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

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# **Supporting Information**

**ABSTRACT:** Restricted motions in proteins (e.g., N–H bond 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 LCs experience nonpolar 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 nonpolar 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 nonpolar axial PDF is pointing along  $\pm z$ . Two-term axial potentials with  $1 \le L \le 3$  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 <sup>15</sup>N-labeled plexin-B1 and thioredoxin, <sup>2</sup>H-, and <sup>13</sup>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.

# 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.<sup>1–3</sup> Such motion may be represented by a 3D diffusion tensor,<sup>3</sup> 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.<sup>4–7</sup> The latter can be expressed in terms of 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.<sup>4–19</sup>

A class of restricted motions of particular interest is intramolecular reorientation processes of segments of proteins

(more generally, biomacromolecules).<sup>10–19</sup> Examples include small moieties such as amide or methyl groups<sup>13–19</sup> that perform localized motions, as well as large loops or domains that perform collective motions.<sup>17–19</sup> 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.<sup>20–25</sup> 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,

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such as explicit forms of local anisotropic potentials and local molecular geometry associated with these potentials, as well as effective local 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.<sup>4–19</sup> They encompass local order parameter and PMFs in a straightforward manner.<sup>6,7</sup> 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 local 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,  $D_{MK}^{L}(\Omega)$ 

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

with  $u(\Omega)$  and the coefficients  $c_{MK}^L$  being dimensionless. The Euler angles,  $\Omega$ , describe the molecular orientation relative to the director frame associated with the orienting surroundings. This general expansion needs to be truncated at some value  $L = L_{\text{max}}$  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.<sup>4–6</sup> The usual assumption is the existence of a uniaxial LC director. Consequently the "quantum number", M, in eq 1 is zero, yielding<sup>4–7</sup>

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

In this case only the two polar angles,  $\theta$  and  $\varphi$ , 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 "nonpolar" ordering. Potentials describing nonpolar ordering comprise only the even-*L* terms of eq 2. This may be thought of as preferential arrangement in the space of double-headed-arrows, a setup often referred to as *alignment*.<sup>27,28</sup> The leading terms here are the functions  $D_{0K}^2$  with |K| = 0, 1, 2 (more generally,  $L = 2, 4, 6, \cdots$ ).

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 the space of single-headed arrows, which is often referred to as *orientation*.<sup>27,28</sup>

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,  $L = 1, 3, 5, \cdots$ ).

Comparison of the polar and nonpolar 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 the  $D_{2h}$  point group.

The comparison between the polar and nonpolar 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.<sup>8</sup> SRLS, and its limit where the protein motion is frozen, the microscopic-ordermacroscopic-disorder (MOMD) approach,<sup>29</sup> have been applied extensively within the scope of ESR spectroscopy.<sup>10-12</sup> Applications to model and biological membranes, complex fluids, nitroxide-labeled proteins, and DNA fragments, and so forth, have been reported.<sup>30,31</sup> We have also applied SRLS extensively to NMR relaxation in proteins dissolved in aqueous solution.<sup>13–19</sup> Recently we have applied MOMD to the analysis of <sup>2</sup>H NMR lineshapes from polycrystalline proteins experiencing internal mobility.<sup>32,33</sup> 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  $D_{0K}^{\bar{L}}(0, \theta, \varphi)$  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.<sup>9,10,14–19,29,32,33</sup> In a few cases the L = 4, K = 0, 2 terms were also included in the analysis.<sup>9</sup> An axial 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.<sup>8</sup> In this study we also take into account the leading axial and rhombic odd-L (L = 1, K = 0, 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 <sup>15</sup>N-labeled plexin-B1 in solution,<sup>25,34</sup> T4 lysozyme in solution,<sup>35</sup> polycrystalline thioredoxin,<sup>36</sup> and discotic and solid phases of benzenehexa-*n*alkanoates (with n = 6 and 7).<sup>37</sup>

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 <sup>31</sup>P spin relaxation.<sup>38</sup> Rhombic polar order parameters have been invoked in the context of twist-bend nematic phases.<sup>39</sup> 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,<sup>40</sup> to treat electric-field-implied polar order,<sup>41</sup> 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

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

$$(3)$$

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.,  $\operatorname{Tr}(c_{ii}^2) = 0$ ) in the same frame as the local ordering tensor,<sup>42,43</sup> 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.

