect of the possible coupling between the rotational and internal degrees of freedom. As mentioned in Sec. I the use of the roto-translational constraints, although statistically mechanically consistent, alters the dynamics in the simulation as the equations of motion are clearly not identical for the constrained and unconstrained systems. This means that the described constrained dynamics method provides ''exact'' results as far as the statistical mechanics is concerned, but not necessarily for the dynamics. However, it is likely that such differences mostly concern the dynamical details of the simulation and are not very relevant for the kinetics (average dynamics) of the system. The application of this method to a molecular system, a large organic molecule in vacuum, showed that it is possible to implement in usual MD codes a numerically stable algorithm and that, at least for the molecule used, the mass tensor correction is negligible even at high temperature. This procedure is expected to be very useful for theoretical studies based on MD simulations, for the development of mean field approach, and to reduce significantly the size of the simulation box in case of large nonspherical solutes, like proteins surrounded by water molecules.

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APPENDIX

In this Appendix we show how the mass tensor, and hence its determinant, can be obtained for each configuration coming from a trajectory with the roto-translational constraints applied. Defining with $\tilde{\Gamma}$ the diagonal mass tensor corresponding to the momenta or velocities of the particles coordinates $\mathbf{r}$ in $(O,\ \mathbf{i},\ \mathbf{j},\ \mathbf{k})$ (see Sec. II A) we have that the mass tensor expressed for the $\mathbf{p}'$ momenta or $\dot{\mathbf{x}}'$ velocities, used in Sec. II D, is $\tilde{M}' = \tilde{B}^T \tilde{\Gamma} \tilde{B}$ where $\tilde{B}$ is the transformation matrix defined by $\dot{\mathbf{r}} = \tilde{B}\dot{\mathbf{x}}'$. Using Eqs. (20)–(25) we can express six coordinates $\mathbf{x}_d$ in $(G, \mathbf{i}_1, \mathbf{i}_2, \mathbf{i}_3)$ as linear combinations of the internal coordinates $\mathbf{x}_{\text{in}}$, corresponding to the left $3N-6$ coordinates, from

$$\widetilde{W}\mathbf{x}_d=\widetilde{Z}\mathbf{x}_{\text{in}}, \tag{A1}$$

where from Secs. II A and II B we have

$$\mathbf{x}_{\text{in}}^T=(z_1\ \ y_3\ \ z_3\ \ x_4\ \ y_4\ \ z_4\ \ \cdots\ \ x_N\ \ y_N\ \ z_N),$$

$$\mathbf{x}_d^T=(x_1\ \ y_1\ \ x_2\ \ y_2\ \ z_2\ \ x_3),$$

and

$$\widetilde{W}=\begin{pmatrix} m_1 & 0 & m_2 & 0 & 0 & m_3 \\ 0 & m_1 & 0 & m_2 & 0 & 0 \\ 0 & 0 & 0 & 0 & m_2 & 0 \\ 0 & -m_1Z_1 & 0 & -m_2Z_2 & m_2Y_2 & 0 \\ m_1Z_1 & 0 & m_2Z_2 & 0 & -m_2X_2 & m_3Z_3 \\ -m_1Y_1 & m_1X_1 & -m_2Y_2 & m_2X_2 & 0 & -m_3Y_3 \end{pmatrix},$$

$$\widetilde{Z}=\begin{pmatrix} 0 & 0 & 0 & -m_4 & 0 & 0 & \cdots & -m_N & 0 & 0 \\ 0 & -m_3 & 0 & 0 & -m_4 & 0 & \cdots & 0 & -m_N & 0 \\ -m_1 & 0 & -m_3 & 0 & 0 & -m_4 & \cdots & 0 & 0 & -m_N \\ -m_1Y_1 & m_3Z_3 & -m_3Y_3 & 0 & m_4Z_4 & -m_4Y_4 & \cdots & 0 & m_NZ_N & -m_NY_N \\ m_1X_1 & 0 & m_3X_3 & -m_4Z_4 & 0 & m_4X_4 & \cdots & -m_NZ_N & 0 & m_NZ_N \\ 0 & -m_3X_3 & 0 & m_4Y_4 & -m_4X_4 & 0 & \cdots & m_NY_N & -m_NY_N & 0 \end{pmatrix}.$$

