A statistical physics based approach to capture spurious solutions in AI-enhanced molecular dynamics

Shashank Pant[a], Zachary Smith[b,d†], Yihang Wang[b,d†], Emad Tajkhorshid[a] and Pratyush Tiwary[c,d*]

[a]*NIH Center for Macromolecular Modeling and Bioinformatics, Beckman Institute for Advanced Science and Technology, Department of Biochemistry, Center for Biophysics and Quantitative Biology,University of Illinois at Urbana-Champaign, Urbana, IL 61801, USA*

[b] *Biophysics Program,* [c] *Department of Chemistry and Biochemistry,* [d]*Institute for Physical Science and Technology, University of Maryland, College Park, 20742, USA*

[†]*These authors contributed equally to this work*

E-mail: ptiwary@umd.edu*

Abstract

Artificial intelligence (AI) based approaches have had indubitable impact across the sciences through the ability to make sense of data. Recently AI has also seen use for enhancing the efficiency of molecular simulations, wherein AI derived slow modes are used to accelerate the simulation in targeted ways. However, while typical fields where AI is used are characterized by a plethora of data, molecular simulations per construction suffer from limited sampling and thus limited data. As such the use of AI in molecular simulations can suffer from a dangerous situation where the AI optimization could get stuck in spurious regimes, leading to incorrect characterization of the reaction coordinate for a given problem at hand. When such an incorrectly characterized reaction coordinate is then used to perform additional simulations or even experiments, one could start to deviate further and further from the ground truth. To deal with this problem of spurious AI solutions, here we report a new and automated algorithm using ideas from statistical mechanics. It is based on the notion that a more reliable AI solution for many problems in chemistry and biophysics will be one that maximizes the time scale separation between slow and fast processes. To learn this timescale separation even from limited data, we use a maximum path entropy or Caliber based framework. We show the applicability of this automatic protocol for 3 classic benchmark problems. Here we capture the conformational dynamics of a model peptide, ligand unbinding dynamics from a protein and the extensive sampling of the folding/unfolding energy landscape of a GB1 peptide. We believe our work will lead to increased and robust use of trustworthy AI in molecular simulations of complex systems.

Introduction

With the development of highly accurate force fields and powerful computers, molecular dynamics (MD) has become a ubiquitous tool to study complex structural, thermodynamic and kinetic process of real-world systems across disciplines. However, the predictive capacity of MD simulations is limited by the large time scale gap between the conformational dynamics of the complex processes and the short periods accessible to simulation.[1,2] This disparity is mostly attributed to the rough energy landscape typically characterized by numerous energy minima with hard to cross barriers between them .[1,3,4] This traps the system in metastable states, leading to an incomplete sampling of the configuration space. Comprehensive sampling of the configuration space not only provides us high temporal and spatial resolution of the complex process but also allows us to compute converged thermodynamic properties, sample physiologically relevant molecular conformations, and explore complex motions critical to numerous biological and chemical processes such as protein folding, ligand binding, energy transfer and countless others.[5–12]

To overcome the limitations of timescales and accurately characterize such complex landscapes, a plethora of enhanced sampling techniques have been developed. We can broadly divide these methods into: (1) tempering based and (2) collective variable (CV) or reaction coordinate (RC) based,[4] either of which can then also be coupled with multiple replica based exchange schemes. In tempering based methods, the underlying landscape is sampled by either modifying the temperature and/or Hamiltonian of the system.[13–18] On the other hand, CV based methods involve enhancing fluctuations along pre-defined low-dimensional modes, through biased sampling approaches like metadynamics,[19–21] umbrella-sampling (US),[22] hyperdynamics,[23] temperature accelerated molecular dynamics (TAMD),[24] adaptive biasing force (ABF)[25–27] and many other.[25,28–30] Although, CV-based methods can be computationally more

efficient than tempering-based approaches, but under poor choice of low-dimensional modes (which not easy to intuit for all the systems), CV biasing can fail miserably.[31] Indeed, one could also argue that the way to make tempering methods more efficient, for example through the REST protocol of Berne and co-workers,[32,33] amounts to selecting a specific part of the system, akin to a CV, which is then subjected to the tempering protocol.

Artificial intelligence (AI) potentially provides a systematic means to differentiate signal from noise in generic data, and thus discover such CVs.[34–38] A large number of such approaches have been proposed recently[34–36,39] and remain the subject of extensive research. A common underlying theme in these methods is to exploit AI tools to gradually uncover the underlying effective geometry, parametrize it on-the-fly, and exploit it to bias the design of experiments with the MD simulator by choosing informative configuration space areas that have not been explored before. This iterative MD-AI-MD-... procedure is repeated until desired sampling has been achieved. Conceptually, these approaches effectively restrain the $3N$-dimensional space to low dimensions (typically 1 or 2) which encode all the relevant slow dynamics in the system and the remaining fast dynamics are effectively discarded. Every round of AI estimates the slow modes given sampling so far, and this information is used to launch new biased rounds. Biasing along the slow modes leads to increased exploration, and any incremental increase in exploration can then be used in another round of AI to estimate the slow mode even more accurately. The use of standard reweighting procedures can then recover unbiased thermodynamic and kinetic information from the AI-augmented MD trajectories so obtained.

However, there is a fundamental problem in such an effort. Most AI tools are designed to be used for data-rich systems. It has been argued[40–43] that given good quality training data and with a neural network with infinitely many parameters, the objective function for associated stochastic gradient optimization schemes is convex. However in enhanced MD, we are firstly per construction in a poorly sampled, data-sparse regime, and secondly, it is impractical to use

a very dense network with too many parameters. The AI optimization function is no longer guaranteed to be convex and can give spurious or multiple solutions for the same data set – in the same spirit as a self-driving car miscategorizing a "STOP" sign as an indication to speed up or some other action.[44] This would happen because gradient minimization got stuck in some spurious local minima or even saddle point type feature on the learning landscape. The slow mode thus derived would be spurious and using it as a biasing CV or RC would lead to incorrect and inefficient sampling. Furthermore, this could lead to one deriving misleading conclusions from the use of AI over a given data set.

While the concerns above and the approach in this work should be applicable to more general instances of AI application in molecular simulations, here we focus on the problem of enhanced sampling through MD-AI iterations. To deal with this spurious solution problem, we report a new and computationally efficient algorithm designed to screen the spurious solutions obtained in AI-based methods. Our central hypothesis is that spurious AI solutions can be identified by tell-tale signatures in the associated dynamics, specifically through poor time-scale separation between slow and fast processes. Thus, if different instances of AI on the same data set lead to different slow mode solutions, they can be ranked on the basis of how much slower is the slow mode relative to the other fast modes. This difference between slow and fast mode dynamics is known as spectral gap. Here we use the "Spectral gap optimization of order parameters (SGOOP)" framework from ref.,[45] which uses notions of maximum caliber or path entropy[46] to cheaply construct spectral gaps along different trial slow modes output from AI. We demonstrate this path entropy based screening procedure in the context of our recent iterative AI-MD scheme "Reweighted auotencoded variational Bayes for enhanced sampling (RAVE)",[37] even though we do expect it to be more generally applicable. Here we show how this automated protocol can be applied to the study of a variety of systems of increasing complexity. These include conformational sampling in a model peptide, ligand unbinding dy-

namics from a protein and the extensive sampling of the folding/unfolding energy landscape of a GB1 peptide. We believe our algorithm marks a big step forward in the use of fully automated AI-enhanced molecular dynamics for the study of complex bio-molecular processes.

Theory

AI can mislead

While the problems identified and solutions proposed in this manuscript are quite generic to the use of AI in molecular science, here our starting point is the recent AI based method RAVE.[34,37,47] This is an iterative MD-AI approach wherein rounds of MD for sampling are alternated with rounds of AI for learning slow modes. Specifically, RAVE begins with an initial unbiased MD trajectory comprising values of some order parameters s. These could be generic variables such as dihedrals or protein-ligand distances.[48] This trajectory is then treated with the past-future information bottleneck (PIB) framework.[49–53] Per construction, the PIB is a low-dimensional representation with the best trade-off between minimal complexity and maximal predictive capability of the trajectory's evolution slightly ahead in future. RAVE uses the PIB as a computationally tractable approximation for the reaction coordinate (RC), which would be the perfect definition of a slow mode.[54] This PIB is then used in an importance sampling framework to perform a next round of biased MD. Assuming that the biased PIB is indeed close enough to the true slow mode or modes of the system, one expects the exploration of configuration space in this new biased round to be greater than in the previous round. The biased MD itself can be performed in many different ways[37,55] - here we do it using the popular enhanced sampling method metadynamics.[56] In order to learn the PIB, RAVE uses an encoder-decoder framework. The PIB or RC χ is expressed as a linear combination of order parameters $\chi = \sum_i c_i^0 s_i$ where the order parameters are $s = (s_1, s_2, ..., s_d)$.[48] The PIB objective function which

is then minimized in every training round can be written down as a difference of two mutual informations: [57]

$$\mathcal{L} \equiv I(\mathbf{s}, \chi) - \gamma I(\mathbf{s}_{\Delta t}, \chi) \tag{1}$$

where $I(.)$ denotes the mutual information between two random variables.[57] The term $I(\mathbf{s}_{\Delta t}, \chi)$ describes the predictive power of the model which is quantified by the amount of information shared by the information bottleneck χ and the future state of the system $\mathbf{s}_{\Delta t}$ when the information bottleneck is decoded back to the order parameters space. To optimize the objective function, the information bottleneck χ should as informative as possible about the future state of the system, quantified through increasing $I(\chi, \mathbf{s}_{\Delta t})$. At the same time, we seek to minimize the complexity of the low dimensional representation. Therefore, when the encoder maps the present state of the system $\mathbf{s}$ to information bottleneck χ, we aim to minimize the amount of information shared between them by decreasing $I(\chi, \mathbf{s})$. The parameter γ is introduced to tune the trade-off between predictive power and the complexity of the model.

