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: ^{1}H; ^{19}F-^{109}Ag, 2Ds
HD500: ^{1}H; ^{31}P-^{109}Ag, 2Ds
AV400: ^{1}H; ^{19}F-^{109}Ag, 2Ds

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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 AV 400 MHz Spectrometer:

Z-Grad BBFO ATM probe: $^{1}H/^{19}F$-^{109}Ag
Variable temperature capability
Automatic sample changer (16 positions)
^{1}H, ^{109}Ag-^{19}F observe 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

Bruker NEO 400 MHz Spectrometer:

Z-Grad BBFO ATM iprobe: $^{1}H/^{19}F$-^{109}Ag
Variable temperature capability
Automatic sample changer (16 positions)
^{1}H, ^{109}Ag-^{19}F observe 1D and various 2D (^{1}H-^{1}H, ^{1}H-^{13}C, ^{1}H-^{19}F, ^{19}F-^{19}F) experiments available
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 AVIIIHD 500 MHz Spectrometer:

Z-Grad BBO ATM probe: $^{1}H/^{31}P$-^{109}Ag
Variable temperature capability
Automatic sample changer (16 positions)
^{1}H, ^{109}Ag-^{31}P observe 1D and various 2D (^{1}H-^{1}H, ^{1}H-^{13}C) experiments available **(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

Which Magnet should you use?

	AV400	NEO400	HD500
^{1}H 1Ds	✓	✓	✓
^{13}C 1Ds	✓	✓	✓
^{31}P 1Ds	✓	✓	✓
^{19}F 1Ds	✓	✓	✗
^{1}H-^{1}H 2Ds	✓	✓	✓
^{1}H-^{13}C 2Ds	✓	✓	✓
^{19}F-^{19}F 2Ds	✓	✓	✗
^{1}H-^{19}F 2Ds	✓	✓	✗
Variable-temp	✗	✓	✗
Solid-State	✗	✓	✗

AV400 NEO400 HD500

^{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~~

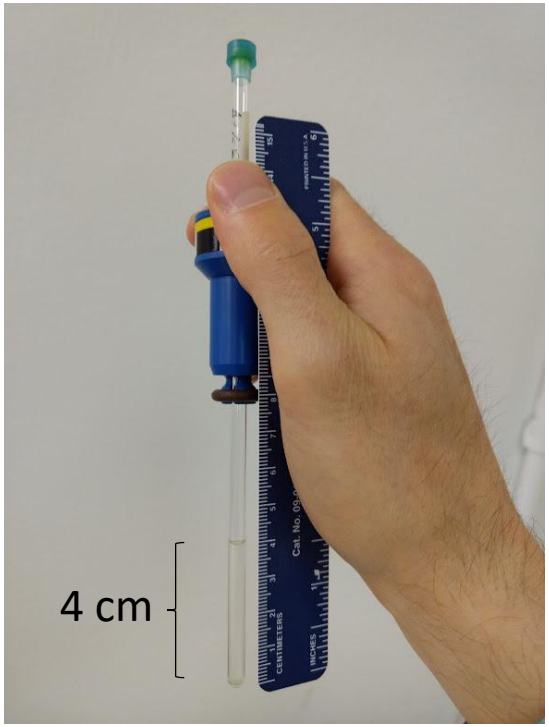
Relative signal-to-noise

Note– no ^{19}F on HD500!

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!

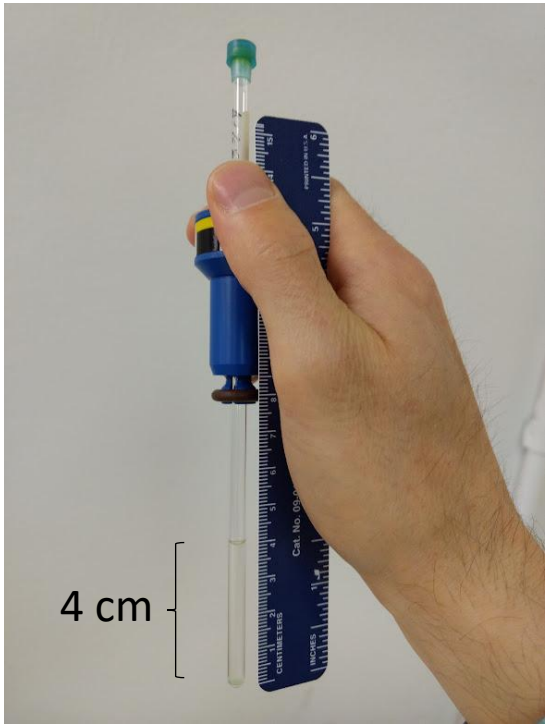


Preparing your NMR sample

- 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)



