Best Practices for Computing Free Energy Profiles and Potentials of Mean Force : v0.1

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Abstract This document provides a starting point for calculating a potential of mean force and free energy profiles from molecular dynamics simulations. There are an increasing number of methods for this type of work. In the present guide, we introduce you to some of the popular current methods, as well as considerations to take into account no matter the method chosen. We point to further resources to gain a deeper understanding into the vibrant topic.

***For correspondence:**

1 Introduction

The goal of this article is help researchers get started productively. The theory is well laid out (add references) and many methods are available. While some considerations are system dependent, we aim to provide overall guidelines. In this document, we will introduce key terms and some common methods.

Note that we will not cover how to perform molecular simulations in this document. We refer readers to other best practices documents for introductory guides:

- MD basics https://github.com/MobleyLab/basic_simulation_training
- MD setup, biomolecular setup https://github.com/michellab/BioMolSetupPaper
- Statistical error and uncertainty analysis https://github.com/dmzuckerman/Sampling-Uncertainty/

You may want to review these first to check if you're comfortable with the material.

Additionally, we will not cover alchemical methods.

Distinguish between methods that choose a coordinate a priori, and those that do not. We will mention those that do not (MSMs, TPS) but leave outside the scope. In the scope will only be combinations of sampling and constructing PMFs from a priori chosen coordinates. Will discuss a first step of learning about the system and metastable states before choosing:

- US/Replica exchange
- Metadynamics
- Adaptive force biasing
- Some on weighted ensemble

Restrict ourselves to a priori choices of RC. Mention that others exist

- approximate continuous function with discrete samples (most of the time)
- Projection onto the state space
- Histograms vs. laying down Gaussians
- Histograms always underestimates barrier heights (not the worst problem)

MSM as an adaptive sampling method (will exclude this from our scope); MSMs gives an RC but hard to relate to any theoretical condition, change temp, anything. What are you going to do: RC for further sampling? If you want to generate

low D representation MSMs are great; if want to discover an RC, TPS

There are many other enhanced sampling methods (multicanonical, Wang-Landau, Adaptive Umbrella Sampling, Tsallis statistics). Can't cover them all. We can suggest reviews.

Discuss analysis of results with committor analysis.

2 Key Terms

Discuss both the definitions and actual use (not always strictly correct!)

2.1 Potential of Mean Force

The configurational portion of the free energy of a system is computed via the partition function

$$Z = \int d\vec{x} \exp -U(\vec{x}/k_B T) \tag{1}$$

as

$$A = -k_B T \ln Z \tag{2}$$

The integrand of Eq. 1 can be thought of as the unnormalized probability, since $\text{prob} \propto \exp -U(\vec{x}/k_B T)$. Thus, if we wish to compute the probability distribution along an arbitrary coordinate, we would write

$$P(x) = \frac{1}{Z} \int d\vec{x'} J(x) \exp -U(\vec{x'}, x/k_B T) \tag{3}$$

where $\vec{x'}$ is all of the degrees of freedom in the system other than $\vec{x'}$, and $J(x)$ is the Jacobian of x (see below). The free energy curve (also called a free energy profile) along x can then be computed as

$$A(x) = -k_B T \ln(P(x)) \tag{4}$$

The Jacobian in Eq. 3 accounts for the variation in the phase space volume associated with the differential $d\vec{x}$ with x. The depends on precisely what $\vec{x}$ is. For example, if $\vec{x} = r$ is a distance in 3-dimensional space,

$$J(\vec{x}) = 4\pi r^2 \tag{5}$$

Physically, this means that specifying r traces out a sphere of radius r, and $J(r)$ is the area of that sphere. Similarly, if $\vec{x} = \theta$, the tilt of a vector relative to the z-axis, then

$$J(\theta) = 2\pi \cos(\theta) \tag{6}$$

which would be the arc length of the circle traced out by a unit vector for a specific value of θ.

