Automated sampling assessment for molecular simulations using the effective sample size

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Abstract

To quantify the progress in development of algorithms and forcefields used in molecular simulations, a method for the assessment of the sampling quality is needed. We propose a general method to assess the sampling quality through the estimation of the number of independent samples obtained from molecular simulations. This method is applicable to both dynamic and nondynamic methods and utilizes the variance in the populations of physical states to determine the ESS. We test the correctness and robustness of our procedure in a variety of systems – two–state toy model, all–atom butane, coarse–grained calmodulin, all–atom dileucine and Met–enkaphalin.

We also introduce an automated procedure to obtain approximate physical states from dynamic trajectories: this procedure allows for sample–size estimation for systems for which physical states are not known in advance.

1 Introduction

The field of molecular simulations has expanded rapidly in the last two decades and continues to do so with the advent of progressively faster computers. Due to this, significant effort is put in the development of more sophisticated algorithms and
forcefields for use in both physical and biological sciences. To quantify progress, it is
critical to answer questions about the efficiency of the algorithms and the accuracy of
the forcefields. This is especially true for large biomolecules that are slow to sample.
Such questions demand a gauge to assess the quality of the generated ensembles that
is applicable regardless of the type of method used to generate the ensembles.

Ensembles are of fundamental importance in statistical mechanical description of
physical systems: thermodynamic properties are obtained from these ensembles. In
addition to the accuracy, the quality of ensembles is governed by the amount of in-
formation present in the ensemble. Due to significant correlations between successive
frames in, say, a dynamic trajectory, the amount of information does not equate to the
total number of frames. Rather, the number of independent samples in the ensemble
(or the effective sample size) is the required gauge for the sampling quality. This
effective sample size (ESS) has remained difficult to assess in general. In this work,
we present an efficient method to determine the ESS of an ensemble – regardless of
the method used to generate the ensemble – by quantifying the variances in physical
states of the system under consideration.

A conventional view of sample size is given by the following equation:

$$N = \frac{t_{\text{sim}}}{t_{\text{corr}}}$$

where $t_{\text{sim}}$ is the simulation time, and $t_{\text{corr}}$ is the largest correlation time in the system.
Thus, significant effort has been invested in developing methods to calculate the corre-
lation time. However, the estimation of the correlation time needs the computation of
a correlation function, which requires a significant amount of data. Other assessment
approaches have, therefore, been proposed. Mountain and Thirumalai\textsuperscript{2,3} introduced
the “ergodic measure”, where the time required for the observable to appear ergodic
is decreased. Flybjerg and Petersen\textsuperscript{4} developed a block averaging method with the
idea that with an increase in size of blocks, adjacent blocks are less correlated.

Most of these methods analyze particular observable, however, different observable
can give different correlation times. For example, in a typical model, bond lengths
are decorrelated faster than dihedral angles. However, bond lengths are never fully
decoupled from the rest of the system: slower motions must ultimately couple to
the fast motions and influence their distributions. Moreover, we are not trying to
compute a particular ensemble average but to generate a truly representative ensemble
of configurations. Thus, there is significant ambiguity in the use of observable to
estimate the correlation times.

In order to overcome this ambiguity, the idea of “structural histograms” was
proposed. In this idea, a dynamic trajectory is divided into structural bins based
on several reference structures. For correlated samples, the average variance of these
structural bins depends upon the time increment between frames used to construct
the structural bins. In the limit of ideal sampling, this variance becomes independent
of this time increment. Accordingly, this method determines the time increment
that results in such an ideal sampling. For most complex systems, the data are
usually limited, leading to significant uncertainties in the estimation of the average
variances, thus, this method gives only logarithmically correct (factor of \( \approx 2 \)) results
for the ESS.

All the methods discussed above require dynamic trajectories (such as those ob-
tained by molecular dynamics (MD), Metropolis Monte Carlo (MC), or Langevin
dynamics) for analyzing the correlations. As such, these methods are not appli-
cable to non–dynamic methods, such as Replica Exchange and its variants and
polymer–growth MC. Thus, an universal method that can estimate the effective
sample size for both dynamic simulations and non–dynamic methods is particularly
important – allowing for, in addition to the sample size estimation for ensembles gen-
erated via non–dynamic method, comparison of efficiency between the two classes of
methods.

