

UC Chemistry Nuclear Magnetic Resonance Facility



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

NEO400: Rm 123A/Crosley **Walk-on, ^1H ; ^{19}F ; ^{31}P - ^{15}N , 2Ds**
AV400: Rm 108/Crosley **Walk-on, ^1H ; ^{19}F ; ^{31}P - ^{15}N**
DMX500: Rm 123B/Crosley **Triple resonance, longer experiments, non-routine**

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:

Walk-up NMR spectrometers

- Two 400 MHz spectrometers are available 24/7 for routine spectroscopy to trained users

Non-Routine experiments

- Instruments can be reserved for non-routine experiments requiring substantial setup/calibration, temperature regulation, or long run times

Consultation

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

Bruker NEO 400 MHz Spectrometer:

Z-Grad BBFO ATM probe: $^1\text{H}/^{19}\text{F}-^{15}\text{N}$

Variable temperature capability

Automatic sample changer

^1H , ^{15}N - ^{19}F 1D and multi-nuclear NMR in one and two dimensions

- 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 400 MHz Spectrometer:

Z-Grad BBFO ATM probe: $^1\text{H}/^{19}\text{F}-^{15}\text{N}$

Variable temperature capability

Automatic sample changer

^1H , ^{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



Bruker DMX 500 MHz Spectrometer:

Z-Grad $^1\text{H}/^{13}\text{C}/^{15}\text{N}$ TXI triple resonance probe

Z-Grad BBI probe: ^1H ; ^{31}P - ^{15}N

Variable temperature capability

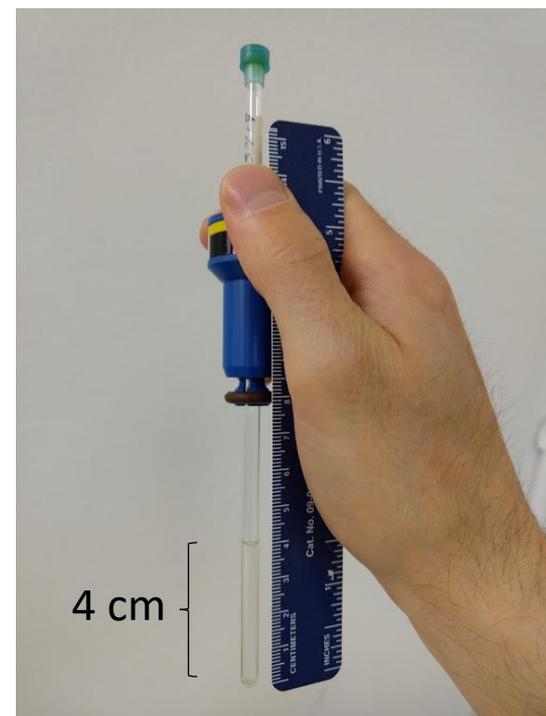
^1H , ^{13}C and multi-nuclear NMR in one, two and three dimensions



- Higher field but older hardware
- Software: XWIN-NMR 3.5
- To operate independently, first get trained on the AV400 and Topspin on the NEO400

Preparing your NMR sample

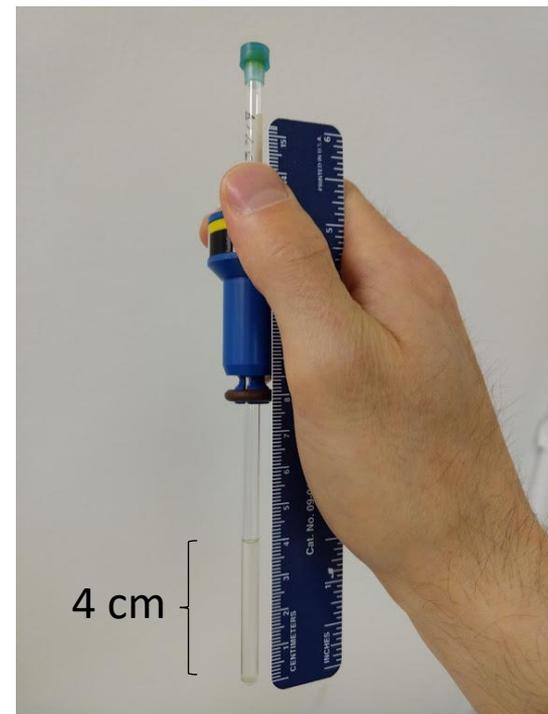
- Use a deuterated solvent (needed for lock)
- Use at least 600 μl (4 cm) for good shimming/linewidths
- Use tubes rated for 400 MHz or 500 MHz (for good shimming/linewidths)
- Mark tubes well and use your lab's designated cap color.
- **Tubes must not be scratched or broken!**
- Tubes should not be dried in ovens hotter than 100 C!



Preparing your NMR sample

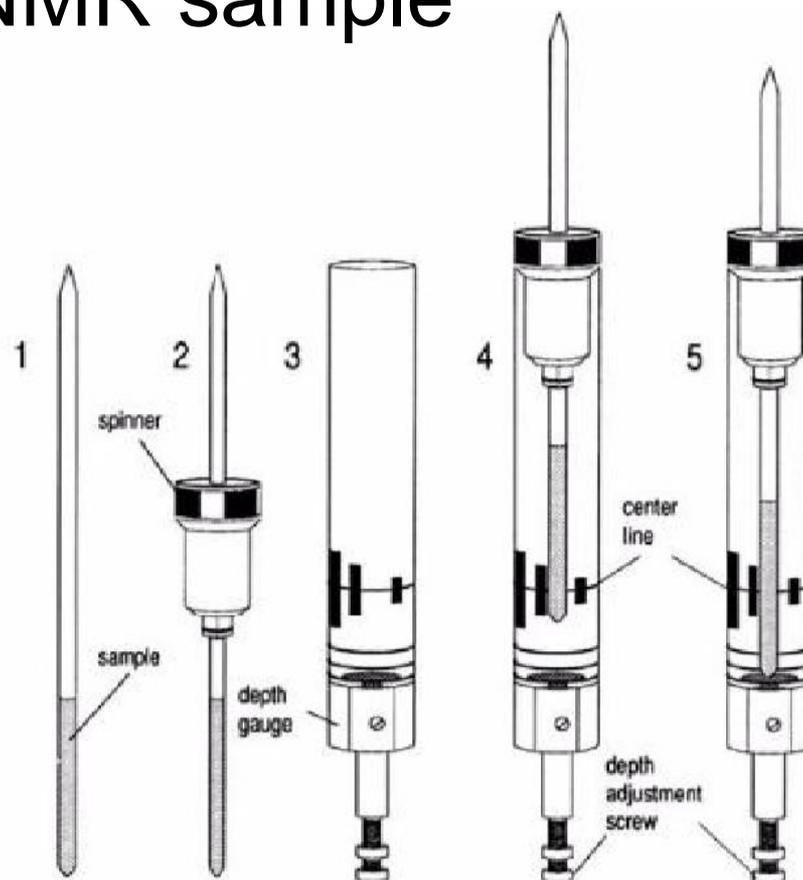
- Solution should be free of particulate— insoluble material will not give signal but **will** disrupt shimming!
- Use appropriate concentration of material!
 - For ^1H 1D: **2 mM** or **~0.25 mg** gives a SNR of 100 in 16 scans
 - For ^{13}C 1D: **35 mM** or **~6 mg** for SNR of 10 at 1024 scans, or **200 mM** or **~25 mg** for SNR of 10 at 32 scans

