How Much Entropy is Contained in NMR Relaxation Parameters?

Falk Hoffmann,^{*,†,‡} Frans A. A. Mulder,^{*,¶} and Lars V. Schäfer^{*,†}

[†]*Center for Theoretical Chemistry, Ruhr University Bochum, D-44780 Bochum, Germany*

[‡]*Current address: Peptone Inc., Northcliff House, Young Street, London, England W8 5, U.K.*

[¶]*Interdisciplinary Nanoscience Center (iNANO) and Department of Chemistry, University of Aarhus, DK-8000 Aarhus, Denmark*

E-mail: falk@peptone.io; fmulder@chem.au.dk; lars.schaefer@ruhr-uni-bochum.de

Phone: +49 (0)234 32 21582

Abstract

Solution-state NMR relaxation experiments are the cornerstone to study internal protein dynamics at atomic resolution on time scales that are faster than the overall rotational tumbling time, τ_R . Since the motions described by NMR relaxation parameters are connected to thermodynamic quantities like conformational entropies, the question arises how much of the total entropy is contained within this tumbling time. Using all-atom molecular dynamics (MD) simulations of T4 lysozyme, we found that entropy buildup is rather fast for the backbone, such that the majority of the entropy is indeed contained in the short-time dynamics. In contrast, the contribution of slow dynamics of side chains on time scales beyond τ_R on the side chain conformational entropy is significant and should be taken into account for the extraction of accurate thermodynamic properties.

Introduction

Molecular recognition between biomolecules guides many biological processes. The enthalpic contribution to the free energy can often be understood based on minimum-energy structures and plays a fundamental role in computer-aided drug design.¹ However, the entropic contribution to the free energy is more difficult to understand at the atomic level and is the topic of intense investigation.^{2–14}

For a protein in solution, both solvent and protein entropy play an important role for the thermodynamics of the system. In pharmaceutical drug design, one is often interested in site-specific modulations that change the conformational entropy of the protein and/or the ligand. An empirical "entropy meter" has been proposed, based on the idea that amide and methyl order parameters from NMR relaxation experiments, which report on the dynamics of protein bonds on ps to ns time scales, can be used as proxies for the backbone and side chain conformational entropy changes in molecular recognition and binding events.^{15–17} Although methyl groups are present only in 6 of the 20 genetically encoded amino acid types in a protein, *i.e.*, residues I, L, V, M, A, T, changes in methyl order parameters provide a reasonably good estimator for the total side chain entropy change of a protein.¹⁵ While the correlation between NMR order parameter changes and conformational entropy changes is promising, obtaining a perfect correlation presupposes that NMR order parameters report on the full dynamics that are responsible for the change in conformational entropy.

Unfortunately, protein relaxation rates in solution are insensitive to dynamics on time scales that are much longer than the global rotational (tumbling) time of the protein,¹⁸ which is typically about 10 ns for a 20 kDa protein in aqueous solution at room temperature. Although it is possible to detect motions on the ns time scale using careful analysis of relaxation data at multiple field strengths,^{19–22} the internal dynamics is convoluted with the overall (and anisotropic) diffusion of the protein to such an extent that it often cannot be separated, particularly in the case of side chains where methyl groups are subject to rotameric transitions.²³ It is therefore of interest to quantify the contribution of conformational entropy

that is accessible to NMR relaxation with respect to the full conformational entropy.

On the computational side, determining the absolute entropy in principle requires counting of all microstates of a system within a given energy range. While this can be possible for small systems, the exceedingly large number of states in large molecules with many degrees of freedom renders the determination of the complete conformational entropy via counting excessively demanding. For complex systems like proteins, the configuration space is therefore usually discretized into coarse bins. If the dynamics inside a bin contribute equally among all bins to the full entropy and the time scale of the intra-bin dynamics is faster than the dynamics of changes between different bins, the contribution from intra-bin dynamics can be estimated as an offset to the conformational entropy from the inter-bin dynamics. While such coarse graining of phase space renders the estimation of the conformational entropy practically feasible, it is inherently connected to a loss of information. Therefore, it is necessary to ensure that motions sampled from transitions between (coarse-grained) bins are still representative for motions of the full system. In the case of protein dynamics, suitable degrees of freedom for the backbone and side chain conformations are the corresponding backbone (ϕ, ψ) and side chain (χ_i) dihedral angles, respectively. Conformational transitions in the protein backbone are directly connected to changes in backbone dihedral angles. Side chains undergo jumps around dihedral angles to interconvert between distinct rotamer states, which can be used for coarse graining the side chain conformational space. These rotamer jumps can occur on a broad range of time scales and also involve rare transitions (slow dynamics) on nanoseconds and beyond. Other side chain motions are usually much faster, such as bond vibrations, angle librations, dihedral vibrations within a single rotamer well, and rotations of methyl groups around their 3-fold symmetry axis.

In the interpretation of experimental NMR relaxation rates, librational motions are widely described by a diffusion-in-a-cone model.^{24,25} With such a simple analytical model, (changes of) NMR relaxation parameters, especially generalized order parameter S^2 , can be transformed into conformational entropy changes, for example due to an amino acid muta-

tion or ligand binding.^{2,26-29} Order parameters of rigid bonds in proteins, such as amide N-H bonds in well-structured regions, can usually be obtained with high accuracy from MD simulations. However, bonds undergoing transitions between states with long residence times, as is the case for many methyl C-H bonds in protein side chains, are more challenging.³⁰⁻³³ Low methyl order parameters are connected to the population of several side chain rotamer states, which interconvert on a broad range of time scales.^{31,34-36} Slow internal dynamics could also occur for the protein backbone, for example in loop regions or (intrinsically) disordered parts.³⁷⁻³⁹ Studies from the Wand lab¹⁵⁻¹⁷ led to the development of an empirical "entropy meter", in which the change in order parameters derived from NMR relaxation experiments is used to estimate the change in conformational entropy. While these approaches are promising and widely used, they can – by construction – only report on the entropy contributions due to motions that can contribute to NMR relaxation, *i.e.*, within time scales that are faster than the rotational tumbling time of the protein.

In an alternative approach, Li and Brüschweiler⁴⁰ created a dictionary of protein side chain entropies from NMR relaxation parameters. It was found that types of amino acids can be grouped together, where the conformational entropy of members of each group correlate similarly to methyl order parameter, but different relations are found between different groups. The effect of dihedral angle correlations on the change of the conformational entropy upon binding is small if the type of motion, *e.g.*, rotameric jumps, is unaffected.⁴¹ This suggests that (changes in) protein entropy can be estimated from a sum of local contributions, although the calibration of the dictionary may vary from protein to protein.⁴² Using a maximum information spanning tree (MIST) approach, Fleck and Zagrovic⁴³ showed that access to the internal correlation function would be adequate to predict entropy, either from backbone or methyl side chain vectors, and showed that the latter two quantities are also strongly correlated, lending ample support for deriving entropy from NMR dynamics.

The convergence of protein simulations has been investigated in numerous studies. Particularly relevant in the context of the present work is the insightful study of Genheden and

Ryde, who systematically analyzed the buildup of conformational entropy as a function of MD simulation time for a few small model proteins.⁴⁴ A striking conclusion of their study was that even the phase space explored in a 1 millisecond trajectory of the small (58 residues) protein BPTI was insufficient to obtain a strictly converged entropy estimate, because all relevant dihedral rotamer states were still incompletely sampled on that time scale. Genheden and Ryde⁴⁴ did not directly link their observations to NMR relaxation and the empirical entropy meter, which is the topic of this work.

We elucidate here how dynamics taking place on various time scales influence the estimation of conformational entropy. MD simulations of T4 lysozyme (T4L) in solution were carried out and the amount of conformational entropy assessed in the simulations due to atomic motions on different time scales was analyzed. Two modern biomolecular force fields were used, namely AMBER ff99SB*-ILDN^{45,46} and CHARMM36,⁴⁷ in each case with reparametrized energy barriers for rotation of methyl groups.^{30,32} It is shown that the majority of the T4L backbone entropy is linked to motions that are faster than the global tumbling time of the protein and should hence be accessible to NMR relaxation experiments in solution. In addition, motions within single rotamer wells are sampled on ps time scales, *i.e.*, much faster than the tumbling time of the protein, although slow motions beyond that time scale are also found to be present for many side chains. Thus, entropy contributions arising from infrequent side chain dihedral angle jumps are incompletely captured by classical NMR relaxation experiments. As a consequence, entropy calibration is sensitive to the protein rotational diffusion time, and thereby to protein size and temperature.

Methods

Simulation Setup

The setup of the MD simulations of wild type T4L with the AMBER force field was described previously.³¹ In brief, the protein was placed in a periodic truncated dodecahedron simulation

cell with a minimum distance between protein and the box boundary of initially 1.2 nm, and solvated by adding hydrogens to the crystal waters and 12081 additional TIP4P/2005⁴⁸ water molecules. For the simulations with the CHARMM36 force field, the protein was centered in a periodic cubic box with an edge length of 8.0 nm, and solvated by adding hydrogens to the crystal waters and additional 15979 TIP3P water molecules (the CHARMM-specific TIP3Pm water model was used). Sodium and chloride ions were added to obtain neutral simulation systems at a salt concentration of 0.15 mol/l.

MD Simulations

All MD simulations were carried out with GROMACS (version 5.0.6).⁴⁹ First, the systems were energy minimized and then equilibrated for 200 ps in the NpT ensemble with harmonic position restraints on all protein heavy atoms (with force constants of 1000 kJ mol⁻¹ nm⁻²). To keep the temperature constant at 300 K, the velocity rescaling thermostat of Bussi and coworkers⁵⁰ was applied, with a coupling time constant of $\tau_T = 0.1$ ps. Isotropic Parrinello-Rahman coupling was used to maintain 1 bar pressure, with a 2 ps coupling time constant and a compressibility of 4.5×10^{-5} bar⁻¹. The SETTLE⁵¹ and LINCS⁵² constraint algorithms were applied to constrain the internal degrees of freedom of water molecules and the bond lengths in the protein, respectively, allowing to integrate the equations of motion with 2 fs time steps. The cutoff for the short range Coulomb and Lennard-Jones 12-6 interactions was set to 1.0 nm. Analytical dispersion corrections were added to energy and pressure to correct for the truncation of the Lennard-Jones interactions. Long-range Coulomb interactions were treated with the smooth particle-mesh Ewald (PME) method⁵³ with a 0.12 nm grid spacing.

The equilibrated configurations were used as starting structures for the subsequent production simulations, during which the position restraints were switched off. Ten individual simulations of length 300 ns, each initialized with different atomic velocities, were carried out. Coordinates were saved to disk every 1 ps. Figure S1 shows that the 1 ps time spacing used to store the atomic coordinates to disk suffices to reliably extract the amount of con-

formational entropy from the simulation trajectories already at fast time scales of about 0.5 to 1 ns.

