5 Heteronuclear Correlation Spectroscopy

H,C-COSY

We will generally discuss heteronuclear correlation spectroscopy for $X = ^{13}$C (in natural abundance!), since this is by far the most widely used application. However, all this can also be applied to other heteronuclear spins, like $^{31}$P, $^{15}$N, $^{19}$F, etc..

In the heteronuclear case, there are some important differences that allow to introduce additional features into the NMR spectra:

- all heteronuclear coupling constants $^1J(^1H,^{13}C)$ are very similar, ranging from ca. 125 Hz (methyl groups) up to ca. 160 Hz (aromatic groups) in contrast to the homonuclear couplings $^2J(^1H,^1H)$ and $^3J(^1H,^1H)$, which can differ by more than an order of magnitude (ca. 1 Hz - 16 Hz). This feature allows to adjust delays for coupling evolution to pretty much their optimum length for all signals.

- r.f. pulses on $^1$H and $^{13}$C can (and actually must!) be applied separately, due to the very different resonance frequencies for different isotopes. Thus, $^1$H and $^{13}$C spins can, e.g., be flipped separately, resulting in refocussing of the heteronuclear coupling. For the same reason, heteronuclear decoupling can also be applied during the acquisition time.

The basic COSY sequence can be readily extended to the heteronuclear case.

\[ \begin{align*}
  ^1H & \quad y \quad t_1 \quad x \\
  ^{13}C & \quad y \quad t_2
\end{align*} \]

Again, during $t_1$ proton chemical shift $\Omega_1$ evolves, as well as heteronuclear coupling $J_{IS}$ will evolve (following the quite illogical convention, we will use $I – insensitive$ – for the proton spins and $S – sensitive$ – for the heteronucleus, i.e., $^{13}$C).
For the simplest case, an I–S two-spin system, we get the following evolution (only shown for the relevant term that will undergo coherence transfer during the 90° pulse pair after $t_1$, i.e., $2 I_y S_z$):

\[
\begin{align*}
90°_y (I) & \quad t_1 \\
I_z & \longrightarrow I_x \longrightarrow 2 I_y S_z \cos (\Omega_I t_1) \sin (\pi J_{IS} t_1) \\
90°_x (I), 90°_y (S) & \quad t_2 \\
& \longrightarrow 2 I_z S_x \cos (\Omega_I t_1) \sin (\pi J_{IS} t_1) \longrightarrow \ldots
\end{align*}
\]

The transfer function is the same as for the $^1$H, $^1$H-COSY. We will get modulation in F1 (from the $t_1$-FT) with the proton chemical shift $\Omega_I$ and the heteronuclear coupling $J_{IS}$, and the coupling is antiphase. Also, in F2 (from the data acquisition during the $t_2$ period) we will get the carbon chemical shift (since we do now have a carbon coherence, $2 I_z S_y$), and it is also antiphase with respect to $J_{IS}$. We will therefore get a signal which is an antiphase doublet in both the $^1$H and $^{13}$C dimensions, split with the $^1$JHC coupling.

However, in the heteronuclear case, we can greatly improve the experiment by decoupling. Depending on the presence or absence of 180° pulses, we can choose to refocus or evolve chemical shift and/or heteronuclear coupling: chemical shift evolution is refocussed, whenever a 180° pulse is centered in a delay. For the refocussing of heteronuclear coupling, the “relative orientation” of the two coupling partners must change, i.e., a 180° pulse be performed on one of them (cf. table).

All these results can be verified by product operator calculations – a good exercise! By inserting a 180° pulse on $^{13}$C in the middle of our $t_1$ period, we can decouple the protons from $^{13}$C, so we won’t get $J_{IS}$ evolution during $t_1$, won’t get a $\sin (\pi J_{IS} t_1)$ modulation and hence no antiphase splitting in F1 after FT, but instead just a singulett at the proton chemical shift frequency.
**(of course, chemical shift evolution of $^1$H or $^{13}$C occurs only when this spin is in a coherent state)**

_Heteronuclear decoupling_ can also be performed during the _direct acquisition_ time. This is done by constantly transmitting a $B_1$ field at the $^1$H frequency. This causes transitions between the $\alpha$ and $\beta$ spinstates of $^1$H (or, rotations from $z$ to $-z$ and back, about the axis of the $B_1$ field). If the rate of these $^1$H spin flips is faster than $J_{IS}$, then heteronuclear coupling will be refocussed before it can develop significantly, and no $J_{IS}$ coupling will be observed. In praxi, heteronuclear decoupling is performed by using – instead of a continuous irradiation – composite pulse sequences optimized for decoupling behaviour, which allow to effectively flip the $^1$H spins over a wide range of chemical shifts with minimum transmitter power, similar to the spinlock sequences used for TOCSY. Some popular decoupling sequences are, e.g., WALTZ or GARP.

The use of decoupling sequences “freezes” spin states with respect to the heteronuclear coupling, i.e., in-phase terms like $S_x$ will stay in-phase and induce a signal in the receiver coil corresponding to a singulet (after FT). Antiphase terms like $2 I_z S_x$ will stay antiphase, won’t refocus to in-phase terms and will not be detectable at all!
With this knowledge, we can remove the heteronuclear coupling from both the F1 and F2 dimension of the H,C-COSY experiment, by decoupling during \( t_1 \) and \( t_2 \):

\[
\begin{align*}
{^1}H & \quad \Delta_1 \quad \Delta_2 \quad \text{decoupl.} \\
{^{13}C} & \quad t_1 \quad \Delta_1 \quad \Delta_2 \\
\end{align*}
\]

Since heteronuclear coupling cannot evolve during \( t_1 \), but we do need a heteronuclear antiphase term for the coherence transfer, we have to insert an additional delay \( \Delta_1 \) before the 90° pulse pair. Also, we need to refocus the carbon antiphase term (after the coherence transfer) to in-phase coherence before acquiring data under \(^1\)H decoupling, which is done during \( \Delta_2 \).

This pulse sequence will give a singulet cross-peak in both dimensions. However, we will also have chemical shift evolution during the two coupling evolution delays \( \Delta_1 \) (\(^1\)H chemical shift) and \( \Delta_2 \) (\(^{13}\)C chemical shift), which will scramble our signal phases in both dimensions, so that we have to process this spectrum in absolute value mode.

We can avoid this be introducing a pair of 180° pulses in the two coupling evolution delays. As shown before, this will not interfere with the \( J_{IS} \) evolution, but refocus chemical shift evolution:

\[
\begin{align*}
{^1}H & \quad \Delta_1 \quad \Delta_2 \quad \text{decoupl.} \\
{^{13}C} & \quad t_1 \quad \Delta_1 \quad \Delta_2 \\
\end{align*}
\]

In this version, the evolution of \(^1\)H chemical shift (during \( t_1 \)) and \(^{13}\)C chemical shift (during \( t_2 \)) are completely separated from the evolution and refocussing of the heteronuclear coupling (during the delays \( \Delta_1 \) and \( \Delta_2 \)):

\[
\begin{align*}
90°_y \; (I) & \quad t_1 \quad \Delta_1 \\
I_z & \rightarrow I_x \rightarrow 2I_z S_z \cos (\Omega_I t_1) \rightarrow 2I_y S_z \cos (\Omega_I t_1) \sin (\pi J_{IS} \Delta_1) \\
90°_x \; (I), \; 90°_y \; (S) & \rightarrow \rightarrow \rightarrow \rightarrow \\
2I_z S_z \cos (\Omega_I t_1) \sin (\pi J_{IS} \Delta_1) & \rightarrow S_y \cos (\Omega_I t_1) \sin (\pi J_{IS} \Delta_1)
\end{align*}
\]
After FT, we get a 2D $^1$H,$^{13}$C correlation spectrum with each cross-peak consisting of a single line, with uniform phase. The factor $\sin (\pi J IS \Delta_I)$ does not contain a $t_1$ modulation (which would lead to a dublet in F1), but merely a constant, which can be maximized by setting $\Delta_I = \frac{1}{2J}$.

