Phase Cycling and Field Gradient Pulses for Coherence Selection in NMR

EMBO Practical Course: Structure Determination of Biological Macromolecules by Solution NMR
July 6 - 13th 2007

Daniel Nietlispach
Department of Biochemistry
University of Cambridge
1. General remarks about NMR experiments
2. Coherence order
3. RF pulses
  3.1 Phase shifting of pulses
4. Coherence transfer pathways
5. Pulse and receiver phase in NMR
6. Basics of phase cycling
   6.1 Selection procedure
   6.2 Selectivity of a phase cycle
7. Frequency discrimination and lineshape requirements in $^n$D experiments
8. Additional considerations for phase cycling
   8.1 180° refocusing pulses
   8.2 Multiple transfer steps
   8.3 Reducing the number of phase cycling steps
   8.4 Axial peak suppression
   8.5 Difference spectroscopy in heteronuclear experiments
   8.6 Advantages and downsides of phase cycling
9. Field gradient pulses
   9.1 How does it work
   9.2 Dephasing by a field gradient
   9.3 How strong do gradients have to be to dephase a coherence
   9.4 Pathway selection using field gradients
   9.4.1 Coherence order selection
   9.4.2 Purging
   9.4.3 180° pulses
9.5 Phase errors
9.6 Heteronuclear experiments
9.7 Sensitivity
   9.7.1 Sensitivity enhancement
10. Practical considerations for biomolecular NMR
   10.1 $H_2O$ samples
   10.1.1 Suppression methods that saturate water
   10.1.2 Non-saturation methods: water flip-back, control of water
   10.1.3 Radiation damping
   10.2 Triple-resonance experiments
11. Summary
1. General remarks about NMR experiments

- 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

Based on this even the simplest \(n\)D experiment will contain an immense range of signals carrying information that has originated on different spin systems and was related via multiple transfer mechanisms to all possible observable spins. The interpretation of such a spectrum would be very difficult and rather confusing. For clarity it becomes therefore necessary to limit the amount of information contained in a spectrum and the development of a particular NMR experiment as such is typically intended to only show a subset of all the possible correlations e.g. only spins that are close in space. This makes it imperative that we can suppress all unwanted pathways. How this can be done will be shown in this chapter.

RF \(t_1 \tau_m\)

\(J, J, \sigma, k_{ex} \ldots\)

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 \(^1\text{H}(^{15}\text{N})\) and \(^1\text{H}(^{13}\text{C})\)?

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
2. 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: \( I_z, 2I_zS_z \) \( p = 0 \)

Multiple-quantum: \( 2I_xS_x \) \( p = \pm 2 \) double quantum

\( p = 0 \) zero quantum

In general:

\[ \text{N spins} \quad \rightarrow \quad \text{coherence order: } -N \ldots 0 \ldots +N \text{ (in integer steps)} \]

Reasoning for this classification:
Different coherences acquire different phases through a \( z \)-rotation (remember: e.g. chemical shift evolution is a \( z \)-rotation due to offset)

Coherence of order \( p \) is described by a density operator \( \sigma^{(p)} \). Evolution of a coherence \( p \) under a \( z \)-rotation of angle \( \phi \) is:

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

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

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

**Raising and lowering operators**

\[
I_+ = I_x + iI_y \quad \quad \quad \quad \quad \quad \quad \quad \quad I_- = I_x - iI_y
\]

\[
I_x = \frac{1}{2}[I_+ + I_-] \quad \quad \quad \quad \quad \quad \quad \quad \quad I_y = -i/2[I_+ - I_-]
\]

Express product operators in terms of raising and lowering operators in order to classify them according to their coherence order. e.g.

\[
2I_xS_z = (I_+ + I_-)S_z
\]

\[
2I_xS_x = 1/2(I_+S_z + I_-S_z + I_xS_z + I_xS_z)
\]

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

\[
\exp(-i\phi F_z) I_z \exp(i\phi F_z) = \exp(\mp i\phi)I_z
\]
3. RF pulses

RF pulses will transfer a particular coherence to all possible coherence orders. There is no selection rule about which transfer is allowed and so many different coherences are generated. On the other hand we know that a particular spin state has to be supported by the spin system otherwise it will not be generated. This somewhat reduces the number of coherences that can be created throughout the pulse sequence. Still the outcome will be rather large.

\[
\begin{align*}
\text{e.g. } I_x & \leftrightarrow DQ & \text{SQ cannot be converted into DQ.} \\
I_x & \rightarrow I_z & \text{Transverse magnetization from equilibrium.} \\
2I_x S_z & \rightarrow DQ, ZQ & \text{Antiphase coherence is transformed into MQ.}
\end{align*}
\]

The outcome of RF pulse conversions are dependent on the flip angle:

\[
I_z \rightarrow I_z \quad \cos \theta I_z + \frac{i}{2} \sin \theta (I_+ - I_-)
\]

Any pulse ≠ 180° generates equal amounts of coherence \(p=\pm 1\)

\[
I_\pm \rightarrow I_\mp \quad \cos^2(1/2\theta) I_+ + \sin^2(1/2\theta) I_- \pm i \sin \theta I_z
\]

an equal transfer to \(I_+\) and \(I_-\) occurs only for a 90° pulse

180° pulse:

reverses the sign of a coherence e.g. \(+1 \rightarrow -1, \quad +2 \rightarrow -2, \quad p \rightarrow -p\)

\[
\begin{align*}
I_+ & \rightarrow I_- \\
I_- & \rightarrow I_+
\end{align*}
\]
3.1 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.

Here is a derivation of this important result:

Assume an RF pulse with phase 0 which transforms coherence of order $p$ into $q$.

