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Sampling Enhancement and Free Energy Prediction by the Flying Gaussian Method

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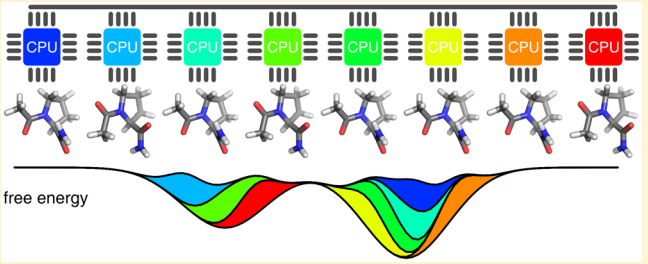
Supporting Information

ABSTRACT: We present a novel sampling enhancement and free energy prediction technique based on parallel simulation of the studied system with a shared bias potential. This history-independent bias potential is defined using selected degrees of freedom (collective variables). Each parallel walker of the system bears a single Gaussian shaped bias potential centered in current values of collective variables. Sampling enhancement is achieved by concentration of multiple walkers in certain free energy minimum. The method was successfully demonstrated on selected molecular systems, and presumed advantages over methods based on a history-dependent bias potential are discussed.



INTRODUCTION

Molecular simulation techniques such as molecular dynamics simulation or Monte Carlo method are widely used in many areas of chemistry. Their application has helped to elucidate dynamics of biomolecules and their complexes. However, these methods are highly computationally expensive. As a result, it is impractical and often even impossible to simulate slow molecular transitions, such as formations of intermolecular complexes, large conformational changes (including protein folding), chemical reactions, and others. Therefore, numerous enhanced sampling techniques have been developed to study slow processes with efficient use of computational resources.[1]

Many enhanced sampling techniques, such as metadynamics,[2−4] umbrella sampling,[5] temperature accelerated molecular dynamics,[6] adiabatic free energy dynamics,[7] logarithmic mean force dynamics,[8] hyperdynamics,[9] or extended adaptive biasing force[10] accelerate a small set of predefined degrees of freedom referred to as collective variables (CVs). Many of these techniques use a bias force or a bias potential that acts on CVs. The Flying Gaussian method introduced here is inspired by a metadynamics method by Laio and Parrinello.[2−4] Metadynamics (Figure 1A) uses a history-dependent bias potential defined in the space of a few (usually one, two, or three) CVs. The bias potential is defined as a sum of Gaussian hills $w \exp(-(s(t)-s')^2/2\delta s^2)$, where $s(t)$ is a collective variable value at time t, s' is a center of a hill, and w and δs are height and width of a hill, respectively. The whole concept can be generalized to two-, three-, and multidimensional hills for two, three, or more collective variables. These hills are deposited in regular intervals, i.e. at the deposition time t' a hill is added and its center s' is set to $s(t')$, until they flood all relevant free energy minima. The free energy surface of the studied system can be predicted from the bias potential.

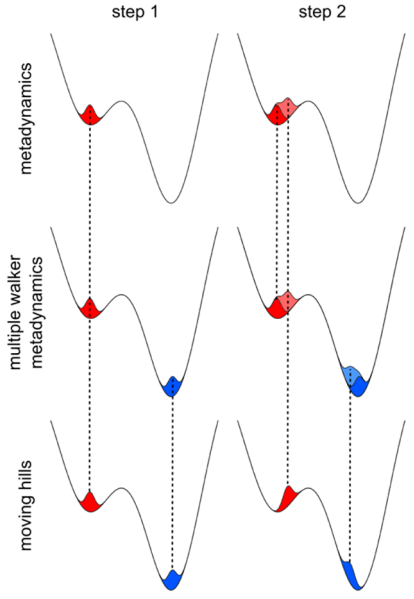


Figure 1. Schematic view of metadynamics (A), multiple walker metadynamics (B), and the Flying Gaussian method (C). Hills from the first and second walker are depicted in red and blue, respectively. Metadynamics adds hills to CV positions in defined intervals, typically every 500 or 1000 microscopic steps. In contrast, the Flying Gaussian method moves hills according to CV evolution in every microscopic step.

A parallel variant of metadynamics known as multiple walker metadynamics has been introduced by Raiteri and co-workers[11]

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(Figure 1B). It simulates the system in multiple replicas (walkers) starting from different starting coordinates. The bias potential is summed along the simulation time as well as across all walkers. This method is efficiently parallelizable due to the fact that simulation nodes exchange only the bias potential, not other molecular data.

The Flying Gaussian method introduced here (Figure 1C) is inspired by ideas of metadynamics and multiple walker metadynamics. Similar to metadynamics the bias potential is constructed as a sum of Gaussian hills defined in the space of preselected collective variables. Similar to multiple walker metadynamics, the system is simulated in multiple walkers. Unlike metadynamics, the bias potential does not accumulate by addition of new hills. Instead, the number of hills is equal to the number of walkers, and their centers move with evolution of collective variables in every microscopic step of the simulation. Filling of free energy basins is achieved by concentration of multiple walkers in the same basin.

