NMR experiments: general remarks

- **Pulse sequence:**
  
  series of RF pulses and delays

- **Observable signals:**
  
  typically many, depend on spin systems

- **Several transfer mechanisms that can act at the same time:**
  
  $J$-coupling, NOE, cross-correlation, exchange

Multitude of transfer mechanism, pathways and spin states create confusion. Many observable terms are generated.

What if e.g. we only want to see the result of a transfer via $\sigma$ between $^1H(^{15}N)$ and $^1H(^{13}C)$?

We need to select for a particular pathway according to the information we intend to obtain. There are two ways how a particular pathway can be separated from others:

- Phase cycling
- Field gradient pulses
Coherence order

Classify coherence according to an order \( p = \pm 1, \pm 2, \pm 3 \ldots \)

transverse magnetization: \( I_x, 2I_xS_z, 4I_xS_zK_z \) \( p = \pm 1 \) single quantum

z-magnetization: \( I_x, 2I_xS_z \) \( p = 0 \)

multiple-quantum: \( 2I_xS_x \) \( p = \pm 2 \) double quantum
\( p = 0 \) zero quantum

in general: \( N \) spins \( \rightarrow \) coherence order: \( -N \ldots 0 \ldots +N \) (in integer steps)

Why this classification?

Different coherences acquire different phases through a z-rotation
(e.g. chemical shift evolution is a z-rotation due to offset)

\[
\exp(-i\phi F_z) \sigma^{(p)} \exp(i\phi F_z) = \exp(-ip\phi) \sigma^{(p)}
\]

Evolution of a coherence \( p \) under a z-rotation of angle \( \phi \)

Coherence of order \( p \) acquires a phase shift of \(-p\phi\)

\[
\sigma^{(p)} \xrightarrow{\text{z-rotation by } \phi} \sigma^{(p)} \exp(-ip\phi)
\]

Raising/lowering operators, z-rotations

\[
l^+ = l_x + il_y \\
l^- = l_x - il_y \\
l_x = 1/2 [ l^+ + l^- ] \\
l_y = -i/2 [ l^+ - l^- ]
\]

Express product operators in terms of raising and lowering operators.

This allows classification according to their coherence order.

\[
e.g. \quad 2I_xS_z = (l^+ + l^-)S_z \\
2I_xS_x = 1/2(l^+S^+ + l^-S^- + l^+S^- + l^-S^+)
\]

Transformation under z-rotation of angle \( \phi \):

\[
\exp(-i\phi l_z) l^\pm \exp(i\phi l_z) = \exp(\mp i\phi) l^\pm
\]
RF pulses

RF pulses transfer coherence:
- no selection rules, many coherences created
- spin system gives upper limit

\[ I_x \rightarrow DQ : \text{SQ cannot be converted into DQ.} \]
\[ I_z \rightarrow I_z : \text{Transverse magnetization from equilibrium.} \]
\[ 2I_zS_z \rightarrow \text{DQ, ZQ : Antiphase coherence is transformed into MQ.} \]

Conversions depend on flip angle:

\[ I_z \xrightarrow{\theta I_z} \cos \theta I_z + i/2 \sin \theta (I^+ - I^-) \]

- Pulses \( \neq 180^\circ \) generate equal amounts of coherence \( p = \pm 1 \)

\[ I_z \pm \theta I_x \xrightarrow{} \cos^2(1/2\theta) I^+ + \sin^2(1/2\theta) I^- \pm i \sin \theta I_z \]
- An equal transfer to \( I^+ \) and \( I^- \) occurs only for a \( 90^\circ \) pulse

\[ I^+ \xrightarrow{\pi I_z} I^- \xrightarrow{\pi I_z} I^+ \]

Phase shifting of pulses

If the phase of a pulse that changes a coherence by \( \Delta p \) is shifted by \( \Delta \phi \), the coherence acquires a phase shift of \( -\Delta p \Delta \phi \).

i.e. different changes in coherence order respond differently to phase changes of pulses.

