Conformational Entropy from Restricted Bond-Vector Motion in Proteins: The Symmetry of the Local Restrictions and Relation to NMR Relaxation

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ABSTRACT: Locally mobile bond-vectors contribute to the conformational entropy of the protein, given by $S_k \equiv S/k = -\int (P_{eq} \ln P_{eq})d\Omega - \ln \int d\Omega$. The quantity $P_{eq} = \exp(-u)/Z$ is the orientational probability density, where $Z$ is the partition function and $u$ is the spatially restricting potential exerted by the immediate internal protein surroundings at the site of the motion of the bond-vector. It is appropriate to expand the potential, $u$, which restricts local rotational reorientation, in the basis set of the real combinations of the Wigner rotation matrix elements, $D_{jk}^L$. For small molecules dissolved in anisotropic media, one typically keeps the lowest even $L$, $L = 2$, nonpolar potential in axial or rhombic form. For bond-vectors anchored at the protein, the lowest odd $L$, $L = 1$, polar potential is to be used in axial or rhombic form. Here, we investigate the effect of the symmetry and polarity of these potentials on $S_k$. For $L = 1$ ($L = 2$), $S_k$ is the same (differs) for parallel and perpendicular ordering. The plots of $S_k$ as a function of the coefficients of the rhombic $L = 1$ ($L = 2$) potential exhibit high-symmetry (specific low-symmetry) patterns with parameter-range-dependent sensitivity. Similar statements apply to analogous plots of the potential minima. $S_k$ is also examined as a function of the order parameters defined in terms of $u$. Graphs displaying these correlations, and applications illustrating their usage, are provided. The features delineated above are generally useful for devising orienting potentials that best suit given physical circumstances. They are particularly useful for bond-vectors acting as NMR relaxation probes in proteins, when their restricted local motion is analyzed with stochastic models featuring Wigner-function-made potentials. The relaxation probes could also be molecules adsorbed at surfaces, inserted into membranes, or interlocked within metal–organic frameworks.

1. INTRODUCTION

Typically, proteins exhibit internal mobility. Within their scope, various structural moieties, notably bond-vectors, move locally in the presence of spatial restrictions exerted by the immediate (internal) protein surroundings. These restrictions result from the anisotropic nature of the local structure. In their presence, the bond-vector orientation is distributed nonuniformly even in cases where the local motion is undetectable, while the local ordering can be measured. The pertinent probability density functions yield conformational entropy, $S_k$, defined (in units of the Boltzmann constant, $k$) as $S_k = -\int (P_{eq} \ln P_{eq})d\Omega - \int d\Omega$. $P_{eq} = \exp(-u)/Z$ is the normalized probability density, where $Z$ is the partition function and $u$ is the restricting local potential. The quantity of interest is the change in conformational entropy, $\Delta S_k$, between two protein states, entailed by a physical process.

Usually, the second term in the expression for $S_k$ cancels out in calculating $\Delta S_k$.

In some cases, the restricted local bond-vector motion is observable (following appropriate isotope labeling) with NMR relaxation. Stochastic models for NMR relaxation analysis feature explicit potentials, which straightforwardly yield $S_k$.

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literature. In the context of ligand binding, Wand et al. devised the empirical relation called “model-independent entropy meter” that features adjustable coefficients, which project the experimentally measured methyl-related changes in motion across the entire protein and ligand.12

Thus, within the scope of the MF conceptualizations, the $S^2$-to-$S_L$ conversion features in some cases simple axial potentials.