Actually, the sequence can be written more elegantly, by combining the two $^{13}$C 180° pulses into a single pulse. Instead of first refocussing the evolution during $t_1$, and then during $\Delta_1$, one can accomplish the same result with a single 180° pulse in the center of $(t_1 + \Delta_1)$:

$$
\begin{array}{c}
t/2 \\
t/2 \\
\Delta/2 \\
\Delta/2
\end{array}
= \begin{array}{c}
t/2 + \Delta/2 \\
t/2 + \Delta/2
\end{array}
$$

This saves us one 180° pulse! No big deal? - well, no pulse is perfect, and this is not only due to sloppy pulse calibration, but even inherent in the pulse: with limited power from the transmitter, our pulse has a finite length (usually $\geq 20 \mu$s for a $^{13}$C 180° pulse). This means, however, that its excitation bandwidth is also limited (cf. the FOURIER pairs), and that the effective flip angle for a “180° pulse” (on resonance) will drop significantly at the edges of the spectral window! This causes not only a decrease of sensitivity, but also an increase of artifacts.

Example: For a 20 µs 180° on-resonance pulse (i.e., 25 kHz $B_1$ field), one gets at $\pm 10,000$ Hz offset (= 80 ppm for $^{13}$C at a 500 MHz spectrometer) an effective flip angle of ca. 135° – which means that instead of going from $z$ to $-z$ (clean inversion), one gets equal amounts of $-z$ and $x,y$ magnetization.

The best pulse sequence for a H,C-COSY spectrum is therefore the following:
An analysis of the rather complicate delays can be quickly done: after the first 90° pulse, $^1$H chemical shift will evolve during $(\Delta_1/2 + t_1/2 + t_{1}/2)$ (the 180° carbon pulse does not affect $^1$H chemical shift evolution!). However, the following 180° proton pulse “reverses” the chemical shift evolution then, and it “runs backwards” during the last part, so that $^1$H chemical shift evolution occurs during $(\Delta_1/2 + t_1/2 + t_{1}/2 - \Delta_1/2) = t_{1} \cdot \Delta_1$.

Evolution of the heteronuclear coupling will also start immediately after the creation of $^1$H coherence and continue during $(\Delta_1/2 + t_1/2 - t_{1}/2 + \Delta_1/2) = \Delta_1$ (coupling evolution is “reversed” by each 180° pulse, on either one of the two coupling spins!).

So, again the chemical shift will only evolve during $t_1$ (and turn up as chemical shift frequency after FT), *not* during $\Delta_1$, and we can easily optimize the delay $\Delta_1 = 1/2J_{IS}$, since $J_{IS}$ evolves only during this delay, *not* during $t_1$.

So far we have limited ourselves to simple I$^S$ two-spin systems. In reality, however, more than one proton can be directly bound to a carbon nucleus: CH / CH$_2$ / CH$_3$. As long as we “are on proton” (i.e., we have a $^1$H coherence), this doesn’t make a difference: each proton is always coupled to just a single carbon ($^{13}$C). However, after the coherence transfer onto $^{13}$C, the carbon couples simultaneously to 1-3 protons (with the same $^1J$ coupling constant).

Let’s look at the refocussed INEPT INEPT (*Insensitive Nuclei Enhancement Polarization Transfer*) sequence, which is the 1D equivalent of our H,C-COSY sequence (i.e., without $t_1$ period): it starts with the creation of $^1$H coherence, the $J_{IS}$ evolves during $\Delta_1$ ($^1$H chemical shift is refocussed), and the resulting antiphase term undergoes a coherence transfer onto $^{13}$C with the 90° pulse pair.
We can easily optimize $\Delta_1$ by setting it to $1/2J_{IS}$, so that the sine factor will be 1 for all $^{13}$C-bound protons. However, once we do have a carbon antiphase coherence and try to refocus it, we have to deal with all protons directly bound to the same carbon:

- for a CH group:

$$\Delta_2$$

$$2 I_z S_x \rightarrow 2 I_z S_x \cos (\pi J_{IS} \Delta_2) + S_y \sin (\pi J_{IS} \Delta_2)$$

(shown in bold face is the detectable in-phase term, antiphase terms cannot be observed under $^1$H decoupling during the acquisition period $t_2$)

- for a CH$_2$ group:

we now have two (equal) couplings, $J_{IS}$ and $J'_{IS}$, to the two $^1$H spins I and I’:

$$\Delta_2$$

$$2 I_z S_x \rightarrow 2 I_z S_x \cos (\pi J_{IS} \Delta_2) \cos (\pi J'_{IS} \Delta_2) + S_y \sin (\pi J_{IS} \Delta_2) \cos (\pi J'_{IS} \Delta_2)$$

$$+ 2 I_z S_y I'_z \cos (\pi J_{IS} \Delta_2) \sin (\pi J'_{IS} \Delta_2) + 2 S_x I'_z \sin (\pi J_{IS} \Delta_2) \sin (\pi J'_{IS} \Delta_2)$$

In order to end up with detectable in-phase terms, we have to refocus the antiphase coupling $J_{IS}$ and not evolve the other coupling $J_{IS}$!

- for a CH$_3$ group:

similar to the CH$_2$ case, we can only get in-phase $^{13}$C magnetization, if we refocus the antiphase coupling to the first proton I and not evolve the two other couplings $J'_{IS}$ and $J''_{IS}$ to the other two methyl protons,

$$\Delta_2$$

$$2 I_z S_x \rightarrow \cdots S_y \sin (\pi J_{IS} \Delta_2) \cos (\pi J'_{IS} \Delta_2) \cos (\pi J''_{IS} \Delta_2) \cdots$$

All other combinations will be either single, double or even triple antiphase terms.
Generally, we get – for the observable term $S_y$ – a factor $\sin(\pi J_{IS} \Delta_2) \cos^{n-1}(\pi J_{IS} \Delta_2)$ for a $\text{CH}_n$ group, and we have to choose our delay $\Delta_2$ wisely to get a signal from all groups!