Now expressing in Eq. (5) $\mathbf{x}_d$ in terms of $\mathbf{x}_{\text{in}}$ via Eq. (A1) and considering that for a system with roto-translational constraints we can always assume, as in Sec. II B without loss of generality,[18] that $(G, \mathbf{i}_1, \mathbf{i}_2, \mathbf{i}_3)$ coincide with $(G, \mathbf{i}, \mathbf{j}, \mathbf{k})$, we can obtain the matrix $\widetilde{B}$ as

$$\widetilde{B}=\begin{pmatrix} 1 & 0 & 0 & 0 & z_1 & -y_1 & C_{1,1} & C_{1,2} & C_{1,3} & C_{1,4} & C_{1,5} & C_{1,6} & \cdots & C_{1,3N-8} & C_{1,3N-7} & C_{1,3N-6} \\ 0 & 1 & 0 & -z_1 & 0 & x_1 & C_{2,1} & C_{2,2} & C_{2,3} & C_{2,4} & C_{2,5} & C_{2,6} & \cdots & C_{2,3N-8} & C_{2,3N-7} & C_{2,3N-6} \\ 0 & 0 & 1 & y_1 & -x_1 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & \cdots & 0 & 0 & 0 \\ 1 & 0 & 0 & 0 & z_2 & -y_2 & C_{3,1} & C_{3,2} & C_{3,3} & C_{3,4} & C_{3,5} & C_{3,6} & \cdots & C_{3,3N-8} & C_{3,3N-7} & C_{3,3N-6} \\ 0 & 1 & 0 & -z_2 & 0 & x_2 & C_{4,1} & C_{4,2} & C_{4,3} & C_{4,4} & C_{4,5} & C_{4,6} & \cdots & C_{4,3N-8} & C_{4,3N-7} & C_{4,3N-6} \\ 0 & 0 & 1 & y_2 & -x_2 & 0 & C_{5,1} & C_{5,2} & C_{5,3} & C_{5,4} & C_{5,5} & C_{5,6} & \cdots & C_{5,3N-8} & C_{5,3N-7} & C_{5,3N-6} \\ 1 & 0 & 0 & 0 & z_3 & -y_3 & C_{6,1} & C_{6,2} & C_{6,3} & C_{6,4} & C_{6,5} & C_{6,6} & \cdots & C_{6,3N-8} & C_{6,3N-7} & C_{6,3N-6} \\ 0 & 1 & 0 & -z_3 & 0 & x_3 & 0 & 1 & 0 & 0 & 0 & 0 & \cdots & 0 & 0 & 0 \\ 0 & 0 & 1 & y_3 & -x_3 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & \cdots & 0 & 0 & 0 \\ 1 & 0 & 0 & 0 & z_4 & -y_4 & 0 & 0 & 0 & 1 & 0 & 0 & \cdots & 0 & 0 & 0 \\ 0 & 1 & 0 & -z_4 & 0 & x_4 & 0 & 0 & 0 & 0 & 1 & 0 & \cdots & 0 & 0 & 0 \\ 0 & 0 & 1 & y_4 & -x_4 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & \cdots & 0 & 0 & 0 \\ \vdots & \vdots & \vdots & \vdots & \vdots & \vdots & \vdots & \vdots & \vdots & \vdots & \vdots & \vdots & & \vdots & \vdots & \vdots \\ 1 & 0 & 0 & 0 & z_N & -y_N & 0 & 0 & 0 & 0 & 0 & 0 & \cdots & 1 & 0 & 0 \\ 0 & 1 & 0 & -z_N & 0 & x_N & 0 & 0 & 0 & 0 & 0 & 0 & \cdots & 0 & 1 & 0 \\ 0 & 0 & 1 & y_N & -x_N & 0 & 0 & 0 & 0 & 0 & 0 & 0 & \cdots & 0 & 0 & 1 \end{pmatrix},$$

