

Heteronuclear 2D Experiments in NMR

Kyra Bothwell

Almac Sciences, 20 Seagoe Industrial Estate, Craigavon, Northern Ireland, BT63 5QD

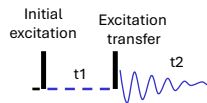
Introduction

Heteronuclear 2D experiments describe experiments featuring 2 different nuclei.

Compared to 1D NMR, the additional dimension is added by varying the length of time (t_1) the system is allowed to evolve following the first pulse.

Instead of a single FID, a series of incremented 1D experiments (typically several hundreds) are acquired at the incremented t_1 time.

Use of a 2nd dimension usually allows for separation of peaks which are unresolved by a 1D spectrum alone.



HSQC

Heteronuclear Single Quantum Correlation

- ^1H - ^nX correlation experiment – where ^1H is in the direct dimension (f_2) and ^nX in the indirect (f_1).
- Single bond correlation between a directly connected ^1H and ^nX atom (through bond coupling aka J coupling).
- Decoupled HSQC experiment typically default (increases sensitivity, simplifies spectral output).
- Most common applications found with ^1H - ^{13}C , ^1H - ^{15}N and ^1H - ^{31}P .
- HSQC phase sensitive experiments that are multiplicity edited are useful – giving phasing info represented in 2D for example as CH & CH₃ blue and CH₂ green; or the other way round if there are more CH₂ groups than CH & CH₃.
- Modern techniques typically rely on inverse detection e.g. in an ^1H - ^{13}C experiment the carbon dimension is acquired by proton observation, resulting in a projected spectrum in the indirect axis which can be replaced with a separately acquired ^{13}C spectrum if required.

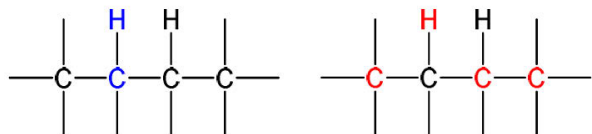


Figure 1: Representation of single bond correlation in a ^1H - ^{13}C HSQC/HMQC (left) vs 2-3 bond correlation in HMBC (right).

HMQC

Heteronuclear Multiple Quantum Correlation

- During a HMQC experiment magnetisation of both nuclei is allowed to evolve (i.e. multiple), whereas in HSQC only ^nX magnetization evolves (i.e. single).
- Therefore HMQC is affected by homonuclear proton J -coupling – manifesting as broadening in the X direction (indirect, f_1).
- This gives better resolution in the projection of the HSQC compared to the HMQC.
- There are more pulses in an HSQC compared to HMQC which means systems with poorer probe tuning or pulse calibration are more susceptible to S:N losses in HSQC compared to HMQC.

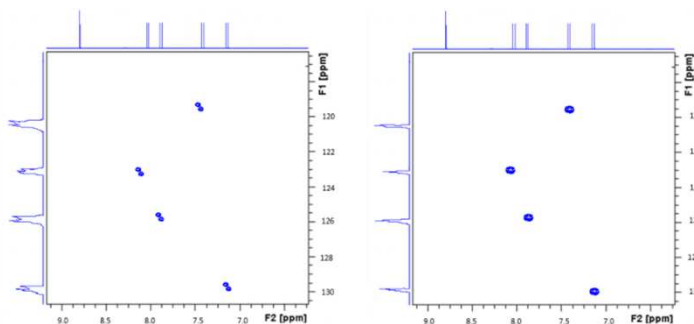


Figure 2: HMQC (left) vs HSQC (right).

Common oddities

- Default ^1H - ^{13}C HSQC cuts off at ~ 150 ppm in the indirect dimension (^{13}C) due to time and limited applications beyond this (aldehydes and some aromatic protons). This can result in some missing peaks however combination with DEPT and HMBC data is typically sufficient.
- HSQC experiment assumes a linear relationship between carbon chemical shift and the size of the proton carbon coupling therefore atypical J -couplings between ^1H and ^{13}C give atypical results.
 - Strained rings (3 member rings) will often be weak, missing or will show up with the opposite phasing expected (i.e. opposite colour cross peak).
 - Alkynes have very large 2-bond J -coupling ($\sim 50\text{Hz}$) and can show up as weak correlations.

