Weighted ensemble dynamics simulations for detecting metastable states

Linchen Gong\textsuperscript{1,2} and Xin Zhou\textsuperscript{1,3}

\textsuperscript{1}Asia Pacific Center for Theoretical Physics, Pohang, Gyeongbuk 790-784, Korea
\textsuperscript{2}Center for Advanced Study, Tsinghua University, Beijing 100084, China
\textsuperscript{3}Department of Physics, Pohang University of Science and Technology, Pohang, Gyeongbuk 790-784, Korea

(Dated: December 2, 2009)

Based on multiple short molecular dynamics simulation trajectories that start from dispersively selected initial conformations, we have designed the weighted ensemble dynamics (WED) method [Phys Rev. E 80, 026707 (2009)] to robustly and systematically explore complex conformational spaces through the spectral analysis of covariance matrix of trajectory-mapped vectors. In WED, the non-degenerate ground state of the covariance matrix directly predicts the ergodicity of simulation data, and the ground state can be adopted as the statistical weights of trajectories to correctly reconstruct equilibrium properties, even though each trajectory only explores part of the conformational space. Otherwise, the degree of degeneracy simply provides the number of the kinetically isolated meta-stable states of the system under the time scale of the individual trajectory. Manipulations on the eigenvectors of the matrix with the first a few small eigenvalues leads to the classification of trajectories into non-transition ones within the metastable states and transition ones between these states. The transition state ensemble may also be predicted only based on these eigenvectors without a priori knowledge of the system. Here, we have further improved the WED method. Several schemes have been designed respectively for depressing the statistical error, systematically selecting the physical variables in analysis, and determining the only adjustable parameter of WED. Besides, we illustrate the application of WED in more complicated examples, including a system with a transition network among multiple states, a system with entropy-dominated states, and a solvated alanine dipeptide system at low temperature. WED is found to correctly capture the topology of the transition network. It can also identify the solvent effect for the solvated alanine dipeptide system, as well as the diffusion-like dynamical behavior both in the entropy-dominated system and in the low-temperature solvated alanine dipeptide. These examples will serve as the references for the application of WED to more complicated biological macromolecules.

PACS numbers: 02.70.Ns; 87.15.A-; 82.20.Wt

I. INTRODUCTION

Exploring the high-dimensional conformational space of molecular systems has long been the focus of computer simulation studies. Two challenges exist under this topic, i.e., how to thoroughly sample the conformational space, and how to understand the equilibrium (and dynamic) properties based on lots of simulation data in hand. Although standard Monte-Carlo (MC) and molecular-dynamics (MD) simulation methods have been widely applied to sample the equilibrium conformational distribution, for many complex systems, such as glassy systems, phase-transition systems, and solvated biopolymers, sufficient sampling is by no means an easy task. With increased degrees of freedom and finite (low) temperature, the potential energy landscapes of these systems are characterized by exponentially growing local energy minimums that are separated by large energy barriers (compared to thermal energy $k_B T$, where $k_B$ is the Boltzmann’s constant and $T$ is the temperature). A rough energy landscape frequently traps the simulation trajectories in a small area of the conformational space, disapproving the thorough exploration of the whole space within limited simulation time. Once this happens, the ergodicity of the simulation data is effectively broken, e.g., the time average of some physical quantities can not converge to their average values within the equilibrium ensemble. To overcome these difficulties, beyond the standard MD and MC sampling algorithms, various advanced techniques\cite{1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22,23} have been developed to more efficiently sample systems with rugged energy (or free-energy) surface. Reviews of the advanced simulation techniques can be found in reference\cite{24,25,26,27}. These methods can prominently accelerate the exploring in conformational spaces, while preserving the ability to reproduce the correct equilibrium distributions. Despite the success already achieved, it is still difficult to thoroughly investigate a practically interesting system even based on these advanced techniques. Based on multiple parallel generated trajectories, the ensemble dynamics (ED) method\cite{28,29,30} provides an alternative way for abridging the gap between simulation power and practical interest. Benefiting from the growing power of massively distributed computation, the ED method can reduce the wall-clock time required for data production. Furthermore, if the ED trajectories were spawned from dispersively selected conformations, then compared to single-trajectory simulations, larger area in conformational space might be covered by the ED simulations within the same CPU time. Besides, the multiple simulation trajectories that start from different conformational regions enable the ergodicity test of the simulation data. For example, the measure of ergodicity
could be defined as $\bar{R}^A(t)$ and $\bar{R}^B(t)$ are the time average of $R_i$ over the beginning $t$ time length of the trajectories that respectively start from conformation $A$ and $B$. For ergodicity-broken simulations, $d(t)$ will converge to a non-zero value without prominent decreasing in a limited simulation time, while for an ergodic and converged simulation, this function will relax to zero.

The proper analysis and understanding of the simulation data may be as important as the data sampling itself, particularly for complex systems with lots of degrees of freedom. Except for testing the ergodicity of simulation data, the major purpose in data analyzing is to search for a coarse-grained description of the conformational space. In the traditional way, a few (usually only one or two) knowledge-based reaction coordinates (or order parameters) are first defined, then the equilibrium (and dynamic) properties of the system are achieved by mapping sampled conformations in the low-dimensional space spanned by the presumed reaction coordinates. However, this method may suffer from the arbitrariness in the selection of the reaction coordinates and the oversimplified description of the system. Recently, a more systematic, conformation-network-based view of dynamics brings prominent improvement. In the kinetic network models, the conformational space is divided into some meta-stable regions. If the transition events between these regions were approximated with first-order reactions, the whole system could be coarse-grained into a Markov transition network. In the meantime, the Markov network can be found in the current review. Being theoretically transparent and inclusive of nearly all the useful information, the network model has received much attention.

The three aspects of sufficient sampling, ergodicity testing, and data mining are quite relevant to each other. To establish such a network, it is necessary to thoroughly sample the conformational space and to suitably analyze the simulation data. The understanding of metastable states is definitely helpful for guarding more efficiently sampling. In the previous work, we have proposed a general method, weighted ensemble dynamics (WED), as an integrated view of these challenges. WED is based on multiple short MD (or MC) simulation trajectories, which are started from dispersively-chosen initial conformations, and generated under the same simulation condition (such as the same Hamiltonian, temperature, pressure, in one word, the same equations of motion). Some of these trajectories may reach local equilibrium within metastable states, the other may happen transition between these states. Although each single trajectory is too short to reach the global equilibrium, the trajectories as a whole may have covered whole the conformational space, and should be able to be combined to reproduce the equilibrium distribution of the system. The combined analysis of parallel trajectories already exists, such as the weighted histogram analysis method (WHAM)\cite{36}. In WHAM, the energetic density of state (DOS) of the simulation system is first estimated by analyzing several trajectories simulated at different temperatures, then the physical properties are derived out with the DOS. For trajectories simulated under the same condition, WED provides a simpler way for combining trajectories, i.e., specifying each trajectory a statistical weight. Besides, WED is capable to establish the network of metastable states, as well as to test the ergodicity of simulation data.

In current paper, we will further improve and validate the WED method by: (i) discussing the approximations in WED and providing a practical scheme to select the unique adjustable parameter in WED; (ii) providing an improvement of WED to depress the statistical errors; (iii) illustrating the application of WED to some more extremal examples, such as, a system with a transition network among multiple states, a system with an entropic state (i.e., a state with large entropy and long intrastate equilibration time), and the solvated alanine dipeptide simulated both at room temperature and at low temperature. We find that WED can correctly detect the topology of the transition network, predict the diffusion-like dynamical property for both the system with an entropic state and the dipeptide simulated at low temperature. More importantly, we apply WED to separately analyze effects of different aspects (degrees of freedom, interactions etc.) in metastable states and dynamics of complex systems, such as the solvent effects in the solvated dipeptide. These examples will serve as the references for the application of WED to more complicated systems.

The article is organized as follows. In sect. II we rederive the central results of WED which was briefly presented in our previous paper\cite{32}. In sect. III we discuss approximations of WED, such as, the completeness of basis functions, the effects of the unique free parameter, and present some tactics for facilitating applications of WED to improve statistical errors. In sect. IV we discuss the application of WED in solved dipeptide at room temperature and low temperature. A short conclusion is given in sect. V. Finally, different trajectory re-weighting methods, and application of WED in a system with multiple-state transition network and a system with entropic-dominated states are presented in the Appendix.

II. THEORY OF WED

Most of the simulation algorithms can fit into the framework of probability evolution,

$$P(\vec{q},t) = \int G(\vec{q},t;\vec{q}',0)P(\vec{q}',0)d\vec{q}' \quad (2)$$
Here $P(\vec{q}, t)$ is the probability density function of the system at time $t$, with $\vec{q}$ (and $\vec{q}'$) denoting the generalized conformational coordinates. $G(\vec{q}, t; \vec{q}', 0)$ is the propagator of the system under the applied simulation algorithm. The initial probability density function, $P_{\text{init}}(\vec{q}) \equiv P(\vec{q}, 0)$, is propagated to $P(\vec{q}, t)$ by $G(\vec{q}, t; \vec{q}', 0)$, and will reach the stationary distribution, $P_{\text{stat}}(\vec{q}) = P(\vec{q}, \infty)$, with long enough $t$.

For instance, $P_{\text{init}}(\vec{q})$ can be written as $\delta(\vec{q} - \vec{q}_0)$ for a single-trajectory MD simulation. Here $\delta(\vec{q} - \vec{q}_0)$ denotes a Dirac $\delta$-function, and $\vec{q}_0$ is the initial conformation of the simulation. If the trajectory is simulated in the canonical ensemble with temperature $T$, the stationary distribution turns out to be the canonical equilibrium distribution $P_{\text{eq}}(\vec{q}) \propto \exp[-\beta V(\vec{q})]$. Here $\beta = \frac{1}{k_B T}$, and $V(\vec{q})$ is the potential energy of the system. In WED, multiple MD (or MC) trajectories are independently generated from different initial conformations, $\vec{q}_{0i}, i = 1, \cdots, N_t$, and with the same simulation condition, the initial distribution should be expressed as $P_{\text{init}}(\vec{q}) = \frac{1}{N_t} \sum_{i=1}^{N_t} \delta(\vec{q} - \vec{q}_{0i})$. In the following, we will focus on reconstructing the canonical equilibrium distribution $P_{\text{eq}}(\vec{q})$ with multiple MD trajectories that are all with the $\tau$—time length and are generated with the same equations of motion but arbitrarily chosen different initial conformations.

