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Assessing the Reliability of the Dynamics Reconstructed from Metadynamics

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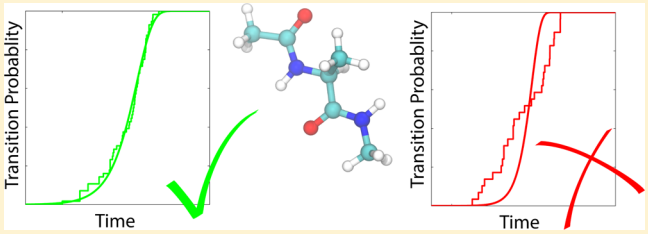
Supporting Information

ABSTRACT: Sampling a molecular process characterized by an activation free energy significantly larger than k_BT is a well-known challenge in molecular dynamics simulations. In a recent work [Tiwary and Parrinello, *Phys. Rev. Lett.* **2013**, *111*, 230602], we have demonstrated that the transition times of activated molecular transformations can be computed from well-tempered metadynamics provided that no bias is deposited in the transition state region and that the set of collective variables chosen to enhance sampling does not display hysteresis. Ensuring though that these two criteria are met may not always be simple. Here we build on the fact that the times of escape from a long-lived metastable state obey Poisson statistics. This allows us to identify quantitative measures of trustworthiness of our calculation. We test our method on a few paradigmatic examples.



INTRODUCTION

The range of the accessible timescale is a central issue in atomistic molecular dynamics (MD). This descends from the serial nature of time evolution and the fact that the size of the integration time step is dictated by the fastest atomic motions which are of the order of femtoseconds. Therefore reaching the time scale of millseconds, seconds, and beyond is extremely costly or outright impossible. Unfortunately, this is the time scale of very many interesting physicochemical processes. Thus, despite the remarkable development in purpose-built computers,[1] the limited time-scale still remains a major limitation of MD simulations. Several enhanced sampling approaches have been proposed to address this issue.[2−15] Many of them, typically based on the application of an external bias potential or force, are focused on the reconstruction of equilibrium probability distributions, therefore losing information on the real dynamical evolution of the system.[2−4]

Recently, Tiwary and Parrinello have removed this limitation showing that from suitably engineered well-tempered metadynamics (WTMT) runs[16] the transition rates can be evaluated if a rare event scenario holds. Namely, if after a long residence time in a metastable state in which memory of the previous history is lost, the system undergoes a very rapid transition to another basin. For the approach of Tiwary and Parrinello[16] to work it is essential that a set of collective variables (CVs) able to distinguish between the different metastable states is chosen and that no bias is deposited in the transition region such that the rapid well-to-well dynamics is unaffected. The experience accumulated in metadynamics simulations allows in many cases to ensure that these two conditions are met. However it would be very valuable to have quantitative criteria that can verify whether or not these necessary conditions are satisfied. To this effect we note that under the very same condition of rare events in which the approach of Tiwary and Parrinello is valid, the statistics of transition times must follow a Poisson distribution, the so-called *law of rare events*.[17] In fact, if the transition between two basins is of the rare event type, the transitions are independent from one another. Thus the statistical analysis of the distribution of transition times provides a powerful quantitative tool to assess whether or not in a simulation the assumptions of ref 16 have been met and provide a robust estimation of the average transition time. We shall demonstrate the power of our analysis on the very same systems considered in ref 16. We establish that an incorrect choice of the CVs can be clearly identified by our approach. We refer the reader to ref 16 for details of the metadynamics simulations and for a detailed explanation of the correspondence between the metadynamics time and the real time.