We test this approach on model energy surfaces, alanine dipeptide (Ace-Ala-Nme) in vacuum and water, capped oligo-proline previously studied by Moradi and Tajkhorshid,[12] and Met-enkephalin studied by Sutto, D'Abramo, and Gervasio.[13]

METHODS

The Flying Gaussian Method. The bias potential acting on the j-th system in the Flying Gaussian method (in the version with one CV, it can be easily generalized for multiple CVs $\mathbf{s}_j$) is defined as

$$V_{bias,j}(t) = \sum_{i=1}^{N} we^{-(s_i(t)-s_j(t))^2/(2\delta s^2)} \quad (1)$$

where N is the number of walkers. For the sake of simplicity a hill also acts on its "own" system ($i = j$). The algorithm of the Flying Gaussian method is as follows:

1. For each walker calculate values of CV $s_j(t)$, where i is the index of the walker
2. Place a Gaussian hill centered at $s_j(t)$ to each walker
3. For each walker calculate forces from the force field and from the bias potential (sum of hills)
4. Make one microscopic step in each walker
5. Go to step 1

Occasionally, in this work every 100 steps, CV values, and the bias potential are saved to a file for calculation of free energy surfaces. The Flying Gaussian method was implemented using Message Passing Interface (MPI). Practically, in the first microscopic step the hill of the first walker is added, in the second step the hill of the first walker is updated and the hill of the second walker is added, and so forth until the number of microscopic steps reaches the number of walkers. Since then hills are being updated every microscopic step.

Unlike in metadynamics, the bias potential cannot be directly used as an estimate free energy surface of the simulated system because it does not approximate the free energy surface and it is highly dynamic. Instead, the free energy surface can be obtained by on-the-fly reweighting[14−16] as

$$F(s) = -kT \log\left(\frac{\sum_{i=1}^{N}\sum_t \delta(s_i(t) - s)e^{+V_{bias,i}(t)/(kT)}}{\sum_{i=1}^{N}\sum_t e^{+V_{bias,i}(t)/(kT)}}\right) \quad (2)$$

where δ is one- or multidimensional Dirac delta function.

As an alternative to on-the-fly reweighting we also tested the Weighted Histogram Analysis Method (WHAM).[9,17]

COMPUTATIONAL DETAILS

Simulations on a model energy surface were done with an *ad hoc* program written in Python (see the Supporting Information). All molecular simulations were done with Gromacs 5.0.4[18] patched by a modified version of Plumed 2.2.[19] Alanine dipeptide (Ace-Ala-Nme) was simulated in the AMBER99SB-ILDN force field[20] in vacuum or in water (periodic box containing 863 TIP3P water molecules). Simulations in vacuum used a stochastic simulation integrator with a 1 fs time step without constraints. Bonds were not constrained, and all noncovalent interactions were modeled without cutoffs. Simulations in water used a molecular dynamics integrator with a 2 fs time step with all bonds being constrained using the LINCS algorithm.[21] Electrostatic interactions were modeled by the particle-mesh Ewald method.[22] Temperature in simulations in vacuum as well as in water was kept constant (300 K) by a Parrinello-Bussi thermostat.[23] Ramachandran torsions φ and ψ were used as collective variables with widths of hills set to 0.3 rad.

Simulations of capped oligo-proline (Ace-$(Pro)_n$-Nme) mostly followed the study of Moradi and Tajkhorshid.[12] It was modeled in the CHARMM27 force field[24] by a stochastic dynamics integrator with 1 fs in implicit solvent (generalized Born-surface area,[25] relative dielectric constant set to 78.5). Bonds were not constrained, and all noncovalent interactions were modeled without cutoffs. Temperature in simulations in vacuum as well as in water was controlled by a Parrinello-Bussi thermostat.[23] Collective variable was defined as a sum of $\cos^2(\omega_i/2)$, where ω_i is peptide bond torsion angle preceding the i-th proline residue. This collective variable approximates the number of *cis* peptide bonds. Widths of hills were set to 0.08 and height to 10 kJ/mol.

Simulations of Met-enkephalin were performed following the study of its free energy landscape done by Sutto, D'Abramo, and Gervasio.[13] The AMBER99SB-ILDN force field[20] was used, and Met-enkephalin was placed in a cubic box containing 1,283 water molecules (TIP3P water model[26]). Bonds were constrained using the LINCS algorithm.[21] The simulation time step was set to 2 fs. The Particle-Mesh Ewald algorithm[22] was used for calculation of electrostatic interactions, and a Berendsen thermostat[27] was used to keep the temperature at 300 K. Three distances (d_1: N[Tyr1]-Cα[Met5], d_2: Cζ[Tyr1]-Cζ[Phe4], and d_3: Cζ[Phe4]-Sδ[Met5]) were used as CVs with height and width of a hill set to 3 kJ/mol and 0.02 nm.

