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Physical Interpretation of Residual Dipolar Couplings in Neutral Aligned Media

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Residual dipolar couplings (RDCs), induced by aligned cosolutes, have revolutionized biomolecular three-dimensional structure prediction.[1] In the absence of strong electrostatic effects, steric interactions are sufficient to explain the alignment,[2] e.g., when using DMPC−DHPC bicelles. This has led to the development of methods for predicting alignment tensors a priori, making residual dipolar couplings easier to interpret, and increasing the applicability of the approach to more complex structural problems. Although alignment can be predicted by tedious simulations,[3] no methods have been proposed which are simple to use, highly predictive, and consistent with these simulations. This restricts our intuition and limits the use of residual dipolar couplings in interpreting dynamic molecular structure, for example.

A novel approach is used here to derive an expression for the alignment tensor induced by a neutral and dilute cosolute, based on hydrodynamic shape. The derived expression is simple, highly predictive, and consistent with simulations of alignment. It is compared and tested against recently reported methods of alignment prediction.

The Saupe order matrix defines the degree of alignment of a molecule and establishes the relationship between measured residual dipolar couplings and their directions in the molecular frame,[4] often referred to as the alignment tensor. When induced by a neutral and dilute cosolute, the alignment tensor is dependent on the shape of the aligned molecules. A suitable descriptor of molecular shape is the second moment of the atomic distribution, eq 1, also known as the gyration tensor, for N atoms at positions $\mathbf{x}^{(r)}$ (uniform mass assumed).

$$R_{ij}^2 = \frac{1}{N}\sum_{r=1}^{N} x_i^{(r)} x_j^{(r)} \qquad (1)$$

Use of eq 1 allows aligned molecules to be represented as equivalent ellipsoids, which are known to provide very effective hydrodynamic descriptions of compact molecular shapes, e.g., globular proteins.[5] Equivalent ellipsoids share characteristic lengths (ρ_1, ρ_2, and ρ_3) with the molecules they describe, which are the square roots of the eigenvalues of R^2. Since alignment is primarily dependent on shape, a dimensionless scalar δ was defined as the ratio between differences in characteristic length, eq 2, where

$$\delta = \frac{(\rho_2 - \rho_3)}{(\rho_1 - \rho_3)} \qquad \sigma(r) = \frac{(r - \rho_3)}{(\rho_1 - \rho_3)} \qquad (2)$$

$\rho_1 > \rho_2 > \rho_3$. By similarity, a parameter σ was defined from the distance between the molecular center of geometry and the alignment surface, r. It is hypothesized that δ describes the form of the alignment tensor but not its magnitude.

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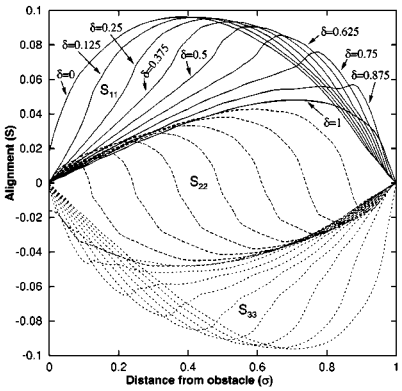

Figure 1. Simulated diagonal components (S_{11},S_{22},S_{33}) of the order matrix for ellipsoids, of shape δ, at varying distances (σ) from a planar obstacle.

Order matrices were calculated numerically for the case of an ellipsoid obstructed by a planar wall.[3,6] Computationally, ellipsoids were represented as a mesh of points distributed evenly over its surface, and orientations in which its surface intersects the obstacle were rejected. In this case, the eigenvectors of R^2 are linearly related to the semi-axes of the ellipsoid, and although S is most generally a tensor of rank 2, by symmetry S is reducible to a tensor of rank 1 in the eigenbasis of the gyration tensor.[7−9] Thus, it can be described by three components, with two degrees of freedom (traceless). These three diagonal components of the order matrix were calculated throughout the aligned region, and for a range of δ values, to yield $S(\sigma,\delta)$. For $\sigma < 0$ the molecule is excluded, and for $\sigma > 1$ no alignment is possible, and thus $S = 0$. Figure 1 shows the order matrix for a range of fixed δ (i.e., shape) at varying distances from the plane. The graphs show that maximum contribution to alignment occurs in the middle region, where $\sigma \approx 0.5$, and although states where the molecule is extremely close to the surface produce the most alignment, they are rarely populated.

The average of $S(\sigma,\delta)$ over σ is the scale-independent alignment tensor, i.e., that which depends on relative rather than absolute lengths. Figure 2 shows that the three simulated components of S are linear functions to a good approximation. Linear (affine) fitting allows the approximation of eq 3 to be obtained, which has a simple

$$(S_{11},S_{22},S_{33}) \propto \left(1 - \frac{1}{2}\delta,\delta - \frac{1}{2}, -\frac{1}{2} - \frac{1}{2}\delta\right) \qquad (3)$$

dependence on δ, in the eigenbasis of R^2. For most purposes eq 3 is sufficient to provide the alignment tensor required for the residual dipolar coupling experiment, since S must always be scaled by a suitable constant, dependent on the concentration of the aligned media.