In recent years, we developed the two-body coupled-rotator slowly relaxing local structure (SRLS) approach18-19 for the analysis of NMR relaxation in proteins. In SRLS, the local potential is expanded in the basis set of the real linear combinations of the Wigner rotation matrix elements, $D_{0k}^L(\Omega)$ (in brief, real Wigner functions).16,19 In accordance with typical experimental data, the lowest even $L$ terms, and in some cases the lowest odd $L$ terms, have been kept, yielding $u = -c_0 D_{00} - c_1 D_{01} - c_2 D_{02}$ for nonpolar ordering $^2$ and $u = -c_0 D_{00} - c_1 D_{01}$ for polar ordering.23-26

Nonpolar ($L = 2$) ordering prevails when there is inversion symmetry with respect to the origin of the director (preferred probe orientation) frame for rigid molecules dissolved in anisotropic media. Polar ($L = 1$) ordering prevails when there is no inversion symmetry with respect to the origin of the director frame. This is the case for bond-vectors anchored at the protein23,24 (or any other relaxation probe anchored at the entity that represents the local director). In view of the various approximations, admixtures are most appropriate. The fact that MF, and in many cases SRLS, have treated local ordering from the nonpolar ($L = 2$) perspective is due to the fact that the theories for proteins originate in theories for small molecules.

In principle, the two-term potentials depicted above can be enhanced in SRLS by adding terms to its expression. In practice, this is often hindered by limited experimental data. We pursued the idea of potential enhancement outside of the scope of SRLS25,26 as follows. Linear combinations of real Wigner functions with $L = 1-4$ were created and optimized against the corresponding potential of mean force (POMF) obtained with MD simulations. Comparison between the best-fit Wigner function and the POMF indicated that the set of terms with $L = 1-4$ suffices for obtaining good agreement. Moreover, using such optimized potentials, new insights into the dimerization of the Rho GTPase binding domain of plexin-B1 (in brief, plexin-B1 RBD) were gained.26 In future work, we plan to incorporate these potentials unchanged into SRLS data-fitting schemes. This will improve the picture of structural dynamics to be obtained due to better potentials, and better characterization of this picture as additional parameters can now be determined with data fitting.

The POMFs are themselves restricting potentials that can yield conformational entropy. However, they are statistical functions and as such cannot be utilized in the development promoted in this study, which is based on explicit potentials.

Thus, we have at hand explicit axial and rhombic, polar and nonpolar, fairly accurate Wigner-function-made local potentials. This constitutes a rich source for conformational entropy derivation. To optimize this process in terms of the suitability of the local potential and the accuracy of the pertinent conformational entropy, it is important to determine the relation between potential form, symmetry, parity, etc. and conformational entropy. We do this here for the $L = 1$ and 2 potentials depicted above. Correlation graphs are provided, and their utilization is illustrated with several applications. NMR relaxation analysis using SRLS directly can benefit from this study.

A theoretical summary is given in Section 2. Our results and their discussion are described in Section 3, and our conclusions appear in Section 4.

2. THEORETICAL BACKGROUND

2.1. Restricting Potentials. The orienting potential, $U(\Omega)$, associated with restricted rotational reorientiation, is typically given by the expansion in Wigner rotation matrix elements, $D_{0k}^L(\Omega)^4,5$

$$u(\Omega) \equiv U(\Omega)/kT = \sum_{l=1}^{\infty} \sum_{m=-l}^{l} \sum_{k=-l}^{l} c_{MK}^l D_{MK}^l(\Omega)$$

where the Euler angles, $\Omega$, describe the orientation of the probe relative to the director, which is the direction of preferential orientation in the restricting surroundings. Note that $u$ and the coefficients featured by eq 1 are dimensionless.

It is usually assumed that the director is uniaxial (see ref 17 for the introduction of biaxiality in a manner involving one additional variable angle). Consequently, the “quantum number” $M$ in eq 1 is zero and $\Omega = (0, \theta, \varphi)$. One has to ensure that the potential is real; this is achieved by expanding $u(\Omega)$ in the basis set of the real combinations of the Wigner rotation matrix elements (the real Wigner functions). Finally, the infinite expansion in eq 1 has to be truncated. Keeping only the lowest even $L$ terms, one has

$$u(\theta, \varphi) = -c_0^2 D_{00}^2(\theta, \varphi) - c_1^2 D_{02}^2(\theta, \varphi)$$