Nest 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  $B_{1w}$ ,  $B_{3w}$ , and  $B_{2w}$ . These functions are similar in character to the atomic orbitals  $p_{z}$ ,  $p_{xy}$ , and  $p_{yy}$ , respectively.<sup>26</sup> 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:

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

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

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

The normalized probability density function is given by

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

The part of the Character Table of the  $D_{2h}$  point group relevant to this study is given in Table S1 of the Supporting Information.<sup>26</sup> 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.<sup>26</sup> These symmetries are also helpful in determining the nonzero matrix elements of the potential terms in the Stochastic Liouville Equation (SLE) developed within the scope of standard theoretical methods.

The polar and nonpolar scenarios are compared in the following manner. 3D potential surfaces are generated as a function of the spherical coordinates,  $\theta$  and  $\varphi$ . In this representation the value of the potential,  $u(\theta, \varphi)$ , 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 = $\sin \theta \cos \varphi | u |, y = \sin \theta \sin \varphi | u |$  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 Cartesian<sup>44</sup> ( $x = \sin \theta \cos \varphi \exp(-u)$ ,  $y = \sin \theta \sin \varphi \exp(-u)$ , and  $z = \cos \theta \exp(-u)$ ) and spherical coordinates ( $\theta$  and  $\varphi$ ). 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  $u(\theta) = -c_0^2[3/2 \cos^2 \theta - 1/2]$  and the order parameter,  $S_{0}^2$  is also given by eq 4a.

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.<sup>36,37</sup> 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 precalculating 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,



**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 4a–d. The respective functional forms are given in Table S2 of the Supporting Information.

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  $-15 \le c_0^L \le 0$  all four order parameters differ. For  $c_0^L < 15$  (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 Figure 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  $\theta = \cos^{-1}\left(\frac{-1}{\sqrt{5}}\right)$  ( $\cong 116.6^\circ$ ) (Figure 2C).

Figure 2D–F shows 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 strong local ordering.<sup>13–19</sup> 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

$$\theta_{\min} = \frac{1}{2} \cos^{-1} \left[ \frac{4R+9}{-15} \right]$$
(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  $-6 \le R \le 1.5$ ; consequently  $0^{\circ} \le \theta_{\min} \le 60^{\circ}$ . We found empirically that the range of  $60^{\circ} < \theta_{\min} \le 70^{\circ}$  ( $70^{\circ} < \theta_{\min} \le 90^{\circ}$ ) 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 often one has to account for structural asymmetry in order to obtain physically relevant result.<sup>14-16</sup> This can be accomplished in a straightforward manner by allowing for rhombic potentials.<sup>14–16</sup> 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.<sup>45–47</sup> 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 be suitable, obviating the need to separate the local ordering and local diffusion frames. Such a scenario is both effective and economical.

*PDFs.* To gain further insight into multiterm 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 small values of  $\theta$ , which dominate the PDF one may approximate  $u(\theta)$  by its Taylor expansion around  $\theta = 0$ . This yields  $u(\theta) \cong -10 \times (1 - \theta^2/2)$ , which has been used in the past to describe wobble-in-a-cone.<sup>48</sup> 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



**Figure 2.** Axial potentials,  $u(\theta)$ , shown as a function of  $\theta$  (we show 3D rather than 2D plots for easy comparison with rhombic potentials,  $u(\theta,\varphi)$ ). 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  $c_0^1 = 10$ 10,  $c_0^2 = 10$ , and  $c_0^3 = 10$  (*F*), and the functional forms given in Table S2 of the Supporting Information.

0

Table 1. Potential Coefficients,  $c_0^L$ , Associated with the PDFs of Figures 3A–F, and Angle  $\theta_{\max}$ 

0

		L				
	1	2	3			
Figure	$c_0^1$	$c_0^2$	$c_0^3$	$\theta_{ m max}$		
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.

may be considered as representing a generalized version of the wobble-in-a-cone PDF.

The PDFs shown in Figure 3C-E (corresponding to the potentials C-E in Table 1) are cone-shaped, with various vertex angles and thicknesses. The vertex angle is given by  $heta_{\max}$ which is the same as  $\theta_{\min}$  of the corresponding potential.  $\theta_{\max}$  is approximately 21°, 57°, and 72° for the PDFs of Figure 3C,D,E, 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.<sup>49</sup> The potentials underlying the PDFs shown in Figure 3C-E represent generalizations of Woessner's model. The PDF shown in Figure 3B corresponds to  $\theta_{max} \cong 7^{\circ}$ ; one may consider the underlying potential as representing a generalization of either wobble-in-a-cone<sup>48</sup> or Woessner's model.49

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  $0 \le \theta \le 90^\circ$  can be accessed with appropriate combinations of the L = 1-3, K = 0axial spherical harmonics.

