Today's Experiments: HETERONUCLEAR CORRELATION

1. **HSQC** – ^{1}H-^{13}C Heteronuclear Single Quantum Coherence ($^1J_{CH}$ coupling)
2. **HSQC-INEPT** –combines HSQC + DEPT information into one experiment
3. **HMBC** with NUS (non-uniform sampling) – ^{1}H-^{13}C Heteronuclear Multiple Bond Correlation ($^2J_{CH}$, $^3J_{CH}$ & sometimes $^4J_{CH}$ coupling, while suppressing $^1J_{CH}$)

For the lab, we'll use **2% 2-ethyl-1-indanone in CDCl$_3$** (white capped NMR tube).

Note: 2D experiments are powerful techniques, but are often a compromise between the amount of information we want and how much spectrometer time we can afford (also, your sample concentration!).
   a. Any of the 2D experiments done in class can utilize the Non-Uniform Sampling (NUS) functionality of Bruker's TopSpin. We are using NUS for some 2D applications, but not all. See the NUS handout on Canvas for more info.
   b. 1D Selective Experiments: these focus on only one specific region of the spectrum or a specific correlation. In class, we are not utilizing 1D selective experiments for heteronuclear coupling applications.

1 & 2. Gradient-Selected, Phase-Sensitive HQSC & Multiplicity-Edited HSQC-DEPT

Fig 1: HSQC gives a 2D correlation map between directly-bonded ^{1}H and heteronuclei like ^{13}C and ^{15}N.w

The ^{1}H-^{13}C heteronuclear single quantum coherence (HSQC) experiment provides correlation between a carbon and its attached protons (Fig 1). The basic scheme involves transfer of magnetization from ^{1}H→^{13}C. After a variable time delay, the magnetization is transferred back to proton and the ^{1}H signal is recorded. HSQC was first developed [*Chem. Phys. Lett.*, **69**, 185-189 (1980)] for transfer of polarization from ^{1}H→^{15}N, but has since been applied to ^{1}H-^{13}C systems.

HSQC is widely used because it is based on proton detection, which offers much higher sensitivity compared to the conventional 2D HETCOR experiment (also called CH-COSY), which observes the ^{13}C signal. Another popular experiment is the multiplicity-edited HSQC-DEPT experiment, which combines the information you get from an HSQC experiment with information you would get from a DEPT.

As an aside, there is yet another similar experiment, called the HMQC (heteronuclear multiple quantum coherence). Like the HSQC, this is a proton-detect experiment; however it is lower in resolution (lines are broader, see below). However, HMQC is more robust to experimental imperfections compared to HSQC, so it is sometimes preferred on older spectrometers.
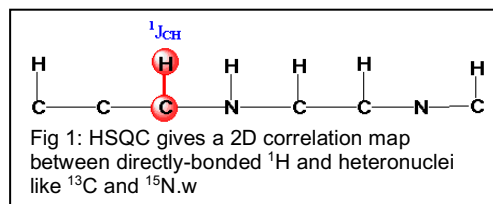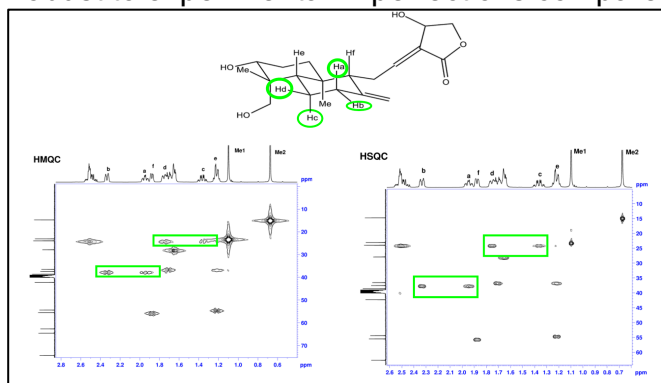
The HSQC-DEPT experiment has become a routine experiment due to its superior resolution and spectral-editing capabilities. It is a proton-detect experiment, so it is recommended that you use an inverse probe or a cryoprobe for maximum sensitivity.

Similar to COSY, there are many different versions of the HSQC experiment. In class today we'll use the gradient-selected, HSQC and HSQC-DEPT experiments with sensitivity improvement and selective pulses.

