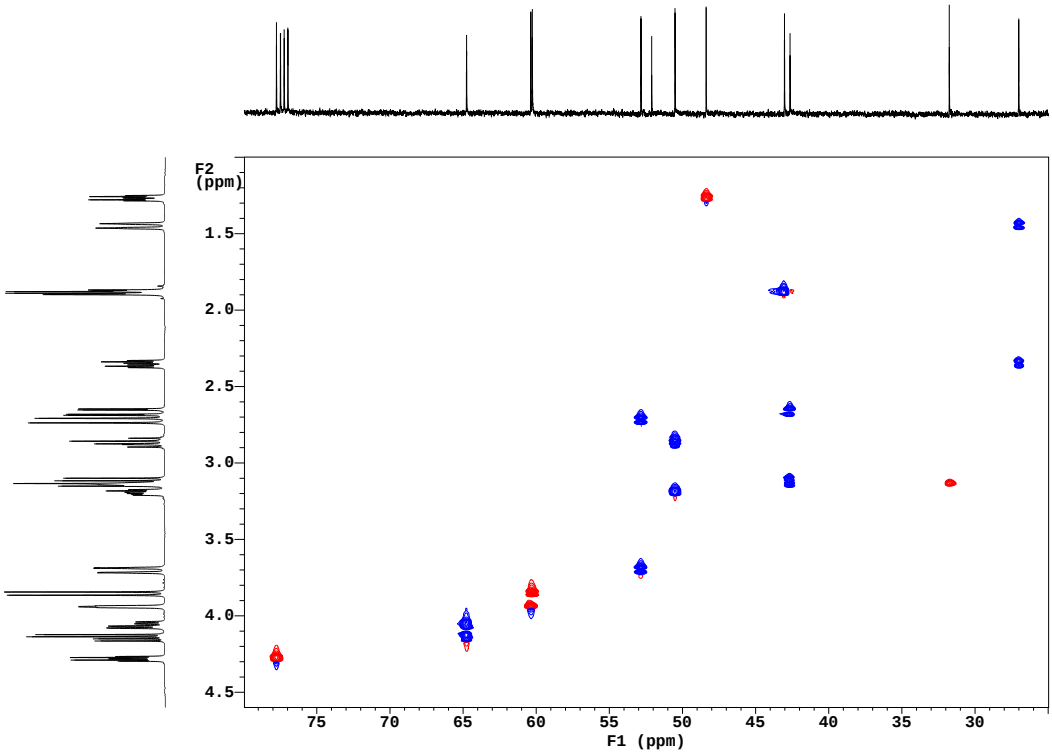
NMR Facility User Guide

Analytical Instrumentation Center

School of Pharmacy

University of Wisconsin–Madison



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Chapter 1

Introduction

Welcome to the *NMR Facility User Guide*, sometimes referred to as the NMRFUG or, simply, the FUG.[1] This document is a guide to the NMR Spectroscopy Facility in the UW–Madison School of Pharmacy's Analytical Instrumentation Center. Key goals of the NMRFUG are to provide the user community with information that is (1) important to know and understand, (2) specific to our facility, and (3) difficult to find or comprehend from other sources. The NMRFUG is also intended to supplement, but not replace, pertinent primary documentation written by the instrument and software vendors. The excellent Varian manuals are available in the lab as bound volumes. The cumbersome and poor Bruker documentation is most readily accessed electronically from within the TopSpin software itself. In addition to the Varian *VNMR* and Bruker TopSpin software, our facility also provides MNova software (Section 2.8), which has its own self-contained documentation. Note also that the user community is expected to be familiar with the resources found on the NMR Facility web pages.

The NMRFUG generally does not aim to provide "cheat sheets" or other "NMR for Dummies" documentation; those kinds of resources can be found elsewhere. It is this author's opinion that such superficial information resources tend to stand in the way of developing the higher level of understanding and critical thought that are expected and required of PhD scientists, the audience for which this document is written.

1.1 NMR Spectrometers

The facility is equipped with two NMR spectrometers: (1) a Bruker Avance III HD 400 MHz instrument (AV-400), installed in 2017, and (2) a Varian $^{\text{UNITY}}$*INOVA* 500 MHz instrument (UI-500), installed in 2001. Both spectrometers have actively shielded magnets and represent modern instrument design with experimental capabilities suitable for analysis of small molecules.

The AV-400 is equipped with a 60-sample robot for automated sample handling, with data acquisition controlled by IconNMR automation software; a BBO SmartProbe provides excellent detection sensitivity for a wide range of nuclides. This general-access instrument is suitable for many experimental needs on a first-come, first-served basis.

The UI-500 is configured for greater capabilities in certain respects, and is typically used in manual or semi-automated mode for projects that require a greater level of user interaction. It does not have an automated sample handler; instrument time must be reserved in advance, making the UI-500 well-suited for projects that require specific timing of events (e.g., reaction kinetics), user interaction (e.g.,

[1] As in "Didn't you read the FUG?!!"

relaxation, diffusion, or selective experiments) or iterative decision making, etc.

Each spectrometer's capabilities and management philosophy is described in more detail below.

1.1.1 AV-400 Spectrometer

The AV-400 provides the facility's routine NMR capabilities, accounting for about 2/3 of total instrument time utilized in the facility. The management philosophy is based on open (or "walk-up") access for essentially first-come, first-served priority. Details are documented elsewhere[2] for the user community, and are subject to periodic review and revision but, in summary, submitted experiments are assigned to either the day queue or night queue, according to experiment duration, while maintaining first-come, first-served priority.

Because of the open-access spectrometer management policy, users are not permitted to reserve instrument time for the AV-400. NMR facility staff, however, do periodically reserve spectrometer time for routine maintenance (e.g., cryogen fills, spectrometer calibrations or tests), diagnostics, user training, etc. It is therefore recommended that users check the instrument reservation schedule before going to the lab to submit samples. The AV-400 has the following general capabilities; SmartProbe performance data are shown in Table 1.1.

- NanoBay console with 2 transmitter channels
- SampleXpress 60-sample automation robot
- SmartProbe, for 5 mm diameter sample tubes, is capable of ^{1}H{^{19}F}, ^{19}F{^{1}H}, ^{1}H{X}, and X{^{1}H} experiments, with the X circuit tunable between ^{109}Ag and ^{31}P (e.g., ^{7}Li, ^{11}B, ^{13}C, ^{31}P)
- Automated tune and match (ATM) module
- z-axis pulsed field gradient (PFG) equipped (hardware and probe)
- Temperature controller maintains sample temperature at 25 °C

1.1.2 UI-500 Spectrometer

With 25 percent greater field strength, three transmitter channels, a 28-channel room-temperature shim set and two complementary probes, the UI-500 is better suited than the AV-400 for certain types of NMR experiments. For example, time-intensive studies such as acquiring a suite of experimental data for full structure elucidation are best done on the UI-500, as are reaction kinetics, translational diffusion or spin relaxation studies. Since triple-resonance experiments are not possible on the AV-400, these experiments must be performed on the UI-500. Basic spectrometer features include:

- Three transmitter channels and waveform generators enable triple-resonance experiments with shaped pulses on all channels
- z-axis pulsed field gradient (PFG) equipped (hardware and probes)
- Two complementary probes provide a range of experiment options
- Digital signal processing (DSP) is available and enabled by default
- Temperature controller maintains the default sample temperature at 25 °C
- FTS Systems VT air pre-cooler for temperatures down to about –10 °C

The capabilities and performance specifications provided by the two probes are itemized in the following subsections and in Table 1.2 and Table 1.3.

[2] Spectrometer sign-up rules and related details are documented within the on-line AIC Instrument Reservation System software.

Varian HCX Tunable Triple-Resonance Probe

* Indirect detection (ID) of ^{13}C and X is achieved via the high-sensitivity ^{1}H circuit
* Triple-resonance capability allows for ^{1}H detection with decoupling of ^{13}C and/or X
* Dedicated circuits are ^{1}H and ^{13}C; X is tunable from ^{15}N to ^{31}P
* Direct detection or decoupling of ^{2}H is possible via the lock circuit
* The probe has a working temperature range from –100 to +130 °C
* Sample tube diameter is 5 mm

Nalorac QN Probe

* Quad nucleus (QN) capability for detection of ^{1}H, ^{13}C, ^{19}F and ^{31}P without user intervention to tune the probe
* The probe has a working temperature range from –60 to +130 °C
* Sample tube diameter is 5 mm

Table 1.1 Bruker SmartProbe specifications

Parameter	Specification
^{1}H line shape, spinning	0.22/2.9/5.2 Hz
^{1}H line shape, static	0.46/3.0/6.3 Hz
^{13}C line shape, spinning	0.06/0.9/2.7 Hz
^{1}H pw90	9.8 μs at 15.8 W
^{13}C pw90	9.8 μs at 69.9 W
^{15}N pw90	20.6 μs at 64.7 W
^{19}F pw90	17.5 μs at 12.8 W
^{31}P pw90	7.9 μs at 50.5 W
^{2}H pw90	373.4 μs at 3.1 W
^{1}H sensitivity	$S/N = 535$
^{13}C sensitivity (ASTM)	$S/N = 242$
^{13}C sensitivity (EB)	$S/N = 260$
^{15}N sensitivity	$S/N = 43$
^{19}F sensitivity	$S/N = 317$
^{19}F sensitivity (with $\{^{1}$H$\}$)	$S/N = 556$
^{31}P sensitivity	$S/N = 265$
^{31}P sensitivity (with $\{^{1}$H$\}$)	$S/N = 335$

Table 1.2 Varian HCX probe specifications

Parameter	**Specification**
^{1}H line shape, spinning	0.36/2.5/4.8 Hz
^{1}H line shape, static	0.65/6.4/9.2 Hz
^{1}H pw90	7.6 μs at tpwr = 61
^{7}Li pw90	17.0 μs at tpwr = 63
^{11}B pw90	16.5 μs at tpwr = 63
^{13}C pw90 (^{13}C circuit)	22.7 μs at tpwr = 63
^{13}C pwx (^{13}C circuit)	23.2 μs at pwxlvl = 63
^{13}C pw90 (X circuit)	12.2 μs at tpwr = 63
^{13}C pwx (X circuit)	13.7 μs at pwxlvl = 61
^{15}N pw90 (X circuit)	25.0 μs at tpwr = 63
^{15}N pwx (X circuit)	26.4 μs at pwxlvl = 62
^{31}P pw90 (X circuit)	15.8 μs at tpwr = 63
^{31}P pwx (X circuit)	15.8 μs at pwxlvl = 63
^{1}H sensitivity	S/N = 800
^{13}C sensitivity[a] (ASTM)	S/N = 95 (^{13}C circuit)

[a] This non-standard specification is shown for illustration only; this probe is neither designed nor specified for direct detection of any nuclide except ^{1}H.

Table 1.3 Nalorac QN probe specifications

Parameter	**Specification**
^{1}H line shape, spinning	0.37/2.9/4.8 Hz
^{1}H line shape, spinning	0.48/5.6/9.0 Hz
^{13}C line shape, spinning	0.10/1.4/2.9 Hz
^{1}H pw90	15.8 μs at tpwr = 63
^{13}C pw90	7.8 μs at tpwr = 55
^{19}F pw90	14.7 μs at tpwr = 62
^{31}P pw90	7.9 μs at tpwr = 55
^{1}H sensitivity	S/N = 275
^{13}C sensitivity (ASTM)	S/N = 265
^{19}F sensitivity	S/N = 355
^{31}P sensitivity	S/N = 275

1.2 Supporting Computers and Software

NMR Facility needs are supported by the computers and software indicated below.

- Sun Ultra 10 computers running the Solaris 8 (SunOS Release 5.8) UNIX operating system: one hosts the UI-500 spectrometer and the other is for off-line data processing
- Varian *VNMR* 6.1C software for UI-500 spectrometer control and Varian data processing
- Hewlett-Packard Z440 AV-400 host computer running the CentOS 7 Linux operating system
- Bruker TopSpin software for AV-400 spectrometer control and Bruker data processing
- Dell Optiplex 780 PC (Microsoft Windows 7) for independent data processing, archiving, and other tasks (Refer to Table 2.1 on page 24 for a list of software available on this computer.)
- Sun NFS protocol and Samba server provide connectivity between Microsoft Windows clients and NMR Facility data disk partitions
- HP LaserJet network-accessible printer

1.3 NMR Facility Policies

This section enumerates the current policies governing NMR Facility usage. These policies are subject to periodic review and revision; suggestions from the NMR user community for revisions are welcome. If you have comments or suggestions, please submit them to the NMR Facility Director. **Facility users are required to know and understand these policies**, and are encouraged to provide feedback for the benefit of the entire NMR user community.

1.3.1 Access and Use

The NMR laboratory is **a restricted-access facility for authorized users only**. Access is gained through direct authorization on an individual basis, obtained via user training and subsequent checkout procedures designed to ensure that all users are capable of safe and appropriate use of the NMR spectrometers and ancillary equipment. Authorized users will have individual (1) key-card access to the NMR Facility and (2) active computer accounts to operate the equipment for which they are granted access. Only those with authorized access are allowed to use the Facility equipment; **sharing of computer accounts and/or key access is explicitly prohibited**.

Taking or allowing *guests* into the laboratory is not permitted without prior approval by Facility staff. Approval can be arranged, in advance, for a variety of cases; contact the NMR Facility Director for more information. University security personnel patrol the School of Pharmacy, including the AIC, and users may be asked to show their UW identification and after-hours building permits. As a restricted-access facility, the NMR laboratory doors are to be closed and locked under normal circumstances, not left open or ajar; security personnel check this also.

1.3.2 User Training

Training is provided only by NMR Facility staff. If necessary, the standard training objectives may be expanded to better meet individual needs according to previous experience and anticipated research objectives. A variety of training events are offered periodically, usually on an *ad hoc* basis. Refer to the NMR Training web page for detailed information and a link to the on-line training registration form.

1.3.3 Fees and Services

The NMR Facility is primarily a user-operated laboratory in which individuals acquire, process and analyze their own data. Monthly accounting of and billing for spectrometer usage is in effect. A flat rate of $4.00 per hour for both the AV-400 and UI-500 NMR spectrometers currently applies to internal (UW) customers who operate the instruments themselves. Direct access or NMR spectroscopy services are available to external (non-UW) customers at higher rates. Refer to the AIC Fees web page for more information. The fee structure is subject to periodic review and modification; the user community will be notified about rate changes.

1.3.4 Reserving Instrument Time

An Instrument Reservation System (IRS) is available for authorized NMR Facility users to reserve time on the UI-500 spectrometer. (The IRS provides similar capabilities for MS Facility users.) Detailed Help Notes and Sign-Up Rules are available within the IRS after logging on via your user name and password.

1.3.5 Laboratory Safety and Health Issues

Access to the NMR laboratory (room 1411) is restricted to only those individuals who have either (1) successfully completed an NMR training course by Facility staff, which includes discussing and completing a safety checklist, or (2) have otherwise received training, from Facility staff, regarding the potential dangers inherent in a magnetic resonance facility. Requests for access authorization must be made through the NMR Facility Director. These restrictions apply to all personnel: NMR users, custodians, maintenance workers, etc.

The potential dangers inherent in a magnetic resonance facility involve the presence of strong magnetic and radio-frequency fields and cryogenic fluids (liquid nitrogen and helium), plus the general hazards of handling chemicals and glassware (primarily NMR tubes). Because only Facility personnel handle cryogens in the NMR lab, related precautions are not discussed further in this document; common hazards regarding other topics are described below. Note, however, that the following examples are in no way all-inclusive; it is always the responsibility of each individual to ensure that safe practices are followed. When in doubt, consult with the NMR Facility Director before proceeding.

Refer to the Laboratory Incident Examples web page for select descriptions of real-life laboratory accidents, near misses, etc.

Preliminary Considerations

- **Authorized Access Only** The NMR Facility (room 1411) is a restricted-access laboratory. Only those directly authorized are allowed into the lab, and the doors are to be shut and locked at all times except during entry and exit.

- **Food and Drink** Neither food nor drink is allowed in the NMR laboratory. Period!

Proper Attire Loose-fitting or high-heeled shoes should not be worn in the NMR laboratory. Such footwear greatly increases the risk of losing balance or falling when using the step platforms to insert or remove sample tubes from the magnets. Open-toed shoes of any kind are prohibited in laboratories by campus policy. You are responsible: Be safe, not sorry.

Hazards Related to Super-Conducting Magnets

WARNING: Persons with implanted or attached medical devices such as pacemakers or prostheses are not allowed to enter the NMR Facility (room 1411) without authorization from a physician.

WARNING: High-field super-conducting magnets produce very strong, fringe magnetic fields that extend **in all directions** beyond the magnet canister, presenting invisible yet very real dangers related to the forceful attraction of ferromagnetic objects. These magnets are always on and cannot simply be turned off. The UI-500 magnet has its radial 5-Gauss perimeter marked out on the floor with red tape, and the AV-400 magnet's 5-Gauss perimeter falls within an imaginary circle circumscribing the magnet legs. Ferromagnetic objects must be kept outside these 5-Gauss perimeters at all times.

WARNING: Although fairly common during the initial energization of super-conducting magnets, the violent quench of a stable magnet does occasionally occur. Violent quenches can cause the liquid helium (e.g., 120 L in the UI-500's Oxford AS500 magnet when full) to boil off in a matter of seconds, venting spectacularly through safety check valves at the top of the magnet canister. If this happens, evacuate the lab immediately — after recovering from the initial scare. The very real danger associated with a violent quench lies in the risk of asphyxiation due to the displacement of oxygen in the room.[3] Normal building ventilation will flush the helium gas out of the room after some time (about 15 minutes); there is no other danger and no real need to evacuate the building (although it would be okay to do so). Inform the NMR Facility Director of the news.

CAUTION: Magnetically encoded media (e.g., ATM cards), mechanical watches and some electronic devices may be damaged or destroyed if subjected to strong magnetic fields; keep such items outside the 5-Gauss perimeters.

Fire Extinguisher A non-ferromagnetic, CO_2 fire extinguisher is located at the right-hand end of the laboratory bench in room 1411.

Chemical and Glassware Hazards

Chemical Hazards NMR Facility users are responsible for knowing the chemical hazards of their compounds, and for taking proper steps to ensure their own and others' safety at all times, e.g., in the event of sample tube breakage and subsequent spill. It is the user's responsibility to completely clean up any spill, broken glass, etc., to the extent possible.

Radio-Nuclides No. Samples containing enriched quantities of radio-nuclides are not permitted in the NMR Facility.

[3] The rapid boil-off of 120 L of liquid helium would produce approximately 90 m^3 of gaseous helium, which is roughly 1/3 the total volume (at 310 m^3) of the NMR lab.

☞ **Sample Preparation** Whenever possible, NMR samples should be prepared in advance in the user's laboratory. If sample preparation must be done in the NMR Facility (as is typically the case for kinetics studies, for example), it is to be done only on the laboratory bench in room 1411; the computer desks or spectrometer consoles are never to be used for such purposes.

☞ **Toxic or Unpleasant Substances** Such substances shall be addressed responsibly according to their nature. For example, flame sealing a sample within an NMR tube may be required to contain toxic vapors or an offensive smell.

☞ **Sample Disposal** Facility users must promptly remove their samples and related materials from the laboratory when their experiments are completed. Arrangements can be made for those with special needs to store samples/tubes in the lab to facilitate their work; however, unlabeled or unclaimed NMR sample tubes or related goods persisting in the laboratory will be discarded.

☞ **Gloves** If needed for extra protection, gloves (e.g., latex, nitrile) may be worn only while preparing or handling NMR samples. Gloves are **never** to be worn while operating computers or handling other community property.

☞ **Glassware Hazards** Routine precautions should be observed when handling glassware, especially when inserting and withdrawing NMR tubes into and from the spinner turbine. Some spinner turbines use rubber O-rings to grip the NMR tubes, and the fit can be quite snug, depending upon the condition of the O-ring and the specific NMR tube used. Grip the tube firmly near the spinner and use a twisting motion while inserting or withdrawing the tube. Carelessness has resulted in puncture wounds. ***Ouch!*** ☹

Miscellaneous Considerations

☞ **Be Careful!** Users must carefully insert and remove their NMR samples into/from the magnets, positioning themselves to maneuver the glass tube straight up or down — not at an angle — out of, or into, the upper barrel. Glass does not bend well at room temperature, and we have had far too many users snap a sample tube by catching it at an angle at the top of the upper barrel. These events are distracting and time-consuming to deal with, are potentially damaging and costly to the equipment, and **are easily and completely preventable**. If you think you're in a hurry in the NMR lab, go away and come back after you've adjusted your attitude; this is no place for reckless or irresponsible behavior!

☞ **Hands Off!** Please keep your hands off the magnet canisters. If you feel compelled to support yourself while inserting or removing samples from the magnets, then you are probably doing something else wrong.

☞ **Common Sense** It is apparently necessary to remind some users to wash their hands and wipe their feet. Come on folks, this is a research laboratory, not kindergarten! Winter in Wisconsin involves snow and ice and sand and salt; these all belong outside, not in the NMR lab, so please do not track this crap into the lab. Let's keep our laboratory space and community property — keyboards, mice, work desks, floor, etc. — clean.

☞ **Temperature Control** Variable-temperature (VT) work may be performed **only** after an individual has completed specific, on-site training by NMR Facility staff.[4] Users are responsible for

[4] VT work may be performed only on the UI-500; sample temperature on the AV-400 is maintained at 25 °C at all times.

knowing and observing the temperature limitations of both their NMR samples and the Facility instrumentation, and must work safely within these limitations. Facility personnel are available for consultation and other assistance in these matters; refer to Section 7.3 for further information.

☞ **Eye Protection** Users must provide and use their own eye protection as needed.

☞ **Consequences** Unsafe, irresponsible or otherwise inappropriate use of the NMR Facility may result in sanctions up to and including loss of access privileges.

1.3.6 AV-400 Sample-Management Policies

The sample workflow for an NMR spectrometer without an automated sample handler (e.g., the UI-500) is relatively uncomplicated: Individuals use the instrument serially, each person having one or more samples that they manually manage in sequence; consequently, the samples are essentially under control by their owner from start to finish. Because investigators are charged for instrument usage according to log-on time, individuals are usually motivated to log off from the instrument and remove their samples as soon as the experiments are completed.

Sample workflow and sample management are quite different for systems, such as the AV-400, with automated sample handlers. Here, the very core of the idea is to free the individual from the routine task of manually managing samples one after the other. This is great! (At least for many types of routine systems and experiments that lend themselves to this treatment.) It allows multiple users to each submit multiple samples in an *ad hoc* manner, with subsequent sample handling and other spectrometer functions executed under control of the automation software. Today's automation software is typically capable of sending users their spectral data via email. Wow! What is there to not love about this?

Automated sample handlers are available in a range of models accommodating from a few to hundreds of NMR samples; pick the model that best suits your goals and budget. An institution's particular needs and conditions determine its sample-management policy. The workflow in a Big Pharma research lab is obviously much different than that in an undergraduate teaching lab. In our NMR Facility, data show that a few tens of NMR samples are run during a typical 24-hour period.[5]

Considering that our SampleXpress automation robot has a capacity of 60 samples, and our currently typical throughput is approximately half of that (give or take), completed samples need to be removed from the sample handler on a daily basis to make room for incoming samples. It is the responsibility of individual users to remove their NMR samples from the SampleXpress unit in a timely manner. If necessary, individual users may remove others' completed samples to make holders available for incoming samples. Finally, in addition to all the other well-known reasons for properly labeling and documenting things in scientific research, this discussion should make clear the importance of legible and meaningful labeling of NMR sample tubes. Specific sample-management policy rules follow:

- If left in the NMR lab, all flasks or other containers for transporting NMR samples must be clearly and legibly labeled with the user's full name (not initials, etc.) so that ownership can be determined. Any such item that is not clearly identified will be discarded.

- The step unit has an integrated work table to facilitate adding and removing multiple samples from the SampleXpress sample changer. Spinner turbines, a depth gauge and tissues are kept on the table, which also provides a temporary place for a flask or other NMR sample-transport

[5] Roughly speaking, 30 ± 10 samples per day is a fairly typical throughput, although examples of more or fewer samples per day exist. A daily throughput of more than 60 samples is virtually unprecedented in our laboratory at the time this was written.

container during the work-flow process. When done adding or removing NMR samples at the sample changer, the container is to be removed from the work table — no exceptions.

- Users may, if desired, temporarily leave their clearly labeled container on the NMR lab bench if they have one or more samples in the sample changer. There is a designated area for this purpose.
- Each NMR facility user is responsible for removing his or her samples and related equipment from the lab in a reasonably timely manner. The term "reasonably timely" here means, for example, the next day for weekday sample submissions, and Monday for weekend sample submissions. This is not currently an absolute rule; instead, the goal is to foster responsible behavior.
- It is occasionally necessary for Facility staff to remove all the NMR samples from the SampleXpress cassette for system maintenance or repair. Users must therefore be able to identify their own samples from a collection of several.

1.3.7 Incident Report Form

This chapter ends with important comments and information about how and when to report problems — either real or perceived — related to the NMR Facility. Problems, issues and conditions appear in all manner of shape, size and significance; the term *incident* is used here in reference to such phenomena. It is the responsibility of the user community to promptly and properly report incidents they experience — or *cause*, as the case may be. During normal working days and hours, please contact directly either the NMR Facility Director or the Project Assistant (PA). For non-emergency incidents outside normal working hours (or if the Director or PA are otherwise unavailable), make a formal report via the on-line Incident Report Form; this method ensures the most timely and meaningful reporting and response, no matter what time or day the incident occurs. Emergencies should, of course, be reported immediately via the proper channels, depending on the details; emergency contact information sheets are posted outside the laboratory doors and at the telephone within the NMR lab itself.

Many years of experience dealing with these kinds of issues prompts the following comments:

- If you experience an incident, report it. Some kinds of problems are real and universal, while others are imagined or isolated; therefore, unless a particular incident is reported, it may be unknown and remain unknown to facility staff.
- Do not assume that a particular incident you experience has already been reported by someone else; this is a corollary to the preceding point.
- Even if you know that a particular incident has been reported, report it again. Perhaps the problem is thought to have been fixed but has actually recurred, which is important to know. Intermittent problems can be exceedingly difficult to diagnose and repair, and it is important to know how frequently they occur; relatively minor issues that occur infrequently are assigned lower priority than if they occur frequently.
- Be responsible! In some circumstances, it may be necessary for you to personally take immediate action to prevent equipment damage or ensure the safety of others. For example, imagine what could happen if someone broke an NMR sample tube in the magnet, then simply walked off without taking measures to prevent another person from subsequently inserting a sample into the broken glass on top of the probe. (Yes, this really happened! What would **you** do?)

Chapter 2

Computers and Software

This chapter contains useful information about NMR Facility computer- and software-related issues. Bruker and Varian each has their own software for spectrometer control, post-acquisition data processing, etc. Third-party vendors such as Acorn NMR Inc. (NUTS), Advanced Chemistry Development Inc. (ACD/Labs), Mestrelab Research (Mnova; refer to Section 2.8), and others, produce commercial software for post-acquisition processing and analysis of NMR data.[1] Many of these programs also include modules to simulate or predict NMR spectra as a function of user input; some have the capability to perform structure elucidations from analysis of several experimental data sets.

The NMR Facility maintains ancillary software packages and tools to provide (1) network access to NMR data (Samba server), (2) terminal emulation for remote log-on and virtual desktop (X-Win32), and (3) data archiving. These are discussed in detail below, following specific information for Bruker and Varian spectrometer users.

2.1 Information for Bruker Users

The Bruker Avance III HD 400 MHz NMR spectrometer, as a complete system, is referred to as the AV-400. A Hewlett–Packard Z440 workstation functions as the AV-400 spectrometer host computer, and is referred to as the *av400* computer. The *av400* runs under the CentOS 7 Linux operating system, and the Bruker TopSpin (version 3.5pl6) and IconNMR (version 5.0.6) software control the spectrometer. The entire spectrometer system is supported by an APC Smart-UPS (model SRT5KXLT) uninterruptible power supply, to provide battery back-up in case of an electrical power outage.

Only NMR Facility staff have log-on accounts to the *av400* computer; one of these log-on accounts, the *nmr* account, initiates first the TopSpin program, then IconNMR, and is thus the owner of all NMR data sets acquired under IconNMR. The general user community have special, restricted accounts known as "Additional User" accounts that allow them to individually log on *only* to the IconNMR user interface, through which they can submit samples for a wide variety of experimental measurements.

The TopSpin software is available for post-acquisition data processing under Linux, Macintosh or Microsoft Windows operating systems. Bruker offers licensed versions free of charge for academic use; a copy is installed and available for use on the Dell *nmr05* PC in the NMR lab.[2] Similarly licensed copies

[1] Several NMR data-processing programs are also available on a free or trial basis, in some cases from commercial vendors providing special or introductory products.

[2] Refer to Section 2.7 for further information about the *nmr05* computer and software.

of TopSpin can be installed on the computers of individuals or research groups in our user community; if interested, contact the NMR Facility Director for more information.[3]

2.1.1 IconNMR Web View

The IconNMR Web View feature allows IconNMR users to view the live IconNMR queue details remotely via a web browser. As configured in our laboratory, users cannot perform any spectrometer control operations, although they can use this interface to download and view PDF versions of their own spectral data.

A link to the IconNMR Web View log-on screen can be found on the AV-400 Reservation Schedule page of the Instrument Reservation System.[4] Users may also make direct connections from web browsers on computers in the School of Pharmacy using the URL https://128.104.114.54:8016. For security reasons, this works only for wired network connections from within Rennebohm Hall.

Note that when first attempting to connect to IconNMR Web View, you may receive a warning about an insecure connection and/or an invalid security certificate. This is because the encryption certificate and key do not come from a known certification authority.[5] Simply add an exception for your web browser (the details will depend on the browser) and proceed to the IconNMR Web View log-on screen.

2.2 Information for Varian Users

The Varian $^{\text{UNITY}}$*INOVA* 500 MHz NMR spectrometer system is referred to as the UI-500. A Sun Ultra 10 workstation serves as the UI-500 spectrometer host computer, and is referred to as the *ui500* computer. The *ui500* runs under the Solaris 8 UNIX operating system, and the Varian *VNMR* (version 6.1C) software controls the spectrometer. The *ui500* computer is supported by an APC Smart-UPS (model 700) uninterruptible power supply, to provide battery back-up in case of an electrical power outage. The spectrometer console is *not* protected by an uninterruptible power supply, and consequently will shut down if a power failure occurs; the console will not automatically restart after the power is restored.

Supporting the UI-500 is another Sun Ultra 10 workstation, *nmr03*, which is used primarily for post-acquisition data processing using the *VNMR* 6.1C software. UI-500 users can make remote connections from their lab computers to *nmr03* via the X-Win32 software, as described in detail in Section 2.5 below. All UI-500 users have log-on accounts on both the *ui500* and *nmr03* workstations, and their account environments are configured for them at the time their computer accounts are created. The menu sequence Main Menu → Custom Macros → NewUserSetup can be executed at any future time to ensure use of the most recent configuration and parameter settings.

2.2.1 *VNMR* 6.1C Users Take Note!

- Start the *VNMR* software by **single-clicking** the icon on the CDE toolbar.
- Be sure to exit the *VNMR* software before logging off from the Sun workstations; do so either by entering **`exit`** at the *VNMR* command line, or by using the Main Menu → More → Exit VNMR menu sequence.

[3] There appears to be little demand for this software from our user community, and TopSpin versions, installation and licensing details change continually.

[4] A reason for putting the link in this particular location is because it's important for users to check to see if the instrument is reserved (for periodic maintenance, user training, etc.) before making a trip to the lab. Refer to Subsection 1.1.1.

[5] I (Thomas C. Stringfellow) created the encryption certificate and key.

- UNIX file names can be up to 256 characters in length and may contain upper- and lower-case letters, numerals, and several special characters; however, due to compatibility issues with Microsoft Windows, use ***only these*** special characters: **.** (**period**), **_** (**underscore**), **-** (**hyphen**). Do not use spaces or any other characters in file names; spaces will prevent the creation of the desired file or directory. There is no distinction between file names and directory names. (Refer to Section 2.6 and Subsection 5.1.1 for additional information about file and directory names.)

- **Never attempt to reboot or restart the Sun Ultra 10 computers!** Only the UNIX system administrator is capable of performing a proper system reboot; any other user who might attempt to do so runs the risk of irreparably damaging the file system — and suffering the consequences of his or her action.

A good rule of thumb is "When in doubt, ask the NMR Facility Director for assistance **before** something unfortunate happens."

2.2.2 Sun Desktop Environments: CDE versus OWD

Sun Microsystems offered two desktop environments with the Solaris 8 version of their UNIX operating system: the OpenWindows Desktop (OWD) and the Common Desktop Environment (CDE). The OWD is an older environment[6] and the CDE is newer and more fully featured. Users' Sun computer accounts in our NMR Facility are initially configured to use the CDE, and this selection **should** persist over subsequent log-on sessions. For unknown reasons, the expected persistence across log-on sessions has been observed to fail for some users. Facility instructions and documentation, where relevant, are written from the perspective of the CDE not the OWD;[7] it is therefore useful to be able to recognize and recover if you find yourself trapped in the OWD.

How does one recognize or identify which desktop environment is in use? After logging on, the most prominent feature of the CDE is the presence of a toolbar and icon box across the bottom of the display; the default desktop screen[8] selection (One of Four) is blue and wallpapered with the Solaris logo. By contrast, the OWD environment has no toolbar or icon box, and the default wallpaper is an ugly green color tiled with the Varian logo. The next section describes how to determine and select, while logging on, which desktop environment you are about to receive.

Exiting from the OWD

If you find yourself inexplicably trapped within the OWD, simply use the mouse to right-click on the desktop wallpaper; you will be presented with a typical pull-down menu, from which you can select the desired Exit... option.

Explicitly Selecting the CDE

For some undetermined reason(s), users occasionally find themselves faced with the OWD whether they want it or not. The following instructions describe how to select the desired desktop environment.

1. Enter your user name at the Sun log-on screen where it says Please enter your user name. You should see the Welcome 'username' message, and directly below it should be displayed either:

[6] The OpenWindows Desktop is not supported in Solaris 9 or later.
[7] With the obvious exception of this particular section of the *NMR Facility User Guide*.
[8] This is technically referred to as a virtual desktop.

(a) Common Desktop Environment (CDE), or

(b) OpenWindows Desktop

2. If you see message 1a, then continue by entering your password.

3. If you see message 1b, use the mouse to specify the CDE via the Options ▽ → Session ▷ → Common Desktop Environment (CDE) path; continue by entering your password.

2.3 NMR Facility Computer Network

The local area network (LAN) for the NMR Facility provides users with convenient and secure access to their data from computers within the School of Pharmacy. Due to the heterogeneous nature of the facility — a mix of Bruker and Varian spectrometers with newer Linux and older UNIX operating systems, plus Microsoft Windows and Apple Macintosh client computers thrown in for good measure — it makes logical sense to discuss separately the two areas where the user community needs to have a working knowledge of the LAN: (1) All users need to know how to access and back up their NMR data, over the network, from client computers elsewhere in the building; this topic is discussed in Section 2.4. (2) Varian users should find it useful to remotely process their NMR data using the native *VNMR* 6.1C software; this is the subject of Section 2.5.

2.4 Samba Server Connections to NMR Facility Disk Shares

Samba is open-source software that provides file and print services for Microsoft Windows and other clients that use the SMB/CIFS protocol.[9] The NMR Facility Samba server makes the Linux (Bruker) and UNIX (Varian) directories `/av400/data`, `/ui500/export/home`, `/nmr03/export/home`, and `/nmr03/data` accessible to School of Pharmacy Microsoft Windows and Macintosh OS X computers over the network. Such shared directories are referred to as *shares* in Samba parlance; the share names `av400`, `ui500`, `nmr03`, and `data`, respectively, refer to the partitions named in the preceding sentence. This connectivity via Samba is illustrated in Figure 2.1.

