

Indirect Detection

Indirect detection is the detection of a heteronucleus through direct detection on proton signals. Detection on proton allows for a much higher sensitivity. Typical experiments detect carbon or nitrogen (usually labeled in biomolecules). Keep in mind that the amount of sample required for a heteronuclear experiment is much larger than a purely proton experiment. HMBC is less sensitive than HMQC and will require longer signal averaging.

HMQC (Heteronuclear Multiple Quantum Coherence)

A proton-carbon HMQC will detect all carbons with a proton attached. Quaternary carbons will not appear in the 2-D spectrum. To set up a HMQC, calibrate the **pw90** for proton and for carbon along with appropriate sweep widths and transmitter offsets for both. Pulse widths for carbon and nitrogen are typically found in the tables in the back of the logbooks. The basic HMQC requires a probe file, has a presat option, and uses gradients.

Setting up a basic HMQC (for carbon):

1. Acquire a 1-D proton spectrum. Find the 90-degree pulse width and set **pw90=pw**. Adjust the **tof** and **sw**.
2. Call the macro **>HMQC**. Take a look at the pulse sequence **>dps**.
3. Set up the parameters with the console **>su**. Now tune the carbon channel (carbon is channel 2, proton is channel 1). The instrument should be left cabled for proton detection (proton cable into preamp and carbon cable into the filter).
4. Set the **sw**=proton sweep width and set the **tof**=proton transmitter offset.
5. Set **sw1**=carbon sweep width. Use a carbon sweep width large enough to encompass all the carbons attached to protons (don't worry about quaternary carbon shifts). Set the **dof**=decoupler offset for carbon. If certain what **dof** to use, a chart of transmitter offsets for carbon on the 500 and 600 are found on the wall. Set the **dof** to be in the center of where the carbon shifts are expected.
6. Set the **at**= acquisition time. *The acquisition time should be short (<0.2 seconds) due to high decoupling power on carbon during this time.*
7. To set up the decoupler for carbon, set **dpwr**= decoupling power (suggested **dpwr=48-50 dB**), set **dmf**= 1/90deg pwC at the above **dpwr** (i.e. **dpwr=48**, pw90C at 48dB=52µsec, **dmf**=1/0.000052=19230). Set **dmm**=**'ccg'**.
8. Set **pwX**= 90 deg hard pulse on carbon and **pwlv1**=power level for hard pulse on carbon (typical=60dB). Set **phase=1,2**.
9. Use **>dps** again to check the pulse sequence before starting. Set **nt**, **gain**, and **ni** and adjust delays to fit the time allowed.
10. Start the experiment with **>go**. Check the first increment with **wft(1)**.
11. Processing:
 - >setLP1**
 - >gaussian**
 - >wft2da**

If this is a long experiment, set **proc1='ft'** to turn off the linear prediction and omit the setLP1, otherwise the processing will take a very long time.

Alternative processing,

[Process]

[Phase F2]

The first increment will appear. Phase the baseline as a 1-D spectrum.

[Adjust Weighting]

Use any weighting function desired, but a common one for a HMQC is a gaussian (**gf**).

[Return] [Transform F2]

A spectrum will appear. Place the cursor on a FID (not solvent) that has a reasonable signal and **[Adjust Weighting]**. Again use a gaussian function or other function.

[Return] [Transform F1]

The spectrum should appear on the screen. If an error about being outside of range occurs, use **>f full dconi**.

