THE JOURNAL OF PHYSICAL CHEMISTRY B

Article

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Pitfall in Quantum Mechanical/Molecular Mechanical Molecular Dynamics Simulation of Small Solutes in Solution

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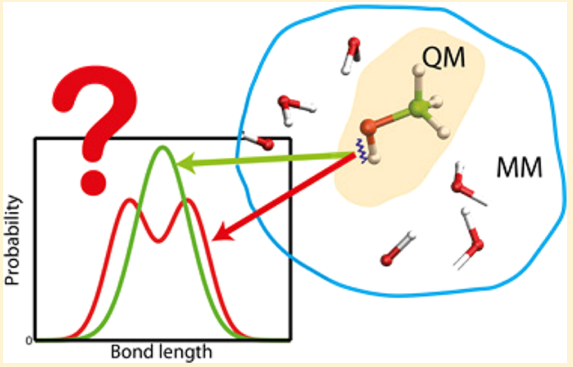
Supporting Information

ABSTRACT: Developments in computing hardware and algorithms have made direct molecular dynamics simulation with the combined quantum mechanical/molecular mechanical methods affordable for small solute molecules in solution, in which much improved accuracy can be obtained via the quantum mechanical treatment of the solute molecule and even sometimes water molecules in the first solvation shell. However, unlike the conventional molecular mechanical simulations of large molecules, e.g., proteins, in solutions, special care must be taken in the technical details of the simulation, including the thermostat of the solute/solvent system, so that the conformational space of the solute molecules can be properly sampled. We show here that the common setup for classical molecular mechanical molecular dynamics simulations, such as the Berendsen or single Nose−Hoover thermostat, and/or rigid water models could lead to pathological sampling of the solutes' conformation. In the extreme example of a methanol molecule in aqueous solution, improper and sluggish setups could generate two peaks in the distribution of the O−H bond length. We discuss the factors responsible for this somewhat unexpected result and evoke a simple and ancient technical fix-up to resolve this problem.



INTRODUCTION

Steady developments in algorithms and computer hardware have made the combined quantum mechanical/molecular mechanical (QM/MM) methods very popular in recent years. With the promises of providing higher accuracy and broader applicability than classical molecular mechanics, QM/MM in combination with molecular dynamics (MD) or Monte Carlo methods have been applied to the modeling of the mechanism and thermodynamics of many complex reaction processes.[1−6] For a small molecule of less than 50 atoms and described by density functional theory (DFT),[7] current community computational resources allow brute force molecular dynamics sampling of ∼10^2 ps for each well-defined conformational state.[8]

MD simulations with QM/MM methods usually follow the same protocol as purely classical molecular mechanics (MM) force field simulations. In a typical MM-based MD simulation of macromolecules, e.g., proteins and DNA in solution, high-frequency vibrations such as those of the bonds involving hydrogen atoms are removed by constraining the bond lengths with SHAKE[9] or similar methods.[10,11] The purpose of the constraint is to increase the integration time step for more efficient phase space sampling.[12] The assumptions underlying the application of constraints are that the bond vibrations are so fast as compared with other "slower" motions that the respective modes can be approximately represented as "running averages". In the meantime the bonds are so stiff that the variations of such "running averages" can be ignored, thus the bonds can be constrained to fixed lengths specified by MM force fields. Usually the solvent water molecules are also described with a model with a rigid geometry. Results of numerous MM MD simulations with this typical setup have proved the effectiveness and correctness for this constraining scheme, despite that the details of bond vibrations are completely zapped out.

