Dipolar Couplings and Residual Dipolar Couplings

BCMB/CHEM 8190
Liquids v. Solids
One can collect similar spectra but some tricks are required

$^{13}$C solution, sat’d glucose, 8 min

$^{13}$C CP-MAS, 30 mg cellulose, 9 min
The Classical Dipole-Dipole Interaction:

\[
E = \left( \frac{\mu_0}{4\pi} \right) \left( \frac{\mu_1 \cdot \mu_2}{r^3} - 3(\mu_1 \cdot r)(\mu_2 \cdot r)/r^5 \right)
\]

\[
r = i \ r_x + j \ r_y + k \ r_z = i \ r \sin\theta\cos\phi + j \ r \sin\theta\sin\phi + k \ r \cos\theta
\]
Quantum Mechanical Dipolar Coupling

\[ \mu = (\gamma \hbar / 2\pi)(i \mathbf{I}_x + j \mathbf{I}_y + k \mathbf{I}_z) = (\gamma \hbar / 2\pi)f(I_z, I_{+,-}) \]

\[ H_D = (\mu_0/4\pi)((\mu_1 \cdot \mu_2)/r^3 - 3(\mu_1 \cdot r)(\mu_2 \cdot r)/r^5) \]

\[ H_D = (\mu_0 \gamma_1 \gamma_2 \hbar^2)/(16\pi^3 r^3)(A + B + C + D + E + F) \]

A, B, C .. Grouped by type of operator, 0, 1, 2 Quantum

A = \(- I_{z1} I_{z2}(3\cos^2 \theta - 1)\), B = \(1/4)(I_{+1} I_{-2} + I_{-1} I_{+2})(3\cos^2 \theta - 1)\)

\[ \text{.............} \]

E = \(-(3/4)(I_{+1} I_{+2})\sin^2 \theta \exp(-2i\phi)\), F = ............
To First Order Only $I_{z1}I_{z2}$ Term is Important

A doublet would result – much like scalar coupling but large: as much as -60,000 Hz for a $^{13}\text{C}^{1}\text{H}$ pair.

Splitting are angle dependent – ranging from -60,000 to +30,000. In a solid all possibilities superimpose: The result is a powder pattern.

Points at 90° on a sphere are most abundant.
Other Anisotropies in NMR

\[ H = H_{\text{CSA}} + H_D + H_Q \ldots \]

All share the following property:

Solution: \( < 3 \cos^2 \theta \ ' – 1 > = 0 \)

Solids: \( (3 \cos^2 \theta \ ' – 1) \neq 0 \)

CSA powder pattern
Magic Angle Spinning

- All interactions can be written in terms of $Y^2_0(\theta) = (3\cos^2(\theta) - 1)/2$
- $Y^2_0(\theta)$ can be transformed to another frame using Wigner Rotation elements: $Y^2_0(\theta) = \sum_{m=-2}^{2} D^2_{m0}(\theta'',\phi'') Y^2_m (\theta',\phi')$
- $D^2_{m0}(\theta'',\phi'') = (4\pi/5) Y^2_m (\theta'',\phi'')$
- With rapid averaging over $\phi''$, all terms except $Y^2_0(\theta'')$ go to zero
- Selecting $\theta'' = 54.7^\circ$, all interactions, regardless of $\theta'$ value, are zero
- $(3\cos^2(\theta) - 1) = (3\cos^2(\theta') - 1) <3\cos^2(54.7^\circ) - 1> = 0$

Dipolar couplings
CSA = 0
Quadrupolar couplings
Cellulose

(10 minute spectra)

What is this peak?
Spinning Sidebands are Frequently Seen

When rotation rate is not >> anisotropies
Resonance position is modulated by rotation
Sidebands at the spinning frequency are produced

There are tricks that remove these:
TOSS – Total Suppression of Spinning Sidebands
180° pulses during rotor cycle dephases sideband magnetization but preserves center band magnetization
Reviews

The Dipolar Interaction Between Two Spins

\[
D = \frac{C}{r^3} \left\langle \frac{3 \cos^2 \theta - 1}{2} \right\rangle I_{NZ} I_{HZ}
\]

Brackets denote averaging – goes to zero without partial orientation.
Inducing Order Using Liquid Crystalline Media

$B_0$
Measurement of Dipolar Couplings – Coupled HSQC

Isotropic

Aligned

\[ J + D \]

\[ J \]
Polyacrylamide Gels
another alignment medium

Yizhou Liu, J. Prestegard (2010)
*J. Biomol NMR*, **47**: 249-258.
\begin{align*}
\langle \frac{3\cos^2\theta - 1}{2} \rangle &= \begin{bmatrix}
\cos \phi_i \cos \phi_j & \cdots & \\
\cdots & \cdots & \\
\cdots & \cdots & \\
\end{bmatrix} \begin{bmatrix}
3\cos \rho_k \cos \rho_l - d_{kl} \\
\cdots \\
\cdots \\
\end{bmatrix}.
\end{align*}
Finding a Principal Order Frame

\[
\begin{bmatrix}
S_{xx} & S_{xy} & \cdots \\
S_{yx} & S_{yy} & \cdots \\
\cdots & \cdots & \cdots
\end{bmatrix}
= \begin{bmatrix}
A & & \\
& S_{x'x'} & \\
& & S_{y'y'}
\end{bmatrix}
\begin{bmatrix}
& & \\
& & \cdots
\end{bmatrix}
A^{-1}
\]
Strategy for Protein Fold Determination