Conformational entropy

The quasi-harmonic approximation (QHA) as formulated by Schlitter⁵⁴ was used to estimate the backbone conformational entropy from the atomic fluctuations. The QHA provides an upper bound to the true entropy,

$$S_{\text{conf}} = \frac{k_B}{2} \ln \det \left(\mathbf{1} + k_B T e^2 \hbar^{-2} \mathbf{M}^{1/2} \mathbf{C} \mathbf{M}^{1/2} \right) \geq S_{\text{true}}, \quad (1)$$

where k_B is the Boltzmann constant, T is the temperature, e is Euler’s number, $\hbar$ is the reduced Planck constant, and $\mathbf{M}$ is the $3N$ -dimensional diagonal mass matrix of the N particles. $\mathbf{C}$ is the covariance matrix characterizing the fluctuations of particle positions,

$$\mathbf{C} = \left\langle (\vec{x} - \langle \vec{x} \rangle) (\vec{x} - \langle \vec{x} \rangle)^T \right\rangle, \quad (2)$$

where the $3N$ -dimensional vector $\vec{x}$ represents the Cartesian coordinates of the N backbone C_α -atoms for which the entropy was calculated. The initial configuration was used as a reference structure for a least-squares fit of trajectory configurations to remove overall translational and rotational motion. The averaging, indicated by $\langle \cdots \rangle$, was carried out over all trajectory frames up to a certain time t to yield the entropy buildup over time.

As described, the QHA was carried out in Cartesian coordinates. The choice of Cartesian or internal coordinates (bonds, angles, dihedrals) has been discussed in the literature, and both have advantages and disadvantages.^{55–57} In principle, the coordinates that yield the lower entropy estimate is the preferable one. However, it is not clear *a priori* which one is better for a given system. In the present work, the main focus is on the entropy buildup over time. Chang and coworkers⁵⁵ have shown for a small model system (*n*-heptane) that the rate of conformational entropy buildup is similar for Cartesian and internal coordinates.

To calculate backbone and side chain entropies on a per-residue basis, the dihedral angle probability distributions were histogrammed.^{58,59} Early work has shown that the contributions of bond and angle fluctuations (but not necessarily of the coupling between angles and dihedrals) are negligible.^{59,60} Backbone ϕ and ψ dihedral angles and side chain χ dihedral angles were extracted from the MD trajectories and binned in increments of 20° and 120° for ϕ/ψ and χ , respectively. For the side chain χ dihedral angles, the discretization was chosen such as to represent the three main rotamer wells *trans*, *gauche*⁺, and *gauche*⁻.⁶¹ For methyl-containing side chains, additional analyses with a finer 3° binning of the χ dihedral angles were carried out. For each backbone group, the two-dimensional (2D) ϕ/ψ histogram was accumulated and the 2D joint probability distribution $P_{\text{bb}}(\phi, \psi)$ was computed. Similarly, for the side chains, rotamer probability distributions $P_{\text{sc}}(\chi)$ of all M bins were constructed from the χ -angle histograms. Here, $M = 3^{N_\chi}$ are the different rotamer states of each side chain for 120° bins, $M = 120^{N_\chi}$ for 3° bins, and N_χ is the number of side chain χ dihedral angles. The backbone and side chain entropies were calculated according to

$$S_{\text{bb}} = -k_B \sum_{i=1}^{18} \sum_{j=1}^{18} P_{\text{bb}}(\phi_i, \psi_j) \ln P_{\text{bb}}(\phi_i, \psi_j) \text{ and} \quad (3)$$

$$S_{\text{sc}} = -k_B / N_\chi \sum_{i=1}^M P_{\text{sc}}(i) \ln P_{\text{sc}}(i) , \quad (4)$$

respectively, where the sum is over all bins i and j for the backbone, or over all $M = N_{\text{rot}}^{N_\chi}$ rotamer combinations of the N_χ side chain dihedral angles with N_{rot} bins per dihedral angle (*i.e.*, $N_{\text{rot}} = 3$ for 120° bins and $N_{\text{rot}} = 120$ for 3° bins). As suggested by Kasinath and coworkers,¹⁵ the side chain entropies were "normalized" by the number of χ -angles in the respective side chain, N_χ . The entropies calculated by Eqs. 3 and 4 depend on the bin size.⁵⁸ On the one hand, this is intended in the context of this study, especially for side chains, because we aim at separating the entropy due to fast intra-bin dynamics (*i.e.*, motions inside a single rotamer well) from the entropy associated with slower inter-bin dynamics (*i.e.*, linked to jumps between different rotamer wells by crossing dihedral angle barriers).

The former dominates the value of the entropy obtained via Eq. 4 with 3° bins whereas the latter dominates for 120° bins. On the other hand, the bin-size dependence might also be a bit puzzling because for 3° bins a large fraction of the total entropy is naturally sampled within very fast time scales (much faster than τ_R), which might lead to the impression that only an insignificant part of the entropy is missed in NMR relaxation. However, this would be misleading because one is often interested in entropy *differences*, *e.g.*, between two (or more) biophysical states, and the contributions due to changes in the width of the dihedral angle rotamer wells are negligible compared to the contributions due to changes in the populations of the distinct rotamer wells.^{15,62,63} Therefore, to provide an estimate that is less bin-size dependent, a normalized side chain entropy was additionally calculated according to⁴⁴

$$\frac{S_{\text{norm}}}{N_\chi k_B} = \frac{1}{2N_\chi} - \ln N_{\text{rot}} - \frac{1}{N_\chi} \sum_{i=1}^M P_{\text{sc}}(i) \ln P_{\text{sc}}(i) \quad (5)$$

The first two terms are normalization factors such that Eq. 5 yields entropies ranging between that of a free rotor in which all dihedral angle bins of the side chain are equally populated (uniform distribution, maximum entropy) and that of a frozen state in which only a single bin is occupied (delta distribution, minimum entropy). The first two terms are constant (for a given bin size) and cancel for entropy differences, ΔS .

The absolute values of the entropy calculated with Eqs. 3 to 5 have no physical meaning because they lack the contributions of other degrees of freedom as well as the Jacobian and momentum contributions.⁵⁸ However, this is not of major importance here, because (i) we are interested in the *rate* of entropy buildup, and (ii) the former are expected to cancel to a large extent and the latter two cancel completely in entropy differences, ΔS . Furthermore, higher order interangle correlations are neglected, *e.g.*, due to correlated motions of ϕ and ψ angles that do not flank the same backbone amide or between backbone and side chain dihedral angles. Likewise, the quasiharmonic entropy (Eq. 1) does not include higher order correlations beyond pairwise linear correlations (*i.e.*, pairwise supralinear and higher order)

and anharmonicities (*i.e.*, non-Gaussian probability distributions). Although these can affect absolute entropies and also entropy differences,^{15,64–66} they are not expected to dramatically change the rate at which the entropy is built up, *i.e.*, the speed at which a certain part of configuration space is explored. If present, correlations would be expected to rather slow down convergence. For example, Baron and coworkers⁶⁴ showed that in simulations of the cc β peptide in water, 99% of the final entropy estimate of the standard quasiharmonic approximation were obtained within 0.7 μ s of simulation time, whereas 1.1 μ s were needed to reach the same percentage when anharmonicities and pairwise supralinear correlations between the quasiharmonic modes were included. Taking these aspects into account, and also considering that our "long-time limit" entropy of T4L, as obtained from the accumulated 3 μ s of MD simulation time carried out in the present work, will likely underestimate the true value because an even larger part of phase space will be sampled on even longer simulation times,⁴⁴ the difference between the entropy sampled within the overall rotational tumbling time of T4L and the long-time limit entropy reported in this work is a lower bound.

Results and Discussion

Backbone entropy from quasi-harmonic approximation

The buildup of the conformational entropy of the T4L backbone was obtained from the Schlitter QHA approach⁵⁴ (Figure 1). As described in the Methods section above, the QHA captures the collective motions of all backbone C^α atoms and provides an upper bound to the true conformational entropy. A principal limitation of QHA is that it overestimates S_{conf} due to the neglect of anharmonicity and higher-order correlations. However, the present study is less concerned with the absolute values than with the buildup rate, *i.e.*, the *relative* percentage of entropy that is linked to motions sampled within a certain time scale. The entropy buildup profiles obtained with both the AMBER ff99SB*-ILDN and the CHARMM36 force field are highly comparable and predict that about 95% of the total conformational entropy,

as obtained from the complete aggregated simulation time of $3 \mu s$, is sampled within the tumbling time τ_R , which is about 11 ns for T4L.³¹

Figure 1: Cumulative conformational entropy of the T4L backbone, calculated with the quasi-harmonic approximation, in the MD simulations with the AMBER ff99SB*-ILDN (A) force field, the CHARMM36 (B) force field and its difference (C). The thick blue line shows the average, while the thin lines show the data from the 10 individual simulations. The light gray area represents the protein tumbling time τ_R , which is about 11 ns for T4L in aqueous solution at room temperature.³¹ The dark gray area displays the conformational entropy due to motions beyond τ_R , which are sampled in MD but not accessible in NMR relaxation experiments in solution. The red and black dashed lines show the average entropy at τ_R and the long-time limit, respectively. The entropy difference in C) is calculated from the averaged buildup curves of the AMBER and CHARMM simulations. The red and black dashed lines in C) show ΔS at τ_R and in the long-time limit, respectively.

Interestingly, about 90% of the long-time limit entropy is already sampled within 1 ns, which is faster than the tumbling time of any folded protein in aqueous solution at ambient conditions. This means that the majority of the thermodynamic information related to backbone motion is contained in NMR relaxation data, although about 5% of the conformational entropy is due to slow motions and can only be detected on time scales longer than τ_R .

Often one is interested in entropy differences, ΔS , between two biophysical states, such as the folded and the unfolded state or the ligand-bound and -unbound state of a protein. How relevant is a difference of 5% between the absolute entropies at τ_R and the long-time limit, as seen in Figure 1A and B? To obtain an idea of the magnitude that one might expect in a real-life application, the difference between the entropy buildup curves from the AMBER and CHARMM simulations is plotted in Figure 1C. The plot shows that ΔS depends on the sampling time, with a difference between τ_R and the long-time limit of ca.

$4 k_B$, corresponding to 10 kJ/mol at 300 K. Such a free energy contribution is not negligible, *e.g.*, for ligand binding or protein folding.

Figure 1 shows that the entropy estimate keeps on growing linearly with the logarithm of time even for long time scales. This behavior was also found in the study by Genheden and Ryde.⁴⁴ They showed that such a time dependence can be explained by a simple model in which the different conformational minima are separated by uniformly distributed barriers that lie within a certain energy range. With the characteristic barrier crossing times being exponentially distributed according to an Arrhenius equation, the time scale associated with crossing of the highest energy barrier present in the system will dictate the time needed for the entropy to converge. This model provides valuable physical insights, but it remains somewhat unclear to what degree the underlying assumptions and simplifications are realistic for a protein such as T4L. In any case, the continuously raising curves in Figure 1 indicate that on the μ s time scale of the simulations new regions of phase space keep on being accessed, and hence our estimate of the long-time limit entropy underestimates the true value, as is also discussed at the end of the Methods section. Thus, the amount of undetected conformational entropy extracted from our analyses represents a lower bound.

