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Enhanced Sampling in Molecular Dynamics Using Metadynamics, Replica-Exchange, and Temperature-Acceleration

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Abstract: We review a selection of methods for performing enhanced sampling in molecular dynamics simulations. We consider methods based on collective variable biasing and on tempering, and offer both historical and contemporary perspectives. In collective-variable biasing, we first discuss methods stemming from thermodynamic integration that use mean force biasing, including the adaptive biasing force algorithm and temperature acceleration. We then turn to methods that use bias potentials, including umbrella sampling and metadynamics. We next consider parallel tempering and replica-exchange methods. We conclude with a brief presentation of some combination methods.

Keywords: collective variables; free energy; blue-moon sampling; adaptive-biasing force algorithm; temperature-acceleration; umbrella sampling; metadynamics

1. Introduction

The purpose of molecular dynamics (MD) is to compute the positions and velocities of a set of interacting atoms at the present time instant given these quantities one time increment in the past. Uniform sampling from the discrete trajectories one can generate using MD has long been seen as

synonymous with sampling from a statistical-mechanical ensemble; this just expresses our collective wish that the ergodic hypothesis holds at finite times. Unfortunately, most MD trajectories are not ergodic and leave many relevant regions of configuration space unexplored. This stems from the separation of high-probability “metastable” regions by low-probability “transition” regions and the inherent difficulty of sampling a $3N$ -dimensional space by embedding into it a one-dimensional dynamical trajectory.

This review concerns a selection of methods to use MD simulation to enhance the sampling of configuration space. A central concern with any enhanced sampling method is guaranteeing that the statistical weights of the samples generated are known and correct (or at least correctable) while simultaneously ensuring that as much of the relevant regions of configuration space are sampled. Because of the tight relationship between probability and free energy, many of these methods are known as “free-energy” methods. To be sure, there are a large number of excellent reviews of free-energy methods in the literature (e.g., [1–5]). The present review is in no way intended to be as comprehensive. As the title indicates, we will mostly focus on enhanced sampling methods of three flavors: tempering, metadynamics, and temperature-acceleration. Along the way, we will point out important related methods, but in the interest of brevity we will not spend much time explaining these. The methods we have chosen to focus on reflect our own preferences to some extent, but they also represent popular and growing classes of methods that find ever more use in biomolecular simulations and beyond.

We divide our review into three main sections. In the first, we discuss enhanced sampling approaches that rely on *collective variable biasing*. These include the historically important methods of thermodynamic integration and umbrella sampling, and we pay particular attention to the more recent approaches of the adaptive-biasing force algorithm, temperature-acceleration, and metadynamics. In the second section, we discuss approaches based on *tempering*, which is dominated by a discussion of the parallel tempering/replica exchange approaches. In the third section, we briefly present some relatively new methods derived from either collective-variable-based or tempering-based approaches, or their combinations.

2. Approaches Based on Collective-Variable Biasing

2.1. Background: Collective Variables and Free Energy

For our purposes, the term “collective variable” or CV refers to any multidimensional function θ of $3N$ -dimensional atomic configuration $\mathbf{x} \equiv (x_i | i = 1 \dots 3N)$. The functions $\theta_1(\mathbf{x}), \theta_2(\mathbf{x}), \dots, \theta_M(\mathbf{x})$ map configuration $\mathbf{x}$ onto an M -dimensional CV space $\mathbf{z} \equiv (z_j | j = 1 \dots M)$, where usually $M \ll 3N$. At equilibrium, the probability of observing the system at CV-point $\mathbf{z}$ is the weight of all configurations $\mathbf{x}$ which map to $\mathbf{z}$:

$$P(\mathbf{z}) = \langle \delta[\theta(\mathbf{x}) - \mathbf{z}] \rangle \quad (1)$$

The Dirac delta function picks out only those configurations for which the CV $\theta(\mathbf{x})$ is $\mathbf{z}$, and $\langle \cdot \rangle$ denotes averaging its argument over the equilibrium probability distribution of $\mathbf{x}$. The probability can be expressed as a *free energy*:

$$F(\mathbf{z}) = -k_B T \ln \langle \delta[\theta(\mathbf{x}) - \mathbf{z}] \rangle \quad (2)$$

Here, k_B is Boltzmann’s constant and T is temperature.

Local minima in F are metastable equilibrium states. F also measures the energetic cost of a maximally efficient (*i.e.*, reversible) transition from one region of CV space to another. If, for example, we choose a CV space such that two well-separated regions define two important allosteric states of a given protein, we could perform a free-energy calculation to estimate the change in free energy required to realize the conformational transition. Indeed, the promise of being able to observe with atomic detail the transition states along some pathway connecting two distinct states of a biomacromolecule is strong motivation for exploring these transitions with CVs.

Given the limitations of standard MD, how does one “discover” such states in a proposed CV space? A perfectly ergodic (infinitely long) MD trajectory would visit these minima much more frequently than it would the intervening spaces, allowing one to tally how often each point in CV space is visited; normalizing this histogram into a probability $P(z)$ would be the most straightforward way to compute F via Equation (2). In all too many actual cases, MD trajectories remain close to only one minimum (the one closest to the initial state of the simulation) and only very rarely, if ever, visit others. In the CV sense, we therefore speak of standard MD simulations failing to overcome *barriers* in free energy. “Enhanced sampling” in this context refers then to methods by which free-energy barriers in a chosen CV space are surmounted to allow as broad as possible an extent of CV space to be explored and statistically characterized with limited computational resources.

In this section, we focus on methods of enhanced sampling of CVs based on MD simulations that are directly biased on those CVs; that is, we focus on methods in which an investigator must identify the CVs of interest as an input to the calculation. We have chosen to limit discussion to two broad classes of biasing: those whose objective is direct computation of the gradient of the free energy ($\partial F/\partial z$) at local points throughout CV space, and those in which non-Boltzmann sampling with bias potentials is used to force exploration of otherwise hard-to-visit regions of CV space. The canonical methods in these two classes are *thermodynamic integration* and *umbrella sampling*, respectively, and a discussion of these two methods sets the stage for discussion of three relatively modern variants: the Adaptive-Biasing Force Algorithm [6], Temperature-Accelerated MD [7] and Metadynamics [8].

2.2. Gradient Methods: Blue-Moon Sampling, Adaptive-Biasing Force Algorithm, and Temperature-Accelerated Molecular Dynamics

2.2.1. Overview: Thermodynamic Integration

Naively, one way to have an MD system visit a hard-to-reach point z in CV space is simply to create a realization of the configuration x at that point (*i.e.*, such that $\theta(x) = z$). This is an inverse problem, since the number of degrees of freedom in x is usually much larger than in z . One way to perform this inversion is by introducing external forces that guide the configuration to the desired point from some easy-to-create initial state; both targeted MD [9] and steered MD [10] are ways to do this. Of course, one would like MD to explore CV space in the vicinity of z , so after creating the configuration x , one would just let it run. Unfortunately, this would likely result in the system drifting away from z rather quickly, and there would be no way from such calculations to estimate the likelihood of observing an unbiased long MD simulation visit z . However, there is information in the fact that the system drifts away; if one knows *on average* which direction and how strongly the system would like to move if initialized at z ,

this would be a measure of negative gradient of the free energy, $-(\partial F/\partial z)$, or the “mean force”. We have then a glimpse of a three-step method to compute F (i.e., the statistics of CVs) over a meaningfully broad extent of CV space:

- (1) visit a select number of local points in that space, and at each one,
- (2) compute the mean force, then
- (3) use numerical integration to reconstruct F from these local mean forces; formally expressed as

$$F(z) - F(z_0) = \int_{z_0}^z \left(\frac{\partial F}{\partial z} \right) dz \quad (3)$$

Inspired by Kirkwood’s original suggestion involving switching parameters [11], such an approach is generally referred to as “thermodynamic integration” or TI. TI allows us to reconstruct the statistical weights of any point in CV space by accumulating information on the gradients of free energy at selected points.