Therefore, using the theory above, a prediction of the alignment tensor is made in the following way. The characteristic lengths for the molecule in question are calculated using the eigenvalues of

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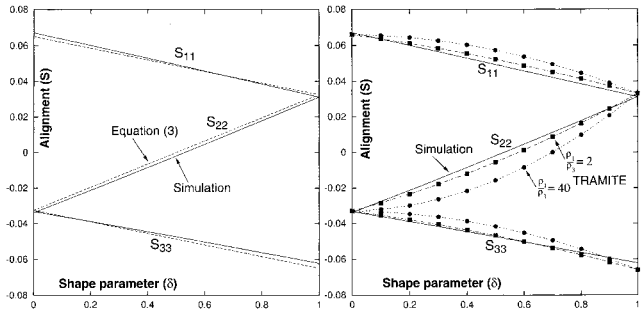

Figure 2. Predicted diagonal components of the alignment tensor from (left) the model presented here and (right) TRAMITE,[9] compared against those obtained from a full simulation approach (solid lines).

eq 1, allowing δ to be evaluated using expression 2. This permits calculation of the scale-independent alignment tensor S, eq 3. Finally, this S is scaled by a suitable constant to best fit the experimental data. Predictions based on this method have been performed for a series of proteins found in the Protein Data Bank (PDB), as used by Fernandes et al.[8] For these proteins, residual dipolar couplings have been measured previously under conditions of a neutral aligning cosolute.[8] Correlation coefficients for the fit against experimental data are given in Table 1, confirming that eq 3 provides consistently excellent predictions of alignment for these proteins.

It is also possible to calculate the scale-dependent form of the alignment tensor by introducing a multiplicative factor $\propto (\rho_1 - \rho_3)$ to all expressions above. This is required when comparing different aligned molecules at the same concentration and type of alignment media, for example. The relevant expressions for cyclic i,j,k are given in eq 4, again subject to a scaling, linearly dependent on aligning media concentration.

$$S_{ii} \propto \rho_i - \frac{1}{2}(\rho_j + \rho_k) \tag{4}$$

Recently, other methods have been proposed which predict alignment tensors in neutral dilute liquid crystal solutions.[3,8,9] A consistency check shows that expression 3 is in excellent agreement with the SSIA algorithm[3] for compact protein shapes. In these cases, eq 3 can obtain values within a few percent of SSIA at a fraction of the processing power. Therefore, the cumbersome SSIA method can be avoided in all but the most problematic of situations.

An alternative method (TRAMITE) has been proposed for calculating the alignment tensor from the molecular inertia tensor (I).[9] The central expression of this method can be rewritten in terms of length, eq 5, since I and mR^2 share the same set of eigenvectors (see Supporting Information; m is molecular mass). However, based

$$S_{ii} \propto m\rho_i^2 - \frac{1}{2}m(\rho_j^2 + \rho_k^2) \tag{5}$$

on simulation, this expression should be linear in length and have no dependence on mass. To illustrate the consequences of nonlinear lengths present in eq 5, consider two ellipsoids with (ρ_1,ρ_2,ρ_3) of (4,3,2) and (8,6,4). It can be shown by simulation that they possess the same alignment tensors, subject to a linear scaling of 2. However, eq 5, dependent on square distances, does not provide this; only eq 4, being linear in length, gives the correct prediction, allowing the relative magnitude of alignment of different molecules to be predicted. Due to this nonlinearity, predictions from eq 5 become progressively worse for highly prolate or oblate anisotropic molecules. For example, Figure 2 shows predictions made with TRAMITE[9] for molecules with low and high ρ_1/ρ_3. For high values

Table 1. Capacity of Different Models To Predict Experimental Residual NH Dipolar Coupling Data

		correlation coefficients		
PDB code[a]	shape δ	eq 3	TRAMITE[9]	analytical[8,b]
2ezx	0.119	0.9899	0.9896	0.98
2ezm	0.107	0.8277	0.8415	0.68
1khm	0.310	0.9127	0.9105	0.83
1cmz	0.173	0.9511	0.9537	0.92
1d3z	0.309	0.8737	0.8892	0.72
3gb1	0.244	0.9891	0.9885	0.96
1e8l	0.100	0.9418	0.9414	0.90

[a] Citations to the original experimental data are contained in Fernandes et al.[8] [b] Values taken directly from original publication.

it deviates markedly from the simulation predictions, particularly when full anisotropy is present ($\delta \approx 0.5$). Although predictions from eqs 4 and 5 are almost indistinguishable for axial ratios likely to be present in biomolecules, only the constant of proportionality between predictions made using eq 4 and experimental data has physical significance, being linearly dependent on both aligned cosolute concentration and molecular length. This is physically intuitive because it is well known that residual dipolar couplings are linearly dependent on aligning media concentration.

The expression derived by Fernandes et al.[8] uses analytical methods to study the alignment of ellipsoids by a planar wall. However, the main derivation is restricted to the case of an axially symmetric ellipsoid. Generalization to full anisotropy is achieved using a "rhombicity correction", which employs the eigenvalues of R^2 directly, i.e., nonlinear lengths. Their expression[8] is therefore a mixture of linear and nonlinear dependencies on length, again not in agreement with simulation. The effect of this combination is a method with less predictive capacity than eq 3, as shown by Table 1.

Therefore, eq 3 is the only expression to date which can provide simple, accurate predictions of the alignment tensor for neutral and dilute alignment media, while being consistent with simulations of alignment. It provides predictions in a fraction of the time of a simulation approach, while aiding physical intuition by providing a direct link between hydrodynamic shape and the alignment tensor. Not only is this physically gratifying, but it also permits residual dipolar couplings to be applied in demanding situations where simulations of alignment are not desirable, such as in studies of molecular dynamics.

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Supporting Information Available: Mathematics showing how the eigenvalues of the inertia and gyration tensors are related (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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