RESULTS

It is practical to test newly developed enhanced sampling methods on simple model energy surfaces. For this purpose we used a one-dimensional double-well energy surface illustrated in Figure 2. Although the major goal of enhanced sampling techniques is to explore and reconstruct a free energy surface, it is possible to test such techniques on a model potential energy surface, because the potential and free energy surfaces are almost identical in the absence of other degrees of freedom and at low temperature. The Flying Gaussian method with 20 Monte Carlo walkers, all starting from the deeper minimum, was used to explore this simple function. Figure 2 shows the original function ($V = -1.5\exp(-(s-3.5)^2) - 0.5\exp(-(s-6.5)^2) + const.$) and its prediction by on-the-fly reweighting of

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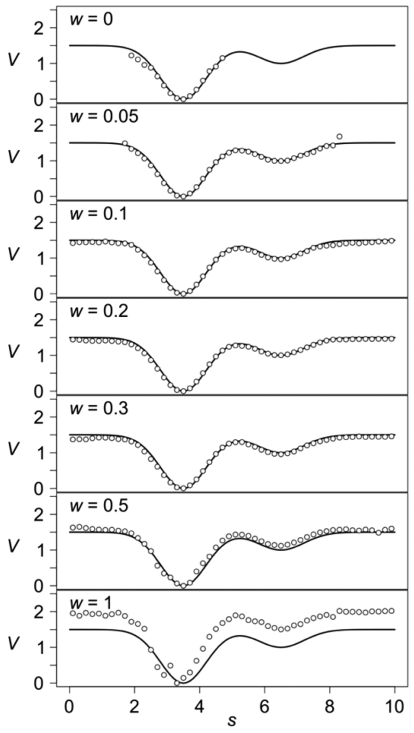

Figure 2. Flying Gaussian method on a model energy surface ($V = -1.5\ \exp(-(s-3.5)^2) - 0.5\ \exp(-(s-6.5)^2)$ + *const.*, line) and its prediction by on-the-fly reweighting (circles).

the results from the Flying Gaussian method. It shows that the simulation with height of hills set to zero (i.e., unbiased Monte Carlo method) explored only the starting basin. Sampling enhancement was achieved by hills of height set to 0.05 in energy units. Complete energy surface was sampled with heights of 0.1 and 0.2. Excessive hill heights (0.5 and 1.0) lead to inaccuracy and noise in predicted free energy surfaces, which is common to other enhanced sampling techniques. Inaccurate and noisy free energy estimates were also observed for narrow hills (data not shown), which can be explained by high gradients on the sides of narrow hills.

The free energy surface of alanine dipeptide in vacuum contains a large energy barrier between C7eq and C7ax conformations, which can be used as a test case for newly developed enhanced sampling techniques. First, the system was simulated by a 200 ps unbiased simulation to generate starting conformations. Twenty snapshots (sampled by 10 ps) were dissected from the trajectory and used as starting structures of the Flying Gaussian method. All these snapshots belonged to the C5/C7eq minima. The Flying Gaussian simulation was performed with 20 walkers each running for 100 ns. Height of a hill was set to 10 kJ/mol. Collective variables were sampled every 100 fs, and these snapshots were used to calculate free energy surface by on-the-fly reweighting (20 × 999,901 snapshots, first 100 snapshots were discarded from each walker). The results are presented in Figure 3. After less than 200 ps three of the 20 walkers jumped to the minimum C7ax. For the rest of the simulation there were usually 15−17 walkers in the minimum C5/C7eq and 3−5 in C7ax. There were 301 transitions between C5/C7eq and C7ax in the whole simulation. The free energy difference between C7ax and C7eq was predicted as 7.4 kJ/mol. This is close to the results of metadynamics (6.6 kJ/mol, see Figure S1A). The free energy barrier was estimated as 36 kJ/mol, close to 37 kJ/mol predicted by metadynamics. It can be shown that an accurate free energy surface can be obtained when the simulation starts from different minima, which was demonstrated by analysis of the second half of the simulation (Figure S1 in the Supporting Information).

Equally a long unbiased simulation was carried out as a Flying Gaussian simulation with hill height set to zero. This simulation stayed in the C5/C7eq minimum and did not explore C7ax (Figure S1B). Results for simulations with different hill heights and numbers can be obtained in the Supporting Information (Figures S2 and S3). Surprisingly, relatively good free energy estimates were obtained for low hill heights even though the number of C5/C7eq - C7ax transitions was low.

Besides on-the-fly reweighting we also tested WHAM.[9,17] The advantage of WHAM is that it can accurately reconstruct the free energy surface albeit individual walkers explore the CV space partially with some overlap. A major disadvantage is in the computational cost of WHAM due to its iterative nature. The result of WHAM analyses can be found in the Supporting Information (Figure S4).

It is important to show how the Flying Gaussian method performs in a different number of walkers. For this purpose we carried out Flying Gaussian simulations of alanine dipeptide in water with 8, 16, 32, and 64 walkers with hill heights set to 2.5, 2.5, 1.25, and 0.625 kJ/mol, respectively. Systems were simulated for 10 ns in each walker. On-the-fly reweighting was done on $N \times 49{,}901$ samples. Resulting free energy surfaces are depicted in Figure 4. It clearly shows excellent agreements between free energy surfaces calculated with a different numbers of walkers.