### A. Central results of WED

Except for the possible effect of insufficient sampling, the difference between the conformational distribution of all the multiple short trajectories and the equilibrium distribution $P_{\text{eq}}(\vec{q})$ solely comes from the particular selection of the initial conformations. Since every trajectory is generated by the same simulation algorithm that keeps the desired equilibrium distribution, different conformations in a trajectory should contribute equally to the final stationary property. Thus, we may specify each trajectory $i$ a statistical weight $w_i$ to mimic the equilibrium samples. Then, the average of any physical quantity $A(\vec{q})$ over the equilibrium distribution can be written as

$$\langle A(\vec{q}) \rangle_{eq} = \sum_i w_i \langle A(\vec{q}) \rangle_i \sum_i w_i.$$  

Here $\langle A(\vec{q}) \rangle_i$ and $\langle A(\vec{q}) \rangle_{eq}$ denote the average of $A(\vec{q})$ over the $i$th trajectory and the equilibrium ensemble, respectively. Summation is over all the trajectories. In practice, it is reasonable to discard the initial short (local equilibrating) segment of each trajectory in calculating averages in trajectories. With this scheme, it is possible to estimate equilibrium properties of the system without demanding the equilibration of each trajectory. When distributed computation facility is available, we can independently generate lots of short trajectories in different computer at the same time, thus the wall-clock time required for converged results greatly decrease. Of course, the examination of the convergence and ergodicity of simulation data, and a proper estimation of $\{w_i\}$ are crucial in application.

While the length of simulation trajectories, $\tau$, is not very short, the conformational space could be divided into a few meta-stable regions, wherein a single $\tau$—length trajectory could reach local (inner-region) equilibrium. In other words, a $\tau$—length trajectory may visits one or more conformational regions, but it always reach local equilibrium in each of its visited regions, the inter-region equilibrium may not be reached due to the not long $\tau$. Thus, for trajectories that started from the same meta-stable region, the specified weights - although dependent on the initial conformations - should be approximately identical when reproducing the equilibrium properties. Therefore, we could write

$$w_i \propto \frac{\int_{\alpha(i)} P_{\text{eq}}(\vec{q}) d\vec{q}}{\int_{\alpha(i)} P_{\text{init}}(\vec{q}) d\vec{q}},$$  

where $\alpha(i)$ denotes the meta-stable region, in which the $i$th trajectory is started. Instead of explicitly using Eq. (4), we simply construct a new ensemble of conformations $\{X\} - \{X\}$ - in practice. $X$ is constituted by the conformations in the initial $\tilde{t}$—length of all the simulation trajectories. Within short enough $\tilde{t}$ (compared to $\tau$), each trajectory is supposed to be still exploring the same meta-stable conformational region which it started from, leading to the quite plausible conclusion that $\int_{\alpha} P_X(\vec{q}) d\vec{q} \approx \int_{\alpha} P_{\text{init}}(\vec{q}) d\vec{q}$ for all the meta-stable regions. Here, $\alpha$ denotes the meta-stable region in consideration; $P_X(\vec{q})$ denotes the probability density function of the conformations in $X$. Consequently, $P_X(\vec{q})$, instead of $P_{\text{init}}(\vec{q})$, could be used to estimate the trajectory weights as follows,

$$w_i = \langle \Omega_{X,eq}(\vec{q}) \rangle_i^+.  \quad \quad (5)$$

Here $\Omega_{X,eq}(\vec{q}) \equiv \frac{P_{\text{eq}}(\vec{q})}{P_X(\vec{q})}$, $\langle \cdots \rangle_i^+$ denotes the average over the initial $\tilde{t}$—length segment of the $i$th trajectory, i.e.,

$$\langle A(\vec{q}) \rangle_i^+ \equiv \frac{1}{\tilde{t}} \int_0^\tilde{t} A(\vec{q}_i(t')) dt',  \quad \quad (6)$$

for any conformational function $A(\vec{q})$. Here $\vec{q}_i(t')$ denotes the conformation at $t'$ time in the $i$th trajectory. Heuristically, by averaging $\Omega_{X,eq}(\vec{q})$ over the initial short segment of each trajectory, we are calculating $\{w_i\}$ according to the initial regions of the trajectories, rather than solely by the initial conformations or explicitly by metastable region which the trajectory start from.

Although the analytical expression of $P_X(\vec{q})$ is unknown, considering the general relation

$$\langle \Omega_{X,eq}(\vec{q}) A(\vec{q}) \rangle_X = \langle A(\vec{q}) \rangle_{eq}$$  

for any conformational function $A(\vec{q})$, we could linearly expand $\Omega_{X,eq}(\vec{q})$ with a complete set of conformational
functions (also referred to as basis functions in the following) \( \{ A^\mu(\vec{q}) \} \), as
\[
\Omega_{X,eq}(\vec{q}) = 1 + \sum_\mu a_\mu \delta_X A^\mu(\vec{q})
\]
with the expanded coefficients as
\[
a_\mu = \sum_\nu g_{\mu\nu}(P_X) \langle \delta_X A^\nu(\vec{q}) \rangle_{eq}.
\]

Due to the non-orthogonalization of basis functions
\[
\delta_X A^\mu(\vec{q}) = A^\mu(\vec{q}) - \langle A^\mu(\vec{q}) \rangle_X, \quad g_{\mu\nu}(P_X) = \langle \delta_X A^\mu(\vec{q}) \delta_X A^\nu(\vec{q}) \rangle_x
\]
which is the inverse of the variance-covariance matrix
\[
g_{\mu\nu}(P_X) = \langle \delta_X A^\mu(\vec{q}) \delta_X A^\nu(\vec{q}) \rangle_x
\]
with \( \langle \cdot \rangle_x \) denoting the average over \( X \) samples, should be included. In Eq. (8), the equilibrium ensemble average \( \langle \delta_X A^\mu(\vec{q}) \rangle_{eq} \) actually relies on the trajectory weights \( \{ w_i \} \) through Eq. (3). Thus, substituting Eq. (3) into Eq. (8) gives out the following self-consistent linear equations of \( \{ w_i \} \),
\[
\vec{G}^i \cdot \vec{w} = 0, \quad i = 1, \ldots, N_t.
\]

Here \( \vec{G}^i = (G_{i1}, \ldots, G_{iN_t})^T \), which can be thought as a vector mapped from the \( i \)th trajectory, \( G_{ij} \equiv \Gamma_{ij} - \delta_{ij} + \frac{1}{N_t} \), and
\[
\Gamma_{ij} = \frac{1}{N_t} \sum_\mu \sum_{\nu'} g_{\mu\nu'}(P_X) \langle \delta_X A^\nu(\vec{q}) \rangle_{\mu'} \langle \delta_X A^\nu(\vec{q}) \rangle_{j},
\]
where \( i, j = 1, \ldots, N_t \), and \( \sum_i w_i = N_t \), \( \vec{w} = (w_1, \ldots, w_{N_t})^T \) is the vector of trajectory weights. All the parameters in Eq. (10) could be calculated simply by averaging basis functions over simulation trajectories. Since the current formulation is exempted from any information about \( P_{init}(\vec{q}) \), initial conformations could be arbitrarily selected from different sources, such as coarse-grained simulation, high-temperature simulation, experimental knowledge, or even theoretical conjecture (possibly with short local-relaxation running before using as initial conformations of WED), then the conformational space could be more efficiently traversed and structured.

By minimizing the residual term
\[
I = \sum_i |\vec{G}^i \cdot \vec{w}|^2,
\]
Eq. (10) can be written in a symmetric form, which is better for analytical treatment,
\[
H \vec{w} = 0.
\]

Thus, \( H = \sum_i \vec{G}^i \vec{G}^i \) is the variance-covariance matrix of the trajectory-mapped vectors \( \{ \vec{G}^i \}, (i = 1, \ldots, N_t) \), with at least one zero-eigenvalue as the ground state of \( H \), since \( \sum_i \vec{G}^i = 0 \). If the ground state of \( H \) is non-degenerate, \( \vec{w} \) is uniquely determined by Eq. (12), and can be subsequently applied to reconstruct the equilibrium distribution of the system. However, it is possible the ground state of \( H \) is degenerate, it indicates non-unique weights of trajectories for reconstructing equilibrium distribution. If the simulation trajectories separately visit some separated conformational regions, and there is not any trajectory connect with different regions by transition, the relative weights of the separated regions will be indeterminable from the trajectories. It is indeed the reason the weights of trajectories are non-unique. Thus it is easy to know that the degree of degeneracy of the ground state equals to the number of the kinetically separated states of the system under the current simulation timescale. In practice, we usually align the eigenvectors of \( H \) in terms of their corresponding eigenvalues with an increasing order. The eigenvalues of \( H \) will grow from zero to 1.0, and the number of the eigenvalues that are obviously smaller than 1.0 gives the number of kinetically isolated (or partial-isolated) states in the system under the timescale of the individual trajectory. The eigenvalues closed to 1.0 corresponds to the statistical fluctuations of these simulation trajectories. By projecting the vectors, \( \{ \vec{G}^i \}, (i = 1, \ldots, N_t) \), to the \( H \) eigenvectors with zero (or near zero) eigenvalues, the trajectories are mapped into a low-dimensional space, where the non-transition trajectories in different states and the transition trajectories between these states can be identified easily.

III. DISCUSSION AND IMPROVEMENT OF WED

A. Completeness of basis functions

Although the expansion in Eq. (8) is exact only while the basis set is complete, it is not necessary to include too many basis functions in WED. This is because only the mean values of \( \Omega_{X,eq}(\vec{q}) \) over a large number of conformations rather than whole the high-dimensional function \( \Omega_{X,eq}(\vec{q}) \) itself are required in Eq. (12). In practice, only physically relevant and important quantities of the system need to be selected as basis functions so that the different meta-stable conformational states are effectively distinguished. The selection does not demand much foreknowledge. For biological macromolecules, the important inner coordinates, such as dihedral angles and some interesting atomic pair distances, could be chosen as basis functions to characterize the conformational motion. Various physical quantities, such as the potential energy of the system, solute-solvent interactions, could also be included for the searching of related kinetic or thermodynamic phenomena. The variance-covariance matrix \( g_{\mu\nu} \) will ensure the consistent consideration of different classes of basis functions, and provide the measure of their relative importance (after orthogonalizing the basis functions based on \( g_{\mu\nu} \)). In addition, the number of independent basis functions is also limited by the finite size of \( X \), thus more selected basis functions will be discarded after considering the matrix \( g_{\mu\nu} \), then do not significantly contribute to final results. Therefore, the errors due to finite basis functions might be relevant to the statistical errors of the finite samples. In principle, \( \Omega_{X,eq}(\vec{q}) \) is a (high-dimensional) continuous function
in the conformational space $\vec{q}$, in practice, it should be thought as discrete values of the function in the finite-size $X$ sample, $\{\Omega_{X,eq}(\vec{q}^k)\}$, where $\{\vec{q}^k\}, k = 1, \ldots, M_X$, are conformations in the $X$ sample with the size $M_X$. If supposing the conformational space can be divided into some meta-stable regions, since both $P_X(\vec{q})$ and $P_{eq}(\vec{q})$ are proportion to the equilibrium distribution inside each meta-stable region, $\Omega_{X,eq}(\vec{q})$ is almost step function, which is constant inside each meta-stable conformational region. Therefore, even no many basis functions in expansion of $\Omega_{X,eq}$ still may provide a good approximation of weights of trajectories on basis on Eq. [12].

