Potentials of mean force (PMFs)—free energies along a selected set of collective variables—are ubiquitous in molecular simulation studies. Applications of potentials of mean force include determining the kinetics of a reaction using the free energy along the reaction path [1–3], understanding the behavior of collective interactions such as hydrophobicity [4–6], elucidating transport mechanisms through molecular pores [7–11], and the parameterization of low-dimensional (generalized) Langevin or Fokker-Planck equations as effective reduced models of the system dynamics [12–16]. PMFs are typically estimated from unbiased or biased molecular simulation trajectories using a variant of histogramming techniques, most commonly a type of multiple histogram reweighting technique such as the weighted histogram analysis technique (WHAM) [17]. However, the process of histogramming in order to obtain the PMF obscures two important issues. First, the true distribution of observations along the desired collective variable or variables in the infinite limit is virtually never actually a histogram but rather a continuous function, so the process of histogramming inherently introduces unnecessary discretization errors. Second, what we actually observe when we perform a simulation is neither a histogram, nor a continuous function, but a discrete set of delta functions, at the observed values of the collective variables. Approximating the “true” PMF attained in the limit of infinite sampling of the discrete observations as a histogram inherently entails a loss of information. What is required to resolve these problems is improved approaches to estimate a continuous PMF along collective variables directly from the discrete set of empirical observations collected in the simulations, without the unnecessary introduction of approximation and information loss that histogramming incurs.

We are certainly not the first to observe the disadvantages of histogramming approaches. A number of recent studies have proposed histogram-free methodologies to estimate PMFs. Westerlund et al. [18] have presented an approach that builds PMFs based on Gaussian mixture models, outperforming histogramming, k-nearest neighbors (kNN) and kernel density estimators (KDE). Schofield [19] presented an adaptive parameterization scheme for a variety of different possible continuous functions for PMFs. Lee and co-workers [20, 21] presented a variational approach (variational free energy profile, or vFEP) to minimize likelihoods of observations from trial continuous free energy surfaces. Stecher et al. [22] have discussed reconstructing free energy surfaces from umbrella sampling using Gaussian process regression that comes inherently equipped with uncertainty estimates. Schneider et al. [23] discuss fitting higher-dimensional PMFs using artificial neural networks. The umbrella integration method of Käster and Thiel [24–26] constructs the PMF by numerical integration of a weighted average of the derivative of the free energy with respect to the order parameter. Meng and Roux presented a multivariate linear regression...
framework to link the biased probability densities of individual umbrella windows to yield a global free energy surface in the desired collective variables, though it uses histograms for some of the intermediate steps [27].

The present work shares particular similarities with the vFEP approach of Lee and co-workers [20, 21] and the adaptive parameterization approach of Schofield [19], but builds upon and goes beyond these works in two main aspects. First, as we detail in our mathematical development, we use the multistate Bennett acceptance ratio (MBAR) approach to furnish the provably minimum variance estimators of the free energy differences required to align independent biased sampling run, and then use these values to compute the maximum likelihood estimate of the unbiased PMF. Second, we show how this approach can easily placed in a fully Bayesian framework that enables transparent incorporation of Bayesian priors, Bayesian uncertainty quantification, and Bayesian model selection.

The calculation of PMFs parameterized by a small number of collective variables is largely motivated by the “curse of dimensionality”. Molecular systems are intrinsically exceedingly high-dimensional (with numbers of degrees of freedom in the tens or hundreds of thousands), which makes study of the system properties in the full configuration space of limited use in understanding and controlling molecular behaviors. Instead, system microstates are frequently projected into a handful of collective variables motivated by the physics of the problem at hand, and PMFs are then constructed over this reduced dimensional space to further analyze.

There are a number of ways to estimate PMFs in these collective variables. One could in theory run a simulation and simply calculate the probability of visiting a representative set of the collective variable. However, free energy barriers in collective variable space exceeding several $k_B T$ in height—where $k_B$ is Boltzmann’s constant and $T$ is temperature—are crossed with exponentially small probability in standard (unbiased) simulations, resulting in non-ergodic kinetic trapping and the inability to sample transition states and mechanisms. A number of methods have been proposed to overcome this problem, which typically involve introducing some form of bias or artificial smoothing of the underlying free energy landscape to enhance sampling of low probability (high free energy) regions and accelerate transitions between high probability (low free energy) metastable states.

Perhaps the most popular and straightforward way to perform biased sampling and PMF estimation is to run an ensemble of $K$ independent simulations, each of which biases the collective variable using a—usually, but not necessarily, harmonic—biasing potential. Each biasing potential forces the simulation to spend the majority of its time visiting locations with specific ranges of the collective variables consistent with the biases. Assuming sampling orthogonal to the collective variables is sufficient fast, good sampling of the thermally-relevant domain of the collective variable can be achieved by tiling collective variable space sufficiently densely with biasing potentials such that neighboring biased simulations sample overlapping configuration spaces. The unbiased PMF can then be determined using a range of mathematical approaches based in importance sampling [17, 28–30]. Provided the collective variables employed are “good” in the sense that they adequately separate out the relevant metastable states, this methodology, which goes by the name umbrella sampling [31], is a very straightforward and popular approach that works in as many dimensions as one can adequately cover the space with biasing potentials with sufficient configurational overlap. Assuming the potential only depends on the difference in collective variable from the restraint point, then the unbiased PMF can be estimated by post hoc analysis of the collective variable at each frame of each biased simulation trajectory without requiring records of the total energies, forces, or any other information from the simulation [17].

In this paper, we establish a mathematical framework to relate a discrete observed empirical distribution determined in a set of biased simulations to the unknown and typically continuous “true” potential of mean force in the collective variables one would expect in the limit of infinite sampling. We present a Bayesian treatment of this formalism to enable the incorporation of regularizing priors, uncertainty quantification, and model selection techniques. We demonstrate our approach in the analysis of umbrella sampling simulations for the $\chi$ torsion of a valine sidechain in lysozyme L99A with benzene bound in the cavity. The focus of the paper is to present analysis methodology, and so we assume that the data collected from biased simulations is sufficient to provide robust estimates of the PMF using reasonable methods. As such, it is our goal to calculate the best estimate of the PMF given a set of sampled data from umbrella sampling simulations, where appropriate definitions of “best” are explored within this paper.

Although we do not do so here, we observe that it is possible to use current best estimates of the PMF to adaptively direct additional rounds of sampling, thereby iteratively improving and refine the PMF. Such adaptive methods include metadynamics [32–34], adiabatic free energy dynamics [35], temperature accelerated dynamics [36], temperature accelerated molecular dynamics [37] / driven adiabatic free energy dynamics [38], adaptive biasing force approaches [39], variationally enhanced sampling [40], and conformational flooding [41]. This class of method has both significant advantages, such as optimally directing computational effort towards under-sampled regions of collective variable space and efficiently reducing uncertainties in the PMF, and significant additional challenges, such as under-sampling slow degrees of motion, and the problems of analyzing simulations that are history-dependent and thus only asymptotically approach equi-
librium sampling. For the purposes of this paper we will therefore consider only equilibrium sampling as the way to generate biased sampling trajectories for the purposes of PMF estimation. However, the approach we present is extensible to any collective variable biasing enhanced sampling technique that generates equilibrium samples, and is independent of the type of shape of biasing potential, as long as the potential is not time-dependent. Importantly, we also note that our approach is also applicable to data generated with temperature, restraint, or Hamiltonian exchange [42–46], or expanded ensemble [47, 48]. The only requirement on the data is that samples are collected at equilibrium with respect to a time-independent (i.e., stationary) probability distribution, and the biased samples cover the range of interest of the collective variable.

II. THEORY: PMF ESTIMATION FROM UMBRELLA SAMPLING DATA

Consider $K$ umbrella sampling simulations with different biasing potentials tiling a collective variable space and enforcing good sampling of all thermally-relevant system configurations with desired values of the collective variable. Typically, the collective variable is 1–3 dimensional, but the formalism holds for arbitrary dimensionality provided the space can be sufficiently densely sampled and sufficient overlaps achieved between neighboring biased distributions. For clarity of exposition, in the present work we will assume the usual case that the biased simulation data are collected at a single temperature and this temperature is the one at which we wish to estimate the unbiased PMF. However, the approach we outline here can be easily adapted to work with simulations performed at multiple temperatures [30, 48–50] or Hamiltonians [51, 52], or indeed performed without biasing potentials.

The reduced potentials $u_{B,k}$ of these states—where we express energies in terms of reduced quantities, $u(\vec{x}) = (k_B T)^{-1} U(\vec{x})$—are written in terms of the original potential $u(\vec{x})$ as:

$$ u_{B,k}(\vec{x}) = u(\vec{x}) + b_k(\Phi(\vec{x}) - \xi_{0,k}) $$

where the subscript $k$ indexes the biased simulation, the subscript $B$ reminds us that the potential is biased, and $b_k(\vec{\xi})$ is a user-defined biasing potential—most commonly a harmonic potential although other forms are perfectly acceptable—as a function of the collective variables $\vec{\xi}$ in which the umbrella sampling was performed.

The value of the collective variables corresponding to a particular system configuration $\vec{x}$ is defined by a low-dimensional mapping $\Phi(\vec{x}) = \vec{\xi}$, and the restraint point of the biasing potential in the collective variables is defined by $\xi_{0,k}$. The biasing potentials are then chosen so that the set of all simulations with biasing potentials give roughly equal sampling across the relevant range of $\vec{\xi}$ and neighboring biased simulations share overlap in configurational space.

We note two features of our description of umbrella sampling that are germane to our subsequent mathematical developments. First, we do not use the term “windows” as is frequently done when discussing umbrella sampling, as this word possesses significant ambiguity. “Window” could refer to either a specific interval of values of the collective variable $\vec{\xi}$, or it could refer one of the $k$ simulations run with biasing potential $b_k$. These two concepts are related in that simulations with a biasing potential generally sample values in a relatively restricted volume around $\xi_{0,k}$, but they are certainly not the same thing. A biased simulation can, in principle, yield any value of $\xi$ (although values far from any of the bias minima are highly unlikely) so the simulation results are not strictly within any finite “window” of $\vec{\xi}$ if run for long enough.

Second, we do not make the problematic assumption that the free energy of biasing a particular simulation is equal to the value of the PMF at the restraint point $\xi_{0,k}$ of the $k$th biasing potential. This approximation is often called the “stiff spring” approximation, as it assumes the collective variable sampling remains very close to the equilibrium position $\xi_{0,k}$ of the bias. But the value of the free energy of biasing is a weighted average over all configurations visited by the biasing potential, and so this approximation deteriorates with increasingly weak biasing potentials. Because one has to include biasing potentials of finite width to sufficiently sample the entire volume of $\vec{\xi}$ of interest, there is always a tradeoff between the strength and number of biasing potentials used: fewer biasing potentials require weaker biases, and weaker biases result in less accurate approximations to the free energy at $\xi_{0,k}$ under the “stiff spring” approximation. An analysis of this approximation (in the non-equilibrium pulling case) can be found in [53], but the approach presented in the present work completely avoids this particular problem.