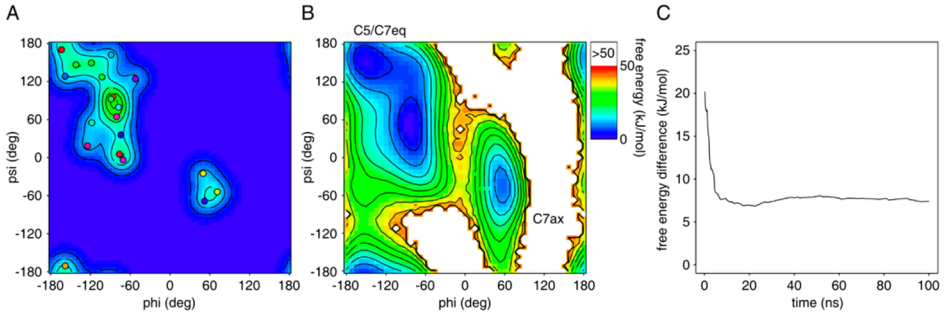

Figure 3. Flying Gaussian simulation of alanine dipeptide in vacuum. A - a sample bias potential (at the end of the simulation). Centers of hills are depicted as circles in different colors. B - free energy surface calculated by on-the-fly reweighting. C - evolution of estimated ΔG between C7ax and C7eq during the simulation.

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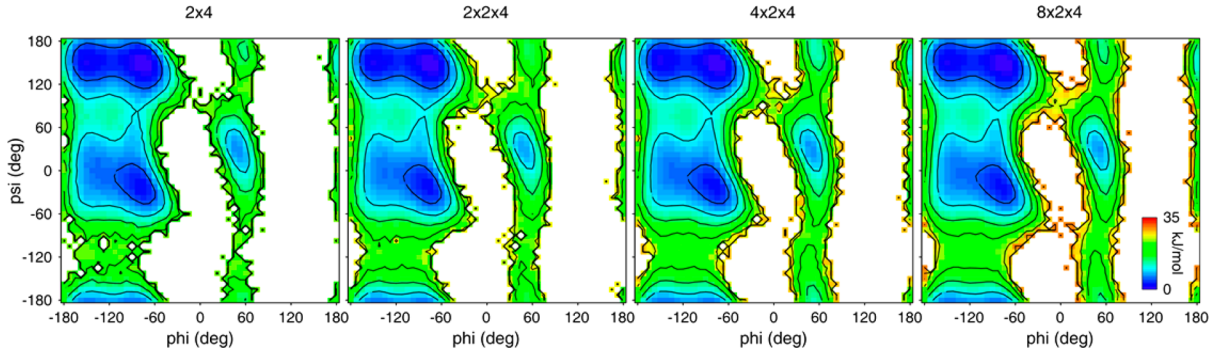

Figure 4. Free energy surface of alanine dipeptide in water calculated by the Flying Gaussian method with 8, 16, 32, and 64 walkers.

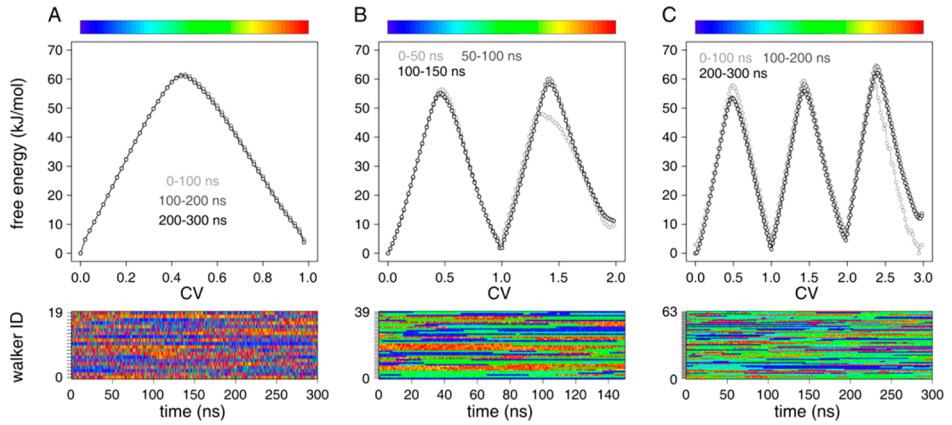

Figure 5. Free energy surfaces (top) and collective variable evolution (bottom) of Ace-$(Pro)_n$-Nme for n = 1 (A), 2 (B), and 3 (C). Collective variable value is depicted in color from blue (all-*trans*) to red (all-*cis*) conformation.

