Structure and Function Elucidation Through NMR Spectroscopy

Burke Group Literature Seminar
Hosted by Seiko Fujii
Sept 17, 2010
Elucidation of Structure and Intermolecular Interactions of a Cyclic-Peptide Inhibitor

Synthesis

NMR

Structure Determination

Synthesis

2D NMR solution structure and spatial orientation determined

Structure

Interactions with the proteasome binding pockets elucidated

Function

I. Basics of NMR

Dave, Brandon, Steve, Erin, Stephanie
Basic NMR Theory

Nucleus must have magnetic moment (spin quantum number other than 0)

Resonance:
Energy is absorbed when the frequency of $B_1$ is the same as the Larmor frequency of the nucleus

Common nuclei with spin 1/2

$^1$H  $^{13}$C  $^{15}$N  $^{19}$F  $^{29}$Si  $^{31}$P

Common Quadrupolar nuclei

$^{11}$B  $^{17}$O  $^{33}$S  $^{35}$Cl

Spin state energy causes popualtional difference
Pulsed NMR

Pulsed NMR: all protons excited and allowed to decay simultaneously
Fourier Transform (FT) allows resolution of frequency domain

Application of pulse ($B_1$) causes precession of magnetic moment around $B_1$ vector

Relaxation releases radiowaves corresponding to nuclear resonance frequencies
Chemical shifts

Moving charges (electrons) create an *induced magnetic field* opposed to the applied magnetic field.

Nuclei are magnetically shielded—magnetic field felt by nuclei is less than $B_0$. Based mostly on electron density around the nucleus, but other factors are also at play.

Nuclei surrounded by weaker electron clouds are referred to as *deshielded*. Therefore, protons near electron withdrawing groups typically have larger chemical shifts.

Shielding contributions due to the magnetic anisotropy of CC triple bonds, CC and CO double bonds and CC single bonds.

Ring current effect in arenes.

Image source: Friebolin, 4th ed
# Heteronuclear NMR

<table>
<thead>
<tr>
<th>Isotope</th>
<th>( v / \text{mHz for } H_0 = 1 \text{ T} )</th>
<th>% Abundance</th>
<th>Relative Sensitivity</th>
<th>Major Applications</th>
</tr>
</thead>
<tbody>
<tr>
<td>(^{13}\text{C})</td>
<td>10.7081</td>
<td>1.10</td>
<td>0.01591</td>
<td>Structure elucidation</td>
</tr>
<tr>
<td>(^{31}\text{P})</td>
<td>17.2510</td>
<td>100</td>
<td>0.06652</td>
<td>Biological and organometallic studies</td>
</tr>
<tr>
<td>(^{15}\text{N})</td>
<td>4.3172</td>
<td>0.366</td>
<td>0.00104</td>
<td>Protein NMR</td>
</tr>
<tr>
<td>(^{11}\text{B})</td>
<td>4.5751</td>
<td>80.1</td>
<td>0.16522</td>
<td>Structure elucidation</td>
</tr>
</tbody>
</table>

Chalk talk

Coupling constants and the Karplus relationship
II. Advanced Techniques for Structure Determination in Solution Phase NMR

Eric G., Kaitlyn, Stevie, Pulin, Graham, Betsy
Chalk talk

Basics of 2D NMR

It’s Pulin Time
Determining Structure from NMR Data

Alternative to X-ray Crystallography
Resolution comparable to 2-2.5 Å

General Process:

• Assign 1H, 13C, etc spectra
• Obtain geometric information from through-bond and through-space interactions
• Input restraints into modeling software
• Energy minimize from randomized starting conformations
• Overlay energy minimized structures for qualitative and quantitative analysis

Lends itself to complex proteins of greater than 100 residues (A 150 residue protein has 900-1500 protons)

Structure of B1 Domain of Protein G

Table 2. Atomic rms differences.

<table>
<thead>
<tr>
<th>Atomic rms differences (Å)</th>
<th>Backbone atoms</th>
<th>All atoms</th>
<th>All atoms excluding disordered side chains*</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;SA&gt; versus &gt;SA</td>
<td>0.27 ± 0.03</td>
<td>0.65 ± 0.05</td>
<td>0.39 ± 0.03</td>
</tr>
<tr>
<td>&lt;SA&gt; versus &lt;SA&gt;r</td>
<td>0.29 ± 0.03</td>
<td>0.74 ± 0.06</td>
<td>0.45 ± 0.041</td>
</tr>
<tr>
<td>&lt;SA&gt;r versus &gt;SA</td>
<td>0.12</td>
<td>0.36</td>
<td>0.22</td>
</tr>
</tbody>
</table>

*The disordered surface side chains that were excluded are: Met1 from the CB position onward, Lys4 from C8, Lys10 from Cγ, Lys13 from C8, Glu15, Glu19 from Cγ, Lys28 from Cγ, Glu32 from Cγ, Lys34 from C8, Glu42 from C8, Glu56 from C8.

![Diagram](image)

Fig. 1. Stereoview showing best-fit superposition of the backbone (N, Ca, and C) atoms of the 60 SA structures of the B1 domain.

