NMR Experiments for Assignment

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Introduction

Selecting experiments for assignment:

1. what type of information do you want?
   • chemical shifts
   • coupling constants
   • correlations

2. what type of spectrum do you want?
   • quick
   • sensitive
   • high-resolution
   • information-rich

3. choose/modify/invent an experiment
1. 1D Carbon NMR
   - 1D carbon spectrum
   - 1D carbon spectrum (proton decoupled)*
   - APT*
   - DEPT*

2. 2D proton NMR
   - COSY
   - DQF COSY*
   - long-range COSY
   - TOCSY

3. 2D carbon NMR
   - INADEQUATE

4. 2D proton-carbon NMR
   - HMQC
   - HMQC (proton-decoupled)*
   - multiplicity-edited HSQC*
   - HMBC*
   - HSQC
   - HETCOR

5. The rest
   - 1D analogues
   - macromolecules, solids, multinuclear
1D Carbon NMR

1D spectrum

• reveals $^{13}$C chemical shift interaction
  AND $J_{CH}$ interactions (1-bond and multiple-bond)
1D Carbon NMR

1D spectrum

• high information content but low resolution and sensitivity

• spectrum reproduced from *Modern NMR Spectroscopy*, by Sanders and Hunter
1D Carbon NMR
proton-decoupled 1D spectrum

- reveals $^{13}$C chemical shift interaction
- $J_{\text{CH}}$ interactions are removed (decoupled)
1D Carbon NMR

proton-decoupled 1D spectrum

• high resolution and sensitivity but lower information content

**spectrum reproduced from Modern NMR Spectroscopy, by Sanders and Hunter**
1D Carbon NMR

APT

• Use $^1J_{CH}$ interaction to change sign of peaks and provide multiplicity information.
• Decouple $^1J_{CH}$ interaction during acquisition for high resolution and sensitivity.

<table>
<thead>
<tr>
<th></th>
<th>C</th>
<th>CH</th>
<th>CH$_2$</th>
<th>CH$_3$</th>
</tr>
</thead>
<tbody>
<tr>
<td>APT</td>
<td>negative</td>
<td>positive</td>
<td>negative</td>
<td>positive</td>
</tr>
</tbody>
</table>
1D Carbon NMR

APT

• spectrum reproduced from 100 and more Basic NMR Experiments, by Braun, Kalinowski and Berger
1D Carbon NMR

**DEPT**

- alternative to APT
- better sensitivity than APT
- Quaternaries not visible

<table>
<thead>
<tr>
<th>DEPT</th>
<th>C</th>
<th>CH</th>
<th>CH$_2$</th>
<th>CH$_3$</th>
</tr>
</thead>
<tbody>
<tr>
<td>DEPT-90</td>
<td>absent</td>
<td>+100%</td>
<td>absent</td>
<td>absent</td>
</tr>
<tr>
<td>DEPT-135</td>
<td>absent</td>
<td>+70%</td>
<td>−50%</td>
<td>+35%</td>
</tr>
</tbody>
</table>
1D Carbon NMR

**DEPT**

![1D Carbon NMR DEPT](image)

*spectra reproduced from *Structure Elucidation by Modern NMR (workbook)*, by Duddeck and Dietrich*
# 1D Carbon NMR

## Summary

<table>
<thead>
<tr>
<th>1D spectrum</th>
<th>1D spectrum (proton decoupled)</th>
<th>APT</th>
<th>DEPT</th>
</tr>
</thead>
</table>
| • low sensitivity  
• information-rich  
• crowded spectra | • high sensitivity and resolution  
• low information content | • low sensitivity  
• multiplicity information  
• quaternaries visible | • high sensitivity  
• multiplicity information  
• no quaternaries |
1. 1D Carbon NMR
   • 1D carbon spectrum
   • 1D carbon spectrum (proton decoupled)*
   • APT*
   • DEPT*

2. 2D proton NMR
   • COSY
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4. 2D proton-carbon NMR
   • HMQC
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   • HSQC
   • HETCOR

5. The rest
   • 1D analogues
   • macromolecules, solids, multinuclear
2D Proton NMR

**COSY**

- Use $J_{HH}$ interaction to correlate protons connected by 2–3 bonds
- Correlations represented by *cross peaks* in 2D spectrum

<spectrum reproduced from *Modern NMR Spectroscopy*, by Sanders and Hunter>
2D Proton NMR

DQF COSY

• Improved lineshapes and resolution
• Singlet (uncoupled) peaks suppressed

*spectra reproduced from Modern NMR Spectroscopy, by Sanders and Hunter*
2D Proton NMR

long-range COSY

• correlations via small $J_{HH}$ enhanced
  e.g. allylic coupling, $W$ and para coupling in aromatic rings
• short-range correlations may be weakened

