

Introduction to Free Energy Methods

Why Do This?

- The free energy of a system is perhaps the most important thermodynamic quantity, and is usually taken as the Helmholtz or Gibb's free energy
- Techniques to calculate the free energy (or relative free energy) of a system are very useful studying phase transitions, critical phenomena or other transformations
- We can never calculate absolute free energies (since we don't have an appropriate reference state), however relative free energies can be found using several different computational techniques

Calculating Free Energies

- We know from statistical mechanics that we can calculate the free energy (here the Helmholtz free energy) by evaluating integrals like

$$A = k_B T \ln \left(\iint dp^N dr^N \exp \left[-\beta H(p^N, r^N) \right] \right)$$

where H is the Hamiltonian.

- In practice it is very difficult to evaluate such integrals using MC or MD since we do not adequately sample high energy regions

Calculation of Free Energy *Differences*

- Although our simulation methods cannot give us absolute free energies, free energy differences are much more tractable
- Consider two states X and Y
- Since the free energy is a state function, the difference in energy between these two states is simply

$$\Delta A = -k_B T \ln \left(\frac{\iint dp^N dr^N \exp [-\beta H_Y (p^N, r^N)]}{\iint dp^N dr^N \exp [-\beta H_X (p^N, r^N)]} \right)$$

Free Energy Differences

- If we multiply the numerator by the factor

$$\exp(\beta H_X) \exp(-\beta H_X) \equiv 1$$

we get

$$\begin{aligned}\Delta A &= -k_B T \ln \left(\frac{\iint dp^N dr^N \exp[-\beta H_Y] \exp[\beta H_X] \exp[-\beta H_X]}{\iint dp^N dr^N \exp[-\beta H_X]} \right) \\ &= -k_B T \ln \left(\frac{\iint dp^N dr^N \exp[-\beta(H_Y - H_X)] \exp[-\beta H_X]}{\iint dp^N dr^N \exp[-\beta H_X]} \right)\end{aligned}$$

Free Energy Difference

- Since we are clever, we notice that this is nothing more than an ensemble average taken over the state X

$$\Delta A = -k_B T \ln \langle \exp [-\beta(H_Y - H_X)] \rangle_X$$

- Equivalently we could write the reverse process

$$\Delta A = -k_B T \ln \langle \exp [-\beta(H_X - H_Y)] \rangle_Y$$

Overlapping States

- In order to evaluate an ensemble average like

$$\Delta A = -k_B T \ln \langle \exp [-\beta (H_Y - H_X)] \rangle_X$$

we could run a simulation either state X or Y and collect statistics

- Problems arise however when the states X and Y do not overlap such that simulating one state does a poor job of sampling the other

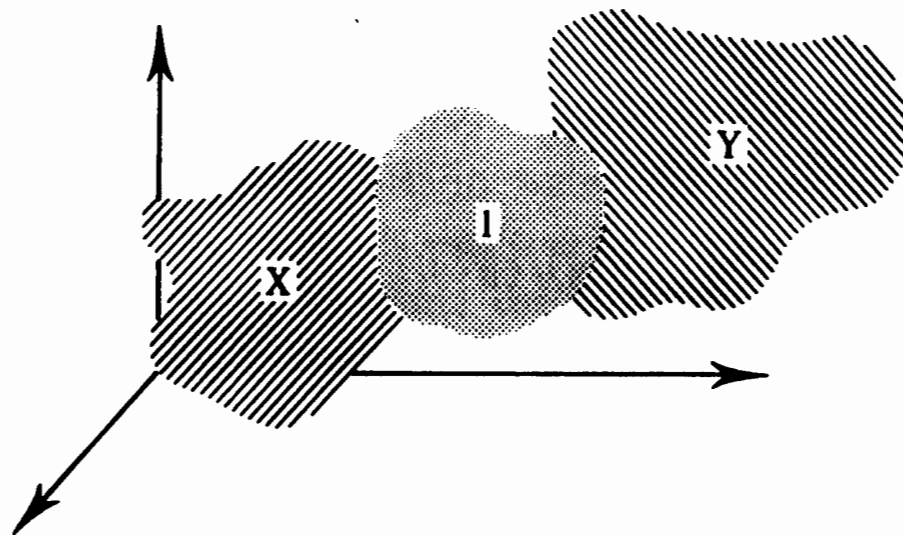
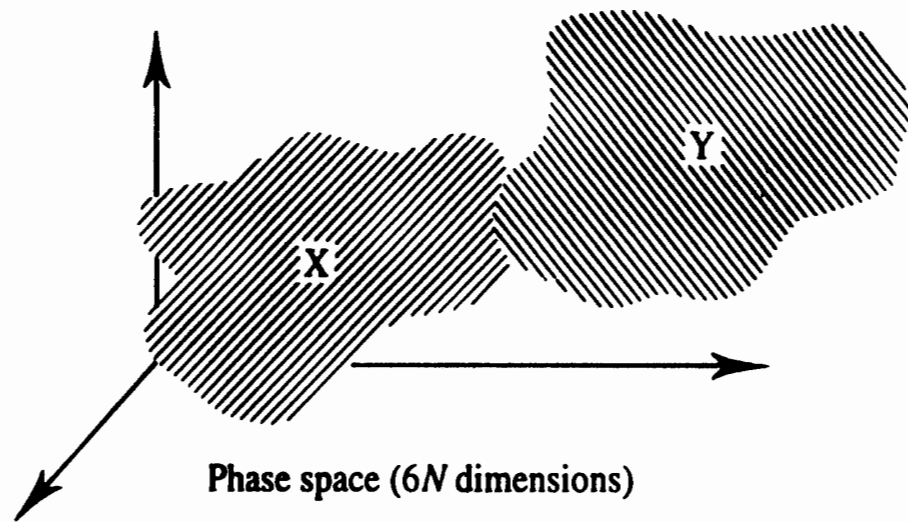


Fig. 9.1 An intermediate state (labelled 1) can improve the degree of overlap in phase space and lead to improved sampling.

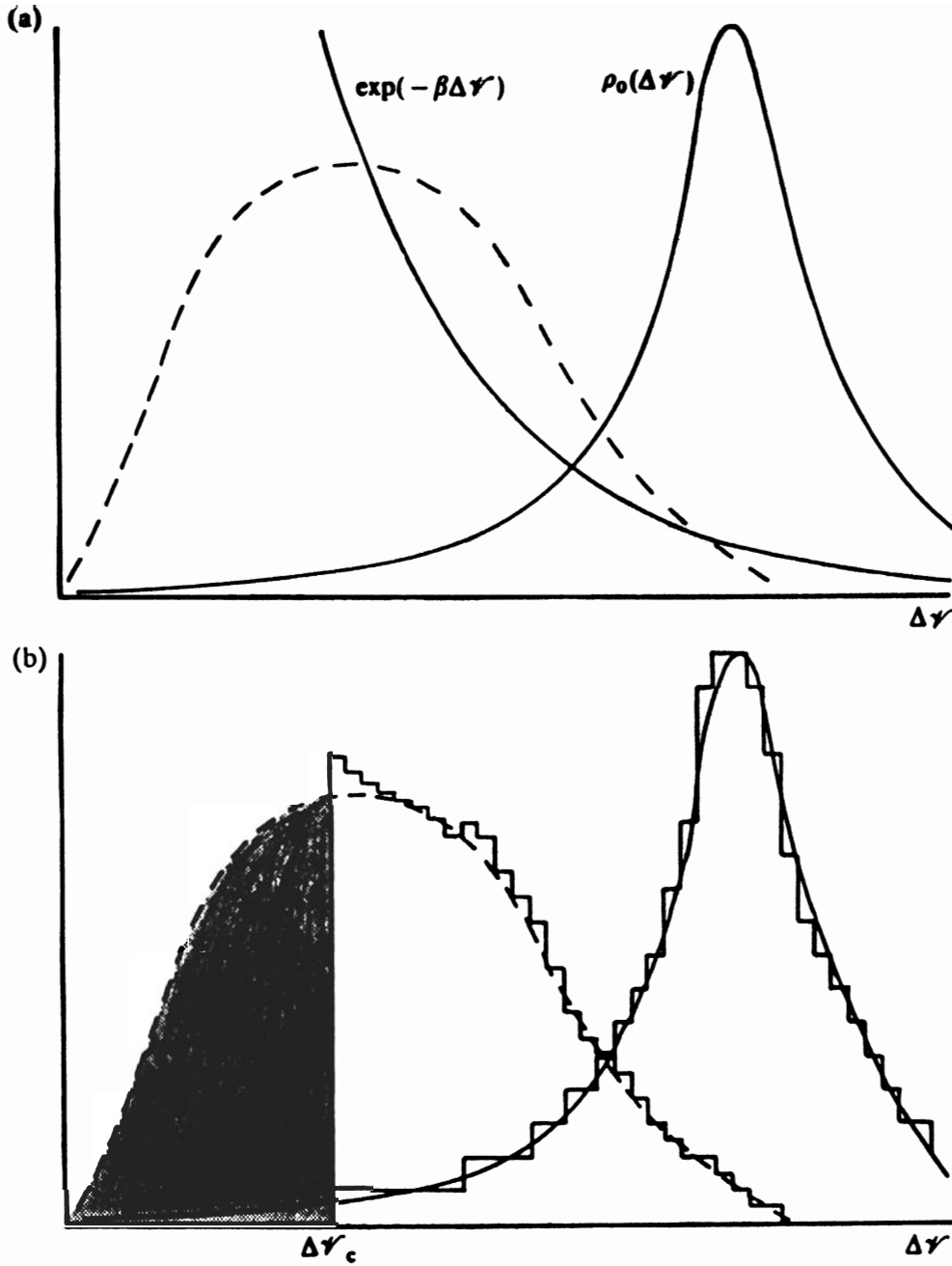


Fig. 7.1 The problem in estimating free energy differences. (a) The functions $\rho_0(\Delta\mathcal{V})$ and $\exp(-\beta\Delta\mathcal{V})$ are shown as solid lines. The product of these two functions, the integrand in eqn (7.3), is shown as a dashed line.

(b) The histogram shows the distribution of $\Delta\mathcal{V}$ values in a finite-length simulation. The shaded area represents the portion of the distribution used for estimation.

