A Practical Method for Calculating Relative Free Energies of Binding. Chiral Recognition of Peptidic Ammonium Ions by Synthetic Ionophores

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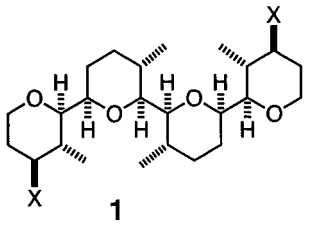
A new free energy simulation method is described and applied to compute the relative binding free energies (enantioselectivity) of enantiomeric guests (**2**) for several chiral host molecules (**1**). The new simulation method is based on a previously described smart Monte Carlo method (MC(JBW)) that is here modified to interconvert diastereomeric complexes as well as to make more traditional changes in conformation. Thus the new method simulates an equilibrium between enantiomeric guests in the binding site of a host molecule and leads directly to the relative free energies of the diastereomeric complexes in a single simulation. Here we show that the MC(JBW) method originally developed for simulations of single molecules can also be applied to simulations of molecular complexes. We describe a further extension of this MC(JBW) method that allows it to interconvert diastereomeric complexes along with all other conformational degrees of freedom. We then use the extended method (termed SME for *simulated mutational equilibration*) to compute the free energies of enantioselection of various alanine derivatives (**2**) binding to ionophore **1** using the AMBER* force field and the GB/SA model for chloroform solvent. One form of the method is found to be more than an order of magnitude faster than traditional free energy perturbation (FEP) calculations on the same system and gives free energies of enantioselection that are in close agreement with experiment. The speed of the new method makes it a practical tool for use in designing new enantioselective host molecules.

Calculating free energies of binding has long been the goal of researchers interested in rational molecular design. Unfortunately, however, free energy calculations are difficult in many real applications and consequently have been used only rarely to guide molecular design efforts.[1] One reason such calculations are troublesome is known as the *sampling problem* and follows from the fact that accurate free energy results are obtained only if the contributions of all significantly populated states (including configurational, conformational, and vibrational states) are included in the calculation.[2] The problem is that including all such states with the required Boltzmann weighting can be difficult if not problematic with complex molecular systems. This is because the number of important states can be very large and because some sets of states (e.g., different conformations) are often separated by energy barriers that traditional molecular simulation methods such as Monte Carlo (MC) or molecular dynamics (MD) have trouble crossing.[3] To make matters worse, traditional methods (e.g., free energy perturbation (FEP)) for calculating relative or absolute binding free energies are multistage procedures that require the sampling problem to be solved repeatedly, once for each stage of which there may be 10−50.[4]

To deal with such problems, we recently described several new simulation methods (MC(JBW) and MC(JBW)/SD) that address the sampling problem for conformationally flexible molecules through the use of a conformation-hopping Monte Carlo procedure we term *jumping between wells* or *JBW*.[5] In this paper, we describe an adaptation of the JBW method that allows the computation of relative free energies of binding by a direct, single-stage procedure that we term *simulated mutational equilibration* or *SME*. Because SME yields relative free energies from a single molecular simulation, it has a significant speed advantage over multistage methods such as FEP. While SME should in principle allow calculation of the relative binding of any two molecules for a third, it is particularly simple in the case of the relative binding of stereoisomers. Here we describe such an application by using SME to compute the relative binding free energies of enantiomeric substrates (**2**, X = OMe, NHMe) for several ionophoric receptors (**1**, Y = H, NHAc, NHAc-butenolide) whose enantioselectivities are known experimentally.[1b]



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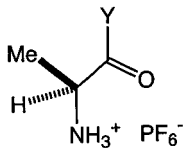

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Simulated Mutational Equilibration (SME). SME is based on a standard approach to evaluating conformational free energy differences in which a thermodynamically equilibrated ensemble of conformational states

[⊗] Abstract published in *Advance ACS Abstracts,* December 1, 1997.

(1) Examples of free energy-directed molecular design: (a) Mertz, K. M.; Kollman, P. A. *J. Am. Chem. Soc.* **1989**, *111*, 5649. (b) Burger, M. T.; Armstrong, A.; Guarnieri, F.; McDonald D. Q.; Still, W. C. *J. Am. Chem. Soc.* **1994**, *116*, 3593.

