

# Using binding free energy to guide ligand design

Charles F. F. Karney,\* Jason E. Ferrara, and Clay D. Spence

Sarnoff Corporation, Princeton, NJ 08543-5300

(Dated: November 7, 2005)

The molecular distributions obtained from canonical Monte Carlo simulations can be used to find an approximate interaction energy. This serves as the basis of a method for estimating the binding free energy for a ligand to a protein which enables the free energy to be used to direct the design of ligands which bind to a protein with high affinity.

Keywords: ligand design, binding free energy, molecular distribution, EM-algorithm, molecular symmetry

## I. INTRODUCTION

The goal of structure-based drug design is to create a ligand which binds with high affinity to a protein target. An exciting prospect is the ability to carry out this design process computationally and thereby obtain a series of drug leads which are potent and which can be subsequently optimized for drug-like properties. In an earlier paper, we presented the wormhole method [1] which, subject to some limitations, allows the binding affinity of a given drug ligand to a protein to be computed. For the purposes of structure-based drug design, we might imagine using the wormhole method to screen a large number of known molecules against the protein. This suffers from two serious drawbacks: only a tiny fraction of feasible drug-like chemicals can be assessed in this way; and it is not initially known where or how the compounds are likely to bind to the protein.

One strategy for alleviating these problems is to use a fragment-based approach [2]. Here fragments are small (usually rigid) organic molecules. By a judicious choice of fragments, a large and diverse set of drug-like molecules may be built *in silico* by forming bonds between them. Because the number of fragments is small,  $O(100)$ , and because they are relatively simple, we can compute maps of where the fragments bind to the protein. These data then serve as the building blocks to create larger drug-like molecules. The process of constructing these molecules, of necessity, provides the binding mode to the protein. The key is, of course, to build the large molecules in such a way as to optimize the binding affinity.

We face two challenges here. Given a partially built candidate molecule, can we quickly assess how a particular fragment can be added? Having grown the molecule with the addition of a fragment, how can we rapidly compute the resulting binding affinity to evaluate whether the new molecule is acceptable?

In this paper we describe possible solutions to both these problems. Let us start by giving an overall description of the method. Our computational model consists of a protein which is either kept rigid or is allowed to have a small number of degrees of freedom interacting with a ligand. We assume that the ligand is “made” by connecting several simpler organic fragments together and, for simplicity, we take the fragments to be rigid and assume that they are joined by rotatable bonds; however, the method is easily generalized to remove these restrictions. This system is described by a conventional force field such as Amber [3] with the effects of the solvent captured by an implicit solvent model such as GB/SA [4; 5]. In this model, we can limit the number of degrees of freedom of the system to a manageable number,  $O(10)$ . For the purposes of this discussion, we assume that we have identified a binding site on the protein. Our goal is to design a set of ligands (created from the fragments) which bind to the protein with high affinity. It would be possible to build additional criteria into the design process, e.g., synthesizability, solubility, etc.; however, these considerations are beyond the scope of this paper. Our standard for success is that the approximate techniques we develop lead to ligands with high affinity as predicted by the full force field outlined above. For this purpose it is convenient to regard the full computational model as “exact”. The degree of agreement with experimental data, while crucial, entails validation of the force field which, again, is beyond the scope of this work.

We begin by performing wormhole Monte Carlo simulations [1] of each of the various fragments binding to the protein. These calculations give the binding affinity of each fragment to the protein and equilibrium distributions of the fragment-protein system. We next fit an analytic function, a Gaussian mixture, to these molecular distributions. The fits for two fragments are then used for two purposes: to find feasible ways to form a bond between the fragments, creating a larger ligand and to give an approximate interaction energy for the newly created ligand with the protein which, in turn, allows for rapidly computing its binding affinity via the wormhole method.

The first part of this paper describes techniques for fitting

---

\*Electronic address: ckarney@sarnoff.com

a Gaussian mixture to a distribution of molecular configurations. We adopt the well-known EM method [6] for this purpose; however, we have to adapt the method to deal with two peculiarities of molecular distributions: firstly, in the presence of constraints, the distributions lie on a sub-manifold of Cartesian space; secondly, for symmetric molecules, we can make a better fit by respecting the symmetry.

Two applications of Gaussian mixtures for molecular distributions are described next. They may be used to define suitable portals for the wormhole method; this provides a more robust method than the use of ellipsoidal portals given in [1]. They may also be used to provide an approximation for the energy of a molecular system.

Finally, we describe how these tools may be combined to compute an approximate binding affinity which allows the binding free energy to be used to direct the design of ligands.

## II. GAUSSIAN MIXTURES

There is often an interest in fitting some observed data with a “model”, an analytic function which approximates the data. One important category of data is the set of configurations of a molecular system given, for example, by the results of a Monte Carlo simulation. An analytic fit then provides an approximate but compact representation of the observed data. Because the samples from a canonical ensemble Monte Carlo simulation are drawn from a distribution which is proportional to  $\exp(-\beta E(\mathbf{x}))$ , where  $E(\mathbf{x})$  is the energy of the system in configuration  $\mathbf{x}$ ,  $\beta = 1/(kT)$ ,  $k$  is the Boltzmann constant, and  $T$  is the temperature, the analytic fit can also be used to give an approximate expression for the energy of a molecular configuration.

An important class of models is the mixture of Gaussians and the EM (expectation-maximization) algorithm [6] is frequently used to optimize this model based on the maximum likelihood. We begin by reviewing an iteration of the standard EM algorithm including the straightforward extension of allowing the samples to have a statistical weight. Assume that our data is

$$[\mathbf{x}_1, \mathbf{x}_2, \mathbf{x}_3, \dots, \mathbf{x}_n],$$

where  $\mathbf{x}_i$  is a point in  $\mathbb{R}^d$  and that associated with each of the samples  $\mathbf{x}_i$  is a scalar weight  $w_i$ . This weight might arise from coalescing consecutive identical samples from a Monte Carlo simulation (because of a run of rejected moves) or because the Monte Carlo sampling is carried out with a non-physical energy  $E^*$  in which case we have  $w_i = \exp[-\beta(E(\mathbf{x}_i) - E^*(\mathbf{x}_i))]$ .

Let the current fit be

$$f(\mathbf{x}) = \sum_{j=0}^{m-1} \alpha_j G(\mathbf{x}; \mathbf{y}_j, \mathbf{C}_j),$$

where  $\sum_{j=0}^{m-1} \alpha_j = 1$  and  $G(\mathbf{x}; \mathbf{y}_j, \mathbf{C}_j)$  is a  $d$ -dimensional Gaussian with unit volume and mean,  $\mathbf{y}_j$ , and covariance,  $\mathbf{C}_j$ . The goal is to find the set  $\{\alpha_j, \mathbf{y}_j, \mathbf{C}_j\}$  which maximizes the

log-likelihood

$$L = \langle \ln f(\mathbf{x}_i); w_i \rangle_i, \quad (1)$$

where  $\langle \cdot; \cdot \rangle_i$  denotes the weighted arithmetic mean,

$$\langle X_i; w_i \rangle_i = \frac{\sum_{i=1}^n w_i X_i}{\sum_{i=1}^n w_i}. \quad (2)$$

An EM iteration proceeds as follows:

$$g_{ij} = \frac{\alpha_j G(\mathbf{x}_i; \mathbf{y}_j, \mathbf{C}_j)}{f(\mathbf{x}_i)}, \quad (3a)$$

$$\alpha_j^{\text{new}} = \langle g_{ij}; w_i \rangle_i, \quad (3b)$$

$$\mathbf{y}_j^{\text{new}} = \langle \mathbf{x}_i; g_{ij} w_i \rangle_i, \quad (3c)$$

$$\mathbf{C}_j^{\text{new}} = \langle (\mathbf{x}_i - \mathbf{y}_j^{\text{new}})(\mathbf{x}_i - \mathbf{y}_j^{\text{new}})^T; g_{ij} w_i \rangle_i. \quad (3d)$$

With this procedure, the log-likelihood is guaranteed to converge to a local maximum [6]. The quantity  $g_{ij}$  gives the responsibility of the Gaussian component  $G(\cdot; \mathbf{y}_j, \mathbf{C}_j)$  for  $\mathbf{x}_i$ .

