Published in final edited form as:

J Phys Chem B. 2016 March 24; 120(11): 2886–2898. doi:10.1021/acs.jpcc.6b00524.

Local Ordering at Mobile Sites in Proteins from Nuclear Magnetic Resonance Relaxation: the Role of Site Symmetry

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Abstract

Restricted motions in proteins (e.g., N–H bonds dynamics) are studied effectively with NMR. By analogy with restricted motions in liquid crystals (LC), the local ordering has in the past been primarily represented by potentials comprising the $L = 2$, $|K| = 0, 2$ spherical harmonics. However, probes dissolved in LC's experience non-polar ordering, often referred to as *alignment*, while protein-anchored probes experience polar ordering, often referred to as *orientation*. In this study we investigate the role of local (site) symmetry in the context of the polarity of the local ordering. We find that potentials comprising the $L = 1$, $|K| = 0, 1$ spherical harmonics represent adequately polar ordering. It is useful to characterize potential symmetry in terms of the irreducible representations of D_{2h} point group, which is already implicit in the definition of the rotational diffusion tensor. Thus, the relevant rhombic $L = 1$ potentials have B_{1u} and B_{3u} symmetry whereas the relevant rhombic $L = 2$ potentials have A_g symmetry. A comprehensive scheme where local potentials and corresponding probability density functions (PDFs) are represented in Cartesian and spherical coordinates clarifies how they are affected by polar and non-polar ordering. The Cartesian coordinates are chosen so that the principal axis of polar axial PDF is pointing along the z -axis, whereas the principal axis of the non-polar axial PDF is pointing along $\pm z$. Two-term axial potentials with $1 \leq L \leq 3$ exhibit substantial diversity; they are expected to be useful in NMR-relaxation-data-fitting. It is shown how potential coefficients are reflected in the experimental order parameters. The comprehensive scheme representing local potentials and PDFs is exemplified for the $L = 2$ case using experimental data from ^{15}N -labeled plexin-B1 and thioredoxin, ^2H -, and ^{13}C -labeled benzenehexa- n -alkanoates, and nitroxide-labeled T4 lysozyme. Future prospects for improved ordering analysis based on combined atomistic and mesoscopic approaches are delineated.

TOC Figure

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Supporting Information Available: Table S1, showing the relevant Part of the D_{2h} Point Group Character Table, and Table S2, showing the functions $D_{00}^L(0, \theta, 0)$ for $L = 1-4$, are provided. In addition figure S1, analogous with Figure 6 except that for $c_0^1 = -10$ and $c_1^1 = 10$ in Figure S1A; $c_2^2 = -10$ and $c_2^2 = 10$ in Figure S1B; $c_0^1 = -10$ and $c_1^1 = -10$ in Figure S1C; $c_0^2 = -10$ and $c_2^2 = -10$ in Figure S1D, is provided. This material is available free of charge via the internet at <http://pubs.acs.org>.

Keywords

restricted motions; symmetry of local ordering; alignment versus orientation; spherical harmonics

1. Introduction

Molecules may be characterized by 3D structures, and dynamics which are often associated with motional rates. We consider, for example, a rigid molecule reorienting in an isotropic medium.^{1–3} Such motion may be represented by a 3D diffusion tensor,³ whose principal values are a function of molecular shape. However, when the molecule is reorienting in an anisotropic medium, or parts of a flexible molecule are reorienting in anisotropic local surroundings, the description of the motion also has to include proper representation of the local spatial constraints.^{4–7} The latter can be expressed in terms of orienting potentials,^{4–7} which represent the local structures. Orienting potentials are associated with the preferential arrangement of the molecule in the surrounding local space, and thus reflect the local geometric arrangement. In other words, structure-related and geometry-related kinetic features can be elucidated by studying motions that take place in anisotropic environments.^{4–19}

A class of restricted motions of particular interest are intra-molecular reorientation processes of segments of proteins (more generally, bio-macromolecules).^{10–19} Examples include small moieties such as amide or methyl groups^{13–19} that execute localized motions, as well as large loops or domains that execute collective motions.^{17–19} These processes are associated with the internal mobility/flexibility of the protein. In this study we focus on structure-related and geometry-related aspects of the local ordering in proteins in terms of their respective symmetries.

Restricted motions are often treated within the scope of atomistic molecular dynamics (MD) simulations. Usually this is done by taking into account sets of relatively slow coordinates.^{20–25} Using effective sampling and appropriate analysis of MD trajectories, probability density functions (PDFs) yielding approximate potentials of mean force (PMFs) have been determined (e.g., ref 25). To our knowledge, the more mesoscopic (collective) local features, such as explicit forms of local anisotropic potentials and local molecular geometry associated with these potentials, as well as effective diffusion tensors, have not been provided by MD methods.

On the other hand, mesoscopic models for treating restricted motions are convenient for interpreting magnetic resonance experiments.⁴⁻¹⁹ They encompass order parameters and PDFs in a straightforward manner.^{6,7} These stochastic approaches utilize a tensorial representation of diffusion and ordering. Using NMR or ESR spectroscopy as reporters of the structural dynamics, one is able to determine the structure-related principal values and the geometry-related orientations of the ordering and diffusion tensors.

The orienting potential, $U(\Omega)$, is typically given in mesoscopic stochastic models by the expansion in the Wigner rotation matrix elements, $D_{MK}^L(\Omega)$:

$$u(\Omega) \equiv U(\Omega)/k_B T = - \sum_{L=1}^{\infty} \sum_{M=-L}^{+L} \sum_{K=-L}^{+L} c_{MK}^L D_{MK}^L(\Omega) \quad (1)$$

with $u(\Omega)$ and the coefficients c_{MK}^L being dimensionless. The Euler angles, Ω , describe the molecular orientation relative to the director frame associated with the orienting surroundings. This general expansion needs to be truncated at some value $L = L_{\max}$ before attempting to reproduce experimental data, thus leaving just a few leading terms. However, the “quantum numbers” M and K are just restricted to $|M| \leq L$ and $|K| \leq L$, which still yields too many c_{MK}^L fitting parameters for the typically limited experimental data available. We thus need to make sensible choices whereby a sufficiently reduced number of parameters are used in the fitting process.

Let us first take a hint from the symmetry of the rotational diffusion tensor, which is a second rank symmetric tensor consistent with D_{2h} point group symmetry. That is, however complex the shape of a molecule, we are approximating its diffusive properties in terms of D_{2h} point group symmetry. Thus, it seems reasonable that we impose similar effective point group symmetry on the orientational potential of eq 1. Let us examine what has been done in this context in the past.

Stochastic models for studying restricted motions of rigid molecules dissolved in liquid crystalline (LC) media by ESR and NMR have been developed in early work.⁴⁻⁶ The usual assumption is the existence of a uniaxial LC director. Consequently the “quantum number”, M , in eq 1 is zero, yielding:⁴⁻⁷

$$u(\theta, \varphi) = - \sum_{L=1}^{\infty} \sum_{K=-L}^{+L} c_K^L D_{0K}^L(0, \theta, \varphi) \quad (2)$$

In this case only the two polar angles, θ and φ , are needed to describe the molecular orientation relative to the director frame. This is a typical simplification, in that it requires just the $D_{0K}^L(0, \theta, \varphi)$ functions, which are proportional to the corresponding spherical harmonics, $Y_{LK}(\theta, \varphi)$ (e.g., see ref 26).