2.2.2. Blue-Moon Sampling

The discussion so far leaves open the correct way to compute the local free-energy gradients. A gradient is a local quantity, so a natural choice is to compute it from an MD simulation localized at a point in CV space by a constraint. Consider a long MD simulation with a holonomic constraint fixing the system at the point z . Uniform samples from this constrained trajectory $\mathbf{x}(t)$ then represent an ensemble at fixed z over which the averaging needed to convert gradients in potential energy to gradients in free energy could be done. However, this constrained ensemble has the undesired property that the velocities $\dot{\boldsymbol{\theta}}(\mathbf{x})$ are zero. This is a bit problematic because virtually none of the samples plucked from a long unconstrained MD simulation (as is implied by Equation (1)), would have $\dot{\boldsymbol{\theta}} = 0$, and $\dot{\boldsymbol{\theta}} = 0$ acts as a set of M unphysical constraints on the system velocities $\dot{\mathbf{x}}$, since $\dot{\theta}_j = \sum_i (\partial \theta_j / \partial x_i) \dot{x}_i$. Probably the best-known example of a method to correct for this bias is the so-called “blue-moon” sampling method [12–15] or the constrained ensemble method [16,17]. The essence of the method is a decomposition of free energy gradients into components along the CV gradients and thermal components orthogonal to them:

$$\frac{\partial F}{\partial z_j} = \langle \mathbf{b}_j(\mathbf{x}) \cdot \nabla V(\mathbf{x}) - k_B T \nabla \cdot \mathbf{b}_j(\mathbf{x}) \rangle_{\boldsymbol{\theta}(\mathbf{x})=z} \quad (4)$$

where $\langle \cdot \rangle_{\boldsymbol{\theta}(\mathbf{x})=z}$ denotes averaging across samples drawn uniformly from the MD simulation constrained at $\boldsymbol{\theta}(\mathbf{x}) = z$, and the $\mathbf{b}_j(\mathbf{x})$ is the vector field orthogonal to the gradients of every component k of $\boldsymbol{\theta}$ for $k \neq j$:

$$\mathbf{b}_j(\mathbf{x}) \cdot \nabla \theta_k(\mathbf{x}) = \delta_{jk} \quad (5)$$

where δ_{jk} is the Kroenecker delta. (For brevity, we have omitted the consideration of holonomic constraints other than that on the CV; the reader is referred to the paper by Ciccotti *et al.* for details [15].) The vector fields $\mathbf{b}_j$ for each θ_j can be constructed by orthogonalization. The first term in the angle brackets in Equation (4) implements the chain rule one needs to account for how energy V changes with z through all the ways z can change with $\mathbf{x}$. The second term corrects for the thermal bias imposed by the constraint.

Although nowhere near exhaustive, below is a listing of common types of problems to which blue-moon sampling has been applied with some representative examples:

- (1) sampling conformations of small flexible molecules and peptides [18–20];
- (2) environmental effects on covalent bond formation/breaking (usually in combination with *ab initio* MD) [21–27];
- (3) solvation and non-covalent binding of small molecules in solvent [28–32];
- (4) protein dimerization [33,34].

2.2.3. The Adaptive Biasing Force Algorithm

The blue-moon approach requires multiple independent constrained MD simulations to cover the region of CV space in which one wants internal statistics. The care taken in choosing these quadrature points can often dictate the accuracy of the resulting free energy reconstruction. It is therefore sometimes advantageous to consider ways to avoid having to choose such points ahead of time, and adaptive methods attempt to address this problem. One example is the adaptive-biasing force (ABF) algorithm of Darve *et al.* [6,35]. The essence of ABF is two-fold: (1) recognition that external bias forces of the form $\nabla_{\mathbf{x}}\theta_j(\partial F/\partial z_j)$ for $j = 1, \dots, M$ exactly oppose mean forces and should lead to more uniform sampling of CV space; and (2) that these bias forces can be converged upon adaptively during a single unconstrained MD simulation.

The first of those two ideas is motivated by the fact that “forces” that keep normal MD simulations effectively confined to free energy minima are mean forces on the collective variables projected onto the atomic coordinates, and balancing those forces against their exact opposite should allow for thermal motion to take the system out of those minima. The second idea is a bit more subtle; after all, in a running MD simulation with no CV constraints, the constrained ensemble expression for the mean force (Equation (4)) does not directly apply, because a constrained ensemble is not what is being sampled. However, Darve *et al.* showed how to relate these ensembles so that the samples generated in the MD simulation could be used to build mean forces [35]. Further, they showed using a clever choice of the fields of Equation (4) an equivalence between (i) the spatial gradients needed to compute forces, and (ii) time-derivatives of the CVs [6]:

$$\frac{\partial F}{\partial z_i} = -k_B T \left\langle \frac{d}{dt} \left(M_\theta \frac{d\theta_i}{dt} \right) \right\rangle_{\theta=z} \quad (6)$$

where M_θ is the transformed mass matrix given by

$$M_\theta^{-1} = J_\theta M^{-1} J_\theta \quad (7)$$

where J_θ is the $M \times 3N$ matrix with elements $\partial\theta_i/\partial x_j$ ($i = 1 \dots M$, $j = 1 \dots 3N$), and M is the diagonal matrix of atomic masses. Equation (7) is the result of a particular choice for the fields $\mathbf{b}_j(\mathbf{x})$. This reformulation of the instantaneous mean forces computed on-the-fly makes ABF exceptionally easy to implement in most modern MD packages. Darve *et al.* present a clear demonstration of the ABF algorithm in a pseudocode [6] that attests to this fact.

ABF has found rather wide application in CV-based free energy calculations in recent years. Below is a representative sample of some types of problems subjected to ABF calculations in the recent literature:

- (1) Peptide backbone angle sampling [36,37];
- (2) Nucleoside [38], protein [39] and fullerene [40,41] insertion into a lipid bilayer;
- (3) Interactions of small molecules with polymers in water [42,43];
- (4) Molecule/ion transport through protein complexes [44–47] and DNA superstructures [48];
- (5) Calculation of octanol-water partition coefficients [49,50];
- (6) Large-scale protein conformational changes [51];
- (7) Protein-nanotube [52] and nanotube-nanotube [53] association.

2.2.4. Temperature-Accelerated Molecular Dynamics

Both blue-moon sampling and ABF are based on statistics in the constrained ensemble. However, estimation of mean forces need not only use this ensemble. One can instead relax the constraint and work with a “mollified” version of the free energy:

$$F_{\kappa}(\mathbf{z}) = -k_B T \ln \langle \delta_{\kappa} [\boldsymbol{\theta}(\mathbf{x}) - \mathbf{z}] \rangle \quad (8)$$

where δ_{κ} refers to the Gaussian (or “mollified delta function”):

$$\delta_{\kappa} = \sqrt{\frac{\beta\kappa}{2\pi}} \exp \left[-\frac{1}{2} \beta\kappa |\boldsymbol{\theta}(\mathbf{x}) - \mathbf{z}|^2 \right] \quad (9)$$

where β is just shorthand for $1/k_B T$. Since $\lim_{\beta\kappa \rightarrow \infty} \delta_{\kappa} = \delta$, we know that $\lim_{\beta\kappa \rightarrow \infty} F_{\kappa} = F$. One way to view this Gaussian is that it “smoothes out” the true free energy to a tunable degree; the factor $1/\sqrt{\beta\kappa}$ is a length-scale in CV space below which details are smeared.