### A. Non-Cartesian space

When simulating complex molecules it is important to reduce the dimensionality of configuration space by imposing, for example, constraints on bond lengths and bond angles. For example, when simulating biphenyl (two benzene rings linked by a single bond), the energetics of the molecule allows us to treat it as two rigid rings connected by a bond that permits only torsional movement. The complete configuration of the molecule is then given by the position and orientation of one of the rings together with the torsion angle of the connecting bond.

We will represent torsion angles as a point on the circle  $\mathbb{S}^1$ . Orientations are conveniently represented as unit quaternions [7]; however, because  $q$  and  $-q$  represent the same orientation, orientations are defined as a pair of opposite points on  $\mathbb{S}^3$ . In Mardia and Jupp [8], a distinction is made between a directed line through the origin, a *direction* (which can represent the torsion angle), and an undirected line through the origin, an *axis* (which can represent orientations of general molecules). The orientation of a diatomic molecule, for example CO, would be given by a unit vector, i.e., a direction on  $\mathbb{S}^2$ . The full configuration  $\mathbf{x}$  is then a mixture of Cartesian coordinates and “angle-like” coordinates in  $\mathbb{S}^l$ . Our strategy for applying Gaussian fits to points in this mixed topology is to replace eq. (3c) by

$$\mathbf{y}_j^{\text{new}} = \langle \langle \cdot; \cdot \rangle_i; \mathbf{x}_i; g_{ij} w_i \rangle_i,$$

where  $\langle \langle \cdot; \cdot \rangle_i$  is the appropriate weighted “physical” mean of  $\mathbf{x}_i$ . For the angle-like coordinates, we find the mean by embedding  $\mathbb{S}^l$  in  $\mathbb{R}^{l+1}$ . The mean direction is given by the direction of the weighted sum of the unit vectors [8], while the mean axis is given by the axis about which the moment of inertia of the weighted axes is minimum [7; 8].

Similarly, we replace eq. (3d) by

$$\mathbf{C}_j^{\text{new}} = \langle \mathbf{d}(\mathbf{x}_i, \mathbf{y}_j^{\text{new}}) \mathbf{d}(\mathbf{x}_i, \mathbf{y}_j^{\text{new}})^T; g_{ij} w_i \rangle_i,$$

where  $\mathbf{d}(\mathbf{x}, \mathbf{y})$  is a displacement in  $\mathbb{R}^d$  from  $\mathbf{y}$  to  $\mathbf{x}$ . The operation of  $\mathbf{d}$  is to map configurations into a local Cartesian space centered at  $\mathbf{y}$ . In order to make such a mapping for the angle-like coordinates, we project the sphere (in the case of directions) or hemisphere (in the case of axes) onto a ball in  $\mathbb{R}^l$  using a generalization of the Lambert azimuthal equal-area projection [1] with the pole of the projection given by the mean. It is important that the projection preserve area so that its Jacobian is constant; in this way, integrals in the projected space are the same (up to a multiplicative constant) as integrals in the original space.

In carrying out this extension of Gaussians to non-Cartesian geometries, we have lost an important property of the Gaussian. In  $\mathbb{R}^d$ , if we fit a single Gaussian to arbitrary data, then the log-likelihood is maximized by choosing the mean and covariance of the Gaussian equal to those of the data. We are not aware of a generalization of the Gaussian which preserves this property for our more complex geometries. However, the prescription given above presumably nearly preserves this property provided that the covariances of the individual components in the mixture are sufficiently small that the Gaussians do not “wrap around”  $\mathbb{S}^l$  to any great degree. We will address this issue later.

In the discussion above, we have implicitly assumed that a uniform measure on  $\mathbb{S}^l$  is the natural metric for angle-like coordinates. This is the case for the orientation of a molecule and for torsion angles. However, the situation is more complex for molecules whose bond angles can vary (for example to treat the common conformations of cyclohexane). A full treatment of such cases is beyond the scope of this paper. However, the strategy would be the same as given here: determine a suitable mean and then map the samples to a locally Cartesian space centered at the mean in such a way that configuration space integrals can be expressed in the transformed space with a constant Jacobian.

## B. Incorporation of symmetries

The use of symmetries allows the simplification of many problems. In describing molecular configurations, we encounter both discrete and continuous symmetries. Examples of the latter are translational and orientational invariance when simulating a solute molecule in a large volume of solvent, rotational invariance about the axis in a diatomic molecule, etc. Such symmetries are best treated by expressing the molecular configurations in a lower dimensional space thereby ignoring the symmetry coordinates. Thus the “orientation” of a diatomic molecule can be expressed as a direction on  $\mathbb{S}^2$  rather than as an axis on  $\mathbb{S}^3$ .

Let us describe some typical discrete symmetries that arise in molecular systems. A molecule of methane,  $\text{CH}_4$ , may be oriented in 12 different ways (the order of the tetrahedral group  $T$ ) that leave like atoms in the same positions. We do not treat the reflection symmetry of methane as an additional symmetry because such inversions do not occur under normal conditions.

A more complex example is biphenyl. When bound to a

protein, this has 8 symmetries made up of combinations of  $180^\circ$  rotations of the benzene rings about the connecting bond and an interchange of the two rings. When biphenyl is placed in any of its 8 symmetric positions the resulting system energy and hence the equilibrium distribution is the same. If, on the other hand, the biphenyl is free in solution, then we remove the continuous symmetries by fixing the position and orientation of one of the rings. There are then 4 symmetries given by  $\psi \mapsto \pm\psi$  and  $\psi \mapsto \pm\psi + \pi$  where  $\psi$  is the torsion angle. These correspond to rotating the free ring by  $180^\circ$  and changing the sign of the torsion angle. The latter operation places the biphenyl into its mirror symmetric conformation (but *not* by inverting the molecule). This symmetry is normally excluded when biphenyl is bound to a protein, because the protein binding pocket will not exhibit the same symmetry (because proteins are chiral).

We shall suppose that the system has a  $k$ -order symmetry, which can be described by a symmetry operator  $S(\cdot, l)$  where  $0 \leq l < k$  and

$$\mathbf{x} \mapsto S(\mathbf{x}, l)$$

maps the configuration into one of the  $k$  symmetric configurations. We can compose symmetry operations with  $S(S(\mathbf{x}, l'), l) = S(\mathbf{x}, l \oplus l')$ . Clearly  $\oplus$  defines a group of order  $k$ . We will take identity element to be 0 and define the inverse of  $l$  to be  $\bar{l}$  (thus,  $l \oplus \bar{l} = 0$ ).