In a simple sense, the ordering of a large solute probe reorienting in a macroscopically aligned liquid crystal (LC) solvent which exhibits inversion symmetry with respect to the origin of the director frame may be taken as reflecting this inversion symmetry. Such a scenario represents “non-polar” ordering. Potentials describing non-polar ordering comprise

only the even- L terms of eq 2. This may be thought of as preferential arrangement in space of double-headed-arrows, a setup often referred to as *alignment*^{27,28} The leading terms here are the functions D_{0K}^2 with $|K|=0, 1, 2$ (more generally, $L = 2, 4, 6 \dots$).

Envision a smaller probe residing in one leaflet of a lipid bilayer. The local environment is most certainly polar, requiring odd- L terms to be retained in eq 2. A similar requirement would apply to a probe dissolved in a thermotropic liquid crystal formed of polar molecules (unless the probe is much larger than the liquid crystal molecule). One may think of polar ordering as preferential arrangement in space of single-headed arrows, which is often referred to as *orientation*.^{27,28}

Now consider a probe reorienting in the anisotropic internal space of a protein while anchored at it. The fact that the probe is bonded to the protein implies a single-headed-arrow-type, i.e., polar, ordering. We represent this scenario by taking the local director to be uniaxial and polar. In this case eq 2, with odd- L terms retained, should be used. The leading terms here are D_{0K}^1 with $|K|=0, 1$ (more generally, $L = 1, 3, 5 \dots$).

Comparison of the polar and non-polar scenarios then clarifies the distinction between *orientation* (visualized as a vector) and *alignment* (visualized as a symmetric tensor), as we have defined them above. The respective potentials may then be classified in terms of the irreducible representations of D_{2h} point group.

The comparison between the polar and non-polar cases and their admixture is carried out within the scope of a comprehensive scheme which includes potential shapes, and forms of the associated PDFs, represented in both Cartesian-coordinate and spherical-coordinate systems. For axial probes ($K=0$), the terms with $L=3$ and $L=4$ are also considered.

Unlike the LC director which is fixed in space (although fluctuations do occur), a local director such as prevailing in internally mobile proteins is itself tumbling in solution, being defined in terms of the protein axes. This represents a two-body (protein and probe) mesoscopic coupled-rotator problem. Freed et al. solved this problem within the scope of the slowly relaxing local structure (SRLS) approach.⁸ SRLS, and its limit where the protein motion is frozen, the microscopic-order-macroscopic-disorder (MOMD) approach,²⁹ have been applied extensively within the scope of ESR spectroscopy.^{10–12} Applications to model and biological membranes, complex fluids, nitroxide-labeled proteins and DNA fragments, etc., have been reported.^{30,31} We have also applied SRLS extensively to NMR relaxation in proteins dissolved in aqueous solution.^{13–19} Recently we have applied MOMD to the analysis of ^2H NMR lineshapes from polycrystalline proteins experiencing internal mobility.^{32,33} All of these approaches use potentials expanded in the basis set of the $D_{0K}^L(0, \theta, \varphi)$ functions. Thus, the ordering-related analysis in this work is relevant to SRLS, MOMD, and any other stochastic model for analyzing NMR and ESR lineshapes and/or relaxation parameters in proteins utilizing the $D_{0K}^L(0, \theta, \varphi)$ functions (i.e., the spherical harmonics) as basis-sets for the expansion of the local potential. We also describe ties to atomistic MD methods, as described below.

In previous SRLS-based and MOMD-based studies the potential typically included the leading even- L ($L = 2$) terms.^{9,10,14–19,29,32,33} In a few cases the $L = 4$, $K = 0$, 2 terms were also included in the analysis.⁹ A potential given by the $L = 1$, $K = 0$ term has been considered by Polimeno and Freed in ref 8. It was found that using potentials given by the $L = 1$, $K = 0$ term or the $L = 2$, $K = 0$ term yields results for motional correlation functions that are similar in nature (although the potential coefficients determined have different magnitudes) when, as in ESR or NMR, the effect of the motion consists of averaging second-rank magnetic tensors.⁸ In this study we also take into account the leading odd- L ($L = 1$) terms; this constitutes a substantial enhancement (currently within the scope of designing the local potential) of the SRLS and MOMD potentials.

Given that order parameters rather than potential coefficients are typically determined experimentally, we show how the latter are reflected by the former. The comprehensive potential/PDF/order parameter description delineated above is exemplified for $L = 2$ potentials using previously-determined order parameters or potential coefficients from ¹⁵N-labeled plexin-B1 in solution,^{25,34} T4 lysozyme in solution,³⁵ polycrystalline thioredoxin,³⁶ and discotic and solid phases of benzenehexa- n -alkanoates (with $n = 6$ and 7).³⁷

Our results are expected to be relevant to a broad range of experiments. Liquid-crystal phases of phospholipids require odd- L potentials for proper analysis of ³¹P spin relaxation.³⁸ Rhombic polar order-parameters have been invoked in the context of twist-bend nematic phases.³⁹ However, they have been associated with the conical angle and the pitch of the medium, rather than potentials formed of odd- L terms of eq 2. Potentials given by the $L = 1$, $K = 0$ term have been used to analyze dielectric relaxation of ferroelectric systems,⁴⁰ to treat electric-field-implied polar order,⁴¹ and interpret the orientation of asymmetric paramagnetic molecules in strong magnetic fields. To our knowledge, applications where the $L = 1$, $|K| = 1$ terms, or both $L = 1$ and $L = 2$ terms, were also included in the expression for the potential, as required for nonaxial cases, have not been reported.

2. Theoretical Background

As pointed out above, we take the local director to be uniaxial. Next we have to ensure that the potential is real; this requires that $c_K^L = (-)^K (c_{-K}^L)^*$ (ref 9). The potential which is real is given by:

$$u(\theta, \varphi) = - \sum_L^\infty \left\{ c_0^L D_{00}^L(0, \theta, 0) + \sum_{K>0}^L c_{K\pm}^L \left[D_{0K}^L(0, \theta, \varphi) + (-)^j D_{0-K}^L(0, \theta, \varphi) \right] \right\} \quad (3)$$

where $j = 1$ for odd- L and $j = 0$ for even- L , and $c_{K\pm}^L \equiv c_K^L \pm c_{-K}^L$.

Let us focus on the $L = 2$ potential and let us assume for convenience, as is usually done, that the local ordering tensor defined in terms of this potential is diagonal in the same molecular frame as the diffusion tensor. The coefficients $c_0^2, c_{2\pm}^2 \equiv c_2^2 \pm c_{-2}^2$ are themselves irreducible tensor components. The Cartesian tensor with components $c_{ij}^2 = c_{ji}^2$ is diagonal (i.e., $Tr(c_{ii}^2) = 0$) in the same frame as the local ordering tensor,^{42,43} with complete

specification given by c_0^2 and c_{2+}^2 (for simplicity, the plus sign in c_{2+}^2 will be omitted below). The corresponding D_{00}^2 and $(D_{02}^2 + D_{0-2}^2)$ terms in eq 3 have symmetry A_g in D_{2h} point group.

