# Efficient and Flexible Algorithm for Free Energy Calculations Using the λ-Dynamics Approach

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*Received: August 19, 1997; In Final Form: November 7, 1997*

We have developed an efficient and flexible algorithm that simultaneously calculates free energies for multiple compounds either reporting quantitative results to a desired precision or rapidly generating a qualitative ranking. When applied to a test case analogous to competitive binding of multiple ligands to a common protein, our algorithm quickly (within a few picoseconds) identifies the ligand possessing the most favorable binding affinity. In a detailed iterative calculation of the free energy changes, our method is approximately twice as efficient as the standard FEP method. In fact, improved biasing potentials may further enhance the efficiency of the protocol.

## I. Introduction

Free energy calculations have important applications in chemical and biophysical research, such as in the study of solvent effects on conformational stability, solvation free energy of molecules, and ligand binding affinity to proteins and nucleic acids.[1−8] In the standard free energy calculations using the coupling parameter approach,[9] such as free energy perturbation or thermodynamic integration methods,[10−12] the initial state is smoothly mapped to the final one through the coupling parameter $\lambda$. In these calculations the value of $\lambda$ either remains fixed at a value between 0 and 1 or changes slowly and monotonically within this interval. Although encouraging results have been obtained in applications of these methods, all are computationally intensive. The development of methods to reduce the computational cost and enhance the sampling efficiency in free energy calculations is still an area of active research. Recently, a semiempirical method using a linear approximation procedure has been applied to biological systems with promising results.[13,14] This method requires only simulations at the two end states ($\lambda = 0$ and 1) and therefore increases the computational efficiency. Various means of enhancing the sampling of the available phase space in order to speed up the convergence of free energy calculations have also been proposed.[15,16]

In addition to its use in calculations of specific changes in free energy between two states, free energy calculations have important applications in the process of drug discovery. When designing pharmaceutical agents, knowledge of the relative free energy of binding of a single modified inhibitor is of little use. In contrast, the prediction of free energy changes for an ensemble of slightly varying inhibitors would be of considerable help in guiding synthetic strategies. Although attempts have been made to use the information of free energy derivatives to pursue this type of predictive calculation,[17,18] more effort is needed to refine this idea and build a practical tool.

Recently, we have proposed a new computational tool that rapidly partitions favorable binders from unfavorable ones.[15] In this $\lambda$-dynamics approach, which is inspired by the work of Liu and Berne[19] and Tidor,[20] the coupling parameter $\lambda$ is treated as a dynamic variable and rather than a single coupling parameter, a set of variables $\{\lambda\}$ is used to scale different interaction terms. A prototype calculation demonstrating the potential application of the $\lambda$-dynamics approach for competitive binding calculations was presented.[15] Furthermore, the treatment of $\lambda$'s as dynamic variables provides great control over sampling of the phase space of interest and therefore facilitates the convergence of free energy calculations. The robustness of this methodology for free energy calculations also has been demonstrated by Kong and Brooks.[15]

In this work, we continue to develop the $\lambda$-dynamics methodology. One of our objectives is to further investigate the $\lambda$-dynamics approach for competitive binding calculations. By including a reference state free energy in the system Hamiltonian, we were able to obtain the relative binding free energy of the ligands corresponding to the full thermodynamic cycle and therefore rapidly identify those ligands with favorable binding affinities. Another objective is to continue developing the potential of $\lambda$-dynamics for detailed free energy calculations by implementing an iterative procedure. By including a biasing potential, barriers between different states are significantly reduced. Therefore, this method provides better sampling of the phase space within a single simulation. WHAM[21,22] is used to combine data from different simulations to obtain an optimal estimate of the free energy. To validate our approach, we also compare our results with those from conventional FEP calculations using the same force field.

## II. Description of the Method

**(1) Theory.** Consider a set of chemically distinct species. Let us construct the hybrid potential as follows

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S1089-5647(97)02699-0 CCC: $15.00 
Published on Web 02/24/1998

$$V(\{\lambda\}, (x)) = \sum_{i=1}^{L} \lambda_i^2 (V_i(x) - F_i) + V_{\text{env}}(x) \qquad (1)$$

$$\text{where} \qquad \sum_{i=1}^{L} \lambda_i^2 = 1$$

As in standard free energy calculation methods, $V_{\text{env}}(x)$ is the interaction involving the environmental atoms only (e.g., solvent, protein, and the part of the molecule that is invariant among all ligands), $V_i(x)$ is the interaction involving any of the atoms in the distinct group of molecule $i$ ($i = 1, ..., L$), $\lambda_i$ is the coupling parameter, and $F_i$ is the reference free energy or biasing potential. Note that there is no interaction among atoms in distinct groups. Unlike standard FEP calculations, each $\lambda_i$ is treated as a fictitious particle with mass $m_i$. The dynamics of the system is governed by the extended Hamiltonian[15]

$$H(\{\lambda\}, x) = T + T_{(\lambda)} + \sum_{i=1}^{L} \lambda_i^2 (V_i(x) - F_i) + V_{\text{env}}(x) \quad (2)$$