Solution NMR structure of five representative glycosylated polyene macrolide antibiotics with a sterol-dependent antifungal activity

Laurent Volpon and Jean-Marc Lancelin

Pimaricin

Nystatin A1

Rimocidin

Candidin

Vacidin A

III. Function/Dynamics/Intermolecular Interaction Elucidation through Solution Phase NMR

Ian, Arjun, Eric W., Jenna, Matt, Greg
Function/Dynamics/Intermolecular Interaction Elucidation via Solution Phase NMR

VT $^1$H NMR signal of cyclohexane & VT $^{13}$C NMR of cyclic disilane and rate constants (s$^{-1}$)

(b) (b) Mazzanti et al., Tetrahedron, 1998, 13181-84.
Dynamic Exchange Process & Variable Temperature NMR

• **Classic case:** Cyclohexane rapidly equilibrates at room temperature

• To prevent exchange from occurring, all we have to do is cool the sample to a sufficiently low temperature. At -90 °C, the axial and equatorial protons of cyclohexane no longer interchange and are resolved as two separate resonances. But as we raise the temperature, the two peaks move together and broaden, indicating that there is some exchange, a regime we call **slow exchange.**

• **In case II:** Ring carbon of cyclohexane upon substitution with dimethyl silicon moiety results in more flexible and faster equilibrium due to lower energy barrier.

• That is due to longer C-Si bond

• Ring inversion barrier of tetramethyl disilacyclohexane has $\Delta G^\# = 5.4 \text{ kcal/mol}$ from cyclohexane $\Delta G^\# = 10.8 \text{ kcal/mol}$

• NMR technique proved to be pivotal in understanding the dynamic components of molecules and also in intermolecular/biophysical processes.
No antifungal activity compared to nystatin.
Halipeptin A
Gomez-Paloma et. al.
2001
Incorrect
Correct
Verified by total synthesis

Neomarinone
Fenical et. al. Moore et. al.
2002 2003
Incorrect Correct
Verified by total synthesis

Diazonamide A
14 years
12 research groups
3 assignments
at least 2 wrong total syntheses

Originally proposed structure
Fenical and Clardy, 1991

Revised structure,
Nicolaou, 2003

Solution Phase Peptide/Protein NMR

- Size constraint (<50kDa)
- Experiments: COSY, NOESY ($^{13}$C; $^{15}$N), TOCSY, HSQC ($^{1}$H-$^{15}$N) of $^{15}$N-enriched proteins
- Additional Methods:
  - Semi-automated interpretation
    - Calculation of torsional angle dynamics (DYANA), NOE assignment (CYANA, CANDID), torsional angles (TALOS+)
    - Homology models for “initial conditions”
    - Allows researcher to screen structures rapidly
  - Specific Labeling (SAIL)
  - 3D NMR (HSQC + addn’l nucleus: $^{1}$H-$^{15}$N-$^{13}$C correlation)
    - Can correlate connectivity across peptide bonds by spin-transfer

SAR by NMR

Fragment-based drug design

- fragmentation of drug leads into smaller pieces
- provides an alternative to computer modeling

- 2D isotope edited NMR is used to look for binding to the targeted protein

IV. Solid State NMR
Principles and Applications
Dan, Justin, Brice, Junqi, Anna Jean, Tom
Heteronuclear Dipolar Coupling

One of the factors leading to broad peak shape is the heteronuclear dipolar coupling.

Dipolar coupling is dependent upon the orientation of the spins relative to the magnetic field; thus, MAS spectra is one way to reduce linewidth.

Linewidth is proportional to $3\cos^2 \Theta - 1$, thus the “magic angle” of 54.74°.
Heteronuclear Dipolar Decoupling

The “pake doublet” arises from the parallel and antiparallel alignment of the two interacting spins.

Continuous wave decoupling can eliminate many of the effects of heteronuclear dipolar coupling.
Chalk talk

Chemical Shift Anisotropy
Homonuclear Dipolar Coupling

Because of the large gyromagnetic ratio, $^1\text{H}$ dipolar couplings routinely approach 100 kHz, giving broad uninterpretable peaks that MAS often cannot spin away.
CP takes advantage of the highly polarizable protons to transfer magnetization to more resolvable nuclei such as $^{13}$C, $^{15}$N and $^{31}$P.
Ensemble of the 10 lowest energy structures of GB1, calculated from a total of 7,826 $^{13}$C–$^{13}$C, $^{15}$N–$^{15}$N, and $^1$H–$^1$H distance restraints. The bbRMSD for all residues is $1.01 \pm 0.13$ Å, and the heavy atom RMSD is $1.52 \pm 0.12$ Å.

Ensemble of 10 GB1 structures determined from distance, TALOS (chemical shift), and VEAN restraints (bbRMSD $0.31 \pm 0.06$ Å, heavy atom RMSD $1.06 \pm 0.07$ Å).

SSNMR based structure determination of the membrane protein GB1

Fig. 5. Structural alignment of high-resolution SSNMR ensemble (cyan) and the trigonal form crystal structure (2QMT; red). (a) With all residues aligned, the bbRMSD is 1.4 Å. (b) Alignment excluding residues 1, 9–14, and 39–41 (1.1 Å bbRMSD), demonstrating that residues in the β1-β2 turn and helix-β3 loop disrupt the relative positioning of helix and four-stranded β-sheet.

Acknowledgements

• References and Organization
  Marty, Tom, Dr. Jacob Lopez

• Presentation
  Group I: Erin, Steve, Dave, Stephanie, Brandon
  Group II: Stevie, Pulin, Graham, Betsy, Kaitlyn, Eric
  Group III: Matt, Ian, Arjun, Jenna, Eric W., Greg
  Group IV: Justin, Tom, Dan, Brice, Anna Jean, Junqi

• Food
  Mac and Cheese entry 1: Justin
  Mac and Cheese entry 2: Dave
  Spiral Ham: Brice and Dave
  Chocolate Cake: Justin