Next we focus on the $L = 1$ potential. Three real potentials, given by the spherical harmonic

functions Y_{10} , $\sqrt{\frac{1}{2}}(Y_{1-1} - Y_{11})$ and $i\sqrt{\frac{1}{2}}(Y_{1-1} + Y_{11})$, may be defined. The respective symmetries in the D_{2h} point group are B_{1u} , B_{3u} , and B_{2u} . These functions are similar in character to the atomic orbitals p_z , p_x , and p_y , respectively.²⁶ When the primary polar axis is taken as the z-axis of the diffusion tensor only the term Y_{10} survives in eq 3. When Y_{10} is

linearly combined with $\sqrt{\frac{1}{2}}(Y_{1-1} - Y_{11})$ ($i\sqrt{\frac{1}{2}}(Y_{1-1} + Y_{11})$) with positive coefficients, the primary polar axis is tilted in the zx (zy) plane (negative coefficients change the sign/signs of

the x , y and/or z coordinates). We choose Y_{10} linearly combined with $\sqrt{\frac{1}{2}}(Y_{1-1} - Y_{11})$ as polar orientational potential (i.e., we assume that in the rhombic case the principal polar axis is tilted in the $|zx|$ plane rather than the $|zy|$ plane). The plus sign in the designation of the

coefficient c_{1+}^1 of $\sqrt{\frac{1}{2}}(Y_{1-1} - Y_{11})$ will be omitted below.

The relevant order parameters are thus:

$$S_0^L = \langle D_{00}^L(0, \theta, 0) \rangle, \quad L=1, 2, 3, 4 \quad (4a)$$

$$S_1^1 = \langle (D_{0-1}^1(0, \theta, \varphi) - D_{01}^1(0, \theta, \varphi)) \rangle \quad (4b)$$

$$S_2^2 = \langle (D_{0-2}^2(0, \theta, \varphi) + D_{02}^2(0, \theta, \varphi)) \rangle \quad (4c)$$

The normalized probability density function is given by:

$$\frac{\int D_{0K}^L(0, \theta, \varphi) e^{-u(\theta, \varphi)} \sin\theta d\theta d\varphi}{\int e^{-u(\theta, \varphi)} \sin\theta d\theta d\varphi} \quad (5)$$

The part of the Character Table of D_{2h} point group relevant to this study is given in Table S1 of the Supporting Information.²⁶ It depicts the symmetry operations that characterize the various potential functions considered.

As pointed out above, for probes with axial symmetry the functions $D_{00}^1(0, \theta, 0)$, $D_{00}^2(0, \theta, 0)$, $D_{00}^3(0, \theta, 0)$, and $D_{00}^4(0, \theta, 0)$, proportional to Y_{10} , Y_{20} , Y_{30} , Y_{40} , respectively, are also considered; they are given in Table S2 of the Supporting Information. The odd- L functions have B_{1u} symmetry whereas the even- L functions have A_g symmetry (Tables S1 and S2 of the Supporting Information).

The real spherical harmonics Y_{10} , $\sqrt{\frac{1}{2}}(Y_{1-1} - Y_{11})$, $i\sqrt{\frac{1}{2}}(Y_{1-1} + Y_{11})$, Y_{20} and $\sqrt{\frac{1}{2}}(Y_{2-2} + Y_{22})$ are given in Table S3 of the Supporting Information. Note that Y_{20} and $\sqrt{\frac{1}{2}}(Y_{2-2} + Y_{22})$ are similar in character to the atomic orbitals d_{z^2} and $d_{x^2-y^2}$, respectively.²⁶ These symmetries are also helpful in determining the non-zero matrix elements of the potential terms in the Stochastic Liouville Equation (SLE) developed within the scope of standard theoretical methods.

The polar and non-polar scenarios are compared in the following manner. 3D potential surfaces are generated as a function of the spherical coordinates, θ and ϕ . In this representation the value of the potential, $u(\theta, \phi)$, is depicted on the z-axis, and color-coding is used to illustrate variations. 3D surfaces of u are also shown in Cartesian coordinates, $x = \sin \theta \cos \phi |u|$, $y = \sin \theta \sin \phi |u|$ and $z = \cos \theta |u|$. In this representation the value of the potential is given by the distance from the origin of the coordinate frame to any point on the 3D surface. Positive and negative values are colored red and blue, respectively, by analogy with positive and negative phases of the p-orbitals for the $L = 1$ case, and the d_{z^2} and $d_{x^2-y^2}$ orbitals for the $L = 2$ case. Note, however, that “blue” potential values are populated preferentially. The utility of the Cartesian representation of the potential will be realized in cases of compounded potential forms.

PDFs are shown in both Cartesian⁴⁴ ($x = \sin \theta \cos \phi \exp(-u)$, $y = \sin \theta \sin \phi \exp(-u)$ and $z = \cos \theta \exp(-u)$) and spherical coordinates (θ and ϕ). Because of the PDF exponential functional dependence on u , large positive potential values hardly contribute to the visual display of the PDF. It is useful to examine simultaneously the Cartesian representations of corresponding potential and PDF pairs. The information provided by the four representations is thus complementary.

3. Results and Discussion

3.1. Axial Ordering ($K = 0$)

Order Parameters—Let us consider axially symmetric molecules described by potentials comprising a single term. The $L = 1$ and $K = 0$ potential is $u(\theta) = -c_0^1 \cos \theta$ and the corresponding order parameter, S_0^1 , is given by eq 4a. The $L = 2$ and $K = 0$ potential is

$$u(\theta) = -c_0^2 \left[\frac{3}{2} \cos^2 \theta - \frac{1}{2} \right] \text{ and the order parameter, } S_0^2, \text{ is also given by eq 4a, etc.}$$

Thus, in these simple cases the order parameter, S_0^L , is obtained from the corresponding potential coefficient, c_0^L , in a straightforward manner by solving eq 4a. However, in many cases order parameters rather than potential coefficients are obtained experimentally.^{36,37} It is of interest to derive c_0^L from S_0^L , given that potential forms are substantially more sensitive to details of the local ordering than order parameters (see below).

This can be accomplished by pre-calculating graphs of S_0^L as a function of c_0^L , and using them subsequently to derive c_0^L from known S_0^L values. Figure 1 shows such graphs for $L = 1, 2, 3, 4$, with c_0^L ranging from -30 to 30 . The trigonometric expressions underlying the potentials for $L = 1-4$ are given in Table S2 of the Supporting Information.

For any given positive value of c_0^L the extent of ordering increases along the series S_0^4, S_0^3, S_0^2 and S_0^1 , with $S_0^1 \cong S_0^2$ somewhat larger than $S_0^3 \cong S_0^4$. All of the order parameters converge to 1 for large and positive values of c_0^L . For large and negative values of c_0^L the order parameters S_0^1 and S_0^3 converge to -1 , S_0^2 converges to $-1/2$, and S_0^4 converges to $-3/7$. For any given $c_0^L < 0$ the extent of ordering increases along the series $|S_0^4|, |S_0^2|, |S_0^3|$ and $|S_0^1|$. In the region $-15 \leq c_0^L \leq 0$ all four order parameters differ. For $c_0^L \leq -15$ (very strong perpendicular ordering), all four order parameters converge to their respective lower limits.