What happens if the phase of the pulse changes to $\phi$??

effect of RF pulse phase 0: $U_0 \sigma^{(p)} U_0^{-1} = \sigma^{(q)}$

with phase changed to $\phi$: $U_\phi = \exp(-i\phi F_z) U_0 \exp(i\phi F_z)$

corresponding transformation: $U_\phi \sigma^{(p)} U_\phi^{-1} = \exp(-i\phi F_z) U_0 \sigma^{(q)} U_0^{-1} \exp(i\phi F_z)$

\[= \exp(i\phi) \exp(-i\phi F_z) U_0 \sigma^{(q)} U_0^{-1} \exp(i\phi F_z)\]

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

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

\[= \exp(-i\Delta p \phi) \sigma^{(q)}\]
4. Coherence transfer pathways

Draw out the required coherence order beneath the pulse sequence diagram. Select the pathway(s) (there might be more than one) and indicate the $\Delta p$ values caused by the RF pulses. Finally take also the following points into consideration:

- The pulse sequence has to support the desired pathway
- RF pulses change the coherence order while $p$ does not change during delays
- Sequence starts with equilibrium magnetization $p = 0$
- The observed signal has $p = -1$ (convention)

Homonuclear examples:

- **NOESY**

```
  I_1 | T_{MN} | I_2
  \downarrow p=+1 \downarrow \downarrow \downarrow p=-1
```

- **DQF**

```
  I_1 | T_{d1} | I_2
  \downarrow p=+1 \downarrow \downarrow p=-1
```

- **DQ spectroscopy**

```
  I_1 | T_{d2} | I_2
  \downarrow p=+1 \downarrow \downarrow p=-1
```

Heteronuclear examples:

Draw separate coherence levels for different nuclei

- **HMOC**

```
  \downarrow T_1 | \downarrow A | \downarrow T_2
  \downarrow S_1 \downarrow I_1 \downarrow I_2
  \downarrow p_1 \downarrow 0 \downarrow -1
  \downarrow p_2 \downarrow 0 \downarrow -1
```

$t_1: p_1 + p_2 = \pm 2, 0$ (DQ and ZQ $\Rightarrow$ MQ)
5. Pulse and receiver phase in NMR

The signal phase is determined by the relative phase difference between RF pulses and the receiver. We are only interested in relative phase changes or differences.

Phase difference: 0°  90°  270°  180°
6. Basics of phase cycling

6.1 Selection procedure

Remember: 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 \(-\Delta p \cdot \Delta \phi\).

\[
\begin{align*}
\text{phase:} & \quad 1) \phi_1 \quad \Delta \phi = \phi_2 - \phi_1 \\
\text{RF} & \\
\Delta p = p_2 - p_1
\end{align*}
\]

- Pathways with different \( \Delta p \) will acquire different phase shifts \(-\Delta p \cdot \Delta \phi\)
- Phase shifts acquired by a particular pathway persist through the sequence to the final observable signal
- Change the receiver phase accordingly so that:
  - signals from wanted pathways add up
  - signals from unwanted pathways cancel out

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>

[.] = in multiples of 90°
As expected the signals of the 4 steps add up.

How about a pathway which has $\Delta p = +2$?

<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>

Our phase cycle selects the pathway with $\Delta p = -3$ and rejects $\Delta p = 2$.

- Signal adds up if receiver follows pulse by $-\Delta p \cdot \Delta \phi$
- Total cancellation of other pathways if all steps of the phase cycle are completed

(Wanted signal adds up, even if the cycle is not completed, but suppression of unwanted parts requires the completion of the full cycle. See example above.)
How about a pathway which has $\Delta p = +1$?

<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°</td>
</tr>
</tbody>
</table>

Next to $\Delta p = -3$ our phase cycle also select $\Delta p = +1$.

### 6.2 Selectivity of a phase cycle

In our example the phase cycle selected a $\Delta p$ of −3 and +1 and rejected e.g. −2.

$\Delta p: \quad (-4) \quad -3 \quad (-2) \quad (-1) \quad (0) \quad (1) \quad (2) \quad (3) \quad (4) \quad 5 \quad \ldots$

General rule for the selectivity of a phase cycle:

N step phase cycle with phases: 0, $\Delta$, 2$\Delta$, 3$\Delta$, …, (N-1)$\Delta$

where $\Delta = \frac{360°}{N}$

selects $\Delta p \pm nN$ with $n = 0, 1, 2, 3, \ldots$

The selectivity rule allows us to select more than one pathway at the time. There are certain situations where this is required to maintain maximal sensitivity or to allow frequency discrimination without compromising the lineshape of a signal.
7. Frequency discrimination and lineshape requirements in nD experiments

The choice of the coherence transfer pathway selected during $t_1$ has implications on the lineshape and frequency discrimination in multidimensional NMR experiments. In brief, for best performance we must make sure that absorptive lineshapes and frequency discrimination can be obtained for all indirect dimensions.

Evolution during $t_1$ can be encoded as an amplitude or phase modulated time domain signal.

