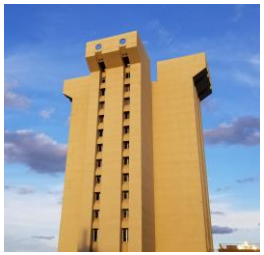
UC Chemistry Nuclear Magnetic Resonance (NMR) Facility



Three NMR instruments available to UC faculty and students as well as universities and industry in the Cincinnati area:

NEO400:	Rm 123A/Crosley	^{1}H; ^{19}F; ^{31}P-^{15}N, 2Ds
AV500:	Rm 123B/Crosley	^{1}H; ^{31}P-^{15}N, 2Ds
AV400:	Rm 108/Crosley	^{1}H; ^{19}F; ^{31}P-^{15}N

Alex Greenwood
Office: Rm 123C/Crosley;
Phone: 513-556-9211;
Email: greenwa2@ucmail.uc.edu

For user training, technical assistance, NMR questions and discussions.

NMR lab services:

NMR On-Demand

- Three spectrometers (two 400 MHz and one 500 MHz) available 24/7 for routine spectroscopy to trained users, ***no reservation required***

Non-Routine experiments

- Instruments can be reserved for non-routine experiments requiring extra setup/calibration, temperature regulation, or long run times
- Solid state experiments on the convertible NEO400 available upon request

Consultation

- Experiment design/planning
- Data interpretation/troubleshooting
- Structure/stereochemistry determination

Bruker NEO 400 MHz Spectrometer:

Z-Grad BBFO ATM iprobe: $^{1}H/^{19}F$-^{15}N
Variable temperature capability
Automatic sample changer
^{1}H, ^{15}N-^{19}F 1D and multi-nuclear NMR in one and two dimensions
Solid-state capabilities

- State-of the art console, probe and software
- Walk-up instrument, 24/7 availability
- Submit experiment and leave-- data is collected automatically and accessed remotely
- Software: Topspin 4 running ICON-NMR

Bruker AV 500 MHz Spectrometer:

Z-Grad BBFO ATM probe: $^{1}H/^{31}P$-^{15}N
Variable temperature capability
Automatic sample changer
^{1}H, ^{15}N-^{31}P 1D NMR and multi-nuclear NMR in one and two dimensions **(no ^{19}F)**

- Walk-up instrument, 24/7 availability
- Submit experiment and leave-- data is collected automatically and accessed remotely
- Software: Topspin 3 running ICON-NMR

Bruker AV 400 MHz Spectrometer:

Z-Grad BBFO ATM probe: $^{1}H/^{19}F$-^{15}N
Variable temperature capability
Automatic sample changer
^{1}H, ^{15}N-^{19}F 1D NMR

- Walk-up instrument, 24/7 availability
- Submit experiment and leave-- data is collected automatically and accessed remotely
- Software: Topspin 2 running ICON-NMR

Which Magnet should you use?

	AV400	NEO400	AV500
			
^{1}H	1.0	**1.4**	**1.4**
^{13}C	1.1	1.0	**1.5**
^{31}P	1.1	**1.5**	1.0
^{19}F	1.0	**1.2**	~~NA~~

Relative signal-to-noise

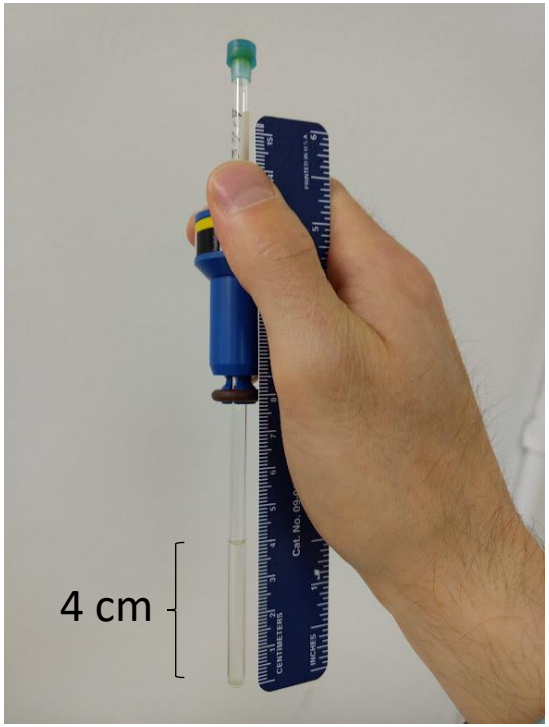
Note– no ^{19}F on AV500!

2D spectra	**NEO400 and AV500**
High-NS ^{1}H 1Ds:	**NEO400 and AV500**
Variable-temp:	**NEO400**
^{13}C 1Ds:	**AV500**
^{31}P and ^{19}F:	**NEO400**
Most cases:	**Whichever is available!**

Whichever one is available is usually best!

Preparing your NMR sample

- Use a deuterated solvent if you want ^{1}H spectra completely free of solvent signals (but solvent suppression works pretty well!).
- Use at least 600 µl (4 cm, or 3 fingers) for good shimming/linewidths
- Use tubes rated for 400 MHz or 500 MHz (for good shimming/linewidths)
- Mark tubes well and use your lab's designated cap color.
- **Tubes must not be scratched or broken!**
- Tubes should not be dried in ovens hotter than 100 C!

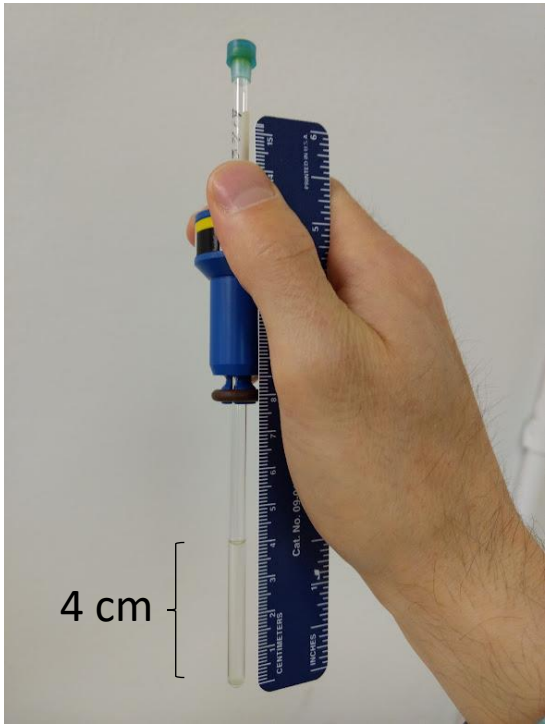


Preparing your NMR sample

- Solution should be free of particulate– insoluble material will not give signal but **will** disrupt shimming!

- Use appropriate concentration of material!
  - For ^{1}H 1D: **2 mM** or ~**0.25 mg** gives a SNR of 100 in 16 scans
  - For ^{13}C 1D: **35 mM** or **~6** mg for SNR of 10 at 1024 scans, or **200 mM** or **~25 mg** for SNR of 10 at 32 scans

  (masses assume molecular mass of 200 Da)



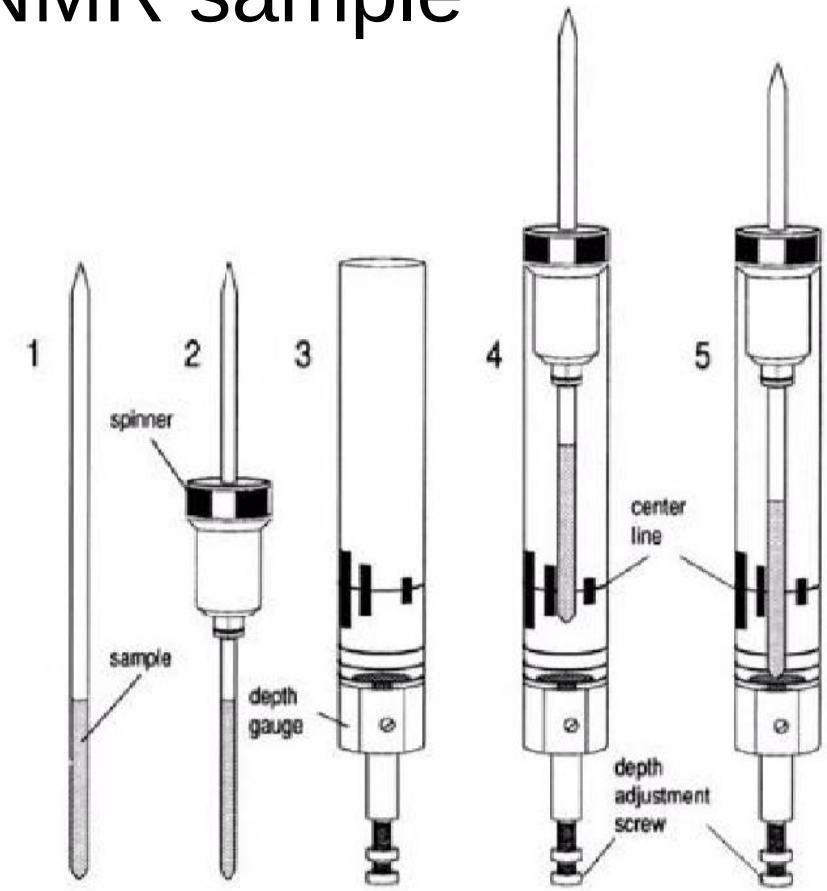
Submitting your NMR sample

1) Put tube in spinner

2) Clean tube and spinner with kimwipe

3) Position tube with depth gauge– make sure spinner is flush with top

4) Small sample volumes should be centered in coil by bringing tube back up a bit