We can now choose different values for $\Delta_2$ and thus select only certain proton multiplicities:

Relative signal intensities in INEPT spectra as a function of $\Delta_2$

<table>
<thead>
<tr>
<th></th>
<th>$\Delta_2 = \frac{1}{4J}$</th>
<th>$\Delta_2 = \frac{1}{2J}$</th>
<th>$\Delta_2 = \frac{3}{4J}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>CH</td>
<td>$\frac{1}{\sqrt{2}}$</td>
<td>1</td>
<td>$\frac{1}{\sqrt{2}}$</td>
</tr>
<tr>
<td>CH$_2$</td>
<td>$\frac{1}{2}$</td>
<td>0</td>
<td>$-\frac{1}{2}$</td>
</tr>
<tr>
<td>CH$_3$</td>
<td>$\frac{1}{2\sqrt{2}}$</td>
<td>0</td>
<td>$\frac{1}{2\sqrt{2}}$</td>
</tr>
</tbody>
</table>
By adding and subtracting two INEPT spectra acquired with different $\Delta_2$ settings, one can also select exclusively CH or CH$_3$ groups:

for CH only:  
$$\Delta = \frac{1}{2}J$$

for CH$_2$ only:  
$$\Delta = \frac{1}{4}J \ - \ (\Delta = \frac{3}{4}J)$$  
(CH and CH$_3$ are symmetric about $\Delta_2 = \frac{1}{2}J$, but not CH$_2$)

for CH$_3$ only:  
$$\Delta = \frac{1}{4}J \ + \ (\Delta = \frac{3}{4}J) \ - \ \sqrt{2} \ (\Delta = \frac{1}{2}J)$$  
removes CH$_2$  
removes CH

The multiplicity selection of the INEPT editing scheme is quite sensitive to misset $\Delta_2$ values. However, since the $^1J_{HC}$ values vary ca. $\pm 10\%$ from the average 140 Hz, it is impossible to set $\Delta_2$ exactly to its theoretical values for all carbon resonances simultaneously. As a result, suppression of the unwanted multiplicities in an INEPT editing experiment is far from perfect.

As an improvement for multiplicity editing, the DEPT (*D*istortionless *E*Nhancement via *P*olarization *T*ransfer) experiment has been developed (and is still the most widely used technique for that purpose).

![DEPT sequence diagram](image)

The analysis of the DEPT sequence shows how even rather confusing techniques can be understood or at least described in a quantitative way. After a first glance at the DEPT sequence, we see that we can safely skip any chemical shift evolution for $^1$H or $^{13}$C, since both will be refocussed during the times where they are in a coherent state (between the first 90° pulse and the θ pulse for $^1$H; between the first $^{13}$C 90° pulse and acquisition for $^{13}$C). All three delays $\Delta$ are set to $\frac{1}{2}J$, so that $\cos (\pi J \Delta) = 0$ and $\sin (\pi J \Delta) = 0$.

$$I_z \rightarrow I_x \rightarrow 2 I_y S_z \rightarrow 2 I_y S_x$$
For a CH group, this heteronuclear multi-quantum coherence is not affected by coupling evolution, since the $^1\text{H}$ and $^{13}\text{C}$ spin are “synchronized” in a common coherence and do not couple to each other in this state. Other coupling partners are not available, so that this terms just stays there during the delay $\Delta$:

$$\begin{align*}
  \Delta & \quad \theta_x (I) \\
  2\, I_y S_x & \rightarrow 2\, I_y S_x \quad \rightarrow \quad 2\, I_y S_x \cos \theta \quad \rightarrow \quad 2\, I_y S_x \cos \theta \\
  & + 2\, I_z S_x \sin \theta \quad \rightarrow \quad + S_y \sin \theta
\end{align*}$$

During the following acquisition time, only the in-phase $^{13}\text{C}$ coherence term will be detected.

For a CH$_2$ group, however, there will be a coupling partner available during the second $\Delta$ delay: the second proton, $I'$. The $J_{IS}$ coupling will cause the $^{13}\text{C}$ part of the MQC ($S_x$) to evolve into antiphase with respect to $I'$:

$$\begin{align*}
  \Delta & \quad \theta_x (I), \, 180^\circ_x (S) \\
  2\, I_y S_x & \rightarrow 4\, I_y S_y \, I'_z \quad \rightarrow \quad -4\, I_y S_y \, I'_z \cos \theta \cos \theta \quad - 4\, I_z S_y \, I'_z \sin \theta \cos \theta \\
  & + 4\, I_y S_y \, I'_y \cos \theta \sin \theta \quad + 4\, I_z S_y \, I'_y \sin \theta \sin \theta
\end{align*}$$

(the $180^\circ_x (S)$ pulse reverses the sign of all terms, $S_y \rightarrow S_y$)

From these terms, only one is a (double antiphase) $^{13}\text{C}$ single-quantum coherence that can refocus to detectable $^{13}\text{C}$ in-phase magnetization during the last delay $\Delta$. Both couplings (to I and I') refocus simultaneously:

$$\begin{align*}
  \Delta & \\
  4\, I_z S_y \, I'_z \sin \theta \cos \theta & \rightarrow \quad \{ 2\, S_x \, I'_z \sin \theta \cos \theta \rightarrow \quad \} \quad S_y \sin \theta \cos \theta
\end{align*}$$

For a CH$_3$ group, there are two additional protons (I' and I'') coupling to the carbon:

$$\begin{align*}
  \Delta & \\
  2\, I_y S_x & \rightarrow -8\, I_y S_x \, I'_z \, I''_z
\end{align*}$$

The $\theta$ pulse can only convert this double antiphase MQC term into $^{13}\text{C}$ SQC (which will then refocus during $\Delta$) pulse in a single way:

$$\begin{align*}
  \theta_x (I) & \quad \Delta \\
  8\, I_y S_x \, I'_z \, I''_z & \rightarrow -8\, I_z S_x \, I'_z \, I''_z \sin \theta \cos \theta \rightarrow \quad S_y \sin \theta \cos \theta \cos \theta
\end{align*}$$
For a CHₙ group, we get a signal with the amplitude $\sin\theta \cos^{(n-1)}\theta$ in the DEPT experiment, compared to $\sin(\pi \Delta_2) \cos^{(n-1)}(\pi \Delta_2)$ in the INEPT. So the dependence of the DEPT spectrum on the flip angle of the $\theta$ pulse is the same as the dependence of the INEPT on the length of $\Delta_2$.

However, the DEPT is much less sensitive to varying $^1J_{HC}$ values and therefore the preferred experiment for multiplicity editing (usually with a set of three $\theta$ values, $\theta = 45^\circ$, $90^\circ$, $135^\circ$; corresponding to the INEPT with $\Delta_2 = 1/4J$, $1/2J$, $3/4J$).

**Inverse heteronuclear spectroscopy**

Proton detection

Today, most of the heteronuclear experiments are performed in a $^1H$ detected version, also called “inverse detection” (in contrast to the classical X nucleus detection described so far). If the proper equipment is available (re-wired spectrometer console; inverse detection probe!), then inverse detection offers such an immense gain in sensitivity that there is (almost) no reason to run any “conventional” heteronuclear correlation experiments anymore.

**Theoretical relative sensitivities (S/N) for H,X correlation spectra ($X=^{13}C$, $^{15}N$)*.**

<table>
<thead>
<tr>
<th>Method</th>
<th>$\gamma_{exz,\gamma_{det.}}^{3/2}$</th>
<th>$^{13}C$</th>
<th>$^{15}N$</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>direct detection</em></td>
<td>$\gamma_X\gamma_X^{3/2}$</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td><strong>INEPT / DEPT</strong></td>
<td>$\gamma_H\gamma_X^{3/2}$</td>
<td>4.0</td>
<td>9.9</td>
</tr>
<tr>
<td><em>reverse INEPT</em></td>
<td>$\gamma_X\gamma_H^{3/2}$</td>
<td>7.9</td>
<td>31.0</td>
</tr>
<tr>
<td></td>
<td>(relative to INEPT=1)</td>
<td>2.0</td>
<td>3.1</td>
</tr>
<tr>
<td><strong>invers</strong></td>
<td>$\gamma_H\gamma_H$</td>
<td>31.6</td>
<td>306.0</td>
</tr>
<tr>
<td></td>
<td>(relative to INEPT=1)</td>
<td>7.9</td>
<td>31.0</td>
</tr>
</tbody>
</table>

* not taking into account other factors, e.g., $T_1$, heteronucl. NOE, linewidths etc.