- Express $^{15}$N labeled protein
- Identify secondary structure elements
- Assign backbone resonances
- Orient protein in LC medium
- Collect residual dipolar data
- Orient individual elements
- Assemble protein fold
Dipolar Interaction Vectors

In an Idealized α-Helix

$^{15}$N Labeling Only
## Data Used in ACP Fold Determination

### A. Dipolar couplings in $\alpha$-helices

<table>
<thead>
<tr>
<th>Couplings (Hz)</th>
<th>Helix 1</th>
<th>Helix 2</th>
<th>Helix 3</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Amide Couplings</strong> (N$_i$-H$_N^i$)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I3</td>
<td>1.4</td>
<td>L37</td>
<td>Q66</td>
</tr>
<tr>
<td>E4</td>
<td>0.4</td>
<td>D38</td>
<td>A67</td>
</tr>
<tr>
<td>E5</td>
<td>3.4</td>
<td>T39</td>
<td>I69</td>
</tr>
<tr>
<td>V7</td>
<td>-1.0</td>
<td>V40</td>
<td>D70</td>
</tr>
<tr>
<td>K8</td>
<td>0.8</td>
<td></td>
<td>N73</td>
</tr>
<tr>
<td>I10</td>
<td>2.0</td>
<td></td>
<td>G74</td>
</tr>
<tr>
<td>I11</td>
<td>-0.3</td>
<td></td>
<td>H75</td>
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<tr>
<td>G12</td>
<td>2.1</td>
<td></td>
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</tr>
<tr>
<td>E13</td>
<td>2.1</td>
<td></td>
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<tr>
<td>Q14</td>
<td>0.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L15</td>
<td>-1.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Amide-Alpha Couplings</strong> (H$<em>N^i$ - H$</em>{\alpha i}$)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>R6</td>
<td>3.0</td>
<td>L37</td>
<td>Q66</td>
</tr>
<tr>
<td>V7</td>
<td>-3.5</td>
<td>L42</td>
<td>A68</td>
</tr>
<tr>
<td>K9</td>
<td>4.5</td>
<td>V43</td>
<td>H75</td>
</tr>
<tr>
<td>L15</td>
<td>0.0</td>
<td>M44</td>
<td></td>
</tr>
<tr>
<td>L46</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>(H$<em>N^i$ - H$</em>{\alpha i\pm 1}$)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>V43$<em>\alpha$L42$</em>\alpha$</td>
<td>2.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M44$<em>\alpha$A45$</em>\alpha$</td>
<td>-2.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Amide/Amide Couplings</strong> (H$_N^i$ - H$_N^{i\pm 1}$)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D38</td>
<td>2.0</td>
<td>N73</td>
<td>2.0</td>
</tr>
<tr>
<td>T39</td>
<td>2.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>V40</td>
<td>2.0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Orientation Maps for Three ACP Helices

Helix 1

Helix 2

Helix 3

Red = $S_{zz}$; Black = $S_{yy}$; Blue = $S_{xx}$
ACP Dipolar Fold vs. NOE Structure

Some Experiments for RDC Data Acquisition


Measurable Dipolar Couplings in a Dipeptide
Define an Order Frame

Coupled HSQC
Soft HNCA-E.Cosy
HNCO
Soft HNCA – E.COSY

Weisemann, Ruterhans, Schwalbe, Schleucher Bermel, Griesinger, J. Biomol. NMR, 4, 231-240, 1994
Soft HNCA E-COSY Spectra of $^{15}$N-Labeled $^{13}$C Natural Abundance Rubredoxin

$\mathrm{C^\alpha}$ chemical shift, $\mathrm{C^\alpha_i}$ to $\mathrm{C^\alpha_{i-1}}$ connectivity, $^{3}J$-$\mathrm{H^N\mathrm{H^\alpha}}$ coupling, $\mathrm{C^\alpha$-$\mathrm{H^\alpha}}$, $\mathrm{H^\mathrm{N\mathrm{H^\alpha}}}$ and $\mathrm{H^\alpha_{i-1}$-$\mathrm{H^N}$ dipolar coupling
Multiple Peptide Segments Oriented to Superimpose Order Frames Yield Structures

More Recent $^{15}$N-$^1$H Depositions use a J-modulation Experiment:
Also can be used for $^{15}$N-$^{13}$C', $^{15}$N-$^{13}$C\textalpha
Data shown are on a 70kDa protein