We also note that the problem of approximating the PMF using free energy of the biasing potential is exacerbated by histogramming—as is done in WHAM—which introduces additional bias into the free energy calculation itself through binning of the energies as well as the free energies. Any sort of averaging of the PMF can be problematic because it tends to artificially lower barriers, which are frequently some of the most critical features of the PMF that we wish to accurately resolve.

Given umbrella sampling data from biased simulations, we seek the statistically optimal estimate of the PMF over the collective variables $F(\vec{\xi})$. This distribution contains exactly the same information content and is essentially interchangeable with the unbiased probability distribution $P(\vec{\xi})$. These two distributions are simply related through the logarithm:

$$ P(\vec{\xi}) = e^{-\beta F(\vec{\xi})} $$

(2)
and we will work with whichever of the pair is most natural for the discussion at hand. It is typically the case in molecular simulation that we work with relative, rather than absolute, free energies, in which case \( F(\tilde{\xi}) \) is only defined up to an arbitrary additive constant. In this case our estimate of the unbiased probability distribution \( P(\tilde{\xi}) \) is only defined up to an arbitrary multiplicative constant, but this can be set by enforcing normalization.

When we perform a simulation, the observed, empirical probability distribution, given a set of samples \( \{\tilde{x}_n\}_{n=1}^N \) distributed over the space of our collective variables \( \tilde{\xi} \):

\[
P_E(\tilde{\xi} | \{\tilde{x}_n\}) = \sum_{n=1}^{N} W(\tilde{x}_n) \delta(\Phi(\tilde{x}_n) - \tilde{\xi}) \tag{3}
\]

Where \( W(\tilde{x}_n) \) are weights associated with each sample. This is the most precise description of our sampled probability density that we can express after a simulation, because it only involves non-zero probability where we actually have measurements, and has zero probability at values of \( \tilde{\xi} \) that are not observed. If we only perform a single, unbiased simulation on a continuous space, then \( W(\tilde{x}_n) = 1/N \) for every sample, where \( N \) is the number of samples, since—in continuous space with arbitrarily high resolution of system configurations and collective variable mapping—each observation occurs only once. However, as we describe in the next section, if we have \( K \) biased simulations, we can incorporate data from all \( \sum_{k=1}^{K} N_k = N \) points gathered over all of the \( K \) states to better estimate \( P_E(\tilde{\xi}) \) [29].

A. MBAR and the empirical PMF

The multistate Bennett acceptance ratio (MBAR) is the statistically optimal approach to estimate the reduced free energies \( f_k = \int e^{-u_\tilde{\xi}(\tilde{x})} d\tilde{x} \), from \( \{\tilde{x}_1, \tilde{x}_2, \ldots, \tilde{x}_N\} \) observations at \( K \) thermodynamic state points [29]. These \( K \) thermodynamic states are defined by the reduced potentials \( \{u_1, u_2, \ldots, u_K\} \), and we assume that the \( \{\tilde{x}_n\}_{n=1}^N \) are distributed according to the Boltzmann distribution corresponding to the reduced potential of the state they are collected from. With these assumptions, the MBAR estimate for the reduced free energy differences between these \( K \) states is [29]:

\[
e^{-\hat{f}_i} = \sum_{n=1}^{N} \frac{e^{-u_i(\tilde{x}_n)}}{\sum_{k=1}^{K} N_k e^{f_k - u_k(\tilde{x}_n)}} \tag{4}
\]

where \( N_k \) is the number of samples taken from state \( K \). This system of equations must be solved self-consistently for the estimated reduced free energies \( \hat{f}_i \). Since the reduced free energies are typically only defined up to an additive constant, we usually choose to pin exactly one of the estimated free energies \( \hat{f}_i \) equal to any constant value we choose and the rest follow as relative free energy differences. We note that MBAR may be considered a binless estimator of free energy differences that can be derived from WHAM in the limit of zero-width bins [29, 54, 55].

After we have solved for these \( \hat{f}_i \), then we can calculate the weight \( W_i \) of sample \( \tilde{x}_n \) in any state \( i \) as [29, 55]:

\[
W_i(\tilde{x}_n) = \frac{e^{f_i - u_i(\tilde{x}_n)}}{\sum_{k=1}^{K} N_k e^{f_k - u_k(\tilde{x}_n)}} \tag{5}
\]

The weight \( W_i(\tilde{x}_n) \) of sample \( \tilde{x}_n \) at thermodynamic state point \( i \) represents the contribution to the average of an observable \( A \) in state \( i \) under a reweighting from the mixture distribution, consisting of all samples collected from all \( K \) state points, to the state \( i \) [28]. The probability of each sample in the mixture distribution is \( p(\tilde{x}_n) = \sum_{k=1}^{K} \frac{N_k}{N} p_k(\tilde{x}_n) = \sum_{k=1}^{K} \frac{N_k}{N} e^{f_k - u_k(\tilde{x}_n)} \) in other words, simply the average of all of the individual \( p_k \) probability distributions weighted by the number of samples \( N_k \) drawn from each [28]. It can be easily checked from eq. 5 that the \( W_k(\tilde{x}_n) \) are normalized such that [29]:

\[
\sum_{k=1}^{K} N_k W_k(\tilde{x}_n) = 1 \tag{6}
\]

and also from eq. 4 and eq. 5 that [29]:

\[
\sum_{n=1}^{N} W_i(\tilde{x}_n) = 1 \tag{7}
\]

The expectation value of the observable \( A \) estimated over all samples at all state points may then be written as:

\[
\langle A \rangle_i = \sum_{n=1}^{N} W_i(\tilde{x}_n) A(\tilde{x}_n) \tag{8}
\]

as discussed in eqs. 9 and 15 of the original MBAR paper [29]. We denote the weight of sample \( \tilde{x} \) as obtained via MBAR in the unbiased state as \( W(\tilde{x}_n) \), and in each of the \( k = 1 \ldots K \) biased states as \( W_k(\tilde{x}_n) \).

By eq. 2, the exponential of minus the potential of mean force \( F_i \) in state \( i \) is a probability density. By combining eq. 2 and eq. 8 under the particular choice for the observable \( A(\tilde{x}_n) = \delta(\Phi(\tilde{x}_n) - \tilde{\xi}) \), we have within the MBAR framework that:

\[
e^{-F_i(\tilde{\xi})} = \langle \delta(\Phi(\tilde{x}_n) - \tilde{\xi}) \rangle_i = \sum_{n=1}^{N} W_i(\tilde{x}_n) \delta(\Phi(\tilde{x}_n) - \tilde{\xi}) \tag{9}
\]

where \( \Phi(\tilde{x}) \) maps from the full coordinate space to the lower dimensional collective variable space of interest, and we have implicitly placed the PMF in reduced form so that it is a pure number. We will maintain
this convention throughout the remainder of this paper. To change into real energy units we simply multiply through by $k_B T$ so that $F_{\text{units}} = (k_B T) F$. We will use $F(\vec{\xi})$ to refer to the unbiased PMF and $F_k(\vec{\xi})$ to the biased free energy PMF obtained from each of the $k = 1 \ldots K$ biased states.

Eq. 9 makes clear that the MBAR estimate of the probability density distribution is a weighted sum of delta functions at the observed points. (Technically, it’s a distribution, not a function, since it is a sum of delta functions, which are themselves are distributions, but this formal distinction doesn’t affect any of the development in this paper.) It is instructive to compare this to the empirical distribution function when collecting samples from a single state where $W_i(\vec{x}_n) = 1/N$:

$$e^{-F(\vec{\xi})} = \frac{1}{N} \sum_{n=1}^{N} \delta \left( \Phi(\vec{x}_n) - \vec{\xi} \right)$$

(10)

from which it can be seen that the empirical distribution $P_E(\vec{\xi}|\vec{x}_n)$ generated using MBAR in eq. 3 is a weighted empirical distribution function using data from all states.

The representation of the empirical probability distribution function $P_E(\vec{\xi}|\vec{x}_n)$ of delta functions has both advantages and disadvantages. Estimating expectation values of observables that are a function of $\vec{\xi}$ is becomes simply a weighted sum over all observations

$$\langle A \rangle_i = \int A(\vec{\xi}) e^{-F(\vec{\xi})} d\vec{\xi} = \sum_{n=1}^{N} W_i(\vec{x}_n) A(\vec{x}_n).$$

(11)

However, it is very complicated to interpret or visualize this delta function representation. Neither can we work with this empirical representation in logarithmic form $F(\vec{\xi}) = -\ln e^{-F(\vec{\xi})}$ because the logarithm of a sum of delta functions isn’t defined, so only the exponential form has a well-defined mathematical meaning. The empirical cumulative distribution function is defined as

$$\text{CDF}(\vec{\xi}) = \int_{\vec{\xi}_{\text{low}}}^{\vec{\xi}} e^{-F(\vec{\xi})} d\vec{\xi}$$

(12)

where $\vec{\xi}_{\text{low}}$ is some arbitrarily defined lower bound for the integral over the collective variables, but this is only well-defined in one dimension.

To reiterate, expectations of quantities of interest can be computed by eq. 11 without recourse to $F_i(\vec{\xi})$ directly, but representing $F_i(\vec{\xi})$ as a continuous function is valuable for interpretation and understanding of the underlying molecular PMF. Developing statistically optimal representations of $F_i(\vec{\xi})$ that can be visualized and exploited to understand and engineer molecular behaviors is the key motivator of the remainder of this work.

B. Representations of $F(\vec{\xi})$ as a continuous function

In most cases, to visualize either a $P(\vec{\xi})$ or $F(\vec{\xi})$, or to use them in some other type of mathematical modeling, we need to choose how to represent them as continuous functions. Additionally, in the infinite sampling limit for molecular systems, they generally should be continuous functions due to the inherent continuity of the distribution supported by non-pathological choices of $\vec{\xi}$. We now proceed to describe a number of possible choices for continuous representations of $F(\vec{\xi})$. Most of the mathematical machinery that we develop can, in principle, be deployed in arbitrarily high dimensionalities of $\vec{\xi}$ although the capacity to achieve sufficient sampling will always present an issue. We note at appropriate junctures in the text any special considerations that may arise when generalizing to high-dimensional parameterizations.

1. Represent the PMF at specific locations $\vec{\xi}_0$ as the free energy of imposing each of the biasing restraints centered at $\vec{\xi}_0$. Assuming we have well-localized biasing potentials, then the free energy difference between the biased simulation and the unbiased simulation can be estimated as the free energy to restrain the simulation by each of the biasing functions, and is the “stiff spring” approximation. As described above, this method entails significant drawbacks in overestimating valleys and underestimating peaks, and in a lack of resolution between umbrella centers. We do not pursue this further.