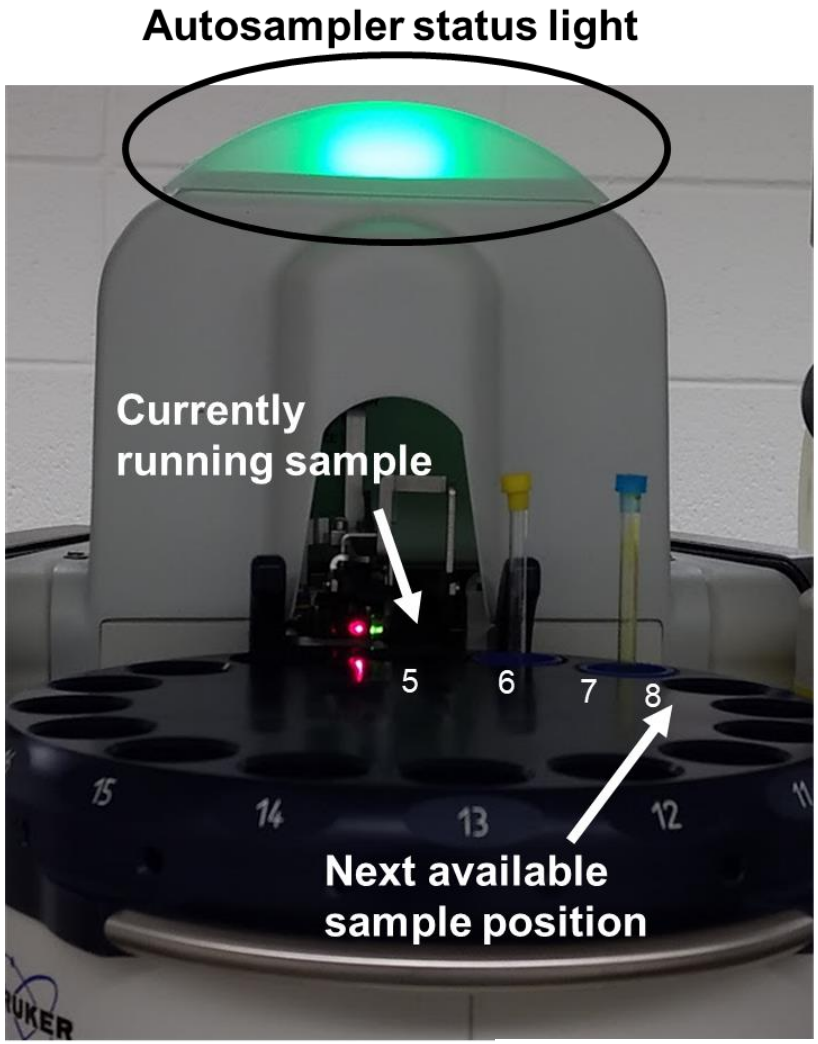


AVANCE Beginners Guide, Bruker

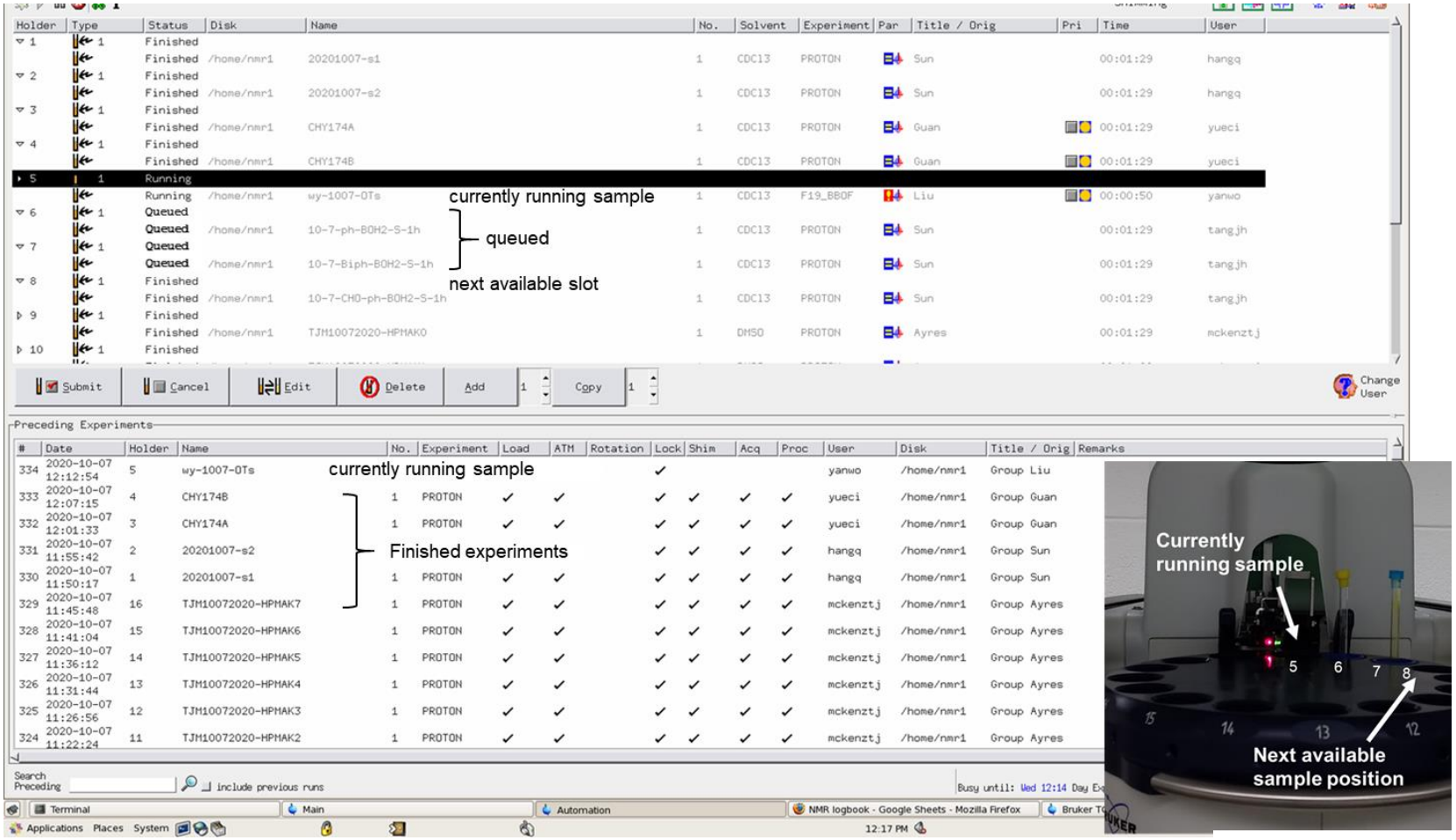
Submitting your sample in the autosamplers

5) Identify the next available position in the autosampler and insert your sample

6) Define your experiment in that slot and press “submit”



ICON-NMR Interface



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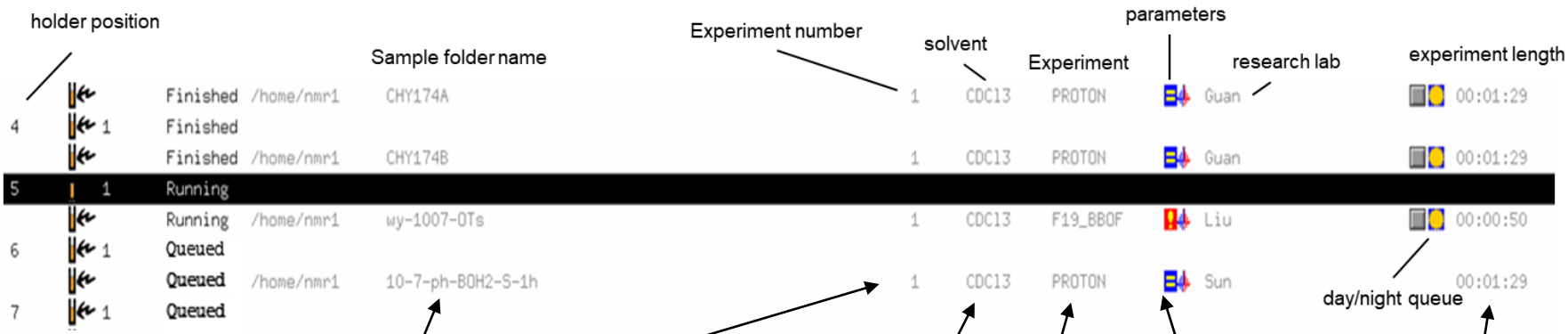


Name your sample folder– if you run multiple experiments on the same sample, they will go into numbered subfolders

Do not start or end the name with a period. Do not use previously-used names or the data will go into the old folder.