For $c_0 > 0$ (the main ordering axis preferentially parallel to the director; this is termed parallel ordering). For $c_0 < 0$, the main ordering axis preferentially perpendicular to the director; this is termed perpendicular ordering.4,5 Keeping only the lowest odd $L$ terms, one has

$$u(\theta, \varphi) = -c_0^2 D_{00}^2(\theta, \varphi) - c_1^2 D_{02}^2(\theta, \varphi)$$

For $c_0 > 0$ (the primary polar axis is parallel to the $+z$ axis of the local ordering frame. For $c_0 > 0$ (the primary polar axis is tilted in the $+z$ (−$z$) plane of the local ordering frame).2,23

Exploring the relationship between these (and further enhanced) potentials and the conformational entropy, $S_u$ is very broad in scope. The connection with SRLS25-29, which applies to proteins in solution, has been delineated above. The SRLS limit where the protein motion is frozen is the very broad in scope. The connection with SRLS,19-26 our discussion are described in Section 3, and our conclusions appear in Section 4.

2.2. Parameters of Interest Defined in Terms of the Restricting Potentials. 2.2.1. Order Parameters. Order parameters are ensemble averages of real Wigner functions defined in terms of restricting potentials. Here, we are using the order parameters

$$S_0^L = \langle D_{00}^L(\theta, 0, 0) \rangle, \quad L = 1, 2$$

and

$$S_1^L = \langle D_{01}^L(\theta, \varphi) D_{01}^L(\theta, \varphi) \rangle$$
The change in conformational entropy is given by1

\[ S_k = \pi [1 - \cos(\beta_0)]^{1/2} \]

The parameter \( A \) is adjusted to suit the individual potentials. This equation was obtained with a different parameterization in ref 14. Only positive values of \( S \) (which, as pointed out above, correspond to parallel ordering) are considered. Empirical relations for methyl groups were developed within the scope of a dictionary-type framework in ref 14 (see above). A comprehensive empirical relation was developed in ref 12 (see above).

3. RESULTS AND DISCUSSION

3.1. Axial Potentials. The first axial \((k = 0)\) even \( L \) term in the Wigner function expansion yields the potential \( u = -c_0 D_{00}^2 \), the order parameter \( S_0 = \langle D_{00}^2 \rangle \), and the squared order parameter \( (S_0^2) \) for \( (0 < c_0 < 1) \). Figure 1a–c shows \( S_k \) as a function of the potential \( u = -c_0 D_{00}^2 \) and \( S_0 = \langle D_{00}^2 \rangle \), respectively. A comparison with MF may be conducted given that both \( S \) and \( S_0 \) range from 0 to 1. Note that \( S_0 = 0 \) corresponds to \( c_0 = 0 \) and \( S_0 = 1 \) corresponds to \( c_0 \rightarrow \infty \). We use \( 0 \leq c_0 \leq 50 \); any number greater than approximately 20 is virtually in the scope of a dictionary-type framework in ref 14 (see above). The parameter \( A \) is adjusted to suit the individual potentials. This equation was obtained with a different parameterization in ref 14. Only positive values of \( S \) (which, as pointed out above, correspond to parallel ordering) are considered. Empirical relations for methyl groups were developed within the scope of a dictionary-type framework in ref 14 (see above). A comprehensive empirical relation was developed in ref 12 (see above).

For wobble-in-a-cone and one-dimensional (1D) harmonic oscillator, analytical expressions connecting \( S^2 \) with \( S_k \) were developed.1,12 For several simple axial potentials, the following empirical expression was developed\(^1\)

\[ S_k = A + \ln [3 - (1 + 8S)^{1/2}] \]

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Figure 1c shows \( S_k \) as a function of \( (S_0^2) \). The conformational entropy, \( S_k \), was calculated according to eq 6; \( S_0 \) was calculated in terms of the potential \( u = -c_0 D_{00}^2 \). All of the curves in Figure 1 of ref 1 agree qualitatively with the curve in Figure 1c; none agrees with it quantitatively, although many of the simple potentials considered in ref 1 are limiting cases of \( u = -c_0 D_{00}^2 \).