In Eq. 1, the encoder is a linear combination of the input coordinates, thereby keeping it interpretable and relatively robust to overfitting. The decoder is a deep artificial neural network (ANN). Due to the principal of variational inference[37,58] wherein optimizing the decoder is guaranteed to lead to a convex optimization problem, we are not concerned with over-fitting in the decoder. It is fitting the encoder, which directly leads to an interpretable RC, that is of concern to us in this work. This can be best illustrated through a simple numerical example involving protein conformational dynamics, which we show in detail in **Methods** section and in Fig. 1. We performed 6 different separately initialized trials of learning the PIB on the same input trajectory for a model peptide, each running for same number of epoches. The RC was expressed as a linear combination of the sines and cosines of various Ramachandran dihedral

angles. As can be seen in Fig. 1A, we obtained different RCs with different trials stopped at the same very low value of the loss function within 6 decimal digits of precision. Given the use of an interpretable linear encoder, one can see a sense of symmetry even in the at first glance different looking RCs in Fig. 1A. However as we show in succeeding sections, the situation described here exacerbates quickly with more complicated systems, and we expect this degeneracy to get only worse in more ambitious AI based applications where even the encoder is non-linear,[34,38,59] and where one does not really know *a priori* when to stop the training.

A

Trials	$\cos\phi$	$\sin\phi$	$\cos\psi$	$\sin\psi$	loss function
1	0.11	-0.59	-0.51	-0.62	0.2803
2	-0.07	0.63	0.48	0.60	0.2803
3	-0.05	0.63	0.47	0.61	0.2803
4	-0.12	0.58	0.52	0.62	0.2803
5	0.11	-0.59	-0.51	-0.61	0.2803
6	-0.11	0.58	0.52	0.62	0.2803

B

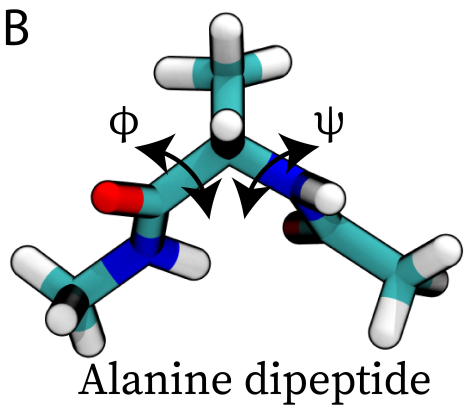

Figure 1: **Spurious AI solutions for model peptide**. (A) Table highlights the insensitivity of the objective function towards the changes in the weights of the order parameters. (B) Molecular representation of alanine dipeptide showing relevant Ramachandran dihedral angles.

Instead the above numerical example demonstrates the problem at heart of what we wish to tackle in this manuscript: how does one screen through spurious solutions resulting from attempts to optimize an objective function in AI applications to molecular simulations, and more broadly in chemistry and other physical sciences? The problem is especially difficult in two situations. Firstly, this will be in problems where one does not know the ground truth against which different AI solutions could be ranked, as can be expected to be true in any application where one seeks to gain new insight. Secondly, as is the case in AI-augmented MD, this problem will have critical, unquantifiable ramifications in iterative learning scenarios when

any such AI-derived insight is used to make new decisions and drive new rounds of simulations or experiments. For instance in RAVE, we have yet another parameter that is not obvious how to select, namely the choice of the predictive time-delay Δt in Eq. 1. As shown in ref.,[60] theoretically speaking the method is robust to the choice of this parameter as long as it non-0 yet small enough. In practice, it can be hard to judge if it is indeed small enough or not.

Path entropy model of dynamics can be used to screen AI solutions

In order to rank a set of AI-generated putative reaction coordinates, we appeal to the fundamental notion of timescale separation, which is ubiquitous across physics and chemistry for example through concepts such as Born-Oppenheimer approximation[61] and Michaelis-Menten principle.[62] We posit that given a basket of RC solutions generated through AI, we can rank them as being closer to the true but unknown RC if they have a higher timescale separation between slow and fast modes. Thus, a spurious AI solution should have a tell-tale signature in its dynamics, with poor separation between slow and fast modes. Indeed, one of the many definitions of a reaction coordinate in chemistry is one that maximizes such a timescale separation.[63] To estimate this timescale separation efficiently and rank a large number of putative AI based solutions for the true RC or PIB, here we use the "Spectral gap optimization of order parameters (SGOOP)" framework from ref.[45] SGOOP uses a maximum path entropy or Caliber model[46,64] to construct a minimal model of the dynamics along a given low-dimensional projection. To construct such a model, SGOOP requires two key inputs. First it needs the stationary probability density along any putative RC, which we directly have after any round of RAVE.[37,60] Second, it needs estimates of unbiased dynamical observables which could be obtained by short MD simulations. With these two key sets of inputs, SGOOP can construct a matrix of transition rates along any putative RC. Diagonalizing this matrix gives us the eigenvalues for the dynamical propagator. The spectral gap from these eigenvalues is then our estimate of the timescale

separation.[65] Improving the quality of the dynamical observables can lead to increasingly accurate eigenvalues.[66] Here we use a computationally cheap dynamical observable denoted $\langle N \rangle$ and defined as the average number of nearest neighbor transitions in unit time along any RC. We use p_n to denote the stationary probability density along any suitably discretized putative RC at grid index n. With these inputs, the SGOOP transition probability matrix K for moving between two grid points m and n is given by:[46,65]

$$K_{mn} = \frac{< N >}{\sum \sqrt{p_n p_m}} \sqrt{\frac{p_n}{p_m}} \tag{2}$$

Our net product is an iterative framework that leverages the predictive power of RAVE and the fundamental notion of timescale separation of SGOOP to generate an optimal reaction coordinate. The use of AI in RAVE allows to generate several possible candidate reaction coordinates, and by constructing a minimal path entropy based dynamical model we efficiently screen out spurious solutions generated from AI. The RC so identified is used as a biasing variable in enhanced sampling, and the biased trajectory itself is fed back to the AI module to further optimize the RC. The iteration between this framework and sampling continues until multiple transitions between different intermediate states are achieved. We also apply this framework to cleanly select the best choice of predictive time-delay in Eq. 1 - the optimal predictive time-delay in our model for PIB is the one that achieves highest timescale separation.