Specify solvent– incorrectly specified solvent will cause lock to fail and/or spectrum to be badly referenced

Set Experiment—Contact NMR manager to run experiments not available on your account

Some parameters can be adjusted, but be careful!

Check experiment length before pressing Submit!

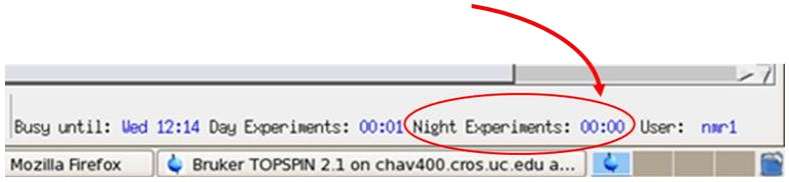
The Night Queue

- On the AV400 and AV500, ^{13}C spectra are automatically placed in the night queue. If the experiment time is < 20 min, they will run during idle daytime. Otherwise, they will run starting at 9 PM.

- On the NEO400, 2D spectra and C13CPD experiments will default to the night queue. C13CPD32 will default to the day queue, so either **do not adjust the experiment length longer than 30 minutes** or switch it to the night queue.

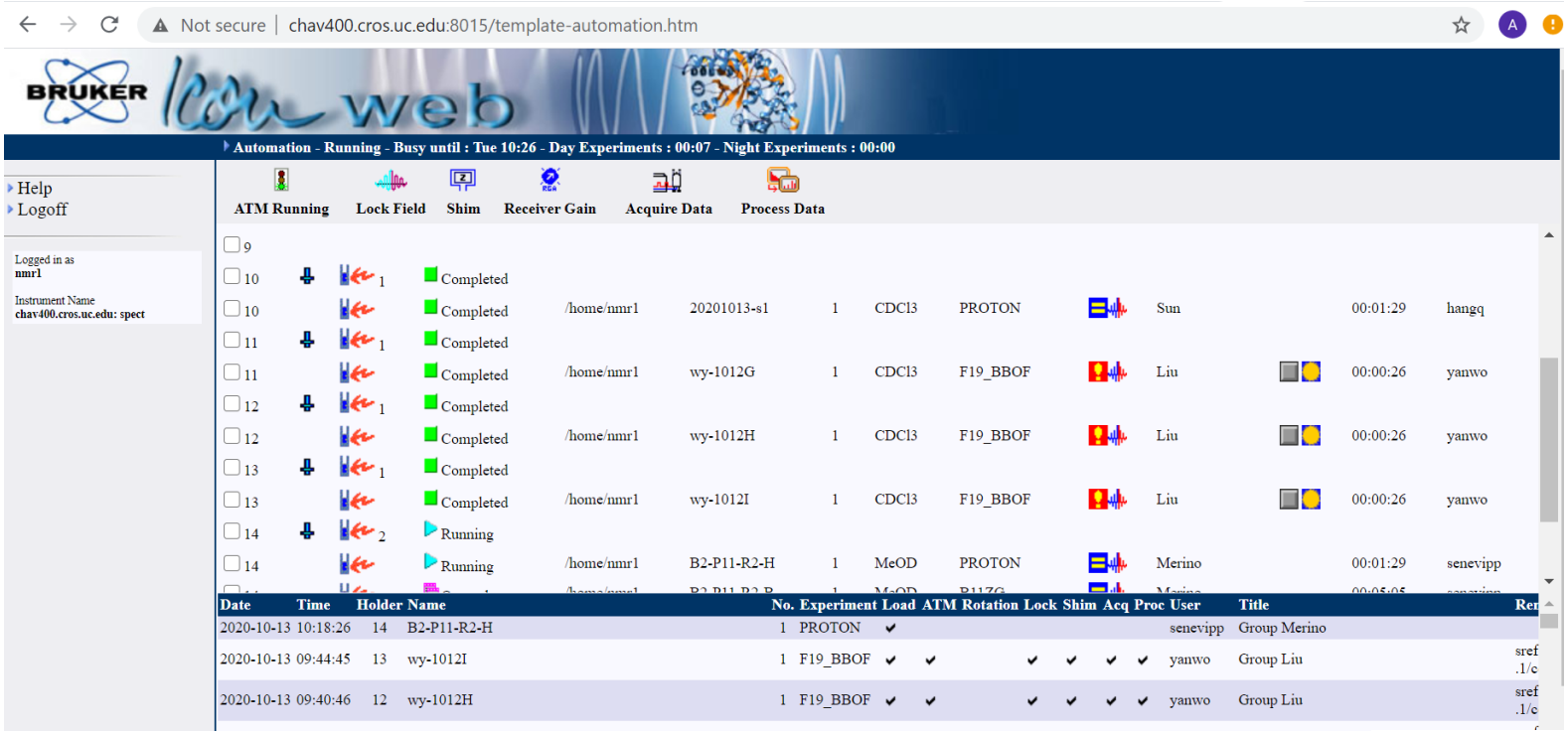
- Day-queue experiments (such as ^{1}H 1D) made to run long (> 20 min on AV400 and AV500, > 30 min on NEO400) should be set to the night queue by clicking on the sun icon:  It should switch to a moon: 

- Mind the total length of the night queue: 9PM-9AM on NEO400, 9PM-10:30 AM on AV400 and AV500. **Your experiment will not run if it can not finish within this window.** Allow approximately 5 extra minutes per experiment for lock/atm/shimming. Before submitting, check the current night queue length (from already-submitted experiments) in the bottom right corner:



Checking Status of NMR Instruments

- Type http://chav400.cros.uc.edu:8015 or http://chneo400.cros.uc.edu:8015 in web browser while on campus (links on NMR lab website)
- Username is your ICON-NMR username, password is “chemistry”
- “Read only” interface



General Rules

1) Wait 10 seconds after your sample is ejected before retrieving it.
   - Removing your sample before the sample changer light turns green again *will freeze the autosampler and halt data collection.*

2) Clean tubes with kimwipes and measure their depth with the depth gauge.
   - Tubes with small sample volumes can be placed higher than bottom of gauge to center the sample in the coil.
   - **Never** place the tube lower than the bottom of the gauge—**it may break in the probe.**

3) Retrieve your samples from the room in a reasonable amount of time.
   - Failure to do so results in our having to dispose of the samples as “unknowns” which is costly and involved.

4) Mark your tubes, using a marker on the glass or cap (no tape)
   - Doing so will allow us to contact you if something happens to your tube, or if it becomes forgotten. Initials should be fine in most cases.

5) Log out when you are done
   - This prevents other users from accidentally submitting jobs as you.

Instrument-Specific Rules

NEO400

Queue experiments in numerical order

- This is necessary due to a glitchy autosampler.
- Especially important when the instrument is idle**.** Failure to do so can cause automation to stop.
- The night queue is still operational.

Do not adjust the time of C13CPD32 experiments longer than 30 minutes.

- They will run during the daytime regardless of their length!
- For long ^{13}C, use C13CPD instead.
- Alternatively you can put these in the night queue by clicking the sun icon: 

AV400

Put experiments with experiment times exceeding 20 minutes in the night queue.