In this work we propose a novel method for the estimation of the effective sample
size that is applicable universally to ensembles generated using both dynamic and
non–dynamic methods. The main idea in this work is to quantify the variance of
populations in approximate physical states, and estimate the effective sample size
from this variance by mapping the problem into a binomial distribution: either a configuration is in one particular physical state or is not. The use of physical states is intuitive: transitions between physical states represent the slowest timescales in a system, and the simulation must be long enough to show several such transitions for the generated ensemble to be.

This method differs from the structural histograms method in various crucial ways. First, the use of population variances allows the present work to estimate the ESS for ensembles generated using both the dynamic and non-dynamic methods. Second, the use of physical-state variances allows us to probe the slowest timescale in the system, and the effective sample size of an ensemble must be governed by this timescale.

Accordingly, a important prerequisite for the estimation of ESS is the determination of physical states. In this work, we use a particularly simple method for the determination of physical states that uses information present in a dynamic trajectory regarding the transition rates between different regions – regions showing high transition rates with each other belong in the same physical state. Further, this procedure also highlights the hierarchical nature of the energy landscape. Our approach to determine the physical states is based on ideas of Chodera et al. who developed an automated procedure to determine approximate physical states in a system from a dynamic trajectory by determining a division of the total configuration space that maximizes the self transition probabilities (i.e., the divisions represent metastable states).

The manuscript is organized as follows. First, we describe in detail the procedure we use to estimate the effective sample size. Then, we present results for several models with different levels of complexity – a two-state toy model, butane, calmodulin, di-leucine, and Met-enkaphalin. This is followed by a discussion of the practical aspects of the procedure. And, finally, we present conclusions. Further, in the Appendix, we show a simple, automated way to determine approximate physical states that are required for obtaining reliable estimates of the effective sample size.
2 Method

State populations are the most fundamental descriptors of the equilibrium ensemble, and any algorithm (either dynamic or non dynamic) must sample state populations correctly. Hence, we claim that variances in populations in “regions” of configurational space form a fundamental basis for estimating the ESS. We shortly define the most appropriate definitions of “regions” and how to compute the variances, but first, we introduce the main idea relating these variances to the ESS.

The key idea that we use in determining the ESS, \( N_{\text{eff}} \), is that the occurrence of a configuration (irrespective of whether generated using a dynamic or a non–dynamic method) in a region (or bin) of the configuration space can be mapped onto the binomial distribution: the configuration is either in bin \( j \) with probability \( p_j \), or not in that bin with probability \( 1 - p_j \). The value of \( p_j \) equals the fractional bin population that is formally and uniquely given by the partition function. In the limit of all such Bernoulli trails being independent, the variance in the fractional population of bin \( j \) is

\[
\sigma_j^2(N) = \frac{p_j(1-p_j)}{N}
\]

where \( N \) is the number of independent trials.

Equation 2 forms the basis for estimating ESS as follows. In essence, independent estimates of populations (of bins or, more accurately, of physical states), \( p_i \), are available, then the computed variances in these populations lead to estimating \( N_{\text{eff}} \) (\( \equiv N \)) by inverting eq 2. In this work, we use Voronoi bins: the details of binning procedure, and the subsequent determination of approximate physical states, are given in the appendix.

The above discussion naturally leads to several issues. Firstly, it is not immediately obvious how to obtain independent estimates of \( p_j \) to compute the bin variances from typical dynamic and non–dynamic simulations. Secondly, what is the best way to define the bins or regions such that we obtain meaningful estimate of the ESS? Thirdly, it is likely that different bins give different values of the ESS. Below, we
discuss these issues separately.

2.1 Population variances from independent probability estimates

First, we discuss how independent estimates of fractional populations in the bins can be obtained in both dynamic and non–dynamic simulations. As discussed in Introduction, non–dynamic methods have no intrinsic correlation time, unlike dynamic simulations. Thus, the only statistical analysis possible for both types of simulations is via several independent simulations. Average fractional bin populations and bin population variances can be computed from these independent simulations leading to an estimate of $N_{\text{eff}}$ using eq 2.

