Absolute and Relative Entropies from Computer Simulation with Applications to Ligand Binding

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A comparison between two related methods, Schlitter's formula and quasiharmonic analysis, for calculating absolute entropies from the covariance matrix of atomic fluctuations using molecular dynamics (MD) simulations is presented. Calculations for a set of organic compounds in the gas phase are compared to the corresponding statistical thermodynamics results for translational and rotational entropies and to experimental data for vibrational entropies. Encouraging agreement is obtained for translational entropies, but for the rotational contribution, both methods fail to reproduce the theoretically calculated values. Absolute and relative vibrational entropies are found to be better reproduced using quasiharmonic analysis compared to Schlitter's formula. For rotational entropies, we propose a method based on the variances in Euler angles, which gives good agreement with theory. Alternative methods for estimating translational entropies based on principal root mean-square (rms) fluctuations of the center of mass are also presented, and these reproduce theoretically calculated values well. These methodologies are applied to the binding of benzene to T4-lysozyme, where close agreement with the literature is obtained for translational and rotational entropies.

1. Introduction

Estimation of the entropic contributions to protein folding and protein−protein and protein−ligand binding, as well as enzyme catalysis, has been and still is the focus of many studies in theoretical, computational, and experimental biochemistry.[1−7] When a ligand or protein binds to a protein, a significant amount of entropy is lost as a result of the restriction of translational, rotational, and conformational motion in the complex. Consequently, this can have a large impact on the free energy of association, and for these reasons, accurate and reliable calculations of absolute and relative entropies are of major interest. Thus, even though solvent entropy effects may actually dominate overall binding or activation entropies, understanding the contributions from ligands or substrates themselves is of considerable importance. In drug design, for instance, intuitive reasoning with "rules of thumb" about the effects of rigidifying the bonds of ligands, performing cyclizations, and so forth, are quite common, although more robust quantitative measurements of such entropy contributions are scarce.

The molecular dynamics (MD) method is a valuable tool in free energy calculations, and computer simulations can often provide impressive agreement with experimental free energies. It is obviously interesting to partition the free energy into entropic and enthalpic contributions, and the use of force field approaches in this context was demonstrated earlier.[8,9] Unfortunately, it has been shown that entropies are very difficult to estimate, and the errors in calculations of this quantity are often several orders of magnitude larger than those for the free energy.[10] This stems from the fact that, unlike free energies, the entropy depends on the complete phase space of a molecule and cannot be calculated as a simple average from an MD simulation. Instead, extensive sampling of all degrees of freedom is required.

Schlitter[11] proposed an ad hoc approximation for estimating an upper limit to the absolute entropy of a macromolecule from MD simulations using the covariance matrix of atomic fluctuations. This method has been used to estimate the absolute translational, rotational, and conformational entropy, as well as the change in entropy upon folding of a β-heptapeptide,[12,13] side chain entropies of α-lactalbumin,[14] conformational entropy of trialanine,[15] and absolute and relative rotational and conformational entropy contributions to fatty acid binding to a fatty-acid-binding protein.[16] Unfortunately, in some of these applications, the calculated entropies were not fully converged, and furthermore, none of them performed a proper validation of Schlitter's formula.

Levy et al.[17] introduced a method based on the quasiharmonic approximation, which also connects the absolute entropy of the atoms to the covariance matrix. This approach was initially used to calculate vibrational entropies using internal coordinates, but Andricioaei and Karplus[18] showed that the method can be extended to estimate the total entropy using the covariance matrix of Cartesian coordinates. They also showed that quasiharmonic analysis should provide a tighter upper bound to the entropy compared to Schlitter's formula, but did not further quantify this statement.

Here, Schlitter's formula and quasiharmonic analysis are tested and compared on the basis of reproducing translational, rotational, and vibrational entropies. In addition, we propose new approaches to estimate the absolute and relative entropies of molecules on the basis of the variances in translational and rotational degrees of freedom for a molecule. A test set of relatively rigid molecules in the gas phase are simulated using MD, and the entropy is then calculated from the covariance matrix of atomic fluctuations. The estimated entropies are compared to entropies calculated from statistical thermodynam-

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ics and experimental values. We also apply the studied methodologies to estimate the change in rotational and translational entropy for a benzene molecule upon binding to a mutant of the T4-lysozyme protein.

2. Theory

Schlitter's Formula. Schlitter[11] derived an approximate expression for the absolute entropy, S_{Schl}, where every degree of freedom is treated as a quantum harmonic oscillator. By connecting the quantum mechanical frequency of the system with the classical variance by the equipartition theorem, an upper limit to the entropy can be obtained as

$$S_{\mathrm{Schl}} = \frac{1}{2} R \ln \det\left[\mathbf{1} + \frac{kTe^2}{\hbar^2}\mathbf{M}^{1/2}\boldsymbol{\sigma}\mathbf{M}^{1/2}\right] = \frac{1}{2} R \ln \det\left[\mathbf{1} + \frac{kTe^2}{\hbar^2}\mathbf{M}\boldsymbol{\sigma}\right] \quad (1)$$

where k is Boltzmann's constant, R is the molar gas constant, T is the temperature, e is Euler's number, $\hbar$ is Planck's constant divided by 2π, $\mathbf{M}$ is the mass matrix with the masses of the atoms on the diagonal and all off-diagonal elements equal to zero, $\mathbf{1}$ is the unit matrix, and $\boldsymbol{\sigma}$ is the covariance matrix of the $3N$ Cartesian coordinates where N is the number of atoms in the considered molecule or molecules. The covariance matrix has the elements

$$\sigma_{ij} = \langle (x_i - \bar{x}_i)(x_j - \bar{x}_j) \rangle \quad (2)$$

Both expressions in eq 1 are valid and yield the same result, but note that, in general, $(kTe^2/\hbar^2)\mathbf{M}\boldsymbol{\sigma} + \mathbf{1}$ is not symmetric as proposed by Andricioaei and Karplus.[18]

The translational contribution to the entropy of a given molecule can be separated from rotation and vibration by performing a translational fit of all frames of the molecule to the origin. Rotational and vibrational movement cannot be separated exactly for flexible molecules, but by carrying out a geometric or mass-weighted least-squares fit of the molecule, the rigid-body rotational and translational contributions are approximately separated from the vibrational movement. For the translational contribution to the entropy, Schlitter's formula can be further simplified by representing each frame by the center of mass of a single molecule instead of its $3N$ Cartesian coordinates (later referred to as $S_{\mathrm{Schl,cm}}$). In this case, each of the center of mass coordinates is weighted with the total mass of the molecule. This reduces computation time, because the dimension of the covariance matrix decreases from $3N$ to 3 for a single molecule.