A typical MD simulation of a small QM subsystem in a MM environment with QM/MM often adopts a similar scheme. That is, the same constrained rigid water model will be used. The MM parts of the solute, if there are any, can have their bonds either constrained or not, while the QM subsystem usually evolves freely during MD simulations with the high-frequency vibrations unsuspended. This limits the MD integration time step to 1 fs or less in Born−Oppenheimer MD simulations with QM/MM or QM methods. This hybrid scheme with mixed constrained and free bond vibrations was very common in QM/MM simulations, and numerous applications have been reported. Nevertheless this scheme may create a pitfall in MD simulations: the couplings between the high-frequency motions of the (QM) solute and the (MM) environment are partially removed with the corresponding

Received: January 28, 2013
Revised: May 3, 2013

   dx.doi.org/10.1021/jp4024662 | J. Phys. Chem. B XXXX, XXX, XXX−XXX

natural energy-exchanging channels destroyed since some motions are free to evolve while others are completely frozen. The altered energy exchange pattern did not become a serious problem in MM-based MD simulations in which lower-frequency motions make dominant contributions to concerned properties (e.g., bond vibration energies are not considered as interesting). However, this argument may not apply to QM/MM simulations in which QM total energies cannot be decomposed into components, and details of a small QM subsystem are often of ultimate interest.

Here we show how the above common hybrid MD setup can produce totally unanticipated sampling for some stiff degrees of freedom of the QM subsystem. We first report the distribution of the O−H bond length in the methanol molecule sampled in a QM/MM simulation and then test several common thermostats with a pure MM force field. We show that using heavier atoms for these stiff bonds in the QM subsystem can remove the artifacts and provide accurate sampling of the QM subsystem.

COMPUTATIONAL DETAILS

The QM/MM simulations were carried out using an in-house QM[4]D program.[13] A methanol molecule, treated by B3LYP/6-31G*,[14,15] was placed in the center of a 40 × 40 × 40 Å^3 box filled with TIP3P water molecules.[16] A 10 Å cutoff was used for nonbonded interactions. The geometry of water molecules was kept fixed with the SETTLE method.[10] The integration time step was 1 fs with the nonbonded pairlist updated every 10 steps. The Berendsen thermostat was employed with the solute and solvent molecules separately coupled to heat baths at 300 K.[17] The length of QM/MM MD simulation is 200 ps.

MD simulations were carried out for the same molecular system using the OPLS force field.[18] A double-precision version of GROMACS 4.5.5 was employed.[19] Three different thermostats were examined: the Berendsen thermostat of weak coupling,[17] the Nose−Hoover method,[20,21] and the velocity-rescaling as a variant of the Berendsen coupling.[22] For the relaxation time in all methods we used a value of 0.1 ps. Each test set contained eight parallel simulations of length of 500 ps, in which the same initial conformations were used but with different initial velocities. Like the QM/MM simulations, the leapfrog method was used with an integration time step of 1 fs.[23] A single cutoff of 10 Å was used for nonbonded interactions, except for the NVE simulations where the PME method was used for long-range electrostatic interactions.[24,25] Unless otherwise noted, a rigid TIP3P water model was used.

RESULTS AND DISCUSSION

QM/MM Simulation. The distributions of the bond length of the O−H bond and one of the three C−H bonds are plotted in Figure 1. The C−H bond-length distribution shows a single Gaussian peak, as is expected. The distribution of O−H bond length, however, displays an unexpected two peaks. Numerical errors have been excluded from the possible factors responsible. So it must have resulted from either biased potential energy or noncanonical sampling. It has been questioned initially if factors such as the rotameric states of the O−H bond with respect to the methyl group or the hydrogen bonding interaction with the MM solvent water molecules could alter the effective potential energy surface of the O−H bond and subsequently lead to multiple states for the O−H bond length. This possibility has been excluded by detailed inspections of the

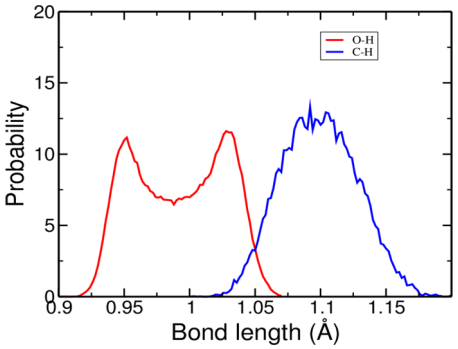


Figure 1. Distribution of the O−H and C−H bond length of methanol obtained from QM/MM simulations.

time evolution of the O−H bond length and its correlations with changes in such environmental factors. In fact, if one considers the very different degrees of stiffness between the O−H bond and these other interactions, it is unlikely that the latter could make significant perturbation to the length of the O−H bond.

