Bruker DRX 600 MHz NMR Spectrometer

- Purchased Used in 1999 from MR Resources, a recycler of NMR Instruments, for $550K; Formerly in Laboratory of James Prestegard, University of Georgia
- Majority of Funding from Office of Naval Research (former Chemistry faculty Seth Marder)
- Magnet: Actively Shielded Demo from Magnex
- Two-Bay Console
  - Three RF Channels (e.g., ^{1}H, ^{13}C, ^{15}N)
  - Gradients on 3 axes (x, y and z)
  - Shaped (Selective) Pulses
  - Oversampling and Digital Filtering
- Software: XWinNMR 3.1
- Gradient Probe:
  - "**txi**" Bruker XYZ-gradient $^{1}H(^{13}C,^{15}N)$ 5mm
- SGI UNIX computer "drx600.chem.arizona.edu"
- Continuous Control of Sample Temperature: Air Dryer (shared with DRX-500) and Air Chiller

Introduction to the Bruker Avance DRX-600

The Bruker DRX-600 spectrometer was purchased used from MR Resources in 1999 at a total cost of $550,000, with the majority of funding coming from an Office of Naval Research grant (former Chemistry faculty member Seth Marder). The Magnex shielded superconducting magnet was a new demo model and the console was used for 3 years by James Prestegard at the University of Georgia. An old Bruker MSL-200 solid-state NMR instrument was decommissioned and moved out to make room for the new instrument.

I. Features.

A. **Pulsed Field Gradients.** The ability to "bend" the magnetic field in a linear fashion has many exciting and powerful applications. Orignally developed for NMR imaging (MRI), gradients disrupt the homogeneity of the magnetic field so that field strength is dependent on position within the sample in a linear fashion. We can apply a gradient in the X, Y or Z (sample tube) direction, or any linear combination, for a short period of time (typically 1 millisecond) and then turn it off. The field homogeneity is re-established within a period of 100 microseconds after the end of the gradient pulse. Gradients can be used for the following applications:

1. Elimination of artifacts: Unwanted signals can be destroyed by using a gradient pulse at a time in the pulse sequence when the desired magnetization is aligned along the z axis, since z-magnetization is not affected by gradients. For example, during the mixing period of a NOESY experiment only z-magnetization is desired.

2. Reduction of 2D experiment time: In 2D experiments the number of scans (NS) acquired and summed for each FID is often determined by the phase cycle rather than the quantity of sample. For many experiments (COSY, HSQC, etc.) a sample containing 10 or 15 mg of a medium-sized (MW ~400) organic molecule would only need one scan (NS=1) per FID to get a good signal-to-noise ratio. But without gradients, you may need 4 or 8 scans to select the correct pathway for magnetization transfer. Gradients make it possible to collect only one scan and still select the pathway and eliminate the undesired signals. Thus for these concentrated samples, an excellent DQF-COSY can be acquired in 15 minutes and a good HMQC may require only 25 minutes of acquisition. For more dilute samples (1-5 mg) this advantage is lost.

3. Water Signal Suppression. For many biological molecules, especially peptides and proteins, the amide NH signal is important to NMR assignment and conformational studies. This signal can only be observed in 90% H_2O / 10% D_2O solution. The problem of observing signal from a 2 mM sample in 100 M water is extremely challenging. A new method called "Watergate" eliminates the water signal using gradient pulses and reduces the water peak height to a level similar to the sample peaks. In addition, the NH signals are not attenuated as they are in older

methods such as solvent presaturation. Even better water suppression is obtain by placing the gradient axis at the "magic angle" (54.74^o from the z axis) using a combination of the three axes available (x, y and z).

4. Selective Excitation. Gradients can work together with shaped pulses (see below) to allow discreet pulses (e.g., 90^o) to be applied only to a single peak in the spectrum. This means that many 2D experiments can be reduced to a single dimension since we can select which nucleus within a molecule we want to study.

B. Shaped Pulses. On older spectrometers the only way to selectively excite one peak in a spectrum is to have a long (one second or more) irradiation at low power and at the specific resonant frequency of the peak. This can only be used to equalize the populations of the two NMR energy levels (for NOE or presaturation) or with a bit higher power to cause the spins to rapidly "boil" or jump back and forth between the upper and lower energy states (selective decoupling). But what if we could create coherent magnetization on one nucleus in a molecule, delivering a 90^o or 180^o pulse only to nuclei at a specific chemical shift? This is now possible with shaped pulses.

The older spectrometers can only deliver RF pulses in a rectangular (on/off) fashion. These pulses are delivered at high power for very short duration and they are non-selective: essentially all of the nuclei of a given type (e.g. ^{1}H) are excited equally regardless of their chemical shift. If we decrease the power of the RF signal delivered to the probe, we need to increase the duration ("length") of the pulse if we want the amount of rotation of magnetization (the "flip angle") to stay the same. As the power is decreased, the selectivity is increased: signals (peaks) which are near the center of the spectrum (the "carrier frequency") might receive a 90^o rotation, but signals farther away from the center might receive only a 45^o or less rotation.