Single-residue entropies: backbone

In an NMR relaxation experiment, one usually measures the dynamics of a local bond or interaction, and order parameters S^2 describe the degree of motional restriction of a specific bond. The aim of an entropy meter is to quantitatively connect these local dynamic probes to conformational entropies. Thus, single-residue conformational entropies were calculated from the distributions of the corresponding backbone or side chain dihedral angles (see Eqs. 3 and 4). These single-residue entropies complement the above QHA entropies, in the sense that (i) they are linked to local motions of individual residues, and (ii) they are based on internal coordinates (dihedral angles) instead of Cartesian coordinates used in the QHA (see Eqs. 1 and 2). The configurational entropies at the tumbling time and in the long-time limit

obtained from the dihedral angle distributions are provided in Tables S1 and S2 for all 158 non-proline residues of T4L (except MET1), and are shown in Figure 2 for the backbone and in Figure 3 for the protein side chains, respectively.

Figure 2: Conformational entropy sampled within the tumbling time τ_R (blue data points) and in the long-time limit (red data points) for the T4L backbone in the MD simulations with the AMBER (left) and CHARMM (right) force field. The entropy values are shown in panels A) and B). The distribution of the values is shown in panels C) and D). The ratio of the conformational entropies sampled within τ_R and in the long-time limit are plotted in panels E) and F). The distribution of the ratios is shown in panels G) and H). The errors plotted are the standard deviations from the 10 individual MD simulations.

Figure 3: Conformational entropy sampled within the tumbling time τ_R (blue data points) and in the long-time limit (red data points) for side chains with 120° binning of the dihedral angles. The entropy values are shown in panels A) and B). The distribution of the values is shown in panels C) and D). The ratio of the conformational entropies sampled within τ_R and in the long-time limit are plotted in panels E) and F). The distribution of the ratios is shown in panels G) and H). The errors plotted are the standard deviations from the 10 individual MD simulations.

Figure 4: Correlation between conformational entropies sampled after the tumbling time τ_R and in the long-time limit for the T4L backbone in the simulations with the AMBER (A) and CHARMM (B) force field.

The data show that the backbone entropy buildup is rather fast for most residues. For 146 of the 158 residues, more than 90% of the long-time conformational entropy is already reached within the tumbling time in the simulations with the ff99SB*-ILDN force field, and only three residues sample less than 80% of the long-time conformational entropy, GLY12 (72%), PHE67 (77%) and GLU11 (78%). This result is consistent with the interpretation that fast librational motions of backbone bonds rapidly sample the conformational space within a restricted region of the Ramachandran map. As long as there are no barrier-crossing transitions to alternative metastates, entropy buildup is fast. Except for PHE67, all residues with less than 90% of the long-time limit conformational entropy are located in the β -region encompassing residues 10 to 30 (see Figure 2). This region is highly conserved among different lysozyme families and is key to the enzyme’s catalytic activity.⁶⁷ Interestingly, the catalytic residues GLU11 and ASP20 are among those with the slowest entropy buildup, suggesting that their motional restriction and structural preorganization may be tuned for enzyme action. Although details vary, a similar overall picture emerges from the simulations performed with the CHARMM force field (Figure S2). The entropy buildup is slightly slower with the CHARMM36 force field than with AMBER ff99SB*-ILDN (see Tables S1 and S2). Especially for some residues close to the active site, the entropy buildup rate is

apparently lower for CHARMM36 than for AMBER ff99SB*-ILDN. This could indicate that the energy landscape of CHARMM36 is more rugged, a point that might warrant further investigations.⁶⁸

Even so, Figure 4 shows that there is a good correlation between the backbone configurational entropies sampled at τ_R and in the long-time limit, with Pearson correlation coefficients of 0.97 and 0.93 for AMBER and CHARMM, respectively. This shows that a simple relation between relaxation parameters and entropies, which underpins an empirical entropy meter, can be expected to work well for backbone motions, because the estimated entropies on short time scales are highly correlated to the full configurational entropies.

Figure 5 shows the entropy buildup of those T4L residues that exhibit the lowest and highest amount of backbone conformational entropy sampled at the tumbling time relative to the long-time limit. The sampling of the conformational backbone entropy with a binning of 20° for the backbone dihedral angles ϕ and ψ contains motions within core regions of the Ramachandran map, *e.g.*, within the α helical region, which are fast. This is in line with the notion that backbone motions in structured regions comprise of librational motions on ps time scales. On the other hand, conformational changes involving barrier crossings between regions, *e.g.*, a helix-coil transition, happen on much longer time scales and would lead to a slower entropy buildup. This did not occur in our simulations of T4 lysozyme.

Figure 5: Backbone entropy buildup of residues GLY12 (top) and ALA97 (bottom) in the simulations with the AMBER (A and B) and CHARMM (C and D) force fields. The results show an average of 15 non-overlapping 20 ns blocks for every of the 10 individual 300-ns simulations (*i.e.*, 150 blocks in total). The thin colored lines and the thick blue line represent the entropy buildup of the 10 different simulations and their average, respectively. The red and black dashed lines show the averaged entropy at τ_R and the long-time limit entropy (after 3 μ s accumulated sampling), respectively.

Single-residue entropies: side chains

Next, the residual conformational entropy of the side chains was investigated. Figure 3 shows the side chain configurational entropies sampled within the tumbling time and in the long time limit for all side chains with a 120 degree binning of the χ dihedrals. Figure 6A and B show the correlation between these two entropy values. The individual values of all side chains and both force fields can be found in Tables S3 and S4 in the Supporting Information.

Figure 6: Correlation between conformational entropies sampled after the tumbling time τ_R and in the long-time limit for side chains with 120° binning for the AMBER (A) and CHARMM (B) force fields.

The comparison between backbone and side chain conformational entropies with 120° bins shows that variations in the backbone entropy are more closely linked to specific parts of the protein than is the case for the side chains. For example, 31 of the 36 residues with a long-time limit backbone conformational entropy of more than $2 k_B$ have at least one neighbouring residue that also has a long-time limit backbone entropy of more than $2 k_B$. For the backbone, the motions of a particular dihedral angle are coupled to the neighboring backbone dihedrals. This is not the case for side chains, where such dynamic couplings between consecutive dihedral angles are not observed (Figure 3A and B). While backbone dihedral angles of neighboring residues are directly connected along the main chain, side chain dihedral angles of neighboring residues are spatially and morphologically separated and thus move largely independent of each other.

The motions of side chains are characterized by fast fluctuations within individual rotamer states and slower jumps between these. Thus, we next investigated how much of the conformational entropy due to intra-rotamer well motions is sampled within the tumbling time. Figure 7 shows that with a fine 3° binning of the χ dihedral angles the entropy buildup is nearly completed within the tumbling time, with a high correlation between short- and

long-time entropy ($R_P = 0.95$), similar to the backbone motions. In contrast, Figure 6A shows that a significant amount of long-time limit side chain conformational entropy is not sampled within the tumbling time for the same force field with a 120° binning, *i.e.*, if only the jumps between the main rotamer states (and not the intra-well motions) are considered. This means that the intra-rotamer well fluctuations are sampled within the tumbling time, but that a significant amount of inter-rotamer motion occurs on slower time scales. This finding has implications, because one is usually interested in entropy differences between two states, ΔS . One could object here that in an NMR ensemble experiment, the entire equilibrium distribution of dihedral angle fluctuations within all populated conformations is sampled, which is not necessarily the case in an MD simulation because it may not sample all conformations. Thus, NMR captures the full entropy associated with the intra rotamer-well dynamics, which may not be equal among different rotamers or conformations. However, for most biophysical processes the widths of the rotamer energy wells do not change.^{15,62,63} In other words, the intra-well entropy can be seen as an (almost) constant offset, and the contributions to ΔS due to changes within the rotamer wells are negligible compared to those linked to changes in the populations of the distinct rotamer wells (see also the discussion at the end of the Methods section).

Figure 7: A) Conformational entropy of methyl-containing side chains with 3° binning, sampled within the tumbling time τ_R and in the long-time limit (blue and red data points, respectively), and B) the correlation between the two.

About 20% of the residues in T4L have a long-time limit side chain conformational entropy of less than $0.2 k_B N_\chi$. These side chains display very limited rotamer jump dynamics on the sub- μ s time scale and effectively occupy a single dominant rotamer state. Thus, their contribution to the total side chain rotamer entropy is small in comparison to other residues. The dynamics of these side chains is dominated by fast librational motions within single rotamer wells on ps time scales.

We next focus on those side chains in T4L for which less than 90% of the long-time limit conformational entropy is sampled within the tumbling time (Figure 3). These display a slow buildup rate and contain at least one additional rotamer state that is frequently visited with very short residence times during the MD. As an example, Figure 8F shows the time evolution for χ_1 of MET106 during a representative trajectory. There are frequent transitions from and to the trans rotamer, but this state is only populated transiently with a residence time in the sub-ns regime.

The entropy buildup rate associated with intra-rotamer well motions is fast if all states relevant to the total conformational entropy have a mean residence time long enough to explore the intra-rotamer well phase space, which is the case for most occupied rotamer wells of protein side chains. Furthermore, we record the fact that for a given χ angle the contribution of intra-rotamer motions to the conformational entropy does not change among different rotamer wells and can be seen as an offset to the total conformational side chain entropy.

Next, we turn to the slower and hence more challenging inter-rotamer motions and the related entropy contributions, S_{inter} . These are counted by jump transitions between distinct rotamer wells. For residues with a long-time limit side chain conformational entropy larger than $0.2 k_B N_\chi$ in the AMBER ff99SB*-ILDN simulations, less than 90% of the long-time limit entropy was sampled within τ_R for most side chains (Figure 3C and D), showing that the desired equilibrium propensity of all rotamer states is not fully sampled within the tumbling time of the protein.

Figure 8: Side chain entropy buildup with 3° bins (A, B) and with 120° bins (C, D) of residues THR142 (top) and MET106 (bottom). Panels E) and F) show representative trajectories of the side chain dihedral angle χ_1 in THR142 (E) and MET106 (F). The thin lines and the thick blue line represent the entropy buildup of the 10 different simulations and their average, respectively. The red and black dashed lines show the entropy at τ_R and the long-time limit entropy after 3 μs , respectively.

Figure 9: Side chain entropy buildup with 120° binning of residues TYR139 (top) and TRP126 (bottom) for the AMBER ff99SB*-ILDN (A and B) and CHARMM36 (C and D) force fields. The results show an average of 15 non-overlapping 20 ns blocks per simulation from all 10 simulations. The thin lines and the thick blue line represent the entropy buildup of the 10 individual simulations and their average, respectively. The red and black dashed line show the averaged entropy at τ_R and the long-time limit entropy after 3 μ s, respectively.