In fitting a Gaussian mixture to data, we can use  $S$  both to symmetrize the samples and to symmetrize the fit. However, by using the properties of  $S$  the computational complexity increases only by  $k$  (instead of  $k^2$ ). We begin by symmetrizing the fit,

$$f(\mathbf{x}) = \sum_{j=0}^{m-1} \alpha_j \frac{1}{k} \sum_{l=0}^{k-1} G(S(\mathbf{x}, l); \mathbf{y}_j, \mathbf{C}_j).$$

For simplicity, we apply the symmetry operation through the configuration argument of  $G$  rather than via  $\mathbf{y}_j$  or  $\mathbf{C}_j$ . From this definition, it is easy to show that

$$f(S(\mathbf{x}, l)) = f(\mathbf{x}).$$

(This follows from the group properties of  $\oplus$ .) In forming the responsibility matrix, we start by computing the responsibility of the component  $G(S(\cdot, l'); \mathbf{y}_j, \mathbf{C}_j)$  for the symmetrized data point  $S(\mathbf{x}_i, l)$ ,

$$\begin{aligned} g_{ilj'l'} &= \frac{1}{k} \frac{\alpha_j G(S(S(\mathbf{x}_i, l), l'); \mathbf{y}_j, \mathbf{C}_j)}{f(S(\mathbf{x}_i, l))}, \\ &= \frac{1}{k} \frac{\alpha_j G(S(\mathbf{x}_i, l' \oplus l); \mathbf{y}_j, \mathbf{C}_j)}{f(\mathbf{x}_i)}, \\ &= g_{ij(l' \oplus l)} \end{aligned}$$

where

$$g_{ijl} = \frac{1}{k} \frac{\alpha_j G(S(\mathbf{x}_i, l); \mathbf{y}_j, \mathbf{C}_j)}{f(\mathbf{x}_i)}.$$

We can now update the components using

$$\begin{aligned} \alpha_j^{\text{new}} &= \langle g_{ijl}; w_i \rangle_{i,l}, \\ \mathbf{y}_j^{\text{new}} &= \langle \langle S(\mathbf{x}_i, l); g_{ijl} w_i \rangle \rangle_{i,l}, \\ \mathbf{C}_j^{\text{new}} &= \langle \mathbf{d}(S(\mathbf{x}_i, l), \mathbf{y}_j^{\text{new}}) \mathbf{d}(S(\mathbf{x}_i, l), \mathbf{y}_j^{\text{new}})^{\text{T}}; g_{ijl} w_i \rangle_{i,l}, \end{aligned}$$

Here in forming  $\langle \cdot; \cdot \rangle$  and  $\langle\langle \cdot; \cdot \rangle\rangle$ , we sum over  $i \in (0, n]$  and  $l \in [0, k)$ .

### C. Extension of the greedy algorithm

In the foregoing, we have supposed that the number of components in the fit is known. In general, this is not the case and various algorithms have been proposed to grow the number of components in such a way that a fit close to the global maximum for the log-likelihood is tracked. Here we adapt the greedy EM algorithm [9] for adding components so that symmetries can be included. We determine the optimal number of components by minimizing a cost function involving the minimal description length [10, §7.4.2],

$$C = -L + \frac{p}{2} \left[ m \left( 1 + d + \frac{d(d+1)}{2} \right) - 1 \right] \ln n. \quad (4)$$

The term in brackets gives the number of free parameters in an  $m$ -component fit and  $p$ , which is normally unity, is a parameter that can be adjusted to penalize the addition of more components.

Let us review the greedy algorithm [9]. After the EM algorithm has converged for an  $m$ -component fit, we attempt to add a new component (with index  $m$ ) as follows. Initially, each data point  $\mathbf{x}_i$  is assigned to the component  $j$  for which  $g_{ij}$  is maximum. In this way the data is partitioned into  $m$  sets  $A_j$ . We make several splits of each component  $j$  by selecting two random samples from  $A_j$  and partitioning  $A_j$  into two subsets based on closeness to the two random samples. A tentative new component is added with  $\alpha_m = \alpha_j/2$  and mean and covariance given by one of the two subsets. The resulting tentative fit undergoes partial EM iterations where  $\alpha_m$ ,  $\mathbf{y}_m$ , and  $\mathbf{C}_m$  are adjusted and the  $\alpha_j$  for  $0 \leq j < m$  are merely scaled by  $1 - \alpha_m$  (with the corresponding  $\mathbf{y}_j$  and  $\mathbf{C}_j$  held fixed). This procedure is repeated several times for each of the  $m$  components and the fit with the maximum log-likelihood (following the partial EM updates) is selected as the  $(m+1)$ -component fit which is then subjected to full EM updates.

When treating weighted samples, we modify the procedure above by selecting the two components from  $A_j$  with probabilities proportional to their weights. Because we do this several times, we use the Walker algorithm [11] to make these selections.

In order to include symmetries, we generalize  $A_j$  above to  $A_{jl'}$  which contains those  $S(\mathbf{x}_i, l)$  for which  $g_{ilj'l'} = g_{ij(l' \oplus l)}$  is maximum. It is only necessary to consider splitting the  $m$  unsymmetrized components of the existing fit; thus we only need to determine  $A_{j0}$ . We can do this by assigning an unsymmetrized sample  $\mathbf{x}_i$  to a symmetrized component by finding the  $j$  and  $l'$  which maximizes  $g_{ilj'l'}$ , and then adding  $S(\mathbf{x}_i, l = l')$  to  $A_{j0}$ .

As before, we partition each  $A_{j0}$  into two subsets by picking two random samples from  $A_{j0}$  (according to the sample weights) and using the distance as defined by  $\mathbf{d}$  as the closeness metric. For each subset we use an initial  $\alpha_m = \alpha_j/2$  and  $(\mathbf{y}_m, \mathbf{C}_m)$  computed from the data in the subset.

The partial EM update then consists of updating the responsibilities for the new component,

$$g_{iml} = \frac{\alpha_m G(S(\mathbf{x}_i, l); \mathbf{y}_m, \mathbf{C}_m)}{(1 - \alpha_m) k f(\mathbf{x}_i) + \alpha_m \sum_{l'} G(S(\mathbf{x}_i, l'); \mathbf{y}_m, \mathbf{C}_m)},$$

where we need to evaluate  $g_{iml}$  for  $l \in [0, k)$  and for  $i \in A_{j0}$ , i.e., for all  $i$ , for which  $S(\mathbf{x}_i, l) \in A_{j0}$  for some  $l$ . The update of the  $m$ th component is then

$$\begin{aligned} \alpha_m^{\text{new}} &= \langle g_{iml}; w_i \rangle_{i^*, l}, \\ \mathbf{y}_m^{\text{new}} &= \langle\langle S(\mathbf{x}_i, l); g_{iml} w_i \rangle\rangle_{i^*, l}, \\ \mathbf{C}_m^{\text{new}} &= \langle \mathbf{d}(S(\mathbf{x}_i, l), \mathbf{y}_m^{\text{new}}) \mathbf{d}(S(\mathbf{x}_i, l), \mathbf{y}_m^{\text{new}})^T; g_{iml} w_i \rangle_{i^*, l}, \end{aligned}$$

where the subscript  $i^*$  indicates that the sums over  $i$  should include only  $i \in A_{j0}$ .

### D. Loose ends

Finding the 1-component fit with non-symmetric data is a simple matter of computing the mean and covariance of the data. However, if we are performing a symmetric fit, we need to apply EM iterations to obtain a converged one-component fit. To determine a starting point for these EM iterations we pick a random ‘‘central’’ sample, and transform the other samples using the symmetry operator so that they are as close as possible to the selected sample. The resulting  $n$  symmetry-transformed samples are used to define a tentative  $(\mathbf{y}_0, \mathbf{C}_0)$ . This procedure is repeated several times with different central samples and the  $(\mathbf{y}_0, \mathbf{C}_0)$  which yields the maximum log-likelihood is used as the initial guess for the first component.

The EM algorithm can fail with poorly conditioned samples. For example, one Gaussian component might converge to a group of samples which are in a lower dimensional space. We avoid this problem by placing a lower limit on the maximum eigenvalue of the covariance matrix and by placing a lower limit on the ratio of the minimum to maximum eigenvalues. This makes the EM algorithm more robust possibly at the cost of requiring more components to maximize the log-likelihood

In Monte Carlo applications, we may wish to avoid Gaussian components where any of the angle-like coordinates ‘‘wrap around’’. The presence of such wrapping may destroy detailed balance because a transition to a wrapped sample drawn from such a Gaussian is not balanced by a reverse process. We can limit the effect of the wrapping by checking those diagonal elements of the covariance matrix corresponding to the angle-like coordinates. If these are so large that wrapping occurs within 3 standard deviations of the mean, for example, then we can scale the corresponding rows and columns of the covariance matrix appropriately so that wrapping is limited to the small fraction of samples beyond 3 standard deviations. Here again, the algorithm can adjust to this constraint with additional Gaussian components. In the next section, we will show how detailed balance can be maintained exactly for wormhole moves even in the face of wrapped angle-like coordinates.