(2) Dealing adequately with the sampling problem in necessary but insufficient to get accurate free energy results—one also needs an accurate potential energy force field which realistically includes effects of solvent.

(3) For comments on the difficulty of the sampling problem with molecules, see: (a) Mitchell, M. J.; McCammon, J. A. *J. Comput. Chem.* **1991**, *12*, 271. b) van Gunsteren, W. F.; Mark, A. E. *Eur. J. Biochem.* **1992**, *204*, 947. (c) van Gunsteren, W. F. *J. Am. Chem. Soc.* **1994**, *116*, 6293. (d) Balbes, L. M.; Mascarella, S. W.; Boyd, D. B. *Rev. Comput. Chem.* **1994**, *5*, 337. (e) Reference 4.

(4) Review: Kollman, P. *Chem. Rev. (Washington, D.C.)* **1993**, *93*, 2395.

(5) (a) Senderowitz, H.; Guarnieri, F.; Still, W. C. *J. Am. Chem. Soc.* **1995**, *117*, 8211. (b) Senderowitz, H.; Still, W. C. *J. Comput. Chem.* **1997**, submitted.

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Table 1. Potential Energy Moments (kcal/mol) and Conformational Populations of the 1(X = H)·$EtNH_3^+$ Complex at 300 K by Various Simulation Methods[a]

	SD[b,d]	MC[c,d]	MC(JBW)[c,d]	MC(JBW)/SD[b,d]
average energy	−4.197 ± 0.018	−4.199 ± 0.018	−4.234 ± 0.042	−4.197 ± 0.015
standard deviation	4.177 ± 0.006	4.183 ± 0.004	4.180 ± 0.037	4.186 ± 0.012
population (conf 1)	0.159 ± 0.021	0.157 ± 0.010	0.152 ± 0.008	0.156 ± 0.004
population (conf 2)	0.123 ± 0.007	0.122 ± 0.006	0.130 ± 0.002	0.123 ± 0.001
population (conf 3)	0.060 ± 0.002	0.061 ± 0.003	0.062 ± 0.003	0.061 ± 0.002
population (conf 4)	0.073 ± 0.003	0.073 ± 0.005	0.073 ± 0.003	0.073 ± 0.002
population (conf 5)	0.056 ± 0.006	0.058 ± 0.004	0.055 ± 0.007	0.058 ± 0.001
population (conf 6)	0.030 ± 0.002	0.030 ± 0.001	0.029 ± 0.002	0.030 ± 0.001

[a] Calculations at 300 K using the AMBER* force field in vacuo [b] 50 ns dynamics simulation. [c] Average results from three 10^8-step Monte Carlo simulations. [d] Error limits = 1 standard deviation of the measured quantity evaluated from three simulations starting with different initial conditions.

is generated and the populations of the various conformations are simply counted. Along similar lines, SME is a procedure which simulates the thermodynamic equilibration of two different molecules (e.g., alternative substrates), which in the relevant case would be interacting with a third molecule (e.g., a receptor). During an SME simulation, chemically different substrates (e.g., **A** and **B**) as well as conformations interconvert with one another and the relative binding free energy of two different substrates follows simply from their populations at equilibrium ($\Delta G_{\mathbf{A-B}} = -RT$ ln(population$_{\mathbf{A}}$/population$_{\mathbf{B}}$). While this simple idea is appealing and has been applied in calculations of relative solvation energies of halide ions by Tidor,[6] it has not been used to calculate relative binding energies because single-step mutations from one polyatomic chemical species to another are not readily accomplished using traditional simulation procedures. New simulation methods that utilize single-step mutations are however under development and have been used to calculate relative solvation free energies of simple molecules.[7] While mutations between molecules having different numbers of atoms in a simulation generally require special techniques (e.g., involving invisible "dummy atoms"), mutations between stereoisomers are straightforward because the differences between stereoisomers are purely geometrical. Even with stereoisomeric mutations, there is still a problem that such mutational events would be expected to be accompanied by large energy increases and thus lead to poor sampling, especially in dense systems such as molecular complexes. To deal with this problem, we employ extensions of our recently described JBW simulation methods (MC(JBW) and MC(JBW)/SD)[5] that use a conformation-hopping, smart Monte Carlo procedure to jump from one low-energy region of conformational space to another and thus to deal with the sampling problems that often plague simulations of conformationally flexible molecules. In the context of the current SME calculations, we have extended our JBW simulation method so that it will interconvert not just the low-energy conformers of single molecules but also low-energy conformers of different stereoisomeric molecules (here the enantiomers of **2**) within the binding site of a chiral third molecule (**1**). As we will show, these interconversions can be made highly efficient and result in rapid equilibration of a system of two diastereomeric complexes. The relative free energy of binding of the two enantiomers (enantioselectivity) then follows from the equilibrium ratio of times the system spends in the two diastereomeric states.