Quasiharmonic Analysis. Quasiharmonic analysis[17,18] assumes that fluctuations in the motions of the system can be approximated by a Gaussian probability distribution. Frequencies, ω, can be calculated from the determinant

$$\det\left(\mathbf{M}^{1/2}\boldsymbol{\sigma}\mathbf{M}^{1/2} - \frac{kT}{\omega^2}\mathbf{1}\right) = 0 \quad (3)$$

The molar quasiharmonic entropy, S_{qh}, is calculated from the frequencies through the formula for the entropy of the harmonic oscillator

$$S_{\mathrm{qh}} = R\sum_i \frac{\hbar\omega_i/kT}{\exp(\hbar\omega_i/kT) - 1} - \ln[1 - \exp(-\hbar\omega_i/kT)] \quad (4)$$

Quasiharmonic analysis can be used to calculate translational, rotational, and vibrational entropies in the same manner as with Schlitter's formula by using rotational and translational fits. In these calculations, three eigenvalues correspond to translational movement, three eigenvalues to rotational movement, and $3N - 6$ (nonlinear molecule) to vibrational motions.

Translational Entropies from Principal rms Fluctuations. Another interesting approach to calculate translational entropies from simulations is to start from the classical expression for the entropy. If we assume that the probability distributions corresponding to translational, rotational, and vibrational motions are independent (i.e., $p(\mathbf{r}, \mathbf{p}) = p_{\mathrm{transl}}(\mathbf{r}, \mathbf{p})\, p_{\mathrm{rot}}(\mathbf{r}, \mathbf{p})\, p_{\mathrm{vib}}(\mathbf{r}, \mathbf{p})$) we can consider each contribution to the entropy separately. The translational contribution to the entropy can be written as an integral over probabilities

$$S_{\mathrm{transl}} = -\frac{R}{h^3}\int p_{\mathrm{transl}}(\mathbf{p}, \mathbf{r}) \ln p_{\mathrm{transl}}(\mathbf{p}, \mathbf{r})\, \mathrm{d}\mathbf{p}\, \mathrm{d}\mathbf{r} \quad (5)$$

where $p_{\mathrm{transl}}(\mathbf{r}, \mathbf{p})$ is the probability density of positions and momenta for the center of mass of a molecule in phase space. The expression has also been corrected with the inverse of Planck's constant for each degree of freedom, $1/h^3$, to yield the correct quantum mechanical entropies.[19] For translational entropies the momenta can be integrated out, yielding (disregarding units)

$$S_{\mathrm{transl}} = R \ln\left[\left(\frac{2\pi emkT}{h^2}\right)^{3/2}\right] - R\int p_{\mathrm{transl}}(\mathbf{r}) \ln p_{\mathrm{transl}}(\mathbf{r})\, \mathrm{d}\mathbf{r} \quad (6)$$

The probabilities in space can, in principle, be estimated from histogram analysis using an MD simulation.[20,21] Because it can be difficult to sample the tails of distributions in a simulation, it can also be useful to assume a specific form of $p_{\mathrm{transl}}(\mathbf{r})$. For a freely translating molecule, a uniform distribution of the motions is appropriate (i.e., $p_{\mathrm{transl}}(\mathbf{r}) = 1/V$ where V is the volume available to the molecule). This results in the familiar Sackur–Tetrode equation for an ideal gas of distinguishable particles[19]

$$S_{\mathrm{transl}} = R \ln\left[\left(\frac{2\pi emkT}{h^2}\right)^{3/2} V\right] \quad (7)$$

To connect the volume, V, to a quantity that can be measured in an MD simulation, we use the statistical result for the variance of a uniform distribution, $\langle x^2 \rangle = L^2/12$, where L is the side of the box in which the molecule moves. For three degrees of translational freedom, the principal variances for three orthogonal directions can be found from the eigenvalues of the covariance matrix for the center-of-mass motions. The entropy can then be expressed

$$S_{\mathrm{transl,uniform}} = R \ln\left[\left(\frac{24\pi emkT}{h^2}\right)^{3/2} \sigma_x\sigma_y\sigma_z\right] \quad (8)$$

where σ_x, σ_y, and σ_z are the principal rms fluctuations for the center of mass of a molecule.

In some cases, for example, the motion of a ligand in a protein binding site, a uniform probability distribution could be inappropriate, and a Gaussian distribution of $p_{\mathrm{transl}}(\mathbf{r})$ might be a better approximation. This yields the probability distribution

$$p_{\mathrm{transl}}(\mathbf{r}) = \frac{1}{(2\pi)^{3/2}\det(\boldsymbol{\sigma})^{1/2}} \exp\left[-\frac{1}{2}(\mathbf{r} - \bar{\mathbf{r}})\boldsymbol{\sigma}^{-1}(\mathbf{r} - \bar{\mathbf{r}})\right] \quad (9)$$

where $\boldsymbol{\sigma}$ again is the covariance matrix for the center of mass of a molecule. The corresponding entropy then becomes

$$S_{\text{transl,gauss}} = R \ln\left[\left(\frac{4\pi^2 e^2 mkT}{h^2}\right)^{3/2} \sigma_x \sigma_y \sigma_z\right] \qquad (10)$$

A Gaussian form for the probability distribution has previously been used by Karplus and Kushick to calculate differences in the conformational entropies using the $(3N - 6)$ internal coordinates of a molecule.[22] Here, we will use the same approach to approximate the probability distribution for the translation of a ligand in a protein-binding site.

Reference Translational, Rotational, and Vibrational Entropies. Reference gas-phase translational and rotational entropies are calculated here for some simple organic molecules from statistical thermodynamics.[19] The Sackur−Tetrode formula (eq 7) can be used to calculate the molar translational entropy for noninteracting, distinguishable molecules from the mass, temperature, and volume available to the molecule.[19] For a freely rotating, rigid molecule, the rotational contribution to the molar entropy can be expressed as

$$S_{\text{theor,rot}} = R \ln\left[\frac{8\pi^2}{\sigma_s}\left(\frac{2\pi ekT}{h^2}\right)^{3/2} (I_A I_B I_C)^{1/2}\right] \qquad (11)$$

where I_A ,I_B, and I_C are the principal moments of inertia and σ_s is the symmetry number.[19] The vibrational entropy can be estimated from experimental values of the absolute entropy after subtracting the values of translational and rotational entropies calculated from eqs 7 and 11.

3. Methods

Molecular Dynamics Protocol and Test Set of Molecules. MD simulations were carried out for a set of compounds consisting of methane, ethene, benzene, cyclohexane, naphthalene, and bromobenzene. Relatively rigid molecules, without any freely rotating groups, were intentionally chosen to be able to make a reasonable comparison to theoretically calculated rotational and experimental vibrational entropies. It should therefore be noted that entropies related to more complex internal rotations are not explicitly investigated in this study. The MD simulations were carried out with the molecular dynamics package Q[23] using the OPLS-AA force field[24] in a periodic box with 64 molecules and a volume of 2 387 300 Å^3, which corresponds to a standard-state concentration (22.42 L/mol). All simulations were carried out at a constant temperature of 300 K with a 1-fs time step and a 10-Å cutoff.