(masses assume molecular mass of 200 Da)



Submitting your NMR sample

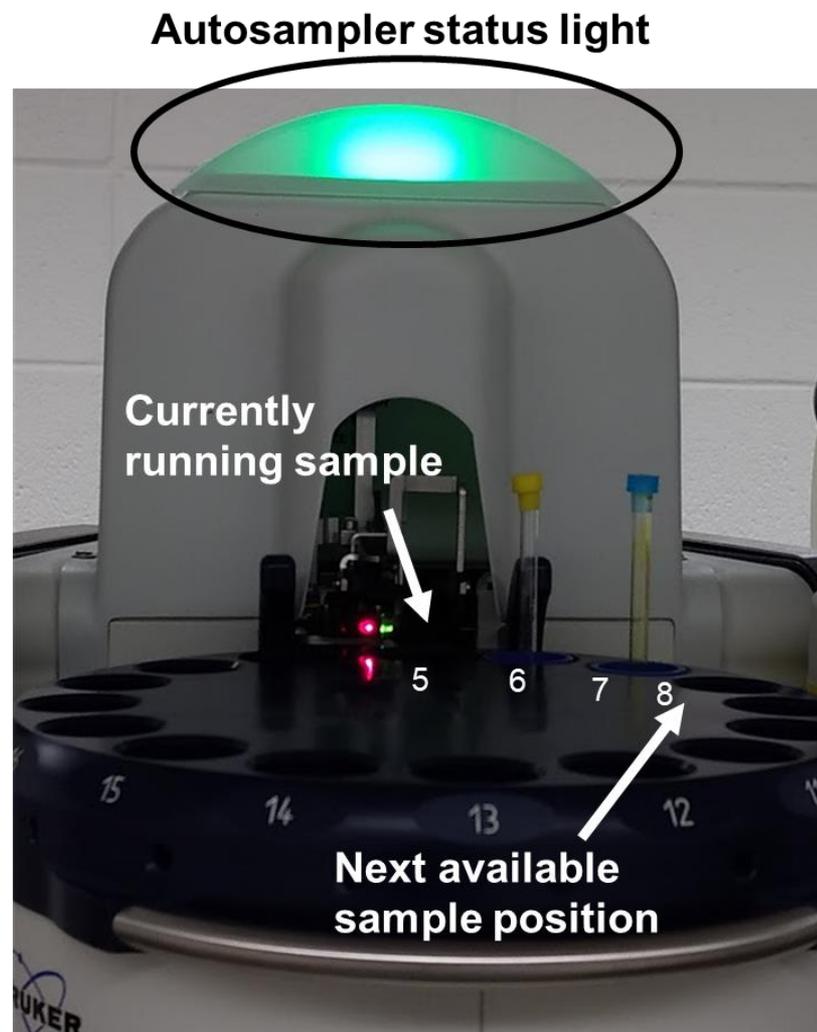
- 1) Put tube in spinner
- 2) Clean tube and spinner with kimwipe
- 3) Position tube with depth gauge—make sure spinner is flush with top
- 4) Small sample volumes should be centered in coil by bringing tube back up a bit



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

The screenshot displays the ICON-NMR interface with two main tables and a photograph of the NMR spectrometer.

Top Table (Queue):

Holder	Type	Status	Disk	Name	No.	Solvent	Experiment	Par	Title / Orig	Pri	Time	User
1	1	Finished	/home/nmr1	20201007-s1	1	CDC13	PROTON		Sun		00:01:29	hangq
2	1	Finished	/home/nmr1	20201007-s2	1	CDC13	PROTON		Sun		00:01:29	hangq
3	1	Finished	/home/nmr1	CHY174A	1	CDC13	PROTON		Guan		00:01:29	yueci
4	1	Finished	/home/nmr1	CHY174B	1	CDC13	PROTON		Guan		00:01:29	yueci
5	1	Running	/home/nmr1	wy-1007-0Ts	1	CDC13	F19_BB0F		Liu		00:00:50	yanwo
6	1	Queued	/home/nmr1	10-7-ph-B0H2-S-1h	1	CDC13	PROTON		Sun		00:01:29	tangjh
7	1	Queued	/home/nmr1	10-7-Biph-B0H2-S-1h	1	CDC13	PROTON		Sun		00:01:29	tangjh
8	1	Queued	/home/nmr1	10-7-CHO-ph-B0H2-S-1h	1	CDC13	PROTON		Sun		00:01:29	tangjh
9	1	Finished	/home/nmr1	10-7-CHO-ph-B0H2-S-1h	1	CDC13	PROTON		Sun		00:01:29	tangjh
10	1	Finished	/home/nmr1	TJM10072020-HPHAK0	1	DMSO	PROTON		Ayres		00:01:29	mckenztj

Annotations for the queue table:
 - Row 5: currently running sample
 - Rows 6-8: queued
 - Row 8: next available slot

Bottom Table (Preceding Experiments):