HMBC

Heteronuclear Multiple Bond Correlation

- Longer range correlations - typically optimised for 2-3 bond however 4 and 5 bond correlations can be observed depending on the electronics of structures (e.g. ring structures/heterocyclics).
- Similar to HSQC but essentially tuned to detect smaller couplings around 10Hz (pulse sequence suppresses 1 bond correlations).
- Also an inverse experiment.
- Can often get indirect information on a 3rd nuclei, e.g. ^{19}F splitting observed in HMBC correlations.

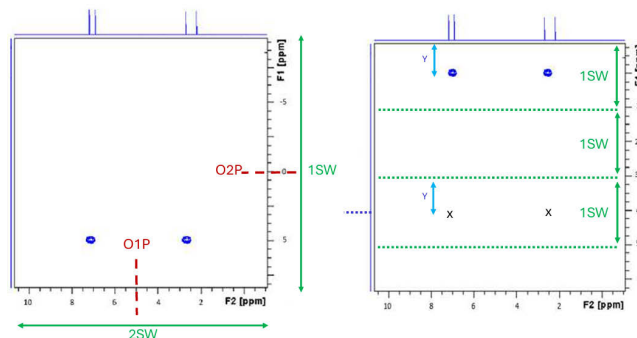


Figure 3: a) Spectrum with cut off f_1 range with folded signals (left).
b) Visual representation of aliasing/peak folding (right).

Common oddities

When setting up HMBC experiments for nuclei with wider frequency ranges than ^1H - ^{13}C (e.g. ^1H - ^{31}P), the default experiment conditions will usually need adjustment of the midpoint (O2P) and spectral width (1SW) of the indirect dimension.

- Figure 3a shows a HMBC experiment where the correlations do not have the expected corresponding projected signal in the f_1 dimension.
- 1D data shows the expected signal is at $\sim X\text{ppm}$ (f_1), which is outside of the range as set by O2P and 1SW in the 2D experiment.
- This effect is known as aliasing or folding.
- Figure 3b illustrates how this occurs; the correlations appear at the distance Y they have been displaced from the start of the spectral width window.
- Ideally first acquire the 1D spectra of the intended indirect nuclei and base the midpoint and spectral width on this for the 2D experiment. However, all is not lost if these parameters have not captured the required detection window as the corrected frequency of the aliased peak can easily be calculated.
- Digital filters can be used to remove this effect (for example the default ^1H - ^{13}C HSQC experiment), however it can be useful in certain applications.
- Also aliasing can be used intentionally to decrease acquisition time, which can be particularly useful in peptide/protein NMR.

Quantitative Heteronuclear 2D NMR

- Whilst 2D NMR is ubiquitous in qualitative NMR, it has so far found less application in quantitative NMR compared to traditional qNMR.
- However, 2D qNMR presents an opportunity for increased resolution for complex mixtures and unresolved peaks.
- Traditional 2D NMR experiments are inherently non-quantitative, as parameters such as relaxation delays and pulse sequence delays are not optimised due to time restraints, and therefore cannot ensure the full relaxation required for quantitative data.
- Increased experimental duration is more susceptible to spectrometer instability over time, generating additional t_1 noise, as well as 2D requiring multiple pulses, all present additional challenges compared to traditional qNMR.
- One such solution to emerge has been "ultrafast" (UF) 2D which allows 2D data to be acquired in a single scan, instead of hundreds of incremented scans.
- However, this has limitations in terms of reduced resolution in the indirect dimension and reduced sensitivity in the 2D spectrum.
- Other examples: Perfect HSQC, HSQC₀ and Q-HSQC.