On the insufficient sampling problem in the free energy perturbation

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We re-examine the underlying theory of the widely-employed free energy perturbation (FEP) method, which is also known as the Zwanzig equation. FEP frequently fails to converge, and this poor performance reflects the problem of insufficient sampling inherent in the theory. Using a harmonic oscillator model, we are able to prove analytically that the deviation between the exact solution and the falsely-converged FEP result obtained from a finite sampling is related to a relaxation energy that the system would release, if it were allowed to relax, after the perturbation is introduced. Upon including this relaxation energy as a correction term to the standard FEP, we are able to obtain exact free energy difference using only trapped finite sampling, i.e. where standard FEP fails even when using Bennett’s acceptance ratio method. Our new approach of augmenting the free energy theory with the relaxation process hence sheds a new light on the insufficient sampling problem in free energy calculations.

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I. INTRODUCTION

Owing to promising applications in drug-design, free energy calculations have become one of the major tools for computational biology. Applications range from ligand (drug-like molecules) solubility, protein-ligand, and protein-protein binding affinities, to ligand permeation across membranes or lipid bilayers. These great achievements of applying free energy calculations in biological systems reflect the advances and numerous efforts in constructing accurate force fields as well as in developing enhanced sampling techniques, to resolve the insufficient sampling issue. Owing to dramatic improvements of computational hardware (e.g. GPUs) during the past decade, regularly applying free energy calculations for drug design is now within reach, yet the computational cost is still rather challenging for large protein systems, such as penicillin-binding proteins. Therefore, any development on theory that may further shorten the necessary simulation length is always desirable.

Traditionally, the insufficient sampling problem is associated with the difficulty in capturing a relevant configuration change upon performing the perturbation, such as the receptor’s conformational change upon a ligand insertion, or the water molecules’ movement following the ligand insertion. Those examples all deliver an important concept. Namely, the system has to reorganize itself to accommodate the perturbation. However, as this reorganization is normally difficult to be captured during a finite sampling, it might be worth the effort to re-examine the insufficient sampling problem encountered in applying the theory for free energy calculations. For this reason, we revisit the widely-applied free energy perturbation (FEP) method, also known as the Zwanzig equation. FEP states that the Helmholtz free energy difference (\(\Delta A\)) between the reference state \(R\) and the perturbed state \(P\) can be obtained via a single ensemble average. The equation reads

\[
e^{-\beta \Delta A} = \langle e^{-\beta u} \rangle_R ,
\]

where \(\beta = 1/k_B T\) with the Boltzmann constant denoted by \(k_B\) and the temperature by \(T\). The term \(u\) is a function of configuration and is defined as the difference between the potential of the perturbed state \(P\) and the reference state \(R\). In Eq. 1 the ensemble average is collected from the equilibrium of the state \(R\) as denoted by \(\langle \cdots \rangle_R\). Although Eq. 1 is theoretically exact, practically it is known to fail, owing to the fact that a large perturbation requires infinite sampling for the calculation to converge. When this occurs, one can perform another calculation for \(\Delta A\), in which the ensemble average is collected over the perturbed state \(P\), i.e.

\[
e^{\beta \Delta A} = \langle e^{\beta u} \rangle_P .
\]

To distinguish the two different FEP sampling approaches, Eq. 1 is often termed the forward FEP and Eq. 2 the backward FEP. Combining both forward and backward FEP results using Bennett’s acceptance ratio (BAR) to minimize the variance of expected error is the standard approach when utilizing Zwanzig’s equation with a single, large perturbation. If this standard approach (FEP-BAR) still fails, then one must construct several intermediate states to bridge the two end states \(R\) and \(P\), and apply FEP along these intermediate states (windows). For instance, if one intermediate is introduced, one can choose its potential to be the average of potentials from state \(R\) and state \(P\). This approach (multi-step FEP) relies on splitting the sampling difficulty into many windows, and within each window the
“perturbation becomes smaller” such that a sufficiently long sampling would work. Today, similar divide-and-conquer strategies are widely-practiced with all major free energy theories, including the FEP [3], thermodynamic integration [2], and Jarzynski equation [22]. In this work, however, we would like to present an alternative approach that can extract the correct free energy from the falsely-converged FEP results, without going through such a divide-and-conquer approach.