Amplitude modulation: selection of $p = +1$ AND $-1$ during $t_1$. $(I_z = 1/2(I_z + I_−))$

$$\cos\Omega_1 t_1 \exp(+i\Omega_2 t_2)I$$

after FT: peak at $F_1 = -\Omega_1$, $F_2 = \Omega_2$ and $F_1 = +\Omega_1$, $F_2 = \Omega_2$

no frequency discrimination in $F_1$

absorptive lineshape

Phase modulation: selection of $p = +1$ OR $-1$ during $t_1$.

$$\exp(-i\Omega_1 t_1) \exp(+i\Omega_2 t_2)I$$

after FT: peak at $F_1 = -\Omega_1$, $F_2 = \Omega_2$ N-type (echo) frequency discrimination in $F_1$

$$\exp(+i\Omega_1 t_1) \exp(+i\Omega_2 t_2)I$$

after FT: peak at $F_1 = +\Omega_1$, $F_2 = \Omega_2$ P-type (anti-echo) frequency discrimination in $F_1$

Unfavorable phase-twist lineshape
To obtain frequency discrimination while maintaining pure-phase absorptive lineshapes both coherence pathways ±p need to be kept during t₁. This must be taken into account when writing the phase cycle so that the achieved selectivity agrees with this.

Typically we record cos and sin modulated data sets for every time point in t₁ and store them separately. With these we construct a new data set according to:

\[
\text{time domain data:} \quad \begin{align*}
(1) \cos \Omega_1 t_1 \exp(i \Omega_2 t_2) \\
(2) \sin \Omega_1 t_1 \exp(i \Omega_2 t_2)
\end{align*}
\]

after FT in t₂:

\[
\begin{align*}
(1) \cos \Omega_1 t_1 \{A_2^+ + i D_2^+\} \\
(2) \sin \Omega_1 t_1 \{A_2^+ + i D_2^+\}
\end{align*}
\]

zero imaginary part:

\[
\begin{align*}
(1) \cos \Omega_1 t_1 A_2^+ \\
(2) \sin \Omega_1 t_1 A_2^+
\end{align*}
\]

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

FT in t₁:

\[
\begin{align*}
(A_1^+ + i D_1^+) A_2^+ \rightarrow \text{Re}: A_1^+ A_2^+
\end{align*}
\]

Finally we have a signal that is frequency discriminated and is purely absorptive.

8. Additional considerations for phase cycling

8.1 180° refocusing pulses

A perfect refocusing pulse inverts the order of a coherence from \( p \) to \( -p \). Here phase cycling is typically used to select transverse magnetization and to remove contributions from pulse imperfections. One of the first phase cycling schemes was EXORCYCLE where the 180° pulse cycles \{0 1 2 3\} with the receiver following \{0 2 0 2\}.

The EXORCYCLE scheme selects all symmetrical pathways that start with an odd coherence order. Additional coherences are created through pulse imperfections which are suppressed by EXORCYCLE. Sometimes a reduced 2 step scheme is used (pulse \{0 2\} receiver \{0 0\} or pulse \{0 1\} receiver \{0 2\}). Note that with the shortened version pulse imperfections are only incompletely suppressed. (see chapter 6)
8.2 Multiple transfer steps

To select a particular pathway every pulse needs to be cycled independently. With many pulses in the sequence this soon turns into a very lengthy procedure where more scans may need to be recorded than dictated by the S/N.

\[ \Delta p = \pm 1 \]

\[ \Delta p = \mp 2 \]

To select a particular pathway every pulse needs to be cycled independently. With many pulses in the sequence this soon turns into a very lengthy procedure where more scans may need to be recorded than dictated by the S/N. e.g. \(4^n\) independent steps required for \(n\) pulses (4 step cycle per pulse).

8.3 Reducing the number of phase cycling steps

- **First pulse of the sequence**
  
  Starting with equilibrium magnetization \(\Delta p\) can only be \(\pm 1\).

- **Last pulse before detection**
  
  Creates observable coherence \(p = -1\). If the coherence before the last pulse has already been selected there is no need for further cycling of the last pulse.

- **Keep step number \(N\) small**
  
  Keep number of steps \(N\) small to shorten the phase cycle. Selectivity \(\Delta p \pm nN\). Higher-order MQ are only present if they can be generated by the spin system (large number of \(J\)-coupling partners is unlikely). In many cases \(N = 2\) or \(4\) is enough to achieve sufficient selectivity.

- **Group pulses together**
  
  To reduce the length of the phase cycle we can group together a set of pulses that achieves the overall transformation \(\Delta p\). The phases of all of these pulses need to be changed by \(\Delta \phi\).

The pulse element generates double quantum coherence which is selected by cycling all three pulses together.

\[ \Delta p = \pm 2 \]

pulses: \[0 \ 1 \ 2 \ 3\]
receiver: \[0 \ 2 \ 0 \ 2\]
8.4 Axial peak suppression

Axial peaks originate from magnetization that recovers during the pulse sequence due to e.g. relaxation or ends up oriented along $z$ due to pulse imperfections. As such contributions are not modulated during $t_1$ they appear at $\omega_1 = 0$.

Such components can be removed by cycling of the first pulse and receiver \{0 2\}.

\[
\begin{align*}
\text{pulse phase of first pulse:} & & 0' & & 180' \\
\text{receiver phase:} & & 0' & & 180'
\end{align*}
\]

\[
\begin{array}{c}
\text{wanted signal} \\
\text{unwanted signal}
\end{array}
\]

\[
\begin{align*}
+ & & +
\end{align*}
\]

Taking these different points into account we are now able to come up with suggestions of some minimal length phase cycles for some basic homonuclear experiments.