These Samba connections enable users to copy their Bruker or Varian NMR data from the host computers' directories directly to their personal computers in the usual fashion; however, data transfer in the other direction (i.e., writing to the Linux or UNIX disks) is prohibited. The ability to view the shares by simply browsing the network is disabled; however, the shares can be accessed by explicit mapping to appear on the client computer as network drives. Connection instructions are provided below for both Microsoft Windows and Macintosh OS X clients.

Be aware of these security related issues before attempting connection:

- Authentication credentials are required for access; users are provided with these credentials as part of the basic access training process.

- IP address restrictions allow connections to be made **only** from the School of Pharmacy's wired network, and **not** the wireless network.

[9] Refer to https://www.samba.org for details, if interested.

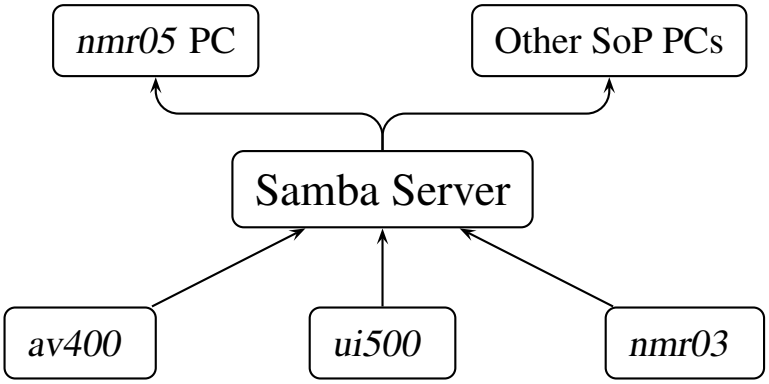

Figure 2.1 NMR Facility Samba shares connectivity illustration. This arrangement allows users to access their NMR data from other computers (Microsoft, Apple, etc.) in the School of Pharmacy, for post-acquisition data backup and processing. Refer to the text for details.

2.4.1 Connection to Samba Shares from Microsoft Windows Clients

The precise, step-by-step details depend on the Microsoft Windows version in use. The procedure shown below is for Windows 7 Enterprise; the procedure for other versions should follow similarly, although some details may differ.

1. From the Windows Explorer graphical file manager (not *Internet* Explorer) select Tools from the tool bar, then Map Network Drive....
2. Select an unused drive letter in the Drive: field.
3. In the Folder: field, enter the IP address and desired share name in the UNC format `\\128.104.115.234\sharename`. For example, to connect to the `av400` share (specifically, the `/av400/data` disk), enter `\\128.104.115.234\av400` exactly as shown.
4. Check the Reconnect at logon box if you want a connection to be made automatically each time you log on to the PC.
5. Note: The authentication credentials for the Windows computer's active log-on account will almost certainly not be the same as those required for Samba access. In this case, select the Connect using different credentials option.
6. Click on Finish to proceed.
7. If you are not using different credentials (and if there are no other issues), the requested connection should be authenticated and the new drive mapping should appear in Windows Explorer.
8. If you are connecting using different credentials:
   (a) Select Use another account and enter the Samba authentication credentials provided during your Basic Access Training session.

(b) You may select the Remember my credentials option to have this Samba share mapped automatically in the future.

(c) Click the OK button.

(d) The requested connection should be authenticated (if there are no other issues) and the new drive mapping should appear in Windows Explorer.

Confounding situations exist that can prevent the preceding instructions from being successful. If you find that these instructions do not work, please contact NMR Facility staff for assistance.

2.4.2 Connection to Samba Shares from Mac OS X Clients

1. From the Finder graphical file manager, select Go from the tool bar, then Connect to Server... (or use the ⌘K shortcut).

2. Enter `smb://128.104.115.234/sharename` into the Server Address: field. For example, to connect to the `av400` share (specifically, the `/av400/data` disk on the av400), enter `smb://128.104.115.234/av400` exactly as shown.

3. Click on Connect then wait for the authentication page.

4. Select Registered User, then enter the Samba authentication credentials provided to you during your Basic Access Training session.

5. Optional: Check the Remember this password in my keychain box to avoid manually authenticating future connections to the same share. Performing this optional step offers a level of convenience; however, it may also present a security risk if the client computer is shared with others.

6. Click on Connect to complete the connection; the contents of the requested share will be presented in a new Finder window.

2.5 X-Win32 Connectivity to Sun Computers

Terms such as *X-server* and *X-terminal* describe a server/client relationship in which a local terminal, such as a PC, runs *server* software that sends requests to a remote computer, typically a UNIX- or Linux-based workstation, to run *client* applications on the remote computer but with user input and output occurring at the local computer.[10] We use X-server software to connect laboratory PCs to the *nmr03* Sun computer for post-acquisition processing and analysis of Varian NMR data. Although several X-server software products are available, the NMR Facility maintains a site license for StarNet X-Win32. This connectivity is illustrated in Figure 2.2.

Because user authentication is performed at the remote (Linux or UNIX) computer, it is necessary to have a valid log-on account on the remote host to successfully log on. For security reasons, X-server sessions are allowed **only** from PCs that are assigned an IP address associated with the School of Pharmacy's wired network domain;[11] wireless network connections are **not** permitted.

[10] Unfortunately, this use of the terms *server* and *client* is backward compared with typical cases where the server is a remote machine and the client is the local machine, as in the discussion about the Samba server is Section 2.4.

[11] IP address assignment is accomplished automatically via DHCP server and is thus generally transparent to the user.

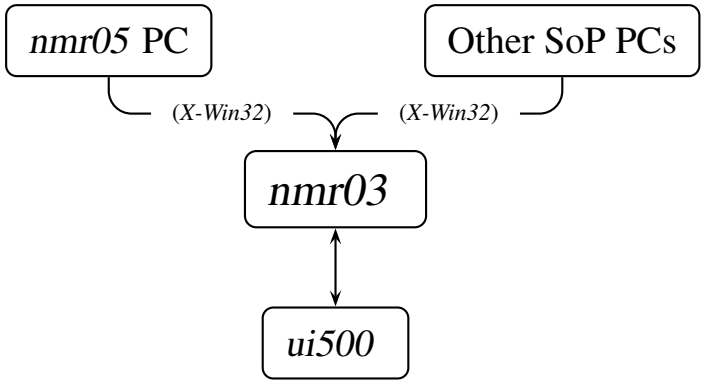

Figure 2.2 NMR Facility X-Win32 connectivity illustration. The X-Win32 server software runs on a PC to provide remote log-on sessions to the *nmr03* computer for post-acquisition processing of Varian NMR data via the *VNMR* 6.1C software. Refer to the text for details.

The following example illustrates the procedure and some important details. Suppose that user *vnmr1* wants to process and analyze NMR data acquired on the UI-500, and he wants to do this from the comfort of his office, where he can enjoy a cup of good coffee ☕.[12] To do this, *vnmr1* first logs on to his office PC using his authentication credentials for that computer; these credentials are completely independent from those for the NMR lab Sun computers. *vnmr1* then starts the X-Win32 software and initiates a connection to the *nmr03* Sun computer, which serves up its normal log-on screen. At this point, *vnmr1* logs on to *nmr03* using his authentication credentials for that computer, and the remote desktop environment is virtually identical to what would be experienced if logging on directly to *nmr03* at its own keyboard. The *nmr03* computer now behaves exactly like a local device, and the *ui500* computer like a remote device. Within the Sun computer environment, the local home directory is therefore `/export/home/vnmr1`, and the remote home directory is `/ui500/export/home/vnmr1` from this perspective. To access NMR data files in his default *VNMR* data directory on the *ui500*, *vnmr1* must look in the remote directory `/ui500/export/home/vnmr1/vnmrsys/data`. Data processing may now commence on *nmr03*, with that computer executing the work and sending the graphical output back to the display on the PC.

Lastly, note that the disk partition named `/data` on *nmr03* is an entire 20 GB hard disk available as a convenience for users to *temporarily* store copies of their NMR data; however, this disk should not be regarded as a substitute for proper data archiving (cf. Section 2.6 below).

2.5.1 Installing X-Win32

The X-Win32 X-server application must be installed on the local PC from which the user wants to connect to the remote host. After obtaining the executable installation file and license key from the NMR

[12] As we know, neither food nor drink is allowed in the NMR laboratory. Also, there is no reason or desire to incur fees for directly using the UI-500 host computer unnecessarily.

Facility Director, perform the following steps to install X-Win32 on your computer.[13]

1. Uninstall any previous versions of X-Win32 from your computer, and delete corresponding directories or shortcuts that remain after the uninstallation process. (This step may not be strictly necessary but is good housekeeping practice and may help to avoid confusion later if errors arise.)
2. Download or copy the installation file, *x-win180-7sf.exe*, and license key to the computer desktop or a temporary directory. (Alternatively, the installation may be run directly from a removable medium, such as a flash drive.)
3. Run the executable file (e.g., by double-clicking) to initiate the installation program; note that administrator privileges are required to install software. Read and click through the panels as necessary, and accept the license agreement when it appears.
4. Under Destination Folder, accept the default installation directory, i.e., `C:\Program Files\StarNet\X-Win32 18`. (Post-installation errors have been observed in the past when specifying a directory different than the default. You've been warned!)
5. Select Install and installation proceeds.
6. Select Finish to complete the software installation.

2.5.2 Configuring X-Win32

Proper configuration is crucial for trouble-free implementation of X-Win32; the necessary steps are indicated below. Most observed failures of proper X-Win32 operation can be traced to either configuration problems or issues related to networking.

1. Select Start → All Programs → X-Win32 18 to start both the X-Win32 server and the configuration utility.
2. From the Connections tab in the X-Win32 Configuration interface:
   (a) Click the Manual tab under New Connection (on the right-hand side).
   (b) Select XDMCP, then Next.
   (c) Under the General tab:
      i. Enter a meaningful Connection Name (e.g., *nmr03*) to identify the session for later use.
      ii. Set the XDMCP Mode field to Query.
      iii. In the Host field, enter the IP address for *nmr03*: **`128.104.115.93`**.
   (d) Under the Advanced tab:
      i. Select Never for the Start New Instance field.
      ii. Tick the Hide on Start check box located below the Monitor field.
   (e) Click on Save.
   (f) Under Other at the bottom of the Connections screen, select Exit when all connections have closed.

[13] These instructions specifically describe installation of X-Win32 18, build 7; details for subsequent versions may differ, although the basic steps should be similar.

(g) Click the Apply button at the bottom.

3. From the Window tab:

   (a) In the lower area, select the Disable Xinerama Extension and Disable Composite Extension options. (The former is critical for use with Sun's Common Desktop Environment, and the latter to properly display colors in the *VNMR* spectral display window.

   (b) Deselect the Use Direct2D and Display Splash Screen on Startup options.

   (c) Click the Apply button at the bottom again to save the latest changes.

4. The default settings and values for the configuration parameters under the other headings (Network, Input, Font and Security) should be appropriate.

2.5.3 Configuring the PC Monitor Resolution

It is important to set the PC monitor resolution suitably for use with X applications such as *VNMR*. If set incorrectly, the results may range from poor viewing to complete failure of the application. For example, if the PC screen resolution is set too low (say at 800 × 600 pixels, as might be the case for a laptop computer) to display a particular *VNMR* window (perhaps set at 960 × 660 pixels), then that window cannot be displayed on the PC monitor and will consequently close; this behavior may be misinterpreted as a software problem when it is actually a screen resolution issue.

1. Right click on the PC desktop, then select Screen Resolution (or its equivalent) to access the display properties interface.

2. Set the active monitor resolution as desired; the available values depend on the display, graphics hardware and drivers.

3. Click on Apply, then select OK to test the new settings.

4. Select either Keep changes or Revert (or their equivalents), as appropriate from the system test results, to either retain the new settings or revert to the original screen resolution. See the table below for empirical guidelines to setting the screen resolution; the comments are based upon trials using a 17-inch monitor except as noted otherwise.

Resolution	**Comment**
1024 × 768	Resolution is too poor for use with *VNMR*.
1152 × 864	Resolution is a good compromise.
1280 × 800	Resolution is good for a laptop with a 15-inch, wide-screen display.
1280 × 1024	Resolution is good for use with *VNMR* but may be poor for text on the PC; this is a good overall choice for use with a 19-inch display.

5. Close the Screen Resolution utility once the setting is finalized.

2.5.4 X-Win32 License Configuration

Upon starting X-Win32 for the first time after installation, the License Wizard GUI opens to guide the process of licensing the software; this GUI may be hidden behind the main, inactive, X-Win32 window. You may also need to grant Microsoft Windows Firewall exceptions at this time, to allow X-Win32 communications over the network. If the Windows Firewall is turned on — as it should be! — a Windows Security Alert GUI should appear and ask if you want to continue blocking the X-Win32 PC X Server program; select Unblock to proceed. Now, back to the X-Win32 license:

1. Select the License your copy using an activation key radio button, then click Next to continue.
2. Enter the *node-locked* license activation key string, contained in the *ActivationKey.txt* file, into the Activation Key field and click Next. (You may, of course, simply copy and paste the license activation key string instead of typing it in manually.)
3. You should see the message: "License activation successful." Click Finish to complete.

Our site license has historically been renewed annually, with each term beginning December 1st and ending November 30th of the following year, and a new license activation key issued at the start of each annual term. To update an expired license, perform the following steps after starting X-Win32:

1. Right-mouse click on the top of the X-Win32 application border.
2. Select Help → Activate License.
3. Follows the instruction above for installing a new license.

2.5.5 Using X-Win32

Once it has been installed, configured and licensed, X-Win32 is simple to use.

1. Select Start → All Programs → X-Win32 18 to start X-Win32; the X-Win32 configuration utility starts simultaneously. A connection is made to the targeted remote computer (*nmr03*), and the familiar log-on display will appear on the PC's monitor.
2. Log in and use the remote computer as usual. Depending upon your PC's screen resolution, the desktop environment on the X server may appear slightly different than during a local session on the *ui500*; this is particularly true when multiple X-application windows are open simultaneously, as when running *VNMR*. You may need to experiment with screen resolution to find the setting that works best for your PC and your sense of aesthetics.
3. You can switch between X-Win32 and other applications running locally on the PC. Copying and pasting can be done from X-Win32 to local applications; see Subsection 5.8.5 for details.
4. When finished with the X-Win32 session, first close the remote applications (i.e., *VNMR* and the UNIX log-on session) as you would normally do at the Sun computer; you may then exit X-Win32 if desired. **DO NOT** terminate an active *VNMR* session by clicking the ☒ icon in the upper-right-hand corner of the X-Win32 application, as this will effectively cause *VNMR* to crash and you will be locked out of *VNMR* as a consequence.[14]

[14] Refer to Subsection 5.11.1 when this happens.

For further ease of use, you can put an X-Win32 application shortcut on the desktop or quick-start toolbar, and configure X-Win32 to automatically launch the desired connection when started.

1. Use the Start → All Programs → X-Win32 18 path to highlight the X-Win32 18 program selection.
2. Select X-Win32 18 with the left mouse button, then drag the icon to a clear area of your desktop or to the quick-start area of the toolbar; release the mouse button at the desired location.
3. Under the X-Win32 18 Configuration → Connections tab, select the desired connection with the left mouse button, then drag it into the Autostart folder.

2.6 Data Archiving

Data archiving is a straightforward task today. Because computer disk and other hardware failures can occur without warning, perhaps causing a loss of valuable data, routine archiving of important data is essential to minimize the loss from such failures, and should be adopted as a routine practice. Remember: **NMR Facility users are responsible for regularly archiving their own data**.

Data archiving "best practices" have evolved over the years. As data volume continues to increase, it becomes less practical to store data on removable media such as CDs or DVDs. (When was the last time you even saw a floppy disk?) Flash drives are convenient for many tasks but are ill-suited for archival usage. Network-accessible storage (NAS) devices, which are clusters of hard drives redundantly configured for robust operation (cf. RAID), provide convenient and affordable solutions for large-volume data storage. Commercially available cloud storage options are also common these days.

For the majority of NMR Facility users, the most convenient method for backing up their NMR data will be to simply copy their sample directories over the network, via Samba connections, to their group's NAS device or other computer drive(s). For archival purposes, however, the data storage will need to be systematically organized and managed for long-term integrity.[15]

The UNIX *tar* command[16] has served a central role in data back-up and archiving for many years. The name originates from the term ***t**ape **ar**chive*, because magnetic tapes provided large and portable storage capacity long before optical devices or large storage arrays were available. Because *tar files* (also known as 'tarfiles') remain a popular and common way to package many data types, a practical discussion of its use is provided in the following section. Applications are available for working with tar files under non-UNIX/Linux operating systems such as Microsoft Windows.

2.6.1 Using the UNIX `tar` Command

The `tar` command, with user-specified options and arguments, works with files and directory structures in general (e.g., to distribute and install computer programs, or archive NMR data directories); it is not limited to use with a physical tape medium. Some of the more common ways to use `tar` are illustrated below, primarily in the context of preparing an archive for subsequent writing to a CD, NAS, or other medium. Consult your favorite UNIX or Linux reference text or manual pages (e.g., enter **`man tar`** from a UNIX terminal window) for more information.[17] Note that the term *tar file* refers to such an

[15] How many years, do you think, is a sufficient time to retain your NMR data?

[16] The `tar` command is a standard component of Linux distributions, too.

[17] Be sure, however, to consult a source that treats the version of `tar` you intend to use; this is best done via the UNIX/Linux manual pages on the computer where you will execute the `tar` command.

archive,[18] which by convention is named with a *tar* extension (e.g., `MyArchive.tar`).

The archiving procedure can be divided into two separate steps: (1) A preparation step executed on the Sun computer involves first packaging the desired directories and files via the `tar` command, followed by an optional data-compression step to reduce the total size in order to occupy less space on the storage medium. (2) A writing step occurs when the data package (tar file) is written to a suitable, long-term storage medium. Optical disks and redundant hard disks are suitable media; portable flash drives are a poor choice for archival storage. It is generally recommended to make at least two archival copies and store them in physically different locations.

The following sections provide reference information and examples, first for the `tar` command, then the `compress` command. A discussion of data archiving using the software and CD/DVD drive installed on the NMR Facility *nmr05* PC follows; details may vary for other burning software, but these operations are common and most users are familiar with writing to optical disks. Regardless of the details, always verify that your method creates a robust and accessible data archive on the target medium.

- **Syntax** `tar` [*`options`*] [*`filespecs`*]

  Copy data to or restore data from a tarfile according to specified *`options`* and *`filespecs`*. If directories are involved, `tar` operates recursively (i.e., it acts on the entire directory sub-tree).

- **Function Options** Choose exactly one option to perform the desired type of operation.

`c`	Create a new archive.
`r`	Replace (append) specific files in an existing archive.
`t`	Display a table of contents of the archive.
`u`	Update the archive with specific files if they are not already in the archive (add) or if they have been modified since originally archived (replace).
`x`	Extract specific files from the archive, or extract all files if particular files are not specified.

- **Function Modifiers** Choose according to context and intentions.

`f arch`	Specifies the location and name of the target tarfile, where `arch` is equivalent to `</path/>tarfile`.
`v`	Verbose display option, useful for monitoring progress or viewing details of tarfile contents.
`w`	Wait for confirmation (i.e., `y` or `n`) from the user.

- **Examples**

  The command

  ```
  tar cvf </path/>tarfile file1 file2 ... filen
  ```

  creates an archive of the specified files (*file1, file2, . . . , filen* — which can be a combination of files and/or directories), where `/path/` is optional and specifies a relative or absolute route to the tarfile; omitting a path causes the tarfile to be written to the current working directory. Another

[18] Readers coming from the PC universe are likely to be familiar with the concept of "zipped" files (e.g., `filename.zip`), which are essentially tar-like files on a different platform.

example is

```
tar cvf </path/>tarfile .
```

which similarly creates an archive of the current directory (as specified by the lone period) and all subdirectories. Use the command

```
tar tvf </path/>tarfile
```

to list the tarfile contents in the UNIX `ls -l` format. It is sometimes convenient to use the variation

```
tar tvf </path/>tarfile -C directory_name
```

to restrict the listing to a particular directory within the tarfile. To include updated or additional files or directories in an existing archive, use

```
tar rvf </path/>tarfile file1 file2 ... filen
```

or

```
tar uvf </path/>tarfile file1 file2 ... filen
```

depending on the context and intended effect. To extract the contents of an archive use

```
tar xvf </path/>tarfile </path/>dest_dir
```

where `dest_dir` represents the desired *destination directory*; otherwise

```
tar xvf </path/>tarfile .
```

extracts the archive contents to the current directory (as specified by the lone period).

2.6.2 File Compression

Individual files or entire directory structures can be compressed. The degree of reduction depends upon the file type and the compression algorithm used. Text files typically can be more effectively compressed (at 50–60 percent compression) than can binary files (at about 20–30 percent). The UNIX command

```
compress -v </path/>filename.ext
```

compresses `filename.ext` and writes a new file `filename.ext.Z`; the `-v` option tells the compress utility to report the percent compression obtained. If the file cannot be compressed, a message is produced and no new file is written. Compressing a tar file can a useful option for fitting more data onto a CD. To uncompress and restore the original file, use the UNIX command

```
uncompress </path/>filename.ext.Z
```

where the `.Z` extension is expected and can be omitted.

2.6.3 Writing to a CD/DVD Drive

The Dell PC *nmr05* (Section 2.7) has a re-writable CD/DVD drive and Nero software suite for archiving NMR data. Three important points deserve emphasis:

- For portability, data should be written to CD/DVD as a CD/DVD-R device and not CD/DVD-RW (specifically, do not make the disk rewritable); otherwise, the drive on the target computer may be

unable to read the disk.

- Also for portability, archiving should be performed as a single session and that session should be closed to complete the process.

- The fact that file-naming conventions vary across different platforms and operating systems can lead to confusion and difficulty in accessing the data if proper preparatory steps are not taken.

The first two points are straightforward; the third, with respect to this discussion, requires further consideration. UNIX file and directory names (which can be up to 256 characters per name), including valid special characters,[19] must be preserved throughout the CD/DVD-writing process that occurs on another platform, e.g., under Microsoft Windows. This is readily accomplished by first using the UNIX `tar` command to archive the data under a single filename that conforms to both UNIX and Microsoft standards; the archival data package thus appears as a single file to the CD/DVD writer, with the UNIX-specific filenames buried inside.

The basic approach is to first create the desired tar file from *nmr03*, then access it via the appropriate Samba share (Section 2.4) and burn it to CD/DVD on *nmr05* or whichever computer you are using to access the tar file. Detailed instructions for burning data to CD/DVD are omitted here, since burning to optical disks is routine these days. Some very serious advice bears repeating: **Always verify that your archived data can be read back to the intended device from the source medium** (e.g., the CD) **before deleting the original data from the computer disk!** The responsibility rests entirely on the users to verify that their archived data can be completely recovered, accessed and used as intended.

2.7 NMR Facility PC

The Dell Optiplex 780 PC, *nmr05*, in room 1411 is available to NMR Facility users. Table 2.1 below lists the software currently installed on *nmr05*. Contact the Facility Director to suggest or request additional software that would be useful to the user community.

Table 2.1 Software available on computer *nmr05*

Software	Functionality
ACD Labs Suite 12	ChemSketch, HNMR and CNMR Predictors, etc.
Adobe Acrobat XI Pro	Create and work with Adobe PDF files
Microsoft Office 2010	Word, Excel and PowerPoint
Mnova	NMR data processing software
Mozilla Firefox	Web browser
Nero Multimedia Suite 10	Software suite for writing to CD/DVD
TopSpin 3.6.0	Bruker NMR data processing software
X-Win32	X-terminal emulation software

[19] Refer to Subsection 5.1.1 for information about special characters under UNIX and Microsoft Windows.

2.8 Mnova Notes

The UW-Madison Department of Chemistry maintains and supports a campus-wide site license for Mestrelab Research Mnova software. Mnova is available for Linux, Macintosh OS X, and Microsoft Windows. Installation instructions are provided at https://nmr.chem.wisc.edu under User Guides → Software Guides.

Chapter 3

NMR Sample Preparation

3.1 Sample Tube Selection and Care

Selection of the appropriate NMR sample tubes and their subsequent care are important factors toward consistently obtaining high-quality NMR data. The following items briefly compare four grades of NMR tubes manufactured by Wilmad-LabGlass, and the notes provide important information to bear in mind; please consult the manufacturer's product details for additional information. Note that the School of Pharmacy stockroom normally stocks both Wilmad WG-1000 and 528-PP tubes.

- **WG-1000**
  These High Throughput series NMR tubes (ca. $1 each) are intended as an economical choice for applications where optimal resolution is not a primary consideration (e.g., high-throughput screening or studies of polymers, crude reaction mixtures or quadrupolar nuclei).
- **527-PP**
  These Precision series NMR tubes (ca. $12 each) are designed for high-resolution work with instruments up to 400 MHz.
- **528-PP**
  These Precision series NMR tubes (ca. $15 each) are of higher quality and are designed and rated for high-resolution work with instruments up to 500 MHz.
- **535-PP**
  These Precision series NMR tubes (ca. $20 each) are of higher quality yet and are intended for high-resolution work with instruments up to 600 MHz.
- **Notes and Discussion**

  1. Use of a particular quality NMR tube in a higher-field instrument than it was designed for may result in sub-optimal resolution, manifested by shimming difficulties and/or poor line shapes. The degree of performance degradation will, of course, depend on how far afield[1] one deviates with respect to the intended application.
  2. If your NMR research demands the highest possible resolution and you plan to use both the AV-400 and UI-500, then you should consider purchasing 528-PP (or equivalent quality from another manufacturer) tubes for use with both instruments.

[1] Yes, pun intended.

3. Treat NMR sample tubes with care and protect them at all times. A tiny scratch can destroy the tube's high quality properties, making it useless for high-resolution experiments, and potentially act as a stress point to initiate breakage when you least expect it.
4. Consult the Wilmad-LabGlass Support web pages for valuable technical information related to tube quality and selection, tube cleaning, etc.

Here's a multi-part thought experiment for the interested reader:[2] (1) How would you actually know whether or not a particular data set suffered from shimming difficulties or poor line shapes? (2) How would you go about determining the cause of such poor-quality data? (3) How would you design an experiment to test your hypotheses? (4) How would you design an experiment to test the performance of NMR tubes of different quality levels.

3.2 Shigemi NMR Microtubes

Important notes regarding proper care and use of Shigemi NMR Microtubes are presented in this section. (Shigemi tubes are discussed further in Section 6.2.) This information is from Shigemi technical assistance in direct response to multiple instances in our laboratory where the tubes were discovered to be warped and subsequently abraded from long-term rotational contact with the inside of the NMR probe. Shigemi tubes may be used on the UI-500, and the details specific to volume (related to solution height) in the following notes are specific to that instrument. Do not use these tubes on the AV-400 without prior discussion with and approval from the NMR Facility Director.

- Never put these tubes into an oven! The tube walls are extremely thin, especially in the sample region, and are easily deformed by heating, thus degrading their performance and risking costly damage to the NMR probe.
- Keep the tubes oriented vertically when washing and drying them, and when they contain sample solution; store the clean tubes in their original packaging when otherwise not in use. These steps will help to maintain the tubes' original condition and prevent damage from improper storage and handling.
- Never spin these tubes in the probe! Any sensitivity gains from sample spinning come at the expense of greater sensitivity loss due to tuning modulation. Two-dimensional NMR experiments in our facility are, by default, performed with sample rotation turned off; however, sample rotation is the default for 1D experiments. To explicitly turn sample rotation off (e.g., for 1D ^{13}C experiments), do so either graphically via the 'acqi' interface or manually by setting `spin=0` before initiating the acquisition.
- Sample solution height should be 18–20 mm and the tube must be positioned so that the sample solution is centered vertically within the detection region of the probe coil. Further sensitivity gains with sample height less than about 18 mm will not be observed, owing to a fixed number of spins within the detection volume, and sensitivity may even decrease due to less effective gradient shimming — translating to poorer line shapes, thus poorer signal-to-noise.
- Further sensitivity improvement may be obtained by manually optimizing the radial shims (those involving x and y character) after gradient shimming, although few users in our facility will know how to do this.

[2] If you're reading this document, you *should* be interested.

3.3 Sample Preparation Guidelines

Proper and consistent sample preparation is an important but often overlooked component of acquiring quality NMR data. The tips below will help to consistently produce good data. Refer to Figure 3.1 on page 29 for illustration of critical dimensions.

- Select the proper NMR tube for the instrument you will use and the type of work you plan to do. Exercise proper care in handling and cleaning the tube (*vide supra*).
- Handle and store NMR (lock) solvents appropriately to avoid contaminations that may obscure the spectral data of the solute.
- Avoid the presence of solids in the final NMR sample solution; filter, if necessary, to remove particulate matter. (Wilmad-LabGlass sells a variety of tools to assist in NMR sample preparation.)
- Also avoid the inclusion of gas bubbles, no matter how tiny they may seem. As with particles dispersed or settled in the NMR tube, gas bubbles will absolutely wreak havoc on the ability to optimize the magnetic field homogeneity.
- Fill the NMR tube with the correct volume of solution. For the probes in use on both the AV-400 and UI-500, that volume is approximately 0.7 mL, which is enough to produce a sample column of about 45–50 mm height. Prepare the sample based on either volume or solution height in the NMR tube: much less than about 45 mm sample height requires more time to shim and will generally lead to poor line shapes; more than about 50 mm is wasteful in terms of both solvent and solute. Refer to section 6.1 of the Varian *VNMR Getting Started* manual for an excellent discussion.
- NMR samples prepared for long-term use should be sealed to prevent loss of solvent or introduction of contaminants. Obviously, such samples should be otherwise stored appropriately with respect to temperature, light, etc.

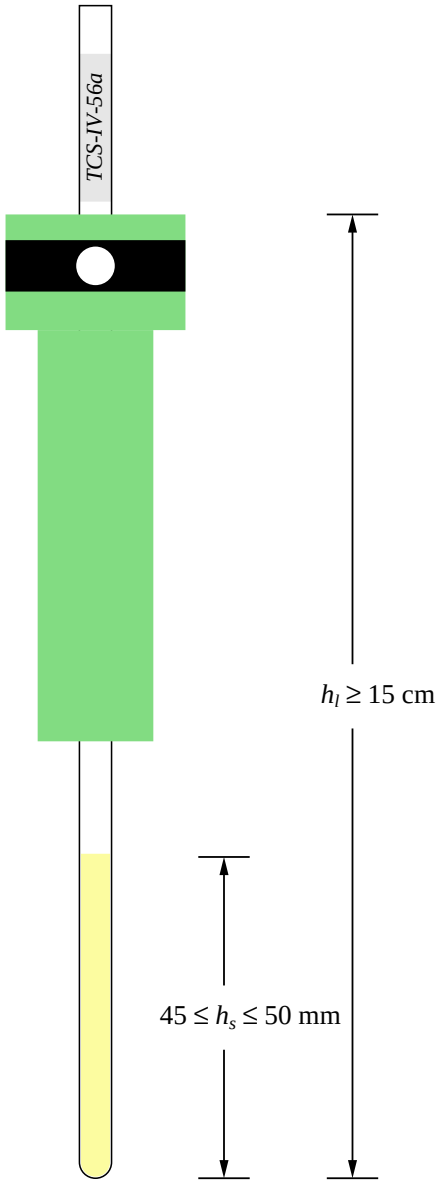

Figure 3.1 NMR tube and Varian spinner specifications for sample preparation in 5 mm NMR tubes as used in the UI-500 HCX and QN probes. This information is provided as a guide to optimal and trouble-free sample preparation. Solution height should be prepared to $45 \leq h_s \leq 50$ mm, corresponding to approximately 650–730 μL volume. Label placement at the top of the tube should be $h_l \geq 15$ cm to allow for depth adjustment of the tube in the spinner turbine without interference from the label. Although the spinner turbine height and profile are different for the Bruker AV-400 spectrometer, details regarding solution volume and height are essentially the same as shown here; the label position is slightly lower, however, at $h_l \geq 14$ cm.

Chapter 4

Guide to TopSpin and IconNMR

This chapter provides additional information that may be useful or interesting to some users in our NMR Facility. Presented in Section 4.1 are two- and three-letter codes related to Bruker pulse sequence (experiment) names. These codes are useful to those wanting to use experiments beyond the basic ones, since Bruker's Rube Goldberg approach to pulse sequence programming provides an object lesson in under-intelligent and over-complicated design. A consequence is the need for an unwieldy and obscure naming system that requires a four-page Rosetta Stone for translation. Section 4.2 may be of mild interest to select individuals with a curiosity for behind-the-scenes details, and it should be helpful to anyone wishing to install and maintain the TopSpin software on a lab computer for post-acquisition data processing.

4.1 TopSpin Experiment Codes

The number and complexity of NMR experiments today present many management challenges for developers and system administrators. Of the very few such challenges actually seen by the general spectrometer user community, one is related to naming conventions for the hundreds of available experiments.

Bruker's approach to this problem relies on a naming system that appends a sequence of two-character codes (there are also a few 3-character codes) to each experiment's base name. The codes are arranged in alphabetical order if more than one code is appended. The experiment base names have no particular fixed length, but tend to take on their commonly used names; examples are COSY, DEPT, HSQC, HMQC, HSQC, NOESY, etc. Note that upper- or lower-case letters are used, depending on whether the name refers to an experiment (upper-case), a parameter set (upper-case), or a pulse sequence (lower-case).

The table and examples below are taken from or inspired by information in the `$TS/exp/stan/nmr/lists/pp/Pulprog.info` file.[1] A complication arises due to the fact that these naming conventions are not applied uniformly across the categories of experiment, parameter set, and pulse program names. For example, some experiment names use codes such as CMC, LC, MLEV, SEL, and SW; these and other codes not identified in the `Pulprog.info` file are indicated by a † symbol in Table 4.1. Hello? Bruker! Consistency?

[1] The `$TS` notation represents the TopSpin installation directory, as explained in Section 4.2.