II. THEORY

To keep the physics simple, we begin by considering the free energy difference between two harmonic oscillators that only differ in their minimum energy, see the vertically shifted parabola example in Fig. 1. Initially, the system is at the equilibrium of the reference state $R$, whose potential is labeled as $U_R$. For simplicity, $U_R$ is chosen as $U_R(x) = \frac{1}{2} k x^2$, where $k$ denotes the force constant. The potential of perturbed state is also chosen to be centered at $x = 0$ with a minimum energy $U_P$, i.e. $U_P(x) = \frac{1}{2} k x^2 + U_0$. Following the usual definition of the Helmholtz free energy in an NVT-ensemble via the associated partition functions ($Z_P$ and $Z_R$ for state $P$ and $R$, respectively), the analytic exact $\Delta A_{\text{Exact}}$ can be evaluated by,

$$e^{-\beta \Delta A_{\text{Exact}}} = \frac{Z_P}{Z_R} = \int \frac{e^{-\beta U_P(x)} dx}{e^{-\beta U_R(x)}} = e^{-\beta U_0} \quad (3)$$

Hence $\Delta A_{\text{Exact}} = U_0$. Note that the kinetic energy partition functions have been integrated out analytically and cancel out in the fraction of Eq. (3) so only the potential contributions are kept in $Z_R$ and $Z_P$. For the cases where the two parabola are further shifted apart, like the displaced parabola scenario depicted in Fig. 1, the exact free energy change is still given by $\Delta A_{\text{Exact}} = U_0$, as long as the two parabola have the same force constant $k$. Eq. (2) also demonstrates that the exact $\Delta A_{\text{Exact}}$ does not change when the temperature varies.

Now, if the potential $U_P$ centers at a different position $x_0$, instead of zero, viz. $U_P(x) = \frac{1}{2} k (x - x_0)^2 + U_0$, the sampling approach via Eq. (1) will fail, because the equilibrium distributions of $R$ and $P$ are now well separated. Using 50,000 steps of Monte Carlo (MC) sampling, and a force constant $k = 0.1$ (kcal/mol/Å$^2$) and $U_0 = 10$ (kcal/mol), one can find the sampled $\Delta A$ deviating from $\Delta A_{\text{Exact}}$ when increasing $x_0$. Plotted in Fig. 2(a) are the average of $\Delta A$ over 50 independent MC simulations and the associated standard deviation indicating the magnitude of error (error bar). Here the exact solution is obtained by Eq. (3) which holds only for parabola with identical force constant. For general harmonic oscillator model, one should employ associated analytical exact equation or by numerical integration over the partition functions. While the failure of the FEP sampling approach is expected, we notice that the sampling yields a $\Delta A$ approaching a 0 K analytical model at the low temperature limit, see e.g. the blue and black curves in Fig. 2(b). At this limit, the probability distribution of state $R$, which is a Gaussian according to the associated Boltzmann weights, will have a very narrow width such that it behaves like a Dirac delta function,

$$P_R(x) = \lim_{\beta \to \infty} \frac{1}{\sqrt{2 \pi} / k \beta} e^{-(\frac{x^2}{2k \beta})} = \delta(x) \quad (4)$$

With this approximation at the low temperature limit, we obtain the sampled $\Delta A$ from

$$e^{-\beta \Delta A} = \langle e^{-\beta (U_P(x) - U_R(x))} \rangle_R \approx \int e^{-\beta \left(\frac{1}{2} k (x - x_0)^2 + U_0 - \frac{1}{2} k x^2\right)} \delta(x) dx = e^{-\beta \left(\frac{1}{2} k x_0^2 + U_0\right)} \quad (5)$$

Thus, at the low temperature limit, the deviation between FEP sampling and the exact solution is given by $\Delta A - \Delta A_{\text{Exact}} = \frac{1}{2} k x_0^2$, which is nothing but the potential difference between $U_P(0)$ and $U_P(x_0)$, i.e. $\Delta A - \Delta A_{\text{Exact}} = U_P(0) - U_P(x_0)$. Interestingly, the analytical model demonstrates that the difference between the FEP-sampled $\Delta A$ and the exact $\Delta A_{\text{Exact}}$ is equivalent to the difference of $U_P$ at the center of the equilibrium probability distribution of state $R$ and at the one
indicated by the black line. As a very low temperature 0.1 K. ∆

FIG. 2: (a) The FEP results obtained using Eq. 1 (ford) and
from sampling (via Eq. 1 and 2) and the exact free energy
that so far we have always distinguished ∆