$H_2$–$H_4$ allylic 4-bond correlation

$^4J_{HH} \sim 2$ Hz

• spectrum reproduced from *100 and more Basic NMR Experiments*, by Braun, Kalinowski and Berger
2D Proton NMR

long-range COSY

H$_2$–H$_5$ para 5-bond correlation

$^5J_{HH} < 0.5$ Hz

*spectrum reproduced from Modern NMR Spectroscopy, by Sanders and Hunter*
2D Proton NMR

COSY – inorganic example

• $^{11}\text{B} - ^{11}\text{B}$ COSY spectrum of [As$_2$B$_9$H$_{10}$]$^-$ ion
• $^1\text{H}$ decoupled

• spectrum reproduced from *Modern NMR Spectroscopy*, by Sanders and Hunter
2D Proton NMR

**TOCSY**

- correlations between all protons within spin system (not just those directly coupled)
- useful when key COSY cross-peaks are obscured
- useful for assigning resonances in side-chains of proteins

\[ \begin{align*} &1H_A \quad J_{AB} \quad 1H_B \quad J_{BC} \quad 1H_C \\
\end{align*} \]

- COSY cross peaks: A—B, B—C
- TOCSY cross peaks: A—B, B—C and A—C
2D Proton NMR

TOCSY

• TOCSY spectrum of \( n \)-butyl acetate
• 2 spin systems

*spectrum reproduced from Modern NMR Spectroscopy, by Sanders and Hunter*
# 2D Proton NMR

**Summary**

<table>
<thead>
<tr>
<th>COSY</th>
<th>DQF COSY</th>
<th>long-range COSY</th>
<th>TOCSY</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^1$H–$^1$H through-bond correlations &lt;br&gt;mostly 2-bond / 3-bond correlations</td>
<td>the <strong>best</strong> standard COSY experiment &lt;br&gt;high resolution and narrow lineshapes &lt;br&gt;singlet peaks suppressed</td>
<td>• good for obtaining correlations when $J_{HH}$ is small &lt;br&gt;• useful for seeing <strong>long range correlations</strong>, e.g. 4-bond allylic, and “W” and “para” correlations in aromatics</td>
<td>• shows correlations between <strong>all</strong> protons in same spin system &lt;br&gt;• narrow lineshapes</td>
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   - HSQC
   - HETCOR

5. The rest
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2D Carbon NMR

• Use the $J_{cc}$ interaction to correlate different carbon nuclei.

• A nice way to trace out the carbon skeleton of an organic molecule

• … but to do this you need pairs of $^{13}$C nuclei
  …the chances of this are $1.1\% \times 1.1\% = 0.0121\%$
  …so sensitivity is low!
2D Carbon NMR

INADEQUATE

• Use $^1J_{CC}$ interaction to correlate neighbouring carbons
• Useful when sensitivity is not an issue

"double quantum" shift

carbon shift

• spectrum reproduced from *100 and more Basic NMR Experiments*, by Braun, Kalinowski and Berger
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2D Carbon NMR

INADEQUATE

• Useful for distinguishing a variety of “topologies”, e.g. linear, cyclic etc.

• Figure reproduced from Spin Choreography, by Ray Freeman
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2D Proton-Carbon NMR

**options**

- Use the $J_{HC}$ interaction to correlate proton and carbon shifts.

![Diagram](1H --> J_{HC} --> 13C)

- Great variety of experiments
  - Do we want 1-bond ($J_{HC} \sim 130$ Hz) correlations or just multiple-bond correlations ($J_{HC} \sim 0\text{-}20$ Hz)?
  - Detect on proton or carbon?
  - Switch $J_{HC}$ interaction off during acquisition for higher resolution?
  - Leave $J_{HC}$ interaction on during acquisition to retain information?
  - Do we need to *measure* $J_{HC}$ values or just see the correlations?
  - Remove $J_{HH}$ interactions in spectrum?
2D Proton-Carbon NMR

$HMQC$

• Use $^{1}J_{HC}$ interaction to correlate protons with neighbouring carbons
• Proton detection for high sensitivity

*spectrum reproduced from *100 and more Basic NMR Experiments*, by Braun, Kalinowski and Berger
spectrum reproduced from *100 and more Basic NMR Experiments*, by Braun, Kalinowski and Berger
2D Proton-Carbon NMR

**HMQC with decoupling**

- Use $^1J_{HC}$ interaction to correlate protons with neighbouring carbons…
- …but decouple $^1J_{HC}$ interaction during acquisition for simpler spectrum and enhanced sensitivity

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*spectrum reproduced from *100 and more Basic NMR Experiments*, by Braun, Kalinowski and Berger
2D Proton-Carbon NMR

