Thermodynamic Sampling of Molecular Conformations

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Abstract

Torsional-space Monte Carlo simulations of flexible molecules are usually based on the assumption that all values of dihedral angles have equal probability in the absence of atomic interactions. In the present paper it is shown that this assumption is not valid. Thermodynamic sampling using dihedral angles or other internal coordinates has to account for both the correct metric in conformational space and the conformation-dependence of the moment of inertia tensor. Metric and moment of inertia terms appear as conformation-dependent factors in the partition function and are obtained by proper separation of internal and rotational degrees of freedom. The importance of both factors is discussed for a number of short peptides as well as for the folded and unfolded states of a protein. It is concluded that thermodynamic Monte Carlo simulations of protein folding that neglect these correction factors tend to underestimate the stability of the folded state.

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1 Introduction

Many organic molecules are able to adopt a large number of different conformations at room temperature which has far reaching consequences for their thermodynamic and biochemical properties. In order to separate rotational and translational degrees of freedom, molecular conformations are generally expressed in terms of internal coordinates where it is often sufficient to describe the conformational state of a molecule by a set of dihedral angles since the effect of high-frequency bond angle and length fluctuations can approximately be included in the potential energy [1].

Dihedral angle coordinates are frequently used to sample molecular conformations in Monte Carlo simulations of biomolecules (see for example [2, 3, 4, 5]). These thermodynamic simulations are usually based on the assumption that the volume element appearing in the partition function is given by $\prod_{i=1}^{M} d\phi_i$ where $\phi_i$ are dihedral angles and $M$ is the number of rotatable bonds. This means that in the absence of atomic interactions all values of dihedral angles are sampled with equal probability. In the present paper it is shown that this underlying assumption is generally not valid, and that the proper metric in internal coordinate space as well as effects arising from the conformation-dependence of the moment of inertia tensor have to be taken into account in order to accurately calculate thermodynamic quantities.

The main mathematical problem in a formulation of the statistical mechanics in the space of molecular conformations (which we will call briefly shape space or shape manifold in the following) poses the proper separation of motions in internal coordinates (or shape coordinates) and rotations. This is non-trivial since it is not possible to define a body frame of reference in a unique way as it can be done for a rigid body. In fact, one can choose an arbitrary coordinate system for each different conformation. It is known that this freedom in the choice of the coordinate system can be expressed in terms of a gauge potential [6, 7] where local gauge transforms correspond to independent rotations of coordinate systems, hence the gauge symmetry group is SO(3). An interesting property of systems with internal degrees of freedom is the possibility to generate a change in orientation solely by the variation of shape without the application of an external torque, i.e., by moving through a closed path in shape space. The most prominent example for this effect is the ability of a falling cat to land on its feet starting from an upside-down position while the total angular momentum is zero. Another example is the observation of a slow rotation of the whole system over time in zero-angular momentum molecular dynamics simulations of proteins [8]. This net rotation of the system is an example of a so-called geometric phase [9] which is independent of the parametrization of the closed path in shape space and also gauge-invariant. In the case of three-dimensional molecules the practical calculation of these geometric phases is complicated by the fact that the group of rotations, SO(3), is non-Abelian, however, this will not be important for the following discussion.

A deeper mathematical foundation of the subject as well as generalizations can be formulated using fiber-bundles [10]. From a classical mechanical standpoint all these rotation-related effects can of course also be discussed in terms of Coriolis forces. A comprehensive
recent review on the separation of internal motions and rotations in the $N$-body problem (with emphasis on gauge fields) has recently been given by Littlejohn and Reinsch [11].

In the following section we discuss the classical canonical partition function in shape space (its detailed derivation is given in the Appendix) and show that it introduces two shape-dependent correction factors which are not present in the usual Cartesian-space partition function involving atom coordinates. The first factor transforms the volume element in shape coordinates to the true volume element on the non-Euclidean shape manifold and involves the metric tensor. The second term reflects the conformation-dependence of the moment of inertia tensor. Using dihedral angles as shape coordinates the importance of these correction terms is discussed in Section 3 for a number of small peptides, Ace-(Ala)$_n$-Nme with $n = 1, 2, 3$, and the pentapeptide Met-enkephalin. In Section 4 we estimate and compare the correction terms that arise in the folded and unfolded states of a protein.

2 Statistical mechanics on the shape manifold

We consider a molecule consisting of $N$ atoms with masses $m_\alpha$ for which a conformation is uniquely described by $M$ shape coordinates $q^i$, $i = 1, \ldots, M$. The Cartesian atom coordinates are given as functions of the shape coordinates,

$$\tilde{c}_\alpha = \tilde{c}_\alpha(q^1, \ldots, q^M)$$

with $\alpha = 1, \ldots, N$. The vectors $\tilde{c}_\alpha$ are taken to be center of mass coordinates, i.e. we assume that for all tuples $(q^1, \ldots, q^M)$ it is $\sum_\alpha m_\alpha \tilde{c}_\alpha(q^1, \ldots, q^M) = 0$. The choice of the functions $\tilde{c}_\alpha$ is of course not unique because the atom coordinates for a given conformation are only determined up to an overall rotation of the molecule. In fact we could replace the functions $\tilde{c}_\alpha$ by their arbitrarily rotated versions,

