Nuclear Magnetic Resonance

Lectures for
CCB 538
James Aramini, PhD.

CABM 014A
jma@cabm.rutgers.edu
Outline

1. Introduction / Spectroscopy Overview
   April 21

2. NMR Spectroscopy – Theory and Practice
   April 23

3. Protein NMR & Resonance Assignment
   April 28

4. NMR Structure Determination & Biological NMR Applications
Lecture #3 Overview

1. Protein NMR Structure Determination
   i. NMR data -> Restraints
   ii. Structure Calculation & Validation

2. Some Biological NMR Applications
   i. Residual Dipolar Couplings
   ii. NMR of large proteins
   iii. Relaxation and Dynamics

3. Special NMR Applications
Protein NMR Structure Determination

OVERVIEW

1. Target selection/design
2. Sample preparation
3. NMR data collection
4. Backbone assignment
5. Sidechain assignment
6. Assignment verification
7. Secondary structure determination
8. RDC constraints
9. NOE assignment
10. Torsion angle constraints

Structure Calculation/Refinement

SPECIFICATIONS:

- 2D, 3D, 4D NMR
- $^{13}C, ^{15}N$-protein
Protein NMR Structure Determination

THE LEVELS OF PROTEIN STRUCTURE

1°

COO⁻

Peptide bond

Amino acid

+H₃N⁻

β (beta) sheet

(a) Primary structure

2°

α (alpha) helix

Disulfide bond

Hydrogen bond

(b) Secondary structure

3°

(c) Tertiary structure

4°

(d) Quaternary structure
Protein NMR Structure Determination

Experimental NMR Constraints

Distance
- NOE
- H-bonds

Dihedral Angle
- $^{13}$C $\delta$
- $J$-couplings

Orientational
- RDCs

Other
- PRE/PCS

NMR “ensemble”

Structure Program
- CYANA
- XPLOR
- UNIO
- ARIA
- CNS
- CNS
- AMBER
- Rosetta
NMR Observables:

**NOE (d)**
- a through space correlation (<5 Å) distance restraint

**Coupling Constant (J)**
- through bond correlation dihedral angle restraint

**Chemical Shift (δ)**
- very sensitive to local changes in environment dihedral angle restraint

**Residual Dipolar Coupling Constant (D)**
- X-Y bond vector orientation in weak alignment medium orientational restraint
I. Distance Restraints: $^1$H-$^1$H NOEs

**Nuclear Overhauser Effect:** change in intensity of 1 NMR signal is another is irradiated.

“Through-space” dipole-dipole interaction

\[ \eta \eta_i = (I - I_o)/I_o \]

\[ \text{NOE depends on motion} \]

Figure 3.46 Biochemistry, Seventh Edition © 2012 W. H. Freeman and Company

Estrada et al. (2011)
I. Distance Restraints: $^1$H-$^1$H NOEs

Nuclear Overhauser Effect: 3D X-filtered $^1$H-$^1$H NOESY

i.e., Glycophorin A 40-aa domain dimer

2D

3D

http://www.bioc.rice.edu/~mev/spectra3.html
Protein NMR Structure: A Short Course

I. Distance Restraints: $^1$H-$^1$H NOEs

“through-space” $\text{noe} \propto \frac{1}{r_{ab}^6}$

Protein NMR Structure: A Short Course
I. Distance Restraints: $^1$H-$^1$H NOEs

**Nomenclature**

- NOE patterns
- 2° structure

- <5Å “weak”
- <4Å “medium”
- <2.5Å “strong”

**Distances & 2° structure**

<table>
<thead>
<tr>
<th>Distance</th>
<th>α-helix</th>
<th>3_10-helix</th>
<th>β</th>
<th>β_p</th>
<th>turn Iα</th>
<th>turn IIα</th>
</tr>
</thead>
<tbody>
<tr>
<td>$d_{\alpha N}$</td>
<td>3.5</td>
<td>3.4</td>
<td>2.2</td>
<td>2.2</td>
<td>3.4</td>
<td>2.2</td>
</tr>
<tr>
<td>$d_{\alpha N}(i,i+2)$</td>
<td>4.4</td>
<td>3.8</td>
<td></td>
<td></td>
<td>3.6</td>
<td>3.3</td>
</tr>
<tr>
<td>$d_{\alpha N}(i,i+3)$</td>
<td>3.4</td>
<td>3.3</td>
<td></td>
<td></td>
<td>3.1-4.2</td>
<td>3.8-4.7</td>
</tr>
<tr>
<td>$d_{\alpha N}(i,i+4)$</td>
<td>4.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$d_{\beta N}$</td>
<td>2.8</td>
<td>2.6</td>
<td>4.3</td>
<td>4.2</td>
<td>2.6</td>
<td>4.5</td>
</tr>
<tr>
<td>$d_{NN}(i,i+2)$</td>
<td>4.2</td>
<td>4.1</td>
<td></td>
<td></td>
<td>3.8</td>
<td>4.3</td>
</tr>
<tr>
<td>$d_{NN}(i,i+3)$</td>
<td>2.5-4.1</td>
<td>2.9-4.4</td>
<td>3.2-4.5</td>
<td>3.7-4.7</td>
<td>2.9-4.4</td>
<td>3.6-4.6</td>
</tr>
<tr>
<td>$d_{NN}(i,i+4)$</td>
<td>2.5-4.1</td>
<td>2.9-4.4</td>
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<td>2.9-4.4</td>
<td>3.6-4.6</td>
</tr>
</tbody>
</table>