As expected, in all three panels of Figure 1, the entropy decreases with increasing potential or ordering strength. In the region of interest for proteins, where \( 1 \leq c_0 \leq 10 \), the \( S_k \) versus \( c_0 \) curve is substantially steeper, hence more sensitive, than the other two curves.

\[ \int D_{\theta \phi}(0, \theta, \phi) e^{-u(\theta,\phi)} \sin \theta \, d\theta \, d\phi 
\]

where \( u(\theta,\phi) \) is the restricting potential.

\[ \Delta S_k = \Delta \left[ -\int (P_{\theta \phi} \ln P_{\theta \phi}) \, d\Omega \right] \]

In this study, \( u \) is given by eqs 2 or 3 and \( \Delta S_k \) is calculated using eqs 6 and 7. The coefficients \( c_{00}, c_{01}, c_{02} \) and \( c_{11} \) may be positive or negative.

Let us relate to the model-free treatment of eq 7. The local spatial restrictions are implicit in the squared generalized order parameter, \( S^2 \), defined as\(^13\)

\[ S^2 = \sum_{K=0,\pm 1,\pm 2} \langle D_{00}^2 (D_{00}^2 - K) \rangle \]

where \( \langle \ldots \rangle \) denotes ensemble average. The functions in eq 8 are the (complex) Wigner rotation matrix elements with \( L = 2 \) and \( K = -L, \ldots, L \). We have shown that\(^10\)

\[ S^2 = (S_0^2)^2 + 0.5 (S_0^2)^2 \]

For wobble-in-a-cone and one-dimensional (1D) harmonic oscillator, analytical expressions connecting \( S^2 \) with \( S_k \) were developed.1,12 For several simple axial potentials, the following empirical expression was developed\(^1\)

\[ S_k = A + \ln [3 - (1 + 8S)^{1/2}] \]

The parameter \( A \) is adjusted to suit the individual potentials. This equation was obtained with a different parameterization in ref 14. Only positive values of \( S \) (which, as pointed out above, correspond to parallel ordering) are considered. Empirical relations for methyl groups were developed within the scope of a dictionary-type framework in ref 14 (see above). A comprehensive empirical relation was developed in ref 12 (see above).

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Let us focus on $c_0$. As indicated, nonpolar ($L = 2$) local ordering may be parallel or perpendicular. We and others found that typically the main ordering axis at N–H sites in proteins is $C^\alpha$–$C^\alpha$. Recalling that the director is given by the equilibrium orientation of the N–H bond, it may be deduced that, within a good approximation, perpendicular ordering prevails at these sites. In general, the sign of the coefficient, $c_0$, in eq 2, obtained with data fitting, determines whether the local ordering is parallel or perpendicular. This information enters the expression for $S_k$ straightforwardly (eq 6). When MF is used, data fitting determines $S^T$. For perpendicular ordering to enter $S_k$, one has to use $-S$ in expressions such as eq 10. This has not been done, with implications illustrated below.

Figure 2a–c is analogous to Figure 1a–c, except that now both parallel and perpendicular orderings are featured by considering the full parameter range of $c_0$. For parallel ordering, one has $0 \leq c_0^2 < \infty$ and $0 \leq S_k^2 < 1$; for perpendicular ordering, one has $0 > c_0^2 > -\infty$ and $0 \geq S_k^2 \geq 0.5$. The $S_k$ patterns for $c_0^2 < 0$ and $c_0^2 > 0$ differ; so do the $S_k$ patterns for $S_k^2 < 0$ and $S_k^2 > 0$. Figure 2c shows $S_k$ as a function of $(S_k^2)^2$. A second branch yielded by negative $S_k^2$ values is featured in the 0–1 range; this branch is missing in MF.

Let us focus on the simplest axial polar case. $L = 1$ potentials are treated in refs 23, 26. In ref 24, we analyzed the $^{15}$N–H relaxation data from the third immunoglobulin binding domain of streptococcal protein G (GB3) with rhombic $L = 1$ or 2 potentials and found that the results differ. Importantly, we found that the process by which GB3 binds to its cognate Fab fragment has polar character. Thus, potential parity is both influential and important.