ANALYSIS OF THE TRANSITION TIMES DISTRIBUTION

Consider a set of independent simulations that sample the $A \rightarrow B$ transition, in which A and B are states separated by an energy barrier significantly higher than k_BT. The process of counting the number of transitions $A \rightarrow B$ can be interpreted as a stochastic process in which the probability of observing n

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events in time interval $(0, t)$ is distributed according to a Poisson distribution with a characteristic transition time τ:[18]

$$P_n = \frac{1}{n!}\left(\frac{t}{\tau}\right)^n \exp\left(-\frac{t}{\tau}\right) \quad (1)$$

By construction, the evolution of each system in its state space can be represented by a Markovian process and independent simulations of the same activated event provide mutually independent estimates of the transition time. The Poisson process is defined to be homogeneous if its characteristic time τ does not depend on the history of the system. The treatment developed in this paper does not depend on the number of alternative escape pathways that could be taken to accomplish the $A \rightarrow B$ transition. This follows from the fact that the sum of an arbitrary number of Poisson random variables also follows a Poisson distribution.[17] Thus if there were n transition mechanisms with transition times $\tau_1, \tau_2, ..., \tau_n$, then eq 1 will still hold, provided that a term $\tau_{\text{eff}} = (1/\sum_{i=1}^{n} \tau_i^{-1})$ is considered in the right-hand side.[18,19] A simple example of Poisson statistics applied to a transition characterized by multiple pathways is provided in the Supporting Information.

To evaluate the average transition time of a given $A \rightarrow B$ transition we perform M independent simulations and for each event we record the corresponding transition time t_i with $i = 1, ..., M$.

Modeling the transitions in this set of simulations as a Poisson process implies that the waiting time T_n associated with the occurrence of n transition events is distributed according to a Γ distribution $f_{T_n}(t) = \Gamma(n, \tau)$.[18,19] In the above-mentioned case of simulations designed to observe only one transition, the waiting time T_1 to observe a single transition is distributed as $f_{T_1}(t) = \Gamma(1, \tau)$ which corresponds to the exponential distribution $f_{T_1}(t) = \tau^{-1} \exp(-t/\tau)$. If the M waiting times recorded from our set of simulations are generated by a Poisson process, $f_{T_1}(t)$ corresponds to their probability density function (PDF) and τ corresponds to the average transition time $\mu = (1/M)\sum_1^M t_i$. Moreover, the distribution of waiting times has the property that its mean μ and standard deviation σ are equal $(\sigma/\mu = 1)$ and that its median t_m and mean μ are related through the expression $t_m = \mu \ln 2$.

Monitoring the average, standard deviation, and median of the set of M transition times obtained from simulation could in principle yield a qualitative estimate of the goodness of the Poisson model in representing faithfully the data collected from simulations. However both (σ/μ) and $(t_m/(\mu \ln 2))$ are extremely sensitive to insufficient sampling, being influenced by single points falling in the long-times tail of the distribution.

An alternative approach to the evaluation of the characteristic time associated with the set of $A \rightarrow B$ transitions obtained from rare event simulation is the construction of an *empirical* cumulative distribution function (ECDF). For a Poisson process, the cumulative distribution function (CDF) can be derived as the probability to observe at least one transition by time t as[18]

$$P_{n\geq 1} = 1 - P_0 = 1 - \exp\left(-\frac{t}{\tau}\right) \quad (2)$$

Using eq 2 to fit the ECDF provides an estimation of the theoretical average rate associated with the $A \rightarrow B$ transition. Estimating τ from a fit of the ECDF with eq 2 is more advantageous, since it avoids considering the fraction of simulations that do not undergo $A \rightarrow B$ transition within a prescribed simulation time. Moreover CDFs can be used to compare quantitatively the theoretical and empirical transition times distributions. To this aim we perform a two-sample Kolmogorov–Smirnov (KS) test.[20,21] The KS test is particularly advantageous as it is nonparametric and does not require a priori knowledge of the distribution. We use it to test the null hypothesis that the sample of transition times extracted from metadynamics and a large sample of times randomly generated according to the theoretical probability density $f_{T_1}(t) = \tau^{-1} \exp(-t/\tau)$ reflect the same underlying distribution. The KS test is based on a measure of the maximum distance between the CDF of the two samples, which is known as KS statistic and defined as follows: $\sup|F_1(t) - F_2(t)|$, where F_1 and F_2 are the two CDFs. Given this value, one can theoretically extract the probability that F_1 and F_2 are drawn from the same distribution. This is expressed in the so-called *p-value*. The null hypothesis is rejected if p-value $< \alpha$. The conventional assumption is to choose $\alpha = 0.05$. The KS test has been performed as implemented in Matlab R2012b.[22,23] A Matlab implementation of the analysis is provided in the Supporting Information.

