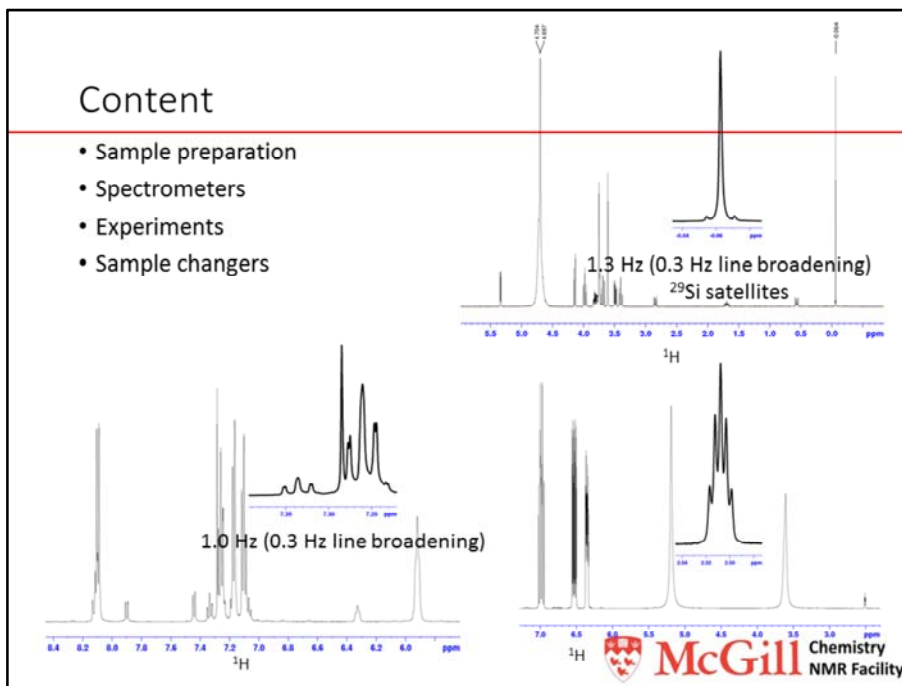


# Intro to *the NMR Facility*



How do you know if a spectrum looks good (meaning, if the spectrometer has worked properly)? You look at a known peak and you check whether it looks as expected. Three examples are shown here. In the upper right is a spectrum of sucrose in 90%  $\text{H}_2\text{O}$  + 10%  $\text{D}_2\text{O}$ , acquired using solvent suppression in automation. The peak at 0 ppm is from TSP (trimethylsilylpropionic acid), which contains a  $\text{Si}(\text{CH}_3)_3$  group, like TMS, but which is soluble in water. The peak width is measured to be 1.3 Hz (remember, to convert Hz to ppm, divide by the resonance frequency; for example, for  $^1\text{H}$  in a 500 MHz magnet, divide by 500 Hz/ppm). This include 0.3 Hz of applied exponential linebroadening, so the intrinsic linewidth is 1.0 Hz—a good number. Below about 1.1 or 1.2 Hz is OK (the shimming is good). Similarly, in the bottom left, the  $\text{CHCl}_3$  is shown, and it has an intrinsic linewidth of only 0.7 Hz, which is very good. On the bottom right is a DMSO peak, showing the well-resolved quintet shape.

Another point to look out for is the presence of satellite peaks where expected. The TSP (or any TMS) peak shows “silicon satellites”. These arise from the 4.6% of the silicon atoms which are  $^{29}\text{Si}$  (spin-1/2). The rest of the silicon is  $^{28}\text{Si}$  or  $^{30}\text{Si}$ , which have no spin. For the 4.6% of the protons which are in the molecule containing  $^{29}\text{Si}$ , there is J-coupling to the  $^{29}\text{Si}$ . This splits the signal of those protons in two (because half the  $^{29}\text{Si}$  is in the spin-up state and the other half is in the spin-down state, giving rise to two different magnetic environments for the protons). The splitting of this two-bond H-Si J coupling is about 6 Hz,

which can be measured from the spectrum.