Figure 3 refers to the potential $u = -c_0^2 D_{00}$ for $0 > c_0$, the order parameter, $S_k^2$ as a function of $c_0^2$, and the minimum, $u_{\text{min}}$ of $u = -c_0^2 D_{00}$, as a function of $c_0^2$. Conformational entropy, $S_k$ (blue), and the minimum, $u_{\text{min}}$, of $u = -c_0^2 D_{00}$ (red), as a function of $c_0^2$ (d).

We extend the analysis by including in it order parameters and the minima of the $L = 1$ and 2 potentials. Figure 4a shows $S_k^2 = (D_{00})$ as a function of $c_0^2$ for $-30 \leq c_0^2 \leq 30$, and Figure 4b shows $S_k^2 = (D_{00})$ as a function of $c_0^2$ for $-30 \leq c_0^2 \leq 30$. For $L = 1$, $S_k^2$ is the same for $c_0^2 = +c_0^2$ (primary polar axis pointing along $z$ and $-z$); for $L = 2$, $S_k^2$ is not the same for $c_0^2$ (parallel ordering) and $-c_0^2$ (perpendicular ordering).

Figure 4c shows $S_k$ as a function of $c_0^2$ (blue), superposed on the minimum of the $u = -c_0^2 \cos \theta$ potential, denoted $u_{\text{min}}$ as a function of $c_0^2$ (red). The corresponding scales are depicted on the left and right ordinates. As one would expect, primary polar axes pointing along $z$ or $-z$ yield the same patterns.

Figure 4d shows $S_k$ as a function of $c_0^2$ (blue), superposed on $u_{\text{min}}$ of $u = -c_0^2 (1.5 \cos \theta - 0.5)$ as a function of $c_0^2$ (red). The corresponding scales are depicted on the left and right ordinates. Both patterns differ for parallel ($c_0^2 > 0$) and perpendicular ($c_0^2 < 0$) ordering. In particular, if the potential...
respectively. Figure 5a,b shows the conformational entropy, $S_k$, as a function of the coefficients, $c_0$ and $c_1$, of the potential $u = -c_0 D_0 - c_1 (D_{1,1} - D_{0,1})$ (a). Conformational entropy, $S_k$, as a function of the coefficients, $c_2$ and $c_3$, of the potential $u = -c_2 D_2 - c_3 (D_{2,2} + D_{2,0})$ (b). The minimum, $u_{\text{min}}$, of $u = -c_0 D_0 - c_1 (D_{1,1} - D_{0,1})$ as a function of $c_0$ and $c_1$ (c). The minimum, $u_{\text{min}}$, of $u = -c_2 D_2 - c_3 (D_{2,2} + D_{2,0})$ as a function of $c_2$ and $c_3$ (d). Color codes are on the right of each panel. A total of 1681 data points were used in generating each panel of this figure.

Figure 6. Conformational entropy, $S_n$, as a function of the order parameters, $S_n^0$ and $S_n^1$, defined in terms of the potential $u = -c_0 D_0 - c_1 (D_{1,1} - D_{0,1})$ (a). Conformational entropy, $S_n$, as a function of the order parameters, $S_n^0$ and $S_n^1$, defined in terms of the potential $u = -c_2 D_2 - c_3 (D_{2,2} + D_{2,0})$ (b). $u_{\text{min}}$ of $u = -c_0 D_0 - c_1 (D_{1,1} - D_{0,1})$ as a function of $S_n^0$ and $S_n^1$ (c). $u_{\text{min}}$ of $u = -c_2 D_2 - c_3 (D_{2,2} + D_{2,0})$ as a function of $S_n^0$ and $S_n^1$ (d). Color codes and number of data points as in Figure 5.

minimum is $-a$ (at $\theta = 0^\circ$) for parallel ordering, it will be $-a/2$ (at $\theta = 90^\circ$) for perpendicular ordering.