■ ANALYSIS OF CONFORMATIONAL TRANSITION RATES FOR ALANINE DIPEPTIDE

The above proposed analysis has been applied to two cases: namely the conformational transition between conformers α and β of alanine dipeptide in vacuum and in water. The details regarding the simulation protocol followed to obtain both the MD and the WTMT sets of data are reported in ref 16. A third case study, regarding transitions between minima in a 2D mutiwell potential[7,16] is discussed in the Supporting Information.

Alanine Dipeptide in Vacuum. The conformational transition of this peptide is a much studied example of a rare event, whose activation barrier has been estimated to be ∼8 kcal/mol.[5] We consider three data sets produced respectively by unbiased molecular dynamics simulations (MD) and WT metadynamics performed depositing bias along two sets of collective variables (CVs). The first set of WT metadynamics simulations has been carried out using both torsional angles Φ and Ψ (see ref 16 for the definition of these angles). This represents a best case scenario, in which the CVs are able to discriminate properly between the stable states of the system. The second distribution comes from WT metadynamics simulations in which only the Ψ angle is used as a collective variable. WT metadynamics simulations in this case were carried out following the same protocol implemented in ref 16 to obtain the WTMT(Φ, Ψ) data set. Namely we used a Langevin thermostat[24] to enforce the temperature, a time step of 0.2 fs,[4] AMBER03 forcefield,[25] and GROMACS4.5[26] molecular dynamics code patched with PLUMED 1.3.[27] Well-tempered metadynamics was performed with a bias factor of 5. The initial Gaussian height was 0.3 kcal/mol, the width was 0.25 rad, and the deposition stride was 20 ps. A single alanine dipeptide molecule was kept in a periodic cubic box of edge 0.25 nm. The LINCS algorithm[28] handled bond constraints while the particle-mesh Ewald[29] scheme was used to treat long-distance electrostatic interactions. The nonbonded van der Waals cutoff radius was 0.8 nm. In this case it is known that the Ψ variable alone does not allow discrimination between free energy basins for the conformers α and β.[5,16] It is used here to provide an example of a typical distribution of times

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reconstructed from a series of WT metadynamics simulations in which a slow CV is missing and the deposited bias corrupts the transition state region, not allowing for a correct recovery of transition times. In all WT metadynamics simulations the transition rate has been calculated through the expression of the acceleration factor reported in ref 16.

As discussed earlier a first qualitative assessment of the quality of the transition times distribution can be obtained from the characteristic ratios μ/σ and $t_m/(\mu \ln 2)$, that should be 1 in an exponential distribution. A more rigorous test comes from analyzing the statistical significance of fitting the ECDF with eq 2, performed with a least-squares method in order to estimate the average transition time τ. The results of this analysis are summarized in Table 1, and a comparison between the ECDF and the theoretical CDF is reported in Figure 1. It is to be seen that for both MD and WTMT(Φ, Ψ) data the expected transition time of the Poisson process τ is in excellent agreement with the average calculated transition rate obtained from the sample, both μ/σ and $t_m/(\mu \ln 2)$ ratios are close to one, and moreover both data sets pass satisfactorily the KS test. These findings allow us to conclude that the distribution of transition times obtained from both MD and WTMT(Φ, Ψ) can be considered compatible with a theoretical transition times distribution generated from a homogeneous Poisson process with a p-value equal or higher than 0.9, well above the usual significance threshold of 0.05.