Figure 1 is useful in cases where one seeks ascribing appropriate single-term axial potentials to specific systems.

Potentials—The potential forms corresponding to $L = 1, 2, 3$ are shown in Figures 2A–C for $c_0^L = 10$. Interestingly, the potential with $L = 3$ and $K = 0$ exhibits two minima: one for $\theta = 0$, and the other for $\theta = \cos^{-1} \left(\frac{-1}{\sqrt{5}} \right) (\approx 116.6^\circ)$ (Figure 2C).

Figures 2D–F show examples of potentials comprising the $L = 1$ and $L = 2$ (part D), $L = 1$ and $L = 3$ (part E), and $L = 1, L = 2$ and $L = 3$ terms (part F) spherical harmonics. All of the coefficients are equal to 10, representing thus strong local ordering.^{13–19} Figure 2 indicates that even within the scope of axial symmetry diverse potential forms can be devised, although the corresponding order parameters are quite similar (Figure 1).

Let us focus on case E, where the potential is given by the $L = 1$ and $L = 3$ spherical harmonics. The angle where the minimum with $\theta \neq 0$ occurs (call it $\theta_{\min}$) is given by the ratio $R \equiv c_0^1/c_0^3$ according to the expression:

$$\theta_{\min} = \frac{1}{2} \cos^{-1} \left[\frac{4R+9}{-15} \right]. \quad (6)$$

Thus, one can control $\theta_{\min}$ by varying the ratio R . The requirement that the absolute value of the cosine function be smaller than, or equal to, 1, implies that $-6 \leq R \leq 1.5$; consequently $0^\circ < \theta_{\min} < 60^\circ$. We found empirically that the range of $60^\circ < \theta_{\min} < 70^\circ$ ($70^\circ < \theta_{\min} < 90^\circ$) can be accessed by combining the axial $L = 1, L = 2$ and $L = 3$ ($L = 2$ and $L = 3$) spherical harmonics.

Given the diversity in their forms, axial potentials of the kind examined above are expected to be useful for analyzing (with SRLS or other stochastic models) NMR relaxation in proteins. We found previously that one has to account for structural asymmetry in order to obtain physically relevant result.^{14–16} This can be accomplished in a straightforward manner

by allowing for rhombic potentials.^{14–16} However, the respective data-fitting calculations are demanding. The computation will become substantially more effective if structural asymmetry is accounted for within the scope of axial potentials by allowing for separate local ordering and local diffusion frames.^{45–47} This strategy, although less straightforward than allowing for rhombic potentials, was found to be appropriate. In refs 45–47 the potential comprises a single term with $L = 2$, $K = 0$. The axial potentials considered above comprise at least two terms. Using them in data-fitting might obviate the need to separate the local ordering and local diffusion frames, while maintaining the axial symmetry of the potential. Such a scenario is both effective and economical.

PDFs—To gain further insight into multi-term axial potentials, $u(\theta)$, we examine this scenario within the scope of the associated PDFs given in Cartesian coordinates. The underlying potentials are specified by the coefficients, c_0^L , shown in rows *A–F* of Table 1. All of these potentials are strong.

Figure 3A shows the PDF corresponding to the strong potential $u(\theta) = -10 \cos \theta$. For the implied small values of θ one may approximate $u(\theta)$ by its Taylor expansion around $\theta = 0$. This yields $u(\theta) \approx -10 \times (1 - \theta^2/2)$, which has been used in the past to describe wobble-in-a-cone.⁴⁸ Thus, Figure 3A may be considered as representing the wobble-in-a-cone PDF.

Figure 3B shows the PDF corresponding to the potential given by $c_0^1 = 30$ and $c_0^3 = -5.1$. Based on its shape, it may be considered as representing a generalized version of the wobble-in-a-cone PDF.

The PDFs shown in Figures 3C–3E (corresponding to the potentials *C–E* in Table 1) are cone-shaped, with various vertex angles and thicknesses. The vertex angle is given by $\theta_{\max}$, which is the same as $\theta_{\min}$ of the corresponding potential. $\theta_{\max}$ is approximately 21, 57 and 72° for the PDFs of Figures 3C, 3D and 3E, respectively (Table 1). A simple model for describing internal motions is Woessner's model, where the probe is confined to reside on the infinitely thin surface of a cone with variable vertex angle.⁴⁹ The potentials underlying the PDFs shown in Figures 3C–3E represent generalizations of Woessner's model. The PDF shown in Figure 3B corresponds to $\theta_{\max} \approx 7^\circ$; one may consider the underlying potential as representing a generalization of either wobble-in-a-cone⁴⁸ or Woessner's model⁴⁹.

Finally, the PDF of Figure 3F may be considered a generalization of ideal perpendicular ordering, where the probe is confined to lie in the *xy* plane of the local director frame.

As pointed out above, the entire range of $0 \leq \theta \leq 90^\circ$ can be accessed with appropriate combinations of the $L = 1–3$, $K = 0$ axial spherical harmonics.

Orientation versus Alignment—Figure 4 shows results for polar and non-polar scenarios, with large positive potential coefficients representing strong parallel ordering, and large negative potential coefficients representing strong perpendicular ordering. The polar case with strong parallel ordering is shown in Figure 4A. It is represented by the potential $u(\theta) = -10 \cos \theta$. Part *a* shows the Cartesian representation of u which (as pointed out above) resembles the atomic orbital p_z . Part *c* shows the spherical-coordinate-representation of u with minimum at $\theta = 0$ maximum at $\theta = \pi$. The PDF is shown in Cartesian coordinates in

part *b*. It is directed along $+z$, as implied by c_0^1 being positive. In addition, it is lobe-shaped as compared to the sphere-shaped potential of Figure 4Aa, because (as pointed out above) for large $c_0^1 > 0$ potential values that are positive virtually do not contribute to $|\exp(-u)|$, while they contribute to $|u|$. The spherical-coordinate-representation of the PDF appears in part *c*; it shows the function values on the z -axis, and illustrates their variations.

The non-polar case with strong parallel ordering is shown in Figure 4B. It is represented by

the potential $u(\theta) = -10 \left[\frac{3}{2} \cos^2 \theta - \frac{1}{2} \right]$. Part *a* shows its Cartesian-coordinate-representation which resembles the atomic orbital d_{z^2} .²⁶ Part *c* shows the spherical-coordinate-

representation of the potential with minima at $\theta = 0$ and π , and maximum at $\theta = \frac{\pi}{2}$. The PDF is shown in Cartesian coordinates in part *b*. It comprises two lobes disposed symmetrically along $+z$ and $-z$. They are thinner than the PDF lobe corresponding to $L = 1$ (Figure 4Ab), indicating reduced spatial distribution, as implied by the functional form of non-polar Y_{20} as compared to polar Y_{10} . The spherical-coordinate-representation of the PDF appears in Figure 4Bd, illustrating the variations in PDF magnitude; comparison with Figures 4Ad shows larger spatial distribution in the $L = 1$ case.