Interestingly, the few side chains for which nearly all of the long-time conformational entropy is sampled within the tumbling time are typically aromatic rings. The rigidity of these side chains constrains the available side chain dihedral angle phase space. For example, more than 80% of the long-time limit side chain conformational entropy of TRP126 is sampled within 100 ps with the AMBER ff99SB*-ILDN force field (Figure 9B), and even already within 10 ps with the CHARMM36 force field (Figure 9D). Rotamer jumps of dihedral angles in aromatic rings are slower than in other side chains. For example, Kulkarni and Söderhjelm⁶⁹ used enhanced sampling techniques to show that the flip rates of the aromatic rings of the 4 tyrosine and the 4 phenylalanine side chains in BPTI lie between 280 μ s and

3.5 s, *i.e.*, they are much slower than the simulation times typically reached in all-atom MD simulations. Our data show that for most side chains (see also Figure 3), rotamer jumps on time scales between τ_R and the MD simulation time are present. The slow entropy buildup might point to differences in side chain dynamics among the independent MD simulations. For example, Figures 9A and 9C show that even for TYR139, the side chain with the lowest amount of relative conformational entropy at τ_R with respect to its long-time limit entropy in the AMBER ff99SB*-ILDN force field, all 10 simulations show nonzero side chain conformational entropy as a consequence of rotamer transitions, but the cumulative conformational entropy as an average over the 15 blocks in a simulation does not exceed $0.2 k_B N_\chi$ at τ_R in any of these simulations. However, the cumulative conformational entropy of this side chain from the concatenated trajectory is $0.5 k_B N_\chi$, which is 2.5 times higher than their largest individual contribution. This suggests that, even in the presence of transitions between rotamers, not all relevant phase space is sampled sufficiently within time scales shorter than τ_R and may not be detected by a dynamical observable in an NMR relaxation experiment in solution.

To more closely investigate this hypothesis, we next look at the side chain dihedral angle motions in more detail. Our previous analyses of all methyl groups of T4 lysozyme³¹ showed that LEU66-C $^{\delta,2}$ is the methyl group with the highest order parameter. One might expect that such high order parameter is connected to a population of a single rotamer well.^{34,70,71} Figure 10A shows the side chain dihedral angle χ_2 of LEU66 for three representative simulations. The trajectories for all simulations are shown in Figure S3. It can be seen that at least two states are occupied in every simulation, but the mean residence time within the rotamer wells is typically longer than τ_R and the distributions from the individual simulation trajectories are quite different.

Figure 10: A) Side chain dihedral angle χ_2 of LEU66 during three representative simulations. B) Entropy buildup for LEU66 for a 3° (top) and 120° (bottom) binning.

In general, different distributions of rotamer states with varying residence times are sampled. Single trajectories can be trapped in a specific region of the energy landscape, which might lead to misleading interpretations of the frequency of jumps between states and the average residence time within a state. This sampling problem can be reduced somewhat by running multiple shorter simulations. For example, Allnér and coworkers⁷² showed that the number of conformational clusters of backbone dihedral angles sampled within 100 ns can be doubled if the simulation length is divided into blocks of 10×10 ns or 5×20 ns with different initial atomic velocities. However, the sampling in any single trajectory has to be long enough to capture processes on all relevant time scales. Here we chose a simulation length of 300 ns for every individual simulation, which exceeds the tumbling correlation time of T4L by about a factor of 30 and hence should be long enough to capture motions on time

scales faster than τ_R . The different residence times among the individual simulations show that many side chain rotamer transitions occur infrequently and that complete sampling of the side chain conformational entropy might require additional and/or longer simulations. For the present study, in which we investigate the rate of entropy buildup over time, the broad distribution of transition frequencies sampled from 10 individual 300 ns simulations provides a reasonable estimate.

The frequency of the transitions between rotamer states has a large impact on order parameters S^2 . Frequent transitions on time scales faster than τ_R decrease the TCF and are contained in the experimental NMR relaxation data; few transitions between states with long residence times lead to a similar canonical rotamer distribution in the long-time limit, but do not contribute to the NMR relaxation data because the TCF has already decayed to zero due to overall tumbling. In consequence, contributions to the conformational entropy due to slow rotamer interconversion are usually not present in the relaxation data. Figure 10B shows the side chain entropy buildup of LEU66. One can see that the fast exploration of the rotamer wells leads to a fast entropy buildup with the 3° binning, but the entropy buildup with 120° binning is indeed relatively slow, with only 38% of the long-time limit entropy being sampled up to the tumbling time. Jumps from and to states with long residence times in the trajectories shown in Figure 10A are directly linked to the entropy buildup steps of the same trajectories in Figure 10B. As discussed above, the intra-well entropy, which dominates for the 3° binning, is an almost constant offset that does not change much between different biophysical states, and the entropy contribution due to changes in the rotamer distributions thus plays the major role in entropy differences ΔS . Transitions between rotamer states with residence times longer than the tumbling time that are not probed in relaxation experiments in solution but can be captured by residual dipolar couplings (RDCs)⁷³ or J-couplings.^{35,74} For instance, Farès and coworkers⁷³ measured NMR order parameters from relaxation experiments and RDC experiments for ubiquitin. They showed that the correlation between the two data sets is reasonable (correlation coefficient of

0.73), but that order parameter from relaxation experiments are systematically overestimated because they do not include the effect of "supra- τ_R " motions. We conclude that one has to be cautious if conformational entropies are extracted from side chain NMR relaxation parameters because entropy contributions from rotamer transitions between states with long residence times will be missing.

Side chain entropy and order parameter

In the previous section, the different types of motions that influence the conformational entropy of proteins were discussed. It was found that some part of conformational entropy is not contained in NMR order parameters if rotamer jumps on slow time scales are present. In this section, we address the question how big the influence of this missing entropy might be on an (empirical) entropy meter. Therefore, the side chain conformational entropy of methyl-bearing side chains is compared with the methyl order parameter, S_{axis}^2 . Figure 11 shows the side chain conformational entropy as a function of the order parameter from the simulation with the AMBER ff99SB*-ILDN force field for all methyl groups. First, we focus on the effect of intra-rotamer well motions on S_{axis}^2 . Regression lines for entropies at τ_R and in the long-time limit with a binning of 120° converge for $S = 0$ to similar methyl order parameter $S_{axis}^2(S = 0) \approx 0.84$ and 0.88 , respectively (Figure 11A). That means that methyl groups without rotamer jumps ($S = 0$ with 120° binning) display motions within a single rotamer well that decrease the methyl order parameter S_{axis}^2 from 1.0 to 0.88. The conformational entropy with a binning of 3° for $S_{axis}^2 = 0.88$ is $S \approx 2.4 k_B N_\chi$ (Figure 11B). As described above, this vibrational entropy can be seen as an offset to the full conformational entropy and is uncoupled from the slow jump motions between rotamer wells. This result can be illustrated by the THR152 side chain, the methyl group with the slowest rotamer transitions ($S=0.014 k_B N_\chi$ in the long-time limit with 120° binning), which indeed has a similar order parameter and 3° binning entropy (0.87 and $2.22 k_B N_\chi$) as estimated from the linear regression.

Figure 11: Side chain entropy with 120° binning (A,C) and with 3° binning (B,D) for all non-alanine methyl groups of T4L as a function of the methyl order parameter S_{axis}^2 from the simulations with the ff99SB*-ILDN force field. The entropy from equation 4 is plotted in panels A) and B) and the normalized entropy from equation 5 is plotted in panels C) and D). The red triangles and blue dots represent the side chain entropy within the tumbling time and in the long-time limit, respectively. The red and blue lines are linear regression lines to the respective data. Black triangles and yellow dots represent the values of the ILE- C^γ methyl groups that were not included for fitting the regression line. Methyl order parameters of the two methyl groups in VAL and LEU were averaged.

Next, the influence of rotamer jumps on the entropy meter is discussed. An entropy meter describes the parametric relation between conformational entropy from the explored phase space and order parameters as dynamical proxies. If this relationship is linear, the slope of the regression line fully describes the entropy meter. The slope of the regression line in the long-time limit is about 30% larger than the slope of the regression line of entropies at τ_R . This is the case both for the 3° and for the 120° binning. In addition, the ratio

depends on the choice of side chain dihedral angles. Normalizing the entropy according Eq. 5 reconciles the differences in the values between the two bin sizes used and supports the above conclusions. The normalized entropies for 120° and 3° bins (Figure 11C and D, respectively) are still somewhat different from each other, with lower entropies for the smaller bins. This is attributed to the sparseness of the data in the small 3° bins,⁵⁸ especially in the lowly populated ones at the energy barriers.

The spread of the data in Figure 11 suggests that the linear decrease in residual conformational entropy as a function of methyl order parameter does not include the chemical composition of the side chains involved, as was done in other approaches.⁴⁰ Furthermore, the spread of the data around the regression line increases more strongly from τ_R to the long-time limit than the slope does, suggesting that conformational entropy from slow dynamics beyond τ_R , which are not captured in methyl order parameter, are not *linearly* related to methyl order parameter. As described above, the additional configurational space not contained in relaxation parameters results from exploring states with residence times beyond τ_R . Because the entropy due to intra-rotamer well dynamics is an offset, the small amount of additional dynamics from intra-rotamer dynamics at long time scales for these kind of motions is not enough to capture the relatively large portion of additional configurational space. The presence of rotamer jumps between states with long mean residence times is therefore linked to higher conformational entropy, without an equal effect on methyl order parameter. Such nonlinear behavior cannot be remedied by adjusting the prefactor of the entropy meter but calls for the implementation of other functional forms.

The results of this study show that concerning the interpretation of entropy differences between two states, ΔS , the connection between NMR relaxation parameters and ΔS will – by construction – rely on an assumed compensation, which is that the relevant dynamics on time scales $\leq \tau_R$ are absent or present to a similar extent in the two states. For illustration, let’s consider a case in which the equilibrium distributions, concerning the populations of the rotameric minima, hardly differ between the two states, and thus the ΔS contribution

should be close to zero. However, if the energy *barriers* that determine the rate of rotamer interconversion do differ in such a way that in one state (*e.g.*, the unbound state) rotamer averaging occurs on fast sub- τ_R time scales but in the other state (*e.g.*, the ligand-bound state) it is shifted to slow supra- τ_R time scales, this assumption will be violated. In the above example, the entropy meter would predict an apparent (but fallacious) reduction of protein conformational entropy upon ligand binding. In summary, we conclude that the contribution of slow dynamics of side chains on time scales beyond τ_R on the side chain conformational entropy is significant and should be considered for the extraction of accurate thermodynamic properties.

General discussion

Going forward, experimental avenues could involve the slowing of molecular tumbling to a degree that is sufficient for the sampling of infrequent transitions across higher barriers, such that these would also drive nuclear spin relaxation rates. To achieve this, one might add viscogens to the protein solution, such as ethylene glycol (EG) or glycerol. For example, Balbach and coworkers followed the backbone dynamics of cold shock protein B upon the addition of EG by ^{15}N NMR relaxation.⁷⁵ They found that the presence of EG slowed overall tumbling in concert with solvent viscosity by a factor four, but at the same time it rigidified the backbone as judged by Lipari-Szabo order parameters. In addition, at the highest amount of co-solvent (70%) the overall tumbling time did not follow the viscosity, which was interpreted to result from preferential hydration by water over EG. In another study, Skrynnikov and coworkers investigated the effect of water/glycerol mixtures on internal dynamics, focusing on the side chain methyl groups of the chicken α -spectrin SH3 domain.⁷⁶ They found that the protein tumbling time could be increased by an order of magnitude, which permitted the observation of nanosecond time scale jumps for Val23. Unfortunately, the presence of a large amount of glycerol also led to an overall 'stiffening' of the protein molecule, thereby changing the variable under observation. As these studies show, viscogens

may not be the best choice for the purpose of slowing molecular tumbling. As an alternative to increasing solvent viscosity, Brüscheiler and coworkers recently took a different approach, where they study proteins that exchange between solution and the corona of a nanoparticle.^{77,78} They set up a system where the protein spends a marginal amount of time (ca. 1%) in the bound state, but this stopover leaves a signature of the internal dynamics of the protein-nanoparticle complex. Assuming that the interaction is non-specific and weak and does not itself affect the protein dynamics and that the exchange kinetics fall in the right window, motion beyond τ_R can be revealed because the nanoparticle has a very long (μs) rotational correlation time. The authors found no dynamics beyond the tumbling times for human ubiquitin, whereas Im7 displayed a clear hallmark of supra- τ_R dynamics in a loop region. Going one step further, the tumbling dynamics can be completely eliminated by studying protein dynamics in the solid state,⁷⁹⁻⁸¹ although this introduces the new problem of inferring the dynamics of proteins in the liquid state from an experiment in a restricting environment, where the protein may also be subject to rocking motions.⁸² This notwithstanding, a similarity between liquid and solid dynamics has been clearly revealed.⁸³ On the simulation side it will be important to address the sampling problem. As shown for example by Genheden and Ryde,⁴⁴ Baron and coworkers,⁶⁴ and Bowman,⁸⁴ convergence in MD simulations can be excruciatingly slow and lead to an underestimation of dynamics and entropy due to insufficient exploration of all relevant states in a reasonable simulation time. Although post-simulation block selection/reweighting schemes can generate improved agreement with experimental data,^{37,85} they do not overcome the underlying problem in case of poor sampling. Starting multiple simulations from slightly different starting structures and/or starting velocities can mitigate this problem somewhat.^{86,87} Ultimately, advanced enhanced sampling methods that allow for a more complete exploration of phase space will be essential to systematically investigate this important issue and to provide sampling error-free estimates from simulations.

Conclusions

Using T4 lysozyme as a case example, it was shown that almost all of the conformational entropy connected to motions of backbone atoms is sampled within times shorter than the overall tumbling correlation time τ_R . A very large fraction of the residual conformational backbone entropy in structured regions of T4L result from sampling inside single wells on the Ramachandran map. These individual wells are explored rapidly and the fast intra-well motions will be reflected in NMR relaxation data. Slow motions observed for a few residues close to the active site of T4L may point to dynamically stable (long-lived) metastable states in this region, with transitions between them on time scales longer than τ_R . At the level of individual residues, amino acids with slower backbone entropy buildup are usually consecutive residues in the protein. In particular, the active site of T4L contains residues with a remarkably slow entropy buildup.

Intra-rotamer motions in the side chains of T4L are sampled very fast and lead to an offset to the total conformational entropy. These fast vibrations decrease side chain order parameters from 1.0 to about 0.84 - 0.88, while jumps between different rotamer wells in side chains lead to a further decrease to lower methyl order parameters and are the main source for the proposed relation between conformational side chain entropy and methyl order parameter in form of an "entropy meter". The changes in the dihedral angle probability distributions resulting from the modulation of such slow rotamer jump dynamics are decisive for side chain entropy differences, ΔS , and hence for the entropy meter. Our data suggest that the relation depends on the local environment of the side chain, which has a significant influence on the rotamer jump frequency and mean residence time within a rotamer well. Motions slower than the global rotational tumbling time of the protein lead to a conformational entropy that is on average about 30% higher than the conformational entropy without these motions and is not detected in conventional NMR relaxation in solution. We observe that the relation between total conformational entropy and methyl order parameter is nonlinear in comparison to the linear relation between entropies captured on time scales accessible

by NMR relaxation parameter in solution and methyl order parameter. We anticipate that the results of this study can help estimate accurate thermodynamic quantities from NMR relaxation parameters and guide a way to recalibrate an empirical entropy meter with the help of molecular dynamics simulations.

Supporting Information Available

QHA entropy buildup analyzed by resampling from the simulation trajectories with different time steps. Correlation between backbone entropy at τ_R and in the long-time limit. Time traces of the χ_2 side chain dihedral angle of LEU66 in the 10 individual MD trajectories. Tables with all entropy values and estimates of their statistical uncertainties. This Information is available free of charge via the Internet at <http://pubs.acs.org>

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Graphical TOC Entry

Supporting Information:

How Much Entropy is Contained in NMR Relaxation Parameters?

Falk Hoffmann,^{*,†,‡} Frans A. A. Mulder,^{*,¶} and Lars V. Schäfer^{*,†}

[†]*Center for Theoretical Chemistry, Ruhr University Bochum, D-44780 Bochum, Germany*

[‡]*Current address: Peptone Inc., Northcliff House, Young Street, London, England W8 5,
U.K.*

[¶]*Interdisciplinary Nanoscience Center (iNANO) and Department of Chemistry, University
of Aarhus, DK-8000 Aarhus, Denmark*

E-mail: falk@peptone.io; fmulder@chem.au.dk; lars.schaefer@ruhr-uni-bochum.de

Phone: +49 (0)234 32 21582

Figure S1: QHA backbone entropy build-up obtained from the AMBER ff99SB*-ILDN simulations with 1 ps (blue), 2 ps (red), and 10 ps (green) time spacing between the trajectory frames plotted on a logarithmic (A) and linear (B) time scale.

Figure S2: Correlation of the backbone entropy of all non-alanine residues at the tumbling time (orange) and in the long-time limit (blue) between the simulations with the AMBER ff99SB*-ILDN and the CHARMM36 force field.

Figure S3: Side chain dihedral angle χ_2 of LEU66 during the 10 individual simulations.

Table S1: Backbone entropy S/k_B within the tumbling time τ_R and in the long-time limit with the AMBER ff99SB*-ILDN force field. STD and SEM are the standard deviation and the standard error of the mean, respectively.

Residue number	S/k_B [τ_R]	S/k_B [t_{sim}]	S [τ_R] / S [t_{sim}]	STD	SEM
2	1.7222	1.8622	0.925	0.0268	0.014
3	1.3733	1.4190	0.968	0.0053	0.004
4	1.5726	1.6902	0.930	0.0184	0.011
5	1.7826	1.9060	0.935	0.0245	0.013
6	1.4700	1.5225	0.966	0.0094	0.006
7	1.4353	1.5145	0.948	0.0076	0.005
8	1.6734	1.8366	0.911	0.0247	0.013
9	1.8814	2.0461	0.920	0.0115	0.006
10	1.9911	2.0935	0.951	0.0100	0.005
11	2.2378	2.8524	0.785	0.0219	0.008
12	2.2539	3.1326	0.719	0.0471	0.015
13	2.4462	2.9379	0.833	0.0490	0.017
14	2.3334	2.6007	0.897	0.0317	0.012
15	2.1936	2.2877	0.959	0.0110	0.005
16	2.0810	2.1558	0.965	0.0084	0.004
17	1.5613	1.5904	0.982	0.0025	0.002
18	1.9644	2.0495	0.958	0.0228	0.011
19	2.1371	2.3698	0.902	0.0150	0.006
20	2.0416	2.5180	0.811	0.0189	0.008
21	1.8590	2.1543	0.863	0.0263	0.012
22	2.1684	2.5335	0.856	0.0216	0.009
23	2.4648	2.8739	0.858	0.0241	0.008
24	2.2289	2.4683	0.903	0.0117	0.005
25	1.6859	1.8868	0.894	0.0191	0.010
26	1.5041	1.5730	0.956	0.0090	0.006
27	1.5443	1.6084	0.960	0.0079	0.005
28	1.6799	1.7797	0.944	0.0215	0.012
29	2.1859	2.6926	0.812	0.0420	0.016
30	1.9963	2.3162	0.862	0.0310	0.013
31	1.8094	1.9418	0.932	0.0086	0.004
32	1.8599	1.9176	0.970	0.0111	0.006
33	1.9310	2.1167	0.912	0.0224	0.011
34	1.4631	1.5545	0.941	0.0278	0.018
35	1.8012	1.9088	0.944	0.0387	0.020
36	1.8650	1.9254	0.969	0.0205	0.011
38	2.4738	2.5531	0.969	0.0285	0.011
39	1.3820	1.3951	0.991	0.0078	0.006
40	1.5195	1.5292	0.994	0.0023	0.002
41	1.5615	1.5869	0.984	0.0046	0.003
42	1.5778	1.6013	0.985	0.0069	0.004
43	1.5223	1.5424	0.987	0.0047	0.003
44	1.6310	1.6656	0.979	0.0088	0.005
45	1.5373	1.5849	0.970	0.0063	0.004
46	1.4109	1.4411	0.979	0.0041	0.003
47	1.4899	1.5130	0.985	0.0048	0.003
48	1.8286	1.8548	0.986	0.0145	0.008
49	1.8551	1.8737	0.990	0.0164	0.009
50	1.9702	2.0718	0.951	0.0087	0.004
51	2.0743	2.1250	0.976	0.0065	0.003
52	1.8354	1.9338	0.949	0.0121	0.006