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q

proach works for the vertically shifted parabola scenario,
and by the heat bath. As the analytical 0 K model states
∆A = ∆A_{Exact} = U_P(0) − U_P(x_{P0}), one immediately
arrives at the correction at the low temperature limit,
\[ e^{-\beta \Delta A_{Exact}} = e^{-\beta \Delta A} \cdot e^{-\beta q} = \langle e^{-\beta u} \rangle_R \cdot e^{-\beta q} = \langle e^{-\beta (u+q)} \rangle_R . \] (6)

Because the term q only depends on k and x_{P0} and not on
x, it can be moved inside the ensemble average in Eq. 6.
The validity of this equation can be confirmed using the
same delta-function model as before. We stress again that
Eq. 6 only holds at the low temperature limit (0 K).
Nevertheless, it opens a new possibility for calculating
the exact free energy difference (ΔA_{exact}) through a fin-
ite sampling method, even when distributions of state
R and P are well separated and FEP sampling fails. This
demonstrates an entirely new concept of sampling stra-

tegy: instead of relying on an infinite sampling for FEP to
converge correctly, adding a reasonable correction term
to the finite sampling FEP result may deliver an accurate
result much more efficiently.

Encouraged by Eq. 6 we then ask, is it possible to
further generalize this correction to the FEP method for
any examples under any temperature, such that an ex-
act solution can be obtained within a finite sampling
time? Again we start from including the relaxation
energy q that should be released during the relaxation
process. However, q now can be any value around
U_P(x_{P0}) − U_P(0), owing to the fluctuation introduced
by the finite temperature. To be more specific, the sys-

tem initially can be at any position x around the equi-

librium of state R, and after the perturbation followed
by a subsequent relaxation the system can be at any
position x′ around the equilibrium of state P. Thus
q = U_P(x′) − U_P(x), where x′ is independent from x be-
cause the two equilibrium configurations are determined
by their own potentials. In contrast to the low tempera-

ture limit, q is now no longer the constant that depends
only on the choice of k and x_{P0}. Even starting from the
same microstate of state R, one will have many differ-
ent q as there are various possible relaxation trajectories,
ending at different configurations of state P. Naturally,
an additional average over all the possible trajectories is
required. That is, for each microstate of state R, one
extra ensemble average over all microstates of state P is
required, since the probability of finding a specific mi-
crostate of P at equilibrium through all possible relax-
ation trajectories only depends on its Boltzmann weight.

of state P. See Fig. 1 for a clear graphical illustration of
this difference (q). Under this picture, the biggest prob-
lem of perturbation u at a fixed position x is that this
position x may not correspond to the equilibrium posi-
tion x′ of the perturbed state P! However, if one allows
the system to propagate on state P after the perturba-
tion, one would expect the system quickly relaxes and
reaches the equilibrium on state P. The energy released
during this relaxation process, i.e. the relaxation energy
q of magnitude ∆A − ∆A_{Exact}, will then be absorbed by
the heat bath.

Inspired by the fact that a finite FEP sampling ap-
proach works for the vertically shifted parabola scenario,
and by the derivation of q using the 0 K model, we
then investigate whether it is possible to introduce a cor-
rection term into the FEP method to yield a good ap-
proximation of ∆A_{Exact}, using only finite sampling. In
other words, whether it is possible to bring the deviation
∆A − ∆A_{Exact} in the 0 K model, as shown in Fig. 2(b),
down to zero. Before proceeding our discussion, we stress
that so far we have always distinguished ∆A obtained
from sampling (via Eq. 1 and 2) and the exact free energy
difference ∆A_{Exact} obtained from evaluating the parti-
tion functions (Eq. 3). Hereafter we will continue this
distinction: ∆A only refers to the free energy result ob-
tained via sampling and might be non-converged, while
∆A_{Exact} refers only to the free energy calculation via ex-
plicitly evaluating the ratio between two partition func-
tions. Now, at the low temperature limit, we have shown
that −q = \(\frac{1}{2}kT_{P0} = U_P(0) - U_P(x_{P0})\), where the mi-
lus sign denotes a net energy flowing from the system
into the heat bath. As the analytical 0 K model states
∆A = ∆A_{Exact} = U_P(0) − U_P(x_{P0}), one immediately
arrives at the correction at the low temperature limit,
\[ e^{-\beta \Delta A_{Exact}} = e^{-\beta \Delta A} \cdot e^{-\beta q} = \langle e^{-\beta u} \rangle_R \cdot e^{-\beta q} = \langle e^{-\beta (u+q)} \rangle_R . \] (6)