Because the Gaussian has continuous gradients, it can be used directly in an MD simulation. Suppose we have a CV space $\boldsymbol{\theta}(\mathbf{x})$, and we extend our MD system to include variables $\mathbf{z}$ such that the combined set $(\mathbf{x}, \mathbf{z})$ obeys the following extended potential:

$$U(\mathbf{x}, \mathbf{z}) = V(\mathbf{x}) + \sum_{j=1}^M \frac{1}{2} \kappa |\theta_j(\mathbf{x}) - z_j|^2 \quad (10)$$

where $V(\mathbf{x})$ is the interatomic potential, and κ is a constant. Clearly, if we fix $\mathbf{z}$, then the resulting free energy is to within an additive constant the mollified free energy of Equation (8). (The additive constant is related to the prefactor of the mollified delta function and has nothing to do with the number of CVs.) Further, we can directly express the gradient of this mollified free energy with respect to $\mathbf{z}$: [54]

$$\nabla_{\mathbf{z}} F_{\kappa} = -\langle \kappa [\boldsymbol{\theta}(\mathbf{x}) - \mathbf{z}] \rangle \quad (11)$$

This suggests that, instead of using constrained ensemble MD to accumulate mean forces, we could work in the *restrained* ensemble and get very good approximations to the mean force. By “restrained”, we refer to the fact that the term giving rise to the mollified delta function in the configurational integral is essentially a harmonic restraining potential with a “spring constant” κ . In this restrained-ensemble approach, no velocities are held fixed, and the larger we choose κ the more closely we can approximate the true free energy. Notice however that large values of κ could lead to numerical instabilities in integrating equations of motion, and a balance should be found. (In practice, we have found that for

CVs with dimensions of length, values of κ less than about 1,000 kcal/mol/Å² can be stably handled, and values of around 100 kcal/mol/Å² are typically adequate.)

Temperature-accelerated MD (TAMD) [7] takes advantage of the restrained-ensemble approach to directly evolve the variables $\mathbf{z}$ in such a way to accelerate the sampling of CV space. First, consider how the atomic variables $\mathbf{x}$ evolve under the extended potential (assuming Langevin dynamics):

$$m_i \ddot{x}_i = -\frac{\partial V(\mathbf{x})}{\partial x_i} - \kappa \sum_{j=1}^m [\theta_j(\mathbf{x}) - z_j] \frac{\partial \theta_j(\mathbf{x})}{\partial x_i} - \gamma m_i \dot{x}_i + \eta_i(t; \beta) \quad (12)$$

Here, m_i is the mass of x_i , γ is the friction coefficient for the Langevin thermostat, and η is the thermostat white noise satisfying the fluctuation-dissipation theorem at physical temperature β^{-1} :

$$\langle \eta_i(t; \beta) \eta_j(t'; \beta) \rangle = \beta^{-1} \gamma m_i \delta_{ij} \delta(t - t') \quad (13)$$

Key to TAMD is that the $\mathbf{z}$ are treated as slow variables that evolve according to their own equations of motion, which here we take as diffusive (though other choices are possible [7]):

$$\bar{\gamma} \bar{m}_j \dot{z}_j = \kappa [\theta_j(\mathbf{x}) - z_j] + \xi_j(t; \bar{\beta}) \quad (14)$$

Here, $\bar{\gamma}$ is a fictitious friction, $\bar{m}_j$ is a mass, and the first term on the right-hand side represents the instantaneous force on variable z_j , and the second term represents thermal noise at the fictitious thermal energy $\bar{\beta}^{-1} \neq \beta^{-1}$.

The advantage of TAMD is that if (1) $\bar{\gamma}$ is chosen sufficiently large so as to guarantee that the slow variables indeed evolve slowly relative to the fundamental variables; and (2) κ is sufficiently large such that $\theta(\mathbf{x}(t)) \approx \mathbf{z}(t)$ at any given time, then the force acting on $\mathbf{z}$ is approximately equal to minus the gradient of the free energy (Equation (11)) [7]. This is because the MD integration repeatedly samples $\kappa [\theta(\mathbf{x}) - \mathbf{z}]$ for an essentially fixed (but actually very slowly moving) $\mathbf{z}$, so $\mathbf{z}$ evolution effectively feels these samples as a mean force. In other words, the dynamics of $\mathbf{z}(t)$ is effectively

$$\bar{\gamma} \bar{m}_j \dot{z}_j = -\frac{\partial F(\mathbf{z})}{\partial z_j} + \xi_j(t; \bar{\beta}) \quad (15)$$

This shows that the $\mathbf{z}$ -dynamics describes an equilibrium constant-temperature ensemble at *fictitious* temperature $\bar{\beta}^{-1}$ acted on by the “potential” $F(\mathbf{z})$, which is the free energy evaluated at the *physical* temperature β^{-1} . That is, under TAMD, $\mathbf{z}$ conforms to a probability distribution of the form $\exp[-\bar{\beta}F(\mathbf{z}; \beta)]$, whereas under normal MD it would conform to $\exp[-\beta F(\mathbf{z}; \beta)]$. The all-atom MD simulation (at β) simply serves to approximate the *local gradients* of $F(\mathbf{z})$. Sampling is enhanced by taking $\bar{\beta}^{-1} > \beta^{-1}$, which has the effect of attenuating the ruggedness of F . TAMD therefore can accelerate a trajectory $\mathbf{z}(t)$ through CV space by increasing the likelihood of visiting points with relatively low physical Boltzmann factors. This borrows directly from the main idea of adiabatic free-energy dynamics [55] (AFED), in that one deliberately makes some variables hot (to overcome barriers) but slow (to keep them adiabatically separated from all other variables). In TAMD, however, the use of the mollified free energy means no cumbersome variable transformations are required. (The authors of AFED refer to TAMD as “driven”-AFED, or d-AFED [56].) It is also worth mentioning in this review that TAMD borrows heavily from an early version of metadynamics [57], which was formulated

as a way to evolve the auxiliary variables z on a mollified free energy. However, unlike metadynamics (which we discuss below in Section 2.3.3.), there is no history-dependent bias in TAMD.

Unlike TI, ABF, and the methods of umbrella sampling and metadynamics discussed in the next section, TAMD is not a method for direct calculation of the free energy. Rather, it is a way to overcome free energy barriers in a chosen CV space quickly without visiting irrelevant regions of CV space. (However, we discuss briefly a method in Section 4.2.2. in which TAMD gradients are used in a spirit similar to ABF to reconstruct a free energy.) That is, we consider TAMD a way to efficiently explore relevant regions CV space that are practically inaccessible to standard MD simulation. It is also worth pointing out that, unlike ABF, TAMD does not operate by opposing the natural gradients in free energy, but rather by using them to guide accelerated sampling. ABF can only use forces in locations in CV space the trajectory has visited, which means nothing opposes the trajectory going to regions of very high free energy. However, under TAMD, an acceleration of $\bar{\beta}^{-1} = 6$ kcal/mol on the CVs will greatly accelerate transitions over barriers of 6–12 kcal/mol, but will still not (in theory) accelerate excursions to regions requiring climbs of hundreds of kcal/mol. TAMD and ABF have in common the ability to handle rather high-dimensional CVs.

Although it was presented theoretically in 2006 [7], TAMD was not applied directly to large-scale MD until much later [58]. Since then, there has been growing interest in using TAMD in a variety of applications requiring enhanced sampling:

- (1) TAMD-enhanced flexible fitting of all-atom protein and RNA models into low-resolution electron microscopy density maps [59,60];
- (2) Large-scale (interdomain) protein conformational sampling [58,61,62];
- (3) Loop conformational sampling in proteins [63];
- (4) Mapping of diffusion pathways for small molecules in globular proteins [64,65];
- (5) Vacancy diffusion [66];
- (6) Conformational sampling and packing in dense polymer systems [67].

Finally, we mention briefly that TAMD can be used as a quick way to generate trajectories from which samples can be drawn for subsequent mean-force estimation for later reconstruction of a multidimensional free energy; this is the essence of the single-sweep method [68], which is an efficient means of computing multidimensional free energies. Rather than using straight numerical TI, single sweep posits the free energy as a basis function expansion and uses standard optimization methods to find the expansion coefficients that best reproduce the measured mean forces. Single-sweep has been used to map diffusion pathways of CO and H₂O in myoglobin [64,65].

2.3. Bias Potential Methods: Umbrella Sampling and Metadynamics

2.3.1. Overview: Non-Boltzmann Sampling

In the previous section, we considered methods that achieve enhanced sampling by using mean forces: in TI, these are integrated to reconstruct a free energy; in ABF, these are built on-the-fly to drive uniform CV sampling; and in TAMD, these are used on-the-fly to guide accelerated evolution of CVs. In this

section, we consider methods that achieve enhanced sampling by means of controlled bias potentials. As a class, we refer to these as *non-Boltzmann sampling* methods.

Non-Boltzmann sampling is generally a way to derive statistics on a system whose energetics differ from the energetics used to perform the sampling. Imagine we have an MD system with bare interatomic potential $V(\mathbf{x})$, and we add a bias $\Delta V(\mathbf{x})$ to arrive at a biased total potential:

$$V_b(\mathbf{x}) = V(\mathbf{x}) + \Delta V(\mathbf{x}) \quad (16)$$

The statistics of the CVs on this biased potential are then given as

$$\begin{aligned} P_b(\mathbf{z}) &= \frac{\int d\mathbf{x} e^{-\beta V_0(\mathbf{x})} e^{-\beta \Delta V(\mathbf{x})} \delta[\boldsymbol{\theta}(\mathbf{x}) - \mathbf{z}]}{\int d\mathbf{x} e^{-\beta V_0(\mathbf{x})} e^{-\beta \Delta V(\mathbf{x})}} \\ &= \frac{\int d\mathbf{x} e^{-\beta V_0(\mathbf{x})} e^{-\beta \Delta V} \delta[\boldsymbol{\theta}(\mathbf{x}) - \mathbf{z}]}{\int d\mathbf{x} e^{-\beta V_0(\mathbf{x})}} \frac{\int d\mathbf{x} e^{-\beta V_0(\mathbf{x})}}{\int d\mathbf{x} e^{-\beta V_0(\mathbf{x})} e^{-\beta \Delta V(\mathbf{x})}} \\ &= \frac{\langle e^{-\beta \Delta V(\mathbf{x})} \delta[\boldsymbol{\theta}(\mathbf{x}) - \mathbf{z}] \rangle}{\langle e^{-\beta \Delta V(\mathbf{x})} \rangle} \end{aligned} \quad (17)$$

where $\langle \cdot \rangle$ denotes ensemble averaging on the unbiased potential $V(\mathbf{x})$. Further, if we take the bias potential ΔV to be explicitly a function only of the CVs $\boldsymbol{\theta}$, then it becomes invariant in the averaging of the numerator thanks to the delta function, and we have

$$P_b(\mathbf{x}) = \frac{e^{-\beta \Delta V(\mathbf{z})} \langle \delta[\boldsymbol{\theta}(\mathbf{x}) - \mathbf{z}] \rangle}{\langle e^{-\beta \Delta V[\boldsymbol{\theta}(\mathbf{x})]} \rangle} \quad (18)$$

Finally, since the unbiased statistics are $P(\mathbf{z}) = \langle \delta[\boldsymbol{\theta}(\mathbf{x}) - \mathbf{z}] \rangle$, we arrive at

$$P(\mathbf{z}) = P_b(\mathbf{z}) e^{\beta \Delta V(\mathbf{z})} \langle e^{-\beta \Delta V[\boldsymbol{\theta}(\mathbf{x})]} \rangle \quad (19)$$

Taking samples from an ergodic MD simulation on the biased potential V_b , Equation (19) provides the recipe for reconstructing the statistics the CVs *would* present were they generated using the *unbiased* potential V . However, the probability $P(\mathbf{z})$ is implicit in this equation, because

$$\langle e^{-\beta \Delta V} \rangle = \int d\mathbf{z} P(\mathbf{z}) e^{-\beta \Delta V[\boldsymbol{\theta}(\mathbf{x})]} \quad (20)$$

This is not really a problem, since we can treat $\langle e^{-\beta \Delta V} \rangle$ as a constant we can get from normalizing $P_b(\mathbf{z}) e^{\beta \Delta V(\mathbf{z})}$.

How does one choose ΔV so as to enhance the sampling of CV space? Evidently, from the standpoint of non-Boltzmann sampling, the closer the bias potential is to the negative free energy $-F(\mathbf{z})$, the more uniform the sampling of CV space will be. To wit: if $\Delta V[\boldsymbol{\theta}(\mathbf{x})] = -F[\boldsymbol{\theta}(\mathbf{x})]$, then $e^{\beta \Delta V(\mathbf{z})} = e^{-\beta F(\mathbf{z})} = P(\mathbf{z})$, and Equation (19) can be inverted for P_b to yield

$$P_b(\mathbf{z}) = \frac{1}{\langle e^{\beta F(\mathbf{z})} \rangle} = \frac{1}{\int d\mathbf{z} P(\mathbf{z}) e^{\beta F(\mathbf{z})}} = \frac{1}{\int d\mathbf{z} e^{-\beta F} e^{\beta F}} = \frac{1}{\int d\mathbf{z}} \quad (21)$$

So we see that taking the bias potential to be the negative free energy makes all states z in CV space equiprobable. This is indeed the limit to which ABF strives by applying negative mean forces, for example [6].

We usually do not know the free energy ahead of time; if we did, we would already know the statistics of CV space and no enhanced sampling would be necessary. Moreover, perfectly uniform sampling of the entire CV space is usually far from necessary, since most CV spaces have many irrelevant regions that should be ignored. And in reference to the mean-force methods of the last section, uniform sampling is likely not necessary to achieve accurate mean force values; how good an estimate of ∇F is at some point z_0 should not depend on how well we sampled at some other point z_1 . Yet achieving uniform sampling is an idealization since, if we do, this means we know the free energy. We now consider two other biasing methods that aim for this ideal, either in relatively small regions of CV space using fixed biases, or over broader extents using adaptive biases.

2.3.2. Umbrella Sampling

Umbrella sampling is the standard way of using non-Boltzmann sampling to overcome free energy barriers. In its debut [69], umbrella sampling used a function $w(\mathbf{x})$ that weights hard-to-sample configurations, equivalent to adding a bias potential of the form

$$\Delta V(\mathbf{x}) = -k_B T \ln w(\mathbf{x}) \quad (22)$$

w is found by trial-and-error such that configurations that are easy to sample on the unbiased potential are still easy to sample; that is, w acts like an “umbrella” covering both the easy- and hard-to-sample regions of configuration space. Nearly always, w is an explicit function of the CVs, $w(\mathbf{x}) = W[\boldsymbol{\theta}(\mathbf{x})]$.

Coming up with the umbrella potential that would enable exploration of CV space with a single umbrella sampling simulation that takes the system far from its initial point is not straightforward. Akin to TI, it is therefore advantageous to combine results from several independent trajectories, each with its own umbrella potential that localizes it to a small volume of CV space that overlaps with nearby volumes. The most popular way to combine the statistics of such a set of independent umbrella sampling runs is the weighted-histogram analysis method (WHAM) [70].

To compute statistics of CV space using WHAM, one first chooses the points in CV space that define the little local neighborhoods, or “windows” to be sampled and chooses the bias potential used to localize the sampling. Not knowing how the free energy changes in CV space makes the first task somewhat challenging, since more densely packed windows are preferred in regions where the free energy changes rapidly; however, since the calculations are independent, more can be added later if needed. A convenient choice for the bias potential is a simple harmonic spring that tethers the trajectory to a reference point z_i in CV space:

$$\Delta V_i(\mathbf{x}) = \frac{1}{2} \kappa |\boldsymbol{\theta}(\mathbf{x}) - \mathbf{z}_i|^2 \quad (23)$$

which means the dynamics of the atomic variables $\mathbf{x}$ are identical to Equation (12) at fixed $\mathbf{z} = \mathbf{z}_i$. The points $\{\mathbf{z}_i\}$ and the value of κ (which may be point-dependent) must be chosen such that $\boldsymbol{\theta}[\mathbf{x}(t)]$ from any one window’s trajectory makes excursions into the window of each of its nearest neighbors in CV space.

Each window-restrained trajectory is directly histogrammed to yield apparent (*i.e.*, biased) statistics on θ ; let us call the biased probability in the i th window $P_{b,i}(z)$. Equation (19) again gives the recipe to reconstruct the unbiased statistics $P_i(z)$ for z in the window of z_i :

$$P_i(z) = P_{b,i}(z) e^{\frac{1}{2}\beta\kappa|z-z_i|^2} \left\langle e^{-\beta\frac{1}{2}\kappa|\theta(x)-z_i|^2} \right\rangle \quad (24)$$

We could use Equation (24) directly assuming the biased MD trajectory is ergodic, but we know that regions far from the reference point will be explored very rarely and thus their free energy would be estimated with large uncertainty. This means that, although we can use sampling to compute $P_{b,i}$ knowing it effectively vanishes outside the neighborhood of z_i , we cannot use sampling to compute $\left\langle e^{-\beta\frac{1}{2}\kappa|\theta(x)-z_i|^2} \right\rangle$.