Jumping between Wells (JBW) Simulations with Molecular Complexes. The JBW simulations we use are of two types, MC(JBW) and MC(JBW)/SD. MC(JBW) is a state-hopping modification of simple Metropolis Monte Carlo[8] while MC(JBW)/SD is a related hybrid algorithm that alternates between MC(JBW) and SD steps.[5,9] As originally described, both methods relied on variations in intramolecular internal coordinates (bond lengths, bond angles, and torsion angles) for conformational jumps and therefore were applicable only to single-molecule simulations. Thus for binding energy calculations, both MC(JBW)-based methods had first to be extended to handle systems having more than one molecule. This extension was accomplished by adding internal coordinates defining intermolecular degrees of freedom to the intramolecular internal coordinate arrays that were varied by the MC procedure. These additional coordinates consisted of translation vectors and rotation matrices that, in combination with intramolecular coordinate variations, made possible the efficient transformation of one configuration of a molecular complex into another. Details of the MC(JBW) simulation method for multimolecular complexes are provided as an Appendix.

To establish that our new JBW simulation method produced the desired thermodynamic ensemble with relevant molecular complexes, we compared MC(JBW) and MC(JBW)/SD results with those from established simulation methods (Metropolis Monte Carlo (MC) and stochastic dynamics (SD)) for the bimolecular complex of **1**(X = H) and $EtNH_3^+$. This simplified complex was chosen because, unlike the **1·2** systems, the energy barriers separating the various conformations of **1**(X = H)·$EtNH_3^+$ are small enough to be crossed at a reasonable rate by simple MC and SD at 300 K. Our JBW simulations began with a 30000-step low-mode[10] conformational search that located 12 conformational minima within the lowest 2 kcal/mol. Due to the C_2 symmetry of **1**, these 12 minima actually corresponded to 6 pairs of chemically distinct conformers but all 12 minima were used as input data for our JBW simulations so that the entire potential energy surface would be efficiently explored and convergence could be assessed by comparing the populations of symmetry-equivalent conformations. In these simulations, both intermolecular and intramolecular degrees of freedom were varied by the JBW algorithm (see Appendix) as noted above. Comparisons of the simulation results obtained using SD, MC, MC(JBW), and MC(JBW)/SD under otherwise identical conditions are shown in Table 1.

These data show that the first two potential energy moments and the populations of the six chemically

(6) Tidor, B. *J. Phys. Chem.* **1993**, *97*, 1069.
(7) Radmer, R. J.; Kollman, P. A. *J. Comput. Chem.* **1997**, *18*, 902.
(8) Metropolis, N.; Rosenbluth, A. W.; Rosenbluth, M. N.; Teller, A.; Teller, E. *J. Chem. Phys.* **1953**, *21*, 1087.
(9) Guarnieri, F.; Still, W. C. *J. Comput. Chem.* **1994**, *15*, 1302.
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Table 2. Free Energies (kcal/mol) of Enantioselective Binding for Chiral Ionophore 1 and Enantiomeric Alanine Derivatives 2 by Various Simulation Methods[a] and by Experiment

receptor (**1**)	substrate (**2**)	FEP[b]	MC(JBW)[c]	MC(JBW)/SD[d]	expt[1b]
1. X = H	Y = OMe	0.3 ± 0.1	0.5 ± 0.7	0.3 ± 0.1	0.4 ± 0.1
2. X = NHAc	Y = OMe	0.6 ± 0.1	0.6 ± 0.4	0.5 ± 0.1	0.8 ± 0.1
3. X = NHAc	Y = NHMe	1.0 ± 0.2	0.7 ± 0.6	0.6 ± 0.2	1.1 ± 0.2
4. X = NHAc-butenolide	Y = NHMe	1.9 ± 0.2	2.0 ± 1.3	2.0 ± 0.2	1.7 ± 0.1
	CPU time =	200 h	126 h[e]	13 h[e]	