From the partition function of the hybrid system

$$Z(\{\lambda\}, x) = \int \exp(-\beta(\sum_{i=1}^{L} \lambda_i^2 (V_i(x) - F_i)) + V_{\text{env}}(x))\, dx \qquad (3)$$

the free energy difference between two molecules, $i$ and $j$, with reference free energy $F_i$ and $F_j$, respectively, can be calculated as

$$\begin{aligned} \Delta\Delta A_{i,j} &= \Delta A_i - \Delta A_j \\ &= -\frac{1}{\beta}[\ln \int dx \exp(-\beta V_i(x)) - \beta F_i] - \frac{1}{\beta} \\ &\qquad [\ln \int dx \exp(-\beta V_j(x)) - \beta F_j] \\ &= -\frac{1}{\beta} \ln \frac{Z(\lambda_i = 1, \{\lambda_{m\neq i}\} = 0)}{Z(\lambda_j = 1, \{\lambda_{l\neq j}\} = 0)} \\ &= -\frac{1}{\beta} \ln \frac{P(\lambda_i = 1, \{\lambda_{m\neq i}\} = 0)}{P(\lambda_j = 1, \{\lambda_{l\neq j}\} = 0)} \end{aligned} \qquad (4)$$

where $P(\lambda_i = \lambda_{m\neq i} = 0\}$is the probability that the hybrid system is in a state dominated by molecule $i$. Therefore, from the probability distribution of states dominated by $\lambda_i = 1$ and $\lambda_j = 1$, the difference in free energy between the two molecules can be obtained.

The function of $F_i$ in the above equations is 2-fold. First, $F_i$ serves as the reference free energy. In a closed thermodynamic cycle, $F_i$ may correspond to the free energy difference for half of the cycle, e.g., the solvation/desolvation component in a binding calculation. Since $V_i(x)$ is the Hamiltonian for the other half, the resulting free energy change from the simulations according to eq 2 corresponds to the difference in free energy change ($\Delta\Delta A$) for the full cycle, the quantity of interest in most applications. If $F_i$ is taken as zero for all species, then the resulting free energy difference corresponds to that of a single state, or half the thermodynamic cycle. Second, $F_i$ serves as a biasing potential. As will be discussed in detail next, one can perform controlled sampling in certain regions of phase space by varying $F_i$. Our aim here is to completely sample the reaction coordinates, $\{\lambda\}$, within a single simulation. We accomplish this by reducing the barrier height between different states along the reaction coordinates.

To better estimate the free energy using the available data set, we use the weighted histogram analysis method[21,22] to combine data from different simulations. We developed an iterative procedure to improve sampling of the phase space and therefore to make free energy calculations converge more rapidly.

**(2) The Iterative Technique.** Since $\{\lambda\}$ is treated as a dynamic variable just as the atomic coordinates, we can use $X$ to denote the phase space that encompasses $\{\lambda\}$ and $\{x\}$. Thus the Hamiltonian in eq 1 can be rewritten as

$$V(\{F\}, X) = V_0(X) - \sum_{i=1}^{L} F_i \lambda_i^2 \qquad (5)$$

$$\text{where} \qquad V_0(X) = \sum_{i=1}^{L} \lambda_i^2 V_i(x) + V_{\text{env}}(x)$$

The WHAM equations[22] for multiple reaction coordinates and at constant temperature can be readily applied to obtain the best estimate of free energy using data from $n$ simulations:

$$P^n_{\{F\}}(\{\lambda^2\}) = \frac{\sum_{k=1}^{n} N_k(\{\lambda^2\}) \exp(-\beta \sum_{i=1}^{L} (-F_i)\lambda_i^2)}{\sum_{m=1}^{n} n_m \exp(f_m - \beta \sum_{i=1}^{L} (-F_i)\lambda_i^2)} \qquad (6)$$

$$\exp(-f_m) = \sum_{(\lambda^2)} P^n_{\{F\}}(\{\lambda^2\}) \qquad (7)$$

After the $n$th iteration, the estimated free energy relative to reference free energy $\{F\}$ is

$$G_{i\{F\}} = -\frac{1}{\beta} \ln P^n_{\{F\}}(\lambda_i^2 = 1, \{\lambda^2_{m\neq i}\} = 0) \qquad (8)$$