**NOESY**

\[
\begin{array}{c}
0 & 0 & 0 & 0 \\
1 & 1 & 1 & 0 \\
2 & 2 & 0 & 0 \\
3 & 3 & 0 & 0 \\
2 & 0 & 0 & 2 \\
3 & 1 & 0 & 2 \\
0 & 2 & 0 & 2 \\
1 & 3 & 0 & 2 \\
\end{array}
\]

**DQF COSY**

\[
\begin{array}{c}
0 & 0 & 0 \\
1 & 1 & 2 \\
2 & 2 & 0 \\
3 & 3 & 2 \\
\end{array}
\]

\[
\begin{array}{c}
0 & 0 \\
1 & 3 \\
2 & 2 \\
3 & 1 \\
\end{array}
\]

\[
\begin{array}{c}
0 & 0 & 0 & 0 \\
1 & 1 & 1 & 2 \\
2 & 2 & 2 & 0 \\
3 & 3 & 3 & 2 \\
\end{array}
\]

or

\[
\begin{array}{c}
0 & 0 \\
1 & 3 \\
2 & 2 \\
3 & 1 \\
\end{array}
\]

**DQ spectroscopy**
8.5 Difference spectroscopy in heteronuclear experiments

In combination with isotope labeled samples simple \{0 2\} cycling of heteronuclear pulses to spin \(S\) and the receiver selects for the wanted coherence while canceling the contributions from unlabelled components or from components that are not \(J\)-coupled to \(S\) and thus follow other unwanted pathways that are not affected by the first \(S\) pulse.

\[ \Delta \rho_S = \pm 1 \]

Phase cycle for HMQC

90° \(S\) pulse: phase \{\(x, -x\)\}

receiver: phase \{\(x, -x\)\}

Cycling of the pulse phase \(\phi\) only affects coherences coupled to the \(S\) spin that as such change sign when the phase of the \(I\) pulse is inverted. Signals not coupled to \(S\) are orthogonal to the antiphase term and are cancelled by the difference procedure. Alternatively the same net result can be achieved when the \(S\) pulse phase is cycled \{0 2\}, as shown in the preceding example.

<table>
<thead>
<tr>
<th>(\phi = y)</th>
<th>(\phi = -y)</th>
<th>receiver ((x, -x))</th>
</tr>
</thead>
<tbody>
<tr>
<td>(I_y)</td>
<td>(I_y)</td>
<td>0</td>
</tr>
<tr>
<td>(-2I_zS_y)</td>
<td>(2I_zS_y)</td>
<td>(-2I_zS_y)</td>
</tr>
</tbody>
</table>
8.6 Advantages and downsides of phase cycling

Phase cycling is a difference method where frequently large signals have to be subtracted from each other in order to allow the observation of small residual contributions of the wanted signal. High stability of the spectrometer system is crucial to guarantee good signal cancellation. Variations in experimental reproducibility from scan to scan will lead to imperfect cancellation, spurious signals and increased noise levels. The completion of a phase cycle can take from a few seconds to several minutes. The different groups of phase steps which make up the full phase cycle should therefore be executed in order of decreasing priority. This could be e.g. that we start out a particular phase cycle with a heteronuclear S spin selection 2-step routine which is then followed by a 2-step axial peak suppression which is followed by a 4-step cleanup of a 180° pulse, in this case leading to a total of 16 scans. Analyze the outcome of an experiment not only in terms of the coherences of interest but also focusing on the unwanted terms and make sure that the steps of the phase cycle eliminate them.

The complete suppression of contributions from unwanted pathways requires the completion of all steps of a phase cycle. This can be much longer than what is required by the sensitivity of an experiment and can lead to excessively long experiment times.

On a scan by scan basis the contributions of many pathways are observed. The dynamic range can therefore be very large (e.g. include large solvent signals) requiring to keep the receiver gain low.

Solely using phase cycling for signal selection, solvent suppression or manipulation methods are somewhat limited.

Phase cycling is nucleus selective.

As you will see from the following sections many of these limitations have been removed through the introduction of field gradient pulses. As a matter of fact in most of our NMR experiments a combination of phase cycling and field gradient pulses are used in a complementary way.
9. Field gradient pulses

9.1 How does it work

For a short period of time (sub-ms to ms) the magnetic field $B_0$ in the sample volume area is made inhomogeneous. The inhomogeneity is introduced in a controlled way by the application of an additional magnetic field that varies linearly in the $z$-direction and that is made to be zero in the middle of the sample. The additional field leads to a $z$-rotation of the spins which depends in size on the distance of the position of the spins from the center of the sample. The effect is equivalent to the introduction of a \textbf{spatially dependent phase} so that after a field gradient the transverse magnetization in the active volume of the sample adds up to zero.

The effect from the $z$-rotation is reversible and can be undone by the application of a second gradient of e.g. opposite size and strength. This way the phase encoding from a field gradient can be carried through a pulse sequence before it is undone at a later stage by another gradient. Gradients therefore offer an alternative way to select coherence order. In analogy to the phase evolution due to chemical shift offset the amount of dephasing through a gradient is proportional to the gyromagnetic ration $\gamma$ of a nucleus. This way it becomes not only possible to select particular ratios of coherence but also to select particular combinations of nuclei. This is particularly useful for the suppression of very intense signals e.g. solvent.

![Probe head with a field gradient coil arrangement](image)

left: No gradient is applied and the entire sample volume feels the effect of the highly uniform main field $B_0$. NMR signals are thus very sharp (Hz). Due to their offset they appear over a range of a few ppm (a few thousand Hz).

right: The presence of an additional field gradient $B_G$ makes the magnetic field inhomogeneous. The additional $z$-rotation due to the gradient gives spins in different parts of the sample different frequencies. Typically these will cover hundreds of kHz making the signal after a gradient unobservably broad.
Introduction of a spatially dependent phase through the application of a field gradient. Dephasing of magnetization in the transverse plane: We can think of the sample as split into thin slices. The additional field introduced by the gradient varies as we move along the z-axis but within each of the slices spins feel the same field. The stronger the gradient the thinner the slices would become. Provided that the slices are thin enough this will eventually lead to cancellation of the magnetization over the entire sample volume. At the end of the gradient the coherence over the total sample volume is completely dephased and is unobservable.