[a] Calculations at 300 K using the AMBER* force field and the GB/SA continuum model for chloroform. [b] Data from 21 window, 500 ps/window free energy perturbation simulation using MC/SD as an ensemble-generating procedure as previously reported in ref 1b. [c] 10^8-step simulation. [d] 10 ns (10^7 step) simulation. [e] CPU time of simulation (~1 ns) on a single R10000 processor necessary to give free energy of enantioselection that is stable to within 0.2 kcal/mol of final result for the last (X = NHAc-butenolide, Y = NHMe) calculation. These times do not include the initial conformational search times which are ~2 CPU hours for finding representative minima or 10 CPU hours for finding all minima within the lowest 10 kcal/mol (see footnote 11).

distinct conformations from the room-temperature ensembles of **1**(X = H)·$EtNH_3^+$ generated by classical SD and MC and by our conformation-jumping MC(JBW) and MC(JBW)/SD methods are indistinguishable from one another within the limits of the convergence that could be achieved. As indicated by the small standard deviations of the conformational populations and by comparisons of populations of symmetry-equivalent conformations (data not shown), all four simulations appear well converged after 10^8 steps or 50 ns, though the convergence of the MC(JBW)/SD is particularly good. These results imply that all four methods generate the same thermodynamic ensemble and indicate that no detectable error has been introduced into our JBW methods by the inclusion of intermolecular degrees of freedom.

Free Energies of Enantioselection by SME. To use such JBW simulation methods in an SME procedure to compute the relative binding free energies of enantiomers, it is necessary that the MC-based jumps previously used to interconvert different conformational states also include jumps that interconvert enantiomers (here alanine derivatives (D)**2** and (L)**2**). Such molecule-mutating jumps are particularly easy in the case of enantiomers (or other stereoisomers) because they are purely geometrical mutations and may be effected by altering relevant improper torsion angles controlling the chirality of **2** in the Z-matrix that defines the system. Thus, in our simulations of **1·2**, we treat the chirality of **2** just like any other geometrical degree of freedom.

To use this methodology to compute the relative binding free energies of the two enantiomers of **2** for ionophore **1**, we first carried out low-mode conformational searches of both diastereomeric complexes (**1**·(D)**2** and **1**·(L)**2**). Resulting lists of coordinates for the low-energy (<10 kcal/mol of the ground state) conformers[11] of both complexes were combined and provided as input data (the X_i list in the Appendix) for the MC(JBW) or MC(JBW)/SD algorithms that simulated the equilibrium between the two diastereomeric complexes **1·2**. The MC(JBW) (10^8 steps) and MC(JBW)/SD (10 ns) simulations of four different **1·2** systems were each carried out at 300 K using the same AMBER* force field and GB/SA solvation model in MacroModel V5.5 and previously described for these systems.[1b] At each step in these simulations, the current structure was compared by Cartesian coordinate superimposition[12] with the conformers in the input data lists (X_i), assigned to the family of the most similar conformer (and diastereomer, see Appendix, step 5) and the assignments were accumulated to provide ensemble-averaged populations. The equilibrium populations of all conformations of the diastereomeric **1**·(D)**2** and **1**·(L)**2** complexes thus obtained provided the relative free energy of enantioselective binding simply from $\Delta G = -RT \ln(\text{population}_{\mathbf{1}\cdot(\text{D})\mathbf{2}}/\text{population}_{\mathbf{1}\cdot(\text{L})\mathbf{2}})$.

The SME free energy results from four **1·2** systems having known experimental enantioselectivities are given in Table 2 along with previously reported FEP-derived results.[1b] First we note that all simulations give free energies of enantioselection that are in good and comparable agreement with experiment. Second we see that the SME calculations (using both MC(JBW) and MC(JBW)/SD methods) are in excellent agreement (1σ error bars touch or overlap) with each other and with the FEP results. Among the three simulation methods we compare, the hybrid MC(JBW)/SD algorithm is by far the fastest. Indeed, CPU time comparisons indicate that MC(JBW)/SD converges an order of magnitude faster than either MC(JBW) or FEP in these systems.