Cis/*trans* isomerization of a peptide bond preceding proline plays an important role in protein folding,[28] HIV life cycle,[29] and many other biological processes. In this study we used a system previously studied by Moradi and Tajkhorshid.[12] Different combinations of *cis*/*trans* peptide bonds in Ace-$(Pro)_n$-Nme (n = 1−3) can be explored by enhancing of a single collective variable defined as a sum of $\cos^2(\omega_i/2)$, where ω_i is a torsion angle preceding the i-th proline residue. Results of simulations are presented in Figure 5.

The *trans* conformation of the bond preceding proline residue is slightly favored over the *cis* conformation, which favors the all-*trans* conformation of the chain. All-*trans* as well as all-*cis* conformations may form stable helical structures, which favor all-*cis* and all-*trans* conformations over mixed *cis*/*trans* forms. Finally, mixed *cis*/*trans* conformations are slightly favored entropically due to the fact that there is only one all-*cis* and one all-*trans* form but multiple combinations of mixed *cis*−*trans* conformations (e.g., for n = 2, CV = 0 corresponds to the *trans*−*trans*, CV = 2 corresponds to the *cis*−*cis*, but CV = 1 corresponds to two forms *cis*−*trans* and *trans*−*cis*).

Flying Gaussian simulations were carried out in 20 (n = 1), 40 (n = 2), and 64 (n = 3) walkers. All simulations started from the all-*cis* conformation. Figure 5 shows free energy surfaces calculated in the first, second, and last third of each simulation. It shows that the free energy surfaces calculated in the first third were inaccurate (with exception of n = 1) due to lack of time necessary to equilibrate. Snapshots taken every 10 fs were used to determine free energy surfaces by on-the-fly reweighting. Free energy surfaces in the second and the last third of the simulation did not differ significantly, which, together with the fact that *cis*/*trans*-isomerization events were seen in the simulation, indicates good convergence of the free energy surfaces.

Alanine dipeptide and oligo-prolines represent systems of low complexity with high energy barriers. In order to test the Flying Gaussian method on a more complex system with lower energy barriers we selected Met-enkephalin in explicitly modeled water as an example of a system with diffuse kinetics of conformational changes. The free energy surface obtained by 15 ns simulation in 20 replicas is depicted in Figure 6. Snapshots taken every 10 fs were used to determine free energy surfaces by on-the-fly reweighting. It is in good agreement with 2 μs unbiased simulation.[13]

DISCUSSION

The behavior of the bias potential in the Flying Gaussian method is in some aspects similar and in some aspects dissimilar to well established enhanced sampling methods. For example, metadynamics (in its original non-well-tempered variant[2,30]) uses a bias potential to flatten the free energy surface of the simulation system. Once the sum of free energy and a bias potential becomes flat, the system can freely diffuse between its states.

Unlike the original metadynamics, the Flying Gaussian method does not flatten the free energy surface. Metadynamics flattens the free energy surface and thus makes sampling uniform. Biasing in the Flying Gaussian method is proportional to sampling. Uniform sampling would therefore cause a uniform bias potential with no sampling enhancement. We can therefore expect a bias potential as a compromise between

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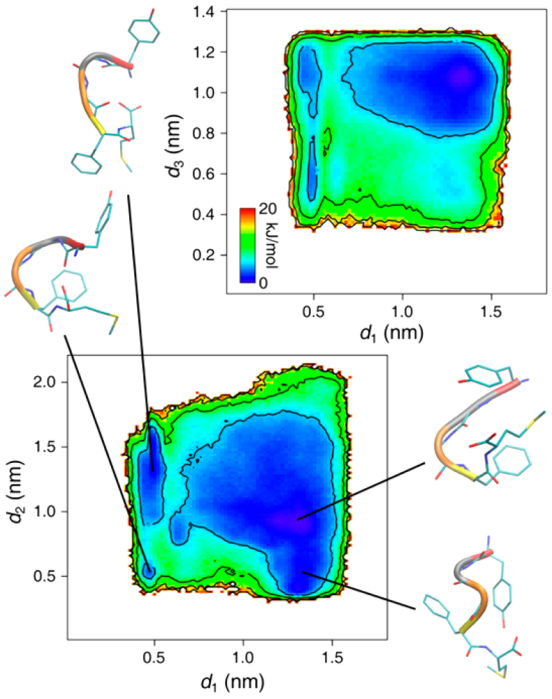

Figure 6. Free energy surfaces of Met-enkephalin in the space of two pairs of CVs together with representative structures.

uniform and cannonical sampling. The Flying Gaussian method is similar in this aspect to well tempered metadynamics[31] where the bias potential is a compromise between a flattened free energy surface (zero "bias factor") and a uniform bias potential (infinite "bias factor").

Another difference between Flying Gaussian and other enhanced sampling methods is in the dynamics of the bias potential. The bias potential in metadynamics accumulates steadily by addition of small hills, usually below 1 kJ/mol. In contrast, the Flying Gaussian bias potential is highly dynamic (see Figure 3A for a snapshot of the bias potential).