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Coherence transfer pathways

Graphical representation of pulse sequence and coherence transfer pathways
- wanted coherence order(s)
- changes \( \Delta p \)

Homonuclear

- NOESY
- DQF
- DQ spectroscopy

Heteronuclear

- HMQC

separate coherence levels for \( I \) and \( S \)

\( t_i; p_I + p_S = \pm 2, 0 \) (DQ and ZQ \( \Rightarrow \) MQ)
Pulse and receiver phase in NMR

Relative phase difference between pulse and receiver is important

Phase difference: 0°  90°  270°  180°

Phase cycling

Selection procedure

phase: 1) $\phi_1$  $\Delta \phi = \phi_2 - \phi_1$
2) $\phi_2$

phase shift: $-\Delta p \Delta \phi$

- Phase changes carry through the sequence
- Different pathways $\Rightarrow$ different phase shifts

Example: Select coherence transfer from +2 to −1. $\Delta p = -3$

<table>
<thead>
<tr>
<th>step</th>
<th>Pulse phase</th>
<th>Phase shift for $\Delta p = -3$</th>
<th>Receiver shift for $\Delta p = -3$</th>
<th>difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0° [0]</td>
<td>0° [0]</td>
<td>0° [0]</td>
<td>0°</td>
</tr>
</tbody>
</table>

$\phi_{rec} = -\Delta p \Delta \phi$

pathway with $\Delta p = -3$ is selected

[.] = in multiples of 90°
Phase cycling

Another example: pathway with \( \Delta p = +2 \)

<table>
<thead>
<tr>
<th>step</th>
<th>Pulse phase</th>
<th>Phase shift for ( \Delta p = +2 )</th>
<th>Receiver shift for ( \Delta p = -3 )</th>
<th>difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0° [0]</td>
<td>0° [0]</td>
<td>0° [0]</td>
<td>0° [0]</td>
</tr>
</tbody>
</table>

pathway with \( \Delta p = +2 \) is rejected

\[ \phi_{\text{rec}} = -\Delta p \Delta \phi \]

Selectivity of phase cycles

One more: How about \( \Delta p = +1 \)?

<table>
<thead>
<tr>
<th>step</th>
<th>Pulse phase</th>
<th>Phase shift for ( \Delta p = +1 )</th>
<th>Receiver shift for ( \Delta p = -3 )</th>
<th>difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0° [0]</td>
<td>0° [0]</td>
<td>0° [0]</td>
<td>0° [0]</td>
</tr>
</tbody>
</table>

pathway with \( \Delta p = +1 \) is selected

Selectivity of a phase cycle

\( \Delta p: \) \( ( -4 ) \) \( -3 \) \( ( -2 ) \) \( ( -1 ) \) \( ( 0 ) \) \( 1 \) \( ( 2 ) \) \( ( 3 ) \) \( ( 4 ) \) \( 5 \) ....

General rule for the selectivity of a phase cycle:

N step phase cycle:

\[ \Delta = \frac{360°}{N} \]

with phases 0, \( \Delta \), 2\( \Delta \), 3\( \Delta \),..... (N-1)\( \Delta \) \( \Rightarrow \) selects \( \Delta p \pm nN \) with \( n = 0, 1, 2, 3, \ldots \)

several pathways can be selected simultaneously

\( \Rightarrow \) sensitivity

\( \Rightarrow \) frequency discrimination, line shape
Frequency discrimination with pure-phase absorptive lineshape

Amplitude modulation in $t_1$:

selection of $p = +1$ AND $-1$ during $t_1$. ($I_1 = 1/2(I_1 + I_2)$)

$\cos \omega_1 t_1 \exp(+i \omega_2 t_2)$

after FT: peak at $F_1 = -\omega_2$, $F_2 = \omega_2$ and $F_1 = +\omega_2$, $F_2 = \omega_2$

no frequency discrimination in $F_1$

absorptive lineshape

Phase modulation:

N-type (echo)