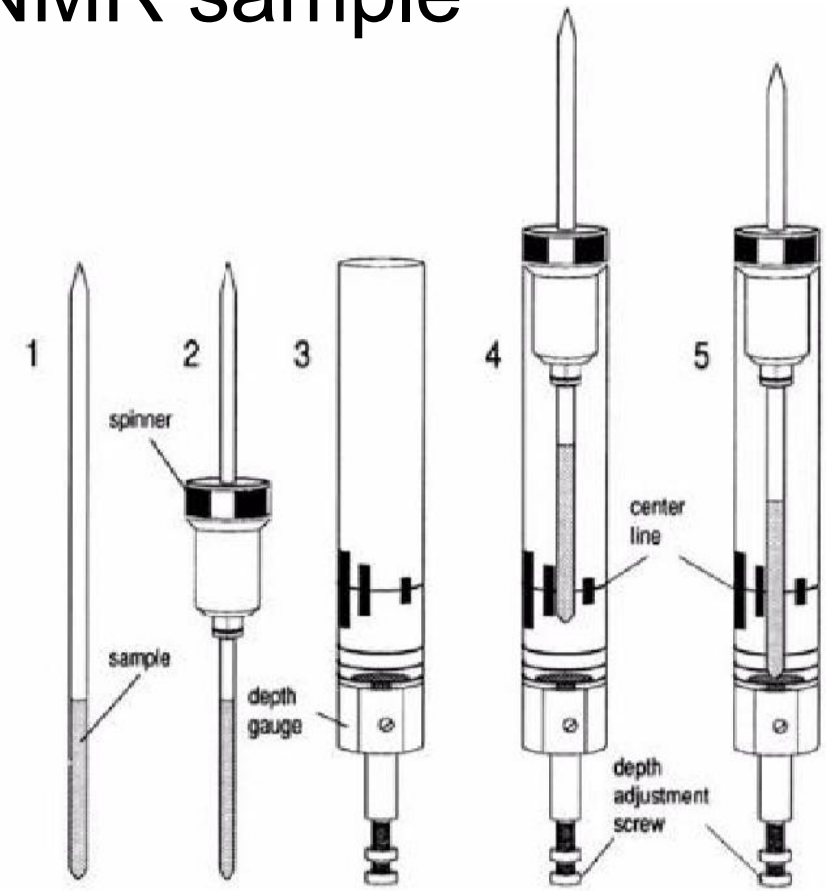
Submitting your NMR sample

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

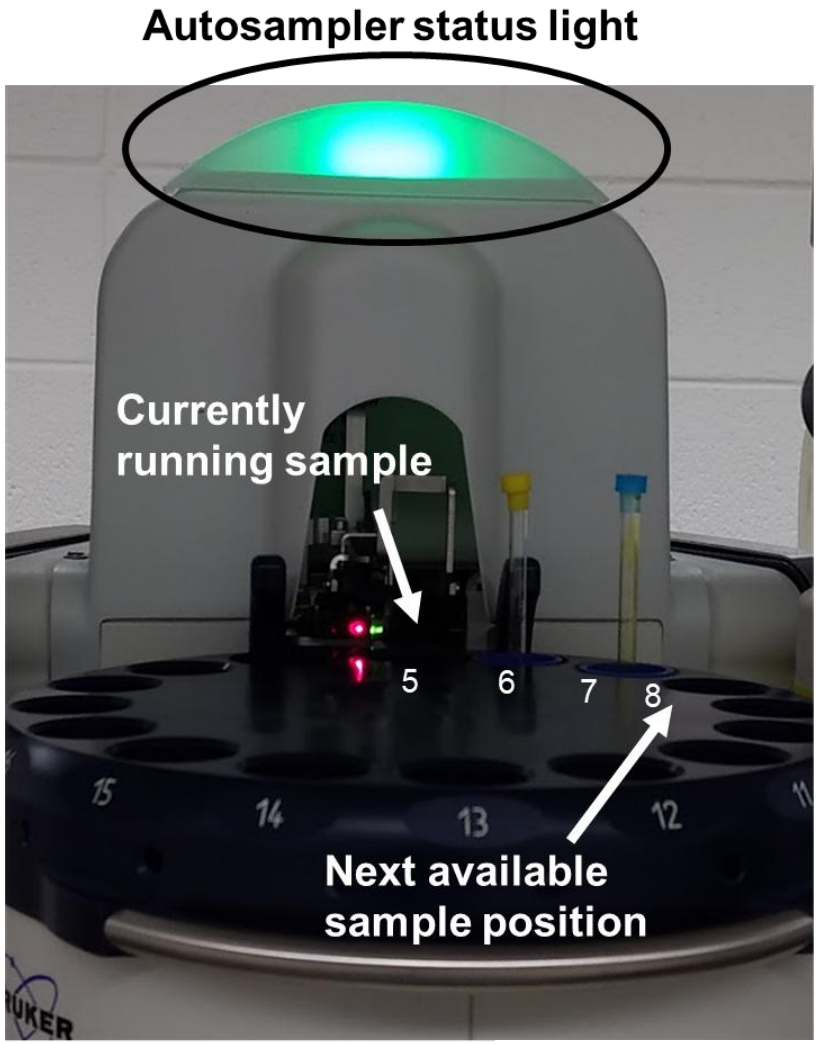


AVANCE Beginners Guide, Bruker

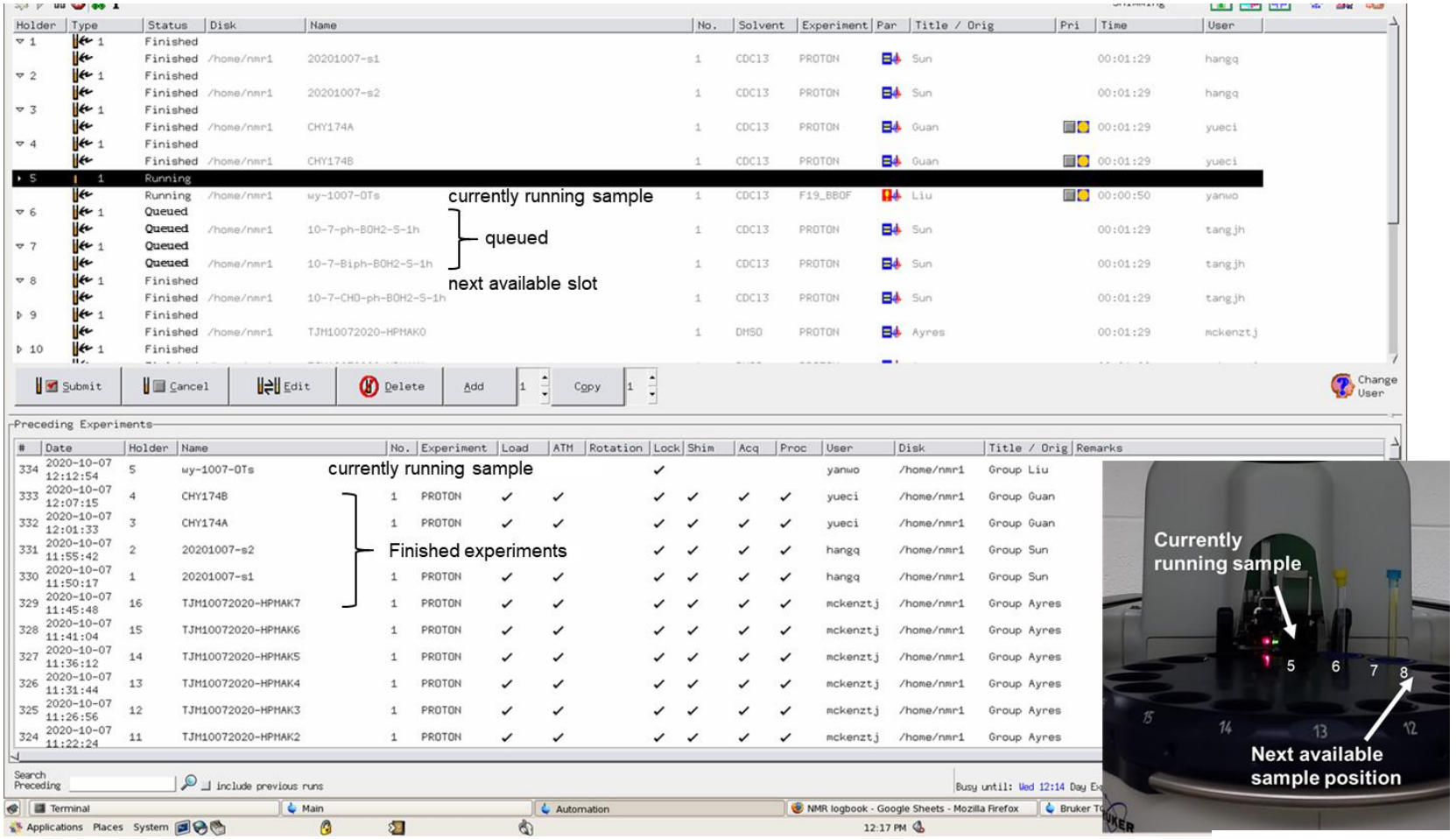
Submitting your sample in the autosamplers