where the matrix $\tilde{C}$ is defined by $\tilde{C}=\tilde{W}^{-1}\tilde{Z}$. Hence the mass tensor elements are readily obtained from

$$M'_{i,j}=\boldsymbol{\eta}_i^T\tilde{\Gamma}\,\boldsymbol{\eta}_j\,, \tag{A2}$$

where $\boldsymbol{\eta}_i$ and $\boldsymbol{\eta}_j$ are, respectively, the ith and jth columns of the $\tilde{B}$ matrix. Equation (A2) was used to construct the mass tensor $\tilde{M}'$ from which we could calculate its determinant.

As a simple example we can use the definition of the matrix $\tilde{B}$ to obtain the mass tensor of a biatomic molecule with identical masses. If we choose to align the molecule in the reference structure along the zed direction ($\mathbf{i}_3$), with hence zero x and y coordinates in (G, $\mathbf{i}_1$, $\mathbf{i}_2$, $\mathbf{i}_3$) for both atoms, we have $z_2=-z_1$. Moreover the sixth column of the $\tilde{B}$ matrix, corresponding to ω_3, involves only zero elements and hence can be removed. It is also clear that x_1, y_1, x_2, y_2 are independent of z_1 being fixed at zero. The $\tilde{B}$ matrix then reduces to

$$\tilde{B}=\begin{pmatrix} 1 & 0 & 0 & 0 & z_1 & 0 \\ 0 & 1 & 0 & -z_1 & 0 & 0 \\ 0 & 0 & 1 & 0 & 0 & 1 \\ 1 & 0 & 0 & 0 & -z_1 & 0 \\ 0 & 1 & 0 & z_1 & 0 & 0 \\ 0 & 0 & 1 & 0 & 0 & -1 \end{pmatrix}$$

and from Eq. (A2) the mass tensor $\tilde{M}'$ is

$$\tilde{M}'=\begin{pmatrix} 2m & 0 & 0 & 0 & 0 & 0 \\ 0 & 2m & 0 & 0 & 0 & 0 \\ 0 & 0 & 2m & 0 & 0 & 0 \\ 0 & 0 & 0 & 2mz_1^2 & 0 & 0 \\ 0 & 0 & 0 & 0 & 2mz_1^2 & 0 \\ 0 & 0 & 0 & 0 & 0 & 2m \end{pmatrix},$$

where m is the atomic mass. Note that for a biatomic molecule $\tilde{G}_{x_{\mathrm{in}},x_{\mathrm{in}}}$ reduces to the element $M'_{6,6}=2m$, as in a biatomic molecule there is only one internal coordinate (z_1). As previously mentioned $\tilde{G}_{x_{\mathrm{in}},x_{\mathrm{in}}}$ involves only coordinate independent elements and indeed this is what we obtain for the simple biatomic molecule where $\tilde{G}_{x_{\mathrm{in}},x_{\mathrm{in}}}=M'_{6,6}=2m$. From $\tilde{M}'$ follows the kinetic energy and the mass tensor correction

$$K=m\mathbf{r}_G\cdot\mathbf{r}_G+mz_1^2(\omega_1^2+\omega_2^2)+m\dot{z}_1^2,$$

$$(\det\tilde{M}')^{1/2}=2^3m^3z_1^2.$$

The same results can be also obtained directly from Eqs. (8)–(15). Note that as expected the mass tensor correction is not a constant but depends on the internal coordinate z_1. Only in the case in which the internal coordinate has very limited fluctuations with respect to its average value (as it happens in usual small molecules but not necessarily in large and flexible ones) does the correction become irrelevant, as the mass tensor determinant can be considered a constant.

[1] A. D. Mc Lachlan, J. Mol. Biol. **49**, 128 (1979).

[2] G. Gallavotti, *The Elements of Mechanics* (Springer-Verlag, New York, 1983).