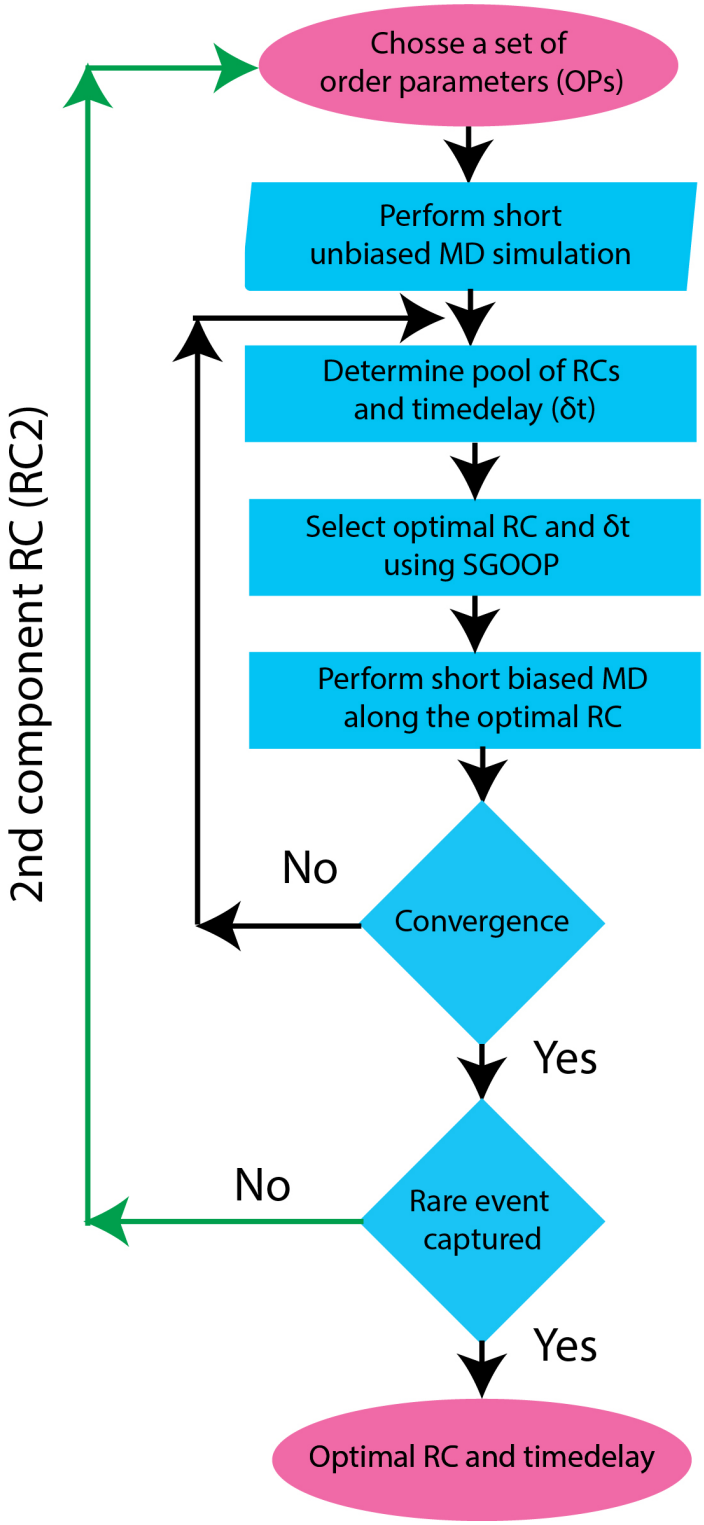

Figure 2: Flowchart illustrates our novel protocol to screen AI solutions.

Results

In the previous section we describe a path entropy and timescale separation based paradigm to capture spurious solutions in AI-enhanced molecular dynamics. In this section we illustrate our framework through three examples of increasing complexity from biochemistry and biophysics. We do however expect our framework will be applicable to more generic problems in molecular systems. Specifically, we consider (A) conformational sampling of a model peptide in vaccum, (B) dissociation of a millimolar affinity ligand from FKBP protein, and (C) folding of GB1 peptide. All simulations are in all-atom resolution, including explicit water in (B) and (C). In all three of these systems, starting with an initial unbiased MD trajectory comprising of generic order parameters s, we perform iterative rounds of RAVE and enhanced sampling, using SGOOP to screen RC candidates generated in RAVE as well as select the optimal time-delay Δt in Eq. 1. Apart from the starting choice of order parameters which is kept quite generic, all simulations are carried out with minimal use of human intuition. To display the versatility of our framework, we patched it with two different enhanced sampling algorithms.[67] In the first two systems, we employed a static bias capable of extensive conformational sampling of the model peptide and ligand dissociation via the reaction path. This static bias was directly obtained by inverting the probability distribution learnt during RAVE.[37,47] In the third system, we implemented time-dependent biasing through well-tempered metadynamics[21,56] to capture folding of 16-residue long GB1 peptide.

A. Conformational dynamics of alanine dipeptide

The first system we consider here is the well-studied case of alanine dipeptide in vacuum. It can exist in multiple conformations separated by intermediate barriers and characterized by differing values of its dihedral angles ϕ and ψ (Fig. 1B). Enabled by the small size of the system, we performed 3 independent simulations, each 2 μs long. The corresponding trajectories along

Table 1: **Optimal weights of OPs obtained through a combination of RAVE and SGOOP**

Transitions	cosϕ	sinϕ	cosψ	sinψ
1	-0.71	-0.68	0.09	-0.15
2	0.35	0.81	0.32	0.34
4	0.63	-0.65	0.03	0.42

the dihedral angles ϕ and ψ are provided in Supplementary Information (SI) in Fig. S1. In line with standard practice,[68,69] we used the sines and cosines of these two dihedral angles as the input order parameters (OPs) $s = (\cos\phi, \sin\phi, \cos\psi, \sin\psi)$ for RAVE, which then learns the RC χ as a linear combination of these 4. Due to the relatively rare and stochastic nature of transitions, even with such long simulation times we capture only 1, 2, and 4 transitions in the three trials. Using such input trajectories with different number of transitions helps us ascertain robustness of the protocol of this work. Each trajectory was used to perform RAVE with 11 different choices of the predictive time-delay Δt in Eq. 1, ranging from 0 to 40 ps. Furthermore, 10 different trials were performed for each Δt value corresponding to different input trajectories. This amounts to a total of 330 RAVE calculations, with 110 for each input trajectory. Each trial was stopped after the same training time, and the loss value after the training as well as the RC so-obtained were recorded.

As hinted at in the **Introduction** section, we obtain very different RCs for the different Δt values and for different independent trials. Furthermore, different trials that were stopped at very similar loss functions gave very different RCs and spectral gaps (Fig. 3 and SI). However our protocol of using spectral gaps to rank these different solutions works well in screening out an optimal RC. In Figs. 3(A-C) we demonstrate the very noisy correlation that we find between the loss and the spectral gap for all 3 input trajectories. In SI (Fig. S2B), we provide illustrative figure for one particular trajectory showing how same loss function results in RCs

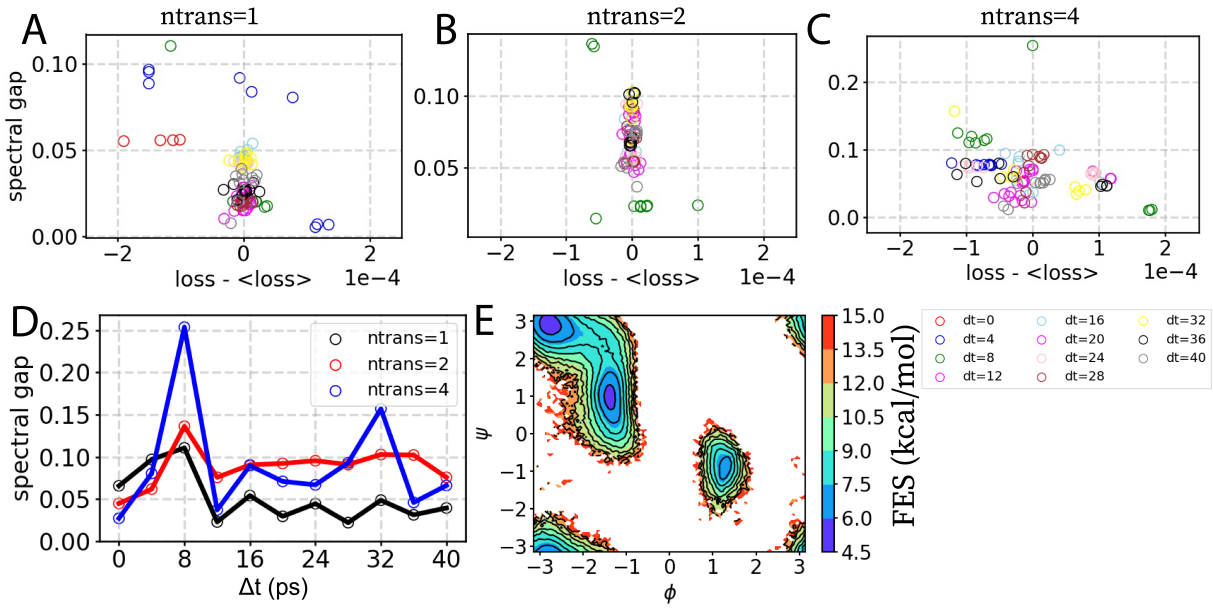

Figure 3: **Conformational dynamics of alanine dipeptide.** Spectral gap and loss was calculated for each of the three unbiased trajectories at multiple time-delays Δt between 0 and 40 ps, indicated using circles of different colors in the bottom right side of the figure. (A-C) show noisy correlation between the loss and the spectral gap for number of transitions $ntrans$ equaling 1,2,4 respectively. Different circles denote different independent trials, with color denoting Δt. For visual clarity, for every $ntrans$ we have plotted a mean-free version of the loss function by subtracting out the average of all losses. (D) Maximum spectral gap (out of 10 different trials of RAVE) vs. Δt was plotted for 3 different unbiased trajectories. (E) Free energy surface (FES) along the two dihedrals Φ and Ψ, obtained from 20 ns-long simulation in the presence of static bias. Energy contours every 1.5 kcal/mol.

with different free energy profiles, and that the one with highest spectral gap stands out with clearly demarcated metastable states. Similarly, spectral gap captures the most optimal RC not just from the set of multiple trials at each time-delay, but it is also able to select the most optimal time-delay, corresponds to the maximum spectral gap from the set of 10 different trials at each time-delay, as shown in (Fig. 3D). Irrespective of the choice of input trajectory, we find that the optimal RC shows higher weights for ϕ (Table 1), in line with previous studies which highlighted ϕ to be a more relevant degree of freedom than ψ.[37,70] Using this RC and its probability distribution as a fixed bias,[37] we then explored the conformational space of the peptide. The two-dimensional free-energy landscape along the two dihedrals ϕ and ψ from this biased run after 20 ns of simulation is shown in (Fig. 3E). This is in excellent agreement with the previous studies.[21,37]

