Enhanced Sampling for Biomolecular Simulations

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Abstract. The use of computer simulations as "virtual microscopes" is limited by sampling difficulties that arise from the large dimensionality and the complex energy landscapes of biological systems leading to poor convergences already in folding simulations of single proteins. In this chapter, we discuss a few strategies to enhance sampling in bimolecular simulations, and present some recent applications.

1 Introduction

Proteins are crucial components of the molecular machinery in cells, responsible for transporting molecules, catalyzing biochemical reactions, or fighting infections. Despite the remarkable progress in experimental techniques for producing and characterizing proteins a detailed understanding of folding and interaction of proteins is still missing. Hence, there is a need for reliable computational tools that can complement experiments in describing protein folding and function from physical interactions within a protein, and between a protein and the surrounding environment. Such tools could lead to new insights into the molecular working of cells as needed in many medical and biotechnological applications. Shaw and co-workers [1] have demonstrated that it is possible to study reversible folding of small proteins in atomistic detail at the time scale observed in experiments. However, their study was based on specialized hardware, and the extensive usage of CPU is out of reach for most academic institutions. In addition, the size of proteins that can be studied with such brute-force approach is limited. This is because the complex form of the forces leads to a rough energy landscape with a vast number of local minima acting as traps, and as a result the computational requirements for sampling the energy landscape increase exponentially with size of the system [2].

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In principle one can think of two approaches to overcome these numerical difficulties. One is to utilize simplified or coarse-grained models since they lead by design to an energy landscape with reduced number of valleys. However, while such models allow a much faster evaluation of energy, the problem of poor sampling and slow convergence will likely reappear for sufficiently large proteins as roughness is an intrinsic characteristics of protein energy landscapes. The other approach to obtain sufficient sampling of the conformational space is the use of enhanced sampling techniques that can quickly find local minima but avoid trapping. Such methods will (flatten) the energy landscape by reducing barriers. While they will change the dynamics and therefore often do not allow to study directly the kinetics of protein folding, association, or aggregation, this is a small price to pay for faster and more accurate calculation of thermal averages and free energy landscapes.

This chapter is organized as follows: we start with a short review of a number of advanced simulation techniques before discussing shortcomings and open problems. Recent applications demonstrate what can be done when using these approaches on high-performance computing systems. We finish this short review with a summary and outlook.

2 Advanced Simulation Techniques

The sampling difficulties in protein simulations at physiological temperature are due to the roughness of the protein energy landscape where crossing of an energy barrier of height ΔE is suppressed by a factor $\propto \exp(-\Delta E/k_B T)$ (k_B is the Boltzmann constant and T is the temperature of the system). Hence, raising the temperature T makes it easier for a protein to cross energy barriers, but at the same time it becomes more difficult to find low energy configurations. Simulations at high temperature can induce thermal unfolding of a protein, which is sometimes interpreted as time - reversed folding [3, 4]. While this approach has been used in the past with some success [3, 4], it is not clear whether it is in general a valid approach. For instance, the C-fragment of TOP7 folds by a non-trivial pathway that involves caching of a N-terminal segment in an adjunct helix. Only when all other part of the proteins are folded and in place, the N-terminal segment unfolds and refolds to a strand that completes the final structure in a three-stranded sheet. Time-reversed unfolding trajectories at high temperature do not show the caching mechanism that governs folding of this protein. An interpretation of unfolding as time-reversed folding may be restricted to simple two-state folder and associated with a nucleation mechanism as observed, for instance, for CI2 [3, 4].

One possibility to ensure sampling of low-energy configurations and avoid trapping in local minima are improved updates that guide the simulation and/or allow for larger time steps in the integrator in molecular dynamics simulations, or collective moves in Monte Carlo. One example is hybrid Monte Carlo [5, 6] where a short molecular dynamics run provides a trial configuration, which is then accepted or rejected according to the Metropolis criterion. This allows a larger step size in the molecular dynamics trajectory as the Metropolis step corrects for the discretization

errors. Another example is the *Rugged Metropolis (RM)* which uses informations from a simulation at a higher temperature to bias a Monte Carlo simulation at a low temperature. Assume a range of temperatures

$$T_1 > T_2 > \ldots > T_r > \ldots > T_{f-1} > T_f \,. \tag{1}$$

Results from the simulation at the highest temperature, T_1, are used to construct an *estimator* of the probability density function

$$\overline{\rho}(x_1,\ldots,x_n;T_1)$$

that biases the simulation at T_2. In turn, this simulation provides a bias for the one at T_3, and iteratively continued down to T_f. Here, one uses the approximation

$$\overline{\rho}(x_1,\ldots,x_n;T_r) = \prod_{i=1}^{n} \overline{\rho}_i^1(x_i;T_r) \,, \tag{2}$$

where $\overline{\rho}_i^1(x_i;T_r)$ are estimators of reduced one-variable probability densities

$$\rho_i^1(x_i;T) = \int \prod_{j\neq i} dx_j \, \rho(x_1,\ldots,x_n;T) \,. \tag{3}$$