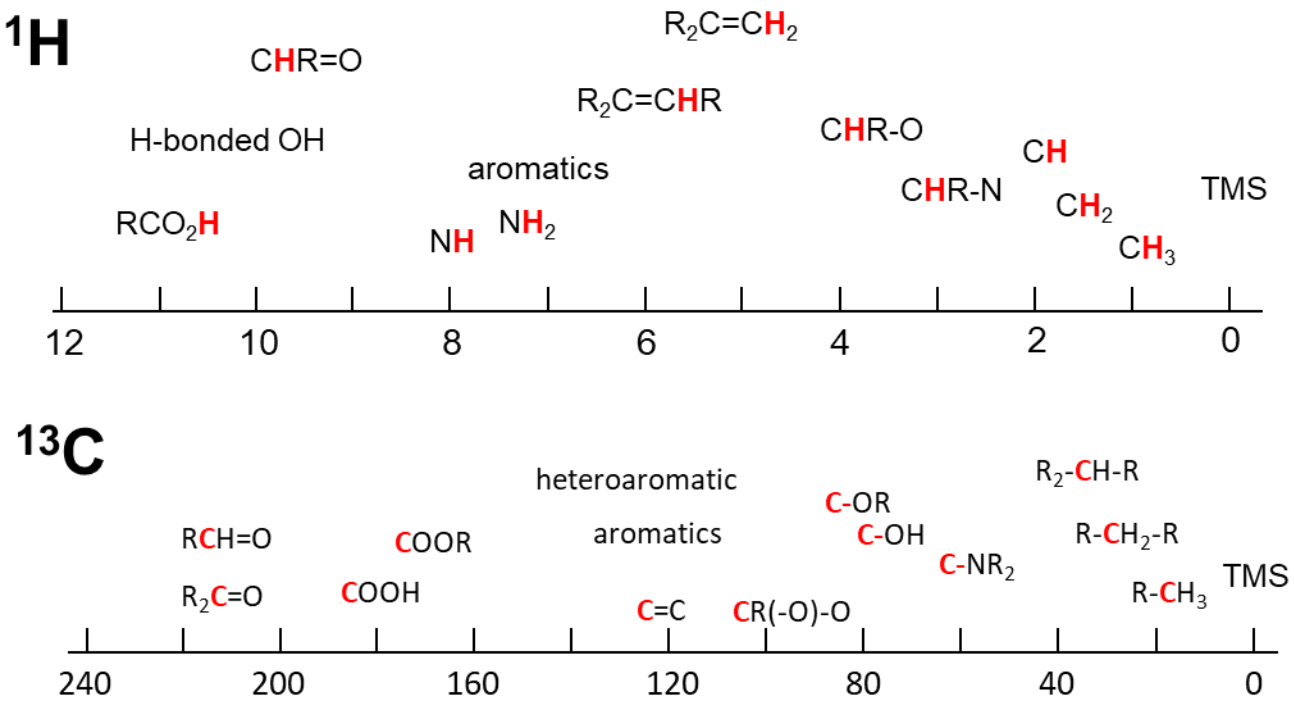
- Large numbers of moderately-long experiments should also be put in the night queue. If they are individually under 20 minutes they will run during idle daytime.
- See me if your account does not allow you to put experiments in the night queue.

AV500

Never carry phone/wallet/keys/headphones past the yellow chain!

- Magnet has large stray field! Keep possessions at desk.
- Go around yellow chain rather than stepping over.
- Do not move posts.

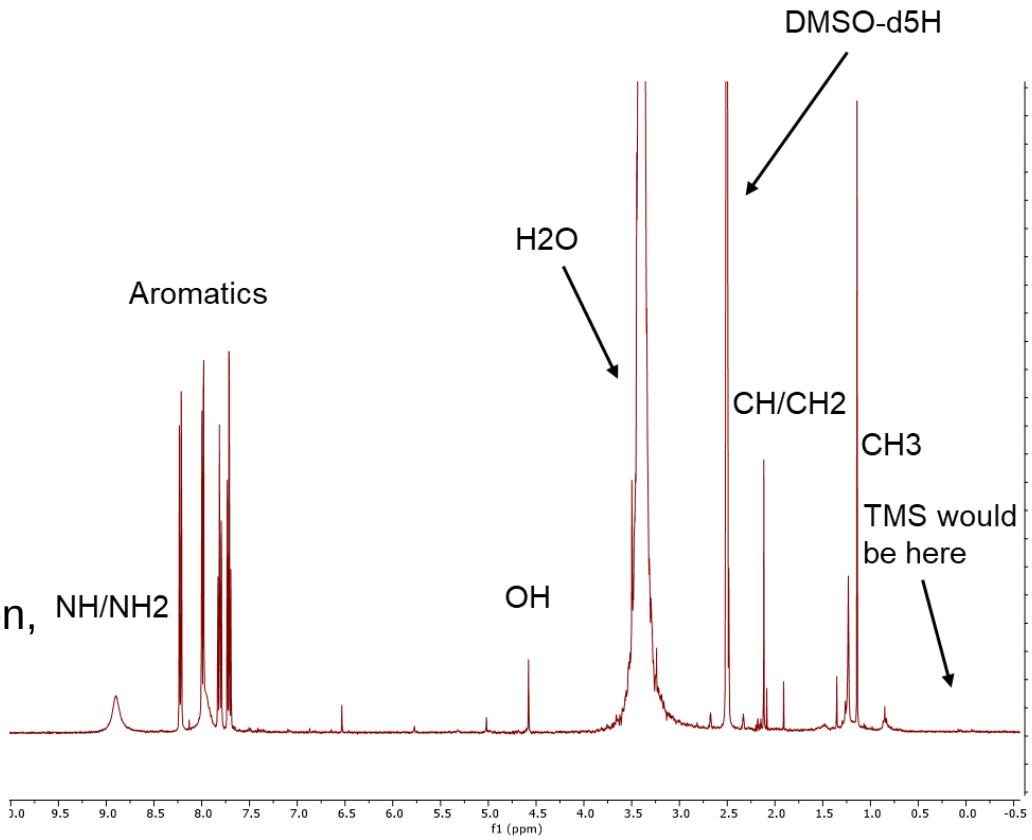
Chemical Shift Ranges



Note that the ^{13}C and ^{1}H trends tend to match each other! Chemical shift is affected by the same electrons in each case!

^{1}H zg30 (PROTON)

- High sensitivity
  - Impurities usually evident if present
- Shows multiplicity, aids in assignments
- Commonly acquired with 100% deuterated solvent, but not always necessary
- Relatively fast T1 relaxation, pulse delay can be ~ 2 s
- Overlap sometimes an issue



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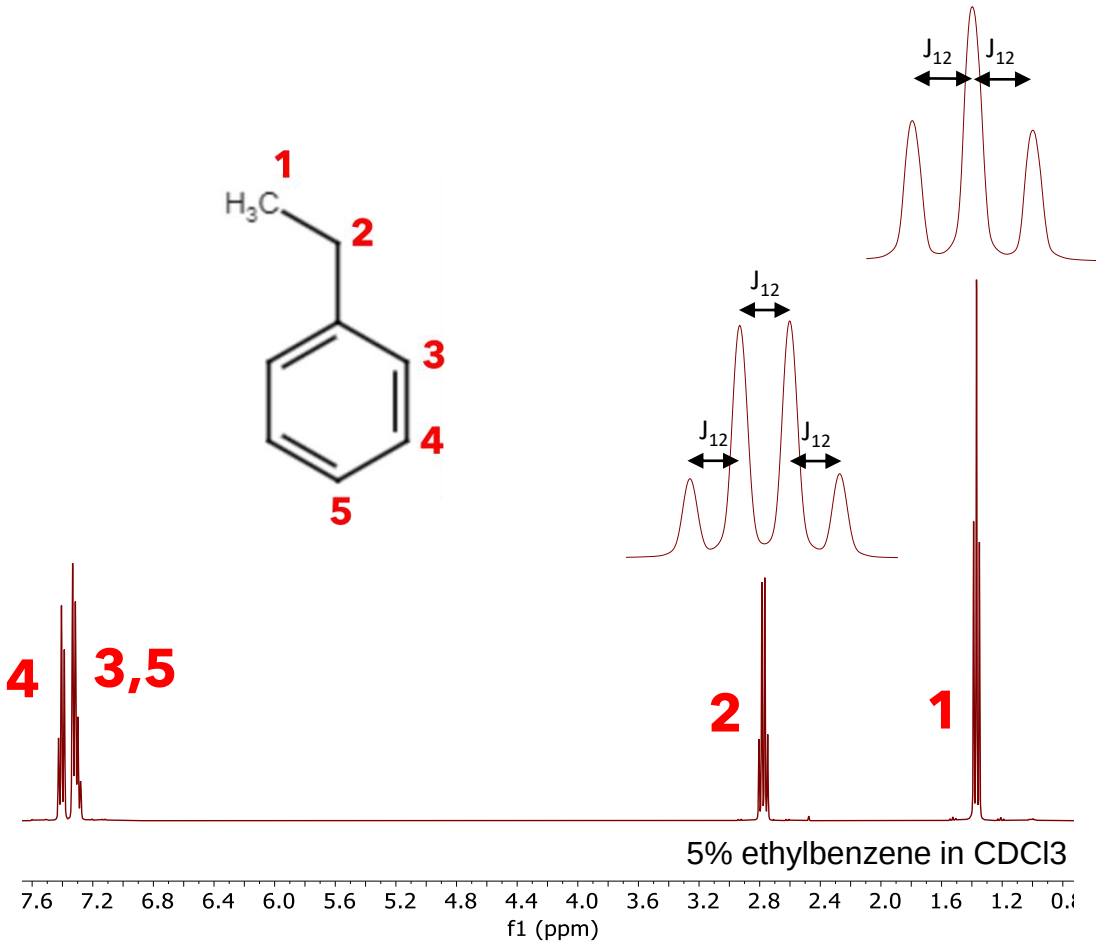
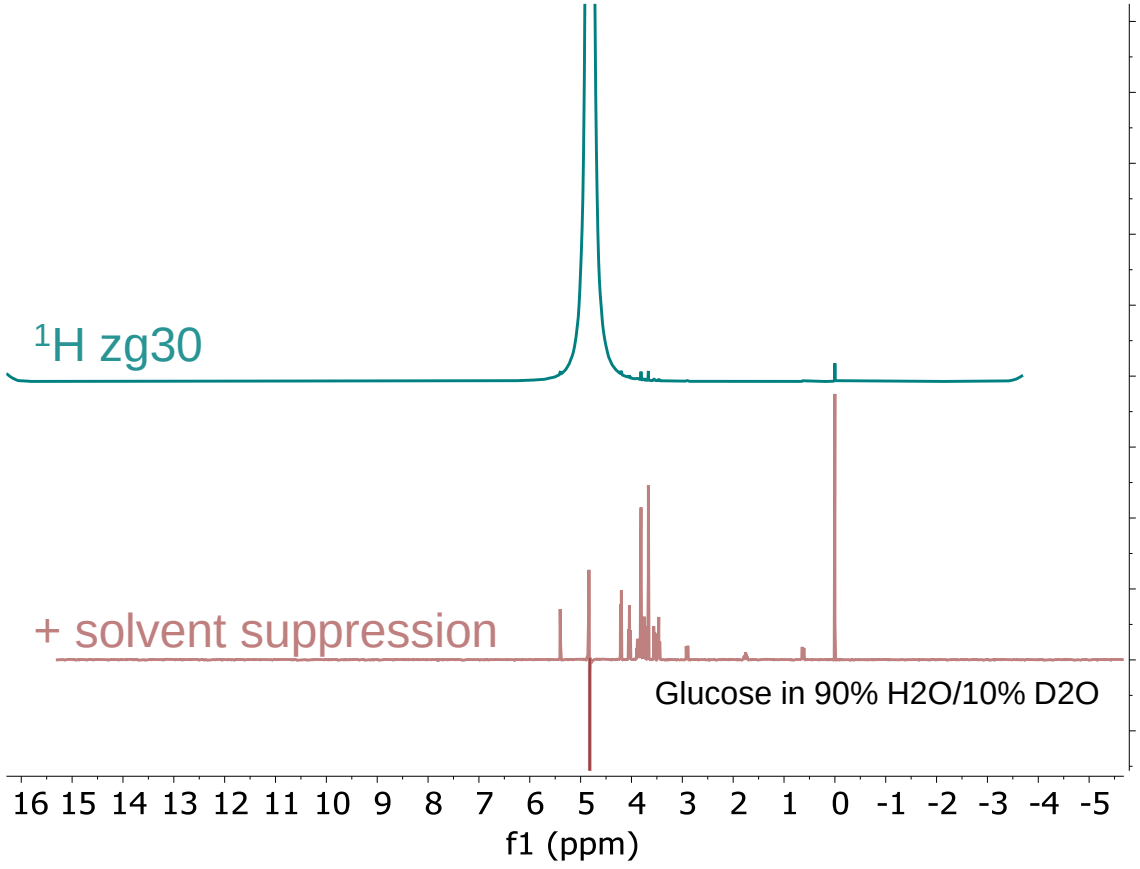