A new biasing potential for the next iteration can be estimated as

$$F_i^{n+1} = G_{i,\{F=0\}} \qquad (9)$$

The above procedure can be used to extract the free energy $A_i$ of each molecule. Here the estimated free energy of each molecule is chosen as the biasing potential for that molecule in the next iteration. Sometimes, an additional term $\Delta_i$ could also be added to eq 9 to either enhance or reduce sampling of the state dominated by molecule $i$. As in all iterative procedures, an initial trial value of $F_i$ must be given. If a poor initial free energy $F_i$ is used, then the state with $F_i < A_i$ will be sampled less frequently than it would with $F_i = A_i$. Similarly, states with $F_i > A_i$ will be sampled more frequently than they would with $F_i = A_i$. In our iterative approach, the free energy barrier is reduced each successive iteration and therefore produces complete sampling of important configurations along the reaction coordinates.

## III. Model and Simulation Details

We examined benzamidine (BENZ) and three of its para-derivatives, namely, p-aminobenzamidine (*p*-NH2), *p*-methyl-

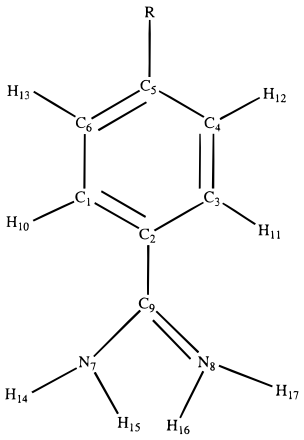


**Figure 1.** Structure of benzamidine inhibitors. R represents the variant groups. R = H for benzamidine, $NH_2$ for *p*-aminobenzamidine, $CH_3$ for *p*-methylbenzamidine, and Cl for *p*-chlorobenzamidine.

**TABLE 1: Atomic Charges of Benzamidine and Three of Its Para-Derivatives**

| | variant atoms | | invariant atoms | |
|---|---|---|---|---|
| R | name | charge | name | charge |
| H | C5 | −0.115 | C1, C3, C4, C6 | −0.115 |
| | H | +0.115 | | |
| $NH_2$ | C5 | +0.120 | C2 | +0.200 |
| | N | −0.740 | N7, N8 | −0.800 |
| | H1, H2 | +0.310 | C9 | +0.560 |
| $CH_3$ | C5 | 0.000 | H10, H11, | +0.115 |
| | C | −0.270 | H12, H13 | |
| | H1, H2, H3 | +0.090 | | |
| Cl | C5 | +0.460 | H14, H15, | +0.460 |
| | CL | −0.460 | H16, H17 | |

benzamidine (*p*-CH3), and *p*-chlorobenzamidine (*p*-CL), as model systems for this work. The variable groups are R = H, $NH_2$, $CH_3$, Cl, as well as the carbon atom in the phenyl ring that is attached to R (Figure 1). We use the CHARMM version 22 parameters and topologies[23] except for the charges, which were built from the QUANTA parameter set and modified slightly to confer identical charges on all invariant ligand atoms. The molecular structure and the corresponding charges are shown in Figure 1 and Table 1, respectively.

The hybrid molecule was solvated in a 24 Å octahedral volume of TIP3P water.[24] Water molecules whose oxygen overlapped within 2.8 Å of any non-hydrogen atoms in the solute molecule were removed. The final simulation system contained 237 water molecules. We employed a nonbond cutoff of 12.4 Å along with van der Waals switching and electrostatic shifting functions. The force shifting method was employed for electrostatic cutoffs at 10.5 Å, and the van der Waals switching was over a range of 8.0 to 10.5 Å. In all the simulations, periodic boundary conditions were employed and the temperature was maintained near 300 K by coupling the non-hydrogen atoms to a Langevin heat bath using a frictional coefficient 50 $ps^{-1}$.[25] All bonds containing hydrogen atoms were fixed using the SHAKE algorithm.[26] The time step used in the simulations was 1.5 fs. The masses of the fictious $\lambda$ degrees of freedom were chosen to be comparable to the mass of a carbon atom; the $\lambda$ masses used were all 20 amu·$Å^2$. All calculations were done using the CHARMM molecular dynamics package.[23]

## IV. Applications

When making comparisons with experimental results, accurate free energy calculations are demanded. In molecular design, however, specific changes in free energy may not be pertinent. Rather, what matters is the ability to qualitatively identify those bearing favorable free energies. We demonstrate below that our $\lambda$-dynamics methodology can be applied to perform both qualitative and quantitative tasks.