3.2. Rhombic Potentials. Equations 2 and 3 show the functional forms of the rhombic $L = 2$ and 1 potentials, respectively. Figure 5a,b shows $S_k$ as a function of the potential coefficients $c_0$ and $c_1$ ($c_2$ and $c_3$). Figure 5c,d shows $u_{\text{min}}$ as a function of the coefficients $c_0$ and $c_1$ ($c_2$ and $c_3$). In all of the Figure 5 simulations, and in the simulations of Figures 6 and 7, 1681 data points were used; the same results were obtained with a larger number of data points.

The curves depicted in Figure 5a represent the group of points with coordinates ($c_0^1$, $c_1^1$) that yield the same conformational entropy, $S_k$; those depicted in Figure 5b represent the group of points with coordinates ($c_0^2$, $c_2^2$) that yield the same conformational entropy. We call these curves $S_k$ isolines. Figure 5c,d shows the $u_{\text{min}}$ isolines for the $L = 1$ and 2 potentials, respectively. The color codes for the values of $S_k$ and $u_{\text{min}}$ are given on the right of each figure. In Figure 5a,b, intense orange corresponds to large entropy and intense blue corresponds to small entropy. In Figure 5c,d, intense orange corresponds to shallow potentials and intense blue corresponds to deep potentials.

$L = 1$ potentials (Figure 5a,c), which are shallow and nearly axial (small $c_0^1$ and $c_1^1$), yield large entropy; those which are deep and highly rhombic (large $c_0^1$ and $c_1^1$) yield small entropy. In-between the changes are less monotonic for $S_k$.****
c). The isolines of Figure 6 are much more dispersed in conformation space than the isolines of Figure 5. Good certainty in $S_{k}$ forms.9,36,37 The minima of these potentials (in units of rhombicity, as the intermediate, strength, and relatively great, and intermediate, sensitivity of the outer region, in a distinctive manner. Note that the high shapes of the $L$ are more sensitive in the outer region. The situation is more complicated for $L = 2$, which is associated with asymmetric shapes of the $S_k$ and $u_{\text{min}}$ isoline patterns (Figure 5b,d). While $+c_2$ and $-c_2$ yield the same isoline patterns, $+c_0$ and $-c_0$ yield different isoline patterns. The $S_k$ patterns are more sensitive in the middle, and the $u_{\text{min}}$ patterns are more sensitive in the outer region, in a distinctive manner. Note that the high sensitivity of the $S_k$ isoline patterns ensures good certainty in $S_k$.

Figure 6a–d is analogous to Figure 5a–d, with the coordinates being order parameters instead of potential coefficients. $(S_0', S_2')$ are defined in terms of $(c_0', c_2')$, and $(S_0, S_2)$ are defined in terms of $(c_0, c_2)$ (cf. Equations 2, 3 and 4a–c). The isolines of Figure 6 are much more dispersed in conformation space than the isolines of Figure 5. Good certainty in $S_k$ is expected for potentials of relatively great, and intermediate, strength, and relatively great, and intermediate, rhombicity, as the $S_k$ isolines vary most in these regions.

$N$–$H$ bonds in well-structured regions of the protein conformation feature relatively strong and highly rhombic potentials.20,21 In this case, it is preferable to use the correlation graphs of Figure 6. C–CH$_3$ bonds in proteins feature relatively weak potentials.20,21 In that case, it is preferable to use the correlation graphs of Figure 5 (see examples below).

Figure 7a shows superposed $S_k$ and $u_{\text{min}}$ isolines as a function of the coefficients $c_0$ and $c_1$ of the $L = 1$ potential. The objective is to examine the correlation between $S_k$ and $u_{\text{min}}$. One can recognize a one-to-one correspondence; its precise form is revealed by Figure 7c, where $S_k$ is depicted as a function of $u_{\text{min}}$. Figure 7b shows superposed $S_k$ and $u_{\text{min}}$ isolines as a function of the coefficients $c_0$ and $c_2$ of the $L = 2$ potential. The relation between $S_k$ and $u_{\text{min}}$ is intricate. Indeed, Figure 7d shows that, in general, multiple $S_k$ values correspond to a given value of $u_{\text{min}}$.