Rephasing of magnetization through a gradient of equal strength but opposite sign. After an additional spatially dependent phase of opposite sign the originally dephased signal becomes again observable. Strictly speaking this is only true provided the spins stay in the same slice and don’t change their position along the z-axis in the time between the two gradient pulses. (In other words, gradients provide an excellent means to assess translational diffusion.)
### 9.2 Dephasing by a field gradient

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

\[ B_0: \text{ main field} \]
\[ Gz: \text{ spatially dependent part of field} \]

\[ \gamma B_z = \gamma B_0 + \gamma Gz \]
\[ \text{this is the spatially dependent Larmor frequency} \]

\[ \omega_z = \omega_0 - \gamma Gz \]
\[ \omega_0: \text{ evolution due to main field} \]

\[ \Omega_z = -\gamma Gz \]
\[ \text{spatially dependent part of frequency} \]

In analogy to the evolution of coherence due to the shift offset we can describe the effect due to the spatially dependent frequency. The effect of a gradient of length \( t \) is:

\[ I_\pm \rightarrow I_\pm \exp(i\Omega_z t) \]
\[ \phi_z = \pm \Omega_z t \]

With the gradient strengths typically used (0-50 Gauss/cm) the spatially dependent part of frequency is much bigger than the frequency due to the chemical shift offset (100’s of kHz vs a few thousand Hz). With this, it can be safely assumed that the spatial encoding is independent of the offset and just depends on the coherence order. With this the dephasing of e.g. DQ coherence is:

\[ I_{1,2} \rightarrow I_{1,2} \exp(-i2\Omega_z t) \]

To generalize this result:

The spatially dependent phase acquired by a coherence of order \( p \) is:

\[ \phi_{z\tau} = s \cdot p \cdot \gamma G z \tau \]

\( s: \text{ shape factor} \)
\( p: \text{ coherence order} \)
\( \gamma: \text{ gyromagnetic ratio} \)
\( G: \text{ gradient strength} \)
\( \tau: \text{ duration of gradient} \)

shape factor:

Despite the less efficient dephasing of sinebell shaped gradients their smooth on/off switching properties makes them in general superior to e.g. square gradients.
9.3 How strong do gradients have to be to dephase a coherence

We assume that the extra field varies linearly along the z-direction and that the sample volume extends from +0.5z to –0.5z, with zero dephasing occurring in the middle of the sample.

A coherence is dephased according to: \( I \rightarrow I \exp(i\gamma Gzt) \)

Since the phase of the signal varies with its position along z, we need to integrate the different signal contributions along the z-direction in order to obtain the total observable signal of the sample.

\[
\text{signal}(t) = \int_{-0.5z}^{+0.5z} \exp(i\gamma Gzt) dz
\]

After integration and normalization we can see that the total signal dephases with a \( \sin x/x \) function:

\[
\text{signal}(t) = \frac{\sin(0.5\gamma Gzt)}{0.5\gamma Gzt}
\]

The oscillations of the function decay with 1/x, where x=0.5\( \gamma Gzt \)

\[
\text{signal}(t) \sim \frac{2}{|\gamma| Gzt}
\]

\( G \): gradient strength (G/cm or Tesla/m)
\( z \): active window (e.g. 2 cm)
\( t \): duration of gradient (ms)
\( g \): gyromagnetic ratio (\(^1\text{H}: 26.75 \times 10^7 \text{T}^{-1}\text{s}^{-1})

Example: Gradient of 25 G/cm and 1 ms duration:

\( G \): 25 G/cm = 0.25 T m\(^{-1} \)
\( z \): 0.02 m
\( t \): 10\(^{-3}\) s

The gradient reduces the intensity of a proton coherence \( p=\pm 1 \) by a factor of 670x. \(^{15}\text{N} \) transverse magnetization on the other hand will only be reduced by a factor of 67x, leaving some residual signal on the order of 1.5%.
9.4 Pathway selection using field gradients

9.4.1 Coherence order selection

\[ \sum_{i} \phi_i = 0 \]
\[ \phi_i = s_i p_i \gamma_i G_i \tau_i \]

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 \( G_2 = 2G_1 \)

refocusing of \( p_I \rightarrow p_S \): \( \phi_I + \phi_S = 0 \)
\[ \frac{s_I G_I \tau_I}{s_S G_S \tau_S} = \frac{p_S}{p_I} \]

e.g. \( I = ^1H, S = ^13C \)

for refocusing of \( +1(I) \rightarrow -1(S) \):
\[ \tau_S = 2\tau_I, G_S = -2G_I \]
\[ \text{or} \]
\[ \tau_S = \tau_I, G_S = -4G_I \]

etc
\[ \sum_i s_i p_i \gamma_i G_i \tau_i = 0 \]

There are many combinations of \( G_i \) and \( \tau_i \) which allow the selection of this pathway.

**Multiple pathways**

It is impossible to select both pathways at the same time. A particular gradient pair can only select one pathway at a time. We loose 50\% of the signal through the selection procedure.

Frequently we would like to retain both pathways e.g. in order to achieve pure-phase frequency discrimination in nD experiments.