To facilitate exploration of important parts of the configuration space, we generate the independent simulations from different starting configuration. Further, all the independent simulations should be of the same length (dynamic simulations) or generate same number of configurations (non–dynamic simulations). This requirement is due to our assumption that all the independent simulations have same $N_{\text{eff}}$. Alternately, it is possible to perform a very long simulation, divide it into 10 or 20 equal—length segments, and consider these segments as independent simulations. However, care should be taken in this case, as we discuss later.

2.2 Appropriate division of configuration space: physical states

Equation 2 can, in principle, be applied to any decomposition of the configurational space. However, the separation of timescales in a system – fast relaxation within a physical state and slow relaxation between two physical states – leads to the use of physical states as the most appropriate decomposition to use with Figure 2.

Due to a separation of the timescale, state populations are slow to converge compared to sampling within a state. However, a prerequisite for a correct determination of state populations is a proper sampling within the states. Thus, an algorithm must
sample correctly within each state of interest. The ratio of partition functions for states \(i\) and \(j\), with configurational–space volume \(V_i\) and \(V_j\), respectively, is

\[
\frac{\text{prob}(i)}{\text{prob}(j)} = \frac{Z_i}{Z_j} = \frac{\int_{V_i} d\mathbf{r} \exp(-U(\mathbf{r})/k_B T)}{\int_{V_j} d\mathbf{r} \exp(-U(\mathbf{r})/k_B T)}
\]

where \(Z_i\) is the partition function for State \(i\), \(U\) is the potential energy of the system, \(T\) is the temperature, and \(\mathbf{r}\) are the configurational–space coordinates. This ratio is converged only if both the integrals are converged.

Variances in the physical states probe the sampling quality within a state, and, thus, are appropriate measures to determine the sampling quality using eq \(2\).

Further, bins much smaller than the physical states lead to incorrect estimates of the sample size using correlated trajectory that is typically obtained from a molecular simulation. We discuss this in more detail in Section 3.5 using results from a butane trajectory.

In the Appendix, we describe in detail a simple and efficient way to determine physical states in a system. We emphasize that our procedure to calculate ESS is independent of the procedure used to determine physical states. Briefly, we first decompose the total configurational space into bins (Voronoi bins, based on reference structures). Subsequently, we combine bins based on rates between the bins: bin pairs with high transition rates are expected to be part of one physical state (\(i.e.,\) devoid of significant internal barriers). This approach naturally leads to a hierarchical picture of the energy landscape, as we discuss in more detail in the Appendix.

### 2.3 Multiple estimates of ESS

Here, we discuss the practical scenario that each physical state gives its own estimate of the ESS. Although it is possible that the sample sizes in different regions are different from each other, we investigate the slowest timescale, which corresponds to the highest energies barrier in the systems. In other words, the sample size we are looking for is the overall sample size in the whole trajectory. The simulation could
sample the space better in one region than the others, however the region that gives the smallest sample size reflect sampling quality and is the sample size we use.

Additionally, taking the slowest time scale has the advantage that the estimated ESS is insensitive to whether a physical state is determined inaccurately. Such an inaccurate physical state can arise, for example, if the bin combination procedure discussed in the Appendix results in a physical state that straddles a barrier.

3 Results

3.1 Toy two–state system

First, we establish the correctness of our method for estimating $N_{\text{eff}}$. For this purpose, we choose a system in which all the samples are independent and, hence, we have a prior knowledge of the exact answer. The system has two “states” defined such that the first state consists of numbers below 0.5, and the second state consists of numbers above 0.5. The sampling consists of independent drawing of random numbers between 0 and 1, and the sample size is the number of independent draws, $N$.

To determine whether an accurate estimate of $N_{\text{eff}}$ ($\equiv N$ in this system) is obtained, we also compute both the mean value and standard deviation of $N_{\text{eff}}$. As suggested by eq 2, this required computation of variances of both the mean population and the population variance (these quantities are equal across the states for a two–state system). Further, care must be taken to account for the nonlinear dependence of $N_{\text{eff}}$ on the state variance. The details of this computation are given in the supplementary information.