The utilization of the correlation graphs of Figures 1–6 is illustrated below.

3.3. Applications. 3.3.1. Example 1. Statistical potentials of mean force (POMFs) can be derived directly from MD trajectories.25,26,36,37 Figure 8a,b shows images of two POMFs representing two protein states before and after a physical event. They belong to residue G73 of plexin-B1 RBD in monomer and dimer forms.26 but we consider them representative of a general situation where the only information available consists of POMFs.
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The estimated minima are (in units of $kT$) 8.4 and 7.8. One could use eq 7 to determine $\Delta S_k$. However, at this stage, it is not known whether the local ordering is parallel or perpendicular. Taking $u = -\frac{c_2}{D_0}$ as a reasonable approximation, and using jointly the graphs of Figure 2a–c, it might be possible to distinguish between these two situations. Figure 2c is likely to be particularly useful in this context. With this information in hand, one could proceed effectively with detailed analysis, where rhombic symmetry is allowed for.

3.3.2. Example 2. $^{15}$N relaxation of the major urinary protein 1 (MUP-1) and its complex with the pheromone 2-sec-butyl-4,5-dihydrothiazol were studied with MF at 300 K in early work. The authors of ref 38 found that pheromone binding brings about increase in conformational entropy. We studied this system with SRLS in the 283–308 K range using $u = -\frac{c_2}{D_0}$ and assuming parallel ordering, to find that below approximately 300 K, $S_k$ indeed increases, but above that temperature, it decreases, upon pheromone binding.

At 308 K, $c_2$ is on the order of 15–17 in both forms of MUP-I. At 283 K, the majority of the $c_2$ values are on the order of 25 in the free form and between 15 and 20 in the bound form (Figure 7 of ref 9). $\Delta S_k$ is small at both temperatures. The $S_k$ versus positive $c_2$ curve in Figure 2a shows that the dependence of $S_k$ on $c_2$ is nearly linear for large $c_2$ and much steeper for smaller $c_2$. Therefore decreasing the pressure at 283 K would lower $c_2$ differentially increasing $\Delta c_2$ hence $\Delta S_k$. Thereby, the change in conformational entropy upon pheromone binding will be determined with enhanced certainty in the interesting temperature range.

3.3.3. Example 3. Table 1 shows the average values of the potential coefficients $\langle c_2 \rangle$ and $\langle c_2 \rangle$ for all of the methyl moieties of the complex of Ca$^{2+}$–calmodulin with the peptide smMLCLp at 288 and 308 K (Rows 1 and 2), and $\langle c_2 \rangle$ and $\langle c_2 \rangle$ of Alanine (A) and Methionine (M) at 295 K (Rows 3 and 4).

Table 1. Average Potential Coefficients, $\langle c_2 \rangle$ and $\langle c_2 \rangle$, of All of the Methyl Groups of the Complex of Ca$^{2+}$–Calmodulin with the Peptide smMLCLp at 288 and 308 K (Rows 1 and 2), and $\langle c_2 \rangle$ and $\langle c_2 \rangle$ of Alanine (A) and Methionine (M) at 295 K (Rows 3 and 4)

| $T$, K | $\langle c_2 \rangle$ | $\langle c_2 \rangle$ | $S_k$ |
|-------|------------------|------------------|------|
| 1     | 288              | 0.92             | 0.68 | 1.69 |
| 2     | 308              | 0.39             | 0.74 | 1.77 |
| 3     | 295              | 0.22             | 0.98 | 1.72 |
| 4     | 295              | 0.65             | 0.50 | 1.76 |

The following situation is envisioned for the methyl groups of a given protein designated for SRLS analysis. One selects representative methyl moieties and determines $c_2$ and $c_2$ with SRLS data fitting. Using Figure 5b, the corresponding isolines are identified. For small $c_0$ and small $c_0$, typical of methyl moieties in proteins, every $S_k$ isoline has two points with $c_2 \approx 0$. In some cases, $c_2$ of such points will be similar to the $c_2$ data of the representative residues. For residues with data similar to those of the representative residues (appropriate criteria will have to be specified), it will be useful to use in SRLS calculations $c_2$ and $c_2 \approx 0$. The geometric information will have to be updated accordingly.