[3] L. D. Landau and E. M. Lifshitz, *Course of Theoretical Physics, Vol.1*, 3rd ed. (Pergamon, New York, 1980).

[4] D. J. Evans and G. P. Morris, Comput. Phys. Rep. **1**, 297 (1984).

[5] D. Brown and J. H. R. Clarke, Mol. Phys. **51**, 1243–1252 (1984).

[6] S. Nosé, Mol. Phys. **52**, 255 (1984).

[7] W. G. Hoover, Phys. Rev. A **31**, 1695 (1985).

[8] C. Eckart, Phys. Rev. **47**, 552 (1935).

[9] Note that for N particles confined in a line, once the center of mass defined in the line by one coordinate is set in the origin, there are only $N-1$ independent coordinates left among the $3N-5$ possible internal coordinates. In the case of N particles confined in a plane, once the center of mass defined in the plane by two coordinates is set in the origin and the rotational angle in the plane is defined, there are only $2N-2-1$ independent coordinates left among the $3N-6$ possible internal coordinates. In the paper, according to the standard analytical mechanical definition, for internal coordinates we mean only the independent ones.

[10] J. P. Ryckaert, G. Ciccotti, and H. J. C. Berendsen, J. Comput. Phys. **23**, 327–341 (1977).

[11] T. Mulders and W. Swegat, Mol. Phys. **94**, 395–399 (1998).

[12] Note that when the total linear momentum is fixed at zero during the dynamics and no explicit constraints for this are used, such a condition implies the presence of three implicit holonomic constraints for the center of mass position. These implicit constraints can be used for the definition of the $\boldsymbol{\xi}$ coordinates and hence in the $\boldsymbol{\xi}$, $\boldsymbol{\pi}$ phase space no constants of motion due to the center of mass constraints are present. The case where the total linear momentum is a nonzero constant can be reduced to the previous one using in the definition of the full phase-space Cartesian coordinates relative to the center of mass position and their conjugated momenta.

[13] L. D. Landau and E. M. Lifshitz, *Course of Theoretical Physics, Vol. 5*, 3rd ed. (Pergamon, New York, 1980).

[14] A. Amadei, M. E. F. Apol, and H. J. C. Berendsen, J. Chem. Phys. **109**, 3004–3016 (1998).

[15] A. Amadei, M. E. F. Apol, and H. J. C. Berendsen, J. Chem. Phys. **106**, 1893–1912 (1997).

[16] W. K. den Otter and W. J. Briels, J. Chem. Phys. **109**, 4139–4146 (1998).

[17] M. Fixman, Proc. Natl. Acad. Sci. USA **69**, 1527 (1974).

[18] The mass tensor $\tilde{M}'$ is independent of the center of mass coordinates and for any rigid body rotation of the molecule given by the Eulerian angles that define the rotation of $(G,\mathbf{i}_1,\mathbf{i}_2,\mathbf{i}_3)$ with respect to $(G,\mathbf{i},\mathbf{j},\mathbf{k})$, the determinant of $\tilde{M}'$ is invariant. This clearly means that $\det\tilde{M}'$ is a function only of the internal coordinates $\mathbf{x}_{\mathrm{in}}$.

[19] Using the coordinates g_4,g_5,g_6 the full mass tensor determinant and hence the Fixman determinant are pure constants.

[20] Note that although the coordinates g_4,g_5,g_6 constrain completely the three Eulerian angles when fixed at zero, they cannot properly describe rotational motions as for their nonzero values rigid body rotations and internal deformations are mixed.

[21] David van der Spoel, Herman J. C. Berendsen, Aldert R. van Buuren, Emile Apol, Pieter J. Meulenhoff, Alfons L. T. M. Sijbers, and Rudi van Drunen, *Gromacs User Manual*. Nijenborgh 4, 9747 AG Groningen, The Netherlands. Internet: http://rugmd0.chem.rug.nl/~gmx (1995).

[22] M. J. Frisch *et al.*, GAUSSIAN 94 (Gaussian, Inc., Pittsburgh, PA, 1994).