For the MC(JBW)/SD-based SME enantioselectivity calculations, Figure 1 shows how the free energies of enantioselection evolve in time. Though we carried out these simulations for a total of 10 ns, it can be seen that the free energy stabilizes within 0.2 kcal/mol of its final value by 2 ns in all four of the **1·2** systems we studied. Since 2 ns simulations on these systems can be carried out in less than a day on a single R10000 processor, these results suggest that free energy calculations can be a practical tool in the design of organic host molecules of contemporary complexity.

The reason MC(JBW)/SD calculations converge so well is that they efficiently interconvert all the known conformers and diastereomers of the **1·2** complexes in a single simulation. Thus the speed advantage over FEP calculations largely stems from the multistage nature of FEP which in our systems required 21 different simulations (windows) to slowly mutate (D)**2** into (L)**2** within the binding cavity of **1**. As noted previously,[1b] attempts to speed the FEP results by using fewer windows or shorter simulation times led only to insufficient convergence. Why MC(JBW) is not as efficient as MC(JBW)/SD is less obvious as both are single-stage methods. However, by monitoring the conformer/diastereomer (from the X_i list) which the two simulations generated

(11) While most of our simulations employed every minimum energy conformer located by the conformational searches, we found that virtually identical free energy results could be obtained when families of closely related conformations were replaced by a single representative example of the family. Closely related conformations are ones that are rapidly interconverted by standard simulation methods (e.g., simple MC or SD) at the temperature of the simulation. In practice, we distinguish such families by graphical examination of superimposed family candidates. Thus while it is important to include a representative example from each significantly populated conformational well as input to the JBW algorithm, it is not necessary to carry out extensive conformational searches to locate each minimum energy form.

(12) Kabsh, W. *Acta Crystallogr.* **1976**, *A32*, 944. Kabsch, W. *Acta Crystallogr.* **1978**, *A34*, 837.

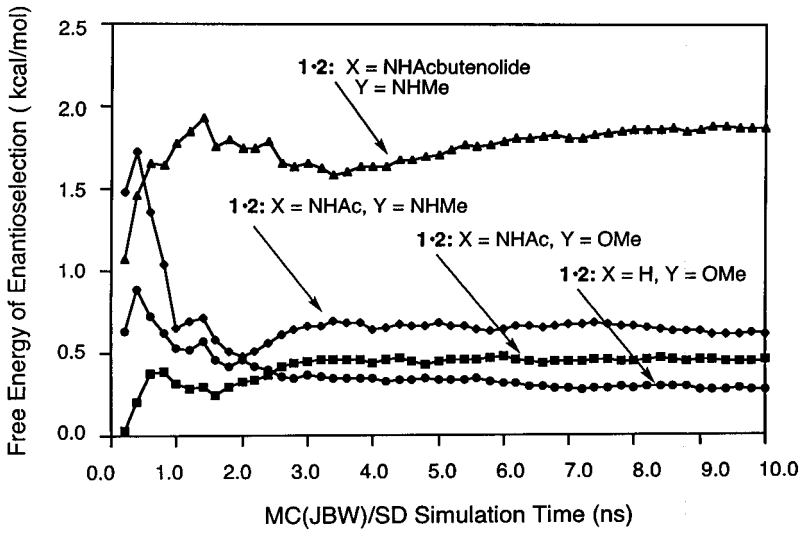

Figure 1. Convergence of free energies of enantioselection of **1**·**2** derivatives using the MC(JBW)/SD-based SME method.