However, it is not always convenient to work in terms of probability, since this contains information not only about the system, but also the chosen coordinate. To separate the two, one can also work with the probability density

$$\rho(x) = \frac{1}{Z} \int d\vec{x'} \exp -U(\vec{x'}, x/k_B T) \tag{7}$$

The equivalent to Eq. 4 defines the potential of mean force (PMF)

$$W(x) = -k_B T \ln(\rho(x)) \tag{8}$$

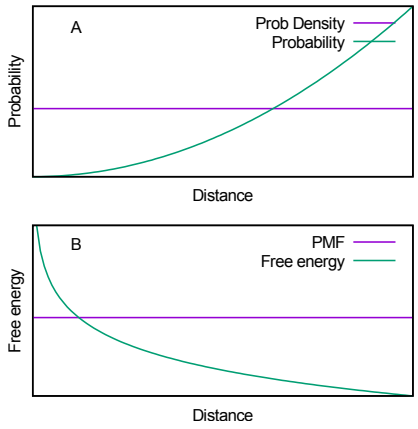

Figure 1. Free energy curves vs. potentials of mean force. Panel A shows the probability density and probability curves for the case where the chosen variable is the distance between 2 ideal gas particles. The probability density is constant, while the probability increases with distance. Panel B shows the equivalent free energy quantities, as computed by Eqs. 4 and 8.

Comparing these two quantities, it is clear that, although the terms "PMF" and "free energy curve" are often used interchangeably, they are not by definition the same unless $J(x) = 1$. Figure 1 demonstrates this difference for the case of a pair of ideal gase particles.

PMF (analogous to probability density) vs free energy surface (analogous to probability); Only ABF gives a PMF, umbrella sampling and metadynamics give free energy curves.

PMF is a free energy as a function of almost, but not all the coordinates:

- PMF(x0,x1. . .) = -kb T log x x0,x1 exp(- beta U(x)) dx (all but x0, x1)
- Only defined up to a constant.
- write equation to show distinction between PMF and free energy
- Jacobian correction
  - Common jacobians – distance ($J = 4\pi r^2$), angles (J = 1)
  - Other common rxn coords J is unknown (native contacts, RMSD)

  - Make a figure showing free energy and PMF for a couple of simple systems (2 ideal gas particles, 2 LJ particles?)

PMF is a fundamentally a continuous function (unless the rxn coord is intrinsically discrete), but we have to approximate the continuous function when we estimate it. Two approaches to approximate:

- Expectations of histograms or other indicator functions $\Delta A(xi) = \sum_{k=1}^{K} \sum_{n=1}^{N} \sum W_i(x_n) I_k(x_n)$, where I_k is some local indicator function, like a top hat function centered at x_n, or a gaussian centered at x_n.
- Some sort of fit from the data to a continuous function with different parameters, for example by a least squares fit or Kullbeck-Liebler divergence. (refs) Useful if you're using the curves as input to simpler calculations

2.2 What is the reaction coordinate/OP/CV?

- **Potential of mean force (PMF)** is formally the "potential" derived by integrating the mean force along a path or coordinate,[4] analogous to the probability density along that coordinate. Often used interchangeably with **Free energy curve**, but strictly the PMF does not contain entropic contributions from the Jacobian of the chosen coordinate.
- **Free energy curve** is the free energy as a function of a particular coordinate or coordinates. It is directly related to the probability distribution along that coordinate, and implicitly includes the entropic contributions from the Jacobian.
- **Collective variable (CV):** any variable composed of displacements of multiple particle coordinates. It could be as simple as the distance between two particles, or complex like the fraction of native contacts present in a protein folding simulation.
- **Order parameter:** a collective variable that distinguishes between two or more stable states
- **Reaction coordinate (RC):** the pathway followed by a chemical reaction or conformational change. Often used interchangeably with collective variable.