For $N = 2000$, we obtain a mean value of $N_{\text{eff}}$ as 2004, with a standard deviation of 57.4. Similarly, for $N = 4000$, we obtain a mean $N_{\text{eff}}$ as 4041 with a standard deviation 117.6. Clearly, our basic premise of using the binomial distribution inherent in eq 2 is correct.
3.2 Systems with \textit{a priori} known physical states

In contrast to sampling in the toy system discussed above, sampling in molecular systems is not typically independent, and, thus, the sample size is not known \textit{a priori}. For this purpose we compute $N_{\text{eff}}$ using the procedure outlined above using sampling obtained from a typical molecular simulation. Furthermore, a knowledge of physical states allows an independent estimate of the effective sample size: a trajectory reentering a physical state is expected to be independent of its previous history in that particular state, and the counting transitions in and out of a physical state gives an estimate of the effective sample size. Although physical states determined using the analysis described in the Appendix can be used to “count” transitions, \textit{a priori} knowledge of physical states makes this estimate especially straightforward.

The particular systems we use are those obtained for an all–atom butane model and a coarse–grained calmodulin model. The trajectories for the all–atom butane model are obtained at 298 K using OPLSAA force field in vacuum with Langevin dynamics (as implemented in Tinker v. 4.2.2). This system has three well–known physical states: trans, gauche+, and gauche–. For calmodulin, the trajectories were generated by employing Monte Carlo simulations using a backbone alpha–carbon double–Gö model stabilizing the two known physical states (apo – PDB ID. 1CFD and the holo – PDB ID. 1CLL forms) – as described in greater detail elsewhere.

We can check the accuracy of $N_{\text{eff}}$ via a few different strategies that we have mentioned above – computing the ESS via the correlation times in the structural histograms, and via counting transitions to and from a known physical state from a dynamic simulation. Further, we check the robustness of our procedure via different reference structure for Voronoi bins in determining the physical states via the method explained in the Appendix (leading to possibly slightly different physical states).

Table 1 shows the results for $N_{\text{eff}}$ obtained for trajectories of butane and calmodulin. Columns 2 to 4 show three different estimates of $N_{\text{eff}}$ from eq 2 using independent binning, Column 5 shows $N_{\text{eff}}$ obtained from eq 2 using known physical states, and
Column 6 shows the range of effective sample size obtained using time correlation analysis. For both butane and calmodulin, the procedure is very robust in estimating \( N_{\text{eff}} \), as different binning procedures give similar estimates. Further, these estimates agree with the range of sample sizes suggested by the correlation time analysis. For butane, the first 10 ns of simulation results in 60 transitions among the three states, and, thus, the whole trajectory (of 1 \( \mu \text{s} \)) has about 6000 independent configurations. For calmodulin, the total number of transitions is 80. These results also agree with the estimates in Table 1.

We further use the knowledge of exact physical states in these two systems to obtain estimates of the sample size using eq 2, instead of using the procedure described in the Appendix to determine the physical states. For butane, analysis of population variances in the three known physical states lead to estimating the sample size as 5865 (as the lowest estimate). A similar analysis using two physical states of calmodulin leads to an estimate of \( N_{\text{eff}} = 91 \). Thus, for both these systems, using known physical states give effective sample sizes consistent with Table 1.

One interesting feature emerging from the above analysis is that the ESS calculated from eq 2 is somewhat larger than the total number of transitions (80) from one state to other in calmodulin. A possible reason could be that calmodulin is a large flexible molecule and stays in a particular state long enough to become decorrelated even before making a transition.

### 3.3 Systems with unknown physical states

For most biomolecular systems, physical states are not known in advance. For this reason, we test our method on two systems, leucine dipeptide (acetaldehyde–(leucine)\textsubscript{2}–n–methylamide) and Met–enkaphalin, for which physical states are not known in advance. We use OPLSAA force field for both systems, and use overdamped Langevin dynamics (in Tinker v 4.2.2) at 298 K with a friction constant of 5/\( \mu \text{ps} \) for both. For leucine dipeptide we use an uniform dielectric of 60, and the BS/SA solvation for Met–enkaphalin. For both the systems, a total of 1 \( \mu \text{s} \) simulation is performed with
frames stored every 1 ps.