As we argued that the potential energy was unlikely to be the cause, we suspected that the erroneous two-state distribution of the O−H bond length was due to technical details of the setups we employed in our MD simulations, though they were very similar to many other reported QM/MM MD simulations. Specifically, we considered two factors that could contribute to the incorrect results: the temperature-controlling scheme and the rigid MM water model. Furthermore, we noticed that the limited simulation length also might be partially responsible.

Because of its simplicity and high efficiency of equilibrating the target system at the desired temperature, the Berendsen thermostat is popular in MD simulations of chemical and biological molecular systems. It is known that the Berendsen thermostat can lead to significant deviations from a canonical ensemble for small systems: it does not generate correct fluctuations for the kinetic energy since the method always suppresses the fluctuations.[26]

In the current case, however, we are also concerned with another issue which might not be unique to the Berendsen thermostat, that is, the redistribution or exchange of energies between different degrees of freedom. This problem is associated with the use of "global" versus "local" thermostats. A normal setup for MD simulations of proteins and DNA usually adopts separated baths for solvents and solutes. For the solute molecule, an instantaneous global "temperature" is defined based on the total kinetic energy of all its atoms. The temperature rescaling is made according to this global temperature without considering the detailed distribution of the kinetic energies among different degrees of freedom and structural inhomogeneity. It is therefore possible, at least in principle, to introduce unnatural hot and cold spots in large molecules. It was hoped that the intramolecular interactions in large molecules would practically fix this problem by providing efficient energy exchange between different degrees of freedom. Nonetheless, the practical achievability of canonical sampling depends on the various time scales relative to each other and to the length of the simulation. Even if the self-rectifying process is practically sufficient to eventually accomplish a canonical distribution, the minimum time it takes starting from an arbitrarily assigned initial state is system dependent and usually unknown.

dx.doi.org/10.1021/jp4024662 | J. Phys. Chem. B XXXX, XXX, XXX−XXX

It might be slightly better if effective energy exchange can occur between bond vibrations of solute and solvent molecules. Unfortunately, the customary use of rigid water models removes the internal vibrations and leaves only low-frequency tumbling of the water molecules. The coupling between the floppy overall motions of water molecules and stiff bond vibrations of the solute could be so weak that the energy exchange between the high-frequency O−H bonds and the rest of the system is insufficient to achieve thermo-equilibration.

We propose that the incorrect sampling of the O−H bond comes from the lack of efficient coupling of the O−H bond to any thermostats, which results from the combined factors of the MD thermostat and the use of rigid water molecules. On one hand, there lacks effective coupling between O−H bond vibrations and other intramolecular motions of the methanol molecule, unlike the three C−H bonds. The vibrations of the three C−H bonds mutually couple to each other, providing efficient energy exchange between them, even if the initial energy of one bond deviates significantly from the expected equilibrium value. On the other hand, the rigid water model eliminates the efficient coupling between bond vibrations of solute and solvent molecules. As such the O−H bond vibration becomes a *semi-isolated* motion within the simulation time scale. It will suffer a severe problem if the (initial) amount of energy contained in this degree of freedom deviates significantly from the equilibrium amount in a canonical ensemble. This analysis also implies that the resulting distribution of the O−H bond length will exhibit dependences on the initially assigned states of velocity or kinetic energy of the specific modes. This implication will be clearly demonstrated with the results presented in the next section.