#	Date	Holder	Name	No.	Experiment	Load	ATM	Rotation	Lock	Shim	Acq	Proc	User	Disk	Title / Orig	Remarks
334	2020-10-07 12:12:54	5	wy-1007-0Ts										yanwo	/home/nmr1	Group Liu	
333	2020-10-07 12:07:15	4	CHY174B	1	PROTON	✓	✓	✓	✓	✓	✓	✓	yueci	/home/nmr1	Group Guan	
332	2020-10-07 12:01:33	3	CHY174A	1	PROTON	✓	✓	✓	✓	✓	✓	✓	yueci	/home/nmr1	Group Guan	
331	2020-10-07 11:55:42	2	20201007-s2	1	PROTON	✓	✓	✓	✓	✓	✓	✓	hangq	/home/nmr1	Group Sun	
330	2020-10-07 11:50:17	1	20201007-s1	1	PROTON	✓	✓	✓	✓	✓	✓	✓	hangq	/home/nmr1	Group Sun	
329	2020-10-07 11:45:48	16	TJM10072020-HPHAK7	1	PROTON	✓	✓	✓	✓	✓	✓	✓	mckenztj	/home/nmr1	Group Ayres	
328	2020-10-07 11:41:04	15	TJM10072020-HPHAK6	1	PROTON	✓	✓	✓	✓	✓	✓	✓	mckenztj	/home/nmr1	Group Ayres	
327	2020-10-07 11:36:12	14	TJM10072020-HPHAK5	1	PROTON	✓	✓	✓	✓	✓	✓	✓	mckenztj	/home/nmr1	Group Ayres	
326	2020-10-07 11:31:44	13	TJM10072020-HPHAK4	1	PROTON	✓	✓	✓	✓	✓	✓	✓	mckenztj	/home/nmr1	Group Ayres	
325	2020-10-07 11:26:56	12	TJM10072020-HPHAK3	1	PROTON	✓	✓	✓	✓	✓	✓	✓	mckenztj	/home/nmr1	Group Ayres	
324	2020-10-07 11:22:24	11	TJM10072020-HPHAK2	1	PROTON	✓	✓	✓	✓	✓	✓	✓	mckenztj	/home/nmr1	Group Ayres	

Annotations for the preceding experiments table:
 - Row 334: currently running sample
 - Rows 332-334: Finished experiments

Photograph: Shows the NMR spectrometer with a sample in the center. An arrow points to the sample with the text "Currently running sample". Another arrow points to the next available sample position (holder 8) with the text "Next available sample position".

ICON-NMR Interface

holder position	Sample folder name	Experiment number	solvent	Experiment	parameters	research lab	experiment length
4	Finished /home/nmr1 CHY174A	1	CDC13	PROTON	☒☒	Guan	☒☒ 00:01:29
	Finished /home/nmr1 CHY174B	1	CDC13	PROTON	☒☒	Guan	☒☒ 00:01:29
5	Running /home/nmr1 wy-1007-OTs	1	CDC13	F19_880F	☒☒	Liu	☒☒ 00:00:50
6	Queued /home/nmr1 10-7-ph-80H2-S-1h	1	CDC13	PROTON	☒☒	Sun	☒☒ 00:01:29
7	Queued						☒☒ 00:01:29

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

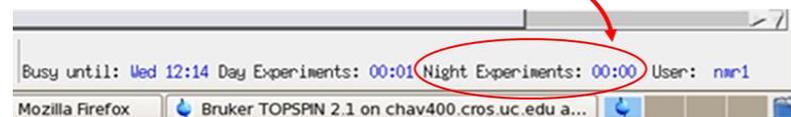
Set Experiment—Contact NMR manager to run experiments not available on your account

Some parameters can be adjusted, but be careful!

Check experiment length before pressing Submit!

The Night Queue

- On the AV400, ^{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 ^1H 1D) made to run long (> 20 min on AV400, > 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. **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

Automation - Running - Busy until : Tue 10:26 - Day Experiments : 00:07 - Night Experiments : 00:00

Help
Logoff

Logged in as nmr1
Instrument Name chav400.cros.uc.edu: spect

Date	Time	Holder Name	No.	Experiment	Load	ATM	Rotation	Lock	Shim	Acq	Proc	User	Title	Res
2020-10-13	10:18:26	14 B2-P11-R2-H	1	PROTON	✓							senevipp	Group Merino	
2020-10-13	09:44:45	13 wy-1012I	1	F19_BBOF	✓	✓		✓	✓	✓	✓	yanwo	Group Liu	sref .1/c
2020-10-13	09:40:46	12 wy-1012H	1	F19_BBOF	✓	✓		✓	✓	✓	✓	yanwo	Group Liu	sref .1/c

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
 - 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.

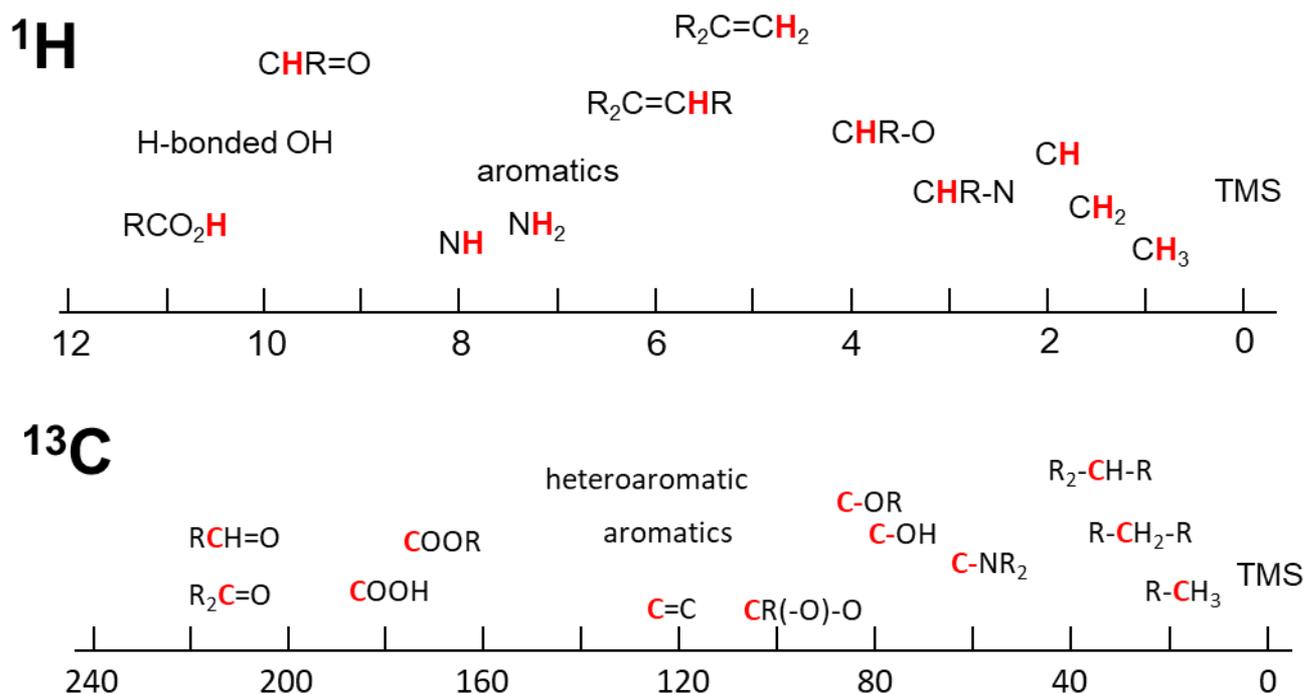
- They will run during the daytime regardless of their length!
- For long ^{13}C , use C13CPD instead.
- Alternatively you can put these in the night queue by clicking the sun icon: 