Table 4.1 Bruker TopSpin two- and three-character experiment codes

Code	Description
AC	Accordion-type experiment
AD	Adiabatic spin lock
AR	Experiment for aromatic residues
AT	Adiabatic TOCSY
BI	Bird pulse for homonuclear decoupling
BP	Bipolar gradients
CC	Cross-correlation experiment
CMC†	Complete Molecular Confidence, a suite of Bruker software products
CN	^{13}C- and ^{15}N-dependent information in different indirect dimensions
CO	COSY transfer
CP	Composite pulse
CPD†	Composite-pulse decoupling
CT	Constant time
CV	Convection compensated
CW	Decoupling via the `cw` command
CX	Using CLEANEX-PM element
DC	Decoupling via the `cpd` command
DF	Double-quantum filter
DI	DIPSI mixing sequence
DH	Homonuclear decoupling in the indirect dimension
DW	Decoupling via the `cpd` command only during the WET sequence
DQ	Double-quantum coherence
EA	Phase-sensitive via the echo/anti-echo method
EC	E.COSY transfer
ED	Multiplicity editing
ES	Excitation sculpting
ET	Phase-sensitive via the echo/anti-echo TPPI method
FB	Using the F_2 and F_3 channels
FD	Using the F_1 and F_3 channels (for presaturation)
FR	With presaturation using a frequency list
FT	Using the F_1, F_2 and F_3 channels (for presaturation)

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Code	Description
FH	^{19}F observe with ^{1}H decoupling
FP	Using a flip-back pulse
FL	For ^{19}F ecoupler (except when it means something else, like FLOPSY or . . .)
FW	Forward-directed type experiment
F2	Using the F_2 channel (for presaturation)
F3	Using the F_3 channel instead of F_2
F4	Using the F_4 channel instead of F_2
GD	Gated decoupling via the `cpd` command
GE	Gradient echo experiment
GP	Using gradients, via the `':gp'` syntax
GR	Using gradients
GS	Using shaped gradients
HB	Hydrogen-bond experiment
HC	Homonuclear decoupling of a region using a CPD sequence
HD	Homonuclear decoupling
HF	^{1}H observe with ^{19}F decoupling
HS	Homospoil pulse
IA	In-phase / anti-phase (IPAP) experiment
ID	Isotopically discriminated spectroscopy (IDIS)
IG	Inverse-gated decoupling
II	Inverse (invi/HSQC) sequence
IM	Incremented mixing time
IN	INEPT transfer
IP	In-phase
I4	Inverse (inv4/HMQC) sequence
JC	For determination of J-coupling constants
JD	Homonuclear decoupled
JR	With jump-return pulse
JS	Jump symmetrized (roesy)
LC†	Liquid chromatography, as in LC-NMR
LD	Low-power `cpd` decoupling
LP	With low-pass J-filter
LQ	With Q-switching (low Q)
LR	For long-range couplings

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Code	Description
L2	With 2-fold low-pass J-filter
L3	With 3-fold low-pass J-filter
MF	Multiple-quantum filter
ML	with MLEV mixing sequence
MLEV†	A class of composite pulses originating from Malcolm Levitt
MQ	Multiple quantum
NC	^{15}N- and ^{13}C-dependent information in different indirect dimensions
ND	No decoupling
NO	With NOESY mixing sequence
PC	With presaturation and composite pulse
PE	Using perfect echo
PG	Power-gated
PH	Phase-sensitive detection using States-TPPI, TPPI, States or QSEQ
PL	Preparing a frequency list
PN	Presaturation using a 1D NOESY sequence
PP	Using purge pulses
PR	With presaturation
PS	With presaturation using a shaped pulse
QF	Absolute-value mode
QN	QNP operation
QS	Phase-sensitive DETECTION using QSEQ mode
RC	For determination of residual dipolar (RDC) and J-coupling constants
RD	Refocussed
RE	Relaxation optimized (H-flip)
RL	With relay transfer
RO	With ROESY mixing sequence
RS	With radiation-damping suppression using gradients
RT	Real time
RU	Radiation-damping compensation unit
RV	With random variation
R2	With 2-step relay transfer
R3	With 3-step relay transfer
SE	Spin-echo experiment
SEL†	Selective experiment

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Code	Description
SH	Phase-sensitive detection via the method of States, *et al.*
SI	Sensitivity improved
SM	Simultaneous evolution of X and Y chemical shift
SP	Shaped pulse
SQ	Single quantum
SS	Spin-state selective experiment
ST	Phase-sensitive detection via the States-TPPI method
SW†	Spectral window (SW) optimized
SY	Symmetric sequence
S3	S3E experiment
TC	Temperature compensation
TF	Triple-quantum filter
TP	Phase-sensitive detection via the TPPI method
TR	TROSY sequence
TZ	Zero-quantum (ZQ) TROSY
UL	Using a frequency list
US	Updating shapes
WG	Watergate using a soft-hard-soft sequence
WT	With WET water suppression
W5	Watergate using a W5 pulse
XF	x-filter experiments
XY	With XY CPMG sequence
X1	x-filter in F_1
X2	x-filter in F_2
X3	x-filter in F_3
ZF	With z-filter
ZQ	Zero-quantum coherence
ZS	Using a gradient/rf spoil pulse
1D	1D version
1S	Using 1 spoil gradient
11	Using 1-1 pulse
19	Using 3-9-19 pulse
19F	For ^{19}F
2H	Using ^{2}H lockswitch unit

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Code	Description
2S	Using 2 spoil gradients
3D	3D sequence
3N	for E.COSY (3 spins, negative correlation)
3P	for E.COSY (3 spins, positive correlation)
3S	Using 3 spoil gradients
30	Using a 30-degree pulse flip angle
45	Using a 45-degree pulse flip angle
90	Using a 90-degree pulse flip angle
135	Using a 135-degree pulse flip angle
180	Using a 180-degree pulse flip angle

Codes marked with a † symbol are not identified in the `Pulprog.info` file.

4.1.1 Conventions and Examples

Here are general naming conventions and some examples for illustration and pleasure:

- Some two-character codes may be omitted if they represent redundant information.
- For heteronuclear experiments, H or X decoupling should be considered as the default configuration (in other words, decoupling is not indicated explicitly — except when it is).
- For 2D experiments, the mode (absolute-value, phase-sensitive, echo/anti-echo) is always indicated explicitly.
- The names of 1D experiments that are analogues of 2D experiments by virtue of a selective pulse begin with '`sel`'. Example: `selhsqcgpsp` = `sel+hsqc+gp+sp`
- Semi-selective 2D experiments have the same base name as the non-selective version, but begin with the letter '`s`'. Example: `scosyphrd` = `s+cosy+ph+rd`
- Phase-sensitive NOESY with presaturation: `noesy+ph+pr` → `noesyphpr`
- Deconstruction fun (decipher the codes to determine the experiment):
  - `hmbcgplpndqf` = `hmbc+gp+lp+nd+qf` = ?
  - `selhsqcgpndnosp` = ?
  - `hsqccoetgpiajclrndsp` = ?
  - `hsqcetgpiajclrndsp_bshd` = ?

4.2 TopSpin and IconNMR Directory Structure

The AV-400 TopSpin installation directory is `/opt/topspin3.5pl6`,[2] represented here in shorthand notation by `$TS`. Other shorthand names are: `$HOST` represents the computer's host name; `$USER` represents an individual's user name (for TopSpin and/or IconNMR); and `$HOME` represents an individual's home directory, `/home/$USER`, for those with a log-on account. `$NAME` represents a unique sample directory; in our facility this is set to the form `%Y-%m-%d.%H%S-$HOLDER.$USER`, where `$HOLDER` is a predefined Bruker variable (as is `$USER`).[3]

Owing apparently to legacy Bruker conventions, confusion arises due to the fact that there is both a predefined, mandatory *nmr* user account and several completely unrelated and independent `nmr` directories. Also potentially confusing is the fact that, for our AV-400, IconNMR users' data are stored in three different locations (in different directories on two different hard drives). An additional hard drive (the entire disk is partitioned as `/data`) was installed (1) for data integrity in case of a primary disk failure, and (2) to provide convenient and secure network access to data without exposing the primary disk to the LAN. The more important directory names and their content are listed below in Table 4.2.

Table 4.2 TopSpin and IconNMR directories and their purpose

Directory path	Usage
`$HOME/.$HOST/prop/*`	TopSpin configuration directory (Linux)
`$HOME/.topspin1/prop/*`	TopSpin configuration directory (Mac)
`$HOME/.topspin-$HOST/prop/*`	TopSpin configuration directory (Windows)
`$TS/data/$USER/nmr`	Legacy TopSpin and IconNMR data directory
`$TS/$USER`	Modified TopSpin and IconNMR data directory
`/data`	Additional disk for secondary data storage
`/data/nmr`	Modified IconNMR data directory for user *nmr*
`/data/$USER`	Modified IconNMR data directory for other users
`$TS/$USER/$NAME`	Modified IconNMR sample directory for all users
`$TS/nmr/$NAME`	Modified IconNMR sample directory for user *nmr*
`/data/$USER/$NAME`	Modified IconNMR sample directory for all users
`/data/nmr/$NAME`	Modified IconNMR sample directory for all users
`$TS/exp/stan/nmr/par`	Parameter sets
`$TS/exp/stan/nmr/au/src`	AU programs
`$TS/exp/stan/nmr/lists/bsms`	Shim files
`$TS/exp/stan/nmr/lists/f1`	F_1 frequency lists
`$TS/exp/stan/nmr/lists/pp`	Pulse programs
`$TS/exp/stan/nmr/lists/wave`	RF and PFG waveform shape files

[2] The `topspin3.5pl6` directory name indicates TopSpin version 3.5 at patch-level 6. It is common for a given version to advance through multiple patch levels before a new version is introduced.

[3] For details about the date and time elements in the format string, refer to the Linux manual documentation (`$ man date`), or similar resource, for the `date` command.

Bruker has demonstrated a total disregard for contemporary best practices in their organization of disk partitions, directory structures, etc. User-owned files and directories should *never* be mixed together with system-wide software directories and files. At best, such practices unnecessarily complicate system administration and maintenance; in worst-case scenarios, this is a recipe for disaster. For example, it is ridiculous that all users' data are stored in the *nmr* user's `/data/nmr` directory, as it is totally unnecessary and results in a useless jumble of all user data into a single directory. It is also redundant (in a bad way) because user data are organized logically in the `/data/$USER` directory.[4]

Note that the six indicated subdirectories below the `$TS/exp/stan/nmr` base directory also contain a jumble of TopSpin and log-on users' files. The only way to distinguish individual files within a given subdirectory is by examining the file ownership; individual file permissions are set so that anyone can read (and use) any file, but can modify or delete only their own files. Sigh.

[4]This logical organization used in our laboratory is a configuration option in addition to, rather than instead of, the default data dump into `/data/nmr`.

Chapter 5

Guide to *VNMR* 6.1C

VNMR version 6.1C was the last release of this software series before the introduction of the *VnmrJ* series circa 2002, which marked the beginning of the end for Varian NMR. Ill-conceived, poorly developed and maintained, *VnmrJ*[1] was the metaphorical "tail wagging the dog" of marketing glitter over substance; it progressed through many half-baked versions over the years, during which Varian NMR was purchased by Agilent, who after a few years shut down the magnetic resonance divisions. May you rest in peace, Varian NMR.

Back to the story: The *VNMR* software comprises a full-featured and powerful package. There is, therefore, a large amount of information to master for those aspiring to high-level utilization. On the other hand, much effort has gone into software development to the extent that many complex operations can be routinely performed in a fully or partially automated fashion, thus sparing most users the burden of those pesky behind-the-scenes details that are executed as if by magic. These automated processes *usually* work very well.

5.1 *VNMR* User Interfaces

A brief history about the development of NMR software in general — and Varian's *VNMR* software in particular — may help put things into perspective. Not many years ago, NMR users controlled the spectrometer's operation by manual entry of parameters, commands, etc. Progressively, the software began to incorporate macro-activating menu buttons as a complement to the manual interface. Varian's first mouse-driven user interface was called *GLIDE* and was effective but quite limited by current standards, offering limited capabilities and designed primarily for entry-level spectrometer operation (similar in functionality to the Walkup interface). *VNMR* version 6.1B introduced the set of Tcl/dg menu panels that include Walkup, CustomQ and Setup EXP; although *GLIDE* is included in version 6.1C, it is vastly inferior to the newer Tcl/dg interfaces.

5.1.1 CustomQ

Of the three *VNMR* 6.1C graphical user interfaces (*Walkup*, *CustomQ* and *Setup EXP*), CustomQ is the *de facto* standard in our laboratory, due primarily to its balance between ease-of-use and allowing user input

[1] The *'J'* in *VnmrJ* indicates that the software was written in the Java programming language. Who knows why this was thought to be important enough to include in the name? Written in the C programming language, the previous series was called simply *VNMR*.

in limited measure. Both Walkup and CustomQ provide fully automated data acquisition, processing, plotting, and saving. Automated file saving must, of course, utilize a standardized protocol to prevent overwriting existing data. This protocol relies on four conventions:

1. Every user has on the spectrometer host computer a *data directory* in his or her *VNMR system directory* under the *home directory*. A local data directory for user *vnmr1* looks like this under Sun Solaris and *VNMR* 6.1C: `/export/home/vnmr1/vnmrsys/data/`.

2. A *sample directory* is created automatically each time an automated data acquisition is initiated. The locally modified format for a *VNMR* sample directory name has the form `yyyymmdd.TEXT`, where *TEXT* is an optional text string provided by the user during experiment setup; if a text string is not specified, the individual's user name is used instead. The date stamp preceding the text string ensures that sample directories are automatically displayed in chronological order through the year, month and day levels; within a given day, though, sample directories are further sorted according to their text strings.

   To illustrate this convention, data acquired by *vnmr1* on March 17, 2018 under the sample text string *Strychnine* will be saved in the `/export/home/vnmr1/vnmrsys/data/20180317.Strychnine/` sample directory.

   Sample directory names (thus text strings) must not contain spaces! UNIX file names can be up to 256 characters in length and may contain upper- and lower-case letters, numerals, and particular (not all!) special characters. Because Microsoft Windows cannot handle some of the special characters that are valid for UNIX, it is important to use only those that are valid with both operating systems. Specifically, use only these special characters:

   . (**period**), **_** (**underscore**), **-** (**hyphen**).

   Remember: Do not use spaces or any other special characters in file or directory names.

3. If an identical sample directory text string is reused on the same date, a time stamp in *HMS* 24-hour, 2-digit format is appended to the sample directory name to ensure uniqueness. To illustrate this feature, if additional data are acquired by *vnmr1* at 4:57:49 PM on March 17, 2018 under the sample text string *Strychnine*, the data will be saved in the `.../20180317.Strychnine.165749/` sample directory. A period character (`.`) is added to concatenate the base name and time stamp.

4. Finally, NMR data sets themselves are uniquely named, in an obvious manner, and written in the corresponding sample directory according to their experiment type. Table 5.1 illustrates this with common examples. Note that an NMR data set is not simply a single file but is actually a predefined *directory structure* containing several files. The special `.fid/` extension[2] denotes this fact and serves as a flag to *VNMR* that the entity is expected to be a valid NMR data set.

5.2 Basic *VNMR* Parameters and Commands

NMR Facility users with access to the UI-500 need to know how to perform routine tasks (such as file and data handling, data processing and plotting) at the spectrometer host computer or elsewhere using

[2]The slash character (`/`) is the UNIX directory separator.

Table 5.1 *VNMR* data set naming convention examples

Data Set Name	Experiment Type
PROTON.fid/	1D ^{1}H acquisition
CARBON.fid/	1D ^{13}C acquisition
FLUORINE.fid/	1D ^{19}F acquisition
PHOSPHORUS.fid/	1D ^{31}P acquisition
NOESY1D.fid/	1D NOESY acquisition
gCOSY.fid/	2D gCOSY acquisition
TOCSY.fid/	2D TOCSY acquisition
NOESY.fid/	2D NOESY acquisition
gHSQC.fid/	2D gHSQC acquisition
gHMBC.fid/	2D gHMBC acquisition

the *VNMR* software. Tables 5.2–5.6 below provide new users with a guide to some of the most common *VNMR* commands and parameters. Consult the Varian *VNMR Command and Parameter Reference* manual for detailed information.

Table 5.2 Useful *VNMR* data and file handling commands

Command	Description
`clear`	Clears the contents of the *VNMR* text window
`pwd`	Gives the full path of the present working directory
`ls`	Lists the contents of the present working directory
`mkdir('newdir')`	Creates a new directory with the name `newdir`
`rmdir('olddir')`	Deletes the (empty) directory with the name `olddir`
`cd('pathname')`	Changes the present working directory to `pathname`
`svf('filename')`	Saves (writes) the NMR data set with the name `filename`
	Note the convenient `svf('part1'+seqfil+'part2').`
`rt('filename')`	Retrieves (reads) the NMR data set with the name `filename`
`exit`	Exits from the *VNMR* application (use before logging off)

5.3 Data Processing Tips

Refer to chapters 8 (Data Processing) and 9 (Display, Plotting, and Printing) of the Varian *Getting Started* manual for step-by-step instructions for basic operations. The Varian *User Guide: Liquids NMR* and *VNMR Command and Parameter Reference* documents contain additional information; the latter is an alphabetized reference. The Varian documents are cross-referenced with respect to topic, thus simplify-

Table 5.3 Useful *VNMR* data processing and display parameters

Parameter	Description
`lb`	Defines the "line broadening" decay rate
`sp, sp1`	Defines the start point of the display region (default units of Hz)
`wp, wp1`	Defines the width (not the end point) of the display region
`sc, sc2`	Define the start position (mm) of the chart for 1D and 2D data
`wc, wc2`	Define the width (mm) of the chart for 1D and 2D data
`wcmax, wc2max`	Define the maximum width (mm) of the chart for 1D and 2D data

Table 5.4 Useful *VNMR* data processing and display commands

Command	Description
`ft`	Performs a Fourier transform of the FID
`wft`	Performs a weighted Fourier transform of the FID (cf. `lb`)
`df`	Displays the FID
`ds`	Displays the spectral data
`dss, dssh`	Displays arrayed spectral data in a stacked format
`aph, aph0`	Automatically phase corrects the spectrum
`vsadj<(height)>`	Automatically scales the spectrum vertically

ing the process of finding the target information or learning a new subject. The information in Table 5.7 on page 43 should help guide new users to the appropriate sections of the Varian documentation for processing and plotting directions.

5.4 Spectral Display and the *VNMR* Graphics Window

The *VNMR* graphics window is that window to which spectral data, file information, etc. are displayed. There exist several parameters and commands to control the appearance of both the window and its contents. A common question is how to make the spectral data fill the entire graphics window, rather than occupy a small portion. It is important to understand that several parameters exist to control the layout of spectral and related data on the plotted page. The spectral display scaling in the graphics window may or may not be directly related to the hardcopy output, depending upon the parameter `wysiwyg`: If `wysiwyg='y'`, then the output in the graphics window is scaled according to plot-control parameters such as `sc` and `wc`; if `wysiwyg='n'`, this display scaling is not performed, thus allowing the spectrum to occupy the full width of the graphics window. The default behavior has been modified to set `wysiwyg='n'`.

Users should also be aware of commands to toggle the horizontal size of the graphics window and to reposition the spectral data within the window. The commands `large` and `small` perform the former task, while `left`, `center` and `right` (also available via menu buttons at the Main Menu →

Table 5.5 Useful *VNMR* plotting parameters and commands

Param/Command	Description
`vp`	Defines the vertical position of the spectrum
`vs`	Defines the vertical scale of the data
`pl`	Sends the spectral data to the plotter buffer
`pscale`	Sends the axis scale to the plotter buffer
`pir`	Sends the integral data to the plotter buffer
`pirn`	Sends the normalized integral data to the plotter buffer
`ppf`	Sends the peak frequency data to the plotter buffer
`pap`	Sends the parameters ("all") to the plotter buffer
`ppa`	Sends the parameters ("in plain English") to the plotter buffer
`plcosy`	Plots 2D homonuclear data to plotter or file, respectively
`plhxcor, plot2D`	Plots 2D homo- or heteronuclear data
`page`	Plots the previously composed graphics data to the defined plotter
`page('filename')`	Plots the previously composed graphics data to file `filename`
`page('clear')`	Removes graphics data from the plotter buffer (cf. `killplot`)

Display → Size level) perform the latter. The `f` command, equivalent to the Main Menu → Display → Interactive → Full button, displays the entire spectrum in the graphics window; the `full` command, equivalent to the Main Menu → Display → Size → Full Screen button, causes the selected spectral subregion to be displayed (nearly) full-width across the graphics window.

Users should know the basic parameters for spectral plot control: `sc`, `wc` and `wcmax` define, respectively, the start of the chart, the width of the chart and the maximum width of the chart; `sc2`, `wc2` and `wc2max` perform similar functions for the second dimension in 2D plots. The parameter `vp` controls the vertical position of the spectrum and `vs` controls the vertical scale for 1D plots; `vs2d` sets the vertical scale for 2D plots. These parameters (except `vs` and `vs2d`) have units of millimeter. These and other parameters and plotting commands make it possible to compose complex and elegant plots. Users who frequently need to perform complicated plotting operations are advised to write macros to help automate the procedures. See the *VNMR Command and Parameter Reference* manual for further information.

5.5 Plotting 2D Spectra

Many methods and macros are available for plotting 2D spectra from *VNMR*. To better choose which to use, it is necessary to understand and compare the possibilities. This section will focus only on plotting via the macros `plcosy`, `plcosyeps`, `plhxcor` and `plot2D`. Users should consult the *VNMR Command and Parameter Reference* manual for details where applicable; see that document's Introduction for a description of the syntax used, and look closely at the examples. Known corrections and additions to the *VNMR* documentation are given below.

Table 5.6 Comparison of commonly used *VNMR* display and plotting commands

Display	Plotting	Description
`df, dfid`	`plfid`	Displays or plots an FID
`ds`	`pl`	Displays or plots spectral data
`dscale`	`pscale`	Displays or plots the axis scale
`dpf`	`ppf`	Displays or plots peak frequencies
`dll`	`pll`	Displays or plots a line listing as text
`dpir, dpirn`	`pir, pirn`	Displays or plots normalized integrals
`dps`	`pps`	Displays or plots the pulse sequence
`dcon`	`pcon`	Displays or plots 2D contours

Table 5.7 Useful references to *VNMR* documentation

Topic	Refer to
Weighting Functions	Getting Started, page 209
Linear Prediction	Getting Started, page 215
Fourier Transformation	Getting Started, page 211
Spectral Phasing	Getting Started, page 212
Referencing	Getting Started, page 235
Peak Picking	Getting Started, page 234
Integration	Getting Started, page 238
Plotting	Getting Started, page 237

5.5.1 The `plcosy` Macro

This is the standard plotting macro for COSY- and NOESY-type homonuclear spectra (e.g., ^{1}H–^{1}H, ^{19}F–^{19}F, ^{31}P–^{31}P); it can be used alone or with optional arguments. The default behavior is to plot both positive and negative contours, with projections plotted along both axes; a high-resolution spectrum can be specified by indicating the experiment number in which the 1D data reside, or the 1D traces can be suppressed. Note that to plot a high-resolution 1D spectrum, one must enter values for `levels`, `spacing` and the *numeral* corresponding to the experiment number in which the 1D data (spectrum or FID) reside. The macro prints parameters via `ppa` and issues a `page` command at the end.

The local macro `plcosyeps`[3] is essentially the `plcosy` macro without the internal `page` command; use it for plotting to a file, as discussed in Subsection 5.8.4.

[3]The *eps* part of the macro name is a mnemonic device, referring to the Adobe Encapsulated PostScript (EPS) file format.

5.5.2 The `plhxcor` Macro

As the name suggests, this macro is designed for plotting spectra from heteronuclear correlation experiments such as HSQC, HMBC and HETCOR (e.g., ^{1}H–^{13}C, ^{1}H–^{15}N, ^{19}F–^{13}C). The macro's default behavior has been modified to plot both positive and negative contours, as does `plcosy`; however, plotting of the two 1D spectra can be controlled separately. This macro also prints parameters via `ppa` and issues a `page` command.

5.5.3 The `plot2D` Macro

This macro is a more generic version for 2D spectral plotting. Before revision of `plhxcor`, the primary advantage of `plot2D` was the ability to plot phase-sensitive data with the negative contours represented by a single level, a standard way to indicate such data on a monochrome plotter or display. The syntax shown in the *VNMR Command and Parameter Reference* manual is incomplete and incorrect; a more consistent and accurate representation of the syntax is

```
plot2D<(<'resize'><,><'pos'|'neg'|'both'><,> \
        <levels<,spacing<,top1D<,side1D>>>>)>
```

where the backslash character (\) at the end of the first line is not a part of the command itself, but indicates that the desired command is continued on the next line. The following option definitions supplement those described in the *VNMR* documentation. The `resize` option automatically resizes the current display to fit other options chosen and make full use of the paper; the default behavior is to not resize. `top1D` can take on one of the parameters `'top'`, `'expN'`, `'proj'` or `'notop'`; similarly, `side1D` can be `'side'`, `'expN'`, `'proj'` or `'noside'`. The parameter `'expN'` tells which experiment workspace contains the high-resolution 1D spectrum to be plotted on the axis. Note that this syntax is different than for the two macros above; for example, to use a 1D spectrum in experiment workspace 4, one must enter `'exp4'` with the `plot2D` macro, but simply `4` with the `plcosy` macro.

Other differences with respect to the two macros described above are (1) 1D data to be plotted along the axes can exist either in other experiment workspaces (e.g., exp3 and exp4) or as other experiments (e.g., CARBON or PROTON) in the sample directory currently in use in the active experiment workspace; (2) if it is desired to have parameters printed on the plot, the appropriate command (e.g., `pap` or `ppa`) must be entered explicitly, as should (3) the `page` command to complete the plotting operation. This flexibility allows the user to exercise more control over the plot results.

The `plot2D` macro has been revised to correct for an error that was responsible for occasionally plotting a ^{13}C spectrum along one of the axes of a ^{1}H COSY spectrum — or a ^{1}H spectrum along a ^{13}C axis. The revision should resolve errors of this type (tests cases were successful); however, please inform the NMR Facility Director if you observe this or other anomalies.

5.6 Using Other Networked Printers

The default *local* configuration of *VNMR* gives users access to the HP LaserJet networked printer in room 1411 for both printer and plotter functionality. Note that *VNMR* makes a technical distinction between printing (fixing text in portrait orientation) and plotting (fixing graphics in landscape orientation) operations. Other network-accessible printers or plotters can also be used; in practical terms this means most modern devices with their own network cards, and therefore their own IP addresses. To be accessible, each physical printer or plotter to which access is desired must be individually configured by the *VNMR* system administrator as a printer and/or a plotter device on the Sun computers. Please forward requests

for networked printer access to the NMR Facility director; include your research group name, the printer brand and model, its IP address and the room number where the device is located.

To use a printer or plotter device that is configured for *VNMR*, the appropriate *VNMR* parameter(s) must first be set by the user. For example, a Mecozzi group member desiring to print and plot directly to the Mecozzi lab printer in room 7216 would set

`printer='Mecozzi_7216_prnt'` for printing, and
`plotter='Mecozzi_7216_plot'` for plotting.

Note that these settings are retained as an individual user's *global parameters* until explicitly changed by some method. Users sometimes forget to reset the parameter appropriately, then print to an unintended device before figuring out why the desired printer "isn't working." To prevent this from happening, the normal configuration in our NMR Facility sets the printer and plotter to a default device (i.e., Null Plotter for *ui500* log-on sessions, and the HP LaserJet device in the NMR lab for *nmr03* log-on sessions) each time a user starts *VNMR*. Those who would like to set a different printer as their default device should read the next section.

5.6.1 Customizing the Default Printer and Plotter

Suppose you would like to sit at the computer in your 7th-floor office, enjoying your morning coffee while analyzing the NMR data for your latest synthesis project — destined to bring you fame and fortune as a vital, new, blockbuster drug. You hate to go all the way down to the NMR lab to get your plot output, and you grow weary of manually resetting the plotter configuration every time you start a new *VNMR* session on *nmr03*. Or perhaps you want the default plot output from CustomQ to go directly to your office printer. What to do? Simply submit an e-mail request to the NMR Facility Director (thomas.stringfellow@wisc.edu) indicating which printer/plotter and Sun host computer combinations you would like as defaults; you will be notified once your request is completed.

5.7 Suppressing Automatic Printing

Automatic plotting via the CustomQ and Walkup interfaces is turned off as the default behavior in our facility, primarily to save paper. If you want the default hardcopy plot, or if you want to plot manually yourself, simply enable plotting. From the Main Menu → Custom Macros menu level under *VNMR* are located two menu buttons, Null Plotter and Default Plot/Print. Use Default Plot/Print to send plot output to the printer in the NMR lab, and use Null Plotter to disable this behavior.

5.8 Advanced Graphics Handling Techniques

5.8.1 Introduction to Graphics Images

It is useful to have a basic understanding of computer graphics and how different graphics formats compare with one another before embarking on a project involving graphics — especially if it's an extensive project. For the purpose of this condensed discussion,[4] there are two classes of electronic formats used for graphics: bitmapped images and vector representations.

[4] Ignored, for example, are discussions of many formats developed for use with web browsers, and discussions of the different color-space models or color-depth issues.

In bitmapped images, the graphics information is encoded point-by-point according to a grid of pixels (picture elements); for a given physical output size, more grid squares provide a greater density of points and a corresponding higher resolution — at the expense of larger file size. To achieve aesthetically pleasing results — or even usable results, in extreme cases — it is necessary to consider in advance the intended size and use of the final image, then to ensure that the final output is generated at a suitable resolution. The details of how to accomplish this goal depend upon the method and/or application used to create the bitmapped image. Even with interpolation algorithms, bitmapped graphics enlarged or reduced much beyond their original size can appear grainy or distorted.

In vector graphics, the information is encoded mathematically, i.e., in terms of lines (vectors), curves and scalable typeface. In vector graphics, the ultimate resolution depends only upon the resolution of the output device itself (e.g., the printer or monitor), not upon the file size. Scaling can be performed indefinitely, without loss of resolution or distortion of the output.

Examples of bitmapped image formats include Microsoft Windows Bitmap (BMP), Windows Metafile (WMF), Enhanced Metafile (EMF), and Graphic Interchange Format (GIF). If you copy a graphics selection to the clipboard, then paste it elsewhere, you are usually working with a bitmapped image format. As for vector graphics formats, Adobe PostScript (PS) and Encapsulated PostScript (EPS) are well-established industry standards; Scalable Vector Graphics (SVG) is a more recent addition to this group; popularity of the Hewlett Packard Graphics Language (HPGL), as used for many years with Hewlett Packard pen plotters, has dwindled due to the proliferation of relatively inexpensive, high-resolution laser printers.

The formats discussed above are useful for graphics images consisting primarily of reasonably basic systems of lines, curves and text — so-called *line art*; color is typically supported as well. Other specialized formats, such as Joint Photographic Experts Group (JPEG or JPG) and Tagged Image File Format (TIFF or TIF), are designed to handle the additional complexities inherent in photographic images.

As an aside, when text is copied/pasted from within a text editor or word processor, it is the (ASCII) characters themselves that are dealt with directly, and not a graphical representation in the sense of the previous discussions. It is the opinion of this author that a scalable vector graphics format is usually the best choice for publication-quality graphics, offering the flexibility that once an image has been created and saved to file, that same image can later be rescaled without the need to produce another image at a different resolution.

In summary, one should carefully consider in advance the intended use for the graphics, then select an appropriate format. Another important consideration has to do with the availability of software for subsequent editing and processing of the image.

5.8.2 Setting Colors Under *VNMR*

Users can set their own color selection schemes for both display and plotter. Enter `color` at the *VNMR* command line to open a color palette application from which the desired settings can be configured, saved, loaded, etc. This is particularly convenient for setting colors suitable for printer output or for slide or poster presentations. If the color scheme is intended for use with a particular plotter or pseudo-plotter, save the color scheme with a name identical to that of the plotter for which the output will be used; in this manner, the saved color definition file (in the `~/vnmrsys/templates/color` directory) will be loaded automatically when that plotter is selected.

The procedure is illustrated with an example. The local NMR guru, *vnmr1*, wants to produce color plots of phase-sensitive 2D data as Encapsulated PostScript files via the pseudo-plotter named EPS1200RC_file. He therefore configures a suitable color scheme and saves the definitions under

the name EPS1200RC_file. The next time he sets the plotter to this particular device, i.e., by setting `plotter='EPS1200RC_file'`, the corresponding color definition will be automatically loaded, and output subsequently sent to the device — in this case, a file — will be written with the color information specified by the color scheme.

When a printer or plotter is selected and no identically named color definition file has been defined for that device, the user's default color definition is loaded. This particular default is exactly the color scheme most users see each time they use *VNMR*.

5.8.3 Setting Up the Page Layout

Achieving exactly specified and therefore reproducible page layouts for plotting is a simple matter if we understand and make use of the power inherent in the *VNMR* plotting and display functions. Adding to the beauty of this design is the capability to incorporate individual page-layout parameters (and plotting commands, too, if desired) as macro commands, easily executed when needed.

Recall that the main page-layout parameters are `sc`, `wc` and `wcmax` for 1D spectra, and their analogs `sc2`, `wc2` and `wc2max`, which describe the additional dimension in 2D plots. The command `vsadj(height)` scales a 1D spectrum to the specified height, in mm units.

Table 5.8 illustrates example page-layout parameters that would be appropriate for plotting 2D homo- and heteronuclear contour spectra, making maximum use of a sheet of letter-sized paper. These parameters result in margins of about 1.5 cm and allow 5.0 cm for plotting projections or high-resolution 1D spectra at the top and side of the contour plot; also, no additional room is provided for printing the associated data parameters.

Table 5.8 Example page-layout parameters[a] for 2D homo- and heteronuclear plots. Use the custom macro `playout` (cf. Table 5.9) to quickly set all these parameters to the values shown in this table.

Parameter	Homo 2D	Hetero 2D
`sc`	0	0
`sc2`	0	0
`wcmax`	180	240
`wc`	130	190
`wc2max`	180	180
`wc2`	130	130

[a] The numerical values are in units of mm.

5.8.4 Plotting to a File from *VNMR*

The procedure described here allows one to save a composed spectral plot as a file rather than send the information directly to a plotter for immediate hardcopy output. The graphics file formats currently supported are Encapsulated PostScript (EPS) and Hewlett Packard Graphics Language (HPGL). Users should select the appropriate format based upon their intended use of the data and the software available to manipulate the file; refer to the discussion in Subsection 5.8.1. Encapsulated PostScript is the more

generally useful of the two formats these days; many graphics applications can work with EPS, and a savvy individual can edit these files, if necessary, using a text editor. To facilitate subsequent importing of the graphics file into an application, it is helpful to save the original file with the `eps` extension for Encapsulated PostScript, and either the `hpg` or `plt` extension for HPGL.

The administrative work to set up the necessary *pseudo-plotters* has been performed in our NMR Facility; users need to know only how to redirect their desired output to a file instead of a print/plot device. There are two ways to do this: (1) manually, where the user executes the necessary commands from the command line, or (2) via the customized Plot to File menu interface. For either method, once the plot device has been set to a *pseudo-plotter*, it must be restored to a physical plot device (e.g., `plotter='nmr_plot'`) after writing data to file before subsequent hardcopy output can be obtained.

- **Manual method**

  1. Define the active plot device by entering[5]
     `plotter='EPS1200R_file'|'EPS1200RC_file'|'HPGL1200R_file'`
  2. Execute the desired plot-related commands.
  3. Enter the command **`page('<path/>filename.ext')`** to write the graphics data to disk according to the filename, extension and optional path specified.
  4. When finished, set the active plot device back to the default plotter by entering
     `plotter='nmr_plot'`
     (or set to another available plotter, if desired).

- **Menu method**

  1. Use the Main Menu → Display → Plot to File sequence to access the new menu.
  2. Choose Select File Type then specify the desired file format.
  3. Select the desired plot-related menu buttons (or execute the commands manually).
  4. Choose Save File then specify a path (optional) and filename.

Refer to Subsection 5.9.4 for additional discussion regarding the menu method. On-line descriptions of the various menu levels are available from any menu level via the Help button. Figure 5.1 illustrates a graphics image created by plotting to a file. The desired spectral layout was plotted from *VNMR* to a file in EPS format; the file was then copied to the hard disk of another computer via the Samba server, and used (with rotation and scaling) as a graphics image in this document.

5.8.5 Copying from *VNMR* and Pasting into Other Applications

Several graphics-handling methods are available to manipulate images for inclusion in documents, etc. Examples include importing data into a document via the Microsoft Windows clipboard, or output directly to a graphics file in a specific format, as described in Subsection 5.8.4.

[5] The vertical bar means to use *one* ***or*** *another* of the multiple choices shown.