Table 1. Simulations of the $\beta \rightarrow \alpha$ Conformational Transitions of the Alanine Dipeptide Molecule[16] in Vacuum[a]

	MD	WTMT(Φ, Ψ)	WTMT(Ψ)
μ [ns]	108 ± 12	102 ± 12	$3.9 \times 10^4 \pm 1.2 \times 10^4$
σ [ns]	106	90	6.3×10^4
t_m [ns]	75	86.5	3.6×10^3
μ/σ	1.02	1.13	0.6
$(\mu \ln 2)/t_m$	1.00	0.82	7.6
τ [ns]	110.2	106.3	1.73×10^4
p-value	0.98	0.89	2.17×10^{-4}

[a]Mean transition time μ, standard deviation σ, and Poisson process characteristic time τ are reported together with the μ/σ and $(\mu \ln 2)/t_m$ ratios. The p-value associated with the KS statistic is also reported.

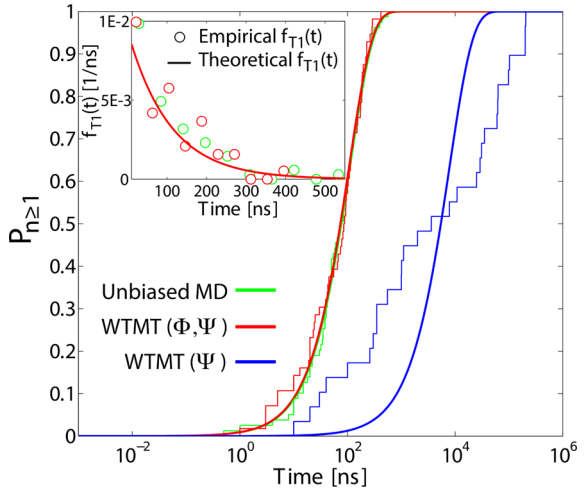

Figure 1. Alanine dipeptide in vacuum. $P_{n\geq1}$ directly evaluated from the simulations (thin lines) and fitted as a Poisson process (solid lines) for each of the three mthods: MD, WTMT(Φ, Ψ), and WTMT(Ψ). In the inset, both empirical (○) and theoretical (solid line) exponential probability density functions of transition times $f_{T_1}(t)$ are displayed.

For the WTMT(Ψ) data set, the indicators highlight a completely different scenario. A large discrepancy between μ and τ can be observed, accompanied by a p-value close to zero. These results show that is extremely unlikely to obtain the distribution of times actually recorded in the WTMT(Ψ) data set from an underlying distribution of waiting times coming from a homogeneous Poisson process. An analysis of the p-value dependence on the number of simulations used to construct the ECDF is reported in the Supporting Information.

This information, accompanied by the evident overestimation of the average transition time points out that when a poorly chosen set of CVs is used, the intrinsic dynamics of the transition between basins is corrupted. Moreover, the shape of the ECDF reconstructed for the WTMT(Ψ) data set suggests that the dynamics follows a nonhomogeneous Poisson process in which τ increases with time, as a consequence of the fact that the free energy barrier that separates the α and β conformers changes due to the undesired deposition of bias in the transition state region. On the other end, this analysis shows that when a good set of CVs is used, as in the case of the WTMT(Φ, Ψ) data set, the distribution of times is practically indistinguishable from that recovered by unbiased MD, as shown in the quantile−quantile plot reported in Figure 2, where quantiles of the time distribution obtained from the WTMT(Φ, Ψ) and MD sets are plotted against one another.

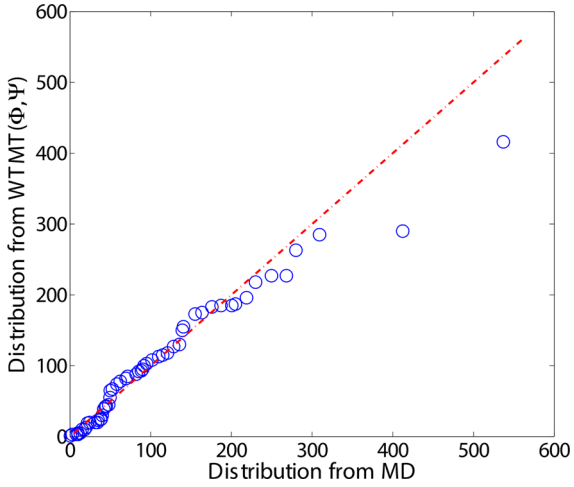

Figure 2. Quantile−quantile plot of the transition times distribution obtained from the MD set against that obtained from the WTMT(Φ, Ψ) set. The plot displays how the two distributions are virtually undistinguishable, with points almost completely aligned on the $y = x$ axis. The p-value associated with the KS statistic obtained comparing the WTMT(Φ, Ψ) and the MD one is 0.874.