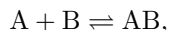
The result of a canonical Monte Carlo simulation is a set of configurations  $\mathbf{x}_i$  drawn from the underlying Boltzmann distribution proportional to  $\exp(-\beta E(\mathbf{x}))$ , together with the corresponding energies  $E(\mathbf{x}_i)$ . In the foregoing discussion, we make the fit to the configurations, essentially ignoring the energies. This is an appropriate use of the data from a Monte Carlo simulation where the sample configurations constitute the “primary” data. One application where we could make use of the energies is when making fits to several independent Monte Carlo runs of the same system. In this case, we can adjust the overall weight of each independent run so that the difference of  $\beta\langle E(\mathbf{x}_i) \rangle$  and  $\langle \ln f(\mathbf{x}_i) \rangle$  is approximately the same across the runs. (Here,  $\langle \cdot \rangle$  denotes an average over a single run.) This adjustment is important when the individual runs are not sufficiently long to sample configuration space fully.

### III. APPLICATIONS OF GAUSSIAN MIXTURES

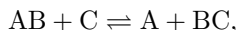
We use the procedure for fitting molecular distributions with a Gaussian mixture in two ways. The first is as a method of defining the portals for wormhole Monte Carlo [1]. In this case we are fitting the data from several independent Monte Carlo runs and Gaussian mixtures then offer a robust way of “clumping” the data with each component of the mixture then providing a portal for the wormhole method. The other application provides an approximation to the energy of the system. Here we are more concerned about the accuracy of the fit, and we also need to establish that the arbitrary constant that connects the energy to the logarithm of the fit drops out when forming physically relevant quantities.

#### A. Portals for wormhole Monte Carlo

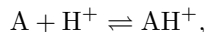
The original description of wormhole Monte Carlo [1] was specialized to the treatment of molecular dissociation,



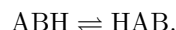
where we sought the equilibration between the bound and unbound states of molecules A and B. This procedure can be generalized to deal with other types of interaction, e.g., molecular exchange,



protonation,



or tautomerization,



(In practice, the free proton in the second case would be handled by an implicit solvent held at constant pH.) We therefore consider the equilibrium of  $\Lambda$  “systems” indexed by  $\lambda$ . Each of the systems is made of  $\Phi_\lambda$  independent molecular “complexes” indexed by  $\phi$  and each complex is made up of 1 or

more interacting molecules. (Thus with molecular dissociation we have  $\Lambda = 2$ . The unbound system  $\lambda = 0$  consists of  $\Phi_0 = 2$  independent complexes, each consisting of a single molecule, A or B, while the bound system  $\lambda = 1$  consists of  $\Phi_1 = 1$  complex, AB.)

If the configuration of complex  $\phi$  in system  $\lambda$  is  $\mathbf{x}_{\lambda\phi}$ , then the full phase space is given by  $\Upsilon = \{\lambda; \mathbf{x}_{\lambda 0}, \mathbf{x}_{\lambda 1}, \dots, \mathbf{x}_{\lambda\Phi_\lambda}\}$ . Here, we have added a set of “ignorable” coordinates,  $\mathbf{x}_{\lambda 0}$ ; the energy of the system, and hence the equilibrium distribution function, is strictly independent of these coordinates. For example, in simulating a molecule in a solvent bath,  $\mathbf{x}_{\lambda 0}$  would include the position and orientation of the molecule; or, when a molecule is deprotonated, it would include the coordinates of the “missing” proton. Inclusion of these ignorable coordinates is dictated by the requirement that  $\Upsilon$  span the same phase space volume for each  $\lambda$ . In practice, we do not keep track of  $\mathbf{x}_{\lambda 0}$ , because the integral over this coordinate is trivial (the integrand is constant!) and we write

$$\int d\mathbf{x}_{\lambda 0} = v_{\lambda 0}.$$

Wormhole Monte Carlo moves allow the state to switch between different systems preserving detailed balance. This allows the determination of the ratios,

$$W_0 : W_1 : W_2 : \dots,$$

where  $W_\mu$  is the statistical weight of system  $\mu$ ,

$$W_\mu = \exp(-\beta F_\mu) = \int \delta_{\lambda\mu} \exp(-\beta\Upsilon) d\Upsilon,$$

and  $F_\mu$  is its free energy. In particular, in the case of protein-ligand binding, the dissociation constant is given by

$$K_d = \frac{1}{V_0} \frac{W_0}{W_1},$$

where  $V_0$  is the system volume.

In this more general framework, the wormhole move [1] is defined as follows. We define a set of “portal functions,”  $w, w', w'', \dots$ , on  $\Upsilon$ , with properties

$$0 \leq w(\Upsilon) \leq 1/v < \infty, \\ \int d\Upsilon w(\Upsilon) = 1,$$

where  $v$  is a representative phase-space volume of the portal function. A wormhole move consists of the following steps: select a pair of portals ( $w, w'$ ) with probability  $p_{ww'}$ ; reject the move with probability  $1 - vw(\Upsilon)$ , where  $\Upsilon$  is the current state; otherwise, with probability  $vw(\Upsilon)$ , pick a configuration  $\Upsilon'$  with probability  $w'(\Upsilon')$ ; and accept the move to  $\Upsilon'$  with probability

$$P_{ww'}(\Upsilon, \Upsilon') = \min\left(1, \frac{p_{w'w} \exp(-\beta E^*(\Upsilon')) v'}{p_{ww'} \exp(-\beta E^*(\Upsilon)) v}\right), \quad (5)$$

where  $E^*(\Upsilon) \approx E(\Upsilon)$  is the “sampling” energy of configuration  $\Upsilon$ , which is used also for the conventional Monte Carlo

moves (within a system). We term  $w$  and  $w'$  the source and destination portals, respectively. The test involving  $w(\Upsilon)$  determines whether the current configuration is “in” the source portal—note, however, that this test is “fuzzy”. If the test succeeds, a move is attempted to the destination portal, and the move is accepted according to a standard Boltzmann factor modified by the ratio of the portal volumes. In the limit of a long Markov chain, we then have

$$W_\mu \rightarrow C \langle \delta_{\lambda\mu} \exp(-\beta[E(\Upsilon) - E^*(\Upsilon)]) \rangle,$$

where  $C$  is independent of  $\mu$  and  $\langle \cdot \rangle$  is the average over the Markov chain.

Although the choice of portal functions is arbitrary, the method is only effective if  $v w(\Upsilon)$  is sufficiently large to allow wormhole moves. For simplicity, we restrict each portal function to a particular system  $\lambda = \mu$ . Because the complexes making up a system are independent it is natural to consider  $w(\Upsilon)$  as product of density functions for each complex. Thus the typical portal function is

$$w(\Upsilon) = \delta_{\lambda\mu} \frac{1}{v_{\mu 0}} \prod_{\phi=1}^{\Phi_\mu} w_{\mu\phi}(\mathbf{x}_{\mu\phi}),$$

where

$$\int w_{\mu\phi}(\mathbf{x}_{\mu\phi}) d\mathbf{x}_{\mu\phi} = 1,$$

and the factor  $1/v_{\mu 0}$  arises from an implicit constant density in  $\mathbf{x}_{\mu 0}$ . For a particular complex  $\phi$  in system  $\lambda$  we need to determine a set of portal functions  $w_{\mu\phi}(\mathbf{x}_{\mu\phi})$ ,  $w'_{\mu\phi}(\mathbf{x}_{\mu\phi})$ ,  $w''_{\mu\phi}(\mathbf{x}_{\mu\phi})$ ,  $\dots$ , which reflect the probable configurations for this complex. We obtain these portal functions using the results of several conventional canonical Monte Carlo runs on the complex. We make a Gaussian fit to the resulting sets of configurations. If the fit contains  $m$  components, then we obtain  $m$  portal functions, indexed by  $j$ , for this complex each of which is a symmetrized Gaussian of the form

$$w_{\lambda\phi j}(\mathbf{x}_{\lambda\phi}) = \frac{1}{k_{\lambda\phi}} \sum_{l=0}^{k_{\lambda\phi}-1} G(S_{\lambda\phi}(\mathbf{x}_{\lambda\phi}, l); \mathbf{y}_{\lambda\phi j}, \mathbf{C}_{\lambda\phi j}), \quad (6)$$

where  $k_{\lambda\phi}$  is the symmetry order for the complex,  $S_{\lambda\phi}$  is the corresponding symmetry operator, etc. We take the “volume” of this portal function to be

$$v_{\lambda\phi j} = k_{\lambda\phi} / G(\mathbf{y}_{\lambda\phi j}; \mathbf{y}_{\lambda\phi j}, \mathbf{C}_{\lambda\phi j}).$$