B. Unbinding of millimolar drug fragments from FKBP

In the second example, we applied our framework to calculate the absolute binding affinity of BUT (4-hydroxy-2-butanone), a millimolar affinity ligand, from the FKBP protein (Fig. 4A). Force-field parametrization and other MD details are provided in the SI. Here our objective was to use RAVE to learn RC on-the-fly as well as the absolutely binding free energy of this protein-ligand complex. This is a difficult and important problem for which many useful methods already exist with varying levels of success.[71] At this stage at least, our intention is not to compete with these other existing methods, but instead validate our framework works well on a standard benchmark problem. We begin by performing four independent MD simulations of FKBP starting from the bound pose (PDB:1D7J).[72] The training rounds were stopped when the ligand unbound, specifically when it was 2 Å away from the binding pocket (Fig. 4B). All trajectories were expressed in terms of a dictionary of 8 OPs comprising various distances between the center of mass (COM) of the ligand and the COM of different residues in the binding pocket (Table S2). We mixed the four independent trajectories to perform RAVE with

11 different choices of predictive time-delay Δt in Eq. 1, ranging from 0 to 40 ps. At each Δt, 10 different trials were performed which amount to a total of 110 RAVE calculations. Each trial was stopped after the same training time, and the loss value after the training as well as the RC so-obtained were recorded. Different RCs were screened using a path entropy based model as discussed in the **Theory** and done for alanine dipeptide. We again find noisy correlation between the loss function and the spectral gap, shown in Fig.4C, for the case of Δt = 40 ps (additional plots are given in Fig. S3). The same value of loss function gives rise to very different values of the spectral gap and of the RC (Fig. S3). Furthermore, spectral gap not only captures the most optimal RC, it is also able to select the most optimal time-delay as shown in (Fig 4D). By using this RC and its probability distribution as a fixed bias,[37] we then performed 800 ns long biased simulations starting from the bound pose, but allowing the ligand to re-associate (Fig. S4A,B). Through this we then calculate the absolute binding affinity of the protein-ligand complex to be 6.6 kJ/mol (Fig 4E), in good agreement with values reported through metadynamics[48] and ultra-long unbiased simulations on ANTON supercomputer.[73] It is worth pointing out that the ANTON simulations took 39 μs while we obtained converged estimates in around 800 ns, reflecting roughly 48 orders of magnitude speed-up with minimal use of prior human intuition.

The use of a linear encoder in RAVE allows us to directly interpret the weights of the different OPs in the RC (Fig. 4F). The highest weight corresponds to the OP d_5, which is the ligand separation from the residue I56. This residue forms direct interactions with the bound ligand in the crystal structure. Interestingly, previous studies[72] have highlighted the importance of I56 as it forms hydrogen bonding interactions with the carbonyl group of the bound ligand, thus highlighting the significance of this residue which our algorithm also captured as the most significant order parameter. Followed by this highest weight component, the second and third highest components are for d_1 and d_2, denoting respectively distances from the residues V55

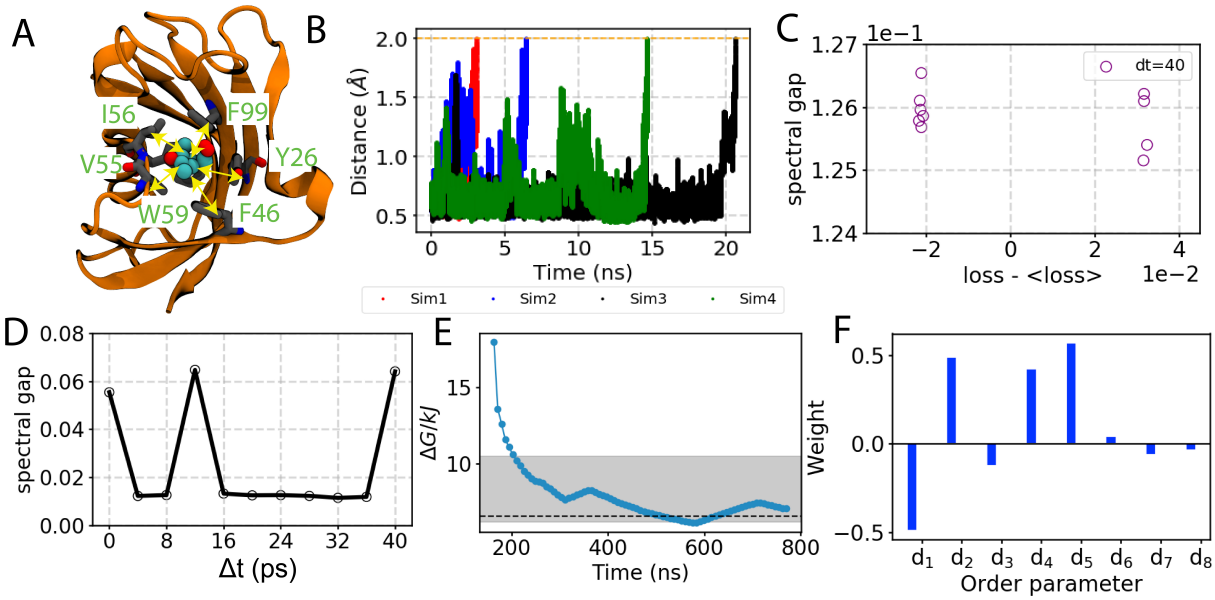

Figure 4: **Unbinding of 4-hydroxy-2-butanone from FKBP.** (A) Molecular image of the bound FKBP/BUT protein–ligand complex, with highlighted residues. The dashed lines show the 8 different OPs detailed in Table 2, that were then used to construct an optimal RC. (B) Four independent MD trajectories of the protein-ligand complex in explicit water. It is to be noted that in order to avoid entropy dominant process, only the ligand-bound trajectories were considered in our protocol. (C) Spectral gap and loss (at time-delay of 40 ps) for 10 different trials was calculated after combining all the four independent trajectories at multiple time-delay Δt between 0 and 40 ps, indicated using circles. Rest of the time-delays are shown in Fig. S3. For visual clarity, at each iteration, we have plotted a mean-free version of the loss function by subtracting out the average of all losses. (D) Plot of maximum spectral gap (out of 10 different trials of RAVE) vs time-delay (Δt). (E) Absolute binding free energy G in kJ/mol of FKBP/BUT system as a function of simulation time with static external bias. The dotted black line shows the reference value reported through metadynamics[48] and the shaded region shows the free energy estimate from long unbiased MD simulations performed on Anton supercomputer by Pan et. al.[73] The shaded region denotes the ± 0.5 kcal error reported with the final ref.[73] ΔG. (F) A visual depiction of the order parameter weights.

and W59. These are roughly equal in magnitude, reflecting that the ligand moves closer to V55 and W59 as it moved away from I56.

C. Folding/unfolding dynamics of GB1 peptide

Finally we tested our method on the folding/unfolding dynamics of C-terminal domain of GB1, which is known to adopt a β-hairpin structure.[74–78] Force-field parametrization and other MD details are provided in the SI. The free-energy landscape of this peptide has been extensively explored by performing Replica-Exchange Molecular Dynamics simulations and clustering based methods.[74,78,79] These studies reported the presence of multiple intermediate conformations by projecting the simulation data along multiple OPs, such as Radius of gyration (RG), root-mean-squared deviation (RMSD), fraction of native contacts (NC), and native state hydrogen bonds (NHB). These OPs on their own were not able to distinguish between intermediate conformations with proper energy barriers. However, using these OPs as input in advanced slow mode analysis methods such as TICA recovers a more superior two-dimensional description.[74,80–82] That work however used more than 12 μs of enhanced sampling, specifically replica exchange MD trajectories for this purpose. Here instead we use just 1.6 μs of unbiased trajectories as our starting point. From that point onwards, using the same OPs as in ref.,[74] our work provides a semi-automated solution in deriving the optimal two-dimensional RC for GB1 peptide, which is capable of resolving the intermediate conformations. Here, in contrast to the previous two examples we use well-tempered metadynamics[21,56] simulations as the choice of enhanced sampling engine coupled with RAVE.