The distinction between *orientation*, implied by $L = 1$ with symmetry B_{1u} , and *alignment*, implied by $L = 2$ with symmetry A_g , is illustrated with particular clarity by the Cartesian PDFs (Figures 4Ab and 4Bb): for $L = 1$ one has directionality (only positive z -values are featured) whereas for $L = 2$ there is no directionality (both positive and negative z -values are featured).

Figure 4C shows the results obtained for $L = 1$ and $c_0^1 = -10$. Rendering c_0^1 negative implies conversion of z into $-z$ for both the Cartesian-, and the spherical-coordinate-representations.

Figure 4D shows the results obtained for $L = 2$ and $c_0^2 = -10$. Rendering c_0^2 negative implies conversion of z into $-z$ for the spherical-coordinate-representations. For the Cartesian-coordinate-representations this change consists of u being converted into $-u$.

3.2. Rhombic Ordering

Order Parameters—Axial orienting potentials, $u(\theta) = -c_0^L D_{00}^L(0, \theta, 0)$, are defined by a single potential coefficient, c_0^L . The derivation of c_0^L from S_0^L , based on pre-calculated profiles (Figure 1), is unambiguous. The rhombic orienting potentials and the two-term axial orienting potentials considered in this study are defined by two potential coefficients, from which two order parameters are derived. It is not obvious that two order parameters, e.g., S_0^2 and S_2^2 , correspond to a unique potential form, i.e., a unique pair of potential coefficients c_0^2 and c_2^2 .

We examine this matter below for the following combinations: c_0^1 and c_1^1 derived from S_0^1 and S_1^1 ; c_0^2 and c_2^2 derived from S_0^2 and S_2^2 ; and c_0^1 and c_0^3 derived from S_0^1 and S_0^3 . The coefficients c_0^1 and c_1^1 have been varied from -20 to 20 in steps of 0.5 . For each point with coordinates

(c_0^1, c_1^1) we calculated S_0^1 and S_1^1 according to eq 3 with $j = 1$, and eqs 4a, 4b and 5. This yielded the 3D surfaces $S_0^1(c_0^1, c_1^1)$ and $S_1^1(c_0^1, c_1^1)$. Figure 5A displays the contours of these surfaces in the c_0^1/c_1^1 plane. This procedure was also carried out for c_0^2 and c_2^2 (using eq 3 with $j = 0$, and eqs 4a, 4c and 5) to obtain the surfaces $S_0^2(c_0^2, c_2^2)$ and $S_2^2(c_0^2, c_2^2)$ with contours shown in Figure 5B. Finally, c_0^1 and c_0^3 yielded the surfaces $S_0^1(c_0^1, c_0^3)$ and $S_0^3(c_0^1, c_0^3)$, with contours shown in Figure 5C.

It can be seen that if two “isolines” intersect they will do so only once. Thus, if a solution exists, it is unique. The “isolines” are very dense for c_0^1 and c_1^1 smaller than approximately 5 in absolute value. This implies c_0^1 and c_1^1 being determined with good certainty in this parameter range. The isolines become much sparser for c_0^1 and c_1^1 of approximately $|10|$, and very sparse for c_0^1 and c_1^1 larger than $|10|$. This implies c_0^1 and c_1^1 being determined with reduced certainty when their absolute values are large. Note that Figure 5A is symmetric with respect to both the horizontal and the vertical lines through the point $(c_0^1, c_1^1) = (0, 0)$. The designations *a–d* refer to the extreme values of $|S_0^1|$ and $|S_1^1|$ in the $(-20, 20)$ parameter range for c_0^1 and c_1^1 . The extreme limits of S_0^1 are given in section 3.1. above; the extreme limit of $|S_1^1|$ is $\sqrt{2}$.

Figure 5B shows the “isolines” of S_0^2 and S_2^2 as a function of c_0^2 and c_2^2 . The parameter range where potential coefficients can be determined with good certainty include relatively small values of $|c_0^2|$ and $|c_2^2|$, as in the $L = I$ case. In addition, it also includes large values of $|c_0^2|$ and $|c_2^2|$, provided they are similar in magnitude. Figure 5B is only symmetrical with respect to the vertical line through the point $(c_0^2, c_2^2) = (0, 0)$. The designations *a–d* refer to the extreme values of $|S_0^2|$ and $|S_2^2|$ in the $(-20, 20)$ parameter range for c_0^2 and c_2^2 . The extreme limits of S_0^2 are given in section 3.1 above; the extreme limit of $|S_2^2|$ is $\sqrt{\frac{3}{2}}$.

Figure 5C shows “isolines” of S_0^1 and S_0^3 as a function of c_0^1 and c_0^3 . Here the parameter ranges where potential coefficients can be determined with good certainty include $-5 \leq c_0^1 \leq 5$ and $-20 \leq c_0^3 \leq 20$. This figure is asymmetric. The designations *a–d* refer to the extreme values of $|S_0^1|$ and $|S_0^3|$ in the $-20, 20$ parameter range for c_0^1 and c_0^3 . The extreme limits of $|S_0^1|$ and $|S_0^3|$ are given in section 3.1. above.

The information inherent in Figure 5 is important for predicting the certainty of the results to be obtained from scenarios with different symmetries.

Orientation versus Alignment—Figure 6 shows results for rhombic polar and non-polar scenarios represented by potential coefficients $c_0^1 = c_1^1 = 10$ (part *A*) and $c_0^2 = c_2^2 = 10$ (part *B*).

An additional example, represented by polar-potential coefficients $c_0^1=10$, $c_1^1=-10$ (part *C*) and non-polar-potential coefficients $c_0^2=10$, $c_2^2=-10$ (part *D*), is provided.

Let us focus on Figure 6A, associated with the linear combination of Y_{10} and

$\sqrt{\frac{1}{2}}(Y_{1-1} - Y_{11})$ with coefficients $c_0^1=c_1^1=10$; the respective potential is given by $u(\theta, \varphi) = -10 \cos \theta - 10 \sqrt{2} \sin \theta \cos \varphi$ (Table S3 of the Supporting Information). Figure

6B is associated with the linear combination of Y_{20} and $\sqrt{\frac{1}{2}}(Y_{2-2} - Y_{22})$ with coefficients $c_0^2=c_2^2=10$; the respective potential is given by

$u(\theta, \varphi) = -10 \left(\frac{3}{2} \cos^2 \theta - \frac{1}{2} \right) - 10 \sqrt{\frac{3}{2}} \sin^2 \theta \cos(2\varphi)$ (Table S3 of the Supporting Information). The 4-panel representations illustrating these scenarios are shown in Figures 6A and 6B, respectively.

The Cartesian $L = 1$ potential of Figure 6Aa has negative values in the xyz quadrant and positive values in the $(-x)(-y)(-z)$ quadrant. Both this function and the Cartesian PDF (Figure 6Ab) have the primary polar axis in the zx plane. The spherical-coordinate representation of the potential has minimum, whereas the spherical-coordinate representation of the PDF has maximum, for $\theta = \tan^{-1} \sqrt{2} \cong 0.955 \text{ rad} (\cong 54.7^\circ)$ and $\varphi = 0$ (Figures 6Ac and 6Ad).