To validate our implementation of Schlitter's formula and to compare our results to earlier work, two additional simulations were carried out. Palmatic acid in aqueous solution was simulated in a periodic box using the GROMOS96 force field, as in ref 16, in a 42 875-Å^3 box. The space that was not occupied with solute was filled with SPC water molecules. The second system consisting of 256 argon atoms was simulated, as in ref 13, in a periodic box with volume 12 200 Å^3 using the van der Waals parameters $\epsilon/k = 119.8$ K and $\sigma = 3.41$ Å. A short equilibration where the temperature was gradually raised to 300 K using a 2-fs time step was carried out for both systems. Nonbonded pair lists were updated every 25 steps, and the cutoff was set to 10 Å.

For the simulation of the binding of benzene to a T4-lysozyme mutant (C54T, C97A, L99A), a crystal structure of the protein in a complex with benzene at 1.9-Å resolution was used (PDB code 181L).[25] Two amino acids in the protein crystal structure were missing, but they are far from the binding cavity and therefore probably do not affect binding. A simulation sphere of 18 Å was centered on the ligand, and the space that was not occupied by solute was filled with TIP3P water. A nonbonded cutoff of 10 Å was used, and the local reaction field multipole expansion treatment[26] was used for long-range electrostatic interactions involving nonligand atoms beyond the direct cutoff. In the complex, all atoms outside the simulation sphere were highly restrained to their starting positions and excluded from nonbonded interactions. Water molecules at the surface of the sphere were subjected to radial and polarization surface restraints according to our version of the SCAAS model.[23,27] The simulation was carried out using the OPLS-AA force field using a 1-fs time step.[24] The structure was relaxed at 1 K for 4000 steps with a 25 kcal/mol Å^2 restraint on all solute atoms. The restraints were then gradually released on the benzene molecule, and the temperature was raised to 300 K. Finally, it was equilibrated at 300 K for 50 ps. A 2-ns production run was then performed. In this simulation, a positional restraint of 50 kcal/mol Å^2 was put on all protein atoms in order to provide similar conditions to a recently published study on this system.[28] An additional 2-ns simulation without restraints on T4-lysozyme was also carried out in order to analyze the effects of a flexible protein on the binding entropy of a benzene molecule. We also simulated the free benzene molecule in water using the same sphere size and simulation setup as for the benzene−T4-lysozyme complex. In the simulation, the benzene molecule was positioned in the center of the sphere by using a restraint of 25 kcal/mol Å^2 to its geometrical center.

Entropy Calculations. Entropies according to Schlitter's formula[11] and quasiharmonic analysis[18] were implemented in the MD package Q. The translational, rotational, and conformational contributions to the entropy for Schlitter's formula and quasiharmonic analysis were calculated by making three calculations for each molecule and method. The translational entropy was calculated from the difference between making one calculation in which no fitting of the molecule was performed and one calculation in which the center-of-mass motions of the molecule were removed. The rotational entropy was calculated in a similar fashion to the difference between a calculation where the translational contribution had been removed and a calculation where a mass-weighted rms fit of all atoms in the molecule to the first frame had also been performed. The calculated rotational entropy is further corrected for symmetry, which contributes with $-R \ln(\sigma_s)$, where σ_s is the symmetry number of the molecule.[19] The vibrational entropy was estimated as the entropy of the latter calculation with the translational and rotational contributions removed. $S_{\text{Schl,cm}}$ was computed by calculating the covariance matrix for the center of mass of each frame. For methane and ethane, 20 000 snapshots from a 10-ns simulation were used to calculate the covariance matrix of atomic fluctuations. For all other compounds, 40 000 snapshots from a 20-ns simulation were used to calculate the covariance matrix. In all cases, the error was estimated by the rms deviation of the results of the entropy calculations for 10 different molecules in the simulation box. For the methods based on principal rms fluctuations of the center of mass, the same number of snapshots as for Schlitter's formula and quasiharmonic analysis were used to construct the covariance matrix. The principal variances were then determined as the eigenvalues of this matrix.

To compare our study to an earlier examination of Schlitter's formula,[13] we also simulated a system of 256 argon atoms in a periodic box. The entropy was calculated using Schlitter's formula for all 256 atoms in the box and for 10 individual

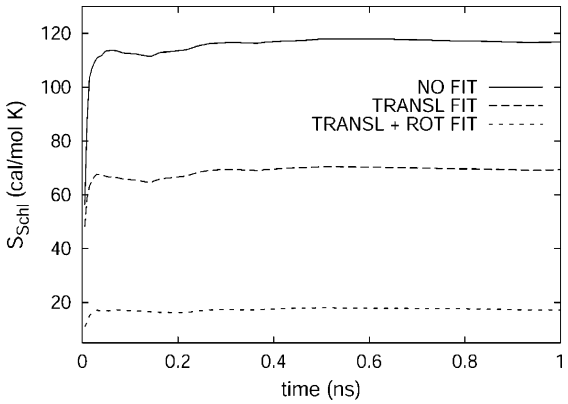

Figure 1. Convergence of the entropy (cal/mol K) for naphthalene calculated from Schlitter's formula using translational, rotational, or no fit of the atoms.

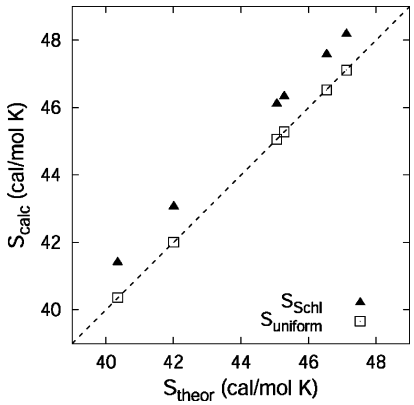

Figure 2. Calculated and theoretical translational entropies (cal/mol K) using Schlitter's formula, $S_{Schl,cm}$, and $S_{transl,uniform}$.

molecules using 50 000 frames from a 5-ns simulation. The absolute entropies of palmatic acid in water were calculated from a 4-ns simulation, from which 20 000 snapshots were collected.

The entropy of benzene in a complex with T4-lysozyme was calculated from 20 000 frames from a 2-ns simulation. The translational contribution to the entropy was separated from rotation and vibration by moving the center of mass of the ligand in all frames to the origin. Because the protein is fixed outside the 18-Å simulation sphere, the benzene molecule moves in the protein frame of reference, and the translational entropy in the bound state can therefore be calculated directly from the simulation trajectory. That ligand rms fluctutations are well-reproduced by the present simulation model has been demonstrated by Almlöf et al.[29] Rotational and vibrational movement were separated by carrying out a mass-weighted least-squares fit of all atoms of the molecule to the first snapshot from the trajectory. We calculate the change in translational entropy compared to the gas phase (in accordance with ref 28) and use the Sackur−Tetrode equation[19] as the reference for the free state. Principal rms fluctuations of the center of mass and fluctuations in Euler angles were determined from a covariance matrix constructed from all 40 000 snapshots. The vibrational entropy of a solvated benzene molecule was calculated using 1500 frames from a 750-ps simulation.