Let us begin with a less taxing problem than that of estimating eqn (7.1), specifically the calculation of a free energy difference. Consider two fluids characterized by potentials $\mathcal{V}(\mathbf{r})$ and $\mathcal{V}_0(\mathbf{r})$. If the free energy of the reference fluid, A_0 , is known, then the free energy of the fluid of interest, A , can be determined from

$$A - A_0 = -k_B T \ln \left(\frac{Q}{Q_0} \right) = -k_B T \ln \left(\langle \exp(-\beta\Delta\mathcal{V}) \rangle_0 \right) \quad (7.2)$$

where $\Delta\mathcal{V}(\mathbf{r}) = \mathcal{V}(\mathbf{r}) - \mathcal{V}_0(\mathbf{r})$ and the ensemble average $\langle \dots \rangle_0$ is taken in the reference system \mathcal{V}_0 . Unless the two fluids are very similar, and $\beta\Delta\mathcal{V}$ is small for all the important configurations in this ensemble, the average in eqn (7.2) is difficult to calculate accurately. The reason for this becomes clear if we rewrite the configurational density function $\rho_0(\mathbf{r})$ as a function, $\rho_0(\Delta\mathcal{V})$, of the energy difference. Then

$$\frac{Q}{Q_0} = \int_{-\infty}^{\infty} d(\Delta\mathcal{V}) \exp(-\beta\Delta\mathcal{V}) \rho_0(\Delta\mathcal{V}). \quad (7.3)$$

Intermediate States

- If the energy difference between the two states is large

$$|H_X - H_Y| \gg k_B T$$

we can introduce an intermediate state between X and Y

$$\begin{aligned}\Delta A &= A(Y) - A(X) \\ &= (A(Y) - A(I)) + (A(I) - A(X)) \\ &= -k_B T \ln \left[\frac{Q(Y)}{Q(I)} \times \frac{Q(I)}{Q(X)} \right]\end{aligned}$$

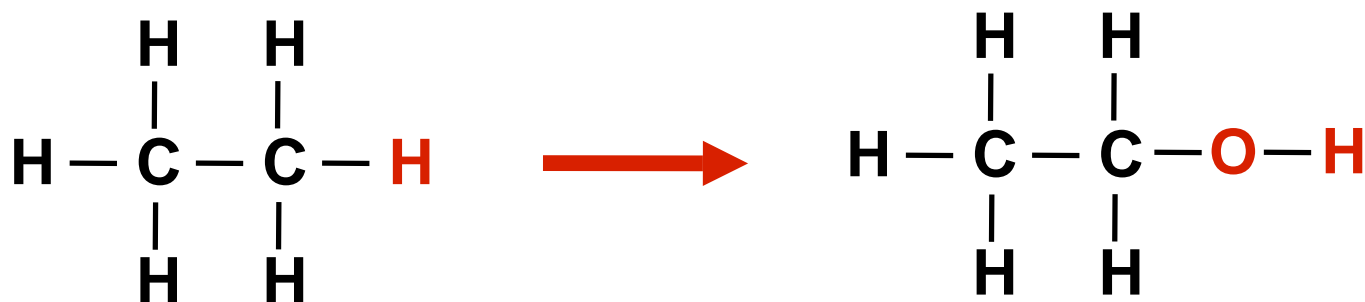
Intermediate States

- We can obviously extend this treatment to include multiple intermediate states with increasing overlap

$$\begin{aligned}\Delta A &= A(Y) - A(X) \\ &= (A(Y) - A(N)) + (A(N) - A(N-1)) + \dots \\ &+ (A(2) - A(1)) + (A(1) - A(X)) \\ &= -k_B T \ln \left[\frac{Q(Y)}{Q(N)} \times \frac{Q(N)}{Q(N-1)} \dots \frac{Q(2)}{Q(1)} \times \frac{Q(1)}{Q(X)} \right]\end{aligned}$$

Intermediate States

- One key to this method is that intermediate states do not need to correspond to actual physical states (consider changing ethane to ethanol)



- Using molecular mechanics we can smoothly interpolate between these two states

Implementation

- If we have an empirical force field (like we do in molecular mechanics) we can write all of the force field terms as a linear combination of the values for X and Y
 - Bonds: $k(\lambda) = \lambda k(Y) + (1-\lambda)k(X)$
 $l_o(\lambda) = \lambda l_o(Y) + (1-\lambda)l_o(X)$
 - Angles: $k_\theta(\lambda) = \lambda k_\theta(Y) + (1-\lambda)k_\theta(X)$
 $\theta_o(\lambda) = \lambda \theta_o(Y) + (1-\lambda)\theta_o(X)$
 - Charges: $q(\lambda) = \lambda q(Y) + (1-\lambda)q(X)$
 - VDW: $\varepsilon(\lambda) = \lambda \varepsilon(Y) + (1-\lambda)\varepsilon(X)$
 $\sigma(\lambda) = \lambda \sigma(Y) + (1-\lambda)\sigma(X)$
 - etc.

$$\begin{aligned}
 v_{ij}^{\text{LJ}}(\lambda) = & 4(1 - \lambda)\epsilon_X \left(\frac{\sigma_X^{12}}{[\alpha_{\text{LJ}}\lambda^2\sigma_X^6 + r_{ij}^6]^2} - \frac{\sigma_X^6}{[\alpha_{\text{LJ}}\lambda^2\sigma_X^6 + r_{ij}^6]} \right) \\
 & + 4\lambda\epsilon_Y \left(\frac{\sigma_Y^{12}}{[\alpha_{\text{LJ}}(1 - \lambda)^2\sigma_Y^6 + r_{ij}^6]^2} - \frac{\sigma_Y^6}{[\alpha_{\text{LJ}}(1 - \lambda)^2\sigma_Y^6 + r_{ij}^6]} \right)
 \end{aligned} \tag{11.31}$$

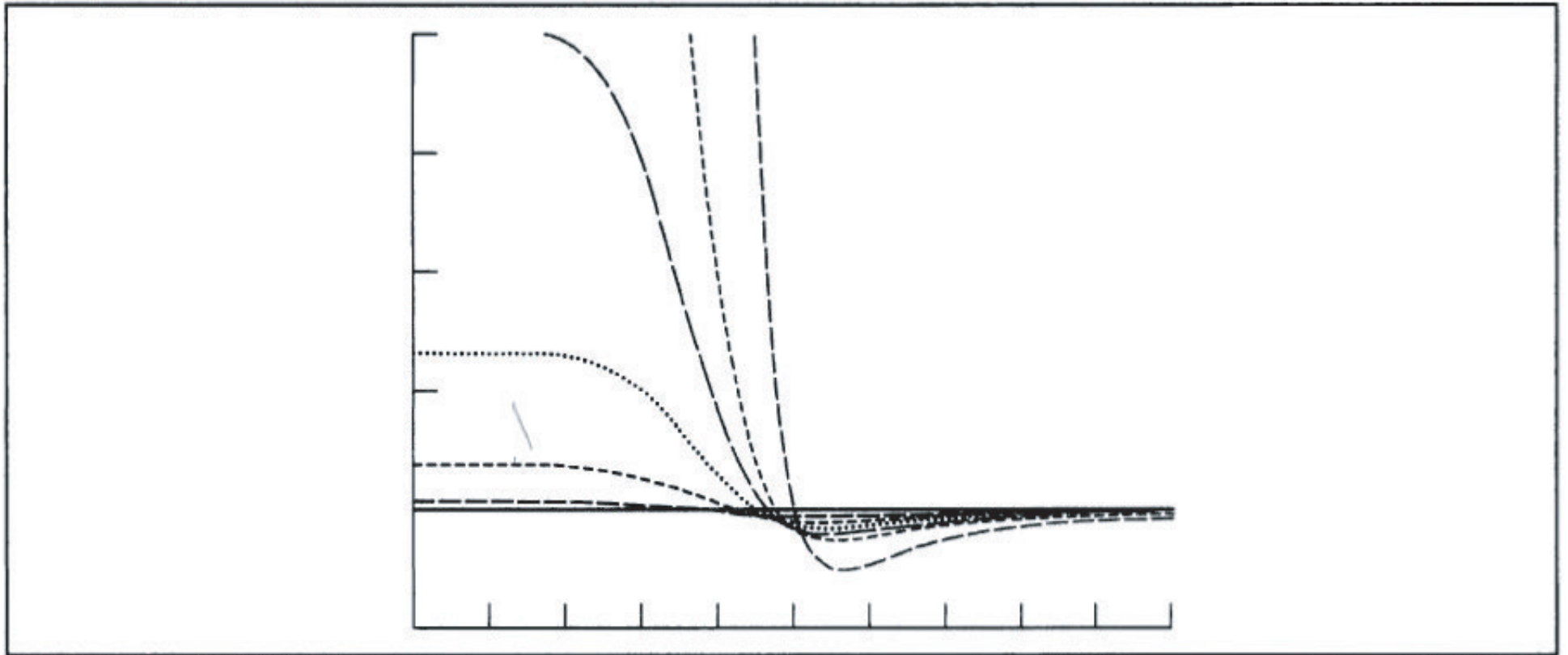


Fig. 11.14: Comparison of scaled and unscaled Lennard-Jones potentials (Equation (11.31)) for the case where a particle disappears at $\lambda = 0$. As λ decreases the curves get progressively closer to the x axis.