Our examples clearly show that the p-value associated with the KS statistic computed comparing a theoretical homogeneous Poisson distribution of transition times with characteristic time τ, with the ECDF obtained directly from the set of WTMT simulations, can be used as a quantitative measure of the quality of the reconstructed dynamics.

Artificially Stiffened Alanine Dipeptide in Water. The same analysis has been extended to the other examples treated

in ref 16, namely both the $\alpha \rightarrow \beta$ and $\beta \rightarrow \alpha$ conformational transitions of an *artificially stiffened* alanine dipetide in water. Since the actual alanine dipeptide molecule in water has a free energy barrier between its two main basins of only around 2 kcal/mol,[30] the transitions in that system can easily be sampled using ordinary unbiased molecular dynamics, and no enhanced sampling scheme is needed. In order to reproduce also in this case the scenario of a rare event transition in solution we have artificially stiffened the terms in the force field associated with the dihedral angles Φ and Ψ as described in ref 16. Also in these cases the averages obtained from the raw data μ are coherent with the transition time τ and the ratios μ/σ and $(\mu \ln 2)/t_m$ are remarkably close to one both for the MD and WTMT data sets. The KS test is positive for all data sets displaying p-values associated with the KS statistic 1 order of magnitude larger that the standard significance level of 5%. Statistical indicators calculated for these data sets are summarized in Table 2. An analysis of the dependence of the p-value on the

Table 2. Simulations of the $\alpha \rightarrow \beta$ and $\beta \rightarrow \alpha$ Conformational Transitions of the Artificially Stiffened Alanine Dipeptide Molecule[16] in Water[a]

	$\alpha \rightarrow \beta$		$\beta \rightarrow \alpha$	
	MD	WTMT(Φ, Ψ)	MD	WTMT(Φ, Ψ)
μ [ns]	12 ± 2	11 ± 2	60 ± 8	63 ± 12
σ [ns]	12	10	49	79
t_m [ns]	9	9	48	30
μ/σ	1.00	1.02	1.22	0.80
$(\mu \ln 2)/t_m$	0.94	0.82	0.87	1.45
τ [ns]	11.8	10.3	66.5	51.5
p-value	0.76	0.53	0.90	0.45

[a]Mean transition time μ, standard deviation σ, and Poisson process characteristic time τ are reported together with the μ/σ and $(\mu \ln 2)/t_m$ ratios. The p-value associated with the KS statistic is also reported.

number of simulations considered to construct the ECDF is reported in the Supporting Information. In Figure 3, the ECDF, the fitted CDF, and the corresponding probability density function of transition times $f_{T_1}(t)$, are displayed for both $\alpha \rightarrow \beta$ and $\beta \rightarrow \alpha$ conformational transitions. We underline that even when the complexity of the system increases, and a solvent is introduced, a Poisson process model to describe the distribution of transition times remains valid. Also in this case, as already shown in ref 16, the agreement between the average rate obtained by unbiased MD and that obtained from the WTMT(Φ, Ψ) data sets are in excellent agreement. Moreover it is important to notice that the choice of a good set of CVs leads also in this case to a coherent description of the dynamics for both $\alpha \rightarrow \beta$ and $\beta \rightarrow \alpha$ transitions. One can also see that the p-values got lower for the dipeptide in water case compared to the other cases, while still well within the statistical threshold. This reflects a general trend that relatively poorer p-values might be obtained as the height of the barrier goes down and as friction induced barrier-recrossing events become more significant in solvated systems, leading to an increased possibility of bias deposition in the transition state region. Vibrational energy relaxation after crossing the barrier can be expected to be especially inefficient in a small molecule like the dipeptide we considered, but we still obtain p-values well within the statistical significance limit. Thus we expect the situation to not worsen, and possibly only improve, for systems