The Cartesian $L = 2$ potential has negative values in a relatively thin region centered at the zx plane (the coordinates have both positive and negative values) with maximum $|z|$ exceeding maximum $|x|$, and positive values in lobe-shaped regions along the y -axis (the coordinates have both positive and negative values) (Figure 6Ba). The Cartesian PDF is *aligned* preferentially along $\pm z$ and distributed in a relatively thin regions centered at the zx plane (Figure 6Bb). The spherical-coordinate-representation of the potential has minimum value for $\theta = 0$ and any φ , as well as $\theta = \pi$ and any φ ; it has maximum value for $\theta = \varphi = \pi/2$; the spherical-coordinate-representation of the PDF has reversed extrema (Figures 6Bc and 6Bd).

The difference between *orientation* and *alignment* is demonstrated with particular clarity by the Cartesian representation of the PDF (Figures 6Ab and 6Bb). Figure 6A illustrates comprehensively a polar ordering scenario based on one of the two lowest odd- L rhombic potentials. Figure 6B illustrates comprehensively a non-polar ordering scenario based on the lowest even- L rhombic potential.

We examine now the rhombic ordering scenario represented by the polar potential given by $c_0^1=10$ and $c_1^1=-10$, shown in Figure 6C. The Cartesian potential has negative values in the $(-x)yz$ quadrant and positive values in the $x(-y)(-z)$ quadrant (Figure 6Aa). Both this function and the Cartesian PDF (Figure 6Cb) have the primary polar axis in the $z(-x)$ plane. Figure 6Ca can be obtained from Figure 6Aa, and Figure 6Cb from Figure 6Ab, by inversion with respect to the origin followed by reflection with respect to the xy plane. The spherical-

coordinate representations of the potential and the PDF are shown in Figures 6Cc and 6Cd, respectively.

The rhombic ordering scenario represented by the non-polar potential given by $c_0^2=10$ and $c_2^2=-10$ is shown in Figure 6D. The Cartesian potential and PDF (Figures 6Da and 6Db) are obtained from their counterparts in Figures 6Ba and 6Bb by a $\pi/2$ clockwise rotation around z . The spherical-coordinate representations of the potential and the PDF are shown in Figures 6Dc and 6Dd, respectively.

Figure S1 of the Supporting Information is analogous with Figure 6 except that $c_0^1=-10$ and $c_1^1=10$ in Figure S1A; $c_0^2=-10$ and $c_2^2=10$ in Figure S1B; $c_0^1=-10$ and $c_1^1=-10$ in Figure S1C; $c_0^2=-10$ and $c_2^2=-10$ in Figure S1D.

Let us assume that a given protein site with largely known site symmetry is subject to investigation. As shown above, well-defined symmetry operations convert parts *a–d* of Figure 6A (6B) into the corresponding parts of Figure 6C, S1A and S1C (6D, S1B and S1D). Thus, one can devise a potential form that largely matches the known site symmetry. Further refinement can then be carried out with analysis of NMR relaxation data.

3.3. Applications

To illustrate the generality of our approach we select four essentially different examples. They include ^{15}N relaxation from a protein dissolved in aqueous solution, ^{15}N relaxation from a polycrystalline protein, ESR lineshape analysis from a nitroxide-labeled protein dissolved in aqueous solution, and ^2H and ^{13}C relaxation in one of the phases of a medium-size molecule exhibiting polymorphism in the solid-state.

^{15}N relaxation in proteins in the liquid-state: We studied previously with SRLS ^{15}N spin relaxation of N–H bonds in plexin-B1 in aqueous solution.²⁵ The order parameters S_0^2 and S_2^2 have been determined as the plateau values of time correlation functions calculated from a 110 ns MD trajectory, after eliminating the global motion. This is a commonly used strategy, based on the assumption that large timescale separation prevails between the global and local motions. Let us denote these parameters as $S_0^2(\text{MD})$ and $S_2^2(\text{MD})$. The potential coefficients c_0^2 and c_2^2 were obtained from $S_0^2(\text{MD})$ and $S_2^2(\text{MD})$ by solving the set of non-linear equations:²⁵

$$S_0^2(c_0^2, c_2^2) - S_0^2(\text{MD}) = 0 \quad (7a)$$

$$S_2^2(c_0^2, c_2^2) - S_2^2(\text{MD}) = 0 \quad (7b)$$

Values of $c_0^2 = -3.56$ and $c_2^2 = -3.47$ were obtained in this manner for the local potential of the N–H bond of Gln56. We show the 4-panel representation for this residue (Figure 7A); PDF contours instead of 3D surfaces are shown.

PDF contours we call “atomistic” have also been calculated in ref 25 for Gln56 directly from the MD trajectory. Let us compare them with the “mesoscopic” PDF contours associated with the analytical potential with coefficients $c_0^2 = -3.56$ and $c_2^2 = -3.47$. Figure 8(c) shows the “atomistic” PDF contours;²⁵ Figure 8(d) (which is the same as Figure 7Ad) shows the “mesoscopic” PDF counterparts. Figure 8(c) is considerably more asymmetric than Figure 8(d). We show in Figure 7B the 4-panel representation associated with the “enhanced mesoscopic” potential given by $c_0^2 = -3.56$, $c_2^2 = -3.47$, $c_0^3 = 3.00$, and $c_1^3 = -5.00$. The “enhanced mesoscopic” PDF contours (Figure 7Bd) are considerably more asymmetric than the “mesoscopic” PDF contours (Figure 7Ad), and quite similar to the “atomistic” PDF contours (Figure 8(c)).

Thus, one can improve mesoscopic PDF contours by adjusting their forms so as to match the corresponding atomistic PDF contours. This is accomplished by linearly combining different spherical harmonics. The best-fit mesoscopic potential underlying the best-fit mesoscopic PDF may be considered a good approximation to the atomistic potential implicit in the atomistic PDF. Enhanced mesoscopic potentials obtained in this manner may be compared among sites, providing new insights. Importantly, they may be used without further adjustment in data-fitting schemes, reducing thereby the number of parameters allowed to vary.

The following comments are in order. (1) One of the future prospects of this work is to develop computational methods⁵⁰ for extracting the best-fit mesoscopic PMF directly from the MD-based PDF. In this process the form of the PMF will be enhanced by adding spherical-Harmonics-based terms systematically; error estimations on the potential coefficients will emerge automatically. (2) In the context of protein dynamics by NMR relaxation there are previous reports on MD-derived PMFs used to interpret order parameters. For example, it was shown that a parabolic PMF (given by $-c_0^2 Y_{20}$ in the limit of strong axial potential) applies to rapidly fluctuating N–H bonds.⁵¹ However, this PMF would not apply to more general cases, such as illustrated by Figure 8(c). On the other hand, in our approach the PMF may comprise even- L and/or odd- L spherical harmonics of axial and/or rhombic symmetry; clearly, this is a general approach.