The difference between two samples (or the corresponding probability density functions), such as $P_X(\vec{q})$ and $P_{eq}(\vec{q})$, can be measured as

$$L^2_{X,eq} = \langle (P_{eq}(\vec{q}) - P_X(\vec{q}))^2 \rangle_X. \quad (13)$$

Here the difference $L^2_{X,eq}$ is non-negative. A nearly zero $L^2_{X,eq}$ indicates the equivalence between $P_X(\vec{q})$ and $P_{eq}(\vec{q})$, and a larger $L^2_{X,eq}$ claims the further necessity for re-weighting the simulation trajectories. It is worth to mention, since $L^2_{X,eq}$ is asymmetric between $P_X(\vec{q})$ and $P_{eq}(\vec{q})$, it is not a normal distance between the two probability density functions (or samples) [44].

Considering Eq. (8), an approximation of $L^2_{X,eq}$ can be written as

$$L^2_{X,eq} \approx S^2_{X,eq} = \sum_{\mu,\nu} g_{\mu\nu} \langle P_X(\vec{q}) \delta_X A^\mu(\vec{q}) \delta_X A^\nu(\vec{q}) \rangle_{eq} \quad (14)$$

with a finite-size set of basis functions $\{A^\mu(\vec{q}), \mu = 1, \ldots, n_m\}$. With enough basis functions included, $S^2_{X,eq}$ will approach $L^2_{X,eq}$, but will never exceed it. Since $L^2_{X,eq}$ is usually undeterminable, the reasonable method to assess the completeness of basis functions is to observe the behavior of $S^2_{X,eq}$ itself. If enough basis functions in certain class, such as the functions of the dihedral angles of a protein molecule, have been included in calculation, $S^2_{X,eq}$ should no longer increase with the number of basis functions, showing a saturation behavior. The reasoning is the same when multiple classes of basis functions are included.

To calculate $S^2_{X,eq}$, the sample for equilibrium distribution $P_{eq}(\vec{q})$, thus the trajectory weights $\{w_i\}$, are necessary. We propose two ways to bypass this difficulty. First, before re-weighting, $P_{eq}(\vec{q})$ can be approximated by the trajectories with equally weighting, i.e., all $\{w_i\}$ are set to 1 at this stage. With this approximation, we roughly select basis functions. Second, after re-weighting, $S^2_{X,eq}$ can be calculated with the resulting trajectory weights. This procedure can be used to test the former selection. If the rigorously calculated $S^2_{X,eq}$ has reached its saturation region with previously roughly selected basis functions, the results should be satisfiable. However, this posterior examination is restricted to the cases that the trajectory weights can be uniquely determined. In practice, the distribution by equally weighting trajectories, denotes as $\tilde{P}$, has the same local equilibrium characteristics in each meta-stable region as $P_X$ and $P_{eq}$, $\Omega_{X,\tilde{P}} \equiv \frac{\tilde{P}(\vec{q})}{\tilde{P}_X(\vec{q})}$ is similarly a step function, which is also constant in each meta-stable region, only the constant values are different from those of $\Omega_{X,eq}$. Thus we believe the a priori test is already enough, and the following results are all calculated by setting $\{w_i\}$ to 1.

Since the statistical errors exist due to the finite sample sizes, $S^2_{X,eq}$ would not strictly stop growing with the number of basis functions, it just increases slowly compared to the initial growing phase with increasing basis functions. Similarly, when large error occurs, $S^2_{X,eq}$ may abnormally increase within the saturation region. To prevent large statistical errors, we design a scheme to get rid of the statistically unreliable basis functions. We can orthogonalize the functions $\{\delta_X A^\mu(\vec{q})\}$, leading to the explicitly identifiable value and error contribution from the orthogonalized basis functions to $S^2_{X,eq}$. In current paper, we implement the Gram-Schmidt orthogonalization method to one-by-one derive out the set of orthogonalized basis functions $\{\delta_X \tilde{A}^\mu(\vec{q})\}$.

$$S^2_{X,eq} = \sum_{\mu} s^2_{\mu}, \quad (15)$$

where

$$s^2_{\mu} = \frac{\langle \delta_X \tilde{A}^\mu(\vec{q})^2 \rangle_{eq}}{\langle \delta_X A^\mu(\vec{q})^2 \rangle_X}. \quad (16)$$

To estimate the error of $S^2_{X,eq}$, we approximately estimate the error of $s_{\mu}$, and combine them by

$$Err(S^2_{X,eq}) = \sqrt{\sum_{\mu} 4s^4_{\mu} Err^2(s_{\mu})}. \quad (17)$$

Here, $Err(\cdot)$ denotes the statistical error of the quantity in brackets. $Err(s_{\mu})$ can be estimated from $Err(\langle \delta_X \tilde{A}^\mu(\vec{q})^2 \rangle_{eq})$ and $Err(\langle \delta_X A^\mu(\vec{q})^2 \rangle_X)$, which are the standard error of sample averaged quantities, and can be estimated by normal statistical methods. For each orthogonalized basis function, $\delta_X \tilde{A}^\mu$, if $Err(s_{\mu})$ is larger than $1|s_{\mu}|$, i.e., the relative error of $s_{\mu}$ is larger than 1.0, this basis function will not be selected in expansion. One-by-one selection will give out the final basis function set and the estimated error of the completeness measure $S^2_{X,eq}$.

The scheme introduced here provides us the knowledge about how many basis functions are enough for WED calculation. After orthogonalization, the contribution of the basis functions to $S^2_{X,eq}$ clearly reflect their relative importance in trajectory re-weighting. The basis functions that contribute more are more important for portraying the difference between $P_X(\vec{q})$ and $P_{eq}(\vec{q})$, and the ones that contribute quite small can be linearly expanded by other existing basis functions. Furthermore, various kinds of basis functions can be safely added to investigate the related phenomena. For illustration, we will show that the basis functions of dihedral angles and those of
inter-atomic distances can be consistently treated in calculation, and the effect of solvent on the conformation of biological macromolecules can be studied by additional basis functions characterizing the solute-solvent relation.

B. Effects in ˜t

There is only one free parameter in WED method, ˜t∗ = t/τ, for collecting the X samples. Large ˜t∗ may bring systematical error to the estimated equilibrium properties, and small ˜t∗ will reduce the sample number in X, leading to a larger statistical uncertainty. ˜t∗ = 0.01 ∼ 0.1 is usually applied in the current work with satisfiable results obtained. We also point out that, for the purpose of discovering the states in conformational space, the results are not very sensitive to ˜t∗.

˜t actually reflects the timescale of the process that we are interested in. If it were set to the single trajectory length τ, then the trajectory weights solved by Eq. (12) will be equal to each other. In this case, we are focusing on the processes with a timescale much longer than τ, thus all the trajectories are still in the relaxation to local equilibrium, and should have the same weight. Usually, if a system can be divided into several metastable states under the focused timescale, ˜t should be selected comparable to or smaller than the smallest life time of these states. Given that the kinetic states of the system are frequently unknown, ˜t should be selected relatively short. At the zero limit of ˜t, the WED method turns into the classical method described by Eq. (A1) with larger weight fluctuation. In practice, it is possible to use shorter and shorter ˜t and check the convergence of the re-weighted free energy surface. Thus, a proper ˜t may be found as a compromise between correctness and statistical reliability (small fluctuation of trajectory weights). Another practical consideration is that, a few percent of τ would be the safe choice for ˜t. This is because the transition events with timescales prominently smaller than τ have reached equilibrium in single trajectory (thus do not need re-weighting), we only need to focus on the ones with time-scales similar to or slightly smaller to τ. In here and former applications, we usually choose ˜t/τ in the interval of [0.01, 0.1], and the results are satisfiable. Supposing the longest τ-comparable inter-state transition time is τm, if τ ≥ τm by a large margin, the simulation time would be longer than necessary, thus, the trajectories can be shortened to realize the considered case (although it is not necessary). If τ ≤ τm by a large margin, it is quite possible that the conformational space is disconnected somewhere. With current simulation data, we can only obtain the knowledge of the state structure in conformational space, instead of the intact equilibrium properties of the system. The considered case will be true for connected sub-regions in the conformational space. We also notice that the state structure of the system is directly reflected from the eigenvalues of H matrix, which is not sensitive to ˜t selection. Thus, to detect the kinetic states of the system, ˜t need not to be very small.

C. Tactic for statistics improvement

For this purpose, we can generate a few trajectories from each initial conformation, and specify the same weight to the trajectories from the same initial conformation. Suppose there are n0 initial conformations (denoted as q10, q20, ..., qn0,0 in the following), and m1, m2, ..., mn0 trajectories initiated respectively from these initial conformations (the total number of trajectories is still Nt), Eq. (10) can be rewritten as

\[ \sum_{k=1}^{n_0} (\sum_j G_{ij} \tilde{T}_{jk}) w_k = 0 \]  

(18)

where wk is the weight for all the mks trajectories initiated from qks. \( \tilde{T} \) is a Nt-by-n0 matrix with zero and unity elements. \( \tilde{T}_{jk} \) is unity only if the jth trajectory starts from the kth initial conformation. Since the number of equations is more than the number of trajectory weights, we estimate Eq. (18) by minimizing I = |GTw|^2. The vector of trajectory weights, \( \tilde{w} \), corresponds to the ground state of matrix H = \( \tilde{T} \tilde{T}^T \). The eigenvalue of the ground state should be zero in principle, but in practice, it might be a small positive value due to statistical errors. For the newly defined H matrix, the number of the degenerated ground states still corresponds to the number of disconnected metastable states under the timescale of the individual trajectory. The existence of a small fraction of transition trajectories can slightly split the ground state of H. We name this method as SI1 in the following of this paper.

Alternatively, we can merge the trajectories with the same initial conformations to a single one, and perform the standard WED analysis. Concretely speaking, we can calculate the average of certain variable in the initial-segment sample set, X, by,

\[ \langle A \rangle_X = \frac{1}{n_s} \sum_{i=1}^{n_s} \langle A \rangle_{q_{a_i}} \]  

(19)

where \( \langle A \rangle_{q_{a_i}} \) means the average of A over the initial segments of all the trajectories with the initial conformation qa. By doing so, more samples are included in calculation, and effectively, both the initial region and the whole area in the conformational space which the trajectory explores become statistically more reliable. This method is named as SI2 in the following. We will illustrate the application of SI1 and SI2 for depressing the statistical noise in the systems with entropic states, where the inter-trajectory difference in the same metastable state is large.
IV. RESULTS AND DISCUSSION

A. Alanine dipeptide at room temperature

For the system of explicitly solvated alanine dipeptide, we generated 500 MD trajectories at 300 K temperature, each with $\tau = 600$ ps length in the NAMD package$^{10}$ with CHARMM27 force field. In the simulations, a few potential barriers were added on the dihedral energy terms of the standard force field to kinetically separate the conformational space at room temperature (about 300 K). The detailed simulation settings can be found in Ref. [12]. The alanine dipeptide molecule, together with the initial conformations of simulation are shown in Fig. 1 where various notations of the atoms, dihedral angles and free energy wells of this molecule are introduced. Here, by selecting different classes of basis functions, we will focus on the effects of different physical variables on the observed substate structure of the system and the improvement of statistical errors.