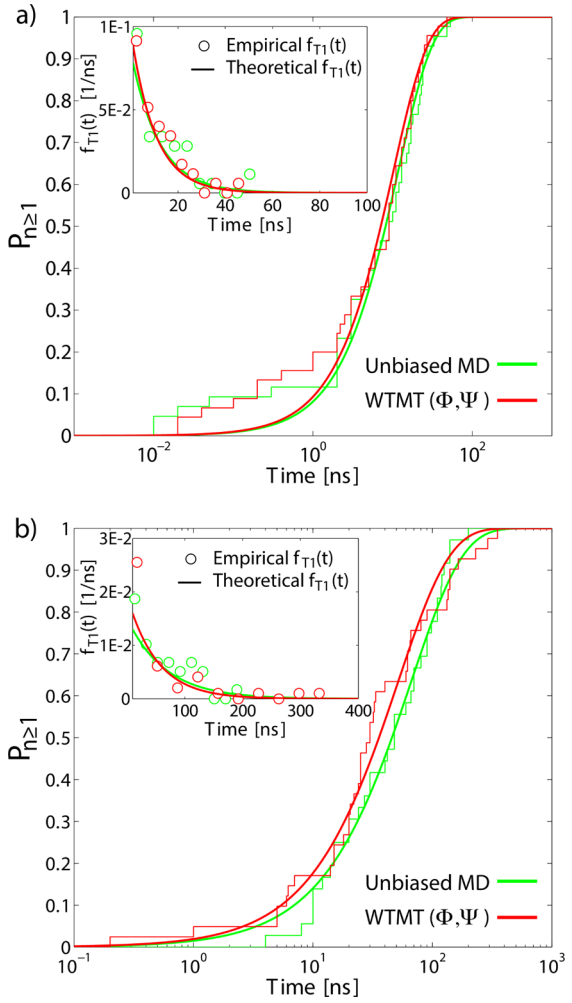

Figure 3. (a) Alanine dipeptide in water, α to β transition. $P_{n\geq1}$ directly evaluated from the simulations (thin lines) and fitted as a Poisson process (solid lines) for each of the MD and WTMT(Φ, Ψ) data sets. In the inset both empirical (○) and theoretical (solid line) exponential probability density function of transition times $f_{T_1}(t)$ are displayed. (b) Alanine dipeptide in water, β to α transition. $P_{n\geq1}$ directly evaluated from the simulations (thin lines) and fitted as a Poisson process (solid lines) for each of the MD and WTMT(Φ, Ψ) data sets. In the inset, both empirical (○) and theoretical (solid line) exponential probability density functions of transition times $f_{T_1}(t)$ are displayed.

with higher barriers and larger molecules. Our initial results on a large protein in water system confirm this picture.

Some Practical Considerations. The use of the Poisson statistics is of great help when one sends out M different simulations in parallel and only m of them undergo a transition within the preassigned simulation time. If one were to estimate the average transition time from the only m successful runs, this would lead to an underestimate since the long time tails have not yet been properly sampled. If in contrast we use eq 2, then the estimator τ converges much faster than the mean μ (see Figure 4a). Similarly susceptible to the poor sampling of the tails would be a fit of the data to the exponential distribution $f_{T_1}(t)$. Use of eq 2 circumvents this problem and solves the practical issue of accounting for those simulations in which the $A \rightarrow B$ transition is not observed in the allotted simulation time. If instead we consider a set of m simulations collected