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by the finite temperature. To be more specific, the sys-
tem initially can be at any position x around the equi-

\[ q = U_P(x) - U_P(x'), x' \text{ independent from } x \]
Thus, we begin with
\[ e^{-\beta \Delta \hat{A}} = \langle e^{-\beta (u + q)} \rangle_R^P, \]  
(7)
where \( \Delta \hat{A} \) is not the exact \( \Delta A_{\text{Exact}} \), and their relation under the finite temperature will be revealed soon. Combining the definition of \( u(x) = U_P(x) - U_R(x) \) as in Eq. [1] and the new definition of \( q = U_P(x') - U_R(x) \), the term \( u + q \) becomes \( u + q = U_P(x') - U_R(x) \), which is the difference between the final potential obtained at the equilibrium of state \( P \) with configuration \( x' \) and the initial potential obtained at the equilibrium of state \( R \) with configuration \( x \). As \( x' \) and \( x \) are independent, the double layer sampling over \( R \) (integral over \( x \)) and \( P \) (integral over \( x' \)) can be further separated into a product of two ensemble averages, i.e.
\[ e^{-\beta \Delta \hat{A}} = \langle \langle e^{-\beta (U_P(x') - U_R(x))} \rangle_R \rangle_P \]
(8)
where the term \( \langle e^{\beta U_R} \rangle_R \) stands for the ensemble average over a Boltzmann factor with negative energy \( -U_R \). By this definition, this term reads,
\[ \langle e^{\beta U_R} \rangle_R = \int e^{\beta U_R(x)} P_R(x) dx \]
(9)
where \( P_R(x) \) stands for the equilibrium probability distribution of state \( R \). In theory, \( P_R(x) \), is given by the Boltzmann distribution \( e^{-\beta U_R}/Z_R \). This leads to a result of \( \langle e^{\beta U_R} \rangle_R = V/Z_R \), where \( V \) denotes the volume of the entire configuration space. While this result is true in an infinite sampling scenario, a finite sampling is normally rather restricted around the equilibrium because of the underlying potential trap. That is, by finite sampling the trajectory can only cover part of the configuration space, and the area with high potential energy is practically never explored. Consequently, it is more reasonable to approximate the probability distribution \( P_R(x) \) under the finite sampling as
\[ P_R(x) \approx \frac{e^{\beta U_R(x)}}{Z_R} \cdot \theta(U_R^* - U_R(x)), \]
(10)
where \( \theta(U_R^* - U_R(x)) \) denotes the Heaviside step function with a nonzero probability requirement that the potential \( U_R(x) \) should be smaller than an upper bound energy \( U_R^* \), which is the initial energy measured within a trajectory. In principle the approximated \( P_R(x) \) in Eq. [10] requires a normalization factor different from the partition function \( Z_R \), but because \( e^{-\beta U_R} \) with \( U_R > U_R^* \) is usually very small (otherwise the associated configurations would have been sampled), \( Z_R \) is still a good estimation for the normalization factor.

Upon introducing the approximated \( P_R(x) \) into Eq. [6], we then arrive at the value of \( \langle e^{\beta U_R} \rangle_R \) that one would obtained using a finite sampling; it reads,
\[ \langle e^{\beta U_R} \rangle_R \approx \int e^{\beta U_R(x)} e^{-\beta U_R(x)} Z_R \cdot \theta(U_R^* - U_R(x)) dx \]
\[ = \frac{V_R}{Z_R}, \]
(11)
with \( V_R = \int_{U_R(x) < U_R^*} 1 dx \), which is just the volume of the configuration space that is accessible under a given temperature during a finite sampling. This finite sampling approximation (approximating the probability distribution as a product of the Boltzmann distribution and a Heaviside function) is a way to introduce a clear cutoff in the probability distribution under a given temperature, and mathematically it defines a support, i.e. an area within which the probability is practically nonzero during the sampling. This support can be smaller than the entire domain, i.e. the configuration space, when a potential trap is present. In contrast, the traditional Boltzmann distribution employs a theoretical support which is equivalent to the entire domain, regardless of the sampling difficulty caused by the potential trap. For the current examples with harmonic oscillators, \( V_R \) can be read directly from the MC trajectory as the farthest distance between two positions of the trajectory. For more advanced systems, further development of a numerical integration of \( V_R \) might be necessary.