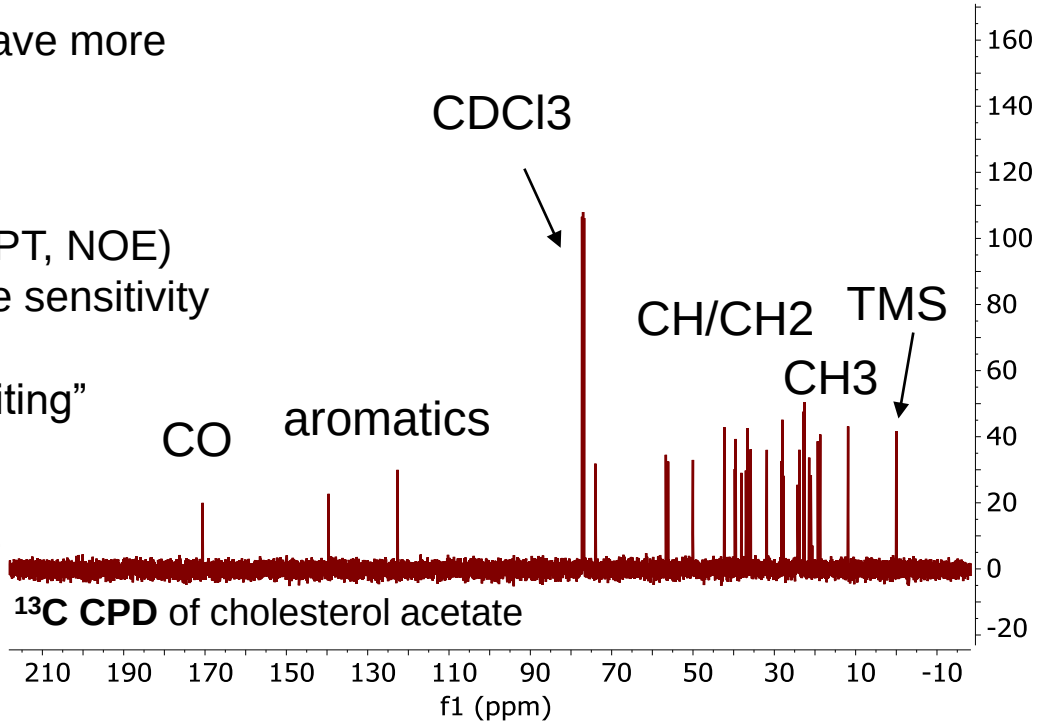
^{1}H with solvent suppression (WATERSUP1, PROTON_1HSOLV)

- Improves sensitivity
- Can be run with lock off (no deuterated solvent required)
- Residual signal at solvent frequency remains
- Peaks near solvent frequency will also be suppressed



^{13}C 1D (C13CPD, C13CPD32, C13DEPT135)

- Lower sensitivity
  - Protonated carbons usually have more signal
  - Use >10 mM if possible!
- Magnetization transfer (DEPT, INEPT, NOE) from attached protons can enhance sensitivity
- Multiplicity can be inferred with “editing” (DEPT)
- Slower T1 relaxation, especially for quaternary/unprotonated carbons
- Overlap not usually a problem



^{13}C CPD of cholesterol acetate

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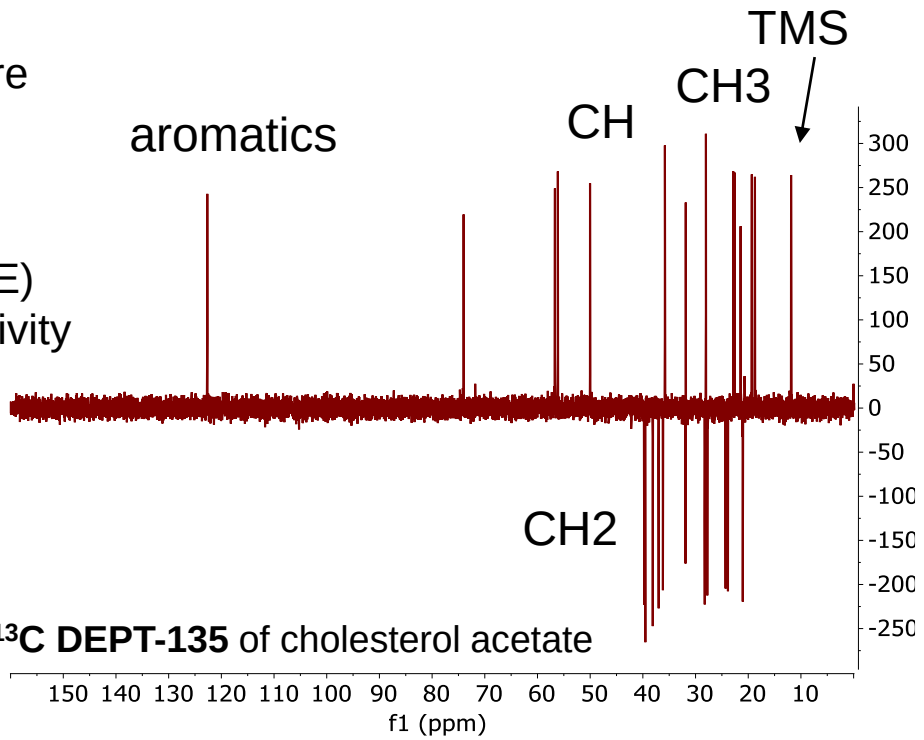
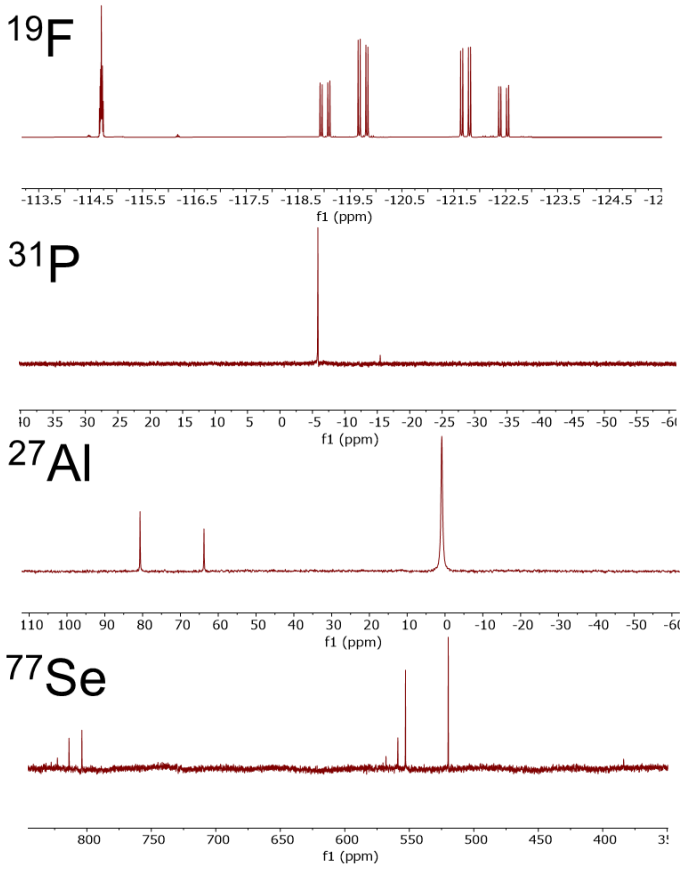