It has to be remembered that the number of scans (~spectrometer time) required goes up with the square of the sensitivity ratio. Thus, a simple 1D $^{13}C$ spectrum might well need almost 1000 times the measuring time of an inverse 2D $^1H,^{13}C$-correlation!
The first $^1\text{H}$ detected correlation experiment was performed in 1977 by Maudsley & Ernst: just the basic 2D $\text{H,C}$ three-pulse correlation experiment (antiphase crosspeaks in both dimensions!) “reversed” to start on $^{13}\text{C}$ and end on $^1\text{H}$ (the $^1\text{H}$ irradiation boosts the $^{13}\text{C}$ magnetization by the heteronuclear NOE).

The term “inverse” is usually reserved for experiments that start on $^1\text{H}$ and detect $^1\text{H}$, giving the maximum sensitivity. There are basically two inverse $^1\text{H},X$ correlation experiments, the HSQC and the HMQC sequence.

**HSQC (Heteronuclear Single Quantum Correlation) Experiment.**

The HSQC experiment consists essentially of the elements (INEPT – $t_1$ – reverse INEPT – $t_2$); the delay $\tau$ is set to $\tau = (4J_{\text{CH}})^{-1}$.

With product operators, the transfer goes as follows (chemical shift is refocussed during $2\tau$):

\[
\begin{align*}
I_z & \xrightarrow{90^\circ_x} I_y \xrightarrow{\Delta} 2 I_x S_z \xrightarrow{90^\circ(I,S)} 2 I_x S_y \xrightarrow{t_1} 2 I_x S_y \cos(\Omega t_1) \\
& \quad \xrightarrow{90^\circ(I,S)} 2 I_x S_z \cos(\Omega t_1) \xrightarrow{\Delta} I_y \cos(\Omega t_1)
\end{align*}
\]

To select only $^{13}\text{C}$ bound protons, a phase cycling scheme has to be used on the $^{13}\text{C}$ $90^\circ$ pulses. A $180^\circ$ phase shift on one of these pulses will flip the sign of the detected term, e.g.: 
Protons that are not directly bound to $^{13}$C will not develop into $2 I_z S_z$ terms and therefore not be affected by phase changes of the $^{13}$C pulses. By subtracting two subsequent scans acquired with a 180° phase shift on a $^{13}$C 90° pulse, the $I_y \cos(\Omega t_1)$ signal will actually add up (due to the sign flip), while signals from non-$^{13}$C bound protons will cancel.

In the HSQC experiment, during $t_1$ only $^{13}$C chemical shift develops ($^1$H-$^{13}$C coupling is refocussed by the $^1$H 180° pulse). During $t_2$, $^1$H chemical shift and $^1$H-$^1$H coupling will develop, heteronuclear coupling is again suppressed by the decoupling sequence run on $^{13}$C. For a proton display a triplet pattern in the $^1$H spectrum, the HSQC cross-peak will look like this (if run with sufficient resolution):

HMOC (Heteronuclear Multi-Quantum Correlation)

This experiment resembles the DEPT transfer, since the coherence transfer doesn’t go from $^1$H to $^{13}$C coherence, but rather to $^1$H-$^{13}$C multiquantum coherence ($\Delta = \frac{1}{2} J$). $^1$H chemical shift evolution during the whole sequence is refocussed by the 180° pulse:
\[ I_z \longrightarrow I_x \longrightarrow 2 I_y S_z \longrightarrow 2 I_y S_x \]

The term \( 2 I_y S_x \) describes a combination of \(^1\text{H}, ^{13}\text{C}\) double- and zeroquantum coherence (as evident when transformed into the \( I^+ / I^- \) base). The DQC part will evolve with the *sum* of the chemical shifts \((\Omega_S + \Omega_I)\), while the ZQC component evolves with the *difference* \((\Omega_S - \Omega_I)\). However, the 180° \(^1\text{H}\) pulse right in the center of \( t_1 \) reverses the \(^1\text{H}\) part, so that the DQC now becomes a ZQC and *vice versa*. At the end of \( t_1 \), both parts have evolved with \((\Omega_S + \Omega_I)\) during \( t_1/2 \), and with \((\Omega_S - \Omega_I)\) during another \( t_1/2 \), so that the \( \Omega_I \) contribution cancels and we get effectively chemical shift evolution only with the \(^{13}\text{C}\) chemical shift during \( t_1 \):

\[
\begin{array}{cccc}
\text{t}_1 & 90°_y(S) & \Delta \\
2 I_y S_x & \rightarrow & 2 I_y S_x \cos(\Omega t_1) & \rightarrow & -2 I_x S_x \cos(\Omega t_1) & \rightarrow & -I_y \cos(\Omega t_1)
\end{array}
\]

Again we get \(^{13}\text{C}\) chemical shift evolution during \( t_1 \), heteronuclear coupling is refocussed; during \( t_2 \), we get \(^1\text{H}\) chemical shift and homonuclear coupling evolution, heteronuclear coupling is again suppressed by the \(^{13}\text{C}\) decoupling sequence. However, we *really* had \(^1\text{H}, ^{13}\text{C}\) MQC evolving during \( t_1 \), with the \(^1\text{H}\) chemical shift contribution refocussed by the 180° pulse. A 180° pulse cannot refocus homonuclear coupling, so the \(^1\text{H}, ^1\text{H}\) couplings which also evolved with the MQC are *not* refocussed and yield another factor \( \cos (\pi J t_1) \).

As a result, we will see the \(^1\text{H}\) multiplet pattern as (in-phase) splitting in the \(^{13}\text{C}\) dimension! Due to the specific nature of the HMQC sequence (the spin states of the \(^1\text{H}\) coupling partners are not disturbed by any non-180° pulse on \(^1\text{H}\)), the \(^1\text{H}\) multiplicity pattern appears as a diagonal slant in the HMQC cross-peaks (if the \(^{13}\text{C}\) resolution is sufficiently high!):
The HMQC version has the advantage of having fewer pulses. This makes it less insensitive to pulse calibration errors. Especially important is the lack of any 180° $^{13}$C pulses, which tend to be pretty much off at the edges of a large $^{13}$C spectral window, even when properly calibrated (on-resonance).

However, the resolution in the $^{13}$C dimension is limited by the $^1$H multiplet pattern, which can be up to 30-40 Hz broad (depending on the $^1$H spin system), while the resolution in an HSQC experiment is only limited by the $^{13}$C linewidths.

Problems of inverse experiments

The very significant sensitivity increase of inverse experiments vs. “forward” $^1$H,X correlation experiments has been discussed already. However, there are some other features that should be mentioned:

- in “forward” correlation experiments (e.g., H,C-COSY), the $^1$H resolution – in the indirect dimension – is usually low (since it depends on the number of increments run), while it is very easy to reach a high $^{13}$C resolution (direct dimension!). In an inverse experiment, $^{13}$C is the indirect dimension, with usually lower resolution (higher resolution requiring more increments = more spectrometer time) – on the other hand, $^1$H resolution is “free”.