### 9.4.2 Purging

Coherence of order \( p = 0 \) is not affected by a gradient and so cannot be dephased. e.g. \( I_z, 2I_zS_z \). Strictly speaking therefore we cannot select \( p = 0 \). Instead we choose a position in the pulse sequence where we can store the wanted coherence along \( z \) while keeping all the unwanted terms still in the transverse plane. Execution of a strong gradient will then dephase all the terms except the ones kept along the \( z \)-axis. Such gradients can easily be accommodated as part of INEPT transfers or during a NOE mixing period. We call them purge or crusher gradients.
9.4.3 180° pulses

**Refocusing:**

<table>
<thead>
<tr>
<th>RF</th>
<th>δ</th>
<th>180°</th>
<th>δ</th>
</tr>
</thead>
<tbody>
<tr>
<td>g</td>
<td>τ</td>
<td></td>
<td>τ</td>
</tr>
<tr>
<td>p</td>
<td></td>
<td></td>
<td>−p</td>
</tr>
</tbody>
</table>

ideal 180° pulse: changes sign of coherence $p \rightarrow −p$ : rephased

Both symmetrical pathways are selected!

imperfect 180°: $p \rightarrow \neq −p$ : dephased

**Inversion:**

<table>
<thead>
<tr>
<th>RF</th>
<th>δ</th>
<th>180°</th>
<th>δ</th>
</tr>
</thead>
<tbody>
<tr>
<td>g</td>
<td>τ</td>
<td></td>
<td>τ</td>
</tr>
<tr>
<td>$ρ_p=0$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$ρ_2$</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

ideal 180°: $z \rightarrow −z$  $p=0$
imperfect 180°: $z \rightarrow$ others dephased

note: since $S$ is transverse the two gradients must have opposite signs.

9.5 Phase errors

Gradient durations are on the order of ms. Chemical shift and $J$-coupling evolution during the gradients leads to phase errors. To avoid this make sure to accommodate the gradients in existing delay periods or during spin-echo periods. Often spin-echo periods exist already in pulse sequences so that no additional pulses and delays are required to accommodate a gradient.

<table>
<thead>
<tr>
<th>RF</th>
<th>180°</th>
<th>180°</th>
</tr>
</thead>
<tbody>
<tr>
<td>g</td>
<td>τ</td>
<td>τ</td>
</tr>
</tbody>
</table>

single gradient: offset evolution is refocused

<table>
<thead>
<tr>
<th>RF</th>
<th>180°</th>
<th>180°</th>
</tr>
</thead>
<tbody>
<tr>
<td>g</td>
<td>τ/2</td>
<td>τ/2</td>
</tr>
</tbody>
</table>

split gradient: offset evolution is refocused

The same amount of dephasing is achieved in half the time.

Coherence selection gradient pair that is accommodated during existing constant-time periods or refocusing delays.
9.6 Heteronuclear experiments

**HSQC with gradient selection**

excellent solvent suppression through selection of heteronuclear coherence order.
e.g. for a $^1$H/$^{13}$C correlation

**I/S single quantum coherence order selection**

**HSQC with purge pulses ($zz$-HSQC)**

solvent suppression is acceptable

**zz-periods with purge pulses**

**HMOC with gradient selection**

**I/S coherence order selection**


9.7 Sensitivity

We need to record our nD data in such a way that it is frequency discriminated and gives absorptive lineshapes in all indirect dimensions. This can be achieved following several sets of different recipes which have implications on the experimental sensitivity.

Data: (1) Collect amplitude modulated data in $t_1$. Get separate cos and sin modulated data sets.

or

(2) Collect separate P- and N-type data in $t_1$. Combine to construct 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 coherence order selection

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

additional processing: construct cos and sin modulated data

$\cos: S_c(t_1,t_2) = 0.5 \{ S_P(t_1,t_2) + S_N(t_1,t_2) \}$

$\sin: S_s(t_1,t_2) = -i \{ 0.5 [ S_N(t_1,t_2) - S_P(t_1,t_2) ] \}$

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

This is frequently used in heteronuclear experiments and relies on the conversion of both orthogonal components that are shift modulated in $t_1$ into observable magnetization. It allows to improve the sensitivity of experiments that use gradient coherence order selection.

Transfer of both orthogonal shift labelled components ($x$ and $y$):

\[
\ldots \rightarrow t_1 \rightarrow 2S_xI_z \cos \Omega t_1 + 2S_yI_z \sin \Omega t_1 \rightarrow 90^\circ_y (S) \rightarrow 90^\circ_x (S)
\]

\[\text{HSQC} \quad \text{P-} / \text{N-type data}\]

For smaller proteins sensitivity enhanced experiments combine gradient selection and improved signal-to-noise. Best performance is achieved for IS 2-spin systems where in the absence of relaxation the theoretical enhancement of $\sqrt{2}$ can be achieved.
10. Practical considerations for biomolecular NMR

10.1 H$_2$O samples

The concentration of water protons is ca. 100000x that of an amide proton in a 1 mM protein sample therefore it is necessary to dramatically reduce the intensity of the water signal to be able to observe the protein resonances. There are many ways how the intensity of the water signal can be reduced. Not all methods are equally well suited and all have some strengths and weaknesses so that commonly we need to find conditions which lead to acceptable solvent suppression without compromising the performance of the particular NMR experiment. There are a few simple considerations to make when choosing a method. e.g. The achievable suppression factor should be as high as possible and should remain robust during all increments of an NMR experiment. The suppression method should not reduce the sensitivity per unit time of the experiment. The water suppression method should not bleach signals of interest in particular those which are underneath the water or should not cause saturation transfer etc etc. In the following sections a few suppression techniques and features related to the presence of the water signal in the NMR sample are discussed.