4. Results and Discussion

Translational Entropies. The translational contribution to the entropies converged relatively quickly for all compounds, and the convergence of Schlitter's formula for naphthalene is shown in Figure 1 as an example. All three calculations using translational, rotational, or no fit are fully converged after 0.5 ns (1000 snapshots). Because quasiharmonic analysis is based on the covariance matrix as well, it displayed similar convergence to Schlitter's formula. The translational gas-phase entropy results for the six-compound test set from quasiharmonic analysis (S_{qh}), Schlitter's formula (S_{Schl}), Schlitter's formula using the center of mass of a molecule ($S_{Schl,cm}$), $S_{transl,uniform}$, and $S_{transl,gauss}$ are shown in Table 1 and Figure 2.

The results for quasiharmonic analysis and Schlitter's formula are very similar, and the two methods overestimate the translational entropy with, on average, 1.05 cal/mol K. Hence, they are both close to the result calculated from the Sackur−Tetrode equation and provide a tight upper bound to the absolute translational entropy. An alternative way to use Schlitter's formula is to represent a molecule by its center of mass instead of all $3N$ coordinates. This does not change the results significantly and might speed up convergence for larger, more flexible, molecules. Estimating the translational entropies by calculating the available volume in the molecule and assuming a uniform distribution of the motions of a molecule and using eq 8 yields an average unsigned error of only 0.008 cal/mol K, which is considerably better than for Schlitter's formula and quasiharmonic analysis. Using a Gaussian distribution for the center-of-mass motions of a molecule for the translational entropy, $S_{transl,gauss}$, yields a similar overestimation of the entropy as Schlitter's formula and quasiharmonic analysis.

The calculated translational entropies using quasiharmonic analysis, Schlitter's formula, and $S_{transl,gauss}$ are all based on a Gaussian distribution for the motions of the atoms. The good agreement for a Gaussian distribution is by intuition somewhat surprising, because the motion of a molecule in the gas phase is probably not described well by a Gaussian distribution. The difference between assuming a Gaussian distribution and assuming a uniform distribution, $\Delta S_{gauss-uniform} = S_{transl,gauss} - S_{transl,uniform}$, could be considered a measure of the error made when assuming a Gaussian distribution for a freely translating molecule. This error is $\Delta S_{gauss-uniform} = {}^3/_2 R \ln(2\pi e/12) \approx 1.05$ cal/mol K, which agrees perfectly with the average error obtained using quasiharmonic analysis, Schlitter's formula, $S_{Schl,cm}$, and $S_{transl,gauss}$. The error is independent of the volume in which the particle moves and will only contribute with a constant offset to the correct value. Relative entropies for

TABLE 1: Translational Entropies (cal/mol K) from Quasiharmonic Analysis, Schlitter's Formula, and Principal rms Fluctuations[a]

compound	S_{theorr}	S_{Schl}	S_{qh}	$S_{Schl,cm}$	$S_{transl,gauss}$	$S_{transl,uniformm}$
methane	40.348	41.408 ± 0.050	41.408 ± 0.05	41.415 ± 0.049	41.412 ± 0.095	40.361 ± 0.095
ethene	42.012	43.057 ± 0.070	43.057 ± 0.070	43.059 ± 0.069	43.055 ± 0.036	42.004 ± 0.036
benzene	45.062	46.112 ± 0.047	46.113 ± 0.047	46.113 ± 0.047	46.110 ± 0.064	45.059 ± 0.064
cyclohexane	45.284	46.335 ± 0.059	46.335 ± 0.059	46.339 ± 0.059	46.336 ± 0.042	45.285 ± 0.042
naphthalene	46.537	47.575 ± 0.064	47.575 ± 0.064	47.578 ± 0.064	47.574 ± 0.053	46.523 ± 0.053
bromobenzene	47.123	48.184 ± 0.050	48.184 ± 0.050	48.186 ± 0.050	48.164 ± 0.028	47.113 ± 0.028

[a] Theoretical results were calculated from classical statistical thermodynamics.

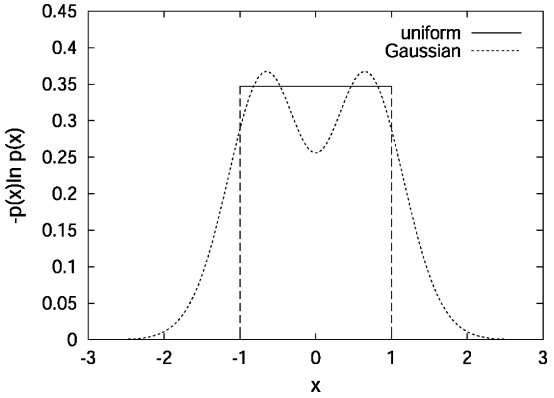

Figure 3. Plot of $-p(x) \ln p(x)$ for a uniform and Gaussian probability density with variance $\langle x^2 \rangle = {}^1/_3$.

TABLE 2: Calculated and Theoretical (eqs 6 and 11) Entropies (cal/mol K) for a 16-amu Ideal Gas, the Argon System of ref 10, and Palmatic Acid in Water

Ideal Gas			
	S_{theor}	S_{Schl}	error (%)
100 K	137.528	138.580	0.76
200 K	139.593	140.644	0.75
300 K	140.800	141.852	0.75

Argon			
	S_{theor}	S_{Schl}	error (%)
	32.594	32.892	0.91

Palmatic Acid			
	S_{theor}	S_{calcd}	error (%)
translation	40.460	39.885 (S_{Schl})	1.5
		39.806 ($S_{uniform}$)	1.6
rotation	34.859	49.334 (S_{Schl})	41.5
		34.927 ($S_{rot,uniform}$)	0.2
vibration		207 (S_{Schl})	
		206 (S_{qh})	

Schlitter's formula and quasiharmonic analysis are hence in very good agreement with theory. The difference between the two methods can also be illustrated by plotting the $S/R = -p(x) \ln p(x)$ function for a uniform and Gaussian distribution in one dimension with the same variance. This is displayed in Figure 3, where $\Delta S_{gauss-uniform}$ is equal to the difference between the areas under the graphs corresponding to a Gaussian and a uniform distribution.