- in $^{13}$C-detected “forward” experiments, an excess of non-$^{13}$C bound protons doesn’t matter, since the $^{13}$C detection automatically selects only the interesting ones.

In inverse experiments, however, all $^1$H signals are detected in the first place, which means for non-enriched samples:

- 1.1 % $^{13}$C-$^1$H
- 98.9 % $^{12}$C-$^1$H
- $10^6$-$10^7$ % $^{12}$C-$^1$H, O-H etc. solvent protons (in protonated solvents)

In theory, phase cycling should remove all non-$^{13}$C bound protons. However, there are two severe restrictions to this:

- phase cycling requires combination of signals from subsequent scans, i.e., it only takes place after digitization of the signals from individual scans. Thus, it does not reduce dynamic range problems from an excess of unwanted $^1$H signals.
- perfect cancelation can never be achieved in an imperfect world! Due to small instabilities (voltage fluctuations in the electronics, temperature changes in the sample or amplifiers, etc.), the subtraction won’t be 100 % complete – but even 0.1-1 % residual from the much more intense non-$^{13}$C bound protons will affect the spectrum!

As a result, inverse correlation spectra in deuterated solvents usually show severe $t_1$ noise ridges at all $^1$H chemical shift frequencies. in protonated solvents, the $t_1$ ridge of the solvent usually completely obscured the interesting $^{13}$C-$^1$H signals!

In the following, two methods will be explained that help reduce these $t_1$ artifacts in inverse correlation spectra.

**BIRD – Bilinear Rotational Decoupling**

The BIRD modul consists of the following pulses, separated by a delay tuned to $\Delta = \frac{1}{2J}$:

Let’s calculate the effect of the BIRD$_y$ modul on magnetization of protons bound to $^{13}$C. Since it has a $^1$H 180° pulse in the center, we can safely ignore chemical shift evolution:

\[
\begin{align*}
I_x \quad &\quad 90^\circ_x \quad \Delta \quad 180^\circ_y(I), 180^\circ_x(S) \quad \Delta \quad 90^\circ_x \\
I_x \quad &\quad 2I_yS_z \\
I_x \quad &\quad -2I_yS_z \\
I_x \quad &\quad I_x
\end{align*}
\]

\[
\begin{align*}
I_y \quad &\quad 90^\circ_x \quad \Delta \quad 180^\circ_y(I), 180^\circ_x(S) \quad \Delta \quad 90^\circ_x \\
I_z \quad &\quad I_z \\
I_z \quad &\quad -I_z \\
I_y \quad &\quad I_y
\end{align*}
\]

\[
\begin{align*}
I_z \quad &\quad 90^\circ_x \quad \Delta \quad 180^\circ_y(I), 180^\circ_x(S) \quad \Delta \quad 90^\circ_x \\
-I_y \quad &\quad 2I_xS_z \\
2I_xS_z \quad &\quad 2I_xS_z \\
I_y \quad &\quad I_z
\end{align*}
\]
So, after the BIRD\textsubscript{y} pulse, all \textsuperscript{1}H magnetization components are unchanged! What happens to a proton \textit{not} bound to \textsuperscript{13}C?

\[
\begin{align*}
90^\circ_x & \quad \Delta & 180^\circ_y(I), 180^\circ_x(S) & \quad \Delta & 90^\circ_x \\
I_x & \longrightarrow I_x \longrightarrow I_x & \longrightarrow -I_x \longrightarrow -I_x \\
90^\circ_x & \quad \Delta & 180^\circ_y(I), 180^\circ_x(S) & \quad \Delta & 90^\circ_x \\
I_y & \longrightarrow I_z \longrightarrow I_z & \longrightarrow -I_z \longrightarrow I_y \\
90^\circ_x & \quad \Delta & 180^\circ_y(I), 180^\circ_x(S) & \quad \Delta & 90^\circ_x \\
I_z & \longrightarrow -I_y \longrightarrow -I_y & \longrightarrow -I_y \longrightarrow -I_z
\end{align*}
\]

For these protons, the \textit{x} and \textit{z} components are inverted, which is exactly the same effect as of a 180\textsuperscript{o}\textsubscript{y} pulse! Thus, the BIRD\textsubscript{y} pulse can distinguish between \textsuperscript{13}C-bound and non-\textsuperscript{13}C-bound protons.

For the BIRD\textsubscript{x} version, the result is just the other way round: it acts on \textsuperscript{13}C-\textsuperscript{1}H spins like a 180\textsuperscript{o}\textsubscript{y} pulse, but does \textit{not} affect protons not bound to \textsuperscript{13}C.

How can a BIRD pulse help to suppress non-\textsuperscript{13}C bound proton signals in inverse correlation experiments? Imagine the effect of a BIRD\textsubscript{y} modulation \textit{between} two scans (e.g., of an HMQC):
After the pulse sequence itself, at the beginning of the acquisition time \( t_2 \) (point “a”), we will have essentially all protons in a coherent state, i.e., the \( z \) component is zero (relaxation during the short duration of the pulse sequence is negligible!). During \( t_2 \) and the following relaxation delay all protons will undergo \( T_1 \) relaxation, bringing them (partially) back to \( I_z \). A BIRD\(_y\) modul now (point “b”) will invert only the non-\(^{13}\)C bound protons, flipping them back to \(-I_z\), while the \(^{13}\)C-\(^1\)H spins will continue to relax back to \( I_z \). After another delay (point’c’), the non-\(^{13}\)C bound protons will have a vanishing \( z \) component, while the \(^{13}\)C-\(^1\)H spins are essentially back to \( I_z \). If we start the next scan of our experiment now, signals from non-\(^{13}\)C bound protons will be effectively suppressed.

For best performance, the time between “a” and “b” (including the acquisition time \( t_2 \)!) should be ca. 0.85 \( T_1 \), and the time between “b” and “c” ca. 0.45 \( T_1 \) (which also means we only have to wait ca. 1.3 \( T_1 \) – including \( t_2 \)! – between scans and can acquire data much faster!).

When the \( T_1 \) times of the protons vary a bit (as is usually the case), then the shortest \( T_1 \) time should be used for the calculation of the delays (curve “A”). All other protons, relaxing more slowly, will then still be very close to the zero crossing (curves “B” and “C”). However, when the \( T_1 \) times vary by an order of magnitude or more, then the BIRD suppression will perform poorly.

The BIRD version of HSQC and HMQC usually suppress \(^{12}\)C-bound protons good enough to eliminate \( t_1 \) noise ridges. However, in the case of protonated solvents with their ca. \( 10^4 \) times more intense signal, other methods have to be used for improved suppression. In recent years, the availability of pulsed field gradients (PFG) has really revolutionized solvent suppression.
**Pulsed field gradients (PFG)**

Field gradients can be used to destroy the homogeneity of the magnetic field (the result of shimming) in a controlled way. This is done by placing an additional pair of coils inside the probe on both sides of the sample (for a z gradient, i.e., above and below the sample). A d.c. current is then send through this coil pair in opposite direction, so that the resulting magnetic field is parallel to the static \( B_0 \) field on one side of the sample, and antiparallel on the other side. The result is a fairly linear field gradient over the sample volume:

In reality, the gradient coils shown are combined with a second pair of “compensating” coils (with reverse polarization, not shown) that help to reduce the induction of eddy currents in the metallic parts of the probe: “shielded gradients”. This allows to increase the gradient field strength without the need for overly long recovery delays after a gradient (for eddy current ring-down = restoration of the field homogeneity). Typical values for high-resolution probes with shielded gradients are:

- maximum gradient strength: \( 50 \text{ G/cm} \)
- gradient length: \( 1 \text{ ms} \)
- eddy current ring-down delay: \( 100-500 \mu\text{s} \)
The phase twist \( \Delta \phi_G \) caused by the gradient field can be easily calculated:

\[
\Delta \phi_G = \Delta \omega_G \tau_G = \gamma B_G \tau_G
\]

with \( \tau_G \) = gradient duration, \( \Delta \omega_G \) = change in precession frequency caused by the gradient field, \( B_G \) = gradient field strength and \( \gamma \) = magnetogyric constant of the spin.

For protons one gets for a 1 ms gradient of 50 G/cm:

- a 220 kHz/cm gradient field, which causes (after 1 ms duration) a twist of 220 full revolutions per cm sample volume (in \( z \) direction), i.e., one coil winding has less than 50 \( \mu m \) height!

- with dedicated gradient probes (gradient strength up to several hundred G/cm) and longer gradients of 10-20 ms the spacing between the “windings” can be as small as 10-100 nm!

A \( I_x \) magnetization is twisted several hundred times after such a gradient, and the detectable net magnetization is essentially zero, because the transverse components cancel over the sample volume. This effect requires a high gradient strength and/or duration, because a “weaker” twist of just a few revolutions will lead to imperfect cancelation – the residual signal is proportional to sinc(\( \gamma B_G \tau_G \)).

Gradients don’t have to be rectangular in shape, they can be of trapezoid or sinusoidal shape to further reduce eddy currents and inductive distortions. As long as all gradients used in an experiment have the same shape, the degree of “twisting” will always depend on the product of gradient duration and gradient strength (i.e., maximum strength for non-rectangular gradients).

Important properties of pulsed field gradients:

- twisting effect proportional to \( (\gamma B_G \tau_G) \)

- only \( x \) and \( y \) components of the magnetization are dephased by \( z \) gradients, all \( z \) components are not affected

- since the dephasing is done in a very defined and reproducible way, the phase twist can be refocused by applying a gradient of equal strength \( (\gamma B_G \tau_G)! \), but opposite polarization:
Some simple gradient “applications”: 

1. 

The gradient does not affect polarization, so – if the delay $\tau$ is long enough for complete ring-down of the eddy currents induced by the gradient – the result of this sequence will be a normal 1D spectrum.

2. 

The gradient is performed after creating the $^1$H coherence (there is no delay necessary before a gradient!). If the gradient is strong enough, $^1$H magnetization will be completely dephased and gone!
The gradient is performed before and after a 180° pulse on a coherence. Since the 180° pulse “reverses” the twist caused by the first gradient, a second gradient of *equal strength and equal sign* is needed to refocus the signal. A gradient pair like this serves to clean up all magnetization components that were *not* refocussed by the 180° (due to pulse miscalibration or offset effects).

With a longer delay $\tau$, there is a marked difference between the two shown sequences. When the two gradients are separated by a long delay, the efficiency of the refocusing is diminished by diffusion. Perfect refocusing can only be accomplished when all molecules stay in the same place after the first gradient, so that the phase twist from the second gradient can exactly compensate the effects of the first gradient. If a molecule moves to a different position in the sample tube (in $z$ direction), then its spins will experience the „wrong“ field strength during the second gradient pulse.

This leads to two consequences:

- a refocusing gradient should be as close as possible (in time) to the gradient whose phase twist it is supposed to compensate. Obviously, this doesn’t matter for purge gradients that are simply dephasing all (unwanted) coherences.

- the dependence of the signal intensity on the separation between a gradient pair can be used to directly measure the diffusion constants of molecules in solution (from which, e.g., the effective molecular weight can be estimated, which depends also on the aggregation state).

One way of implementing gradients is as *purge gradients*, e.g., in a HSQC sequence:
After the $2\tau$ delay and the $90^\circ_y$ $^1$H pulse, the magnetization of all $^{13}$C-bound protons is oriented in $z$, while all other protons are in $y$ coherence:

\[
^1$H-$^{13}$C: \quad 90^\circ_x(I) \quad 2\tau \quad 90^\circ_y(I) \\
I_z \rightarrow -I_y \rightarrow 2I_xS_z \rightarrow -2I_zS_z
\]

\[
^1$H-$^{12}$C: \quad 90^\circ_x(I) \quad 2\tau \quad 90^\circ_y(I) \\
I_z \rightarrow -I_y \rightarrow -I_y \rightarrow -I_y
\]

The following gradient pulse therefore selectively dephases the non-$^{13}$C bound protons. It can be used to suppress them without affecting the $^{13}$C-bound protons contributing to the wanted cross-peaks.

Except for purge gradients and gradient pairs flanking $180^\circ$ pulses, gradients can also be used for coherence selection.

The phase twist caused by a gradient depends not only on the gradient’s length and field strength, but also on the type of coherence it affects (i.e., it’s magnetogyric ratio $\gamma$):

- $^1$H coherences dephase four times as fast as $^{13}$C coherences under the same gradient pulse
- $^1$H-$^1$H double-quantum coherences evolve with twice the speed than $^1$H single-quantum coherences, i.e., they are dephased twice as fast under a gradient pulse

These features can be used to selectively refocus only specific combinations of coherences with a pair of gradients:

For a gradient ratio of 4:1, only magnetization components will be completely refocussed after the second gradient that were a $^{13}$C coherence during $\Delta$ and a $^1$H coherence during $\tau$ – because of the four times higher sensitivity of $^1$H coherence to gradients. For all other combinations, there will be a net twist left after the second gradient.
Gradient pulses, however, select for coherences in the $I^*/I$ coordinate system, not in the $I_x/I_y$ basis. In our last sequence, if we assume that we have a term $2I_zS_x$ at the end of $\Delta$, and this is then converted into $2I_xS_z$ by the 90° pulse pair, our gradient pair will select the combinations $S^+/I$ and $S^-/I^+$ (during $\Delta/\tau$, resp.), i.e., the combinations with opposite sign / rotation sense. If we choose our two gradients in the ratio 4:(-1) – with opposite sign –, then we will refocus the S/I combinations with equal sign during $\Delta/\tau$, i.e., $S^+/I^+$ and $S^-/I^-$. Because of $S_x = \frac{1}{2}(S^+ + S^-)$ and $I_x = \frac{1}{2}(I^+ + I^-)$, all these combinations are actually present in $2I_zS_x$ and $2I_xS_z$!

This feature of gradient coherence selection has some important consequences when we implement it in a real pulse sequence, e.g., in the HSQC experiment:

The first gradient G1 serves as a purge gradient. The second and third gradient, G2 and G3, form a pair with G2 acting on $^{13}\text{C}$ coherence (during $t_1$) and G3 on $^1\text{H}$ coherence (after the coherence transfer). Note that we have to introduce an additional delay $\tau'$ and a 180° $^{13}\text{C}$ pulse to compensate for $^{13}\text{C}$ chemical shift evolution during G2! G3 usually fits into the existing delay $\tau = \frac{1}{4J} \approx 1.7$ ms.

Now only the part of the $^1\text{H}$ magnetization that actually was a $^{13}\text{C}$ coherence during G2 (i.e., $t_1$) will be refocussed by G3 (at the beginning of $t_2$). This gradient-selected HSQC gives a great solvent suppression, as well as complete suppression of $t_1$ noise caused by $^{12}\text{C}$ bound protons!