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.

Chemical Shift Ranges

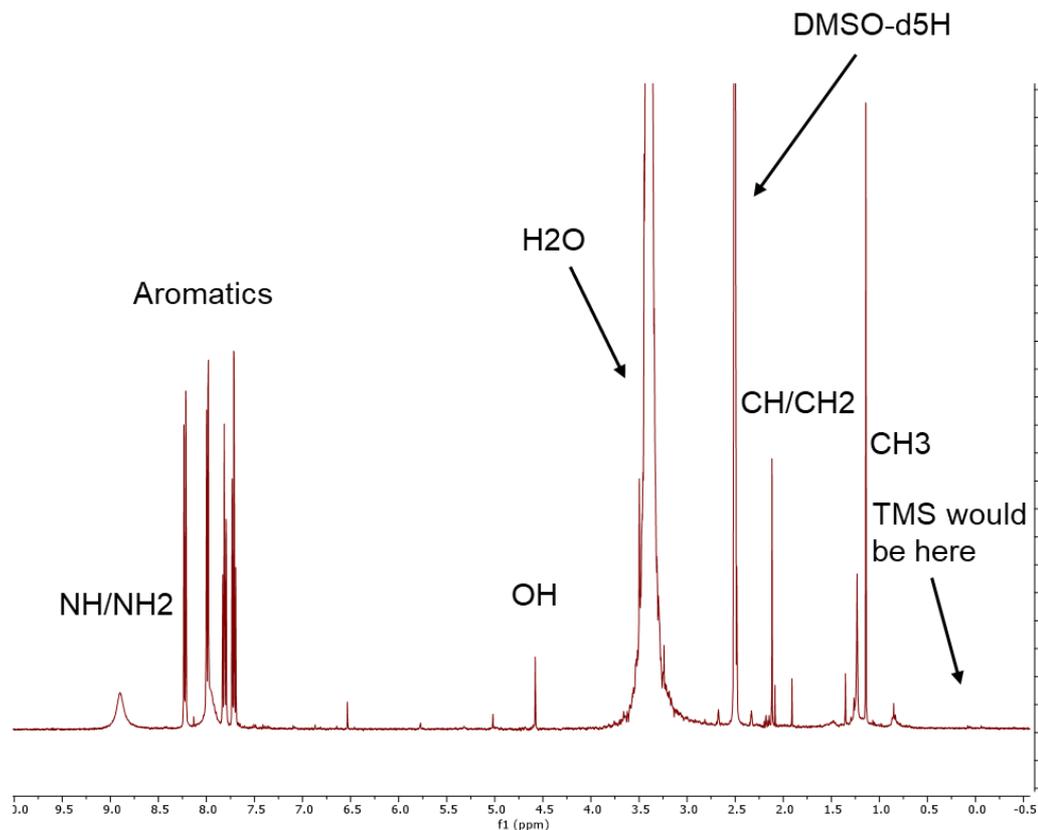


Note that the ¹³C and ¹H trends tend to match each other! Chemical shift is affected by the same electrons in each case!

^1H zg, zg30

Standard Experiments

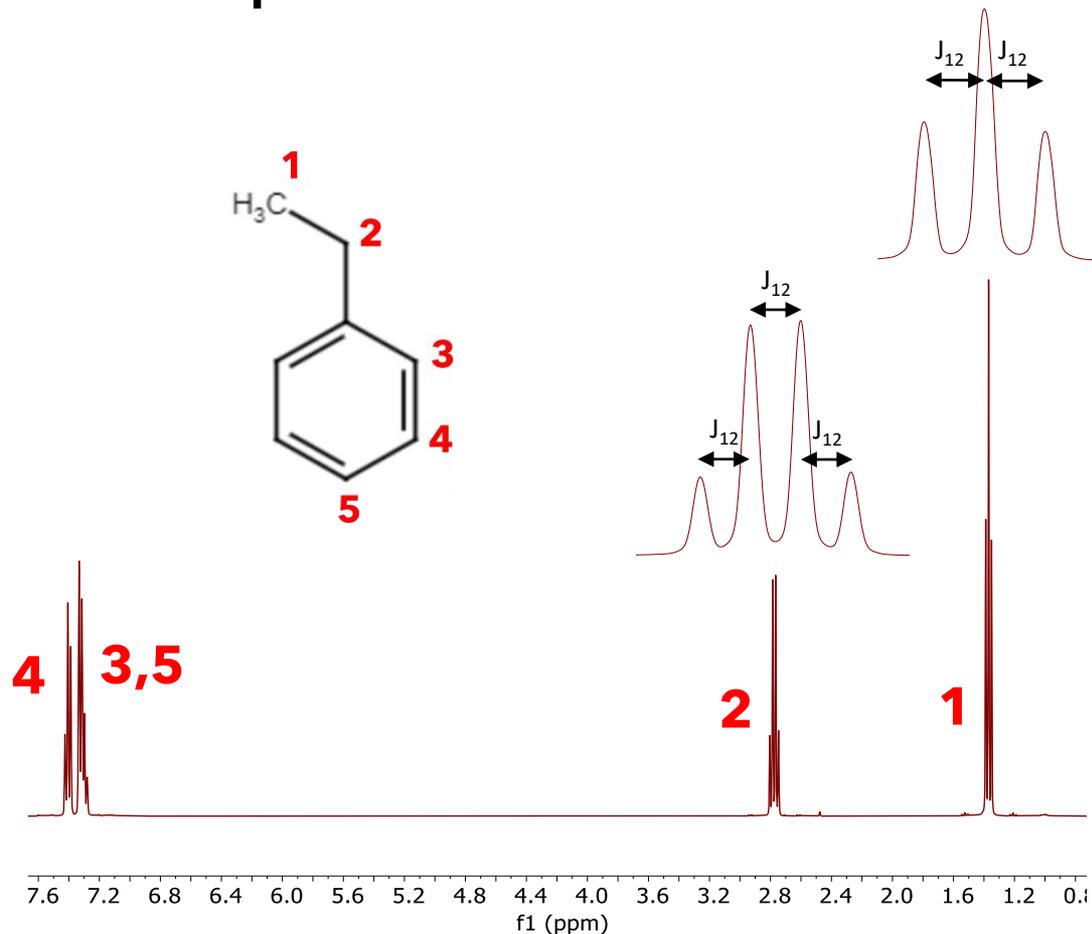
- High sensitivity
 - Impurities usually evident if present
- Shows multiplicity, aids in assignments
- Typically requires 100% deuterated solvent
- Relatively fast T1 relaxation, pulse delay can be ~ 2 s
- Overlap sometimes an issue



