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Efficient and Rapid Structure Determination by NMR
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Building blocks and some practical aspects of
NMR pulse sequences

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Prog. NMR Spectrosc. (1999) 34, 93-158.
http://www.embl.de/nmr/sattler
Outline

• Basic tools and building blocks of NMR pulse sequences

• RF pulses, selective pulses, and pulse calibration

• Sensitivity enhancement

• Issues with cryoprobes:
  • B1 inhomogeneity
  • radiation damping
  • importance of water-flip back
Structure determination by NMR
$^{1}J$- and $^{2}J$-couplings in proteins

\[ 
\begin{align*}
^{13}C_{\alpha} & \quad ^{13}C' \quad ^{15}N \quad ^{13}C_{\alpha} \\
H_{\alpha} & \quad H' \quad H' \quad H_{\alpha} \\
^{13}C_{\beta} & \quad ^{13}C' \quad ^{13}C_{\gamma} \\
& \quad 35 \text{ Hz} \quad 130 \text{ Hz} \quad 35 \text{ Hz}
\end{align*} \]
Speed/efficiency of magnetization transfer

Homonuclear magnetization transfer via $^3J$ coupling

Heteronuclear magnetization transfer via $^1J$ couplings

$S/N \sim N \gamma_{exc} \gamma_{det}^{3/2} B_0^{3/2} N S^{1/2} T_2$
Assignment based on J-correlations

side chain assignments
Multi-dimensional NMR experiments

- To resolve signal overlap with increasing molecular weight
- $1/\sqrt{2}$ loss of S/N per indirect dimension
- Potential problems: time needed for sampling

![3D FT NMR](image)

$\Omega_T \rightarrow \Omega_S \rightarrow \Omega_I$

$I \rightarrow S \rightarrow T \rightarrow I$

- Preparation
- Mixing
- Mixing
- Mixing
- Detection

$\phi_S \rightarrow \phi_T$

$\Omega_1, \Omega_2, \Omega_3$

$\Omega_T, \Omega_S, \Omega_I$

$1^H, 13^C, 15^N$
Basic heteronuclear correlations: HMQC

HMQC: heteronuclear multiple quantum correlation

\[ \phi = x - x \]
\[ \psi = x \ x \ -x \ -x \]
\[ \phi_{\text{rec}} = x \ -x \ -x \ x \]

\[ \delta^{(1)H}: \text{refocused} \]
\[ \delta^{(13)C}: \text{evolution during } t_1 \]
\[ J^{(1H,13C)}: \text{active during } \Delta \]
\[ J(H,H): \text{active !} \]
\[ J(C,C): \text{active !} \]

Relaxation during \( t_1 \): multiple quantum line-narrowing
Basic heteronuclear correlations: HSQC

HSQC: heteronuclear single quantum correlation

$\delta^{(1)}(1H)$: refocused
$\delta^{(13)}(C)$: evolution during $t_1$
$J^{(1)}(H,13C)$: active during $\Delta$
$J(H,H)$: not active
$J(C,C)$: active!
Relaxation during $t_1$: $T1^{(1)}H$, $T2^{(13)}C$
Basic building blocks: heteronuclear correlation

Relaxation during $t_1$

$T_{2MQ}(I_xS_y) > T_2(S)$

$\Rightarrow$ Methyl TROSY

$T_1$ I-spin ($^1H$)

$T_2$ S-spin ($^{13}C$)

$T_2$ S-spin ($^{13}C$)

$\phi = x - x; \psi = x - x - x; \phi_{rec} = x - x - x - x$.

Bax et al JMR (1990) 86, 304-318
Basic building blocks: spin-state-selective filters

IPAP HSQC

$^{1}H$  $^{15}N$  $^{13}C_{\alpha}$  $^{13}C'$

$\phi_1$ $\phi_2$ $\phi_3$ $\phi_4$

$\frac{\Delta}{2}$ $\tau$ $\frac{\tau}{2}$ $\frac{\tau}{2}$ $\frac{\Delta}{2}$ $\frac{\Delta}{2}$

$\Delta$ $\Sigma$

Pulses marked * are applied for the antiphase expt (AP) only.