For the reasons explained in the previous two paragraphs we cannot use the bias potential to directly estimate the free energy surface. Instead we used on-the-fly reweighting or WHAM. On-the-fly reweighting combines sampling of collective variables with values of the bias potential. This method can be used provided that sampling can adapt to changes of the bias potential. It must be carefully assessed whether this condition is fulfilled for a dynamic bias potential of the Flying Gaussian method. This is supported by agreement of predicted energy surfaces with the target energy surface (Figure 2) and with the results of reference simulations done by metadynamics or unbiased molecular dynamics simulation. The advantage of on-the-fly reweighting is the fact that the free energy surface can be calculated even for degrees of freedom that were not biased.[16]

The condition of adaptation of sampling to the bias potential is probably not fulfilled at the beginning of the Flying Gaussian simulation when all walkers start from the same minimum. This causes a very high bias potential at the beginning of the simulation. For this reason we removed the initial phase of Flying Gaussian simulations when predicting free energy surfaces by on-the-fly reweighting. Moreover, we observed noisy free energy surfaces for the simulation setup with high or narrow hills (for example Figure 2 with w = 0.5 or 1), i.e. with high bias potential gradients. Similar to other enhanced sampling techniques it is not possible to use an excessive bias potential or a bias force.

In summary, we observed a good agreement between free energy surfaces calculated by Flying Gaussian and reference methods. Nevertheless, we plan to further evaluate the accuracy of this method to study the effect of sampling adaptation to the bias potential described above, as well as other potential sources of error, for example deformation of the phase space by a time-dependent Hamiltonian (i.e. a time-dependent bias potential).[32]

Dynamics of the bias potential may be slowed down by application of extended Lagrangian. The extended Lagrangian formalism may be also used to further theoretically investigate and evaluate the relationship between sampling and free energy surface in the Flying Gaussian method. However, it turned out to be difficult to find suitable parameters for accurate free energy prediction. The results of the extended Lagrangian variant of the Flying Gaussian method are provided in the Supporting Information.

Accuracy of the Flying Gaussian simulation was assessed by comparison of free energy surfaces predicted at different stages of a simulation. A stable free energy surface can be interpreted as accurate. However, it must be kept in mind that free energy predictions may become frozen due to lack of transitions between minima. In case the bias potential is too low it may happen that the walkers distribute between the minima but stay there for the rest of the simulation. This leads to a stable free energy surface, but it does not ensure its accuracy. For an accurate estimate it is necessary to increase heights of hills or to increase the number of walkers in order to increase the number of transitions between the minima.

Let us discuss possible advantages and disadvantages of the new method. Its parallel nature may be an advantage as well as a disadvantage. The method can be easily parallelized to a high number of CPUs, because communication of hill positions is relatively inexpensive compared to communications between nodes in a parallelized evaluation of a noncovalent interaction. On the other hand, other methods such as unbiased molecular dynamics simulation or metadynamics allow users to choose to run simulations on one or multiple CPUs, depending on their needs and resources. In this study we used a relatively low number of walkers (8 to 64) to demonstrate that the method performs well even without a huge numbers of walkers. The fact that it is necessary to prepare multiple starting structures as well as a necessity to use on-the-fly reweighting may be viewed as a disadvantage of the Flying Gaussian method.

A potential disadvantage of Flying Gaussian comes from the fact that CV-based biased simulations in highly complex (for example biomolecular) systems usually cannot fully describe all slow motions by CVs and must therefore rely on sampling. For such systems it is useful to perform one long biased simulation (e.g., in a single-walker metadynamics) instead of multiple short ones (like in multiple walker metadynamics or the Flying Gaussian method). However, there are simulation techniques where this disadvantage can be overcome. For example, path collective variables[33] with numerous successful applications on a complex biomolecular system[34,35] require generation of a series of landmark structures along the studied process. Since it is necessary to somehow generate a series of structures representing the whole process, it is possible to use them as starting structures of the Flying Gaussian method and thus improve the chance of getting converged free energy surface with efficient use of computational resources.

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Advantages of the Flying Gaussian method, compared to metadynamics, include the fact that the bias potential data do not accumulate with the progress of the simulation. This effect may slow down metadynamics simulations when huge numbers of hills must be evaluated. More importantly, it is possible to control the bias potential so that it does not overflood energy minima, and it samples physically relevant states. In this aspect the Flying Gaussian method is similar to well tempered metadynamics.[31] The accuracy of a free energy surface calculated by on-the-fly reweighting is increasing with the progress of a simulation due to better sampling of the system, again analogously to well tempered metadynamics.[31] On-the-fly reweighting can be applied not only to the biased CVs but also to other degrees of freedom.

An interesting feature of the Flying Gaussian method is an autonomous nature of the bias potential. The fact that the bias potential is not history-dependent makes it possible to change the definition of collective variables during the simulation. This opens opportunities for new variants of the Flying Gaussian method with an adaptive self-learning definition of collective variables, which are being intensively studied.[15,36] Furthermore, combinations of the Flying Gaussian method with parallel tempering[37] and bias exchange[38] will be subjects of future studies. Finally, it seems to be possible to use the Fying Gaussian method in a way that each walker represents a slightly different system, for example different drug-like molecules binding to the same protein, with retaining the possibility to predict the free energy surface for each system separately. This is in fashion of our newly developed Altruistic metadynamics[39] and could be useful in fields dealing with parallel testing of multiple systems, e.g. in drug discovery.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jctc.6b00551.

