Reconstructing Potential Energy Functions from Simulated Force-Induced Unbinding Processes

M. Balsera, S. Stepaniants, S. Izrailev, Y. Oono, and K. Schulten

Beckman Institute and Department of Physics, University of Illinois, Urbana, Illinois 61801 USA

ABSTRACT One-dimensional stochastic models demonstrate that molecular dynamics simulations of a few nanoseconds can be used to reconstruct the essential features of the binding potential of macromolecules. This can be accomplished by inducing the unbinding with the help of external forces applied to the molecules, and discounting the irreversible work performed on the system by these forces. The fluctuation-dissipation theorem sets a fundamental limit on the precision with which the binding potential can be reconstructed by this method. The uncertainty in the resulting potential is linearly proportional to the irreversible component of work performed on the system during the simulation. These results provide an a priori estimate of the energy barriers observable in molecular dynamics simulations.

INTRODUCTION

Atomic force microscopy (AFM) and similar micromanipulation techniques allow researchers to measure macromolecular adhesion forces that stabilize the multitude of supramolecular structures found in all life forms. In the simplest case, adhesion forces bind ligands to proteins. The force necessary to unbind a single ligand-receptor complex has been determined recently for the streptavidin/avidin-biotin system (Florin et al., 1994; Moy et al., 1994a, b; Chilcotti and Stayton, 1995; Chilcotti et al., 1995). Naturally, one wishes to relate the observed rupture forces to the potential energy surfaces that govern the adhesion between ligands and proteins.

A natural approach to interpret AFM experiments in terms of potential energy surfaces and to develop insights into the process of dissociation of macromolecules is furnished by molecular dynamics (MD) simulations (Grubmüller et al., 1996; Izrailev et al., 1997). Unfortunately, the mechanisms by which dissociation is induced in AFM experiments and in MD simulations are markedly different: on the millisecond time scale of AFM experiments, unbinding is a thermally activated process; molecular dynamics simulations, on the other hand, can cover only nanosecond time scales and need to apply large forces, which abolish all relevant potential energy barriers to induce a sufficiently rapid dissociation (Izrailev et al., 1997). The force needed to induce unbinding within time τ_R depends sensitively on this time scale (Evans and Ritchie, 1997; Izrailev et al., 1997). In the extreme case that τ_R is chosen longer than the natural dissociation time of a molecular complex, no external force is needed, i.e., a zero rupture force would be measured. In AFM experiments τ_R values are ~ 1 ms; in the case of the

avidin-biotin complex, the rupture forces associated with such τ_R measure ~ 160 piconewtons (pN) (Florin et al., 1994). In molecular dynamics simulations, by necessity, one chooses τ_R values of ~ 1 ns or even shorter; the forces that rupture the same complex are several hundred pN (Izrailev et al., 1997), i.e., are significantly larger than those observed in AFM experiments. This increase of the rupture force can be related in part to the need of abolishing the potential barriers through superposition of an external potential, while the major contribution stems from the dissipative processes that accompany the unbinding (Izrailev et al., 1997). The question arises in how far one can discount the contribution of dissipation and relate the rupture forces observed in MD simulations to the potential energy surfaces that govern natural dissociation, as well as dissociation-induced in AFM experiments.

In this paper we investigate, in the framework of one-dimensional stochastic models, the feasibility of reconstructing the potential energy function along the path of force-induced unbinding in MD simulations, as has been suggested by Evans (Evans and Ritchie, 1997). The effect of dissipation is represented by a friction coefficient γ, to be obtained from MD simulations. Once γ is known it can be used to estimate the amount of irreversible work produced during forced unbinding. The model introduced below describes adequately a situation in which the forced unbinding reaction is much slower than the relaxation of all other degrees of freedom in the system. This condition is difficult to satisfy on the time scales achievable in MD simulations. It is, however, the most favorable case for the reconstruction of the energy landscape without the assumption of thermodynamic reversibility. The introduction of γ, even in this idealized model, imposes limitations on the accuracy with which the potential can be reconstructed.