$\exp(-i \Omega t_1) \exp(+i \Omega t_2)$

after FT: peak at $F_1 = -\Omega$, $F_2 = \Omega$

frequency discrimination in $F_1$

P-type (anti-echo)

$\exp(+i \Omega t_1) \exp(+i \Omega t_2)$

after FT: peak at $F_1 = +\Omega$, $F_2 = \Omega$

frequency discrimination in $F_1$

Unfavorable phase-twist lineshape

Frequency discrimination with pure-phase absorptive lineshape

- keep both coherence pathways $\pm p$ during $t_1$ selectivity of phase cycle
- record cos and sin modulated data sets for every $t_1$ increment
- combine data sets according to:

<table>
<thead>
<tr>
<th>time domain data</th>
<th>after FT in $t_2$</th>
<th>after FT</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1) $\cos \Omega_1 t_1 \exp(i \Omega_2 t_2)$</td>
<td>$\cos \Omega_1 t_1 {A_2^+ + iD_2^\ast}$</td>
<td>$\cos \Omega_1 t_1 A_2^+$</td>
</tr>
<tr>
<td>(2) $\sin \Omega_1 t_1 \exp(i \Omega_2 t_2)$</td>
<td>$\sin \Omega_1 t_1 {A_2^+ + iD_2^\ast}$</td>
<td>$\sin \Omega_1 t_1 A_2^+$</td>
</tr>
</tbody>
</table>

Construct new data set: $\text{Re}(1) + i\text{Re}(2) = \exp(+i \Omega_1 t_1) A_2^+$

FT in $t_1$:

$(A_1^+ + iD_1^\ast) A_2^+ \rightarrow \text{Re} A_1^+ A_2^+$

Frequency discrimination with pure-phase absorptive lineshapes:
Basic phase cycles: EXORCYCLE, CYCLOPS

EXORCYCLE: 180° refocusing pulses

![Diagram]

pulse $\{0\ 1\ 2\ 3\}$
$\phi_{rec} \{0\ 2\ 0\ 2\}$

$\Delta p = \pm 2, N=4$

selects symmetrical pathways which go $p_{odd} \Rightarrow p_{odd}$

reduced 2-step scheme:

pulse $\{0\ 2\}$; receiver $\{0\ 0\}$
or $\{0\ 1\} \{0\ 2\}$

$\Rightarrow$ incomplete suppression

CYCLOPS: Suppression of quadrature detection imbalances

![Diagram]

pulse $\{0\ 1\ 2\ 3\}$
$\phi_{rec} \{0\ 1\ 2\ 3\}$

$\Delta p = -1, N=4$

selects $\Delta p = -1$ for the pulse sequence, starting from $I_z \Rightarrow I$ is selected for detection

Reducing the length of phase cycles

Problem: in multi-pulse sequences there are multiple transfer steps

![Diagram]

phase cycle grows very quickly

number steps phase cycle $> \frac{S}{N}$

First pulse of the sequence

starting with $I_z \Delta p$ can only be $\pm 1$

Last pulse before detection

creates observable coherence $p = -1$

no need for additional steps if selection happens earlier on

Keep step number $N$ small

selectivity: $\Delta p \pm nN$

Higher-order MQ are unlikely (spin system limited J network)

$\Rightarrow$ in many cases $N = 2$ or $4$ is sufficient

Group pulses together

overall transformation $\Delta p$

change phases of all of pulses involved

![Diagram]
Phase cycles: axial peak suppression

Contributions that are not modulated during \( t_1 \) appear at \( \omega_1 = 0 \).