2. Create a histogram out of the empirical distribution. This was default choice made in the pymbar package’s computePMF function, which has occasionally been erroneously called the “MBAR estimate of the PMF” in the literature. As we have shown, the use of MBAR is completely independent of the determination of the PMF, although it can be used in various algorithms to estimate the PMF.

We can calculate the expectation of the binning function $I_i(\vec{\xi}_i, \vec{\xi}, \vec{x}) = 1$ if $\Phi(\vec{x}) > (\vec{\xi}_i - \delta/2)$ and $\Phi(\vec{x}) < (\vec{\xi}_i + \delta/2)$ and $I_i(\vec{\xi}_i, \vec{\xi}, \vec{x}) = 0$ otherwise, where the $\vec{\xi}_i$ are the centers of the histogram bins and with some abuse of notation $\delta$ denotes the multidimensional bin widths, which—for clarity of exposition—we select to be equal in all dimensions. The binning function is used to essentially assign a fractional count to each bin according to the value of $W(\vec{x}_n)$ for $\vec{x}_n$ within the bin. The potential of mean force with $J$ total indicator functions is the expectation:

$$e^{-F(\vec{\xi})} = \sum_{i=1}^{J} \sum_{n=1}^{N} W(\vec{x}_n) I_i(\vec{\xi}_i, \vec{\xi}, \vec{x}_n)$$

(13)

where the second sum, as discussed above, is over all $N$ samples collected from all biased simulations. Since we are calculating an expectation of a function, MBAR gives a straightforward estimate for the error in the uncertainties, as outlined in the original MBAR paper [29].
If the bin widths chosen adaptively with the number of samples, the uncertainty becomes more complicated, since a different data set would have a different set of bin widths. If we wished, we could fit this histogram to a smooth function, using a least square fitting method, choosing the function to balance variance and bias. However it is better to avoid any histogramming steps altogether due to the inherent, unnecessary, and often uncontrolled bias that they introduce. This is especially true with multidimensional histograms, where the curse of dimensionality causes the number of bins required, and thus the number of samples for equal resolution, to scale exponentially with dimensionality.

When WHAM is employed to perform the PMF estimation [17], the histograms used to compute the free energies are the same as the ones used to calculate the PMF, which has a tendency to smooth out the PMF [56]. With MBAR, one can choose exactly how wide to make the histograms, since the histograms can be of any width that one chooses to best represent the underlying data, and are not constrained by the choice of separation in $\vec{\xi}$ between biasing functions $b_k(\vec{\xi})$ [29].

3. Employ a kernel density approximation. We can replace each delta function in the empirical PMF with a smooth function with weight centered at each sample and scaled by the weight. The most common choice is an isotropic Gaussian kernel $K(\vec{\xi}, \delta, \vec{\xi}) = (2\pi\delta^2)^{-1/2}e^{-\frac{\|\vec{\xi} - \vec{\xi}_i\|^2}{2\delta^2}}$, where $\delta$ now plays the role of the kernel bandwidth, but anisotropic Gaussians, “top hat,” and triangle functions are also frequently used. We observe that histogramming can be considered a form of kernel density estimation using indicator functions, with the center of the mass the preassigned bin center rather than the location of the sample. The bandwidth $\delta$ can be calculated in a number of ways, although the optimal choice is frequently not obvious [57–60]. For example, the maximum likelihood approach with the empirical distribution shrinks $\delta$ to zero, so other approaches must be used. The PMF in the kernel density approximation then becomes:

$$e^{-F(\vec{\xi})} = \sum_{n=1}^{N} W(\vec{x}_n) K(\Phi(\vec{x}_n), \delta, \vec{\xi})$$  \hspace{1cm} (14)

and $F$ is calculated by taking the logarithm, which is non-zero everywhere.

4. Identify a parameterized continuous probability distribution that best represents the empirical distribution. The fundamental difficulty with this approach is that there is no unambiguous “best” continuous distribution that stands independent of any other assumptions beyond those made so far. Specifically, the closest parameter-independent continuous function to a set of $\delta$ functions, for any reasonable definitions of close, are continuous functions that are essentially indistinguishable from the $\delta$ functions themselves. It is necessary, therefore, to instead impose some constraints upon the family of continuous functions that that represent our understanding of the empirical distribution as a discrete finite-data sampling of what should be a smooth and continuous distribution in the limit of infinite samples. This is an extremely flexible and generic point-of-view which allows for a variety of ways to represent the function with minimal bias and which naturally admits Bayesian formulations. The examination of this fourth perspective is our focus for the remainder of the paper. We now proceed to present a number of possible “best” choices for the representation for this continuous function along with proposed quantitative definitions of “best”.

C. Kullback-Leibler divergence as a measure of distance

Before we start examining mathematical forms of the trial PMF, we need to decide how we will evaluate how “close” a (continuous) trial function $P_T(\vec{\xi}|\vec{\theta})$ of some arbitrary parameters $\vec{\theta}$ is to the empirical distribution $P_E(\vec{\xi}|\{\vec{x}_n\})$. For the purposes of the present mathematical development we will leave the form of $P_T(\vec{\xi}|\vec{\theta})$ abstract, but it can be useful to consider that a number of possible parameterizations for the trial function are possible, including linear interpolants, cubic splines, or piecewise cubic Hermite interpolating polynomial (PCHIP) interpolations. For non-pathological continuous representations of $P_T(\vec{\xi}|\vec{\theta})$, the corresponding PMF is simply $F(\vec{\xi}|\vec{\theta}) = -\ln P_T(\vec{\xi}|\vec{\theta})$.

One logical definition of “closeness” is the Kullback-Leibler (KL) divergence from the empirical distribution in the state of interest (the one without any biasing distribution) to our trial distribution $P_T(\vec{\xi}|\vec{\theta})$, over the volume $\Gamma$ of collective variables. The Kullback-Leibler divergence from $Q$ to $P$, denoted $D_{KL}(P||Q)$, can be interpreted as a measure of the information lost when $Q$ is used to approximate $P$, and is defined as:

$$D_{KL}(P||Q) = \int_{\Gamma} \ln \frac{P(x)}{Q(x)} dx$$  \hspace{1cm} (15)

In later usage, we will generally omit the explicit reference to the volume $\Gamma$ over the collective variable space. We will develop several different formulations of the KL divergence that each consist of a weighted sum of the function evaluated at each sampled point, and the integral of the simulation over all the entire PMF (or sum of several integrals). We present them here and then later report the results of numerical tests to demonstrate their performance.

C.1. Unbiased state Kullback-Leibler divergence.
The KL divergence from $P_T(\tilde{\xi}|\tilde{\bar{\theta}})$ to $P_E(\tilde{\xi}|\tilde{x}_n)$ is:

$$D_{\text{KL}}(\tilde{\bar{\theta}}) = \int P_E(\tilde{\xi}|\tilde{x}_n) \ln \frac{P_E(\tilde{\xi}|\tilde{x}_n)}{P_T(\tilde{\xi}|\tilde{\bar{\theta}})} d\xi$$

$$\quad = \int \left[ P_E(\tilde{\xi}|\tilde{x}_n) \ln P_E(\tilde{\xi}|\tilde{x}_n) \right] - P_E(\tilde{\xi}|\tilde{x}_n) \ln P_T(\tilde{\xi}|\tilde{\bar{\theta}}) d\xi$$

(16)

The first term in the integral is somewhat problematic, in that it has a factor of $\ln P_E(\tilde{\xi}|\tilde{x}_n)$, which is not well-defined for delta functions. Even taking Gaussian approximations for the delta functions and allowing them to shrink to zero-width fails to yield a well-defined value since the entire integral $\int P_E(\tilde{\xi}) \ln P_E(\tilde{\xi})$ is unbounded in the positive direction as the width of the $\delta$ function goes to zero. Fortunately, whatever the value may be, it is independent of the parameters $\tilde{\bar{\theta}}$. Accordingly, we may neglect the first term in our minimization with respect to $\tilde{\bar{\theta}}$ and focus only on minimization of the second term. For the purposes of functional optimization we will—with some abuse of terminology—use $D_{\text{KL}}(\tilde{\bar{\theta}})$ to stand for the second, $\tilde{\theta}$-dependent term, with the dropping of the first parameter-independent term understood.

Using eq. 2, the normalized trial probability distribution can be equivalently expressed in terms of a trial potential of mean force $F_T(\tilde{\xi}|\tilde{\bar{\theta}})$:

$$P_T(\tilde{\xi}|\tilde{\bar{\theta}}) = \frac{e^{-F_T(\tilde{\xi}|\tilde{\bar{\theta}})}}{\int_T e^{-F_T(\tilde{\xi}|\tilde{\bar{\theta}})} d\xi}$$

(17)

If we set $W(\tilde{x}) = W_{\text{unbiased}}(\tilde{x})$ to be the weighting function for our unbiased reduced potential energy $u(\tilde{x})$, and seek the trial potential of mean force in the unbiased state $F_T(\tilde{\xi}|\tilde{\bar{\theta}}) = F(\tilde{\xi}|\tilde{\bar{\theta}})$, the function to be minimized reduces to:

$$D_{\text{KL}}(\tilde{\bar{\theta}}) = \int -P_E(\tilde{\xi}|\tilde{x}_n) \ln P_T(\tilde{\xi}|\tilde{\bar{\theta}}) d\xi$$

$$\quad = \int P_E(\tilde{\xi}|\tilde{x}_n) F(\tilde{\xi}|\tilde{\bar{\theta}}) d\xi + \int P_E(\tilde{\xi}) \ln \int e^{-F(\tilde{\xi}|\tilde{\bar{\theta}})} d\xi d\xi$$

$$\quad = \int P_E(\tilde{\xi}|\tilde{x}_n) F(\tilde{\xi}|\tilde{\bar{\theta}}) d\xi + \ln \int e^{-F(\tilde{\xi}|\tilde{\bar{\theta}})} d\xi d\xi$$

$$\quad = \sum_{n=1}^{N} W(\tilde{x}_n) F(\tilde{\xi}_n|\tilde{\theta}) + \ln \int e^{-F(\tilde{\xi}|\tilde{\bar{\theta}})} d\xi$$

(18)

Between the 2nd and 3rd steps we can integrate out the $P_E(\tilde{\xi}|\tilde{x}_n)$ term as $P_E(\tilde{\xi}|\tilde{x}_n)$ is normalized, is independent of the dummy variable $\tilde{\xi}$, and $\tilde{\xi}_n = \Phi(\tilde{x}_n)$, and between the 3rd and 4th steps we employ eq. 11 to estimate the expectation value over the data. Minimization of eq. 18 presents a prescription to adjust $\tilde{\bar{\theta}}$ to find the potential of mean force $F(\tilde{\xi}_n|\tilde{\theta})$ which is the logarithm of the closest distribution to the empirical delta function distribution calculated from MBAR.