According to the fluctuation-dissipation theorem, the friction coefficient γ is related to the magnitude of random forces that perturb the ligand. As a consequence, the work needed to displace the ligand exhibits an inherent randomness. The accuracy of the reconstruction of the potential

energy function is determined by the fluctuations of the potential energy function estimated from the work done on the ligand. We will demonstrate that these fluctuations are proportional to the amount of irreversible work deposited into the system during enforced unbinding. The error estimate derived is useful for the development of MD simulation protocols to determine energy barriers between different states of macromolecular systems. The estimate can also be used to select simulation conditions adequate to attain a given accuracy in the reconstruction of a potential energy profile.

Our approach resembles the conventional free energy perturbation techniques for the reconstruction of the potential energy surfaces as furnished by the umbrella sampling method (McCammon and Harvey, 1987). Unlike the umbrella sampling technique, however, our approach strives to explicitly discount dissipation due to fast manipulation. Relying on the ideal of reversibility of the unbinding process, the umbrella sampling requires equilibration at each step and is computationally demanding. This equilibration is practically impossible in computer simulations if the system undergoes major transformations. In addition, it is known that breakage of the bonds in biopolymers as well as in industrial adhesives is accompanied by dissipative work, which can be much larger than the work due to the underlying thermodynamic potentials (Baljon and Robbins, 1996). Many non-covalent bonds and adhesive linkages (Evans et al., 1995) gain their strength through dissipation.

In the next section the stochastic model describing forced unbinding is introduced. We illustrate the method by reconstructing an energy barrier for the case of a one-dimensional unbinding path. We then discuss the predictions and shortcomings of the model.

THEORY

Let us consider a protein-ligand dissociation reaction induced by external forces. We assume that the dissociation can be described by a single reaction coordinate. The unbinding path does not, therefore, change when a force is applied to the system. This assumption cannot be valid in general and needs to be reconsidered in a future, more general, treatment of the problem. As mentioned in the introduction, we will also assume that the unbinding reaction takes place on a time scale long compared to the relaxation times of all other degrees of freedom of the system. We can then consider the effective friction coefficient γ between the ligand and the protein to be independent of time, simplifying the model significantly. The model, even under such idealized assumptions, allows us to investigate the limitations of the reconstruction method.

In the strong friction limit, when the time scale of unbinding is much longer than velocity relaxation, i.e., much longer than 1 ps, the motion of the ligand is governed by the

stochastic differential equation (e.g., Gardiner, 1985)

$$\gamma \dot{x} = -\frac{dU}{dx} + F(x, t) + \sigma N(t). \tag{1}$$

Here x is the coordinate of the ligand along the unbinding path, $U(x)$ is the associated thermodynamic potential, $F(x, t)$ is the force applied along the path, γ is the friction coefficient, $N(t)$ represents a Gaussian white noise of unit amplitude with zero mean, i.e., with correlation function $\langle N(t + t_0)N(t_0)\rangle = \delta(t)$, and σ is the amplitude of the fluctuating forces. According to the fluctuation-dissipation theorem, γ and σ are related through temperature as $\sigma^2 = 2k_B T \gamma$. For a protein-ligand complex in solution with constant temperature and pressure we can identify $U(x)$ with the Gibbs free energy of the system.

For the present description we choose for the applied force the functional form

$$F(x, t) = K(vt - x), \tag{2}$$

where K is a positive constant. This force corresponds to the ligand being pulled by a harmonic spring of stiffness K with its end moving with velocity v. In computer simulations this procedure can be implemented through restraining a molecule harmonically to a point, and moving this point with velocity v. The position fluctuations associated with the restraint, according to the well-known Boltzmann distribution of a harmonically bound particle, are given by

$$\delta x \sim (k_B T/K)^{1/2}, \tag{3}$$

and the fluctuations of the applied force are related to K through $\delta F \sim (K k_B T)^{1/2}$. A stiff restraint confines the ligand to fluctuate in a small region of the binding pocket, so that only local properties of the binding potential are sampled, while the fluctuations of the force become large. For a soft restraint, on the other hand, the ligand is able to fluctuate in a large region of the binding pocket, and the fluctuations of the force are small.