We assume that the choice of source and destination portals is independent so that the portal probability  $p_{ww'}$  can be factored into probabilities for  $w$  and  $w'$ ; furthermore, we assume that these probabilities may in turn be factored into choices for the source and destination systems and for the portals for the respective complexes for each system. In this case, the wormhole moves can implemented as follows. Pick a source portal system  $\mu$ ; if  $\lambda \neq \mu$ , the move fails; otherwise consider each complex in the system  $\mu$  in turn; for complex  $\phi$ , pick a

random portal function  $j$  and pick a random symmetry index  $l$ ; with probability

$$1 - v_{\mu\phi j} G(S_{\mu\phi}(\mathbf{x}_{\mu\phi}, l); \mathbf{y}_{\mu\phi j}, \mathbf{C}_{\mu\phi j}) / k_{\mu\phi},$$

reject the move; if none of these tests cause the move to be rejected, the “in” test succeeds and we proceed with choosing the destination portal by picking the destination system  $\mu'$ , picking a portal function  $j'$  and a symmetry  $l'$  for each complex  $\phi'$ , and setting the configuration for the complex to  $S_{\mu'\phi'}(\mathbf{x}_{\mu'\phi'}, l')$  where  $\mathbf{x}_{\mu'\phi'}$  is selected from  $G(\mathbf{x}_{\mu'\phi'}; \mathbf{y}_{\mu'\phi'j'}, \mathbf{C}_{\mu'\phi'j'})$ . In evaluating the acceptance probability, eq. (5), we express  $p_{ww'}$  as the product of the individual probabilities (of selecting source and destination systems and of selecting particular portals for the source and destination complexes). Similarly the volume of the portal is given by the product of  $v_{\mu 0}$  and the volumes of the portal functions for the separate complexes.

In this formulation, the symmetry of a complex is incorporated into the portal function, eq. (6). However the test for being in the portal and the operation of selecting a configuration from it are decomposed into picking a random symmetry (with equal probabilities) followed by a test or selection on a unsymmetrized Gaussian.

If, when sampling from the destination portal, any of the angle-like coordinates are wrapped around, then we immediately reject the whole move. This is necessary in order to maintain detailed balance, because the test on the source portal never involves wrapped coordinates. This effectively replaces the Gaussians in the definition of the portal functions by clipped versions which evaluate to zero for wrapped coordinates.

There is a great deal of flexibility in the choice of portal probabilities offered by the scheme outlined here. Because the test of being in the source portal is typically very inexpensive, it is desirable to arrange that the source portal probabilities are roughly equal. In addition, we usually adjust the ratio of conventional to wormhole moves so that, on average, each configuration is tested against all the portals for every attempted conventional move. On the other hand, the probabilities for the destination portals would usually be adjusted to reflect the statistical weight of the portal.

Let us turn to the details of making the Gaussian fits to define the wormhole portals. Because the individual Monte Carlo runs performed for each complex are independent, it is natural to consider scaling the overall weight to the results from each run in order to match the energy samples. In practice, this procedure results in rather poor fits with too many components. In this application, Gaussian fitting may viewed merely as a robust clumping technique and for this purpose it suffices to attach the same weight to all the samples. For the same reason, we increase  $p$  to 5 in eq. (4) so that a smaller number of components is used to make the fit.

Gaussian portals offer advantages over the use of ellipsoidal portals proposed in [1]. With a given number of components, the EM method does a “global” optimization and is thus likely to obtain a better fit and than the somewhat *ad hoc* scheme for choosing ellipsoids. Also the Gaussian fit to a configuration of independent complexes naturally factors into a product of

Gaussians for each complex. Thus Gaussian portals reflect the independence of complexes properly. Gaussian portals, combined with the mean energy for a portal (which can be readily estimated from the energies of the samples) also offer a rough *a priori* estimate of  $W_\lambda$ . This, in turn, allows us to adjust  $v_{\lambda 0}$  to maximize the probability of transitions between systems and hence to reduce the error in the eventual estimate of  $W_\lambda$ . In the case of ligand-protein binding, where we simulate single ligand and protein molecules in a system of physical volume  $V_0$ , this procedure entails adjusting  $V_0$  so that the fraction of time the molecules are associated is roughly  $\frac{1}{2}$ .

Finally, we remark that when performing a conventional Monte Carlo move for a particular system, it is preferable to select randomly a single complex to move. This will result in a higher acceptance rate compared to attempting to move all the complexes simultaneously.

## B. Obtaining the energy from the fit

The result of a wormhole Monte Carlo simulation is a set of configurations sampled from  $\exp(-\beta E^*(\Upsilon))$ . If we fit an analytic function to these samples weighted by  $\exp(-\beta[E(\Upsilon) - E^*(\Upsilon)])$ , then the fit can serve as a basis for approximating  $E(\Upsilon)$ . Here we detail how we can use Gaussian mixtures to carry out this fit and we show how to obtain approximations for the energies of the individual complexes and how the arbitrary offsets for energies cancel whenever energy differences are computed using the approximate energies.

We begin by making normalized fits,  $f_{\lambda\phi}$ , to all the complexes in all the systems. If the same complex appears in multiple systems, the samples may be aggregated in order to permit a fit using more data. The energy of the system is taken to be the sums of the energies of the complexes, i.e.,

$$E(\Upsilon) = \sum_{\phi=1}^{\Phi_\lambda} E(\mathbf{x}_{\lambda\phi}),$$

and similarly for  $E^*(\Upsilon)$ . The sampled configurations for each complex are assigned weights of  $\exp(-\beta[E(\mathbf{x}_{\lambda\phi}) - E^*(\mathbf{x}_{\lambda\phi})])$ .

The normalized fit for a particular system is then given as the product of the fits for the contributing complexes, multiplied by  $1/v_{\lambda 0}$  and these can be combined weighted by  $W_\lambda$  to provide a fit in  $\Upsilon$  space as

$$f(\Upsilon) = \frac{W_\lambda}{W} \frac{1}{v_{\lambda 0}} \prod_{\phi=1}^{\Phi_\lambda} f_{\lambda\phi}(\mathbf{x}_{\lambda\phi}),$$

where  $W = \sum_\lambda W_\lambda$ , and we have

$$-\beta E(\Upsilon) \approx D + \ln f(\Upsilon),$$

where  $D$  is an arbitrary adjustable constant. This provides an approximation of the energy of a system. In addition, we can approximate the energies of the individual complexes by

$$-\beta E(\mathbf{x}_{\lambda\phi}) \approx D_{\lambda\phi} + \ln f_{\lambda\phi}(\mathbf{x}_{\lambda\phi}),$$

where  $D_{\lambda\phi}$  are adjustable constants which satisfy

$$\sum_{\phi=1}^{\Phi_\lambda} D_{\lambda\phi} = D + \ln(W_\lambda/W) - \ln v_{\lambda 0},$$

for all  $\lambda$ .