Recursively, the estimated probability density function

$$\overline{\rho}(x_1,\ldots,x_n;T_{r-1})$$

is generated as an approximation of $\rho(x_1,\ldots,x_n;T_r)$. The acceptance step in the (biased) Metropolis procedure at temperature T_r is now given by

$$P_{RM} = \min\left\{1, \frac{\exp(-\beta E')}{\exp(-\beta E)} \, \frac{\overline{\rho}(x_1,\ldots,x_n;T_{r-1})}{\overline{\rho}(x'_1,\ldots,x'_n;T_{r-1})}\right\} \tag{4}$$

Improved updates such as rugged Metropolis have been tested successfully in simulations of small peptides. While in general the gain in efficiency is not enough to make folding simulations of protein domains (usually consisting of 50–200 residues) feasible, they can be combined readily with the generalized-ensemble techniques described in the following sections further increasing their efficiency.

2.1 Generalized-Ensemble Techniques

2.1.1 Energy Landscape Paving

The idea behind all generalized-ensemble techniques can be seen most easily for the global optimization method energy landscape paving (ELP) [7] which relies on low-temperature Monte Carlo simulations with an effective energy:

$$w(\tilde{E}) = e^{-\tilde{E}/k_B T} \quad \text{with} \quad \tilde{E} = E + f(H(q,t)) \; . \tag{5}$$

Here, T is a (low) temperature and $f(H(q,t))$ is a function of the histogram $H(q,t)$ in a pre-chosen "order parameter" or "reaction coordinate" q. The weight of a local minimum state decreases the longer the system stays in that state until the local minimum is no longer favored, after which the system will again explore higher energies. We have evaluated the efficiency of ELP in simulations of the 20-residue *trp*-cage protein whose structure we could "predict" within a root-mean-square deviation (rmsd) of 1 Å [8]. Energy landscape paving allows also the possibility of zero-temperature simulations [8]. For $T \to 0$ only moves with $\Delta\tilde{E} \leq 0$ will be accepted. If one chooses: $\tilde{E} = E + cH(E,t)$, the acceptance criterion is given by:

$$\Delta E + c\Delta H(q,t) \leq 0 \leftrightarrow c\Delta H(q,t) \leq -\Delta E \tag{6}$$

where E is the "physical" energy. Hence, energy landscape paving can overcome even at $T = 0$ any energy barrier. The waiting time for such a move is proportional to the height of the barrier that needs to be crossed. The factor c sets the time scale, and in this sense the $T = 0$ form of ELP is parameter-free.

However, the weight factor is time dependent, and therefore ELP violates detailed balance. Hence, the method can not be used to calculate thermodynamic averages. Detailed balance is fulfilled only for $f(H(q,t)) = f(H(q))$ in which case ELP reduces to one of the *generalized-ensemble* methods [9] generating a random walk through order parameter space (energy, for instance), control parameter space (temperature), or model space (i.e. different energy functions).

2.1.2 Random Walks in Order Parameter Space

We first consider generalized-ensemble techniques that realize random walks in order parameter space leading to a broad distribution of a pre-chosen physical quantity. This allows one to sample both low and high energy states with sufficient probability. For simplicity only ensembles that lead to flat distributions in *one* variable will be considered. Extensions to higher dimensional are straightforward [10]. One of the earliest realization of this idea is *umbrella sampling* [11], but now more common is multicanonical sampling [12] and methods derived of it. The first application of these techniques to protein simulations can be found in Ref [13] where a Monte Carlo technique was used. Later, it was also adapted to molecular dynamics [14].

In multicanonical simulations configurations with energy E are assigned a weight $w(E)$ such that the distribution of energies

$$P_{mu}(E) \propto n(E) w_{mu}(E) = \text{const}, \tag{7}$$

where $n(E)$ is the spectral density. Since all energies appear with the equal probability, a free random walk in the energy space is enforced and the simulation can overcome *any* entrapment in one of the many local minima. For a wide range of temperatures it is now possible to obtain a canonical distribution by reweighting techniques [15]:

$$P_B(T,E) \propto P_{mu}(E)\, w_{mu}^{-1}(E)\, e^{-\beta E}\ , \tag{8}$$

since a large range of energies is sampled. This allows one to calculate the expectation value of any physical quantity $\mathcal{O}$ at temperature T by

$$< \mathcal{O} >_T \ = \frac{\int dE\ \mathcal{O}(E) P_B(T,E)}{\int dE\ P_B(T,E)}\ . \tag{9}$$

The drawback of multicanonical sampling is that the weights $w_{mu}(E) \propto n^{-1}(E)$ are not *a priori* known and one needs their estimates for a numerical simulation. Calculation of the weights is usually done by an iterative procedure [13, 16, 17]. For instance, the so-called Wang-Landau sampling [18] where the transition probability between two conformations with energy E_1 and E_2 is given by the ratio of the (time-dependent) estimators $n(E)$ of the density of states

$$p(E_1 \to E_2) = \min\left[\frac{n(E_1)}{n(E_2)}, 1\right]\ . \tag{10}$$

Each time an energy level is visited, the estimator is updated according to

$$n(E) \to n(E)\, f \tag{11}$$

where, initially, $n(E) = 1$ and $f = f_0 = e^1$. Once the desired energy range is covered, the factor f is refined,

$$f_1 = \sqrt{f},\ f_{n+1} = \sqrt{f_n}\ , \tag{12}$$

until some small value is reached.