Coupling Parameter

- As we change the coupling parameter λ from 0 to 1, we move from state X to Y
- At each intermediate step λ_i we perform a simulation (Monte Carlo or MD) by first performing a short equilibration run (since our point of equilibrium has changed) and then a “production” run where we calculate

$$\Delta A(\lambda_i \rightarrow \lambda_{i+1}) = k_B T \ln \langle \exp(-\beta \Delta H_i) \rangle$$

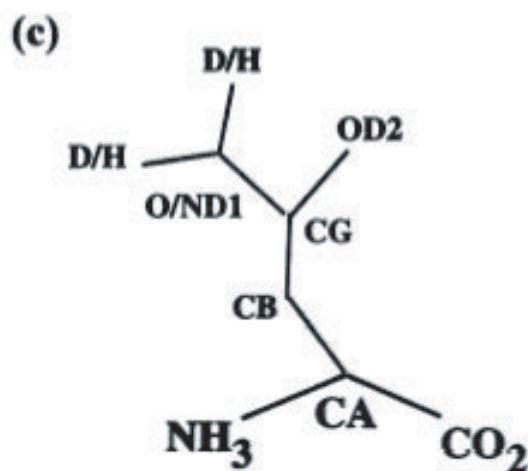
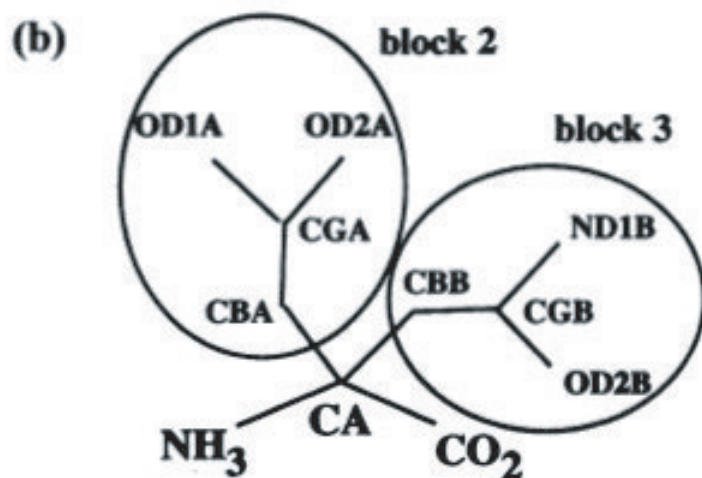
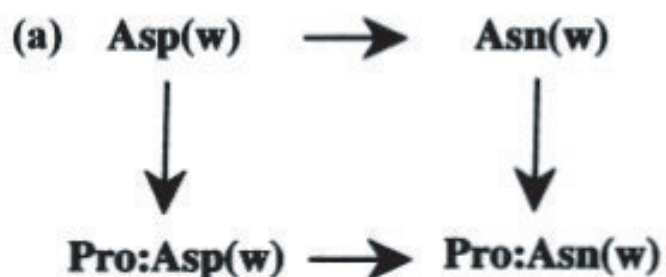
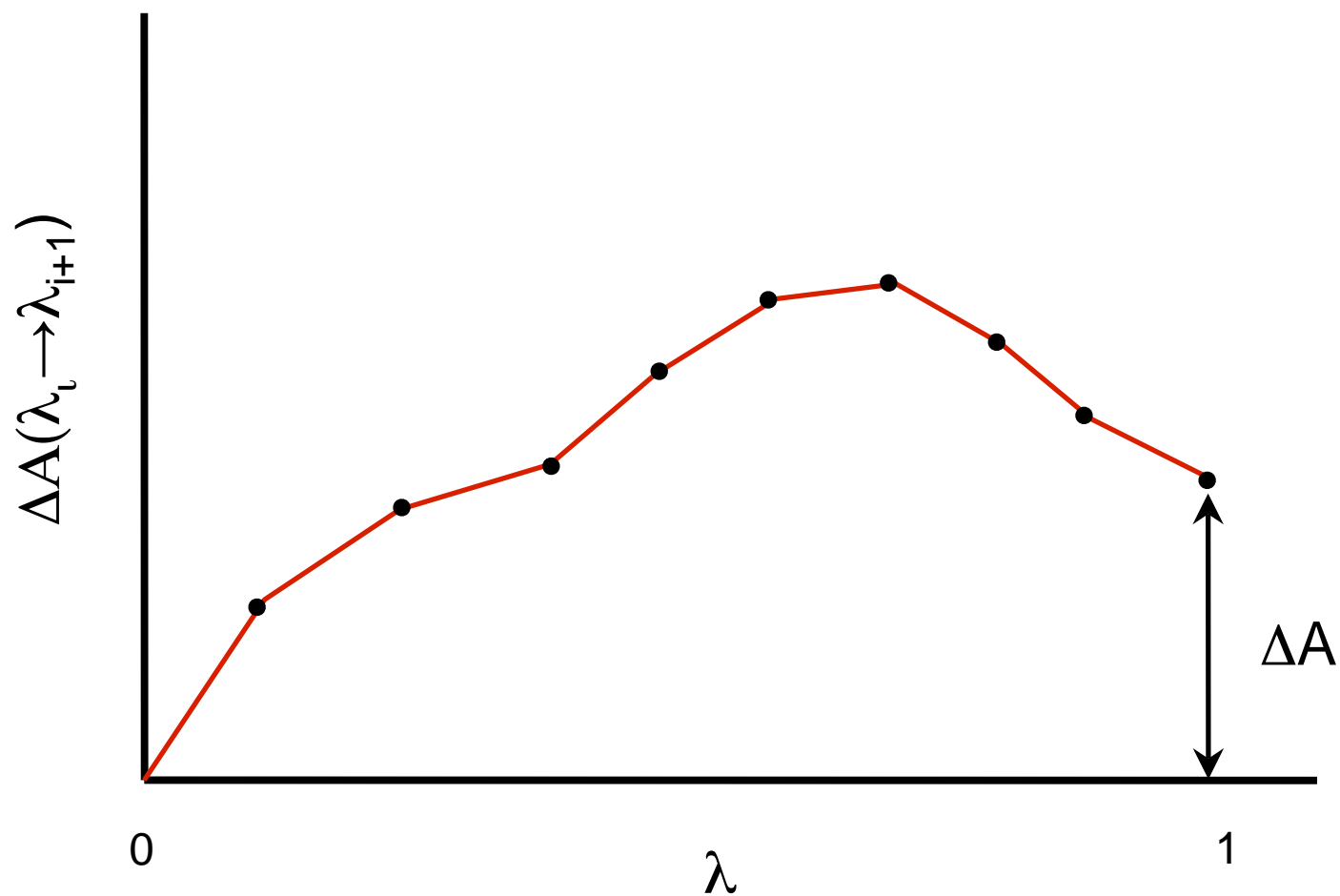


Figure 3 Mutation of a ligand Asp into Asn in solution and bound to a protein. (a) Thermodynamic cycle. (b) Dual topology description: a hybrid ligand with two side chains. “Blocks” are used to define the hybrid energy function [Eq. (14)]. Only the ligand is shown; the environment is either solvent or the solvated protein. (c) Single-topology description.

Free Energy Perturbation



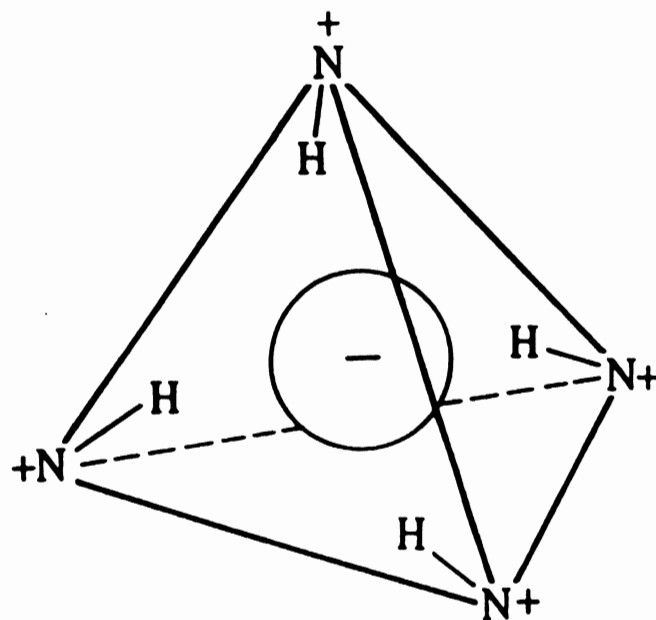
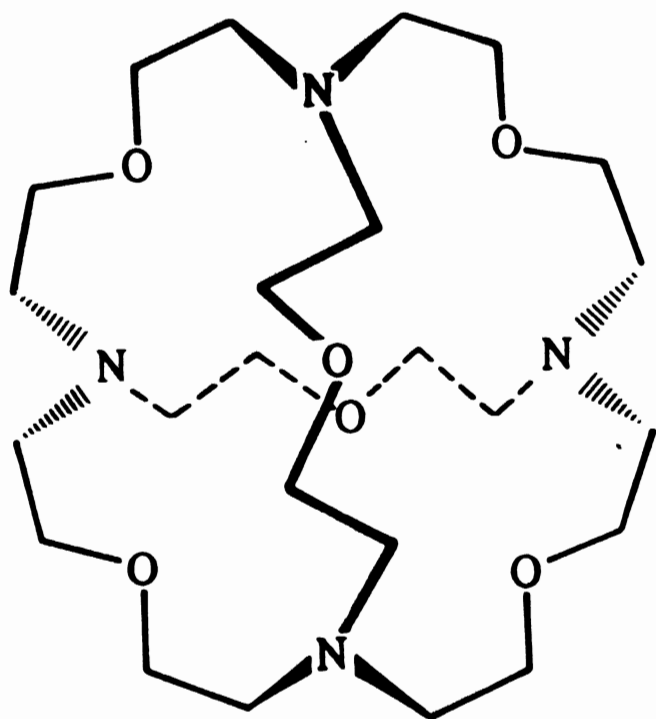
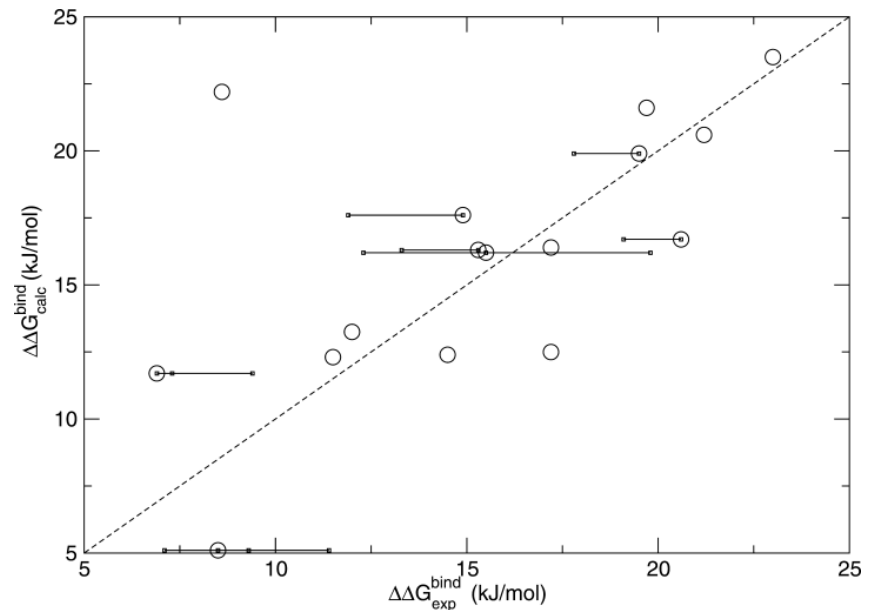
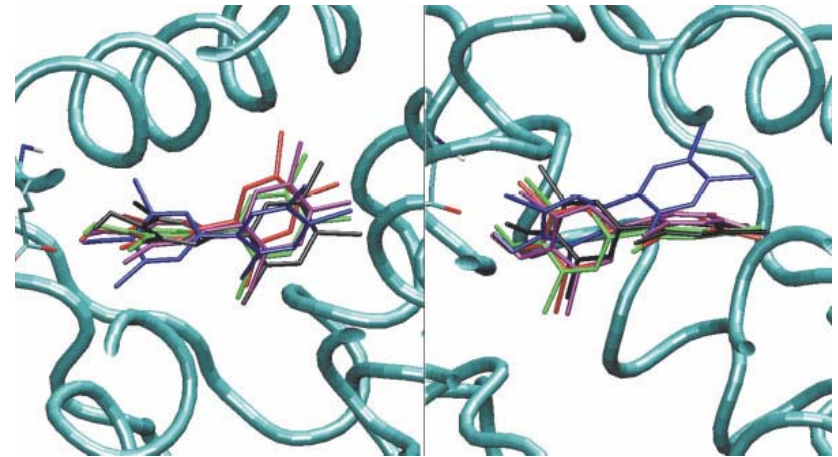