Cycle first pulse and receiver with: \{0 2\}

Pulse phase of first pulse: 
\[ 0^\circ \quad 180^\circ \]

Receiver phase \( \phi_{\text{rec}} \): 
\[ 0^\circ \quad 180^\circ \]

Wanted signal
\[ - \quad - \quad = \]

Unwanted signal
\[ - \quad + \quad = \]

Phase cycling: homonuclear examples

**NOESY**

- \( \Delta p = 0 \)
- Axial peak suppression

**DQF COSY**

- \( \Delta p = 0 \)
- \( \Delta p = \pm 2 \)

**DQ spectroscopy**

- \( \Delta p = 0 \)
- \( \Delta p = \pm 2 \)

Or

- \( \Delta p = -3, +1 \)
Phase cycling: selection of X-bound signals

- Isotope labelled samples
- J-coupling evolution

⇒ suppression of unlabelled contributions

\[ S \text{ pulse: } \{ x, -x \} \]
\[ \phi_{\text{rec}} : \{ x, -x \} \]

2-step phase cycle

\[ \Delta \phi_{s} = \pm 1 \]

\[ \begin{array}{c}
\text{Spin system} \\
I & \text{unwanted} & I_{y} & I_{y} & 0 \\
I_{z} & \text{wanted} & -2I_{z}S_{y} & 2I_{z}S_{x} & -2I_{z}S_{y} \\
\end{array} \]

unwanted signals cancel

Pros and cons of phase cycling

- Difference method ⇒ stability !
- Suppression ⇒ complete all steps
- Large dynamic range of signals
- Solvent suppression is limited

- Nucleus selective (via RF pulses)
**Pulsed field gradients (PFGs)**

**normal situation:**
- homogeneous main field $B_0$

**field gradient active:**
- additional contribution $B_G$
- inhomogeneous field
- spatially dependent phase

$$B_{\text{total}} = B_0 + B_G$$

Phase encoding by pulsed field gradients

Phase encoding according to the position along the z-axis

Additional field contribution changes linearly along z-axis

Dephasing

The effect of a gradient is reversible

Opposite field gradient

Rephasing
Dephasing by pulsed field gradients

\[ B_z = B_0 + B_G, \text{ where } B_G = G_z \]

\( B_0 \): main field  
\( G_z \): spatially dependent part of field

\( \gamma B_z = \gamma B_0 + \gamma G_z \)  
spatially dependent Larmor frequency

\( \omega_z = \omega_0 - \gamma G_z \)  
Frequency across the sample  
\( \omega_0 \) : evolution due to main field

\( \Omega_z = -\gamma G_z \)  
spatially dependent part of frequency

Dephasing of coherence:  
\[ I^\pm \rightarrow I^\pm \exp(i\phi_z) = I^\pm \exp(i\Omega_z t) \]

\( \phi_z = \mp \Omega_z t \)

\[ s \text{: shape factor} \]
\[ p \text{: coherence order} \]
\[ \gamma \text{: gyromagnetic ratio} \]
\[ G \text{: gradient strength (G/cm or Tesla/m)} \]
\[ z \text{: coordinate along z} \]
\[ \tau \text{: duration of gradient} \]

How much gradient dephasing is required?

\[ I^\pm \rightarrow I^\pm \exp(i\gamma G z t) \]

signal\( (t) = \int_{-0.5z}^{0.5z} \exp(i\gamma G z t) \frac{dz}{0.5\gamma G z t} \)

signal\( (t) \approx \frac{2}{|\gamma G z\tau|} \)

Example:

dephasing of proton magnetization to 0.1% \((10^{-3})\) over a sample region of 1 cm with a 30 Gauss/cm gradient requires a gradient pulse of \( \tau = 2.5 \text{ ms} \) duration:

\( \gamma = \gamma_1 \text{H}, \ G= 30 \text{ Gauss/cm} = 0.3 \text{ T/m}, \ r = 1 \text{ cm} \rightarrow \tau = 2.5 \text{ ms} \)

\( \rightarrow \text{if} \ r = 3.25 \text{ cm (NMR sample tube)} \tau = 770 \mu\text{s} \)
Coherence order selection by pulsed field gradients

\[ \sum \phi_i = 0 \quad \phi_i = s_i p_i \gamma_i G_i \tau_i \]