The splitting in the DMSO peak comes from splitting of the observed proton ( $^1\text{H}$ ) signal by deuterium ( $^2\text{H}$ , spin 1). Although deuterated DMSO is used in NMR, some very small amount ( $\ll 1\%$ ) is present as  $\text{CD}_2\text{H-S(=O)-CD}_3$ . Two-bond J coupling of 1-2 Hz in size between  $^1\text{H}$  and the two  $^2\text{H}$  of the same methyl group gives rise to a 1:2:3:2:1 quintet. (If there had been only one  $^2\text{H}$  that the proton was coupled to, there would be a 1:1:1 triplet. Note that such a triplet is present in the  $^{13}\text{C}$  spectrum of  $\text{CDCl}_3$ .)

## Sample preparation

- Sample should be
  - Clear (filter if necessary, for example through a ball of cotton at the neck of a glass pipette)
  - Homogeneous
  - 5 cm long
  - Labeled on top with marker only
- NMR tube should be
  - Clean
  - Dry (use oven up to 50 C, keep tubes standing as upright as possible)
  - Free of chips
  - Wiped with a Kimwipe immediately before insertion in magnet
- Why? *Shimming*
  - Better observation of small J-couplings
  - Better S/N
- *Avoiding finger grease in probe*
  - The grease contaminates your sample and other people's, too



If your shimming isn't good (meaning, your lines are broad and/or misshapen), then there are two possible explanations: the sample is not good, or the instrument hasn't performed properly.

Narrow lines appear because the sample being studied is in a homogeneous environment. Small magnetic fields called "shims" are adjusted to maximize homogeneity. They can only do so much, however. The sample itself should be well dissolved and not have particles floating in it (although precipitate that has settled down at the bottom of the NMR tube is probably OK). Also, the sample should be sufficiently long that all the sample in the "active area" (about 2 cm long—see slide about depth gauges) sees the same environment (ie other sample) both above and below it. If there is an abrupt transition between sample and air in the active area, because your sample is too short, then shimming will be poor. Thus, you should have at least 5 cm of sample (4.5 cm for the Brukers).

To avoid dirt getting into the instruments, which shows up both as a contaminant in the spectrum and which prevents shimming, wipe the NMR tube with a Kimwipe before placing it in the magnet, and do not use marker anywhere besides at the very top of the NMR tube. Otherwise, ink gets inside the spinners and eventually works its way down into the NMRs.

NMR tube should be made of glass with a consistent thickness all the way around. Tubes

can be warped by drying at high temperatures.

Avoid using tubes that are so chipped on top that a cap will not fit securely on it. When the tube is removed by the spinner (and you might not be the person removing it!), then if the cap is not on securely, it can come off, exposing the person's fingers to the jagged glass at the top.

## Positioning sample in depth gauge

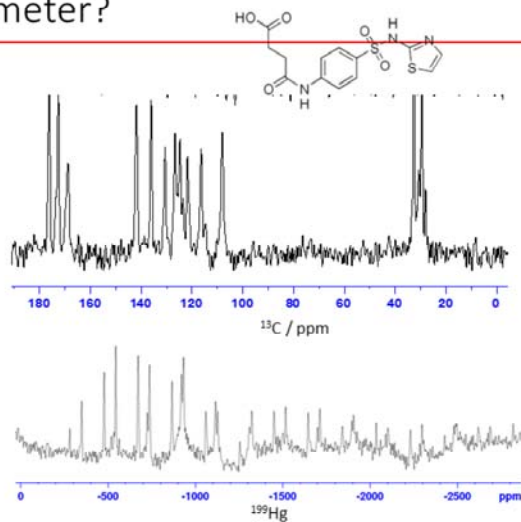
- It is better to dilute sample to correct depth than to run with a short sample (because concentrated, short sample will have a hump at the bottom of the peak; the added concentration won't increase peak intensity)
- But, if you have a short sample anyway...