In contrast to our results, Schäfer et al.[13] obtained significantly larger deviations for an ideal gas at a set of different temperatures. They found that for a 16-amu ideal gas the deviation in molar entropy for Schlitter's formula was about 28.5 cal/mol K at 300 K, which results in a 16.9% deviation from the value obtained from the Sackur−Tetrode equation. For the same set of values, we, however, find different results using exactly the same analytical forms of the Sackur−Tetrode equation and Schlitter's formula (eqs A3 and A4 in ref 13), and these are displayed in Table 2. The deviation in all these cases is 1.05 cal/mol K, as calculated in the previous section. This results in an error of less than 1%. Schäfer et al. also calculated the translational entropy for an argon Lennard-Jones system and found that Schlitter's formula approximated the translational entropy within 3−6% of an ideal gas estimate that was corrected for nonideal behavior. These results were later found by Andricioaei and Karplus to be based on nonconverged trajectories.[18] Our 5-ns simulation shows that the converged result is 32.9 cal/mol K, which is within 1% of the value calculated using statistical thermodynamics (Table 2). Using only 1 atom instead of all 256 atoms results in significantly faster convergence, and the result from 10 different calculations on individual, randomly chosen argon atoms is 33.644 ± 0.070 cal/mol K. This is only slightly higher than that determined by considering the full covariance matrix, indicating that the difference between using 1 molecule in the calculation instead of 256 is not more than about 1 cal/mol K. Also for palmatic acid in solution, the calculated translational entropies are very close to the theoretical gas-phase result (Table 2).

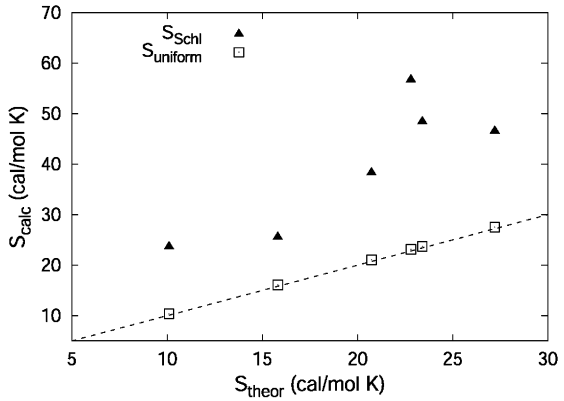

Figure 4. Rotational entropies (cal/mol K) for the test set of six compounds using Schlitter's formula and from rms fluctuations in Euler angles.

Rotational Entropies. Because Schlitter's formula and quasiharmonic analysis are based on harmonic approximations, a freely rotating molecule should represent a difficult case for both methods. But given the good results obtained for translational entropies, one could perhaps expect reasonable results for the rotational contribution also. On the contrary, we find that the deviations from theoretical values are very large for both methods. The calculated values for the gas-phase rotational entropies of our test set are shown in Figure 4 and Table 3, where very large overestimations of S_{rot} can be seen.

Schlitter's formula has previously been used to calculate the absolute rotational entropy for a β-heptapeptide.[13] More recently, Bakowies et al.[16] have used Schlitter's formula to calculate the rotational entropy contribution to the binding of palmatic acid to a fatty-acid-binding protein. Their results show that, using Schlitter's formula, palmatic acid has 52 cal/mol K of rotational entropy in aqueous solution. Assuming that ideal gas statistics are also valid for a solvated molecule, this entropy can again be estimated using statistical thermodynamics (eq 11), which yields 34.9 cal/mol K. Hence, the calculation[16] using Schlitter's formula overestimates the absolute entropy by 49%. We also obtain a similar overestimation of the rotational term for this case, as can be seen from Table 2. A small increase of this entropy could arise from the difficulties of separating the rotational and internal motions or fluctuations in the moments of inertia, but note that the statistical rotational entropy only has a small dependence on structure (i.e., doubling all three moments of inertia only contributes 2.1 cal/mol K to the entropy). Some cancellation of the error can perhaps be expected for relative entropies, but it is difficult to estimate the error in such a calculation. In this context, it should also be mentioned that, while one could claim that ideal gas results are not applicable to solvated systems,[3,30] it is unreasonable to suggest higher values of the rotational entropy for solvated molecules than in the ideal-gas state. Also, from a theoretical viewpoint, the probability distributions for a ligand or protein freely translating in a solvent should basically be uniform, because the solvent does not favor any position or orientation of the

TABLE 3: Calculated Rotational Entropies (cal/mol K) for the Set of Selected Compounds Using Quasiharmonic Analysis, Schlitter's Formula, and Principal rms Fluctuations[a]

compound	σ_s	S_{theor}	S_{Schl}	S_{qh}	$S_{rot,gauss}$	$S_{rot,uniform}$
methane	12	10.095	23.669 ± 0.047	23.684 ± 0.05	11.415 ± 0.094	10.364 ± 0.094
ethene	4	15.810	25.543 ± 0.019	25.591 ± 0.015	17.171 ± 0.035	16.119 ± 0.035
benzene	12	20.734	38.282 ± 0.130	38.000 ± 0.136	22.131 ± 0.061	21.080 ± 0.061
cyclohexane	6[b]	22.800	56.718 ± 0.105	56.154 ± 0.107	24.184 ± 0.034	23.132 ± 0.034
naphthalene	4	23.403	48.425 ± 0.189	48.220 ± 0.206	24.789 ± 0.044	23.738 ± 0.044
bromobenzene	2	27.207	46.537 ± 0.096	46.438 ± 0.101	28.587 ± 0.028	27.535 ± 0.028

[a] Theoretical values are calculated from statistical mechanics, and σ_s is the rotational symmetry number. [b] Based on chair conformation.

molecule.[31] Because a uniform distribution of the motions is the basis of eqs 7 and 11, it should therefore also be valid for solvated systems.

The discrepancy between theoretical estimates and quasiharmonic analysis and Schlitter's formula for rotational entropies could arise from the fact that the projection of rotational movement on the Cartesian coordinates is not described well by a Gaussian distribution. A transformation to coordinates that more effectively describe the rotational degrees of freedom, such as Euler angles, would appear to be a better descriptor of rotational movement. By assuming a uniform distribution of Euler angles and making a mean angle approximation, the absolute entropy can be expressed (see Appendix 1 for a detailed derivation of eqs 12 and 13)

$$S_{rot,uniform} = R \ln\left[\frac{(12)^{3/2}}{\sigma_s}\left(\frac{2\pi ekT}{h^2}\right)^{3/2}(I_A I_B I_C)^{1/2}\sigma_\phi \sigma_\psi \sigma_\theta \sin\bar{\theta}\right] \quad (12)$$

where σ_ϕ, σ_ψ, and σ_θ are rms fluctuations in the three Euler angles ($-\pi \le \phi < \pi$, $-\pi \le \psi < \pi$, and $0 \le \theta < \pi$), and θ is the average of θ in the simulation. For a Gaussian distribution of Euler angles, we instead obtain the following expression for the absolute entropy

$$S_{rot,gauss} = R \ln\left[\frac{(2\pi e)^{3/2}}{\sigma_s}\left(\frac{2\pi ekT}{h^2}\right)^{3/2}(I_A I_B I_C)^{1/2}\sigma_\phi \sigma_\psi \sigma_\theta \sin\bar{\theta}\right] \quad (13)$$

For very flexible molecules, an average moment of inertia can be used in eq 12, but for rigid molecules, as the set chosen here, only one calculation on a minimized structure is necessary. Hence, only rms fluctuations in Euler angles have to be evaluated to calculate the entropy in eq 12. Using the approach of eq 12 for freely rotating molecules, we obtain an excellent agreement with theoretical values calculated from eq 11 and get an average unsigned error of only 0.320 cal/mol K for the test set of six compounds. These values are presented in Table 3 and Figure 4. Also for palmatic acid, discussed already, the theoretical value of the rotational entropy of 34.9 cal/mol K is now reproduced with impressive accuracy by eq 12, as shown in Table 2. One can once again note that translational entropy is described well by eq 8. When taken together, the results for the test set and palmatic acid thus clearly demonstrate that the absolute rotational entropies, both in the gas phase and in solution, can be accurately calculated from the rms fluctuations in Euler angles, assuming a uniform distribution of these.