3 Considerations before you start computing

3.1 Checklist for designing a free energy profile calculation

The first key to a successful outcome is appropriate experimental design. That is, you first need to ensure that the calculation is plausibly doable given the resources available, and that the question is is well-posed. The following is a list of items that must be considered before beginning such a calculation. Answering these questions will help you decide which techniques

- **What collective variable or variables will be used as reaction coordinate?** The statistical physics of free energy curves gives us considerable leeway in choosing our reaction coordinates — in principle, any variable could be used, and if the calculation is performed correctly the resulting free energy curve will be "true". However, as a practical matter, interpreting the curve will be challenging (or even deceptive) unless the reaction coordinate is a good approximation to the true mechanism.
- **What are the other relevant motions in the system? On what timescale do they take place?** The derivation of a free energy curve involves computing a thermodynamic average over all degrees of freedom in the system other than the chosen path. In practical terms, this means that any slow degrees of freedom in your system that aren't explicitly biased or tracked must be sampled for the results to converge statistically. This is particularly problematic if there are multiple slowly exchanging states not explicitly spanned by the chosen collective variable.
- **What is the expected "lengthscale" of features on the reaction coordinate?** Over what range will the collective variables be tracked? How finely do we need to determine the free energy curve to be able to answer the scientific question?
- **Can the reaction coordinate be used to calculate a biasing energy or force?** Many techniques, such as metadynamics and umbrella sampling, make direct use the chosen collective variables by adding additional biasing forces to the simulation. Thus, when using one of these techniques, one is restricted to collective variables that can easily be computed on the fly in the simulation, preferably without greatly reducing computational performance.
- **How many collective variables do you plan to bias?** There's a challenging tradeoff here: If there are slow degrees of freedom you don't bias, you may effectively just be doing brute force sampling. On the other hand, the number of trajectories typically increases exponentially with the number of collective variables biased. As a result, it is very rare that you see more than 1 or 2 dimensional enhanced sampling.
- **Are the barriers expected to enthalpic, entropic, or both? Are there major entropic differences between states?** Different enhanced sampling methods do better with different kinds of biases. For example, conventional replica exchange is great for purely enthalpic barriers (raising the temperature exponentially increases

the rate of barrier crossing) but is less helpful with entropic ones (the only gain at high temperature is an increase in the "diffusion" constant). Thinking about the kinds of barriers involved should inform your choice of enhanced sampling technique.

4 Selecting collective variables for sampling

[Andy F]

- Intuition / experience based selection of CVs
- "Perils of projection" – hidden barrier problem, artificial collapse of kinetically distinct states
- Number of CVs and curse of dimensionality – exponential increase in sampling cost with number of CVs
- Post-hoc projection into CVs other than those in which sampling was conducted[2]
- Requirement for explicit (MC) and differentiable (MD) expressions for CVs in terms of atomic coordinates
- Systematic techniques for CV discovery (PCA, Isomap, LLE, dMaps, ANNs)

5 Sampling techniques

Mention what they do.

- Restrict ourselves to conventional/T Rep EX, US / HREx , Metadynamics, and adaptive force biasing; add strengths and weaknesses of each method (dynamic range to be surmounted)
- Conventional MD / T Rep Ex (conventional histogram; MSM to analyze)
- Direct counting/MSM: Usually bad, because only samples the minima, not the barriers. Will fail even in the absence of barriers – just need a significant (> 5 kT, maybe) range of free energies
- Umbrella sampling / Ham Rep Ex (need weighted)
- Design simulation so it can be tested (Prefer running each window multiple times, construct as independently as possible, best way to get error bars)
- Tests for convergence and self-consistency. For Ham Rep Ex[7]
- Uncertainty quantification and error estimation – bootstrap resampling, Gibbs / MC sampling of Bayes posterior

6 Computing the free energy/PMF from US/Ham Rep Ex

6.1 Computing free energy profiles using WHAM

The most common way to extract a free energy profile from umbrella sampling data is the weighted histogram analysis method, or WHAM.[5;6;9] For the most common case, where all trajectories were run at the same temperature and the bias is solely a function of the collective variable, the two key WHAM equations are

EQUATIONS HERE, USING MY OLD NOTATION

where DEFINE TERMS HERE. Each trajectory is histogrammed along the chosen collective variable, and thus produced a biased estimate of the probability distribution. If there were only one trajectory, the WHAM equations would reduce to simple thermodynamic reweighting, but with multiple estimates from the various trajectories one must compute some kind of weighted average. The F_i values serve precisely this purpose; the WHAM derivation shows that iteratively solving these equations minimizes the variance in the resulting probability distribution.