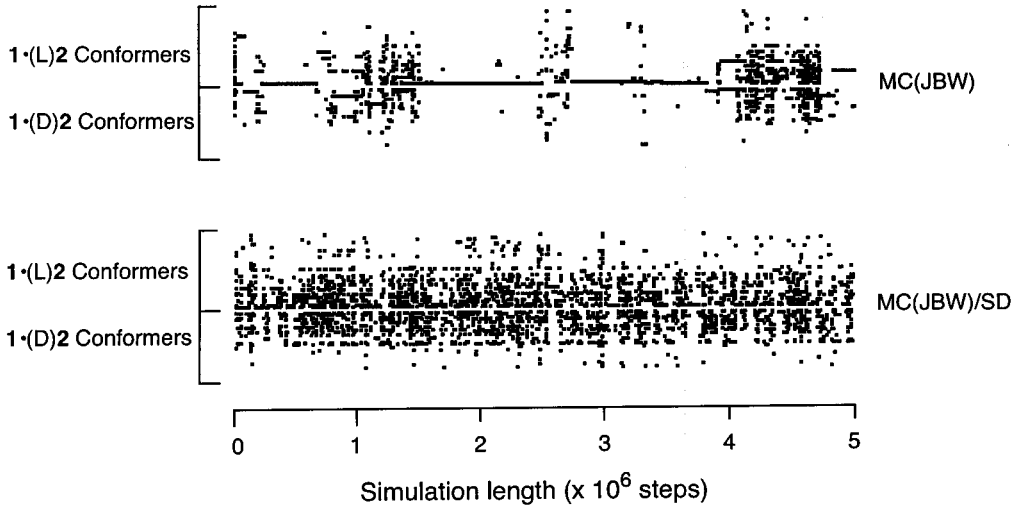

Figure 2. Conformers and diastereomers of **1**(X = H)·**2**(Y = OMe) sampled during MC(JBW) and MC(JBW)/SD simulations (see text).

in time, we could see that MC(JBW)/SD interconverts different structures (both different conformers and diastereomers) much more efficiently than does MC(JBW). Figure 2 shows such monitoring for both simulation methods on **1**(X = H)·**2**(Y = OMe). The various conformers the simulations visited over time are indicated on the vertical axes with the lowest energy conformers being closest to the center lines. Above the center lines are conformers of (L)**2** complexes while the diastereomeric (D)-**2** complexes are shown below the center lines. Thus Figure 2 indicates that the simple MC(JBW) method sporadically spends long periods of time in the vicinity of the same structure between periods when the structure-hopping JBW algorithm rapidly interconverts conformers and diastereomers. Such periods of infrequent structure interconversion occur when simulation structures stray and remain far from the bottoms of energy wells (where JBW structure-to-structure jumping is most efficient). In comparison, the hybrid MC(JBW)/SD algorithm interconverts structures efficiently throughout the entire simulation implying that the SD portion of the algorithm favors more frequent passes near energy well bottoms. Whether these results reflect a less than optimal randomization protocol in our MC procedure or some inherent advantage of SD in efficiently exploring single-energy wells is not clear at this time.

Conclusion

In this paper we have shown how a Monte Carlo method (JBW) can be used to simulate an equilibrium between two different molecules (here stereoisomers) and thus yield the relative binding free energies of those molecules for a third. Because the new method (SME) involves only a single simulation, it is more efficient than traditional multistage simulation methods such as free energy perturbation (FEP). The speed advantage of SME is roughly equal to half the number of stages (FEP windows) that must be used to obtain comparable degrees of free energy convergence. SME takes approximately twice as long to converge as a single stage in an FEP simulation because SME must fully explore the populated conformational space of two significantly different molecules. In contrast, a single FEP stage must fully explore the (smaller) conformational space of two molecular systems that are generally very similar to one another ($\Delta\lambda$ is typically small).

For the ionophoric complexes studied here, we found one type of SME simulation (based on the MC(JBW)/SD

method) to be particularly fast. In fact, this method converged roughly an order of magnitude faster than analogous (21-stage) FEP calculations on the same molecular systems. Other attractive features of the SME approaches are that they provide immediate estimates of total free energy differences that grow more precise with increasing simulation time and can readily be broken down into a number independent subsimulations (each having different initial conditions) for running in parallel when more rapid results are desired. Even on a single R10000 processor, the simulations described here give free energy results that are converged to within 0.2 kcal/mol within 1 CPU day and thus are fast enough to be used as a practical tool for molecular design (e.g., in designing chiral resolving agents or enantioselective catalysts). We use SME here to compute the free energy difference between two molecular complexes, but the same procedure can be applied to compute the relative free energies of many different of complexes in a single simulation though the simulation convergence time will increase accordingly. A limitation of our SME method stems from the large atomic movements that characterize all JBW-like procedures—they require that solvation be handled by continuum models (e.g., GB/SA[13]) until some practical way can be found to make large movements of both solute and solvent molecules.