$\phi_1 = -y/y$; $\phi_2 = 2(x), 2(-x)$ (IP); $2(-y), 2(y)$ (AP) + TPPI($t_i$);

$\phi_3 = 4(x), 4(y), 4(-x), 4(-y) + TPPI(t_i)$ (AP); $\phi_4 = 8(x), 8(-x)$.

$\phi_{\text{rec}} = x, 2(-x), x, -x, 2(x), -x$.

Basic building blocks: TROSY

\[ \phi_1 = y, -y; \phi_{\text{rec}} = x, -x. \]

E/AE selection: \( \psi = x/-x; \kappa = +10/-10. \)

\[ \phi_1 = x, -x; \phi_{\text{rec}} = x, -x. \]

E/AE selection: \( \psi_1 = y/-y; \psi_2 = x/-x; \kappa = +5/-5. \)
Pulse calibration

**Problem:** no phase cycle \( \rightarrow \)

\(^1H\)-\(^{12}C\) signals not suppressed!

**Phase cycling:** \( \rightarrow \)

\(^1H\)-\(^{12}C\) signals are suppressed!

**Determination of \(90^\circ(X)\)**

\[ 1^H \quad \Delta \quad \Delta \quad t_2 \]

\[ ^{13}C \]

\[ 2I_{x,y}S_z \quad 2I_{x,y}S_z \cos\beta \]

\[ I_y \cos\beta \]

**Determination of \(180^\circ(X)\)**

\[ 1^H \quad \Delta \quad \Delta \quad t_2^{\phi_{\text{rec}}} \]

\[ ^{13}C \]

\[ 2I_{x,y}S_z \quad 2I_{x,y}S_z \sin\beta \sin2\beta \cos\delta \]

\[ I_y \sin\beta \sin2\beta \cos\delta \]

\[ \phi = x - x \]

\[ \psi = x \quad x - x - x \]

\[ \phi_{\text{rec}} = x - x - x \quad x \]
Pulse calibration: considerations

• What sample to use for pulse calibration – H₂O, protein, urea?
  • H₂O: 😊 large signal, very sensitive, optimize on FID
    ☹ radiation damping, different NMR properties than biomolecule
  • protein: 😊 NMR signals of interest
    ☹ poor S/N
  • urea: 😊 high sensitivity, isolated signals
    ☹ different NMR properties than actual sample

• Calibration of 90°, 180°, 360° pulse?

• Effects of B1 inhomogeneity
  • room temperature probe vs. cryoprobes
Pulse calibration: B1 inhomogeneity

- Calibration of 180° or 360° pulse
  - off-resonance effects
- B1 inhomogeneity
  Pulse calibration:
  Center: [Z-shim detuned] Bulk [Z tuned] ERROR
  90deg: 61us (expected) (app. 90)
  180deg: 123us (2*90=122us) 132us (66.0us) 8%
  360deg: 248us (4*90=244us) 257us (64.3us) 5%

- Optimization of RF pulses in actual pulse sequence

1D $^{13}$C spectrum of a protein

$\delta^{13}$C 160.0 120.0 80.0 40.0 [ppm]

$C'$  $C_{\text{arom.}}$  $C_{\text{aliph.}}$
Band-selective RF pulses: rectangular vs. shaped pulses

Selective excitation

Selective inversion

\[ \Delta \Omega / 2\pi \text{ [kHz]} \]

\[ M_{xy} \]

\[ M_{z} \]
Band-selective RF pulses

<table>
<thead>
<tr>
<th>Selective excitation (90°)</th>
<th>Selective inversion, refocusing (180°)</th>
<th>Adiabatic inversion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gaussian (90° / 270°)</td>
<td>I-BURP</td>
<td>Hyperbolic secant</td>
</tr>
<tr>
<td>E-BURP</td>
<td>RE-BURP</td>
<td>WURST</td>
</tr>
<tr>
<td>G4</td>
<td>G3</td>
<td>CHIRP</td>
</tr>
<tr>
<td>Q5</td>
<td>Q3</td>
<td></td>
</tr>
</tbody>
</table>