Fig. 9.6 The SC24/halide system. Figure adapted from Lybrand T P, J A McCammon and G Wipff 1986. Theoretical Calculation of Relative Binding Affinity in Host-Guest Systems. *Proceedings of the National Academy of Sciences USA* **83**:833–835.

Free energy perturbation example

- Oostenbrink C, van Gunsteren WF. *Proteins* **54** (2) 234-246, 2004.
- Poly-chlorinated biphenyl binding to estrogen receptor
- “Fast” FEP on 17 compounds
- Good agreement with experiment
- Insight into structural and dynamic aspects of ligand binding



GROMOS ΔG_{solv} in Water

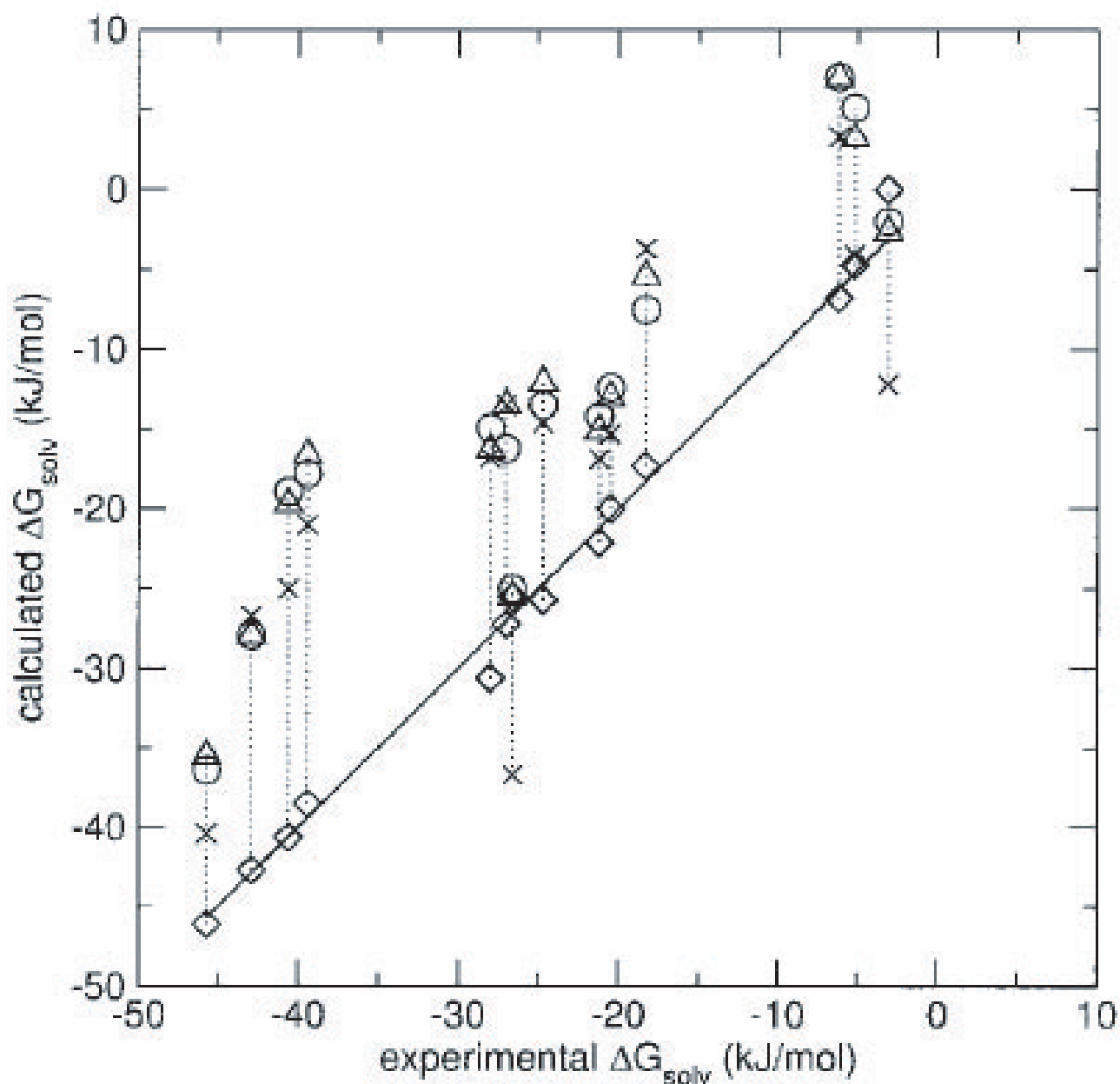
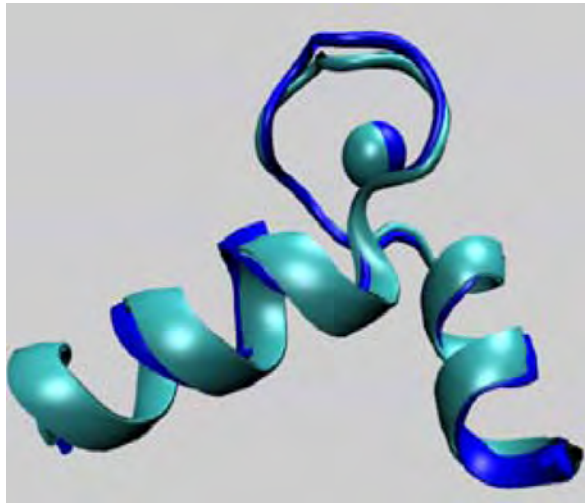


Figure 4. Free enthalpies of solvation in water. Comparison of experimental free enthalpies of solvation to calculated values obtained using parameter sets 43A2 (circles), 45A3 (triangles), 53A5 (crosses), and 53A6 (diamonds) for the 14 compounds listed in Table 18. Diagonal line corresponds to perfect agreement with experiment. Dotted lines are drawn to facilitate comparison between values for the same compound obtained with different parameter sets.

AMOEBA Binding Free Energies

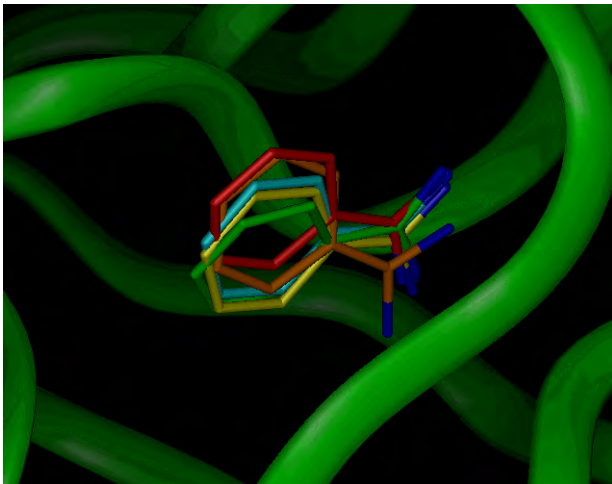
EF Hand: Relative $\text{Ca}^{+2}/\text{Mg}^{+2}$ Binding Affinity



Wild Type: $\sim 10^4 \times$ (expt)
6.6 kcal/mol (calc)

Glu \rightarrow Asp: $\sim 10 \times$ (expt)
1.3 kcal/mol (calc)

Trypsin-Benzamidine: Absolute Binding Affinity



6.3 to 7.3 kcal/mol (expt)

6.7 \pm 0.6 kcal/mol (calc)