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NMR Facility

The “depth gauges” show the “active areas”—the portion of the sample that is actually visible to the NMR instrument. On the Bruker depth gauges, the rectangle labeled “3/5/8 mm” is the appropriate one (we use 5 mm NMR tubes). On the Varian depth gauges, the box drawn with dashes at the back of the gauge is the active area. If you have a standard length sample (or one which is longer), then the sample should touch the bottom of the gauge. If the sample is shorter, then raise it in its spinner slightly so that it is centered on the active area indications. This will give you the best possible results—but they will not be as good as diluting the sample a bit and positioning it normally.

Which spectrometer?



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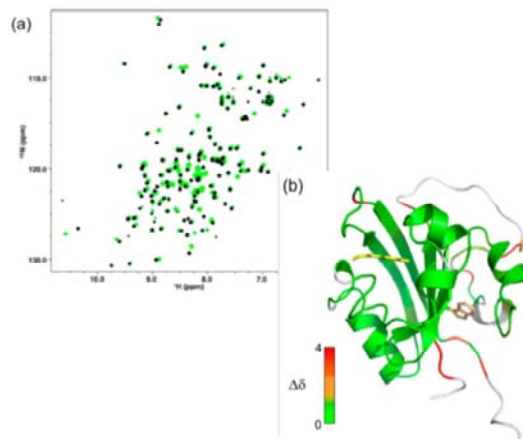
This probably *isn't* the spectrometer you are looking for...it's a solid-state NMR instrument capable of examining solid (usually powder) samples. Keep it in mind for samples that you are interested in examining in powder form.

## Bio?



QANUC 500

...also an 800 and??



This is probably also not the instrument you want! It is a 500 MHz that belongs to the regional biological NMR facility, QANUC. If you want to study proteins or ligand binding or similar, contact them. They also have an 800 MHz NMR (subject to the rebuilding of the pulp and paper annex).



More prosaically...I want it now!



400 MHz  
Varian NMR

$^1\text{H}$ ,  $^{31}\text{P}$

nucleus	SNR
$^1\text{H}$	358
$^{31}\text{P}$	30



500 MHz Varian  
NMR

$^1\text{H}$ ,  $^{13}\text{C}$

nucleus	SNR
$^1\text{H}$	420
$^{13}\text{C}$	260



400 MHz  
Bruker NMR

nucleus	SNR
$^1\text{H}$ , $^{13}\text{C}$ , $^{31}\text{P}$ , $^{11}\text{B}$ , $^{19}\text{F}$ , $^{15}\text{N}$ , etc....	476
$^{13}\text{C}$	266
$^{31}\text{P}$	253
$^{19}\text{F}$	250



These, however, probably are the instruments you want! They are the three non-sample changer instruments that we have. You have to book time on each of them. The SNR (signal-to-noise ratio) figures listed are values achieved on sealed test samples in a single scan.

The Varian Mercury 400 can do  $^1\text{H}$  and  $^{31}\text{P}$  (only). It's not great for  $^{31}\text{P}$ , but that may not matter, since  $^{31}\text{P}$  is an intrinsically sensitive nucleus (100% abundant, in contrast with, for example,  $^{13}\text{C}$ , which is 1% abundant). You can do VT on this instrument up to about 50 °C.

The Varian VNMR5 500 can do  $^1\text{H}$  and  $^{13}\text{C}$  by default. It can be booked for long periods of time, so it is good for VT experiments (down to -5 °C, up to +90 °C) or kinetics runs, or for long  $^{13}\text{C}$  experiments. You can learn to do  $^{19}\text{F}$ ,  $^{11}\text{B}$ , or other nuclei on it, with a bit of training. Or, if it's not busy, you can run a quick  $^1\text{H}$  on it. Note that you must tune it if you are running  $^{13}\text{C}$  or 2D experiments.

The Bruker Avance III HD 400 can do many nuclei in full automation. It takes a bit longer for shimming than the others. If necessary, you can do VT experiments on it (up to 80 °C without additional training).

I want it later!