$$\tilde{c}_\alpha \rightarrow R(q^1, \ldots, q^M) \cdot \tilde{c}_\alpha,$$

where the rotation matrix $R$ is an arbitrary function of the shape coordinates. The only assumption we make is, that $R(q^1, \ldots, q^M)$ and $\tilde{c}_\alpha(q^1, \ldots, q^M)$ are sufficiently well-behaved w.r.t. the existence of derivatives. Because of the shape-dependence of the atom coordinates, the moment of inertia tensor $\bar{M}$ (Appendix Eq. (19)) is also a function of the shape coordinates. It is convenient to write $\bar{M}$ as a dimensionless quantity,

$$\bar{M} = \bar{M}(q^1, \ldots, q^M) = \sum_\alpha \Lambda_\alpha^{-2} \left( |\tilde{c}_\alpha|^2 I - \tilde{c}_\alpha \otimes \tilde{c}_\alpha \right)$$

where

$$\Lambda_\alpha = \left( \frac{2\pi \hbar^2 \beta}{m_\alpha} \right)^{1/2}$$

is the thermal de Broglie wavelength of atom $\alpha$ and $\beta = 1/k_B T$ is the inverse temperature. Here, $\otimes$ denotes the outer product of three-dimensional vectors. We also define the gauge
potential (see Eq. (23) in the Appendix)

$$\vec{A}_i = \vec{A}_i(q^1, \ldots, q^M) = \vec{M}^{-1} \sum_\alpha \Lambda_\alpha^{-2} \vec{c}_\alpha \times \frac{\partial \vec{c}_\alpha}{\partial q^i},$$

(4)

which is dimensionless by definition (assuming that the shape coordinates $q^i$ are dimensionless), and the metric tensor $g_{ij}$ on the shape manifold (Appendix Eq. (25)) that can be written in a dimensionless form as

$$\tilde{g}_{ij} = \tilde{g}_{ij}(q^1, \ldots, q^M) = \sum_\alpha \Lambda_\alpha^{-2} \frac{\partial \vec{c}_\alpha}{\partial q^i} \cdot \frac{\partial \vec{c}_\alpha}{\partial q^j} - \vec{A}_i \cdot \vec{M} \cdot \vec{A}_j.$$  

(5)

As discussed in the Appendix, infinitesimal distances $ds = \sqrt{g_{ij}dq^idq^j}$ correspond (up to a constant prefactor) to mass-weighted root mean square deviations (RMSD) minimized w.r.t. rotations. This fact can in principle be used to approximate distances in shape space without referring to underlying coordinates.

The behavior of the gauge potential $\vec{A}_i$ under the gauge transform (2) is given by

$$\vec{A}_i \rightarrow \vec{R} \cdot \left(\vec{A}_i + \vec{\gamma}_i\right),$$

(6)

where $\vec{\gamma}_i$ is defined by the partial derivative of the rotation $\vec{R}$ w.r.t. the shape coordinates, $\partial \vec{R}/\partial q_i = \vec{R} \cdot \vec{\gamma}_i \times$. With (2) and (6) it is straightforward to see that the quantity $\partial \vec{c}_\alpha/\partial q_i - \vec{A}_i \times \vec{c}_\alpha$ and hence the metric tensor $g_{ij}$ (see Eq. (25) in the Appendix) is gauge-invariant, i.e. independent of the choice of the rotation functions $\vec{R}(q^1, \ldots, q^M)$. As noted in the introduction it can be shown that closed paths in shape space are associated with a change in orientation of the molecule. According to Eq. (22) in the Appendix the (gauge-dependent) infinitesimal rotation vector $d\vec{\phi}_0$ associated with the variation of shape coordinates $dq^i$ is given by $d\vec{\phi}_0 = -\vec{A}_i dq^i$. The net rotation $\mathbf{S}$ generated by moving through a closed path in shape space can be calculated by accumulating these infinitesimal rotations along the path. However, because of the non-Abelian nature of the rotation group SO(3), i.e. the fact that rotations do not commute if their rotation axes are different, it is only possible to express $\mathbf{S}$ in terms of a path-ordered product $\prod$. As a measurable quantity, $\mathbf{S}$ is of course gauge-invariant and also independent of the parametrization of the closed path. It is possible to derive an explicit expression for $\mathbf{S}$ in the case of infinitesimal small loops where a generalized version of Stokes’ theorem can be applied $\prod$. A consequence of the existence of orientational changes associated with closed loops in shape space is the fact that the shape manifold defined by the metric $g_{ij}$ cannot be embedded in the $3N$-dimensional coordinate space as a single-valued function, i.e. the shape manifold does not only exhibit curvature but also torsion $\prod$.

It is shown in the Appendix that the classical canonical partition function $Z$ in shape space reads

$$Z = 8\pi^2 \int dq^1 \cdots \int dq^M (\det \tilde{g}_{ij})^{1/2} (\det \tilde{M})^{1/2} e^{-\beta V(q^1, \ldots, q^M)},$$

(7)
where the prefactor $8\pi^2$ stems from the integration over all possible orientations of the molecule. Note, that no translational degrees of freedom have been taken into account in deriving $Z$ since the atom coordinates $\vec{c}_\alpha$ are defined as center of mass coordinates. However, because of the separability of center of mass movements in the equation of motion, translational effects can simply be included by an additional prefactor of $Z$. In the case of orientational constraints the prefactor $8\pi^2$ would have to be modified accordingly.