HSQC & Multiplicity-Edited HSQC-DEPT with NUS Data Acquisition

1) From your ^{1}H proton data previously acquired, determine an optimized spectral window (**sw**), i.e., the region containing your peaks with at least one ppm on either side. Note that all of the peaks outside of this chemical shift range will be eliminated from your 2D spectrum. Write down the following parameters:

     a. **sw** [ppm]: the spectral width of your optimized window
     b. **o1p** [ppm]: the center of the optimized window
     c. **sr** [Hz]: this parameter gets set automatically, it's usually 0
   d. You also need to calibrate your 90° pulse and get the **p1** value [us]

2) From the ^{13}C spectrum you acquired earlier, determine an optimized spectral window (**sw**), i.e., the region containing your peaks with at least one ppm on either side. *Unlike* the ^{1}H dimension, the peaks outside of this chemical shift range *will not* be eliminated from your 2D spectrum; instead they may fold over in an effect called aliasing. Write down the following parameters:

     a. **sw** [ppm]: the spectral width of your optimized window
     b. **o1p** [ppm]: the center of the optimized window
     c. **sr** [Hz]: this parameter gets set automatically, it's usually 0

3) Create a new experiment and read in the gHSQC parameter set (e.g., **rpar ghsqcNUS.av500**). Lock, tune the probe, shim, etc.

- Note: the name of the **pulprog** is **hsqcetgpsisp2.2**, which implies it is an HSCQ (hs<u>q</u>cetgpsisp2.2) sequence with an <u>E</u>cho/Antiecho-<u>T</u>PPI acquisition mode in F1 (hsqc<u>et</u>gpsisp2.2), <u>S</u>ensitivity <u>I</u>mprovement (hsqcetgp<u>si</u>sp2.2), and uses <u>G</u>radient <u>P</u>ulses and <u>S</u>haped (selective) <u>P</u>ulses (hsqcet<u>gp</u>si<u>sp</u>2.2).

3) Type **eda** to view the Acquisition Parameters. There are two columns: on the left is the **F2 dimension** and on the right is the **F1 dimension**. Check the setup for the parameters:
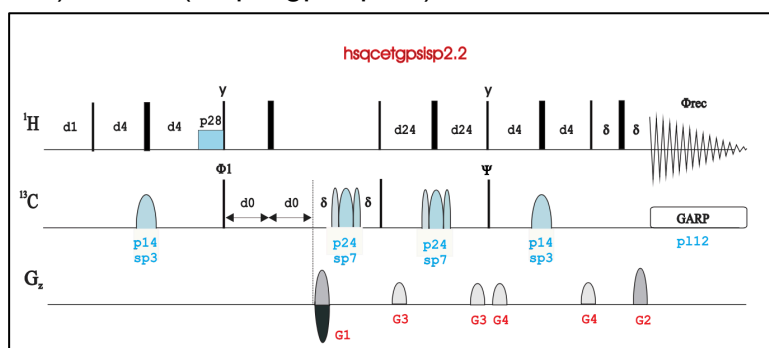
Parameter	F2 dimension	F1 dimension
td [# of points]	4k (4096) **see note below	256
o1p [ppm]	use the ^{1}H o1p from step 1b	use the ^{13}C o1p from step 2b
sw [ppm]	use the ^{1}H sw from step 1a	use the ^{13}C sw from step 2a

You'll also want to set **p1** to your calibrated 90° pulse length.
** Note: For small molecules with sharp linewidths, you can start with **td(F2)** = 4k. Ensure that **aq** does not exceed 0.5 seconds. If it does, lower **td(F2)** accordingly.

4) Click on the "**PulseProg**" tab and check the pulse program text for suggested values for **ns** and **ds**. In 2D experiments, these parameters frequently depend on something called "phase-cycling."

5) Set **ns** = 2, **ds** = 16, and **d1** = 2. We will enable non-uniform sampling (NUS) for this experiment. Set the **FnTYPE** to non-uniform sampling and set **NusAMOUNT** to 50%. (Reduces scan time by a factor of 2 so that expt ≈ 10-15 minutes). Check how long the experiment will take with **expt**.

6) Under the **ased** menu, the default value of **CNST2** = 145 Hz. This is the "generic" or "average $^1J_{CH}$ for directly bonded C-H. Setting **CNST2** = 145 Hz is a compromise for the wide range of $^1J_{CH}$ values that can exist in a sample. You can modify this value for different purposes.

7) Before you start acquiring make sure the probe is tuned to both proton and carbon. Set the receiver gain with **rga** and then **zg** to start acquiring.