We start by performing four independent 400 ns of unbiased MD simulations of the peptide in explicit solvent. All the simulated systems were observed to be fairly stable when projected along a library of OPs comprising RMSD, NC, RG, and NHB, with their detailed construction described in SI (Fig. 5, Fig. S5). All the unbiased trajectories were mixed and fed into RAVE for learning the RC. We performed 10 different trials of RAVE for different time-delays Δt

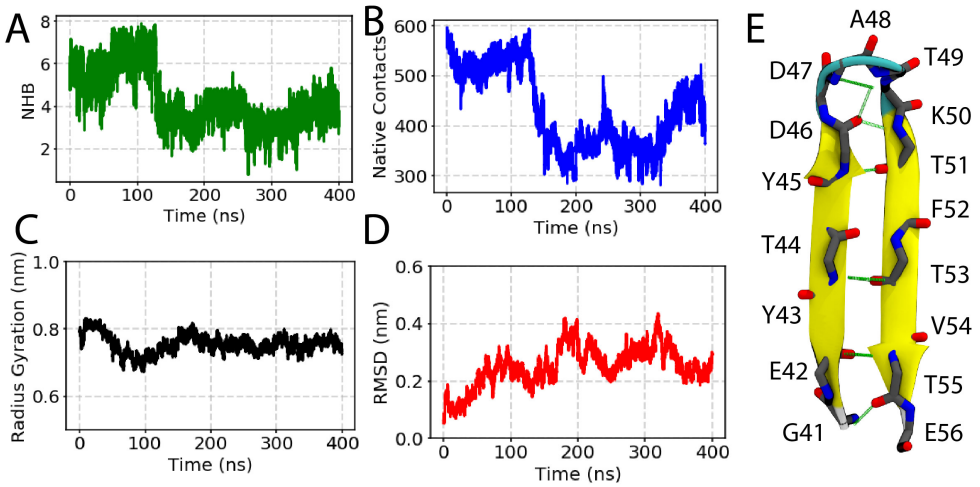

Figure 5: **Dynamics of GB1-peptide captured from unbiased MD.** One of the four representative trajectory of GB1 peptide in explicit solvent is projected along different order parameters (A) number of hydrogen bonds (NHB), (B) native contacts (NC), (C) radius of gyration (RG), and (D) root-mean square displacement (RMSD). (E) Molecular image of the GB1 peptide. Native backbone hydrogen bonds are highlighted with green lines.

in Eq. 1, ranging from 0 to 20 ps which amounts to a total of 110 RAVE calculations. Different putative RCs learnt from RAVE were screened using the path entropy based model as discussed in the **Theory** and as done for the other two systems. Similar to the previous systems, we find noisy correlation between the loss function and the spectral gap, shown in Fig. S8. The most optimal RC was selected for performing biased simulations using well-tempered metadynamics (Fig. S8A,B). Based on the maximum spectral gap, we chose Δt=8 ps for the next this round of 50 ns-long metadynamics simulation. We then alternatively iterate between the rounds of learning improved RC, using our framework, and running metadynamics using the optimal RC in every iteration. After two rounds of iterations, we did not find any further improvement in sampling with this one-dimensional RC, which we call χ_1, we were unable to attain back and forth movement between different metastable states. This suggested the presence of missing/orthogonal degrees of freedom not encapsulated by χ_1. In order to learn these other degrees of freedom through the second component of the RC, which we call χ_2, we used

the protocol from ref.[83] For practical purposes, this corresponds to ignoring the already learnt χ_1 and treating the biased trajectory without any consideration of the bias along χ_1. We then performed 50 ns long 2D metadynamics simulations (Fig. S8 E,F), which was used to train χ_2. The most optimal 2-dimensional RC obtained after three iterations of training χ_2 is detailed in (Fig. 6B). The backbone heavy atom RMSD contribute the most toward the construction of slowest dimension χ_1, whereas radius of gyration contributed more towards the second slowest dimension.

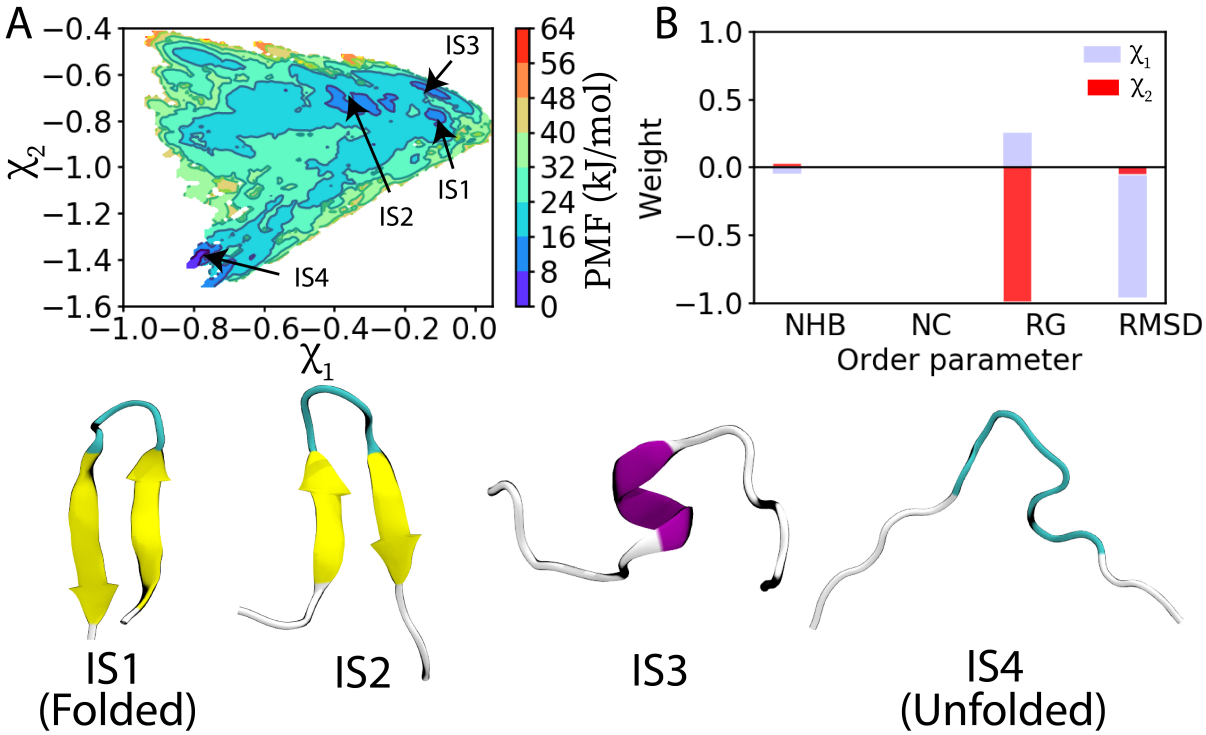

Figure 6: **Free-energy landscape and OPs contribution.** (A) A highly rugged 2-dimensional free-energy landscape of GB1 folding/unfolding. We were able to capture multiple intermediate states, corresponding to the folded (IS1), unfolded (IS4), and multiple intermediate states (IS2 and IS3). Interestingly, we were able to capture partial helical state (IS3), which otherwise was not easy to distinguish solely using traditional OPs. (B) Contribution of the different OPs to the 2-dimensional reaction coordinaate χ. The two components χ_1 and χ_2 are shown in blue and red bars respectively.

The two-dimensional RC is then used in longer well-tempered metadynamics to facilitate

movement between different metastable states and obtained converged free energy surfaces. We performed 1.2 μs-long metadynamics simulations at 300 K, starting from the crystal structure (Fig. S6). The 2-dimensional metadynamics simulation was performed with initial hill height 0.5 kJ, bias factor = 10, Gaussian widths 0.03 for both χ_1 and χ_2, and bias added every 4 ps. Additional restraint potential was applied along the RMSD order parameter preventing very high values from being attained (see details in SI). In principle this step is not necessary as the simulation would eventually return back to low RMSD states, but in practice, due to the entropic nature of the high RMSD states, such a restraint significantly helps with computational efficiency. Fig. 6A shows the 2D free energy landscape as a function of the two RC components, at 300 K. We find that the system shows multiple energy basins corresponding to the different stable and metastable intermediates. Interestingly, we captured a helical conformation of this peptide, which was not was not easy to distinguish using conventional OPs.[84] The two-dimensional free-energy landscape when projected along the pair of order parameters, yield results consistent with the previous studies and suggest the presence of two metastable states (Fig. S7).

Conclusion

To conclude, we have introduced a new approach to sieve-out the spurious solutions from artificial intelligence augmented enhanced sampling simulations. The need for this work arises because AI can lead to spurious or multiple solutions based on the same training data set. To deal with this our solution is motivated by the fundamental notion of timescale separation and that this can be practically implemented on the basis of Maximum Caliber based SGOOP.[45,46] The approach developed here is quite generic and should be applicable to many recent methods (ref.[34] and references therein) which involve iterating between MD and AI for sampling and

learning respectively. Here we demonstrate its usefulness through our recent integrated AI-MD algorithm RAVE.[37] We illustrate the applicability of our algorithm through three illustrative examples, including the complex problem of capturing the energetic landscape of GB1 peptide folding in all-atom resolution. In this last case, we started from a library of 4 order parameters that are generic for folding/unfolding processes and demonstrated how to semi-automatically learn a 2-dimensional RC, which we then used in well-tempered metadynamics protocol to obtain folding/unfolding trajectories. This directly allow us to gain atomic level insights into different metastable states relevant to the folding/unfolding process. We believe our method marks a new and useful step forward in increasing the utility of machine learning and artificial intelligence-based methods in context of enhanced sampling and arguably for molecular simulations in general. The use of ideas such as path entropy, maximum caliber and spectral gap optimization from statistical physics is demonstrated to make AI more trustworthy, thereby reducing the chances of performing misleading molecular simulations when AI is used to speed them up.