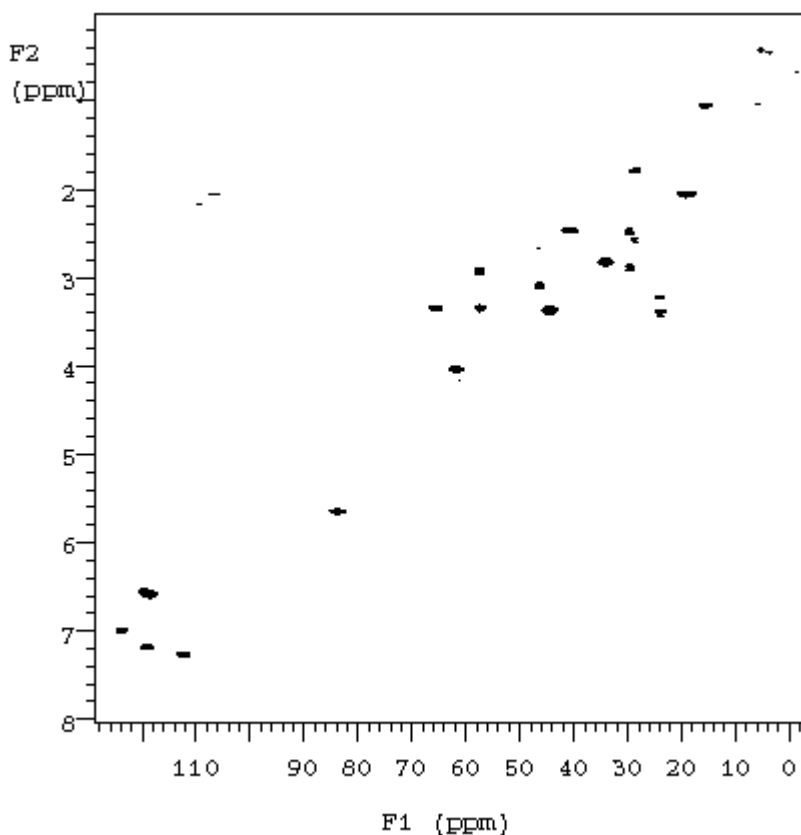


Figure 1: A gHMQC of a small molecule is asymmetrical and has a proton and carbon axis indicating carbons with their attached protons.

Versions of HMQC

gHMQC: Similar to HMQC, use **phase=1,2**.

hmqc – lower case version of hmqc, see **man('hmqc')** for setup instructions, but basic parameters: **pw/tpwr** – 90 deg. pulse on proton, **pwX/pwXlvl** – 90 deg. pulse on heteronucleus, **J** – average one bond coupling constant, **null** selects for BIRD option, presat options, **phase=1,2**

ghmqc – gradient version of hmqc, see **man('ghmqc')** for details on optimizing gradients.

Note: Sometimes altering the value of **j1xh** can be helpful in finding peaks that are weak or do not show up because the carbon-proton coupling is too far from the default (**j1xh=140**). Typical aliphatic one-bond couplings range from 125-155 Hz, but alkenes range from 155-170, aromatics from 155-165, and alkynes from 240-250 Hz. Moving the **dof** nearer the peaks in question can also strengthen their signals.

HMBC (Heteronuclear Multiple Bond Coherence)

HMBC is a heteronuclear 2-D experiment that will pick up carbons (non-isolated) without a proton attached. This experiment can be very useful to see quaternary peaks when there isn't enough sample for a 1-D carbon. HMBC is very similar to HMQC except there is no decoupling on carbon.

Setting up a basic HMBC (for carbon):

1. Acquire a 1-D proton spectrum. Find the 90-degree pulse width and set **pw90=pw**. Adjust the **tof** and **sw**.
2. Call the macro **>HMBC**. Take a look at the pulse sequence **>dps**.
3. Set up the parameters with the console **>su**. Now tune the carbon channel (carbon is channel 2, proton is channel 1). The instrument should be left cabled for proton detection (proton cable into preamp and carbon cable into the filter).
4. Set the **sw**=proton sweep width and set the **tof**=proton transmitter offset.
5. Set **sw1**=carbon sweep width. Use a carbon sweep width large enough to encompass *all* possible carbons in the molecule. Set the **dof**=decoupler offset for carbon. If uncertain what **dof** to use, a chart of transmitter offsets for carbon on the 500 and 600 are found on the wall. Set the **dof** to be in the centered on the expected carbon shifts.
6. Set the **at**= acquisition time. Acquisition time can be longer here since the decoupler is off during this experiment.
7. Set **pwX**= 90deg hard pulse on carbon and **pwXlvl**=power level for hard pulse on carbon (typical=60dB). Set **phase=1,2**. The coupling constants for the one-bond is set by **j1xh** and the multiple bond by **jnxh**.
8. Use **>dps** again to check the pulse sequence before starting. Set **nt**, **gain**, and **ni** and adjust delays to fit the time allowed. Set up presat option if desired.
9. Start the experiment with **>go**. Check the first increment with **wft(1)**.