1. Dihedral angles as basis functions

With thorough investigation, the two dihedral angles $\phi$ and $\psi$ have been recognized as the major coordinates that characterize the conformational motion of alanine dipeptide molecule. We adopted the first-to-fourth order two-dimensional trigonometrical functions of $\phi$ and $\psi$ as the basis functions in our WED analysis. Calculating with this set of basis functions, the 10 smallest eigenvalues of $H$ are shown in Fig. 2(a). The smallest zero eigenvalue is intrinsic due to the construction of $H$. The second eigenvalue corresponds to the kinetic separation between the region with $\phi > 0$ (containing $C_7^{\alpha\phi}$ and $\alpha_L$) and the region with $\phi < 0$ (containing $C_7^{\beta\phi}$ and $\alpha_R$). Consequently, by projecting vectors $\vec{G}_i$ to the second eigenvector of $H$ (i.e., through $\{L_3^7\}$), the trajectories can be clearly classified into the above mentioned two regions. The third eigenvalue of $H$ corresponds to the partially kinetic connection between the $C_7^{\beta\phi}$ and $\alpha_R$ states. Only a fraction of the trajectories in $C_7^{\beta\phi}$ can climb over the free energy barriers to the $\alpha_R$ state, and vice versa. Thus, we may use the $\{L_3^7\}$ values to classify the trajectories that are confined in the super-state involving $C_7^{\beta\phi}$ and $\alpha_R$ into three groups, i.e., the non-transition trajectories in the two potential wells, and the transition trajectories between them. Since the two potential wells are well defined with their internal relaxation time smaller than the transition timescale, we can manipulate the trajectories to predict the transition times and locate the transition state ensemble $^{[12]}$. The fourth smallest eigenvalue of $H$ is also smaller than 1.0. Although it no longer indicates new state in the conformational space and should be taken as the reflection of statistical difference between trajectories, we recently find that, by observing $\{L_4^7\}$, two trajectories that transiently walk into the $\alpha_L$ region can be picked out. One of the two trajectories initially located in the $\alpha_L$ region, then quickly moved into $C_7^{\alpha\psi}$, another just occasionally visited $\alpha_L$. Actually, at the temperature of 300 K, the $\alpha_L$ region seems not very stable with the applied force field. The other eigenvalues of $H$ are very close to 1.0 without observable meaningful information. In the previous paper $^{[12]}$, we also pointed out that the results here can be reproduced only with the twelve first-to-second order two-dimensional trigonometrical functions, i.e., the trigonometrical functions of $\psi$ and $\phi$ (without multiplication to any integer), and the one-time multiplication between these functions of the two adjacent dihedral angles.

2. Inter-atomic distances as basis functions

After introducing the major results obtained by analyzing with the functions of dihedral angles, we can begin to test the case that the inter-atomic distances of alanine dipeptide are taken as the basis functions. We will compare two aspects, i.e., the eigenvalues of $H$ and the manipulations on $\{L_3^7\}$ for extracting the kinetic information between $C_7^{\alpha\phi}$ and $\alpha_R$. There are 10 non-hydrogen heavy atoms in alanine dipeptide. Except for the directly bonded atoms, 36 inter-atomic distances exist between the non-hydrogen heavy atoms. Together with the two distances for the possible intramolecular hydrogen bonds (see Table I), we select 38 distances as basis functions. The resulting eigenvalues of $H$ are shown in Fig. 2(a). While the three smallest eigenvalues are almost the same with the results obtained with the functions of dihedral angles, the fourth eigenvalue is prominently lifted to a value closer to 1.0. However, the discrimination of the two $\alpha_L$-visited trajectories is still possible. We plot the $\{L_3^7\}$ values calculated with the trajectories truncated to 80-percent length (i.e., the 0.27 ending segment of the trajectories are discarded in analysis) versus the ones calculated with full trajectories $^{[12]}$ in Fig. 2(d). For comparison, the same figure generated with the first-to-second order two-dimensional trigonometrical functions are shown in Fig. 2(c). The two figures have quite similar structure. The points corresponding to the non-transition trajectories in $\alpha_R$ and $C_7^{\alpha\phi}$ are plotted as upward and downward (black) triangles, respectively. The points corresponding to the transition trajectories that start from $\alpha_R$ and $C_7^{\alpha\phi}$ are plotted as (red) squares and (blue) circles, respectively. The points along the inclined dashed lines correspond to the trajectories with only one time transition, and the points along the horizontal dotted lines correspond to the trajectories that do not happen transition within the initial 0.8$t$. Thus the intersection between the dotted and dashed lines correspond to a single-transition trajectory with its transition happened exactly at 0.8$t$. These points can be used to predict the transition times of the single-transition trajectories, consequently the transition state ensemble can be constructed. All the other points correspond to the tra-
jectories with even number of transitions which all happen within 0.8τ, or with early transitions occurred within τ, or the multiple transition trajectories with transition happened both within 0.8τ and after 0.8τ. More details about detecting transition time thus transition conformation ensemble from the truncated-trajectory plots are presented in Ref. [42]. The similarity between Fig. 2(c) and Fig. 2(d) suggests that the inter-atomic distances can also be applied as basis functions for the current system.

3. Completeness of basis functions

When the functions of dihedral angles and the inter-atomic distances are both included as basis functions, we get similar results on the eigenvalues of $H$ and the projection values. This property can be predicted by $S^2_{X, eq}$. The $S^2_{X, eq}$ calculated with different number of basis functions are plotted in Fig. 2(b). Two curves are shown. In one of the two curves, the first-to-fourth order trigonometrical functions are taken into account before the 38 distances, the order between these two classes of basis functions is reversed in another curve. As can be seen, the two curves reach the same $S^2_{X, eq}$ within statistical error. Apparently, before changing to the other group of basis functions, the functions of dihedral angles account for larger value of $S^2_{X, eq}$ than the inter-atomic distances. This is because the inter-atomic distances are not the intrinsic coordinates of the system, although they can also be adopted to detect the various states in the conformational space, but the characterization is not so definite as the dihedral angles do. Thus, even the distances has been taken into account, there is still independent and informative part in the trigonometrical functions, leading to the further increase of $S^2_{X, eq}$. Instead, when the trigonometrical functions have been considered, the distances become non-informative (due to the orthogonalization process), this explains the fact that lots of basis functions are discarded by error analysis in this case [see the different number of basis functions in the two curves in Fig. 2(b)]. From Fig. 2(b), we also find that either with the first 12 basis functions of dihedral angles (first-to-second two dimensional trigonometrical functions of $\phi$ and $\psi$) or the first 12 inter-atomic distances (the distances between main chain heavy atoms separated by two atoms, the distances between main chain and side chain heavy atoms separated by two atoms, and the distances between side chain heavy atoms), $S^2_{X, eq}$ has already entered into the phase with slower increase. This explains why the results obtained by 12 trigonometrical functions are similar with the results with 40 ones. We also plot the eigenvalues of $H$ calculated by 12 inter-atomic distances in Fig. 2(a). The fourth eigenvalue is further lifted, consequently the identification of $\alpha_4$-visited trajectories by $\{L^4_i\}$ is not possible. However the behavior of $\{L^2_i\}$ and $\{L^3_i\}$ is qualitatively similar to the previous results (data not shown).

We also try to add the dihedral angle $\omega$ (see Fig. 1) into analysis, which has been found to occasionally happen cis-trans transition at high temperature [40]. By adding the sine and cosine functions of $\omega$ itself and its correlation to $\phi$, no difference is found in analysis. Indeed, in our simulation, the conformations are all found to have trans-$\omega$ angles.

As a summary, we have compared the results of the WED analysis with basis functions either of the dihedral angles or the inter-atomic distances. The previous results obtained with dihedral angles can be reproduced with inter-atomic distances. However, both from the spectral properties of $H$ and from the $S^2_{X, eq}$ term, we conclude that the distances are not as accurate as the dihedral angles for describing the structure of current model system. The results obtained with the inter-atomic distances may get worse when studying larger protein molecules, since the selection of characteristic inter-atomic distance becomes more complicated. Thus, for the application of WED method to proteins, we suggest to use the functions of the dihedral angles as the preferential basis functions. For two adjacent main chain dihedral angles, the first-to-second order two-dimensional trigonometrical functions are the proper choice. If only the correlation between adjacent dihedral angles were taken into account, the number of basis functions would be proportional to the chain length of the simulated molecule, which is practical for implementation.

4. The effects of solvent molecules

For solvated biological macro-molecules, the conformational change is sometimes correlated to the surrounding solvent molecules. In WED, since the solvent-related properties can also be selected as basis functions, it becomes possible to systematically one-by-one check and filter out the variables that may affect the thermodynamic and kinetic properties of the solvated molecule. Here, for illustration, we study the solvated alanine dipeptide system with 91 basis functions to search for the solvent-related effects. These basis functions are classified into three groups, which respectively reflect the internal freedom of alanine dipeptide (denoted as P), the solute-solvent relation (denoted as PW) and the structure of bulk solvent (denoted as W). The details of these basis functions are listed in Table 1. The eigenvalues of $H$ are shown in Fig. 3(a). As can be seen, at 300K temperature, the inclusion of the solvent-related basis functions does not affect the spectral property of $H$. Particularly, the eigenvalues that are prominently smaller than 1.0 do not change, reflecting the unchanged physical division of the conformational space. Only a few eigenvalues pretty close to 1.0 are slightly lowered due to the added basis functions. The above results suggest that, for the current system, the solvent related properties have almost reached equilibrium in single trajectory. Consequently, these properties contribute quite a little to the inter-trajectory difference, and are unlikely to be strongly
coupled with the dynamic process of the solute molecule under the simulated timescale.

**B. Alanine dipeptide at low temperature**

We also apply the WED method to the low-temperature simulations of the solvated alanine dipeptide. First, the 500 conformations selected from a 600 K simulation in the NAMD package[46] are first relaxed by simulating at 150 K for 200 ps, then continuously simulate at 150 K for 200 ps, and collect conformations every 0.2 ps for subsequent analysis. Standard CHARMM27 force field is adopted in all the simulations.

We note that, at this temperature, the physical phenomena are quite complicated; the force field and the simulation procedure that we adopt may not reflect the physical reality. Here, we are only meant to illustrate the application of the WED method in the extreme condition, instead of providing physical insight into the system.

The eigenvalues of the \( H \) matrix are calculated with the 91 basis functions listed in Table I and are plotted in Fig. 2(b). Different from the results at 300K, at \( T = 150K \), there are lots of eigenvalues deviating from 1.0, which gradually grows from zero to larger values. Besides, the solvent-related quantities do affect the eigenvalues of \( H \) now, leading to even more small eigenvalues. Correspondingly, the \( S_{X,eq}^2 \) as shown in Fig. 3(d), almost always increases with the number of basis functions. At such a low temperature, the free energy surface becomes quite rough, the diffusive dynamics \([47]\) in the conformational space slows down seriously. As a result, the simulation trajectories may only explore a small region in the conformational space. Some degrees of freedom, like the solvent motion around the solute molecule and the conformational motion perpendicular to the \( \phi - \psi \) plane, are no longer equilibrated in single trajectory. Thus, the inter-trajectory difference becomes larger, more states are detected automatically, and more basis functions are required to expand the rugged distribution of the samples, leading to the abnormal behavior of the eigenvalues of \( H \) and \( S_{X,eq}^2 \).