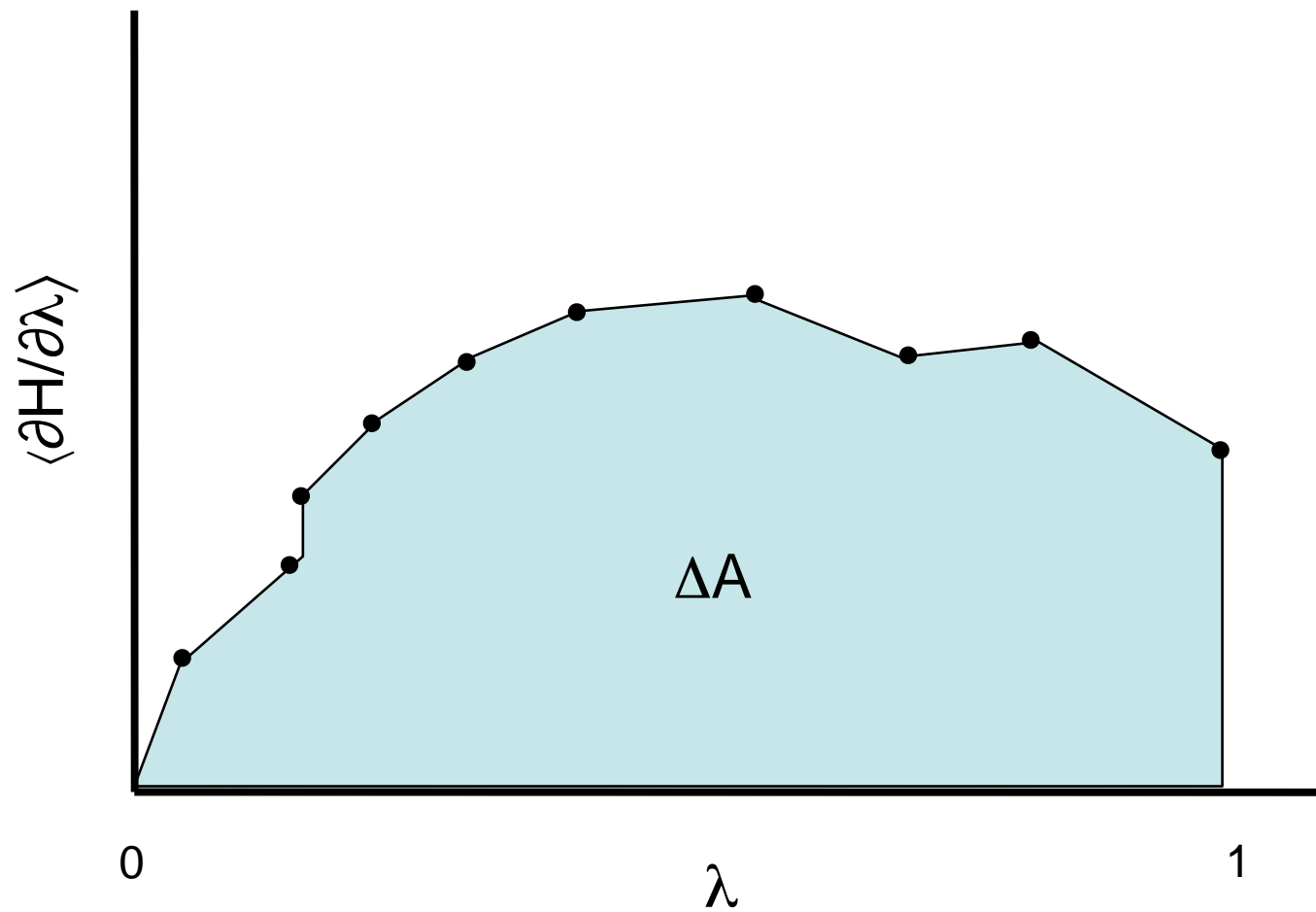
Thermodynamic Integration

- Instead of evaluating the difference in the free energy between subsequent states, we could also calculate the derivative of the Hamiltonian

$$\Delta A = \int_{\lambda=0}^{\lambda=1} \left\langle \frac{\partial H}{\partial \lambda} \right\rangle_{\lambda} d\lambda$$

- In this case, the free energy difference is the area under the curve

Thermodynamic Intergration



Appendix 9.1 Calculating free energy differences using thermodynamic integration

If the free energy, A , is a continuous function of λ then we can write:

$$\Delta A = \int_0^1 \frac{\partial A(\lambda)}{\partial \lambda} d\lambda \quad (9.81)$$

Now

$$A(\lambda) = -k_B T \ln Q(\lambda) \quad (9.82)$$

Thus

$$\Delta A = -k_B T \int_0^1 \left[\frac{\partial \ln Q(\lambda)}{\partial \lambda} \right] d\lambda = \int_0^1 \frac{-k_B T}{Q(\lambda)} \frac{\partial Q(\lambda)}{\partial \lambda} d\lambda \quad (9.83)$$

From the definition of Q (section 5.1.1):

$$Q_{NVT} = \frac{1}{N!} \frac{1}{h^{3N}} \iint dp^N dr^N \exp \left[-\frac{\mathcal{H}(p^N, r^N)}{k_B T} \right] \quad (9.84)$$

we can write the following for $\partial Q(\lambda)/\partial \lambda$:

$$\frac{\partial Q(\lambda)}{\partial \lambda} = \frac{1}{N!} \frac{1}{h^{3N}} \iint dp^N dr^N \frac{\partial}{\partial \lambda} \exp \left[-\frac{\mathcal{H}(p^N, r^N)}{k_B T} \right] \quad (9.85)$$

Applying the chain rule:

$$\frac{\partial Q(\lambda)}{\partial \lambda} = -\frac{1}{N!} \frac{1}{h^{3N}} \frac{1}{k_B T} \iint dp^N dr^N \frac{\partial \mathcal{H}(p^N, r^N)}{\partial \lambda} \exp \left[-\frac{\mathcal{H}(p^N, r^N)}{k_B T} \right] \quad (9.86)$$

Substituting back into the expression for $\partial A/\partial \lambda$ gives:

$$\begin{aligned} \frac{\partial A(\lambda)}{\partial \lambda} &= \frac{1}{N!} \frac{1}{h^{3N}} \frac{1}{Q(\lambda)} \iint dp^N dr^N \frac{\partial \mathcal{H}(p^N, r^N)}{\partial \lambda} \exp \left[-\frac{\mathcal{H}(p^N, r^N)}{k_B T} \right] \\ &= \iint dp^N dr^N \frac{\partial H(p^N, r^N)}{\partial \lambda} \left\{ \frac{\exp \left[-\frac{\mathcal{H}(p^N, r^N)}{k_B T} \right]}{Q(\lambda)} \right\} \\ &= \left\langle \frac{\partial \mathcal{H}(p^N, r^N, \lambda)}{\partial \lambda} \right\rangle_\lambda \end{aligned} \quad (9.87)$$

Thus

$$\Delta A = \int_{\lambda=0}^{\lambda=1} \left\langle \frac{\partial \mathcal{H}(p^N, r^N, \lambda)}{\partial \lambda} \right\rangle_\lambda d\lambda \quad (9.88)$$

Slow Growth Method

- If the changes in the system are gradually made such that the Hamiltonian is nearly constant, we can expand the exponential and ln terms to get

$$\begin{aligned}\Delta A &= -k_B T \sum \ln \langle \exp(-\beta [H(\lambda_{i+1}) - H(\lambda_i)]) \rangle \\ &\simeq -k_B T \sum \ln \langle 1 - \beta [H(\lambda_{i+1}) - H(\lambda_i)] \rangle \\ &\simeq \sum [H(\lambda_{i+1}) - H(\lambda_i)]\end{aligned}$$

More Reading

- Many references and papers that cover these topics. In the texts for this class consider:
 - Leach Chapter 11 (watch for errors!!)
 - Frenkel & Smit Chapter 7

Umbrella Sampling and Histogram Methods

The Sampling Problem

- By now you realize that the major problem in simulations is that of sampling
- We have an exact method of computing a partition function and associated thermodynamic quantities, however this is dependent on us accurately sampling the entire conformational space
- In general (i.e. the way most people run simulations) MD simulations *do not* do an adequate jobs of sampling configurational space unless run for a very, very long time

Let's Force the System to Sample

- The basic idea behind *Umbrella Sampling* is that we can bias or force the system to sample a particular region(s) (based on some reaction coordinate)
- If we were interested in the free energy difference between two systems X and Y , we should sample the conformational space associated with both conformations

Free Energy Perturbation

- Recall from our discussion of FEP that the free energy difference between two systems can be expressed as

$$\Delta U = -k_B T \ln \left(\frac{\iint dr^N \exp[-\beta U_Y]}{\iint dr^N \exp[-\beta U_x]} \right)$$

or equivalently

$$\langle \exp(-\beta \Delta U) \rangle_X = \frac{\iint dr^N \exp[-\beta U_Y]}{\iint dr^N \exp[-\beta U_x]}$$

A New Weight Function

- In order to sample both X and Y spaces, we now introduce a new weight function $\pi(r^N)$ to replace the Boltzmann factor

$$\langle \exp(-\beta \Delta U) \rangle_X = \frac{\iint dr^N \pi(r^N) \exp[-\beta U_Y] / \pi(r^N)}{\iint dr^N \pi(r^N) \exp[-\beta U_x] / \pi(r^N)}$$

which using our shorthand notation becomes

$$\langle \exp(-\beta \Delta U) \rangle_X = \frac{\langle \exp(-\beta U_Y) / \pi(r^N) \rangle_\pi}{\langle \exp(-\beta U_X) / \pi(r^N) \rangle_\pi}$$

Umbrella Sampling Considerations

- In order that both the numerator and denominator are non-zero, the weight function $\pi(r^N)$ should have considerable overlap between the spaces of X and Y
- This property gives rise to the name *Umbrella Sampling*
- Although it appears we could sample the entire space with a single choice of $\pi(r^N)$, this is not optimal. It is still best to perform several sampling runs using overlapping windows

Choosing a Weight Function

- In order for Umbrella Sampling to work well we need to make a good choice for $\pi(r^N)$ – it is not known *a priori*
- A common choice is to make the biasing potential quadratic