Vibrational Entropies. In contrast to translational and rotational entropies, there are significant differences between the absolute vibrational entropies calculated using Schlitter's formula and quasiharmonic analysis. This can be seen from Figure 5, where the entropies for Schlitter's formula and quasiharmonic analysis as a function of frequency, ω, are

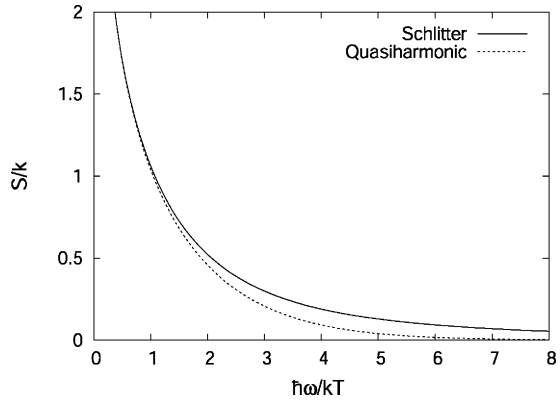

Figure 5. The difference in entropy (S/k) between quasiharmonic analysis and Schlitter's formula. For definitions, see eqs 1 and 4.

TABLE 4: Calculated Vibrational Entropies (cal/mol K) Using Schlitter's Formula and Quasiharmonic Harmonic Analysis Compared to Experimental Values

compound	S_{exp}	S_{Schl}	S_{qh}
methane	0.329[41]	0.257 ± 0.025	0.001 ± 0.001
ethene	0.990[41]	0.356 ± 0.041	0.014 ± 0.009
benzene	4.816[41]	4.815 ± 0.238	2.437 ± 0.240
cyclohexane	9.468[41]	13.992 ± 0.303	8.390 ± 0.268
naphthalene	12.900[34]	15.593 ± 0.33	9.942 ± 0.280
bromobenzene	9.592[42]	9.203 ± 0.259	6.305 ± 0.199

displayed. For frequencies corresponding to translational movement, the two methods are similar, but for high-frequency motions, such as vibrations, quasiharmonic analysis always gives a lower entropy than Schlitter's formula. The results using Schitter's formula and quasiharmonic analysis for gas-phase vibrational entropies for our test set are displayed in Table 4 and Figure 6. Absolute entropies are in fair agreement with experimental data for both quasiharmonic analysis (rms deviation of 2.1 cal/mol K) and Schlitter's formula (rms deviation of 2.2 cal/mol K). Note, however, that the vibrational contribution is bound to be more sensitive to force field parameters (force constants) than the translational and rotational terms. In a recent study, Mu et al.[15] compared the conformational entropy between extended and helical conformations of trialanine and found that Schlitter's formula overestimated the conformational entropy by 6% compared to quasiharmonic analysis. Calculated relative entropy differences, however, remained the same.

To determine how well relative entropies are reproduced, the best fit to the experimental data using the addition of a constant offset (λ) to the calculated entropies was calculated here for both Schlitter's formula and quasiharmonic analysis. The results calculated using quasiharmonic analysis ($\lambda = 1.8$ cal/mol K) yields an rms deviation of 1.1 cal/mol K and $R^2 = 0.94$, which is slightly better than $R^2 = 0.83$ (rms deviation of 1.9 cal/mol K) obtained with Schlitter's formula ($\lambda = -1.0$ cal/mol K). The relative entropy errors can probably be expected to be even smaller for a comparison between two different states of the same molecule (e.g., the difference in entropy between a ligand

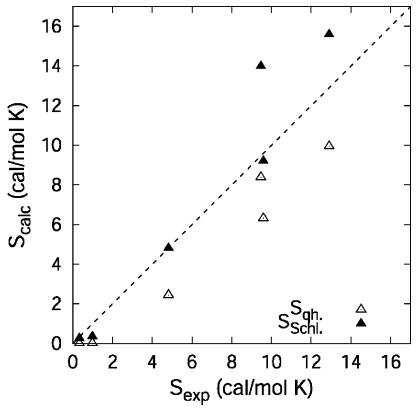

Figure 6. Vibrational entropies (cal/mol K) for the test set of six compounds obtained from Schlitter's formula and quasiharmonic approximation compared to experimental values.

free in solution and one bound to a protein). In the case of palmatic acid, we find that the difference between Schlitter's formula and quasiharmonic analysis for the conformational (vibrational) is less than 1% for the free molecule in water (Table 2), and one could perhaps expect the differences between Schlitter's formula and quasiharmonic analysis to be smaller for larger molecules.[15] However, the accuracy of Schlitter's formula and quasiharmonic analysis for conformational entropies of molecules with freely rotating bonds has not been investigated, and it is not clear whether reliable results would be obtained for very flexible molecules. This could, for example, be addressed by exmaining entropy differences between alkanes in their cyclic and linear forms.

T4-Lysozyme Binding of Benzene. Theoretical estimates of the loss of entropy upon ligand binding have been pursued for over 40 years.[32] As a first approximation, it has often been assumed that a molecule completely loses its gas-phase translational and rotational entropy upon complex formation.[33] This difference can then easily be estimated from statistical thermodynamics.[19] It is, however, known that ligands can preserve a significant amount of rotation and translation when bound to a protein-binding site, and more advanced methodologies try to take these motions into account. Finkelstein and Janin[34] assumed that the movements of molecules in complexes are similar to the motions of molecules in crystals and estimated the loss of translational and rotational entropies from principal rms fluctuations in three orthogonal directions, σ_i. Translational and rotational entropies were then estimated from the expressions, $\Delta S = R \ln(\sigma_\theta \sigma_\psi \sigma_\varphi / 8\pi^2)$ and $\Delta S = R \ln(\sigma_z \sigma_y \sigma_z / 1660)$, where 1660 Å^3 represents the available volume at 1 M concentration and $8\pi^2$ corresponds to the free rotational "volume". The theoretical basis for choosing this expression is, however, not entirely clear, because rms fluctuations are not identical to the relevant volumes, and it seems that the accessible volumes in the complex are underestimated in these expressions, which has been noted in several recent publications.[1,2]