The terms $(\det \tilde{g}_{ij})^{1/2}$ and $(\det \tilde{M})^{1/2}$ can be included in the Boltzmann factor as effective conformation dependent energy terms $F_G$ and $F_M$,

$$Z = 8\pi^2 \int dq^1 \cdots \int dq^M e^{-\beta(V + F_G + F_M)}, \quad (8)$$

where

$$F_G(q^1, \ldots, q^M) = -\frac{k_B T}{2} \log(\det \tilde{g}_{ij}) \quad (9)$$

and

$$F_M(q^1, \ldots, q^M) = -\frac{k_B T}{2} \log(\det \tilde{M}). \quad (10)$$

Below, we will calculate $F_G$ and $F_M$ for a number of molecules. In the remainder of the paper it is assumed that the shape coordinates $q^1, \ldots, q^M$ are given by the dihedral angles $\phi_1, \ldots, \phi_M$, where $M$ is the number of rotatable bonds.

The effect of the correction factors $(\det \tilde{g}_{ij})^{1/2}$ and $(\det \tilde{M})^{1/2}$ becomes visible in the probability distributions $P_n(\phi)$ of the dihedral angles $\phi_n$ in the absence of atomic interactions. If the correction factors are omitted in Eq. (7) values of the dihedral angles are uniformly distributed with $P_i(\phi) = (2\pi)^{-1}$, a fact which is sometimes used to test microreversibility in Monte Carlo simulations with complicated concerted-rotation move sets \[4\]. Here, these distributions are given by

$$P_n(\phi) = \frac{1}{Z} \int d\phi_1 \cdots \int d\phi_{n-1} \int d\phi_{n+1} \cdots \int d\phi_M [\det \tilde{g}_{ij}(\phi_1, \ldots, \phi_{n-1}, \phi, \phi_{n+1}, \ldots, \phi_M)]^{1/2} \times [\det \tilde{M}(\phi_1, \ldots, \phi_{n-1}, \phi, \phi_{n+1}, \ldots, \phi_M)]^{1/2}, \quad (11)$$

where

$$Z = \int d\phi_1 \cdots \int d\phi_M [\det \tilde{g}_{ij}(\phi_1, \ldots, \phi_M)]^{1/2} [\det \tilde{M}(\phi_1, \ldots, \phi_M)]^{1/2}.$$

### 3 Correction factors for small molecules

In this section we calculate the conformation-dependent correction factors in the partition function \[7\] and the related quantities $F_G$ and $F_M$ at $T = 300$ K for the peptides Ace-(Ala)$_{1,2,3}$-Nme as well as for the pentapeptide Met-enkephalin with the sequence Tyr-Gly-Gly-Phe-Met. We only consider dihedral angles at fully rotatable bonds ($\omega$-angles are set to $180^\circ$) that do not connect to methyl- or NH$_3^+$-groups. Conformations of the polyalanines are parametrized by 2, 4, and 6 dihedral angles, while 17 dihedral angles have to be taken into
account for Met-enkephalin. Potential energies have been calculated with the Tinker software package \[13\] using the OPLS all-atom forcefield \[14\] (without electrostatic interaction cut off and with $\epsilon = 1$) in conjunction with a GB/SA implicit-solvent term \[15\].

Fig. 1 shows the terms $(\det \tilde{g}_{ij})^{1/2}$ and $(\det \tilde{M})^{1/2}$ as functions of the dihedral angles $\phi$ and $\psi$ for alanine dipeptide (Ace-Ala-Nme). It is seen that both terms show relative variations of about 25% and 30% respectively. The corresponding variations of $F_G$ and $F_M$ are 0.17 kcal/mol and 0.21 kcal/mol. Note that only these relative variations matter for the calculation of thermodynamic quantities. The distributions of the effective energies $F_G$ and $F_M$ for Ace-(Ala)$_2$-Nme and Ace-(Ala)$_3$-Nme are shown in Fig. 2, 3, and 4, where each point $(F_G, F_M)$ corresponds to a low-energy molecular conformation. For the polyalanines conformations have been obtained on an equidistant grid in dihedral angle space with grid spacing $\Delta \phi = \pi/5$. In total $10^4$ conformations have been sampled for Ace-(Ala)$_2$-Nme, and $10^6$ conformations for Ace-(Ala)$_3$-Nme. Of these conformations, Fig. 2 (Fig. 3) shows those 200 (1000) with the lowest potential energies. The insets in Fig. 2 and 3 give the corresponding cumulative energy distributions.

Conformations of the larger and more flexible molecule Met-enkephalin cannot be obtained by explicit enumeration. In this case conformations have been generated by randomly choosing dihedral angles and subsequently applying an annealing and energy minimization procedure that uses short stochastic dynamics runs at decreasing temperatures followed by steepest-descend minimization, and thereby mostly avoiding steric clashes and other high-energy situations. This way, 2000 conformations have been generated, of which those 200 with the lowest potential energies have been plotted in Fig. 4 along with the cumulative energy distribution shown in the inset. It is known that Met-enkephalin does not adopt a single conformation in aqueous solution at room temperature \[16\], last but not least because of its biological function as a neuro-transmitter binding to a number of different receptors. One may therefore expect, that many conformations generated by the procedure described above can actually be assumed by the molecule under biological conditions.