In multicanonical simulations the computational effort increases with the number of residues like $\approx N^4$ (when measured in Metropolis updates) [19]. In general, the computational effort in simulations increases with $\approx X^2$ where X is the variable in which one wants a flat distribution. This is because generalized-ensemble simulations realize by construction of the ensemble a $1D$ random walk in the chosen quantity X. In the multicanonical algorithm the reaction coordinate X is the potential energy $X = E$. Since $E \propto N^2$ the above scaling relation for the computational effort $\approx N^4$ is recovered. Hence, multicanonical sampling is not always the optimal generalized-ensemble algorithm in protein simulations. A better scaling of the computer time with size of the molecule may be obtained by choosing more appropriate reaction coordinate for our ensemble than the energy.

This is the motivation behind the various other existing realizations of the generalized-ensemble approach. All aim at sampling a broad range of energies in order that the simulation will overcome energy barriers and allow escape from local minima. For instance, in Ref. [20] it was proposed that configurations are updated according to a special choice of the Tsallis generalized mechanics formalism [21] (the Tsallis parameter q is chosen as $q = 1 + 1/n_F$):

$$w(E) = \left(1 + \frac{\beta(E - E_0)}{n_F}\right)^{-n_F} . \tag{13}$$

Here E_0 is an estimator for the ground-state energy and n_F is the number of degrees of freedom of the system. The weight reduces in the low-energy region to the canonical Boltzmann weight $\exp(-\beta E)$. This is because $E - E_0 \to 0$ for $\beta \to 0$ leading to $\beta(E - E_0)/n_F \ll 1$. On the other hand, high-energy regions are no longer exponentially suppressed but only according to a power law, which enhances excursions to high-energy regions.

In stochastic tunneling [22]), conformations are weighted by $w(E) = \exp(f(E)/k_B T)$. Here, $f(E)$ is a nonlinear transformation of the potential energy onto the interval $[0,1]$ and T is a low temperature. The energy in the stochastic tunneling technique is transformed dynamically dependent on the simulation history. The transformation is designed so that the system is automatically cooled down near the local minima, and heated up at the high energy region allowing efficient tunneling through the barriers [22]. Such a transformation can be realized by

$$f(E) = e^{-(E-E_0)/n_F} , \tag{14}$$

where E_0 is again an estimate of the ground state and n_F is the number of degrees of freedom of the system. Note that the location of all minima is preserved. The efficiency of this algorithm for protein-folding simulations was demonstrated in Ref. [23]. As a broad range of energies is sampled, one can use again re-weighting techniques [15] to calculate thermodynamic quantities over a large range of temperatures. In contrast to other generalized-ensemble techniques, the weights are explicitly given. One needs only to find an estimator for the ground-state energy E_0 which is easier than the determination of weights for other generalized ensembles.

In the context of molecular dynamics the generalized-ensemble idea is utilized in the *metadynamics method* where gaussian-shaped repulsive potentials $U_{bias}(\mathbf{s},t) = \sum_{t_i} h \exp\left(-\frac{|\mathbf{s}-\mathbf{s}(t_i)|^2}{2w^2}\right)$ are added iteratively to the energy function. These potentials are centered at updated points $\mathbf{s}(t_i)$ of the reaction coordinates in order to discourage the system from revisiting the configurations [24]. The overall contribution from these auxiliary potentials flattens the underlying curvatures of the free energy wells, therefore leading to a random walk. The original free energy potentials are recovered by $-U_{bias}(\mathbf{s},t)$.

2.1.3 Random Walks in Control Parameter Space

Another way of generating a generalized ensemble is through enforcing in the simulation a random walk in a control parameter, most often temperature. For instance, in simulated tempering, temperature is treated as an independent dynamic variable [25] and is sampled uniformly by updating both temperature and configuration with a weight:

$$w_{ST}(T,E) = e^{-E/k_B T - g(T)} . \tag{15}$$

Here, the function $g(T)$ is chosen so that the probability distribution of temperature is given by

$$P_{ST}(T) = \int dE\, n(E)\, e^{-E/k_BT - g(T)} = \text{const.} \tag{16}$$

Physical quantities have to be sampled for each temperature point separately and expectation values at intermediate temperatures are calculated by reweighting techniques [15].

As with the previously discussed generalized-ensemble methods, the weight $w_{ST}(T,E)$ is not *a priori* known, since it requires knowledge of the parameters $g(T)$ and their estimator has to be calculated. It can be again obtained by an iterative procedure. In the simplest version the improved estimator for $g^{(i)}(T)$ for the i-th iteration is calculated from the histogram of *temperature* distribution $H_{ST}^{(i-1)}(T)$ of the preceding simulation as follows:

$$g^{(i)}(T) = g^{(i-1)}(T) + \log H_{ST}^{(i-1)}(T)\ . \tag{17}$$

In this procedure one uses that the histogram of the i-th iteration is given by

$$H_{ST}(T) = e^{-g_{i-1}(T)} Z_i(T)\ , \tag{18}$$

where $Z_i(T) = \int dE n(E) \exp(-E/k_BT)$ is an estimate for the canonical partition function at temperature T. Setting $exp(g_i(T)) = Z_i(T)$ leads to the iterative relationship of Eq. 17.