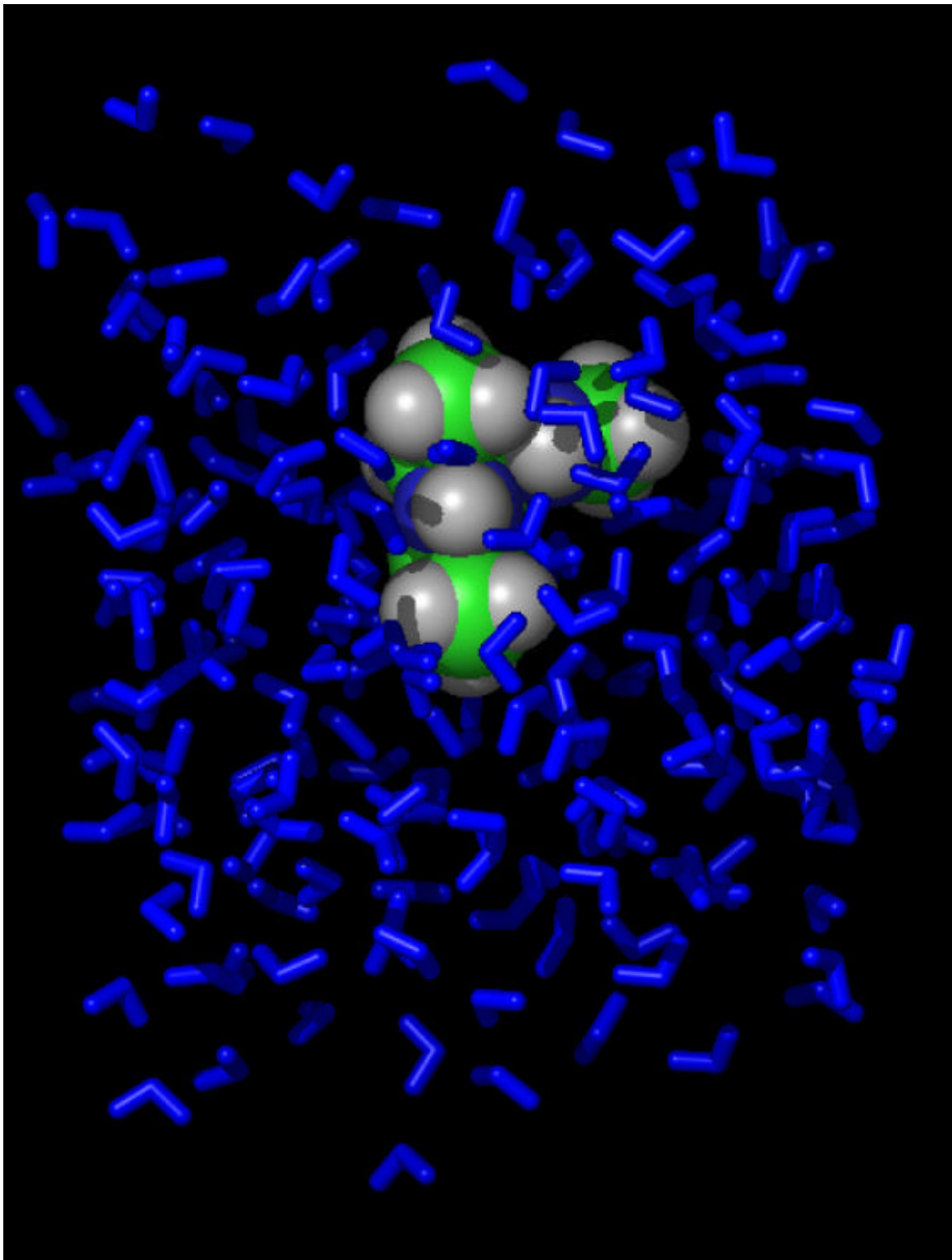
$$\begin{aligned}U'(r^N) &= U(r^N) + W(r^N) \\ &= U(r^N) + k_w(r^N - r_o^N)^2\end{aligned}$$

so that the biasing potential is simply

$$\pi(r^N) = \exp[-\beta U'(r^N)]$$

Weighted Histogram Analysis Method (WHAM)

- Umbrella Sampling is valid in theory, but the implementation is often difficult since the “windows” of overlap must be carefully chosen to minimize the error (since the errors from the individual simulations add quadratically)
- WHAM is a useful method for combining sets of simulations with different biasing potentials in a manner such that the unbiased potential of mean force (PMF) can be found



**Periodic Box Simulation
(alanine dipeptide and
206 water molecules)**

**Stochastic Dynamics
(576 trajectories of
200 picoseconds each)**

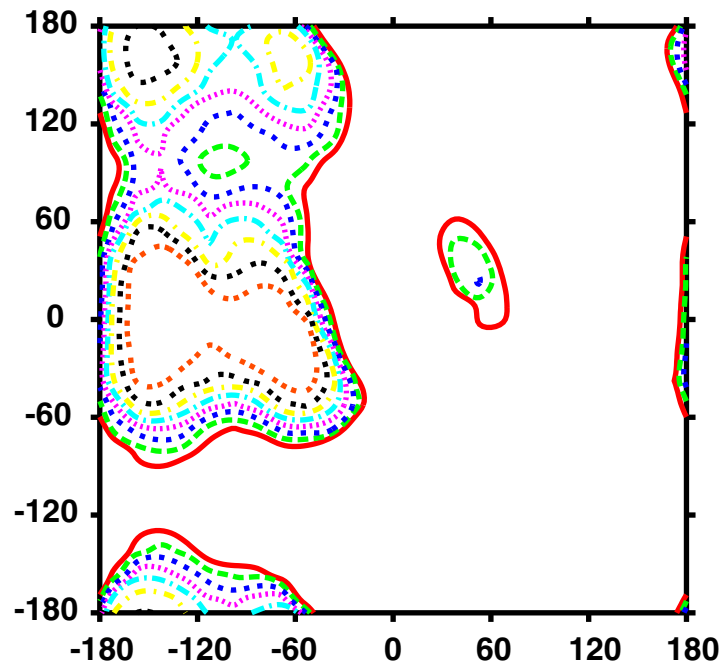
**Free Energies *via*
Umbrella Sampling
and 2D-WHAM:**

$$r(\mathbf{f}, \mathbf{y}) = \frac{\sum_{i=1}^{N_w} n_i r_{w_i}(\mathbf{f}, \mathbf{y})}{\sum_{i=1}^{N_w} n_i e^{-w_i(\mathbf{f}, \mathbf{y}) - F_i} / k_b T}$$

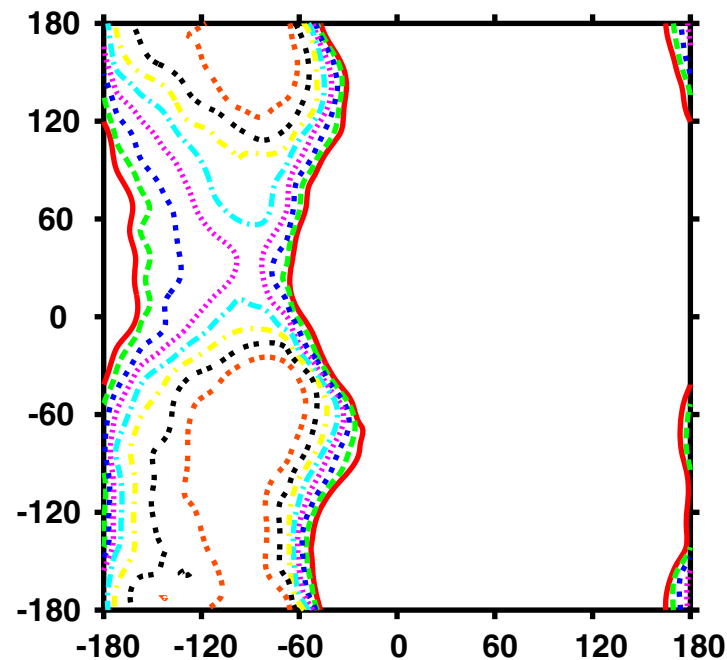
$$F_i = -k_b T \ln \left[\sum_{\mathbf{f}} \sum_{\mathbf{y}} e^{-w_i(\mathbf{f}, \mathbf{y}) / k_b T} r(\mathbf{f}, \mathbf{y}) \right]$$

$$\Delta G(\mathbf{f}, \mathbf{y}) = -k_b T \ln r(\mathbf{f}, \mathbf{y})$$

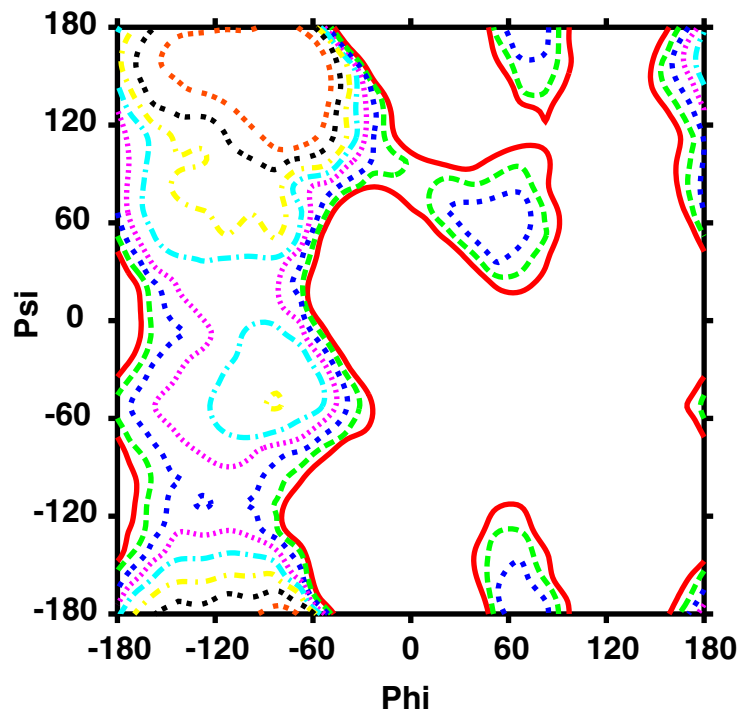
AMBER ff99



CHARMM27



OPLS-AA



*Solvated
Alanine
Dipeptide*

*Free
Energy
Surfaces*

Conformational Populations

	<i>Alpha</i>	<i>Pass</i>	<i>Beta</i>	<i>Other</i>
Amber ff94	68	5	26	1
Amber ff99	77	10	13	1
CHARMM27	46	2	52	0
OPLS-AA	13	9	75	3
OPLS-AA/L	23	8	65	4
SCCDFTB (Amber)	27	16	48	9
SCCDFTB (CHARMM)	33	14	48	4
SCCDFTB (CEDAR)	27	12	61	0
AMOEBA (Polar Water)	29	16	54	1
AMOEBA (Fixed Water)	32	13	54	1

Examples and Further Reading

- Leach has some details on Umbrella Sampling in Ch. 11
- Frenkel & Smit discusses Umbrella Sampling and WHAM (disguised as the self-consistent histogram method) in Ch. 7
- There are many papers using these methods. (See Ron Levy paper that uses both techniques)

Jarzynski's Method

Jarzynski's equality is a relation between *equilibrium* free energy differences and work done through *nonequilibrium* processes. Consider a process that changes a parameter λ of a system from λ_0 at time zero to λ_t at time t . The second law of thermodynamics states that the average work done on the system cannot be smaller than the difference between the free energies corresponding to the initial and the final values of λ :

$$\Delta F = F(\lambda_t) - F(\lambda_0) \leq \langle W \rangle, \quad (1)$$

where the equality holds only if the process is quasi-static (see, e.g., Ref. 17). According to this inequality, a nonequilibrium process provides only an upper limit for the free energy difference. However, Jarzynski discovered an equality that holds regardless of the speed of the process:

$$e^{-\beta \Delta F} = \langle e^{-\beta W} \rangle. \quad (2)$$

This equality has been tested against computer simulations and experiments.

The major difficulty is that the average of exponential work appearing in Jarzynski's equality is dominated by the trajectories corresponding to small work values that arise only rarely. An accurate estimate of free energy hence requires suitable sampling of such rare trajectories. Therefore, although Jarzynski's equality holds for processes of any speed, practical applications are currently limited to slow processes for which the fluctuation of work is comparable to the temperature.