The position of the ligand at time t can be written as $x(t) = x_0(t) + \delta x(t)$, where $x_0(t)$ represents the trajectory in the limit of zero noise, and $\delta x(t)$ is the deviation of the position relative to x_0. Equation 1 can then be written as

$$\gamma(\dot{x}_0 + \dot{\delta x}) = -\frac{dU(x_0 + \delta x)}{dx}$$
$$+ F(x_0 + \delta x, t) + \sigma N(t). \tag{4}$$

We can solve Eq. 4 by expanding in powers of δx. To 0th order we have

$$\gamma \dot{x}_0 = -\frac{d}{dx} U(x_0) + K(vt - x_0), \tag{5}$$

while the first-order correction is governed by the stochastic differential equation

$$\gamma \dot{\delta x} = -\left[K + \frac{d^2}{dx_0^2} U(x_0)\right]\delta x + \sigma N(t). \tag{6}$$

For a stiff spring, satisfying $K \gg |d^2U/dx^2|$, the fluctuations δx are small according to Eq. 3, so that we may neglect higher-order corrections as well as the $d^2U(x_0)/dx_0^2$ term in Eq. 6. The fluctuations $\delta x(t)$ obey, then, the Langevin equation for an overdamped harmonic oscillator

$$\gamma \delta \dot{x} = -K \delta x + \sigma N(t), \tag{7}$$

the solutions of which are known to relax with a characteristic time τ given by, e.g., Gardiner (1985)

$$\tau = \gamma/K. \tag{8}$$

We introduce $\bar{g}(t) = 1/\Delta t \int_t^{t+\Delta t} dt' g(t')$ to represent a running time average of $g(t)$. Here Δt is assumed to be much smaller than the overall time of unbinding, but much larger than τ defined in Eq. 8. The fluctuations δx are small and average to zero for times longer than τ. We can then replace $x_0(t)$ in Eq. 5 by the average position of the ligand $\bar{x}(t)$ and obtain

$$x \approx vt - \frac{1}{K}\frac{dU(x)}{dx} - \frac{\gamma}{K}\dot{x}. \tag{9}$$

For a stiff restraint under the overdamped condition assumed in Eq. 1 follows $\dot{x} \sim v$. The latter allows us to rewrite Eq. 5 as

$$\bar{F} = \frac{dU}{dx} + \gamma v, \tag{10}$$

where $\bar{F} = K(vt - \bar{x})$.

Equation 10 implies that for a stiff restraint the average applied force measures the local slope of the binding potential plus a frictional contribution that depends linearly on the pulling velocity. This dependence was observed in the MD simulations of the biotin-streptavidin complex by Grubmüller and co-workers (1996). For a soft restraint, on the other hand, the condition $\dot{x} \sim v$ does not hold and no linear scaling of the measured force should be observed with the pulling rate (Izrailev et al., 1997).

In the simulations of Grubmüller et al., the maximum averaged force was identified with the rupture force observed in AFM experiments. However, the stiffness of the cantilever in the actual experiments $K = 6$ pN/Å (Moy et al., 1994a) is much smaller than the spring constant $K = 280$ pN/Å employed in the computer simulations of these authors. The spatial fluctuations of the ligand as given by Eq. 3, in the case of AFM measurements, are ~ 3 Å, comparable to the size of the binding pocket of ~ 9 Å. Under these conditions AFM does not sample the local properties of the binding energy profile and the measured force cannot be related to the maximum slope of the binding potential.

For a soft restraint and unbinding times ($\tau_R \sim 1$ ms) of AFM experiments (Florin et al., 1994), fluctuations aid the exit of the ligand from the binding pocket, i.e., the unbinding is thermally activated. Therefore, the unbinding time depends exponentially on the height of the energy barrier

$\Delta U^\dagger$ reduced by the applied force F (Bell, 1978),

$$\tau_R = \tau_d \exp\left(\frac{\Delta U^\dagger - FL}{k_B T}\right), \tag{11}$$

where L is the size of the binding pocket and $\tau_d \sim L^2/D$ is the diffusion time of the ligand in the binding pocket [for biotin $D \sim 1$ Å^2/ns and $\tau_d \sim 25$ ns (Izrailev et al., 1997)]. Expression 11 can be rewritten as

$$F_{AFM} = \frac{\Delta U^\dagger}{L} - \frac{k_B T}{L} \ln \frac{\tau_R}{\tau_d}. \tag{12}$$

The rupture force measured in AFM experiments is given, therefore, by the average slope of the energy profile minus a correction related to the effects of thermal fluctuations. Equation 12 demonstrates that the rupture force measured in AFM experiments is linearly proportional to the activation energy of the system (Chilcotti et al., 1995). A comparison of Eqs. 10 and 12 shows that the unbinding induced in MD simulations and that induced by AFM differ drastically, and that the forces measured by both techniques cannot be readily related.

In order to fully account for the kinetics of binding and unbinding of biomolecules without applied forces, a knowledge of the binding potential $U(x)$ is necessary. According to Eq. 10, the average applied force, for a stiff restraint, samples the slope of the binding potential. We can, therefore, reconstruct the potential $U(x)$, integrating Eq. 10 over $\bar{x}$ and explicitly discounting frictional contributions to the applied force.

$$\bar{U}(x) - U(0) = \int_0^x dx' (\bar{F} - \gamma v). \tag{13}$$

We wish to estimate the limitations imposed on the accuracy of the reconstruction of the potential $U(x)$ due to the presence of dissipation in the problem. According to Eq. 13, we can relate the fluctuations in the time averaged potential $\delta \bar{U}(x)$ to the fluctuations in the time averaged applied force $\delta \bar{F}$,

$$\delta \bar{U}(x) = \int_0^x dx' \, \delta \bar{F}[t(x')], \tag{14}$$

where $t(x) = x/v$. Using Eq. 2, we can express $\delta \bar{F}$ in terms of the fluctuations of the position, δx,

$$\delta \bar{F}(t) = -\frac{1}{\Delta t} \int_t^{t+\Delta t} dt' K \delta x(t'). \tag{15}$$

The fluctuations of the force are, therefore, expressed as an integral over the trajectory of an overdamped harmonic oscillator. The error in the estimate of the slope of the potential is given by the variance in the applied force (Allen

and Tildesley, 1987)

$$\sigma_F^2(t) \equiv \langle (\delta \bar{F}(t))^2 \rangle = 2Kk_BT\left(\frac{\tau}{\Delta t}\right), \tag{16}$$

where $\langle \cdot \rangle$ stands for the ensemble average and $\Delta t \gg \tau$. Using Eq. 8, we can finally write

$$\sigma_F^2(t) = 2\frac{\gamma k_BT}{\Delta t}. \tag{17}$$

It should be noted that, although the instantaneous fluctuations of the force are proportional to K, the error in the applied average force does not depend on the stiffness of the restraint, but only on the averaging time Δt.

To resolve the spatial features of the binding potential, the variance of the force should be much smaller than the characteristic slope of the binding potential, $\delta_F \ll \Delta U/\Delta x$. This condition determines the averaging time Δt and, according to $v \ll \Delta x/\Delta t$, the velocity of pulling. Molecular dynamics simulations for a system of the size of avidin-biotin are limited to time scales of nanoseconds. The size of the binding pocket of avidin is $L \sim 10$ Å, which limits the pulling velocity to $v \sim 0.01$ Å/ps. In order to reconstruct the slope of the binding potential with a spatial resolution of $\Delta x \sim 1$ Å, the averaging time Δt can then be at most 100 ps. Using Eq. 17 and assuming a friction coefficient $\gamma \sim 4 \cdot 10^4$ pN ps/Å (Izrailev et al., 1997), we estimate the error in the force to be $\sigma_F \sim 170$ pN. In the near future we can expect simulations as long as 10 ns. In this case the error in the force measured will be reduced to 50 pN.