It is easy to see that the factor $g(T)$ drops out once one considers more than one copy of the system. This is the idea behind replica exchange method (or parallel tempering)[26], which was first applied to protein science in Ref. [27]. Assuming we have N *non-interacting* replicas of the molecule, each at a different temperature T_i, standard MC or MD moves are performed in parallel and independently at these N temperatures. At certain time points, conformational exchanges occur between neighboring temperatures T_i and T_{i+1}, and the exchange moves are accepted or rejected with probability

$$w(\mathbf{C}^{old} \to \mathbf{C}^{new}) = \min(1, \exp(-\beta_i E(C_j) - \beta_j E(C_i) + \beta_i E(C_i) + \beta_j E(C_j))) \tag{19}$$

$$= \min(1, \exp(\Delta\beta \Delta E)\ . \tag{20}$$

The result of the exchange of conformations is the faster convergence of the Markov chain than in regular canonical simulations since the resulting random walk in temperatures allows the configurations to move out of local minima and to cross energy barriers. Hence, the temperature distribution should be chosen such that any relevant energy barrier can be crossed at the highest temperature.

There is no clear consensus on the optimal frequency of exchange attempts. One opinion is that exchanges should be performed often, but no more often than the potential energy autocorrelation time [28, 29]. The other argument is that exchange moves should be attempted every few steps [30, 31]. It has been also suggested to use multiplexed layers of replicas (n layers, each with M temperatures). In this

multiplexed replica exchange method, replicas are exchanged both within and between layers [32]. This offers a way of using more computing units on massively parallel computers without the need of adding more temperatures.

Expectation values of a physical quantity A are calculated as usual according to:

$$\langle A \rangle_{T_i} = \frac{1}{MES} \sum_{k}^{MES} A(C_i(k)) \ , \tag{21}$$

where MES is the number of measurements taken for the i-th temperature. Values for intermediate temperatures are calculated using reweighting techniques [15]. Note that parallel tempering does not require Boltzmann weights. The method can be combined easily with *generalized-ensemble* techniques [27]. Obviously, the method is also not restricted to temperature but can be used with any control parameter, for instance, pH [33] or pressure.

2.1.4 Random Walks in Model Space

Finally, one can enhance sampling of low energy configurations also by performing a random walk through an ensemble of systems with altered energy functions. In that way, information is exchanged between varying stages of coarse graining or different local environments. This is the idea behind "model hopping" [34], "hamilton exchange method" [35], and related approaches. Consider, for instance, that the energy function can be separated into two terms: $E = E_A + aE_B$. As in parallel tempering, "model hopping" considers N non-interacting copies of the molecule, but adjacent copies are now exchanged with probability

$$w(\mathbf{C}^{old} \to \mathbf{C}^{new}) = \min(1, \exp\{-\beta \left[E_A(C_j) + a_i E_B(C_j) + E_A(C_i) + a_j E_B(C_i)\right. \tag{22}$$

$$\left. - E_A(C_i) - a_i E_B(C_i) - E_A(C_j) - a_j E_B(C_j)\right]\}) \tag{23}$$

Here, $\Delta a = a_j - a_i$ and $\Delta E_B = E_B(C_j) - E_B(C_i)$. Configurations perform a random walk on a ladder of models with $a_1 = 1 > a_2 > a_3 > .... > a_N$ that differ by the relative contributions of E_B to the total energy E of the molecule.

Take as an example the barriers in the energy landscape of proteins that arise from van der Waals repulsion between atoms that come too close. Assuming that such barriers are a main reason for slow sampling in protein simulations, we have considered a version of "model hopping" where the contributions from the van der Waals energy become successively smaller. While the "physical" system is on one side of the ladder (at $a_1 = 1$), the (non-physical) model on the other end of the ladder (at $a_N << 1$) allows in the extreme atoms to share the same position in space. As the protein "tunnels" through van der Waals energy barriers, sampling of low-energy configurations is enhanced in the "physical" model (at $a_1 = 1$). With this realization of "model hopping" we have "predicted " the structure of a 46-residue protein A in an all-atom simulation within a root mean square deviation (rmsd) of 3.2 Å [34].

Model Hopping also allows guiding a simulation by information obtained from homologous structures [36]. Usually, such spatial constraints introduce an additional

roughness into the energy landscape which often leads to extremely slow convergence of the simulation. This problem is circumvented in our approach through a random walk in an ensemble of replicas that differ by the strength of the constraints which are coupled to the system. We have demonstrated the usefulness of this approach on some examples of the CASP6 competition [36].

2.2 Advancing Generalized-Ensemble Techniques

While there has seen much progress in advancing the generalized-ensemble approach, folding simulations are still limited in their scope. Aggregation, oligomer assembly and intra-oligomer conformational rearrangements are examples of systems with a need for faster algorithms: the sampling process poses even for relatively simple systems such as polyglutamine repeats a formidable challenge [37, 38]. The importance and severity of the problem motivates our search for further methodological advances.