Before proceeding to do so, it is instructive to make three observations about eq. 18. First, we note that the biasing functions only appear through the weights $W(\tilde{x}_n)$, which penalize points $\tilde{x}_n$ with values of $\Phi(\tilde{x}_n) = \xi_n$ inconsistent with the given bias. The calculation of the PMF does not otherwise include the biasing functions. Second, the contribution $F(\tilde{\xi}|\tilde{\bar{\theta}}) = -\ln \int e^{-F(\tilde{\xi}|\tilde{\bar{\theta}})} d\xi$ independent of the samples can be considered to penalize PMFs that are simply low everywhere. Third, low free energy regions of the PMF contribute more to the integral $F(\tilde{\xi}|\tilde{\bar{\theta}}) = -\ln \int e^{-F(\tilde{\xi}|\tilde{\bar{\theta}})} d\xi$ than high free energy regions. Accordingly, we should expect better estimates at the low values of $F$ (high probability states), but may sacrifice accuracy at large values of $F$ (low probability states).

C.2 Summed biased state Kullback-Leibler divergence. We can measure closeness to the KL divergence in a slightly different way, and try to find a single function that minimizes the sum of KL divergences from the $K$ empirical distribution functions observed at each biased sample state to the trial function with the biased potential added. The motivation for this ansatz is that it will force the trial function close to the potential of mean force in all regions the biased simulations have high density and therefore good sampling. When summing over the $K$ different biased simulations, we elect to weight the KL divergence proportional to the number of samples $N_k$ from that state. The motivation for this choice is that states with few samples should contribute less information than states with many. We will see that this assumption leads to particularly simple results.

Under these choices we define the sample-weighted sum of Kullback-Leibler divergences and function to be minimized as:

$$\sum_{k=1}^{K} N_k D_{\text{KL}}(\tilde{\bar{\theta}}) = \sum_{k=1}^{K} N_k \left( \sum_{n=1}^{N} W_k(\tilde{x}_n) F_k(\tilde{\xi}_n|\tilde{\theta}) \right)$$

$$\quad + \ln \int e^{-F_k(\tilde{\xi}|\tilde{\theta})} d\xi$$

$$= \sum_{k=1}^{K} N_k \left( \sum_{n=1}^{N} W_k(\tilde{x}_n) \left( F(\tilde{\xi}_n|\tilde{\theta}) + b_k(\tilde{\xi}_n) \right) \right)$$

$$\quad + \ln \int e^{-F(\tilde{\xi}|\tilde{\theta}) - b_k(\tilde{\xi})} d\xi$$

$$= \sum_{n=1}^{N} \left( \sum_{k=1}^{K} N_k W_k(\tilde{x}_n) \right) F(\tilde{\xi}_n|\tilde{\theta})$$

$$\quad + \sum_{k=1}^{K} N_k \ln \int e^{-F(\tilde{\xi}|\tilde{\theta}) - b_k(\tilde{\xi})} d\xi$$

$$= \sum_{n=1}^{N} F(\tilde{\xi}_n|\tilde{\theta})$$

$$\quad + \sum_{k=1}^{K} N_k \ln \int e^{-F(\tilde{\xi}|\tilde{\theta}) - b_k(\tilde{\xi})} d\xi$$

(19)
where $F_k(\tilde{\xi})$ is the potential of mean force of the $k$th biased state, $F(\tilde{\xi}_n)$ and $F_k(\tilde{\xi}_n)$ are the values of $F$ and $F_k$ at $\Phi(\tilde{x}_n) = \tilde{\xi}_n$, $b_k(\tilde{\xi}_n)$ is the value of the biasing potential associated with biased simulation based state, $F(\tilde{\xi}) = b_k(\tilde{\xi})$. We note that in moving from the second to third line we dropped the term $\sum_{k=1}^{K} \left( \sum_{n=1}^{N} W_k(\tilde{x}_n) b_k(\tilde{\xi}_n) \right)$ because it is independent of the $\tilde{\theta}$, and thus does not affect the minimization, and in moving from the third to fourth line we appeal to the normalization condition for $W_k(\tilde{x}_n)$ in eq. 6. The latter operation eliminates the weights from each individual state, leaving as the first term in our final expression an unweighted sum over the trial functions at the empirical data points. The second term is a weighted sum over an integral over the trial functions and biasing potentials and contains significant contributions only where the biasing potential is low. Large biasing potentials result in small contributions and essentially free variations of the trial function. However, as long as the trial function has significant weight in one of the biasing functions, then it will be constrained over that region of space. In our numerical tests discussed below, it appears that eq. 19 gives additional accuracy in the densely sampled regions by sacrificing accuracy in the sparsely sampled regions, but provides superior global fits compared to those achieved by minimization of eq. 18.

C.3. Summed sampled biased state Kullback-Leibler divergence. The final alternative we consider is to sum the KL divergences from the $K$ empirical distribution functions with the biased potential added as we do in the preceding section, but only using the $N_k$ actual samples from each biased state. In this case, each weight will be simply $1/N_k$, as each of the $N_k$ samples will be equally weighted. We will continue to weight each state by the number of samples $N_k$ collected from the state, as states with more samples contribute proportionally more information to the KL divergence. Following a similar development to that which led to eq. 19 and again dropping terms that are not dependent on $\tilde{\theta}$ yields the expression to be minimized as:

$$
\sum_{k=1}^{K} N_k D_{\text{KL}}(\tilde{\theta}) = \sum_{k=1}^{K} N_k \left( \sum_{n=1}^{N_k} \frac{1}{N_k} F_k(\tilde{\xi}_n | \tilde{\theta}) \right) + \ln \int e^{-F_k(\tilde{\xi} | \tilde{\theta})} d\tilde{\xi}^2
$$

$$
= \sum_{k=1}^{K} N_k \left( \sum_{n=1}^{N_k} \frac{1}{N_k} \left( F(\tilde{\xi}_n | \tilde{\theta}) + b_k(\tilde{\xi}_n) \right) \right) + \ln \int e^{-F(\tilde{\xi} | \tilde{\theta}) - b_k(\tilde{\xi})} d\tilde{\xi}^2
$$

$$
= \sum_{k=1}^{K} N_k \ln \int e^{-F(\tilde{\xi} | \tilde{\theta}) - b_k(\tilde{\xi})} d\tilde{\xi}^2
$$

$$
= \sum_{n=1}^{N} F(\tilde{\xi}_n | \tilde{\theta}) + \sum_{k=1}^{K} N_k \ln \int e^{-F(\tilde{\xi} | \tilde{\theta}) - b_k(\tilde{\xi})} d\tilde{\xi}^2 \quad (20)
$$

Somewhat surprisingly, this result is exactly the same as eq. 19. This emerges due to the normalization condition for $W_k(\tilde{x}_n)$ defined by eq. 6. Accordingly, whether we sum the contribution to the KL divergence of each sample over all states using the MBAR weights, or simply sum the contribution of each sample to its biased state, we will be minimizing the same function, provided we weight by the number of samples $N_k$ from each distribution.

We could, in principle, also choose to sum over the $K$ KL divergences without weighting each biased distribution by $N_k$. Doing so and following the steps leading to eq. 20 yields the expression:

$$
\sum_{k=1}^{K} D_{\text{KL}}(\tilde{\theta}) = \sum_{k=1}^{K} \frac{1}{N_k} \sum_{n=1}^{N_k} F(\tilde{\xi}_n | \tilde{\theta}) + \sum_{k=1}^{K} \ln \int e^{-F(\tilde{\xi} | \tilde{\theta}) - b_k(\tilde{\xi})} d\tilde{\xi}^2 \quad (21)
$$

which is both less mathematically elegant and less intuitively satisfying than eq. 20 since simulations conducted at a state point with small $N_k$ contribute equally to those with large $N_k$. Likewise, if we follow the logic of eq. 19 but employing equal weightings, we end up with a similarly unsatisfying result:

$$
\sum_{k=1}^{K} D_{\text{KL}}(\tilde{\theta}) = \sum_{n=1}^{N} \left( \sum_{k=1}^{K} W_k(\tilde{x}_n) \right) F(\tilde{\xi}_n | \tilde{\theta}) + \sum_{k=1}^{K} \ln \int e^{-F(\tilde{\xi} | \tilde{\theta}) - b_k(\tilde{\xi})} d\tilde{\xi}^2 \quad (22)
$$
which is not only more complicated than eq. 19, but also differs (as numerical tests confirm) from eq. 21 unless all $N_k$ are equal, in which case $\sum_{k=1}^{K} W_k(\vec{x}_n) = K/N = 1/N_k$, and equality is restored. Due to these mathematically and intuitively unsatisfying features, we will not pursue eq. 21 and eq. 22 further.

D. Likelihood as a measure of distance

As an alternative to the Kullback-Leibler divergence, we can measure distances using likelihoods. Specifically, we can take our trial probability distribution $P_T(\vec{\xi} | \vec{\theta})$ and compute the likelihood of one of our $N$ observations by evaluating the $P_T$ associated with that observation. The observations taken together comprise our data $D$. Assuming the samples are independent and identically distributed (i.i.d.) observations, then we can calculate the total likelihood as the product of the individual likelihoods. The trial probability distribution as a function of $\theta$ that maximizes this likelihood will be the one closest to the empirical distribution. In a similar manner to the KL divergence, we may construct this distribution in a number of ways. We shall show that the two choices we propose contain the same information as the KL divergence expressions, but offer greater interpretability and amenability to a Bayesian treatment.

D.1. Product over unbiased state likelihoods. Perhaps the simplest choice is to consider the joint likelihood of each weighted sample in the unbiased state. In this case, since we can consider each sample to be observed according to its weight $W(\vec{x}_n)N$ (the expected number of counts at $\vec{x}_n$ given the empirical distribution), then the overall likelihood as a function of $\vec{\theta}$ is:

$$\ell(\vec{\theta} | \{\vec{x}_n\}) = \prod_{n=1}^{N} P_T(\vec{\xi}_n | \vec{\theta})^W(\vec{x}_n)N$$

(23)

and the log likelihood is:

$$\ln \ell(\vec{\theta} | \{\vec{x}_n\}) = \sum_{n=1}^{N} NW(\vec{x}_n) \ln P_T(\vec{\xi}_n | \vec{\theta})$$

$$= \sum_{n=1}^{N} NW(\vec{x}_n) \left( -F(\vec{\xi}_n | \vec{\theta}) - \ln \int e^{-F(\vec{\xi} | \vec{\theta})} d\vec{\xi} \right)$$

$$= -N \sum_{n=1}^{N} W(\vec{x}_n) F(\vec{\xi}_n | \vec{\theta}) - N \ln \int e^{-F(\vec{\xi} | \vec{\theta})} d\vec{\xi}$$

(24)

In going from the second to the third line, we employ normalization condition in eq. 7. As expected [61], we quickly verify that eq. 24 is identical to eq. 18 up to a factor of $(-N)$, so maximizing this log likelihood is the same as minimizing the unbiased state KL divergence.

