Residual Dipolar Couplings

BCMB/CHEM 8190
Recent Reviews

• Tolman, Curr. Opin. Struct. Biol. 11, 532-539 (2001)
• Fushman et al., Prog. NMR Spect. 44, 189-214 (2004)
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
Measurement of Dipolar Couplings – Coupled HSQC

Isotropic

Aligned

$J$

$J + D$
Polyacrylamide Gels
another alignment medium

Yizhou Liu, J. Prestegard
J. Biomol NMR, submitted
Order Matrix Analysis

\[
\begin{bmatrix}
\cos \phi_i \cos \phi_j & \cdots \\
\cdots & \cdots & \cdots \\
\cdots & \cdots & \cdots \\
\cdots & \cdots & \cdots \\
\end{bmatrix}
\times
\begin{bmatrix}
3 \cos \rho_k \cos \rho_l - \delta_{kl} \\
\cdots & \cdots \\
\cdots & \cdots \\
\cdots & \cdots \\
\end{bmatrix}
\]

\[
\left\langle \frac{3 \cos^2 \theta - 1}{2} \right\rangle
\]

\[
\rho_x \quad \rho_z
\]

\[
\phi_z \quad \theta
\]

\[
x \quad y \quad z
\]
Finding a Principal Order Frame

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

• Express $^{15}\text{N}$ labeled protein
• Identify secondary 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 $\alpha$-Helix
$^{15}\text{N}$ Labeling Only
## Data Used in ACP Fold Determination

### A. Dipolar couplings in α-helices

<table>
<thead>
<tr>
<th>Helix Couplings</th>
<th>Helix 1</th>
<th>Helix 2</th>
<th>Helix 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>I3 (N_i-H_i)</td>
<td>I3 1.4</td>
<td>L37 -2.6</td>
<td>Q66 8.2</td>
</tr>
<tr>
<td>E4</td>
<td>E4 0.4</td>
<td>D38 1.6</td>
<td>A67 7.7</td>
</tr>
<tr>
<td>E5</td>
<td>E5 3.4</td>
<td>T39 -0.3</td>
<td>I69 6.8</td>
</tr>
<tr>
<td>V7</td>
<td>V7 -1.0</td>
<td>V40 -2.6</td>
<td>D70 6.1</td>
</tr>
<tr>
<td>K8</td>
<td>K8 0.8</td>
<td></td>
<td>N73 7.4</td>
</tr>
<tr>
<td>I10</td>
<td>I10 2.0</td>
<td></td>
<td>G74 5.5</td>
</tr>
<tr>
<td>I11</td>
<td>I11 -0.3</td>
<td></td>
<td>H75 7.7</td>
</tr>
<tr>
<td>G12</td>
<td>G12 2.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E13</td>
<td>E13 2.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Q14</td>
<td>Q14 0.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L15</td>
<td>L15 -1.9</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Amide-Alph Couplings</th>
<th>Helix 1</th>
<th>Helix 2</th>
<th>Helix 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>R6 (H_i - H^N_i)</td>
<td>R6 3.0</td>
<td>L37 0.4</td>
<td>Q66 0.0</td>
</tr>
<tr>
<td>V7</td>
<td>V7 -3.5</td>
<td>L42 0.0</td>
<td>A68 -8.5</td>
</tr>
<tr>
<td>K9</td>
<td>K9 4.5</td>
<td>V43 3.5</td>
<td>H75 -9.0</td>
</tr>
<tr>
<td>L15</td>
<td>L15 0.0</td>
<td>M44 2.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>L46 -2.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>V43N-L42α</td>
<td>2.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>M44N-A45α</td>
<td>-2.0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Amide-Amide Couplings</th>
<th>Helix 1</th>
<th>Helix 2</th>
<th>Helix 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>D38</td>
<td>D38 2.0</td>
<td>N73 2.0</td>
<td></td>
</tr>
<tr>
<td>T39</td>
<td>T39 2.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>V40</td>
<td>V40 2.0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### B. NOEs and distances used to position helices in POSE:

- I3 H^N - F50 H_α, V7 H^N - F50 H_δ, V7 H_α - F50 H_δ, V7 methyl - A68 H^N, I3 methyl - N73 H^N
- 6 Å, 8 Å, 8 Å, 8 Å, 8 Å
Orientation Maps for Three ACP Helices

Helix 1

Helix 2

Helix 3

Red = Szz; Black = Syy; Blue = Sxx
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

$C^\alpha$ chemical shift, $C^\alpha_i$ to $C^\alpha_{i-1}$ connectivity, $3J$-$HNH^\alpha$ coupling, $C^\alpha$-$H^\alpha$, $HNH^\alpha$ and $H^\alpha_{i-1}$-$H^N$ dipolar coupling
Automated Analysis Through NMRPipe:
HNCA E.COSY on isotropic Pfu-1016054

http://spin.niddk.nih.gov/bax/software/NMRPipe/
Multiple Peptide Segments Oriented to Superimpose Order Frames Yield Structures

Proof of principle: rubredoxin structure overlays X-ray structure to 1.6Å
Novel targets in process: PF1496972 (8.9kDa), PF1016054(8.6kDa)
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$_\alpha$
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

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

exp RDC vs calc RDC for refined structure after sa
Q-factor = 0.33

exp RDC vs calc RDC for 1jsb
Q-factor = 0.23
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>#residue</th>
<th>nmr Q</th>
<th>xray Q</th>
<th>RMSD*</th>
</tr>
</thead>
<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>
</tr>
<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>
</tr>
<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>
</tr>
<tr>
<td>MbR242E</td>
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<td>2kko</td>
<td>3gw2</td>
<td>peg</td>
<td>100</td>
<td>0.36</td>
<td>0.29</td>
<td>1.05</td>
</tr>
<tr>
<td>PfR193A</td>
<td>16385</td>
<td>2kl6</td>
<td>3idu</td>
<td>phage</td>
<td>114</td>
<td>0.36</td>
<td>0.30</td>
<td>0.86</td>
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<tr>
<td>SgR42</td>
<td>15604</td>
<td>2jz2</td>
<td>3c4s</td>
<td>peg</td>
<td>58</td>
<td>0.42</td>
<td>0.23</td>
<td>0.58</td>
</tr>
<tr>
<td>SoR77</td>
<td>15456</td>
<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.

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

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

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