WHAM solves this problem by renormalizing the probabilities in each window into a single composite probability. Where there is overlap among windows, WHAM renormalizes such that the statistical variance of the probability is minimal. That is, it treats the factor $\left\langle e^{-\beta\frac{1}{2}\kappa|\theta(x)-z_i|^2} \right\rangle$ as an undetermined constant C_i for each window, and solves for specific values such that the composite unbiased probability $P(z)$ is continuous across all overlap regions with minimal statistical error. An alternative to WHAM, termed “umbrella integration”, solves the problem of renormalization across windows by constructing the composite mean force [71,72].

The literature on umbrella sampling is vast (by simulation standards), so we present here a very condensed listing of some of its more recent application areas with representative citations:

- (1) Small molecule conformational sampling [73–76];
- (2) Protein-folding [77–79] and large-scale protein conformational sampling [80–83];
- (3) Protein-protein/peptide-peptide interactions [84–92];
- (4) DNA conformational changes [93] and DNA-DNA interactions [94–96];
- (5) Binding and association free-energies [97–107];
- (6) Adsorption on and permeation through lipid bilayers [108–117];
- (7) Adsorption onto inorganic surfaces/interfaces [118,119];
- (8) Water ionization [120,121];
- (9) Phase transitions [122,123];
- (10) Enzymatic mechanisms [124–132];
- (11) Molecule/ion transport through protein complexes [133–140] and other macromolecules [141,142].

2.3.3. Metadynamics

As already mentioned, one of the difficulties of the umbrella sampling method is the choice and construction of the bias potential. As we already saw with the relationship among TI, ABF, and TAMd, an adaptive method for building a bias potential in a running MD simulation may be advantageous. Metadynamics [8,143] represents just such a method.

Metadynamics is rooted in the original idea of “local elevation” [144], in which a supplemental bias potential is progressively grown in the dihedral space of a molecule to prevent it from remaining in one region of configuration space. However, at variance with metadynamics, local elevation does not

provide any means to reconstruct the unbiased free-energy landscape and as such it is mostly aimed at fast generation of plausible conformers.

In metadynamics, configurational variables $\mathbf{x}$ evolve in response to a biased total potential:

$$V(\mathbf{x}) = V_0(\mathbf{x}) + \Delta V(\mathbf{x}, t) \quad (25)$$

where V_0 is the bare interatomic potential and $\Delta V(\mathbf{x}, t)$ is a time-dependent bias potential. The key element of metadynamics is that the bias is built as a sum of Gaussian functions centered on the points in CV space already visited:

$$\Delta V[\boldsymbol{\theta}(\mathbf{x}), t] = w \sum_{\substack{t' = \tau_G, 2\tau_G, \dots \\ t' < t}} \exp\left(-\frac{|\boldsymbol{\theta}[\mathbf{x}(t)] - \boldsymbol{\theta}[\mathbf{x}(t')]|^2}{2\delta\boldsymbol{\theta}^2}\right) \quad (26)$$

Here, w is the height of each Gaussian, τ_G is the size of the time interval between successive Gaussian depositions, and $\delta\boldsymbol{\theta}$ is the Gaussian width. It has been first empirically [145] then analytically [146] demonstrated that in the limit in which the CVs evolve according to a Langevin dynamics, the bias indeed converges to the negative of the free energy, thus providing an optimal bias to enhance transition events. Multiple simulations can also be used to allow for a quicker filling of the free-energy landscape [147].

The difference between the metadynamics estimate of the free energy and the true free energy can be shown to be related to the diffusion coefficient of the collective variables and to the rate at which the bias is grown. A possible way to decrease this error as a simulation progresses is to decrease the growth rate of the bias. Well-tempered metadynamics [148] used an optimized schedule to decrease the deposition rate of bias by modulating the Gaussian height:

$$w = \omega_0 \tau_G e^{-\frac{\Delta V(\boldsymbol{\theta}, t)}{k_B \Delta T}} \quad (27)$$

Here, ω_0 is the initial “deposition rate”, measured Gaussian height per unit time, and ΔT is a parameter that controls the degree to which the biased trajectory makes excursions away from free-energy minima. It is possible to show that using well-tempered metadynamics the bias does not converge to the negative of the free-energy but to a fraction of it, thus resulting in sampling the CVs at an effectively higher temperature $T + \Delta T$, where normal metadynamics is recovered for $\Delta T \rightarrow \infty$. We notice that other deposition schedules can be used aimed, e.g., at maximizing the number of round-trips in the CV space [149]. Importantly, it is possible to recover equilibrium Boltzmann statistics of *unbiased* collective variables from samples drawn throughout a well-tempered metadynamics trajectory [150]; it does not seem clear that one can do this from an ABF trajectory. Finally, it is possible to tune the shape of the Gaussians on the fly using schemes based on the geometric compression of the phase space or on the variance of the CVs [151].

In the well-tempered ensemble, the parameter ΔT can be used to tune the size of the explored region, in a fashion similar to the fictitious temperature in TAMd. So both TAMd and well-tempered metadynamics can be used to explore *relevant* regions of CV space while surmounting *relevant* free energy barriers. However, there are important distinctions between the two methods. First, the main source of error in TAMd rests with how well mean-forces are approximated, and adiabatic separation,

realizable only when the auxiliary variables z never move, is the only way to guarantee they are perfectly accurate. In practical application, TAMD never achieves perfect adiabatic separation. In contrast, because the deposition rate of decreases as a well-tempered trajectory progresses, errors related to poor adiabatic separation are progressively damped. Second, as already mentioned, TAMD alone cannot report the free energy, but it also is therefore not practically limited by the dimensionality of CV space; multicomponent gradients are just as accurately calculated in TAMD as are single-component gradients. Metadynamics, as a histogram-filling method, must exhaustively sample a finite region around any point to know the free energy and its gradients are correct, which can sometimes limit its utility.

Metadynamics is a powerful method whose popularity continues to grow. In either its original formulation or in more recent variants, metadynamics has been employed successfully in several fields, some of which we point out below with some representative examples:

- (1) Chemical reactions [57,152];
- (2) Peptide backbone angle sampling [153–155];
- (3) Protein folding [156–159];
- (4) Protein aggregation [160];
- (5) Molecular docking [161–163] ;
- (6) Conformational rearrangement of proteins [164];
- (7) Crystal structure prediction [165];
- (8) Nucleation and crystal growth [166,167];
- (9) and proton diffusion [168].

2.4. Some Comments on Collective Variables

2.4.1. The Physical Fidelity of CV-Spaces

Given a potential $V(x)$, any multidimensional CV $\theta(x)$ has a mathematically determined free energy $F(z)$, and in principle the free-energy methods we describe here (and others) can use and/or compute it. However, this does not guarantee that F is meaningful, and a poor choice for $\theta(x)$ can render the results of even the most sophisticated free-energy methods useless for understanding the nature of actual metastable states and the transitions among them. This puts two major requirements on any CV space:

- (1) Metastable states and transition states must be unambiguously identified as *energetically* separate regions in CV space.
- (2) The CV space must not contain hidden barriers.

The first of these may seem obvious: CVs are chosen to provide a low-dimensional description of some important process, say a conformational change or a chemical reaction or a binding event, and one can not describe a process without being able to discriminate states. However, it is not always easy to find CVs that do this. Even given representative configurations of two distinct metastable states, standard MD from these two different initial configurations may sample partially overlapping regions of CV space, making ambiguous the assignation of an arbitrary configuration to a state. It may be in this case that the two representative configurations actually belong to the same state, or that if there are two

states, that no matter what CV space is overlaid, the barrier separating them is so small that, on MD timescales, they can be considered rapidly exchanging substates of some larger state.