D.2. Product over biased state likelihoods. We could also calculate the overall likelihood as the product of the likelihoods of the individual samples in each of the biased simulations:

$$\ell(\vec{\theta} | \{\vec{x}_n\}) = \prod_{k=1}^{K} \prod_{n=1}^{N_k} P_T(\vec{\xi}_n | k, \vec{\theta})$$

(25)

where we have denoted the probability distribution resulting from the trial PMF plus the $k$th bias as $P_T(\vec{\xi}_n | k, \vec{\theta})$. The corresponding log likelihood is:

$$\ln \ell(\vec{\theta} | \{\vec{x}_n\}) = \sum_{k=1}^{K} \sum_{n=1}^{N_k} \ln P_T(\vec{\xi}_n | k, \vec{\theta})$$

$$= \sum_{k=1}^{K} \sum_{n=1}^{N_k} \left( -F(\vec{\xi}_n | \vec{\theta}) - b_k(\vec{\xi}_n) \right) - \ln \int e^{-F(\vec{\xi} | \vec{\theta}) - b_k(\vec{\xi}_n)} d\vec{\xi}$$

$$= \sum_{k=1}^{K} \left( \sum_{n=1}^{N_k} -F(\vec{\xi}_n | \vec{\theta}) \right) - b_k(\vec{\xi}_n) d\vec{\xi}$$

$$= -\sum_{n=1}^{N} F(\vec{\xi}_n | \vec{\theta})$$

$$- \sum_{k=1}^{K} N_k \ln \int e^{-F(\vec{\xi} | \vec{\theta}) - b_k(\vec{\xi}_n)} d\vec{\xi}$$

(26)

where in going from the second to third line we drop the $b_k(\vec{\xi}_n)$ term as independent of $\vec{\theta}$ and therefore irrelevant to the maximization. Eq. 26 is identical to eq. 19 up to a minus sign, so maximizing the product of biased state likelihoods is equivalent to minimizing the summed biased KL divergence.

D.3. Weighted product over biased state likelihoods. We could try to construct a likelihood that was consistent with the KL divergence in eq. 21 by constructing a sum of KL divergences over each state weighted by the reciprocal of the number of samples in each state:

$$\ell(\vec{\theta} | \{\vec{x}_n\}) = \prod_{k=1}^{K} \prod_{n=1}^{N_k} P_T(\vec{\xi}_n | k, \vec{\theta}) \frac{1}{N_k}$$

(27)
for which the corresponding log likelihood is:

$$
\ln \ell(\theta) \{ \xi_n \} = \sum_{k=1}^{K} \sum_{n=1}^{N_k} \frac{1}{N_k} \ln P_{\xi_n}(\xi_n | k, \theta) \\
= \sum_{k=1}^{K} \frac{1}{N_k} \sum_{n=1}^{N_k} \left( -F(\xi_n | \theta) - b_k(\xi_n) \right) \\
- \ln \int e^{-F(\xi | \theta) - b_k(\xi_n)} d\xi \\
= \sum_{k=1}^{K} \frac{1}{N_k} \sum_{n=1}^{N_k} -F(\xi_n | \theta) \\
- \sum_{k=1}^{K} \ln \int e^{-F(\xi | \theta) - b_k(\xi_n)} d\xi \\
= \sum_{k=1}^{K} \frac{1}{N_k} \sum_{n=1}^{N_k} F(\xi_n | \theta) \\
- \sum_{k=1}^{K} \ln \int e^{-F(\xi | \theta) - b_k(\xi_n)} d\xi (28)
$$

Eq. 28 is identical to eq. 21 up to a minus sign, and so maximizing the former is equivalent to minimizing the latter. However, as discussed above, there appears to be no real theoretical or practical justification reason to weight samples in the manner expressed in eq. 27 and for this reason we do not advocate the use of this formulation.

E. Least squares as a measure of distance

Finally, we could choose to adopt a functional form, and then perform a least squares fit to the empirical distribution or to the empirical PMF in order to define a distance between the distributions. Although seemingly quite a natural and straightforward approach, it does not give rise to easily interpretable or implementable expressions. Accordingly, we defer an analysis of the least squares approach to the Appendix and do not pursue this further.

F. How does vFEP fit into this framework?

We now examine the correspondence of our development with the variational free energy profile (vFEP) approach developed by Lee and co-workers [20, 21].

We first note the potential ambiguity within vFEP regarding the definition of the term ‘window’, which as described before, could refer to either a biasing potential, the data collected from a simulation run with that biasing potential, or a region of collective variable space within which a biased simulation has high probability density are related, but not equivalent, concepts. In the present comparison with vFEP, we will assume “window” as used in the vFEP definition refers to a biasing potential plus the data collected during simulations with that biasing potential. Under this definition of “window”, samples in the window are not included or excluded based on the associated values of $\xi$, only on the basis of biased simulation from which they were collected.

Using the original vFEP notation, $Z^a = \int e^{-F_{i,a}(\theta, x)} dx$ is the partition function of biased simulation $a$ and $F_{i,a}(\theta, x) = F_i(\theta, x) + W_a(x)$ is the biased trial partition function determined by parameters $\theta$ and collective variable $x$, where $W_a(x)$ is the biasing potential, and vectors in $x$ and $\theta$ are implicit. Since $W_a(x)$ is not a function of $\theta$ and does not affect the minimization, the log likelihood to be maximized with respect to the parameters $\theta$ of the trial function $F$ is:

$$
\ln \ell(\theta) = \sum_a \left[ -\ln Z^a - \frac{1}{N_a} \sum_{i=1}^{N} F_{i,a}(\theta, x_i) \right] \\
= \sum_a \left[ -\frac{1}{N_a} \sum_{i=1}^{N} F_{i}(\theta, x_i) - \ln \int_{\Gamma_a} e^{-F_{i,a}(\theta, x)} dx \right] (29)
$$

If we can assume (i) the substitution of $k$ as a label for biasing potential rather than $a$ as the label of windows, (ii) the recognition that $\Gamma_a$ should be either the same or approximately the same as $\int_{\Gamma} d\xi$, since samples from biased potential will be mostly constrained to subset of $\Gamma$, but can in principle appear anywhere in $\Gamma$, then we can translate this into the terminology of the present paper. The window $a$ becomes the biased simulation $k$, $N_a$ becomes $N_k$, $x$ becomes $\xi$, vectors are noted explicitly, and we obtain:

$$
\ln \ell(\theta) = \sum_{k=1}^{K} \left[ -\frac{1}{N_k} \sum_{i=1}^{N_k} F(\xi_i | \theta) \right] \\
- \ln \int e^{-F(\xi | \theta) - b_k(\xi_n)} d\xi (30)
$$

This expression is identical to eq. 28 and, up to a minus sign, eq. 21. Accordingly, when viewed through the lens of the development presented in this paper—and with the previously mentioned assumptions about the definitions of windows and range of integrals—vFEP corresponds to a particular choice of biased state weighting within a Kullback-Leibler divergence (eq. 21) or likelihood formulation (eq. 28). As discussed above, this expression is hard to justify from a theoretical or practical perspective, but if the direct sum over biasing potentials is changed to one weighted by $N_k$, then it would become the easier-to-work with and better justified eq. 26.
III. A BAYESIAN FRAMEWORK FOR PMF ESTIMATION

Equipped with the prescriptions to calculate likelihood of observations under the different assumptions detailed in Section II D, we can switch to a Bayesian framework to find distributions possessing desirable features of an analytical form, continuity, and smoothness that is most consistent with our understanding of $F(\xi)$. We note that our use of a likelihood formulation, which was shown to be fully consistent with the KL divergence framework, is crucial in opening the door to a Bayesian formulation.

At the first step in this framework, we take a candidate trial distribution $P_T(\xi|\theta)$ and optimize its parameters $\theta$ to form the maximum a posteriori probability (MAP) estimate of $P_T(\xi|\theta)$. This estimate maximizes the Bayes posterior probability of the trial distribution, rather than simply the likelihood, given the collected (biased) samples and MBAR estimates of the relative free energy differences $\Delta f_{ij} = f_j - f_i$ between biased states.

As we introduce our Bayesian formulation, we note that the free energies emerging from the MBAR equations have no free parameters; they are the only estimated normalizing constants satisfying the self-consistent equations in eq. 4. It is possible to employ a Bayesian approach to free energy estimation by sampling of either the density of states [62] or weights of each sample in the unbiased state [63], allowing one to incorporate additional priors about the simulations in addition to priors on the shape of the potential of mean force. However, since the free energy is defined completely by the Boltzmann distribution, and since the MBAR equations provide the lowest variance importance sampling estimator and are asymptotically unbiased, then in the absence of other information about the system, it is the simplest and least biased approach to employ MBAR estimates for $\{f_i\}$.

A difference from previous efforts is that we cast our approach within a Bayesian framework that enables transparent incorporation of Bayesian priors, Bayesian uncertainty quantification, and Bayesian model selection about the functional form of the potential of mean force. Although we do not do so here, this formalism also sets the stage for adaptive sampling, in which regions of the probability distribution containing the most uncertainty are identified for additional biased sampling to optimally direct computational resources. This is similar in spirit to, but would go beyond, the adaptive approach of Schofield, which presents an elegant means to alter the analytical representation of the unbiased probability distribution to minimize uncertainty [19], to actually guiding the collection of additional data to optimally reduce uncertainty in the estimated distribution.

Given the set of biased samples $\{\bar{x}_n\}$ and their collective variable mappings $\{\xi_n\} = \{\hat{\Phi}(\bar{x}_n)\}$ and the associated weights in the (unbiased) thermodynamic state calculated by MBAR $W(\bar{x}_n)$ (eq. 5), we apply Bayes’ theorem [64] to construct an expression for the posterior probability of the parameters $\theta$ given the data $\{x_n\}$, obtaining:

$$\mathcal{P}(\theta|\{x_n\}) = \frac{\mathcal{P}(\{x_n\}|\theta)\mathcal{P}(\theta)}{\mathcal{P}(\{x_n\})} \quad (31)$$

where $\mathcal{P}(\theta|\{x_n\})$ is the posterior probability of the parameters $\theta$ given the sampled data, $\mathcal{P}(\{x_n\}|\theta) = \ell(\theta|\{x_n\})$ is the previously-defined likelihood specifying the probability of the collected samples given the particular choice of parameters, $\mathcal{P}(\theta)$ is the prior probability of the parameters before any data have been collected, and $\mathcal{P}(\{x_n\}) = \int \mathcal{P}(\{x_n\}|\theta)\mathcal{P}(\theta) d\theta$ is the probability of observing the samples that we did (the evidence), serves to normalize the posterior, and contains no dependence on the parameters $\theta$. Importantly, the prior enables us to transparently encode any prior beliefs or knowledge about the system into our analysis that can serve to regularize and stabilize our estimation.