Manipulating the eigenvectors of \( H \) can provide further information of the simulation data. The projections of \( \{ \bar{G}_i \} \) to the second and the third eigenvectors of \( H \) pick out two completely separated states, \( C_7^{eq} \) and the super-state containing \( \alpha_R \) and \( C_7^{eq} \) [Fig. 4(a)], at current temperature, the \( \phi \) and \( \psi \) angles may not be enough to characterize the conformational motion, thus the notations, \( C_7^{eq}, \alpha_R \) and \( C_7^{eq} \), are used mainly for the purpose of illustration. By the aid of the histogram of \( \{ L_i \} \) [see Fig. 4(a), inset], we can roughly classify the trajectories inside the super-state into three groups, i.e., the trajectories in the leftmost two bins, in the rightmost two bins and the remaining ones. The conformational distribution of trajectories in different groups are shown in Fig. 4(c). The two groups of the leftmost or rightmost bins both possess single peak distribution, either in \( C_7^{eq} \) region or \( \alpha_R \) region, just like the non-transition trajectories identified in the simulations at higher temperature. In contrast, the trajectories in the remaining group have a significant occupation in the intermediate region between \( C_7^{eq} \) and \( \alpha_R \). Obviously, these trajectories are trapped at the intermediate region due to slow dynamics, and are different from the transition trajectories identified with the similar procedure at 300K. We plot the \( \{ L_i \} \) calculated with 80 percent trajectory length with the ones with full trajectory length in Fig. 4(c). The behaviors presented in Fig. 2 no longer exist. In terms of kinetics, the transition between the two regions, \( \alpha_R \) and \( C_7^{eq} \), can neither be approximated as Markovian process nor described with first-order rate constant any more. Consequently, with the current simulation data, it is only possible to reconstruct the intrastate equilibrium distribution in the local regions of conformational space, or to study the more localized kinetic behavior.

Due to the numerous local minimums at the low temperature, the \( \alpha_R \) and \( C_7^{eq} \) regions are just like two entropic states. We also test the statistics improvement method in this system. With one-fifth initial conformations and five trajectories from each conformation, the inter-trajectory difference, thus the statistical noise, is indeed suppressed as shown in Fig. 4(d).

**V. CONCLUSION**

In the current paper, we apply the WED method to a few extremal model systems, including a system with multiple-state transition network, a system with entropy-dominated state, and the solvated alanine dipeptide system. The solvent-related basis functions and the low-temperature properties of the dipeptide molecule are also studied. For the first two examples, WED can correctly resolve the topology of the transition network. For the alanine dipeptide system simulated at 300 K with modified potential, we find the redundant basis functions (including functions of both the dihedral angles and the inter-atomic distances) are consistently treated in the WED analysis, and the inclusion of the solvent-related basis functions gives out the expected results. At pretty low temperature of 150 K, the roughness of the free energy surface is reflected in the results of WED. The conformational space can still be roughly divided into parts, however, the kinetics becomes too complicated to be unambiguously characterized. As a systematic method for exploring the conformational space, WED does not require much priori knowledge and approximation, thus its results can honestly reflect the thermodynamic and kinetic information of the system. For biological macromolecules, the usually expected hierarchical structure can be extracted out step-by-step. For these complex systems, the structure of the conformational space may be much more complicated, these examples studied here become quite relevant for physically interpreting the re-
sults. Along with the applications, we design the $S^2_{X,eq}$ quantity for checking the completeness of the selected basis functions, and the schemes for statistics improvement. These tactics are also proved to be helpful for portraying the conformational space.

Acknowledgments

Authors acknowledge the Max Planck Society (MPG) of Germany and the Korea Ministry of Education, Science and Technology (MEST) for the support of the Independent Junior Research Group at the Asia Pacific Center for Theoretical Physics (APCTP) in Korea. L.G. is also supported by National Basic Research Program of China (973 Program) No. 2007CB935903.

APPENDIX A: TRAJECTORY-REWEIGHT SCHEME

1. Direct weighting of trajectories from analyzed distributions

Suppose we already know the initial distribution, $P_{\text{init}}(\vec{q})$, of the simulation trajectories, it is possible to assign each trajectory a weight

$$w_i = \frac{P_{eq}(\vec{q})}{P_{\text{init}}(\vec{q})}_{\vec{q}=\vec{q}_0}, \quad \text{(A1)}$$

where $\vec{q}_0$ is the initial conformation of the $i$th trajectory. If $P_{\text{init}}(\vec{q})$ is the summation of a finite number of $\delta$-functions, this method leads to trajectory weights with singular value. However, if the initial conformations are selected from a known distribution $P_0(\vec{q})$, $P_{\text{init}}(\vec{q})$ can be approximated by $P_0(\vec{q})$ to avoid the singularity. For example, suppose the simulation system has the potential energy $V(\vec{q})$, and the initial conformations are selected from the simulation with the potential energy $U(\vec{q})$, which might be a more flat version of $V(\vec{q})$ for improving the sampling efficiency, then the weight of the $i$th trajectory should be $w_i = \exp[\beta U(\vec{q}_0) - \beta V(\vec{q}_0)]$. This weighting scheme is actually proper for trajectories with arbitrary length $\tau$. In the limit of $\tau \to 0$, it reduces to the classical scheme for regenerating the desired distribution from a simulation with a modified potential [24]. However, since the energy fluctuation for a system with particle number $N_S$ is approximately proportional to $\sqrt{N_S}$ for large systems, the weights in Eq. (A1) suffer from huge fluctuation. It is quite possible that the weights of a few trajectories prominently outweigh the others, then only the samples in these trajectories contribute to the final results, while the significance of the other trajectories has been depressed to nearly zero. Besides, the substitution of the initial distribution function combined by $\delta$-functions by an analytical distribution is only valid when a large number of trajectories are generated for analysis.

2. Weighting of not-so-short trajectories

One should notice that there are actually more than one appropriate weighting schemes for the same set of simulation trajectories. Taking the long time limit of the trajectory length $\tau$ to ensure that every trajectory has been in equilibrium over the whole conformational space, then the trajectories should be assigned with equal weights. This choice brings the largest effective sample size, thus is better than Eq. (A1). When the simulation time is neither zero nor long enough, it is plausible to expect that the proper weights for the trajectories will gradually relax from the ones in Eq. (A1) to the case of equal weights as simulation time increases. This conjecture can be explained with probability evolution. As we all know, the relaxation from $P_{\text{init}}(\vec{q})$ to $P_{eq}(\vec{q})$ is a process with multiple timescales, some events may relax faster than the others. Usually, the temporal hierarchy is highly correlated to the conformational degrees of freedom, i.e., the relaxation processes with short timescales are related to local conformational adjustment, and the ones with long timescales are related to global conformational transformation. Consequently, if each $\tau$-length trajectory is able to reach local equilibrium within certain conformational region (metastable state), then only the overall difference between $P_{\text{init}}(\vec{q})$ and $P_{eq}(\vec{q})$ in each local state need to be considered in weight estimation, making it possible to reduce the fluctuation of trajectory weights.

In practice, within a short time $t$ at the beginning of a simulation trajectory, some fast degrees of freedom of conformational motion may have relaxed, thus, intuitively, the trajectory has approximately reached equilibrium within certain local area, $\alpha$, around its initial conformation. If the initial conformations of two trajectories are so close in the conformational space, that both of the two trajectories explore the same area within $\tilde{\tau}$, then the difference between the two trajectories in terms of the initial conformation should be almost wiped off, leaving the two trajectories contributing nearly the same to the equilibrium properties. Generally speaking, for the trajectories that start and stay in the same area within short $\tilde{t}$, similar trajectory weights should be assigned with prominently reduced fluctuation. The number of trajectories that start in a local area $\alpha$ is actually proportional to $\int_{\alpha} P_{\text{init}}(\vec{q}) d\vec{q}$, i.e., the integration of the initial distribution over $\alpha$. However, if the initial conformations are selected from the desired equilibrium distribution, the number of trajectories that start in this region should be proportional to $\int_{\alpha} P_{eq}(\vec{q}) d\vec{q}$. Consequently, we can specify the weight

$$w_\alpha = \frac{\int_{\alpha} P_{eq}(\vec{q}) d\vec{q}}{\int_{\alpha} P_{\text{init}}(\vec{q}) d\vec{q}} = \frac{\langle P_{eq}(\vec{q}) \rangle^{-1}_{eq,\alpha}}{\langle P_{\text{init}}(\vec{q}) \rangle^{-1}_{eq,\alpha}} \quad \text{(A2)}$$

to all the trajectories from $\alpha$ region. Here, $\langle \rangle_{eq,\alpha}$ denotes the average over the equilibrium distribution restricted in the $\alpha$ region. Considering the plausible local equili-
Considering the general relation is approximately proportional to certain extent. However, for large systems, this is for each trajectory, the fluctuation of. We will further discuss this issue in Sec. III B.