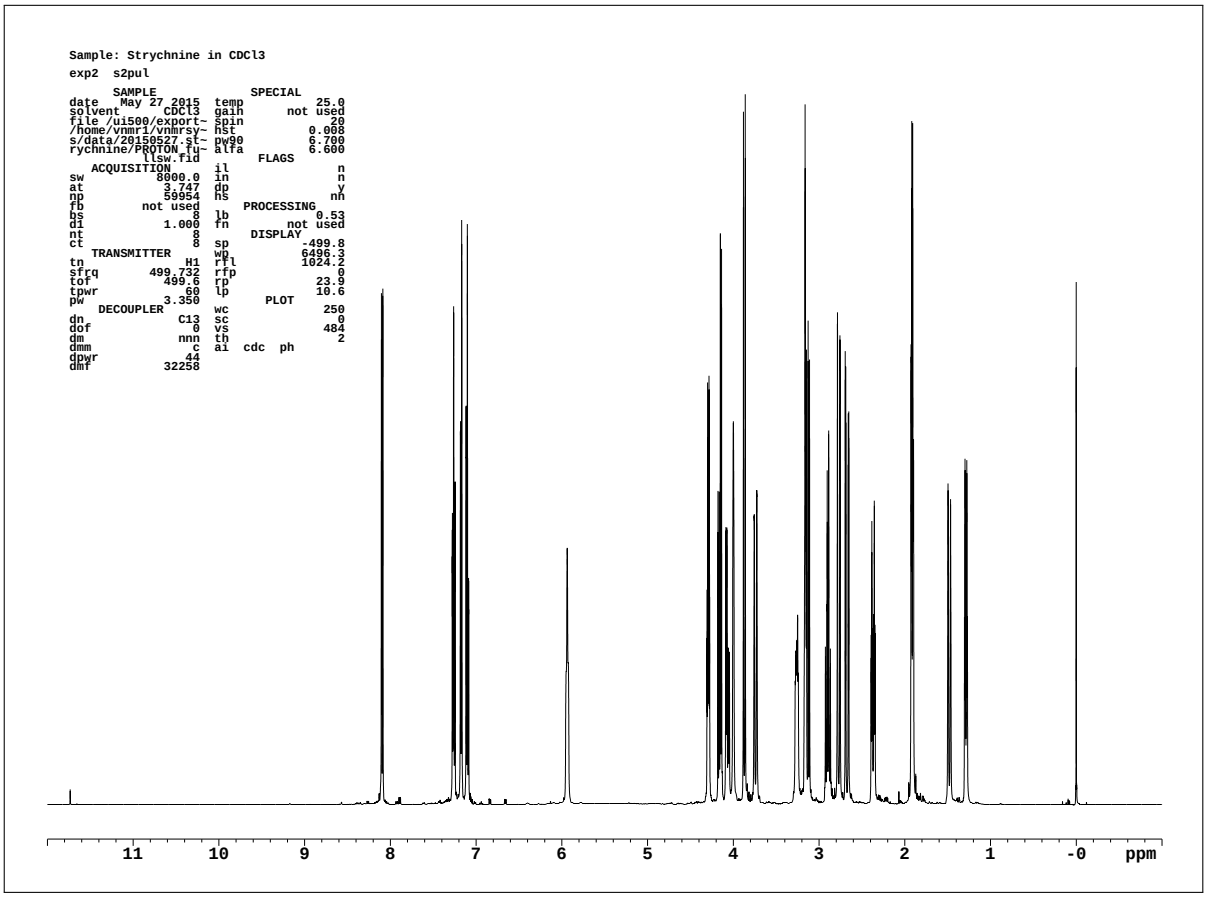

Figure 5.1 Graphics image imported into this LaTeX document as an Encapsulated PostScript file. The image has been scaled and rotated, and is shown with its bounding box for illustration.

Using X-Win32

With the X-Win32 application one can select graphics images from *VNMR* on the Sun (more correctly, from the image of the Sun output on the PC display) and direct the image to the PC's clipboard, a printer or a file. From the clipboard, the graphics data can be pasted into a chosen PC application. As an example, the following directions illustrate how to import spectral data into Microsoft PowerPoint. From an active X-Win32 session running *VNMR*, and with the spectral display composed as desired, perform the following steps:

1. Right-click with the mouse on the top border of the active X-Win32 session window.
2. Select the menu sequence Screen Shot → Rectangle.
3. Using the left mouse button, click and drag to select the desired region, then release the mouse button to capture the image.
4. A graphics preview window will open, giving options to Print, Copy to Clipboard, Save to File, or Close; for the purpose of this example, you would of course select Copy to Clipboard.

5. Open the desired application (e.g., PowerPoint) into which you want to paste the graphics data.

6. Select Paste from the Edit menu (or use the Ctrl+V method) to import the image from the clipboard.

Other options (Full Screen and Window) for graphics handling are visible from the X-Win32 menu at step 1 above; these can be explored by those interested. On-line help documents are available via the X-Win32 configuration utility.

5.9 Custom *VNMR* Menu Items

Several custom macros have been locally developed for implementation via the menu interface, in an effort to simplify and/or streamline spectrometer or workstation use. Clicking the Help button at any menu level brings up a description of the options available at that level. Descriptions of the Facility-specific (i.e., non-standard) menu buttons are shown below; it should be obvious that some of the functions are applicable only to a spectrometer host computer. Users are encouraged to suggest additional macro operations that would be useful to our user community.

5.9.1 From the Main Menu → Custom Macros Menu Level

These custom macros and corresponding menu buttons were developed to facilitate common tasks:

- NewUserSetup Sets up several global parameters for a new user's *VNMR* environment. Print and plot devices are set, `maclibpath` is created and set, `lockphase` is set to assist with autolocking, and parameters are set to enable DSP and PFG operation. Using this function more than once is not harmful. Performing this operation periodically will help ensure that the most recently customized default global settings are activated.

- SetMaclibPath Creates and sets each user's global parameter *maclibpath* to access the custom macros. This operation is normally executed via NewUserSetup rather than independently.

- Default Plot/Print Restores the default plotter functionality to the printer in room 1411.

- Null Plotter Sets the active plotter to a null device to suppress automatic hardcopies.

- Tune H,C Configures channel 1 for ^{1}H and channel 2 for ^{13}C, to facilitate tuning the Varian HCX probe on the UI-500. Refer to Subsection 7.2.1 for information about probe tuning.

- LoadShim Reads in and loads facility-optimized DAC values for the installed probe. This initialization operation is executed automatically before each automated gradient-shimming operation.

- Reset Performs the steps necessary to reset the host-to-acquisition computer communication channel. Refer to Subsection 5.11.2 for more information.

- Return Returns control to the Main Menu level.

5.9.2 From the Main Menu → File → Set Directory Menu Level

The following two menu options[6] complement the standard *VNMR* menu buttons; the other buttons at this level retain their normal functionality.

- nmr03 Data Changes the current working directory to the user's `vnmrsys/data` directory on the computer *nmr03*.
- ui500 Data Changes the current working directory to the user's `vnmrsys/data` directory on the computer *ui500*.

5.9.3 From the Main Menu → Display → Plot Menu Level

The following two menu options complement the standard *VNMR* menu buttons for the 1D plot menu level; the other buttons at this level retain their normal functionality.

- Plot Adjusts `vp`, if necessary, then sends spectral data to the plotter buffer. This is the Plot button *within* the 1D Plot *menu*.
- Integrals Plots the "non-normalized" integral values below the axis. Refer to the *VNMR Command and Parameter Reference* manual for descriptions of the `dpir`, `pir`, `dpirn` and `pirn` commands.

5.9.4 From the Main Menu → Display → Plot to File Menu Level

The specific menu items available at the Display and Plot to File levels depend upon the dimensionality of the data set loaded at the time of menu access: either the 1D or the 2D Display menu-level options will be active. The following descriptions are valid for both 1D and 2D data sets, and the menu levels are designed to complement the standard *VNMR* menu buttons; the plot-command buttons at this level have the same functionality as elsewhere.

- Select File Type The user must first choose a graphics output format (i.e., EPS or HPGL) and color option (color or monochrome) for the file. This step sets the current plotter designation to a physically non-existent plotter, but subsequent graphics output is run through an appropriate printer driver.
- Use the Plot ... Peaks (for 1D) or All Contours ... Params (for 2D) menu buttons to perform the desired and familiar plot-related operations; alternatively, one can manually enter their corresponding commands, if known.
- Save File Allows the user to specify a file name, then writes the graphics data to that file (be aware of the directory location for the file).

After selecting the file type, the current plot device (as specified by the `plotter` parameter) will remain active for plotting to a file until otherwise changed. When you are done plotting to file(s), you may want to reset the plot device to a physical plotter, e.g., via the Default Plot/Print menu button or by explicit assignment such as `plotter='MyPlotter'`; otherwise, subsequent plots intended as hardcopy output to a real plotter will be lost in the ether. Recall that default values are set for both plotter and printer devices each time *VNMR* is started.

[6] For those who prefer typing to mouse-clicking, the underlying macro names are `cd3` and `cd5`, respectively.

5.10 Custom *VNMR* Macros

In a similar effort to simplify and/or streamline use of the spectrometer, many custom macro commands have been developed in our laboratory. The functionality of these macros is initiated by entering the macro name on the *VNMR* command line. For various reasons — which may be discernible — some of the macros described below are not available on both the UI-500 host computer (*ui500*) and the workstation (*nmr03*).

Table 5.9 Custom *VNMR* macros available on the UI-500 host computer and *nmr03* workstation.

Macro Name	Description
`bcarray`	Performs a default baseline correction on arrayed 1D spectra (takes no arguments); refer to the *VNMR* `bc` command.
`bflp`	Configures sequential backward and forward linear prediction in the directly detected dimension (e.g., for ^{19}F 1D data or the t_2 dimension for 2D data), by creating and initializing the required parameters. Refer to the *VNMR* documentation for linear prediction details.
`blp`	Configures backward linear prediction in the directly detected dimension. Syntax: `blp(LP_points<,basis<,coefficients>>)`
`cd3, cd5`	Changes the current working directory used in *VNMR* to the user's data directory on *nmr03* or *ui500*, respectively.
`cnpoint`	Creates and initializes the parameter `npoint`, primarily for use in analyses of spin-relaxation, kinetics or PFG diffusion experiments.
`dcarray`	Performs linear baseline correction on arrayed 1D spectra; refer to the *VNMR* `dc` command.
`dlim`	Sets the spectral display limits for 1D, 2D homonuclear or 2D heteronuclear spectra; 1D and 2D cases are detected automatically. Example 1: `dlim(0.5p,8.5p)` sets the spectral display/plot region from 0.5 to 8.5 ppm for a 1D spectrum or in both F_2 and F_1 dimensions for a 2D homonuclear spectrum. Example 2: `dlim(0.5p,8.5p,20.0d,120.0d)` sets the heteronuclear spectral display/plot regions from 0.5 to 8.5 ppm in F_2 and from 20.0 to 120.0 ppm in F_1. (Note that the unit `d` indicates ppm with respect to the *decoupler* channel.)

Continued on the next page ...

Table 5.9, continued from the previous page

Macro Name	Description
`dodc`	Performs a linear baseline correction on both dimensions of 2D spectra; refer to the *VNMR* `dc2d` command.
`fft`	Applies `lb=1/at wft zpp aph vsadj ds cdc dc dscale`; works for individual or arrayed 1D data.
`invert`	Changes `rp` by 180 (degrees) to invert the phase of a 1D spectrum or the directly detected dimension of an nD data set.
`nbpexpl`	Sets the dimensions appropriately for notebook-sized output, issues the `pexpl` command to send exponential data to the plotter for output, then resets the plot dimensions to their original values.
`playout`	Sets the page-layout parameters for homo- and heteronuclear 2D spectral display or plotting to the values shown in Table 5.8.
`plcosyeps`	Similar to the *VNMR* `plcosy` macro, but without the internal `page` command. This macro is intended for plotting homonuclear 2D spectra to a file. Refer to Subsection 5.5.1 for information about the `plcosy` macro and its variants, and to Subsection 5.8.4 for details about plotting to a file.
`plot2D`	This is a corrected version of the *VNMR* `plot2D` macro; refer to Subsection 5.5.3 for usage details and correct syntax.
`plot2Dps`	Facilitates plotting of phase-sensitive 2D spectra with different contour densities for positive and negative peaks (useful with a monochrome plotter); the default values can be over-ridden by user input.
`plotall`	Plots a horizontal array of 1D spectra with a full parameter listing (`pap`) positioned in the upper right-hand side of the page; this is designed especially for spectra of diminishing intensity, e.g., T_2 and diffusion data.
`plotcosy`	Similar to the *VNMR* `plcosy` macro, but plots the high-resolution traces at a larger scale, performs a `dc` and uses `pap` instead of `ppa`.
`plothomo2D`	Similar to the `plotcosy` macro above, it plots the high-resolution traces at a larger scale and performs a `dc`, but does not include parameters or perform an internal `page` command; it is intended for plotting homonuclear 2D spectra to a file. Refer to Subsection 5.8.4 for details about plotting to a file.
`plothomo2Dvol`	Plots homonuclear 2D spectra with volume integrals via `plothomo2D`.
`read_lifrq`	Reads previously defined integral regions from a file (as written by `save_lifrq`, described below) for reuse with another **similar** data set. File name and directory specifications are as described below for `save_lifrq`.

Continued on the next page . . .

Table 5.9, continued from the previous page

Macro Name	Description
`rsfid`	Calculates the *VNMR* `lsfid` parameter based on acquisition parameters, to right-shift ('negative left-shift') the FID. Useful for data processing in conjunction with the `blp` or `bflp` macros described above.
`save_lifrq`	Writes the currently defined integral regions to a file for subsequent reuse with another **similar** data set. An optional file name can be specified; otherwise, the default file name is `lifrq.dat`. Files are written to, and read from, the user's `vnmrsys/lifrqdir` directory. Refer to `read_lifrq` above.
`setdconi`	Creates the *dconi* parameter and sets `dconi='dpcon,16,1.1'`. The numerical values can subsequently be changed to effect the desired contour density; Refer to the *VNMR* `dconi` macro for related information.
`ssb`	Sets sine-bell apodization functions. Syntax: `ssb(phi,span,'dim')`, where *phi* is the phase angle for shifting, in degrees; *span* is the time- domain span over which the function applies, expressed as a fraction of the applicable time (a negative value applies a sine-bell squared function); *dim* is the dimension in which to apply the function, e.g., *t2* or *t1*. Example: `ssb(45,0.75,'t1')` applies a 45-degree shifted sine-bell over 75% of the total of the t1 increments plus any linear predicted extension that exists.
`tempcal_sop`	Facilitates and improves upon the *VNMR* `tempcal` macro by accurately finding the calibrant resonance maxima, and by displaying and, optionally, plotting the results.
`zpp`	Applies `rp=0 lp=0` to zero the spectral phase parameters.

Table 5.10 Custom *VNMR* macros available on the UI-500 host computer. Post-acquisition processing macros in this table are also available on the *nmr03* workstation. Table 7.4 and Table 7.5 summarize additional experiment driver macros that are not listed here.

Macro Name	Description
`loadshim`	Loads the standard shim file for the currently installed probe.
`noDnmr`	Facilitates setup for experiments using non-deuterated solvents. Refer to Section 7.18 for details.

Continued on the next page ...

Table 5.10, continued from the previous page

Macro Name	Description
`set`	Intended for quick experiment setup, this macro applies `nt=1 ss=0 wshim='n' alock='n' gain=30 gain='n' su`.
`setdec`	Facilitates setup of the first or second decoupler for simple applications by extracting the appropriate parameters from the probe file. Syntax: `setdec<('nucleus'<,channel<,offset>>)>`, where `nucleus` is the nucleus to be decoupled (the default is `C13`), `channel` is the desired decoupler channel (the default is the first decoupler channel), and `offset` is the desired decoupler offset in ppm. Example: `setdec('P31',1,-40)` sets up ^{31}P decoupling on the first decoupler channel with `dof` set to −40 ppm in the ^{31}P spectral range.
`seth1`	Applies `setup('H1','d2o') setsw(9.0,-1.0) pw(10) nt=1 ss=0 wshim='n' alock='n' gain=0 spinoff su`.
`setz0`	Calculates and sets `z0`, e.g., for use with non-deuterated solvents; `setz0` is called automatically by the `noDnmr` macro but may be used independently.
`spinoff`	Applies `spin=0 spin` to turn the spinner off.
`spinon`	Applies `spin=20 spin` to turn the spinner on at 20 Hz.
`sett1`	Sets up an inversion–recovery T_1 relaxation experiment; refer to Section 7.6 for details related to relaxation experiments.
`taucalct1`	Interactively calculates a quadratically spaced array of τ values for an inversion–recovery T_1 relaxation experiment.
`proct1`	Processes inversion-recovery T_1 data for subsequent analysis via the *VNMR* `t1` or `t1s` macros.
`sett2`	Sets up a CPMG T_2 relaxation experiment; refer to Section 7.6 for details related to relaxation experiments.
`taucalct2`	Interactively calculates a quadratically spaced array of τ values for a CPMG T_2 relaxation experiment; it also writes a setup record as a uniquely named file.
`proct2`	Processes CPMG T_2 data for subsequent analysis via the *VNMR* `t2` or `t2s` macros.
`ecosy`	Driver macro to convert an optimized 1D proton data set to an ECOSY experiment; refer to Section 7.9 for details.
`hetloc_gse`	Driver macro to convert an optimized 1D proton data set to a HETLOC experiment; refer to Section 7.14 for details.

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Table 5.10, continued from the previous page

Macro Name	Description
`hetloc_mod`	Driver macro to convert an optimized 1D proton data set to a HETLOC experiment; refer to Section 7.14 for details.
`hetloc_proc`	Macro to process HETLOC data acquired via either the `hetloc_gse` or `hetloc_mod` experiment; refer to Section 7.14 for details.
`PGSE`	Driver macro to configure the PGSE experiment, starting from an optimized 1D spectrum; refer to Section 7.19 for details.
`PGSElog`	Analyzes PGSE or PGStE data and writes the output to a text file for subsequent use in a curve fitting program.
`PGStE`	Driver macro to configure the PGStE experiment, starting from an optimized 1D spectrum; refer to Section 7.19 for details.
`PGStElog`	Executes `PGSElog` to analyze PGSE or PGStE data.
`gzar`	Constructs an array of equally spaced `gz1` values for the PGSE or PGStE experiment; the `gzcalc` macro is typically preferable. Syntax: `gzar<(array_size<,min_value<,max_value>>)>`
`gzcalc`	Constructs an array of quadratically spaced `gz1` values for the PGSE or PGStE experiment; this macro is typically preferable to `gzar`. Syntax: `gzcalc<(array_size,min_value,max_value)>`
`PRESAT_UW`	Driver macro to configure PRESAT solvent suppression, starting from an optimized 1D spectrum. Refer to Subsection 8.3.1 for details related to this family of presaturation experiments.
`PSgDQCOSY`	Driver macro to convert an optimized 1D PRESAT proton data set to a PRESAT gDQCOSY experiment.
`PSgHSQC`	Driver macro to convert an optimized 1D PRESAT proton data set to a PRESAT gHSQC experiment.
`PSNOESY`	Driver macro to convert an optimized 1D PRESAT proton data set to a PRESAT NOESY experiment.
`PSopt`	Master macro to optimize the PRESAT solvent suppression parameters. The `PSoptc` and `PSoptf` slave macros are called internally to perform coarse and fine optimization steps, respectively.
`tunehc`	Configures the hardware for tuning the ^{1}H and ^{13}C probe circuits, channels 1 and 2, on the Varian HCX probe. The Tune H,C menu button executes this macro. Refer to Subsection 7.2.1 for details.
`wet`	Master macro to configure the WET solvent suppression method, starting from an optimized 1D spectrum; the `wet1d` and `wetit` slave macros are used internally. Refer to Subsection 8.3.2 for details.

Continued on the next page . . .

Table 5.10, continued from the previous page

Macro Name	Description
`wetopt`	Master macro to optimize the parameters for the WET solvent suppression method. The `wetoptfine` and `wetoptfinal` slave macros are called internally to perform additional, and critical, optimization steps.

5.11 Miscellaneous Considerations

5.11.1 Recovering After Locking Yourself out of *VNMR*

Despite detailed explanations about the origin, and numerous cautionary warnings for prevention, users inevitably find themselves "locked out" of the *VNMR* software. Typical symptoms include:[7]

(1) *VNMR* warnings about being "`Unable to lock experiment` *n*";
(2) warnings that certain variables, such as `seqfil` or `wc`, are undefined or do not exist;
(3) the entire lower row of menu buttons is missing;
(4) indication of being in `Exp:0` rather than, e.g, `Exp:1`; and
(5) the message "`foreground processing active`".

Getting Locked Out

Getting locked out of *VNMR* is not a software bug or related issue; it usually represents a breakdown of the chair-to-keyboard interface. Common to all computer applications that allow multiple and simultaneous access to the same files or other electronic resources, is the potential that one client (e.g., a human) may alter a particular file that is simultaneously being used — if only in principle — by another client. Imagine two individuals simultaneously editing the same financial report located on a company's server! This potentially disastrous competition can be avoided by the use of a *lock* file: At the first instance of a client (NMR user) accessing a protected resource (a *VNMR* experiment workspace, e.g., `exp1`), the parent application (*VNMR*) creates a lock file (e.g., `lock_1.primary` in the user's `/export/home/username/vnmrsys` directory); the presence of the lock file signals to other instances of the parent application (and possibly other applications as well) that the resource is in use and therefore cannot be accessed by additional clients or applications.[8] When the original client is finished with the resource (e.g., by closing the file), the parent application should delete the lock file, thereby releasing the resource for access by another client. *VNMR* deletes the lock file when it, *VNMR*, is properly exited. If the application is improperly terminated — such as by effectively crashing the program — then the lock file does not get deleted as intended.

Prevention

Recall[9] that a gram of prevention is worth a kilogram of cure! When done working with *VNMR*, remember to exit the application, either by (1) entering **`exit`** on the *VNMR* command line or by (2) using the Main Menu → More → Exit VNMR mouse-clicking route. Only after exiting *VNMR* should the Sun log-on session be terminated. X-Win32 users beware: **Do not** use the Windows application's ☒

[7] These signs are typical; individual experiences may vary.
[8] Alas, this is strictly true only for applications that know and follow the specific rules.
[9] More succinctly expressed by $P(\text{g}) = C(\text{kg})$.

icon in the upper-right screen corner as an alternate way to "close" *VNMR*, as this closes the X-Win32 application and crashes *VNMR* on *nmr03*, causing you to become locked out!

Another way to be caught off guard is via a Microsoft Windows computer configured to perform automatic operating system updates. Such updates commonly require a system reboot to complete the process; if the update and restart occur while a remote session is active — perhaps left unattended during lunch or overnight — the X-Win32 application will be shut down without properly exiting *VNMR* or the remote log-on session, and a mysterious *VNMR* lockout will occur. Disallow fully automatic updates to prevent this from happening. Configure the PC to either (1) automatically download the updates, followed by manual installation and restart, or (2) notify the operator when updates are available, but not automatically download or install them. Each of these options allows the operator to properly exit *VNMR*, log off from the remote computer and close X-Win32 before the computer restart operation. If fully automated updating must be maintained, then active remote sessions should not be left unattended.

Recovery

Despite the foregoing discussion and tips for prevention, suppose you still find yourself locked out of *VNMR*. Now what? One method of several possible is given below, making use of the *VNMR* `unlock` command; refer to the *VNMR Command and Parameter Reference* manual for details of `unlock`. Although it is helpful to know which experiment workspace is locked, this is not strictly necessary.

1. If you can determine which experiment workspace is locked, enter the command **`unlock(`***n***`)`** on the *VNMR* command line, where the integer *n* identifies the locked experiment workspace. (For example, if `exp5` is locked, enter **`unlock(5)`**.)

2. If you do not know which experiment workspace is locked, use the systematic guesswork method (enter **`unlock(1)`**, **`unlock(2)`**, **`unlock(3)`**, etc.) until victory is yours. Pay attention to the messages generated, as a guide to your progress; at some point you should be able to continue on to the next steps.

3. *VNMR* will release the lock and properly *join* the experiment workspace as it normally would, displaying a message that the indicated experiment was unlocked. (Continuing the example, the message will read "`experiment 5 unlocked`".)

4. Next exit then restart *VNMR* to complete the recovery procedure. Normal behavior and functionality should now be restored. Congratulations!

5.11.2 Communication Between the Host and Acquisition Computers

The spectrometer host computer (Sun Ultra 10) and acquisition computer (a dedicated, modular unit in the left-hand console bay) communicate bidirectionally to control the spectrometer operation. Details of the desired NMR experiment are ***su****bmitted* (via the `su` command)[10] to the acquisition computer, which controls data acquisition and periodically transfers data packets of ***b****lock* ***s****ize* (set by the `bs` parameter) signal-averaged FIDs to the host computer for subsequent processing, plotting, saving, etc.

The *VNMR* `Expproc` program family carries out the host-to-acquisition communication details via ethernet connection between the two computers. This communication link occasionally suffers a failure,

[10]Note the mnemonic origin of the *VNMR* `su` command, which can be executed independently but is an integral and transparent component of the `go`, `ga` and `au` commands.

typically of unknown origin. The acquisition computer may periodically require a reboot to recover from an abnormal state. In addition to malfunctioning spectrometer components, external stimuli — such as line voltage drops or power surges — can be the underlying cause of apparent communication problems.

When a host-to-acquisition communication error occurs, communication fails in both directions: from the host computer to the acquisition computer, and from the acquisition computer to the host computer. Such failures are thus discovered through the loss of a normal action or process that involves such communication; examples include:

(1) the sample eject function doesn't work (i.e., the eject air fails to turn on or off);
(2) an acquisition command (e.g., via CustomQ or the `go` command) fails to initiate acquisition;
(3) accumulated data may not transfer to the host computer as expected (more likely to be noticed during a long-term acquisition such as 1D ^{13}C or 2D experiments); and
(4) an "`Acquisition system is not active`" error message may be displayed.

It is generally good practice to pay careful attention to error messages, perhaps even recording their details for future reference. The following two sections provide instructions to execute if faced with an apparently broken communication channel between the host and acquisition computers.

Restarting the Host-to-Acquisition Communication Programs

Restarting the host–acquisition communication programs is a robust, simple, two-step procedure requiring only about 30 seconds; perform these operations first:

1. Enter the command **`reset`** on the *VNMR* command line; alternatively, you can use the menu selections Main Menu → Custom Macros → Reset to achieve the same result. The `reset` command acts as a toggle to kill `Expproc` if it is running or start `Expproc` if it is not running. Upon entering `reset` the first time, the user **should** be presented with a message in the *VNMR* text window that begins with "`Killing Expproc ...`".

   Pay careful attention at this point! If Expproc was actually turned off (e.g., by the person who used the spectrometer before you) before you enter a `reset` command, Expproc will restart at this stage, which is desired. In such a case, **do not** continue with the next step.

2. Once the `Expproc` program has been shut down, restart it by entering a second **`reset`** command, which will result in the message "`Starting Expproc ...`".

3. Enter the **`su`** command to initialize the spectrometer hardware.

This is a good place for a reminder about the *VNMR* Help menu button available next to the Main Menu button; clicking Help from any submenu brings up a corresponding set of brief descriptions in the text-display window. If the previous three-step procedure fails to remedy the initial communication problem, proceed to the instructions enumerated in the next section.

Rebooting the Acquisition Computer in the Spectrometer Console

If the preceding procedure fails to re-establish host–acquisition computer communication, then the acquisition computer in the console may require a reboot, as described in the following steps:

1. Enter a **`reset`** command or its equivalent to stop `Expproc` as described above.

2. Open the left-hand door of the spectrometer console, noting that the doors open outward from the center (i.e., the hinges are on the outsides of the doors) and are held closed by magnetic latches.

3. Locate the acquisition computer card on the far left-hand side in the row of vertically oriented modules; it is labeled 'CPU' near the bottom of the module.

4. Between the two ethernet cables plugged in near the top of the CPU module are two small buttons marked ABT (for ABORT) and RST (for RESET); press the RST button once and wait.

5. You should notice the red FAIL light come on, on the MAGNET/SAMPLE REGULATION (MAG SMPL REG) module located 8 slots to the right of the CPU module, in the 9th position.

6. During the next 45 seconds or so, the acquisition computer will reload instructions, reboot and execute a self-test of the various digital components.

7. When finished, the MAGNET/SAMPLE REGULATION module's red FAIL light will go off and the green READY light will come on.

8. Issue another **`reset`** command to start `Expproc`, followed by the **`su`** command to initialize the spectrometer hardware.

If this procedure fails to remedy the original symptoms, notify the NMR Facility Director or other appropriate staff, and include your recorded details of any error messages. Attempt no further actions with the spectrometer.

Chapter 6

Sensitivity Issues in NMR

6.1 Introductory Definitions and Principles

NMR is a relatively insensitive method compared to other analytical techniques; however, consistent technical advances over the years have made for steady improvements. The sensitivity specification for signal-to-noise ratio (S/N), as used in the field of NMR spectroscopy, is defined as the maximum height of the resonance absorption-mode signal divided by twice the root-mean-square (rms) noise.[1] Peak-to-peak noise and rms noise are related by $N_{\mathrm{pp}} = 5N_{\mathrm{rms}}$, leading to the following definition for what is actually measured when an NMR signal-to-noise ratio is quantified:

$$S/N = R(\omega) = \frac{S(\omega)}{2N_{\mathrm{rms}}(\omega)} = \frac{5S(\omega)}{2N_{\mathrm{pp}}(\omega)}. \tag{6.1}$$

These terms are written implicitly here as functions of angular frequency as a reminder of their frequency dependence. Directly presenting an equation suited for the purposes of the current discussion — e.g., regarding the direct detection of an NMR signal by the pulse–acquire method — we have[2]

$$R(\omega) = \left(\frac{\gamma\hbar^2 I(I+1)}{24k^{3/2}\mu_0^{1/2}}\right)\left(\frac{C'\omega_0^{3/2}(T_2^*)^{1/2}}{T^{3/2}}\right)\left(\frac{2\xi^2\rho Q V_{\mathrm{c}}}{\lambda F}\right)^{1/2} \sin\Theta_{\mathrm{p}}\sqrt{n_{\mathrm{t}}}. \tag{6.2}$$

This expression groups the various terms roughly according to their origins. Those terms within the first set of parentheses are fundamental constants; those within the second set of parentheses relate primarily to characteristics of the sample system itself; those within the third set are instrument-specific (probe, preamplifier, receiver, etc.). The $\sin\Theta_{\mathrm{p}}$ and n_{t} terms are acquisition parameters: $\sin\Theta_{\mathrm{p}}$ describes the projection of the magnetization onto the transverse plane following an RF pulse producing a tip angle Θ_{p}, and n_{t} is the number of transients acquired for signal averaging. Note that the total experimental acquisition time, t_{exp}, is proportional to the number of transients acquired: $t_{\mathrm{exp}} \propto n_{\mathrm{t}}$. The meanings of these symbols are described in Table 6.1.

The typical reader may now be asking "What's the point in all this?".[3] To answer, the points are several and have significant, practical importance; explicit descriptions are outlined within the remainder of this section, and various applicable relationships can be found throughout other parts of this document.

[1] This thermal noise is random, with a near-Gaussian amplitude distribution, and averages to zero when integrated over sufficient time or frequency space.

[2] For a more detailed yet accessible discussion of sensitivity in NMR, see *A Handbook of Nuclear Magnetic Resonance*, 2nd Ed., by Ray Freeman, Addison Wesley Longman, 1997.

[3] The *typical* reader probably skips this material, eh?

Table 6.1 NMR sensitivity-related definitions

Symbol	Description
γ	Magnetogyric (a.k.a. gyromagnetic) ratio of the nuclide
I	Spin quantum number of the nuclide $(1/2, 1, 3/2, \ldots)$
k	Boltzmann's constant ($k = 1.38066 \times 10^{-23}$ J/K)
$\hbar$	Planck's constant divided by 2π ($\hbar = 2.10915 \times 10^{-34}$ J·s)
μ_0	Permeability of free space (SI unit conversion factor)
C'	Number of spins per unit volume (an effective concentration)
ω_0	Larmor angular frequency of the nuclide ($\omega_0 = \gamma B_0$)
T_2^*	Effective T_2 (time constant for transverse relaxation)
T	Absolute temperature (Kelvin)
ξ	Filling factor of the probe receiver coil ($\xi < 1$)
ρ	Ratio of the effective inductance to the total inductance ($L_{\text{eff}}/L_{\text{tot}}$) of the probe receiver coil
Q	Quality factor of the probe coil (depends on design and materials)
V_c	Volume enclosed by the probe receiver coil (the active, or effective, volume); the volume accessible to detection
λ	Nagaoka's constant for the probe receiver coil; depends on the coil geometry and affects only the noise
F	Noise figure of the preamplifier ($F > 1$)
Θ_p	Effective tip (or "flip") angle of the radio-frequency pulse
n_t	Number of transients acquired for signal averaging; proportional to the total experiment time ($t_{\text{exp}} \propto n_t$)

To help make sense of what is often regarded as a complicated and confusing set of relationships, we discuss Equation 6.2 according to groups of terms and individual terms, and describe their influence on sensitivity as quantified by the signal-to-noise ratio. An introductory caveat is that some of the terms can, arguably, be classified in more than one category (fundamental constants, sample-specific, or instrument-specific); this point will be illustrated in the discussions below.

6.1.1 Fundamental Constants

Clearly, k, $\hbar$ and μ_0 are fundamental and beyond our control or influence. Of the other terms in the first parenthetical factor of Equation 6.2, γ is the most important, owing to its ultimate $\gamma^{5/2}$ dependence via the relationship $\omega_0 = \gamma B_0$, and one can refer to tabulated data of theoretical sensitivity to illustrate this influence. The gyromagnetic ratio is a fundamental constant in the sense that its value is invariant; however, the experimentalist typically has some latitude in choosing which nuclide is detected (for example,

detecting ^{1}H versus ^{13}C), and so in this sense γ could be viewed as a sample-dependent factor. This distinction is more academic than practical in the context of 1D experiments, because we typically choose which nuclide(s) to detect according to the desired information content; however, as we shall see later in Subsection 7.1.3, this sensitivity dependence on γ can be used to great advantage in heteronuclear 2D experiments.

Similarly, fundamental versus sample-dependent arguments can be made in regard to the spin quantum number, I, as well. For $I = 1/2, 1,$ and $3/2$, the term $I(I+1)$ yields $3/4, 8/4,$ and $15/4$, respectively, which would appear to translate into impressive sensitivity gains for nuclides with larger spin quantum numbers. However, since nuclides with $I > 1/2$ are quadrupolar and typically have quite small T_2^* values and correspondingly broad lines, any anticipated sensitivity gains are rarely realized. Exceptions to this generalization fall outside the scope of this document.

Other quantities implied by the definition of the term C' of Equation 6.2 are the natural abundance, A_{nat}, and the equivalence of the nuclide under consideration. Since C' represents the concentration of *equivalent spins* per unit volume, it is weighted by both the natural abundance and equivalence number of the nuclide. For example, if we desire spectral information about a methyl group, it is obviously more efficient to detect the proton signal than the carbon signal, given three equivalent protons per methyl group (3:1 equivalence number) and the facts that $\gamma_{^1\text{H}} = 4\gamma_{^{13}\text{C}}$ and $A_{\text{nat}}^{^1\text{H}} = 91 A_{\text{nat}}^{^{13}\text{C}}$. In contrast to viewing the effective concentration, C', as a fundamental property because of a nuclide's natural abundance, we must bear in mind that it is common — although expensive — to improve the detection sensitivity of so-called "rare spins" (e.g., ^{13}C and ^{15}N) through the use of isotopic enrichment.

6.1.2 Sample-Dependent Terms

To illustrate the effect of ω_0 on sensitivity, we use the $\omega_0 = \gamma B_0$ relationship and rewrite the product $\gamma\omega_0^{3/2}$ of Equation 6.2 as $\gamma^{5/2} B_0^{3/2}$ to more clearly emphasize the importance of γ and the relevance of B_0. The magnetic field strength is, of course, a critical property of the instrument, but it is sometimes under the control of the experimentalist, as many modern NMR labs have instruments with different field strengths. Although it is illustrated in Section 7.1.4 that other instrument-specific factors can outweigh the 3/2-power dependence of sensitivity on B_0, the interested reader should notice a theoretical 40-percent sensitivity increase upon going from a 400 to a 500 MHz instrument.[4]

The effective time constant, T_2^*, for transverse relaxation characterizes the lifetime of the FID.[5] From Fourier transform theory there exists an inverse relationship between the FID lifetime and the width of the spectral resonance: rapidly decaying FIDs (short lifetimes) have correspondingly broad line widths. Since the area of a spectral resonance remains proportional to the number of spins from which it originates, narrow resonances are more intense (taller) than their relaxation-broadened counterparts and therefore result in greater signal-to-noise ratios.