Variations $\Delta F_G$ and $\Delta F_M$ of the energies $F_G$ and $F_M$ in Fig. 2, 3, and 4 (here defined by the difference between maximal and minimal values in the data) strongly depend on the size of the molecule. While for Ace-(Ala)$_2$-Nme and Ace-(Ala)$_3$-Nme these variations are small ($\Delta F_G = 0.29$ kcal/mol and $\Delta F_M = 0.21$ kcal/mol in the first case, and $\Delta F_G = 0.53$ kcal/mol and $\Delta F_M = 0.35$ kcal/mol in the latter one), they become more significant for Met-enkephalin, where $F_G$ and $F_M$ vary by $\Delta F_G = 2.45$ kcal/mol and $\Delta F_M = 0.77$ kcal/mol according to the data plotted in Fig. 4. Fig. 5 shows those conformations of Met-enkephalin that correspond to the numbered data points in Fig. 4 where $F_G$ and $F_M$ and therefore $(\det \tilde{g}_{ij})^{1/2}$ and $(\det \tilde{M})^{1/2}$ assume extreme values. Clearly, conformations with small factor $(\det \tilde{M})^{1/2}$ exhibit a small radius of gyration and are therefore more compact than conformations with large values of this term. The interpretation of the term $(\det \tilde{g}_{ij})^{1/2}$ is more difficult and its values depend on the details of the molecule. However, consistent with the pictures shown in Fig. 5 it can be said that more “rigid”, stretched conformations lead to a smaller factor $(\det \tilde{g}_{ij})^{1/2}$ than “sloppier”, curved conformations, where small changes in the dihedral angles result in larger changes in the atom coordinates. Since conformations with
large radius of gyration are usually more stretched than those with small radius of gyration we may explain the weak negative correlation between \( F_G \) and \( F_M \) indicated by the straight lines (obtained by a least squares fit) in Fig. 2, 3, and 4 (correlation coefficient = \(-0.5427, -0.5405, \) and \(-0.1511\)). We do not find correlations between the potential energy \( E \) and \( F_G \) or \( F_M \) except for the case of Met-enkephalin, where \( E \) is negatively correlated with \( F_M \) because more compact conformations forming hydrogen bonds or salt bridges are found to be energetically favored by several kcal/mol.

The probability distributions \( P_n(\phi) \) of dihedral angles in the absence of atomic interactions defined in Eq. (11) can be calculated by first obtaining a series of conformations where all values of dihedral angles are sampled with equal probability. The histograms \( H_n(k) \) approximating \( P_n(\phi) \), are then given by

\[
H_n(k) = \frac{\langle \delta_k(\phi_n)(\det \tilde{g}_{ij})^{1/2}(\det \tilde{M})^{1/2} \rangle}{\langle (\det \tilde{g}_{ij})^{1/2}(\det \tilde{M})^{1/2} \rangle}
\]

where \( \langle \cdot \rangle \) denotes an average over the series of conformations, \( k = 0, \ldots, K - 1 \) (\( K \) being the number of bins), and

\[
\delta_k(\phi) = \begin{cases} 
0 & \text{for } \phi < \frac{2\pi k}{K} \\
1 & \text{for } \frac{2\pi k}{K} \leq \phi < \frac{2\pi (k+1)}{K} \\
0 & \text{for } \frac{2\pi (k+1)}{K} \leq \phi
\end{cases}
\]

It is \( KH_n(k) \approx 2\pi P_n(\phi) \) for \( \phi \in \left[ \frac{2\pi k}{K}, \frac{2\pi (k+1)}{K} \right) \). Fig. 6 shows the so-obtained distributions for all 17 dihedral angles of Met-enkephalin using \( 10^6 \) randomly sampled conformations. It is seen that deviations from the uniform distribution \( P_n(\phi) = (2\pi)^{-1} \) of up to 40% occur for some of the dihedral angles (most prominently for \( \phi_3, \phi_{11}, \) and \( \phi_{16} \), where the indices refer to the bond indices shown in Fig. 5). This again demonstrates that the correction factors \((\det \tilde{g}_{ij})^{1/2}\) and \((\det \tilde{M})^{1/2}\) are significant for Met-enkephalin.

## 4 Protein folding

In this section we discuss the terms \( F_G \) and \( F_M \) in the context of folded and unfolded states of a protein with radii of gyration \( R_{\text{folded}} \) and \( R_{\text{unfolded}} \). At first, an estimate of \( F_G \) is given considering only backbone dihedral angles (\( \phi \) and \( \psi \)-angles) with the assumption that the contributions of side chain dihedrals to the metric tensor \( g_{ij} \) are roughly the same for folded and unfolded states. In order to calculate the determinant of \( g_{ij} \) we have to estimate its eigenvalues \( \lambda \). One may argue, that the corresponding eigenvectors are localized on the peptide chain and span about seven adjacent torsion angles, having in mind that six is the number of angles needed to solve the so-called rebridging problem for polymer chains [17]. This means, that there is one free parameter in the concerted motion of seven adjacent \( \phi/\psi \)-angles leaving the rest of the protein unchanged. We therefore assume that an eigenvector of \( g_{ij} \) corresponds to a loop of about seven residues with length \( \ell \) and with endpoints \( A \)
and $B$. Let $r$ be the average distance of $A$ and $B$, and $\xi$ the length scale of the average lateral fluctuations of such a loop w.r.t. the axis $\vec{AB}$. Since this is a short loop (w.r.t. the persistence length scale) we estimate the scaling behavior of $\xi$ to be $\xi \sim \sqrt{\ell^2 - r^2}$. The corresponding eigenvalue of $g_{ij}$ should scale as $\lambda \sim m \xi^2$, where $m$ is the mass of the loop, and therefore $\det g_{ij} \sim (m \xi^2)^{2N}$, where $N$ is the number of residues. Assuming $r \ll \ell$ and taking $r$ to be proportional to the radius of gyration we obtain

$$\Delta F_G = F_G^{\text{unfolded}} - F_G^{\text{folded}} \approx Nk_B T \left( \frac{r_{\text{folded}}}{\ell} \right)^2 \left[ \left( \frac{R_{\text{unfolded}}}{R_{\text{folded}}} \right)^2 - 1 \right].$$