**TABLE 2: Summary of Free Energy Calculations Using Standard FEP Method**[a]

| R | $\Delta G$ (vacuum) | $\Delta G$ (solution) | $\Delta\Delta G$ |
|---|---|---|---|
| H | 0.0 | 0.0 | 0.0 |
| $NH_2$ | −7.13 | −10.61 | −3.48 |
| $CH_3$ | −3.11 | −4.15 | −1.04 |
| Cl | −7.46 | −1.08 | −8.54 |

[a] Free energy changes are in kcal/mol and relative to benzamidine (R = H); the precision of these calculations is near or below ±0.4 kcal/mol.

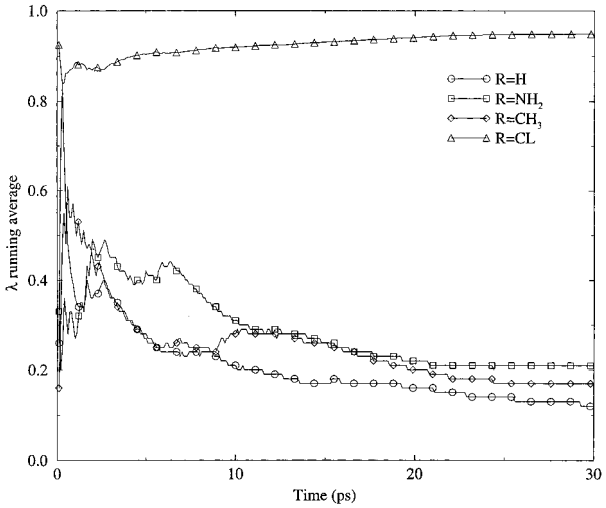


**Figure 2.** Cumulative $\lambda$ running average for benzamidine and three of its para-derivatives. The ranking of the ligands BENZ > *p*-NH2 > *p*-CH3 > *p*-CL is consistent with FEP calculations.

**(1) Rapid Screening of Molecules with Favorable Binding Free Energy.** Many problems of current interest (e.g., drug design) require choosing a ligand with high affinity for a target molecule. The most favorable free energy in either the bound or the free state is not of primary interest because affinity is measured by the difference in free energy between the two. In this demonstration, which is in spirit analogous to competitive binding experiments, the solvent environment is considered as the "protein receptor" that all the ligands are competing for, and the gas phase as the free state. The free energy of the ligands in the "free state" is taken as the reference state free energy, $\{F\}$. In this work, the value of $F$ is obtained from standard FEP calculations (column 2 of Table 2). The ordering of these reference state free energy is *p*-CL < *p*-NH2 < *p*-CH3 < BENZ. In general, the free energy of a molecule in the free state can be obtained from any rapid method that provides a reasonably good estimate of the free energy. The iterative procedure described in section II could also be used for such calculations.

To find ligands possessing favorable binding free energy, a straightforward $\lambda$-dynamics simulation of 30 ps is carried out by incorporating the reference free energy value $\{F\}$ into the Hamiltonian (eq 2). The output of the $\lambda$-trajectory can be analyzed to identify those favorable binders. Figure 2 displays the ranking of the "binding affinity" of all the ligands according to the running average of the corresponding $\lambda$-values. The running average displayed in the figure represents the cumulative average of the value of particular $\lambda$-variables. After only a few picoseconds of simulation, the molecule with the most favorable