Let us apply this to the case of molecular dissociation. The  $\lambda = 0$  (resp.  $\lambda = 1$ ) system contains two complexes (resp. one complex) each of which is free to move within a system of 3-dimensional volume  $V_0$ ; thus, we have  $v_{00} = (\sigma V_0)^2$  (resp.  $v_{10} = \sigma V_0$ ), where  $\sigma$  is the volume of orientation space. In this case, there is one constraint on the choice of energy offsets for the fit energies, namely

$$D_{10} = D_{00} + D_{01} - \ln(K_d/\sigma).$$

As expected, this constraint does not involve  $V_0$ . It is also apparent from the form of this constraint, that differences in energy between the unbound and bound systems will be independent of the choice of offsets.

## IV. MOLECULAR DESIGN

We now have the tools to tackle ligand design. We start the process by computing the binding affinity of the fragments. Fragment-based design works on the principle of building a complex molecule from simpler sub-components. We extend this idea by also computing the binding affinity of the larger molecule using data from the calculation of the binding affinity of the simpler molecules. Finally, we describe the process by which simple molecules can be combined.

### A. The single fragment binding affinity

Our starting point is a protein target for which we know the structure and a set of simple organic fragments. The symmetries of the fragment are determined. In the case of a rigid fragment, this consists of the set of 3-dimensional rotations which leave the molecule invariant. The energy of the system is computed using a conventional force field with an implicit solvent model as described in the introduction. The simulation is focused on a certain portion of the protein by adding a restraint energy which is zero if the fragment is within a region of interest on the protein (e.g., within a binding site) and increases parabolically outside this region. It is possible to define the restraint region to include a few solvent layers about the entire surface of the protein—but this obviously results in a longer simulation. Including a parabolic portion to the restraint potential allows the fragment distribution to fall off gradually and this allows the distribution to be fit with fewer Gaussian components than with a hard restraint. The binding affinity of a molecule will be only weakly dependent on the precise extent of the restraint region providing that it encompasses the true binding site of the protein.

We perform a wormhole calculation to find the binding affinity and to provide the distribution of fragments. In order to determine initial portals for this calculation we systematically search for plausible binding modes by inserting the

fragment randomly into the restraint region with a random orientation and random conformations for the protein and fragment (if these molecules are flexible). This process is most efficiently carried out with a tailored restraint region (which prevents attempts to insert the ligand within the protein) followed by a quick steric check (where configurations are rejected as soon as two clashing atoms are found). This can be followed by a crude energy minimization using the vacuum energy model. We can make an estimate of how many probes need to be made in order to explore the surface of the protein thoroughly and so to find all possible binding pockets. This estimate is based on the volume of the restraint region and the typical length and orientation scales for energy variation. A similar exercise is carried out for the unbound system—this merely consists in finding allowable conformations of the protein and ligand. We then drop any of the bound configurations whose energy exceeds the minimum unbound energy. The resulting bound and unbound configurations are used as starting points for a set of conventional Monte Carlo runs with the full sampling energy. The initial portion of each run should be discarded and any bound run whose energy is stuck close to (or above) the unbound energy should be eliminated. The resulting data from these Monte Carlo runs is then used to determine wormhole portals using a Gaussian mixture and to estimate a starting value of the system volume  $V_0$ .

In addition, we can add “catch-all” portals for the unbound and bound systems. For the unbound system, this will allow the molecules to assume arbitrary conformations (subject to whatever constraints are imposed by the molecular model). For the bound system, the molecules would be allowed to assume arbitrary conformations and in addition the ligands would be selected from the restraint region with an arbitrary orientation. These catch-all portals allow new binding modes to be discovered.

The binding affinity is then calculated using the wormhole Monte Carlo method. During the course of this simulation,  $V_0$  is adjusted to maintain  $W_0 \sim W_1$  and if  $V_0$  is increased (resp. decreased) we reduce  $W_1$  (resp.  $W_0$ ) by the same factor. We may also find that the ligand becomes trapped in a local energy well. Whenever this is detected (by the absence of successful wormhole moves), new Gaussian portals are found by rerunning the Gaussian fit adding recent configurations and restarting the binding affinity calculation.

The process converges with a sufficiently long run without the need to add new portals and with sufficiently frequent wormhole moves between the bound and unbound systems. This process provides an estimate of the dissociation constant  $K_d$  for this fragment-protein interaction and a set of samples for the bound and unbound configurations. From this configurational data (weighted to reflect the difference between the full and sampling energies), we fit a Gaussian mixture to the bound and unbound distributions. This provides an approximation to the energy of the protein and the ligand either unbound or as a bound complex.

## B. Approximate energy of combined molecules

Let us consider the case where we have identified two possible ligands AB and BC and we wish to combine these via the “overlap” portion B to form a ligand ABC. We might form a  $N$ -fragment ligand with A and C being fragments and B being an  $(N - 2)$ -fragment overlap ligand. Alternatively, we might take B to be null and merely add a fragment C to a  $(N - 1)$ -fragment ligand A. (In either case, we form a 2-fragment ligand by taking A and C to be fragments and B to be null.)

Because we are concerned here with the energies of different combinations of fragments, we adopt a notation for the energy where we do not explicitly specify the molecular configuration and where  $E(Z)$  is the energy for a single molecule  $Z$  and  $E(X, Y)$  is the energy for the two interacting molecules  $X$  and  $Y$ . We write

$$E(ABC) = E(AB) + E(BC) - E(B) + \delta E(ABC), \quad (7a)$$

$$E(ABC, P) = E(AB, P) + E(BC, P) - E(B, P) + \delta E(ABC, P), \quad (7b)$$

where we expect  $\delta E$  to be small if the energies are approximately additive. In this and subsequent equations, we understand the configuration of the molecules to be consistent throughout the equation, e.g., A is in the same configuration in all the terms. If B is null, then we can write  $E(B, P) = E(P)$ .

We now make two approximations. We assume that the energies involving the simpler molecules AB, BC, and B, are given by fits to configurations from prior binding affinity calculations and we assume that  $\delta E(ABC, P)$  and  $\delta E(ABC)$  are small. If these assumptions hold, then eq. (7) provides a method of computing the energy of the more complex molecule ABC very rapidly which, in turn, allows its binding affinity to the protein to be determined quickly. We expect the neglect of  $\delta E(ABC, P)$  and  $\delta E(ABC)$  to be most easily justified when the overlap portion B is as large as possible; i.e., when two  $(N - 1)$ -fragment ligands are combined to form an  $N$ -fragment ligand. In carrying out this calculation, the arbitrary constants that enter when converting the fits to energies cancel when considering energy differences between the bound and unbound systems.

In our initial implementation, we build ligands by adding fragments one at a time with no overlap portion (i.e., B is null). Furthermore the approximate expressions for the energy are applied recursively so that the energy of a  $N$ -fragment ligand interacting with the protein is found by summing each of the individual fragment-protein energies.

The accuracy can be improved as follows: Assume that we have computed the binding affinity of the best ligands with up to  $N - 1$  fragments using the full energy. Fits to the distributions of these molecules provide the corresponding approximate energies which can be used to compute the approximate energy, using eq. (7), for  $N$ -fragment ligands either by adding a single fragment or, preferably, by using an  $(N - 2)$ -fragment overlap. This allows us to compute approximate values for the binding affinity of the  $N$ -fragment ligands. The binding affin-

ity of those ligands with the best approximate binding affinities can then be recomputed using the full energy. Because this latter binding affinity calculation is carried out following the similar calculation with the approximate energy, we can use Gaussian fits to the samples from the approximate calculation to define the initial portals for the wormhole method with the full energy. This procedure can be repeated to create ligands with an arbitrary number of fragments.