Notes the ensemble average of for any conformational function. For systems like solvated biological macromolecules, the large fluctuation usually does not come from the physically important degrees of freedom. For example, the solvent molecules account for most of the energetic fluctuation of the system. In the same time, the physically interesting degrees of freedom, say the conformation of a macromolecule, are determined by its own internal coordinates, such as dihedral angles, inter-atomic distances and so on. Thus, we can expand the term only by quantities that are physically interesting to get rid of the irrelevant fluctuations. To fulfill this aim, we define an inner product between two conformational functions, $A(\vec{q})$ and $B(\vec{q})$, as

$$ (A(\vec{q}), B(\vec{q})) = \int d\vec{q} P_{eq}(\vec{q}) A(\vec{q}) B(\vec{q}). $$  

(A4)

Considering the general relation

$$ \left\langle \frac{P_1(\vec{q})}{P_2(\vec{q})} A(\vec{q}) \right\rangle_2 = \langle A(\vec{q}) \rangle_1 $$  

(A5)

for any conformational function $A(\vec{q})$ [where $\langle A(\vec{q}) \rangle_k$ denotes the ensemble average of $A(\vec{q})$ under the probability density $P_k(\vec{q})$], a set of physical quantities $\{A^\mu(\vec{q})\}$ also named as basis functions in the follows of the paper) are selected to approximate $\frac{P_{init}(\vec{q})}{P_{eq}(\vec{q})}$ as

$$ \frac{P_{init}(\vec{q})}{P_{eq}(\vec{q})} \approx 1 + \sum_{\mu,\nu} g_{\mu\nu} \langle \delta_{eq} A^\mu \rangle_{init} \delta_{eq} A^\nu(\vec{q}). $$  

(A6)

Here $g_{\mu\nu}(P_{eq})$ is the inverse of the covariance matrix of the basis functions, $g_{\mu\nu}(P_{eq}) \equiv \langle \delta_{eq} A^\mu \delta_{eq} A^\nu \rangle_{eq}$, and $\delta_{eq} A(\vec{q}) \equiv A(\vec{q}) - \langle A(\vec{q}) \rangle_{eq}$. Substituting Eq. (A6) into Eq. (A3), the trajectory weights are found to satisfy the following self-consistent equations.

$$ w_i = \left\{ 1 + \sum_{\mu,\nu} g_{\mu\nu} (P_{eq}) \langle \delta_{eq} A^\mu \rangle_{init} \delta_{eq} A^\nu(\vec{q}) \right\}_i^{-1}. $$  

(A7)

Here, the time average of the function $\delta_{eq} A^\nu(\vec{q})$ over the initial $t$-length segment of the $i$th trajectory has been written as $\langle \delta_{eq} A^\nu \rangle_{init}$. For any function of conformational coordinates, the average over the distribution function, $P_{init}(\vec{q})$, can be calculated with the samples that are adopted for selecting the initial conformations. The average over distribution $P_{eq}(\vec{q})$, however, relies on the trajectory weights through Eq. (8). Consequently, an iterative method should be invoked to calculate $\{w_i\}, (i = 1, \ldots, N_t)$. Since $\frac{P_{init}(\vec{q})}{P_{eq}(\vec{q})}$ is now projected to the hyperplane spanned by the selected basis functions $\{\delta_{eq} A^\mu(\vec{q})\}$, the calculated trajectory weights can be used to reconstruct the free energy surface along the selected degrees of freedom. Eq. (A7) is already practical for getting reasonable trajectory weights with small fluctuation. However, there are still two drawbacks of this method. First, we need enough samples of the distribution $P_{init}(\vec{q})$. If the initial conformations are chosen from high temperature simulation, then all of the conformations in the high temperature trajectory can be utilized. However, if the initial conformations are chosen from coarse-grained simulation, or simply from arbitrary selection, the estimation of $\langle A(\vec{q}) \rangle_{init} \approx \frac{1}{N_t} \sum_i A(\vec{q}_i)$ might be insufficient if $N_t$ (the number of trajectories) is not large, leading to problematic application of Eq. (A7). Second, the iterative calculation hampers the in-depth analysis of the simulation system. When the conformational space has been separated into different free-energy wells without any intercommunication within the finite simulation time, the iterative method may still provide an incorrect answer without pointing out the broken ergodicity.

4. Weighting scheme based on a linear equation: WED

Considering the shortcomings of Eq. (A7), we design the final framework of WED, which, to a large extent, solves the above mentioned problems, and is still able to find out the fluctuation-reduced trajectory weights.
Here, instead of using the sample of $P_{init}(\vec{q})$, we artificially construct a new sample set $X$ with its distribution symbolically denoted as $P_X(\vec{q})$. $X$ consists of the initial $t$-length segments of all the trajectories. If $t$ is short enough (compared to $\tau$), the trajectories only explore their local area within $\tilde{\tau}$. Thus, the number of conformations in each local area, $\alpha$, does not change after the $t$ relaxation, i.e.,

$$\int_0^\alpha P_{init}(\vec{q})d\vec{q} \approx \text{const.}, \quad \forall \alpha. \quad (A8)$$

Then, substituting $P_{init}(\vec{q})$ with $P_X(\vec{q})$ in calculation, the expression of the trajectory weights will become

$$w_i = \frac{1}{t} \int_0^t \frac{P_{eq}(\vec{q})}{P_X(\vec{q})}|_{\vec{q}=\vec{q}(t)}dt = \left\langle \frac{P_{eq}(\vec{q})}{P_X(\vec{q})} \right\rangle_{i+} \quad (A9)$$

Since the sample set $X$ is supposed to reach local equilibrium in each $\alpha$ region (but may not reach equilibrium among these regions), we are able to get Eq. (A9) without the inverse sign in Eq. (A3). After redefining the inner product between two conformational functions as

$$(A(\vec{q}), B(\vec{q})) = \int d\vec{q} P_X(\vec{q}) A(\vec{q}) B(\vec{q}), \quad (A10)$$

the $\frac{P_{eq}(\vec{q})}{P_X(\vec{q})}$ term in Eq. (A9) can be expanded by a set of basis functions similar to Eq. (A6).

$$\frac{P_{eq}(\vec{q})}{P_X(\vec{q})} \approx 1 + \sum_{\mu,\nu} g_{\mu\nu}(P_X) \langle \delta X A^\mu(\vec{q}) \rangle_{eq} \delta X A^\nu(\vec{q}). \quad (A11)$$

Finally, similar to Eq. (A7), substituting Eq. (A11) into Eq. (A9), and considering the reliance of the average over equilibrium ensemble on the trajectory weights, we have the following set of linear equations,

$$w_i = 1 + \sum_j \left\{ \frac{1}{N_t} \sum_{\mu,\nu} g_{\mu\nu}(P_X) \langle \delta X A^\mu(\vec{q}) \rangle_{i+} \langle \delta X A^\nu(\vec{q}) \rangle_j \right\} w_j, \quad (A12)$$

where $\sum_j w_j = N_t$. It is direct to derive the central equation of WED, Eq. (12), from Eq. (A12).

**APPENDIX B: HEURISTIC DERIVATION OF THE SPECTRAL PROPERTY OF $H$**

We also provide a heuristic derivation of the spectral property of the $H$ matrix. We define the element of a matrix $\Gamma$ as,

$$\Gamma_{ij} = \frac{1}{N_t} \left\langle \frac{P_{s(i)}(\vec{q})}{P_X(\vec{q})} \right\rangle_{s(j)} \quad (B1)$$

where $i+$ (or $j+$) represents the short initial segments ($t$-length) in the $i$th (or $j$th) simulation trajectory. If $\tilde{t}$ is short enough that most of the trajectories are still confined in their local area, and in another sense long enough that local equilibrium is almost obtained, the elements of the matrix $\Gamma$ can be expressed as

$$\Gamma_{ij} = \frac{1}{N_t} \left\langle \frac{P_{s(i)}(\vec{q})}{P_X(\vec{q})} \right\rangle_{s(j)} . \quad (B2)$$

Here $P_X$ is the distribution function of the $X$ sample set, $s(i)$ represents the state in which the initial segment of the $i$th trajectory locates, and $P_{s(i)}$ is defined as

$$P_{s(i)}(\vec{q}) = \frac{P_X(\vec{q}) \chi_{s(i)}(\vec{q})}{\int d\vec{q} P_X(\vec{q}) \chi_{s(i)}(\vec{q})}, \quad (B3)$$

where $\chi_{s(i)}(\vec{q})$ is the characteristic function of state $s(i)$, which is non-vanishing with value 1 only inside the $s(i)$ region. Thus, $\Gamma_{ij}$ is not zero only if $s(i)$ and $s(j)$ are the same state. Suppose that there are $n$ states in the system, i.e., $s_1, s_2, \ldots, s_n$, and there are $m_1, m_2, \ldots, m_n$ trajectories initially locating in these states, respectively. The matrix $\Gamma$ becomes a block diagonalized matrix with proper numbering of the trajectories.
The dimension of the sub-matrix with elements $\frac{1}{m_i}$ is $m_i$. For this matrix, the eigenvalue 1.0 is in fold degenerate and the eigenvalue 0 is $N_1 - n$ fold degenerate. The matrix defined as $H' = (\Gamma - I)^T (\Gamma - I)$ has $n$ eigenvalues equal to 0 (equal to the number of states) and $N_1 - n$ eigenvalues equal to 1.0. Where $I$ is a $N_t$-dimensional unit matrix. Another matrix $\tilde{\Gamma}$ is defined as

$$\tilde{\Gamma}_{ij} = \frac{1}{N_t} \{1 + \Sigma_{\mu,\nu} g_{\mu\nu} (P_X) A^\mu (q) A^\nu (q) \}_{i+} (\delta_X A^\nu (q))_j.$$  

(B4)

The only difference between $\tilde{\Gamma}$ and $\Gamma$ is that the average over the initial segments of trajectory $j$ has been sub-

tuted into the average over the whole trajectory. Consequently, the possible transition events in trajectory $j$ will be reflected in the matrix elements, and $\tilde{\Gamma}$ is a non-symmetric matrix now. For the simplicity in derivation, we suppose that there are two states, $s_1$ and $s_2$, in the system, with $m_1$ and $m_2$ trajectories initially locating in these two states, and there are $x_1$ (or $x_2$) transition ones amid the $m_1$ (or $m_2$) trajectories. Then the matrix $\tilde{\Gamma}$ should be written as

$$G = \tilde{\Gamma} - I.$$  

(B5)

Here, $\beta_i$ (or $\tilde{\beta}_i$) denotes the time proportion of the $s_1$ (or $s_2$)-started trajectory $i$ in state $s_2$ (or $s_1$). For the trajectories that start from the same state, the row vectors of $\tilde{\Gamma}$ are the same. Thus, the transition and non-transition trajectories can not be differentiated by the row vectors of $\tilde{\Gamma}$. For systems with multiple states, the conclusion is similar. In WED, the matrix $G = \{ \tilde{G}^1, \tilde{G}^2, \ldots, \tilde{G}^{N_t} \}^T$ is directly related to $\tilde{\Gamma}$ as

$$G = \tilde{\Gamma} - I.$$  

(B5)

Hence, the row vectors for the trajectories from the same initial state are no longer the same, the deviation of the transition trajectories from the non-transition ones are linearly related to the distribution of the transition tra-
jectories in different states. For the matrix $H \equiv G^T G$, considering the perturbation of the transition trajectories to the matrix $G$, the number of the eigenvalues that are obviously smaller than 1 should still be the same with the number of the disconnected states in the conformational space. The value of these eigenvalues should deviate from zero due to the transition trajectories. The other interesting properties can also be rationalized by this heuristic proof.

APPENDIX C: WED FOR MULTIPLE-STATE TRANSITION NETWORK AND ENTROPY-DOMINATED STATES

We illustrate the application of WED in two extreme model systems, one with a transition network among multiple states, the other with an entropy-dominated state. The first model system which with Two-Dimension-Quad-Well(TDQW) potential has four degenerate states [four potential wells with exactly the same shape, see Fig. 5(a)], and four potential barriers of equal height separating them apart. The second system which with Mexican-Hat(MHAT) potential has two states [see Fig. 5(b)]. The outer state has large entropy, thus, its intrastate relaxation time may be comparable to the time-scale of inter-state transition. We will first analyze the WED trajectories of these two systems under different temperatures. Then, we will discuss the topics about basis function selection, $t$ selection and statistics improvement.