- Cross-peaks overlap HSQC peaks exactly
- Time requirements are similar to TROSY/HSQC
- Based on TROSY detection for application to larger proteins
- Fit gives $T_2$ estimate – used to eliminate data on loops
Analysis of Residual Dipolar Couplings

- Dosset, Hus, Marion & Blackledge (2001), JBNMR, 20: 223-231
Example of Validation and Refinement (MTH1743)

\[ Q = \left( \frac{\sum (D_{\text{obs}} - D_{\text{calc}})^2}{\sum D_{\text{obs}}^2} \right)^{1/2} \]
X-ray Structures fit RDCs Better than NOE-Based NMR Structures

<table>
<thead>
<tr>
<th>neSG</th>
<th>bmr</th>
<th>pdb nmr</th>
<th>pdb xray</th>
<th>alignment media</th>
<th>#residues</th>
<th>nmr Q</th>
<th>xray Q</th>
<th>RMSD*</th>
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<tbody>
<tr>
<td>BeR31</td>
<td>15702</td>
<td>2k2e</td>
<td>3cpk</td>
<td>phage</td>
<td>150</td>
<td>0.52</td>
<td>0.28</td>
<td>1.39</td>
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<tr>
<td>CsR4</td>
<td>15317</td>
<td>2jr2</td>
<td>2ota</td>
<td>peg (and peg+ctab)</td>
<td>68</td>
<td>0.37</td>
<td>0.32</td>
<td>0.52</td>
</tr>
<tr>
<td>Ctr107</td>
<td>16097</td>
<td>2kcu</td>
<td>3e0h</td>
<td>phage (and peg)</td>
<td>158</td>
<td>0.44</td>
<td>0.30</td>
<td>1.84</td>
</tr>
<tr>
<td>Gmr137</td>
<td>15844</td>
<td>2k5p</td>
<td>3cwi</td>
<td>peg</td>
<td>70</td>
<td>0.38</td>
<td>0.21</td>
<td>1.37</td>
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<tr>
<td>Hr3646E**</td>
<td>16250</td>
<td>2khn</td>
<td>3fia</td>
<td>polyacrylamide gel</td>
<td>110</td>
<td>0.53</td>
<td>0.29</td>
<td>1.06</td>
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<td>3gw2</td>
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<td>Pfr193A</td>
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<td>2kl6</td>
<td>3idu</td>
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<td>Sgr42</td>
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<td>2jz2</td>
<td>3c4s</td>
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<td>0.42</td>
<td>0.23</td>
<td>0.58</td>
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<tr>
<td>Sor77</td>
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<td>2juw</td>
<td>2qtI</td>
<td>polyacrylamide gel</td>
<td>72</td>
<td>0.26</td>
<td>0.21</td>
<td>0.91</td>
</tr>
</tbody>
</table>

* PSVS analysis listed structured regions (obtained via PROCHECK)
1st NMR model compared to X-Ray structure for all analysis

** It was difficult to compare the xray and nmr structures for this protein.

\[
Q = \left[ \frac{\sum (D_{obs} - D_{calc})^2}{\left( \sum D_{obs}^2 \right)^{1/2}} \right]^{1/2}
\]
Structure Refinement Using RDCs

Write RDCs in principal alignment frame:
\[ D = \left( \frac{D_a}{r^3} \right) \left\{ (3\cos^2\theta - 1)/r^3 + \frac{3}{2}R\sin^2\theta\cos(2\phi) \right\} \]

Write error function in terms of \( D_{\text{meas}} \) and \( D_{\text{calc}} \):
\[ E_{\text{RDC}} = (D_{\text{meas}} - D_{\text{calc}})^2 \]

Seek minimum in \( E_{\text{RDC}} \) to refine structure –
Need to float alignment axes during search
Refinement with RDCs can Improve Quality CtR107 with and without RDCs

Cyan, X-ray
Red, best refined with RDC
Gray, best refined without RDC

<table>
<thead>
<tr>
<th>Refinement detail</th>
<th>Average RMSD to X-ray (best of 10)</th>
<th>RMSD of the ensemble</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anneal, no RDC</td>
<td>4.4</td>
<td>4.2</td>
</tr>
<tr>
<td>Anneal, with RDC</td>
<td>3.4</td>
<td>2.7</td>
</tr>
<tr>
<td>Refine, no RDC</td>
<td>2.5(2.0)</td>
<td>1.4</td>
</tr>
<tr>
<td>Refine, with RDC</td>
<td>2.0(1.6)</td>
<td>1.4</td>
</tr>
</tbody>
</table>

Alignment carried out by superimposing backbone atoms of residues 19 to 26, 30 to 39, 59 to 61, 69 to 71, 88 to 90, 97 to 102, 111 to 122, 132 to 134 and 149 to 152