10. Processing:

```
>setLP1  
>gaussian  
>wft2da
```

If this is a long experiment, set **proc1='ft'** to turn off the linear prediction and omit the setLP1, otherwise the processing will take a very long time.

Alternative processing,

[Process]

[Phase F2]

The first increment will appear. Phase the baseline as a 1-D spectrum.

[Adjust Weighting]

Use any weighting function desired, but a common one for a HMBC is a gaussian (**gf**).

[Return] [Transform F2]

A spectrum will appear. Place the cursor on a FID (not solvent) that has a reasonable signal and **[Adjust Weighting]**. Use a gaussian function or other function.

[Return] [Transform F1]

The spectrum should appear on the screen. If an error about being outside of range occurs, use **>f full dconi**.

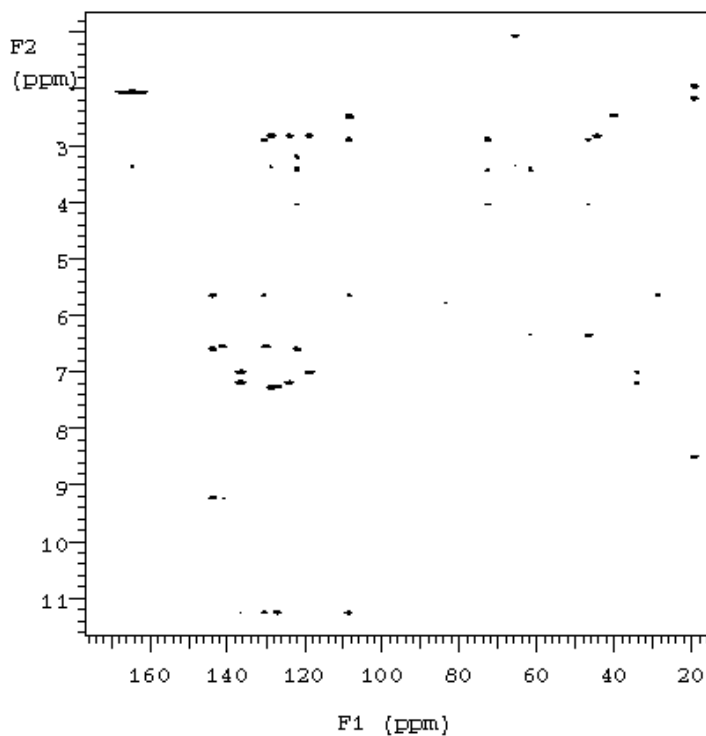


Figure 2: A HMBC is asymmetrical with a proton and carbon axis and can provide the carbon shifts of quaternary carbons when combined with the information from a HMQC.

Versions of HMBC

gHMBC: Gradient selected, absolute value version of upper case HMBC. Uses **phase=0**.

hmqc – lower case hmqc can be set up as hmbc via the **mbond** parameter. Set up using **mbond='y'**, set **taumb, null=0, dm='nnn', phase=1,2**.

HSQC

Proton spectral window is **sw**, the transmitter offset for proton is **tof**.

Carbon spectral width should correspond to the carbons with protons attached (**sw1**) and the decoupler offset, **dof**, should correspond to the transmitter offset for the carbon channel.