2.2.1 Improving the Efficiency of Generalized-Ensemble Sampling

The computational efficiency of replica-exchange techniques and generalized-ensemble is often worse than their theoretical optimum. The reason for this suboptimal efficiency is the bottlenecks and barriers that lead to slow relaxation. In parallel tempering convergence is evaluated by the frequency of statistically independent configurations at lowest temperature. A *lower* bound for this number is the rate of round-trips n_{rt} between the lowest and highest temperature, T_1 and T_N. We define $n_{up}(i)$*and* $n_{dn}(i)$ as the number of replicas at temperature T_i that came from T_1 (T_N). The fraction of replicas moving up is given by:

$$f_{up}(i) = \frac{n_{up}(i)}{n_{up}(i) + n_{dn}(i)} \tag{24}$$

and describes the probability of stationary flow between temperatures T_1 and T_N. Maximizing the number of round-trips n_{rt} results in a linear flow distribution [39]:

$$f_{up}^{opt}(i) = i/N \tag{25}$$

Explicit solvent simulations of proteins are dominated by the water molecules. As a result, the heat capacity C is constant, and the system can be approximated by a $D = 2C$ harmonic oscillator. Based on this approximation, one can find that the optimal temperature distribution is the one with the number of replicas given by

$$N^{opt} \approx 1 + 0.594\sqrt{C}\ln(T_{max}/T_{min}) \tag{26}$$

replicas, and the temperatures distributed according to

$$R_i^{opt} = T_{min}\left(\frac{T_{max}}{T_{min}}\right)^{\frac{i-1}{N-1}} ; \tag{27}$$

where T_{max} is the highest temperature, $T_{min}$is the lowest temperature. Both quantities have to be chosen in advance [40].

If the relaxation at a particular temperature is slower than hopping in temperature, the state space partitions into disjoint free energy basins forming a tree-like hierarchical network. Because of this *broken ergodicity* an optimized temperature distribution needs to be found iteratively [41]

$$\int_{T_1}^{T_j^k} \eta^{(\mathrm{opt})}(T)dT = j/N \,, \tag{28}$$

where $1 < j < N$, the two terminal temperatures T_1 and T_N are kept fixed, and

$$\eta^{(\mathrm{opt})}(T) = C' \sqrt{\frac{1}{\Delta T}\frac{df}{dT}} \,, \tag{29}$$

with the normalization constant C' chosen so that

$$\int_{T_1}^{T_N} \eta^{(\mathrm{opt})}(T)dT = 1 \,. \tag{30}$$

This will again lead to a linear flow distribution, but the acceptance probabilities are *not* any longer constant. One can also show that in the case of broken ergodicity weight optimization of flow through order parameter space (for instance, energy) leads to a distribution that is no longer flat [39, 41].

A direct measurement of the flow distribution is computationally costly as individual replicas have to cross the full ladder of nodes many times. Such "tunneling" events are especially rare in early stages of the control parameter optimization when round trip times are largest. For this reason, we have proposed to estimate the flow distribution from measurements of mean first passage times of replicas crossing only part of the ladder. In our simulations, this procedure led to temperature sets that are more stable upon iteration than those from flows measured directly [42].

Traditionally, temperature replica exchange method is implemented such that the exchanges have been synchronous and this has been a major limiting factor making it highly inefficient. This replica exchange synchronization of attempted moves strategy which results in wasted computation time as the periodic synchronization causes the overall simulation to run at the speed of the slowest processor and the centralized coordination step is not scalable to many processors. In asynchronous replica exchange, one attempts to escape this problem through performing replica exchange moves for pairs of replicas independently from the other replicas, thereby removing the need for processor synchronization found in conventional synchronous implementations [43]. Because it does not involve a centralized synchronization step, the algorithm is scalable to an arbitrary number of processors and it is not limited by the slowest processor. The method is suitable for integration in dynamical simulation environments, such as computational grids, in which processors dynamically join and leave the calculation [43].

2.2.2 Velocity-Rescaling Improved Replica Exchange Molecular Dynamics

In a molecular dynamic simulation, the energy

$$E(x,v) = E_{pot}(x) + E_{kin}(v) \quad \text{with} \quad E_{kin}(v) = \frac{1}{2}\sum_i m_i v_i^2 \tag{31}$$

is the sum of the potential energy E_{pot}, which depends only on the coordinates x, and the kinetic energy E_{kin} that is solely a function of the velocities v. Scaling all velocities by a factor r changes the kinetic energy by:

$$E_{kin}(rv) = r^2 E_{kin}(v)\ . \tag{32}$$

In standard replica exchange molecular dynamics this relation is used by scaling the velocities after a successful exchange with a factor

$$r_{(1,2)} = \sqrt{T_{(2,1)}/T_{(1,2)}}\ , \tag{33}$$

that depends on the temperatures T_1 and T_2 of the two replicas that are exchanged. The rescaling of the velocities leads to $v^{new}_{(1,2)} = v^{old}_{(2,1)}$, and therefore $\Delta E_{kin} = 0$. Hence, the probability for an exchange is given only by the difference of potential energies of the two replicas