In the limit $\Delta t \gg \tau$, the time averaged force fluctuations $\delta \bar{F}(t)$ at times t and $t + \Delta t$ are statistically independent. We can use this fact to estimate the error in the measurement of the energy difference between two points separated by a distance x. Approximating Eq. 14 by a finite sum

$$\delta U \approx \sum_{i=1}^{N} \delta \bar{F}(t_i)\Delta x, \tag{18}$$

where $\bar{F}(t_i)$ is the time averaged force fluctuation at time $t_i = i\Delta t$, $\Delta x = v\Delta t$ is the distance traveled during time Δt, and the number of terms in the sum is $N = x/(v\Delta t)$. The variance of the fluctuations δU of the estimated potential becomes

$$\sigma_U^2 = \langle \delta U^2 \rangle = \sum_{j=1}^{N}\sum_{i=1}^{N} \langle \delta \bar{F}(t_j)\delta \bar{F}(t_i) \rangle \Delta x^2. \tag{19}$$

Using the fact that $\delta \bar{F}$ has zero mean and is uncorrelated for times $t_i \neq t_j$ we can rewrite the previous expression as

$$\sigma_U^2 = \sum_{i=1}^{N}\langle \delta \bar{F}(t_i)^2 \rangle \Delta x^2. \tag{20}$$

Using Eq. 16, $\Delta x = v\Delta t$ and $N = x/(v\Delta t)$ we obtain

$$\sigma_U^2 = 2Kk_BTxv\tau \tag{21}$$

and finally, with the help of Eq. 8,

$$\sigma_U^2(x) \equiv \langle (\delta \bar{U}(x))^2 \rangle = 2k_BT\gamma vx, \tag{22}$$

where we have assumed $t \gg \Delta t \gg \tau$.

The estimated potential $\bar{U}(x)$, therefore, strays away from the actual potential in a diffusive manner as described by a diffusion constant $D_U = k_BT\gamma v$. With the introduction of the work performed by the frictional force $W_{fr} = \gamma vx$, Eq. 22 can be rewritten as

$$\sigma_U^2 = 2k_BTW_{fr}. \tag{23}$$

Thus, the uncertainty in the potential U is determined by the irreversible work done on the system. For the avidin-biotin system, we replace x in Eq. 22 by the size of the binding pocket of avidin $L \sim 10$ Å. Simulation periods of 1 ns and 10 ns that correspond to the pulling velocities v of the order of 10^{-2} and 10^{-3} Å/ps yield $\sigma_U \sim 8$ and 3 kcal/mol, respectively. We can use the potential reconstructed from an MD simulation according to Eq. 13 to estimate the force that would be measured in an AFM experiment, as given by Eq. 12. The uncertainty in the force measured by AFM, F_{AFM}, will be given by $\sigma_{AFM} = \sigma_U/L$. For 1-ns and 10-ns simulations these uncertainties are 50 pN and 20 pN, respectively. It must be stressed that σ_U and σ_{AFM} are the lower bounds for the errors in $\Delta U^{\dagger}$ and F_{AFM} as estimated from MD simulations. The presence of other slowly relaxing degrees of freedom in the system, or the uncertainty in the value of γ, can only increase the errors in $\Delta U^{\dagger}$ and F_{AFM}.