Our MD simulations of T4-lysozyme in a complex with benzene show that the ligand is relatively free to move in the binding pocket of the protein and therefore keeps a significant amount of translational and rotational entropy in the bound state. Previous estimates solely based on a total loss of rigid-body motion calculated from the statistical thermodynamics entropy hence overestimates the positive entropic contribution to the binding free energy[35] (the mass dependence of binding entropies obtained in ref 35 is also incorrect). T4-lysozyme binding of benzene has previously been studied by Hermans and Wang (HW).[28] They used a rigorous approach based on positional restraints where the ligand's translational and rotational degrees of freedom initially are frozen. By gradually removing the restraints on the ligand in the protein-binding site, the translational and rotational (but not vibrational) entropy could be estimated from the change in free energy. These simulations were, however, based on a static protein model and, using a 1 M ideal-gas reference state, gave an estimate of the so-called cratic entropy contribution of $-T\Delta S = 8-9$ kcal/mol.[28] An alternative calculation by HW, again using a static protein, that directly evaluated the entropy contribution as the difference between the free energy and the mean binding energy gave a corresponding value of about 7 kcal/mol. Our approach is considerably easier to apply than the restraint−release approach in this case, because the translational and rotational entropy can be estimated from a single unrestrained simulation of the protein−ligand complex. A variant of the restraint−release method, that treats all ligand degrees of freedom employing more straightforward Cartesian coordinate restraints, has recently been used by Warshel and co-workers to evaluate activation entropies in enzyme reactions.[36,37] However, a general problem with using restraints is that a single reference structure to which these are applied may not suffice for getting reliable statistics, which prompts multiple simulations to use (slightly) different reference structures.[28,36,37] The calculated change in translational and rotational entropies of benzene binding to T4-lysozyme with different methodologies are shown in Table 5. Our data in Table 5 are based on the restrained simulation of the T4-lysozyme complex in order to be comparable with the results of HW. For the change in translational entropies, we find a loss of −17.5 cal/mol K using Schlitter's formula and −17.6 from quasiharmonic analysis, which is in good agreement with the result obtained by Hermans et al., −17.0 to −20.3 cal/mol K. The two different results obtained by HW correspond to different orders in which the positional restraints were released in the simulations[28] and thus gives an indication of the intrinsic uncertainty of that approach. The absolute entropy for benzene in the free translating gas-phase state was calculated using the Sackur−Tetrode equation.

TABLE 5: Change in Entropy (cal/mol K) and Corresponding Free Energy Contributions (kcal/mol at 300 K) for Benzene Binding to (restrained) T4-Lysozyme[a]

	S_{HW1}	S_{HW2}	S_{Schl}	S_{qh}	$S_{uniform}$	S_{gauss}	S_{Finkel}
ΔS_{transl}	−17.0	−20.3	−17.5[b]	−17.6[b]	−18.3	−17.3	−25.7
ΔS_{rot}[c]	−11.1	−11.7	−19.4	−19.1	−7.8	−6.8	
$-T\Delta S_{rot+transl}$	8.4	9.6	11.1	11.0	7.8	7.2	

[a] S_{HW1} and S_{HW2} are the results presented in ref 28. S_{Schl} and S_{qh} are the entropies using Schlitter's formula and quasiharmonic analysis, respectively. $S_{uniform}$ and S_{gauss} are entropies calculated from rms fluctuations. [b] The gas-phase translational entropy was calculated using eq 7.

We also consider the alternative way of estimating the change in translational entropy for the binding of a ligand, where the accessible volume in the binding site is estimated and compared to a reference volume. The reference volume is determined by the chosen standard concentration of 1 M and corresponds to a volume of 1660 Å^3 per molecule. If a uniformly distributed available space for the ligand is assumed for both the free and the bound states, the change in entropy can thus be approximated with

$$\Delta S_{transl,uniform} = R \ln\left[\frac{12^{3/2}\sigma_x\sigma_y\sigma_z}{1660}\right] \qquad (14)$$

For benzene bound to T4-lyzosyme, this yields an entropy loss of $\Delta S_{transl,uniform} = -18.3$ cal/mol K, which also is close to

the result of HW. By assuming a uniformly distributed motion of the ligand in the free state and Gaussian motions of the molecule in the bound state, the principal rms fluctuations can be related to the change in entropy through the expression

$$\Delta S_{\text{transl,gauss}} = R \ln\left[\frac{(2\pi e)^{3/2}\sigma_x\sigma_y\sigma_z}{1660}\right] \qquad (15)$$

A similar expression has been proposed by Swanson et al.[1] Our expression contains an extra factor, $e^{3/2}$, which arises from the quadratic term in the Gaussian distribution function. This expression yields $\Delta S_{\text{transl,gauss}} = -17.3$ cal/mol K, which again is in excellent agreement with ref 28. Finally, we calculated the translational entropy according to Finkelstein et al.[34] and obtained a value of $\Delta S_{\text{transl,Finkel}} = -25.7$ cal/mol K. This is clearly an overstimate of the lost entropy, because more entropy is lost than is present in the free state, which is unphysical.

Hermans and Wang[28] found the rotational binding entropy contribution to be between −11.1 and −11.7 cal/mol K for the benzene−T4-lysozyme complex. The symmetry of benzene makes it possible for the molecule to rotate around its sixfold symmetry axis in the binding pocket, causing the carbons to switch places. This turns out to be problematic for several reasons. First, both the Schlitter and quasiharmonic formulas assume that the ligand fluctuates about a single minimum. Also, even for relatively long simulations, these indistinguishable states are not equally sampled, which was also noted by HW. To sample only one single minimum, each of them were considered separately in the calculation of the rotational entropy. The calculated average is a mean of the three most populated states. The symmetry term associated with these rotations, R ln(12), can then be added to the calculated rotational entropy for one minimum. Once again, one can see that quasiharmonic analysis and Schlitter's formula yield overestimates of the absolute rotational entropy and hence also for the binding differences. The loss of rotational entropy upon binding for benzene was calculated to be −19.4 and −19.1 for Schlitter's formula and quasiharmonic analysis, respectively, and is thus an overestimate compared to ref 28. As noted already, a useful alternative to Cartesian coordinates is to use Euler angles to determine the rotational entropy loss. By assuming a uniform distribution of Euler angles in the bound state, the entropy change can be approximated to be

$$\Delta S_{\text{rot,uniform}} = R \ln\left[\frac{12^{3/2}\sigma_\phi\sigma_\psi\sigma_\theta \sin\bar{\theta}}{8\pi^2}\right] \qquad (16)$$

while a Gaussian distribution instead yields the expression

$$\Delta S_{\text{rot,gauss}} = R \ln\left[\frac{(2e\pi)^{3/2}\sigma_\phi\sigma_\psi\sigma_\theta \sin\bar{\theta}}{8\pi^2}\right] \qquad (17)$$

From these expressions, we find a loss of −7.8 cal/mol K, assuming a uniform distribution and −6.8 cal/mol K for a Gaussian distribution of Euler angles, which is in reasonable agreement with the results obtained by HW. For the Gaussian estimates (eqs 15 and 17) that could perhaps be considered the most reliable, the sum of translational and rotational entropy loss is −24.1 cal/mol K, which corresponds to a free energy ($-T\Delta S_{\text{transl/rot,gauss}}$) of 7.2 kcal/mol. This value is, in fact, quite close to the cratic entropy component estimated from free energy calculations in ref 28, but it should be kept in mind that these calculations are based on a "rigid" protein model.