Source code of the Flying Gaussian simulation on a model energy profile and additional results on alanine dipeptide (PDF)

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) Spiwok, V.; Šućur, Z.; Hošek, P. Enhanced Sampling Techniques in Biomolecular Simulations. *Biotechnol. Adv.* **2015**, *33*, 1130−1140.

(2) Laio, A.; Parrinello, M. Escaping free-energy minima. *Proc. Natl. Acad. Sci. U. S. A.* **2002**, *99*, 12562−12566.

(3) Barducci, A.; Bonomi, M.; Parrinello, M. Metadynamics. *WIREs Comput. Mol. Sci.* **2011**, *1*, 826−843.

(4) Laio, A.; Gervasio, F. L. Metadynamics: A Method to Simulate Rare Events and Reconstruct the Free Energy in Biophysics, Chemistry and Material Science. *Rep. Prog. Phys.* **2008**, *71*, 126601.

(5) Torrie, G. M.; Valleau, J. P. Nonphysical sampling distributions in Monte Carlo free-energy estimation: Umbrella sampling. *J. Comput. Phys.* **1977**, *23*, 187−199.

(6) Maragliano, L.; Vanden-Eijnden, E. A temperature accelerated method for sampling free energy and determining reaction pathways in rare events simulations. *Chem. Phys. Lett.* **2006**, *426*, 168−175.

(7) Rosso, L.; Tuckerman, M. A. An Adiabatic Molecular Dynamics Method for the Calculation of Free Energy Profiles. *Mol. Simul.* **2002**, *28*, 91−112.

(8) Morishita, T.; Itoh, S. G.; Okumura, H.; Mikami, M. Free-energy calculation via mean-force dynamics using a logarithmic energy landscape. *Phys. Rev. E* **2012**, *85*, 066702.

(9) Voter, A. F. Hyperdynamics: Accelerated Molecular Dynamics of Infrequent Events. *Phys. Rev. Lett.* **1997**, *78*, 3908.

(10) Lelièvre, T.; Rousset, M.; Stoltz, G. Computation of free energy profiles with parallel adaptive dynamics. *J. Chem. Phys.* **2007**, *126*, 134111.

(11) Raiteri, P.; Laio, A.; Gervasio, F. L.; Micheletti, C.; Parrinello, M. Efficient Reconstruction of Complex Free Energy Landscapes by Multiple Walkers Metadynamics. *J. Phys. Chem. B* **2006**, *110*, 3533−3539.

(12) Moradi, M.; Tajkhorshid, E. Driven Metadynamics: Reconstructing Equilibrium Free Energies from Driven Adaptive-Bias Simulations. *J. Phys. Chem. Lett.* **2013**, *4*, 1882−1887.

(13) Sutto, L.; D'Abramo, M.; Gervasio, F. L. Comparing the Efficiency of Biased and Unbiased Molecular Dynamics in Reconstructing the Free Energy Landscape of Met-Enkephalin. *J. Chem. Theory Comput.* **2010**, *6*, 3640−3646.

(14) Dickson, B. M. Approaching a Parameter-Free Metadynamics. *Phys. Rev. E Stat. Nonlin. Soft. Matter. Phys.* **2011**, *84*, 037701.

(15) Tribello, G. A.; Ceriotti, M.; Parrinello, M. Using Sketch-Map Coordinates to Analyze and Bias Molecular Dynamics Simulations. *Proc. Natl. Acad. Sci. U. S. A.* **2012**, *109*, 5196−5201.

(16) Bonomi, M.; Barducci, A.; Parrinello, M. Reconstructing the Equilibrium Boltzmann Distribution from Well-Tempered Metadynamics. *J. Comput. Chem.* **2009**, *30*, 1615−1621.

(17) Kumar, S.; Bouzida, D.; Swendsen, R. H.; Kollman, P. A.; Rosenberg, J. M. The weighted histogram analysis method for free-energy calculations on biomolecules. I. The method. *J. Comput. Chem.* **1992**, *13*, 1011−1021.

(18) Abraham, M. J.; Murtola, T.; Schulz, R.; Páll, S.; Smith, J. C.; Hess, B.; Lindahl, E. GROMACS: High Performance Molecular Simulations Through Multi-Level Parallelism from Laptops to Supercomputers. *SoftwareX* **2015**, *1−2*, 19−25.

(19) Tribello, G. A.; Bonomi, M.; Branduardi, D.; Camilloni, C.; Bussi, G. PLUMED 2: New Feathers for an Old Bird. *Comput. Phys. Commun.* **2014**, *185*, 604−613.

(20) Lindorff-Larsen, K.; Piana, S.; Palmo, K.; Maragakis, P.; Klepeis, J. L.; Dror, R. O.; Shaw, D. E. Improved Side-Chain Torsion Potentials for the Amber ff99SB Protein Force Field. *Proteins: Struct., Funct., Genet.* **2010**, *78*, 1950−1958.