Using a value of 1.6 for the ratio of the radii of gyration in the unfolded and folded states from [18], and very roughly estimating $r_{\text{folded}}/\ell \approx 0.4$ from the analysis of C$_\alpha$ distances of protein structures, we find $\Delta F_G \approx 7.5$ kcal/mol at $T = 300$ K for a protein with $N = 50$ residues. One may therefore conclude that energy corrections due to the metric of the dihedral angle shape manifold are a significant energetic contribution and should be taken into account in folding simulations using Monte Carlo moves based on dihedral angles.

We now turn to the term $\det \mathbf{M}$. Since this term arises form the thermal equilibration of angular momentum its contribution is for instance not included in molecular dynamics simulations that enforce $L = 0$ for the protein. In order to estimate $F_M$ we have to consider inertial effects of the solvent as well. While these effects should be negligible for small molecules it is reasonable to assume that in the unfolded state of a globular protein many water molecules are effectively trapped and therefore rigidly coupled. For our estimate we therefore consider two limiting cases: complete viscous coupling of the solvent (i.e. neglecting inertial effects of the solvent at all) and complete rigid coupling, (i.e. all solvent molecules in the sphere defined by the radius of gyration $R$ are rigidly coupled to the protein). In the first case the components $M$ of the diagonalized moment of inertia tensor $\mathbf{M}$ scale as $M \sim m R^2$ where $m = \text{const.}$ is the mass of the protein, while in the latter case the mass $m$ itself scales as $m \sim R^3$, so that $M \sim R^5$ assuming a uniform mass density of protein and solvent. With $\det \mathbf{M} \sim (m R^2)^3$ we derive

$$\Delta F_M = F_M^{\text{unfolded}} - F_M^{\text{folded}} \approx -k_B T \kappa \log \left( \frac{R_{\text{unfolded}}}{R_{\text{folded}}} \right),$$

where $\kappa = 3$ in the case of viscously coupled solvent and $\kappa = 15/2$ for rigid coupling. At $T = 300$ K we get $\Delta F_M \approx -0.85$ kcal/mol in the first case and $\Delta F_M \approx -2.1$ kcal/mol in the second.

Note, that $\Delta F_G$ and $\Delta F_M$ have different signs: Unfolded or stretched conformations have a smaller value of $\det g_{ij}$ because the loops are stiffer and therefore dihedral angle variations result in smaller variations in Cartesian space, while the moment of inertia is the larger the larger the characteristic length scale of the molecule is. This confirms the trend that had already been observed for small molecules discussed in the previous section. It is seen that $|\Delta F_G|$ is by about a factor of 5 larger than $|\Delta F_M|$ for a protein with 50 residues. While $\Delta F_G$ depends linearly on the chain length, $\Delta F_M$ is independent of the size of the protein.
5 Discussion and conclusion

It has been shown in the present paper that statistical mechanical sampling of molecular conformations has to account for the correct metric $g_{ij}$ in conformational space as well as the conformation-dependence of the moment of inertia tensor $M$, both of which can be expressed in terms of effective conformation-dependent energy contributions, $F_G$ and $F_M$. Using dihedral angles as internal coordinates, the distribution of these energy contributions for low-energy conformations has been calculated numerically for a number of short peptides. While their influence is small for molecules with few rotatable bonds, we find variations of $F_G$ and $F_M$ of about 2.45 kcal/mol (for $F_G$) and 0.77 kcal/mol (for $F_M$) for the pentapeptide Met-enkephalin. A rough estimate of both terms in the folded and unfolded states of a protein with 50 residues leads to the significant energy difference of 7.5 kcal/mol for $F_G$ and between -0.85 kcal/mol and -2.1 kcal/mol for $F_M$. This shows that both correction terms should be taken into account when dihedral coordinates are used in thermodynamic Monte Carlo simulations of protein folding in order to accurately calculate thermodynamic quantities. Since $F_G + F_M$ is larger in the unfolded state of a protein, Monte Carlo simulations that omit these corrections lead to free energy differences between unfolded and folded states that are too small. These simulations therefore underestimate the stability of the folded state. The efficient implementation of metric and moment of inertia-related correction terms within a Monte Carlo algorithm will be subject of a future publication.

A related problem, where the consideration of the proper metric in conformational space is important, is the estimate of thermodynamic quantities from a given, finite ensemble of molecular conformations. Such ensembles can be generated for molecules with not too many rotatable bonds and allow for the calculation of free energies and conformational entropies which are otherwise difficult to access in importance sampling-based methods. An example is the calculation of protein side chain free energies [20] from rotamer libraries [21]. In principle it is possible to estimate thermodynamic properties such as entropies from any given ensemble of conformations without referring to underlying internal coordinates. Distances according to the metric $g_{ij}$ can be approximated by the mass-weighted RMSD between two conformations minimized w.r.t. rotations and translations. However, the calculation of the partition function would then require a triangulation procedure in order to calculate volume elements in conformational space.
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Appendix

In the following we will derive the classical canonical partition function $Z$ in shape space.