In order to compute the full energy of the enlarged molecule, we may have to determine the Amber atom types afresh, for example, using the GAFF rules [12; 13]. In addition, we need to determine the partial charges for the new ligand. One simple prescription is as follows: when two fragments, A and C are combined to form a 2-fragment molecule AC, the charge on the hydrogen removed from A is donated to the derivatized atom on C and *vice versa*. This rule, which maintains charge neutrality, can be readily generalized for larger ligands. More realistic charge models could be employed, if necessary. For example, VC/2003 [14] allows partial charges to be computed without the need for a quantum calculation, while AM1-BCC [15; 16] gives the charge on the basis of an relatively inexpensive AM1 quantum calculation. The overall expense of these more detailed charge calculations could be reduced by carrying them out only for the ligands with the best predicted binding affinity. Techniques for making constrained moves of a molecule made up of rigid fragments and for efficiently evaluating the resulting energy (including the solvation free energy) are given in [17].

We can further improve the accuracy of the approximate energy evaluation by including some contributions to  $\delta E$ . For example, we can set  $\delta E(ABC, P) \approx \delta E(ABC)$  and evaluate  $\delta E(ABC)$  using eq. (7a) together with a direct evaluation of  $E(ABC)$  (which, typically, is fast because it does not involve the interaction with the large protein). In this way, we expect to include the main intra-molecular contributions to the energy including the effects of atom removal and charge redistribution. In this approximation, we still neglect three-body effects which enter into the solvation energy for the bound system, e.g., the modification of solvation energy of A interacting with P due to the presence of C [4]. Also neglected are the effects of atom removal and charge redistribution on the ligand-protein energy.

We might make a further simplification to  $\delta E(ABC)$  by including only some terms in energies in eq. (7a). For example, we might include just the torsion energy of the inter-fragment bonds and a “steric” energy, which is infinite if non-bonded atoms in ABC overlap and is zero otherwise.

Finally, note that we do not need to include the chemical bond energies when forming ABC because these energies are the same in the bound and unbound systems, and so cancel in the computation of the binding affinity.

### C. Combining molecules

When forming a complex ligand ABC from ligands AB and BC, we need to generate starting configurations for ABC for the purposes of identifying the wormhole portals [1]. We

consider the problem for the bound ( $\lambda = 1$ ) case; the unbound case ( $\lambda = 0$ ) follows as a straightforward simplification.

We compute Gaussian mixtures for the configurations of AB and BC bound, respectively, to the protein. We draw several sample configurations  $[\Gamma_A, \Gamma_B, \Gamma_P]$  and  $[\Gamma'_B, \Gamma_C, \Gamma'_P]$  from each possible pair of Gaussian components selected from the two fits. Here  $\Gamma_M$  denotes the configuration of molecule M. We form a “bond” constraint term,

$$D^2 = \|\Gamma_B - \Gamma'_B\|^2 + \|\Gamma_P - \Gamma'_P\|^2,$$

where  $\|\Gamma_M - \Gamma'_M\|$  is some suitable measure of the separation of the two configurations of molecule M and we take  $D \geq 0$ . If B is null, then  $\|\Gamma_B - \Gamma'_B\|^2$  is replaced by a constraint term for the new bond between A and C, for example, an appropriately weighted sum of the squared deviations of the bond length and bond angles from their ideal values. In forming  $D$ , we weight the various contributions so that  $D$  is an approximate distance that atoms must move to satisfy  $D = 0$ .

We now seek nearby configurations for the two systems  $[\Gamma_A, \Gamma_B, \Gamma_P]$  and  $[\Gamma'_B, \Gamma_C, \Gamma'_P]$  for which the bond constraint is zero. This is accomplished by gradually decreasing a “target” constraint,  $D_t$ , from the initial value of  $D$  to zero. For a specific  $D_t$ , we randomly perturb the configurations in such a way as to meet the target constraint,  $D \leq D_t$ , and accept the new configuration with a Boltzmann probability  $\min[1, \exp(-\Delta E/(kT'))]$ , where  $\Delta E$  is the change in the (fit) energy and  $T'$  is an annealing temperature. In this way, we attempt to minimize the energy of the combined system subject to the bond constraint. This procedure is attempted at the physiological temperature  $T' = T$  and then at successively higher temperatures until either we achieve  $D = 0$  or an upper temperature,  $T' = 2T$ , is reached.

If the bond constraint can be satisfied and if we include the steric term for ABC in the approximate binding affinity calculation, we repeat the above procedure to satisfy a steric constraint  $S = 0$ , where  $S$  measures the degree of overlap between the non-bonded atoms of ABC. In this case, we perturb the molecule ABC subject to the constraint  $D = 0$  in order to reduce  $S$  to zero following a similar strategy as that used to meet the bond constraint.

If the bond and (if applicable) steric constraints can be satisfied in such a way that both  $[\Gamma_A, \Gamma_B, \Gamma_P]$  and  $[\Gamma_B, \Gamma_C, \Gamma_P]$  are within a few standard deviations of one of the components of their respective Gaussian mixtures, then  $[\Gamma_A, \Gamma_B, \Gamma_C, \Gamma_P]$  gives a configuration for ABC bound to P which serves as one of the starting points for finding portals for the bound system.

The scheme described above is appropriate when we have direct calculations of the fit energies of AB and BC interacting with the protein. However, if these are given by summing the contributions over fragments, then the bond minimization of the new bonds is carried out allowing all the inter-fragment bonds to relax. In this case, the “old” inter-fragment bonds would start in the ideal constrained state; however, in allowing the new bonds to relax, the old bonds are allowed to stretch so that the ligand can find a good pose where all the inter-fragment bonds meet the constraints.

## D. Implementation

The preceding sections describe the physical basis for using binding free energy to direct ligand design. The implementation entails additional challenges. There is a need for book-keeping to associate a molecule with the smaller molecules out of which it was created. Care must be taken to match up the configurations of the molecules in order to implement the approximate energy evaluations. In order to avoid building the same molecule multiple times (e.g., putting the fragments together in a different order), we use the USMILES representation [18] as a unique tag for the molecule. (Unfortunately, this representation has shortcomings for our purposes. It does not, in fact, provide a unique representation of a molecule; e.g., C1C2CC2CC3CC13 and C1C2CC3CC3CC12 are two different USMILES representations of the same molecule. Furthermore, USMILES only deals with the 2-dimensional structure of a molecule and for the purposes of binding affinity, stereoisomers should be treated as distinct.)

In our current implementation, we build molecules by adding one fragment at a time. The approximate binding affinity of the molecules is computed by summing the fit energy of the individual fragments and including the steric energy. Any intermediate molecule meeting a threshold binding affinity is recorded and is used as a base from which to build larger molecules (up to a given size) in a depth-first fashion.

We have tested this procedure by building ligands which bind to botulinum neurotoxin type B [19] starting with 35 organic fragments. A subsequent evaluation of the binding affinity using the full energy shows that good agreement with the approximate binding affinity in the case of 2-fragment ligands, with 90% of the pairs having an approximate binding affinity within 1–2 log units of the full binding affinity. However the agreement is poor for ligands made up of 3–5 fragments. We attribute this to basing the approximate energy of  $N$ -fragment ligands purely on the energies of single fragments. The errors in the use of the approximate energies may become excessive so that the approximate binding affinity is no longer close to the full binding affinity. Alternatively, it’s possible that the approximate energy is still reasonably accurate but that the binding mode is slightly wrong so that using the approximate distributions to provide the initial portals for the full binding affinity calculation may be inadequate; if the binding mode is quite tight, the full binding affinity calculation may never find it. Both of these problems would be largely overcome by basing the approximate energy for  $N$ -fragment ligands on the fit energy for  $(N - 1)$ -fragment ligands.

## V. DISCUSSION

We have described a way to determine the approximate binding affinity of a ligands based on knowledge of the binding affinity of simpler ligands and the associated equilibrium distributions. This procedure correctly accounts for the loss of entropy associated with connecting molecules together to form a larger molecule and allows the binding free energy to be used to direct the design of ligands.