However, there is a problem: normally, we create a $^{13}\text{C}$ coherence with the first $^1\text{H}/^{13}\text{C}$ 90° pulse pair, and then convert it back at the end of $t_1$ with the second one. The experiment is the repeated with a 90° phase shift on the first $^{13}\text{C}$ 90° pulse to yield the cosine and sine components needed for quadrature detection (STATES version):
If we want to understand what happens with gradient coherence selection during $t_1$, then we have to switch to the single element operators:

$$2I_xS_z \rightarrow 2I_zS_x \rightarrow 2I_zS_x \cos \Omega S t_1 \rightarrow 2I_xS_z \cos \Omega S t_1$$

$$2I_xS_z \rightarrow 2I_zS_y \rightarrow -2I_zS_x \sin \Omega S t_1 \rightarrow -2I_xS_z \sin \Omega S t_1$$

Now, our gradient $G_2$ (in combination with $G_3$) selects either $S^+$ or $S^-$ during $t_1$ (here: $S^+$):

$$G_2 \rightarrow \frac{1}{2} 2I_zS^+ \cos \Omega S t_1 - i\frac{1}{2} 2I_zS^+ \sin \Omega S t_1 = \frac{1}{2} 2I_z(S_x + S_y) \cos \Omega S t_1 - i\frac{1}{2} 2I_z(S_x + S_y) \sin \Omega S t_1$$

The 90° pulse pair then only transfers one of the $S_x/S_y$ components back to $^1H$ coherence, e.g., $S_x$:

$$\frac{1}{2} 2I_z(S_x + S_y) \cos \Omega S t_1 - i\frac{1}{2} 2I_z(S_x + S_y) \sin \Omega S t_1 \rightarrow \frac{1}{2} 2I_xS_z \cos \Omega S t_1 - i\frac{1}{2} 2I_xS_z \sin \Omega S t_1$$

So, we loose 50% by introducing gradient coherence selection during $t_1$. In addition, we cannot achieve quadrature detection anymore by flipping the phase of the first $^{13}C$ 90° pulse, since the $G_2/G_3$ gradient pair always selects a combination of sine and cosine terms.

The second problem can be solved by switching the sign of one of the $G_2/G_3$ gradients in the second run, so that we now acquire the $S^+$ and $S^-$ during $t_1$ in separate scans, instead of $S_x$ and $S_y$. $S_x$ and $S_y$ can be recreated from addition/subtraction of $S^+$ and $S^-$ (cf. the conversion rules for single-element operators). This is usually done automatically during the data processing, and this special way of quadrature detection is often referred to as „echo-antiecho“ scheme.

The 50% intensity loss is often accepted, since the gradient selection offers such a superior artifact suppression. However, modifications have been developed to increase the intensity of the experiment, usually called the “sensitivity-enhanced” version:
(in addition, gradient pairs can be added on both sides of each 180° pulse pair, as described above).

The difference to the “normal” HSQC experiment is the double INEPT transfer module at the end, once with a 90°_x pulse pair and the second time with a 90° phase shift, as 90°_y pulses. This version can transfer both the S_x and S_y (i.e., 2I_zS_x and 2I_zS_y) magnetization components back to \(^1\)H coherence. As a result, in spite of the gradient selection, this experiment has theoretically the same sensitivity as a normal HSQC with STATES mode quadrature detection.

In addition, an additional delay 2\(\tau\)' (and a 180° pulse for chemical shift refocusing) is needed at the end to accommodate the G3 gradient on \(^1\)H coherence. During the \(\tau\) delays, the two parts of magnetization (2I_zS_x and 2I_zS_y) undergo different transfer paths, so that the gradient cannot be inserted there. Only after the last \(^1\)H 90° pulse both components are converted back to \(^1\)H SQC. **In praxi**, however, the theoretical sensitivity gain is reduced by

1. pulse imperfections that accumulate from the large number of (esp. 180°) pulses;
2. increased relaxation losses during the longer pulse sequence; and especially
3. a compromise in the length of the \(\tau\) delays in the sensitivity-enhanced INEPT steps, required for CH\(_2\) and CH\(_3\) groups.
**Long-range correlations**

For assignment and connectivity elucidation the direct $J_{\text{HC}}$ correlations are only of limited use. More important is the possibility to connect neighbouring $^1\text{H}-^{13}\text{C}$ units via $^{2,3}J_{\text{HC}}$ long-range couplings, which are in the order of 1-15 Hz. In contrast to the $^1J_{\text{HC}}$ couplings, this leads to two related problems:

- the variation between the different long-range couplings exceeds a factor of 1000 $\%$, while the direct couplings are much more uniform (140 Hz $\pm$ 10 $\%$).

- the $^1\text{H},^{13}\text{C}$ long-range couplings are in the same range as homonuclear $^1\text{H},^1\text{H}$ couplings.

As a result, it is usually impossible to set any delays exactly to, e.g., $1/2J$ for complete antiphase development or refocusing, and the sensitivity of these experiments is therefore drastically reduced, relativ to the $^1J$ $^1\text{H},^{13}\text{C}$ correlation techniques.

For the „normal“ case (i.e., starting on $^1\text{H}$ and detecting the heteronucleus) a very popular sequence is the COLOC experiment (CORrelation via LOng-range Couplings):

The COLOC is a constant-time experiment, i.e., the pulse sequence doesn’t grow gradually longer with the incrementation of $t_1$. Instead, the $t_1$ modulation is achieved by shifting the pair of 180$^\circ$ pulses stepwise out of the center of the constant delay $\Delta_1$.

How does the $^1\text{H}$ magnetization (generated by the first 90$^\circ$ pulse) evolve during this time:

$\Omega_{\text{H}}$: \[(\Delta t/2 + t_1/2) - (\Delta t/2 - t_1/2) = t_1\] (evolution reversed by the $^1\text{H}$ 180$^\circ$ pulse)

$J_{\text{H,H}}$: \[(\Delta t/2 + t_1/2) + (\Delta t/2 - t_1/2) = \Delta_1\] (not affected by $^1\text{H}$ 180$^\circ$ pulse)

$J_{\text{H,C}}$: \[(\Delta t/2 + t_1/2) + (\Delta t/2 - t_1/2) = \Delta_1\] (not affected by 180$^\circ$ pulse pair)
As a result, at the end of $\Delta_1$, the $J_{HC}$ antiphase term we need for the $^1H$, $^13C$ coherence transfer will be modulated as follows:

$$I_x \longrightarrow 2I_y S_z \cos \Omega_{t1} \sin \pi J_{HC} \Delta_1 \cos^n \pi J_{HH} \Delta_1$$

(we will get a cosine term for each one of the $n J_{HH}$ couplings!)

This means that, after FT, we will get only $^1H$ chemical shift frequencies in F1, no homo- or heteronuclear coupling, since these are not modulated with $t_1$. The factors $\sin \pi J_{HC} \Delta_1$ and $\cos \pi J_{HH} \Delta_1$ are mere constants determining the transfer efficiency. For the heteronuclear coupling, the best values for $\Delta_1$ would be around 50-100 ms; however, to avoid $\cos \pi J_{HH} \Delta_1 = 0$, the length of $\Delta_1$ is usually set to 25-30 ms.