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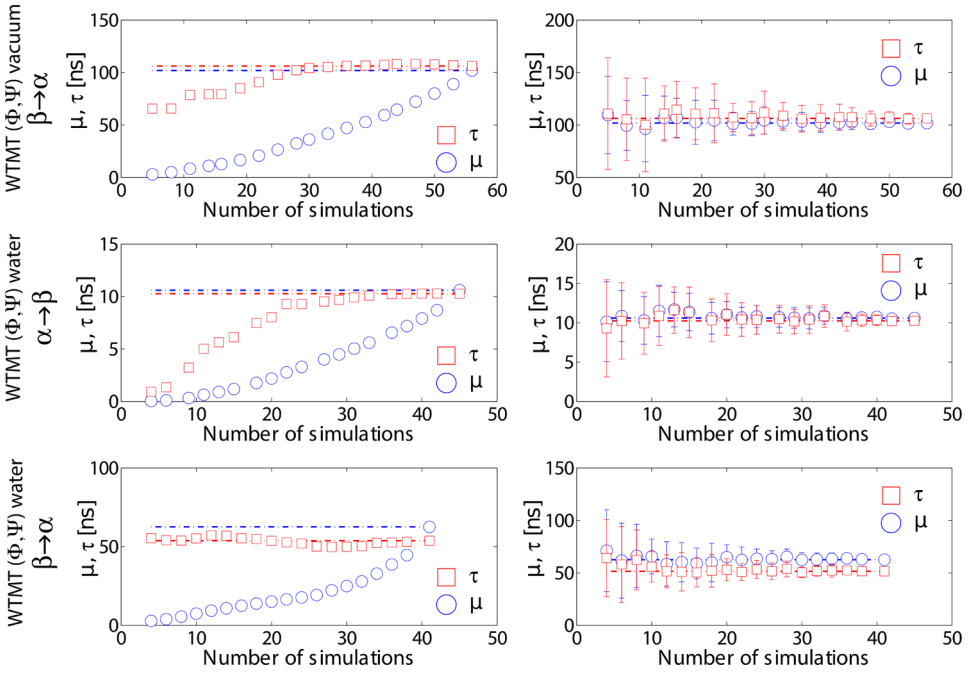

Figure 4. (left column) Average of the transition times μ (blue ○) and characteristic time of the Poisson process τ (red □) calculated from the subset composed of the m fastest transitions and reported as a function of m. (right column) Terms μ and τ as functions of the size of a subsample of transition times randomly extracted from the main complete sample. Errorbars represent the standard deviation of both μ and τ obtained from the extraction of 20 subsamples.

independently irrespective of their τ values, the rate of convergence of both the estimators τ and μ is comparable (see Figure 4b). These considerations are valid not just for transition time extracted from metadynamics but also from brute-force MD simulations.[1]

SUMMARY AND CONCLUSIONS

In this paper, we have shown that constructing an empirical cumulative distribution function provides a robust approach for the estimation of the rate of the Poisson process associated with a generic activated transition $A \rightarrow B$. Compared to the mean time, this estimate is less sensitive to single data points falling in the long time tail of the distribution and allows to use data sets in which not all the simulations have yet shown a transition. This is particularly effective in the case of times recollected from WT metadynamics simulations, as the method itself allows to sample transition time distributions spanning several orders of magnitude. In such cases while for well sampled distributions the average time μ and the characteristic time of the Poisson process τ coincide (see Tables 1 and 2), the latter is more robust.

From our analysis it also emerges that a time estimation obtained from a series of WT metadynamics simulations in which the hypotheses of ref 16 are not fulfilled displays marked deviations from the Poisson process model, that are quantitatively reflected by p-value lower than the confidence threshold. In these cases the empirical cumulative distribution function resembles a CDF obtained from a nonhomogeneous Poisson process in which the rate diminishes with time. This can be considered as the hallmark of an undesired corruption of the transition state ensemble[5,7,16] by the metadynamics biasing potential. To conclude, our analysis shows that for a transition time estimation exploited through WT metadynamics simulations performed along a good set of collective variables (CVs) not only is the average time consistent with unbiased simulations[16] but the whole distribution is for all intents and purposes indistinguishable from that obtained by unbiased MD. We expect this analysis to be extremely useful to asses the quality of the transition times distributions obtained from metadynamics in complex systems, where identifying CVs that do not corrupt the transition dynamics might be more difficult.

ASSOCIATED CONTENT

Supporting Information

Further analysis of the p-values computed for the examples treated in the paper, an analysis of an additional example, and a Matlab implementation of the analysis described in this paper. This material is available free of charge via the Internet at http://pubs.acs.org/.

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Notes

The authors declare no competing financial interest.

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