(21) Hess, B.; Bekker, H.; Berendsen, H. J. C.; Fraaije, J. G. E. M. LINCS: A Linear Constraint Solver for Molecular Simulations. *J. Comput. Chem.* **1997**, *18*, 1463−1472.

(22) Darden, T.; York, D.; Pedersen, L. Particle Mesh Ewald: An *N*.log(*N*) Method for Ewald Sums in Large Systems. *J. Chem. Phys.* **1993**, *98*, 10089−10092.

(23) Bussi, G.; Donadio, D.; Parrinello, M. Canonical Sampling Through Velocity-Rescaling. *J. Chem. Phys.* **2007**, *126*, 014101.

(24) MacKerell, A. D., Jr.; Banavali, N.; Foloppe, N. Development and Current Status of the CHARMM Force Field for Nucleic Acids. *Biopolymers* **2000**, *56*, 257−265.

(25) Qiu, D.; Shenkin, P.; Hollinger, F.; Still, W. The GB/SA Continuum Model for Solvation. A Fast Analytical Method for the Calculation of Approximate Born Radii. *J. Phys. Chem. A* **1997**, *101*, 3005−3014.

DOI: 10.1021/acs.jctc.6b00551
J. Chem. Theory Comput. 2016, 12, 4644−4650

(26) Jorgensen, W. L.; Chandrasekhar, J.; Madura, J. D.; Impey, R. W.; Klein, M. L. Comparison of Simple Potential Functions for Simulating Liquid Water. *J. Chem. Phys.* **1983**, *79*, 926−935.
(27) Berendsen, H. J. C.; Postma, J. P. M.; van Gunsteren, W. F.; DiNola, A.; Haak, J. R. Molecular-Dynamics with Coupling to an External Bath. *J. Chem. Phys.* **1984**, *81*, 3684−3690.
(28) Wedemeyer, W. J.; Welker, E.; Scheraga, H. A. Proline cis-trans Isomerization and Protein Folding. *Biochemistry* **2002**, *41*, 14637−14644.
(29) Thali, M.; Bukovsky, A.; Kondo, E.; Rosenwirth, B.; Walsh, C. T.; Sodroski, J.; Göttlinger, H. G. Functional Association of Cyclophilin A with HIV-1 Virions. *Nature* **1994**, *372*, 363−365.
(30) Laio, A.; Rodriguez-Fortea, A.; Gervasio, F. L.; Ceccarelli, M.; Parrinello, M. Assessing the Accuracy of Metadynamics. *J. Phys. Chem. B* **2005**, *109*, 6714−6721.
(31) Barducci, A.; Bussi, G.; Parrinello, M. Well-Tempered Metadynamics: A Smoothly Converging and Tunable Free-Energy Method. *Phys. Rev. Lett.* **2008**, *100*, 020603.
(32) Evans, D. J.; Searles, D. J. The Fluctuation Theorem. *Adv. Phys.* **2002**, *51*, 1529−1585.
(33) Branduardi, D.; Gervasio, F. L.; Parrinello, M. From A to B in Free Energy Space. *J. Chem. Phys.* **2007**, *126*, 054103.
(34) Limongelli, V.; Bonomi, M.; Marinelli, L.; Gervasio, F. L.; Cavalli, A.; Novellino, E.; Parrinello, M. Molecular Basis of Cyclooxygenase Enzymes (COXs) Selective Inhibition. *Proc. Natl. Acad. Sci. U. S. A.* **2010**, *107*, 5411−5416.
(35) Berteotti, A.; Cavalli, A.; Branduardi, D.; Gervasio, F. L.; Recanatini, M.; Parrinello, M. Protein Conformational Transitions: the Closure Mechanism of a Kinase Explored by Atomistic Simulations. *J. Am. Chem. Soc.* **2009**, *131*, 244−250.
(36) Tribello, G. A.; Ceriotti, M.; Parrinello, M. A Self-Learning Algorithm for Biased Molecular Dynamics. *Proc. Natl. Acad. Sci. U. S. A.* **2010**, *107*, 17509−17514.
(37) Bussi, G.; Gervasio, F. L.; Laio, A.; Parrinello, M. Free-Energy Landscape for β Hairpin Folding from Combined Parallel Tempering and Metadynamics. *J. Am. Chem. Soc.* **2006**, *128*, 13435−13441.
(38) Piana, S.; Laio, A. A Bias-Exchange Approach to Protein Folding. *J. Phys. Chem. B* **2007**, *111*, 4553−4559.
(39) Hošek, P.; Toulcová, D.; Bortolato, A.; Spiwok, V. Altruistic Metadynamics: Multisystem Biased Simulation. *J. Phys. Chem. B* **2016**, *120*, 2209−2215.

DOI: 10.1021/acs.jctc.6b00551
J. Chem. Theory Comput. 2016, 12, 4644−4650