TABLE 6: Change in Entropies (cal/mol K) and Corresponding Free Energy Contributions (kcal/mol at 300 K) for Benzene Binding to T4-Lysozyme Obtained from Unstrained MD Simulation of the Complex

	S_{Schl}	S_{qh}	S_{uniform}	S_{gauss}
ΔS_{transl}	−13.7	−13.7	−14.7	−13.7
ΔS_{rot}	−16.7	−16.4	−6.8	−5.7
ΔS_{vib}	2.8	1.6	1.6	1.6
$-T\Delta S_{\text{rot+transl+vib}}$	8.3	8.5	6.0	5.4

To provide a more reliable estimate of the binding entropy for benzene to T4-lysozyme, we also carried out these calculations, including the vibrational contribution, from unrestrained MD simulations of the protein−ligand complex. These data are shown in Table 6, where it can be seen that the translational and rotational entropy loss upon binding is significantly reduced by employing a fully flexible protein model. This is as expected, because a rigid model is bound to reduce the configurational space of the ligand more than a flexible one does. It may also be noted that the vibrational term is small, but it is predicted to contribute favorably to binding. Hence, our best estimate for the total $-T\Delta S$ term in the benzene−lysozyme case is 5.4 kcal/mol, and this is then based on eqs 15 and 17 for the translational and rotational contributions together with eq 4 (quasiharmonic approximation) for the vibrational term.

It should be noted here that solvent entropy effects are not addressed in the present work or in the HW calculations, but this contribution, in general, can be of major importance for protein−ligand binding affinities. For example, the binding of large nonpolar ligands to proteins can be acompanied by a significant entropy increase,[38] which reflects an overall hydrophobic effect that overshadows other entropy contributions. The calculation of solvent entropies has been addressed recently by Peter et al. using standard methods that involve computing ensemble averages of the entire system Hamiltonian, which is associated with rather severe convergence problems.[39] An alternative method, described by Florian and Warshel, uses the Langevin dipole (LD) model to estimate entropies of solvation for netural and ionic solutes.[40] That method is empirical, or parametrizable, and has also been used to calculate the solvent entropy contribution for chemical reactions.[36,37]

5. Conclusions

In this study, we have compared several methods for calculating molecular entropies from the covariance matrix of atomic fluctuations. We have found that all methods reproduce both absolute and relative translational entropies well and that representing the motion with the center of mass of the solute also gives good agreement with statistical thermodynamics. Rotational entropies are not predicted well by Schlitter's formula or quasiharmonic analysis, and it seems to be inappropriate to approximate rotational movement in Cartesian coordinates with a harmonic oscillator. A new method for estimating rotational entropies based on Euler angles has been presented, which gives considerably better agreement with theoretical values compared to Schlitter's formula and quasiharmonic analysis. We also found convincing agreement with the literature for the change in translational and rotational entropy for benzene when it binds to T4-lysozyme, but the methods used in this work are significantly easier to apply than those employed previously for this type of problem. A major challenge is obviously to be able to evaluate conformational entropies for very flexible molecules where more extensive sampling is required, and in this respect, it is not entirely clear what is the optimal computational method. The strategy used here could nevertheless be useful for estimating ligand entropy changes in binding processes and enzyme catalysis.

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APPENDIX 1

Rotational Entropy from Euler Angles. If the parts that depend on the momenta are integrated out, the rotational partition function for a molecule freely rotating in space is[32]

$$q_{\mathrm{rot}} = \frac{1}{\sigma_s h^3}(2\pi kT)^{3/2}(I_A I_B I_C)^{1/2} \int_{\phi=0}^{2\pi}\int_{\psi=0}^{2\pi}\int_{\theta=0}^{\pi} \sin\theta \, \mathrm{d}\phi \, \mathrm{d}\psi \, \mathrm{d}\theta \quad (18)$$

where I_A, I_B, and I_C are the principal moments of inertia, T is the temperature, and σ_s is the symmetry number of the molecule. For a molecule with restrained rotational freedom, the integral can be interpreted as the "rotational volume", $V_{\mathrm{rot}}(\phi, \psi, \theta)$, available to the molecule

$$V_{\mathrm{rot}}(\phi, \psi, \theta) = \int\int\int_{\substack{\text{allowed}\\ \text{angles}}} \sin\theta \, \mathrm{d}\varphi \, \mathrm{d}\psi \, \mathrm{d}\theta \quad (19)$$

The "rotational" volume for a molecule in term of $\Delta\varphi$, $\Delta\psi$, and $\Delta\theta$ can, using a mean angle approximation ($\sin\theta \approx \sin\bar{\theta}$), be expressed

$$V_{\mathrm{rot}}(\phi, \psi, \theta) \approx \Delta\phi\Delta\psi\Delta\theta \sin\bar{\theta} \quad (20)$$

where $\bar{\theta}$ is the average value of θ. If a uniform distribution is assumed, the volume can be found from principal variances: $\Delta\phi = 12^{1/2}\sigma_\phi$, $\Delta\psi = 12^{1/2}\sigma_\psi$, $\Delta\theta = 12^{1/2}\sigma_\theta$. That the above approximation is reasonable can be seen from the results in Table 2. A change in entropy between a freely rotating molecule and a molecule confined in a small interval of angles is then

$$\Delta S_{\mathrm{rot,uniform}} = R \ln\left[\frac{12^{3/2}\sigma_\phi\sigma_\psi\sigma_\theta \sin\bar{\theta}}{8\pi^2}\right] \quad (21)$$

where we assume that the moments of inertia are constant. The absolute entropy can be expressed as

$$S_{\mathrm{rot,uniform}} = R \ln\left[\frac{12^{3/2}}{\sigma}\left(\frac{2\pi ekT}{h^2}\right)^{3/2}(I_A I_B I_C)^{1/2}\sigma_\phi\sigma_\psi\sigma_\theta \sin\bar{\theta}\right] \quad (22)$$

For a Gaussian distribution of angles, the change in entropy can instead be written

$$\Delta S_{\mathrm{rot,gauss}} = R \ln\left[\frac{(2\pi e)^{3/2}\sigma_\phi\sigma_\psi\sigma_\theta \sin\bar{\theta}}{8\pi^2}\right] \quad (23)$$

and the corresponding absolute entropy is then

$$S_{\mathrm{rot,Gauss}} = R \ln\left[\frac{(2\pi e)^{3/2}}{\sigma}\left(\frac{2\pi ekT}{h^2}\right)^{3/2}(I_A I_B I_C)^{1/2}\sigma_\phi\sigma_\psi\sigma_\theta \sin\bar{\theta}\right] \quad (24)$$

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