Again, we estimate $N_{\text{eff}}$ using eq\[2\] using three different repetitions of the binning procedure, and also using time correlation analysis. The results are shown in Table\[2\]. Again, for both the systems, we obtain a good agreement with independent binnings to determine physical states and also with the range obtained using time–correlation analysis.

3.4 Application to discontinuous trajectories

In the examples so far to compute ESS, we have used dynamic trajectories that allow for good approximations to the physical states (that are based on rates of transitions between regions of configuration space). Here, we show the robustness of our procedure regardless of the actual definitions of the physical states. The motivation behind this exercise is to extend the method efficiently to non–dynamic trajectories. Although the sample size estimation using eq\[2\] is applicable to non–dynamic trajectories as well, the underlying physical states, based on transition rates between regions of configuration space, cannot be calculated from non–dynamic trajectories. For such simulations, instead of performing dynamic simulations as well merely to determine physical states, it is less computationally intensive to run short dynamic trajectories starting from configurations already obtained from non–dynamic simulations.

For this purpose, we divide the dynamic trajectory into smaller segments of equal length. We pick only a fraction of configuration at the beginning of each segment to determine the physical states, as well as population means and variances. Due to absence of some configurations between two segments, the trajectories are discontinuous and reflects, conceptually, the procedure described above for obtaining approximate physical states for non–dynamic simulations. This could lead to physical states being determined inaccurately.

However, we find out that as long as each segment is long enough (with more than 2 independent configurations), we still find physical states that determine the sample size. We studied the 4 systems and analyzed the sample sizes by discontinuous
trajectories. We took the first 300 ps for each butane segment, first 1000 Monte Carlo sweeps for each calmodulin segment, first 1000 ps for each dileucine segment, and first 5000 ps for each met-enkaphalin segment.

Despite the use of discontinuous trajectories to determine the physical states, we obtain good estimates of the effective sample size in each case, as shown in Table 3 (two estimates using different binnings are reported).

### 3.5 Importance of using physical states

We argued above that population variances in physical states are the most important descriptors of the sample size. Here we show an example that quantifies that. The system we use is butane. We divide the configurational space into 10 bins using Voronoi cells, and perform no combination into physical states – i.e., we determine the effective sample size using these arbitrary bins. For this system, we first performed the structural histogram analysis discussed in the Introduction to obtain the correlation time: configurations chosen after this interval are uncorrelated. For uncorrelated configurations, we expect the estimate of the sample size to be independent of particular bins, however, correlated configurations show a different picture.

Table 4 shows estimates of the ESS obtained for the 10 arbitrary bins obtained using uncorrelated and correlated configurations. Clearly, the correlated trajectory shows a dramatic bin dependence for the estimated sample size (note that the full correlated trajectory gives consistent estimates when bins are combined into approximate physical states).

The difference in bin estimates for correlated configurations is because the variance of one bin is a convolution of state variances and fast processes. To elaborate, we consider a double well. Imagine that we divide the space into several bins, and, say, the seventh of which has the observed probability of $p_7$. In ideal sampling, the observed probability should $p_7$ in the bin and $1-p_7$ out of the bin. However in dynamic sampling, where the correlation comes into play, the division of the configuration space is similar to the separation of the timescales. When the system is trapped in
state A, with a fractional population $p_A$, its observed probability in the bin turns out to be $p_7/p_A$ instead of $p_7$ and probability out of the bin is $1 - p_7/p_A$. The increase of observed probability in the bin comes from correlation. For example, in MD simulations, the system most likely remains in state A at the next time increment, unlike ideal sampling. This dual effects from fast process and state variance leads the new observed probability.

On the other hand, dynamic correlations identify states – as discussed in the Appendix.