3.3.4. Example 4. Table 2 shows $S_0$ and $S_2$ obtained with SRLS analysis of the N–H bonds of residues Q2 and A26 of the third immunoglobulin binding domain of streptococcal protein G (GB3) using the rhombic $L = 2$ potential.

The points $(S_0, S_2)$ with values $(−0.49, 1.08)$ and $(−0.42, 1.13)$ are located in the upper left corner of Figure 6b, as they represent strong perpendicular ordering and large rhombicity. Figure 6b refers to the rhombic $L = 2$ potential, whereas Figure 6a refers to the rhombic $L = 1$ potential. Figure 6a shows better sensitivity in the region under consideration than Figure 6b. This indicates that analyzing $^{15}$N relaxation in compact proteins such as GB3 using the rhombic $L = 1$ potentials is likely to yield local potentials, hence pertinent order parameters and conformational entropy, which are determined with enhanced certainty. This is useful information for future work.

3.3.5. Future Prospects. It is of interest to compare for a given NMR relaxation probe dynamic structures associated with the same value of $S_k$. This can be accomplished by the following strategy: Analyze NMR relaxation data of a given probe with SRLS and determine the "experimental" $c_0$ and $c_2$ values; calculate $S_k$ (eq 6) and use Figure 5b to determine the corresponding isoline; select representative pairs of $c_2$ and $c_2$ belonging to this isoline; use these $c_2$ and $c_2$ pairs unchanged in SRLS data fitting and determine the corresponding local motional rates and local geometry; and compare the results of steps 1 and 4.

3.3.6. Comments. (1) Lately, pressure-dependent and temperature-dependent studies have been performed in the context of conformational entropy derivation. The results of such studies might be useful in a project where explicit SRLS potentials and statistical MD-derived POMFs improve one another within the scope of an iterative scheme. We contemplate devising such a scheme in future work. (2) We derive conformational entropy from restricted local motions. In

\[
\begin{align*}
\langle c_2 \rangle & = -\frac{c_0}{2} - \frac{1}{2} \sqrt{\frac{3}{2} c_2^2} \\
\langle c_2 \rangle & = \left( \frac{3}{2} c_0^2 - \frac{1}{2} c_2^2 \right) / 2
\end{align*}
\]
the context of NMR relaxation, the pertinent restrictions are “observed” sources. There exists a different approach pursued, e.g., in ref 44, where the entropy changes are derived using an "entropy meter". The latter is an expression comprising S̃ from observed sources as well as adjustable coefficients that "project the experimentally measured changes in motion across the entire protein and ligand". The projected changes are “unobserved” sources. Such contributions are outside the scope of our study.

4. CONCLUSIONS

The local potentials, u, at the site of mobile bond-vectors in proteins have been expressed in terms of the real linear combinations of the Wigner rotation matrix elements, D_L^L, (in brief, real Wigner functions), with L = 1 or 2. From them, the conformational entropy, S_u, has been derived. To determine the effect of the symmetry (axial or rhombic) and L-parity of the local potential on the associated conformational entropy, correlation graphs between S_u and the coefficients of u, as well as between S_u and the order parameters defined in terms of u, have been created. The S_u patterns obtained are highly specific and exhibit distinctive parameter-range-dependent sensitivity. This lays the groundwork for devising potentials for the determination of S_u that best suit given physical circumstances.

NMR relaxation analysis has been invoked as a physical method that can profit substantially from these results. So can any physical method where the local restrictions are expressed in terms of real Wigner functions.

We use here the amide bond and the methyl moiety of proteins as examples of NMR relaxation probes. Additional examples are molecular moieties adsorbed as surfaces, embedded in membranes, or interlocked in metal–organic frameworks.
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