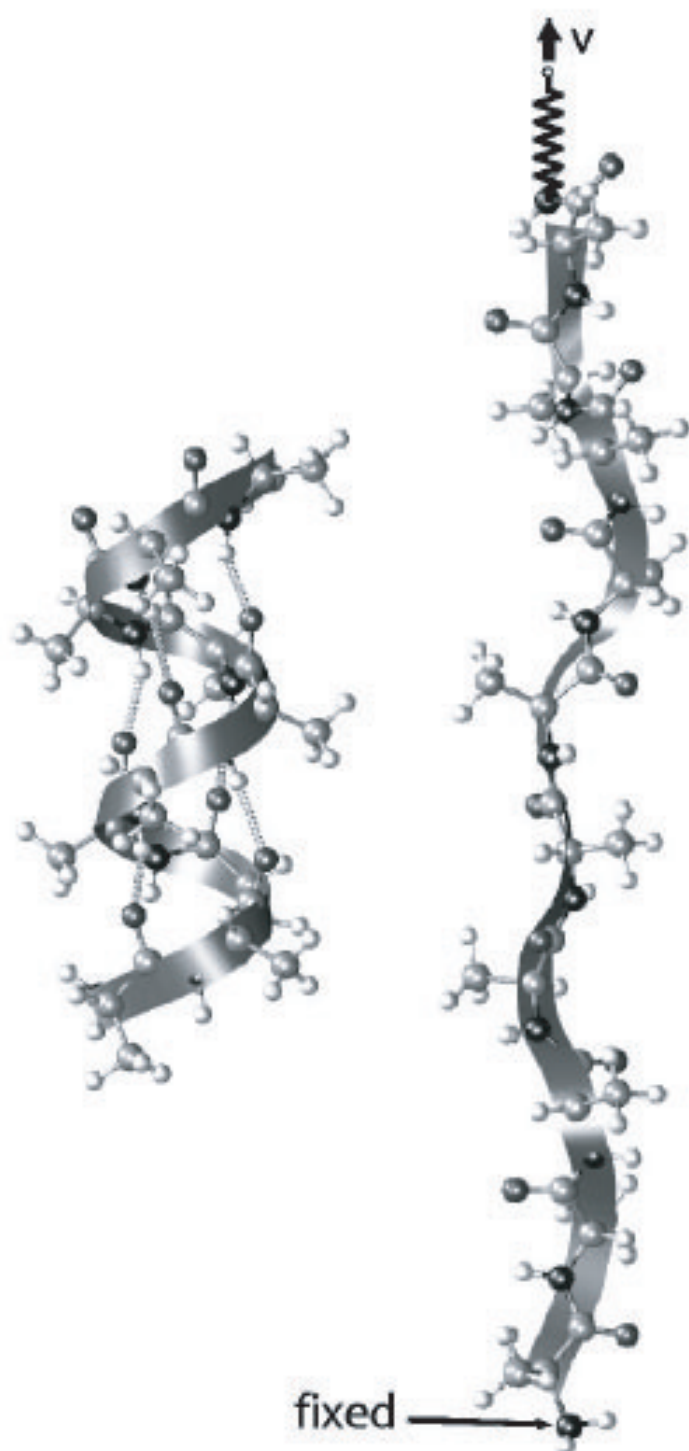


FIG. 1. Unfolding of helical deca-alanine. Left, a folded configuration (α -helix). The six hydrogen bonds that stabilize the helix are shown. Right, an extended configuration (coil). The backbone of the peptide is represented as a ribbon. The N atom of the first residue was fixed during the simulations. The moving guiding potential used in the pulling simulations is represented by a spring which is connected to the C-terminus and pulled with a constant velocity v . Figure made with VMD (Ref. 39).

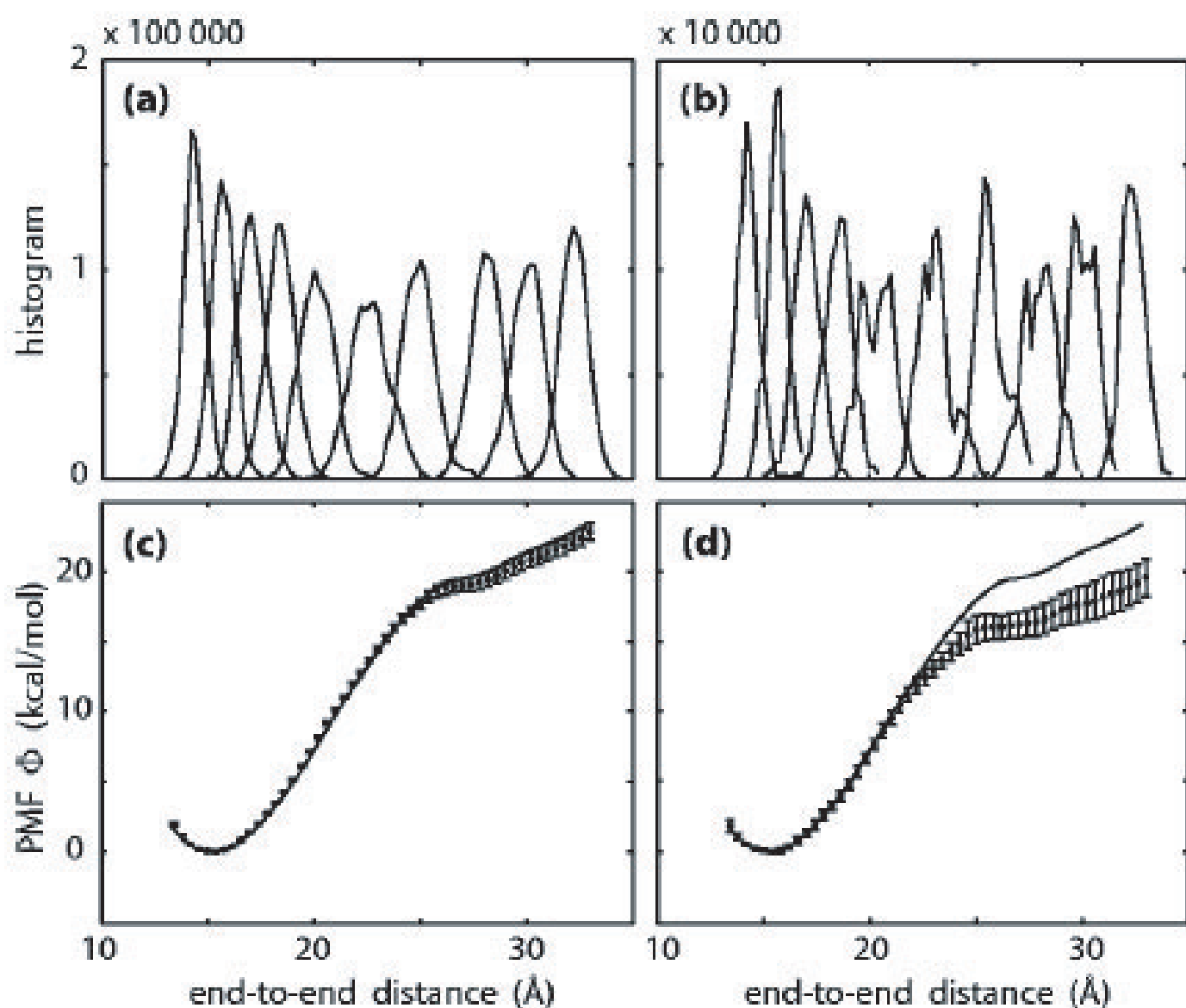


FIG. 8. PMF calculated from umbrella sampling simulations. (a) and (c): 2 ns simulation for each histogram; ten histograms for each block; ten blocks in total. (b) and (d): 0.2 ns simulation for each histogram; ten histograms for each block; ten blocks in total. (a) and (b) show histograms in one block out of the ten blocks. In (c) and (d), the error bars indicate the standard deviation over the blocks, and the exact PMF is plotted as a solid line. The minimum at $\xi = 15.2 \text{ \AA}$ was chosen as a reference point for calculating block averages.