300 MHz Varian  
NMR with  
autosampler

$^1\text{H}$ , $^{13}\text{C}$ , $^{19}\text{F}$	
nucleus	SNR
$^1\text{H}$	152
$^{13}\text{C}$	106
$^{19}\text{F}$	108



500 MHz Bruker  
NMR with  
sample changer

$^1\text{H}$ , $^{13}\text{C}$ , $^{31}\text{P}$ , $^{11}\text{B}$ , $^{19}\text{F}$ , $^{15}\text{N}$ , etc....	
nucleus	SNR
$^1\text{H}$	731
$^{13}\text{C}$	326
Less busy in morning $^{31}\text{P}$	242
$^{19}\text{F}$	550

Busy when CHEM 360/392 is active



The two sample changer instruments are good choices if you don't need your spectra immediately, although they can also return spectra very quickly if they are not busy (eg in the mornings, or out of term time for the 300).

The Varian Mercury 300 can do  $^1\text{H}$ ,  $^{13}\text{C}$ , and  $^{19}\text{F}$  in full automation. Sensitivity is not great compared with the other instruments, but this may not matter. The Varian 300 is the only instrument that can do  $^{13}\text{C}$  with  $^{19}\text{F}$  decoupling in automation (ie without additional setup or training).

The Bruker Avance III HD 500 can do all the same experiments as the Bruker 400.

## I want something special (=extra training...)



500 MHz Varian  
NMR

- VT (+90 C, -10 or so)
- Long-time experiments (eg kinetics)
- Other nuclei
- $^{13}\text{C}$  with  $^{19}\text{F}$  decoupling (also Mercury 300!)
- DOSY



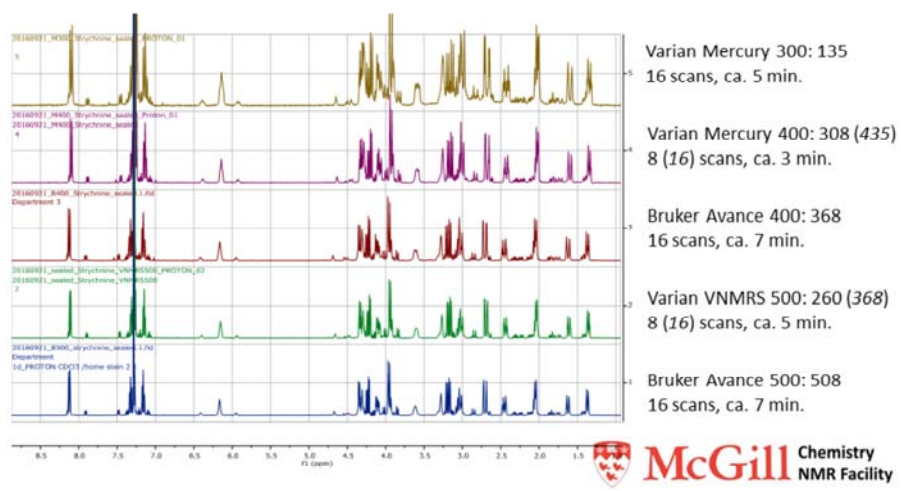
400 MHz  
Bruker NMR

- VT (+150 C, -150 or so)
- Long-time experiments overnight (periodic: Bruker 500 also)
- Other nuclei, including low gamma
- DOSY