The MAP estimate of the parameters follows from maximization of the log posterior:

$$\hat{\theta}^{\text{MAP}}(\{x_n\}) = \arg\max_{\theta} \ln \mathcal{P}(\theta|\{x_n\})$$

$$= \arg\max_{\theta} \left[ \ln \mathcal{P}(\{x_n\}|\theta) + \ln \mathcal{P}(\theta) \right]$$

$$= \arg\max_{\theta} \left[ \ln \mathcal{P}(\{x_n\}|\theta) + \ln \mathcal{P}(\theta) \right]$$

$$= \arg\max_{\theta} \left[ \ln \mathcal{P}(\{x_n\}|\theta) + \ln \mathcal{P}(\theta) \right]$$

Exploiting our previous observation that maximizing a log likelihood is the same as minimizing the corresponding KL divergence from an empirical distribution [61], we can equivalently view maximization of the Bayes posterior (eq. 32) from a frequentist perspective as minimization of the Kullback-Leibler divergence or maximization of the log likelihood subject to regularization by the logarithm of the Bayes prior.

To use eq. 32 we need to adopt a form for the likelihood $\ell(\theta|\{x_n\})$ and prior $\mathcal{P}(\theta)$. The development in Section II D suggests we adopt eq. 23 or 25 as candidates for the likelihood, where we explicitly assumed samples to be i.i.d. distributed. If the samples cannot be treated as i.i.d., then the counts $N$ or $N_k$ should be corrected by an inefficiency factor reflecting the presence of correlations in the sampling procedure [65, 66]. The simplest and most common choice for the prior is a uniform prior $\mathcal{P}(\theta) = 1$. With no dependence on the model parameters $\theta$, it drops out of the maximization in eq. 32 and the MAP estimate $\hat{\theta}^{\text{MAP}}$ becomes coincident with the maximum likelihood (ML) estimate $\hat{\theta}^{\text{ML}}$:

$$\hat{\theta}^{\text{ML}}(\{x_n\}) = \arg\max_{\theta} \ln \ell(\theta|\{x_n\}) \quad (33)$$
In principle, arbitrary priors are admissible—even improper priors that do not have a finite integral—provided the posterior is proper (i.e., integrates to unity) [67]. In a Bayesian sense, we use the prior to encode prior knowledge or belief about the character of the probability distribution (such as smoothness of the splines). In the frequentist sense, the prior serves to regularize the probability estimate, providing bias-variance trade-off and compensating for sparse data. In a practical sense, the appropriate prior to adopt depends on the form of the model selected $P(\xi | \bar{\theta})$, the size and quality of the simulation data, and the degree of prior belief or understanding of the system. Adopting the likelihood eq. 23 the maximization in eq. 32 can be expressed as:

$$
\hat{\theta}^{\text{MAP}}(\{x_n\}) = \arg\max_{\bar{\theta}} \left[ -N \sum_{n=1}^{N} W(x_n) F(\xi_n | \bar{\theta}) - N \ln \int e^{-F(\xi | \bar{\theta})} d\xi + \ln P(\bar{\theta}) \right]
$$

$$
\hat{\theta}^{\text{MAP}}(\{x_n\}) = \bar{\theta} \left[ -N \sum_{n=1}^{N} W(x_n) F(\xi_n | \bar{\theta}) + N \ln \int e^{-F(\xi | \bar{\theta})} d\xi - \ln P(\bar{\theta}) \right]
$$

$$
\arg\min_{\bar{\theta}} \left[ N \sum_{n=1}^{N} W(x_n) F(\xi_n | \bar{\theta}) - \ln P(\bar{\theta}) \right] \text{ s.t. } \int e^{-F(\xi_n | \bar{\theta})} d\xi = 1,
$$

where in going from line 2 to 3 we have appealed to the identity $P(\xi | \bar{\theta}) = e^{-F(\xi | \bar{\theta})}$ (eq. 2) and asserted that this distribution must be normalized.

Adopting the product of likelihoods eq. 25 the maximization in eq. 32 becomes:

$$
\hat{\theta}^{\text{MAP}}(\{x_n\}) = \arg\max_{\bar{\theta}} \left[ -N \sum_{n=1}^{N} F(\xi_n | \bar{\theta}) - \sum_{k=1}^{K} N_k \ln \int e^{-F(\xi | \bar{\theta}) - b_k(\xi_n)} d\xi + \ln P(\bar{\theta}) \right]
$$

$$
\hat{\theta}^{\text{MAP}}(\{x_n\}) = \bar{\theta} \left[ \sum_{n=1}^{N} F(\xi_n | \bar{\theta}) + \sum_{k=1}^{K} N_k \ln \int e^{-F(\xi | \bar{\theta}) - b_k(\xi_n)} d\xi - \ln P(\bar{\theta}) \right]
$$

$$
\arg\min_{\bar{\theta}} \left[ N \sum_{n=1}^{N} F(\xi_n | \bar{\theta}) - \ln P(\bar{\theta}) \right] \text{ s.t. } \int e^{-F(\xi_n | \bar{\theta}) - b_k(\xi_n)} d\xi = 1 \forall k.
$$

There are thus two approaches to find the MAP or ML estimate: an unconstrained minimization enforcing the normalization implicitly (second-to-last lines in eq. 34 and 35), and a constrained minimization enforcing the normalization explicitly (last lines in eq. 34 and 35). The constrained minimization versions of the above expressions can be solved using the method of Lagrange multipliers or through any other constrained optimization method such as the interior point method or sequential quadratic programming (SQP). The relative efficiency of the two approaches will depend on the details of software methods available as well as the particular forms of the biases and $F(\xi_n | \bar{\theta})$.

IV. MODEL SELECTION

The Akaike information criterion (AIC) or Bayesian information criterion (BIC) provide a principled means to discriminate between different possible choices for the Bayes prior and the trial probability distribution. The AIC is defined as [68]:

$$
AIC = 2k - 2 \ln \ell(\bar{\theta} | \{x_n\}),
$$

where $k$ is the number of estimated parameters in the model. The BIC is defined as [69]:

$$
BIC = k \ln N - 2 \ln \ell(\hat{\theta} | \{x_n\}),
$$

where $N$ is the number of data points. If we compute $\hat{\theta} = \hat{\theta}^{\text{MAP}}$ for a number of model choices $i$, we can use these parameter estimates to compute the set of AIC or
BIC values $\{a_i\}$ for the candidate models. The model with the lowest $a_i$ is the single model that is best supported by the data.

A more sophisticated approach to model selection defines the smallest of the $\{a_i\}$ as $a_{\text{min}}$, then assigns the relative likelihood of model $i$ as $r_i = e^{-\Delta_i/2} = e^{-(a_i-a_{\text{min}})/2}$. The model weights follow from the normalized $r_i$ and provide the likelihood of model $i$ [19]:

$$\omega_i = \frac{r_i}{\sum_k r_k} = e^{-\Delta_i/2}.$$  \hspace{1cm} (38)

Adopting a threshold $q = 0.05$ (for example), the $\{r_i\}$ can be used to discard models from consideration and/or determine that there is insufficient evidence to choose one model over the other. The $\{\omega_i\}$ may also be used as weighting factors with which to construct a multi-model composed from the weighted sum of the predictions of each candidate model.

V. BAYESIAN UNCERTAINTY QUANTIFICATION

The $\hat{\theta} = \hat{\theta}^{\text{MAP}}$ estimate represents the single best point estimate of the parameters of the trial distribution $P_T(\xi; \hat{\theta})$ given the data $\{\xi_n\}$ and the prior $P(\theta)$. Uncertainties around these point estimates may be approximated by analytical error expectations or through bootstrap estimation [70]. A fully Bayesian uncertainty estimate is defined by the distribution of $\hat{\theta}$ dictated by the Bayes posterior [30]. Empirical samples of $\hat{\theta}$ from the Bayes posterior may be generated using the Metropolis-Hastings algorithm. This Markov Chain Monte-Carlo (MCMC) approach generates a sequence of parameter realizations that converges to the stationary distribution of the Bayes posterior [71]. Under this approach we propose trial moves in $\theta$ that are accepted or rejected according to the Metropolis-Hastings acceptance criterion [71, 72]:

$$\alpha(\hat{\theta}^\nu | \hat{\theta}^\mu) = \min \left\{ \frac{P(\hat{\theta}^\nu | \{\xi_n\}) \cdot q(\hat{\theta}^\nu | \hat{\theta}^\nu)}{P(\hat{\theta}^\mu | \{\xi_n\}) \cdot q(\hat{\theta}^\mu | \hat{\theta}^\nu)}, 1 \right\}$$

$$= \min \left\{ \frac{P(\{\xi_n\} | \hat{\theta}^\nu) \cdot P(\hat{\theta}^\nu) \cdot q(\hat{\theta}^\nu | \hat{\theta}^\nu)}{P(\{\xi_n\} | \hat{\theta}^\mu) \cdot P(\hat{\theta}^\mu) \cdot q(\hat{\theta}^\mu | \hat{\theta}^\nu)}, 1 \right\}$$

$$= \min \left\{ \frac{\ell(\hat{\theta}^\nu | \{\xi_n\}) \cdot P(\hat{\theta}^\nu) \cdot q(\hat{\theta}^\nu | \hat{\theta}^\nu)}{\ell(\hat{\theta}^\mu | \{\xi_n\}) \cdot P(\hat{\theta}^\mu) \cdot q(\hat{\theta}^\mu | \hat{\theta}^\nu)}, 1 \right\}$$ \hspace{1cm} (39)

where $\alpha(\hat{\theta}^\nu | \hat{\theta}^\mu)$ is the probability of accepting a trial move from parameter set $\hat{\theta}^\mu$ to parameter set $\hat{\theta}^\nu$, and $q(\hat{\theta}^\nu | \hat{\theta}^\mu)$ is the probability of proposing this trial move. We have invoked Bayes’ Theorem (eq. 31) in going from the first line to the second, and observe that (importantly) the evidence has canceled top and bottom. In going from the second line to the third, we employed the identity $P(\{\xi_n\} | \hat{\theta}) = \ell(\hat{\theta} | \{\xi_n\})$. In the event that symmetric trial move proposal probabilities are adopted such that $q(\hat{\theta}^\nu | \hat{\theta}^\mu) = q(\hat{\theta}^\mu | \hat{\theta}^\nu)$, the Metropolis-Hastings acceptance criterion reduces to the Metropolis criterion [71, 73]:

$$\alpha(\hat{\theta}^\nu | \hat{\theta}^\mu) = \min \left\{ \frac{\ell(\hat{\theta}^\nu | \{\xi_n\}) \cdot P(\hat{\theta}^\nu)}{\ell(\hat{\theta}^\mu | \{\xi_n\}) \cdot P(\hat{\theta}^\mu)}, 1 \right\}$$ \hspace{1cm} (40)