In these constant time experiments, the maximum achievable resolution is limited by the length of the delay $\Delta_1$, since the $t_1$ time cannot be extended beyond $t_1/2 = \Delta_1/2$ or $t_1 = \Delta_1$. The maximum $^1H$ resolution in F1 is therefore $1/\Delta_1 = 30-40$ Hz for the COLOC.

After the coherence transfer onto $^13C$, one could start with the acquisition time immediately, having the $^13C$ antiphase terms refocus during $t_2$. However, the acquisitions would have to be performed without $^1H$ decoupling then. For protonated carbons, this would mean a split into a doublet / triplet / quartet by the large $^1J_{HC}$ coupling. Alternatively, a delay $\Delta_2$ can be inserted to enable refocusing before $t_2$, so that the acquisition can be performed with $^1H$ decoupling. A pair of 180° pulses on $^1H$ and $^13C$ in the center of $\Delta_2$ to refocus $^13C$ chemical shift evolution can be left away, since the spectrum is usually FOURIER transformed in absolute value mode.

The COLOC offers very good $^13C$ resolution (direct dimension!), but only very limited (constant time!) $^1H$ resolution. Since only $^13C$ signals are directly acquired in $t_2$, suppression of solvent signals or $t_1$ noise from protons not coupling to a $^13C$ spin is not a problem. However, the low natural abundance in conjunction with the low transfer efficiency through long-range couplings create problems with the overall sensitivity for $^1H$, $^13C$ long-range correlations. Today, inverse experiments are usually preferred for $^13C$, due to their inherent higher sensitivity. COLOC type experiments are however still popular, e.g., for $^1H$, $^31P$ long-range correlations, which are far more sensitive due to the 100% natural abundance of $^31P$ and its higher $\gamma$. 
Inverse C,H long-range correlation — HMBC

The HSQC pulse sequence can be easily changed into the HMBC experiment (heteronuclear multi-bond correlation), essentially by lengthening the delay $\Delta$ for the evolution of the heteronuclear coupling.

As in the HSQC sequence, $^1$H is coherent during the whole sequence, but now the delay $\Delta$ is so long (ca. 40-100 ms) that significant evolution of homonuclear $^1$H coupling occurs. Therefore the $^1$H signals will be phase twisted at the beginning of $t_2$, and a phase-sensitive processing of the $^1$H dimension is not advisable. For an absolute value mode processing, $^1$H chemical shift evolution during the sequence need not be refocussed anymore, so that the second delay $\Delta$ after the $^{13}$C $t_1$ time (in the HSQC sequence) is usually left away in the HMBC version. Refocussing of the $^1$H, $^{13}$C long-range couplings occurs during the acquisition time, and no $^{13}$C decoupling is performed during $t_2$ (due to the low natural abundance of $^{13}$C, most of the protons with long-range couplings to $^{13}$C won’t also have a directly bound $^{13}$C, so that no $^1$J$_{H,C}$ splitting occurs).

In addition, the very intense direct ($^1$J) correlations can be suppressed by a *low-pass J filter*, i.e., an additional $^{13}$C 90° pulse at a time $\delta = 1/(2\ J_{HC})$ (ca. 3.5 ms). At this time, only the large one-bond couplings will be completely in antiphase $2I_xS_z$, and the 90° $^{13}$C pulse will convert them into heteronuclear MQC ($2I_xS_x$) which is removed by the phase cycle. The resulting HMBC sequence then looks as follows:
Although the HMBC experiment is clearly superior in sensitivity, due to its inverse detection scheme, suppression of solvent $^1\text{H}$ signals and excessive $t_1$ noise from protons without correlations to $^{13}\text{C}$ are a major problem. The BIRD trick cannot be exploited here, because it relies on a $^1\text{J}$ coupling to $^{13}\text{C}$ which is not present for most protons with long-range correlations to a $^{13}\text{C}$ spin.

The best solution to this problem is a HMBC with gradient coherence selection (i.e., one gradient during the $t_1$ evolution time and another directly before acquisition). Since the HMBC is not phase sensitive anyway in the $^1\text{H}$ dimension, refocusing of $^1\text{H}$ chemical shift evolution during the gradients is not required, and implementation of the gradients is much easier than in the (phase sensitive) HMQC experiment. A drawback is the 50\% of (absolute) signal intensity during to the gradients’ selection for S$^+$ or S$^-$ (as discussed in the gradient section). However, the perfect $t_1$ noise suppression delivered by the gradients allows to observe much weaker peaks, so that the signal-to-noise ratio is usually improved over the non-gradient HMBC.

**INADEQUATE**

For the elucidation of the carbon skeleton of an organic molecule, the HMBC experiment with its $^2\text{J}$ and $^3\text{J}$ $^1\text{H}$, $^{13}\text{C}$ long-range correlations can be quite useful. However, it requires the presence of a certain amount of protonated carbons. In some sorts of compounds, e.g., condensed aromatic systems, this can be a problem.

Theoretically, the carbon skeleton can be examined by $^{13}\text{C}$, $^{13}\text{C}$ correlation experiments. Due to the low natural abundance of $^{13}\text{C}$ (1.1\% of $^{13}\text{C}$, 0.01\% of $^{13}\text{C}$-$^{13}\text{C}$ pairs), in a $^{13}\text{C}$, $^{13}\text{C}$-COSY experiment the diagonal peaks from isolated $^{13}\text{C}$ spins would prevail.

Like in the $^1\text{H}$ DQF-COSY experiment, $^{13}\text{C}$ DQ coherence can only be generated by $^{13}\text{C}$ pairs. However, historically the INADEQUATE (Incredible Natural Abundance Double QUAntum Experiment) experiment has been the standard for $^{13}\text{C}$, $^{13}\text{C}$ correlations. In contrast to the DQF-COSY, where the DQ coeherence exists only during a very short delay, in the INADEQUATE sequence the DQ coherence is created \textit{at the beginning} of the $t_1$ time, and evolves during $t_1$. During $\Delta = 1/(2 \ J_{\text{C,C}})$ $^{13}\text{C}$, $^{13}\text{C}$ antiphase develops, which is then converted into DQ coherence by the second $^{13}\text{C}$ 90° pulse:
During $t_1$, $^{13}$C chemical shifts develop with the sum of neighbouring (=coupling) $^{13}$C shifts. This leads to a very specific appearance of the spectrum, with the two $^{13}$C chemical shifts of coupling $^{13}$C spins (in F2) correlated to the sum of the two in F1. For a linear system $\text{C}_A - \text{C}_B - \text{C}_C$ one gets the following spectrum (with the peaks arranged pairwise about the "double quantum diagonal"):

While this experiment allows to completely assign any carbon skeleton in principle, the main limitation is its low sensitivity: for a useful $^{13}$C INADEQUATE spectrum within ca. one day of spectrometer time, one needs – as a rule of thumb – a sample concentration yielding a 1D $^{13}$C spectrum in a single scan!

An alternative to this rather INADEQUATE experiment might be the ADEQUATE series of experiments, which consists of HSQC / HMBC experiments combined with a $^1J_{C,C}$ or long-range ($^2J_{C,C}$ or $^3J_{C,C}$) $^{13}$C,$^{13}$C-COSY step. This allows to see $^1$H, $^{13}$C correlations via up to 5-6 bonds. While not as unambiguously to evaluate as the INADEQUATE experiment, the ADEQUATE type experiments gain a sensitivity boost from their inverse detection scheme (cf. Reif et al., J. Magn. Reson. A 118, 282-285 (1996)).