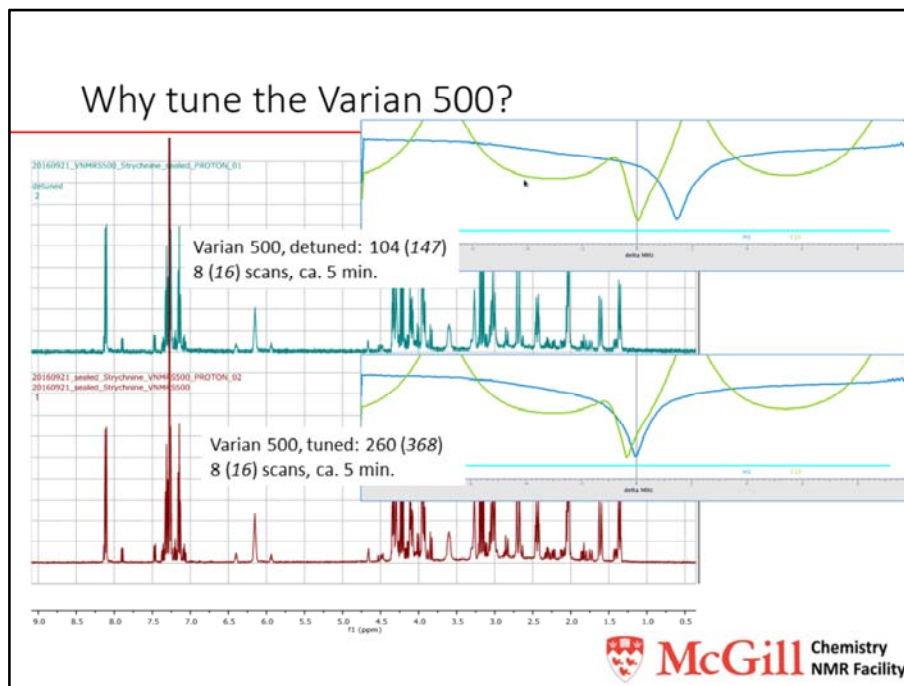
The Varian 500 and the Bruker 400 have the capabilities to do lots of interesting experiments, including diffusion spectroscopy, or DOSY, where components of a mixture are separated according to their diffusion coefficients.

## What spectrometer should I use?

- SNR for peak at 8.1 ppm of same 13.5 mM sealed strychnine sample (approximately 13 mM or 3 mg), using default acquisition parameters



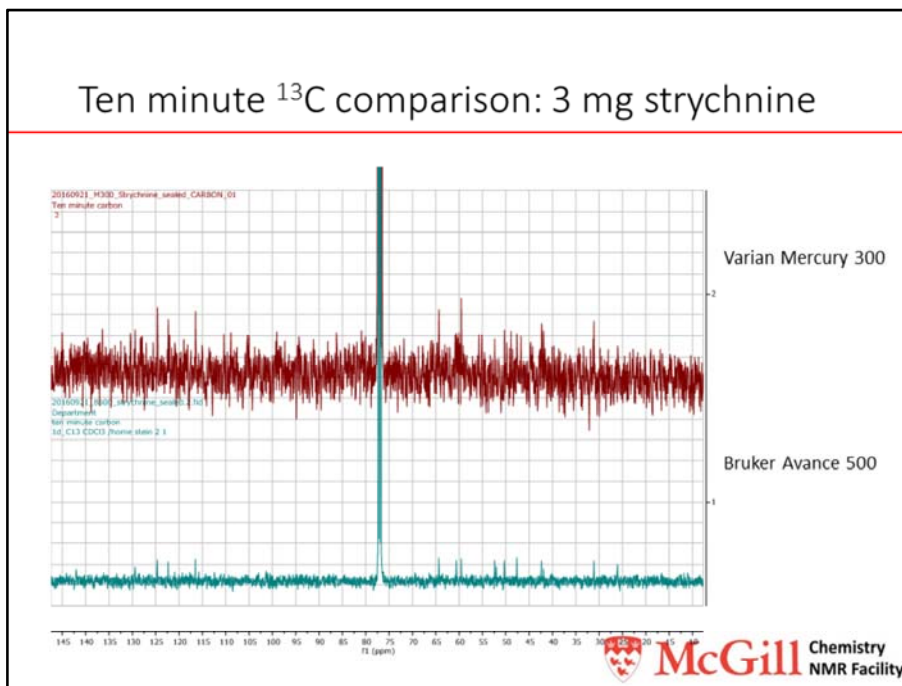
Here is a practical example of the exact same sample used with the default Proton (Varian) or 1d\_PROTON (Bruker) experiments with no changes to acquisition parameters. In all cases, the SNR is more than sufficient to see the peaks and some smaller components. The 300 is less well-resolved (peaks with similar chemical shifts are less separated) than on the 400 or 500, as expected.