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

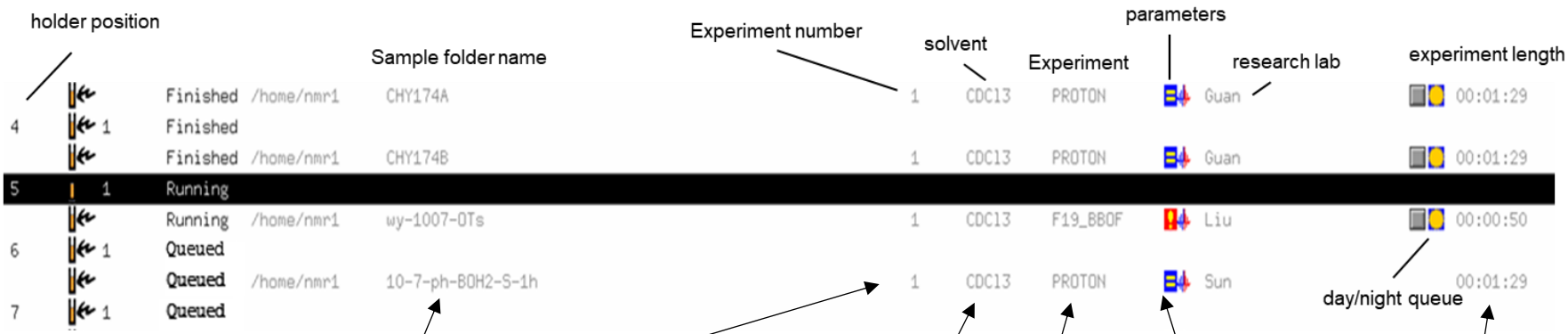
6) Define your experiment in that slot and press “submit”



ICON-NMR Interface



ICON-NMR Interface



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!

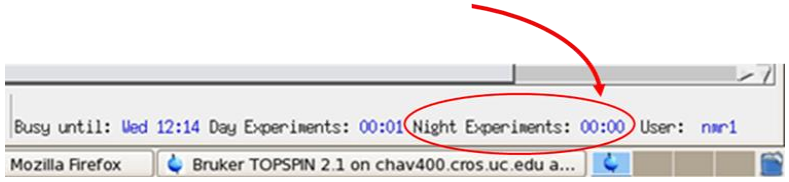
The Night Queue

- On the AV400 and HD500, ^{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 HD500, > 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

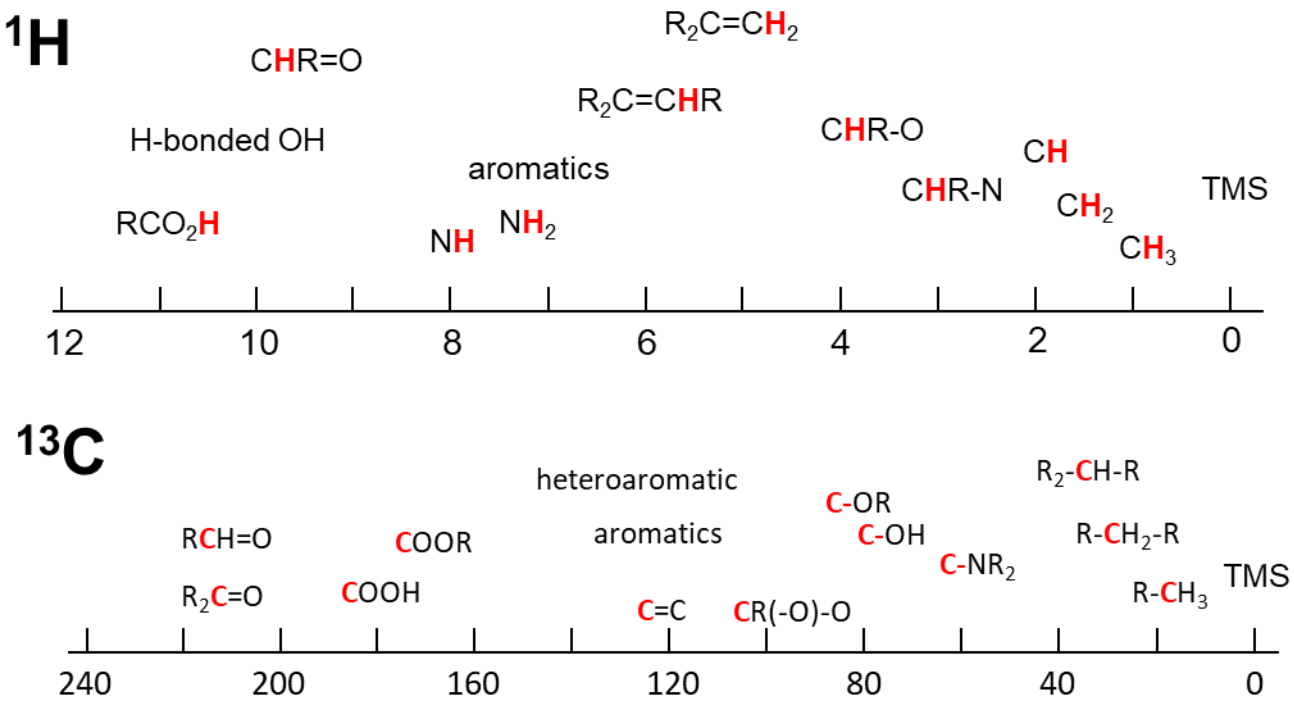
http://chav400.oldchem.uc.edu:8015 (AV400)
http://chhd500.oldchem.uc.edu:8015 (HD500)
http://chneo400.oldchem.uc.edu:8015 (NEO400)

- (links on NMR lab website)
- Username is your ICON-NMR username, password is “chemistry”
- “Read only” interface

General Rules

1) Put experiments with lengths exceeding 20 minutes in the night queue
2) Do not remove/touch sample until autosampler light is green
    - Automation will halt otherwise
3) Spinners go in the designated holder, tubes go in the tube rack
4) No open containers/tubes or syringes permitted in NMR lab
5) Report broken tubes promptly to NMR facility manager
6) Submit experiments in numerical order if possible **(always do this on NEO400)**
7) Clean tubes with kimwipes and measure their depth with the depth gauge
    - Tubes with small sample volumes raised slightly to center the sample in the coil
    - Never place the tube lower than the bottom of the gauge—it may break in the probe
8) Retrieve samples from room in a reasonable amount of time
9) Mark your tubes. Do not use tape
10) Log out when you are done