1. TDQW and MHAT systems

For the two-dimensional systems, TDQW and MHAT, simulations are restricted in the square region of $[-2, 2] \times [-2, 2]$. Reflecting boundary condition is imposed. The over-damped Langevin equations

$$\frac{dx}{dt} = -\frac{1}{\gamma} \frac{\partial U(x, y)}{\partial x} + \sqrt{\frac{2k_B T}{\gamma}} \xi_1(t)$$
$$\frac{dy}{dt} = -\frac{1}{\gamma} \frac{\partial U(x, y)}{\partial y} + \sqrt{\frac{2k_B T}{\gamma}} \xi_2(t)$$

(C1)

is adopted to generate the dynamical trajectories. Where $\gamma$ is the frictional coefficient, $k_B$ is the Boltzmann constant, $T$ is the simulation temperature, $\xi_1(t)$ and $\xi_2(t)$ are the white noises satisfying $\langle \xi(t)\xi(t') \rangle = \delta(t - t')\delta_{ij}$ with $\langle \rangle$ denoting the ensemble average of noise. We simply take $k_B$ and $\gamma$ as unity to get the dimension-reduced units for time, position and temperature.

The potential of TDQW is

$$U(x, y) = \begin{cases} \infty & \text{if } |x|, |y| > 2 \\ 5(\frac{2}{3}x^2 - 1)^2 + 5(\frac{2}{3}y^2 - 1)^2 & \text{if } |x| \leq 2 \text{ and } |y| \leq 2 \\ 0 & \text{otherwise} \end{cases}$$

The potential of MHAT is

$$U(r) = 40 \cdot \left(\frac{1}{24}r^6 - \frac{2}{9}r^4 + \frac{1}{3}r^2\right), \quad r^2 = x^2 + y^2$$

For both systems, 900 initial conformations are randomly selected if not further mentioned, then WED simulation are performed, with each trajectory simulated for 50 dimensionless time units. The coordinates are recorded every 0.01 time units. The $t/\tau$ is selected as 0.04, if not further mentioned.

2. Multiple-state transition network

For TDQW system, WED simulations are performed at three temperatures of 0.30, 0.85 and 1.50. 900 trajectories with $\tau = 50$ dimensionless time units are generated in each set of WED simulation. The eigenvalues of $H$ are plotted in Fig. 5(a). As expected, there are four zero eigenvalues at temperature 0.3, implying the four disconnected states in TDQW system. At higher simulation temperatures, the second to fourth eigenvalues of $H$ deviate from zero to larger values due to the increasing fraction of the inter-state transition trajectories. More illustrative perspective can be obtained from Fig. 5(b), (c), and (d), where the trajectories are mapped into the three-dimensional space by projecting $\{G_i\}$ to the second, third and fourth eigenvectors of $H$ (the projection to the first eigenvector is always zero; the projection values are denoted by $L_{0i} \equiv \vec{G}_i \cdot \vec{u}_0$, where $\vec{u}_0$ is the $0$th eigenvector of $H$). At temperature 0.3, the trajectories agglomerate into four disconnected clusters that locate respectively at the four vertexes of a tetrahedron. Inside each cluster are the trajectories isolated in the same potential well. At temperature 0.85, part of the trajectories can climb over the potential barriers to the other states. As a result, the points corresponding to the transition trajectories are found to gather along the straight lines that shoot out of the vertexes, and the ones corresponding to the non-transition trajectories are still locating on the vertexes. The transition trajectories along a straight line are found to connect the same two states. These two states respectively correspond to the vertex that emits
the line and the vertex that the line approximately points to. Thus, between the two trajectory-connected vertices (states), there are two parallel lines, respectively corresponding to the transition trajectories that start from different states. In Fig. 8(c), each vertex only connects to two neighboring vertexes, correctly reflecting the topology of the transition network of TDQW system. At temperature 1.5, it is relatively easier for the trajectories to transfer between different states. The tetrahedron structure is still preserved, and its interior is filled by multiple transition trajectories which transition among more than two states. The eigenvalues of $H$ actually provide clues for the preserving of the tetrahedron structure. Since the second to fourth eigenvalues are still not close to 1.0, i.e., the conformational space can not be sufficiently explored by single trajectory, the difference between trajectories in terms of their initial conformations still exists, leading to the structure in the projection space. At higher temperature, the inter-trajectory difference will further reduce, leading to the diminishing of the tetrahedron structure. In that case, it is more proper to assert that there is almost only one state in the conformational space in the current simulation timescale (however, each single trajectory might be not sufficient to reach the global equilibrium and the weights of trajectories are still necessary to reproduce the global equilibrium due to the fact that exists three eigenvalues in the $(0,1)$ interval). In shorter time scales, e.g., when each of the trajectories is shortened, the substate structure could emerges.

Considering the tetrahedron structure and the straight lines in Fig. 8(c), and the small second to fourth eigenvalues of $H$, various kinetic information can be directly extracted from the simulation data of TDQW at $T = 0.85$. The procedure can be (1) arbitrarily picking out the non-transition and transition trajectories in two neighboring states from Fig. 8(c), (2) analyzing these trajectories with the WED method (constructing $H$ and analyzing its spectral properties), (3) projecting the trajectories into low-dimensional space and manipulating the projection values. In the paper, we just focus on the abilities of WED in detecting meta-stable structure, the construction of equilibrium properties of the system based on the obtained weights of trajectories is directed [42]. Some interesting information, such as the transition state ensemble, the first order kinetic rates, and the transition times of single trajectories, can be obtained. The proposed kinetic analysis [42] is based on the assumption that most of the transition trajectories only climb the potential barriers a few times. If, in each single trajectory, the transition events that we are interested in can happen for many times (i.e., a single trajectory almost already reached equilibrium), we directly treat whole the visited region as a super states, or perform a shortening of the simulation trajectories (for example, dividing each trajectory into a few shorter ones) to analyze the detailed kinetics in shorter time scales. The WED method provides much flexibility for such manipulations.

### 3. Entropy-dominated states

For MHAT system, WED simulations are performed at four temperatures of 0.05, 0.30, 0.85, and 1.50, with the same settings as the TDQW system. The 70 smallest eigenvalues of $H$ are shown in Fig. 7(a). Different from TDQW, the eigenvalues of $H$ gradually increase from zero at the temperature of 0.05, and lots of small eigenvalues exist. The number of the eigenvalues that obviously deviate from 1.0 are always more than the number of physical states in MHAT system, till the temperature is high enough that there is only one zero-eigenvalue apparently different from 1.0. This behavior simply reflects the entropic nature of the outer potential well. At low temperature, a single trajectory can not reach local equilibrium in that entropic state, consequently a few small eigenvalues exist due to the inter-trajectory difference (it looks like there are many sub-states). At higher temperature, the trajectories that start from the outer potential well usually climb over the potential barrier before reaching equilibrium in the whole state, thus the inter-trajectory difference will not be extinguished, until the two states in MHAT kinetically become one state at high enough temperature. The projections of $\{G_i\}$ onto the second, third and fourth eigenvectors of $H$ are shown in Fig. 8(b), (c), and (d). At both temperatures 0.05 and 0.30, the trajectories are divided into two groups. In one group, the trajectories dispersively locate on the same plane which is perpendicular to the $L_2^2$ axis. These trajectories are found to be isolated in the outer potential well. In the other group, the trajectories concentrate to nearly one point. They are found to be isolated in the inner potential well. These two groups of trajectories are clearly differentiated by the projections along the second eigenvector of $H$, i.e., the one indicating the most important contribution to ergodicity broken. At temperature 0.05, each trajectory only explore very limited region in the entropic state, leading to the distribution on the perimeter of a circle. At temperature 0.30, since each trajectory can explore larger area, the circle has been filled with points. At temperature 0.85, the trajectories are found to locate in a cone. The points in the bottom surface and the vertex of the cone correspond to the non-transition trajectories inside the outer and inner potential wells, respectively, and the other points are transition trajectories between the two states. Once again, the trajectory-projected space clearly provides the topological information of the transition network of this system.

Interestingly, due to the symmetry of the outer state of MHAT, we can even extract the kinetic information of the transition between the inner and the outer states (data not shown), although it might not be general for other systems with entropy-dominated states.
4. Completeness of basis functions

At temperature 0.85, the $S_{X,eq}^2$ analysis is performed for the two-dimensional systems. We apply the following two-dimensional trigonometrical functions

$$
\begin{align*}
\sin [(m+n)x], \cos [(m+n)x], \\
\sin [(m+n)y], \cos [(m+n)y], \\
m + n > 0 \\
\sin (mx) \sin (ny), \sin (mx) \cos (ny), \\
\cos (mx) \sin (ny), \cos (mx) \cos (ny), \\
m \geq 1, n \geq 1
\end{align*}
$$

(C2)

as basis functions. Where, $(x, y)$ are the two-dimensional Cartesian coordinates multiplied by a transformation factor $\tau$, $m$ and $n$ are non-negative integers. We define the summation of $m$ and $n$ in Eq. (C2) as the order of these functions, and adopt the first-to-fourth order functions (totally 40 basis functions) in our analysis. Based on the error analysis mentioned in Sec. IIIA, three and two basis functions are discarded in the TDQW and MHAT systems, respectively. For both of the two systems, $S_{X,eq}^2$ initially grows fast with increasing number of basis functions, then the growth slows down and shows a saturation behavior of $S_{X,eq}^2$ as expected [see Figs. 3(a) and (b)]. Once $S_{X,eq}^2$ has approximately reached the platform region, more basis functions will not change the calculation results any more (data not shown). Thus, for TDQW and MHAT systems, about 24 basis functions, i.e., the first-to-third order two-dimensional trigonometrical functions, are already enough.

5. The effects of $\tilde{t}$

We study the effect of $\tilde{t}$ with the simulation data of MHAT at temperature 0.85. Since there is only one zero eigenvalue of $H$, the whole free energy surface can be reconstructed. The whole conformational space is uniformly divided into small pieces, $k = 1, \ldots, N_p$ ($N_p$ is selected as 1600 here, i.e., 40 segments along each dimension), and the distribution probability in each piece is reconstructed with different $t^* = t/\tau$. We define the function $\delta_{\text{dist}}(t^*)$ as

$$
\delta_{\text{dist}}(t^*) = \sqrt{\frac{1}{N_p} \sum_{k=1}^{N_p} (P_k(t^*) - P_{k}^{\text{theo}})^2}
$$

(C3)

to measure the difference between the reconstructed probability distribution and the theoretical distribution. Here $P_k(t^*)$ is the distribution probability in the $k$th piece which is reproduced by WED method using $t^*$. $P_{k}^{\text{theo}}$ is the theoretical distribution probability in the $k$th piece. As shown in Fig. 3(c), $\delta_{\text{dist}}(t^*)$ indeed decreases with shorter $t^*$ in calculation. For $t^*$ in the $[0.01, 0.1]$ interval, the $\delta_{\text{dist}}(t^*)$ values are almost the same, and are prominently smaller than the value of the non-weighted distribution of the simulation data. In the non-weighted (all $w_i$ is unity) distribution, the major deviation from theoretical distribution resides in the inner potential well of MHAT, where the distribution probability is too high for the non-weighted data. In the re-weighting process, the trajectories that start from the inner potential well are usually specified lower weights [see Fig. 3(c), inset], thus the over occupation in the inner state can be offset. The other variation in trajectory weights further adjust the intrastate distribution for both the inner and the outer states.