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Supporting Information

Methods

Molecular Dynamics All the systems were simulated through GROMACS version 5.0[85] patched with PLUMED version 2.4.2.[86,87] We constrain the bonds involving hydrogen atoms using LINCS algorithm and employed integraion timestep of 2 fs.

(A) **Simulation setup for alanine dipeptide in vacuum.** We followed Ref.[37] to set up our simulations for alanine dipeptide in vacuum. Multiple 2 μs-long independent MD simulations each with randomized velocities were performed to improve the sampling. The temperature was kept constant at 300 K using the velocity rescaling thermostat.[88]

(B) **Simulation setup for FKBP-BUT complex.** As a second system, we studied the dissociation of 4-hydroxy-2-butanone (BUT) from the protein FKBP. For the protein, AMBER99SB-ILDN force field[89–91] was used with TIP3P water and the ligand was parametrized with the generalized amber force field (GAFF).[92] The simulations were initiated from the BUT-bound crystal structure of FKBP (PDB ID: 1D7J). Initial equilibration was performed in NPT ensemble for 1 ns followed by four independent production run with Nosé-Hoover thermostat[93] maintained at 300 K.

(C) **Simulation setup for GB1 peptide.** As a third system, we studied the folding dynamics of GB1 peptide in water. The system was setup and equilibrated following the protocol described in Ref .[74] After this we performed four independent production runs, each lasting for 400 ns.

Metadynamics The central idea behind metadynamics is that by adding a history-dependent biasing potential it encourages the given system to escape the free energy minima, and explores various stable and metastable regions of the energy landscape.[21] Following the reweighting procedure, one can then construct the underlying energy landscape along any RC.[94] To capture the unfolding energy landscape of GB1 peptide, we employed well-tempered metadynamics using PLUMED implementation.[21,86,87] The simulations were performed along the optimal RC obtained through the combination of RAVE and SGOOP. The metadynamics simulations were performed with the biasfactor of 10, initial hill height of 0.5 kJ/mol, and bias deposition rate of 4 ps. The sigma values of the added Gaussian bias was set to the standard deviation of the biasing RC obtained from the unbiased simulation 400 ns.

Once a 50 ns metadynamics run was completed, the biased trajectory was reweighted and used as an input for the second round of RAVE.[37] The system in this 50 ns biased runs explored more conformational space than in initial 400 ns of unbiased simulations. After this, we performed another round of biased simulations, where we

employed 2D metadynamics along the optimal RCs obtained through the combination of RAVE and SGOOP. To prevent the peptide from getting trapped in entropic non-physical conformations due to the accumulated bias, we also restrain the movement of the peptide along the RMSD order parameter by addition of another bias. This bias had the form of Eq. 3. The final round of 2-dimensional metadynamics simulations were performed for 1200 ns.

$$V_{RMSD} = 2000(RMSD - 0.7)^{0.4}(kJ/mol) \tag{3}$$

Definitions of Order Parameters. For each system we first constructed a dictionary of order parameters (OPs), summarized in Table 2.

Table 2: **Details of the Pre-Selected Dictionary of Order Parameters (OPs)**

system	**order parameters**	**definition**
Alanine dipeptide	dihedral angle	cosϕ
	dihedral angle	sinϕ
	dihedral angle	cosψ
	dihedral angle	sinψ
FKBP-BUT	d_1	Y59-BUT
	d_2	V55-BUT
	d_3	F46-BUT
	d_4	Y26-BUT
	d_5	I56-BUT
	d_6	F99-BUT
	d_7	Y59-end A
	d_8	Y59-end B
GB1 peptide	NHB	backbone-backbone
	NC	all native contacts
	RG	backbone heavy atoms
	RMSD	backbone heavy atoms

We have calculated NC and NHB using a switching function

$$S = \sum_i \sum_j s_{ij} \tag{4}$$

$$s_{ij} = \begin{cases} \frac{1-(\frac{r_{ij}-d_o}{r_o})^6}{1-(\frac{r_{ij}-d_o}{r_o})^{12}} & if r_{ij} > r_o \\ 1 & otherwise \end{cases} \tag{5}$$

where d_o= 0.05 nm and r_o = 0.65 nm were used for NC calculations and d_o= 0.0 nm and r_o = 0.25 nm were used for NHB calculations. In the case of NC contacts, r_{ij} is a distance between i and j heavy atoms in the native state. While, in NHB, i and j are the pairs of O and HN (amino group) atoms of the backbone. RMSD of all the backbone heavy atoms with respect to crystal structure and RG of all the backbone heavy atoms were also calculated using PLUMED.

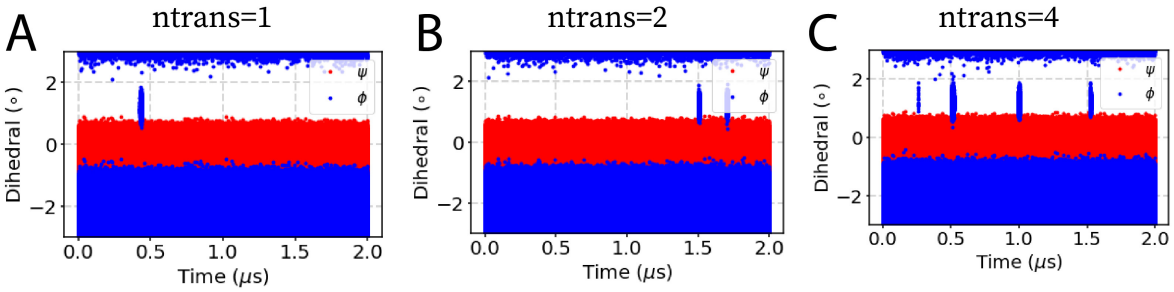

Figure S1: **Unbiased MD simulations of model peptide in vacuum.** (A-B) Three independent MD simulations of alanine dipeptide projected along dihedral (ϕ, ψ) angle space. Differing number of transitions along the ϕ order parameter were captured in the different independent simulations.

A

Trials	$\cos\phi$	$\sin\phi$	$\cos\psi$	$\sin\psi$	loss function	spectral gap
1	-0.695	-0.701	0.067	-0.138	0.3902	0.1121
2	0.691	0.704	-0.076	0.144	0.3902	0.1094
3	0.627	-0.654	0.029	0.423	0.3902	0.2536

B

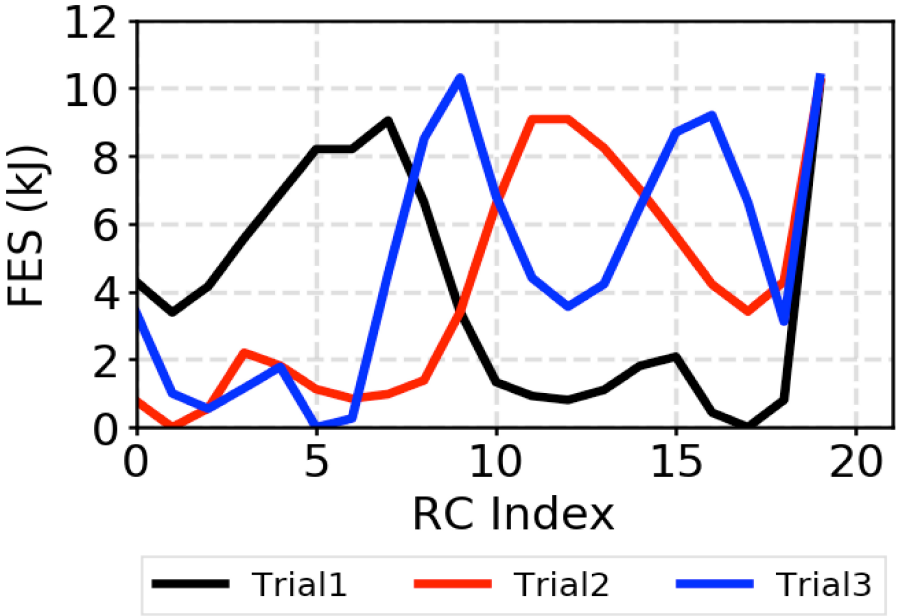

Figure S2: **Same loss function can give differing RCs** (A) Table highlights the sensitivity of spectral gap towards changes in the weights of the order parameters. While objective function remain insensitive to the changes in the weights of the order parameter. (B) Free energy landscape of one particular trajectory showing same loss function results in RCs with very different free energy profiles. The RC with maximum spectral gap highlights distinct metastable state.