¹⁵N relaxation in proteins in the solid-state: The authors of ref 36 studied ¹⁵N spin relaxation in polycrystalline thioredoxin. Assuming that the local motions are in the extreme motional narrowing limit, the experimental powder patterns have been interpreted in terms of effective partially-reduced rhombic ¹⁵N chemical shift anisotropy (CSA) tensors. We calculated from the tensor components of Gly21 the potential coefficients $c_0^2 = 1.71$ and $c_2^2 = 4.21$ using eqs 7a,b, and employed these values to generate the 4-panel representation shown in Figure 9A.

It can be seen that the coefficients of the local potentials at the N–H site of Gln56 of plexin-B1 in the liquid state ($c_0^2 = -3.56$ and $c_2^2 = -3.47$), and the N–H site of Gly21 of thioredoxin in the solid-state ($c_0^2 = 1.71$ and $c_2^2 = 4.21$), differ moderately in their absolute

values, substantially in the ratios c_2^2/c_0^2 , and have opposite signs. Thus, the main difference lies in the symmetry of the potential.

Nitroxide relaxation in T4L Lysozyme in solution: In Figure 9B we show the 4-panel representation for residue 72 of T4 Lysozyme which has been mutated into cysteine and labeled with a nitroxide.³⁵ Liquid-state ESR lineshapes were analyzed with SRLS. Two conformers have been detected at this site; we show conformer 2, for which a local potential with $c_0^2=3.31$ and $c_2^2=-1.67$ was determined at 25 °C.

This case illustrates yet another type of local potential with magnitude similar to, but symmetry different from, the local potentials at the N–H sites of Gln56 of plexin-B1 in solution and Gly21 of thioredoxin in the solid-state.

NMR relaxation in benzenehexa-*n*-alkanoates: Our last example is related to restricted motion in the discotic mesophase of benzenehexa-7-alkanoate, and in phase I of benzenehexa-6-alkanoate. Having been determined to be in their extreme motional narrowing limit, these motions give rise to partially averaged rhombic powder spectra.³⁷ We converted the axial and rhombic components of these tensors into the order parameters S_0^2 and S_2^2 , calculated c_0^2 and c_2^2 using eqs 7a,b,²⁵ and generated the 4-panel representations shown in Figure 10.

In part *A* we show results obtained for the potential with $c_0^2=1.49$ and $c_2^2=1.81$, associated with ^2H relaxation from the β -hydrogen of benzenehexa-7-alkanoate (BHA7) in its discotic mesophase. In part *B* we show results obtained for the potential with $c_0^2=1.28$ and $c_2^2=2.68$, associated with ^{13}C relaxation from the ^{13}C -labeled carbonyl of benzenehexa-7-alkanoate (BHA7) in phase I. Once again, we detect local potentials which differ moderately in magnitude, but quite a bit in symmetry, from the other local potentials discussed above.

Both Gln56 of plexin-B1 and Gly21 of thioredoxin are rather flexible residues. The nitroxide of T4 lysozyme is attached to the protein through a long flexible tether. The side-chains of the benzenehexa-*n*-alkanoates, which have been labeled with ^2H or ^{13}C , are flexible moieties. In accordance, our examples are associated with medium-strength local potentials. N–H sites in proteins residing in well-structured regions of the polypeptide were found to be associated with strong local potentials.^{14–16} On the other hand, side-chain methyl sites in proteins were found to be associated with weak local potentials.^{14–16} Although the strength of the local potentials in our examples might not be typical of many protein N–H and methyl sites, the diversity in symmetry is likely to be general in nature. Further studies in this context are required.

We show in Table 2 the experimental order parameters S_0^2 and S_2^2 of the five examples considered, and the corresponding potential coefficients. We also show the Saupe order parameters S_{xx} , S_{yy} and S_{zz} , related to S_0^2 and S_2^2 as:

$$S_{xx} = +(\sqrt{3/2}S_2^2 - S_0^2)/2 \quad (8a)$$

$$S_{yy} = -(\sqrt{3/2}S_2^2 + S_0^2)/2 \quad (8b)$$

$$S_{zz} = S_0^2 \quad (8c)$$

Table 2, together with the 4-panel representations illustrated in Figures 7, 9 and 10, constitute an insightful and comprehensive description of the local spatial restrictions at mobile sites of molecules, in particular proteins.

Let us focus on the Cartesian potential and PDF representations shown in Figures 7Aa, 9Aa, 9Ba, 10Aa and 10Ba. The potentials and PDFs shown in Figures 9Aa and 10Ba are similar given that both coefficients are positive and the ratio c_2^2/c_0^2 is comparable: 2.46 in Figure 9Aa and 2.09 in Figure 10Ba. The potential in Figure 10Aa also has positive coefficients but $c_2^2/c_0^2 = 1.21$; hence its shape is different. This illustrates the sensitivity of potential and PDF shapes to the relative magnitudes of c_0^2 and c_2^2 .

The signs of c_0^2 and c_2^2 are also important factors in determining potential and PDF shapes. Let us focus on the spherical-coordinate-representations of the potentials (Figures with the designation “c”). Figure 7Ac, where both coefficients negative, shows two potential wells.

Figure 9Ac, where c_0^2 is positive and c_2^2 is negative, shows two potential barriers. Figure 9Bc, where both coefficients are positive, shows three potential barriers.

4. Conclusions

Potentials consisting of linear combinations of the $L = 1$ spherical harmonics Y_{10} and

$\sqrt{\frac{1}{2}}(Y_{1-1} - Y_{11})$ provide a useful description of polar local ordering at mobile sites in proteins. Comparison was made with corresponding non-polar $L = 2$ potentials consisting of linear combinations of the spherical harmonics Y_{20} and $(Y_{22} + Y_{2-2})$.

The shapes of the $L = 1$ and $L = 2$ potentials and associated PDFs differ substantially. The orientational polarity of $L = 1$ potentials is reflected particularly well by the Cartesian-coordinate-representations of the PDF. Thus, for positive (negative) potential coefficients the primary polar axis points in a direction in the zx ($z(-x)$) plane determined by their magnitudes. By contrast, the $L = 2$ PDFs have their main symmetry axis pointing symmetrically along $+z$ and $-z$.

A representation where potentials and probability density functions are shown in both Cartesian and spherical coordinate systems provides an extensive characterization of the local ordering.

Substantial diversity was detected among potentials obtained as linear combinations of $L = 1$, $L = 2$ and/or $L = 3$, and $K = 0$, spherical harmonics. Being multi-term axial functions,

these potentials are expected to help in devising economical and computationally effective data-fitting schemes for NMR relaxation analysis in proteins.

The representation specified above was applied to potential coefficients c_0^2 and c_2^2 from fits to several different experimental systems. For the examples considered we found similarity in potential strength and diversity in potential symmetry.

A method whereby experimental mesoscopic PDFs can be improved by using their atomistic counterparts as a benchmark is proposed. The underlying best-fit mesoscopic potentials may then be considered as reasonable approximations to the atomistic potentials implicit in the atomistic PDFs.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported by the Israel–U.S.A. Binational Science Foundation (Grant No. 2010185 to E.M. and J.H.F.), and the Israel Science Foundation (Grants No. 437/11 and No. 369/15 to E.M.). This work was also supported by NIH/NIGMS grant P41GM103521 to J.H.F.