6. Statistics improvement

We also test the statistics improvement methods, SI1 and SI2. In here and below, we always randomly select one-fifth of the initial conformations, then five trajectories are spawned from each conformation to test the improvement. At $T = 0.85$, we apply both SI1 and SI2 to reconstruct the energy surface of MHAT. From Fig. 3(c), it can be seen that both methods correctly reproduce the energy surface with even smaller deviation from the theoretical one, as compared to the standard WED method. At $T = 0.05$, the eigenvalues of the $H$ matrix are calculated with SI2 method. As shown in Fig. 3(d), no matter one trajectory or multiple trajectories are generated from one initial conformation, the eigenvalues calculated by the standard WED method are almost the same. However, analyzing by SI2 method significantly alters the structure of eigenvalues. By generating more trajectories from single initial conformation, and combining these trajectories together in analysis, the statistics of single trajectory is prominently improved, and the inter-trajectory difference for trajectories in the same state (or near in conformational space) are reduced to large extent. As a result, some of the eigenvalues increases, in particular for the ones deviating but close to 1.0. In other words, the SI2 method has depressed the statistical noise in the spectral analysis of $H$, leaving the physically relevant states recognized.

[1] G. M. Torrie and J. P. Valleau, J. Comput. Phys. 23, 187 (1977).

[2] C. Bartels and M. Karplus, J. Phys. Chem. B 102, 865 (1998).
[3] P. Maragakis, A. van der Vaart, and M. Karplus, J. Phys. Chem. B 113, 4664 (2009).
[4] D. D. Frantz, D. L. Freeman, and J. D. Doll, J. Chem. Phys. 93, 2769 (1990).
[5] B. A. Berg and T. Neuhaus, Phys. Lett. B 267, 249 (1991).
[6] E. Marinari and G. Parisi, Eur. Phys. Lett. 19, 451 (1992).
[7] A. P. Lyubartsev, A. A. Martsinovski, S. V. Shevkunov, and P. N. Vorontsov-Velyaminov, J. Chem. Phys. 96, 1776 (1992).
[8] H. Grubmüller, Phys. Rev. E 52, 2893 (1995).
[9] A. F. Voter, Phys. Rev. Lett. 78, 3908 (1997).
[10] R. A. Miron and K. A. Fichthorn, Phys. Rev. Lett. 93, 128301 (2004).
[11] X. Zhou, Y. Jiang, K. Kremer, H. Ziock, and S. Rasmussen, Phys. Rev. E 74, 035701(r) (2006).
[12] Y. Sugita and Y. Okamoto, Chem. Phys. Lett. 329, 261 (2000).
[13] F. Wang and D. P. Landau, Phys. Rev. Lett. 86, 2050 (2001).
[14] Q. Yan and J. J. Pablo, Phys. Rev. Lett. 90, 035701 (2003).
[15] J. Kim, J. E. Straub, and T. Keyes, Phys. Rev. Lett. 97, 050601 (2006).
[16] X. Zhou and Y. Jiang, arXiv: 0811.1829 (2008).
[17] A. Laio and M. Parrinello, Proc. Natl. Acad. Sci. U.S.A., 99, 12562 (2002).
[18] A. Mitsutake and Y. Okamoto, J. Chem. Phys. 130, 214105 (2009).
[19] P. G. Bolhuis, D. Chandler, C. Dellago, and P. L. Geissler, Ann. Rev. Phys. Chem. 53, 291 (2002).
[20] R. Elber, A. Ghosh, and A. Cardenas, Acc. Chem. Res. 35, 396 (2002).
[21] Weinan E, W. Ren, and E. Vanden-Eijnden, J. Phys. Chem. B 109, 6688 (2005).
[22] A. K. Faradjian and R. Elber, J. Chem. Phys. 120, 10880 (2004).
[23] E. Vanden-Eijnden, M. Venturoli, G. Ciccotti, and R. Elber, J. Chem. Phys. 129, 174102 (2008).
[24] D. Frenkel and B. Smit, Understanding Molecular Simulation: From Algorithms to Applications, (Academic Press, Tokyo, 2002)
TABLE I: Basis Function List

| Group                        | Number | Function                                                                 |
|------------------------------|--------|--------------------------------------------------------------------------|
| Internal freedom of alanine  | 40     | Two-dimensional trigonometric functions of φ and ψ.                      |
| dipeptide                    | 8      | N1 – O1, N2 – O2, N1H – O1, N2H – O2 and their squares*.                |
| Interaction between          | 2      | Interaction** between peptide and water.                                |
| solvent                      | 8      | Interaction between O1, O2, N1, N2 and water.                           |
| and solute                   | 2      | Water number around peptide (3.3Å, 5.5Å).                               |
|                              | 1      | Solvent accessible surface area.                                        |
|                              | 20     | Water number around O1, O2, N1, N2 (3.3Å, 3.7Å, 4.1Å, 4.5Å, 4.9Å).     |
| Solvent structure            | 10     | Integration of the OO radial distribution function, g(r), of the bulk water***, i.e., ∫₀^r₀ r²g(r)dr (r₀ is selected to be 2.9Å, 3.3Å, 3.7Å, 4.1Å, 4.5Å, 4.9Å, 5.3Å, 5.7Å, 6.1Å and 6.5Å). |

* The four atoms O1, O2, N1 and N2 are labelled in Fig. 1 left panel. N1H(N2H) is the hydrogen atom bonded to N1(N2). O1-N1 and O2-N2 may form intra-molecule hydrogen bonds, or may be connected by water bridge [45].

** In here and below, interaction means the electrostatic energy part and the Van de Waals energy part.

*** These functions reflect the packing of bulk water.

FIG. 1: (Color online) Illustration of alanine dipeptide molecule and the initial conformations of WED simulation. The alanine dipeptide is shown in the left panel, with some dihedral angles and atoms labeled. The initial conformations projected to the φ-ψ plane are shown in the right panel. The background is the free energy surface at T = 600 K, with the free energy wells labeled.
FIG. 2: (Color online) WED analysis with the basis functions of dihedral angles and inter-atomic distances. Shown are the results for solvated alanine dipeptide, which is simulated at $T = 300 \text{ K}$ with the modified force field. (a) The eigenvalues of $H$ matrix calculated with different sets of basis functions, including the full set of inter-atomic distances (squares), 12 inter-atomic distances (upward triangles), first-to-fourth order trigonometrical functions of $\phi$ and $\psi$ (circles) and first-to-second order trigonometrical functions of $\phi$ and $\psi$ (stars). (b) The behavior of $S_{X, eq}^2$ is shown either with the inter-atomic distances (Distance-Angle) at first, or with the trigonometrical functions of $\phi$ and $\psi$ at first (Angle-Distance). The $\{L^i\}$ values calculated with truncated trajectories (80 percent length) versus the ones calculated with full trajectories are shown. The results are obtained either with the first-to-second order trigonometrical functions of $\phi$ and $\psi$ (c), or with the inter-atomic distances (d). The arrows label the intersection points between the dashed and dotted lines.
FIG. 3: (Color online) The spectral properties of $H$ calculated with the basis functions including solute-solvent relation. Shown are the results for the system of solvated alanine dipeptide. The Eigenvalues of $H$ calculated with different groups of basis functions are shown in (a) (300 K simulation with modified force field) and (b) (150 K simulation with standard force field). The functions used in calculation are labeled in the legend. $P, PW, W$ means the three sets of basis functions are all included in calculation, with an order of internal freedom of dipeptide, solvent-solute relation and bulk water structure in orthogonalization and error analysis. See the main text for more details. The behavior of $S_{X,eq}^2$ with increasing number of basis functions are shown in (c) (300 K simulation with modified force field) and (d) (150 K simulation with standard force field). The insets of (c) and (d) show the part of $S_{X,eq}^2$ curve that is related to the functions of the bulk solvent structure.
FIG. 4: (Color online) Results for the low-temperature simulation of alanine dipeptide. (a) The projection of trajectories into the space of $L_i^2$ and $L_i^3$. The inset is the histogram of $\{L_i^3\}$ values. The different states, into which the trajectories are roughly classified, are labeled. (b) The distribution function along $\psi$ angle for the groups of trajectories classified by the histogram in (a). Shown are the distributions for the trajectories in the leftmost two bins (downward triangles), the rightmost two bins (upward triangles) and the other trajectories (circles). (c) The $\{L_i^3\}$ values calculated with truncated trajectories (80 percent length) versus the ones calculated with full trajectories. (d) The first 70 eigenvalues of $H$ calculated with normal data set (blue stars), multiple trajectory data set (one-fifth initial conformations and five trajectories for each conformation) and normal method (red circles), multiple trajectory data set and SI2 method (green squares) are shown.
FIG. 5: (Color online) The two-dimensional potentials. (a) The TDQW potential shown as contour map. The darker regions correspond to potential wells, and the lighter regions correspond to potential barriers. (b) The MHAT potential projected along one-dimension. Rotating around the dashed line reproduces the two-dimensional potential.

FIG. 6: (Color online) The eigenvalues and projection maps for TDQW potential under different temperatures. (a) The first ten eigenvalues of $H$ matrix calculated at different temperatures. For the three temperatures of 0.3(b), 0.85(c) and 1.50(d), the projection of $\{G^i\}$ to the second, third and fourth eigenvectors of $H$ are also plotted.
FIG. 7: (Color online) The eigenvalues and projection maps for MHAT potential under different temperatures. (a) The first seventy eigenvalues of $H$ matrix calculated at different temperatures. For the three temperatures of 0.05(b), 0.30(c) and 0.85(d), the projection of $\{\overline{G}_i^j\}$ to the second, third and fourth eigenvectors of $H$ are also plotted.
FIG. 8: (Color online) The $S^2_{X,eq}$ curve, effect of $\tilde{t}$ and illustration of statistics improvement. The behavior of $S^2_{X,eq}$ with increasing number of basis functions are shown in (a) (TDQW system) and (b) (MHAT system). (c) The difference between the reconstructed equilibrium distribution and the theoretical distribution are shown for MHAT potential at $T = 0.85$. The horizontal axis denotes the method for reproducing the equilibrium distribution. The numbers mean the selected $t^*$ values in WED analysis (expressed in percentage ratio). Eq means the results without re-weighting (i.e., $w_i \equiv 1$). SI1 and SI2 label the two methods for statistics improvement. The trajectory weights calculated with $t^*$ equal to 0.01 are plotted as inset. In figure (a), (b) and (c), the statistical errors are also shown (see the main text for more details). (d) The first 40 eigenvalues of $H$ for MHAT potential at $T = 0.05$. The ones calculated with normal data set (stars), multiple trajectory data set (one-fifth initial conformations and five trajectories for each conformation) and normal method (circles), multiple trajectory data set and SI2 method (squares) are shown.