Not just for chemists: high-resolution NMR spectroscopy

ASAS NMR Centre Workshop
Michael Schmitz
Outline

- History of NMR.
- (Very!!) Basic Theory of NMR
- Uses of small molecule NMR.
- Some uses of biomolecular NMR.
- Real life examples.
Nuclear Magnetic Resonance (NMR) Spectroscopy

Edward Purcell
1912 - 1997

Felix Bloch
1905 - 1983

**Nobel Prize in Physics 1952:** for the development of new methods for nuclear magnetic precision measurements and discoveries in connection therewith.

Bloch: “We have thought of various investigations in which this effect can be used fruitfully”
NMR Bare Basics

- Atomic nuclei of many elements have ‘spin’. e.g. $^1\text{H}$, $^{13}\text{C}$, $^{15}\text{N}$, $^{31}\text{P}$

- Nuclei with ‘spin’ are magnetic.

- These nuclei align in discrete orientations or ‘spin states’ in an applied magnetic field. e.g. $^1\text{H}$, spin = $\frac{1}{2}$

No applied field
NMR Bare Basics

e.g. $^1$H, spin = $\frac{1}{2}$

- Nuclei will transition between the two spin states when a radiofrequency pulse in “resonance” with the energy gap is applied.

$\Delta E = h\nu$
Simple NMR experiment

\[ \Delta E = h\nu \]
What determines the energy gap between spin states?

- Strength of the applied magnetic field.

- Nature of the atomic nuclei.
  e.g. NMR frequencies in a 9.4 T magnet

<table>
<thead>
<tr>
<th>Nuclei</th>
<th>Frequency (MHz)</th>
</tr>
</thead>
<tbody>
<tr>
<td>${}^1\text{H}$</td>
<td>400.0</td>
</tr>
<tr>
<td>${}^{13}\text{C}^*$</td>
<td>100.6</td>
</tr>
<tr>
<td>${}^{15}\text{N}^*$</td>
<td>40.5</td>
</tr>
</tbody>
</table>

*: < 100% natural abundance!

- Local chemical environment of the nuclei
  - electron cloud 'shields' nuclei from magnetic field

  $\delta$: chemical shift!
Chemical Shift

- Effect of chemical environment on NMR frequency of nuclei.
- Magnitudes of shifts in frequency are very small (ppm).

E.g. t-butanol in a 9.4 T magnet.

\[
\text{Si(CH}_3\text{)}_4
\]

400,000,000 Hz

1H

1440 Hz

3.6 ppm

480 Hz

1.2 ppm

ppm
Coupling

- NMR signal of a nuclei is effected by the spin states of adjacent magnetic nuclei.

  e.g. $^1$H NMR signals are split by protons on adjacent carbons
### Coupling in $^1$H NMR

- **Spin-spin coupling – (n +1) rule**

The diagram illustrates spin-spin coupling using arrows to represent the coupling of protons, with the chemical shift values in ppm shown on the x-axis.
NMR spectroscopy

- Chemical shifts report on chemical environment of the nuclei
- Coupling reveals which nuclei are neighbours.

⇒ Structures of chemical compounds in a sample can be deduced!!
What is good about NMR spectroscopy?

- Chemical shifts and coupling patterns are exquisitely sensitive to structure.
- Isotopic labeling is often not necessary e.g. $^1$H (natural abundance 99.98%)
- Samples can be directly and continuously analysed in solution.
- Peak intensities are concentration dependent.
- Reports on dynamic processes in solution.
- Non-destructive.
What is bad about NMR spectroscopy?

- Relatively insensitive i.e. > 0.2 mM concentrations required.
- Typically require ~ 600 µl sample.
- All sample components containing magnetic nuclei will produce signals.
  
  e.g. $^1$H NMR – water, buffer reagents, DTT, glycerol etc

- Data for each spectrum must be averaged over a number of scans.
What is special about protein NMR spectroscopy?

- Chemical shifts more important than coupling patterns (due to linewidth).
- Isotopic labeling is almost always necessary e.g. $^{15}\text{N}$, $^{13}\text{C}$
- Severe spectral overlap (see example 1D below): requires spectral editing.
- Multidimensional experiments exploiting known atom connectivity (H-N-C-H …).
- Many specialised, tailored experiments
- Needs days/weeks of instrument time
- Extremely high information content
- Needs expert support
What is special about protein NMR spectroscopy?

Spectral overlap: 2D $^1\text{H}-^1\text{H}$ NOESY spectrum

$^1\text{H}$ [ppm] $^1\text{H}$ [ppm]

$^1\text{H} \leq 5\text{Å}$

NOE intensity $\sim 1/r^6$
What is special about protein NMR spectroscopy?

Resolving overlap: 3D $^{15}$N-edited TOCSY

3 overlapped NH resonances

Same NH, different $^{15}$N

TOCSY HSQC

$^1H$ $^1H$ $^{15}N$

t_1 t_2 t_3

$^1H-^{15}N$ 2D spectrum

$^1H-^1H$ 2D spectrum
What could I use small molecule NMR spectroscopy for?

- Following enzyme catalysed reactions
  
  a) Substrate consumption, product formation and/or intermediates.

- Identifying small molecule ligands bound to a protein

b) Activity of enzyme mutants.
What could I use small molecule NMR spectroscopy for?