However, a third possibility exists: the two MD simulations mentioned above may in fact represent very different states. The overlap might just be an artifact of neglecting to include one or more CVs that are truly necessary to distinguish those states. If there is a significant free energy barrier along this neglected variable, an MD simulation will not cross it, yet may still sample regions in CV space also sampled by an MD simulation launched from the other side of this hidden barrier. And it is even worse: if TI or umbrella sampling is used along a pathway in CV space that neglects an important variable, the free-energy barriers along that pathway might be totally meaningless.

Hidden barriers can be a significant problem in CV-based free-energy calculations. Generally speaking, one only learns of a hidden barrier after postulating its existence and testing it with a new calculation. Detecting them is not straightforward and often involves a good deal of CV space exploration. Methods such as TAMD and well-tempered metadynamics offer this capability, but much more work could be done in the automated detection of hidden barriers and the “right” CVs (e.g., [169–171]).

An obvious way of reducing the likelihood of hidden barriers is to use increase the dimensionality of CV space. TAMD is well-suited to this because it is a gradient method, but standard metadynamics, because it is a histogram-filling method, is not. A recent variant of metadynamics termed “reconnaissance metadynamics” [172] does have the capability of handling high-dimensional CV spaces. In reconnaissance metadynamics, bias potential kernels are deposited at the CV space points identified as centers of clusters detected and measured by an on-the-fly clusterization scheme. These kernels are hyperspherically symmetric but grow as cluster sizes grow and are able to push a system out of a CV space basin to discover other basins. As such, reconnaissance metadynamics is an automated way of identifying free-energy minima in high-dimensional CV spaces. It has been applied the identification of configurations of small clusters of molecules [173] and identification of protein-ligand binding poses [162].

2.4.2. Some Common and Emerging Types of CVs

There are very few “best practices” codified for choosing CVs for any given system. Most CVs are developed ad hoc based on the processes that investigators would like to study, for instance, center-of-mass distance between two molecules for studying binding/unbinding, or torsion angles for studying conformational changes, or number of contacts for studying order-disorder transitions. Cartesian coordinates of centers of mass of groups of atoms are also often used as CVs, as they are functions of these coordinates.

The potential energy $V(\mathbf{x})$ is also an example of a 1-D CV, and there have been several examples of using it in CV-based enhanced sampling methods, such as umbrella sampling [174], metadynamics [175] well-tempered metadynamics [176]. In a recent work based on steered MD, it has been shown that also relevant reductions of the potential energy (e.g., the electrostatic interaction free-energy) can be used as effective CVs [177]. The basic rationale for enhanced sampling of V is that states with higher potential energy often correspond to transition states, and one need make no assumptions about precise physical

mechanisms. Key to its successful use as a CV, as it is for any CV, is a proper accounting for its entropy; *i.e.*, the classical density-of-states.

Coarse-graining of particle positions onto Eulerian fields was used early on in enhanced sampling [178]; here, the value of the field at any Cartesian point is a CV, and the entire field represents a very high-dimensional CV. This idea has been put to use recently in the “indirect umbrella sampling” method of Patel *et al.* [179] for computing free energies of solvation, and string method (Section 4.2.1.) calculations of lipid bilayer fusion [180]. In a similar vein, there have been recent attempts at variables designed to count the recurrency of groups of atoms positioned according to given templates, such as α -helices paired β -strands in proteins [181].

We finally mention the possibility of building collective variables based on set of frames which might be available from experimental data or generated by means of previous MD simulations. Some of these variables are based on the idea of computing the distances between the present configuration and a set of precomputed snapshots. These distances, here indicated with d_i , where i is the index of the snapshot, are then combined to obtain a coarse representation of the present configuration, which is then used as a CV. As an example, one might combine the distances as

$$s = \frac{\sum_i e^{-\lambda d_i}}{\sum_i e^{-\lambda d_i}} \quad (28)$$

If the parameter λ is properly chosen, this function returns a continuous interpolation between the indexes of the snapshots which are closer to the present conformation. If the snapshots are disposed along a putative path connecting two experimental structures, this CV can be used as a path CV to monitor and bias the progression along the path [182]. A nice feature of path CVs is that it is straightforward to also monitor the distance from the putative path. The standard way to do it is by looking at the distance from the closest reference snapshot, which can be approximately computed with the following continuous function:

$$z = -\lambda^{-1} \log \sum_i e^{-\lambda d_i} \quad (29)$$

This approach, modified to use internal coordinates, was used recently by Zinovjev *et al.* to study the aqueous phase reaction of pyruvate to salicylate, and in the CO bond-breaking/proton transfer in PchB [183].

A generalization to multidimensional paths (*i.e.*, sheets) can be obtained by assigning a generic vector v_i to each of the precomputed snapshots and computing its average [184]:

$$s = \frac{\sum_i e^{-\lambda d_i} v_i}{\sum_i e^{-\lambda d_i}} \quad (30)$$

3. Tempering Approaches

“Tempering” refers to a class of methods based on increasing the temperature of an MD system to overcome barriers. Tempering relies on the fact that according to the Arrhenius law the rate at which activated (barrier-crossing) events happen is strongly dependent on the temperature. Thus, an annealing procedure where the system is first heated and then cooled allows one to produce quickly samples which are largely uncorrelated. The root of all these ideas indeed lies in the simulated annealing procedure [185], a well-known method successfully used in many optimization problems.

3.1. Simulated Tempering

Simulated annealing is a form of Markov-chain Monte Carlo sampling where the temperature is artificially modified during the simulation. In particular, sampling is initially done at a temperature high enough that the simulation can easily overcome high free-energy barriers. Then, the temperature is decreased as the simulation proceeds, thus smoothly bringing the simulation to a local energy minimum. In simulated annealing, a critical parameter is the cooling speed. Indeed, the probability to reach the global minimum grows as this speed is decreased.

The search for the global minimum can be interpreted in the same way as sampling an energy landscape at zero temperature. One could thus imagine to use simulated annealing to generate conformations at, e.g., room temperature by slowly cooling conformations starting at high temperature. However, the resulting ensemble will strongly depend on the cooling speed, thus possibly providing a biased result. A better approach consists of the so-called simulated tempering methods [186]. Here, a discrete list of temperatures T_i , with $i \in 1 \dots N$ are chosen *a priori*, typically spanning a range going from the physical temperature of interest to a temperature which is high enough to overcome all relevant free energy barriers. (Note that we do not have to stipulate a CV-space in which those barriers live.) Then, the index i , which indicates at which temperature the system should be simulated, is evolved with time. Two kind of moves are possible: (a) normal evolution of the system at fixed temperature, which can be done with a usual Markov Chain Monte Carlo or molecular dynamics and (b) change of the index i at fixed atomic coordinates. It is easy to show that the latter can be performed as a Monte Carlo step with acceptance equal to

$$\alpha = \min \left(1, \frac{Z_j}{Z_i} e^{-\frac{U(x)}{k_B T_j} + \frac{U(x)}{k_B T_i}} \right) \quad (31)$$

where i and j are the indexes corresponding to the present temperature and the new one. The weights Z_i should be chosen so as to sample equivalently all the value of i . It must be noticed that also within molecular dynamics simulations only the potential energy usually appears in the acceptance. This is due to the fact that the velocities are typically scaled by a factor $\sqrt{\frac{T_j}{T_i}}$ upon acceptance. This scaling leads to a cancellation of the contribution to the acceptance coming from the kinetic energy. Ultimately, this is related to the fact that the ensemble of velocities is analytically known *a priori*, such that it is possible to adapt the velocities to the new temperature instantaneously.

Estimating these weights Z_i is nontrivial and typically requires a preliminary step. Moreover, if this estimate is poor the system could spend no time at the physical temperature, thus spoiling the result. Iterative algorithms for adjusting these weights have been proposed (see e.g., [187]). We also observe that since the temperature sets the typical value of the potential energy, an effect much similar to that of simulated tempering with adaptive weights can be obtained by performing a metadynamics simulation using the potential energy as a CV (Section 2.4.2.).