10.1.1 Suppression methods that saturate water

- **Saturation using a weak RF field:**
  - signal bleaching
  - saturation transfer
  - exchangeable protons are attenuated

- **Gradient coherence order selection in heteronuclear experiments:**
  - very efficient suppression due to $\gamma_I \neq \gamma_S$
  - water is saturated before the detection
  - exchangeable protons are attenuated but increase in recycle time can reduce saturation level of water
  - suitable for non-exchangeable protons. $^1$H/$^{13}$C correlation experiments
• **Watergate and excitation sculpting** (DPFGSE) gradient dephasing methods:
  - can be highly frequency selective
  - saturation method
  - bleaching (selective pulses)

![Watergate and DPFGSE diagrams](image)


10.1.2 Non-saturation methods: water flip-back, control of water

Field gradient methods are very efficient at removing any water signal but since they obviously act through dephasing of the water this results in the saturation of the solvent signal. In return this leads to the attenuation of labile protons that are in chemical exchange with the saturated water. The concomitant loss in sensitivity is particularly pronounced around neutral pH in experiments that involve amide protons.

It turns out that the better approach is not to dephase/saturate the water but instead to return the water magnetization in a controlled way back to the z-axis where it does not contribute to the observable signal. This can be achieved through insertion of frequency selective pulses to the water resonance at pivotal points in the pulse sequence. The selective RF pulses make sure that the water is always put along z before a field gradient is applied so that no saturation can occur (Grzesiek and Bax J. Am. Chem. Soc. 1993, 115, 12593). We say that we control the water. Residual amounts of water that are left in the transverse can successfully be removed with a watergate pulse element before the acquisition. Following this recipe typically less than 5% of the water signal is saturated.

A second advantage of not saturating the water resonance comes from the faster recovery of longitudinal magnetization. Chemical exchange with the huge water component along the z-axis increases the proton recovery rate and so allows for faster repetitions resulting in increased sensitivity per unit time.
Sensitivity comparison of $^{15}$N HSQC experiments using different water manipulation schemes

Sample pH = 7.3

Water saturation using a weak RF field leads to strong signal attenuation.

P/N-type gradient coherence order selection dephases water. Saturation is less severe than when using presaturation leading to slightly improved sensitivity.

Selective flip-back pulses and the watergate element are used to control the water along the z-axis. Due to the low saturation level of the solvent the sensitivity is much improved.
Effect of water saturation on the rate of recovery of $^1$H$_N$ longitudinal magnetization

![Graph showing the effect of water saturation on the rate of recovery of $^1$H$_N$ longitudinal magnetization.](image)

- In the absence of exchange:
- With $\mathrm{H}_2\mathrm{O}/\mathrm{H}_N$ exchange:
  - % non-saturated $\mathrm{H}_2\mathrm{O}$
  - $(R_{\text{ex}}=10 \text{ Hz}, R_1(\mathrm{H}_N)=1.0 \text{ Hz},\ R_1(\mathrm{H}_2\mathrm{O})=0.3\text{Hz})$


Sensitivity as a function of the repetition rate: saturation versus non-saturation

1D $^{15}$N HSQC with RF presaturation

1D zz $^{15}$N HSQC with flip-back and watergate
Examples of $^{15}$N HSQC using different water control schemes

- **zz-HSQC with flip-back pulses and watergate**

- **P-/N-type gradient coherence selection (with flip-back pulses)**

- **Sensitivity enhanced with gradient coherence selection**

- **zz-HSQC with water-dephase/rephase and watergate**

10.1.3 Radiation Damping

The intense signal of the solvent can induce an oscillating current in the coil which accelerates the return of the transverse component of its magnetization towards the $+z$-axis acting in principle similar to a frequency selective pulse to the water resonance. The additional spin life time contribution due to radiation damping is:

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

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

The effect becomes bigger at higher magnetic field and leads to increasingly faster recovery of water and other signals that are in chemical exchange with the solvent. Such signals show therefore typically increased linewidths (short "$T_2$'s") in indirect $^1$H dimensions (e.g. NOESY spectra). Additionally as recovery of magnetization is very rapid the water departs very quickly from the original position it is left in, so that cumulative effects of RF manipulations become increasingly difficult to predict and can vary in their outcome throughout the $t_1$ incrementation.

It turns out that radiation damping can practically be removed by keeping the size of the bulk magnetization small in order to reduce back-coupling to the coil. There are several ways how this can be achieved and one simple way is the use of a weak split field gradient (dephase/rephase) which is applied during periods where the water magnetization is in the transverse. Water is so continually dephased followed by rehasing before the next RF pulse to $^1$H. Such a scheme can be incorporated in incremented fashion for every time point during the $t_1$ evolution.

Alternatively radiation damping can be used in a constructive way by allowing the complete recovery of the water signal to the $+z$-axis within little time (10-50ms) as e.g. during a NOE mixing period.

Time course showing the return of transverse magnetization due to radiation damping after excitation by a 90° pulse. Left: free radiation damping with 1ms increments. Right: control of damping by a time incremented dephase-rephase gradient with 5ms increments.
Effect of radiation damping following an initial 180° pulse applied to equilibrium magnetization. Left: 10 ms increments. After a slow induction period as soon as some transverse components are present recovery becomes increasingly fast. Right: 20 ms increments of dephase–rephase gradient (2 G/cm). The effect of radiation damping is stopped and the recovery of magnetization occurs with the true $T_1$ of water.