Competing temperature dependences generally have a canceling or overall negative effect on sensitivity, as the theoretical enhancement via the Boltzmann factor[6] at lower temperature is typically negated by the line broadening arising from more efficient spin relaxation due to slower molecular motions. For high-resolution studies, additional line broadening is likely to have the deleterious effect of obscuring critically important spectral information.

[4] The truly interested reader might even calculate and tabulate the theoretical results comparing sensitivity enhancement among today's available magnets, say from 400 to 900 MHz.

[5] In modern high-resolution NMR spectrometers, there is often little practical difference between T_2^* and T_2, the *natural* transverse relaxation time constant, owing to the ability to achieve a very high degree of magnetic field homogeneity.

[6] Refer to any standard NMR text for a discussion of the Boltzmann distribution into the eigenstates.

6.1.3 Instrument-Specific Terms

Considering the intended scope of the present discussion, we first note that ξ, ρ, Q, V_c, and λ describe properties of the NMR probe, and that the noise factor, F, is a function of the instrument's preamplifier.[7] The first take-home message emphasizes how important it is to understand the critical nature of the probe itself in determining the detection sensitivity of an NMR spectrometer.

Secondly, we focus on the roles of ξ and V_c, and exclude ρ, Q, and λ from detailed discussion. The filling factor, ξ, is related to how spatially close the probe detection coil is to the sample solution. If d_c and d_s represent the diameters of the coil and sample, respectively, the filling factor is related by $\xi \approx (d_s/d_c)^2$; the difference between these diameters includes the NMR tube wall thickness, air gap, coil form, etc. This mathematical expression describes something that most of us know from our own experiences,[8] that radio or television signal reception degrades when the receiver (the radio or probe coil) is farther from the transmitter (the radio station or nuclear spins). One approach to optimize NMR sensitivity is to manufacture sample tubes with very thin walls (to increase d_s) and coils into which the tubes fit with minimal clearance (to decrease d_c); however, note that $\xi < 1$ by physical constraints.

As for V_c, it may at first seem as though larger probe coil volumes (via greater length and/or larger diameter) would lead to dramatic improvement in sensitivity; however, there are inherent limitations and arguments against this approach. An increase in V_c would require a concomitant increase in solute to maintain the same concentration, C, and it is often desirable or necessary to work with less material rather than with more. Consider, for example, the increase in solution volume and amount of solute needed to change from a 5 mm to a 10 mm NMR tube. This approach to improving sensitivity has been employed in the past (e.g., with NMR tubes of up to 25 mm diameter, or with 5 mm diameter tubes in long probe coils) but has been essentially abandoned today except for special cases such as studies of polymers, which tend to be limited by their solubility. In fact, the opposite trend has been observed over the past several years: There has been a trend toward miniaturization to increase the filling factor term and simultaneously enable the routine detection of smaller amounts of material; this is especially vital in the fields of natural products chemistry, biochemistry and pharmaceutical chemistry, particularly in animal or clinical studies. Today there are a variety of specialty probes available for small-scale applications (e.g., ranging from 5 μL to 100+ μL sample volumes[9]), designed either for use with very small diameter glass tubes, or for injection into a fixed flow cell.

Cryogenically cooled probes (often referred to as *cold probes* or *cryo probes*) have been commercially available for decades, and are today routinely available in many NMR labs; they achieve superior sensitivity[10] by cooling the probe circuits and preamplifiers to near liquid nitrogen or liquid helium temperature, thus immensely reducing thermal noise, which is the predominant origin of the noise component. This approach serves as a reminder that one can improve sensitivity by increasing the signal, by reducing the noise, or by a combination of both.

6.1.4 Acquisition Parameters

Acquisition parameters are the spectrometer settings — nowadays set via computer software — that allow the experimentalist to control the details under which data are acquired; we discuss here only those

[7] On the Varian spectrometer in our laboratory, the reamplifiers are those long, narrow, rectangular boxes from which the RF cables connect to the probe.

[8] At least for those who remember radio and television in the days before cable, satellite or digital transmission.

[9] For comparison, conventional 5 mm tubes require approximately 700 μL of solution.

[10] Cold probes provide an approximately two-fold (nitrogen-cooled) to four-fold (helium-cooled) sensitivity increase for ^{1}H compared to conventional probes.

acquisition parameters having significant influence on detection sensitivity. Commonly referred to as the *pulse width* and typically given the parameter name `pw`, setting of the pulse flip angle, Θ_p, is at the user's discretion only in basic, one-dimensional, directly detected experiments; most if not all other experiments require sequences of pulses having specific flip angles. Discussion of optimizing Θ_p will therefore focus on its role in long-term data acquisition for the purpose of signal averaging to increase the signal-to-noise ratio.

Signal averaging involves the coherent addition of multiple (i.e., n_t) FID signals to achieve a sensitivity enhancement due to the $\sqrt{n_t}$ dependence of the signal-to-noise ratio. Consideration of signal averaging for long-term experiments naturally leads to viewing the signal-to-noise ratio in terms of its related "expense" per unit time; spin relaxation plays a crucial role in this cost analysis because of the time required to recover magnetization along the z axis following a pulse. The interested reader is, at this point, directed to a standard NMR text[11] for a detailed discussion of these issues and discussion of the so-called *Ernst angle*.[12]

6.1.5 Understanding the Relationships

Several important results will be discussed in the following subsections, which are under development as of Version 2020-05-15. Readers are invited and encouraged to work through the incomplete subsections below as exercises; you never know when a related question will appear on an NMR training quiz.

Time Dependence of the S/N Ratio

For basic ^{1}H (or even ^{19}F or ^{31}P) NMR experiments for most organic chemistry research, it is seldom necessary to consider or plan for how long the experiment will take. In most cases the spectrometer overhead (getting the sample into and out of the probe, tuning, field–frequency locking, shimming, etc.) requires more time than does the actual data acquisition. A total experiment time of 5–10 minutes is common for a modern instrument, with about 1–2 minutes required for the data acquisition component. The signal-to-noise ratio is seldom even considered, as a S/N of ca. 1000 is typical for commonly prepared samples.

Carbon-13, in contrast, is the epitome of insensitive nuclides, and it is often the case that some degree of planning is required in order to achieve a desired or acceptable signal-to-noise ratio. For ^{13}C, a S/N of 10 or greater (measuring the least intense signal, of course) is usually adequate, and a S/N greater than about 100 would be considered quite good for most work.

As a practical matter, it is sometimes desirable to first acquire a routine ^{1}H spectrum followed by a quick, or preliminary, ^{13}C spectrum using the same spectrometer overhead. The preliminary ^{13}C spectrum may be sufficient for the immediate needs, or it may be desirable to acquire a follow-up spectrum later, with more signal averaging (more transients) to achieve a greater S/N ratio. For example, a preliminary experiment may be used to assess the sample purity of an important product; if the purity is good, then publication-quality NMR data may be desired. The question is: How long will it take to achieve a specific S/N target?

[11] The discussion by Claridge in *High-Resolution NMR Techniques in Organic Chemistry* is highly recommended.

[12] The Ernst angle is the optimal pulse flip angle for achieving the best S/N per unit time, given a particular combination of T_1 and acquisition recycle time.

The answer is simple, and the following exercise provides a general result that can be used to mentally estimate the answer to similar questions in the future. Considering Equation 6.2 in light of this particular question, it should be clear[13] that we can achieve the simplification $S/N = \kappa\sqrt{n_t} = \kappa'\sqrt{t_{exp}}$, since the only difference between one hypothetical experiment and another is the number of transients accumulated, and the time required, for signal averaging.

Since the number of transients (n_t) and experimental acquisition time (t_{exp}) are proportional, we can choose either as the primary variable, then determine the other, secondary value afterward. Since time is such an important parameter for humans (remember, our goal is to plan how long it will take to perform a second experiment), we shall choose t_{exp} as the primary variable. Let's use t_1 to represent t_{exp} for the first experiment, and t_2 for the second; similarly, we'll use n_1 and n_2 to represent n_t for the two experiments.

Continuing symbolically, we have $(S/N)_1 = \kappa'\sqrt{t_1}$ and $(S/N)_2 = \kappa'\sqrt{t_2}$ to represent the two experiments. Taking the ratio $(S/N)_1/(S/N)_2$ and performing some basic algebra, we have as the result,

$$t_2 = t_1\left(\frac{(S/N)_2}{(S/N)_1}\right)^2 = t_1 f^2. \tag{6.3}$$

If this result or its meaning is not clear, think about it this way: The time required for the second experiment is the time of the first experiment multiplied by the ***square*** of the quotient of the second (target) S/N ratio divided by the first (measured) S/N ratio. If we regard the target S/N ratio as a multiple of the measured value, which is a very practical and common approach, then $(S/N)_2 = f(S/N)_1$, where f is a multiplicative factor by which we want to increase or improve the S/N ratio. Thus, if we want to increase the target S/N ratio by a factor or 2, it takes 4 times longer; a factor of 3 increase requires 9 time longer, etc. It is hopefully clear, too, that the second experiment will require $n_2 = n_1 f^2$ to meet the target S/N ratio.

This important relationship is shown graphically in Figure 6.1. Since the time required to meet a particular S/N ratio depends exponentially on the improvement factor, it rapidly becomes impractical to try to make huge S/N gains via increased experiment time alone. For example, if a preliminary ^{13}C experiment produced a $S/N = 5$ with 15 minutes of acquisition time (t_{exp}), it would require 1 hour to achieve $S/N = 10$, 4 hours to achieve $S/N = 20$, and an 8-hour acquisition time would result in $S/N \approx 28$, all of which would be practical experiments to perform, since it is fairly common to do such experiments within an overnight time period. Continuing with this same example, how long would an acquisition require to achieve a signal-to-noise ratio of 100? Would it be practical to attempt this experiment?

Concentration Dependence of the S/N Ratio

Assignment: Using an approach similar to that in the preceding subsection, derive an analogous equation relating sample concentrations and experiment times. Based on your result, what is the expected time savings (for a given S/N) when using a Shigemi NMR tube compared to a conventional NMR tube? If needed, refer to Section 6.2 for pertinent information about Shigemi NMR tubes.

[13] If it isn't clear, try to figure it out.

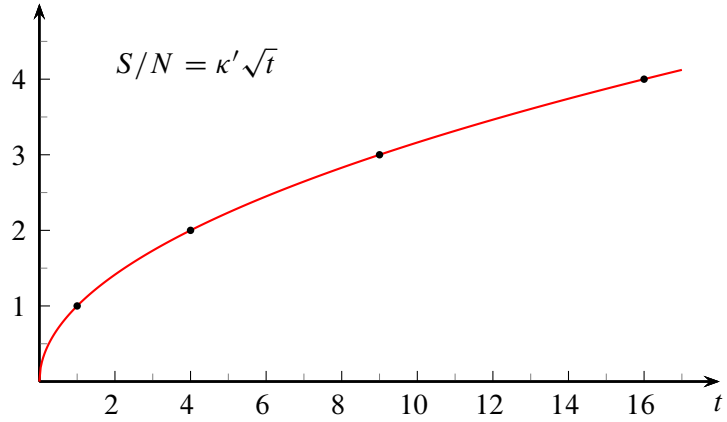


Figure 6.1 Normalized signal-to-noise ratio versus experiment time.

Instrument Dependence of the S/N Ratio

Assignment: Using an approach similar to those in the preceding subsections, derive an analogous equation relating experiment times for different instrument-and-probe combinations. For example, compare the HCX and QN probe on the UI-500, and/or the AV-400 Smart Probe versus the UI-500 QN probe. Based on your results, what are the expected time savings (for a given S/N) when using one instrument–probe combination compared to another? If needed, refer to the tables in Subsection 1.1.1 and Subsection 1.1.2 for pertinent data regarding probe performance.

Putting it All Together

At this point, readers should be able to effectively utilize the information from the preceding subsections to maximize detection sensitivity as needed for their experimental needs. If the detection capabilities of our NMR Facility still fall short of users' needs, be aware that there are several cold probe resources on campus that may be used for critical research.

6.2 Magnetic Susceptibility Matched NMR Tubes and Inserts

In order to optimize the magnetic field homogeneity to obtain truly *high-resolution* quality data, the sample solution volume in the NMR tube should extend above and below the probe coil by an amount approximately equal to that within the probe coil itself.[14] The UI-500 HCX and QN probes have coils that are 16 mm in height; this means that the NMR sample tube should be filled to about 48 mm total height, and that only about one third of the sample solute is actually being detected. This can be a severe restriction indeed when faced with a small and limited amount of sample.

[14] This is the well-known Stringfellow Law of Thirds.

Perusal of this information in light of Equation 6.2 above suggests that if we could confine all the sample solute within the volume of the probe coil, the result would be a 9-fold decrease in the experiment time required to achieve a given signal-to-noise ratio. There exist two approaches to achieve this end, using magnetic susceptibility matched materials in place of the volume of solution that would otherwise occupy the regions above and below the active volume of the probe coil: (1) Doty Scientific manufactures susceptibility matched NMR tube inserts, for use with regular NMR tubes; and (2) Shigemi Co., LTD manufactures high-quality, susceptibility matched NMR tubes. Wilmad Glass is a distributor for both the Doty inserts and Shigemi tubes. Although the Shigemi NMR tubes are somewhat more expensive to purchase[15] and must be handled very carefully (refer to Section 3.2), they are considerably easier to use and will last a long time if cared for properly.[16] Refer to Wilmad's excellent supporting information for NMR and EPR Consumables; this highly recommended resource has all manner of information related to NMR tubes, solvents and reference standards.

The NMR Facility has three sets of Doty magnetic susceptibility inserts and related tools, and a full set of Shigemi NMR tubes, available for trial use by our local NMR community; beyond trial use of this Facility equipment, users are expected to purchase their own supplies as with other NMR sample tubes, solvents, etc.

The three Doty insert sets on hand are compatible with (1) chloroform and water, (2) acetone and MEK, (3) methanol, MEK and ethyl ether. Table 6.2 presents information about these Doty inserts available for use in the NMR Facility.

The Shigemi Advanced NMR Microtubes are manufactured for use with four common NMR solvents ($CDCl_3$, CD_3OD, D_2O and DMSO). Available in 8 mm (Bruker), 12 mm (JEOL), and 15 mm (Varian/Agilent) *bottom lengths* designed specifically for the coil of the NMR probe in which it will be used, one must order the correct tube set to match the desired probe. Table 6.3 shows the part numbers and other information for the Shigemi tubes on hand and suitable for the Varian UI-500 probes.

Users who need to acquire NMR data with limited sample amounts should seriously consider using one of these susceptibility matched methods.

Table 6.2 Doty magnetic susceptibility-matched inserts and accessories

Part No.	Description	Material	$-\chi_V$
SP-GV-5	5 mm plugs, vented (Acetone, MEK)	G-10	0.50
SP-PSV-5	5 mm plugs, vented ($CHCl_3$, H_2O)	PPS	0.73
SP-GPV-5	5 mm plugs, vented (MeOH, MEK, EtOEt)	GFP	0.52
SP-PR-K-5	5 mm rod/clamp set	Kel-F	0.92
SP-PR-SC-5	5 mm sealing clamp		

$-\chi_V$ is reported here in c.g.s. units scaled by 10^{-6}.

[15] The greater expense, at approximately $140 per tube set, is well worth the extra initial investment.
[16] But then, you treat all of your NMR tubes with care, don't you?

Table 6.3 Shigemi Advanced NMR Microtubes

Part No.	**Solvent**	**Color**	$-\chi_V$
CMS-005TV	$CDCl_3$	clear	0.74
BMS-005TV	D_2O	clear	0.70
DMS-005TV	DMSO-d_6	green	0.68
MMS-005TV	Methanol-d_4	blue	0.53

$-\chi_V$ is reported here in c.g.s. units scaled by 10^{-6}.
The xMS-005TV tubes have a 15 mm bottom length, which is correct for the UI-500 probes.

6.2.1 Magnetic Susceptibility Data

Magnetic susceptibility data are reported in several ways; in c.g.s. units, a unifying relationship between the different standards is

$$\chi_M = M\chi_m = M\frac{\chi_V}{\rho}, \tag{6.4}$$

where χ_M, χ_m and χ_V are the molar, mass and volume susceptibility, respectively, M is the molar mass, and ρ is the density. Table 6.4 on page 70 lists molar and volume magnetic susceptibilities for common NMR solvents and other materials, ordered with respect to decreasing volume magnetic susceptibility. Note that deuterated solvents have magnetic susceptibilities very similar to their protonated equivalents.

If one must use a solvent for which an ideally matched sample tube or insert set is not available, determine the tube or material with the closest magnetic susceptibility; a match within about 5 percent should provide acceptable results. It is critical to properly prepare the tube with the solution and to position the sample tube correctly within the probe coil. For probes with a 16 mm coil (e.g., the UI-500 probes), use sufficient volume to make a solution height of 18–20 mm[17] and make sure that no air bubbles are trapped within the sample region; this ensures that shimming can successfully produce good line shapes. For similar reasons, use the sample depth gauge to carefully center the solution vertically within the probe coil detection region.

[17] It is left as an exercise for the curious reader to divine the physical reason why the solution should extend slightly beyond the coil ends.

Table 6.4 Physical properties of common NMR solvents and other materials, ordered by their volume magnetic susceptibility.

Solvent	$-\chi_M$	$-\chi_V$	ρ	η	ε	T_m	T_b
glycerol		0.78	1.26				
$CDCl_3$	59.3	0.74	1.50	0.58	4.8	–64.0	62.0
CD_2Cl_2	46.6	0.73	1.35	0.45	9.1	–95.0	40.0
H_2O	13.0	0.72	1.00	1.00	78.5	0.0	100.0
D_2O	12.8	0.70	1.11		78.5	3.8	101.4
CCl_4	67	0.69	1.58	0.97			
DMSO-d_6		0.68	1.18	2.20	46.7	18.0	189.0
toluene-d_8	66	0.62	0.94	0.59	2.4	–95	110.6
pyridine-d_5	48.8	0.61	1.05	0.95	12.4	–42	115
dioxane-d_8	52	0.61	1.13	1.44	2.2	11.8	101.1
benzene-d_6	54.8	0.61	0.95	0.65	2.3	5.5	80.1
ethanol-d_6	33.6	0.57	0.89		24.5	–114.1	78.5
acetonitrile-d_3	28	0.54	0.84	0.34[a]	37.5	–45	81.6
methanol-d_4	21	0.53	0.89	0.55	32.7	–97.8	64.7
diethyl ether-h_{10}	55	0.53	0.71	0.24			
diethyl ether-d_{10}			0.78			–116.3	34.6
MEK (2-butanone)	46	0.51		0.42			
acetone-d_6	34	0.46	0.87	0.30[a]	20.7	–94	56.5
nitromethane-h_3	21	0.39					100
nitromethane-d_3			1.19			–26	
THF			0.99	0.55	7.6	–109.0	66.0
Kel-F		0.92					
PPS		0.73					
Ultem		0.71					
Aurem		0.71					
Zirconia		0.70					
GFP		0.52					
G-10		0.50					

$-\chi_M$ and $-\chi_V$ are reported here in c.g.s. units scaled by 10^{-6}.
ρ is the density in units of g/mL, reported for 20 °C unless otherwise noted.
η is the viscosity in units of mPa·s, reported for 20 °C except as noted: [a] measured at 25 °C.
ε is the dielectric constant, reported for 20 °C unless otherwise noted.
T_m and T_b are the melting and boiling points, respectively, in °C.
Many data reported here are taken from the CRC Handbook of Chemistry and Physics, 71st Ed., 1990–1991, CRC Press.

Chapter 7

VNMR Experiment Guide

A goal of this chapter is to provide a unified source of important information related to many practical aspects of NMR experimentation. Although the content is directly intended for Varian UI-500 users, some of the information is general and platform independent. Discussed are instrumentation-related sensitivity issues, probe tuning, temperature control, chemical shift referencing, and the selection and execution of several experiments. The approach incorporates discussions of the "whys" in addition to explaining the "hows." Parts of this chapter aim to supplement, rather than replace, the *VNMR* documentation.

7.1 Which Spectrometer or Probe Should I Use?

This question is less relevant since the installation of the Bruker AV-400 (in early 2017) to replace the Varian UI-400, primarily because (1) the Bruker SampleXpress automation robot and IconNMR queue provide *very* convenient and robust data acquisition, and (2) the performance of the Bruker SmartProbe rivals or exceeds that of the UI-500 HCX or QN probes for all but the most challenging or specialized experiments. Note that the AV-400 is, in principle, capable of performing many specialized experiments; however, these tend to fall outside the normal philosophy of a walk-up spectrometer operating under automation. Lastly, it is important to recognize that the principles discussed in this section apply also to other NMR spectrometers and probes; these principles are not limited to comparisons between only our two instruments.

7.1.1 Spectrometer Smackdown

Comparing the sensitivity data from Table 1.1, Table 1.2 and Table 1.3, we see that the only area in which the AV-400 appears deficient compared to the UI-500 is ^{1}H detection. For ^{1}H, the UI-500 HCX probe performs significantly better, at about 1.5 times the detection sensitivity. For ^{13}C, the UI-500 QN probe is only marginally better, in principle, at 1.1 times the sensitivity. For routine work, these differences are unimportant. However, for cases of limited sample material, when the AV-400 time limits are insufficient for adequate data collection, the UI-500 becomes the next logical choice.[1]

[1] On the UW-Madison campus, both NMRFAM and the Chemistry Department NMR Facility have spectrometers with cold probes, and these resources may be available to our user community for their most challenging cases.

The sections below present discussions based upon different scenarios; brief discussions of equipment and terminology are first presented to help establish the proper context.

7.1.2 The Role of the Probe

The probe is unquestionably one of the most critical components of an NMR spectrometer;[2] it is arguably the single component with the greatest potential to provide incredible experimental specialization and versatility. The selection of an NMR probe is nearly always a trade-off between sometimes conflicting criteria, such as optimum performance, cost, degree of specialization, flexibility and convenience.

Common NMR experiments include ^{1}H 1D and 2D homonuclear (e.g., COSY, TOCSY, NOESY); 1D ^{13}C detection with ^{1}H decoupling; 1D detection of other nuclides such as ^{19}F, ^{31}P, ^{29}Si and ^{15}N (with or without ^{1}H decoupling); inverse-detection 2D heteronuclear experiments such as HSQC, HMQC and HMBC; and triple-resonance experiments (usually via inverse-detection) like a ^{1}H,^{13}C gHSQC with ^{31}P decoupling, or ^{1}H,^{13}C,^{15}N 3D experiments. Most probes include a ^{2}H lock circuit and variable temperature capability. A z-axis pulsed-field gradient (PFG) coil is valuable to suppress artifacts and accelerate acquisition times for multi-dimensional experiments, and is typically included in solution-state NMR probes these days. No single probe can perform optimally over a full range of all possible experiments, and probes designed to perform multiple types of experiments (e.g., quad-nucleus probes) usually suffer from performance compromises — although the best modern probe designs are significantly improved in these regards.

Lastly only in the order of this presentation, and not in its importance, the probe represents a rather fragile spectrometer component — a potential "single point of failure," if you will — that can render an NMR spectrometer system completely unusable if damaged by a falling, broken or improperly positioned sample, by mechanical failure (e.g., of the tuning mechanism), or by electrical breakdown of the circuit components from excessive radio-frequency or PFG pulse intensities or times.

7.1.3 Direct versus Indirect Detection

Terms relating to *direct* and *indirect* detection or experiments refer to the manner in which the NMR signal of the X nuclide is detected — where X is commonly used as a generic reference to any nuclide other than ^{1}H or ^{19}F.[3] The heart of the matter concerns the physical design and construction of the NMR probe. Dual- or multiple-detection probes usually have two distinct coils, one arranged within the other in a concentric configuration. Since the coupling of energy between the spin system (in the NMR tube) and the spectrometer depends upon the distance between the sample and the coil, the inner coil typically provides more efficient excitation and subsequent signal detection than the outer coil.[4] Probes that are optimized for direct detection of X have the X- and ^{1}H-circuit coils configured as the inner and outer coils, respectively;

[2] This section was inspired partly by an article in *Nuclei-Spin*, **2**(5), 2003.

[3] Note that there are exceptions to this common usage for X. See if you can understand the reason for the exception that arises in the discussion in Subsection 7.1.4 below.

[4] Recall the *filling factor* from Subsection 6.1.3.

thus, X detection is optimized at the expense of ^{1}H detection, historically a reasonable compromise since ^{1}H is typically much more sensitive anyway. The coil configuration is reversed for indirect-detection probes, with the ^{1}H coil closer to the sample — hence another common term, *inverse* detection.

Why were indirect-detection experiments developed? Besides making life more complicated for NMR spectroscopy neophytes, their existence owes primarily to the fact that they have a significant sensitivity advantage over directly detected experiments. This arises from detection via the more sensitive ^{1}H nuclide, as illustrated by the equation[5]

$$S/N \propto \gamma_{\text{exc}}\gamma_{\text{det}}^{3/2}\left[1-\exp(-R_{1(\text{exc})}t_{\text{rec}})\right], \tag{7.1}$$

where γ_{exc} and γ_{det} refer to the excited and detected spins, respectively; $R_{1(\text{exc})}$ is the longitudinal relaxation rate constant for the excited spins and t_{rec} is the recycle time for one transient (determined by the sum of the relaxation delay and acquisition time, plus other intervening events such as t_1 increments, inter-pulse delays, mixing times, etc.).

Let us now compare indirect- and direct-detection experiments for an IS spin system, where I refers to an insensitive nuclide such as ^{13}C or ^{15}N, and S refers to a sensitive nuclide such as ^{1}H or ^{19}F. In an indirectly detected experiment (e.g., HSQC) we excite S, then transfer magnetization to I and back again to the S spin (often represented schematically as $S \rightarrow I \rightarrow S$), which is detected; thus we have a sensitivity proportional to $n\gamma_S^{5/2}$, where the factor n is the multiplicity of atoms whose signal is measured (e.g., $n = 1, 2, 3$ for CH, CH_2 and CH_3 groups of protons, respectively (of course, $n = 2$ for CH_2 only if the two protons are equivalent)). In a directly detected experiment (e.g., HETCOR) we excite S, then transfer magnetization to the I spin ($S \rightarrow I$), which is detected, and the resulting sensitivity is proportional to $\gamma_S\gamma_I^{3/2}$. Taking the relative sensitivity of the indirect- and direct-detection experiments shows that the former is inherently more sensitive by the factor $n(\gamma_S/\gamma_I)^{3/2}$.

How significant is this result? If we recall that $\gamma_{^1\text{H}} \approx 4\gamma_{^{13}\text{C}}$ and $\gamma_{^1\text{H}} \approx 10\gamma_{^{15}\text{N}}$, we can work out that $(\gamma_S/\gamma_I)^{3/2} \approx 8$ for ^{13}C and 32 for ^{15}N; thus we should expect, e.g., an 8-fold increase in sensitivity for a ^{1}H,^{13}C HSQC experiment compared to HETCOR.

7.1.4 Directly Detected Experiments

Direct- and indirect-detection probes are clearly different; what about the related experiments? Examples of directly detected X experiments include the standard 1D sequences with or without NOE buildup and/or ^{1}H decoupling, the various DEPT and INEPT experiments, the 2D heteronuclear correlation (HETCOR) experiments, etc.

Example: Direct ^{13}C Acquisition

There is an important and multi-faceted topic in NMR spectroscopy, one that should be kept regularly in mind. This topic is easily illustrated in the form of the common question: "How much sample do I need to acquire a carbon spectrum?" The answer depends on a multitude of variables: formula weight, solvent, solubility, temperature, nuclide to be observed, type of

[5] The curious reader — and what is a scientist if not curious? — will no doubt want to work out how this equation relates to Equation 6.2 on page 61. Hmm? That would make a good quiz question.

experiment(s) to be performed, data quality desired, plus several factors concerning the NMR spectrometer. Recall that magnetic susceptibility-matched NMR tubes and inserts were discussed in Section 6.2 as a method to improve detection sensitivity for mass-limited sample quantities.

Here we compare the performance of our AV-400 and UI-500 spectrometers, with respect to direct ^{13}C acquisition. The AV-400's SmartProbe is optimized for X-nuclide detection, specifically ^{109}Ag through ^{19}F, via the inner coil. With no comparative data in hand to either support or refute the claim, a Bruker marketing brochure states that "[t]he SmartProbe™ delivers highest sensitivity on both the multinuclear and proton channel."[6] The UI-500's Varian HCX inverse-detection probe is optimized for ^{1}H-detected experiments and is technically not designed for direct ^{13}C observation; in contrast, the Nalorac QN direct-detection probe is the best choice for direct ^{13}C observation on the UI-500. How do these probes compare quantitatively?

Referring to sensitivity data shown in Table 1.1 and Table 1.3, we see that if the UI-500 Nalorac QN probe performance is compared with the AV-400's SmartProbe probe, we might predict an approximately 10-percent improvement in S/N for the UI-500 ($265/242 = 1.10$). The standard calculations to estimate anticipated performance gains, for either time savings or sensitivity improvements, show that only very modest gains are to be expected. These theoretical gains are very unlikely to be achieved in routine practice, however, because the spectrometer performance specification tests for sensitivity are very carefully executed under optimal conditions. The take-home message, then, is that the AV-400 and the UI-500 with the QN probe perform similarly in a direct comparison for ^{13}C direct acquisiiton.

If the Varian HCX probe is optimized for ^{1}H detection, does that mean it cannot perform ^{13}C detection? No, not exactly. However, let's compare the QN and HCX probes theoretically. If we take the ^{13}C detection sensitivities as 265 for the QN probe and 95 for the HCX probe (Table 1.2), and perform the standard calculation for relative acquisition times needed to achieve a particular S/N value, we see that it would require almost eight times longer with the HCX probe compared to the QN probe. Therefore, using the UI-500 HCX probe for direct ^{13}C acquisition is an unintelligent choice except under certain non-typical conditions.

To conclude this section, consider the previous discussion related to sensitivity enhancement via Shigemi NMR tubes (Section 6.2 on page 67). Suppose our hypothetical example from the paragraphs above involves a limited sample quantity as routinely prepared in a regular NMR tube. Further suppose that we concentrate that sample solution, by a factor of roughly three, to the volume required for use in a Shigemi tube ($\approx$ 250 μL). We could thus reduce a hypothetical experiment time by a factor of 9, and the results of a 12-hour overnight experiment (e.g., UI-500 with the QN probe) could be replicated within 1.5 hours; alternatively, we could perform a 12-hour overnight experiment and anticipate a 3-fold improvement in S/N.

7.1.5 Indirectly Detected Experiments

Common indirectly detected experiments include the 2D heteronuclear single-quantum coherence (HSQC), and the heteronuclear multiple-quantum coherence (HMQC) and multiple-bond correlation (HMBC) experiments. Many additional indirect experiments can be generated by extension to the third and higher dimensions.

[6] If true, then Bruker has achieved the Holy Grail of probe design.

7.2 Probe Tuning

Properly tuned probe circuits are essential for optimal instrument performance. Besides being required to achieve maximum detection sensitivity, most pulse sequences rely on accurately executed pulse flip angles for artifact-free data. An improperly tuned probe circuit is not only inefficient but can indirectly damage the probe components in some circumstances. With these statements in mind, the following NMR Facility policies exist with respect to probe tuning.

- Only NMR Facility staff will perform probe tuning for the UI-500 QN direct-detection probe. These types of probes require advanced knowledge and tuning methods that are beyond the routine capabilities of users without special training.
- Properly tuned, quad-nucleus probes generally deliver good performance with a variety of solvents. Only in cases of lossy samples (e.g., high salt concentrations) should additional adjustments become necessary. If such cases are anticipated, users should discuss the matter with the Facility director, who will tune the probe as needed for such samples.
- The UI-500's HCX triple-resonance, indirect-detection probe has a ^{1}H circuit that is very sensitive to the solvent used; the ^{13}C and X circuits are both relatively insensitive to solvent. For this probe, users **must** adjust the ^{1}H tuning with each NMR sample used; the other circuits should be checked also, if they are used, but adjustments are typically either minor or unnecessary.
- Every person using the Varian HCX indirect-detection probe on the UI-500 is required to know how to tune it properly, and to do so.

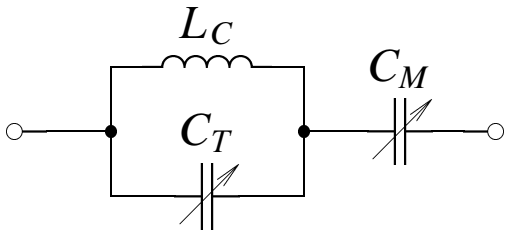


Figure 7.1 Diagram of a parallel LC resonance circuit, where L_C is the (fixed) inductance of the probe coil, C_T is the (variable) capacitance of the tune capacitor, and C_M is the (variable) capacitance of the match capacitor. Probe tuning involves adjusting C_T and C_M until the resonance condition of $\omega_0 = 1/\sqrt{L_C C_T}$ (tune) and a circuit impedance of 50 Ω (match) are met.

7.2.1 Tuning the Varian HCX Probe

Refer to Figure 7.2 below for an illustration of the external tuning-related components of the Varian HCX probe.

1. Use standard procedures to set up the desired experiment(s); make sure the sample is inserted into the magnet/probe.

2. Use **one** of these two methods, 2a or 2b, to configure the hardware for tuning the probe:

   (a) Use the `tunehc` macro — either by entering **`tunehc`** on the *VNMR* command line or via the Main Menu → Custom Macros → Tune H,C sequence — to configure channel 1 for ^{1}H and channel 2 for ^{13}C. This method and nuclide-to-channel correlation[7] is completely independent of the experiments set up or their order; when probe tuning is complete, the hardware configuration will revert to the previous settings. This method provides a simple and robust way to set up for the most common tuning configuration.

   (b) Set `tn`, `dn`, etc., followed by the `su` command, to manually configure the hardware to the desired settings. Determine which spectrometer channels are assigned to the various nuclides. (You may want to do this in a different experiment workspace, e.g. `exp5`, depending upon the application details; if so, remember to rejoin the original experiment workspace after probe tuning is completed.)

3. At the magnet, connect the desired probe cable to the TUNE port on the preamplifier housing TUNE INTERFACE, including any filters normally used in the circuit.

4. Switch the channel selector (CHAN) to the appropriate setting (e.g., to 1 for direct ^{1}H detection).

5. Note the value shown for the reflected power and the sensitivity (ATTEN) setting.

6. The goal is to achieve a reflected power of less than about 5 units at the maximum sensitivity (ATTEN) of 9.

7. Adjust the tune and match capacitors in an iterative or "matched" fashion to achieve the goal — it is usually possible to easily attain $\lesssim 3$ units for ^{1}H and $\lesssim 5$ units for ^{13}C and other X nuclides.

8. Switch the channel selector to 0 to turn off the tune interface, then return the cables to their operational positions.

9. Repeat as necessary for other nuclides and channels.

7.3 Temperature Control

The default behavior in our NMR Facility is for all standard parameter sets to regulate the temperature at 25 °C. How does one change the regulated temperature from the default setting? First, it is necessary to receive proper training, from NMR Facility staff, specifically about temperature control. **There are no exceptions to this rule!** Improper use has the potential to severely damage the equipment, resulting in significant down-time and expenses. Contact the NMR Facility Director to discuss matters relating to temperature control or to schedule the appropriate training.

[7] The user is reminded of the nuclide-to-channel correlation by a friendly yellow banner in the *VNMR* display window.