Low-power long-duration rectangular pulses can provide a certain amount of selectivity, but they can excite signals far from the center of the spectrum as well. We can define the "excitation profile" as a graph or spectrum of the amount of rotation a nucleus receives from the pulse as a function of its chemical shift. The maximum excitation always occurs at the center of the spectral window ("on resonance"), but with rectangular pulses there are "wiggles" of excitation which occur far from the center of the spectral window. These pulses are also limited to exciting at the center of the spectral window, so we have to move the spectral window around to excite the desired peak. If instead of a rectangular (on-off) pulse we use a specific shape (for example, the Gaussian or bell-curve) we can get very specific excitation with no "wiggles" in the excitation profile. A shaped pulse is really just a series of rectangular pulses of very short duration and varying amplitude. If the phase of each rectangular pulse in the series is increased by a fixed amount relative the previous pulse, we can excite nuclei which are not in the center of the spectral window. Thus we can now deliver a pulse of any flip angle to any peak in the spectrum without changing the spectral window.

C. Oversampling and Digital Filtering. The analog-to-digital converter (ADC) samples the FID at regular intervals and converts the voltage to a number which goes into a list. This list of numbers is the digital FID. Modern ADC's can sample very fast:

300,000 samples per second. A typical spectral width for proton on a 500 MHz instrument might be 6250 Hz, which requires a sampling rate of 12,500 samples per second to prevent aliasing of signals. That means that we could be sampling 24 times faster than we really need to. Why not sample at the maximum rate, collecting 24 samples for every one sample we really need, and then take the average of those 24 samples? Accuracy would be greater since we are taking the average of a number of measurements. This method is called oversampling. The decimation factor in this case is defined as 24. Bruker calls this parameter DECIM.

Now we might want to get more sophisticated and instead of taking the simple average of the 24 data points we could take a weighted average, using some kind of weighting function which gives more weight to the samples taken in the middle of the period than to those at the beginning and end of the 24-sample period. The envelope over which we take the weighted average could even be extended over hundreds of samples, so that we are actually running the weighting function over the sampled data and taking a weighted average every 24 data points. This kind of math can be done very rapidly using chips which were devcloped for audio CD players. The end result of this weighted average is a frequency filter: some frequencies in the raw data are discriminated against and others are passed without any effect. If we shape the weighting function correctly, we can make this frequency filter have any desired shape. The shape chosen for NMR results in a flat response for frequencies within the spectral window (e.g., 6250 Hz) and an extremely sharp cutoff blocking all higher frequencies. This shape is called a "brick wall" filter. Older NMR spectrometers use analog audio filters made from simple electronic components to block out the high frequencies (and the high frequency noise which would alias into the spectral window), but these have a relatively gradual cutoff outside the spectral window and are not flat within the window. The "brick wall" shape is perfect for NMR.

Another advantage can be seen for a brick wall filter: we can look at only one part of the spectrum. If, for example, we are only interested in the amide region of a peptide or protein, we can set the spectral window to the region from 7 ppm to 11 ppm. There are peaks in other regions of the spectrum, of course, but they will not alias into the spectral window because the "brick wall" filter blocks them out. This can be dangerous, too: if you set the spectral window to a region with no peaks, there will be no FID!

We always use oversampling to the fullest extent on the DRX-500. This affects the appearance of the FID: if you look closely at the beginning of the FID you will see that there is little or no signal for a short time at the beginning, and then the FID "blossoms" out of this quiet baseline. This delay is called the "group delay" and is basically the time it takes for the weighting function to reach the beginning of the FID as it is passed through the raw oversampled data. If you process this data using software other than XWIN-NMR, you will have to specify that it is oversampled and give the decimation factor so that the software can correct for this delay. In XWIN-NMR, this is done automatically.

D. Three Channels. Older spectrometers have two RF "channels" which are called the transmitter and the decoupler. Sometimes the decoupler is fixed at the 1H frequency and the transmitter can be changed to accomodate the different frequencies of different nuclei,

including the 1H frequency. In newer spectrometers both of these channels are "broad-band" and can be set to any frequency. The DRX-500 continues this trend by having three equivalent channels. Each one can be set to any NMR frequency. For example, we could run an experiment in which we pulse on and detect proton in the FID (channel 1 = 500 MHz) and deliver pulses on ^{13}C (channel 2 = 125 MHz) and 15N (channel 3 = 50.7 MHz). The probe has three RF coils tuned to these three frequencies: it is called a "triple-resonance" probe. Modern biological NMR is dependent on uniformly-labelled samples with 13C at all carbon positions and 15N at all nitrogen positions. The flexibility to connect any of these channels, at any frequency, to any amplifier and route the output to the appropriate coil in the probe is one of the advantages of the DRX-500.