MM Simulations with Different Thermostats. To examine the issues we just raised, we turn to MD simulations with a pure MM force field which allow a longer simulation time. The results of the O−H bond length distributions simulated with the same OPLS/TIP3P force field but different thermostats are shown in Figure 2. Note in all simulations the solute methanol molecule is flexible, and the water molecules are rigid. Like the QM/MM simulations, the Berendsen thermostat led to a clear pathetic two-peak distribution of the O−H bond lengths. The Nose−Hoover thermostat also generated incorrect results as the distribution is too narrow and too sharp if compared to what is expected from the model potential (Figure 6). The velocity rescaling method gave the best results, even though distortions from ideal single-peak distribution could still be observed for some simulations. When the NVE ensemble, instead of the NVT ensemble, was simulated, the bond lengths show similar two-peak distributions (Figure 3). To confirm that numerical error of the MD integration is not a factor responsible for the results, smaller integration time steps (e.g., 0.2−0.5 fs) were used in MD simulation, and it was found that the erroneous situation did not change: Both Berendsen and single-bath Nose−Hoover thermostats lead to two-peak distributions (Supporting Information, Figure S1).

The results of different thermostats and NVE ensemble clearly suggested that the known issues of the Berendsen thermostat are not the main factors responsible for the erroneous distribution of the O−H bond lengths. Instead, deviations from equilibrium distribution of kinetic energy for a small subsystem embedded in a solution environment in up to nanosecond of simulations, rarely noticed in classical MM MD simulations, may be an important factor. With the use of a

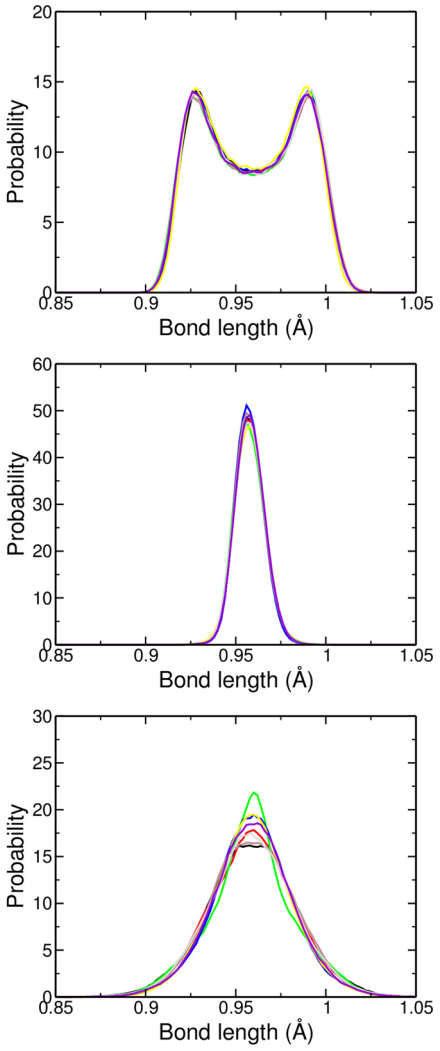


Figure 2. Distribution of methanol O−H bond length simulated with GROMACS using OPLS/TIP3P force fields and a MD time step of 1 fs. (a) Berendsen thermostat; (b) Nose−Hoover thermostat; (c) velocity-rescaling.

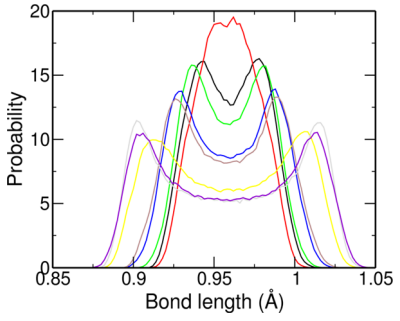


Figure 3. Distribution of methanol O−H bond length obtained from NVE simulations using the rigid TIP3P water model and a MD time step of 1 fs.

single global temperature for the solute, it is then not surprising to see that the Berendsen thermostat failed to generate canonical sampling for the stiff modes that are weakly coupled to other modes. For the Nose-Hoover thermostat, it is well-known that nonergodic sampling is occasionally produced for a simple potential energy surface such as harmonic oscillators.[21] Thus, it seems that our results are somewhat not too

dx.doi.org/10.1021/jp4024662 | J. Phys. Chem. B XXXX, XXX, XXX−XXX

unexpected. We note that using the Nose−Hoover chain method might solve this issue. The reason that the velocity-rescaling method performs the best may lie in the stochastic noise embedded in the method. Still, as long as a "global" thermostat is used, the equilibration of kinetic energy among different degrees of freedom would still be a problem when isolated high-frequency motions exist in the system.