(Similar set up to HMQC)

1. Call the macro **>HSQC**. Take a look at the pulse sequence **>dps**.
2. Set up the parameters with the console **>su**. Now tune the carbon channel (carbon is channel 2, proton is channel 1). The instrument should be left cabled for proton detection (proton cable into preamp and carbon cable into the filter).
3. Set the **sw**=proton sweep width and set the **tof**=proton transmitter offset.
4. Set **sw1**=carbon sweep width. Use a carbon sweep width large enough to encompass all the carbons attached to protons (don't worry about quaternary shifts). Set the **dof**=decoupler offset for carbon. If certain what **dof** to use, a chart of transmitter offsets for carbon on the 500 and 600 are found on the wall. Set the **dof** to be in the center of where the carbon shifts are expected.
5. Set the **at**= acquisition time. Make this time short (<0.2 seconds) due to high decoupling power on carbon during this time. Set **phase=1,2** for HSQC.
6. The TANGO-gradient option (recommended) is selected by **nullflg**. A BIRD-null option is selected by **null**. The HSQC has the option of a presat.
7. The one-bond coupling is selected by **j1xh**.
8. Set **pwxc**= 90 deg hard pulse on carbon and **pwlv1**=power level for hard pulse on carbon (typical=60dB).
9. Start the experiment with **>go**. Check the first increment with **wft(1)**.
10. Processing:
 - >setLP1**
 - >gaussian**
 - >wft2da**

If this is a long experiment, set **proc1='ft'** to turn off the linear prediction and omit the **setLP1**, otherwise the processing will take a very long time.

Alternative processing,

[Process]

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The first increment will appear. Phase the baseline as a 1-D spectrum.

[Adjust Weighting]

Use any weighting function desired, but a common one for a HMBC is a gaussian (**gf**).

[Return] [Transform F2]

A spectrum will appear. Place the cursor on a FID (not solvent) that has a reasonable signal and [Adjust Weighting]. Use a gaussian function or other function.

[Return] [Transform F1]

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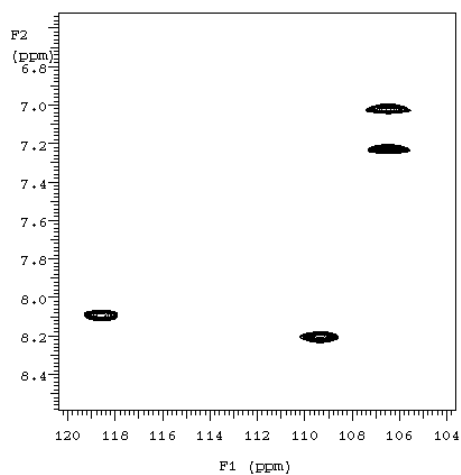
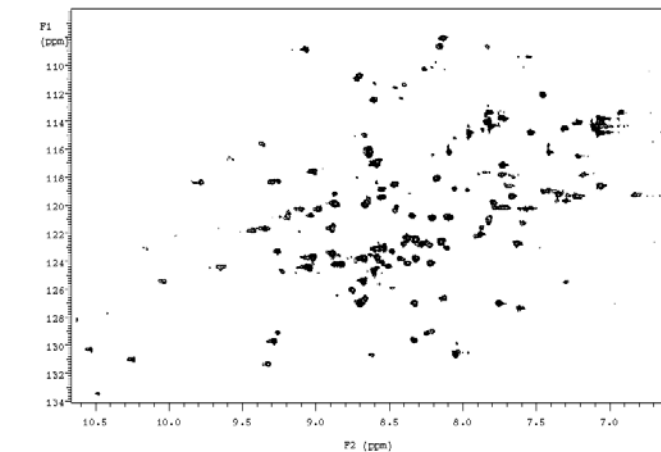


Figure 3: A gNHSQC of a small, ^{15}N -labeled protein (top) has many resonances compared to a HSQC_d2 (^{15}N) of a three-residue peptide at natural abundance.

Versions of HSQC

gHSQC – gradient version of the HSQC, no presat option, TANGO gradient option, no BIRD option. Use **phase=1,2**.

hsqc – lower case version

gNhsqc – Protein Pack version, gradient sens. Enhanced for N15, options for TROSY, T1, T1rho, and T2 relaxation measurements, see **man('gNhsqc')**.

gNfhsqc – Protein Pack version, fast N15 hsqc, same parameters as gNhsqc except has option for 3919 watergate suppression.