References (shaped pulses and band-selective decoupling):
shaped pulse: universal rotation

Simulation

W. Bermel, Bruker
BSP compensating pulse

Bloch Siegert shift: inversion

simulation
$M_z \rightarrow M_z$

Q3

(2 msec, 3300.8Hz, freq: 2kHz, -3kHz)

with BS compens.
without

W. Bermel, Bruker
Sensitivity enhancement

• Optimized coherence transfer: coherence order selective transfers
  • sensitivity enhancement (coherence order selective coherence transfer)
  • double sensitivity enhancement
  • TROSY & MQ line-narrowing (methyl TROSY)

• Simultaneous acquisition, i.e. $^1\text{H}-^{13}\text{C}$, $^1\text{H}-^{15}\text{N}$ correlation

• Water-flip-back (water suppression, reduce saturation transfer)

• Longitudinal relaxation optimization (LHSQC, LTROSY)

• Fast data acquisition
Sensitivity enhancement

Conventional HSQC

$^1\text{H}$

- $x$ \(\Delta/2\)\(\Delta/2\) $y$ \(\Delta/2\) \(\Delta/2\) $t_2^{\phi+\psi}$

- $^1\text{C}$

- $t_1$ \(\phi\) \(\psi\)

- $2I_zS_x - 2I_yS_x - 2I_yS_x$ unobservable

- $2I_zS_y - 2I_yS_z$ $I_x$

Sensitivity enhanced HSQC

$^1\text{H}$

- $x$ \(\Delta/2\)\(\Delta/2\) $y$ \(\Delta/2\) \(\Delta/2\) $\Delta/2$ \(\Delta/2\) \(\Delta/2\) \(\Delta/2\) $t_2^{\phi+\psi}$

- $^1\text{C}$

- $t_1$ \(\phi\) \(\psi\) \(\psi+\pi/2\)

- GARP

- \(\psi=0:\) \[
\begin{align*}
2I_zS_x & - 2I_yS_x - 2I_yS_x & \pm 2I_yS_z & \mp I_x & \mp I_x \\
2I_zS_y & - 2I_yS_z & I_x & - I_z & I_x & - I_y \\
2I_zS^{-} & (\psi+\pi/2) & -I^- & \text{antiecho} \\
2I_zS^{+} & (\psi-\pi/2) & I^- & \text{echo}
\end{align*}
\]

RSH amplitude modulation

Echo/anti-echo

Phase modulation

Double sensitivity enhanced HCCH-TOCSY

3D DE H(C)CH–TOCSY

- $\phi_1$ = $4(x), 4(-x)$; $\phi_{rec} = 4(x), 4(-x)$.
- Four successive scans are stored separately.
- $\Delta' = 3.8$ ms, $\Delta'_1 = 2.3$ ms, $\Delta'_2 = 1.7$ ms.

• can be recorded in H$_2$O due to excellent water suppression by heteronuclear gradient echo

Simultaneous $^{13}\text{C}/^{15}\text{N},^{1}\text{H}$ HSQC

$^{1}\text{H}$

$^{15}\text{N}$

$^{13}\text{C}$

Grad.

$\phi_{1} = x, -x; \phi_{2} = x, x, -x, -x + \text{TPPI}(t_{i}); \phi_{rec} = x, -x, -x, x. \Delta = 5.4 \text{ ms}, \Delta' = 3.7 \text{ ms}.$

$\kappa t_{i} = t_{i}(^{15}\text{N})-t_{i}(^{13}\text{C})$ to allow for different sweep widths.