While relative free energies of different stereoisomers or stereoisomeric complexes are straightforward to calculate using SME, the more general case of computing the relative binding energies of constitutionally different molecules requires an extension to our current implementation, e.g., involving invisible place-holding particles (dummy atoms) as used in FEP for similar purposes. The theoretical groundwork for such an extension has already been laid by Tidor.[6] This limitation notwithstanding, the work described here establishes the potential of the SME approach for practical, single-stage free energy calculations and illustrates its use by computing the relative free energies of several diastereomeric host–guest complexes. Extension to the general case of any two molecular mutants is underway.

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Appendix

MC(JBW) and MC(JBW)/SD. Extension to molecular complexes. The MC(JBW) algorithm has been described previously for conformational free energy simulations of single molecules.[5] To extend the method to molecular complexes requires that conformation-interconverting transformations (T_{ij} of step 3 below) which are used to interconvert different structures on the X_i list (step 1) include intermolecular transformations that control the relative orientation and position of a complex's constituent molecules. Such intermolecular transformations have the form of rotation matrixes and translation vectors. The complete MC(JBW) algorithm for molecular complexes follows:

Step 1. Carry out a conformational search of the complex to find the set of low-energy (typically within 5 kcal/mol of the global minimum) conformers—call these structures X_i.

Step 2. Align each structure on the X_i list by global translation and rotation with the first member of the X_i list using three arbitrarily chosen bonded atoms (*reference atoms*, designate them atoms 1−2−3) from the first molecule of the complex. This alignment superimposes atom 1, places atom 2 along the first member's 1−2 bond axis, and places atom 3 in the first member's 1−2−3 plane.

Step 3. For each pair of structures (i, j) in the X_i list and for a complex consisting of m molecules, calculate m intramolecular transformation sets (each consisting of a nonredundant set of $n - 1$ bond lengths, $n - 2$ bond angles, and $n - 3$ torsions where n is the number of atoms of the mth molecule) and $m - 1$ intermolecular transformation sets (each consisting of a rotation matrix and a translation vector) that when applied to structure i will generate structure j—call these transformation sets T_{ij}.

Step 4. Pick a starting geometry of the complex—call this structure Y_0.

Step 5. Find the conformer on the X_i list that is closest to Y_0—call this conformer X_0. For molecular complexes, we use a least-squares superimposition[12] of the structure in Cartesian coordinates with the members of the X_i list (having the correct chirality, i.e., that of Y_0) to determine which conformer is closest.

Step 6. Randomly choose a conformer from the X_i list—call this conformer X_T.

Step 7. Independently apply each of the intramolecular components of the transformation matrix $T_{X_0X_T}$ to each of the molecules constituting structure Y_0 to generate intermediate structure $Y_{1'}$.

Step 8. Globally translate and rotate structure $Y_{1'}$ to align its 3 reference atoms as defined in Step 2 above with those of the trial conformation X_T.

Step 9. Apply the intermolecular components of the transformation matrix $T_{X_0X_T}$ to intermediate structure $Y_{1'}$ to generate structure Y_1.

Step 10. Apply small random variations to (typically two) randomly chosen degrees of freedom (including both intermolecular and intramolecular coordinates) to structure Y_1 to generate the new trial structure Y_2.

Step 11. Verify that the resulting structure Y_2 is indeed closer to the intended trial structure X_T than to any other stucture on the X_i list. If not, reject the step. Closeness is defined in step 5.

Step 12. Compare energies of Y_0 and Y_2, accepting Y_2 with a probability defined by Metropolis:[8]

$$p = \min\{1, \exp[-(EY_2 - EY_0)/kT]\}$$

Step 13. Define the resulting structure as Y_0 and go back to step 5.

For MC(JBW)/SD, the above MC(JBW) procedure was used as the MC component of the hybrid MC/SD procedure described previously in which simulation steps alternated between single MC and SD steps.[9] No additional changes in the MC/SD procedure were necessary for use with molecular complexes.

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