4 Discussion

First, we discuss the appropriateness of subdividing a dynamic trajectory into smaller, equal segments to estimate the population mean and variances. If the sample size estimate for each of these segments is $O(1)$, then the method does not reliably give the estimate of the sample size of the total trajectory, and likely overestimates it. For example, if the correct total number of independent configurations in the total trajectory is 10, and we subdivide the whole trajectory into 20 equal segments, then each of the segment will give a sample size of 1 (minimum number possible) – leading to an overestimate of the sample size. In such a scenario, division into fewer segments is desirable.

Next, we discuss an appropriate approach in cases that show significant asymmetry in state populations. For example, in a system with two final physical states, separated by a high barrier, it is possible that one state has most of the population. The physical states analysis in the Appendix will correctly give two states, however, the state with significantly smaller population may not be important for determining the sample size. In our examples, we found that if a state has less than 5% of the population, then it is not meaningful for determining the sample size.
5 Conclusions

We have developed a new method to assess the quality of molecular simulation trajectories – effective sample size – using variances of population in the physical states. A major feature of this method is that it is applicable both to dynamic and non-dynamic methods, and gives a tool to compare sampling and efficiencies of different molecular simulation algorithms. Another feature of this procedure is that it is applicable to discontinuous trajectories as well. We also demonstrated that our procedure is robust with respect to actual definition of physical states – a fact that is expected to be of importance for systems for which actual physical states are not known in advance.

To supplement the estimation of the effective sample size, we also developed a procedure for automated determination of physical states. This procedure yields, in a natural way, a hierarchical picture of the configurational space, based on transition rates between regions of configurational space.
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Appendix: Physical states determination from dynamic trajectories

In this Appendix, we give a brief description on our physical state discovery method and its results. In this method, bins in configurational space are combined to give the physical states, as discussed below in more detail. There is no Markovian requirement on the selection of bins. Indeed, a typical bin in a configurational space for a large multidimensional system may itself encompass several separate minima.

Our method is based on the work of Chodera et al., but is simpler. In addition, the following method clearly shows the hierarchical nature of the configurational space, and focuses on the slowest timescale – which is of paramount importance for the estimation of the effective sample size in the main text.

Method

Use of rates to describe conformational dynamics

Rates between regions of configuration space are a fundamental property that emerges uniquely from the natural system dynamics. We use rates between regions of configurational space to determine the physical states. We first decompose the conformational space into multiple bins. Subsequently, we combine some of the bins that have high transition probability between them. This procedure is based on the physical idea of separation of time scales: there is a fast timescale (high transition rates) associated with regions within a single physical state, and a slow time scale (low transition rates) associated with regions of configurational space separated by high barriers. Thus, transition rates between regions of configurational space form a fundamental basis for determination of physical states.
Binning decomposition of the configurational space

We divide the whole configuration space into a large number of bins, and determine the physical states by combination of these regions. The procedure to decompose the whole configurational space (with \( N \) configurations) into \( M \) bins is described as follows:

1. A configuration \( i \) is picked at random from the trajectory.
2. The distance, by use of an appropriate metric as discussed later, of the configuration \( i \) to all remaining configurations in the trajectory is, then, computed.
3. The configurations are rearranged by the distance in an ascending order. The first \( 1/M \times N \) configurations are removed.
4. Steps 1–3 are repeated \( M - 1 \) times on progressively smaller set of remaining configurations, resulting in a total of \( M \) reference configurations, \( i \).

For the distance metric, we select the root-mean squared deviation (RMSD) of the full molecule, estimated after alignment. In contrast, using just the backbone RMSD may be a poor distance metric as it ignores potentially relevant side chain kinetics.

After references are selected, we decompose the whole space into bins based on a Voronoi construction. For each configuration, we calculate the RMSD of this configuration from each reference structure. We assign this configuration to the bin associated with the reference structure, \( i \), with which the configurations has the smallest RMSD.

Calculation of rates among bins and bin combination

We compute the mean first passage time (MFPT) from each bin, \( i \), to every other bin, \( j \), using the full dynamic trajectory. The rate from bin \( i \) to bin \( j \) is the inverse of that MFPT. In general, the rate from bin \( i \) to bin \( j \) is not the same as the rate from bin \( j \) to bin \( i \) – and we take a linear average of these two rates to define the
rate between bin $i$ and bin $j$. Subsequently, we chose a cutoff rate, $k_c$, and combine all pairs of bins that show a rate higher than $k_c$.