^{13}C DEPT-135 of cholesterol acetate

1Ds of other nuclei

- Sometimes have long T1s
- Sometimes have very large chemical shift ranges
- Sensitivities range from very poor to comparable to ^{1}H!

	Spin I	Natural abundance	Receptivity (13C = 1)	Resonance frequency on a 400 MHz magnet (MHz)
Hydrogen	1/2	99.985%	5670	400.00
Deuterium	1	0.015%	0.0082	61.40
Carbon-13	1/2	1.108%	1.00	100.60
Nitrogen-15	1/2	0.370%	0.022	40.56
Fluorine-19	1/2	100.000%	4730	376.36
Aluminum-27	5/2	100.000%	1170	104.32
Silicon-29	1/2	4.700%	2.1	79.48
Phosphorous-31	1/2	100.000%	377	161.92
Selenium-77	1/2	7.630%	3.15	76.29



Got ^{19}F?

On NEO400 & AV400, many options for ^{19}F NMR including:

^{19}F 1D
- with and without ^{1}H decoupling
- with and without echo for improved baseline

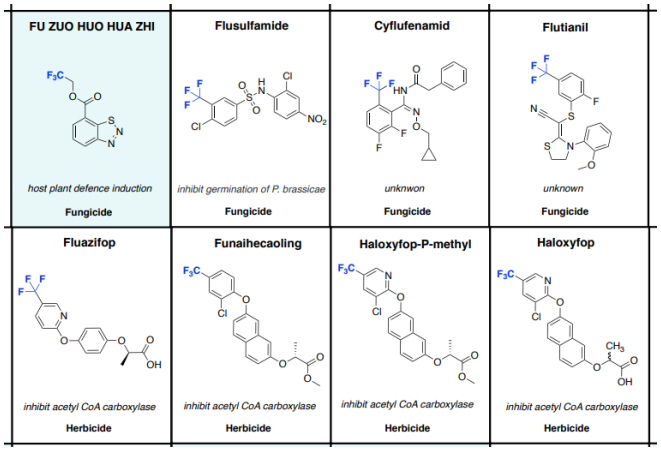
^{19}F-^{19}F NOESY 2D (through-space ^{19}F-^{19}F couplings)

^{19}F-^{1}H HOESY 1D (through-space ^{19}F-^{1}H couplings)

^{19}F-^{1}H SRI (through-bond ^{19}F-^{1}H couplings in a 1D)

^{19}F-^{1}H HMBC (through-bond ^{19}F-^{1}H couplings in a 2D)

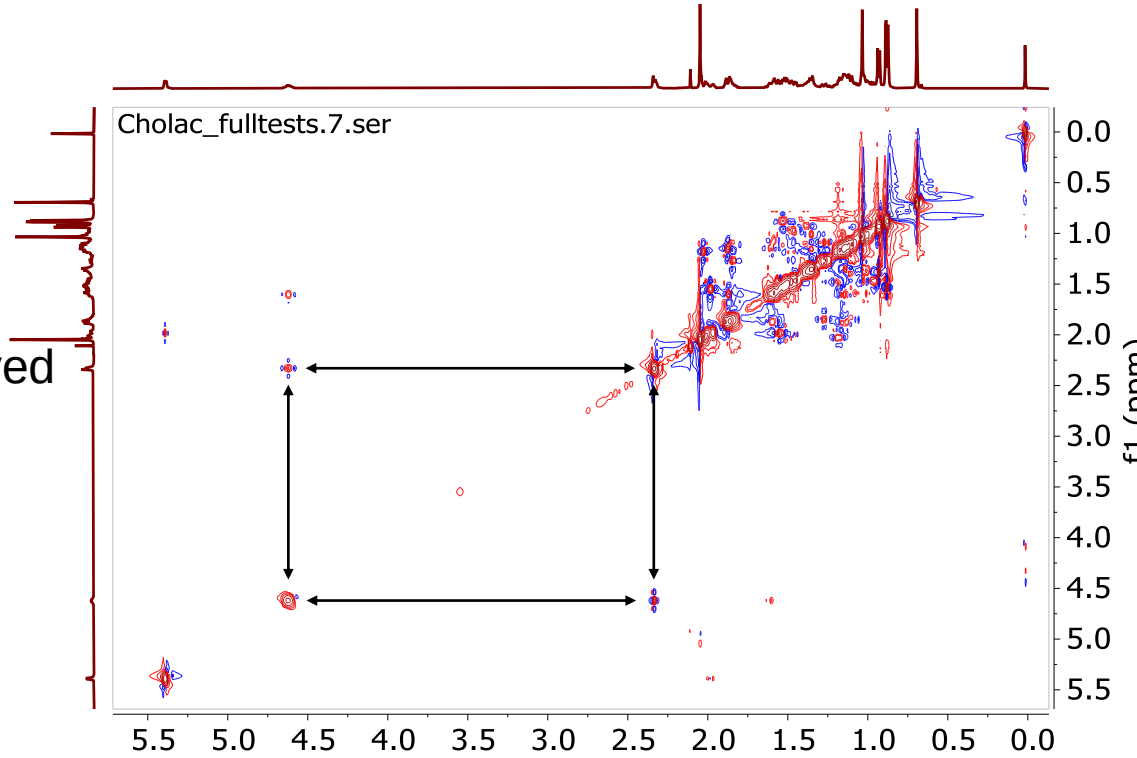
^{19}F T1 inversion-recovery (^{19}F T1 measurement)

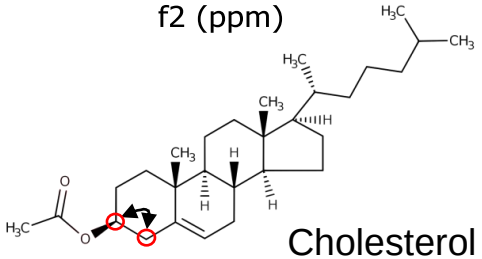


Liu laboratory

^{1}H-^{1}H COSY 2D

- Provides 3-bond ^{1}H-^{1}H correlations
- Complex multiplicies resolved in cross-peaks
- Option for suppression of solvent peak
- Most useful for compounds with many protons!