**Coherence order selection**

- **homonuclear**
  - RF
  - gradient 1: \( \phi_1 = s_1 p_1 G_1 z \tau_1 \)
  - gradient 2: \( \phi_2 = s_2 p_2 G_2 z \tau_2 \)
  - A given gradient pair selects a particular ratio of coherences:
    - refocusing of \( p_1 \rightarrow p_2 \): \( \phi_1 + \phi_2 = 0 \)
    - \( \frac{s_1 G_1 \tau_1}{s_2 G_2 \tau_2} = -\frac{p_2}{p_1} \)
    - e.g. \( p_1 = +2, p_2 = -1 \) with \( \tau_1 = \tau_2 \) and \( G_2 = 2G_1 \)

- **heteronuclear**
  - refocusing of \( p_1 \rightarrow p_S \): \( \phi_1 + \phi_3 = 0 \)
  - \( \gamma_S G_1 \tau_1 = -\frac{p_S}{p_I} \)
  - e.g. \( I = ^1H, S = ^13C \)
  - for refocusing of \(+1(I) \rightarrow -1(S)\) :
    - \( \tau_S = 2\tau_I, G_S = -2G_I \)
    - or \( \tau_S = \tau_I, G_S = -4G_I \)
    - etc

**Multiple pathways**

- **cannot keep symmetrical pathways**
- **loose 50% of signal**
**Coherence selection by gradients**

The phase introduced by a gradient of duration $\tau_G$ to coherence of order $p$ which involves $k$ spins with gyromagnetic ratios $\gamma_k$ is given by:

$$\phi(r) = r G_z \tau_G S_k(p_k \gamma_k)$$

The requirement to obtain a gradient stimulated spin echo after a certain pulse sequence with $j$ gradient pulses is:

$$\Sigma_j \{ (G_z \tau_G) \cdot S_k(p_k \gamma_k) \} = 0$$

**Purging of unwanted signals by PFGs**

- no dephasing for $p = 0$
- place wanted coherence along $z$
- dephase unwanted coherences
Purging of unwanted signals by PFGs

180° pulses

Refocusing:

ideal 180°: \( p \rightarrow -p \) rephased

imperfect 180°: \( p \rightarrow \neq -p \) dephased

keeps symmetrical pathways

Inversion:

ideal 180°: \( z \rightarrow -z \) \( p = 0 \)

imperfect 180°: \( z \rightarrow \text{others} \) dephased

Preventing of phase errors

single gradient

split gradient

use existing echo periods

Gradients in heteronuclear NMR experiments

Spin echo with gradients

Spoil/purge gradients

Coherence selection

Coherence rejection

I: \( I_z - I_y - I_y - I_y - I_x \)

I-S: \( I_z - I_y - 2I_xS_z - 2I_xS_z - 2I_yS_z - 2I_yS_z \)
PFGs in heteronuclear experiments

HSQC with gradient selection

HSQC with purge pulses (zz)

I/S SQ coherence order selection

zz-periods with purge pulses

HMQC with gradient selection

I/S coherence order selection

Gradient coherence selection and sensitivity

Need: absorptive signals and frequency discrimination

(1) amplitude modulated data in $t_1$, separate cos and sin data
or
(2) P- and N-type data in $t_1$. Combine ⇒ pure cos and sin modulated data

• no selection gradients (phase cycled, zz-periods)
  amplitude modulation

  signal: $S$, noise: $N$

  sensitivity: $S/N$

• selection gradient outside $t_1$ evolution
  amplitude modulation

  signal: $S/2$, noise: $N$

  sensitivity: $S/2N$

• selection gradient during $t_1$
  phase modulation (P- and N-type selection)

  signal: $S/2$, noise: $N$

  cos: $S_c(t_1,t_2) = 0.5 \left[ S_P(t_1,t_2) + S_N(t_1,t_2) \right]$

  sin: $S_s(t_1,t_2) = -0.5 \left[ S_N(t_1,t_2) - S_P(t_1,t_2) \right]$

  sensitivity: $(S/2N) \sqrt{2} = S/(N \sqrt{2})$
Sensitivity enhancement

Conventional HSQC

Transfer both orthogonal shift labeled components (x and y):