Figure 7.2 The Varian HCX triple-resonance probe: external tuning mechanisms

There are many critical issues at the heart of temperature control, and this document does not attempt to be all inclusive; rather, the objective is to provide an outline of the main operational steps as a supplement to the **mandatory** hands-on user training. Chapter 8 of the Varian *User Guide: Liquids NMR* manual is devoted to "Variable Temperature Operation" and is required reading for anyone performing temperature control in the NMR Facility.

There are one or two stages possible in the overall temperature control procedure, depending upon the actual sample temperature desired. The working temperature range for both UI-500 probe combinations is from approximately –20 to +100 °C. The sample system of interest may further limit the actual temperature range accessible. An FTS Systems pre-conditioning apparatus is available as a first stage in temperature control, if necessary. In all cases, final temperature regulation is achieved with additional hardware controlled by *VNMR* software and external controls. Operational guidelines are given below.

7.3.1 FTS Systems Preconditioner

The UI-500 NMR spectrometer is equipped with an FTS Systems preconditioning device to control either the pre-cooling or pre-heating of the gas (typically either dried air or nitrogen) used as a temperature control medium. The overall FTS Systems device is comprised of a refrigeration unit, a temperature controller and an 8-foot insulated transfer line with a heater located in the nozzle.

Follow these steps to precondition the VT gas when necessary for overall temperature control:

1. Switch on both the XR 401 Air-Jet refrigeration unit and the TC-84 controller unit; wait a few seconds for the latter device to go through its boot-up process.

2. Use the right-pointing arrow key on the TC-84 controller unit to scroll through the parameters until "SP °C" is selected.

3. Use the up or down arrow keys to select the desired temperature set point of the preconditioned gas supply. Note that this value must be less than the desired temperature to be regulated for the NMR sample; heat losses will be greater as one gets further from ambient temperature.

7.3.2 Temperature Control from *VNMR*

Several methods give equivalent results; example graphical and manual methods are described below. Check that the VT gas flow rate is at 10 L/min. The flow meter is located at the bottom on the front of the large box near the magnet leg; **never** adjust any of the gas pressure regulators on the wall-mounted manifold.

The Graphical Method

1. Before starting the acquisition process, enter the command **`temp`** to start the temperature controller graphical interface; use the slider bar to set the desired temperature.

2. Deselect the option to "Allow temperature control in an experiment with go," then select an option to specify the action to occur in the event of a temperature error.

3. Wait for temperature equilibration, then acquire the desired data. The temperature will be regulated at the specified value throughout the measurements, and that value will be reflected in the experiment parameters.

4. When the measurements are finished, reset the temperature to 25 °C, select the option to "Allow temperature control in an experiment with go," then exit the temperature controller graphical interface.

The Manual Method

1. Before starting acquisition, enter the command string **`temp=x su vttype=0`** (where **`x`** represents the desired temperature in °C) to initiate temperature regulation.

2. Wait for temperature equilibration, then acquire the desired data. The temperature will be regulated at the specified value throughout the measurements, and that value will be reflected in the experiment parameters.

3. When the measurements are finished, enter the command string **`vttype=2 temp=25 su`** to restore the default temperature regulation behavior.

7.3.3 Measuring the Sample Temperature

The temperature value monitored and reported by the VT controller is measured by a thermocouple positioned in the gas stream immediately below the NMR sample region, and is therefore not necessarily an accurate measure of the actual sample temperature. There can be a significant difference between the *set* value and the *actual* value, particularly at extreme temperatures, and the consequences are potentially damaging!

Various methods have been investigated and implemented to determine the actual temperature of the sample region; examples include the use of a thermocouple or other resistive device embedded within the sample volume. For common use, convenient methods have been exploited using NMR samples in which a particular nuclide exhibits a well-characterized, temperature-dependent chemical shielding. Common examples are indicated below.

^{1}H NMR Chemical Shift Thermometers

- **Low Temperature Range: methanol** (with 0.03 % concentrated HCl)

  temperature range: –100 to 55 °C

  $T_{\mathrm{meas}}[\mathrm{C}] = -23.83(\Delta\delta_{\mathrm{M}})^2 - 29.46(\Delta\delta_{\mathrm{M}}) + 129.8,$

  where $\Delta\delta_{\mathrm{M}}$ represents the chemical shift difference (in ppm) between the methyl and hydroxyl peaks. [106]

- **High Temperature Range: ethylene glycol** (neat)

  temperature range: 35 to 165 °C

  $T_{\text{meas}}[\text{C}] = -102.24(\Delta\delta_{\text{EG}}) + 192.6,$

  where $\Delta\delta_{\text{EG}}$ represents the chemical shift difference (in ppm) between the methylene and hydroxyl peaks. [107]

VNMR provides standard macros to facilitate measuring the sample temperature with the methanol and ethylene glycol samples. Refer to the `tempcal` entry in the Varian *VNMR Command and Parameter Reference* manual. A custom macro, `tempcal_sop`, was developed as an improvement over the standard *VNMR* macro; cf. Table 5.9. There exist several other *NMR thermometers* or *thermometric samples* in addition to the methanol and ethylene glycol standards indicated above, and there are particular reasons to use one specific standard instead of another. I have written an independent document[8] to describe and compare about a dozen different standards for NMR thermometers; those details are beyond the scope of this document, but the specialized document is available on request.

7.3.4 VT Checklist

- **Be careful with the equipment!** Temperature standards cost ~ $100. Even relatively minor probe repairs can cost several thousands of dollars. The potential exists for damaging or even destroying a magnet, with the associated repair costs running well over $10,000 for a best-case scenario.

- It is important to understand that the actual sample temperature can be 20 °C or more from the set point! Sample tubes can break and cause much damage in the process. **It is always the user's responsibility to ensure that VT experiments are performed safely.** Some guidelines are:

  1. Never go closer than 5 °C **actual temperature** to the solvent's boiling point.
  2. Never go closer than 25 °C set temperature to the boiling point without performing a temperature calibration *immediately prior* to the measurement in question.
  3. Never go closer than 5 °C actual temperature to the solvent's melting point.

- Never exceed the stated working temperature range of the NMR probe being used.

- The actual sample temperature depends upon the gas flow rate through the system. The relationship is not always what one might expect (e.g., a greater flow rate does not always lead to a more extreme actual temperature), so follow known operation guidelines unless performing a temperature calibration.

- VT users must allow sufficient time for temperature equilibration of the probe after their VT experiments. This typically means allowing a **minimum** of 30 minutes for equilibration from measurements at non-extreme temperatures. Greater temperature extremes

[8] "NMR Thermometric Samples and Calibration Data" by Thomas C. Stringfellow.

require longer equilibration times. It is the responsibility of the user to ensure that the research of others is not negatively affected.

7.4 Detection of Other X Nuclides

It is sometimes desirable and possible to acquire NMR data for nuclides other than ^{1}H, ^{13}C, ^{19}F and ^{31}P, which are routinely investigated in our facility. Other nuclides that have been studied in our lab over the years include ^{7}Li, ^{11}B, ^{15}N, ^{23}Na and ^{119}Sn. Two general questions are critical in determining whether a particular X-nuclide acquisition is possible and feasible: (1) Is the spectrometer (i.e., the probe and filters) capable of detecting the necessary frequency, and (2) does the X nuclide of interest offer sufficient detection sensitivity?

The UI-500 HCX probe X circuit is broad-band tunable from ^{15}N at the lowest frequency to ^{31}P at the highest. The UI-500 QN probe has two limited tuning ranges to accommodate ^{1}H and ^{19}F at high frequency, and two for ^{13}C and ^{31}P at low frequency, and is thus not broad-band tunable. The term *limited tuning range* indicates a range sufficient to optimize the tuning for the desired nuclide in a variety of solvents with a wide range of dielectric constants, but insufficient to observe other nuclides, since they tend to possess Larmor frequencies that fall outside the limited tuning band-width.

In principle, if an instrument is capable of observing a particular X nuclide, detection may be accomplished via either direct or indirect methods; however, the usual detection sensitivity issues should be borne in mind, as well as the nature of the desired chemical information. That stated, setup for X-nuclide detection essentially reduces to a matter of selecting and installing the correct filters and establishing proper cabling, provided that the instrument has first been properly set up and calibrated by the system administrator for such detection. Detailed instructions for ^{11}B and ^{15}N are provided in the subsections below, as examples to assist inexperienced users understand and carry out the process.

7.4.1 ^{11}B Acquisition

The UI-500 has been set up and calibrated by the system administrator for direct detection of ^{11}B using the Varian HCX probe. Default settings include a –120 to 100 ppm spectral window, which covers the entire ^{11}B chemical shift range. Chemical shift referencing is with respect to $BF_3{\cdot}OEt_2$ at 0.0 ppm as described in Subsection 7.5.2.

1. **Preliminary setup:**
   (a) Insert the sample into the magnet/probe.
   (b) Use the Main Menu → Setup → Nucleus,Solvent sequence to select first B11 then the correct solvent.
   (c) Enter **su** on the *VNMR* command line.
2. **Filters and related:**
   (a) Install the BE 175-60-8BB barrel filter (^{7}Li, ^{11}B, ^{31}P) in place of the BE 135-35-8BB filter, which is needed for ^{13}C and represents the default configuration.

(b) Note that the 120–170 MHz *quarter-wavelength* cable/filter should already be installed as part of the default configuration. The band-width of this filter is applicable for both ^{13}C and ^{11}B.

(c) Firmly install the 8-turn (8T) inductor wand.

(d) Adjust the *X*-circuit tune capacitor until the counter reads 56.

3. **Probe tuning:**

(a) Connect the cable to tune the *X* circuit to ^{11}B on channel 1, then tune for a minimum reflected power; a value less than 5 units should be easily achieved. Since the tune capacitor has already been closely adjusted (counter at 56), begin here by adjusting the match.

(b) Tune the ^{1}H circuit on channel 2.

(c) Check the ^{11}B tuning and readjust as needed for minimum reflected power.

(d) Check the ^{1}H tuning and readjust if needed.

4. **Data acquisition:**

(a) In the Setup EXP interface, click on the find z0 button (or enter **`findz0`** on the command line).

(b) Start gradient shimming via Main Menu → Setup → Shim → Gradient Autoshim on Z.

(c) Optional: Perform field–frequency locking under Acqi, then enter **`alock='n'`** on the *VNMR* command line.

(d) Check the acquisition parameters and modify as appropriate.

(e) Enter **`go`** on the *VNMR* command line to start acquisition.

When finished, restore the filter(s) and cabling to their default configuration, remove the 8T wand and retune the probe for ^{13}C (first set the counter to 08, as indicated on the probe, then optimize the tune and match settings).

7.4.2 ^{15}N Acquisition

The UI-500 has been set up and calibrated by the system administrator for both direct and indirect detection of ^{15}N using the Varian HCX probe. A default spectral window from –70 to 530 ppm is set for direct detection, and 90–140 ppm for indirect detection (e.g., HSQC and HMBC). Due to the large range of ^{15}N chemical shift values (approximately 900 ppm), it is imperative that the spectral window be optimized for the compound of interest; refer to the *VNMR* `setsw`, `setsw1` or `setsw2` commands, as appropriate. It is sometimes necessary to perform multiple experiments with different spectral windows to adequately cover the required spectral range. Chemical shift referencing is with respect to liquid ammonia at 0.0 ppm as described in Subsection 7.5.3.

1. **Preliminary setup:**

(a) Insert the sample into the magnet/probe.

(b) Set up the desired direct- or indirect-detection experiment.

(c) Enter **su** on the *VNMR* command line.

2. **Filters and related:**

(a) If using either the transmitter or 1st decoupler channel for ^{15}N, install the BE 53-15-8BB barrel filter (^{15}N) in place of the BE 135-35-8BB (^{13}C) filter which is used for the default configuration. If using the 2nd decoupler channel for ^{15}N, install the BE 53-15-8BB filter appropriately for that configuration.

(b) For direct ^{15}N detection, install the 48–64 MHz *quarter-wavelength* cable/filter in place of the 120–170 MHz cable/filter, which is part of the default ^{13}C configuration. (This step is not needed for indirect-detection experiments.)

(c) Firmly install the 28 pF (28pF) capacitance wand.

(d) Adjust the *X*-circuit tune capacitor until the counter reads 77.

3. **Probe tuning:**

(a) Connect the cable to tune the *X* circuit to ^{15}N on channel 1, 2 or 3, according to the experimental configuration, then tune for a minimum reflected power; a value less than 5 units should be easily achieved. Since the tune capacitor has already been closely adjusted (counter at 77), begin here by adjusting the match.

(b) Tune the ^{1}H circuit.

(c) Check the ^{15}N tuning and readjust as needed for minimum reflected power.

(d) Check the ^{1}H tuning and readjust if needed.

4. **Data acquisition:**

(a) In the Setup EXP interface, click on the find z0 button (or enter **findz0** on the command line).

(b) Start gradient shimming via Main Menu → Setup → Shim → Gradient Autoshim on Z.

(c) Optional: Perform field–frequency locking under Acqi, then enter **alock='n'** on the *VNMR* command line.

(d) Check the acquisition parameters and modify as appropriate.

(e) Enter **go** on the *VNMR* command line to start acquisition.

When finished, restore the filter(s) and cabling to their default configuration, remove the 28 pF wand and retune the probe for ^{13}C (first set the counter to 08, as indicated on the probe, then optimize the tune and match settings).

7.5 Chemical Shift Referencing

In practice, chemical shift referencing tends to fall into one of two categories. Most familiar is the semi-quantitative use for spectral characterization, typically by *internal referencing* using either a primary (e.g., TMS) or secondary (e.g., a residual ^{1}H resonance from the deuterated

solvent) standard to establish the origin of the chemical shift axis. This method is suitable for characterizing the spectrum of an analyte, as the resonance positions are simply measured and reported with respect to the experimental conditions under which the spectrum was acquired. Refer to the 2001 IUPAC recommendations [25] for further discussion and details related to chemical shift referencing.

Less common are quantitative investigations into the effects of solvent, concentration, temperature, pH, etc., on the chemical shielding (measured as chemical shift) of one or more analyte resonances. The motivations for such studies usually fall within the realm of chemical physics. Attempting the highly accurate chemical shift referencing necessary for such work is non-trivial, however, due to the interdependencies of solvent, concentration, temperature, pH, magnetic susceptibility and other complicating effects.[9] Detailed discussion of these topics is beyond the scope of this document, and are not considered further.

High Resolution NMR: Theory and Chemical Applications, by Edwin D. Becker [6], is an excellent source for basic and additional information about chemical shift referencing topics that are omitted from this manual. A useful, practical reference is "NMR Chemical Shifts of Common Laboratory Solvents as Trace Impurities," by Gottlieb, *et al.* [23].

7.5.1 Absolute Referencing via the Unified Scale (Ξ) Method

An important physical principle often overlooked or undervalued relates to the fact that nuclear gyromagnetic ratios are fundamental constants, and all NMR-active nuclides have Larmor frequencies that scale linearly with magnetic field strength. Figure 7.3 illustrates this relationship pictorially for select nuclides in an applied magnetic field of 2.35 T, at which the ^{1}H Larmor frequency is 100 MHz.

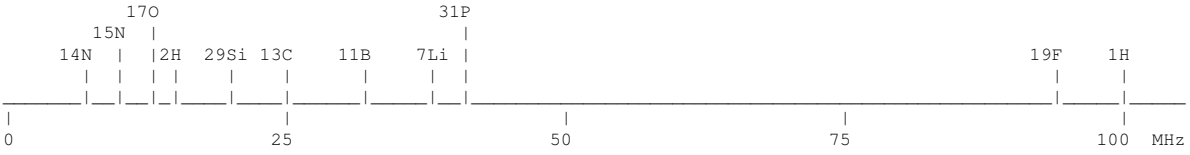


Figure 7.3 The chemical shift axis as a continuum, illustrated for a magnetic field of 2.35 T, at which the ^{1}H Larmor frequency is 100 MHz. Refer to Table 7.1 for numerical values.

Resonances of each nuclide (^{1}H, ^{13}C, ^{31}P, etc.), of course, span a range of chemical shift values determined by the chemical shielding effects due to different electronic environments. The key point is that there exists an underlying, fixed relationship between the chemical shift values of all the NMR-active nuclides in a given sample. Further, the relationship is independent of magnetic field strength when chemical shift, δ, is expressed in dimensionless units (typically as parts per million, ppm):

$$\delta_{X,\text{sample}}[\text{ppm}] = \frac{(\nu_{X,\text{sample}} - \nu_{X,\text{reference}})[\text{Hz}]}{\nu_{X,\text{reference}}[\text{MHz}]} \tag{7.2}$$

[9] From [25]: "Accurate and consistent referencing is easy to visualize but hard to implement."

where the difference in frequency, for nuclide X, between a sample resonance and that of the chemical shift reference (numerator) is given in units of Hz, and the absolute frequency of the reference resonance (denominator) is in MHz units.

How it Works: Part I

The following thought experiment illustrates the concept of how an absolute chemical shift reference scale can be established and utilized. Imagine that we prepare an NMR sample consisting of our analyte of interest in $CDCl_3$, with TMS (Me_4Si, at 1 % concentration by volume; cf. [25]) added as an internal, primary, chemical shift standard. We insert the sample into the UI-500 spectrometer's magnet, then shim, lock and acquire data for ^{1}H, ^{13}C and ^{29}Si, carefully measuring the absolute frequency of the TMS resonance for each of the three nuclides. For completeness, we also carefully measure the absolute frequency of the ^{2}H lock resonance. Lastly, we normalize the four absolute frequencies with respect to the ^{1}H resonance being assigned a value of exactly 100, so that all values are expressed as a (percent) ratio based on the TMS proton resonance. Just for fun, we repeat the same procedures on the AV-400, then compare the normalized ratios with those from the UI-500 to check that they produce the same values.

With the NMR data acquired for this sample, we could now perform chemical shift referencing in the familiar way by centering the cursor on the TMS resonance (e.g., for ^{1}H) and entering the `rl(0)` command to establish the origin of the chemical shift axis. Behind the scenes, the software essentially performs the calculation of Equation 7.2 to construct the chemical shift axis based on $\nu_{X,\mathrm{TMS}}$. Although in this thought experiment we have explicitly measured and recorded the absolute frequencies for the three TMS (^{1}H, ^{13}C and ^{29}Si) and one lock (^{2}H) resonances — for reasons that will be made clear in the next section — we are typically oblivious to exactly what the software is doing for us; those who would like to understand the details are encouraged to consult the *VNMR* documentation, especially the "Referencing" section on pages 235–237 in the *Getting Started* manual.

How it Works: Part II

Consider now a second thought experiment that extends from the preceding one. We prepare another NMR sample nearly identical to the first one, except this time we omit the TMS; we then acquire ^{1}H and ^{13}C NMR data after performing the preliminary shimming, locking, etc. How do we perform chemical shift referencing in this case? We could, of course, use the solvent resonances as secondary, internal standards, but we already know how to do that, so that route offers no new insight for the purpose of this discussion.[10] Instead, let's reflect on what we know from the previous thought experiment results and how that information might be useful.

1. We know the absolute ^{1}H resonance frequency of TMS in $CDCl_3$ for the spectrometer in question (noting the probe used, too, if needed); we can therefore set the chemical shift axis origin directly to this value even though there is no actual resonance at that position.

2. From the normalized ratios of absolute resonance frequencies, we can similarly calculate and set the ^{13}C chemical shift axis origin.

[10] Which is the reason we're performing these thought experiments: to learn something new.

Based on these results, it might seem that we could forego using TMS in our NMR samples in the future, and use this absolute referencing method instead. Although we could, actually, don't throw away your TMS just yet; there are complicating factors to consider (e.g., sample concentration, temperature, pH, magnetic susceptibility), and using a convenient, internal reference relieves us of having to deal with them directly. For example, even considering the same NMR spectrometer and probe, the absolute ^{1}H resonance frequency of TMS differs between solvents (the applied magnetic field strength must be altered slightly to find the on-resonance condition for each lock solvent), and it will gradually change over time due to intrinsic drift (diminution) of the superconducting magnet.

If we measure the absolute chemical shift reference frequency for pertinent nuclides of multiple reference standards in various NMR solvents (i.e., if we measure $\nu_{X,\text{reference,solvent}}$), we could calculate and tabulate a set of ratios (Ξ values[11]) that could subsequently be used for chemical shift referencing, as just described, in the absence of the actual chemical shift standard. Although the absolute frequency for a particular reference–solvent system depends on the spectrometer details and will change over time due to magnetic field drift, the tabulated Ξ values are invariant; it is necessary only to periodically measure the absolute frequency for the base reference standard (1 % TMS in $CDCl_3$) to update its value. This is the concept underlying the method of chemical shift referencing based on an absolute, unifying scale.

How it Works: Part III

Before continuing with the final section on the topic at hand, let's take a moment to briefly describe the *VNMR* 6.1C method as used by `setsw`, `setsw1`, etc. The *setsw* family of macros indirectly use, via the `setref` macro, the absolute resonance frequency and chemical shift of the active lock solvent to calculate the transmitter frequency (or decoupler frequency, for indirect experiments) that defines the center of the requested spectral window. This procedure relies on internal tables of solvent data (`/vnmr/solvents`) and Ξ values (`/vnmr/nuctables/nuctabref`) as discussed and shown in Table 7.1 below.

How it Works: Part IV

Using a convenient, chemical shift standard such as TMS or a residual solvent resonance simplifies routine work and allows us to focus on other things. However, for a variety of reasons,[12] chemical systems of interest do not all lend themselves to convenient chemical shift referencing standards.

Chemical shift referencing of insensitive nuclides such as ^{15}N or even ^{13}C can be problematic for 1D experiments using traditional methods, and is even more difficult when extended to the indirectly detected dimension in multi-dimensional experiments. The details of correctly setting the spectral window in either the directly or indirectly detected dimension[13] are, in fact, closely related to those for setting the chemical shift reference. Further, there are many NMR-active nuclides for which suitable chemical shift standards do not exist, particularly for use

[11] Ξ (pronounced 'ksee') is the upper-case form of the 14th letter of the Greek alphabet; ξ is the lower-case form.

[12] Some reasons have already been stated or implied, and others will be described further on.

[13] Consider, for example, how you would find the correct spectral window when setting the spectrometer to detect a "new" nuclide, such as ^{17}O, for which acquisition parameters do not yet exist.

as primary internal standards. For these and other reasons, a method has been developed and standardized for assigning the chemical shift reference of any nuclide, X, on a relative basis, with respect to the exact resonance frequency of TMS protons, $\nu_0(^1\text{H, TMS})$, as measured at any magnetic field strength, B_0. The absolute frequency of the X chemical shift reference position can then be determined using the appropriate ratio of accurately measured and tabulated Ξ values. Table 7.1 lists relative frequencies for select nuclides.

Table 7.1 Chemical shift references and their Ξ values

Nuclide	Reference	Ξ (MHz)
^{1}H	TMS (1 %) in $CDCl_3$	100.000 000
^{2}H	$(CD_3)_4Si$ (neat)	15.350 609
^{7}Li	LiCl (9.7 m) in D_2O	38.863 797
^{11}B	$BF_3{\cdot}O(C_2H_5)_2$ (15 %) in $CDCl_3$	32.083 974
^{13}C	TMS (1 %) in $CDCl_3$	25.145 020
	DSS in D_2O	25.144 953
^{14}N	CH_3NO_2 (neat/$CDCl_3$)	7.226 317
	NH_3 (liquid)	7.223 569
^{15}N	CH_3NO_2 (neat/$CDCl_3$)	10.136 767
	NH_3 (liquid)	10.132 912
^{17}O	D_2O (neat)	13.556 457
^{19}F	CCl_3F in $CDCl_3$	94.094 011
^{23}Na	NaCl (0.1 M) in D_2O	26.451 900
^{29}Si	TMS (1 %) in $CDCl_3$	19.867 187
^{31}P	H_3PO_4 (85 %) in D_2O	40.480 742
	$(CH_3O)_3PO$ (10 %) in D_2O	40.480 864
	$(CH_3O)_3P$ in $CDCl_3$	40.486 459
^{119}Sn	$(CH_3)_4Sn$ (neat/C_6D_6)	37.290 632

Ξ for ^{1}H of TMS in $CDCl_3$ is assigned to exactly 100 MHz, and the other values have been accurately measured and scaled relative to the ^{1}H standard; the values shown, except that for ^{14}N with respect to liquid NH_3 (calculated by TCS), are taken from reference [25].

Consider the following example to illustrate how this method works in practice. Suppose we want to set the ^{15}N chemical shift reference in the indirectly detected dimension in a ^{1}H,^{15}N HSQC experiment on a 500 MHz spectrometer. We first measure and determine the absolute ^{1}H frequency for TMS as, say, $\nu_0(^1\text{H, TMS}) = 499.889765$ MHz.[14] To determine the absolute frequency, and thence the spectral position, for the ^{15}N chemical shift reference, $\nu_0(^{15}\text{N, NH}_3)$, we calculate

[14] This particular value is for illustration only; the correct value must be measured for each real application.

$$\nu_0(^{15}\mathrm{N},\,\mathrm{NH_3}) = \left(\frac{\Xi(^{15}\mathrm{N},\,\mathrm{NH_3})}{\Xi(^{1}\mathrm{H},\,\mathrm{TMS})}\right)\nu_0(^{1}\mathrm{H},\,\mathrm{TMS}), \tag{7.3}$$

from which the value 50.653390 MHz is obtained, and at which spectral position we set the ^{15}N chemical shift axis to 0.0 ppm according to the previous discussion (Subsection 7.5.3) about ^{15}N chemical shift referencing. To further illustrate this method, suppose we want to center the ^{15}N spectral window in the middle of the amide resonance region (e.g., at 115 ppm) for an HSQC experiment. We simply calculate the decoupler position as $\nu(^{15}\mathrm{N}) = \nu_0(^{15}\mathrm{N},\,\mathrm{NH_3})(1 + 115.0 \times 10^{-6}) = 50.659215$ MHz.

References [6], [24] and [25] are recommended for those interested in further discussion and detail about relative chemical shift referencing via the Ξ scale.

7.5.2 Referencing ^{11}B

The setup, calibration and chemical shift standard for ^{11}B is 15% (v/v) $BF_3{\cdot}OEt_2$ in $CDCl_3$ [25], and is referenced to 0.0 ppm. Standard acquisition parameters for ^{11}B in our NMR Facility (AV-400 and UI-500) include an indirect chemical shift reference to $BF_3{\cdot}OEt_2$ at 0.00 ppm.

7.5.3 Referencing ^{15}N

Although ^{15}N data acquisition is performed infrequently in our laboratory, a few important points are worth noting here, if only for general information, due to the fact that different referencing schemes have been in common use and are reported in the literature. One referencing scale, dating to the early 1980s, sets the chemical shift of nitromethane at 0.0 ppm; an inconvenience of this scheme is that the ^{15}N resonances of most compounds thus have negative values. To correct for this deficiency, another referencing scheme uses liquid ammonia to establish the chemical shift origin. Ignoring practical aspects of working with liquid ammonia,[15] the relationship between these two referencing schemes is a simple difference of 380.5 ppm, and is illustrated in Table 7.2. Note that contemporary ^{15}N chemical shift data for organic compounds are typically expressed, indirectly, with respect to liquid ammonia.

7.5.4 Referencing ^{19}F

The primary chemical shift reference standard for ^{19}F is neat $CFCl_3$ [25] at 0.0 ppm; other secondary reference standards may be used, provided that their chemical shifts are known with respect to the primary reference. The ^{19}F sensitivity standard was tested as a secondary reference on the UI-500; the sample is 0.05% $C_6H_5CF_3$ in C_6D_6, with $\delta = -62.9$ ppm as assigned by the default referencing. A reported value for the ^{19}F chemical shift of $C_6H_5CF_3$ is –63.72 ppm, with no concentration or solvent data specified. Concern about the apparent 0.8 ppm discrepancy between the values of –62.9 and –63.7 ppm should be minimal for most work, absent further information about sample details. Chemical shift values for ^{19}F on the UI-500 are therefore indirectly referenced to $CFCl_3$ at 0.00 ppm.

[15] The vapor pressure of liquid ammonia is 909 kPa at 295 K [CRC Handbook], or approximately 9 atmospheres (132 p.s.i.) at room temperature.

Table 7.2 Chemical shift referencing schemes for ^{15}N

Compound	$\delta(NH_3)$	$\delta(CH_3NO_2)$
Amines	– 49	–429
NH_3	0	–380
$\underline{N}H_4NO_3$	21	–359
NH_4Cl	39	–341
Amides	119	–261
CH_3CN	243	–137
Nitriles	258	–122
Pyridine	319	– 61
Imines	343	– 37
$NH_4\underline{N}O_3$	376	– 4
CH_3NO_2	380	0
Nitrates	388	8

The chemical shift values shown for compound *families* are averages over specific compounds from those families; these values are therefore indicative of general trends.

7.5.5 Referencing ^{31}P

The primary chemical shift standard for ^{31}P is 85% phosphoric acid, H_3PO_4, in D_2O, with $\delta = 0.0$ ppm by definition. Because of the disaster that would result from breaking a sample tube of concentrated phosphoric acid in an NMR probe, it is typical practice to instead use a secondary reference such as 0.0485 M triphenyl phosphate in $CDCl_3$, which has a chemical shift $\delta = -17.9$ ppm with respect to the primary reference.

7.6 Spin Relaxation Measurements: The T_1, T_2, and $T_{1\rho}$ Suite

There are several motivations for measuring spin relaxation:[16] (1) For quantitatively meaningful spectral integrations, the spin states must return to their equilibrium population distributions ("relax") before the start of each transient acquisition. Knowing how long this takes is therefore important to properly setting up these experiments. (2) Since the NOE build-up rate is related to the longitudinal relaxation rate, an estimate of T_1 is necessary for obtaining optimal NOE data. (3) To optimize NMR imaging (MRI) experiments with respect to total acquisition time, it is critical to know the relaxation time constant(s) for the spin system used as a probe. (4) Quantitative spin relaxation studies can provide information about molecular dynamics and inter- and intramolecular parameters such as internuclear distances, chemical shielding tensors and electric field gradient tensors.

[16] Spin relaxation is quantified via a characteristic rate constant, R_k, or its inverse, T_k, a time constant.

7.6.1 General Considerations and Preparation

Two primary considerations are important before undertaking spin relaxation measurements. First, one should have a basic understanding of spin relaxation processes and data analysis procedures such as curve fitting. Second, oxygen gas (triplet electronic state) dissolved in the sample solution can provide a powerful, and often undesirable, external spin-relaxation pathway; therefore, one should determine whether or not it is necessary to remove the $O_2(g)$ by the freeze–pump–thaw method, followed by flame sealing the NMR tube to prevent reintroduction of $O_2(g)$ over time. The underlying reason for making the spin relaxation measurements will drive the decision about degassing the sample.

Table 7.3 provides an overview of the basic relaxation experiments described in this document. Section 1.3 of the Varian *User Guide: Liquids NMR* manual describes the *VNMR* standard-issue procedure for T_1 experiment setup and data analysis. While the *VNMR* T_1 method and procedure is convenient, workable and well documented, the same cannot be said in regard to measuring T_2, as no description of T_2 experiment setup is given, and certain aspects of the underlying process are flawed. I have therefore designed and implemented a robust suite of relaxation experiments that require minimal effort to set up, execute, process and analyze. The methodology closely follows the *VNMR* description for T_1 in procedure and spirit, but with several improvements in design and functionality.

Detailed procedures are given in the subsections below for experiment setup and data acquisition, and for data processing and analysis. These instructions are intended to (1) complement the discussion in the *User Guide: Liquids NMR* manual, while maintaining this document as a self-contained resource for our user community; (2) illustrate the use of locally developed or modified macros, which are improvements over the original *VNMR* versions; and (3) explain some of the important or behind-the-scenes details.

As with all other macro scripts for *VNMR*, anyone interested can examine the code to explore the underlying details. Original *VNMR* macros reside in the `/vnmr/maclib` directory, and locally developed or modified macros that are available to all users are found in `/vnmr/maclib.path`. If a macro script of exactly the same name exists in both of these directories, the one in `/vnmr/maclib.path` takes precedence (i.e., it is executed instead of the one in `/vnmr/maclib`).

Note that the $T_{1\rho}$ experiment and related macros are not available system-wide to all users. Please contact the NMR Facility Director for additional information or to request access to the $T_{1\rho}$ experiment and related macros.

7.6.2 Experiment Setup and Data Acquisition

The starting point for the relaxation experiments described here is the familiar, optimized 1D spectrum. Each setup macro converts the optimized data set and parameters into the desired relaxation experiment. After one or two requests for user input (*vide infra*), the setup macro then calls the appropriate `taucalct`k[17] macro to array the independent variable and optimize related parameters. For T_2 experiments, a report of the generated parameters is written to a uniquely named file in the current experiment directory; this record provides a useful reference for the experiment.

[17] The label k is used throughout this section as a convenient, and hopefully obvious, reference to 1, 2, 1ρ or 1rho.

Table 7.3 NMR spin relaxation experiments

Experiment	Description
Inversion–Recovery	Measures longitudinal relaxation (T_1) via the 180–τ–90 method. The `sett1` macro configures the `s2pul` pulse sequence and calls the `taucalct1` macro to calculate an array of delay values.
CPMG	Measures transverse relaxation (T_2) via the Carr–Purcell–Meiboom–Gill method. The `sett2` macro configures the `CPMG_T2` (default) or `cpmgt2` pulse sequence, as desired, then calls the `taucalct2` macro to calculate an array of delay values and record details of the settings.
$T_{1\rho}$	Measures transverse relaxation (as $T_{1\rho}$) in the rotating frame. The `sett1rho` macro configures the `t1rho` pulse sequence and calls the `taucalct1rho` macro to calculate an array of delay values.

We usually do not know the relaxation time constant (T_k) before making the measurements, which is typically why we do the measurements. Consequently, we must make an initial estimate of T_k, perform the measurements and analyze the data, then refine our estimate for T_k and repeat the process until we achieve a high-quality data set that converges to a T_k value with minimal error. For this reason, the `taucalct`k macros are designed to be used independently of the `sett`k macros; specifically, once the initial, optimized 1D data set has been converted to the desired relaxation experiment, the relaxation experiment's acquisition parameters can subsequently be optimized without completely starting over from the original 1D data set.

Another important thing to know and understand about the `taucalct`k macros is that they set up an array of *quadratically spaced* delay (τ) values as the independent variable. What this means qualitatively is that the time points are more closely spaced initially, and spaced farther apart gradually as the delay times increase. This helps to optimize the data *collection* quality because the early portion of an exponential curve exhibits the greatest point-to-point variation in intensity; in other words, data collection is more heavily weighted where the exponential function is most sensitive. In contrast, the corresponding *VNMR* method uses linearly spaced delay (τ) values.

A final note about these arrays of the independent variable concerns the total experiment time and efficiency. It makes little sense to use a large array (thus longer experiment time) for initial experiments when the preliminary estimate of T_k is likely to be far off. Instead, use smaller data arrays (e.g., 12) for initial experiments, then increase the size (e.g., 18) for measurements that are close to converging on a robust T_k value.

Step-by-Step Procedure

1. Acquire and save a high-resolution 1D spectrum, optimized with regard to spectral window, receiver gain, signal-to-noise, etc.

2. Optional: If desired (and if you know what you're doing!), you may perform a pulse-width calibration at this point; if you do so, save the corresponding data set(s).

3. Enter the name of the `sett`k setup macro for the desired relaxation experiment. Specifically, enter **`sett1`** for T_1, **`sett2`** for T_2, or **`sett1rho`** for $T_{1\rho}$. You will be prompted for various input as appropriate for the experiment requested; the input consists of:

   (a) For either the T_1 or T_2 experiment, you will first be asked if you have performed a pulse-width calibration (calibrated the pw90 value). Answer yes [y] or no [n] as appropriate. If you answer yes, you will be asked to input the value; otherwise, a previously calibrated `pw90` value will be read from the probe file.