Orientation versus Alignment. Figure 4 shows results for polar and nonpolar scenarios, with large positive potential



0

Figure 3. Axial probability densities, exp(-u), given in Cartesian coordinates ( $x = \sin \theta \cos \varphi \exp(-u)$ ,  $y = \sin \theta \sin \varphi \exp(-u)$ ,  $z = \cos \theta$  $\theta \exp(-u)$ ). 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  $c_0^3$  $= -10.0 (D), c_0^2 = -9.0 \text{ and } c_0^3 = -10.0 (E) \text{ and } c_0^2 = -10.0 (F), \text{ and the}$ functional forms given in Table S2 of the Supporting Information.

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



**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.

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-coordinaterepresentation of the PDF appears in part d; it shows the function values on the *z*-axis, and illustrates their variations.

The nonpolar 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  $\pi$ , and maximum at  $\theta = \pi/2$ . 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 nonpolar  $Y_{20}$  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 Figure 4Ad shows larger spatial distribution in the L = 1 case.

The distinction between *orientation*, implied by L = 1 with symmetry  $B_{1w}$  and *alignment*, implied by L = 2 with symmetry  $A_{gr}$  is illustrated with particular clarity by the Cartesian PDFs (Figure 4Ab and Bb): 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



**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 S3 of the Supporting Information.

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 precalculated 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  $S_{22}^2$ , correspond to a unique potential form, i.e., a unique pair of potential coefficients  $c_0^2$  and  $c_2^2$ .

2892

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  $(c_{0i}^1, c_1^1)$  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  $S_0^1(c_{0i}^1, c_1^1)$  and  $S_1^1(c_{0i}^1, c_1^1)$ . 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  $S_0^2(c_{0i}^2, c_2^2)$  and  $S_2^2(c_{0i}^2, c_2^2)$  with contours shown in Figure 5B. Finally,  $c_0^1$  and  $c_0^3$  yielded the surfaces  $S_0^1(c_{0i}^1, c_0^3)$  and  $S_0^3(c_{0i}^1, c_0^3)$ , 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 110l, and very sparse for  $c_0^1$  and  $c_1^1$  larger than 110l. 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 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; 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 = 1 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; 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  $-5 \le c_0^1 \le 5$  and  $-20 \le c_0^3 \le 20$ . 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.

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

Orientation vs Alignment. Figure 6 shows results for rhombic polar and nonpolar 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 nonpolar-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  $u(\theta, \varphi) = -10 \cos \theta - 10\sqrt{2} \sin \theta \cos \varphi$ . 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  $u(\theta, \varphi) = -10\left(\frac{3}{2}\cos^2\theta - \frac{1}{2}\right) - 10\sqrt{\frac{3}{2}}\sin^2\theta\cos(2\varphi)$ . The

4-panel representations illustrating these scenarios are shown in Figures 6A and B, 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  $\theta = \tan^{-1}\sqrt{2} \cong 0.955$  rad ( $\cong$ 54.7°) and  $\varphi = 0$  (Figures 6Ac and Ad).

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  $\varphi$ , as well as  $\theta = \pi$  and any  $\varphi$ ; it has maximum value for  $\theta = \varphi = \pi/2$ ; the spherical coordinate representation of the PDF has reversed extrema (Figures 6Bc and Bd).

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

The rhombic ordering scenario represented by the nonpolar 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 Db) are obtained from their counterparts in Figures 6Ba and Bb by a  $\pi/2$  clockwise rotation around *z*. The spherical coordinate representations of the potential and the PDF are shown in Figures 6Dc and Dd, 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, welldefined symmetry operations convert parts a-d of Figure 6A (B) into the corresponding parts of Figures 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 <sup>15</sup>N relaxation from a protein dissolved in aqueous solution, <sup>15</sup>N relaxation from a polycrystalline protein, ESR line shape analysis from a nitroxide-labeled protein dissolved in



**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 <sup>15</sup>N relaxation from the N–H bond of Gln56 of plexin-B1 in solution<sup>25</sup> (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).

aqueous solution, and <sup>2</sup>H and <sup>13</sup>C relaxation in one of the phases of a medium-sized molecule exhibiting polymorphism in the solid state.