At first, an expression for the Hamiltonian $H$ is obtained which separates shape space and rotational contributions. A more detailed derivation of $H$ and a discussion of the related theory can be found in the review article [11]. In order to keep equations as simple as possible three different notations are used to distinguish between vectors in three-dimensional Cartesian space, sums over atom positions $\vec{c}_\alpha$, $\alpha = 1 \ldots N$ and sums over shape coordinates $q^i$, $i = 1 \ldots M$. Three-dimensional vectors are given in a vector notation with dots ($\cdot$) and crosses ($\times$) for scalar and vector products. The Einstein sum convention is employed for summation over latin indices involving the shape coordinates $q^i$. Sums over atom positions (greek indices) are written explicitly. We also define a $3N$-dimensional vector (denoted in bold face), $\mathbf{c} = (\vec{c}_1, \ldots, \vec{c}_N)$, that contains the (three-dimensional) atom positions as components. Vector operations, $\cdot$ and $\times$, acting on $3N$-dimensional vectors are meant to act on each vector component independently. Finally, $< | >$ is a scalar product in the $3N$-dimensional vector space defined by

$$< \mathbf{u} | \mathbf{v} > = \sum_\alpha m_\alpha \vec{u}_\alpha \cdot \vec{v}_\alpha,$$

where the associated norm is $\| \mathbf{u} \|^2 = < \mathbf{u} | \mathbf{u} >$.

Let a molecular conformation be given by a vector $\mathbf{c}$ and consider a (kinematic) variation of shape coordinates $\{ dq^i \}$ and the resulting variation $d\mathbf{c}$ in Cartesian space,

$$d\mathbf{c} = \left( \frac{\partial \vec{c}_1}{\partial q^i} dq^i, \ldots, \frac{\partial \vec{c}_N}{\partial q^i} dq^i \right).$$

The goal is to separate $d\mathbf{c}$ into a pure shape variation part $\mathbf{c}_\parallel$ and a pure rotational part $\mathbf{c}_\perp$,

$$d\mathbf{c} = d\mathbf{c}_\parallel + d\mathbf{c}_\perp,$$

such that both parts are orthogonal to each other

$$< d\mathbf{c}_\parallel | d\mathbf{c}_\perp > = 0.$$

This can be achieved by defining an infinitesimal rotation $\mathbf{R} = \mathbf{I} + d\vec{\phi} \times$ that acts on $\mathbf{c}$ where $d\vec{\phi}$ is an angular rotation vector. Then,

$$\mathbf{R} \mathbf{c} - \mathbf{c} = d\vec{\phi} \times \mathbf{c}$$

defines a three-dimensional linear subspace $V$ which is parametrized by $d\vec{\phi}$, and $d\mathbf{c}_\perp$ is the orthogonal projection of $d\mathbf{c}$ on $V$. The vector $d\mathbf{c}_\perp$ can be calculated by minimizing the infinitesimal distance

$$D(d\vec{\phi}) = \| d\mathbf{c} + d\vec{\phi} \times \mathbf{c} \|.$$
where the minimum \( D_0 \) is defined by
\[
D_0 = D(d\vec{\phi}_0) = \min_{d\vec{\phi}} \{ D(d\vec{\phi}) \}. \tag{17}
\]
This leads to the result
\[
d\vec{\phi}_0 = M^{-1} \cdot \sum_{\alpha} m_{\alpha} d\vec{c}_\alpha \times \vec{c}_\alpha \tag{18}
\]
where
\[
M = \sum_{\alpha} m_{\alpha} \left( |\vec{c}_\alpha|^2 \mathbf{I} - \vec{c}_\alpha \otimes \vec{c}_\alpha \right) \tag{19}
\]
is the moment of inertia tensor, and we have
\[
d\vec{c}_\perp = -d\vec{\phi}_0 \times \vec{c} \tag{20}
\]
and
\[
d\vec{c}_\parallel = d\vec{c} + d\vec{\phi}_0 \times \vec{c}. \tag{21}
\]
The rotation vector \( d\vec{\phi}_0 \) can be expressed in terms of a gauge potential \( \vec{A}_i \),
\[
d\vec{\phi}_0 = -\vec{A}_i dq^i, \tag{22}
\]
where \( \vec{A}_i \) is defined as
\[
\vec{A}_i = M^{-1} \sum_{\alpha} m_{\alpha} \vec{c}_\alpha \times \frac{\partial \vec{c}_\alpha}{\partial q^i}. \tag{23}
\]
Note, that the variation \( d\vec{c}_\parallel \) is independent of the freedom in the choice of the coordinate functions \( \vec{c}_\alpha(q^1, \ldots, q^M) \) described in Eq. (2) because it has been obtained by minimizing the distance \( D \), while \( d\vec{c}_\perp \) is non-unique and depends on this choice. According to Eq. (16) and (17) the infinitesimal distance \( D_0 \) corresponds to the mass-weighted RMSD between \( \vec{c} + d\vec{c} \) and \( \vec{c} \) minimized w.r.t. rotations.

Let us now use a body frame of reference and assume that the Cartesian coordinates of the system are momentarily described by the vector \( \vec{c} \). Consider a variation of Cartesian coordinates \( d\vec{r} \) which is the sum of a shape contribution \( d\vec{c} \) and an external infinitesimal rotation given by \( d\vec{\phi} \times \vec{c} \),
\[
d\vec{r} = d\vec{c} + d\vec{\phi} \times \vec{c}. \tag{24}
\]
Using Eqs. (20) and (21) we can write
\[
d\vec{r} = d\vec{c}_\parallel + (d\vec{\phi} - d\vec{\phi}_0) \times \vec{c}. \tag{24}
\]
Because the rotational part of \( d\vec{r} \), \( (d\vec{\phi} - d\vec{\phi}_0) \times \vec{c} \), is an element of the linear space \( V \), it is also orthogonal to \( d\vec{c}_\parallel \). This can be independently verified using the definition of the scalar product (12).