**Hierarchy**

An obvious manner in which the hierarchical picture emerges from the above discussion is from the choice of $k_c$. An increase in $k_c$ leads to the combination of fewer bins – resulting in an increase in the final number of states obtained. Starting from a low value of $k_c$, we obtain a hierarchical picture with increasing number of states upon increasing $k_c$.

This hierarchical picture can be significantly affected by the time interval underlying the MFPT calculations. For example, although a trajectory may have a low likelihood (hence a low rate) to cross over the $2kT$ barrier in Figure 1 in time $\tau_1$, it may easily cross that barrier for a long enough time interval, $\tau_2$. Thus, a hierarchical picture at the lowest level can differentiate the two left states of Figure 1 if the rates are computed from the dynamic trajectory at every $\tau_1$ interval. On the other hand, if the rates are computed using the $\tau_2$ interval, $2kT$ barrier cannot be resolved at the lowest hierarchical level. As an extreme case, if $\tau$ is chosen to be longer than the largest decorrelation time in the system, then the rates to a bin from any other bin is simply proportional to the equilibrium population of that particular bin – the application of the procedure described above is not appropriate.

**Results**

Figures 1 and 2 show the hierarchical physical for dileucine and butane, respectively. They both start from 20 bins and combine all the way to single states. We took the second last step (2 states) to calculate sample size. Dileucine is very flexible systems, due to which the transition time goes up smoothly, while butane get 3 states at early stage and have a sharp increase in transition time among those 3 states.
Table 1: Effective sample sizes for butane and calmodulin using eq \(2\) and three different repetitions of the binning procedure in Columns 2–4, using eq \(2\) and known physical states in Column 5, and as obtained by the structural time correlation in Column 6.

| System   | eq \(2\) (1) | eq \(2\) (2) | eq \(2\) (3) | known physical states | Time correlation function |
|----------|---------------|---------------|---------------|----------------------|---------------------------|
| butane   | 6064          | 6236          | 6200          | 5865                 | 5000–10000                |
| calmodulin | 93           | 90            | 92            | 91                   | 80–160                    |
Table 2: Effective sample sizes for di-leucine and Met-enkaphalin using eq 2 and three different repetitions of the binning procedure in Columns 2–4, and as obtained by the structural time correlation.

| System          | eq 2 (1) | eq 2 (2) | eq 2 (3) | Time correlation function |
|-----------------|----------|----------|----------|---------------------------|
| di-leucine      | 1982     | 1878     | 1904     | 1100-2200                 |
| Met-enkaphalin  | 416      | 362      | 365      | 250–500                   |
Table 3: Effective sample sizes for discontinuous trajectories obtained from eq 2 by using two repetitions of the binning procedure.

| System         | eq 2 (1) | eq 2 (2) |
|----------------|----------|----------|
| butane         | 5842     | 6024     |
| calmodulin     | 91       | 92       |
| dileucine      | 1803     | 2016     |
| Met–enkaphalin | 397      | 336      |
Table 4: Butane sample size estimated in 10 different bins using correlated and uncorrelated sampling. The actual sample size is 2000.

| Bin number | uncorrelated | correlated |
|------------|--------------|------------|
| 1          | 1882         | 1256       |
| 2          | 2415         | 61380      |
| 3          | 1837         | 82080      |
| 4          | 2444         | 91820      |
| 5          | 1866         | 292640     |
| 6          | 2172         | 71180      |
| 7          | 1892         | 240200     |
| 8          | 2264         | 5600       |
| 9          | 3936         | 162720     |
| 10         | 2040         | 310260     |
Figure 1: Hierarchical physical states for dileucine shown via the average transition time required for transition among bin pairs. Bin pairs that combine “faster” (i.e., have shorter transition time) are combined at a lower level of the hierarchy.
Figure 2: Hierarchical physical states for butane shown via the average transition time required for transition among bin pairs. Bin pairs that combine "faster" (i.e., have shorter transition time) are combined at a lower level of the hierarchy.