Inserting Eq. [11] into Eq. [8], we find
\[ e^{-\beta \Delta \hat{A}} = \langle e^{-\beta U_P} \rangle_p \cdot \frac{V_R}{Z_R} \cdot \frac{V_P}{Z_P} + \frac{1}{\beta} \ln \frac{V_R}{V_P} \]
(12)
using the exact definition of the Helmholtz free energy via the partition functions and the volume of accessible configuration space for sampling of state \( P \), i.e.
\[ \langle e^{\beta U_P} \rangle_p \cdot \frac{V_R}{Z_R} \cdot \frac{V_P}{Z_P} + \frac{1}{\beta} \ln \frac{V_R}{V_P} \]
(13)
As expected from the definition of a state function, the final form of \( \Delta A_{\text{Exact}} \) in Eq. [13] does not depend on \( q \) nor \( u \), but solely on properties from the two equilibrium states \( R \) and \( P \). Second, as \( V_R \) and \( V_P \) are determined through the sampling trajectories, their values depend implicitly on the temperature. That said, both \( V_R \) and \( V_P \) should approach the same volume \( V \) as temperature increases, and the volume correction term \( \frac{1}{\beta} \ln \frac{V_R}{V_P} \) disappears at the high temperature limit, bringing the equation to the known traditional form of \( \Delta A = \frac{1}{\beta} \ln \langle e^{\beta U_P} \rangle_p - \frac{1}{\beta} \ln \langle e^{\beta U_R} \rangle_R \). This further highlights the difference between the theoretical Boltzmann distribution and our approximated finite sampling distribution: the former leads to an exact solution but introduces an underlying sampling difficulty, while the latter leads to a good approximation of the exact solution and can be efficiently evaluated, through a volume correction term. This volume correction term generally is not zero and contributes to the free energy results pronouncedly. Last but not least, as the derivation of Eq. [13] is initiated
The result of BAR shows an increasing fluctuation as 50 independent replicas of the same calculations as before. The error bar is obtained from the standard deviation over results of BAR performed over those FEP results at 300 K.

FIG. 3: (a) RAFEP results obtained from the same trajectories employed in previous FEP calculations, together with the results of BAR performed over those FEP results at 300 K. The error bar is obtained from the standard deviation over 50 independent replicas of the same calculations as before. The result of BAR shows an increasing fluctuation as $x_{P_0}$ increases, and finally fails to converge when $x_{P_0} \geq 18 \text{ Å}$. In contrast to BAR, RAFEP results always agree well with the exact solution, viz. 10 kcal/mol. (b) Deviation of FEP and RAFEP results from the numerical exact $\Delta A_{\text{Exact}}$ for a displaced parabola scenario with $x_{P_0} = 20 \text{ kcal/mol}$ and various $k_P$. The force constant of the state $R$ is kept the same as 0.1 kcal/mol/Å$^2$ and temperature at 300 K. Again, RAFEP utilizes the same trajectories as those for FEP calculations, and its good performance demonstrate that the theory holds for more general case rather than parabola with identical force constant, echoing the fact that no exact potential form is assumed during the derivation of Eq. 13.

One may wonder how the RAFEP result fluctuates with the number of samples, compared with the FEP result that is known to exhibit a saw-tooth behavior. We employ the most challenging example so far, viz. $x_{P_0} = 20 \text{ Å}$ and $k_P = 1.0 \text{ kcal/mol/Å}^2$ at $T = 300 \text{ K}$, to answer this question. The evolution of a single RAFEP result with respect to the number of MC samples (steps) used is depicted in Fig. 3(a). In contrast to the large fluctuation that can be observed in the forward FEP result (red) and the falsely-converged backward FEP result (magenta), RAFEP (orange) fluctuates relatively close to the exact $\Delta A_{\text{Exact}}$ (black). Since all RAFEP results shown in this paper are obtained based on exactly the same samples as those used for the FEP calculations, Eq. 13 does provide a novel approach to combining forward and backward sampling for a stable result.

Finally, using the same harmonic oscillator model as in Fig. 3(a), we show that the volume correction term will naturally disappear at the high temperature limit. By nature there is no boundary of an harmonic oscillator potential, i.e. the configuration space $V$ is infinite, but here we confine the sampling to between -30 Å and 30 Å to force a finite $V$ for our discussion. As the system can explore a larger space at a higher temper-
from the volume correction (1), and then eventually decreases to zero, since both \(V\) (blue). The latter first increases with increasing temperature, from energy (red) and a contribution from volume correction originates from comparing FEP sampling to the quantification to Eq. 13, the RAFEP result (orange) can be divided into two contributions: the one from the energy (red) and a contribution from volume correction (blue). The latter first increases with increasing temperature, and then eventually decreases to zero, since both \(V_R\) and \(V_P\) cover the entire volume of configuration space. When this happens, RAFEP is reduced to only the energy contribution term, as one would expect from the theory.