53	1.9852	2.1008	0.945	0.0085	0.004
54	2.0459	2.2550	0.907	0.0098	0.004
55	1.5495	1.5843	0.978	0.0123	0.008
56	1.8838	1.9191	0.982	0.0161	0.008
57	1.7764	1.8822	0.944	0.0137	0.007
58	1.8040	1.8627	0.968	0.0084	0.005
59	1.8730	1.9254	0.973	0.0101	0.005
60	1.5927	1.6327	0.976	0.0111	0.007
61	1.7530	1.8187	0.964	0.0190	0.010
62	1.6601	1.7112	0.970	0.0287	0.017
63	1.4712	1.5008	0.980	0.0205	0.014
64	1.5204	1.5938	0.954	0.0310	0.019
65	1.6483	1.7240	0.956	0.0191	0.011
66	1.6292	1.7557	0.928	0.0249	0.014
67	1.5907	2.0594	0.772	0.0343	0.017
68	1.6509	1.7519	0.942	0.0093	0.005
69	1.7985	1.9498	0.922	0.0276	0.014
70	1.5918	1.6401	0.971	0.0081	0.005
71	1.5274	1.5803	0.967	0.0148	0.009
72	1.5551	1.5851	0.981	0.0030	0.002
73	1.4995	1.5299	0.980	0.0068	0.004
74	1.4938	1.5107	0.989	0.0085	0.006
75	1.4699	1.5024	0.978	0.0066	0.004
76	1.5276	1.5503	0.985	0.0072	0.005
77	1.7604	1.8115	0.972	0.0120	0.007
78	1.6397	1.7099	0.959	0.0156	0.009
79	1.9791	2.0206	0.979	0.0089	0.004
80	2.6298	2.7984	0.940	0.0229	0.008
81	2.1342	2.2480	0.949	0.0113	0.005
82	1.7266	1.7348	0.995	0.0020	0.001
83	2.0007	2.0302	0.985	0.0052	0.003
84	1.8779	1.9094	0.984	0.0057	0.003
85	1.4220	1.4461	0.983	0.0049	0.003
87	1.6223	1.6756	0.968	0.0152	0.009
88	1.5377	1.5787	0.974	0.0052	0.003
89	1.8686	1.9120	0.977	0.0066	0.003
90	2.2431	2.3060	0.973	0.0151	0.007
91	2.1287	2.2129	0.962	0.0155	0.007
92	1.7420	1.7712	0.984	0.0109	0.006
93	1.8785	1.8931	0.992	0.0104	0.006
94	1.7132	1.7240	0.994	0.0021	0.001
95	1.1998	1.2037	0.997	0.0013	0.001
96	1.4104	1.4165	0.996	0.0023	0.002
97	1.4347	1.4375	0.998	0.0013	0.001
98	1.4679	1.4755	0.995	0.0017	0.001
99	1.3979	1.4046	0.995	0.0033	0.002
100	1.4050	1.4182	0.991	0.0017	0.001
101	1.4435	1.4634	0.986	0.0057	0.004
102	1.4024	1.4294	0.981	0.0023	0.002
103	1.3088	1.3313	0.983	0.0091	0.007
104	1.7418	1.7929	0.972	0.0167	0.009
105	2.1022	2.1518	0.977	0.0187	0.009
106	2.0314	2.2001	0.923	0.0141	0.006
107	1.7671	1.8000	0.982	0.0065	0.004
108	1.7151	1.7830	0.962	0.0095	0.005
109	1.6246	1.6647	0.976	0.0117	0.007

110	1.9136	1.9477	0.982	0.0114	0.006
111	1.7129	1.7910	0.956	0.0157	0.009
112	1.5829	1.5988	0.990	0.0039	0.002
113	1.8019	1.8115	0.995	0.0030	0.002
114	1.8190	1.8672	0.974	0.0112	0.006
115	1.7909	1.8285	0.979	0.0089	0.005
116	1.6675	1.7133	0.973	0.0106	0.006
117	1.5960	1.6371	0.975	0.0072	0.004
118	1.4668	1.4728	0.996	0.0020	0.001
119	1.5094	1.5212	0.992	0.0031	0.002
120	1.4791	1.4879	0.994	0.0019	0.001
121	1.4549	1.4610	0.996	0.0021	0.001
122	1.6008	1.6159	0.991	0.0046	0.003
123	1.6468	1.6668	0.988	0.0064	0.004
124	1.5032	1.5129	0.994	0.0043	0.003
125	1.7572	1.7904	0.982	0.0132	0.007
126	1.6962	1.7329	0.979	0.0089	0.005
127	1.8162	1.8611	0.976	0.0091	0.005
128	1.8177	1.8492	0.983	0.0066	0.004
129	1.5443	1.6137	0.957	0.0086	0.005
130	1.7274	1.7519	0.986	0.0047	0.003
131	2.0129	2.0993	0.959	0.0092	0.004
132	1.7125	1.7670	0.969	0.0101	0.006
133	1.5462	1.6179	0.956	0.0073	0.005
134	1.7680	1.7833	0.991	0.0045	0.003
135	2.6287	2.6935	0.976	0.0154	0.006
136	2.3388	2.3961	0.976	0.0127	0.005
137	1.5151	1.5351	0.987	0.0061	0.004
138	1.4790	1.4908	0.992	0.0018	0.001
139	1.6347	1.6470	0.993	0.0026	0.002
140	2.2163	2.2577	0.982	0.0051	0.002
141	1.9969	2.0120	0.992	0.0070	0.003
142	1.4814	1.4973	0.989	0.0031	0.002
144	1.6188	1.6455	0.984	0.0033	0.002
145	1.4252	1.4595	0.977	0.0037	0.003
146	1.4462	1.4594	0.991	0.0015	0.001
147	1.4972	1.5127	0.990	0.0015	0.001
148	1.4030	1.5185	0.924	0.0057	0.004
149	1.3583	1.3654	0.995	0.0045	0.003
150	1.3767	1.3829	0.995	0.0012	0.001
151	1.4521	1.4711	0.987	0.0024	0.002
152	1.4549	1.4587	0.997	0.0013	0.001
153	1.4321	1.4399	0.995	0.0033	0.002
154	1.7167	1.7289	0.993	0.0043	0.003
155	1.8281	1.8404	0.993	0.0030	0.002
156	1.6109	1.6192	0.995	0.0027	0.002
157	1.8216	1.8479	0.986	0.0024	0.001
158	1.7890	1.8807	0.951	0.0070	0.004
159	1.8413	1.8883	0.975	0.0084	0.004
160	1.9287	1.9827	0.973	0.0165	0.008
161	2.9010	3.0689	0.945	0.0284	0.009
162	3.6589	3.9553	0.925	0.0195	0.005

Table S2: Backbone entropy S/k_B within the tumbling time τ_R and in the long-time limit with the CHARMM36 force field. STD and SEM are the standard deviation and the standard error of the mean, respectively.

Residue number	S/k_B [τ_R]	S/k_B [t_{sim}]	S [τ_R] / S [t_{sim}]	STD	SEM
2	1.8065	2.1104	0.856	0.0562	0.027
3	1.3168	1.3597	0.968	0.0152	0.011
4	1.1863	1.2160	0.976	0.0269	0.022
5	1.3621	1.4048	0.970	0.0241	0.017
6	1.2736	1.4178	0.898	0.0537	0.038
7	1.4413	1.7408	0.828	0.0630	0.036
8	1.5677	2.0471	0.766	0.0575	0.028
9	1.6117	2.0502	0.786	0.0422	0.021
10	1.7306	2.4578	0.704	0.0827	0.034
11	2.1088	3.3367	0.632	0.0587	0.018
12	2.1805	3.1312	0.696	0.0488	0.016
13	2.1077	2.5106	0.840	0.0399	0.016
14	1.7864	2.1338	0.837	0.0285	0.013
15	1.8917	1.9890	0.951	0.0196	0.010
16	2.0455	2.1519	0.951	0.0140	0.006
17	1.6676	1.7210	0.969	0.0110	0.006
18	1.8090	1.9742	0.916	0.0478	0.024
19	1.9725	2.1309	0.926	0.0239	0.011
20	1.9678	2.5942	0.759	0.0391	0.015
21	1.8831	2.4726	0.762	0.0311	0.013
22	1.9108	2.0204	0.946	0.0359	0.018
23	2.2905	2.4532	0.934	0.0159	0.006
24	2.1015	2.1752	0.966	0.0312	0.014
25	1.6292	1.7543	0.929	0.0509	0.029
26	1.4288	1.5278	0.935	0.0584	0.038
27	1.4400	1.5056	0.956	0.0087	0.006
28	1.7230	1.9242	0.895	0.0131	0.007
29	1.8964	2.2691	0.836	0.0455	0.020
30	1.9130	2.0726	0.923	0.0369	0.018
31	1.7600	1.8569	0.948	0.0513	0.028
32	1.9230	1.9738	0.974	0.0320	0.016
33	2.0413	2.1054	0.970	0.0222	0.011
34	1.5795	1.6014	0.986	0.0128	0.008
35	1.6196	1.6316	0.993	0.0050	0.003
36	1.6183	1.6726	0.968	0.0075	0.004
38	2.3838	2.4585	0.970	0.0068	0.003
39	1.3934	1.4078	0.990	0.0036	0.003
40	1.3764	1.3798	0.998	0.0014	0.001
41	1.2317	1.2331	0.999	0.0031	0.002
42	1.2339	1.2371	0.997	0.0019	0.002
43	1.3030	1.3116	0.993	0.0041	0.003
44	1.2701	1.2870	0.987	0.0039	0.003
45	1.3596	1.3704	0.992	0.0017	0.001
46	1.1617	1.1693	0.993	0.0059	0.005
47	1.3242	1.3266	0.998	0.0018	0.001
48	1.3642	1.3633	1.001	0.0044	0.003
49	1.4156	1.4171	0.999	0.0032	0.002
50	1.8066	1.8318	0.986	0.0053	0.003
51	1.8718	1.8942	0.988	0.0035	0.002
52	1.5072	1.5225	0.990	0.0068	0.004

53	1.7505	1.7726	0.988	0.0053	0.003
54	1.9065	2.0567	0.927	0.0048	0.002
55	1.8632	1.9159	0.973	0.0060	0.003
56	1.9404	1.9692	0.985	0.0042	0.002
57	1.5894	1.6883	0.941	0.0147	0.009
58	1.6640	1.6969	0.981	0.0100	0.006
59	1.5787	1.5989	0.987	0.0108	0.007
60	1.3840	1.4073	0.983	0.0055	0.004
61	1.3910	1.4119	0.985	0.0024	0.002
62	1.3305	1.3517	0.984	0.0081	0.006
63	1.3063	1.3227	0.988	0.0083	0.006
64	1.3371	1.3754	0.972	0.0079	0.006
65	1.3075	1.3194	0.991	0.0041	0.003
66	1.2556	1.2666	0.991	0.0078	0.006
67	1.1944	1.2048	0.991	0.0127	0.011
68	1.3614	1.3745	0.990	0.0030	0.002
69	1.3953	1.4062	0.992	0.0024	0.002
70	1.2973	1.3072	0.992	0.0025	0.002
71	1.2214	1.2253	0.997	0.0024	0.002
72	1.2210	1.2229	0.998	0.0055	0.005
73	1.3952	1.4007	0.996	0.0019	0.001
74	1.1573	1.1599	0.998	0.0030	0.003
75	1.1627	1.1708	0.993	0.0034	0.003
76	1.4689	1.4833	0.990	0.0071	0.005
77	1.4717	1.4845	0.991	0.0099	0.007
78	1.2368	1.2517	0.988	0.0091	0.007
79	1.6549	1.6776	0.986	0.0085	0.005
80	2.0667	2.1558	0.959	0.0141	0.007
81	2.0619	2.0973	0.983	0.0029	0.001
82	1.9456	1.9525	0.996	0.0021	0.001
83	2.1599	2.1783	0.992	0.0052	0.002
84	2.0204	2.0416	0.990	0.0032	0.002
85	1.2086	1.2131	0.996	0.0031	0.003
87	1.1584	1.1657	0.994	0.0056	0.005
88	1.2774	1.2877	0.992	0.0072	0.006
89	1.4398	1.4732	0.977	0.0180	0.012
90	1.6469	1.7476	0.942	0.0298	0.017
91	1.6845	1.7653	0.954	0.0221	0.013
92	1.6783	1.7283	0.971	0.0114	0.007
93	1.8626	1.9083	0.976	0.0229	0.012
94	1.5793	1.6010	0.986	0.0081	0.005
95	1.2464	1.2545	0.994	0.0046	0.004
96	1.2667	1.2699	0.997	0.0032	0.003
97	1.3487	1.3532	0.997	0.0013	0.001
98	1.3576	1.3642	0.995	0.0013	0.001
99	1.2564	1.2605	0.997	0.0019	0.002
100	1.3075	1.3163	0.993	0.0068	0.005
101	1.2501	1.2643	0.989	0.0104	0.008
102	1.1725	1.1870	0.988	0.0073	0.006
103	1.1999	1.2091	0.992	0.0034	0.003
104	1.3529	1.3657	0.991	0.0101	0.007
105	1.7160	1.8302	0.938	0.0245	0.013
106	1.9203	2.1057	0.912	0.0129	0.006
107	1.6556	1.7967	0.921	0.0080	0.004
108	1.4255	1.4863	0.959	0.0086	0.006
109	1.6905	1.7170	0.985	0.0099	0.006