Tuning is the adjustment of the probe to be as sensitive as possible to the frequencies used in NMR for the sample being studied (it is the adjustment performed underneath the magnet on the Varian 500). These two spectra show the advantage of tuning the probe. In the tuning diagrams shown, the blue curve is the  $^1\text{H}$  curve. When the tuning is off by about 1 MHz, as in the top spectrum, the sensitivity is comparable to that of the Varian 300 in 16 scans (147 vs. 135). This may not be important for  $^1\text{H}$ , which is very sensitive, but it can be critical for  $^{13}\text{C}$ .

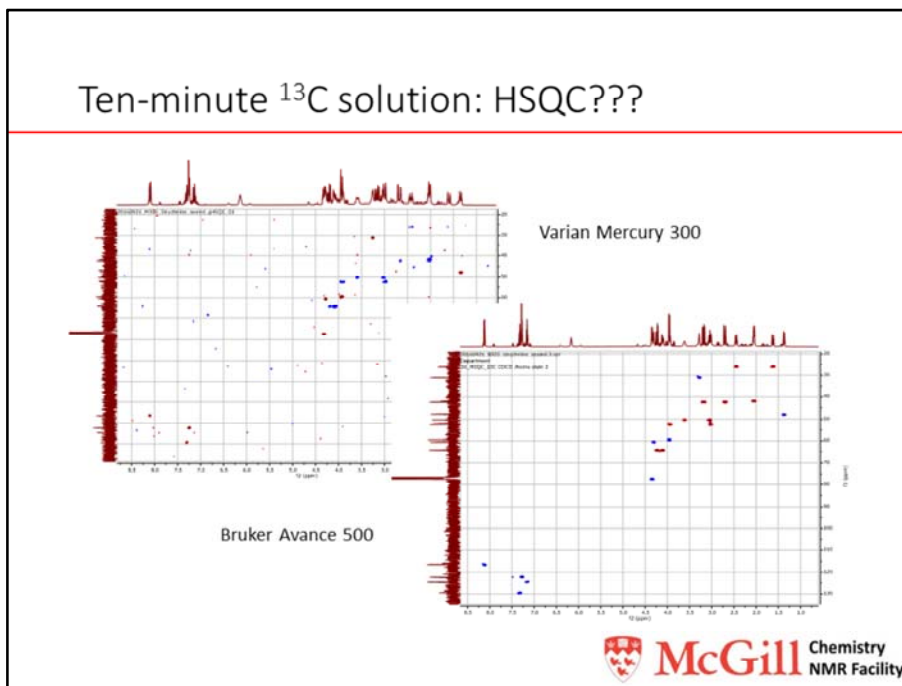
What about the other instruments? The two Bruker machines have small motors which accomplish the same task as people do on the Varian 500. And, the other two Varian machines aren't as sensitive to changes in solvent as the other instruments.

## Ten minute $^{13}\text{C}$ comparison: 3 mg strychnine



These two spectra were acquired on the same sample as for the proton spectra shown above. The 300 isn't really up to the job. Even running 4 or 16 times as many scans in order to double or quadruple the SNR will not suffice. However, the Bruker 500 could give a reasonable spectrum if given enough time. SNR increases as the square of the number of scans.

## Ten-minute $^{13}\text{C}$ solution: HSQC???



However, if you don't have much time to spare and the 300 is the only available instrument, all is not lost. An HSQC, where protons and their directly-bonded carbons are correlated, gives information about protonated carbon sites in much less time than a full carbon spectrum. It would have been better to spend 20 or 40 minutes on this spectrum, on the 300, but it's certainly usable.

The HSQCs used on our instruments are "DEPT-edited": CH and  $\text{CH}_3$  peaks have the opposite phase (or sign or colour in the plots) as  $\text{CH}_2$  peaks.