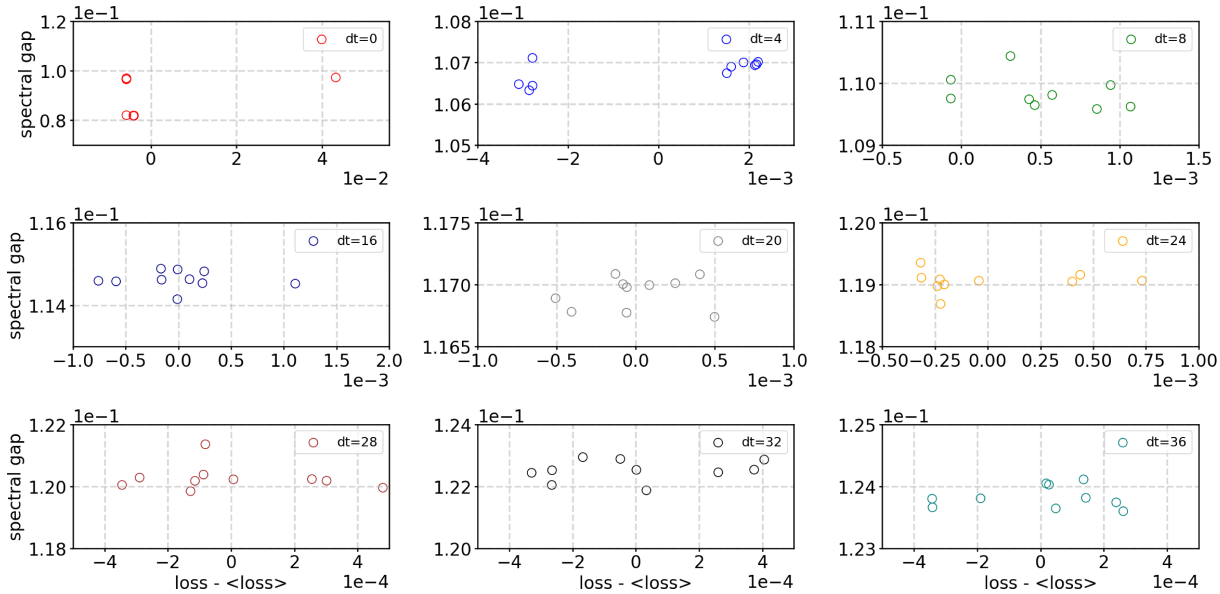

Figure S3: **Loss function versus spectral gap for ligand unbinding from FKBP along the optimal reaction coordinate.** Spectral gap and loss was calculated for the unbiased trajectory at multiple time-delays Δt between 0 and 40 ps, indicated using circles of different colors. At each time-delay we observed a noisy correlation between the loss and the spectral gap. For visual clarity, we have plotted a mean-free version of the loss function by subtracting out the average of all losses.

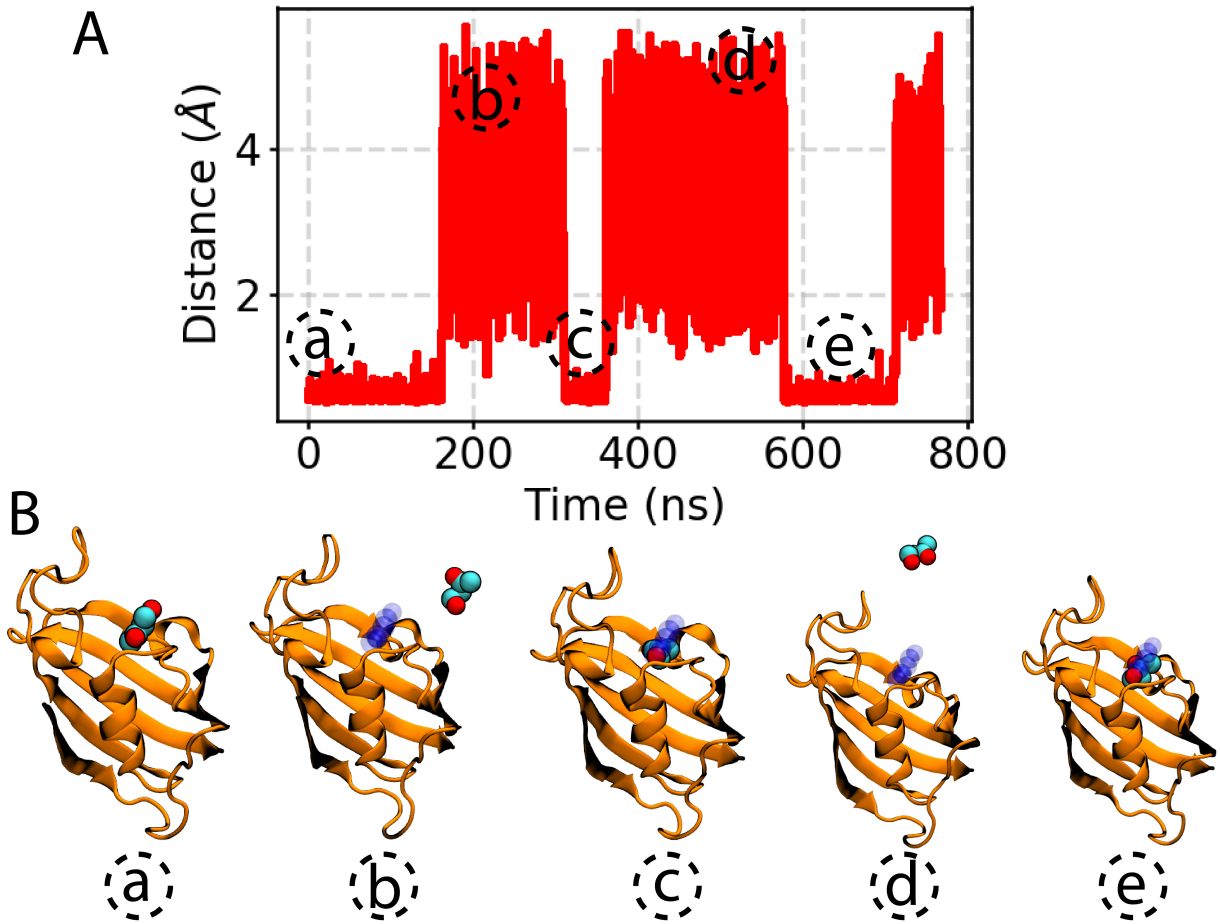

Figure S4: **Biased simulation of ligand unbinding from FKBP along the optimal reaction coordinate.** Biased simulation of ligand unbinding was performed under static bias. (A) Distance between the center of mass of the ligand from its binding pocket highlights multiple unbinding/binding events. (B) Representative snapshots of the trajectory are shown. Ligand is shown in vDW representation and the protein is shown in new cartoon (orange). Starting orientation of the ligand is shown in transparent blue vDW representation.

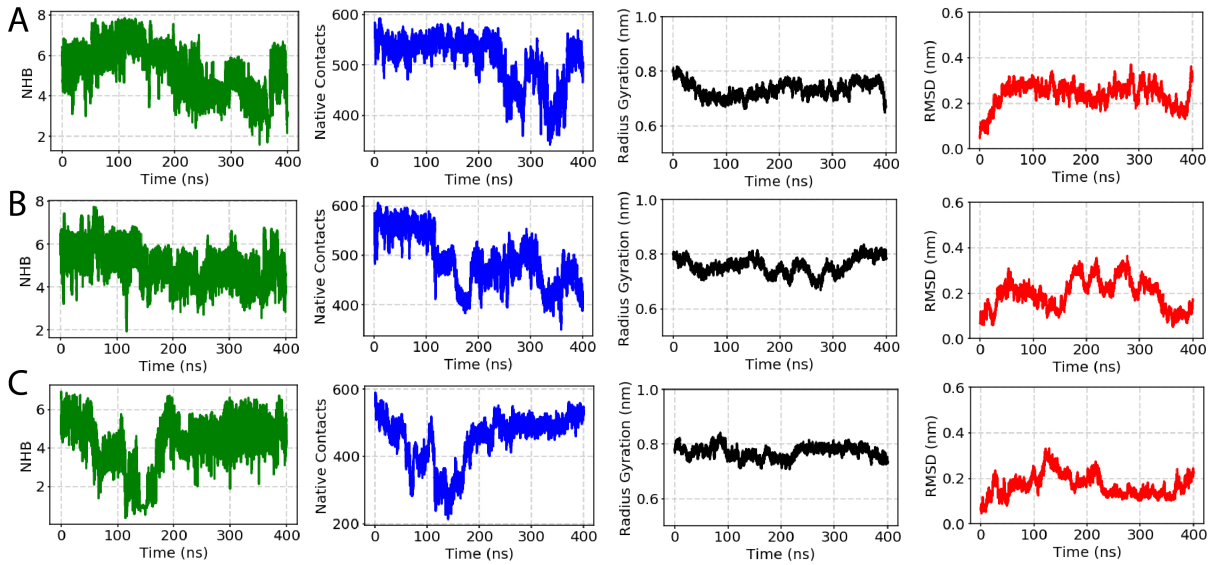

Figure S5: **Unbiased MD simulations of GB1 peptide in explicit solvent.** (A-C) Three independent MD simulations of GB1 peptide projected along different order parameters. Time evolution of root-mean square displacement (RMSD) and radius of gyration suggests that the GB1 peptide was stable in all the simulated replicas.