There are a number of WHAM implementations available. Probably the most commonly used one is due to Grossfield,[3] but there are also version included as part of gromacs[8] and CHARMM.[1] Software implementing Bayesian formulation of WHAM.[2]

Although WHAM is a robust method, there are a number of checks one should apply to any calculation performed with it (this is in addition to the design-level tests discussed in Section 3.1).

- **Check that your units are consistent**. Many WHAM codes are agnostic to the choice of collective variable, but do require that the units be consistent. Specifically, most implementations assume the restraints are harmonic in the reaction coordinate, and thus expect that if the time series are given in units of X (e.g. Å or nm for a distance), the restraint's spring constant is given in units of energy/X^2, and will output the free energy curve in units of energy. There are known issues with some simulation packages. Most notably, when restraining angles or torsions in Amber, the location of the restraint minimum is specified in degrees (as is the output), but the restraint is specified in radians. This mismatch is dramatic enough to cause the WHAM calculation to fail immediately, generally producing all "NaN" values for the free energy curve.
- **Check the functional form of the restraint**. Although most packages use harmonic restraints, some include a prefactor of 0.5, while others do not. To produce correct results, you may need to given a different value to the WHAM code than you did to the simulation package. For example, NAMD and Amber restraints restraints do not use the prefactor, while Grossfield's WHAM does, so if you ran a NAMD simulation with a spring constant of 10 kcal/Å^2, you would put 20 as the spring constant in the WHAM metadata file.
- **Verify the histograms overlap**. If there are "bare" patches

(or even just poorly sampled regions), the free energy profile will not be reliable. This test should be performed *before* running WHAM; WHAM works best when the total number of samples is roughly constant across the whole range of the collective variable.

- **Make sure the iteration is fully converged**. The WHAM equations are generally solved by iteration to self-consistency, so it is crucial to ensure that the iteration has proceeded far enough that the answer is not changing. You should check the manual for your chosen WHAM implementation for a suggested value, but also should (if possible) plot the intermediate free energy curves to verify they've stopped changing. If the range of values on your free energy curve is especially large (hundreds of k_BT), there may be significant issues with numerical precision preventing the curves from converging. In this case, you can use a special-purpose WHAM implementation intended to handle this case, available from https://github.com/dejunlin/gwham.
- **Check that the chosen bin width isn't altering the answer**. Finite-width histogram bins intrinsically smooth the free energy profile by breaking it into regions. This effect can be minimized (and often effectively eliminated) by reducing the bin size, at the cost of reducing the number of samples in each bin, which makes the curve noisier. To verify that the bin width isn't a problem, one should always re-run WHAM several times with different bin width to verify that the resulting curve does not vary systematically. The key is that the bins must be narrower than the relevant "features" of the free energy curve; bins comparable to or broader than these features with obscure them or even smooth them away.

It is also crucial to assess whether other relevant orthogonal degrees of freedom (variables other than the one used as a collective variable) are well-sampled. This is a complex general problem, so we can't give you a simple test. Rather, you have to honestly look at your system, and verify that overlapping trajectories are genuinely similar (and not just similar according to the collective variable). The only automated procedure for looking for problems that we are aware of is due to Neale et al,[?] and only applies to the case where the umbrella sampling was performed using Hamiltonian replica exchange; in essence, the method looks for regions of the collective variable where there is hysteresis in the migration of the trajectories up and down the variable range as evidence for slow orthogonal relaxation.

7 Projection of computed PMF into alternative CVs

[Andy F] Reweighting of PMF into arbitrary CVs other than those in which sampling was conducted[2]

8 Analysis common to all methods

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