^1H zg, zg30

Standard Experiments

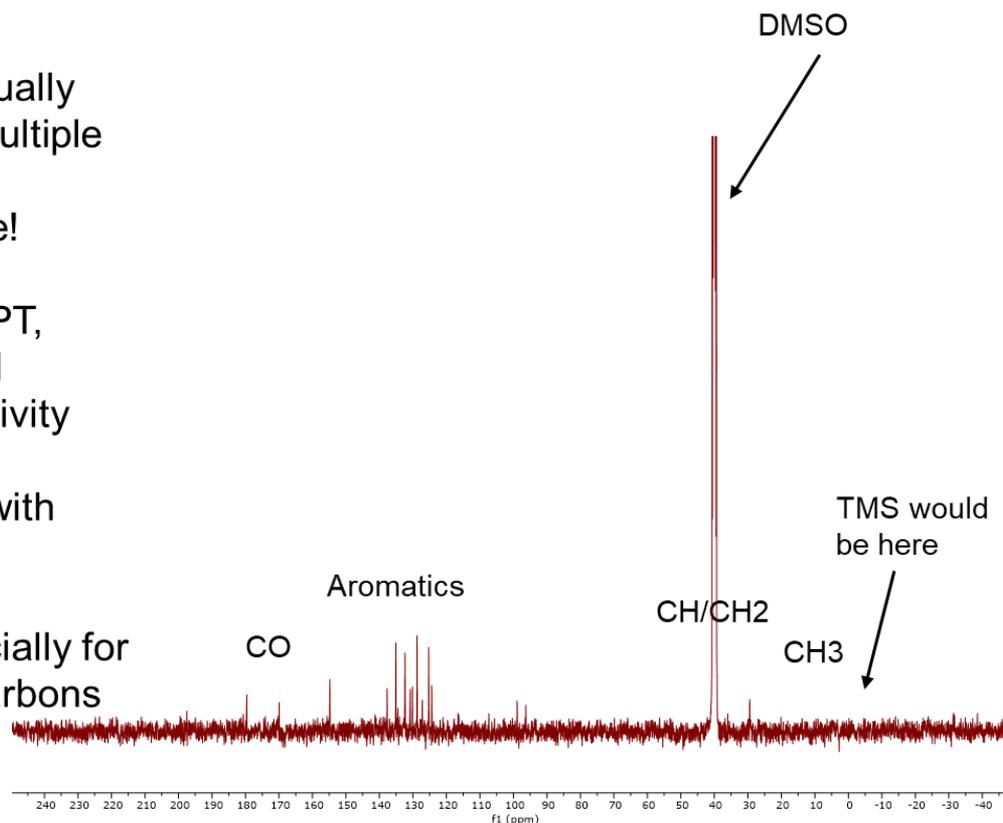
- High sensitivity
 - Impurities usually evident if present
- Shows multiplicity, aids in assignments
- Typically requires 100% deuterated solvent
- Relatively fast T1 relaxation, pulse delay can be ~ 2 s
- Overlap sometimes an issue



Standard Experiments

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

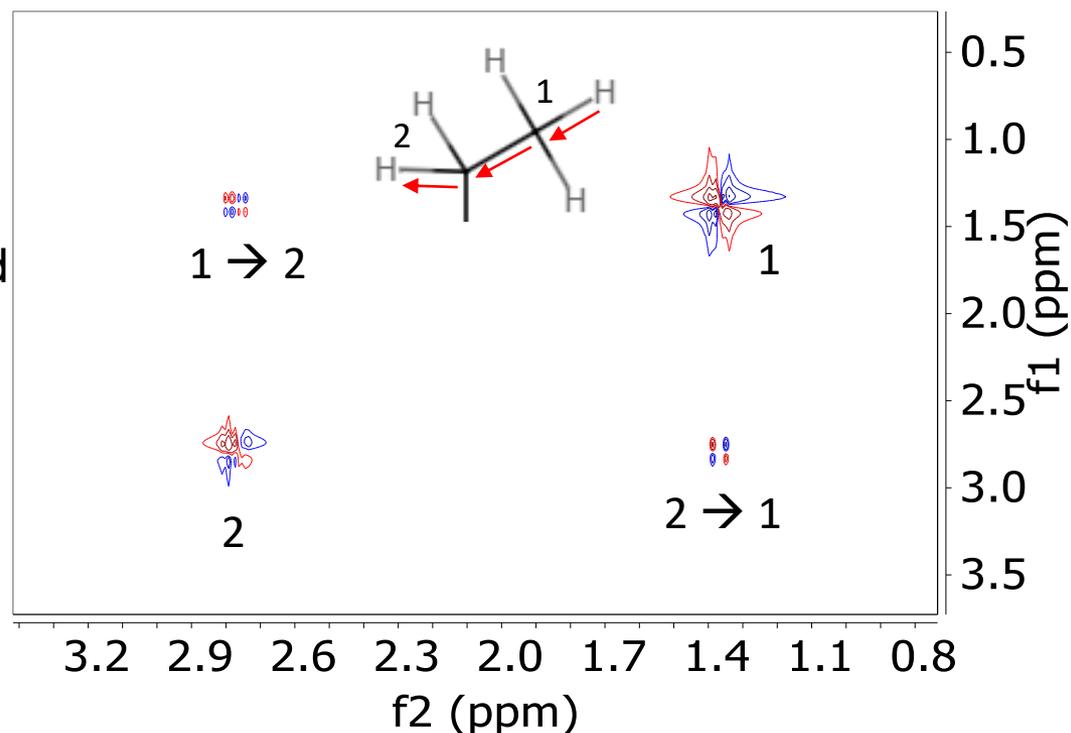
- Lower sensitivity
 - Protonated carbons usually have more signal for multiple reasons
 - Use >10 mM if possible!
- Magnetization transfer (DEPT, INEPT, NOE) from attached protons can enhance sensitivity
- Multiplicity can be inferred with “editing” (DEPT)
- Slower T1 relaxation, especially for quaternary/unprotonated carbons
- Overlap less common