free energy change unambiguously emerges. This molecule clearly populates the region $\lambda = 1$ with the highest probability. For other ligands smaller, or possibly no, sampling of the $\lambda = 1$ region occurs in these short screening simulations. Longer simulations would allow us to determine the correct ordering of binding affinities for all the compounds under consideration. Despite seeing little or no sampling for less-favored ligands, we observe the correct overall trend in the running average. While this observation does not follow from the formalism presented above, it can be anticipated if the free energy landscape in the $\lambda$-dimensions is smooth. In this situation, the ligand striving to reach its lowest free energy state will populate regions of the $\lambda$-space consistent with its relative affinity for the receptor and the constraint that the sum of all species equal unity. We justify this claim by comparing the fast screening results with those from the detailed calculations listed in column 4 of Table 2. Since no biasing potential is used in this experiment, it is possible for the system to become trapped in a local minimum, as happens in all optimization problems. For this reason, we performed several simulations with different initial conditions (e.g., different initial values for each $\lambda$ "coordinate" and velocity). In all of these simulations, *p*-CL, which has the most favorable free energy change, always wins within a short simulation time. Also, the correct ordering for the remaining species (whose free energy differs by only 1−2 kcal/mol) is observed. This provides some empirical evidence for the smoothness of the landscape in this case. While the proper ordering of the rest of the field cannot be guaranteed, nor may it be of practical interest, the most favorable species is clearly identified in every simulation.

**(2) Comparison of Standard FEP Method and $\lambda$-Dynamics Method.** As described in Section II, by applying biasing potentials and an iterative technique, we expect to achieve better sampling of the phase space of interest and therefore faster convergence of the calculations. To evaluate the efficiency of our algorithm for free energy calculations, we compare the $\lambda$-dynamics approach with the standard FEP method. Although the $\lambda$-dynamics methodology enables one to calculate free energy differences among multiple species within a single simulation, for purpose of comparison we restrict our choice to a pair of molecules, *p*-NH3 and *p*-CH3, and consider only half of the thermodynamic cycle—the solvated ligands. The gain in efficiency for multiple compounds can easily be deduced using simple arithmetic. For the FEP calculations, we performed simulations with $\lambda = 0.125$, 0.5, and 0.875, respectively. This choice of three $\lambda$-values is sufficient to ensure that the total free energy change in each simulation is less than 2.0 kcal/mol. The free energy change for each simulation is calculated using the double-wide sampling technique.[27] For each $\lambda$, a 20-ps equilibration period followed by 60 ps of data collection is performed. Therefore a total of 240-ps simulation time is used. The overall free energy change and its standard deviation are obtained, which are $6.46 \pm 0.13$ kcal/mol.

In the $\lambda$-dynamics method, which uses an iterative technique, an initial value of the biasing potential, $\{F\}$, must be given. We use the following procedure to initially guess the free energy. The free energy of the two species are first initialized to zero, and a short (a few picoseconds) simulation run is performed. With this choice of free energy, *p*-NH2 clearly dominates and *p*-CH3 shows little sign of competition. Therefore in the subsequent trial run, we assign a free energy value of zero to *p*-NH2 and of 5 kcal/mol to *p*-CH3 (from our experience, molecules with free energy 5 kcal/mol higher than the lowest one do not compete). This choice of initial free energy is sufficient to render *p*-CH3 competitive because the $\lambda$-value of *p*-CH3 reaches one within a short simulation. Having chosen the initial value of $\{F\}$, several 120-ps simulations, each following a 20-ps equilibration, are performed using the iterative technique described in the previous section. In these simulations, the estimated free energy from previous iterations is used as a bias for the next iteration. Figure 3 indicates that even after the first iteration, the estimated free energy (5.95 kcal/mol with standard deviation 0.15 kcal/mol) differs only by ∼0.3 kcal/mol from the final converged value (∼6.25 kcal/mol). Thus this method is observed to be two times more efficient than the standard FEP calculations for a similar level of precision in this case. We note that the increase in efficiency may depend on the system under study and simulation protocols. However, as more windows are added in the standard FEP calculations, the "cost" of such calculations increases. Thus, we anticipate that the iterative $\lambda$-dynamics approach will increase in efficiency relative to standard FEP in these situations. Figure 4 shows the potential of mean force (pmf) along the $\lambda$-coordinate. It demonstrates that the free energy barrier is significantly reduced when biasing potentials are applied, as compared to the unbiased situation. In our case no further reduction of barrier