In some situations the use of water selective pulses is undesirable and the magnetization has to stay in the transverse. In spin-echo periods gradients are often used to clean up pulse errors. At the same time they can reduce the effects of radiation damping. For this it is of advantage to keep the gradient pair as widely separated as possible.

is better than
10.2 Triple-resonance experiments

Triple-resonance experiments have many coherence transfer steps A ➞ B ➞ C ➞ D ➞ C ➞ B ➞ A e.g. of the type INEPT so that it is impossible and not sensible to phase cycle all pulses. At first glance this may seem to lead to a fundamentally flawed situation but luckily it turns out that we can take advantage of several additional features which help to select a pathway even if we phase cycle only a few selected pulses and use some well positioned field gradient pulses.

Firstly we design a pulse sequence with a particular spin-system in mind. As the J-coupling values are known and normally lie within a well defined narrow range (at least for proteins) we can channel our magnetization through the desired coherence pathway while limiting to a certain extent the generation of unwanted coherences.

This is further aided by the use of band-selective shaped pulses on $^{13}$C which have better off-resonance behavior and split the spectral range into several ‘heteronuclear’ regions. Independent manipulation of several homonuclear J-couplings even simultaneously becomes so possible while the generation of disturbing MQ-coherences can be prevented.

Once this is in place a particular pathway will be selected using a simple ‘difference experiment’ procedure by 2-step cycling one of the 90° pulses of one of the later transfer steps. e.g. C ➞ D. If required this can be repeated for other coherence transfer steps. In each of the steps 90° pulse and receiver are cycled \{0 2\} (for $\Delta p = \pm 1$). The further away from the start a cycled transfer step is the less likely it becomes that unwanted spin systems can support the coherence transfer pathway. Spin systems which can only follow part of the pathways are so successfully eliminated.

In addition we make sure to cycle one of the 90° pulses which surrounds an evolution period. Axial peaks can be shifted through inversion of this pulse together with the receiver for every $t_1$ increment (e.g. States-TPPI).

While 180° pulses are prone to imperfections these can easily be removed when surrounded by a pair of gradients. This comes at no sensitivity loss.

If gradients are used for coherence order selection, remember the implications on sensitivity. Try to use gradient coherence order selection together with sensitivity enhancement methods. Typically this relegates the selection gradient to the end of the last coherence transfer step. As dephase and rephase gradients are so close together this minimizes also losses from translational diffusion.

Decide on the fate of the water signal. Saturate (presat, gradients or both) in the case of HCCH COSY, TOCSY, NOESY experiments. Use flip-back for all HCN & HN experiments involving amide protons. Don’t use flip-back pulses if aliphatic regions are of interest.

Here are a few simple examples to illustrate the points made:
3D HNCA: with presaturation and phase cycling

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

Water saturation makes this experimental implementation only useful at low pH where water exchange is slow. The first phase cycling step \( \phi_1 \) helps with the water suppression. Step two \( \phi_2 \) selects for \( ^{13}\text{C} \) that was selected via \( ^{15}\text{N} \). Step three \( \phi_3 \) is an Exorcycle scheme which functions here to select \( ^{15}\text{N} \) SQ coherence and to help cleaning up the 180° pulse. (note: Cx and CO 180° pulses are band-selective. see notes Sattler, Griesinger)

3D NOESY \( ^{13}\text{C} \) HSQC: purge pulses, zz--fashion

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

Phase cycling is used for axial peak suppression and \(^1\text{H}, ^{13}\text{C}\) selection. Unwanted coherence is destroyed in zz-fashion by purge pulses. Gradient pairs clean-up 180° and help water suppression. The gradients during the mixing time remove antiphase terms and transfer through cross-correlation.
3D CT HNCA: gradient selection with sensitivity enhancement and water-control

\[ \phi_1 = (x, -x) \quad \text{select } H \rightarrow N \text{ transfer} \]
\[ \phi_2 = [x, x, -x, -x] \quad \text{select } N \rightarrow C \text{ transfer} \]
\[ \phi_3 = 4(x), 4(-x)'\text{reduced}' \text{ Exorcycle} \]
\[ \text{rec} = [(x, -x) (-x, x)] \]

The gradient pair \( G_7/G_{10} \) selects in interleaved manner for \(^1\text{H}/^{15}\text{N} \) P- and N-type coherence order through alternation of the sign of gradient \( G_z \). The additional gradient pairs \( G_2, G_3, G_5, G_9 \) clean-up 180° pulses. \(^1\text{H} \) pulses are applied with phases that allow the return of water to the z-axis before the application of gradients or the signal detection. Note that during \( G_z \) \(^1\text{H} \) composite decoupling should be interrupted, otherwise this will result in the water being dephased. \( G_3 \) and \( G_4 \) are applied while the wanted term is stored as \( 2I_zS_z \). The first \(^{15}\text{N} 90° \) pulse and \( G_1 \) eliminate the \(^{15}\text{N} \) equilibrium contribution. (Note that the use of a \(^{13}\text{C} \) CT period in this experiment may require \(^2\text{H} \) labeling and \(^2\text{H} \) decoupling to improve experimental sensitivity.)
11. Summary

**Phase cycling:**
Vary phases of pulses and receiver so that signals from desired pathways add up while the signals from unwanted pathways cancel out. Phase cycling selects changes in coherence order.

- difference method (issues with stability)
- several scans
- dynamic range is limited (per scan, all signals are detected)
- nucleus selective

**Field gradient pulses:**
Short periods where magnetic field is made inhomogeneous in order to dephase coherences, leading to spatial encoding. The effect is reversible and can be used to select particular coherence order ratios.

- single scan selection (experiment time according to required signal-to-noise)
- high dynamic range (only the signals of interest are detected)
- affects all nuclei
Further reading:

• For a general review on pulsed field gradients:

• gradient selection, sensitivity enhancement, sensitivity comparison:

• sensitivity comparison of different triple-resonance implementations: