

Symmetry, Goniometers, and RDC's.

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Abstract

In this tutorial, we discuss some of the results of having extra symmetries in a system where we measure residual dipolar couplings (RDC's). In the first case, we look at RDC's of protein complexes which are trimers, tetramers, or higher. As mentioned to our group by James Chou, the rhombicity of the Saupe matrix will be zero.

Next, we consider the question of using a goniometer with an NMR sample to generate a potentially infinite number of independent weak alignments for measuring RDCs. As described by Ad Bax, if the aligning media is bi-axial, the setup will work and generate a series of independent alignments. However, if the aligning media has only one axis, which we (arbitrarily) call "uniaxial", then rotations by the alignments will not be independent, and in fact, the RDC's will simply be scaled as the goniometer rotates.

While we do not yet fully understand Bax's comment, we can provide some intuition on the matter by showing that if the rhombicity is zero, then the RDC's will just scale linearly as the goniometer angles are changed.

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1 Introduction

In this tutorial, we would like to explain two things:

(i) Why do trimers, tetramers, and higher oligomers have zero rhombicity when in aligning media?

(ii) If an aligning media is uni-axial, why will a goniometer not give us a series of independent alignments?

Aside: During the 2004 Gordon Conference on Biomolecular NMR: Computational Aspects, I briefly dropped by Bax’s table during lunch, and asked him about using a goniometer with RDC’s. As an example, I suggested using stretched polyacrylamide gel which can be rotated mechanically. He and another researcher were actually just talking about this when I came by. Bax briefly mentioned that the set-up wouldn’t work, and that the RDC values would just get scaled as the sample was rotated. However, if the aligning media was bi-axial, then it would work, and you could get a series of independent alignments. I didn’t fully understand Bax’s comments at first, which is why I wrote this tutorial, to help myself figure it out. Jeff Hoch, James Chou, and I believe Prestegard have all thought about this problem, so it seems that it is very much on the mind of researchers in the field.

Both (i) and (ii) involve having additional (ie: externally imposed) symmetries of the Saupe matrix. That is, an extra symmetry of the Saupe matrix that does not occur in the general case. In Section 2 we’ll show the consequences of having a rotation symmetry in a Saupe matrix. These results will be applied to (i) and (ii). In Section 3 we’ll look at (i). First, we’ll give an intuitive explanation, and then follow that with a more formal discussion. In Section 4 we’ll look at (ii), and show why a uni-axial alignment media is not useful with a goniometer.

2 Symmetries and the Saupe Matrix

We consider the case where our system has a rotational symmetry. As always, we start with the RDC equations. Let \mathbf{v} be a bond vector (such as an NH bond vector). Let D be the RDC value associated with \mathbf{v} . Let S be the Saupe matrix. Let K be the dipole interaction constant comprised of physical constants including gyromagnetic ratios, Planck’s constant, and an overall scale factor. Finally, let \mathbf{B} be the external magnetic field. For simplicity of exposition, we’ll initially assume the protein is essentially “rigid” so that a molecular frame is well defined. In the molecular frame, \mathbf{v} is constant (assuming rigidity). Each element of the ensemble will see a magnetic field in its own molecular frame, due to independent tumbling in solution. If we work in the molecular frame, this induces a distribution of magnetic field vectors \mathbf{B} . The standard RDC equations are:

$$D = K \mathbf{v}^T S \mathbf{v} \tag{1}$$

$$S \equiv \frac{1}{2} \langle 3(\mathbf{B}\mathbf{B}^T) - I \rangle \tag{2}$$

The brackets in (2) are an ensemble and time average, and I is the identity matrix. Let $U = \{\mathbf{B}_i\}$ be the set of magnetic fields seen in the molecular frame. Each \mathbf{B}_i is a unit vector and represents the direction of the external magnetic field in the molecular frame of the i th member of the ensemble. Using the set U , we can write the ensemble average:

$$U = \{\mathbf{B}_i\} \quad (3)$$

$$S = \frac{1}{2} \langle 3(\mathbf{B}\mathbf{B}^T) - I \rangle = \frac{1}{2n} \sum_{i=1}^n (3\mathbf{B}_i\mathbf{B}_i^T - I) \quad (4)$$

Where n is some huge number, representing the size of the ensemble. (We're using $\{\mathbf{B}_i\}$ to represent the molecular ensemble of proteins in solution, and the time ensemble as well.) Although it is an abuse of notation and terminology, We'll use $\{\mathbf{B}_i\}$ to represent both the *set* as well as the *distribution* for the magnetic field in the molecular frame. If the set is very large with good sampling, it is virtually equivalent to a probability distribution. One can imagine dividing the histogram of $\{\mathbf{B}_i\}$ by n to get an estimate of the probability distribution.

Notice that the Saupe matrix is function of the set $U = \{\mathbf{B}_i\}$. Explicitly, we can say

$$S = \mathcal{F}(U) = \mathcal{F}(\{\mathbf{B}_i\}) \quad (5)$$

$$\mathcal{F}(\{\mathbf{B}_i\}) = \frac{1}{2n} \sum_{i=1}^n (3\mathbf{B}_i\mathbf{B}_i^T - I) \quad (6)$$

where $\mathcal{F} : P \rightarrow Q$. P the set of all sub-sets of unit vectors, and Q is the set of all real, symmetric, and traceless 3×3 matrices. Equation (5) is significant, because, any operation that leaves the set $U = \{\mathbf{B}_i\}$ unchanged, will consequently also leave the Saupe matrix S unchanged.

Suppose our system has a rotational symmetry. Let R be a rotation which is the symmetry of our system. In that case, a rotation by R leaves everything unchanged. In particular, our distribution $\{\mathbf{B}_i\}$ is unchanged by the rotation.

$$RU = U \quad (7)$$

$$R\{\mathbf{B}_i\} = \{\mathbf{B}_i\} \quad (8)$$

In other words, rotating the distribution $\{\mathbf{B}_i\}$ by R does not change the distribution. Since our Saupe matrix is a function of the distribution $\{\mathbf{B}_i\}$, it follows that our Saupe matrix must obey the same symmetry. Applying \mathcal{F} to both sides of equation (8) we get,

$$\mathcal{F}(R\{\mathbf{B}_i\}) = \mathcal{F}(\{\mathbf{B}_i\}). \quad (9)$$

The RHS is our original Saupe matrix S . The LHS is a *rotated* S . Because S is a direct function of the unit vectors $U = \{\mathbf{B}_i\}$, a rotation on U will induce a rotation on S . So we must have

$$S = \mathcal{F}(\{\mathbf{B}_i\}) \quad (10)$$

$$\text{Rotated } S = \mathcal{F}(R\{\mathbf{B}_i\}) \quad (11)$$

$$\text{Rotated } S = S. \quad (12)$$

Let's consider this explicitly, by rotating the $\{\mathbf{B}_i\}$ by R , and then using \mathcal{F} to construct the

rotated Saupe matrix:

$$R\{\mathbf{B}_i\} = \{\mathbf{B}_i\} \quad (13)$$

$$\text{Rotated } \mathbf{B}_i = R\mathbf{B}_i \quad (14)$$

$$\text{Rotated } \mathbf{B}_i^T = \mathbf{B}_i^T R^T = \mathbf{B}_i^T R^{-1} \quad (15)$$

$$S = \mathcal{F}(U) = \frac{1}{2} \langle 3\mathbf{B}_i\mathbf{B}_i^T - I \rangle \quad (16)$$

$$\text{Rotated } S = \mathcal{F}(RU) = \frac{1}{2} \langle 3R\mathbf{B}_i\mathbf{B}_i^T R^{-1} - I \rangle = R \left(\frac{1}{2} \langle 3\mathbf{B}_i\mathbf{B}_i^T - I \rangle \right) R^{-1} \quad (17)$$

$$\text{Rotated } S = RSR^{-1} \quad (18)$$

This is exactly what we expected, since (18) is the standard formula for rotating an arbitrary matrix (change of basis). Combining equations (12) and (18), we see that S has rotation symmetry R :

$$S = RSR^{-1} \quad (19)$$

If a Saupe matrix S has rotation symmetry R , then there are two consequences. First, the eigenvectors of S must also have R as a symmetry. Second, the eigenvectors of S must be either parallel or perpendicular to the axis of rotation of R . Below, we show these two facts.

Theorem 2.1 *Given:*

1. S is a Saupe matrix (real, symmetric, traceless).
2. S has rotation symmetry R . $S = RSR^{-1}$ where $R = R_{\mathbf{a}}(\theta)$ is a rotation about vector \mathbf{a} by θ radians.
3. \mathbf{x} is an eigenvector of S with eigenvalue λ . $S\mathbf{x} = \lambda\mathbf{x}$.

Then:

$(R^{-1}\mathbf{x})$ is also an eigenvector of S with eigenvalue λ .

PROOF.

$$\begin{aligned} S\mathbf{x} &= \lambda\mathbf{x} \\ S &= RSR^{-1} \\ RSR^{-1}\mathbf{x} &= \lambda\mathbf{x} \\ S(R^{-1}\mathbf{x}) &= \lambda(R^{-1}\mathbf{x}) \end{aligned}$$

So $(R^{-1}\mathbf{x})$ is an eigenvector with eigenvalue λ . \square

Here is a review of some linear algebra. Suppose we have two linearly independent eigenvectors that share the same eigenvalue:

$$M\mathbf{x} = \lambda\mathbf{x} \quad (20)$$

$$M\mathbf{y} = \lambda\mathbf{y} \quad (21)$$

Then *every* vector in the plane spanned by \mathbf{x} and \mathbf{y} is an eigenvector with the same eigenvalue λ . Taking a linear combination of equations (20)-(21) we get:

$$M(ax + by) = \lambda(ax + by) \quad (22)$$

where a and b are scalars.

Similarly, if we had three eigenvectors \mathbf{x} , \mathbf{y} , and \mathbf{z} that share a single eigenvalue λ , then any vector in $\text{Span}\{\mathbf{x}, \mathbf{y}, \mathbf{z}\}$ is an eigenvector with eigenvalue λ . If \mathbf{x} , \mathbf{y} , and \mathbf{z} are linearly independent, then they span is all of \mathbb{R}^3 . That means our matrix S must be a uniform dilation:

$$M = \begin{bmatrix} \lambda & & \\ & \lambda & \\ & & \lambda \end{bmatrix} \quad (23)$$

We'll use this idea for proof by contradiction in the next theorem.

Theorem 2.2 *Given:*

1. S is a Saupe matrix (real, symmetric, traceless).
2. S has rotation symmetry R . $S = RS R^{-1}$ where $R = R_{\mathbf{a}}(\theta)$ is a rotation about vector \mathbf{a} by θ radians.
3. $\theta \neq 0$ and $\theta \neq \pi$. (Non-degenerate rotations.)
4. S is not the zero matrix. (Non-degenerate Saupe matrix.)

Then:

1. The eigenvectors of S are either parallel or perpendicular to \mathbf{a} , the axis of the rotation symmetry.
2. There is one eigenvector parallel to \mathbf{a} .
3. There are two eigenvectors perpendicular to \mathbf{a} , and they share the same eigenvalue.

PROOF. Proof by contradiction. Suppose \mathbf{x} is an eigenvector of S which is neither perpendicular nor parallel to \mathbf{a} .

$$S\mathbf{x} = \lambda\mathbf{x} \quad (24)$$

Then by Theorem 2.1 we know that

$$S(R^{-1}\mathbf{x}) = \lambda(R^{-1}\mathbf{x}) \quad (25)$$

$$S(R^{-1}R^{-1}\mathbf{x}) = \lambda(R^{-1}R^{-1}\mathbf{x}) \quad (26)$$

$$(27)$$

Let $\mathbf{y} = R^{-1}\mathbf{x}$ and $\mathbf{z} = R^{-1}R^{-1}\mathbf{x}$.

Claim: \mathbf{x} , \mathbf{y} and \mathbf{z} are linearly independent. Because \mathbf{y} and \mathbf{z} are generated from \mathbf{x} by the same rotation R , we know that \mathbf{x} , \mathbf{y} and \mathbf{z} all live on a cone. (See Figure 1.) The cone is formed by the revolution of \mathbf{x} around \mathbf{a} the axis of of the rotation. Furthermore, because \mathbf{y} and \mathbf{z} are neither on the axis of rotation, nor perpendicular to it, we know that the cone is non-degenerate (not a thin ray, nor an infinite flat disc).

Consider the plane spanned by \mathbf{x} and \mathbf{z} . This plane slices through the apex of the cone. In the cross section of this arrangement, the cone is represented by a circle, and the plane by a line. A line cannot intersect a circle at more than two points. Therefore, \mathbf{y} is not in the same plane as \mathbf{x} and \mathbf{z} . So \mathbf{x} , \mathbf{y} and \mathbf{z} are not linearly dependent.

(To make this argument complete, we need to mention that \mathbf{x} , \mathbf{y} and \mathbf{z} are all distinct points, because we require that R to be a rotation whose angle is not zero, nor π radians.)

But if this is the case, then any vector in \mathbb{R}^3 is spanned by \mathbf{x} , \mathbf{y} and \mathbf{z} , and is therefore an eigenvector with eigenvalue λ .

$$S(a\mathbf{x} + b\mathbf{y} + c\mathbf{z}) = \lambda(a\mathbf{x} + b\mathbf{y} + c\mathbf{z}) \quad (28)$$

where a , b , and c are scalars. So the three eigenvalues of our Saupe matrix are all λ .

$$S_{xx} = S_{yy} = S_{zz} = \lambda \quad (29)$$

But S is traceless, so $S_{xx} + S_{yy} + S_{zz} = 0$. So we must have $S_{xx} = S_{yy} = S_{zz} = 0$. In which case, S must be the zero matrix. But we assume S is not the zero matrix.

Contradiction.

So \mathbf{x} must be parallel or perpendicular to \mathbf{a} the axis of rotation symmetry.

Furthermore, S is symmetric, so we can always choose three perpendicular eigenvectors. The only way to fit three perpendicular eigenvectors into these constraints, is to have one eigenvector parallel to \mathbf{a} and the other two in the plane perpendicular to \mathbf{a} .

Finally, the two eigenvectors in the plane perpendicular to \mathbf{a} must share the same eigenvalue. This is because, a rotation of one eigenvector in the plane makes a new eigenvector in the plane with the same eigenvalue. Then, every vector in the plane will be an eigenvector with the same eigenvalue. (See Theorem 2.1 and the discussion immediately after it.)

□

In the next two sections, we apply these two theorems to our given problems.

3 RDC's of Trimers, Tetramers, and Higher.

Suppose we have aligning media, such as bicelle, and a trimer which we represent as a triangle. (See Figure 2.) We'll consider the symmetries of our system first in the lab frame, and then move to the molecular frame.

But before we get into the details, we'll discuss the problem intuitively. The essential idea is that if the protein is trimeric, then rotating it by 120 degrees will not change anything. In the molecular frame, this symmetry will show up as a symmetry of the magnetic field's distribution. In turn, this will cause the Saupe matrix to share the same symmetry as well. Once we know that the Saupe matrix has a rotation symmetry, we know right away that at least two of its eigenvalues are identical from Theorem 2.2. If we assume that the principal eigenvector is unique (that is, $S_{xx} > S_{yy} \geq S_{zz}$), then it must be that $S_{xx} = S_{yy}$ and the two other eigenvectors line in the plane of the rotation symmetry. Because $S_{xx} = S_{yy}$, the rhombicity is zero.

To discuss the above in more detail, consider the lab frame. In the lab frame, we have a single fixed magnetic field \mathbf{B}_0 . Consider a single type of NH bond vector. By single type, we mean the NH bond vector of a specific residue number in the protein. For example, the NH bond vector of the 17th residue in the protein. Because our protein is a trimer, we can rotate our protein by $R = R_{\mathbf{a}}(\theta)$ with $\theta = 120$ degrees, and nothing would change. The RDC values would remain the same, and the set of bond vectors in the ensemble would also be the same.

A kind of simplified example is to imagine our bond vectors as being parallel to the three sides of an equilateral triangle. This set of three vectors remains unchanged if we rotate it by a symmetry of the equilateral triangle. Each vector rotates, and turns into a different element of the original set. In the same way, the ensemble of NH vectors has the same rotation symmetry.

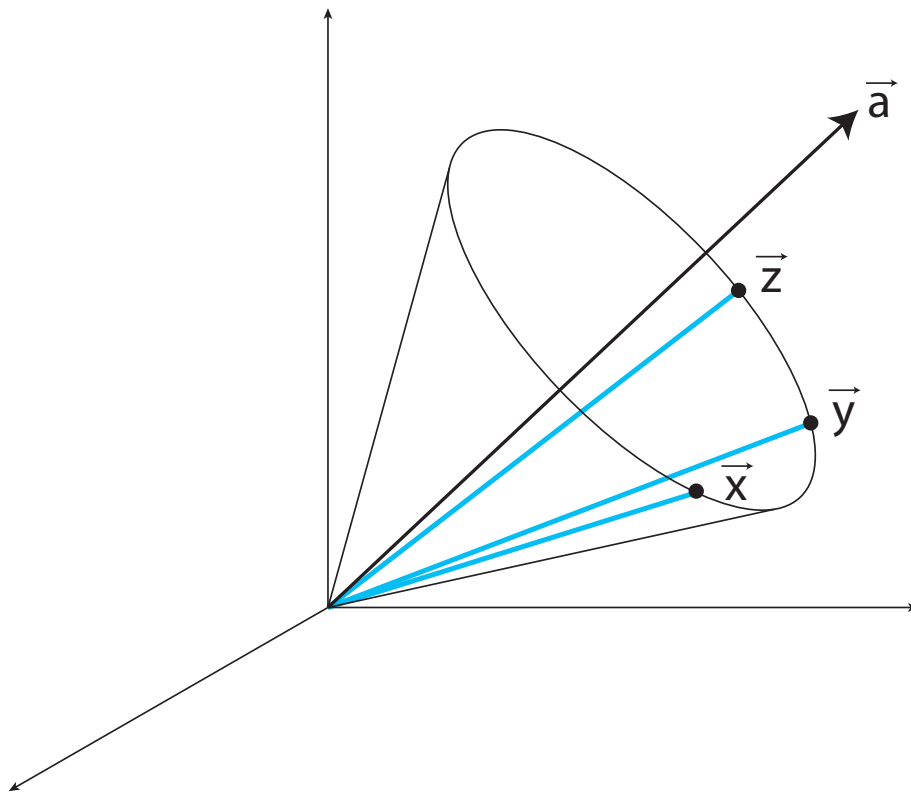


Figure 1: **Cone diagram for \mathbf{x} , \mathbf{y} and \mathbf{z} .** (See Theorem 2.2.) The vector \mathbf{a} is the axis of rotation. The rotation generates \mathbf{y} from \mathbf{x} , and \mathbf{z} from \mathbf{y} . Notice that \mathbf{x} , \mathbf{y} and \mathbf{z} are linearly independent.

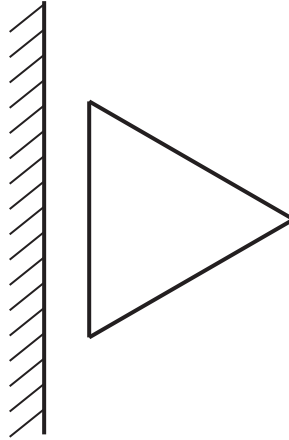


Figure 2: **Trimer model in bicelle medium.** The wall on the left represents a bicelle, and the equilateral triangle represents our protein trimer.

Now let's go to the molecular frame. In the molecular frame, the protein is fixed, and the magnetic field varies. Let $U = \{\mathbf{B}_i\}$ represent the ensemble of magnetic field directions observed in the molecular frame. Now we know rotation by R is a symmetry of our system. Rotating by R in the molecular frame, the protein is unchanged. But distribution of the magnetic field must rotate too.

$$\{\mathbf{B}_i\} \rightarrow R\{\mathbf{B}_i\} \quad (30)$$

Since this rotation is a symmetry of the entire system, we must have

$$\{\mathbf{B}_i\} = R\{\mathbf{B}_i\}. \quad (31)$$

In our simplified example, take our three vectors (which are parallel to the sides of an equilateral triangle), and consider some arbitrary external magnetic field. The direction of the magnetic field does not need to be in the plane of the equilateral triangle, nor perpendicular to it. In the lab frame, the magnetic field is fixed, and we see the triangle “jumping” between each of its symmetries. In the triangle's frame (the molecular frame), the triangle is fixed, and instead, the magnetic field is jumping between three symmetric possibilities. In the ensemble, the molecular frame will see the distribution of the magnetic field as having a three-fold symmetry, which is equation (31).

Given that we have $\{\mathbf{B}_i\} = R\{\mathbf{B}_i\}$, we can conclude that our Saupe matrix has the same symmetry because it is constructed from the $\{\mathbf{B}_i\}$.

$$S = RSR^{-1} \text{ where } R = R_{\mathbf{a}}(\theta) \text{ is a rotation about } \mathbf{a} \text{ by } \theta \text{ radians.} \quad (32)$$

We can now apply Theorem 2.2 to get that one of the eigenvectors is perpendicular to \mathbf{a} and the other two are perpendicular to \mathbf{a} . The two eigenvectors perpendicular to \mathbf{a} share the same eigenvalue.

If we move to the POF of the Saupe matrix S , we have:

$$S = \begin{bmatrix} S_{xx} & & \\ & S_{yy} & \\ & & S_{zz} \end{bmatrix} \quad (33)$$

By convention, we have $S_{zz} \geq S_{xx} \geq S_{yy}$. If we assume that the eigenvector for S_{zz} is uniquely determined, then we must have that S_{zz} is strictly larger than S_{xx} and S_{yy} . In that case, the two equal eigenvalues must be S_{xx} and S_{yy} . With $S_{xx} = S_{yy}$, the rhombicity of S is zero. Finally, we know that the eigenvector for S_{zz} must point in the direction of the axis of the rotational symmetry.

4 RDCs and Goniometers

RDC's are measured in weakly aligning media. Multiple media are interesting because they induce multiple alignments, each of which could provide independent information. A natural idea arises, to place the NMR sample in a goniometer, so that the induced alignment could be controlled manually. This would open up the possibility of having an "infinite" number of independent weak alignments.

According to Ad Bax, this will work only if the aligning media is bi-axial. While we do not understand Bax's statement in full detail, we can give some intuition by considering a *uniaxial* medium. We define a uni-axial medium to be one where the order is defined by a single vector. The medium has a cylindrical type symmetry.

Naively, one might expect stretched polyacrylamide gels to be an example of uni-axial media, where the gel is stretched in only one direction. Another example might be phage, which are essentially long thin cylinders. See Figure 3. *However, in actual experiments stretched gels and phage are not uniaxial media.* Below we will show that uni-axial media cause the Saupe matrix to have zero rhombicity. *In actual experiments (such as [1, 2]), the rhombicity of stretched gel and phage can be large.* In these cases, we speculate that the non-zero rhombicity might be due to charge effects. Bax in [3] simulates RDCs by looking at all protein orientations which do not sterically clash with a phage cylinder. It is our understanding that Bax's simulation is cylindrically symmetric. His predictions do not agree with experiment, and Bax explains this by saying, "This indicates that protein alignment in fd phage is not dominated by obstruction, and presumably contains a large contribution from electrostatic attraction between negatively charged fd and positive surface groups on the protein G domain." (His bicelle simulation had very good agreement with experiment.) Our speculation is that these charge effects might be a cause of non-zero rhombicity in otherwise rotationally symmetric media.

Although we do not fully understand Bax's statement, we speculate that a bi-axial medium breaks the symmetry of the uni-axial medium, by not being rotationally symmetric. For example, if one could somehow stretch the gel in the z -axis by amount b , and then stretch the gel in the y -axis by a different amount c , one could get a bi-axial medium where all three axes are different.

I mentioned to James Chou the idea of stretching/compressing a gel different amounts in each direction. Chou was skeptical that such a gel could be made to work in practice, because gels are like microscopic "spaghetti" and are not easily controllable in practice. One might mechanically apply such a deformation to a gel, but the gel is soft and might reorganize itself into something that isn't significantly bi-axial. In contrast, using a gel to make a uni-axial medium is simple: You squeeze the gel into a thinner tube, and it basically has no choice but to get stretched and become uni-axial.

In a uni-axial medium, the medium has rotational symmetry. Let \mathbf{a} be the axis of the medium's rotational symmetry. Rotating the sample around the axis \mathbf{a} does not change the distribution of protein orientations. The RDC values are also unchanged by such a rotation. It is important

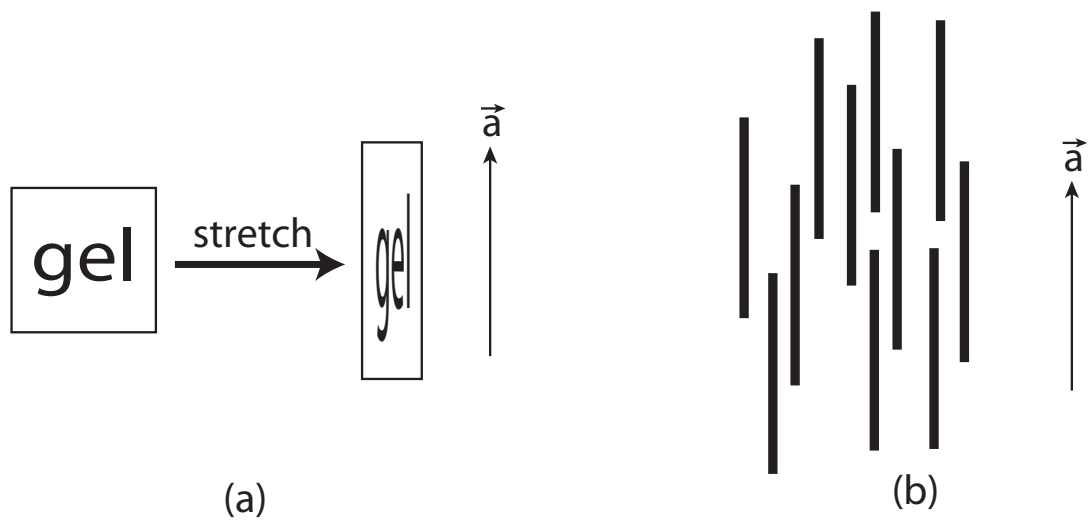


Figure 3: Uni-axial Aligning Media. Naively, one might expect stretched polyacrylamide gel and phage to be uni-axial media. This figure shows why. However, in practice, stretched polyacrylamide gel and phage can be non-uni-axial. (a) In theory, the gel is stretched in only one direction, and contracts uniformly in the other two axes. As a result, the stretched gel has a rotational symmetry around axis \mathbf{a} . Actual experiments contain gel which is not uni-axial. (b) If the aligning media is made of long thin rods, then the aligning media is (averaged over a random distribution) rotationally symmetric about axis \mathbf{a} . One might naively think that phage is such a medium, but in practice phage can be non-uni-axial.

to make a very simple assumption: We assume that the distribution of protein orientations is a function of just the axis \mathbf{a} (and a variety of chemical factors, such as pH, charge, concentration, etc), but *not* a function of the external magnetic field. That is, we assume the protein does not have any magnetic permeability effects, and that its weakly induced alignment is controlled by \mathbf{a} so that if \mathbf{a} is rotated by R , then the distribution of orientations is also rotated by R . This is a fairly reasonable assumption if you assume that the medium induces alignment by simple effects, such as steric interactions, and that the external magnetic field does not induce any alignment. This is symbolically represented in Figure 4.

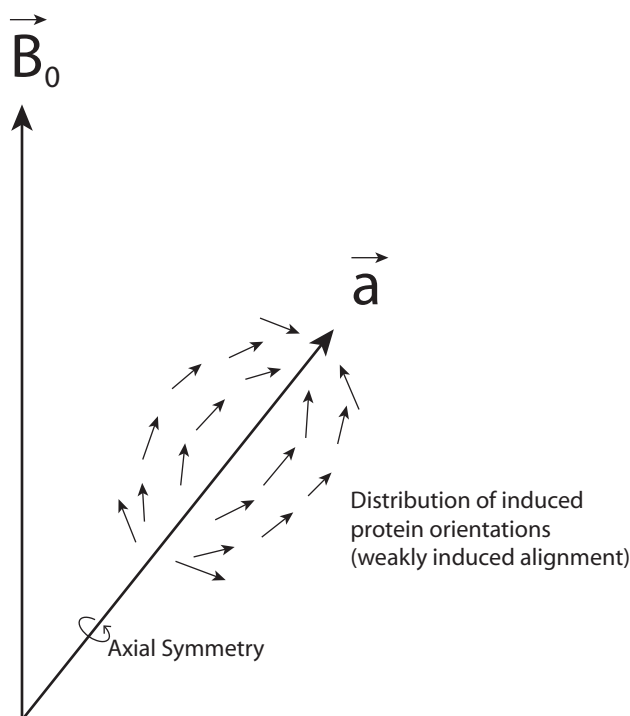


Figure 4: **Cartoon Diagram of Protein Orientations.** This is a cartoon. \mathbf{B}_0 is the direction of the magnetic field. \mathbf{a} represents the axis of the uni-axial alignment medium. The uni-axial medium has axial (rotational) symmetry about \mathbf{a} . The cloud of tiny arrows around \mathbf{a} symbolically represents the weakly-induced alignment of proteins in solution, which is a function of \mathbf{a} . Each small vector represents a molecular frame in the ensemble of proteins in solution. Properly drawn, each molecular frame should be represented by three axes(x, y, z), however to avoid cluttering the diagram, we have drawn only one axis per frame.

Below, we will eventually put ourselves in the molecular frame, and look at the distribution of the direction of the magnetic field. We will conclude that the distribution of the magnetic field will be rotationally symmetric. Because the argument and its visualization is awkward to explain, we state the conclusion as succinctly as possible before continuing:

In the lab frame, all the molecular frames lie in a distribution which is rotationally symmetric about \mathbf{a} . (See Figure 4.) Therefore, in the molecular frame, the magnetic field \mathbf{B}_0 will lie in a distribution which is rotationally symmetric¹.

¹Before we do the NMR experiment, we don't know the axis of rotational symmetry in the molecular frame, for the

We now make a couple of observations, which will become important later. In the lab frame, the magnetic field \mathbf{B}_0 and the medium axis \mathbf{a} are fixed. In solution, we have an ensemble of protein orientations. The distribution of protein orientations has rotational symmetry about \mathbf{a} . If we go to the molecular frame, there is only one orientation for the protein. But the molecular frame sees a distribution of magnetic field directions, and a distribution of directions for the medium's axis. Our first observation is to characterize the distribution of the medium axis \mathbf{a} in the molecular frame. In the lab frame, the axis \mathbf{a} is fixed, and the distribution of molecular orientations is rotationally symmetric. Therefore, in the molecular frame, the protein orientation is fixed, and the distribution of the axis \mathbf{a} is rotationally symmetric. We will define the vector \mathbf{b} to be the axis of rotational symmetry, for the distribution of \mathbf{a} in the molecular frame. A key point is that *the direction of \mathbf{b} is fixed in the molecular frame*, and never changes. This is because the distribution of protein orientations is fully specified by the axis² \mathbf{a} .

As we shall show later, the distribution of the magnetic field, *in the molecular frame*, will also be rotationally symmetric about \mathbf{b} .

Suppose now, we put the uni-axial sample in a goniometer and in an external magnetic field. (See Figure 5.) The goniometer controls the three angles θ , ϕ , and ψ .

We'll consider three different coordinate frames: the lab frame, the sample's frame, and the molecular frame. In the lab frame, we can do things like rotate the sample using the goniometer. In the sample frame, the sample is fixed, and adjusting angles in the goniometer rotates the external world. You can think of the sample frame as a coordinate system tied to the test-tube which is *inside* the goniometer. Finally, we have the molecular frame of the protein, which one can think of as local coordinates sitting on the protein itself, or one can think of it as the PDB coordinate frame.

The main observation is that the sample has rotational symmetry around \mathbf{a} because it is the axis of the uni-axial medium. In the lab frame, the external magnetic field is fixed, and we could physically rotate the sample around \mathbf{a} , and nothing would happen; our RDC values would remain unchanged, and the distribution of protein orientations would also remain unchanged.

Moving to the sample's frame, the sample would remain fixed, but the magnetic field would rotate around \mathbf{a} . Since rotation by $R_{\mathbf{a}}(\psi)$ is a symmetry of the sample, the sample cannot tell it has been rotated.

Start in the lab frame, and consider a specific orientation of the protein in solution. This specific orientation will see a single direction for the medium's axis \mathbf{a} . In the molecular frame, we will use the vector \mathbf{c} to represent the direction of \mathbf{a} for this specific protein orientation. In the entire ensemble of proteins, a subset of them will see the medium axis \mathbf{a} as being in the direction \mathbf{c} in the molecular frame. Let's have a closer look at this subset of the ensemble. The magnetic field is an angle θ away from \mathbf{a} . Therefore, in this subset, we will see a cone of possibilities for the location of \mathbf{B} , where the cone's axis is parallel to \mathbf{c} , and the cone has an angular radius of θ . Because our sample is symmetric about rotations around \mathbf{a} , it must be that the distribution of \mathbf{B}

distribution of the magnetic field. (If the medium is stretched gel, then we know the axis of symmetry in the lab and sample frames, but that has no obvious relationship to the molecular frame.) This is because the axis of symmetry depends on the details of how the medium induces weak alignment of the proteins. A computer simulation, such as [3] could, in theory, compute the axis of symmetry for the magnetic field. In a real experiment, we can compute the Saupe matrix S , and then use Theorem 2.2 to conclude the axis of symmetry (for the distribution of the magnetic field) is along the principal eigenvector of S in the molecular frame.

²Various other biochemical effects determine the distribution of protein orientations, such as pH, charge, concentration, etc. However, we assume that all of these other effects are held constant. If they are constant, then the distribution of protein orientations is determined by the axis \mathbf{a} .

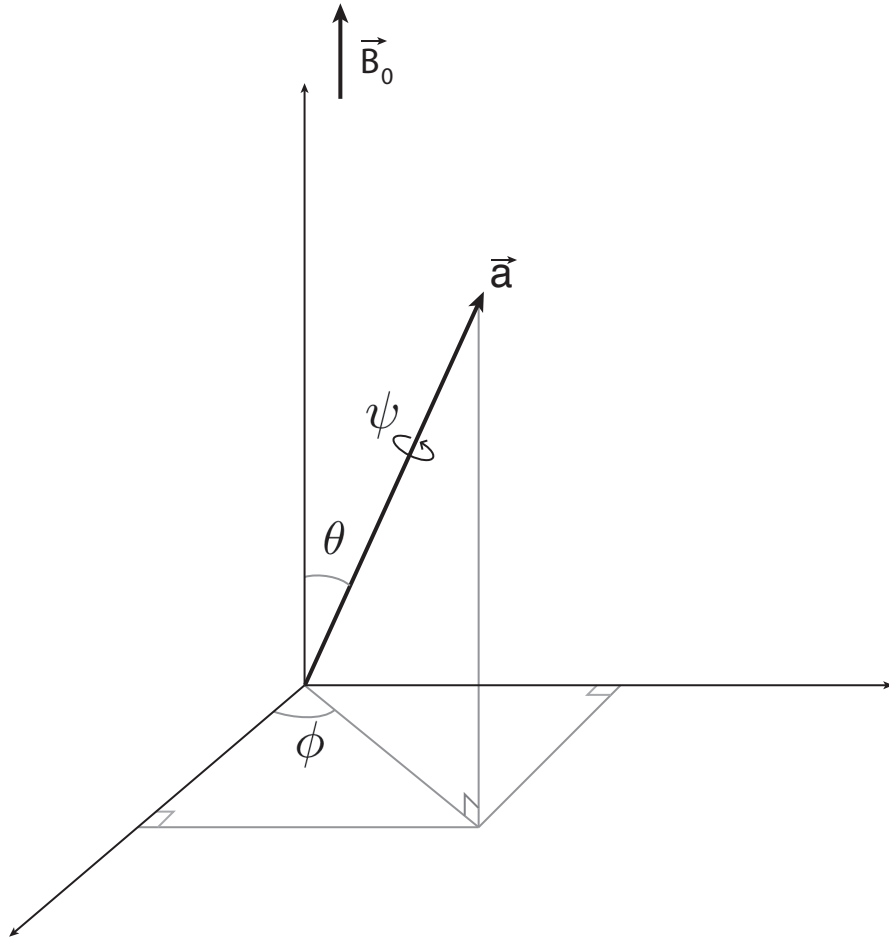


Figure 5: **Uni-axial Medium with Goniometer.** The magnetic field is vertical along the z -axis. The axis of the uni-axial aligning medium is \mathbf{a} . The goniometer controls the angles θ , ϕ , and ψ . The angle ψ is around the vector \mathbf{a} which is the axis of rotational symmetry for the aligning medium.

must be rotationally symmetric around \mathbf{c} in the molecular frame (for this subset of the ensemble).

Now instead of looking at a single direction for \mathbf{a} , we return to the entire ensemble. In the molecular frame, the entire ensemble sees a distribution for the direction of \mathbf{a} . For each specific direction of \mathbf{a} , we have a cone of possibilities for \mathbf{B} where the cone's axis is along \mathbf{a} . Therefore, to find the overall distribution of \mathbf{B} in the molecular frame, we can just convolve the cone with the distribution for \mathbf{a} in the molecular frame.

The cone is a highly symmetrical object, so if the distribution of \mathbf{a} has a symmetry, then the convolution of a cone with the distribution of \mathbf{a} will produce a new distribution with the same symmetry. In the molecular frame, the distribution of \mathbf{a} is rotationally symmetric about \mathbf{b} . Therefore, when we convolve to get the distribution of \mathbf{B} in the molecular frame, we discover that the distribution for \mathbf{B} is also rotationally symmetric around \mathbf{b} . In the molecular frame, let $\{\mathbf{B}_i\}$ represent the set (distribution) of magnetic field directions.

$$R_{\mathbf{b}}(\psi)\{\mathbf{B}_i\} = \{\mathbf{B}_i\} \tag{34}$$

Therefore, in the molecular frame, the distribution of the magnetic field has rotational symmetry. Consequently, the Saupe matrix must have the same rotational symmetric because it is constructed from the distribution of the magnetic field.

$$S = RSR^{-1} \text{ where } R = R_{\mathbf{b}}(\psi) \tag{35}$$

We can now apply Theorem 2.2 and immediately conclude the following. (Let's assume that the principal eigenvector is uniquely determined; that is S_{zz} is strictly greater than the other two eigenvalues.)

1. The rhombicity of S is zero ($S_{xx} = S_{yy}$).
2. The principal eigenvector (S_{zz}) is parallel to \mathbf{b} .

In addition, we argued above that the direction of \mathbf{b} is a *constant* in the molecular frame, independent of how the goniometer has changed the angles θ , ϕ , and ψ . Therefore, *the principal eigenvector (S_{zz}) of S is fixed in the molecular frame.* This means that the alignments we obtain essentially the same, and not independent. (The effective degree of alignment may vary, since the eigenvalues can change, but the eigenvectors are the same.)

Consequently, we can conclude that a goniometer with a uni-axial medium does not produce alignments which are independent.

We present a final result, showing that changing the angles inside the goniometer only scales the RDC values. This result was mentioned by Ad Bax and I believe also Prestegard, but not understood by myself until now. We just concluded that the principal eigenvector of S is fixed in the molecular frame. The direction of the other two eigenvectors is not fully determined, because they share the same eigenvalue. However, we can choose the remaining two eigenvectors to be perpendicular to each other, and for a moment let's pretend they are uniquely determined. The following argument holds in general, but it is easier to explain if we make these additional assumptions.

In the molecular frame, consider a bond vector \mathbf{v} . Since the eigenvectors of the Saupe matrix are fixed in the molecular frame³ we now go to POF of the Saupe matrix. The RDC value for \mathbf{v} will be

³The eigenvectors are fixed up to the choice of direction for the S_{xx} and S_{yy} eigenvectors in the plane perpendicular to the eigenvector for S_{zz} . All three eigenvectors are fixed, or can be chosen to be fixed in the molecular frame.

$$D = D_{\max} \mathbf{v}^T S \mathbf{v} \quad (36)$$

$$D = D_{\max} \begin{bmatrix} v_x & v_y & v_z \end{bmatrix} \begin{bmatrix} S_{xx} & & \\ & S_{yy} & \\ & & S_{zz} \end{bmatrix} \begin{bmatrix} v_x \\ v_y \\ v_z \end{bmatrix} \quad (37)$$

$$D = D_{\max} (S_{xx}v_x^2 + S_{yy}v_y^2 + S_{zz}v_z^2) \quad (38)$$

But we know $S_{xx} = S_{yy}$ because of zero rhombicity. Furthermore, S is traceless, so we also have $S_{xx} + S_{yy} + S_{zz} = 0$. So we get $S_{xx} = S_{yy} = -\frac{1}{2}S_{zz}$. Substituting into the equation above, we get

$$D = D_{\max} S_{zz} \left(-\frac{v_x^2}{2} - \frac{v_y^2}{2} + v_z^2 \right) \quad (39)$$

So we see that the RDC's have a factor of S_{zz} . So as the goniometer rotates the sample, the RDC values will just scale with S_{zz} . It is worth noting that if the direction of the Saupe eigenvectors were not fixed in the molecular frame, this result would not hold.

5 Questions

Before we conclude, we mention a couple of questions which we have not answered, or not fully understood. Our first question is: why would a medium with non-zero rhombicity, such as stretched polyacrylamide gel, only scale the RDC values when it is rotated in a goniometer? Our second question is: why are stretched polyacrylamide gel and phage not a uni-axial media in actual experiments?

6 Conclusion

We have considered consequences of additional symmetry of the Saupe matrix. In particular, we have explored the consequences of additional rotational symmetry of the Saupe matrix, and applied them two two different cases. In the first case, we considered the rhombicity of Saupe matrices for trimers, tetramers, and higher symmetries. We showed that the rhombicity must be zero. In the second case, we looked at the use of a goniometer with uni-axial media, and showed that this setup does not produce independent alignments. However, for bi-axial media, a goniometer will produce independent alignments. Perhaps in the future, some very clever material science will be invented which creates a biaxial mesh or medium for proteins. The resulting continuous series of independent alignments would likely be very useful for assignment and structure determination.

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