The predictive capability of our initial implementation (described above) is limited to ligands made up of just 2 fragments. However, we believe that this limitation could be removed by computing the full binding affinity of the best  $(N - 1)$ -fragment ligands and using this as the basis of building  $N$ -fragment ligands. Such a scheme would allow a breadth-first search which would allow the search to be directed toward the molecules with the greatest binding affinity.

In pruning intermediate molecules, we may wish to retain  $(N + 1)$ -fragment molecules which have a worse binding affinity than their  $N$ -fragment parents, with the expectation that this would enable us to build better  $(N + 2)$ -fragment molecules. This would allow non-functional linkers (with no intrinsic propensity to bind to the protein) to bridge between high-affinity functional groups.

The technique described here covers joining fragments in a simply connected fashion where each added fragment attaches at one point. It would be possible to generalize the method to allow the formation of rings. For example we might overlap the molecules ABC, BCD, CDA, DAB to create a 4-fragment ring (ABCD).

The wormhole technique and the ability to fit distributions of molecular configurations with Gaussian mixtures intrinsically depends on the system having a “small” number of degrees of freedom, because we require that phase space be spanned by a reasonable number of samples. This limits the degree of flexibility that can be allowed for the protein and dictates the use of an implicit solvent model. On the other hand, because we are just interested in describing where samples are concentrated, one might expect the method to continue to function well as the number of degrees of freedom is increased to, say, 20.

Our ability to obtain realistic results is also limited by the accuracy of the force field and the solvent model. There are several areas of concern. The force field, the methods for determining partial atomic charges, and the solvent models have all been developed largely independently, and it’s not clear how consistent these models are. It is also noteworthy that the data validating the GB models is based on comparisons with solutions to the Poisson-Boltzmann equation [20], which requires specification of the atomic radii, or is based on comparisons with experimental data for the absolute solvation energy (from vacuum to solution) [5]. More relevant for our purposes would be a comparison against experimental data for the changes in solvent free energy on molecular association. Another area of uncertainty is the charge state of the protein, which may have a small effect when differences in binding free energy are being computed (e.g., with the free energy perturbation method) but which may have a large effect on the absolute binding free energy. The impact of salt effects is easily incorporated into GB models [21]. More interesting would be a principled treatment of the protonation of charged residues in the protein, proton exchange between the protein and ligand, and tautomerization of the protein or the ligand. The wormhole method offers a natural vehicle for such a treatment avoiding the need to treat protonation as a continuous process [22] and avoiding the need to add an uncharged ghost proton [23].

## Acknowledgment

This work was supported by the U.S. Army Medical Research and Materiel Command under Contract No. DAMD17-03-C-0082. The views, opinions, and findings contained in this report are those of the author and should not be construed as an official Department of the Army position, policy, or decision. No animal testing was conducted and no recombinant DNA was used.

## References

- [1] C. F. F. Karney, J. E. Ferrara, and S. Brunner, *Method for computing protein binding affinity*, J. Comput. Chem. **26**, 243–251 (2005). E-print arXiv:cond-mat/0401348.
- [2] A. Miranker and M. Karplus, *Functionality maps of binding sites: A multiple copy simultaneous search method*, Proteins: Struct. Func. Gen. **11**, 23–34 (1991).
- [3] W. D. Cornell, P. Cieplak, C. I. Bayly, I. R. Gould, K. M. Merz, Jr., D. M. Ferguson, D. C. Spellmeyer, T. Fox, J. W. Caldwell, and P. A. Kollman, *A second generation force field for the simulation of proteins, nucleic acids and organic molecules*, J. Am. Chem. Soc. **117**, 5179–5197 (1995).
- [4] W. C. Still, A. Tempczyk, R. C. Hawley, and T. Hendrickson, *Semianalytical treatment of solvation for molecular mechanics and dynamics*, J. Am. Chem. Soc. **112**, 6127–6129 (1990).
- [5] D. Qiu, P. S. Shenkin, F. P. Hollinger, and W. C. Still, *The GB/SA continuum model for solvation: A fast analytical method for the calculation of approximate Born radii*, J. Phys. Chem. A **101**, 3005–3014 (1997).
- [6] A. P. Dempster, N. M. Laird, and D. B. Rubin, *Maximum likelihood from incomplete data via the EM algorithm*, J. Royal Statist. Soc. B **39**, 1–38 (1977).
- [7] C. F. F. Karney, *Quaternions in molecular modeling*, Technical report, Sarnoff Corp. (June 2005). E-print arXiv: physics/0506177.
- [8] K. V. Mardia and P. E. Jupp, *Directional Statistics* (J. Wiley & Sons, 1999).
- [9] J. J. Verbeek, N. Vlassis, and B. Kröse, *Efficient greedy learning of Gaussian mixture models*, Neural Computation **15**, 469–485 (2003).
- [10] G. Deco and D. Obradovic, *An Information-Theoretic Approach to Neural Computing* (Springer-Verlag, 1996).
- [11] A. J. Walker, *An efficient method for generating discrete random variables and general distributions*, ACM Trans. Math. Software **3**, 253–256 (1977).
- [12] J. Wang, R. M. Wolf, J. W. Caldwell, P. A. Kollman, and D. A. Case, *Development and testing of a general Amber force field*, J. Comput. Chem. **25**, 1157–1174 (2004).
- [13] J. Wang, W. Wang, P. A. Kollman, and D. A. Case, *Antechamber, an accessory software package for molecular mechanical calculations*, J. Chem. Inf. Comput. Sci. (2005). Submitted.
- [14] M. K. Gilson, H. S. R. Gilson, and M. J. Potter, *Fast assignment of accurate partial atomic charges: An electronegativity equalization method that accounts for alternate resonance forms*, J. Chem. Inf. Comput. Sci. **43**, 1982–1997 (2003).
- [15] A. Jakalian, B. L. Bush, D. B. Jack, and C. I. Bayly, *Fast, efficient generation of high-quality atomic charges. AM1-BCC model: I. Method*, J. of Comput. Chem. **21**, 132–146 (2000).
- [16] A. Jakalian, D. B. Jack, and C. I. Bayly, *Fast, efficient generation of high-quality atomic charges. AM1-BCC model: II. Parameterization and validation*, J. Comput. Chem. **23**, 1623–1641 (2002).
- [17] C. F. F. Karney and J. E. Ferrara, *Modeling molecules with constraints*, Technical report, Sarnoff Corp. (August 2005). E-print arXiv:physics/0508116.
- [18] D. Weininger, A. Weininger, and J. L. Weininger, *SMILES: 2. Algorithm for generation of unique SMILES notation*, J. Chem. Inf. Comput. Sci. **29**, 97–101 (1989).
- [19] M. A. Hanson and R. C. Stevens, *Cocrystal structure of synaptobrevin-II bound to botulinum neurotoxin type B at 2.0 Å*, Nature Struct. Biol. **7**, 687–692 (2000).
- [20] M. Feig, A. Onufriev, M. S. Lee, W. Im, D. A. Case, and C. L. Brooks III, *Performance comparison of generalized Born and Poisson methods in the calculation of electrostatic solvation energies for protein structures*, J. Comput. Chem. **25**, 265–284 (2004).
- [21] J. Srinivasan, M. W. Trevathan, P. Beroza, and D. A. Case, *Application of a pairwise generalized Born model to proteins and nucleic acids: Inclusion of salt effects*, Theor. Chem. Acc. **101**, 426–434 (1999).
- [22] M. S. Lee, F. R. Salsbury, Jr., and C. L. Brooks III, *Constant-pH molecular dynamics using continuous titration coordinates*, Proteins: Struct. Func. Bioinf. **56**, 738–752 (2004).
- [23] J. Mongan, D. A. Case, and J. A. McCammon, *Constant pH molecular dynamics in generalized Born implicit solvent*, J. Comput. Chem. **25**, 2038–2048 (2004).