<sup>15</sup>N Relaxation in Proteins in the Liquid State. We studied previously with SRLS <sup>15</sup>N spin relaxation of N–H bonds in plexin-B1 in aqueous solution.<sup>25</sup> 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 time scale separation prevails between the global and local motions. Let us denote these parameters as  $S_0^2$ (MD) and  $S_2^2$ (MD). The potential coefficients  $c_0^2$  and  $c_2^2$  were obtained from  $S_0^2$ (MD) and  $S_2^2$ (MD) by solving the set of nonlinear equations:<sup>25</sup>

$$S_0^2(c_0^2, c_2^2) - S_0^2(MD) = 0$$
(7a)

$$S_2^2(c_0^2, c_2^2) - S_2^2(MD) = 0$$
(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 8c shows the "atomistic" PDF contours;<sup>25</sup> Figure 8d (which is the same as Figure 7Ad) shows the "mesoscopic" PDF counterparts. Figure 8c is considerably more asymmetric than Figure 8d. 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 8c).

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



**Figure 8.** PDF contour plots (in spherical coordinates,  $\theta$  and  $\varphi$ ) for residue Gln56 of plexin-B1 calculated directly from the MD trajectory<sup>25</sup> (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 <sup>15</sup>N relaxation<sup>25</sup> (d). Part d is the same as Figure 7Ad.

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 methods<sup>50</sup> 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  $-c_0^2Y_{20}$  in the limit of strong axial potential) applies to rapidly fluctuating N–H



**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 line shape analysis of nitroxide-labeled T4 lysozyme at position 72, conformer 2 (part B).<sup>36</sup>



**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 <sup>2</sup>H-labeled  $\beta$ -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 <sup>13</sup>C-labeled carbonyl of BHA6 in phase I (part B).

bonds.<sup>51</sup> However, this PMF would not apply to more general cases, such as illustrated by Figure 8c. 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.

<sup>15</sup>N Relaxation in Proteins in the Solid State. The authors of ref 36 studied <sup>15</sup>N 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 <sup>15</sup>N 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 and 7b, 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.<sup>35</sup> Liquid-state ESR lineshapes were analyzed with SRLS. Two conformers have been detected at this site; we show the results associated with conformer 2, for which a local potential with  $c_0^2 = 3.31$  and  $c_2^2 = -1.67$  was determined at 25 °C.

Table 2. Potential Coefficients and	Corresponding Order P	Parameters in Irreducible	Tensor Notation (	$S_0^2$ and $S_2^2$ ) a	nd in Saupe
Tensor Notation $(S_{xx}, S_{yy}, S_{zz})$ (eq	s 3, 4, 5, and 8) <sup>a</sup>				

ref no.	$c_{0}^{2}$	$c_{2}^{2}$	$S_0^2$	$S_2^2$	S <sub>xx</sub>	$S_{yy}$	$S_{zz}$
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

<sup>*a*</sup>The data shown are associated with N–H relaxation of Gln56 of plexin-B1;<sup>25</sup> ESR relaxation of the nitroxide-labeled residue 72 of T4 lysozyme;<sup>35</sup> N–H relaxation of Gly21 of thioredoxin;<sup>36 2</sup>H relaxation of the  $\beta$ -deuterium in the discotic mesophase of BHA6 (penultimate row); and <sup>13</sup>C carbon relaxation of the <sup>13</sup>C-labeled carbonyl of BHA6 in phase I (last row).<sup>37</sup>

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.<sup>37</sup> We converted the axial and rhombic components of these tensors into the order parameters  $S_0^2$  and  $S_{22}^2$ , calculated  $c_0^2$  and  $c_2^2$  using eqs 7a and 7b,<sup>25</sup> 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 <sup>2</sup>H relaxation from the  $\beta$ -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 <sup>13</sup>C relaxation from the <sup>13</sup>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 <sup>2</sup>H or <sup>13</sup>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. <sup>14–16</sup> On the other hand, side-chain methyl sites in proteins were found to be associated with weak local potentials. <sup>14–16</sup> 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  $S_2^2$  associated with the five examples considered, and the corresponding potential coefficients. We also show the Saupe order parameters  $S_{xxy}$   $S_{yy}$  and  $S_{zzy}$  related to  $S_0^2$  and  $S_2^2$  as

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

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

$$S_{zz} = S_0^2 \tag{8c}$$

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,Ba, and 10Aa,Ba. 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 are 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 nonpolar 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 multiterm 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 good approximations to the atomistic potentials implicit in the atomistic PDFs.

## ASSOCIATED CONTENT

#### **Supporting Information**

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jpcb.6b00524.

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. Figure S1, analogous with Figure 6 except  $c_0^1 = -10$  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 (PDF)

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#### Notes

The authors declare no competing financial interest.

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