110	1.6200	1.6686	0.971	0.0140	0.008
111	1.5356	1.6910	0.908	0.0240	0.014
112	1.7748	1.9197	0.925	0.0159	0.008
113	1.8934	2.0234	0.936	0.0209	0.010
114	1.7805	1.9563	0.910	0.0236	0.012
115	1.5026	1.5411	0.975	0.0081	0.005
116	1.3805	1.3932	0.991	0.0071	0.005
117	1.3603	1.3797	0.986	0.0075	0.005
118	1.3442	1.3484	0.997	0.0018	0.001
119	1.3234	1.3419	0.986	0.0063	0.005
120	1.3642	1.3798	0.989	0.0019	0.001
121	1.3542	1.3577	0.997	0.0023	0.002
122	1.5646	1.5852	0.987	0.0090	0.006
123	1.5942	1.6251	0.981	0.0102	0.006
124	1.7649	1.7757	0.994	0.0027	0.002
125	1.7238	1.7870	0.965	0.0277	0.015
126	1.5318	1.5558	0.985	0.0202	0.013
127	1.4148	1.4380	0.984	0.0032	0.002
128	1.4667	1.4995	0.978	0.0046	0.003
129	1.3298	1.3436	0.990	0.0059	0.004
130	1.3904	1.4140	0.983	0.0066	0.005
131	1.4352	1.5491	0.926	0.0101	0.007
132	1.5374	1.6026	0.959	0.0186	0.012
133	1.5999	1.6666	0.960	0.0176	0.011
134	1.9329	1.9570	0.988	0.0054	0.003
135	2.5579	2.6135	0.979	0.0190	0.007
136	2.2655	2.3360	0.970	0.0237	0.010
137	1.4127	1.4388	0.982	0.0036	0.003
138	1.2857	1.2939	0.994	0.0037	0.003
139	1.4584	1.4771	0.987	0.0127	0.009
140	1.6698	1.6944	0.985	0.0180	0.011
141	1.8977	1.9545	0.971	0.0107	0.005
142	1.5728	1.6092	0.977	0.0183	0.011
144	1.3855	1.4056	0.986	0.0129	0.009
145	1.2770	1.3474	0.948	0.0213	0.016
146	1.2902	1.3322	0.968	0.0104	0.008
147	1.3566	1.3839	0.980	0.0048	0.003
148	1.2370	1.2770	0.969	0.0140	0.011
149	1.0306	1.0401	0.991	0.0058	0.006
150	1.2263	1.2317	0.996	0.0031	0.003
151	1.3487	1.3655	0.988	0.0028	0.002
152	1.3061	1.3150	0.993	0.0039	0.003
153	1.3549	1.3671	0.991	0.0044	0.003
154	1.5002	1.5255	0.983	0.0083	0.005
155	1.9227	1.9667	0.978	0.0141	0.007
156	1.5660	1.5840	0.989	0.0136	0.009
157	1.4635	1.7554	0.834	0.0264	0.015
158	1.5202	1.6456	0.924	0.0313	0.019
159	1.8755	1.9276	0.973	0.0156	0.008
160	1.7967	1.8402	0.976	0.0139	0.008
161	2.0250	2.1444	0.944	0.0526	0.025
162	3.5057	3.5650	0.983	0.0225	0.006

Table S3: Side chain entropy $S/(k_B N_\chi)$ within the tumbling time τ_R and in the long-time limit with the AMBER ff99SB*-ILDN force field. STD and SEM are the standard deviation and the standard error of the mean, respectively.

Residue number	$S/(k_B N_\chi)$ [τ_R]	$S/(k_B N_\chi)$ [t_{sim}]	S [τ_R] / S [t_{sim}]	STD	SEM
1	0.3133	0.3620	0.865	0.0091	0.025
2	0.2820	0.4850	0.581	0.0133	0.027
3	0.1147	0.2545	0.451	0.0222	0.087
4	0.2069	0.8371	0.247	0.0439	0.052
5	0.4097	0.5102	0.803	0.0125	0.025
6	0.1424	0.1790	0.796	0.0174	0.097
7	0.1978	0.3471	0.570	0.0397	0.114
8	0.3157	0.6811	0.463	0.0170	0.025
9	0.3086	0.5239	0.589	0.0089	0.017
10	0.0445	0.2547	0.175	0.0052	0.020
11	0.3882	0.6431	0.604	0.0192	0.030
13	0.1765	0.2822	0.626	0.0235	0.083
14	0.1650	0.3534	0.467	0.0188	0.053
15	0.0887	0.1587	0.559	0.0191	0.121
16	0.4947	0.5955	0.831	0.0057	0.010
17	0.0370	0.1332	0.278	0.0076	0.057
18	0.0001	0.0002	0.802	0.0000	0.210
19	0.3959	0.4960	0.798	0.0074	0.015
20	0.4654	0.6920	0.673	0.0089	0.013
21	0.5961	0.6992	0.853	0.0134	0.019
22	0.5965	0.7620	0.783	0.0113	0.015
24	0.0869	0.4499	0.193	0.0189	0.042
25	0.0016	0.0021	0.754	0.0005	0.257
26	0.3279	0.7361	0.445	0.0230	0.031
27	0.2687	0.3464	0.776	0.0148	0.043
29	0.1706	0.3186	0.536	0.0195	0.061
31	0.0418	0.0615	0.679	0.0060	0.098
32	0.3315	0.5142	0.645	0.0102	0.020
33	0.0125	0.0532	0.235	0.0039	0.073
34	0.0409	0.3379	0.121	0.0149	0.044
35	0.3173	0.4047	0.784	0.0133	0.033
36	0.8637	0.9716	0.889	0.0105	0.011
37	0.3201	0.3282	0.976	0.0044	0.014
38	0.4562	0.5090	0.896	0.0140	0.028
39	0.0802	0.1051	0.762	0.0055	0.053
40	0.7217	0.8037	0.898	0.0114	0.014
43	0.2795	0.3402	0.821	0.0078	0.023
44	0.3717	0.4093	0.908	0.0091	0.022
45	0.3788	0.5397	0.702	0.0074	0.014
46	0.0075	0.0213	0.355	0.0049	0.229
47	0.2035	0.5057	0.402	0.0229	0.045
48	0.5587	0.6238	0.896	0.0039	0.006
50	0.0544	0.2147	0.253	0.0105	0.049
52	0.3123	0.5462	0.572	0.0187	0.034
53	0.5849	0.8466	0.691	0.0142	0.017
54	0.0212	0.0441	0.481	0.0079	0.180
55	0.5940	0.7574	0.784	0.0216	0.029
57	0.6333	1.0979	0.577	0.0223	0.020
58	0.0238	0.0316	0.753	0.0029	0.093
59	0.0253	0.0952	0.265	0.0074	0.078

60	0.5196	0.6627	0.784	0.0051	0.008
61	0.6671	1.0105	0.660	0.0123	0.012
62	0.3525	0.7832	0.450	0.0224	0.029
64	0.4679	0.5596	0.836	0.0213	0.038
65	0.5290	0.6387	0.828	0.0059	0.009
66	0.1619	0.4265	0.380	0.0355	0.083
67	0.0496	0.4413	0.112	0.0286	0.065
68	0.4947	0.7064	0.700	0.0141	0.020
69	0.4275	0.7465	0.573	0.0325	0.044
70	0.2205	0.5005	0.441	0.0266	0.053
71	0.4191	0.7599	0.551	0.0426	0.056
72	0.3962	0.5325	0.744	0.0123	0.023
75	0.2328	0.4077	0.571	0.0212	0.052
76	0.3877	0.6277	0.618	0.0128	0.020
78	0.0303	0.0588	0.514	0.0050	0.084
79	0.0810	0.1166	0.695	0.0077	0.066
80	0.3898	0.6561	0.594	0.0183	0.028
81	0.0192	0.0701	0.274	0.0062	0.089
83	0.5318	0.6035	0.881	0.0031	0.005
84	0.0554	0.0845	0.656	0.0063	0.074
85	0.3609	0.4398	0.821	0.0071	0.016
86	0.3190	0.3252	0.981	0.0044	0.014
87	0.1221	0.1878	0.650	0.0133	0.071
88	0.0021	0.0023	0.919	0.0002	0.070
89	0.5918	0.7099	0.834	0.0083	0.012
90	0.3559	0.8405	0.423	0.0231	0.027
91	0.0017	0.0610	0.027	0.0017	0.027
92	0.3910	0.4834	0.809	0.0111	0.023
94	0.0071	0.0134	0.529	0.0047	0.353
95	0.0213	0.0215	0.988	0.0009	0.042
96	0.0249	0.0398	0.626	0.0043	0.107
99	0.0878	0.1059	0.828	0.0084	0.079
100	0.0467	0.0695	0.673	0.0095	0.137
101	0.0887	0.0976	0.910	0.0136	0.139
102	0.2066	0.2363	0.875	0.0052	0.022
103	0.0368	0.0581	0.634	0.0090	0.156
104	0.2997	0.6710	0.447	0.0461	0.069
105	0.0102	0.0157	0.651	0.0019	0.118
106	0.3397	0.5144	0.660	0.0158	0.031
108	0.4969	0.8539	0.582	0.0147	0.017
109	0.6172	0.6988	0.883	0.0070	0.010
111	0.1096	0.2954	0.371	0.0192	0.065
114	0.3353	0.4885	0.686	0.0135	0.028
115	0.5842	0.6999	0.835	0.0101	0.014
116	0.6307	0.7512	0.840	0.0160	0.021
117	0.1590	0.2094	0.759	0.0141	0.068
118	0.0124	0.0328	0.379	0.0063	0.193
119	0.5501	0.7534	0.730	0.0067	0.009
120	0.2731	0.3184	0.858	0.0059	0.018
121	0.0026	0.0085	0.299	0.0025	0.298
122	0.2410	0.3178	0.758	0.0142	0.045
123	0.0665	0.1021	0.652	0.0057	0.056
124	0.3107	0.4108	0.756	0.0062	0.015
125	0.0968	0.2097	0.461	0.0133	0.063
126	0.2565	0.2661	0.964	0.0029	0.011
127	0.7554	0.9614	0.786	0.0108	0.011