   (b) For a T_2 experiment, you will then be asked if you desire the `CPMG_T2` (the default option) or `cpmgt2` variant of the CMPG experiment. Unless you have specific reasons to do otherwise, you should choose the default.

4. The setup macro will next call the `taucalct`k macro (`taucalct1`, `taucalct2`, or `taucalct1rho`) to array the independent variable and optimize related parameters. This internal macro call is transparent, but you will be asked for further input:

   (a) An estimate of the expected relaxation time constant, T_k;

   (b) the number of delay (τ) values to use (τ is the independent variable, and each value corresponds to one FID; *vide supra*); and

   (c) a multiple of the estimated T_k, over which the measurements will be extended; this is typically in the range of 3–10. Choose 3 for preliminary studies when your estimate of T_k is less certain, at least 5 for measurements converging toward a robust T_k value, and near 10 for measurements requiring the highest accuracy.

5. For T_2 experiments, a report of the parameters generated by `taucalct2` is written to a date-and-time-stamped file in the current experiment directory; this record is intended for immediate and future reference.

6. Check that the acquisition parameters are set correctly and as desired. In particular, check the experiment time (enter the **`time`** command before starting the acquisition) and, if desired, modify parameters intelligently to adjust the time. The receiver gain may require adjustment unless the optimized 1D spectrum was acquired using a 90° pulse angle. If the gain is set too high, the first FID will generate a "receiver overflow" warning message; if this occurs, stop the acquisition (enter **`aa`**), set the `gain` parameter to a smaller value, and try again.

7. Initiate data acquisition using **one** of these two methods:

   (a) Enter **`au`** on the *VNMR* command line to begin data acquisition *with* automated processing and plotting, via the `proct`k macro (described below), when the experiment completes.

   (b) Enter **`go`** on the *VNMR* command line to begin data acquisition *without* automated processing and plotting after experiment completion.

8. Save the data set.

9. After running the setup macro once to configure and initialize the desired relaxation experiment, you should thereafter run the corresponding `taucalct`k macro to set up a new array of delay values as desired for subsequent acquisitions. This is commonly necessary as an iterative mechanism because the initial estimate of T_k is seldom close enough.

7.6.3 Data Processing and Analysis

Familiar processing steps such as weighted Fourier transforms, spectral phasing, and the like require no further explanation; steps that are unique to relaxation measurements or are otherwise likely to be unfamiliar are described in some detail. As always, consult the Varian *VNMR Command and Parameter Reference* manual for additional details about *VNMR* commands or macros indicated herein.

The `proct`k macro can be used to prepare the data set for downstream analysis; it may be run automatically when the acquisition completes, as described above, or later by entering **`proct`**k on the *VNMR* command line. Although this automated step usually gives suitable results with "well-behaved" data sets, it is sometimes necessary to perform the individual steps by hand. The `proct`k macros basically automate the individual actions listed under step 1 of the following procedure; examine the macro code to learn exactly what they do.

Step-by-Step Procedure

1. Process and prepare the spectral data for plotting and analysis:
   (a) Perform a weighted FT of the most intense positive peak, phase correct, scale and set the spectral display region as desired.
   (b) Fourier transform and perform a DC-offset correction on the entire array (i.e., enter **`wft dcarray`**).
   (c) Display, inspect and plot the spectral results as desired.
2. Define and measure the peak(s) to be analyzed:
   (a) Display the spectrum with the tallest positive peak, then expand and scale the spectral region of interest as needed to set the threshold to target the peak(s) to be analyzed.
   (b) Enter **`dpf`** to locate and report the peak position(s).
   (c) Enter **`fp`** to measure and record the peak intensities of each spectrum in the array. This step creates the `fp.out` file in the current experiment directory.
3. Analyze the measured peak-intensity data to determine the T_k value(s) and other parameters:
   (a) Enter **`t`**k to perform an exponential curve-fitting analysis of the data in the `fp.out` file as a function of the experimental delay (τ) times. The analysis output includes the T_k value(s), curve-fitting details and an error estimate, and is recorded in the `analyze.list` and `analyze.out` files in the current experiment directory.[18]

[18] This description summarizes a more complicated process involving the `analyze` and `expfit` commands called via `t`k.

The `tks` macro performs a similar analysis but the `analyze.list` output is abbreviated ('s' is for 'short' version).

4. Graph and plot the analytical data:

   (a) Enter **`expl`** to display the graphical results, from data in the `analyze.out` file. Modify the display parameters (e.g., `wc` and `wc2`) as needed for suitable output.

   (b) Enter **`pexpl page`** to plot these results using the current display parameters.

7.7 The DEPT and INEPT Family of Experiments

The superior HSQC-type experiments have essentially replaced the 1D DEPT and INEPT experiments for routine analysis of ^{13}C. The DEPT and INEPT family of experiments are discussed — in varying level of detail — in most basic NMR texts.

7.8 Phase-Cycled versus Gradient Experiments

This section will provide a very terse and introductory description of and comparison between phase-cycled, gradient-assisted (also referred to as gradient-enhanced) and gradient-selected pulse sequences. Reference [42] is recommended to the interested reader as a good source for the next level of information.

Phase cycling has been the traditional method for suppressing or minimizing certain types of spectral artifacts, for achieving spectral editing, and for quadrature detection and coherence pathway selection in multi-dimensional experiments. Around the late 1980s and early 1990s, pulsed field gradients began to be exploited for similar purposes, and have dramatically changed the ways in which most NMR spectroscopy is done today. For example, automated gradient shimming is now routine, and the NOESY1D experiment has essentially replaced the older NOE difference version. Most multi-dimensional experiments today have gradient versions that usually provide superior performance. Note that the term *gradient selected* indicates that coherence pathway selection is performed by pulsed field gradients; *gradient enhanced* usually refers to the suppression of artifacts by use of pulsed field gradients.

By way of illustration, consider the routine COSY experiment. In the phase-cycled version, the minimum number of transients required is 4, and a minimum of 16 transients is necessary for the complete phase cycling needed to achieve optimal data quality. A completely phase cycled COSY experiment would typically require about 15–30 minutes, depending upon other acquisition parameters such as the number of t_2 and t_1 points and the relaxation delay. In contrast, the gradient-selected version, gCOSY, requires an absolute minimum of only 1 transient, and a complete phase cycle takes only 4 transients; thus the data acquisition time for a gCOSY experiment is typically one-fourth that required for a phase cycled COSY.

The main point of the foregoing example applies to other 2D and higher dimensional experiments, although there is no implication that the four-fold time savings applies universally. An obvious question, then, is why would anyone want to perform the phase-cycled variant of an experiment if a gradient-selected version is available? Is there really such thing as a free lunch? Perhaps not. In gradient-selected experiments — compared to their phase-cycled counterparts

— a smaller amount of the total magnetization is detected, and thus the gradient-selected version tends to be less sensitive in absolute terms. However, if one has a "sufficiently concentrated" sample, the gradient-selected version is preferred.

A practical rule of thumb to keep in mind is that if the number of transients required to attain sufficient sensitivity with a particular sample and experiment approaches the requirement for phase cycling, then the phase-cycled experiment is probably the better choice. The details are, of course, highly variable and depend upon the spectrometer and especially the probe; however, for those who like "ball park figures," sample concentrations of less than a few tens of mmol/L are likely candidates for phase-cycled experiments.

7.9 The COSY Family of Experiments

This section briefly describes and compares the COSY, DQCOSY, *p*QCOSY and ECOSY experiments, including their potential for measuring J values. A standard NMR text should be consulted for more information; especially recommended are [7] and [22].

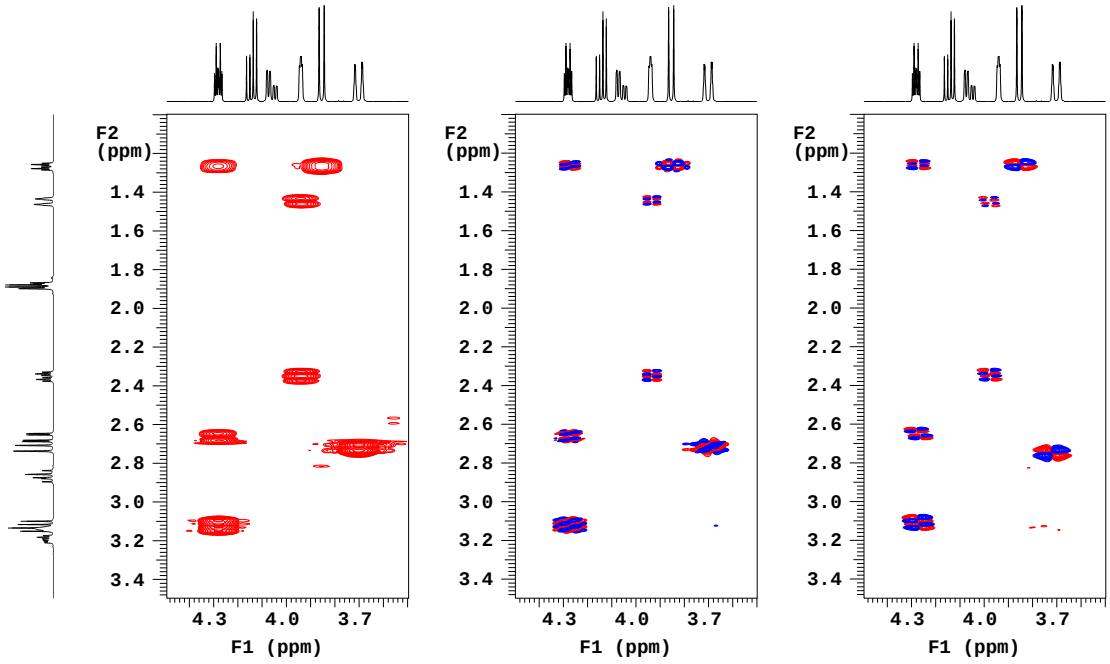
The COSY family of experiments provide homonuclear chemical shift correlation information via the J-coupling al representation similar to a topographical or contour map. The TOCSY experiment described in the following section is a type of relayed COSY experiment, capable of showing similar correlations across 4 to 5 bonds in favorable circumstances. Most common are ^{1}H COSY experiments, but other abundant nuclides such as ^{19}F and ^{31}P are readily investigated; ^{13}C at natural abundance is not suited for such experiments.

7.9.1 General Considerations

Detection sensitivity is generally not an issue; the gradient versions are normally preferred except for cases of very dilute sample concentrations. Refer to Section 7.8 for a related discussion. gCOSY is the standard experiment; it is acquired and processed in absolute value (magnitude) mode and usually requires no more than a few minutes to acquire. Spectral resolution is generally sufficient to observe most correlations, although correlations near the diagonal tend to be obscured by spectral tailing.

The double-quantum filtered COSY experiments, DQCOSY and gDQCOSY, provide better resolution capabilities and are thus recommended for cases in which correlations occur close to the diagonal and cannot be fully resolved via COSY. In addition, the spectrum is simplified due to the fact that singlet signals are suppressed by this technique. In the spirit of higher resolution, DQCOSY data are typically acquired with more t_2 points, usually 4–8 k. Higher order multiple-quantum filtered COSY experiments, *p*QCOSY, take the benefits of DQCOSY to higher levels; e.g., 3QCOSY filters out singlets and doublets.

The *exclusive* COSY experiment, ECOSY, uses multiple-quantum filters and extensive phase cycling to eliminate passive coupling information in the spectrum and thus show only the active coupling information between spin-coupled pairs. This is the preferred experiment for extracting homonuclear J values from 2D data sets. Refer to Cavanagh, *et al.* [22] for excellent discussion on this topic. Figure 7.4 illustrates the differences in appearance between gCOSY, gDQCOSY and ECOSY data acquired for a strychnine sample.



(a) gCOSY spectral expansion **(b)** gDQCOSY spectral expansion **(c)** ECOSY spectral expansion

Figure 7.4 Comparison of gCOSY, gDQCOSY and ECOSY subspectra of strychnine. Red contours indicate positive phase and blue negative. Spectral data were rendered from *VNMR* as an Encapsulated PostScript file, edited for fine-tuning and included into this LaTeX document.

7.9.2 Preliminary Preparations

There is nothing too special here; follow standard good practices for sample preparation.

7.9.3 Experiment Setup and Data Acquisition

For optimal spectral resolution, be sure to minimize the spectral window in a preliminary step; use no more than about 10 percent of baseline on either end of the spectrum so that digital resolution is not wasted recording base-plane information. Prepare for using linear prediction in t_1 during post-acquisition data processing: For normal work, 128–200 or so t_1 points (FIDs) are sufficient; linear prediction will be used later to expand the data set four-fold in t_1. Acquisition time is generally better spent toward achieving greater signal-to-noise than toward increasing digital resolution in t_1.

7.9.4 Data Processing

Use suitable apodization in both dimensions, apply one zero fill in t_2 and at least one zero fill in t_1 (use more to achieve a mathematically square matrix, if desired), set the linear prediction parameters then perform a 2D Fourier transform. Phase as needed for the phase-sensitive experiments, e.g., DQCOSY and ECOSY. Spectral symmetrization is sometimes performed for absolute-value COSY data, requiring a square matrix.

7.9.5 ECOSY Setup, Acquisition and Processing

The ECOSY experiment merits a few brief comments. First off, the ECOSY experiment is not currently accessible via any of the *VNMR* graphical user interfaces such as CustomQ; the experiment must be set up and initiated from the *VNMR* command line — which is a simple matter:

1. Acquire and save a by-now-familiar optimized high-resolution 1D data set (with a minimized spectral window), then display the processed spectrum.
2. Set up the ECOSY experiment by entering **`ecosy`** at the *VNMR* command line.
3. A setup macro configures the experiment and sets default parameters, then displays a graphical representation of the pulse sequence and a descriptive text to assist in further refinement.
4. The help text can be redisplayed by entering **`man('ecosy')`** on the *VNMR* command line.
5. Adjust the acquisition parameters as appropriate for the system under study. Note in particular that this experiment requires multiples of 32 transients for correct phase cycling — this is critical!
6. Initiate data acquisition by entering **`go`** on the *VNMR* command line.

After acquisition, follow normal phase-sensitive data processing procedures.

7.10 TOCSY and TOCSY1D

TOCSY and TOCSY1D are essentially *relayed* or *long-range* COSY-type experiments; TOCSY is a 2D experiment and the one-dimensional TOCSY1D experiment uses selective excitation to acquire 1D data that are high-resolution analogs of 1D slices from a full 2D TOCSY data set.

7.10.1 General Considerations

Refer to the discussion above regarding COSY experiments. One additional consideration worth mention relates to deciding which experiment to perform, the full 2D TOCSY or the TOCSY1D. For small molecules with very few correlation assignments remaining unknown following COSY analysis, the TOCSY1D is probably sufficient, especially if the unassigned spin system involves one or more resonances that are relatively free from overlap; an acquisition with selective irradiation at a couple of key resonances may provide the missing information. On the other hand, if the molecule is large and/or the spectrum excessively crowded or complex, then the full 2D TOCSY is likely the best course; first obtain all the information possible, then analyze the full data set.

7.10.2 Preliminary Preparations

There is nothing too special here; follow standard good practices for sample preparation.

7.10.3 TOCSY

Experiment Setup and Data Acquisition

Pay careful attention to all the items discussed for COSY experiments. The TOCSY mixing time, τ_m, is related to how far the spin–spin relay carries throughout the spin sub-system. In general, longer mixing times allow the spin information to propagate further; however, there are practical limits to what can be achieved. Mixing times in the range of 60–100 ms are typically useful to observe 4- and 5-bond coupling correlations; due to loss of signal in t_1, very little is achieved with mixing times greater than about 150 ms. Strictly speaking, it is not the number of intervening bonds but the magnitude of the J-coupling interaction that bears a relationship with an optimal τ_m value; however, it is generally true that a greater number of intervening bonds correlates with smaller J. For best results, one must often acquire multiple data sets using different mixing times.

Data Processing

Data processing is basically the same as for COSY.

7.10.4 TOCSY1D

Experiment Setup and Data Acquisition

TOCSY1D experiments are easy to set up and execute — thanks to sophisticated *VNMR* software applications that transparently perform several difficult tasks. Setup and acquisition via

CustomQ is trivial; the steps involved for manual acquisition are presented here:

1. Acquire and save an optimized high-resolution 1D data set (with spectral window minimized), then display the processed spectrum.
2. Set up the TOCSY1D experiment either by entering **`TOCSY1D('ds')`**[19] at the *VNMR* command line or by selecting TOCSY1D under the Setup EXP interface.
3. A new menu level is presented, from which one uses the display cursors to graphically set individual bands corresponding to those resonances to be selectively irradiated. When the desired band is set (the center and bandwidth are determined by the left and right cursor positions), click on the Select menu button; a selective pulse is created corresponding to the position and bandwidth specified by the cursors.
4. Repeat the previous step for each additional resonance desired. This generates an array of selective pulses, which in turn leads to an arrayed data set.
5. Left-mouse click on the Proceed menu button to finalize the setup of selective pulses and exit the current menu level.
6. Set the acquisition parameters (typically the mixing time and number of transients) suitably for the system under study. Remember to increase the number of transients significantly, as the goal of this experiment is to detect signals due to long-range magnetization transfer.
7. Initiate data acquisition by entering **`go`** on the *VNMR* command line.

Data Processing

Data processing is straightforward:

1. Set `lb` appropriately then use **`wft(1)`** to Fourier transform and display the first spectrum in the array.
2. Phase the spectrum so that all observed resonances are positive. Those resonances other than the one irradiated appear due to propagation of magnetization throughout the spin subsystem.
3. Use **`wft dss`** to transform and display the arrayed data as vertically stacked spectra. Adjust `vs`, `vp`, `vo` and `ho` as necessary to display the spectra in a desirable manner.
4. The arrayed spectra can be plotted with **`pl('all')`**. Use other plot-related commands as normal.

Plotting the normal 1D spectrum on the same page as the arrayed TOCSY1D spectra is possible (it's actually fairly straightforward) with a bit more effort — and patience — and an understanding of how *VNMR* handles graphics.

[19] A description of the `ds` argument is missing from the *VNMR* documentation but is necessary to invoke the resonance-selection menu.

7.11 The NOE Family of Experiments

The NOE experiment family comprises the NOE difference experiment (now effectively outdated), the one-dimensional NOESY1D, and the two-dimensional NOESY and gNOESY experiments; HOESY is the heteronuclear analog of NOESY. The 2D EXSY (EXchange SpectroscopY) experiment, for detecting the correlation between nuclei undergoing chemical or conformational exchange, is closely related to NOESY. The NOESY and NOESY1D experiments are discussed below. Basic NMR texts (e.g., [7, 10, 13]) or specialized monographs (e.g., [15, 16, 22]) should be consulted for more detailed information.

7.11.1 General Considerations

Molecular weight, sample temperature, solution viscosity and the strength of the applied magnetic field, B_0, all influence the potential to observe a nuclear Overhauser enhancement, and its algebraic sign if the enhancement is observed. Note that the absence of an observable NOE does not necessarily indicate that two nuclei are not spatially close.[20] The ROE experiments (*vide infra*) offer an alternative technique for cases in which the NOE happens to be zero or negative.

A few comments are in order concerning molecular size. It is actually the product $\omega_0\tau_c$ and not molecular size itself that is fundamentally important toward determining whether a particular NOE is positive, negative or zero (unobserved). For this discussion it is sufficient to note that τ_c is related to the rate at which a molecule rotates in solution. Although larger molecules tend to rotate more slowly than smaller molecules at a given temperature, solution viscosity plays an important role as well. With the caveat that one must beware sweeping generalizations, a few rules of thumb follow. *Small* molecules here refers to those with molecular mass, $M \lesssim 1000$ daltons; the NOE is generally positive in such cases. *Large* molecules are those with $M \gtrsim 2000$ daltons; they generally exhibit negative NOE. Medium sized molecules fall within these approximate boundaries and tend to have little or no observable NOE; the ROESY experiments are useful to investigate such systems.

7.11.2 Preliminary Preparations

Because NOE originates from spin relaxation, it is highly advisable to degas the sample solution and flame seal the NMR tube prior to performing NOE experiments. This step eliminates the possibility of electron-induced nuclear spin relaxation arising from interaction with paramagnetic molecular oxygen. Although this step is not strictly necessary for most qualitative applications, it is critical for quantitative studies in which one desires to determine inter-nuclear distances.

[20] This is a specific case of the general statement that "Absence of evidence is not evidence of absence."

7.11.3 NOESY

Experiment Setup and Data Acquisition

Pay attention to all the items discussed for COSY experiments. The NOESY mixing time is indirectly related to the ability to detect correlations between spin pairs based on their internuclear distance, r. The actual physical phenomenon underlying NOE is the dipolar spin relaxation mechanism, which is a function of r^{-6}. (To be technically correct, the dipolar interaction *energy* is proportional to r^{-3} and the dipolar relaxation *rate* is proportional to r^{-6}.) Stated in an oversimplified fashion, longer mixing times *may* allow for detection of correlations between more distant spin pairs; however, there are again practical limits to what can be achieved. Optimal mixing times, τ_m, correspond approximately to the longitudinal relaxation time, T_1, for the spin under study. Mixing times in the range of 500–800 ms are usually sufficient to observe spatial correlations in small molecules, which tend toward less efficient spin relaxation and thus larger T_1 values (e.g., 0.5–2 s). Larger molecules, on the other hand, tend to relax more rapidly (smaller T_1 values) and thus require shorter mixing times, say in the 50–500 ms range.

Correctly setting the recycle delay (also referred to as the relaxation delay) is important for obtaining useful NOESY and ROESY data. For best results, the total recycle time, t_r, for consecutive transient acquisitions should be in the range of 3–5 times the largest T_1 value for the molecule; however, in practice it is common to cheat somewhat and reduce it such that $t_r \approx 2$–3 times T_1. The total recycle time includes the recycle delay, the mixing time and the acquisition time as the major contributors. In Varian parlance, this becomes $t_r \approx \mathrm{d1} + \mathrm{mix} + \mathrm{at}$.

Data Processing

Data processing is basically the same as for phase-sensitive COSY data. Note that the convention for spectral phasing is to adjust for negative phase of the diagonal peaks; in this manner, positive NOE correlations appear with positive phase and negative correlations with negative phase. If present, correlations arising from chemical or conformational exchange appear with negative phase.

7.11.4 NOESY1D

The modern 1D NOE experiment in *VNMR* is referred to as NOESY1D, and is a gradient-assisted experiment that has effectively replaced the older and error-prone NOE difference experiment. Although the nature of many molecular structure elucidation or related questions require the 2D NOESY experiment, the desired information can sometimes be obtained more rapidly via NOESY1D. The NOESY1D experiment can be accessed from the CustomQ H1 & Selective 1D experiment group, or manually, typically following acquisition of an optimized 1D spectrum, by entering **`NOESY1D('ds')`** at the *VNMR* command line.

Experiment Setup and Data Acquisition

Experiment setup and acquisition for NOESY1D follows the procedure for TOCSY1D, as described above:

1. Acquire a high-resolution data set with an optimized spectral window, then display the processed spectrum.
2. Set up the NOESY1D experiment either by entering **`NOESY1D('ds')`** at the *VNMR* command line or by selecting NOESY1D under the Setup EXP interface.
3. A new menu level is presented, from which one uses the display cursors to graphically set individual bands corresponding to those resonances to be selectively irradiated. When the desired band is set (the center and bandwidth are determined by the left and right cursor positions), click on the Select menu button; a selective pulse is created corresponding to the position and bandwidth specified by the cursors.
4. Repeat the previous step for each additional resonance desired. This generates an array of selective pulses, which in turn leads to an arrayed data set.
5. Left-mouse click on the Proceed menu button to finalize the setup of selective pulses and exit the current menu level.
6. Set the acquisition parameters (typically the mixing time and number of transients) suitably for the system under study. Remember to increase the number of transients significantly, as the goal of this experiment is to detect signals as small as perhaps 1 percent of their original size.
7. Initiate data acquisition by entering **`go`** on the *VNMR* command line.

Data Processing

Data processing is straightforward:

1. Set `lb` appropriately then use **`wft(1)`** to Fourier transform and display the first spectrum in the array.
2. Phase this spectrum so that the irradiated resonance is negative. Peaks with positive or negative phase correspond to positive or negative NOE, respectively.
3. Use **`wft dss`** to transform and display the arrayed data as vertically stacked spectra. Adjust `vs`, `vp`, `vo` and `ho` to display the spectra as desired.
4. The arrayed spectra can be plotted with **`pl('all')`**. Use other plot-related commands as normal.

NOESY1D Example

Figure 7.5 below illustrates NOESY1D spectra plotted with the normal 1D spectrum.

7.12 The ROE Family of Experiments

The ROESY1D, ROESY and gROESY experiments follow very much in analogy with previous discussion of their NOE experiment cousins. Read Section 7.11 and apply here.

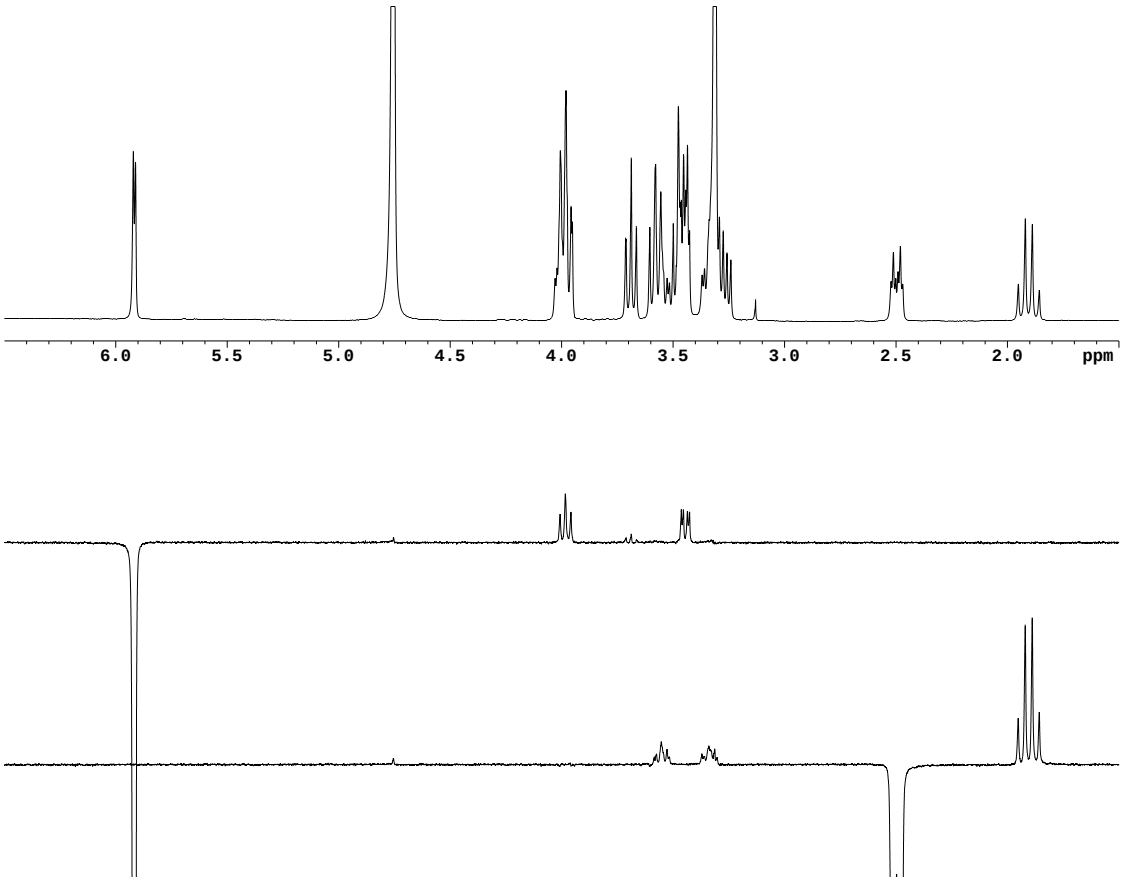


Figure 7.5 Experimental NOESY1D data of neamine in D_2O. Data were acquired on the UI-500 using the Varian HCX probe. The top trace is the normal 1D spectrum; the two lower traces are NOESY1D spectra, where the large negative peak indicates the irradiated resonance.

7.13 ^{1}H-detected Heteronuclear Family of Experiments

HSQC, edited HSQC, HMQC, HMBC

7.13.1 General Considerations

7.13.2 Preliminary Preparations

7.13.3 Experiment Setup and Data Acquisition

Typically, digital resolution of 0.1–0.2 ppm in F_1 is adequate. Recall these commands to aid setup of the desired spectral regions:

```
setsw(downfieldppm,upfieldppm)
setsw1(nucleus,downfieldppm,upfieldppm):offset
```

Refer to the *VNMR Command and Parameter Reference* for detailed explanations. For example, for a gHSQC experiment, one could use `setsw(9.5,-0.5)` to set the F_2 spectral window for ^{1}H, and `setsw1(dn,160,-10):dof` to set the F_1 spectral window for ^{13}C. In both cases, the transmitter (for F_2) or decoupler (for F_1) frequency is set to the center of the specified spectral window.

7.13.4 Data Processing

7.14 The HETLOC Experiment

The *HETLOC* experiment versions described herein utilize pulsed-field gradients for sensitivity enhancement and coherence selection; the full and proper name for this experiment is the *sensitivity- and gradient-enhanced hetero (ω_1) half-filtered TOCSY* experiment [45]. Two versions are set up on the UI-500 spectrometer: *hetloc_gse* and *hetloc_mod*, where *gse* stands for "gradient sensitivity enhanced," and *mod* indicates a "modified" version (revised phase cycling and pulsed field gradient schemes) compared to the former. Those interested in more information are directed to references [43–47, 73].

Preliminary comparative measurements using standard samples revealed no clearly discernible performance differences between the hetloc_gse and hetloc_mod experiments. Although subjective, the hetloc_gse experiment appeared to provide slightly better quality data in regard to artifact suppression. In the absence of more conclusive comparative results or other information, the two experiments are considered to perform equivalently.

7.14.1 General Considerations

The HETLOC experiments provide a way to indirectly measure n-bond heteronuclear spin–spin coupling constants, $^{n}J(I,S)$, which play a vital role in structure elucidation. The typical application to ^{1}H,^{13}C systems will serve as example in the following discussions. The resultant 2D spectral data from this proton-detected experiment appear as a ^{1}H,^{1}H homonuclear spectrum with chemical shift axes in both dimensions; F_2 and F_1 are the directly and indirectly detected dimensions, respectively; refer to Figure 7.6.

Proton magnetization evolves under the influence of both homonuclear and carbon J-coupling during t_1; the heteronuclear coupling (and no carbon decoupling during t_2) thus causes the diagonal peaks to appear as doublets. One-bond heteronuclear J-coupling constants are measured (in Hz) in the F_1 dimension, and multiple-bond coupling constants are measured in F_2; details are described below and are illustrated graphically in Figure 7.7.

7.14.2 Preliminary Preparations

Standard sample considerations and preparation apply here, as with other proton-detected heteronuclear correlation experiments. As an informational example, a test of the hetloc_gse experiment using a 27 mM sample yielded excellent results in 14 hours of acquisition time (64 transients and 256 increments).

7.14.3 Experiment Setup and Data Acquisition

The HETLOC experiments are not accessible via any of the *VNMR* graphical user interfaces such as CustomQ; the desired experiment is set up and acquisition initiated from the *VNMR* command line as follows:

1. Acquire and save an optimized, high-resolution 1D data set (with minimized spectral window), then display the processed spectrum.
2. Enter **`hetloc_gse`** or **`hetloc_mod`** at the *VNMR* command line.
3. The setup macro configures the experiment and sets default parameters, then displays a graphical representation of the pulse sequence and a descriptive text to assist in further refinement of the acquisition parameters.
4. The descriptive help text can be redisplayed by entering **`man('hetloc_gse')`** or **`man('hetloc_mod')`**, as appropriate, on the *VNMR* command line.
5. Adjust the acquisition parameters as needed for the system under study.
6. Initiate data acquisition by entering **`go`** on the *VNMR* command line.
7. Save the data via `svf('<path>filename')`.

7.14.4 Data Processing

Follow normal procedures for processing phase-sensitive data. The `hetloc_proc` custom macro was written to facilitate processing of HETLOC data; it works correctly for data acquired using either the `hetloc_gse` or `hetloc_mod` experiment. HETLOC data may alternatively be processed via the *VNMR* Process Tcl/Tk interface, setting apodization, zero-filling, linear prediction and other processing parameters as desired.

Heteronuclear J-coupling constants can be obtained from the 2D spectrum as follows, where the letters k and l label the atom positions. The one-bond coupling constant, ${}^1J(\mathrm{H}^k,\mathrm{C}^k)$, is measured in the F_1 dimension as the separation of the major splitting of the H^k resonance; this can be evaluated at any conveniently accessible correlation with H^k in F_1.

Long-range J-coupling constants are measured as the offset in F_2 of the ECOSY-type multiplets. For ${}^nJ(H^k,C^l)$, this offset is taken from the H^k,H^l correlation, where the F_2,F_1 coordinate pair convention is used: the intersection of H^k in F_2 and H^l in F_1. Figures 7.6 and 7.7 illustrate, respectively, a full HETLOC spectrum and the preceding verbal descriptions for measuring heteronuclear J-coupling constants.

As a practical matter, recall that the *VNMR* parameters `delta` and `delta1` relate, respectively, to the cursor separations, in Hz, in the directly detected (F_2) and first indirectly detected (F_1) dimensions. The ${}^nJ(H^k,C^l)$ values can therefore be graphically determined by first positioning the cursors on the (correct!) correlation peaks, then querying the value of `delta` or `delta1`: simply enter **`delta?`** or **`delta1?`**, as appropriate.

7.15 Combination Experiments

HSQC-TOCSY, HSQC-COSY, etc.

7.15.1 General Considerations

7.15.2 Preliminary Preparations

7.15.3 Experiment Setup and Data Acquisition

7.15.4 Data Processing

7.16 The ^{1}H,^{19}F Experiment Suite

It is often desirable or necessary to complement standard ^{19}F 1D spectra with other ^{19}F-related NMR data. Examples of such data include ^{1}H 1D and homonuclear 2D experiments acquired with ^{19}F decoupling; ^{19}F 1D and homonuclear 2D experiments with ^{1}H decoupling; ^{13}C 1D experiments with decoupling of ^{1}H and/or ^{19}F; ^{1}H,^{19}F 2D heteronuclear correlation experiments; ^{1}H,^{13}C 2D heteronuclear correlation experiments with ^{19}F decoupling; and ^{19}F,^{13}C 2D heteronuclear correlation experiments with ^{1}H decoupling. Table 7.4 summarizes the experiments available in our local ^{1}H,^{19}F experiment suite.

7.16.1 General Considerations

The 100-percent natural abundance and relatively large gyromagnetic ratio of ^{19}F make it an excellent candidate for NMR experiments;[21] however, the large chemical shift range of fluorine compounds has important ramifications. This chemical shift range spans about 400 ppm (from roughly 150 to –280 ppm for most compounds), which translates to 150 kHz on a 400 MHz spectrometer and 235 kHz on a 500 MHz instrument. The consequences are significant and two-fold.

First, it is helpful to have an idea about the expected ^{19}F chemical shift range for the compound of interest; otherwise, it will be necessary to either enlarge the spectral window or perform multiple experiments with overlapping spectral windows in order to correctly observe the

[21] Specifically, $\gamma_{^{19}\mathrm{F}}/\gamma_{^1\mathrm{H}} = 0.94$.