*multiplicity-edited HSQC*

- HMQC-like spectrum
- contains multiplicity information
- 20 minutes

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<td>me-HSQC</td>
<td>absent</td>
<td>positive</td>
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*spectrum reproduced from *100 and more Basic NMR Experiments*, by Braun, Kalinowski and Berger*
2D proton-carbon NMR

multiplicity-edited HSQC

*spectra reproduced from http://rmn.iqfr.csic.es/guide/tutorials/specdata/spectra/suc_hsqced.html, by Teodor Parella*
2D proton-carbon NMR

HMBC

• Use $^2J_{HC}$, $^3J_{HC}$ etc. interaction to correlate protons with more distant carbons.
• Usually acquired without decoupling
• Low-pass filter to suppress one-bond correlations

*spectrum reproduced from *100 and more Basic NMR Experiments*, by Braun, Kalinowski and Berger
H3–C1 (3 bond)
H3–C4 (2 bond)
H3–C2 (2 bond)
H3–C3 (1 bond)
H3–C1 (3 bond)

*spectrum reproduced from *100 and more Basic NMR Experiments*, by Braun, Kalinowski and Berger
2D Proton-Carbon NMR

\textit{HSQC}

• Similar to HMQC
• $J_{HH}$ splitting absent in $^{13}$C dimension (useful if carbon spectrum is crowded)
• preferred technique in protein NMR

*spectrum reproduced from 100 and more Basic NMR Experiments, by Braun, Kalinowski and Berger*
2D Proton-Carbon NMR

**HETCOR**

- Use $^{1}J_{HC}$ interaction to correlate protons with neighbouring carbons
- Less popular alternative to HMQC
- Carbon signal detected, which means sensitivity is much lower…
- …but useful when high resolution is required in $^{13}$C dimension

*_spectrum reproduced from *100 and more Basic NMR Experiments*, by Braun, Kalinowski and Berger*
2D Proton-Carbon NMR

HMOC – inorganic example

• $^1$H—$^{57}$Fe correlation spectrum
• $\nu_0(^1$H) ~ 300 MHz
• $\nu_0(^{57}$Fe) ~ 9.7 MHz

*spectrum reproduced from *100 and more Basic NMR Experiments*, by Braun, Kalinowski and Berger
### 2D Proton-Carbon NMR

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<th>HMBC</th>
<th>me-HSQC</th>
<th>HSQC</th>
<th>HETCOR</th>
</tr>
</thead>
</table>
| • $^1$H–$^{13}$C one-bond correlations  
• standard method for small-medium size molecules | • $^1$H–$^{13}$C 2/3-bond correlations | • $^1$H–$^{13}$C one-bond correlations  
• with multiplicity information | • $^1$H–$^{13}$C one-bond correlations  
• mainly used for biomolecules | • $^1$H–$^{13}$C one-bond correlations  
• typically lower sensitivity than HMQC  
• high resolution in $^{13}$C dimension |
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Further experiments

1D Analogues

• Almost every 2D experiment has a “selective” 1D analogue, e.g. 1D COSY
• Experiment time can be much shorter than for 2D
• “Target” multiplet must be resolved

*spectra reproduced from http://rmn.iqfr.csic.es/guide/tutorials/specdata/spectra/dis_selco.html, by Teodor Parella
Further experiments

Macromolecules

• 100s of experiments designed for assigning macromolecules, e.g. proteins, carbohydrates

Solids

• plenty of equivalent experiments for use on solid samples, see lecture 4

Multinuclear NMR

• experiments described can be used throughout the periodic table, not just for carbon and proton
Conclusion

• 1000’s of NMR experiments have been developed
• about a dozen are really useful
• the rest are occasionally useful…
• …and nearly all of them are available in the department!
Useful Reading

General and Organic:

• *Modern NMR Spectroscopy* (the workbook is also useful), by J.K.M. Sanders and B.K. Hunter
• *100 and more Basic NMR Experiments*, by Braun, Kalinowski and Berger (a practical guide for Bruker users)
• *Structure Elucidation by Modern NMR (workbook)*, by Duddeck and Dietrich
• *Spectroscopic Methods in Organic Chemistry*, by Williams and Fleming
• *Carbon-carbon and C—H NMR couplings*, by James L. Marshall (coupling constants)
• the literature, e.g. *Magnetic Resonance in Chemistry*

Inorganic:

• *NMR Spectroscopy in Inorganic Chemistry (Oxford Chemistry Primer)*, by Jonathan A. Iggo
• several books in department library (look for “ multinuclear” in the title)

Proteins:

• *Protein NMR Spectroscopy: Principles and Practice*, by Cavanagh, Fairbrother, Palmer and Skelton

Introductory NMR theory:

• *Understanding NMR spectroscopy*, by James Keeler
• *Nuclear Magnetic Resonance (Oxford Chemistry Primer)*, by P.J. Hore
• *NMR: The Toolkit (Oxford Chemistry Primer)*, by P.J. Hore, Jonathan Jones and Stephen Wimperis