The kinetic energy of the system is given by
\[
T = \frac{1}{2} \sum_{\alpha} m_{\alpha} \left( \frac{d\vec{r}_\alpha}{dt} \right)^2
\]
which can be expressed in terms of the scalar product \( \langle \mathbf{d} \mathbf{r} | \mathbf{d} \mathbf{r} \rangle \) as,
\[
T = \frac{1}{2} \left( \frac{d \mathbf{r}}{dt} \right) \cdot \left( \frac{d \mathbf{r}}{dt} \right).
\]
The orthogonality between the shape and rotational part of \( d \mathbf{r} \) described above now allows us to separate both contributions in the expression for \( T \),
\[
T = \frac{1}{2} \sum_{\alpha} m_{\alpha} \left\{ \left( \frac{\partial \mathbf{c}_{\alpha}}{\partial q_i} - \mathbf{A}_i \times \mathbf{c}_{\alpha} \right) \cdot \left( \frac{\partial \mathbf{c}_{\alpha}}{\partial q_j} - \mathbf{A}_j \times \mathbf{c}_{\alpha} \right) \dot{q}_i \dot{q}_j + \left( \mathbf{\omega} \times \mathbf{c}_{\alpha} + \mathbf{A}_i \times \mathbf{c}_{\alpha} \dot{q}_i \right)^2 \right\},
\]
where the angular velocity vector is defined by \( \mathbf{\omega} = \frac{d \mathbf{\phi}}{dt} \) and we inserted the expressions (20), (21), and (22). With the definition of the metric tensor
\[
g_{ij} = \sum_{\alpha} m_{\alpha} \left( \frac{\partial \mathbf{c}_{\alpha}}{\partial q_i} - \mathbf{A}_i \times \mathbf{c}_{\alpha} \right) \cdot \left( \frac{\partial \mathbf{c}_{\alpha}}{\partial q_j} - \mathbf{A}_j \times \mathbf{c}_{\alpha} \right) = \sum_{\alpha} m_{\alpha} \frac{\partial \mathbf{c}_{\alpha}}{\partial q_i} \cdot \frac{\partial \mathbf{c}_{\alpha}}{\partial q_j} - \mathbf{A}_i \cdot \mathbf{M} \cdot \mathbf{A}_j \quad (25)
\]
the kinetic energy \( T \) can finally be written as
\[
T = \frac{1}{2} g_{ij} \dot{q}_i \dot{q}_j + \frac{1}{2} (\mathbf{\omega} + \mathbf{A}_i \dot{q}_i) \cdot \mathbf{M} \cdot (\mathbf{\omega} + \mathbf{A}_j \dot{q}_j). \quad (26)
\]
We now assume that the system is only subject to forces between the atoms, which means that the potential energy \( V = V(q^1, \ldots, q^M) \) is a function of the shape coordinates alone and the Lagrangian can be written as
\[
\mathcal{L} = T - V(q^1, \ldots, q^M). \quad (27)
\]
From Eq. \( (26) \) the angular momentum \( \mathbf{\tilde{L}} \) is obtained by
\[
\mathbf{\tilde{L}} = \frac{\partial T}{\partial \mathbf{\omega}} = \mathbf{M} \cdot (\mathbf{\omega} + \mathbf{A}_i \dot{q}_i), \quad (28)
\]
and the generalized momenta \( p_i \) associated with the shape coordinates \( q^i \) are
\[
p_i = \frac{\partial T}{\partial \dot{q}_i} = g_{ij} \dot{q}_j + \mathbf{\tilde{L}} \cdot \mathbf{A}_i. \quad (29)
\]
In the case of zero angular momentum, i.e. the absence of any external torque, the rotation generated by an internal motion \( \dot{q}_i \) is therefore given by \( \mathbf{\omega} = -\mathbf{A}_i \dot{q}_i \). Comparing this with Eq. \( (22) \) shows that the shape manifold itself is actually defined by the condition \( \mathbf{\tilde{L}} = 0 \) \[11\].

Now, the Hamiltonian \( \mathcal{H} = T + V \) can be obtained by solving \( \mathcal{H} \) and \( \mathcal{L} \) for the angular and shape coordinate velocities and inserting them into the kinetic energy \( (26) \),
\[
H = \frac{1}{2} g^{ij} (p_i - \mathbf{\tilde{L}} \cdot \mathbf{A}_i)(p_j - \mathbf{\tilde{L}} \cdot \mathbf{A}_j) + \frac{1}{2} \mathbf{\tilde{L}} \cdot \mathbf{M}^{-1} \cdot \mathbf{\tilde{L}} + V(q^1, \ldots, q^M). \quad (30)
\]
Here, \( g^{ij} \) is the inverse metric tensor, and \( q^i \) and \( p_i \) are canonically conjugated variables. It is not possible to define coordinates which are canonically conjugated to the angular momentum \( \vec{L} \) [11], because the angular velocity \( \vec{\omega} \) cannot be written as a simple time derivative of angular coordinates. Nevertheless, in order to derive the partition function \( Z \) from \( \mathcal{H} \), it is necessary to describe the rotational part of the Hamiltonian \( \mathcal{H} \) with canonical variables as well. This can be achieved by using Euler angles \( \theta, \varphi, \psi \) and their canonically conjugated momenta \( p_\theta, p_\varphi, p_\psi \). Here, it is convenient to use the principle axes of the moment of inertia tensor \( \mathbf{M} \) as the basis of the underlying coordinate system (where \( \mathbf{M} \) is diagonal). With