HSQC

\[ H_{\text{eff}} = \pi(2I_xS_x + 2I_yS_y) \]

signal: S, noise: N
processing: \( \cdot \sqrt{2} \)

sensitivity: \((S/2)/N\)
Sensitivity enhancement / gradient coherence selection

a) Standard HSQC
no gradient coherence selection
S/N = 1

b) Standard HSQC
gradient coherence selection
S/N = 1/2

c) Sensitivity enhanced HSQC
gradient coherence selection
S/N = \sqrt{2}

S/N = \sqrt{I/n} (I: \Sigma \text{intensities}, n: \# \text{signals})

Water suppression methods

Concentration \([{}^1\text{H}]\) in H\(_2\)O \(\approx 110\) M, concentration biomolecule \(\approx 10^{-3}\) M

PROBLEMS: dynamic range (receiver); radiation damping

- Presaturation
  - depends on \(B_0\) homogeneity (shimming)
  - signals with near solvent frequency are suppressed as well (e.g. H\(_\alpha\) in proteins)
  - reduces S/N of exchangeable protons due to saturation transfer

- Jump-and-return / binominal sequences
  - water-flip-back intrinsic/possible
  - non-optimum excitation profile
  - difficult to combine with triple resonance/multi-pulse sequences

- Spin-lock, gradient spoil pulses, WATERGATE
  - can be combined with water-flip-back
  - suppression of signals near water

- Heteronuclear gradient echoes
  - excellent water-suppression with sensitivity enhancement, combine with water-flip-back

- Post-acquisition
  - apply low-pass filters to eliminate signals at 0 ± \(\omega\), i.e. water on-resonance
  - suppresses signal near water as well
Radiation damping

$$\tau_{\text{rad.damp}} = \frac{1}{2\pi \eta Q \gamma M}$$

$\eta$: filling factor;
$Q$: quality factor of the coil;
$\gamma$: gyromagnetic ratio;
$M$: transverse magnetization.

Recovery of transverse magnetization

full radiation damping

controlled recovery

Chen, Mao, Ye JMR (1997) 124, 490-494
Radiation damping

Recovery of z-magnetization

full radiation damping

controlled recovery

Control of radiation damping during transfer periods

Water suppression

Saturation methods

• weak RF field
  • signal bleaching
  • saturation transfer
  • exchangeable protons ↓↓↓

• Gradient coherence selection
  • efficient suppression: γ_I ≠ γ_S
  • saturation before the detection
  • exchangeable protons ↓
  • suitable for non-exchangeable protons. e.g. 1H/13C

zz-purge 13C HSQC

gradient selection 13C HSQC

for side chain experiments
Water suppression

• Watergate and excitation sculpting

Watergate

\[ \phi_1 - \phi_1 - \phi_{\text{rec}} \]

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

\[ \Delta = 1/[4\Omega_{\text{max.excitation}}] \]

DPFGSE

\[ \phi_1 - \phi_1 - \phi_{\text{rec}} \]

• can be highly frequency selective
• saturation method
• bleaching (selective pulses)

Non-saturation methods

• water flip-back, water control

Grzesiek and Bax J. Am. Chem. Soc. 1993, 115, 12593

- return water back to z-axis
- water never dephased
- exchangeable protons \( \uparrow \uparrow \)
- faster H\( \text{H}_2\text{O} \) recovery

Water-flip-back

1, -1 Jump-return
excitation null on-resonance

\[ \phi_1 - \phi_1 - \phi_{\text{rec}} \]

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

\[ \Delta = 1/[4\Omega_{\text{max.excitation}}] \]

WATERGATE with water-flip-back
Water suppression in HSQC experiments

Water-flip-back WATERGATE HSQC

E/AE gradient selection (with flip-back pulses) (sensitivity “dehanced”!)