3.2. Parallel Tempering

A smart way to alleviate the issue of finding the correct weights is that of simulating several replicas at the same time [188,189]. Rather than changing the temperature of a single system, the defining move

proposal in parallel tempering consists of a coordinate swap between two T -replicas with acceptance probability

$$\alpha = \min \left(1, e^{\left(\frac{1}{k_B T_j} - \frac{1}{k_B T_i} \right) [U(\mathbf{x}_j) - U(\mathbf{x}_i)]} \right) \quad (32)$$

This method is the root of a class of techniques collectively known as “replica exchange” methods, and the latter name is often used as a synonymous of parallel tempering. Notably, within this framework it is not necessary to precompute a set of weights. Indeed, the equal time spent by each replica at each temperature is enforced by the constraint that only pairwise swaps are allowed. Moreover, parallel tempering has an additional advantage: since the replicas are weakly coupled and only interact when exchanges are attempted, they can be simulated on different computers without the need of a very fast interconnection (provided, of course, that a single replica is small enough to run on a single node).

The calculation of the acceptance is very cheap as it is based on the potential energy which is often computed alongside force evaluation. Thus, one could in theory exploit also a large number of virtual, rejected exchanges so as to enhance statistical sampling [190,191]. Since efficiency of parallel tempering simulation can deteriorate if the stride between subsequent exchanges is too large [192,193], a typical recipe is to choose this stride as small as possible, with the only limitation of avoiding extra costs due to replica synchronization. One can push this idea further and implement asynchronous versions of parallel tempering, where overhead related to exchanges is minimized [193,194]. One should be however aware that, especially at high exchange rate, artifacts coming from e.g., the use of wrong thermostating schemes could spoil the results [195,196].

Parallel tempering is popular in simulations of protein conformational sampling [197,198], protein folding [189,199–203] and aggregation [204,205], due at least in part to the fact that one need not choose CVs to use it, and CVs for describing these processes are not always straightforward to determine.

3.3. Generalized Replica Exchange

The difference between the replicas is not restricted to be a change in temperature. Any control parameter can be changed, and even the expression of the Hamiltonian can be modified [206]. In the most general case every replica is simulated at a different temperature (and or pressure) and a different Hamiltonian, and the acceptance reads

$$\alpha = \min \left(1, \frac{e^{-\left(\frac{U_i(\mathbf{x}_j)}{k_B T_i} + \frac{U_j(\mathbf{x}_i)}{k_B T_j} \right)}}{e^{-\left(\frac{U_i(\mathbf{x}_i)}{k_B T_i} + \frac{U_j(\mathbf{x}_j)}{k_B T_j} \right)}} \right) \quad (33)$$

Several recipes for choosing the modified Hamiltonian have been proposed in the literature [207–219]. Among these, a notable idea is that of solute tempering [208,217] which is used for the simulation of solvated biomolecules. Here, only the Hamiltonian of the solute is modified. More precisely, one could notice that a scaling of the Hamiltonian by a factor λ is completely equivalent to a scaling of the temperature by a factor λ^{-1} . Hamiltonian scaling however can take advantage of the fact that the total energy of the system is an extensive property. Thus, one can limit the scaling to the portion of the system which is considered to be interesting and which has the relevant bottlenecks. With solute tempering, the solute energy is scaled whereas the solvent energy is left unchanged. This is equivalent to keeping the

solute at a high effective temperature and the solvent at the physical temperature. Since in the simulation of solvated molecules most of the atoms belong to the solvent, this turns in a much smaller modification to the explored ensemble when compared with parallel tempering. In spite of this, the effect on the solute resemble much that of increasing the physical temperature.

A sometimes-overlooked subtlety in solute tempering is the choice for the treatment of solvent-solute interactions. Indeed, whereas solute-solute interactions are scaled with a factor $\lambda < 1$ and solvent-solvent interactions are not scaled, any intermediate choice (scaling factor between λ and 1) could intuitively make sense for solvent-solute coupling. In the original formulation, the authors used a factor $(1 + \lambda)/2$ for the solute-solvent interaction. This choice however was later shown to be suboptimal [217,220], and refined to be $\sqrt{\lambda}$. This latter choice appears to be more physically sound, since it allows one to just simulate the biased replicas with a modified force-field. Indeed, if one scales the charges of the solute by a factor $\sqrt{\lambda}$, electrostatic interactions are changed by a factor λ for solute-solute coupling and $\sqrt{\lambda}$ for solute-solvent coupling. The same is true for Lennard-Jones terms, albeit in this case it depends on the specific combination rules used. Notably, the same rules for scaling were used in a previous work [209]. As a final remark, we point out that solute tempering can be also used in a serial manner *a la* simulated tempering, in a simulated solute tempering scheme [221].

3.4. General Comments

In general, the advantage of these tempering methods over straightforward sampling can be rationalized as follows. A simulation is evolved so as to sample a modified ensemble by e.g., raising temperature or artificially modifying the Hamiltonian. The change in the ensemble could be drastic, so that trying to extract canonical averages by reweighting from such a simulation would be pointless. For this reason, a ladder of intermediate ensembles is built, interpolating between the physical one (*i.e.*, room temperature, physical Hamiltonian) and the modified one. Then, transitions between consecutive steps in this ladder (or, in parallel schemes, coordinate swaps) are performed using a Monte Carlo scheme. Assuming that the dynamics of the most modified ensemble is ergodic, independent samples will be generated every time a new simulation reaches the highest step of the ladder. Thus, efficiency of these methods is often based on the evaluation of the round trip time required for a replica to traverse the entire ladder.

Tempering methods are thus relying on the ergodicity of the most modified ensemble. This assumption is not always correct. A very simple example is parallel tempering used to accelerate the sampling over an entropic barrier. Since the height of an entropic barrier grows with the temperature, in this conditions the barrier in the most modified ensembles are unaffected [222]. Moreover, since a lot of time is spent in sampling states in non-physical situations (e.g., high temperature), the overall computational efficiency could even be lower than that of straightforward sampling. Real applications are often in an intermediate situation, and usefulness of parallel tempering should be evaluated case by case.

The number of intermediate steps in the ladder can be shown to grow with the square root of the specific heat of the system in the case of parallel tempering simulations. No general relationship can be drawn in the case of Hamiltonian replica exchange, but one can expect approximately that the number

of replicas should be proportional to the square root of the number of degrees of freedom affected by the modification of the Hamiltonian. Thus, Hamiltonian replica exchange methods could be much more effective than simple parallel tempering as they allow the effort to be focused and the number of replicas to be minimized.

Parallel tempering has the advantage that all the replicas can be analyzed to obtain meaningful results, e.g., to predict the melting curve of a molecule. This procedure should be used with caution, especially with empirically parametrized potentials, which are often tuned to be realistic only at room temperature. On the other hand, Hamiltonian replica exchange often relies on unphysically modified ensembles which have no interest but for the fact that they increase ergodicity.

As a final note, we observe that data obtained at different temperature (or with modified Hamiltonians) could be combined to enhance statistics at the physical temperature [223]. However, the effectiveness of this data recycling is limited by the fact that high temperature replicas visit very rarely low energy conformations, thus decreasing the amount of additional information that can be extracted.

4. Combinations and Advanced Approaches

4.1. Combination of Tempering Methods and Biased Sampling

The algorithms presented in Section 3 and based on tempering are typically considered to be simpler to apply when compared with those discussed in Section 2 and based on biasing the sampling of selected collective variables. Indeed, by avoiding the problem of choosing collective variables which properly describe the reaction path, most of the burden of setting up a simulation is removed. However, this comes at a price: considering the computational cost, tempering methods are extremely expensive. This cost is related to the fact that they are able to accelerate all degrees of freedom to the same extent, without an *a priori* knowledge of the sampling bottlenecks. In this sense, Hamiltonian replica exchange methods are in an intermediate situation, since they are typically less expensive than parallel tempering but allow to embed part of the knowledge of the system in the simulation set up.