\[
\vec{\omega} = \begin{pmatrix}
\dot{\theta} \cos \psi + \dot{\psi} \sin \psi \sin \theta \\
\dot{\theta} \sin \psi - \dot{\psi} \cos \psi \sin \theta \\
\dot{\psi} + \psi \cos \theta
\end{pmatrix}
\]

we have

\[
\vec{L} = \frac{1}{\sin \theta} \begin{pmatrix}
\cos \psi \sin \theta p_\theta + \sin \psi (p_\varphi - \cos \theta p_\psi) \\
\sin \psi \sin \theta p_\theta - \cos \psi (p_\varphi - \cos \theta p_\psi) \\
\sin \theta \dot{p}_\psi
\end{pmatrix},
\]

and the rotational part of kinetic energy, \( \frac{1}{2} \vec{L} \cdot \mathbf{M}^{-1} \cdot \vec{L} \), becomes

\[
T = \frac{1}{2} \left\{ \frac{[\varphi - p_\psi \cos \theta] \sin \psi + p_\theta \sin \theta \cos \psi)^2}{M_1 \sin^2 \theta} + \frac{[\varphi - p_\psi \cos \theta] \cos \psi - p_\theta \sin \theta \sin \psi)^2}{M_2 \sin^2 \theta} + \frac{p_\psi^2}{M_3} \right\}.
\]

where \( M_1, M_2, \) and \( M_3 \) are the diagonal components of the moment of inertia tensor. The classical canonical partition function \( Z \) of the system is then given by (no sum convention!)

\[
Z = \int \cdots \int \left( \prod_{i=1}^{M} \frac{dp_i dq_i}{2\pi\hbar} \right) \frac{d\theta d\varphi dp_\theta dp_\varphi dp_\psi}{(2\pi\hbar)^3} e^{-\beta \mathcal{H}(q^1, \ldots, q^M, p_1, \ldots, p_M, \theta, \varphi, \psi, p_\theta, p_\varphi, p_\psi)},
\]

where \( \beta \) is the inverse temperature. The factor \( \left( \frac{1}{2\pi\hbar} \right)^{M+3} \) comes from the standard “coarse-graining” procedure in phase space which is a way to derive the correct quantum-mechanical prefactor of classical partition functions [19]. In this procedure, as a consequence of the Heisenberg uncertainty relation, the phase space is divided into cells with volume \( \Delta p \Delta q = 2\pi\hbar \), where \( p \) and \( q \) are arbitrary pairs of conjugated generalized canonical coordinates and momenta.

In order to obtain the partition function in the shape coordinates \( q^i \) alone we first integrate (31) over the generalized momenta \( p_i \), followed by an integration over the Euler momenta \( p_\theta, p_\varphi, p_\psi \). These integrals are purely Gaussian and can readily be performed analytically. Finally, the integration over all orientations of the system, i.e. the Euler angles themselves, is trivial and results in a numerical factor \( 8\pi^2 \). This leads to the final result

\[
Z = 8\pi^2 \left( \frac{1}{2\pi\hbar \beta} \right)^{M+3} \int dq^1 \cdots \int dq^M (\det g_{ij})^{1/2} (\det \mathbf{M})^{1/2} e^{-\beta \mathcal{V}(q^1, \ldots, q^M)}.
\]
It is seen, that the factor \((\text{det} \, g_{ij})^{1/2}\) which depends on the shape coordinates \(q^i\) results from the equilibration of the momenta in shape space, while the factor \((\text{det} \, \mathbf{M})^{1/2}\) originates from the equilibration of angular momentum. Upon absorbing the factor \(\left(\frac{1}{2\pi \hbar^2 \beta}\right)^{\frac{M+3}{2}}\) in the determinants we arrive at the expression for \(Z\) given in Eq. (7).
Fig. 1. The terms \((\det \tilde{g}_{ij})^{1/2}\) (top) and \((\det \tilde{M})^{1/2}\) (bottom) as function of the dihedral angles \(\phi\) and \(\psi\) for alanine dipeptide (Ace-Ala-Nme).
Fig. 2. Effective energies $F_G$ and $F_M$ for low-energy conformations of Ace-(Ala)$_2$-Nme. The inset shows the cumulative distribution of potential energies of the sampled conformations.
Fig. 3. Effective energies $F_G$ and $F_M$ for low-energy conformations of Ace-(Ala)$_3$-Nme. The inset shows the cumulative distribution of potential energies of the sampled conformations.
Fig. 4. Effective energies $F_G$ and $F_M$ for low-energy conformations of Met-enkephalin. The inset shows the cumulative distribution of potential energies of the sampled conformations.
Fig. 5. Conformations of Met-enkephalin corresponding to the numbered data points in Fig. 4 for which either $F_G$ or $F_M$ take maximal or minimal values.
Fig. 6. Probability distributions of dihedral angles $\phi_i$ for Met-enkephalin in the absence of atomic interactions. The indices $i$ correspond to the bond indices given in Fig. 5.