8) While this is running, you can save time by setting up the HSQC-DEPT experiment. Type **iexpno** to create a new experiment with the exact same parameters as your HSQC.
- Change the **pulprog** to **hsqcedetgpsisp2.2**, which is similar to the regular HSQC, except it has a multiplicity-editing via DEPT (hsqcedetgpsisp2.2) and utilizes Gradient Pulses and Shaped Pulses (hsqcedetgpsisp2.2).
- You also need to pay attention to **CSNT2**, **D21**, and **D24**. By default, **CNST2** = 145 Hz. **D21** is suggested to be 1/(2J) for DEPT135. **D24** is suggested to be 1/(8J) for DEPT135 (all multiplicities) or 1/(4J) for DEPT90 (CH only).

9) While the HSQC-DEPT is running, you can save time by creating a new experiment to set up the HMBC experiment, see instructions below.


HSQC & HSQC-DEPT Data Processing in TopSpin:
1) Type **edp**. Change the following parameters in both **F2** and **F1** dimensions:
   a. **sr**: set to the value determined above.
   b. **window functions**: use **em** for **F2** and **QSINE** for **F1**.
   c. **si**: set SI ≥ TD for both F1 & F2. Note that **SI**(F1) > 512 slows data processing.

2) For NUS data there are two extra steps to perform after 2D Fourier transform **xfb**. TopSpin will need to perform 2D Hilbert transform (**xht1**; **xht2**). This can take a *long* time in TopSpin (but it can be done!), so we recommend just opening it up in Mnova. ☺

HSQC & HSQC-DEPT Data Processing in MestReNova
Mnova will do the data processing for you, although you may want to change the *window function (apodization)* and *zero-filling* parameters, phase the data appropriately, and put up external projections on the F2 and F1 dimensions. HSQC is not a homonuclear correlation experiment; you do not want to *Symmetrize* your data.
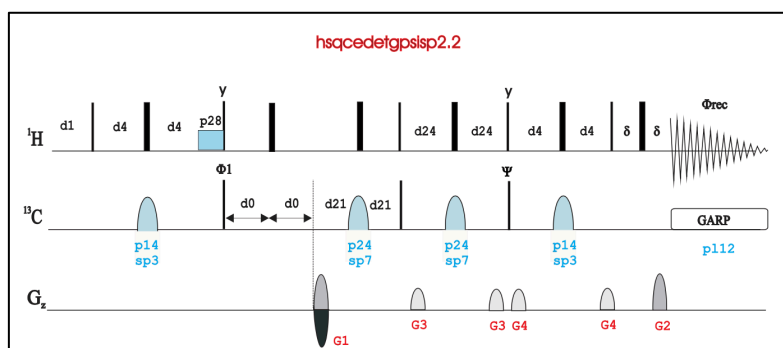
One way to get Mnova to automatically plot the external projections from your 1D ^{1}H and ^{13}C spectra is to first open a 1D proton and carbon spectra in the software. When you open the 2D dataset, it usually automatically puts in the external projections. See https://resources.mestrelab.com/starting-guide-to-mnova-nmr-2d/ for more details.

For the HSQC-DEPT experiment, you may want to look at the traces directly from the 2D spectrum. Use Mnova's "Select Traces Graphically" tool in the "Show Traces" menu. See the Mnova manual for more details.

3. Gradient-Selected HMBC

The Heteronuclear Multiple Bond Correlation (HMBC) experiment gives correlations between carbons and protons that are separated by two, three, and sometimes four bonds (Fig. 2). Direct one-bond correlations are suppressed via a low-pass filter, but can still appear for peaks with large intensities. HMBC is certainly one of the most

Fig 2: HMBC gives a 2D correlation map showing 2- and 3-bond correlations between ^{1}H and ^{13}C (or other heteronuclei like ^{15}N).

important and information rich experiments for structure elucidation. Since this experiment is based on proton detection, use an inverse probe/cryoprobe/inverse cryoprobe for increased sensitivity when possible.
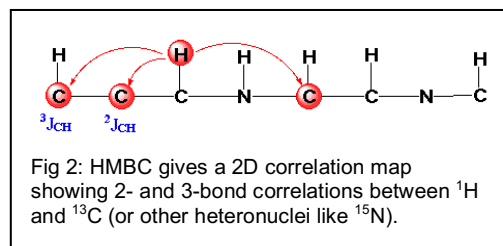
HMBC with NUS Data Acquisition

1) Create a new experiment and read in the gHMBC with NUS parameter set (e.g., **rpar ghmbcNUS.av500**).