Because of the conceptual difference between tempering methods and CV-based methods, these approaches can be easily and efficiently combined. As an example, the combination of metadynamics and parallel tempering can be used to take advantage of the known bottlenecks with biased collective variables at the same time accelerating the overall sampling with parallel tempering [156]. In that work, the free energy landscape for the folding of a small hairpin was computed by biasing a small number of selected CVs (gyration radius and the number of hydrogen bonds). These CVs alone are not enough to describe folding, as can be easily shown by performing a metadynamics simulation using these CVs. However, the combination with parallel tempering allowed acceleration of all the degrees of freedom blindly and reversible folding of the hairpin. This combined approach also improves the results when compared with parallel tempering alone, since it accelerates exploration of phase-space. Moreover, since parallel tempering samples the unbiased canonical distribution, it is very difficult to use it to compute free-energy differences which are larger than a few $k_B T$. The metadynamics bias can be used to disfavor, e.g., the folded state so as to better estimate the free-energy difference between the folded and unfolded states.

It is also possible to combine metadynamics with the solute tempering method so as to decrease the number of required replicas and the computational cost [224]. As an alternative to solute tempering, metadynamics in the well-tempered ensemble can be effectively used to enhance the acceptance in parallel tempering simulations and to decrease the number of necessary replicas [176]. This combination of parallel tempering with well-tempered ensemble can be pushed further and combined with metadynamics on a few selected degrees of freedom [225]. As a final note, bias exchange metadynamics [226] combines metadynamics and replica exchange in a completely different spirit: every replica is run using a different CV, thus allowing many CVs to be tried at the same time. This technique has been successfully applied to several problems. For a recent review, we refer the reader to [227].

4.2. Some Methods Based on TAMD

4.2.1. String Method in Collective Variables

The string method is generally an approach to find pathways of minimal energy connecting two points in phase space [228]. When working in CVs, the string method is used to find minimal free-energy paths (MFEP's) [229]. String method calculations involve multiple replicas, each representing a point z_s in CV space at position s along a discretized string connecting two points of interest (reactant and product states, say). The forces on each replica's z_s are computed and their z_s 's updated, as in TAMD, with the addition of forces that act to keep the z 's equidistant along the string (so-called reparameterization forces):

$$\bar{\gamma}\dot{z}_j(s, t) = \sum_k \left[\tilde{M}_{jk}(\mathbf{x}(s, t)) \kappa[\theta_k(\mathbf{x}(s, t)) - z_k(s, t)] \right] + \eta_z(t) + \lambda(s, t) \frac{\partial z_j}{\partial s} \quad (34)$$

Here, $\tilde{M}_{jk}$ is the metric tensor mapping distances on the manifold of atomic coordinates to the manifold of CV space, η is thermal noise and $\lambda(s, t) \frac{\partial z_j}{\partial s}$ represents the reparameterization force tangent to the string that is sufficient to maintain equidistant images along the string. String method has been used to study activation of the insulin-receptor kinase [63], docking of insulin to its receptor [230], and myosin [231]. In these examples, the update of the string coordinates is done at a lower frequency than the atomic variables in each image.

In contrast, in the on-the-fly variant of string method in CVs, the friction on the z_s 's is set high enough to make the effective averaging of the forces approach the true mean forces, and the z updates occur in lockstep with the x updates of the MD system [232]. Just as in TAMD, the atomic variables obey an equation of motion like Equation (12) tethering them to the z_s . Stober and Abrams recently demonstrated an implementation of on-the-fly string method to study the thermodynamics of the normal-to-amyloidogenic transition of $\beta 2$ -microglobulin [233]. Unique in this approach was the construction of a single composite MD system containing 27 individual $\beta 2$ molecules restrained to points on $3 \times 3 \times 3$ grid inside a single large solvent box. Zinovjev *et al.* used a combination of the on-the-fly string method and of path-collective variables (see Equations (28) and (29)) in a quantum-mechanics/molecular-mechanics approach to study a methyltransferase reaction [234].

4.2.2. On-the-Fly Free Energy Parameterization

Because TAMD provides mean-force estimates as it is exploring CV space, it stands to reason that those mean forces could be used to compute a free energy. In contrast, in the single-sweep method [68], the TAMD forces are only used in the CV space exploration phase, not the free-energy calculation itself. Recently, Abrams and Vanden-Eijnden proposed a method for using TAMD directly to *parameterize* a free energy; that is, to determine the best set of some parameters λ on which a free energy of known functional form depends [235]:

$$F(z) = F(z; \lambda^*) \quad (35)$$

The approach, termed “on-the-fly free energy parameterization”, uses forces from a running TAMD simulation to progressively optimize λ using a time-averaged gradient error:

$$E(\lambda) = \frac{1}{2t} \int_0^t |\nabla_z F[z(s), \lambda(t)] + \kappa [\theta(x(s)) - z(s)]|^2 ds \quad (36)$$

If constructed so that F is linear in $\lambda = (\lambda_1, \lambda_2, \dots, \lambda_M)$, minimization of E can be expressed as a simple linear algebra problem

$$\sum_j A_{ij} \lambda_j = b_i, \quad i = 1, \dots, M \quad (37)$$

and the running TAMD simulation provides progressively better estimates of A and b until the λ converge. In the cited work, it was shown that this method is an efficient way to derive potentials of mean force between particles in coarse-grained molecular simulations as basis-function expansions. It is currently being investigated as a means to parameterize free energies associated with conformational changes of proteins.

Chen, Cuendet, and Tuckermann developed a very similar approach that in addition to parameterizing a free energy using d-AFED-computed gradients uses a metadynamics-like bias on the potential [236]. These authors demonstrated efficient reconstruction of the four-dimensional free-energy of vacuum alanine dipeptide with this approach.

5. Conclusions

In this review, we have summarized some of the current and emerging enhanced sampling methods that sit atop MD simulation. These have been broadly classified as methods that use collective variable biasing and methods that use tempering. CV biasing is a much more prevalent approach than tempering, due partially to the fact that it is perceived to be cheaper, since tempering simulations are really only useful for enhanced sampling of configuration space when run in parallel. CV-biasing also reflects the desire to rein in the complexity of all-atom simulations by projecting configurations into a much lower dimensional space. (Parallel tempering can be thought of as increasing the dimensionality of the system by a factor equal to the number of simulated replicas.) But the drawback of all CV-biasing approaches is the risk that the chosen CV space does not provide the most faithful representation of the true spectrum of metastable subensembles and the barriers that separate them. Guaranteeing that sampling of CV space is not stymied by hidden barriers must be of paramount concern in the continued evolution of such methods. For this reason, methods that specifically allow broad exploration of CV space, like TAMD

(which can handle large numbers of CVs) and well-tempered metadynamics will continue to be valuable. So too will parallel tempering because its broad sampling of configuration space can be used to inform the choice of better CVs. Accelerating development of combined CV-tempering methods bodes well for enhanced sampling generally.

Although some of these methods involve time-varying forces (ABF, TAMD, and metadynamics), all methods we've discussed have the underlying rationale of the equilibrium ensemble. TI uses the constrained ensemble, ABF and metadynamics ideally converge to an ensemble in which a bias erases free-energy variations, and TAMD samples an attenuated/mollified equilibrium ensemble. There is an entirely separate class of methods that inherently rely on *non-equilibrium* thermodynamics. We have not discussed at all the several free-energy methods based on non-equilibrium MD simulations; we refer interested readers to the article by Christoph Dellago and Gerhard Hummer in this issue.

Finally, we have also not really touched on any of the practical issues of implementing and using these methods in conjunction with modern MD packages (e.g., NAMD [237], LAMMPS [238], Gromacs [239], Amber [240], and CHARMM [241], to name a few). At least two packages (NAMD and CHARMM) have native support for collective variable biasing, and NAMD in particular offers both native ABF and a TcL-based interface which has been used to implement TAMD [58]. The native collective variable module for NAMD has been recently ported to LAMMPS [242]. Gromacs offers native support for parallel tempering. Generally speaking, however, modifying MD codes to handle CV-biasing and multiple replicas is not straightforward, since one would like access to the data structures that store coordinates and forces. A major help in this regard is the PLUMED package [243,244], which patches a variety of MD codes to enable users to use many of the techniques discussed here.

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Conflicts of Interest

The authors declare no conflict of interest.

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