Standard Experiments

^1H - ^1H COSY 2D

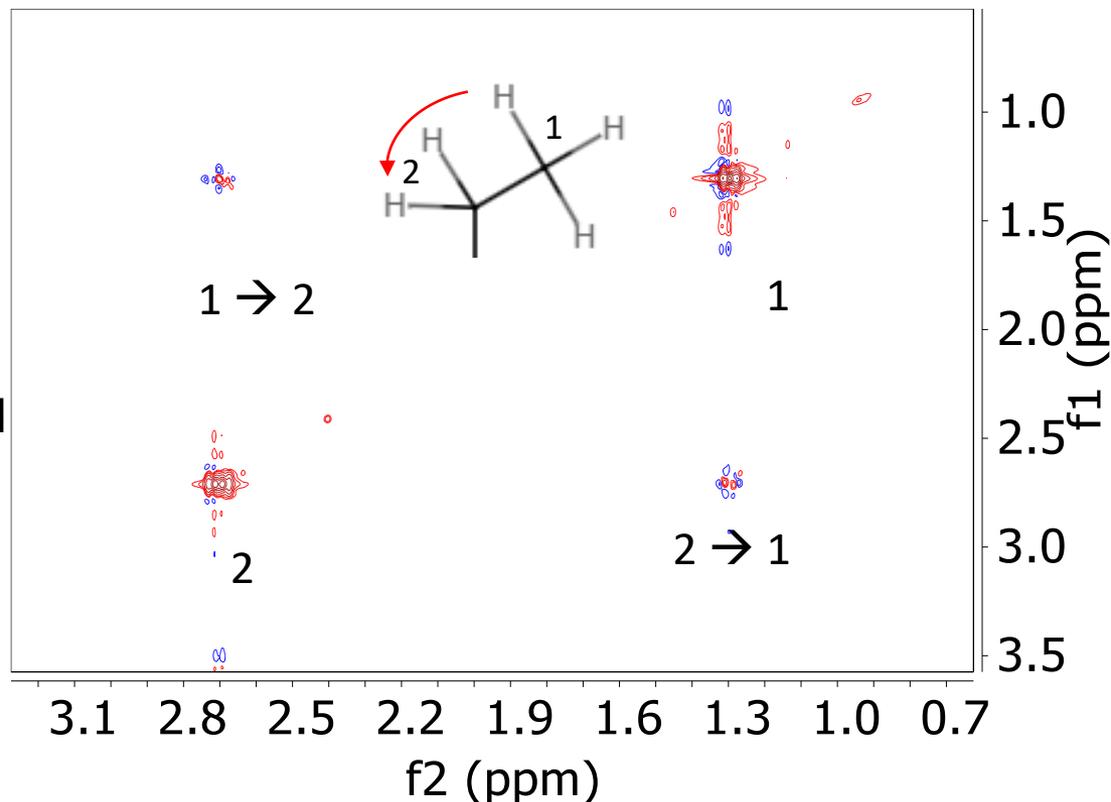
- Provides 3-bond ^1H - ^1H correlations
- Complex multiplicities resolved in cross-peaks
- Option for presaturation of solvent peak
- Most useful for compounds with many protons!



Standard Experiments

^1H - ^1H NOESY 2D

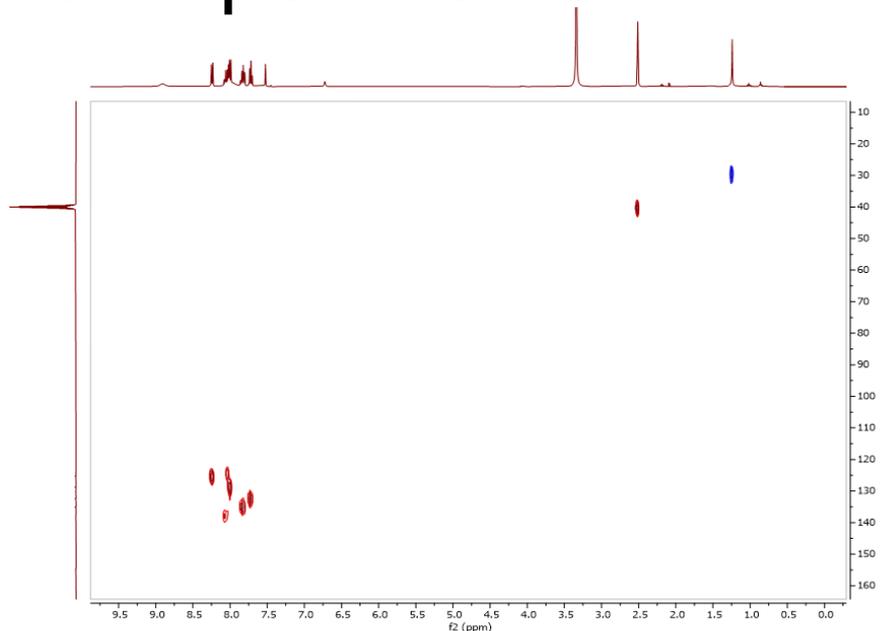
- Provides through-space ^1H - ^1H correlations
- Unsuitable for compounds between ~1-2 kDa (related ROESY should be performed instead for these)
- Requires setting of a mixing time, usually between 500-800 ms.
- Most useful for compounds with many protons!



Standard Experiments

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

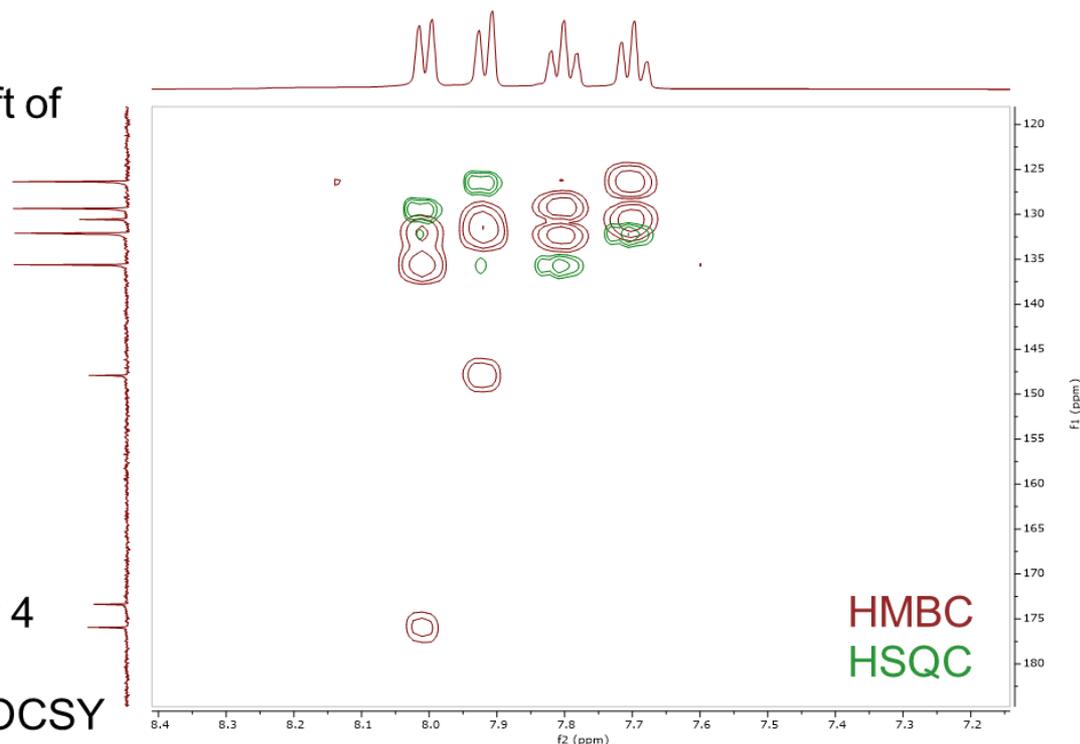
- Correlates ^{13}C shift to ^1H shift of attached proton
- Helps reduce overlap
- Aids in assignments
- Non-protonated carbons are missing!
- Don't let Mnova's "projections" confuse you! They are separate spectra by default!
- Related: ^{13}C - ^1H HMBC 2D shows correlations between carbons and protons that are 2+ bonds apart



Standard Experiments

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

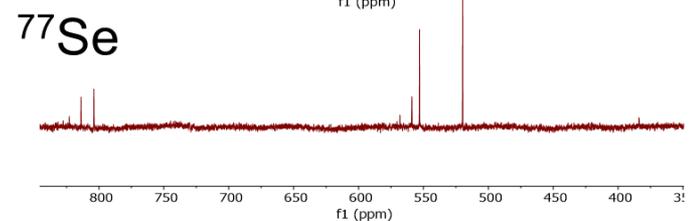
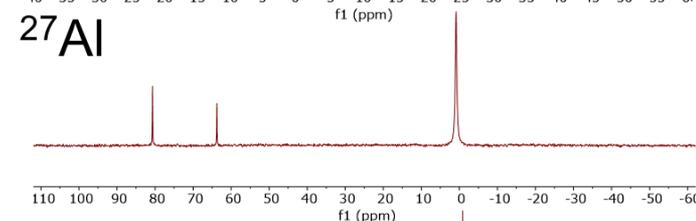
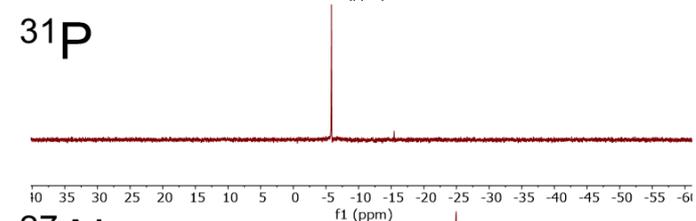
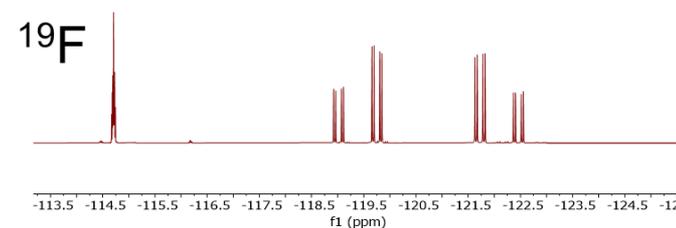
- Correlates ^1H shift to ^{13}C shift of carbons 2+ bonds apart
- Helps reduce overlap
- Aids in assignments
- Shows correlations to non-protonated carbons!
- Ambiguities (are they 2, 3 or 4 bonds apart?)
 - Related selHSQMBC-TOCSY can help resolve ambiguity by providing sign/magnitude of couplings



Standard Experiments

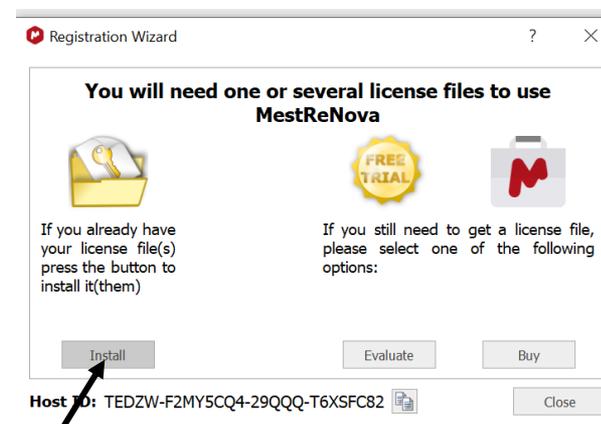
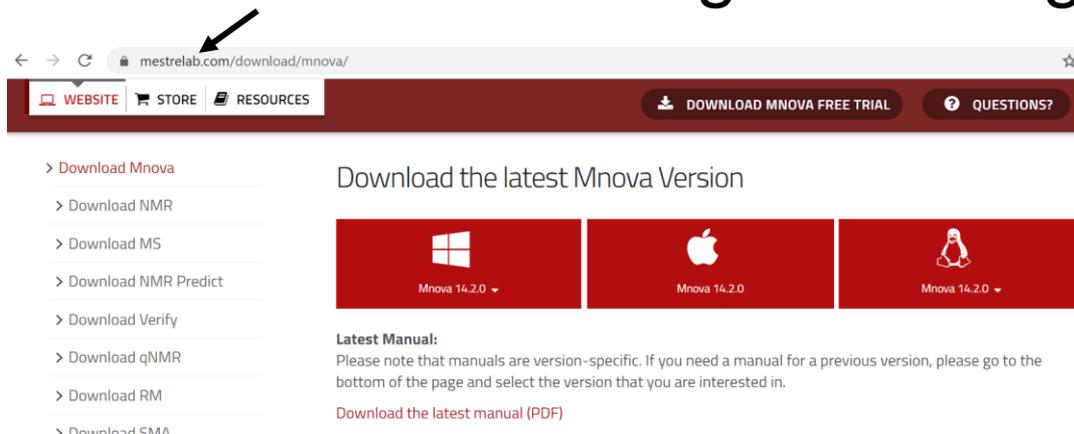
zg 1Ds for other nuclei

- Sometimes have long T1s
- Sometimes have very large chemical shift ranges
- Sensitivities range from worse than ^{15}N to comparable to ^1H !

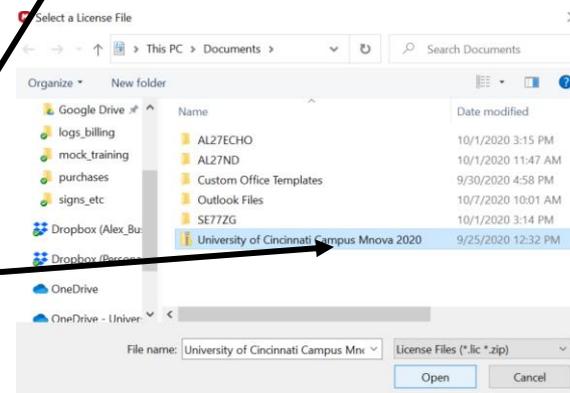


	Spin I	Natural abundance	Receptivity ($^{13}\text{C} = 1$)	Resonance frequency on a 400 MHz magnet (MHz)
Hydrogen	1/2	99.985%	5670	400.00
Deuterium	1	0.015%	0.0082	61.40
Carbon-13	1/2	1.108%	1.00	100.60
Nitrogen-15	1/2	0.370%	0.022	40.56
Fluorine-19	1/2	100.000%	4730	376.36
Aluminum-27	5/2	100.000%	1170	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

Installing/Activating MNova



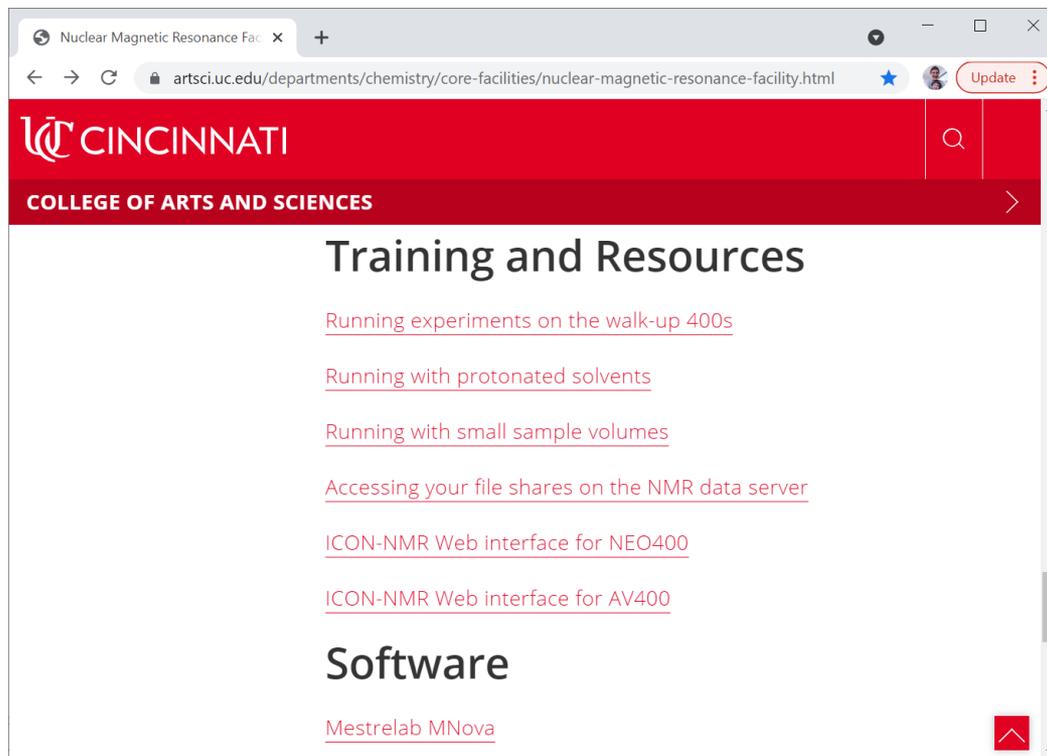
- Download Mnova from mestrelab.com/download/mnova
- Get the campus license file from <https://www.artsci.uc.edu/departments/chemistry/resources/software.html>
- While on campus network, load the license file the first time you use the software:
- The license needs to refresh every few months by running 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





Contact:

Alex Greenwood

Office: Rm 123C/Crosley;

Phone: 513-556-9211;

Email: greenwa2@ucmail.uc.edu

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