128	0.2760	0.4823	0.572	0.0214	0.044
131	0.5977	0.8919	0.670	0.0222	0.025
132	0.3340	0.5931	0.563	0.0185	0.031
133	0.0330	0.1256	0.263	0.0047	0.038
135	0.5690	0.6520	0.873	0.0056	0.009
136	0.0385	0.0979	0.393	0.0168	0.172
137	0.5888	0.7594	0.775	0.0130	0.017
138	0.0000	0.0000	1.029	0.0000	0.261
139	0.0239	0.2498	0.096	0.0095	0.038
140	0.6765	0.8395	0.806	0.0148	0.018
141	0.4442	0.6194	0.717	0.0080	0.013
142	0.0087	0.0130	0.670	0.0018	0.138
143	0.3568	0.3598	0.992	0.0010	0.003
144	0.6221	0.7402	0.840	0.0102	0.014
145	0.0360	0.0873	0.412	0.0045	0.051
147	0.3915	0.4532	0.864	0.0063	0.014
148	0.0923	0.2291	0.403	0.0111	0.048
149	0.0099	0.0151	0.654	0.0048	0.317
150	0.0216	0.0333	0.651	0.0049	0.146
151	0.1649	0.2734	0.603	0.0215	0.079
152	0.0078	0.0140	0.559	0.0046	0.327
153	0.0053	0.0051	1.030	0.0009	0.172
154	0.3247	0.5242	0.619	0.0124	0.024
155	0.0185	0.0523	0.355	0.0071	0.135
157	0.0130	0.0396	0.328	0.0052	0.132
158	0.2381	0.2642	0.901	0.0042	0.016
159	0.6449	0.8818	0.731	0.0078	0.009
161	0.0124	0.0275	0.452	0.0021	0.077
162	0.6297	0.7607	0.828	0.0046	0.006

Table S4: Side chain entropy $S/(k_B N_\chi)$ within the tumbling time τ_R and in the long-time limit with the CHARMM36 force field. STD and SEM are the standard deviation and the standard error of the mean, respectively.

Residue number	$S/(k_B N_\chi)$ [τ_R]	$S/(k_B N_\chi)$ [t_{sim}]	S [τ_R] / S [t_{sim}]	STD	SEM
1	0.4345	0.6057	0.717	0.0148	0.024
2	0.2841	0.4044	0.702	0.0242	0.060
3	0.1362	0.2186	0.623	0.0289	0.132
4	0.1295	0.4621	0.280	0.0121	0.026
5	0.5129	0.6645	0.772	0.0135	0.020
6	0.4274	0.6138	0.696	0.0225	0.037
7	0.0370	0.0637	0.580	0.0078	0.123
8	0.1412	0.6591	0.214	0.0256	0.039
9	0.4565	0.7447	0.613	0.0101	0.014
10	0.2321	0.8071	0.288	0.0354	0.044
11	0.2801	0.6396	0.438	0.0172	0.027
13	0.1265	0.2039	0.620	0.0247	0.121
14	0.0571	0.3695	0.154	0.0070	0.019
15	0.0495	0.0704	0.703	0.0051	0.072
16	0.6102	0.7283	0.838	0.0061	0.008
17	0.2288	0.3887	0.589	0.0260	0.067
18	0.0653	0.4938	0.132	0.0234	0.047
19	0.4240	0.5188	0.817	0.0205	0.039
20	0.2755	0.5393	0.511	0.0146	0.027
21	0.6143	1.0032	0.612	0.0806	0.080
22	0.4426	0.5469	0.809	0.0184	0.034
24	0.2053	0.4128	0.497	0.0226	0.055
25	0.3166	0.3357	0.943	0.0092	0.028
26	0.1328	0.7821	0.170	0.0343	0.044
27	0.2959	0.3406	0.869	0.0104	0.030
29	0.2381	0.4617	0.516	0.0157	0.034
31	0.0260	0.0313	0.829	0.0060	0.192
32	0.3450	0.4111	0.839	0.0264	0.064
33	0.0029	0.0256	0.114	0.0020	0.077
34	0.0002	0.0006	0.334	0.0002	0.323
35	0.4268	0.4902	0.871	0.0070	0.014
36	0.2141	0.5112	0.419	0.0276	0.054
37	0.1704	0.1747	0.976	0.0062	0.035
38	0.0117	0.0239	0.490	0.0049	0.205
39	0.0357	0.0458	0.780	0.0027	0.060
40	0.5524	0.6161	0.897	0.0105	0.017
43	0.2891	0.3400	0.850	0.0032	0.009
44	0.4778	0.8649	0.552	0.0265	0.031
45	0.3154	0.3576	0.882	0.0050	0.014
46	-0.0000	0.0000	-0.000	0.0000	0.000
47	0.2902	0.2951	0.983	0.0076	0.026
48	0.4021	0.5447	0.738	0.0061	0.011
50	0.0412	0.0733	0.563	0.0103	0.140
52	0.0863	0.1258	0.686	0.0085	0.068
53	0.4740	0.7887	0.601	0.0210	0.027
54	0.0010	0.1892	0.005	0.0005	0.003
55	0.5919	0.6549	0.904	0.0115	0.018
57	0.3711	0.7718	0.481	0.0264	0.034
58	0.0339	0.0355	0.954	0.0024	0.068
59	0.0192	0.0520	0.369	0.0129	0.248

60	0.3788	0.6734	0.563	0.0232	0.035
61	0.5404	0.6133	0.881	0.0105	0.017
62	0.0431	0.2439	0.177	0.0109	0.045
64	0.4996	0.6455	0.774	0.0116	0.018
65	0.5755	0.7568	0.761	0.0101	0.013
66	0.1012	0.3644	0.278	0.0149	0.041
67	0.2982	0.4300	0.693	0.0180	0.042
68	0.4759	0.5336	0.892	0.0150	0.028
69	0.3849	0.5600	0.687	0.0091	0.016
70	0.1542	0.3751	0.411	0.0082	0.022
71	0.0141	0.0267	0.527	0.0067	0.252
72	0.5181	0.6156	0.842	0.0087	0.014
75	0.1237	0.1963	0.630	0.0122	0.062
76	0.3865	0.6145	0.629	0.0083	0.014
78	0.0659	0.0759	0.868	0.0098	0.129
79	0.1297	0.1564	0.829	0.0136	0.087
80	0.1637	0.3044	0.538	0.0125	0.041
81	0.0337	0.0783	0.430	0.0051	0.066
83	0.4967	0.5690	0.873	0.0038	0.007
84	0.0135	0.0173	0.778	0.0039	0.226
85	0.1248	0.1946	0.641	0.0132	0.068
86	0.3470	0.3490	0.994	0.0017	0.005
87	0.0527	0.0954	0.552	0.0138	0.144
88	0.1968	0.2146	0.917	0.0121	0.057
89	0.1891	0.5115	0.370	0.0097	0.019
90	0.2922	0.9783	0.299	0.0272	0.028
91	0.0022	0.0163	0.135	0.0022	0.135
92	0.5296	0.6064	0.873	0.0065	0.011
94	0.0104	0.0257	0.406	0.0051	0.198
95	0.1095	0.1213	0.902	0.0051	0.042
96	0.0512	0.1782	0.287	0.0154	0.086
99	0.0243	0.0303	0.802	0.0018	0.060
100	0.0072	0.0128	0.562	0.0020	0.155
101	0.3075	0.4004	0.768	0.0125	0.031
102	0.3238	0.4206	0.770	0.0132	0.031
103	0.0031	0.0046	0.676	0.0009	0.184
104	0.0681	0.2971	0.229	0.0135	0.046
105	0.0920	0.6098	0.151	0.0230	0.038
106	0.3481	0.5440	0.640	0.0110	0.020
108	0.3372	0.6890	0.489	0.0260	0.038
109	0.7495	0.8235	0.910	0.0141	0.017
111	0.0392	0.2469	0.159	0.0145	0.059
114	0.4394	0.5391	0.815	0.0152	0.028
115	0.5721	0.7138	0.801	0.0136	0.019
116	0.6503	0.7780	0.836	0.0111	0.014
117	0.1443	0.3324	0.434	0.0269	0.081
118	0.0201	0.0533	0.378	0.0107	0.201
119	0.2805	0.6302	0.445	0.0245	0.039
120	0.2871	0.3661	0.784	0.0091	0.025
121	0.0069	0.0206	0.333	0.0028	0.138
122	0.5202	0.6226	0.836	0.0101	0.016
123	0.1899	0.3840	0.495	0.0115	0.030
124	0.3418	0.4962	0.689	0.0125	0.025
125	0.0704	0.2593	0.272	0.0134	0.052
126	0.3325	0.3368	0.987	0.0023	0.007
127	0.5034	0.6053	0.832	0.0101	0.017

128	0.3059	0.4992	0.613	0.0426	0.085
131	0.3306	0.7753	0.426	0.0247	0.032
132	0.3708	0.4585	0.809	0.0148	0.032
133	0.0481	0.1690	0.285	0.0136	0.080
135	0.5382	0.6481	0.830	0.0064	0.010
136	0.0052	0.0231	0.225	0.0052	0.225
137	0.2377	0.4936	0.481	0.0147	0.030
138	0.0004	0.0004	0.805	0.0001	0.204
139	0.0273	0.2257	0.121	0.0084	0.037
140	0.6323	0.7427	0.851	0.0108	0.014
141	0.1450	0.2647	0.548	0.0198	0.075
142	0.0402	0.0394	1.020	0.0081	0.205
143	0.1874	0.1895	0.989	0.0051	0.027
144	0.5120	0.6025	0.850	0.0223	0.037
145	0.0677	0.4466	0.152	0.0143	0.032
147	0.3696	0.4794	0.771	0.0125	0.026
148	0.1099	0.3653	0.301	0.0135	0.037
149	0.0154	0.0256	0.602	0.0082	0.320
150	0.1137	0.1438	0.791	0.0093	0.065
151	0.2037	0.3567	0.571	0.0267	0.075
152	0.0118	0.0414	0.285	0.0077	0.185
153	0.2762	0.3716	0.743	0.0102	0.027
154	0.3002	0.4842	0.620	0.0084	0.017
155	0.0290	0.0595	0.487	0.0047	0.079
157	0.0228	0.6068	0.038	0.0107	0.018
158	0.1324	0.2555	0.518	0.0103	0.040
159	0.3963	0.4919	0.806	0.0109	0.022
161	0.0057	0.0324	0.176	0.0026	0.082
162	0.4108	0.5811	0.707	0.0127	0.022