In this article, we have revisited the insufficient sampling problem encountered in the FEP sampling approach using a simple harmonic oscillator model. Based on this model, a finite sampling with a short simulation always provides excellent free energy results for the vertically shifted parabola scenario. Further analytical investigation revealed that it’s possible to add a correction term, coming from allowing the system to relax after perturbation, to bring the falselv-converged FEP results to the exact solution. We then further generalized this relaxation correction to arbitrary potentials and arbitrary temperature, which leads to an unexpected working equation that resembles the known definition of free energy difference between two states, plus an extra volume correction to account for the trapped sampling under a finite temperature. Mathematically, our approach defines a support, within which the probability distribution is nonzero and can be sampled during a finite sampling time. The volume of the support is no greater than the volume of the entire domain. Following the definition of the support, the effect of sampling trapped by the potential is taken into account via the volume of the support. This may provide a new perspective for resolving the issue of low sampling probability at the crucial area in an importance sampling in mathematics. Good agreement between our RAFEP method and the exact analytical and numerical solution is found using harmonic oscillator examples, and we find that RAFEP performs better than BAR. Although not shown here, we would like to mention that RAFEP has already been successfully applied to a system of Ar atoms. Those results and how the implementation can be achieved will be published in a

FIG. 4: (a) FEP and RAFEP results for a single trajectory, based on exactly the same samples. Both forward and backward FEP results fail to converge to the correct answer, and the former shows the well-known saw-tooth structure. The drop in \(\Delta A\) indicates that an important configuration has been sampled. In contrast, the RAFEP result only fluctuates mildly around the exact value \(\Delta A_{\text{Exact}}\) (black line). (b) Free energy difference calculated using RAFEP and using the exact numerical integration at different temperature. The RAFEP \(\Delta A\) (orange) can be further split into a contribution from energy (red) and a contribution from volume correction (blue). The latter first increases with increasing temperature, and then eventually decreases to zero, since both \(V_R\) and \(V_P\) cover the entire volume of configuration space. When this happens, RAFEP is reduced to only the energy contribution term, as one would expect from the theory.

IV. CONCLUSION

temperature, the simulation length is now set to 500,000 MC steps. Results are shown in Fig. 4(b). According to Eq. 13, the RAFEP result (orange) can be divided into two contributions: the one from the energy \((\frac{1}{\beta} \ln \left< e^{\beta U_R} \right>_P - \frac{1}{\beta} \ln \left< e^{\beta U_P} \right>_R)\) shown in red and one from the volume correction \((\frac{1}{\beta} \ln \frac{V_R}{V_P})\) shown in blue. As expected from the theory, the latter approaches zero at the high temperature limit, but we stress that at low and medium temperature its contribution can be up to 30 kcal/mol, proving that the volume correction is indeed indispensable if one would like to obtain a good estimate of \(\Delta A_{\text{Exact}}\) (black), when using only the finite sampling without any enhanced sampling techniques. To end the discussion, we mentioned that our idea of including \(q\) originates from comparing FEP sampling to the quantum molecular dynamics following an electronic excitation (vertical transition) [25]. In light of this comparison, the relaxation process can be a nonequilibrium process, but the initial state \(R\) and the final state \(P\) are both at their equilibrium. Thus, RAFEP can also be viewed as a theory that implicitly goes through a nonequilibrium relaxation to bridge the two equilibrium states \(R\) and \(P\) (see Eq. 13), without calculating the work function as required when employing Jarzynski equation [22]. Although at first glance this contradicts one’s understanding of the nonequilibrium methods [26], a recent article by Ross et al demonstrates that it is indeed possible to utilize non-equilibrium trajectories with relaxation to calculate the free energy difference between two equilibrium states, assuming a Markov state model with Brownian dynamics [27]. In stead of explicitly utilizing the relaxation trajectories [27], RAFEP simply further carries out this idea analytically such that the net result of the theory depends only on the equilibrium of the two end states, viz. the reference state \(R\) that is at its own equilibrium initially and the equilibrium of the perturbed state \(P\) that is discovered by relaxation trajectories.
separate article, and further development of this method for biological systems may pave the way toward a fast free energy scan, using only a very limited amount of sampling.

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