$$w(1 \leftrightarrow 2) = \exp(\Delta\beta\Delta E_{pot}). \tag{34}$$

Microcanonical replica exchange simulations call for a different scaling [44, 45]. By definition of the ensemble, one has to assure that $\Delta E = 0$. Assuming $E_1 < E_2$, and scaling parameters r_1 and r_2 given by

$$\begin{aligned} r_{(1,2)} &= \sqrt{\frac{E_{(2,1)} - E_{pot}(\mathbf{x}_{1,2})}{E_{(1,2)} - E_{pot}(\mathbf{x}_{1,2})}} \\ &= \sqrt{\frac{E_{kin}(\mathbf{v}_{(2,1)}) \pm \Delta E_{pot}}{E_{kin}(\mathbf{v}_{1,2})}} \quad , \end{aligned} \tag{35}$$

two configurations are exchanged with probability one :

$$\begin{aligned} E_1(\mathbf{x}_1, \mathbf{v}_1) &= E_{pot}(\mathbf{x}_1) + E_{kin}(\mathbf{v}_1) \\ &= E_{pot}(\mathbf{x}_2) + r_2^2 E_{kin}(\mathbf{v}_2) \quad . \end{aligned} \tag{36}$$

and

$$\begin{aligned} E_2(\mathbf{x}_2, \mathbf{v}_2) &= E_{pot}(\mathbf{x}_2) + E_{kin}(\mathbf{v}_2) \\ &= E_{pot}(\mathbf{x}_1) + r_1^2 E_{kin}(\mathbf{v}_1) \end{aligned} \tag{37}$$

Such rejection-free moves are possible for $E_{pot}(\mathbf{x}_2) < E_1$, a restriction that does not violate detailed balance. Molecular dynamics time evolution between exchange moves ensures ergodicity. Hence, the sampling will lead for sufficiently long simulation times to the correct distribution:

$$P(E_{pot};E) \propto \Omega_{pot}(E_{pot}) E_{kin}^{n_f/2} \,, \tag{38}$$

where Ω is the density of states and n_f is the number of degrees of freedom.

The above scaling leading to rejection-free sampling has been used in Ref. [45] to study the trp-cage protein with an implicit solvent. However, this approach is not restricted to microcanonical simulations. Instead, it can be generalized to the more commonly used canonical ensemble without changes of the functional form of Eq. 35.

The search for more efficient replica exchange schemes is an active area of research [46, 47], especially for the case of explicit solvent simulations of proteins [48, 49]. Inspired by Okur et al. [48] we have proposed in Ref. [50] to circumvent the problem by a hybrid method. We assume that the potential energy of the system can be written as

$$E = E_{pot} + E_{kin} \quad \text{with} \quad E_{pot} = P_{pp} + P_{pw} + P_{ww} \quad \text{and} \quad E_{kin} = K_p + K_w \,, \tag{39}$$

where P_{pp} marks the contribution from interaction solely between atoms in the protein, P_{ww} denotes the ones arising from water–water interactions, and P_{pw} stands for water-protein interactions. Between exchange moves the system evolves with the energy function given by Eq. 39. However, for exchange moves we utilize in addition an implicit solvent term P_{is} that is an approximation for $P_{ww} + P_{pw}$. The difference between the two solvation terms is given by

$$H = P_{ww} + P_{pw} - P_{is} \,. \tag{40}$$

The "true" potential energy E_{pot} can be approximated by a quantity $Q = P_{pp} + P_{is}$, leading to:

$$E_{pot} = Q + H \,. \tag{41}$$

Exchange moves are as usual accepted with probability

$$w(1 \leftrightarrow 2) = \min(1, \exp(D)) \text{ with } D = \Delta\beta\Delta Q - \beta_1(\hat{E}_{kin}^{(1)} - E_{kin}^{(1)} + \Delta H) - \beta_2(\hat{E}_{kin}^{(2)} - E_{kin}^{(2)} - \Delta H), \tag{42}$$

where $E_{kin}^{(1)}$ and $\hat{E}_{kin}^{(1)}$ are the kinetic energies at temperature T_1 before and after an exchange move, respectively. Rescaling the velocities according to

$$v^{(2)} \leftrightarrow \hat{v}^{(1)} = v^{(2)} \sqrt{\frac{E_{kin}^{(1)} - \Delta H}{E_{kin}^{(2)}}} \quad \text{and} \quad v^{(1)} \leftrightarrow \hat{v}^{(2)} = v^{(1)} \sqrt{\frac{E_{kin}^{(2)} + \Delta H}{E_{kin}^{(1)}}} \tag{43}$$

leads to

$$\hat{E}_{kin}^{(1)} = E_{kin}^{(1)} - \Delta H \quad \text{and} \quad \hat{E}_{kin}^{(2)} = E_{kin}^{(2)} + \Delta H \quad . \tag{44}$$

Exchange moves are now accepted with a probability of the same form as in Okur et al. [48]:

$$w(1 \leftrightarrow 2) = \min(1, \exp(\Delta\beta\Delta Q)) \text{ with } Q = P_{pp} + P_{is}. \quad (45)$$

However, the velocity rescaling improves on that method by relating the solvation energies as measured with the explicit solvent and the one calculated with the implicit solvent. We have shown for the 20-residue Trp-cage protein that the number of replicas in explicit solvent replica exchange molecular dynamics can be reduced from 40 to 10 replicas [50]. As the contribution of solvent–solvent interaction increases faster than protein–protein and protein-solvent terms one can expect a more dramatic improvement for the larger proteins, allowing to evaluate and improve velocity rescaling as a way to advance on explicit solvent simulations and other applications of replica exchange.