We initialize the Markov chain from $\hat{\theta}^{\text{MAP}}$ corresponding to the maximum of the Bayes posterior $P(\hat{\theta} | \{\xi_n, W_n\})$ and propose trial moves that maintain normalization $\int_P P(\xi | \hat{\theta}) d\xi = 1$. By monitoring

\[ \mathcal{L}(\hat{\theta} | \{\xi_n\}) = \ln \left( P(\{\xi_n\} | \hat{\theta}) P(\hat{\theta}) \right) = \ln \ell(\hat{\theta} | \{\xi_n\}) + \ln P(\hat{\theta}) \]—which is proportional to the Bayes posterior up to an additive constant with no $\hat{\theta}$ dependence (eq. 31)—we can determine that the Markov chain has converged when $\mathcal{L}(\hat{\theta} | \{\xi_n\})$ plateaus to fluctuate around a stable mean. At this point we may harvest realizations of $\hat{\theta}$ distributed according to the Bayes posterior. Using these parameter realizations, we can construct realizations of $P(\xi | \hat{\theta})$ to quantify the uncertainties in this estimated distribution.

VI. EXAMPLE: UMBRELLA SAMPLING OF PROTEIN SIDECHAIN TORSION WITHIN BINDING CAVITY

As an illustrative example, we consider the application of our mathematical framework to compute a 1D PMF from an umbrella sampling simulation. Code implementing these methods can be found publicly available in the ‘pmf’ branch.
The data correspond to umbrella sampling simulation data for the \( \chi \) torsion of a valine sidechain in lysozyme L99A with benzene bound in the cavity \([74]\) (fig. 1).

![Image](http://github.com/choderalab/pymbar)

**FIG. 1:** \( \chi \) torsion angle in Lys111 of L99a T4 lysozyme around with the potential of mean force is calculated using umbrella sampling.

We analyze data from 26 biased simulations employing umbrella potentials at a range of dihedral values with harmonic biasing constants of between 100 and 400 kJ/mol/nm\(^2\). A 100 ps simulation was carried out under each umbrella potential with angles and energies saved every 0.2 ps for a total of 500 samples at each state. The data was analyzed for correlations, and approximately every other data point is taken (exact frequency varying with state) for a total of 7446 data points, ranging from 42 to 410 points per umbrella. We examine the histogram approach (with 30 bins, a number chosen to be visually clear—the number of bins can be chosen completely independently of the number of umbrella simulations run), and the kernel density approximation with a Gaussian kernel, with bandwidth parameter half of the bin size, in this case, \( \frac{1}{2} \times 360/30 = 6 \) degrees. We also look at parameterized splines as an representation; in this case, using B-splines with varying numbers of knots placed uniformly, using cubic splines in this example; the theory is independent of these particular choices of spline.

We note that one could use splines to fit to either the PMF \( F(\vec{\xi}|\vec{\theta}) \) or the probability distribution \( P(\vec{\xi}|\vec{\theta}) \). However, we find that it becomes difficult to satisfy the non-negativity condition of \( P(\vec{\xi}|\vec{\theta}) \) when using standard spline implementations, and that large changes in PMF propagate exponentially to the probability distribution making it challenging to fit stably and robustly. For numerical stability, we therefore recommend using splines to approximate \( F(\vec{\xi}|\vec{\theta}) \) rather than \( P(\vec{\xi}|\vec{\theta}) \).

We examine the parameterized spline representations emerging from the optimizations defined by the expressions in eq. 34—corresponding to the unbiased state likelihood in eq. 23, log likelihood in eq. 24, and KL divergence in eq. 18—and eq. 35—corresponding to the product of biased states likelihood in eq. 25, log likelihood in eq. 26, and KL divergence in eq. 19. We will refer to the first as the “unbiased state likelihood”, and the second as the “biased states likelihood,” as it combines samples from all biased states.

Efficient optimization of these expressions requires calculating the gradient and potentially the Hessians. The use of B-splines, which construct the spline in terms of local basis function, makes this calculation relatively efficient, as detailed in the Appendix. For simplicity, we elect to use a uniform distribution of spline knot locations over the domain, but these could be adaptively situated by optimizing their locations to maximize the MAP as proposed by Schofield \([19]\).

For the Bayes prior, where we compute the full posterior, rather than just the likelihood, we adopt an unnormalized Gaussian prior on the difference between successive spline knot values:

\[
P(\vec{\theta}) = \prod_{c=1}^{C-1} e^{-\alpha(\theta_c - \theta_{c+1})^2}
\]

where \( \alpha \) is a hyperparameter that controls the degree of smoothing regularization imposed upon the trial distribution. Selecting \( \alpha = 0 \) corresponds to a uniform prior that drops out of the maximization and \( \vec{\theta}_{\text{MAP}} = \vec{\theta}_{\text{ML}} \). Selecting \( \alpha > 0 \) favors smoother splines with less variation from knot to knot. We examine the effect of priors governed by choice of \( \alpha \), where \( \alpha = k/n \), where \( n \) is the number of spline knots, for some constant \( k \). Uncertainties are estimated by MCMC sampling of the Bayes posterior using the Metropolis-Hastings algorithm and acceptance criteria (eq. 39).

The time limiting factor, both for optimizations and MCMC sampling of the posterior, is the numerical quadrature of the integral \( \int P_T(\vec{\xi}|\vec{\theta}) d\vec{\xi} \). For the log likelihoods from the unweighted state (eq. 24), the integral enforcing the normalization of \( P_T \) is only carried out over the unbiased trial function, whereas for approaches considering all states (eq. 26), the integral is carried out over all \( K \) trial functions with biases and is thus roughly \( K \) times slower.

The AIC and BIC allow us to select the number of spline knots best supported by the data. We plot in fig. 2 the AIC (eq. 36) and BIC (eq. 37) for the unbiased state likelihood and biased states likelihood choices. In the unbiased state case, the AIC exhibits a local minimum at 16 knots and a global minimum at 26, whereas the BIC—which penalizes excessive parameters more strongly than the AIC—possesses a local minimum at 24 knots and a global minimum at 16. In the biased states
FIG. 2: AIC (solid) and BIC (dotted) for splines maximizing PMF likelihoods for the unbiased state estimator (red, eq. 24) and biased states estimator (blue, eq. 26) as a function of the number of spline knots, referenced from the minimum of each method. Although the curves are noisy, and occasionally nonmonotonic, they provide a useful guide towards choosing optimal numbers of parameters for models, as can be seen by comparison to Fig. 3.

We can see how the behavior of PMF changes as a function of the number of knots and how the AIC and BIC help select optimal knot numbers in fig. 3. In this figure, we plot maximum likelihood PMFs under the unbiased state likelihood (eq. 34, in fig. 3a) and biased states likelihood (eq. 35, in fig. 3b) as a function of the number of spline knots, along with the histogram estimate equipped with uncertainties generated from error propagation from the weights via MBAR [29]. As expected, higher numbers of knots provide improved fitting, but overfitting becomes clear for larger numbers of knots, especially in the case of fits using the unbiased state likelihood. However, model complexities corresponding to AIC/BIC minima fit the data relatively well in both cases. We note that the unbiased state PMF fits in fig. 3a, even for the 10-knot spline, are tightly grouped at the various PMF minima, but they vary significantly at the maxima, as there are less constraints on the maxima than the minima using this approach. In contrast, all fits with sufficient functional flexibility (more than 10 spline knots) using the biased states approach agree relatively well across the entire range of the PMF (fig. 3b), even with as few as 14 spline knots, the value corresponding to the minimum of both AIC and BIC for the biased states likelihood.

Adding bootstrapped uncertainty estimates to the PMFs help better show the relationship between the methods and their strengths and weaknesses. We present in Fig. 4 a comparison of the histogram (with 30 bins), kernel density approximation (with Gaussian kernels with $\sigma$ of $6^\circ$), unbiased state likelihood and biased states likelihood splines employing the AIC/BIC optimal number of knots, and vFEP (using with the same number of splines as the biased states likelihood case). Uncertainties all estimates are estimated from an ensemble of 40 bootstrap samples from each of the umbrellas. All methods give relatively similar results, which is to be expected with a well-sampled system and careful selection of parameters. In particular, PMF using vFEP (subject to the assumptions discussed earlier in the text) is...
close to the biased states likelihood approximation. This result is expected because the two approaches coincide in the limit of equal numbers of uncorrelated samples per state.

In fig. 5 we demonstrate the utility of fully Bayesian uncertainty quantification. Uncertainties in the MAP splines are computed from 50,000 (for biased states posteriors) and 200,000 (for unbiased state posteriors) steps of MCMC sampling from the Bayes posterior. Uncertainties represent the 95% confidence intervals at each spline knot. In both cases, we show results for 10, 20, and 30 splines for two different Gaussian priors (eq. 41): (i) \( \alpha = 0.1/n \) in fig. 5a and fig. 5c, where \( n \) is the number of spline knots, and (ii) \( \alpha = 1/n \) in fig. 5b and fig. 5d. We recall that larger values of \( \alpha \) result in smoother posterior distributions. We find that the maximum likelihood calculated only from the biased state (eqs. 24 and 18) has a tendency to underestimate the free energy barriers in collective variable. The product of likelihoods from all the unweighted samples collected from each biased state, weighted by the number of samples collected from each biased state (eqs. 26 and 19), has much better overall performance over the entire PMF range. In contrast, the biased states formulation provides more constraints across the entire PMF (cf. eq. 35), and the MCMC error bounds are smaller over the entire range of the PMF for both choices of \( \alpha \) (fig. 5c and 5d).

VII. CONCLUSIONS

In this paper, we have presented a Bayesian formalism to compute potentials of mean force from the empirical distributions generated by biased sampling. Within this formalism, we avoid any arbitrary choice of histogram in either the definition of the PMF or the calculation of the weights, and provide clear and explicit criteria to decide which continuous potentials of mean force are most consistent with the biased sampling data. The choice and optimization of the representation of the continuous PMF is completely decoupled from the choice of biasing functions and calculation of the relative free energies between the biased simulations. Biasing functions can be chosen to give appropriate sampling along the collective variables of interest, and the samples and their associated Boltzmann weights are used to construct the PMF. The Bayesian formalism allows us to choose the PMF that is sufficiently close to the empirical distribution of the samples we have collected, and explicitly include any prior information that we include by our choice of representation of our PMF functional form. Our development also clearly demonstrates the equivalence of the likelihood-based Bayesian formulation and Kullback-Leibler-based frequentist formulation.