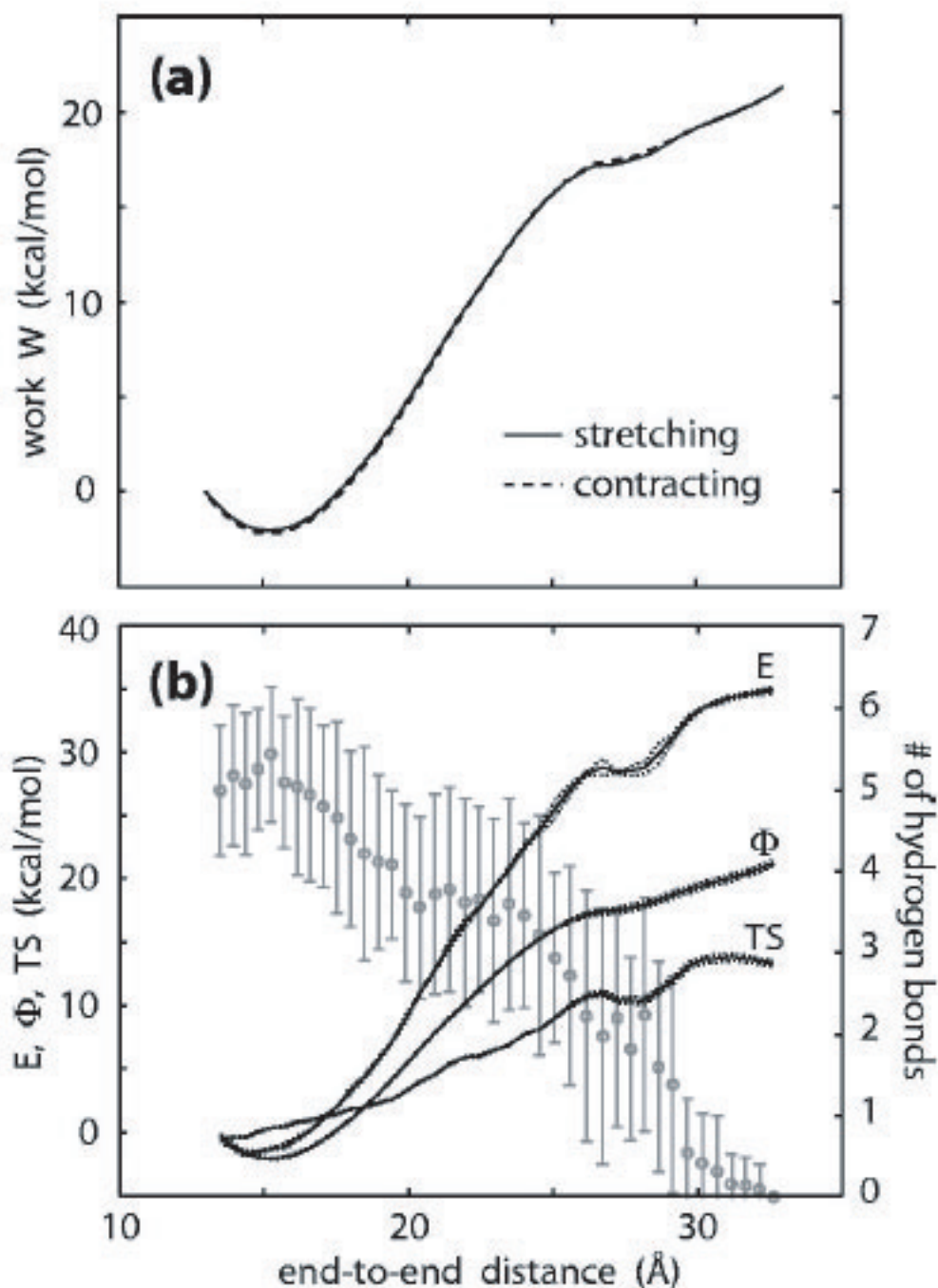


FIG. 3. Reversible pulling ($|v|=0.1$ Å/ns). (a) Work done by forward pulling (stretching) and backward pulling (contracting). For the forward pulling, the position of the constraint center λ is varied from 13 to 33 Å; for the backward pulling, from 33 to 13 Å. For the sake of comparison, the backward-pulling work curve has been shifted vertically so that it coincides with the forward-pulling work curve at $\lambda=33$ Å. (b) Energy E , PMF Φ , and entropy S calculated from four forward pullings. The error bars are shown as dotted lines. Also shown is the number of hydrogen bonds (averaged over time windows) plotted against the end-to-end distance (circles with error bars). A minimum heteroatomic distance of 3.5 Å (between N and O) and a minimum bond angle of 140° (N-H \cdots O) were used for defining a hydrogen bond.

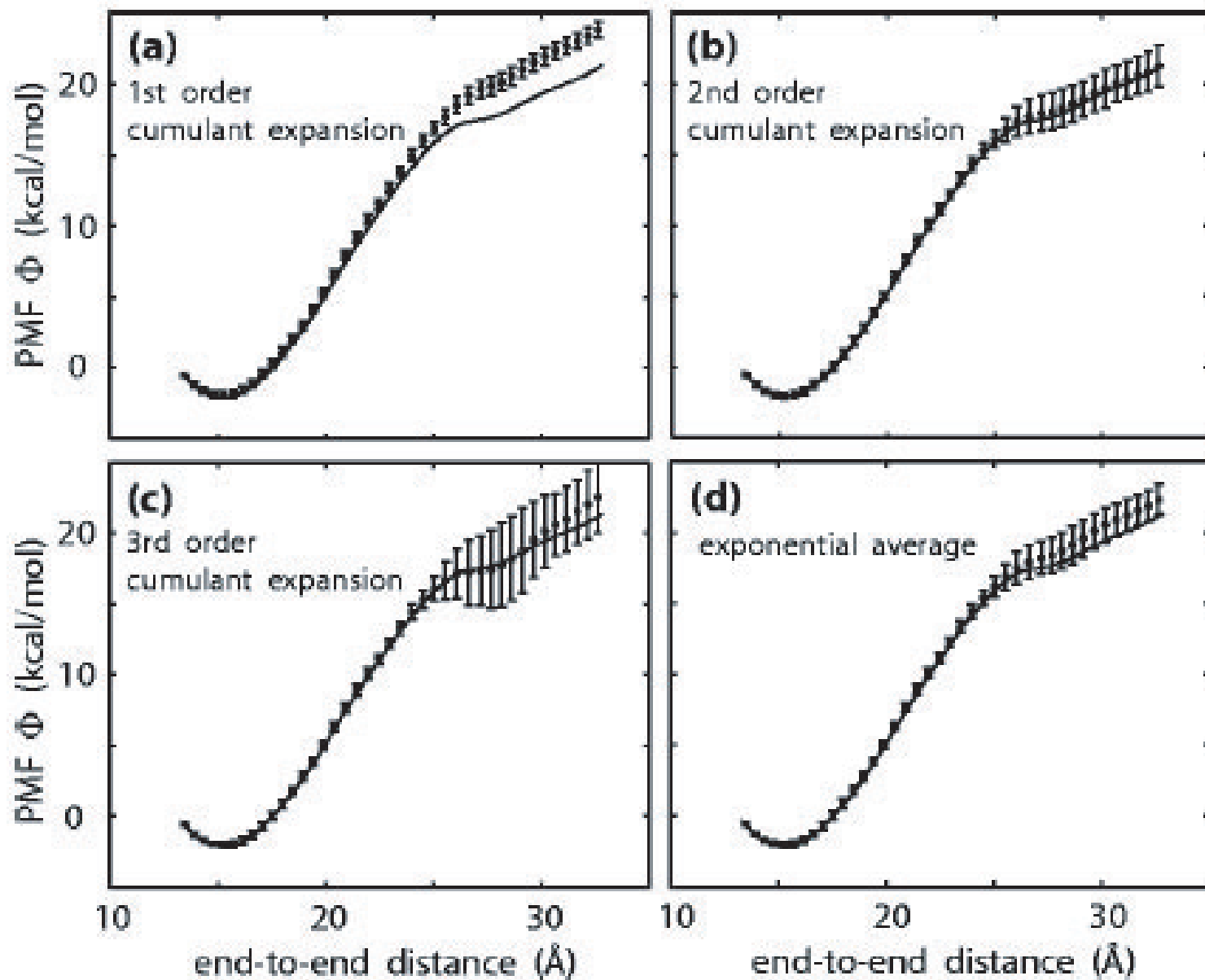


FIG. 4. PMF calculated from irreversible pulling ($v = 10 \text{ \AA/ns}$) through the block average of ten blocks of ten trajectories. The error bars indicate the standard deviation over the blocks. The exact PMF calculated from the reversible pulling is plotted as a solid line in each panel.

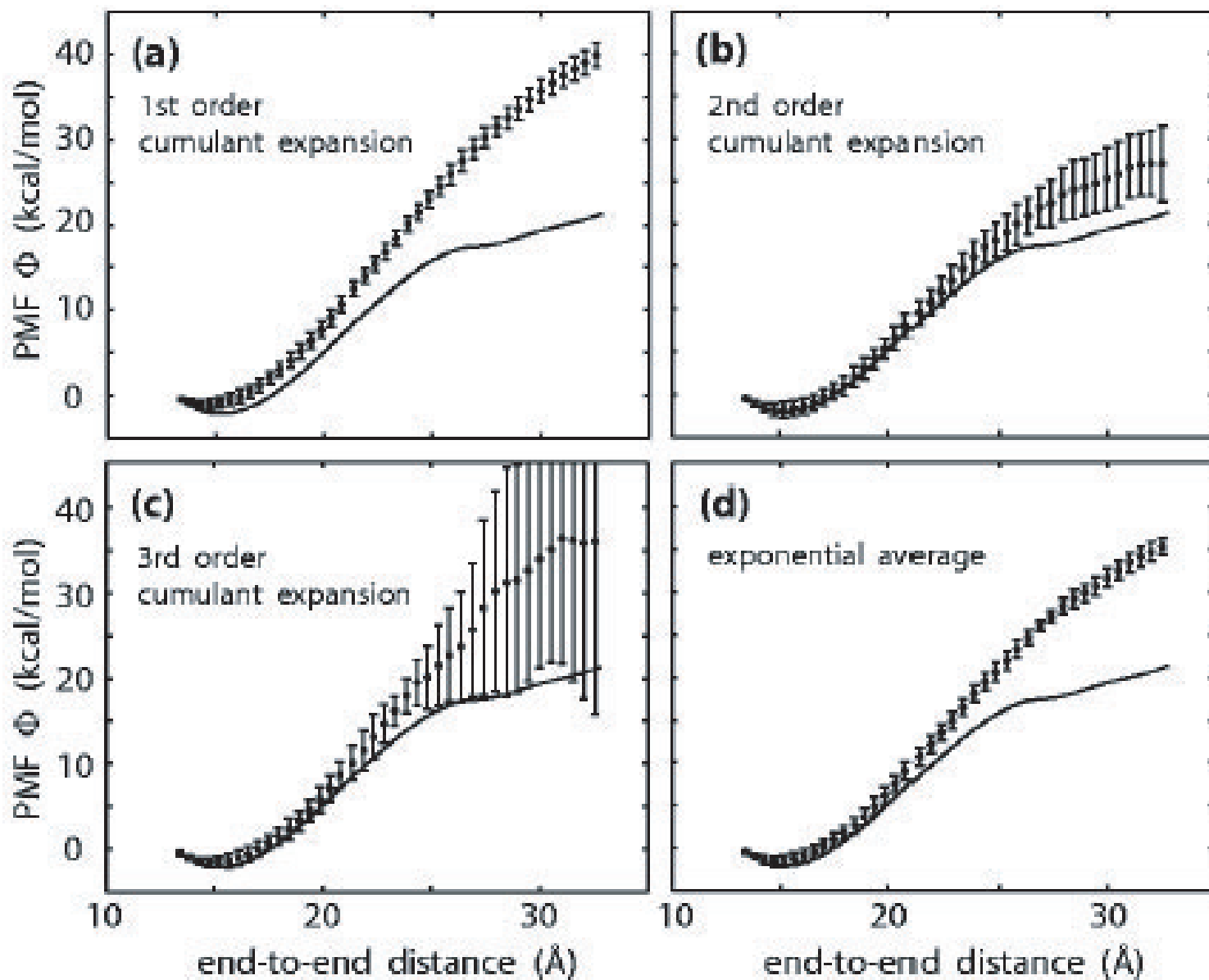


FIG. 5. PMF calculated from irreversible pulling ($v = 100 \text{ \AA/ns}$) through the block average of ten blocks of ten trajectories. The error bars indicate the standard deviation over the blocks. The exact PMF calculated from the reversible pulling is plotted as a solid line in each panel.