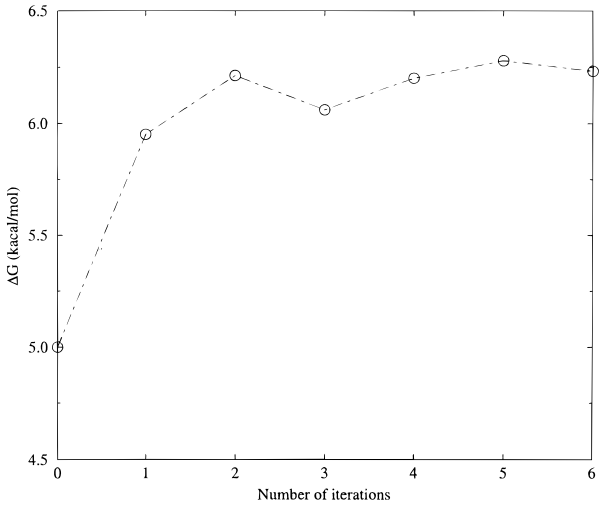


**Figure 3.** Estimate of free energy of solvation difference as a function of the number of iterations. The estimated free energy is quite close to the converged value even after the first iteration.

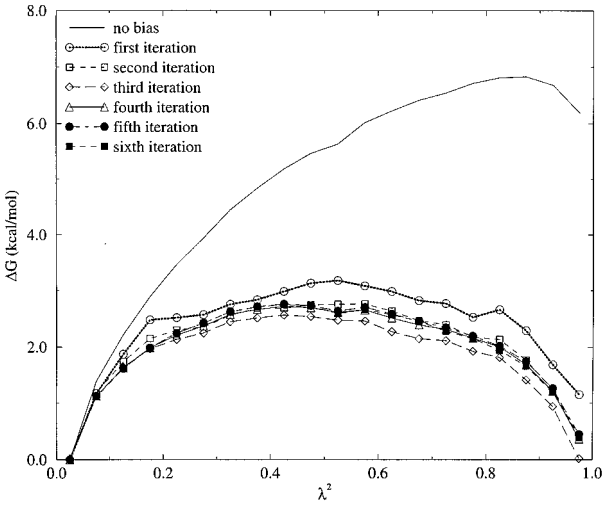


**Figure 4.** Potential of mean force ($\Delta G$) along the coordinate $\lambda$. Without a biasing potential, the barrier between the two end states is 7 kcal/mol. The barrier is reduced to 3 kcal/mol when a biasing potential is applied.

height is obtained after the second iteration. This indicates that the estimated free energy approximates the expected value within statistical uncertainties. Although the free energy barrier is decreased substantially with the choice of biasing potentials, a barrier of about 3 kcal/mol nonetheless still exists, therefore slowing down the transition between the two end states. The efficiency of our method would increase further if this free energy barrier was reduced. This can be accomplished by choosing a more sophisticated biasing potential dependent on $\lambda$ itself.[15]

## V. Conclusions

Our study shows that, with the $\lambda$-dynamics approach, a rather short simulation (on the order of picoseconds) is sufficient to identify molecules with the most favorable binding affinities, though the total simulation time may be system-dependent. In our study, only one species emerges as most favorable because the "binding" free energy of *p*-CL is about 5 kcal/mol more favorable than the next closest competitor. In general, however, more than one species could be identified as a favorable binder if the binding affinities differ by only a few kcal/mol. Various test runs indicate that species whose binding free energies fall at least 5 kcal/mol higher than the most favorable are unable to compete during very short screening calculations: they never reach the $\lambda_i = 1$ state within our simulation time. Therefore, those species can easily be screened out as unfavorable. For molecules with binding affinities that lie within 5 kcal/mol of the best binder, this method can provide the correct ordering if the free energy between the two species exceeds ~0.5 kcal/mol.

Our method for accurate calculation of free energy employs feedback from previous simulations to improve the bias of the current simulation. Such an approach is similar in spirit to the entropy sampling method of Lee and Scheraga.[28,29] The optimal estimate of these biasing potentials is achieved by the multiple reaction coordinate WHAM technique. In these calculations, biasing potentials are derived from constant values that correspond to the estimated free energy of each species. Figure 4 demonstrates that the free energy barrier is reduced significantly as compared to the original, unbiased one. However, there remains a barrier of ~3 kcal/mol between the two end states, an indicatition that a biasing potential of constant value may reduce the free energy barrier, yet may not suffice to flatten the free energy surface. To obtain a smooth surface other types of biasing potentials, such as one constructed as a function of the reaction coordinates, would be more appropriate. A similar idea has been tested by Kumar et al.[30] in the context of Monte Carlo simulations, in which it was shown that the sampling efficiency of the phase space of interest increased and the free energy calculations converged faster.

**Acknowledgment.** Support from the National Institutes of Health (GM37554) is greatly appreciated. We thank Yigal Nochomovitz for helpful comments during manuscript preparation.

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