The energy exchange process is ultimately a quantum process where the bond vibrations must be treated quantum mechanically. In the classical mechanical picture, our situation here is similar to a known case of energy relaxation of high-frequency vibrations embedded in a reservoir of low-frequency translations and rotations of diatomic molecules. It was realized that the relaxation rate of vibrational energy decreases exponentially with the vibrational frequency of the bond, increases faster than a linear function of density, and increases with temperature quantitatively following the Arrhenius law.[27] It then becomes clear why the energy redistribution between an isolated bond, such as the O−H bond of methanol, and the rigid water molecules is so slow that extremely long MD simulation is required to obtain converged results. The diverse distributions observed in NVE simulations (Figure 3) strongly support this picture. In this case, the results critically depend on the initial energy assignment of each motional mode.

In support of the importance of energy transfer in MD simulations, we examined whether using flexible water models could help to relieve this problem. When the water molecules are flexible, the distribution of the O−H bond length is significantly improved (Figure 4 and Figure S2, Supporting Information). For the Berendsen thermostat, a trend of single-peak distribution is clearly indicated. For the Nose−Hoover thermostat, the peaks are broader and lower than those from rigid water models. This observation clearly suggested that, as expected, the similarly high-frequency bond vibrations of many solvent water molecules now established effective coupling with the vibrations of the O−H bond of the methanol molecule. We note however that the use of flexible water would bring out additional problems. Current MM force fields were mostly parametrized with the rigid water models, so the use of a flexible water model might completely alter the dynamics of the molecular system. Therefore, further studies are required to confirm the validity of using flexible water models in MD simulations of proteins and other important macromolecules.

Since most MM simulations employed rigid water models, there will be many isolated or semi-isolated bonds in a typical solute molecule. Like the O−H bond in methanol, these bonds lack effective energy exchange with the rest of the system. The correct modeling of the kinetic energy distribution of those degrees of freedom would become a troublesome issue in QM/MM MD simulations. The issue of "global" and "local" temperatures for different motions of macromolecule has been discussed recently,[28,29] in which the equipartitioning of energy was found to be violated in classical MD simulations. Our results here provided another example to show how subtle this problem could be. The previous work proposed to solve the problem by improving the accuracy of MD integrators and use of the multiple time step algorithm. The additional issue identified in the current work, i.e., the lack of efficient exchange between different motions, would further challenge the popular algorithms for MD simulations.

Considering all these factors, we realized that a simple fix could be made to the problem as the easiest approach to many users in the QM/MM community, even though there might be many more elaborate methods to solve this problem. We seek help from an aged trick in classical statistical mechanics; that is, the phase space distribution is independent to the mass of the particles.[30−32] We can use a heavier proton to slow down the vibrations of the O−H bond, thus allowing sufficient kinetic energy exchange between this and other motional modes. Of course, this approach will vary the dynamics of the bonds and bond angles involving the changed atom, but it is undoubtedly legitimate as long as thermodynamic properties, e.g. free energies, are concerned within the framework of classical statistical mechanics.

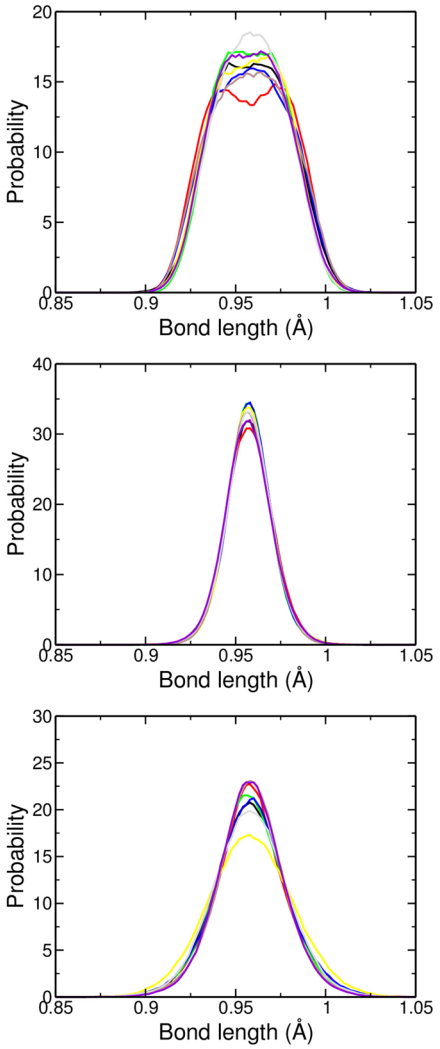


Figure 4. Distribution of methanol O−H bond length simulated using the OPLS force field and a flexible TIP3P water model. (a) Berendsen thermostat; (b) Nose−Hoover thermostat; (c) velocity-rescaling.

When an artificially heavier proton, e.g., of 10 times the normal hydrogen mass, was used to replace the proton in the O−H bond, the distributions obtained from all simulations with the three thermostats both showed satisfactory results (Figure 5). The results could even be compared with the theoretical distribution of O−H bond lengths from the OPLS force field (Figure 6) which validates conventional application of constraints in MD simulations. Note that the shift of the position of the peak must result from other interactions with the two atoms. The correct single modal distribution is the direct result of the stiff O−H bond vibrations.

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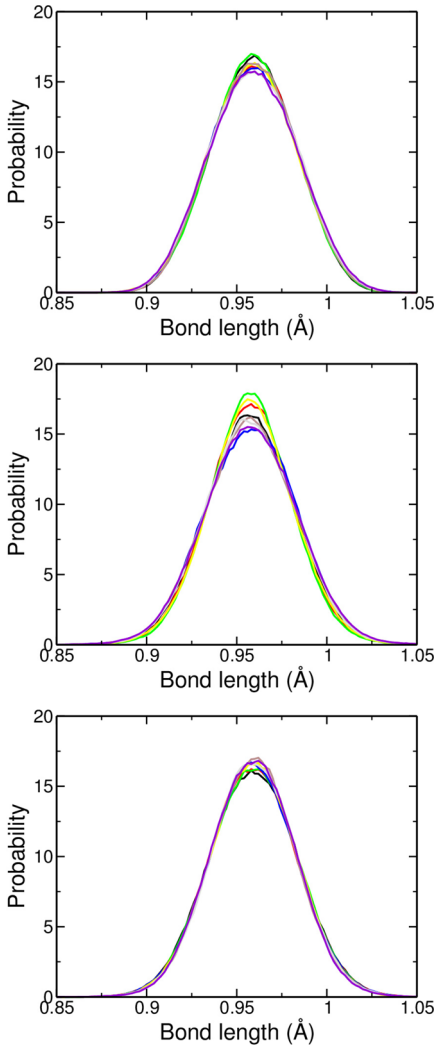

Figure 5. Distribution of methanol O−H bond length simulated with the hydrogen mass set to 10 atomic units. (a) Berendsen thermostat; (b) Nose−Hoover thermostat; (c) velocity-rescaling.

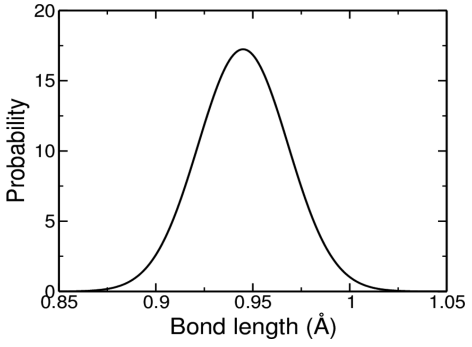

Figure 6. Ideal distribution of methanol O−H bond length from OPLS force field parameters.

Previous QM/MM MD simulations have proposed to use heavier protons for larger integrating time steps.[33] A significantly increased time step of 3 fs was shown to produce a reaction free energy profile in good agreement with that from 1 fs simulations. There are actually many issues that need to be considered when the mass of many hydrogen atoms of the molecular system is increased. Previous work provided an excellent account of them.[34]

The effect of the distorted distribution of a small number of stiff bonds on the reaction free energies simulated by QM/MM methods remains to be determined. Currently many QM/MM free energy simulation methods have been developed so that QM or QM/MM-based direct MD simulation could be avoided.[35−40] In such a case, one probably does not need to worry about this problem. However, if one is interested in simulating the nuclear quantum effects or dynamical properties through Born−Oppenheimer approximation based classical MD approaches, this issue could lead to severe consequences and must be handled carefully. In this regard, simulating dynamical properties such as vibrational spectra with the QM/MM MD methods[41−43] is still a challenging issue. The method proposed here to use increased mass would alter the dynamics and becomes inappropriate. On the other hand, proposals to constrain bond lengths in QM/MM MD simulations[44,45] are also inapplicable. A better solution to these problems might be using flexible water models.[46,47] However, as we argued before, further careful validations and even some reparameterizations must be done before one simply adopts the common rigid water models.

To our best knowledge, the issue of thermalization of isolated stiff bonds in MD simulations with the QM/MM and even MM methods has not been reported before. This issue is so subtle that many researchers probably would not notice it. Even though experienced researchers could have avoided this problem by setting simulations up carefully and making thorough analysis of results,[48] many occasional QM/MM users might not be fully aware of the importance of the correct MD simulation. Therefore, the current report could provide a helpful reference to many users of QM/MM methods.

CONCLUSION

Despite the great potential for providing results with improved accuracy, the use of the combined QM/MM methods with normal MD sampling technique requires extra care. Specifically, the limited simulation length and the explicit inclusion of vibrations of stiff bonds in the dynamic sampling would bring previously unnoticed technical issues to light. This problem might be unique to QM/MM MD simulations because of several facts: (1) Bond vibrations of the QM subsystem are usually not suppressed in QM/MM MD simulations; (2) Rigid water models are often used which prohibit efficient energy transfer between high-frequency bond vibrations of solute molecule and low-frequency global motions of solvent molecules; (3) The length of QM/MM MD simulations is often much shorter compared to MM MD simulations. We believe the combined effect of these factors is responsible for the observed pathetic distribution of semi-isolated stiff bonds such as the O−H bond in the methanol molecule. Even though one should carefully design the setup for MD simulations, for example, use of a chain of baths instead of a single bath in the Nose−Hoover method, the approach we evoke here, i.e., use of heavier hydrogen atoms, seems to be an easy and effective ad hoc method to correct this problem.

ASSOCIATED CONTENT

Supporting Information

Figures for the O−H bond length distributions simulated with a time step of 0.5 fs, using either rigid or flexible water models. This material is available free of charge via the Internet at http://pubs.acs.org.

dx.doi.org/10.1021/jp4024662 | J. Phys. Chem. B XXXX, XXX, XXX−XXX

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Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

H.H. thanks Dr. X. C. Zeng for running some testing simulations. H.H. also thanks the Research Grants Council of Hong Kong, the University Development Fund on Fast Algorithms, Strategic Theme on Computational Sciences, and Seed Funding for Basic Research at the University of Hong Kong for providing financial supports and the high-performance computing facility of the computer center at HKU for providing computing resources. We are grateful to Prof. K. Y. Chan for his proofreading of the manuscript.

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