K. Wüthrich (1986)
## I. Distance Restraints: $^1H-^1H$ NOEs

### $^1H-^1H$ Distances & 2D structure

#### i. $\alpha$-helix

<table>
<thead>
<tr>
<th>Distance</th>
<th>$\alpha$-helix 3$_{10}$-helix</th>
</tr>
</thead>
<tbody>
<tr>
<td>$d_{\alpha N}$</td>
<td>3.5</td>
</tr>
<tr>
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</tr>
<tr>
<td>$d_{\alpha N}(i,i+4)$</td>
<td>4.2</td>
</tr>
<tr>
<td>$d_{NN}$</td>
<td>2.8</td>
</tr>
<tr>
<td>$d_{NN}(i,i+2)$</td>
<td>4.2</td>
</tr>
<tr>
<td>$d_{NN}(i,i+3)$</td>
<td>2.5-4.1</td>
</tr>
<tr>
<td>$d_{NN}(i,i+4)$</td>
<td>2.5-4.4</td>
</tr>
<tr>
<td>$d_{\alpha\beta}(i,i+3)$</td>
<td>2.5-4.4</td>
</tr>
</tbody>
</table>

### Diagnostics:

- 3D $^{15}$N-NOESY
- big sequential $H^N-H^N$ NOEs
- $H^\alpha-H^N$ (i,i+2), (i,i+3), (i,i+4)
- 3D HNHA small $^3J_{HNH\alpha}$
I. Distance Restraints: $^1$H-$^1$H NOEs

$^1$H-$^1$H Distances & $2^\circ$ structure

ii. $\beta$-strands

<table>
<thead>
<tr>
<th>Distance</th>
<th>$\beta$</th>
<th>$\beta_P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$d_{\alpha N}$</td>
<td>2.2</td>
<td>2.2</td>
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<tr>
<td>$d_{\alpha N}(i,i+2)$</td>
<td></td>
<td></td>
</tr>
<tr>
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<td></td>
<td></td>
</tr>
<tr>
<td>$d_{\alpha N}(i,i+4)$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$d_{NN}$</td>
<td>4.3</td>
<td>4.2</td>
</tr>
<tr>
<td>$d_{NN}(i,i+2)$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$d_{\beta N}$</td>
<td>3.2-4.5</td>
<td>3.7-4.7</td>
</tr>
<tr>
<td>$d_{\alpha \beta}(i,i+3)$</td>
<td>9 9 9 9 9 9 1 2 3 4 5 6</td>
<td>$3J_{HN\alpha}$(Hz)</td>
</tr>
</tbody>
</table>

Diagnostics: 3D $^{15}$N-NOESY
- big sequential $H^\alpha-H^N$ NOEs

3D $^{13}$C-NOESY
- big cross-strand $H^\alpha-H^\alpha$ NOEs

3D HNHA
- large $3J_{HNH^\alpha}$
Protein NMR Structure: A Short Course

I. Distance Restraints: $^1$H-$^1$H NOEs

$^1$H-$^1$H Distances & 2D structure

#### iii. $\beta$-turns

<table>
<thead>
<tr>
<th>Distance</th>
<th>turn I $^a$</th>
<th>turn II $^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$d_{\alpha N}$</td>
<td>3.4</td>
<td>2.2</td>
</tr>
<tr>
<td>$d_{\alpha N}(i,i+2)$</td>
<td>3.6</td>
<td>3.3</td>
</tr>
<tr>
<td>$d_{\alpha N}(i,i+3)$</td>
<td>3.1-4.2</td>
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</table>

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<th>Distance</th>
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<th>turn II $^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$d_{\beta N}$</td>
<td>2.9-4.4</td>
<td>3.6-4.6</td>
</tr>
<tr>
<td>$d_{\beta N}(i,i+3)$</td>
<td>3.6-4.6</td>
<td>3.6-4.6</td>
</tr>
</tbody>
</table>

Diagnostics: 3D $^{15}$N-NOESY
- specific sequential
- $H^N-H^N$ and $H^\alpha-H^N$ NOEs