SE HSQC with gradient coherence selection

HSQC water-dephase/rephease and WATERGATE

HSQC with WATERGATE & water-flip-back

Suppress radiation damping of H₂O signal during t₁
Sensitivity-enhanced HSQC with water-flip-back

$H_2O: z \rightarrow y \rightarrow z \rightarrow y \rightarrow z$  
$\phi_1 = \frac{\Delta}{2}, \phi_2 = \frac{\Delta}{2}$  
$1H$  
$t_1 \rightarrow t_2 \phi_{\text{rec}}$

$1H$  
$15N$  
$Gz$

$\phi_2 = x - x; \phi_{\text{rec}} = x - x$.  
E/AE selection: $\psi = y - y; \kappa = +10/10$.

Suppress radiation damping of $H_2O$ signal during $t_1$

Sensitivity comparison SE vs. WATERGATE/WFB

$I$  
$S$  
$t_1 \rightarrow t_2$

$I$  
$S$  
$t_1 \rightarrow t_2$

$\Delta_1 \sim 1/2J, 1/4J \text{ etc}$  
$\Delta_2 \sim 1/2J$

$S_{I_n} : \sim \sin(\pi J\Delta_1) \cos(\pi J\Delta_1)^n-1$

optimize $\Delta_1$ and $\Delta_2$ independently

$I_{SE} \quad I_{WG/WFB}$

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

$0 \quad 5 \quad 10 \quad 15 \quad 20 \quad 25$

correlation time [ns]

relative intensity

NH, CH

NH$_2$, CH$_2$, CH$_3$

$\Delta_1 \sim 1/2J$
Water-flip-back

**SE HSQC**

**WATERGATE HSQC**

2D: S/N*√2

Tips and tricks for triple-resonance experiments

Multiple INEPT: \( A \rightarrow B \rightarrow C \rightarrow D \rightarrow C \rightarrow B \rightarrow A \)

Impossible to cycle each pulse independently.

instead:

- Adapt sequence to \( J \)-spin system
- Band-selective pulses
- Selection of pathway: ‘difference experiment’
  e.g. \( C \rightarrow D \)
- Cycle 90° pulse where evolution period.
- Remove 180° imperfections: gradients (no sensitivity loss)
- Purge while along \( z \).
- gradient coherence selection in \( t_1 \) only with SE
- Spectral purity higher when gradient selection
Tips and tricks for triple-resonance experiments

Example 1: 3D HNCA: with presaturation and phase cycling

\[ \phi_1 = (y, -y) \]
\[ \phi_2 = [x, x, -x, -x] \]
\[ \phi_3 = (x, x, x, y, y) (-x, -x, -x, -x) (-y, -y, -y, -y) \]
\[ \phi_{rec} = [(x, -x) (-x, x)] [(x, -x) (-x, x)] \]

Example 2: 3D NOESY H\textsubscript{13}C HSQC: purge pulses, zz--fashion

\[ \phi_1 = (x, -x) \quad : \quad \text{axial peak suppression} \]
\[ \phi_2 = [x, x, -x, -x] \quad : \quad \text{select H} \leftrightarrow \text{C transfer} \]
\[ \phi_{rec} = [(x, -x) (-x, x)] \]

Tips and tricks for triple-resonance experiments

Example 3: 3D CT HNCA: gradient selection with sensitivity enhancement and water flip-back

\[ \phi_1 = (x, -x) \quad : \quad \text{select H} \leftrightarrow \text{N transfer} \]
\[ \phi_2 = [x, x, -x, -x] \quad : \quad \text{select N} \leftrightarrow \text{C transfer} \]
\[ \phi_3 = 4(x), 4(-x) \quad : \quad \text{’reduced’ EXORCYCLE} \]
\[ \phi_{rec} = [(x, -x) (-x, x)] \]
Summary

Phase cycling:
changes in coherence order
• difference method (issues with stability)
• several scans required
• receiver gain setting low (every scan detects all signals)
• nucleus selective

Pulsed field gradients:
spatially dependent phase
select particular coherence order ratios
• single scan selection (experiment time depends only on required S/N)
• receiver gain setting high (only signals of interest are detected)
• affects all nuclei (provided they are in transverse plane)