- Screening small molecule as protein ligands

  e.g.  
  a) Saturation Transfer Difference NMR  
  b) WaterLOGSY
Real life examples from the LSB

- GlmU – enzyme activity by 1H NMR (Zhening Zhang)
Real life examples from the LSB

- MbtA – ATPase activity by $^{31}$P NMR

ATP

$\gamma$-ATP

$\alpha$-ATP

$\beta$-ATP

$\alpha$-ADP

$\beta$-ADP

$\text{PO}_4^{3-}$

$\text{MbtA} + \text{PO}_4^{3-}$

$\text{MbtA} - \text{ATPase activity by }^{31}\text{P NMR}$

$\text{ATP}$

$\text{PO}_4^{3-}$

$\text{MbtA}$

$\text{ATP}$

$\text{ADP}$

$\text{PO}_4^{3-}$
Real life examples from the LSB

- PurH – identification of bound nucleotide (Jérôme Le Nours)
Real life examples from the LSB

- PurH – substrate binding by WaterLOGSY (Jérôme Le Nours)

No enzyme

+ 10 μM PurH
What could I use protein NMR spectroscopy for?

Is my protein folded?

• Real life $^1$H-$^{15}$N HSQC from the LSB (osmolyte induced folding)

(Richard Kingston, Esther Bulloch)
Real life examples from the Dingley lab

Binding characterization of Cp5 to model membranes

Binding is pH dependent

** Binding is pH dependent **

<table>
<thead>
<tr>
<th>pH dependence</th>
<th>DMPG</th>
<th>DMPC</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>1,2-dimyristoyl-sn-glycero-3-phosphoglycerate</td>
<td>1,2-dimyristoyl-sn-glycero-3-phosphocholine</td>
</tr>
<tr>
<td>5</td>
<td>Anionic headgroups</td>
<td>Neutral headgroups</td>
</tr>
</tbody>
</table>

Binding is headgroup dependent

** Binding is headgroup dependent **

<table>
<thead>
<tr>
<th>Lipid composition</th>
<th>NaCl</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH5</td>
<td>pH5</td>
</tr>
<tr>
<td>GREEN: just Cp5</td>
<td>GREEN: 200mM NaCl</td>
</tr>
<tr>
<td>BLACK: Cp5+DMPG</td>
<td>BROWN: 100mM NaCl</td>
</tr>
<tr>
<td>BROWN: Cp5+DMPC</td>
<td>BROWN: 100mM NaCl</td>
</tr>
</tbody>
</table>

Binding is salt dependent

** Binding is salt dependent **
Real life examples from the Dingley lab

Protonation of amino acid side chains: Cp5 2D HCCO Experiment

\[ \text{pH} \]

\[ \text{1H [ppm]} \]

\[ \text{13C [ppm]} \]

\[ \text{183.0} \]

\[ \text{178.1} \]

Medini K, Li W, In preparation
What is the role of the NMR Centre?

- Provide training and access to NMR instruments 300 MHz - 500 MHz
- Design, run and analyse solid state experiments (Dr. Zoran Zujovic)
- Support and run experiments on 600 MHz instrument (Dr. Michael Schmitz)
- Provide experiment design and initial set-up for novel uses
- Instrument maintenance, performance testing, troubleshooting and repair
- Vendor liaison for parts, service and repairs
- Administration of NMR Centre (billing, user enquiries)
What equipment is available at the NMR Centre?

- 300 MHz AV - liquids (\(^{1}\text{H}/^{13}\text{C}/^{31}\text{P}/^{19}\text{F}\)), solid state, no automation

- 400 MHz AVIII - liquids (\(^{1}\text{H}/^{19}\text{F-109}\text{Ag}\)), VT, automation capable

- 400 MHz AVIII - liquids (\(^{1}\text{H}/^{19}\text{F-109}\text{Ag}\)), full automation only

- 500 MHz AVIIIHD - liquids (\(^{1}\text{H}/^{19}\text{F-109}\text{Ag}\)), VT, automation capable, solid state

- 600 MHz AV - liquids (\(^{1}\text{H}/^{19}\text{F-109}\text{Ag BBI; }^{1}\text{H}/^{13}\text{C},^{15}\text{N TCI}\)), \(^{1}\text{H}\) optimized, VT

- 300-500 MHz : user-run (except solid state), NZ $25 / hour

- 600 MHz : run by NMR Centre staff (or BioNMR researchers), NZ $50 / hour
What equipment is available at the NMR Centre?

300 MHz: 190:1 $^1\text{H}$, 120:1 $^{13}\text{C}$

400 MHz: 310:1 $^1\text{H}$, 190:1 $^{13}\text{C}$

400 MHz: 370:1 $^1\text{H}$, 230:1 $^{13}\text{C}$

500 MHz: 870:1 $^1\text{H}$, 360:1 $^{13}\text{C}$

600 MHz (*): 6000:1 $^1\text{H}$, 860:1 $^{13}\text{C}$

($^1\text{H}$: 0.1% ethyl benzene, $^{13}\text{C}$: ASTM)
Acknowledgements

Thomas Lagautriere  Jérôme Le Nours  Zhening Zhang  Esther Bulloch
Andrew Dingley (FZJ)  Karima Medini (SCS)  Wei Li (FZJ/SCS)

References