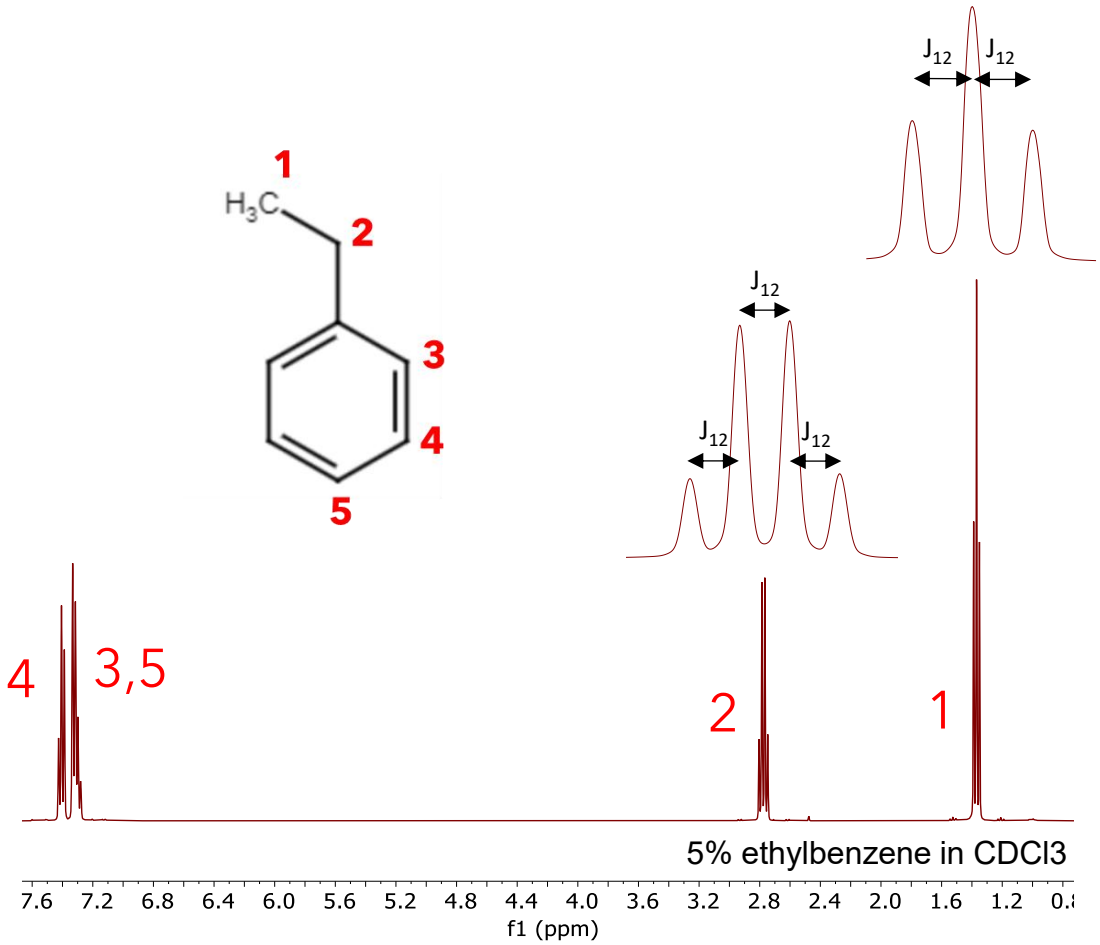
Chemical Shift Ranges



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!

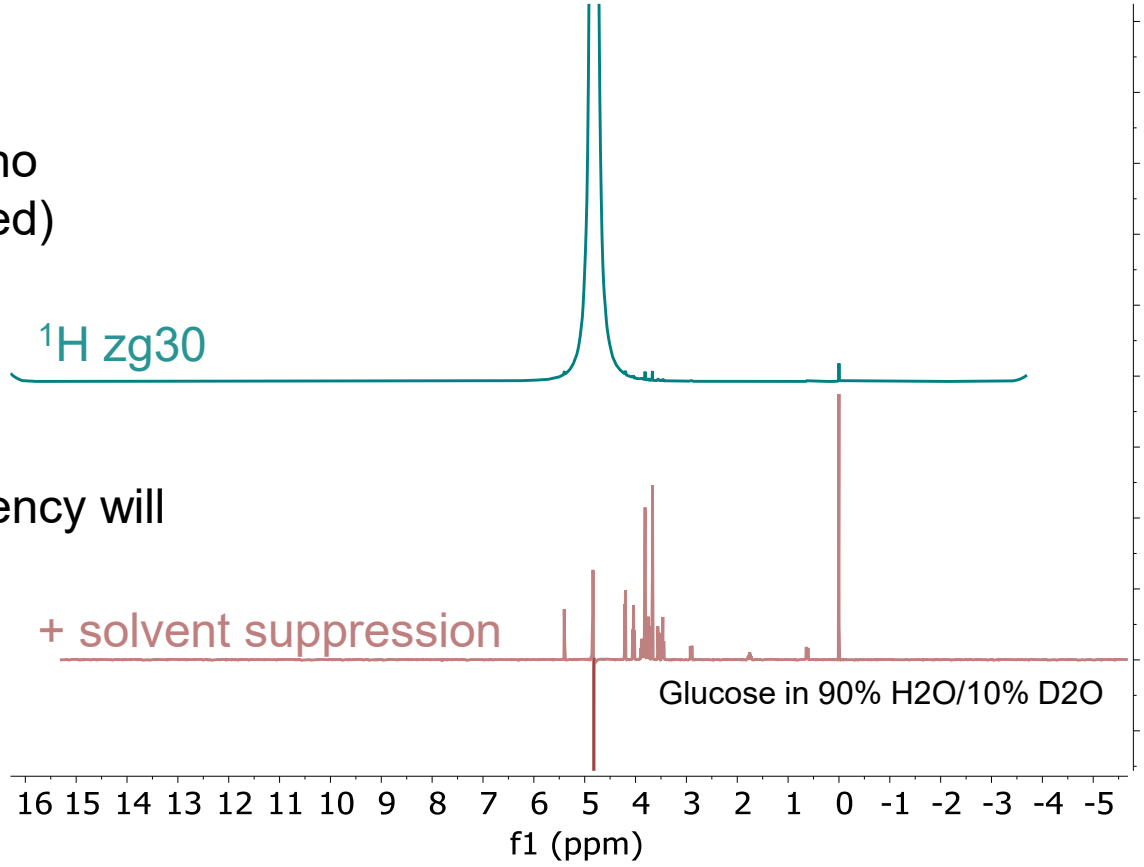
^{1}H zg30

- 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



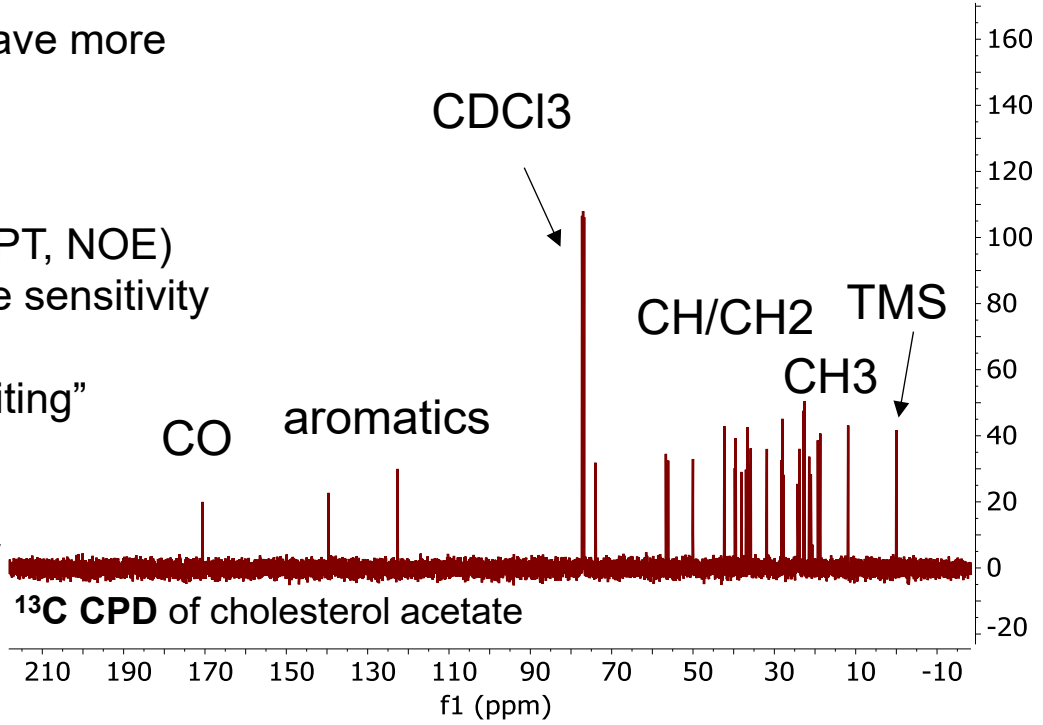
^{1}H with solvent suppression

- 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 CPD, zigig, zgpg, dept

- 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

^{13}C CPD, zigig, zgpg, dept

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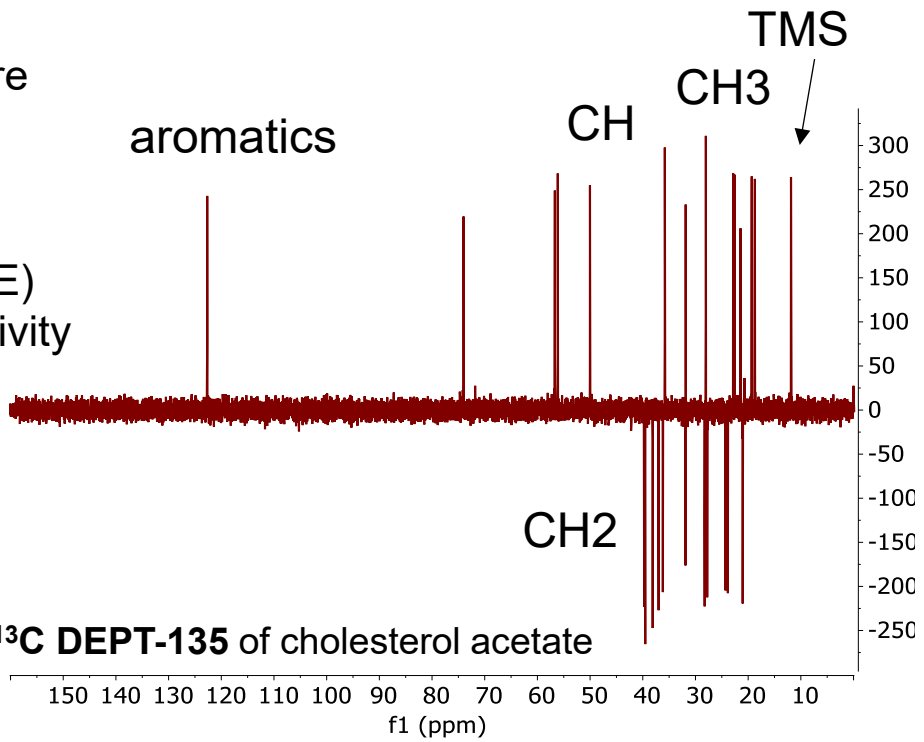
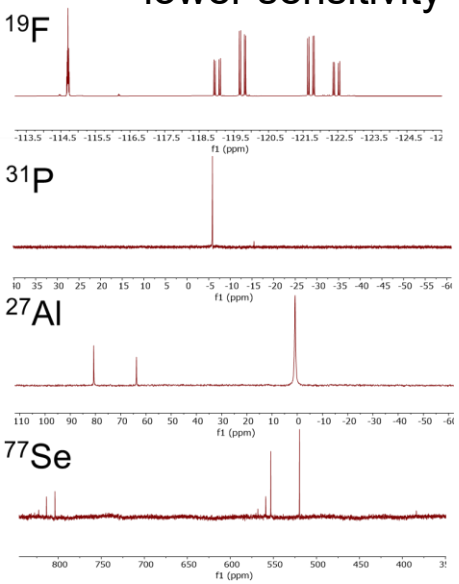
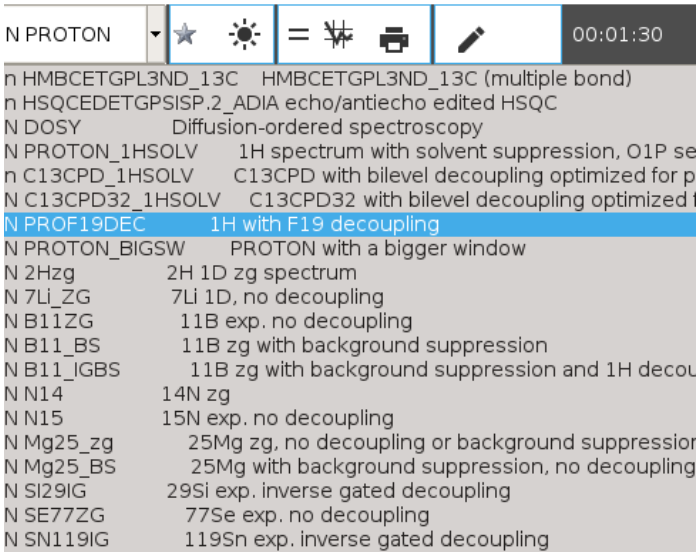


^{13}C DEPT-135 of cholesterol acetate

1D spectra of less-common nuclei

- Our broadband probes can excite any NMR-active nucleus!
- Watch out for unexpected…
  - long T1 relaxation
  - large chemical shift ranges
  - lower sensitivity





	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

^{19}Fun with ^{19}Fluorine

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 COSY 2D (through-bond ^{19}F-^{19}F couplings)
- with and without ^{1}H decoupling

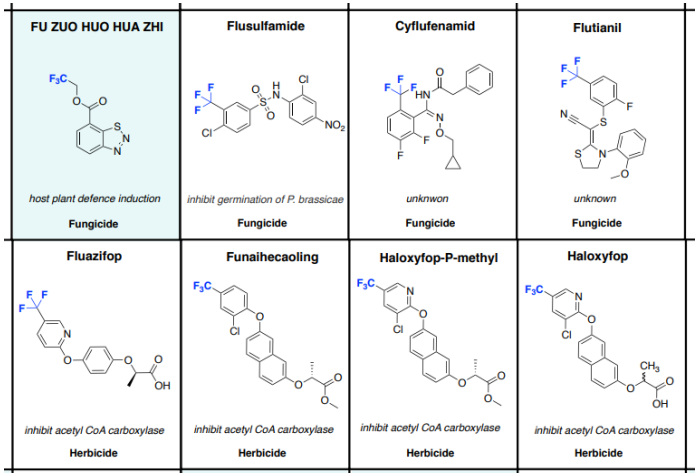
^{19}F-^{19}F NOESY 2D (through-space ^{19}F-^{19}F correlations)
- with and without ^{1}H decoupling

^{19}F-^{1}H HMBC/HSQC 2D (through-bond ^{19}F-^{1}H couplings)
- with and without ^{1}H presaturation

^{19}F-^{1}H HOESY 2D (through-space ^{19}F-^{1}H correlations)
- with and without ^{1}H presaturation

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

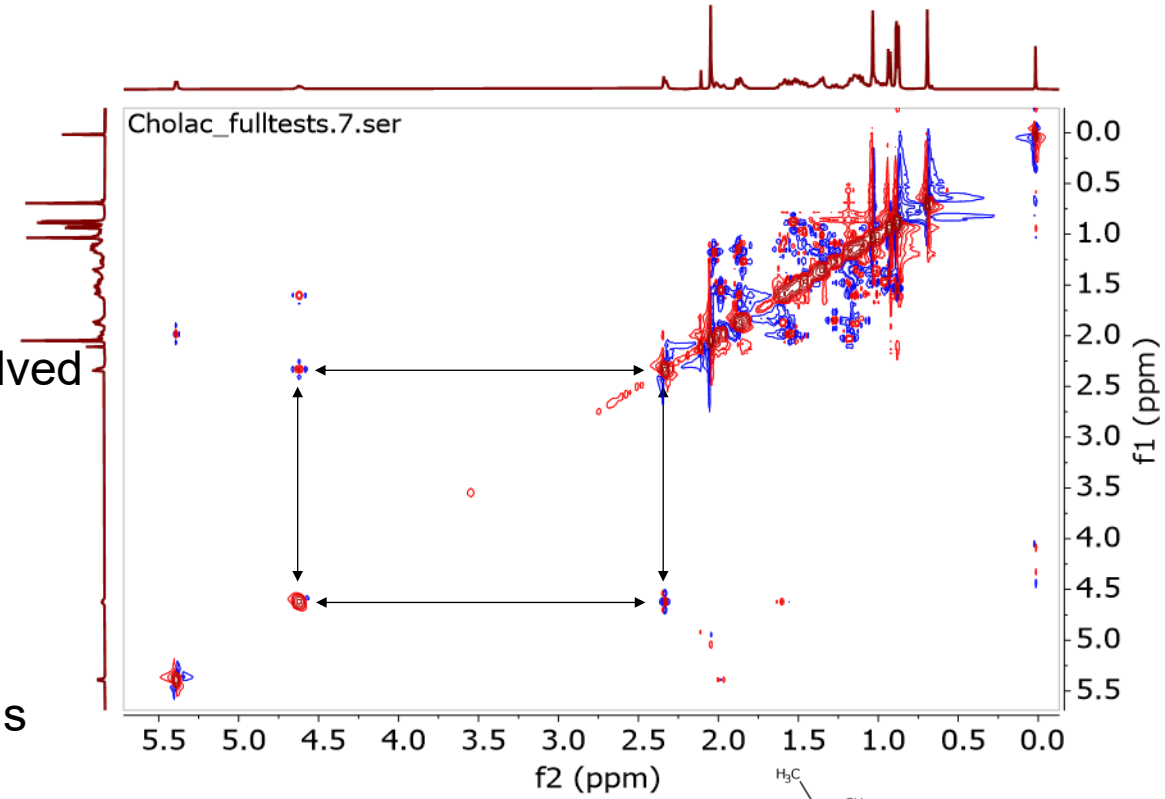
^{19}F DOSY (^{19}F diffusion measurement)

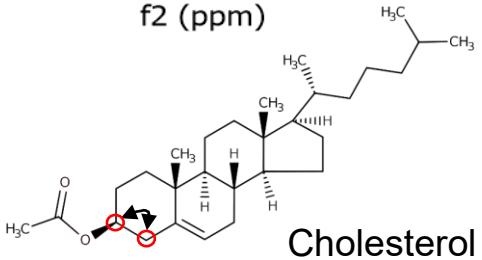


Liu laboratory

^{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!

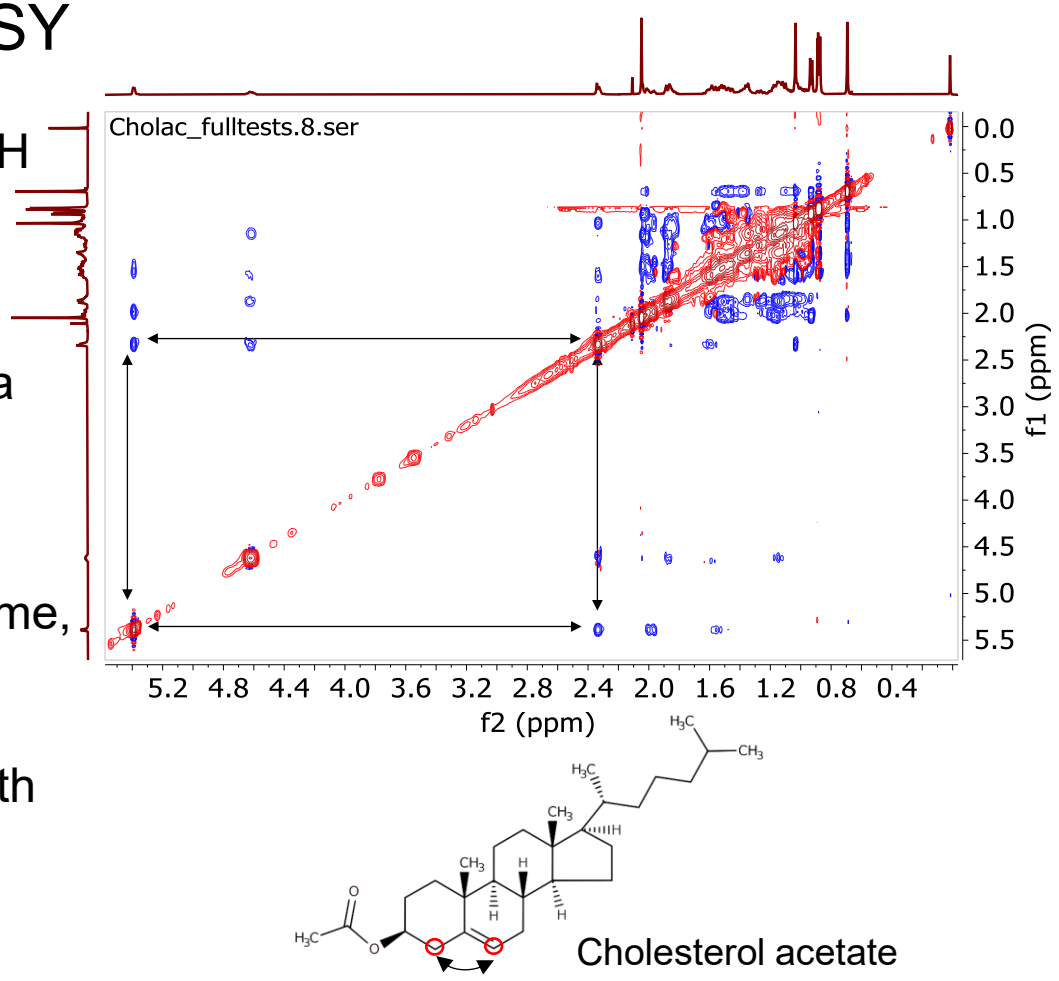




Cholesterol acetate

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

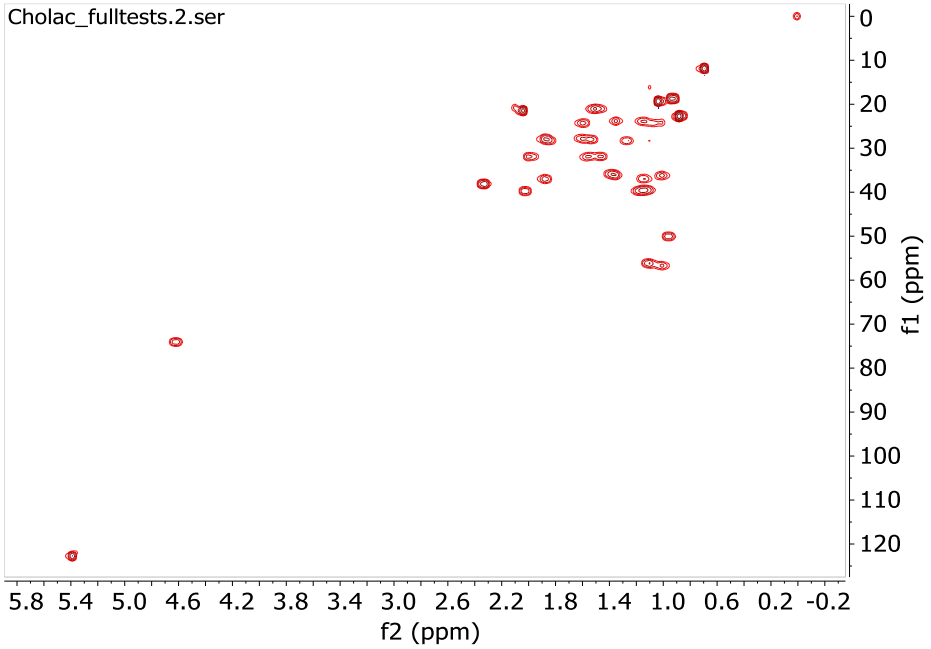
- Provides **through-space** ^{1}H-^{1}H correlations (< 5 Å apart)
- NOESY not suitable for compounds between ~1-2 kDa (ROESY should be performed instead for these)
- Requires setting of a mixing time, usually between 500-800 ms
- Most useful for compounds with many protons!

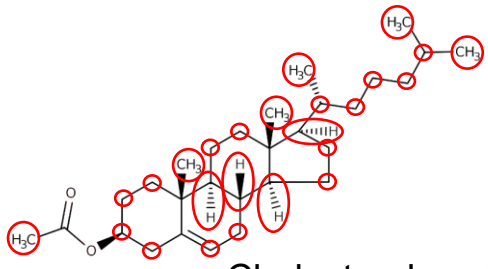


Cholesterol acetate

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

- Correlates ^{13}C shift to ^{1}H shift of attached proton
- Helps reduce overlap
- CH/CH_3 positive, CH_2 negative
- Non-protonated carbons are missing!
- Related: ^{13}C-^{1}H HMBC 2D shows correlations between carbons and protons 2+ bonds apart.

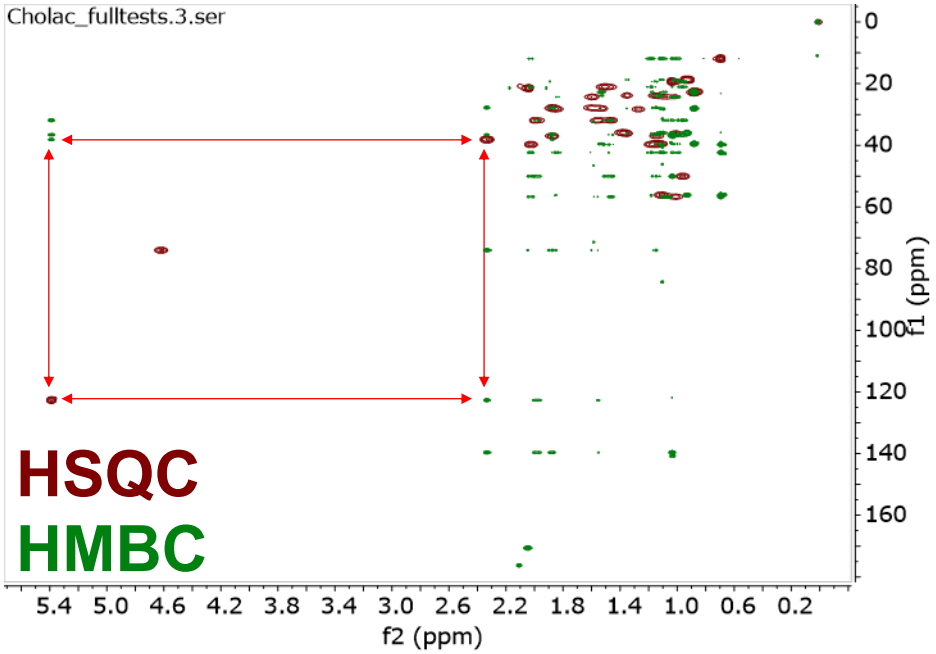


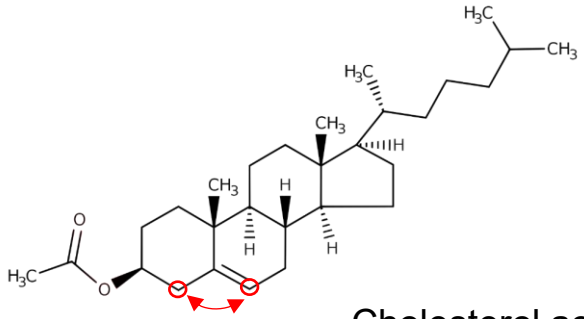


Cholesterol acetate

^{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?)





Cholesterol acetate

Solid-State MAS NMR

4mm HX CPMAS probe

- Paid for in part by a Core Enhancement grant from the UC Office of Research
- Spins 4mm rotors up to 12.5 kHz
- $^{1}H/^{19}F$ and X (tunes up to ^{31}P) channels
- Modern MAS III controller and MAS shuttle system for rotor insert/eject

Solid-State MAS NMR

Samples:

- Insoluble compounds
- Large-MW polymers
- Reactions performed in the solid state
- Should be a powder, 100 µl or greater

Nuclei:

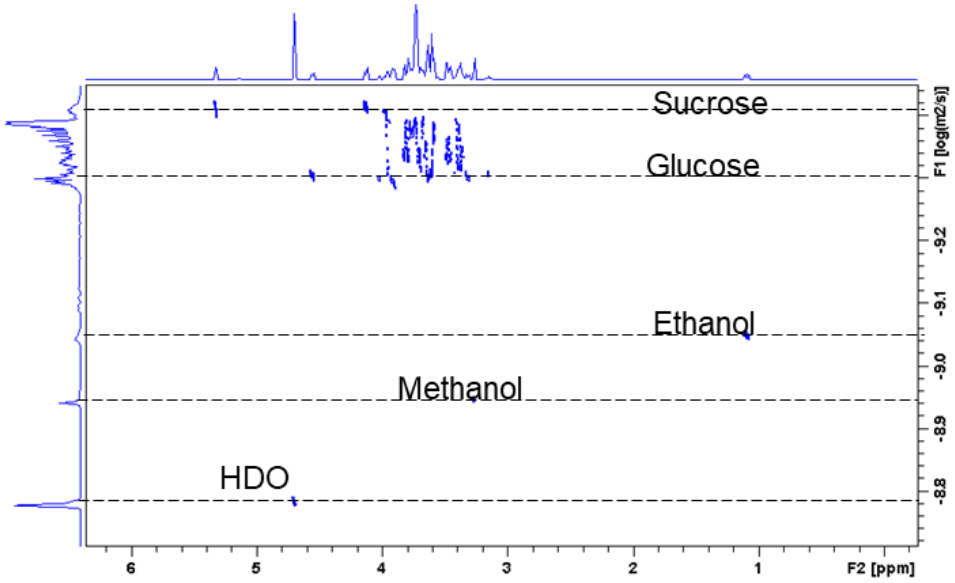
- ^{109}Ag-^{31}P (^{19}F without ^{1}H decoupling) and ^{1}H.
- ^{1}H is of limited use due to very wide lineshapes (high gyromagnetic ratio)
- Most common is direct-polarization ^{13}C with ^{1}H decoupled

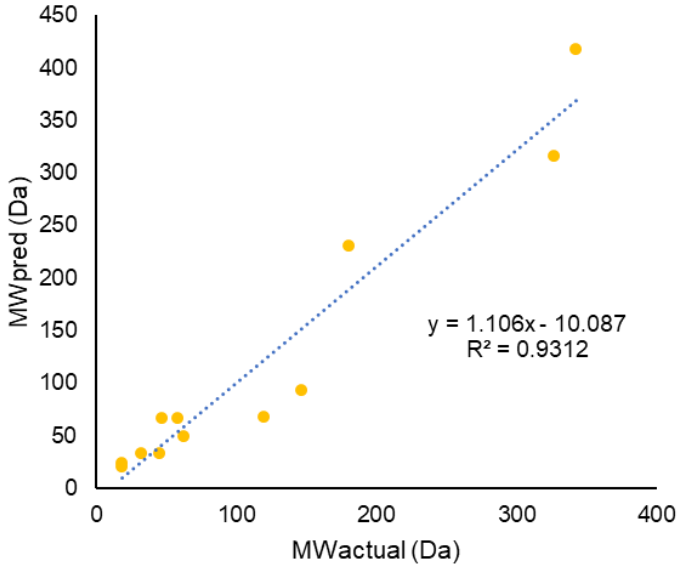
Sensitivity:

- Natural-abundance ^{13}C signal-to-noise of 50-400 with an hour of data collection, depending on complexity of molecule

Diffusion NMR (DOSY)

- 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!
- ^{19}F versions now available

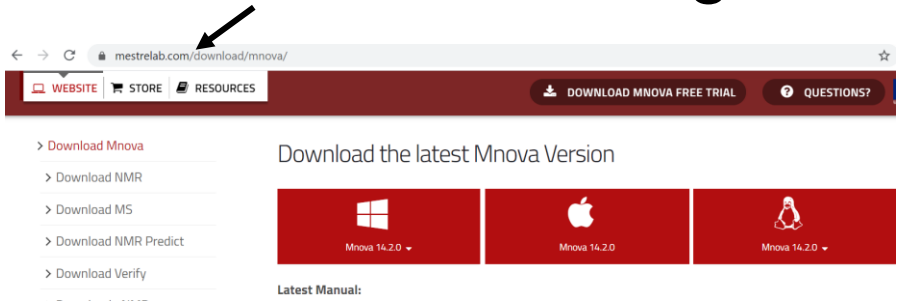




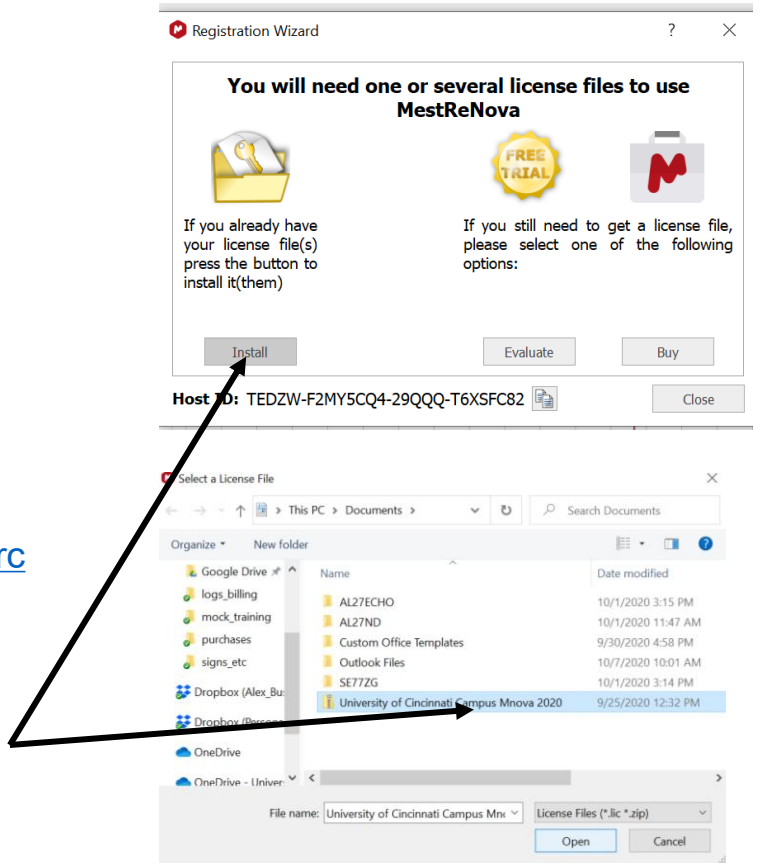
Becoming an NMR Facility User:

1) Become affiliated with a research lab!
2) Contact the facility manager (Alex, greenwa2@ucmail.uc.edu)
3) Read safety guidelines
4) Schedule first training session (submitting on walk-ups) with Alex
5) Set up NMR data access and complete data processing exercise
6) Once (**4**) and (**5**) are complete, receive card access and start collecting data!
7) (Optional) Further training on NEO400 (variable temperature)

Installing/Activating MNova



- Download Mnova from https://mestrelab.com/download/mnova/
  NOTE: DO NOT DOWNLOAD THE LATEST VERSION. Rather, download version **15.1 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 to show current ICON-NMR status
- Link to MNova and Mnova license

Training and Resources

- Running NMR experiments in ICON-NMR
- Running with protonated solvents
- Running with small sample volumes
- Accessing your data on the NMR data server
- ICON-NMR Web interface for NEO400
- ICON-NMR Web interface for AV400
- ICON-NMR Web interface for HD500
- MNova processing exercise
- NMR Experiment Guide

Software

- Mestrelab MNova
- Mnova display properties

Links

NMR Impurity Tables:

- http://pubs.acs.org/doi/pdf/10.1021/jo971176v
- http://pubs.acs.org/doi/pdf/10.1021/om100106e