- Note: the name of the **pulprog** is **hmbcgplpndqf**, which implies it is a magnitude-mode HMBC (hmbcgplpndqf) sequence with a Low-Pass J-filter, uses Gradient Pulses for H-X correlation via heteronuclear zero & double quantum coherence with No Decoupling during acquisition.
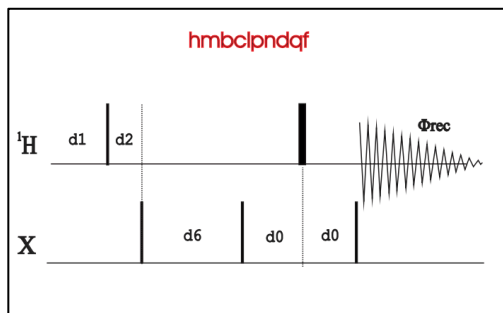
2) Type **eda** to view the Acquisition Parameters. There are two columns: on the left is the **F2 dimension** and on the right is the **F1 dimension**. Check the setup for the parameters:

Parameter	F2 dimension	F1 dimension
td [# of points]	4k (4096) **see note below	512
o1p [ppm]	use the ^{1}H o1p from earlier	use the ^{13}C o1p from earlier
sw [ppm]	use the ^{1}H sw from earlier	use the ^{13}C sw from earlier

You'll also want to set **p1** to your calibrated 90° pulse length.
** Note: For small molecules with sharp linewidths, you can start with **td(F2)** = 4k. Ensure that **aq** does not exceed 0.5 seconds. If it does, lower **td(F2)** accordingly.

4) Click on the "**PulseProg**" tab and check the pulse program text for suggested values for **ns** and **ds**. In 2D experiments, these parameters frequently depend on something called "phase-cycling."

5) Set **ns** = 2, **ds** = 16, and **d1** = 2. We will enable non-uniform sampling (NUS) for this experiment. Set the **FnTYPE** to non-uniform sampling and set **NusAMOUNT** to 50%.

(Reduces scan time by a factor of 2, so that expt ≈ 15-20 minutes.) Check how long the experiment will take with **expt**. If the experiment takes too long, you can reduce **td(F1)** by a factor of 2, i.e., change to 256.

6) Under the **ased** menu, take note of **CNST2** and **CNST3.** The default value of **CNST2** = 145 Hz and CNST3 = 10 Hz (the "generic" long-range J-values for $^2J_{CH}$ and $^3J_{CH}$).

7) Before you start acquiring make sure the probe is tuned to both proton and carbon. Set the receiver gain with **rga** and then **zg** to start acquiring.

HMBC Data Processing in TopSpin:
1) Type **edp**. Change the following parameters in both **F2** and **F1** dimensions:
       a. **sr**: set to the value determined above.
       b. **window functions**: use **QSINE** for **F2** and **F1**. You can use SSB=2(cos).
       c. **si**: set SI ≥ TD for both F1 & F2. Note that **SI**(F1) > 512 slows data processing.

2) For NUS data there are two extra steps after the 2D Fourier transform **xfb**. TopSpin will need to perform 2D Hilbert transform (**xht1**; **xht2**). This can take a *long* time in TopSpin (but it can be done!), so we recommend just opening it up in Mnova. ☺

HMBC Data Processing in MestReNova
Mnova will do the data processing for you, although you may want to change the *window function (apodization)* and *zero-filling* parameters, and put up external projections on the F2 and F1 dimensions.

When you are done in the lab, make sure to log out. Type **CTRL + ALT + BACKSPACE** to log out of the computer.

What to turn in:
   * The full HSQC, HSQC-DEPT, and HMBC spectra, as well as any expanded regions that you think are important. Assign all of the cross peaks.
       o If you use the same plot expansions for HSQC and HMBC, this will simplify the comparison.
   * Answers to the following questions:

Questions

1. In normal 1D proton NMR, we may see a pair of ^{13}C satellites in ^1H peaks of high intensity. What causes these ^{13}C satellites, and what is the dominant signal that we observe?
2. In the HSQC experiment, cross peaks for protons directly bound to a carbon-13 atom (i.e., we are looking at the satellite peaks). So in theory, we ought to see a doublet in our HSQC cross peak, but in practice we only see a singlet. Why? (Hint: In an NMR experiment, how do you make a multiplet "collapse" into a singlet?)
3. What are the assumptions we make for $^1J_{CH}$ and $^nJ_{CH}$ we make in our pulse sequence parameters? Discuss the validity of these assumptions.
4. HMBC shows cross peaks that do not appear in the HSQC. Why?
5. In Lab 4, we were able to "Symmetrize" the COSY and TOCSY data. (In theory, you can do this with a 2D NOESY as well.) Why should you not apply this same processing technique to your HSQC or HMBC.