3D HNHA
- specific $^3J_{HNN\alpha}$ pattern
II. Dihedral Angle Restraints:

Protein chemical shifts depend on fold

Unfolded

“random coil”

δ’s

Folded

Local Environment

Secondary Structure
II. Dihedral Angle Restraints:

$^3 J_{HNH\alpha}$ & backbone dihedral angles

3D HNHA

$J(\phi - 60) = 6.51\cos^2 (\phi - 60) - 1.76\cos (\phi - 60) + 1.60$

II. Dihedral Angle Restraints:

Chemical shifts & backbone dihedral angles

$\text{C}_\alpha$ and $\text{C}_\beta$

$\alpha$-helix:
$\Delta C_\alpha \sim +3 \text{ ppm}$
$\Delta C_\beta \sim -1 \text{ ppm}$

$\beta$-strand:
$\Delta C_\alpha \sim -2 \text{ ppm}$
$\Delta C_\beta \sim +3 \text{ ppm}$

"CSI"
+1, 0, -1 vs. random coil $\delta \pm \text{range}$


Wishart & Sykes (1994) *J. Biomol. NMR* 4, 171
II. Dihedral Angle Restraints:

**TALOS+:** $\Phi,\psi$ restraints from $\delta$’s

$\Phi,\psi$ space using $\delta$’s, residue type, neighbors

**INPUT:** $^{13}\text{C}_\alpha,^{13}\text{C}_\beta,^{13}\text{C}',^1\text{H}_\alpha,^1\text{H}_N,^{15}\text{N}$

**OUTPUT:** $\Phi,\psi$; +/-; $S^2$

*Ex. Ubq*

Protein NMR Structure: A Short Course

Structure Determination:

Manual

Automated: CYANA, AutoStructure, ARIA, UNIO,....

Input Files: assignments (Ω’s), NOE spectral peak lists
TALOS+ Φ/ψ, stereospecific assignments
(Other: H-bonds, J’s, RDCs, etc.)

Protein NMR Structure: A Short Course

Iterative NMR Structure Refinement:

Iterative Cycle

Distance Constraints Assignments
Stereospecific Assignments
Torsion–Angle Assignments

3D Structure Determination

Convergence

Constraints ➔ Convergence

Secondary Structure Backbone rms (Å)

Number of Distance Constraints
Protein NMR Structure: A Short Course

Structure Quality & Validation: Many Programs & Metrics

| Complete resonance assignments (%) | 99.4 |
| Side chain (%)                       | 98.3 |
| Aromatic (%)                         | 96.6 |
| Stereospecific methyl (%)            | 100  |
| Conformationally-restricting constraints | 100  |
| Total distance violations             | 2,478 |
| Intra-residue (i = j)                | 688  |
| Sequential (i-j = 1)                 | 619  |
| Medium range (1 < |i-j| < 5)        | 462  |
| Long range (i-j ≥ 5)                 | 709  |
| Dihedral angle violations             | 162  |
| Hydrogen bond violations              | 0    |
| No. of constraints per residue        | 25.6 |
| No. of long range constraints per residue | 6.8 |

Residual constraint violations:
- Average no. of distance violations per structure
  - 0.1-0.2 Å: 8.75
  - 0.2-0.5 Å: 1.85
  - >0.5 Å: 0
- Average no. of dihedral angle violations per structure
  - 1-10°: 8.75
  - >10°: 0

Model quality:
- RMSD backbone atoms (Å): 0.6
- RMSD heavy atoms (Å): 0.9
- RMSD bond lengths (Å): 0.018
- RMSD bond angles (°): 1.1

MolProbity Ramachandran statistics:
- Most favored regions (%): 96.8
- Allowed regions (%): 3.1
- Disallowed regions (%): 0.1

Global quality scores (Raw/Z-score):
- Verify3D: 0.40, -0.96
- ProsaII: 0.66, 0.04
- Procheck (phi psi): -0.15, -0.28
- Procheck (all): -0.03, -0.18
- MolProbity clash score: 12.51, -0.62

RPF scores:
- Recall/Precision: 0.976, 0.934
- F-measure/DP-score: 0.955, 0.817

BMRRP access number: 17965
PDB ID: 2LJW
III. Residual Dipolar Couplings:

“Global” structural restraints

Weak alignment

RDC ($D$ in Hz)

\[ J_{\text{N-H}} \times \nu_D = 93 \text{Hz} \]

(not decoupled)

- isotropic tumbling: no preferred orientation to $B_0$

- anisotropic

Dipole-Dipole Interaction

late 1990’s
III. Residual Dipolar Couplings:

Many alignment methods

i. Bicelles

ii. Filamentous phage

iii. PAGE

$^2$H NMR splitting

Measure $\Delta J$
III. Residual Dipolar Couplings:

**RDC Theory**

\[ D_{PQ}^{(\theta_{PQ}, \phi_{PQ})} = -S_{LS} \frac{\gamma_P \gamma_Q \hbar \mu_0}{4\pi^2} \left( \frac{3 \cos^2 \theta_{PQ} - 1}{\langle r_{PQ}^3 \rangle} \right) + \frac{3}{2} R \sin^2 \theta_{PQ} \cos 2\phi_{PQ} \]

"alignment tensor"

- \( D_a \) axial
- \( R \) rhombic
III. Residual Dipolar Couplings: Applications

Global Structural Information
Helical Curvature  Angle between 2° Structural Elements

Structure Refinement
IgG-binding domain of protein A

Relative Orientation of Domains

NMR of Large Proteins / Complexes

Techniques for raising the MW Limit in Biomolecular NMR

TROSY – TRansverse Optimized Spectroscopy

Deuteration/Selective Protonation

$[^{2}\text{H},^{13}\text{C},^{15}\text{N},^{1}\text{H}-\text{Ile-}\delta_{1},\text{Leu-}\delta,\text{Val-}\gamma]$}

Pervushin et al (1997) PNAS 94, 12366

Tzeng & Kalodimos (2012) Nature 488, 236


Other Methods

- segmental labeling
- residue-specific labeling
- alternate expression

NMR of Large Proteins / Complexes

Techniques for raising the MW Limit in Biomolecular NMR

Other Current Selective Methyl Approaches

A. \(^{13}\text{CH}_3\text{CD}_2\text{C}\text{COO}^-\) \(\text{\(\alpha\)-ketobutyrate}\)

\[ ^{12}\text{C}, \text{D] glucose} \rightarrow ^{13}\text{CH}_3\text{CD}_2\text{C}\text{COO}^-\text{Ileucine} \]

B. \(^{13}\text{CH}_3\text{CD}_3\text{C}\text{COO}^-\) \(\text{\(\alpha\)-ketoisovalerate}\)

\[ ^{12}\text{C}, \text{D] glucose} \rightarrow ^{13}\text{CH}_3\text{CD}_2\text{C}\text{COO}^-\text{Leucine} \]

C. \(^{13}\text{CH}_3\text{CD}_3\text{C}\text{COO}^-\) \(\text{\(\alpha\)-ketoisovalerate}\)

\[ \text{ammonium chloride} \rightarrow ^{13}\text{CH}_3\text{CD}_2\text{C}\text{COO}^-\text{Valine} \]

D. \(^{13}\text{CH}_3\text{CD}_2\text{C}\text{COO}^-\) \(\text{Alanine}\)

\[ \text{ammonium chloride} \rightarrow ^{13}\text{CH}_3\text{CD}_2\text{C}\text{COO}^-\text{Alanine} \]

E. \(^{13}\text{CH}_3\text{CD}_2\text{C}\text{COO}^-\) \(\text{\(\alpha\)-oxomethionine}\)

\[ \text{ammonium chloride} \rightarrow ^{13}\text{CH}_3\text{CD}_2\text{C}\text{COO}^-\text{Methionine} \]

Tugarinov & Kay (2004)
*J Biomol NMR* 28, 165-172

Ayala *et al.* (2009)
*J Biomol NMR* 43, 111-119.

Gifford *et al.* (2011)
*J Biomol NMR* 50, 71-81

NMR Relaxation and Dynamics

Insights into a broad range of molecular motions

Figure 1. Solution NMR techniques cover the complete range of dynamic events in enzymes.
NMR Relaxation Analysis of Proteins

$T_1$, $T_2$, CPMG relaxation dispersion experiments

$T_1$  inversion recovery

$T_2$  CPMG

$I(T) = I(0) e^{-T/T_2}$

CPMG relaxation dispersion

$I(\tau) = I(0) (1 - 2e^{-\tau/T_1})$

Baldwin & Kay (2009)
NMR Relaxation Analysis of Proteins

ns motion
Backbone Dynamics

ns - μs - ms motion
Conformational Entropy & Molecular Recognition

Oligomerization State

^{13}CH_3 Relaxation

Tzeng & Kalodimos (2012) Nature 488, 236
Special NMR Applications

Histidine tautomeric states

Rossi et al. (2008) Proteins 74, 515
Special NMR Applications

\textbf{\textsuperscript{19}F protein NMR}

Influenza A NS1

Crystal structures ED dimers

Aramini, Hamilton et al. (2014) \textit{Structure} 22, 515

Special NMR Applications

**19F protein NMR**

**19F NMR of Influenza A NS1 Protein**

Aramini, Hamilton et al. (2014) *Structure* 22, 515
THANK YOU & GOOD LUCK!!!