2.3 Multiscale Sampling

Another approach to enhance sampling of protein configurations is multiscale sampling. Simplified or coarse-grained models lead by design to an energy landscape with reduced number of valleys, and allow often in addition for a much faster evaluation of energies. The reduced model allows to observe long time scale changes quickly enough, which could take all-atom models an infeasible simulation time. The so-obtained coarse-grained potentials are designed to reproduce the thermodynamical and structural properties of the corresponding all atom system. But the lost fine details in coarse-grained models are in principle critical to the accurate description of realistic molecular behaviors. For example, structure prediction of a pathologically important enzyme is usually performed by using reduced models for a fast outcome. But the drug screening followed requires more details in side chain arrangement in the active site. Multiscale simulations attempt to overcome this problem by combining coarse-grained with all-atom simulations, altering the fineness of the system studied in a stepwise way.

Obviously, combining different coarse-graining levels requires a scheme for back-mapping to the detailed degrees of freedom. The difficulty of back-mapping is evident — coarse graining in the large part averages a fine-grained model, thus the reversing is not one-to-one, but mapping a single coarse-grained structure to a fine-grained ensemble. The high-resolution ensemble generated in the normal back-mapping mode does not assure necessarily the correct statistical properties. As an extension of parallel tempering, Zuckermann and coworkers developed the Resolution exchange algorithm in which several simulations of differing resolutions are conducted in parallel and exchanges of configurations are attempted periodically between the neighboring resolutions[51]. Instead of using high temperature to smoothen the rugged potential energy landscape, resolution exchange uses coarse-grained model to effectively sample the conformational space. The method guarantees the canonical sampling in the atomic fineness level by using the following exchange acceptance criterion.

$$P_{RM} = \min\{1, \frac{\pi_H(\phi_a, x_b)\pi_L(\phi_b)}{\pi_H(\phi_b, x_b)\pi_L(\phi_a)}\} \tag{46}$$

The configuration of a coarse-grained model is described by a set of coordinates ϕ and that of a fine model is described by a larger set of coordinates including not only ϕ but also x which is for the extra degrees of freedom. If the two configurations before exchange is ϕ_a and $\{\phi_b, x_b\}$, the trial configurations are simply ϕ_b and $\{\phi_a, x_b\}$. Namely, only the coarse-grained part of potential energies are subjected to exchange. Subscripts H and L denote high-resolution and low-resolution respectively and the corresponding potential energy is defined as U_H and U_L. Then the probability of having configuration a and b before exchange is the product of probability of having configuration a, $\pi_H = exp(-\beta_H U_H(\phi_a))/Z_H$ and having b, $\pi_L = exp(-\beta_L U_L(\phi_b, x_b))/Z_L$. Similarly, the probability after exchange is the product of $\pi_H = exp(-\beta_H U_H(\phi_b))/Z_H$ and $\pi_L = exp(-\beta_L U_L(\phi_a, x_b))/Z_L$. Z_H and Z_L are partition functions. In sum, the exchange criterion can be written as equation 46. The criterion satisfies the detailed balance and therefore ensures the canonical distribution at any resolution.

A practical problem of the resolution exchange method is that when the system studied is of larger size than dipeptides, the trial exchanges are rejected easily. Lyman et. al have found that the rejection rate depends on both number and type of the degrees of freedom of coordinates x. They employed an incrementally coarse-graining scheme to coarsegrain one residue each time [52]. In-between the finest and most coarse-grained replica, hybrid models which are partially atomic and for the rest united atoms are used. Finally the acceptance rate of exchange becomes reasonably high (from 0.09% to $> 2\%$). To tackle the same issue, Liu et. al used configurational-bias monte carlo (CBMC) to reconstruct the nascent degrees of freedom [53]. The position of the next interacting site is constructed using a look-ahead algorithm. A set of trial positions are generated and each is assigned a weight $w_i = exp(-\beta U_i)$. The coordinates will be selected based on its Rosenbluth factor, $w_i / \sum w_i$, and the process iterated till the last site is generated.