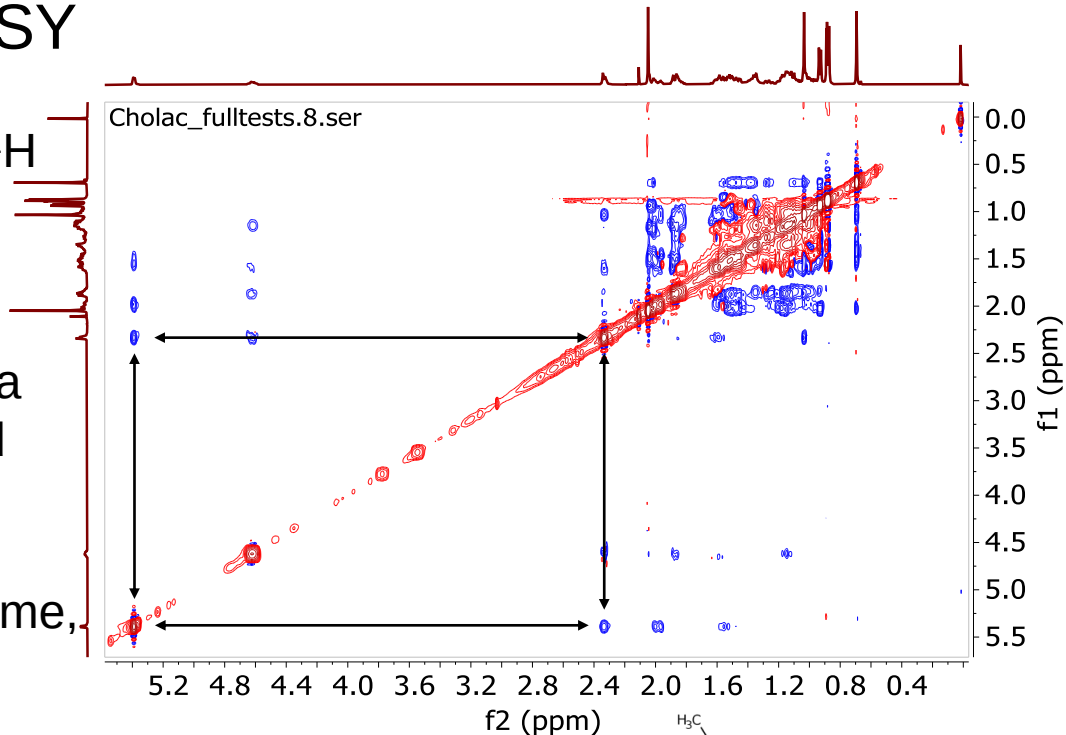


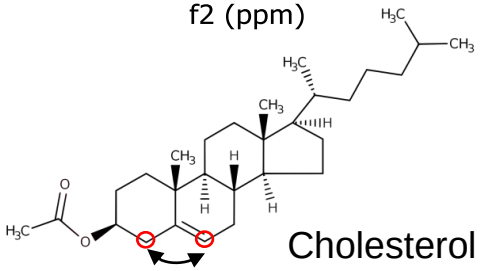


Cholesterol acetate

^{1}H-^{1}H NOESY and ROESY

- Provides **through-space** ^{1}H-^{1}H correlations
- NOESY not suitable for compounds between ~1-2 kDa (ROESY should be performed instead for these)
- Requires setting of a mixing time, usually between 500-800 ms
- Most useful for compounds with many protons!

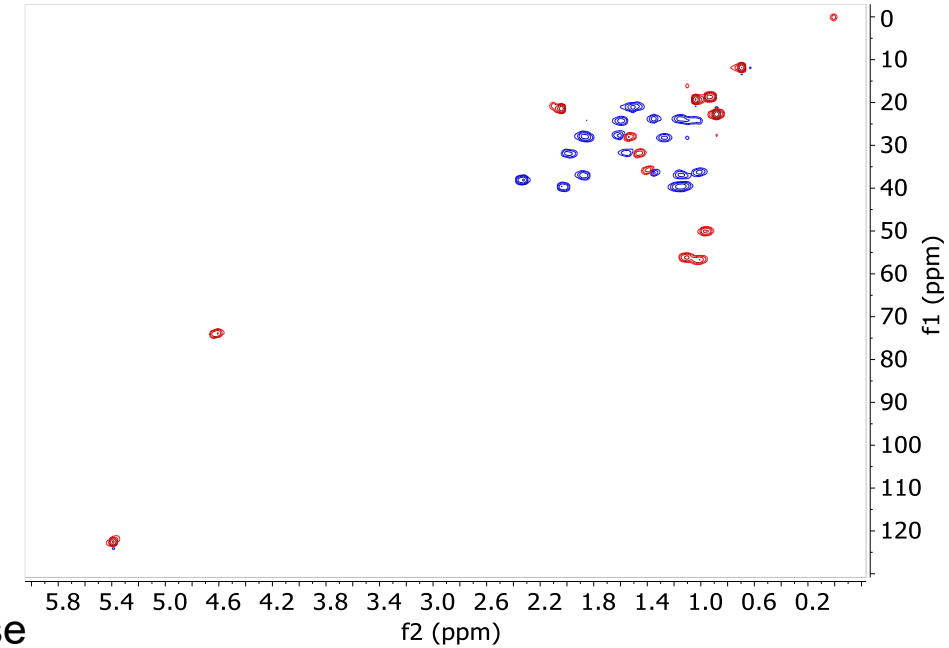


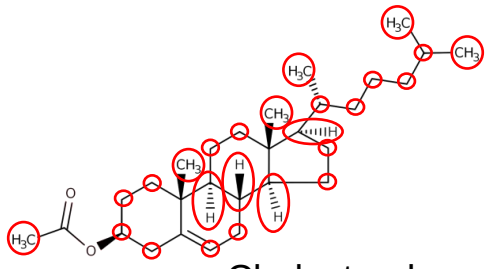


Cholesterol acetate

^{13}C-^{1}H HSQC 2D

- Correlates 13C shift to 1H shift of attached proton
- Helps reduce overlap
- CH/CH3 positive, CH2 negative
- Non-protonated carbons are missing!
- Don't let Mnova's "projections" confuse you! They are separate spectra and may need to be corrected.
- Related: ^{13}C-^{1}H HMBC 2D shows correlations between carbons and protons 2+ bonds apart.

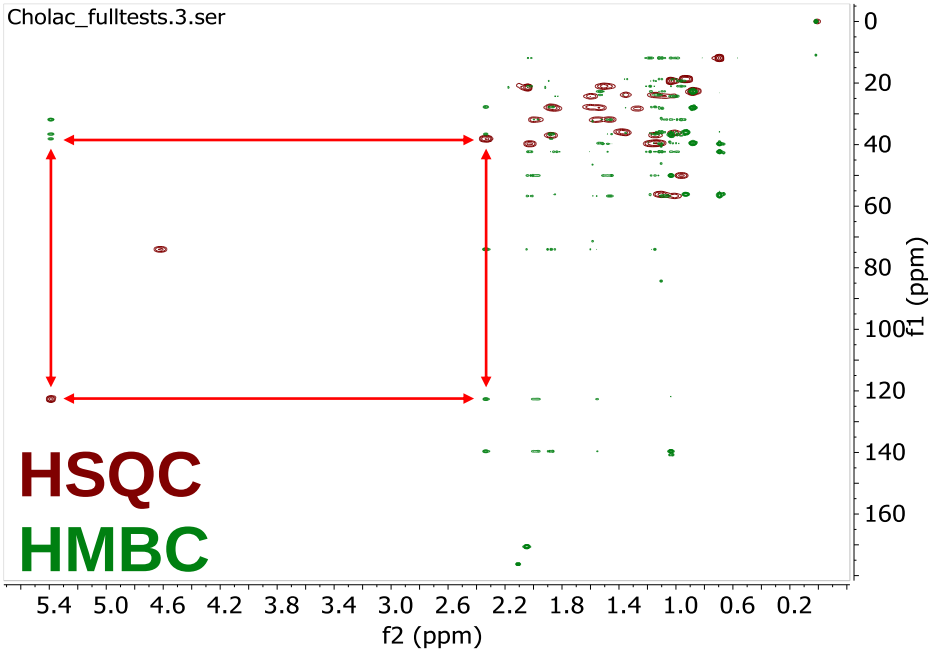


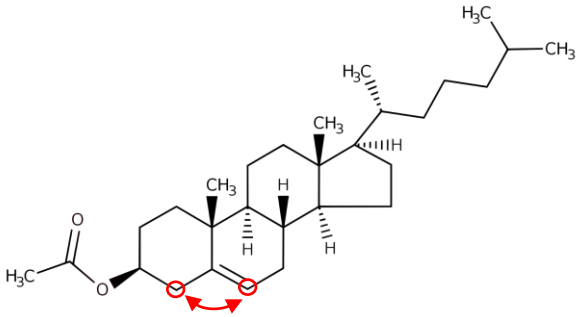


Cholesterol acetate

^{13}C-^{1}H HMBC 2D

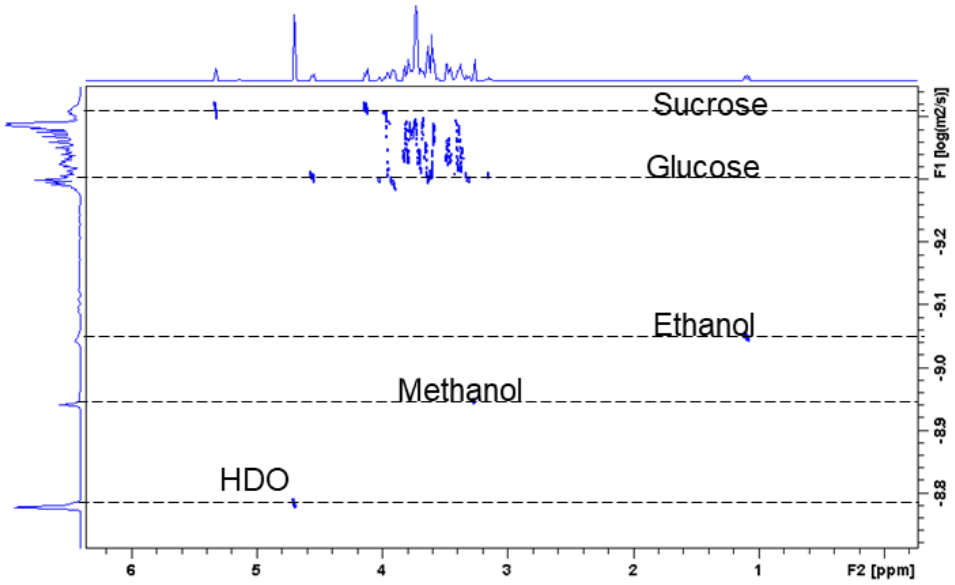
- Correlates ^{1}H shift to ^{13}C shift of carbons 2+ bonds apart
- Helps reduce overlap
- Aids in assignments
- Shows correlations to non-protonated carbons!
- Ambiguities (are they 2, 3, or 4 bonds apart?)