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Figure 1.

Order parameters, S_0^L , shown as a function of potential coefficients, c_0^L , for $L = 1, 2, 3, 4$ and $K = 0$, calculated according to eqs 3 and 4. The respective functional forms are given in Table S2 of the Supporting Information.

Figure 2.

Axial potentials, $u(\theta)$, shown as a function of θ (we show 3D rather than 2D plots for easy comparison with rhombic potentials, $u(\theta, \phi)$). The various potentials are defined by the coefficients $c_0^1=10$ (A), $c_0^2=10$ (B), $c_0^3=10$ (C), $c_0^1=10$ and $c_0^2=10$ (D), $c_0^1=10$ and $c_0^3=10$ (E), and $c_0^1=10$, $c_0^2=10$ and $c_0^3=10$ (F), and the functional forms given in Table S2 of the Supporting Information.

Figure 3.

Axial probability densities, $\exp(-u)$, given in Cartesian coordinates ($x = \sin\theta\cos\phi|\exp(-u)|$, $y = \sin\theta\sin\phi|\exp(-u)|$, $z = \cos\theta|\exp(-u)|$). The potentials, u , are defined by the coefficients $c_0^1=10$ (A), $c_0^1=30$ and $c_0^3=-5.1$ (B), $c_0^1=30$ and $c_0^3=-6.0$ (C), $c_0^1=7$ and $c_0^3=-10.0$ (D), $c_0^2=-9.0$ and $c_0^3=-10.0$ (E) and $c_0^2=-10.0$ (F), and the functional forms given in Table S2 of the Supporting Information.

Figure 4.

4-panel representations of the axial scenarios $L=1$, $K=0$ and $c_0^1=10$ (part A, panels a–d); $L=2$, $K=0$ and $c_0^2=10$ (part B, panels a–d); $L=1$, $K=0$ and $c_0^1=-10$ (part C, panels a–d); and $L=2$, $K=0$ and $c_0^2=-10$ (part D, panels a–d). The potentials, u , are represented in Cartesian coordinates in the panels denoted a, and in spherical coordinates in the panels denoted c. The probability density functions, $\exp(-u)$, are represented in Cartesian coordinates in the panels denoted b, and in spherical coordinates in the panels denoted d.

Figure 5.

Contour plots of the surfaces $S_0^1(c_0^1, c_1^1)$ and $S_1^1(c_0^1, c_1^1)$ (part A), $S_0^2(c_0^2, c_2^2)$ and $S_2^2(c_0^2, c_2^2)$ (part B), and $S_0^1(c_0^1, c_0^3)$ and $S_0^3(c_0^1, c_0^3)$ (part C), as a function of the respective potential coefficients. The designations *a–d* represent the extreme values of the respective order parameters, for potential coefficients varied in the $(-20, 20)$ range.

Figure 6.

4-panel representations of the rhombic scenarios $L = 1$, $K = 0, 1$ and $c_0^1 = c_1^1 = 10$ (part A, panels a–d); $L = 2$, $K = 0, 2$ and $c_0^2 = c_2^2 = 10$ (part B, panels a–d); $L = 1$, $K = 0, 1$ and $c_0^1 = c_1^1 = -10$ (part C, panels a–d); and $L = 2$, $K = 0, 2$ and $c_0^2 = c_2^2 = -10$ (part D, panels a–d). The real spherical harmonics forming the respective potentials are given in Table S3f the Supporting Information.

Figure 7.

4-panel representation associated with the potential given by $c_0^2 = -3.56$ and $c_2^2 = -3.47$, obtained with SRLS analysis of ^{15}N relaxation from the N–H bond of Gln56 of plexin-B1 in solution²⁵ (part A). 4-panel representation associated with the potential given by $c_0^2 = -3.56$, $c_2^2 = -3.47$, $c_0^3 = 3.00$ and $c_1^3 = -5.00$ (part B).

Figure 8.

PDF contour plots (in spherical coordinates, θ and ϕ) for residue Gln56 of plexin-B1 calculated directly from the MD trajectory²⁵ (c). PDF contour plots for residue Gln56 of plexin-B1 corresponding to the potential with coefficients $c_0^2 = -3.56$ and $c_2^2 = -3.47$, obtained with SRLS analysis of ^{15}N relaxation²⁵ (d). Figure 8(d) is the same as Figure 7Ad.

Figure 9.

4-panel representation based on the potential given by $c_0^2=1.71$ and $c_2^2=4.21$, obtained from the order parameters of the N–H bond of Gly21 of polycrystalline thioredoxin (part A). 4-panel representation based on the potential given by $c_0^2=3.31$ and $c_2^2= -1.67$ obtained from SRLS-based ESR lineshape analysis of nitroxide-labeled T4 lysozyme at position 72, conformer 2 (part B).³⁶

Figure 10.

4-panel representation associated with the potential given by $c_0^2=1.49$ and $c_2^2=1.81$ obtained from the order parameters of ^2H -labeled β -hydrogen of BHA7 in its discotic mesophase (part A). 4-panel representation associated with the potential given by $c_0^2=1.28$ and $c_2^2=2.68$, obtained from the order parameters of ^{13}C -labeled carbonyl of BHA6 in phase I (part B).

Table 1

Potential Coefficients, c_0^L , Associated with the PDFs of Figures 3A–3F, and angle $\theta_{\max}^a$.

	L			$\theta_{\max}$
	1	2	3	
Figure	c_0^1	c_0^2	c_0^3	
3A	10.0	0.0	0.0	0.0
3B	30.0	0.0	−5.1	7.2
3C	30.0	0.0	−6.0	21.4
3D	7.0	0.0	−10.0	57.2
3E	0.0	−9.0	−10.0	72.4
3F	0.0	−10.0	0.0	90.0

^a $\theta_{\max}$: Angle between the z -axis of the director frame and the line connecting the origin of this frame with the maximum PDF value.

Potential Coefficients and Corresponding Order Parameters in Irreducible Tensor Notation (S_0^2 and S_2^2) and in Saupe Tensor Notation (S_{xx} , S_{yy} , S_{zz}) (Eqs 3, 4, 5 and 8).^a

ref. no.	c_0^2	c_2^2	S_0^2	S_2^2	S_{xx}	S_{yy}	S_{zz}
25	-3.56	-3.47	-0.416	-1.00	-0.404	0.820	-0.416
35	3.31	-1.67	0.576	-0.151	-0.380	-0.196	0.576
36	1.71	4.21	-0.161	0.821	0.583	-0.422	-0.161
37	1.49	1.81	0.171	0.409	0.165	-0.336	0.171
37	1.28	2.68	-0.020	0.632	0.397	-0.377	-0.020

^aThe data shown are associated with N-H relaxation of Gln56 of plexin-B1²⁵; ESR relaxation of the nitroxide-labeled residue 72 of T4 lysozyme³⁵; N-H relaxation of Gly21 of thioredoxin³⁶; ^2H relaxation of the β -deuterium in the discotic mesophase of BHA6 (penultimate row) and ^{13}C carbon relaxation of the ^{13}C -labeled carbonyl of BHA6 in phase I (last row)³⁷.