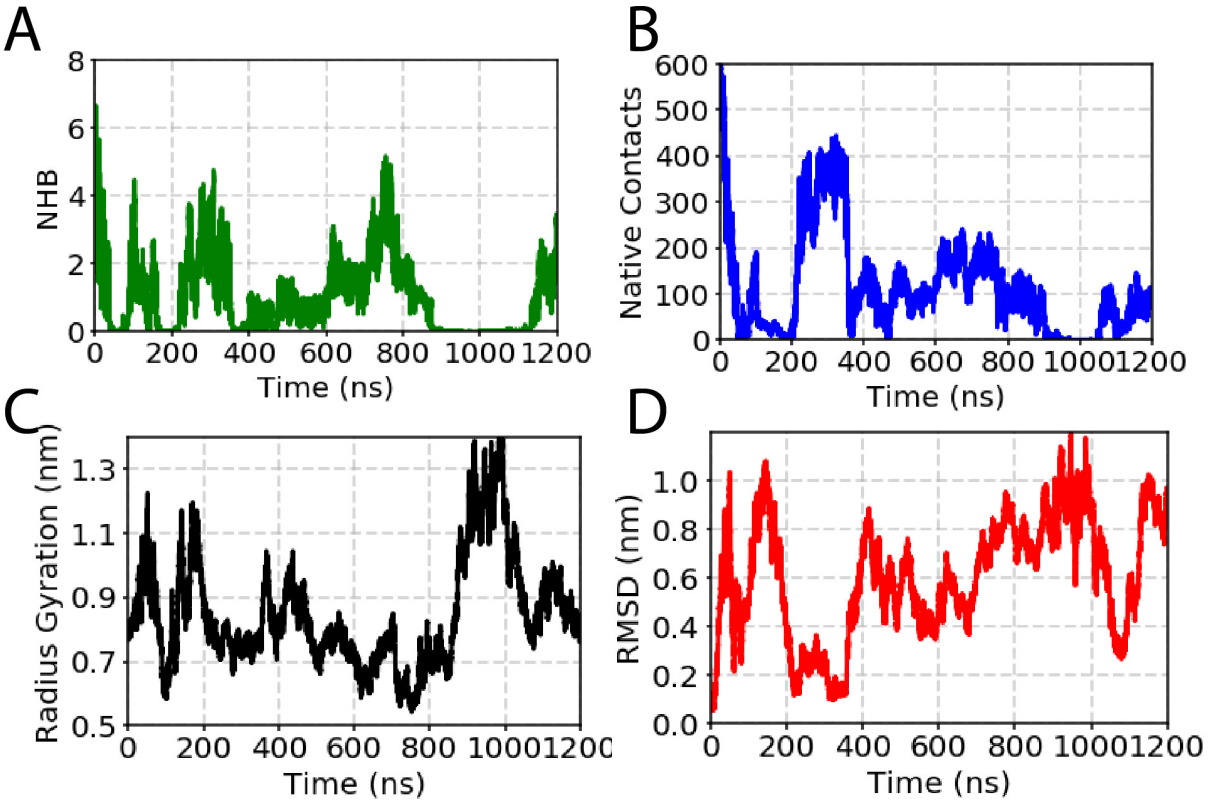

Figure S6: **Biased simulation along the optimal 2-d reaction coordinate.** The biased simulation was projected along the dictionary of order parameters used in this study. We captured multiple back and forth movement of the peptide between folded and unfolded states.

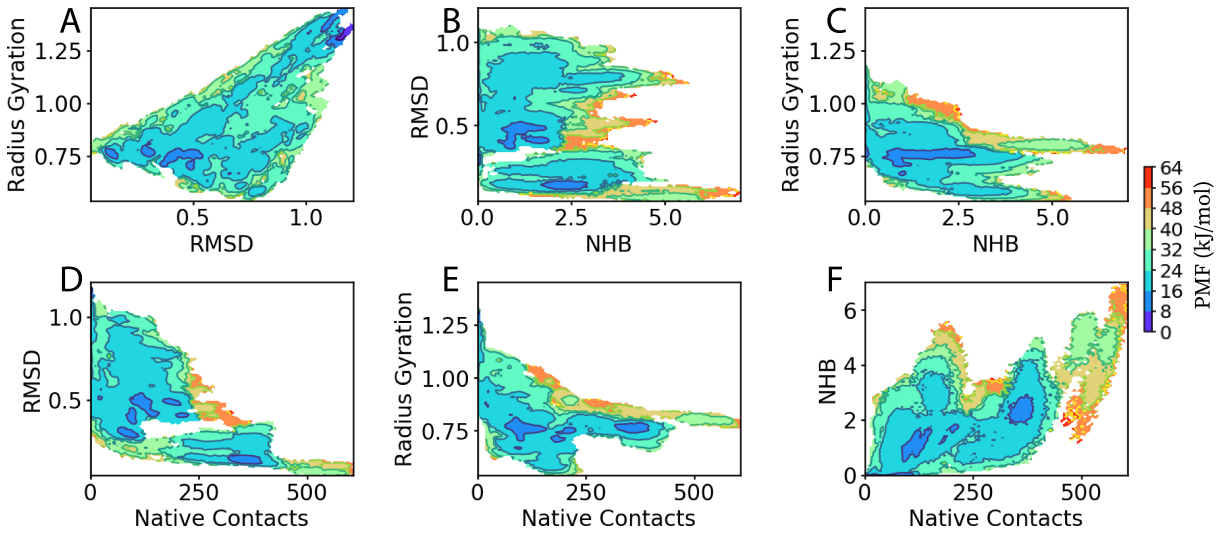

Figure S7: 2-dimensional free-energy profiles along the different order parameters used in the current study.

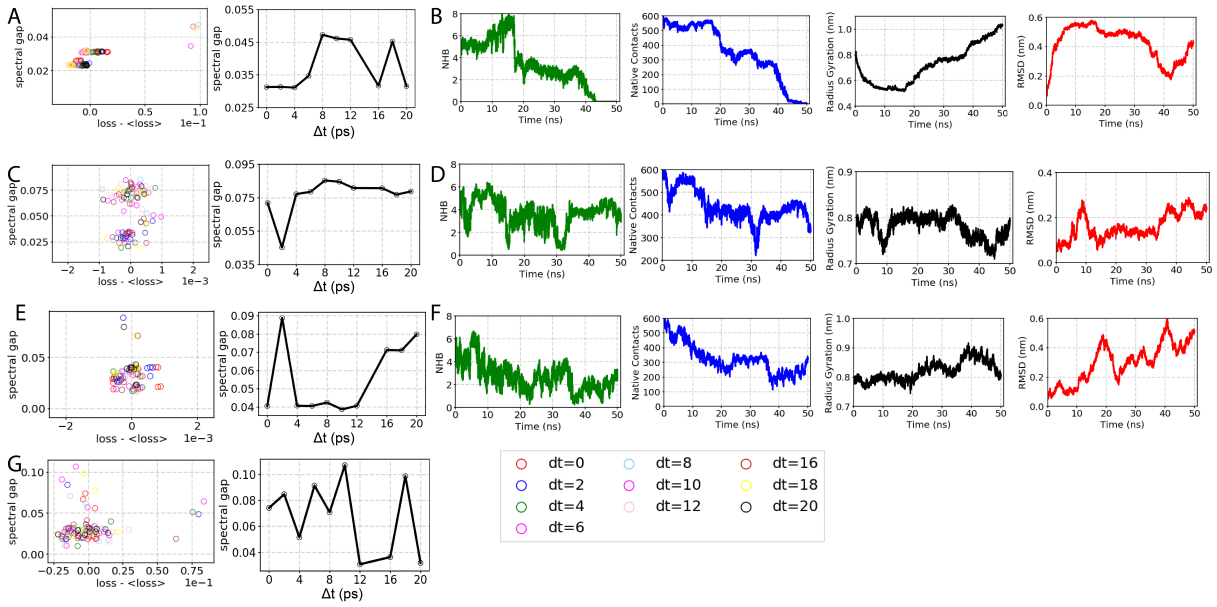

Figure S8: **Capturing optimal reaction coordinate for GB1 peptide.** Using an iterative ML-MD approach which incorporates RAVE and SGOOP, we have constructed an optimal RC to capture folding/unfolding of GB1 peptide. Spectral gap and loss was calculated in each round at multiple time-delays Δt between 0 and 20 ps, indicated using circles of different colors. (A,C,E,G) Show noisy correlation between the loss and the spectral gap at each iteration. Different circles denote different independent trials, with color denoting Δt. For visual clarity, at each iteration, we have plotted a mean-free version of the loss function by subtracting out the average of all losses. Corresponding maximum spectral gap (out of10 different trials of RAVE) vs. Δt was plotted at each iteration. (B,D,F) 50 ns-long biased simulation along the constructed RC (at ieach iteration) was performed and the trajectory was projected along the order different parameters employed in this study.