We find that the maximum likelihood calculated only from the unbiased state (eqs. 24 and 18) has a tendency to underestimate the free energy barriers in collective variable. The product of likelihoods from all the unweighted samples collected from each biased state, weighted by the number of samples collected from each biased state (eqs. 26 and 19), has much better overall performance over the entire PMF range. Surprisingly, this likelihood is exactly equal to the likelihood generated from the product over all states of the reweighted contribution of all samples to each biased state state, again weighted by the number of samples collected from each state (cf. eqs. 19 and 20).

We can then take these likelihoods and directly incorporate them into a Bayesian inference framework. Priors on the parameters of the PMF can then be chosen using whatever criteria is most appropriate; in this study we considered a Gaussian prior enforcing smoothness,
but the selection can be made based on any user-defined criteria, such as tethering free energies to particular values or enforcing similarity to previously estimated distributions. We can then use MCMC sampling of the posterior of the PMF curves to perform uncertainty quantification for arbitrary choices of prior.

We demonstrate our approach in an application to calculation of the PMF for the leucine rotation in the L99A mutant of T4 lysozyme. The unbiased state likelihood has some clear failures in that it insufficiently constrains the PMF at the highest points. This failure shows up in multiple ways. When computing bootstrap uncertainties, the unbiased states approach has very high uncertainty in the barriers. With MCMC sampling, the issues become even clearer, with significant fluctuation in the parameters at the barriers unless a relatively severe prior is imposed. The biased states likelihood, however, behaves much more stably, with a well-constrained PMF over the entire range, even under weak priors.

Code implementing this approach is distributed in pymbar, where the previous potential of mean force functionality, using histograms to represent the PMF, is replaced with a more comprehensive module implementing the formalism presented in this paper.

The Bayesian approach we present here approach is directly extensible to multidimensional potentials of mean force. However, the numerical details of performing the fitting may be challenging in some cases. Both the optimization processes and the MCMC require successive quadrature of the integrals \( \int P_T(\vec{\xi}|\vec{\theta}) d\vec{\xi} \), which in all but the simplest cases cannot be carried out analytically. The authors of vFEP have already noted this challenge [20] in even two dimensions with splines. This approach may also be extensible to other methods that construct biasing functions and PMFs adaptively, though the equations presented above will require modification if the sampling is not strictly stationary.
FIG. 5: Comparison of MAP estimates as a function of the number of spline knots with uncertainty estimates and a Gaussian prior (eq. 41) with (a, c) \( \alpha = 0.1/n \) and (b, d) \( \alpha = 1/n \), where \( n \) is the number of spline knots. We illustrate the MAP distributions for (a,b) the unbiased state likelihood (eq. 34) and (b,d) biased states likelihood (eq. 35). The shading represents the 95% confidence intervals in the MAP estimate evaluated at each spline knot by MCMC sampling of the posterior, and the dashed line represents the ML solution. The MAP and ML solutions are coincident for \( \alpha = 0 \). The choice \( \alpha = 0.1/n \) results in only minor differences between the ML and MAP solutions, whereas \( \alpha = 1/n \) results in a visible difference between the two curves. In the biased states formulation (figs. 5c and 5d), the uncertainties are approximately constant across the range of the PMF, whereas under the unbiased state formulation (figs. 5a and 5b), the uncertainty is largest at the high free energy regions where the likelihood function is least constrained.
APPENDIX

A. Least squares functional fitting

One possibility briefly mentioned in the main text is to minimize a least squares fit of our trial function to the empirical distribution by writing the function to be minimized as

\[
S(\bar{\theta}) = \int \left( P_E(\xi|\bar{x}_n) - e^{-F(\xi|\bar{\theta})} \right)^2 d\xi
\]

\[
= \int P_E(\xi|\bar{x}_n)^2 - 2P_E(\xi|\bar{x}_n)e^{-F(\xi|\bar{\theta})} + e^{-2F(\xi|\bar{\theta})} d\xi
\]

\[
= -2 \sum_{i=1}^N W(\bar{x}_n)e^{-F(\xi_n|\bar{\theta})}
\]

\[
+ \int e^{-2F(\xi|\bar{\theta})} d\xi
\]

where we neglect the terms independent of \(\bar{\theta}\) and employ eq. 11 to estimate the thermal average. However, this integral is problematic as it is strongly biased towards low free energy regions. Large values of \(F\) contribute very little to the sum or the log and are therefore largely unconstrained.

One could consider ameliorating this issue by minimizing over the relative error instead of the absolute. Since we can’t divide by delta functions, we would have to divide by the trial function:

\[
S(\bar{\theta}) = \int \left( \frac{P_E(\xi|\bar{x}_n) - e^{-F(\xi|\bar{\theta})}}{e^{-F(\xi|\bar{\theta})}} \right)^2 d\xi
\]

\[
= \int \left( P_E(\xi|\bar{x}_n) - 2P_E(\xi|\bar{x}_n)e^{-F(\xi|\bar{\theta})} + e^{-2F(\xi|\bar{\theta})} \right) d\xi
\]

\[
=-2 \sum_{i=1}^N W(\bar{x}_n)e^{-F(\xi_n|\bar{\theta})}
\]

\[
+ \int e^{-2F(\xi|\bar{\theta})} d\xi
\]

This integral is, however, even more problematic since squares of integrals of delta functions are not well-defined and the integral over the square of a delta function is infinite. In the direct least squares approach, we didn’t really care, because this undefined function was independent of \(\bar{\theta}\) and could be dropped, but in this case we must maintain this term. This seems an insurmountable deficiency and so we choose to abandon this approach.

Finally, we could consider minimizing over the squared log probabilities (i.e the PMF), instead of the weights. This is not the Kullback-Leibler divergence, but does penalize divergence in the positive as well as the negative direction:

\[
S(\bar{\theta}) = \int P_E(\xi|\bar{x}_n) \left( \ln \frac{P_E(\xi|\bar{x}_n)}{P_T(\xi|\bar{\theta})} \right)^2 d\xi
\]

\[
= \int P_E(\xi|\bar{x}_n) \left( \ln P_E(\xi|\bar{x}_n) - \ln P_T(\xi|\bar{\theta}) \right)^2 d\xi
\]

\[
-2 \ln P_E(\xi|\bar{x}_n) \ln P_T(\xi|\bar{\theta}) + \ln P_T(\xi|\bar{\theta})^2 d\xi
\]

It appears that square minimizing the log weights isn’t really possible, because the logarithm of the empirical distribution of delta functions that occurs in the cross-term is not well defined. However, other least square alternatives to determining similarities of distributions involving the cumulative distribution have been previously presented by Schofield [19].

B. Solving the minimization problem

We briefly describe efficient optimization routines to solve the minimization problems defined in eqs. 34 and 35 in the case of splines. In below, we suppress explicit dependence of \(F\) on \(\bar{\theta}\) for compactness. We start by examining the minimization of eq. 35:

\[
S(\bar{\theta}) = \sum_{n=1}^N F(\xi_n) + \sum_{k=1}^K N_k \ln \int e^{-F(\xi|\bar{\theta}) - b_k(\xi)} d\xi - \ln P(\bar{\theta})
\]

Various minimization approaches are required to compute the gradient and Hessian of this function with respect to the parameter vector \(\bar{\theta}\). For convenience, we define the equilibrium average performed with biasing function \(k\) of some observable \(A\) that is a function of \(\bar{\theta}\) as:

\[
\langle A(\bar{\theta}) \rangle_k = \frac{\int A(\xi|\bar{\theta}) e^{-F(\xi|\bar{\theta}) - b_k(\xi)} d\xi}{\int e^{-F(\xi|\bar{\theta}) - b_k(\xi)} d\xi}
\]

The \(i\) components of the gradient are then:

\[
\nabla S(\bar{\theta})_i = \sum_{n=1}^N \frac{\partial F(\xi)}{\partial \theta_i} + \sum_{k=1}^K N_k \left( \frac{\partial F(\xi)}{\partial \theta_i} \right)_k - \frac{1}{P(\bar{\theta})} \frac{\partial P(\bar{\theta})}{\partial \theta_i}
\]

We note that if we have linear basis functions, the first term is independent of \(\bar{\theta}\) and can be precomputed, as \(\frac{\partial F}{\partial \theta_i}\) is simply the corresponding basis function. Additionally, the integral term will have only limited support for each basis function, so the integrals are relatively easy to carry out, and the calculations scales easily in the number of basis functions.
The $ij$ entries in the Hessian are:

$$\nabla^2 S(\theta)_{ij} = \sum_{n=1}^{N} \frac{\partial^2 F(\xi_n)}{\partial \theta_i \partial \theta_j} - \sum_{k=1}^{K} N_k \left[ \left\langle \frac{\partial^2 F(\xi)}{\partial \theta_i \partial \theta_j} \right\rangle_k - \left\langle \frac{\partial F(\xi)}{\partial \theta_i} \frac{\partial F(\xi)}{\partial \theta_j} \right\rangle_k \right] + \left[ \frac{1}{P(\theta)} \frac{\partial P(\theta)}{\partial \theta_i} \frac{\partial P(\theta)}{\partial \theta_j} \right]$$

(42)

If we assume that we have a trial function that is linear in the parameters, then the initial terms involving mixed second derivatives vanish, leaving only:

$$\nabla^2 S(\theta)_{ij} = \sum_{k=1}^{K} N_k \left[ \left\langle \frac{\partial F(\xi)}{\partial \theta_i} \frac{\partial F(\xi)}{\partial \theta_j} \right\rangle_k \right]$$

(43)

If the function is linear in the parameters (again, such as splines), this will only be nonzero in areas where basis functions have mutual support, essentially just banded along the diagonal, so are relatively inexpensive to compute.

In the case of eq. 34, this becomes:

$$\nabla S(\theta)_i = N \sum_{n=1}^{N} W_n(x_n) \frac{\partial F(\xi)}{\partial \theta_i} - N \left\langle \frac{\partial F(\xi)}{\partial \theta_i} \right\rangle - \frac{1}{P(\theta)} \frac{\partial P(\theta)}{\partial \theta_i}$$

(44)

$$\nabla^2 S(\theta)_{ij} = \sum_{k=1}^{K} N_k \left[ \left\langle \frac{\partial F(\xi)}{\partial \theta_i} \frac{\partial F(\xi)}{\partial \theta_j} \right\rangle_k \right]$$

(45)

Where expectations are now over the unbiased state rather than any of the $K$ biased simulations.

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