E. Temperature Control. We have continuous temperature control on the DRX-500, so that sample temperature is always regulated very accurately to a set value. Building air is dried using a dessicant column and then chilled in a refrigerated chiller. Cold air is delivered to the probe and heated to the desired temperature using a thermocoupled positioned near the sample to regulate temperature. We can operate from 5^{o}C to 40^{o}C without the need for any external cooling device, liquid nitrogen, or dry nitrogen supply. As a user you need to be aware of this feature because the sample temperature will depend on the previous user. You need to check the temperature, adjust it if necessary and note the temperature used on your spectrum. The dessicant dryer has two columns, one is continuously heated and purged with air while the other is being used to dry the air supplied to the spectrometer. Every few minutes the roles of the two columns are switched and there is a surge of air released. Don't panic! The first time you hear it you will think something horrible has gone wrong with the spectrometer, but eventually you get used to it.

II. UNIX File Structure

The DRX-600 is operated by an SGI O2 Unix workstation using the IRIX operating system. If your login ID is jones, your home (login) directory will be **/usr/people/jones**. But all of your nmr data will be located in a directory called **/u/data/jones/nmr**. You can change to this directory using the simple shortcut command **dat**. In this directory you will find the names of your NMR samples:

```
/u/data/jones/nmr
        jh142a16s
        dimer_dmso
        menthol
```

Under each sample heading are a number of experiments, each designated by a number. Typically, experiment number 1 is the 1D proton spectrum. You might have the carbon spectrum in 2, the COSY in 3, the HMQC in 4, etc. Unfortunately, these cannot have descriptive names like "cosy" or "13c"; they must be named with integers. For example:

```
jh142a16s       (sample name)
      1         (proton spectrum)
```

2 (carbon spectrum)
3 (DQF-COSY)
4 (HSQC)

Each of these experiment numbers is a Unix directory containing a number of files related to raw FID and acquisition. For example, the proton spectrum experiment might have:

1
- acqu (original acquisition parameters)
- acqus (acquisition parameters, modified since acquisition)
- fid (actual binary FID data)
- format.temp
- pulseprogram (a copy of the pulse program used)
- pdata (directory for processed data, i.e. spectra)

In the pdata directory is another series of numbers: 1, 2, 3, etc., corresponding to different ways you might try to process the data. Hardly anyone uses this feature, so usually there is only the directory "1". In this directory you might find:

1
- 1i (imaginary spectrum)
- 1r (real spectrum)
- proc (original processing parameters)
- procs (modified processing parameters)

The processed data for a 2D experiment is a little different. For example, in experiment 3 you would find:

/u/data/jones/jh142a16s/3
- acqu (original F_2 acquisition parameters)
- acqus (modified F_2 acquisition parameters)
- acqu2 (original F_1 acquisition parameters)
- acqu2s (modified F_1 acquisition parameters)
- grdprog.r (gradient program- for gradient experiments)
- pulseprogram (copy of the pulse program used)
- ser (the "serial file" - actual binary 2D FID's)
- pdata (directory containing processed data)
  - 1 (directory for first processing method)
    - 2ii (imaginary-imaginary 2D matrix)
    - 2ri (real-imaginary 2D matrix)
    - 2ir (imaginary-real 2D matrix)
    - 2rr (real-real 2D matrix)
    - proc (original F_2 processing parameters)
    - procs (modified F_2 processing parameters)
    - proc2 (original F_1 processing parameters)

proc2s (modifed F_1 processing parameters)

As you can see, Bruker took Varian's idea of using directories to store NMR data to an extreme - there is a menagerie of directories and files. A result of this approach is that the disk fills up very rapidly - XWIN-NMR is a voracious disk hog. For this reason we have created a "daemon" which checks for processed 2D data (2ii, 2ri, 2ir and 2rr) files and deletes them if they are more than 3 days old. This helps somewhat with the proliferation of processed data files which can, after all, be instantly regenerated from the raw data any time you need them.

III. Bruker Parameter Names.

Most of the Bruker parameter names and commands are the same ones you remember from the AM-250 or the AM-500: NS, TD, SI, RG, AQ, O1, O2, SR, FT, EF, RGA, etc. But some have been changed, and there are some new names:

Old Name	New Name	Stands For
SW	SWH	Spectral Width in Hz
---	SW	Spectral Width in ppm
---	O1P	Transmitter Offset in ppm (center of spectral window)
---	O2P	Decoupler (Channel 2) Offset in ppm
IN	IN0	Increment in t_1 (2D experiments)
---	DECIM	Decimation Factor (for oversampling)