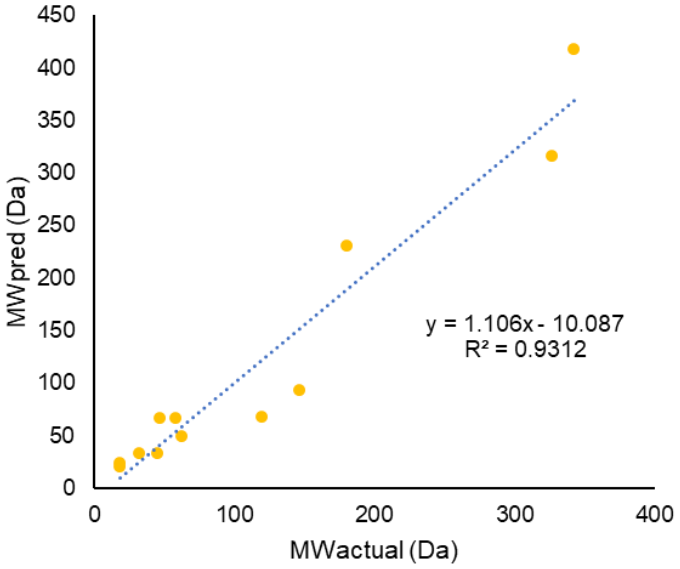




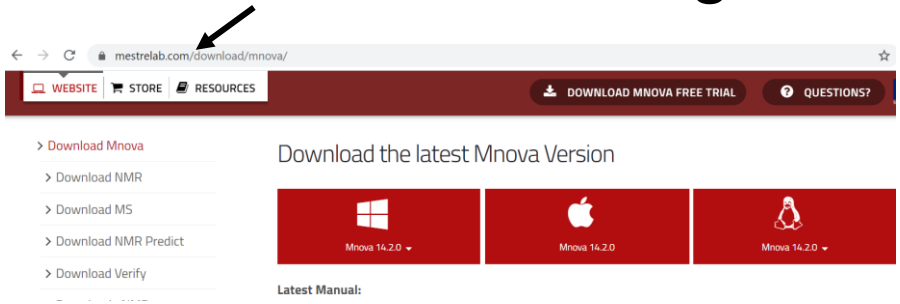
^{1}H-^{1}H DOSY 2D

- Extends a ^{1}H spectrum into a second dimension that informs on diffusion
- Provides approximate diffusion constants/mol weights for each ^{1}H peak
- Enables easy distinguishing between compounds in a mixture!
- Coming soon: ^{31}P and ^{19}F DOSY!





Installing/Activating MNova

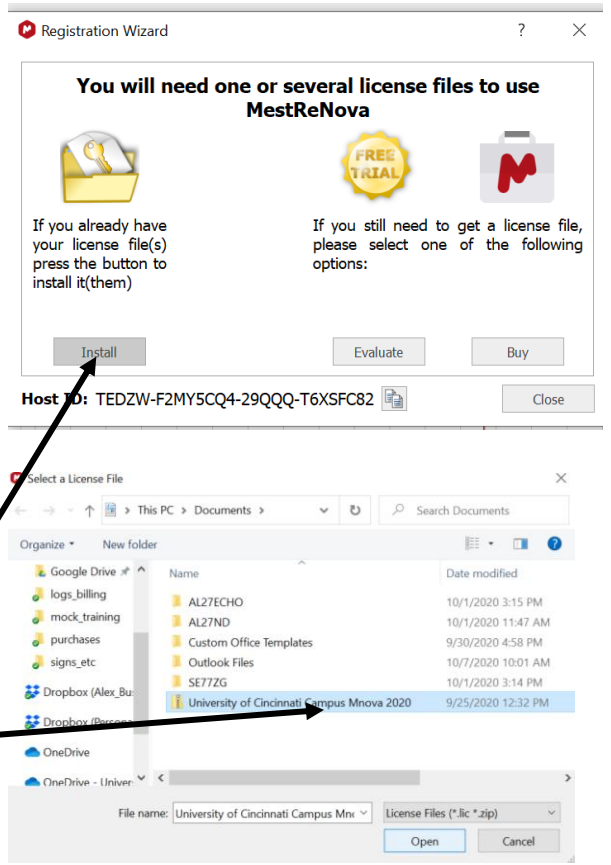


- Download Mnova from https://mestrelab.com/download/mnova/
  NOTE: DO NOT DOWNLOAD THE LATEST VERSION. Rather, download version **14.3.1 or lower.**

- Get the campus license file from https://www.artsci.uc.edu/departments/chemistry/resources/software.html

- While on campus network, load the license file the first time you use the software:

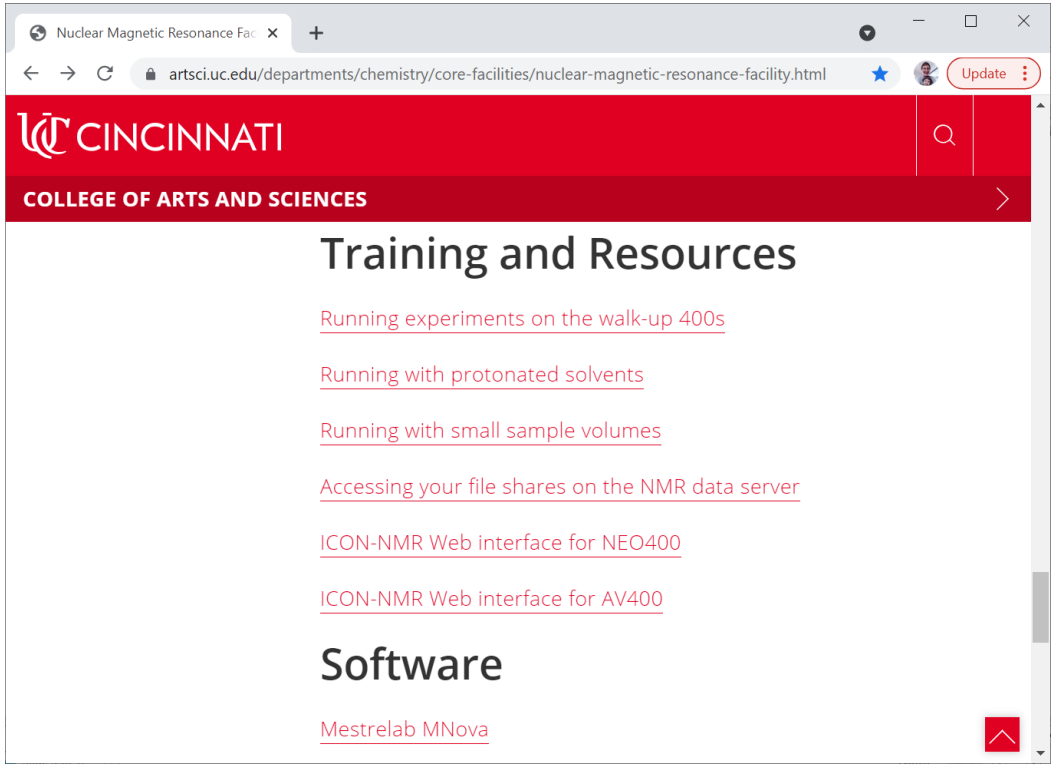
- The license needs to refresh every few months by running the software on the campus network



Resources

On the NMR lab website:

- Instructions for running on the instruments
- Instructions for special samples (protonated solvents, small volumes)
- Instructions for accessing data
- Web interfaces for automated instruments (AV400, NEO400)
- Link to MNova and Mnova license



Department of Chemistry NMR Facility

Contact:
Alex Greenwood
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Phone: 513-556-9211;
Email: greenwa2@ucmail.uc.edu
For user training, technical assistance, NMR questions and discussions.