- $^{1}\text{H} \leftrightarrow X$ transfer can be optimized simultaneously for $^{13}\text{C}$ and $^{15}\text{N}$ but: some relaxation loss for $^{13}\text{C}$ due to longer delay.
- poor water-suppression, since E/AE cannot be implemented without sensitivity loss.
- building block for simultaneous 3D/4D NOESY experiments.
Simultaneous sensitivity enhancement

Simultaneous SE $^{15}\text{N},^{1}\text{H}$ and $^{13}\text{C},^{1}\text{H}$–HSQC

$^{1}\text{H}$

$^{15}\text{N}$

$^{13}\text{C}$

$^{13}\text{C}^\prime$

$G_Z$

$\mu = -6, 6; \lambda = 4, -4; \psi = -y, y; \bar{\psi} = -y, y;$

$\Delta = 5.4\text{ms}, \Delta' = 3.6\text{ms}, \tau_G = 1.8\text{ms}.$

Simultaneous sensitivity enhanced HSQC in H$_2$O

Water-flip-back in HSQC experiments

**WATERGATE–HSQC**

\[ H_{2}O: \begin{array}{c}
\phi_{1} \\
\Delta \\
x \\
\Delta \\
\frac{t_{1}}{2} \\
H_{2}O \\
\phi_{2} \\
\frac{t_{1}}{2} \\
H_{2}O \\
H_{2}O \\
t_{2} \\
\phi_{rec}
\end{array} \]

\[ ^{1}H \]

\[ ^{15}N \]

\[ G_{Z} \]

\[ \phi_{1} = x; \phi_{2} = x, -x + TPPI (t_{1}); \phi_{rec} = x, -x. \]

**Sensitivity–enhanced HSQC**

\[ H_{2}O: \begin{array}{c}
\phi_{1} \\
\Delta \\
x \\
\Delta \\
\frac{t_{1}}{2} \\
H_{2}O \\
\phi_{2} \\
\frac{t_{1}}{2} \\
\tau_{G} \\
\tau_{G} \\
\psi \\
\kappa G_{Z} \\
G_{Z}
\end{array} \]

\[ ^{1}H \]

\[ ^{15}N \]

\[ G_{Z} \]

\[ \phi_{2} = x - x; \phi_{rec} = x - x. \]

E/AE selection: \( \psi = y/-y; \kappa = +10/-10. \)
Water-Flip-back

SE HSQC
2D: S/N*√2

WATERGATE HSQC

H₂O: +z
H₂O: dephased
H₂O: -z
Longitudinal relaxation optimization

- faster longitudinal relaxation
- band-selective inversion of spins, i.e. amide protons
- partially already achieved by water-flip-back
- faster repetition rates

Pervushin et al JACS (2002) 124, 12898
Schanda & Brutscher JACS (2005), 127, 8014
Deschamps & Campbell JMR (2006) 178, 206
Radiation damping

\[ \tau_p = 20.5 \mu s (<180^\circ) \]

\[ \tau_p = 20.8 \mu s (>180^\circ) \]

\[ \Delta \]

- Chen, Mao, Ye JMR (1997) 124, 490-494
Measuring $^{15}$N $T_1$ relaxation on a cryoprobe

Problems with cryoprobes:

- large B1 inhomogeneity
- fast radiation damping (<20ms on a cryoprobe)
- multiexponential decay of NMR signals during the $T_1$ relaxation delay

$\Delta=5.4\text{ms}; \delta=5\text{ms}; \text{relaxation delay}=\delta^*n; \kappa=+/5; \psi= y/-y; \phi_1= x, -x; \phi_2= 2(x), 2(y), 2(-x), 2(-y); \phi_{\text{rec}}= x, -x, -x, x.$
$^{15}$N $T_1$ relaxation, water-flip-back and cryoprobe

- multiexponential decay of NMR signals during the $T_1$ relaxation delay
- amides with fast H/D exchange rates (<50ms) are affected
- ➔ water-flip-back during T1 relaxation delay to overcome this problem
\[ ^{15}\text{N} T_1 \text{ relaxation, water-flip-back and cryoprobe} \]

- water-flip-back during T1 relaxation delay to overcome multiexponential decay related to B1 inhomogeneity and radiation damping

\[ \Delta=5.4\text{ms}; \delta=5\text{ms}; \text{relaxation delay}=\delta^*n; \kappa=\pm/-5; \psi=y/-y; \phi_1=x,{-x}; \phi_2=2(x),2(y),2(-x),2(-y); \phi_{\text{rec}}=x,-x,-x,x. \]
$^{15}$N $T_1$ relaxation, water-flip-back and cryoprobe

No water-flip-back during $T_1$ delay

With water-flip-back during $T_1$ delay
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