Another consequence of Eq. 22 in the present model is that the accuracy in the measurement of ΔU obtained by averaging the potential in n simulations of time length t is the same as for a single simulation of length nt. In the case of averaging over n trajectories the average fluctuation will be $\delta \hat{U} = 1/n\sum_1^n \delta U_i$. Because different simulations are uncorrelated, we have $\hat{\sigma}_U^2(t) = \sigma_U^2/n$, where σ_U^2 is given by Eq. 22. Using $v = x/t$, one obtains

$$\hat{\sigma}_U^2(t) = \frac{1}{n}2\gamma k_BT\frac{x^2}{t}, \tag{24}$$

This coincides with the variance as determined from Eq. 22 for a simulation extruding the ligand with velocity v/n and for a time span nt,

$$\sigma_U^2(nt) = 2k_BTx^2/(tn). \tag{25}$$

This equivalence hinges on the fact that τ, the correlation time in the fluctuations δx, is small relative to t, so that ensemble and time averages are equivalent.

In our model the spatial resolution Δx is limited by $v\Delta t$ and, ultimately, by the distance traveled, on average, during one oscillator relaxation time τ. One may be tempted to increase the stiffness of the spring K to reduce τ (cf. Eq. 8). Expression 22, however, assumes that all other degrees of

freedom in the system relax on a time scale much shorter than τ. If we assume for those degrees of freedom a finite relaxation time $\tau_r > \tau$, the correlation time of the force fluctuations will be of the order of τ_r. If $\Delta t \gg \tau_r$, we can substitute τ_r into Eq. 21 to obtain

$$\sigma_U^2 = 2k_BTK\tau_r vx = 2k_BT\gamma vx \left(\frac{\tau_r}{\tau}\right). \qquad (26)$$

As one can see, the magnitude of the fluctuations increases now as $\sqrt{K}$. The optimal combination of spatial resolution and magnitude of the fluctuations is thus obtained when $\tau = \gamma/K \sim \tau_r$. The best possible spatial resolution achievable for the potential is given by $\Delta x \approx v\tau_r$.

To apply the above method to MD simulations, knowledge of the friction coefficient is required. We can use Eq. 17 to estimate the friction coefficient

$$\gamma(\bar{x}) = \sigma_F^2(t)\Delta t/2k_BT \qquad (27)$$

where the position dependence of γ is expressed explicitly. The friction coefficient can also be obtained from the power spectrum of the force fluctuations under the assumption $\Delta t \gg \gamma/K$. In this case holds (Allen and Tildesley, 1987)

$$\gamma(x) = \frac{1}{k_BT}\int_0^{\Delta t} \langle\delta F(t)\delta F(t + s)\rangle ds. \qquad (28)$$

An Example

To illustrate the formal description outlined above we consider the reconstruction of the sample potential

$$U(x) = \Delta U\left[\left(\frac{x}{L}\right)^4 - 2\left(\frac{x}{L}\right)^2\right] \qquad (29)$$

for $\Delta U = 25$ kcal/mol and $L = 10$ Å. For this potential we simulated a random process governed by the stochastic differential equation (Eq. 1) with friction coefficient $\gamma = 4 \times 10^4$ pNps/Å, restraint coefficients $K = 280$ pN/Å (stiff), 28 pN/Å (soft), and pulling velocities $v = 10^{-2}$ Å/ps, 10^{-3} Å/ps. The position $x(t)$ of the ligand has been evaluated at discrete time steps $j\,dt$, $j = 1, 2, 3\ldots$ according to

$$x(t + dt) = x(t) + \left(-\frac{dU}{dx} + F(x, t)\right)\frac{dt}{\gamma}$$
$$+ \left(2k_BT\frac{dt}{\gamma}\right)^{1/2}R(t), \qquad (30)$$

where $R(t)$ are normally distributed random numbers determined as described in Press et al. (1992). We chose $dt = 1$ ps. The ligand was initially located at $x(0) = -10$ Å.

For the averaging procedure we adopted $\Delta t = L/(10\ v)$ to resolve the spatial features of $U(x)$ within 1 Å. The reconstruction procedure for the slope $dU(x)/dx$ is based on Eq. 10, while for the reconstruction of $U(x)$ Eq. 13 was used.

This leads to the equations

$$\left(\frac{dU}{dx}\right)_{x=vt} = \bar{F}(t) - \gamma v = \frac{dt}{\Delta t}\sum_{j=0}^{\Delta t/dt} F(jdt) - \gamma v, \qquad (31)$$

$$\bar{U}(x = vt) = \sum_{j=0}^{t/dt}(\bar{F}(jdt) - \gamma v)vdt, \qquad (32)$$

where $F(t) = F[x(t), t]$ is the force applied to the ligand at time t. The resulting forces and potentials are presented in Figs. 1 and 2 for a stiff restraint and in Fig. 3 for a soft restraint. In the case of a stiff restraint, the reconstructed forces and potentials are in agreement with the assumed potential and the resolution in the reconstructed potential improves with the length of the simulation as predicted by Eq. 22. For the soft restraint, on the other hand, the reconstruction clearly fails as expected.

DISCUSSION

We employed stochastic models to investigate how the application of external forces to protein-ligand complexes in MD simulations can be used to reconstruct the potential energy surfaces governing ligand binding and unbinding. Our computational approach, inspired by micromanipulation experiments, provides a general tool for the investigation of the mechanisms of binding and unbinding of biomolecules in MD simulations.

We showed that the unbinding force measured in AFM experiments is linearly proportional to the mean slope of the activation energy barrier and does not provide information
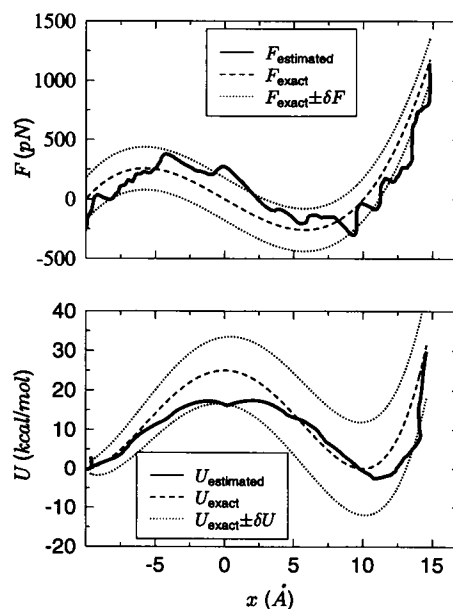

FIGURE 1 Reconstruction of the force and the potential for $K = 280$ pN/Å (stiff restraint) and $v = 10^{-2}$ Å/ps. The computed force and potential fall into the estimated error bounds.
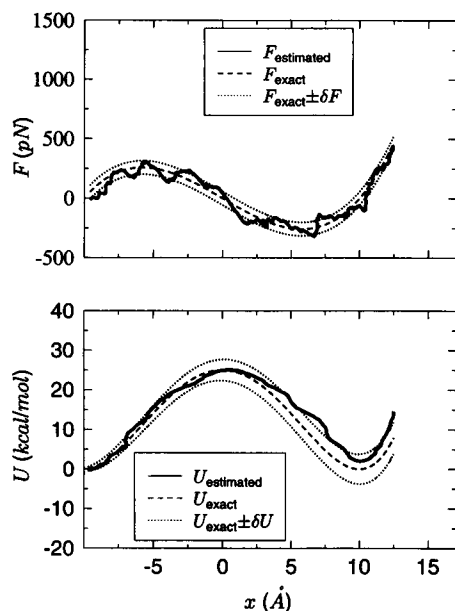
FIGURE 2 Reconstruction of the force and the potential for $K = 280$ pN/Å (stiff restraint) and $v = 10^{-3}$ Å/ps. The computed force and potential fall into the estimated error bounds. The error bounds are smaller than those for the simulation presented in Fig. 1.

on the local properties of the binding potential. Since AFM employs soft harmonic restraints and long (1 ms) observation periods, it allows the ligand to thermally fluctuate in a wide range of the binding pocket and to surmount potential barriers spontaneously so that the observed unbinding event can be considered thermally activated.
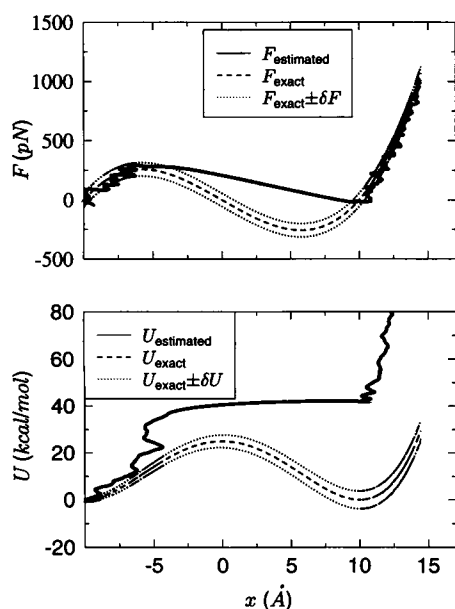

FIGURE 3 Reconstruction of the force and the potential for $K = 28$ pN/Å (soft restraint) and $v = 10^{-3}$ Å/ps. For the soft restraint the reconstruction fails near the top of the potential barrier.

Unbinding induced in MD simulations on a very short (1 ns) time scale proceeds in a dissipative regime where significant irreversible work is being generated. We have shown that the unbinding forces measured in MD are, in general, larger than those measured in AFM experiments and are sensitive to the local distribution of energy barriers. Under these conditions the irreversible work conceals the true thermodynamic potentials and has to be discounted in order to reconstruct the energy landscape of the system.

Our analysis in the framework of one-dimensional stochastic models showed that such reconstruction is possible in MD simulations if stiff harmonic restraints are employed. A stiff harmonic restraint confines fluctuations of the ligand to a small spatial region of the binding pocket of the protein. Pulling the ligand slowly out of the binding pocket and measuring the applied force at all instances of time allows one to sample the local slope of the energy landscape of the system. The irreversibility, in this case, is represented in the form of a viscous friction force proportional to the linear velocity of the ligand. The effect of such irreversibility can be discounted and the profile of the true binding energy can be uncovered by integration of the applied force over the coordinate of the ligand.

The analysis also furnishes limits on the accuracy with which the potential can be determined. We showed that, as a direct consequence of the fluctuation-dissipation theorem, the variance in the measured energy will be proportional to the amount of irreversible work performed on the system.

Some important questions still remain open. The value of the friction coefficient γ that appears in our model can be computed, in principle, by monitoring the force-force or the position-position correlation function of the ligand as noted in the previous section. Assuming that the correlation function depends on a single decay time τ, one can compute γ from MD simulations using Eq. 8. This assumption, however, is valid only if all other processes in the system relax faster than the fluctuations related to the motion of the ligand. In general, this assumption does not hold for proteins that have a relatively soft structure. For example, open loops at the entrance of the binding pocket often serve as regulators of ligand binding. The slow relaxation times of such loops can render the friction coefficient γ highly dispersive. The described reconstruction of the binding potential, using one-dimensional models, will not be valid in this case and a more complete treatment will be required.

A related question is how to choose the stiffness of the restraint K. As we already mentioned, the stiffness of the restraint should be chosen in accordance with $K \gg |d^2U(x)/dx^2|$, but the details of the binding potential are not known a priori. Moreover, the restraint should not be chosen too stiff, since the ligand will then relax too quickly without allowing other degrees of freedom to equilibrate properly. In this case the friction coefficient γ will become strongly dependent on time, which will significantly complicate the analysis of the data obtained from MD simulations.

In spite of the above difficulties, steered molecular dynamics opens new frontiers in the investigation of noncovalent interactions of biopolymers that govern, e.g., the recognition of ligands by receptors, protein-DNA complexes, and protein-protein aggregation. It is desirable to establish the relationship of steered molecular dynamics to the umbrella sampling method as used in free energy perturbation theory in the framework of weighted histogram analysis method (WHAM) (Kumar et al., 1992).

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