3 Recent Applications

Our group has a long-standing interest in mis-folding and aggregation of proteins. A class of proteins where one would expect an increased danger of mis-folding are proteins with end-to-end β-sheet. This is because the N-terminal β-strand is synthesized early on, but it cannot bind to the C-terminus before the chain is fully synthesized. During this time there is a danger that the β-strand at the N-terminus interacts with nearby molecules leading to potentially harmful aggregates of incompletely folded proteins. Using our advanced generalized-ensemble techniques we have recently shown [54, 55] that the 49-residue C-terminal CFr of the artificially designed Top7 [56, 57] avoids this risk by a "caching" mechanism, that relies on chameleon behavior of one of the terminal β-strands, to facilitate folding. In the early phases of folding the N-terminal residues are "cached" as part of the subsequent α-helix. Only after the other parts of the molecules have folded into the correct structure, do the

N-terminal residues unfold and refold to a strand that then forms with a C-terminal hairpin into a three-stranded β-sheet. While "caching" is not in contradiction to the funnel picture, it implies a rather complex energy landscape. We have shown further that mutations which increase the propensity of forming strands and decrease that of forming helices, still lead to the same native structure, but by interfering with the caching mechanism lead to reduced folding rates [58].

We recently started to explore the importance of the caching mechanism in other proteins with an end-to-end β-sheet. For instance, we became interested in the possible mechanisms by which the A629P (alanine to prolin) mutant of ATP7A causes Menkes Disease (a hereditary copper deficiency disease in most cases leads to death in early childhood). The mutation is located in the fourth (and C-terminal) strand of the β-sheet in the sixth domain. The isolated domain consists of 75 residues, with the mutation at position 69, and exists in solution as a monomer. As such it has been characterized by NMR for wild type and mutant, both in the apo and the copper-binding form. Structural differences between wild type and mutant are around 3 Å rmsd and within the variations of the respective NMR ensembles. Hence, the question arises by what mechanism the mutation leads to the outbreak of Menkes disease. Our results indicate that the mutation does not have appreciable effects on the stability of copper-bound states but rather destabilizes the characteristic end-to-end β-sheet [59]. The resulting transient unfolding leads to partial exposure of hydrophobic residues that makes the mutant prone to degradation. In turn this leads to the low effective concentration of the copper transporting protein that is responsible for the pathology of Menke's disease. We further show that the differences in the binding affinities between the two terminal strands alter the folding mechanism for the mutant: the secondary structure elements form contacts between each other in different order than in the wild type [60].

Another example of recent applications of generalized-ensemble techniques are our investigations into the folding of the A and B domain of protein G. Both proteins fold in a two-state way without detectable intermediates, similar to the often studied small protein CI2. They share no significant sequence homology and have different folds: GA is a three-helix bundle, and GB a α-helix on top of a 4-stranded β-sheet. The group of Bryan and Orban (University of Maryland) have studied systematically mutations of these two proteins that increased the homology of the two proteins up while preserving structure and function [61]. The final mutants GA98 and GB98 differ by a single residue that switches between the two folds. Our assumption is that the two proteins and their mutants have both structures as local minima, with the sequence determining their relative weight. We conjecture that the sequence of a protein encodes not only the native fold but also other forms that either are important to the folding process (as in the case of the caching mechanism in CFr) and the protein functions (changes of protein structure upon binding), or reflect an evolutionary history (or future): mutations can accumulate without changing structure and function of a protein until a single mutation finally switches the fold. In the case of GA and GB this process can be studied systematically by comparing the free-energy landscapes of the various mutants.

We have probed this assumption with all-atom Go-model simulations of both the GA and GB wild types and the GA98 and GB98 mutants [62]. While Go-models by construction lead to preselected structures as lowest-energy states, our simulations show a clear difference between sequences that "fits" a certain fold and ones that do not. For the wild type GA and GB, simple all-atom Go-model simulations allow to study the folding mechanism of these proteins. However, such models that by construction have only a single folding funnel will fail when the energy landscape of a protein is more complex. In the case of the mutants GA98 and GB98 that differ only in a single residue but have very different distributions of folded structures we therefore tried a modified Go-model that incorporates folding funnels to both GA and GB fold. This model reproduced not only correctly the experimentally observed distributions but also revealed details on the folding mechanism in these two mutants. The two mutants differ only in residue 45. This difference does not change the frequency of the long range contacts typical for the GB fold. In the case of GA98, the competing structure (resembling the B domain of protein G instead of the A domain) therefore is also accessible, and is indeed also observed experimentally with a certain, but low, probability. On the other hand, 45TYR in GB98 has a much smaller probability to form contacts with residues 32-35 (characteristic for the GA fold) than 45LEU in GA98. Hence, switching from 45LEU to 45TYR decreases the frequency of intra-helical contacts (i.e. disfavoring the GA fold). Hence, unlike in GA98 one does not observe in GB98 the competing structure (resembling the A domain of protein G instead of the B domain).

4 Conclusion

Progress in the development of algorithms over the last two decades has extended the size of peptides and proteins that are accessible in all-atom simulations, and has also allowed to pinpoint the remaining difficulties. The most important open problem in present generalized-ensemble techniques is that they require careful tuning of parameters. Unfortunately, there are no simple and universal rules for this tuning toward optimal sampling. As the described techniques can only reduce the sampling difficulties from an exponential scaling to a power law, it is necessary to have software that is highly adapted to massively parallel computers and modern architectures such as GPUs and cell processors. Further advancements in hardware and algorithms may overcome the remaining sampling problems and establish the use of computer simulations as "microscope" to a point where the whole cells can be explored *in silico*.

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