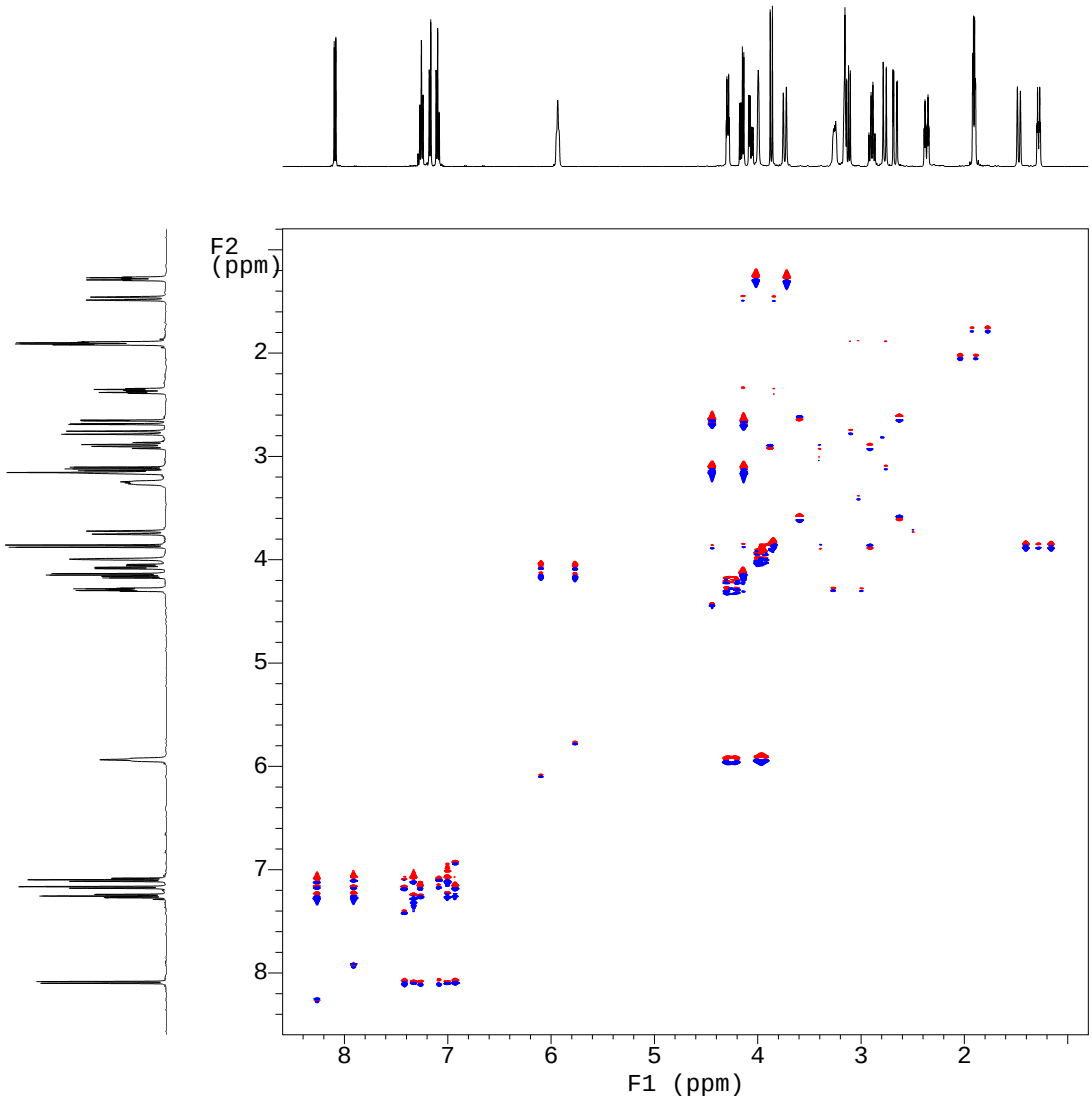


Figure 7.6 Experimental HETLOC data of strychnine in $CDCl_3$, acquired on the UI-500 using the Varian HCX probe. The high-resolution 1D spectrum is shown along F_2 and F_1.

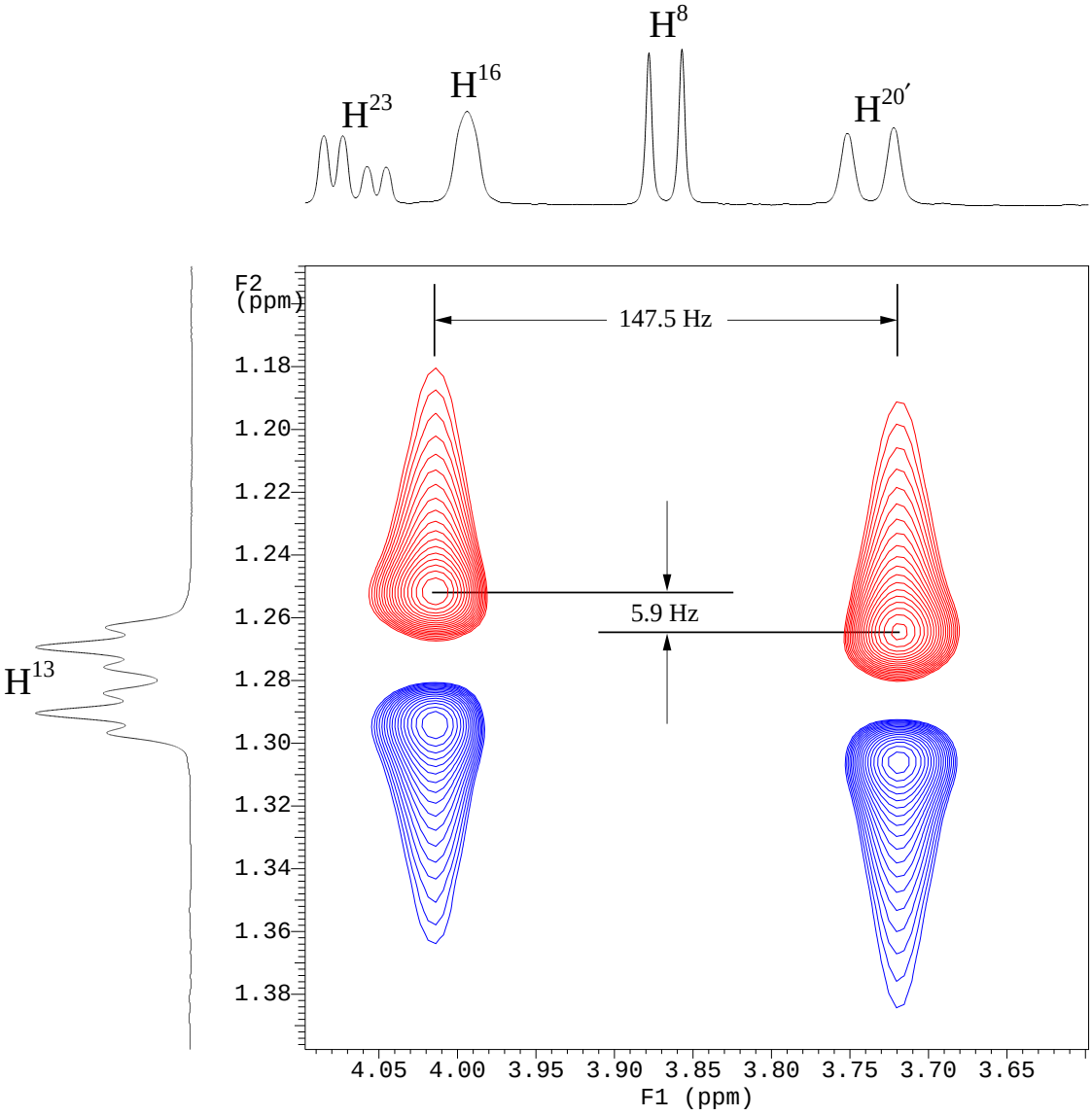


Figure 7.7 HETLOC data analysis of strychnine, illustrating measurement of $^{1}J(H^{8},C^{8})$ = 147.5 Hz in F_1 and $^{2}J(H^{13},C^{8})$ = 5.9 Hz in F_2. Data were acquired on the UI-500 using the Varian HCX probe. High-resolution 1D spectral expansions, rather than projections, are shown along F_2 and F_1 to emphasize that the F_2,F_1 intersection coincides with the correlation *center*, and that the correlation peaks *do not* necessarily coincide with peaks from the high-resolution multiplet structure.

Table 7.4 Experiments available in the ^{1}H,^{19}F suite

Done?	Experiment	Set-up Macro	tn	dn	dn2	Prerequisite
☒	$^{1}H\{^{19}F\}$	`Hobs_Fdec`	^{1}H		^{19}F	Optimized ^{1}H spectrum
☒	$^{19}F\{^{1}H\}$	`Fobs_Hdec`	^{19}F		^{1}H	Optimized ^{19}F spectrum
☒	$^{13}C\{^{1}H,^{19}F\}$	`Cobs_HFdec`	^{13}C	^{1}H	^{19}F	Optimized ^{13}C spectrum
☒	^{19}F,^{1}H gHSQC	`gHSQC_FH_d2`	^{19}F		^{1}H	Optimized ^{19}F spectrum
☒	^{1}H,^{19}F gHSQC	`gHSQC_HF_d2`	^{1}H		^{19}F	Optimized ^{1}H spectrum
☐	^{19}F,^{1}H gHMBC	`gHMBC_FH_d2`	^{19}F		^{1}H	Optimized ^{19}F spectrum
☐	^{1}H,$^{13}C\{^{19}F\}$ gHSQC	`gHSQC_HCF_d2`	^{1}H	^{13}C	^{19}F	Optimized ^{1}H spectrum
☐	^{1}H,$^{13}C\{^{19}F\}$ gHMBC	`gHMBC_HCF_d2`	^{1}H	^{13}C	^{19}F	Optimized ^{1}H spectrum
☒	^{19}F,^{1}H HOESY	`HOESY_FH_d2`	^{19}F		^{1}H	Optimized ^{19}F spectrum
☐	^{1}H,^{19}F HOESY	`HOESY_HF_d2`	^{1}H		^{19}F	Optimized ^{1}H spectrum
☐	^{19}F,^{1}H gHOESY	`gHOESY_FH_d2`	^{19}F		^{1}H	Optimized ^{19}F spectrum

These are locally developed experiments and are not part of the standard *VNMR* 6.1C installation. Available experiments are indicated by ☒; ☐ means the experimental details are not completed.

entire spectrum without spectral aliasing. After finding the resonance(s), one can then typically reset the spectral window to achieve the desired resolution, etc. Note that unusual cases may require obtaining multiple spectra. For example, if a compound has fluorinated groups whose resonances are significantly separated (by, say, 100 ppm), then it may be useful to acquire two data sets, with each spectrum more narrowly focused on the spectral subregion of interest; the desired spectral resolution will typically be the determining factor in such considerations for 1D fluorine spectra.

The second consequence is related but concerns the decoupling of ^{19}F. For efficient decoupling, the decoupler transmitter must be centered within the ^{19}F resonances, which is easily done if their positions are known; therefore, it is necessary to first acquire a 1D ^{19}F spectrum. In addition, the effective ^{19}F bandwidth is about 15 kHz for GARP decoupling,[22] which is 30 ppm on a 500 MHz (^{1}H) spectrometer. It should therefore be clear that compounds with fluorine chemical shifts spanning a range of more than approximately 30 ppm would require multiple acquisitions with the decoupler set appropriately for each.

NMR experiments involving both proton and fluorine are not commonplace because they require an atypical spectrometer configuration: two high-band transmitter channels and a probe capable of detecting both ^{1}H and ^{19}F. Specialized probes with independent proton and fluorine circuits are available and offer the best performance and greatest ease of use; probes with doubly tuned ^{1}H,^{19}F circuits (such as H,F;C,P quad nucleus probes) are more commonly available but require additional radio-frequency (RF) components to combine the two RF cables into the single connector on the probe. In our NMR laboratory, the experiments described in this section can be performed only the UI-500 spectrometer with the Nalorac quad-nucleus probe. Because

[22] GARP decoupling is the best we can do for fluorine-related experiments until I complete the setup and calibrations required for WURST decoupling, which has an approximately five-fold greater bandwidth compared to GARP.

of the non-standard configuration required for these experiments (described in detail below), no menu- or GUI-driven interface (e.g., via CustomQ) has been developed; software setup and data acquisition are easily performed via macro calls on the *VNMR* command line. A consequence of our own hardware 'limitation' is that we cannot perform experiments for which carbon and fluorine simultaneously use the transmitter and first decoupler; for example, we can perform ^{1}H,^{13}C{^{19}F} gHSQC experiments but not ^{19}F,^{13}C{^{1}H} gHSQC.[23]

7.16.2 Notes on Naming Conventions

Notation such as ^{13}C{^{1}H} indicates a carbon acquisition with proton decoupling, and should be familiar to most readers. By extension, ^{13}C{^{1}H,^{19}F} refers to a carbon acquisition with both proton and fluorine decoupling. Our spectrometers have a single (observe) *transmitter* channel, which in a loose sense functions as the *receiver* channel; thus for a ^{13}C{^{1}H} experiment, the transmitter and receiver are both set for carbon via the `tn` (**t**ransmitter **n**ucleus) parameter. Stated another way, the detected signal is always from the nuclide to which the transmitter is set.[24] Our three-channel UI-500 spectrometer therefore has two additional channels that can be used for decoupling; these are astonishingly referred to as the *first decoupler* and *second decoupler*, and are set for the desired nuclides via the parameters `dn` and `dn2`, respectively.

Because of the details in how our spectrometers are designed and configured, channels 1 and 2 can be set for ^{1}H and *Z*, where *Z* is any nuclide, including ^{1}H (this is referred to as a *full-band* channel). Channel 1 can be set to ^{1}H and channel 2 set for *Z*, or *vice versa*. Note that channel 1 represents the transmit/observe channel and channel 2 the first decoupler channel. This is true for both the UI-400 and UI-500. The UI-500 has a third channel — the second decoupler — which is also full-band capable; due to technical reasons it terminates in two physical cables, one of which is for *high-band* applications (i.e., ^{1}H or ^{19}F) and the other for *low-band* applications (basically, anything except ^{1}H or ^{19}F).

Understanding these details is important because the experiments described in this section require the operator to manually configure the RF cables, filters and power divider according to the experiment to be executed. To that end, a naming convention has been established using a mnemonic device to identify which nuclides are associated with each of the three channels. Table 7.4 on page 109 identifies the channel configuration and illustrates this convention. For example, the ^{1}H{^{19}F} experiment, which is set up using the `Hobs_Fdec` macro, observes proton while decoupling fluorine via the second decoupler channel. The ^{13}C{^{1}H,^{19}F} experiment is configured via the `Cobs_HFdec` macro, and decouples proton and fluorine via the first and second decoupler channels, respectively. Note that the 2- or 3-letter *order* in the setup macro names corresponds to the `tn`, `dn` and `dn2` spectrometer configuration *order*, with the implicit understanding that for experiments involving only ^{1}H and ^{19}F, the second decoupler must be used for the 'other' high-band nuclide.

[23] Technically, it *should* be possible to work around this using the `rfchannel` parameter.

[24] Modern spectrometers can be configured with multiple receivers, thus making this issue more complex than stated here; however, since our instruments have single receivers, such discussions are unnecessary and beyond the scope of this documentation.

7.16.3 Preliminary Preparations

No new sample preparation considerations come into play. The operator must understand the discussions in the preceding sections about (1) the broad range of fluorine chemical shift values and the consequent implications, and (2) spectrometer channels and their configuration according to the desired experiments.

7.16.4 Experiment Setup and Data Acquisition

As per the discussions above (Subsection 7.16.1), one should normally first acquire optimized and referenced 1D spectra as dictated by the subsequent experiments desired. This enables the operator to ascertain and correctly set the `dof` and/or `dof2` values for subsequent data acquisition. The general procedure is illustrated by the following specific example.

Suppose our ultimate goal is to acquire a 1D carbon spectrum with both proton and fluorine decoupling; we will therefore ultimately use the `Cobs_HFdec` setup macro to initialize this experiment. However, we begin by first acquiring an initial 1D ^{1}H data set, then optimize the spectral window so that the span of resonances is centered within the spectral window and there is about 10 % of baseline at each end of the spectrum; re-acquire, transform, phase and check/set the chemical shift reference, then save the data. Query the transmitter offset frequency by entering **`tof?`**, and record the value (call it x); this value will be assigned to the first decoupler offset, `dof`, in the $^{13}C\{^{1}H,^{19}F\}$ experiment. Next, perform a similar procedure to acquire an optimized ^{19}F data set, then and query and record its `tof` value (call it y), which will be assigned to the second decoupler offset, `dof2`, in the $^{13}C\{^{1}H,^{19}F\}$ experiment.

Now use a standard method to set up a carbon experiment, then enter the macro name **`Cobs_HFdec`** on the *VNMR* command line. Enter the string "**`dof=`***x* **`dof2=`***y* **`su`**" on the command line. The spectrometer software and console hardware are now initialized. Configure the RF cables, filters and power divider *under the magnet* according to Figure 7.8. Double check the configuration, then check/set additional acquisition parameters according to normal considerations; initiate data acquisition by entering the **`go`** command.

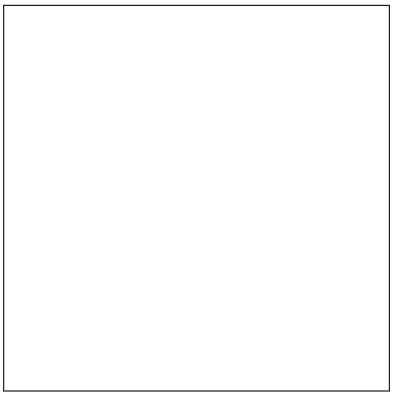


Figure 7.8 (This empty figure is a placeholder for a real figure to illustrate) RF cable and filter configuration for H/F experiments. Refer to Table 7.4 for correspondence to specific experiments.

7.16.5 Data Processing

Data processing typically requires no additional consideration or discussion for these experiments, with the possible exception that spectral referencing or phase correction may be necessary for some of the 2D data.

7.17 The ^{1}H,^{31}P Experiment Suite

In addition to basic ^{31}P 1D spectra, other ^{31}P-related data may at times be desirable. Examples include ^{31}P-decoupled ^{1}H 1D and ^{1}H,^{1}H 2D experiments; ^{13}C 1D experiments acquired with decoupling of both ^{1}H and ^{31}P; ^{1}H,^{31}P 2D heteronuclear correlation experiments; and ^{31}P-decoupled ^{1}H,^{13}C 2D heteronuclear correlation experiments. These various experiments are relatively easy to set up and execute under the *VNMR* software. Table 7.5 summarizes the experiments in this ^{1}H,^{31}P experiment family for which locally developed setup macros are available to facilitate the process. Those with interests or needs beyond these experiments should consult with the NMR Facility Director for assistance.

Table 7.5 Experiments available in the ^{1}H,^{31}P suite

Done?	Experiment	Set-up Macro	tn	dn	dn2	Prerequisite
☒	^{1}H{^{31}P}	`H1_P31`	^{1}H	^{31}P		Optimized ^{1}H spectrum
☒	^{13}C{^{1}H,^{31}P}	`C13_P31_d2`	^{13}C	^{1}H	^{31}P	Optimized ^{13}C spectrum
☒	^{1}H{^{31}P} gCOSY	`gCOSY_P31`	^{1}H	^{31}P		Optimized ^{1}H spectrum
☒	^{1}H{^{31}P} gDQCOSY	`gDQCOSY_P31`	^{1}H	^{31}P		Optimized ^{1}H spectrum
☒	^{1}H{^{31}P} NOESY	`NOESY_P31`	^{1}H	^{31}P		Optimized ^{1}H spectrum
☒	^{1}H{^{31}P} ROESY	`ROESY_P31`	^{1}H	^{31}P		Optimized ^{1}H spectrum
☒	^{1}H,^{31}P gHMQC	`gHMQC_P31`	^{1}H	^{31}P		Optimized ^{1}H spectrum
☒	^{1}H,^{31}P gHSQC	`gHSQC_P31`	^{1}H	^{31}P		Optimized ^{1}H spectrum
☒	^{1}H,^{31}P gHMBC	`gHMBC_P31`	^{1}H	^{31}P		Optimized ^{1}H spectrum

These locally developed experiments are not part of the standard *VNMR* 6.1C installation.

7.17.1 General Considerations

The 100-percent natural abundance and relatively large gyromagnetic ratio of ^{31}P make it a very good candidate for NMR experiments; however, the large chemical shift range of phosphorus compounds has important consequences. This chemical shift range spans about 430 ppm, which translates to 70 kHz on a 400 MHz spectrometer and 87 kHz on a 500 MHz instrument. The consequences of such large chemical shift ranges are essentially two-fold.

First, it is helpful to have an idea about the expected ^{31}P chemical shift for the compound in hand; otherwise, it may be necessary to either enlarge the spectral window or perform multiple experiments with overlapping spectral windows in order to locate the resonance. After finding

the resonance, one can then reset the spectral window to achieve the desired resolution, etc. The second consequence is related but concerns the decoupling of ^{31}P. For efficient decoupling, the decoupler transmitter must be placed near the ^{31}P resonances, which is easy to do if their positions are known; therefore, it is necessary to first acquire a 1D ^{31}P spectrum.

7.17.2 Preliminary Preparations

No new sample preparation considerations come into play.

7.17.3 Experiment Setup and Data Acquisition

Following the discussions above in Subsection 7.16.1 (the considerations for finding and efficiently decoupling the ^{19}F resonances apply here to ^{31}P), one should first acquire an optimized and referenced ^{31}P spectrum. For efficient ^{31}P decoupling in additional experiments, assign the decoupler position by setting `dof=xxxd` in the subsequent experiments of interest. Here, `xxx` specifies the center of the phosphorus resonances in ppm, and `d` specifies that the setting relates to the first decoupler channel. When using the second decoupler channel, as with the ^{13}C{^{1}H,^{31}P} experiment, one must use `dof2=xxx*dfrq2`, as there is no analogous suffix for the second decoupler.[25] This syntax is critical for both `dof` and `dof2`. The default action for the setup macros in Table 7.5 is to decouple phosphorus at 30 ppm. Other aspects of experiment setup and data acquisition typically proceed as normal.

7.17.4 Data Processing

Data processing requires no additional consideration or discussion for these experiments.

7.18 The No-D NMR Experiment

It is sometimes necessary or simply desirable to acquire NMR data using a fully protonated solvent instead of a deuterated solvent, as is typically used. Although the details related to implementing NMR experiments in protonated solvents follow directly and logically from a basic understanding of NMR principles and instrument design features, a brief review of the salient considerations is given below.

7.18.1 General Considerations

There are two primary reasons for using deuterated NMR solvents: (1) Minimizing the intensity of solvent ^{1}H resonance(s) via depletion of hydrogen atoms allows the solute resonances to be more optimally observed. (2) The solvent deuterium resonance can be used for field–frequency locking to compensate for intrinsic drift of the magnetic field, B_0, thus greatly improving spectrometer resolution and detection sensitivity. It is therefore important to understand the related implications when using fully protonated NMR solvents.

[25] The suffixes `p` and `d` signify and convert the preceding numerical value into ppm for, respectively, the transmitter and first decoupler channels; they are exactly equivalent to `*sfrq` and `*dfrq`. Since there exists no corresponding suffix for the second decoupler channel (i.e., no `d2` suffix), one must explicitly specify the conversion to ppm via `dof2=xxx*dfrq2`.

More easily addressed are the consequences of losing the field–frequency locking function. To understand the extent of the potential consequences, it is important to have a measure of the intrinsic drift associated with the spectrometer magnet in question. Although instrument (magnet) manufacturers typically cite a rather lax performance specification (e.g., less than 8 Hz per hour drift), our magnets' intrinsic drift rates have been measured at about 1 Hz per hour or less. Using a 1 Hz per hour drift rate to illustrate, if an acquisition was carried out for one hour, then all resonances would consequently be artificially broadened by approximately 1 Hz; this would of course have deleterious effects on both sensitivity (it would be diminished) and resolution (multiplets or other, closely spaced resonances may become less clearly identifiable).

Here's a challenge exercise for the interested reader: Make quantitative calculations to illustrate the effect on both the resolution and the signal-to-noise ratio of a hypothetical doublet having a 2 Hz spin–spin coupling and, in the absence of field drift, a 1 Hz line width and S/N of 100. Assume a one-hour data acquisition and 1 Hz per hour intrinsic drift rate.

Another consequence of having no deuterium atoms and thus no deuterium signal is that we can no longer use that signal for "shimming" the magnetic field. Gradient or manual shimming must therefore be performed using the solvent proton signal(s).

Three implications follow as a result of the overwhelming signal intensity of solvent proton resonance(s). One is the potential for the partial or complete obscuration of solute resonances due to some degree of overlap with the solvent resonance(s). The second implication — less obvious to the neophyte — involves the spectrometer receiver's dynamic range and how it is able to accommodate the relative intensity differences between the solute and solvent resonances. In qualitative terms, this amounts to the capability to detect relatively small signals (and perhaps also to differentiate those small signals from noise) in the presence of a very intense signal that dominates the total signal detected and digitized in the spectrometer's receiver.

The third implication also relates to the capabilities of the spectrometer receiver circuit, in this case the *receiver gain*, which amplifies the incoming signal for optimal detection and digitization. (Think of using the volume control on your stereo to adjust the intensity of the output for optimal detection by your ears and brain.) Since NMR spectrometers are designed to detect very-small-intensity, analyte (solute) signals, it should not be too difficult to imagine that significantly larger incoming signals — e.g., from a protonated solvent — could present a difficulty. In fact, fully protonated solvents generally present signals so intense that the receiver circuit cannot properly digitize them, even with the receiver gain parameter set to its minimum value. In such cases, the FID and observed spectrum will show characteristic *receiver-gain artifacts*. For routine 1D pulse-and-acquire experiments, it is a straightforward matter to simply reduce the pulse flip angle to consequently diminish the observed signal intensity so that it falls within the limits of the receiver circuit. But what does one do in the case of other experiments that demand 90-degree pulses and/or multiples thereof? We make use of external, in-line attenuators, of course, as described in the experimental setup section below. (Think of using ear plugs to reduce the sound level to your brain via your ears.)

Here's another challenge exercise for the interested reader: (a) Considering your favorite NMR solvent, what would be the relative number of hydrogen atoms for the fully protonated solvent compared to the deuterated solvent at 99.9 atom % D? (b) How much larger would the NMR signal be for the fully protonated solvent compared to the deuterated solvent? (c) What would be the *hydrogen atom* molar concentration for the fully protonated solvent, and how does

that value compare with a typical *solute* concentration for NMR samples?

Finally, note that the No-D NMR experiment setup and acquisition procedure described below **is NOT compatible** with the *VNMR* CustomQ or Walkup interfaces; therefore, No-D NMR experiments must be set up and executed using "manual" methods.

7.18.2 Preliminary Preparations

The number one preliminary preparation is to understand the general considerations presented above; there are several practical trade-offs that need to be seriously considered before heading into the NMR lab to run No-D experiments. It is also possible — at least in principle, if not always in practice — to incorporate solvent suppression elements (PRESAT, WET, etc.) into No-D NMR experiments, and *vise versa*. Otherwise normal sample preparation guidelines, such as filtering out precipitates and using the correct sample volume, should be followed.

7.18.3 Experiment Setup and Data Acquisition

Although the following directions are relatively straightforward, users are advised to request and participate in a hands-on demonstration with NMR Facility staff before attempting to perform the experiments alone for the first time. Please make such requests via the NMR Spectroscopy Training web page and form.

1. Log in, start *VNMR* and insert the sample into the magnet as usual.
2. Initiate No-D NMR experiment setup using the `noDnmr<(nucleus<,solvent>)>` syntax; the following examples illustrate three different syntax levels:
   (a) On the *VNMR* command line, enter `noDnmr(nucleus,solvent)` to specify both the observe nucleus and the solvent. For example, **`noDnmr('H1','DMSO')`** configures for proton data acquisition in DMSO; use **`noDnmr('C13','D2O')`** to acquire carbon data in H_2O, etc. (Yes, use the name of the deuterated solvent even though you are actually using the protonated version. The *VNMR* solvent file that is read by the setup macro consists primarily of entries with names for deuterated solvents; however, the pertinent information extracted from the file is the same for both protonated and deuterated versions of a particular solvent.)
   (b) Entering `noDnmr(nucleus)` allows for specifying the observe nucleus but configures $CDCl_3$ as the solvent by default. For example, entering **`noDnmr('H1')`** configures for proton data acquisition in $CHCl_3$.
   (c) You may enter simply **`noDnmr`** as an alternative to directly specifying the nucleus or solvent information as described in the preceding instructions. This command configures for a proton acquisition in chloroform (i.e., it sets `nucleus='H1'` and `solvent='CDCl3'`).

   In addition to setting parameters for a 1D experiment with the requested nucleus and solvent, the `noDnmr` macro loads the default shim file and sets the parameter `z0` to correctly center the spectral window for the requested solvent. A brief set of reminder instructions is presented in the *VNMR* text panel.

3. Tune the HCX probe as you would normally do.
4. Initiate **proton** gradient shimming ('H1,' not 'H2' or 'lk'); this is most easily executed via the SetupEXP interface. Manual shimming on the proton FID may also be performed, if desired.
5. Begin acquisition of the preliminary 1D experiment by entering the **`go`** (or **`ga`** or **`au`**) command.
6. Inspect the resulting NMR signal and modify the `gain` and/or `pw` values as needed to prevent receiver gain artifacts. If necessary, insert external, in-line attenuators at the high-band (1H/19F) preamplifier output at the port labeled OUTPUT J5302.
7. Once the receiver gain and pulse width (and external attenuation, if used) have been optimized, review and set any additional acquisition parameters (e.g., the spectral window and `nt`) as desired.
8. Begin data acquisition. Data quality can be monitored while the acquisition is in progress, at multiples of `bs`.
9. Save the data set via `svf('<path>filename')`.
10. Additional experiments (gCOSY, gHSQC, CARBON, etc.) can be set up and executed subsequent to acquiring the initial 1D experiment.

7.18.4 Data Processing

Data processing for No-D NMR data is essentially the same as for data acquired using deuterated solvents; however, post-acquisition *solvent subtraction* spectral filtering may be useful or desired to reduce the solvent resonance intensity (cf. the Varian *Getting Started* manual for a discussion and implementation details).

7.19 The Pulsed Gradient Spin Echo (PGSE) Experiment

The pulsed gradient spin echo (PGSE) experiment is the basic method for measuring translational diffusion coefficients by NMR spectroscopy. (This section may be completed in the future if it ever becomes important to a broader audience; in the meantime, contact the NMR Facility Director for information related to this experiment.)

Chapter 8

Solvent Suppression

8.1 Introduction

The realm of solvent suppression includes a variety of techniques to eliminate — or at least to greatly reduce — one or more, usually large, solvent resonances from the NMR spectrum. The usual origin of such large resonances stems from the use of protonated rather than deuterated solvents. Biological NMR applications commonly use mixtures of H_2O/D_2O in the ratio of 90/10, thereby resulting in a single large resonance to suppress. LC-NMR applications typically employ mixed, protonated solvent systems (e.g., acetonitrile/water) and therefore have multiple resonances to suppress. Organic chemists sometimes want to analyze a reaction solution directly, without having to isolate particular components of interest and dissolve them into deuterated solvent.

As discussed in more detail in Section 7.18, the consequences of such large and unwanted resonances are primarily threefold: (1) The peak(s) may simply obscure the solute resonance(s) of interest. (2) A huge solvent signal in the presence of much smaller solute resonances presents a *dynamic range* problem that impedes proper detection of the smaller signals. (3) Additional measures, perhaps external, may be required to reduce the solvent signal to an intensity that the receiver can manage.

NMR spectroscopists have dealt with these issues for many years, and today there exist a variety of methods for the suppression of unwanted signals. There are several important considerations when faced with selecting a solvent suppression technique; therefore, choosing the most appropriate method requires understanding in regard to both the sample system and the spin physics that underlie the suppression techniques. There are two basic philosophies toward suppressing such undesired resonances: (1) The signals are the enemy and therefore must be destroyed. (2) The signals are our friends and we must work nicely together to achieve the goal. The former approach works well for systems in which the solvent protons are not in a state of dynamic exchange with the solute molecules; the latter approach is typically needed when dealing with sample systems in which there is proton exchange between solute and solvent.

8.2 Methods

Some of the methods in contemporary use are listed in this section. A brief description of these methods may be included here in the future, if warranted by the needs and interests of the user community. In the meantime, those interested are encouraged to consult Section 3.5 of Cavanagh, *et al.* [22].

8.2.1 Presaturation (PRESAT)

8.2.2 Binomial Water Suppression (BINOM)

8.2.3 Water Suppression Enhanced Through T_1 Effects (WET)

8.2.4 Water Suppression by Gradient-Tailored Excitation (WATERGATE)

8.3 Implementation under *VNMR*

Optimal suppression of large solvent resonances requires very good field homogeneity, especially in regard to the low-intensity signal area around the peak base; this generally requires optimizing the higher-order axial (e.g., z^6) and low-order radial (e.g., x, y, xy, $x^2 - y^2$) shims. For a good discussion about shimming in preparation for solvent suppression, see pages 146–147 of the Varian *Getting Started* manual.

Only the PRESAT and WET techniques are currently implemented on the Varian UI-500 spectrometer, and both methods require use with the HCX probe.

8.3.1 PRESAT

The following steps outline the procedure for acquiring 1D ^{1}H data with solvent signal(s) suppression using the PRESAT element incorporated into the s2pul sequence.

1. Acquire a preliminary 1D ^{1}H spectrum with optimized spectral window. Phase the spectrum properly, then enter either **`calfa`** or **`crof2`** to optimize `alfa` or `rof2`, respectively,[1] to obtain flatter baselines, which are particularly critical for quality 2D data.

2. Acquire and process a new data set to test the updated parameters. If the result is suitable, save the file for future reference; otherwise, continue until a suitable result has been achieved.

3. Position the cursor on the resonance to be suppressed, enter the **`nl`** command to set the cursor to the peak maximum, then enter **`PRESAT_UW`** (note the upper-case letters) to convert the pulse sequence and set initial parameters according to the installed probe; note that **only** the Varian HCX probe is supported.

4. Enter **`PSopt`** to initiate the automated optimization algorithm. The suppression performance depends critically on the exact positioning of the presaturation frequency, and this is what the `PSopt` macro optimizes.

[1] As usual, refer to the Varian *VNMR Command and Parameter Reference* manual for details related to these commands.

5. Good suppression can typically be achieved with `satfrq` thus optimized and the remaining parameters left at their initial values. In some cases, however, `satdly` and/or `satpwr` may require further optimization as well. **Caution: excessive `satpwr` or `satdly` values can damage the probe!** If suppression does not work reasonably well with `satfrq` optimized via `PSopt`, and `satpwr` and `satdly` at their default values, something else is probably wrong. **Do not blindly forge ahead!**; stop your efforts and seek the NMR Facility Director for assistance.

6. Save the data set corresponding to your optimized parameters, and note the optimized values for the critical parameters.

The `PRESAT_UW` macro configures the experiment for use with a pulse sequence that specifically handles cases in which the presaturation irradiation frequency `satfrq` can differ from the transmitter offset frequency `tof`. This is in contrast to the original *VNMR* `presat` macro (note the lower-case letters here).

If presaturation is desired for subsequent use in a 2D sequence, use the appropriate macro to convert your optimized 1D PRESAT spectrum and parameters to the desired 2D experiment. The macro commands `PSgDQCOSY`, `PSgHSQC` and `PSNOESY` configure PRESAT versions of the 2D DQCOSY, gHSQC and NOESY experiments, respectively. PRESAT versions of additional 2D experiments may be implemented in the future.

8.3.2 WET

The following steps outline the procedure for acquiring 1D ^{1}H data with suppression of solvent signal(s) using the WET element incorporated into the s2pul sequence.

1. Perform the usual pre-acquisition procedures, such as field–frequency locking, gradient shimming, etc.

2. Set up for and acquire 1D data with the spectral window optimized for your analyte; save the data set. Do not modify the position of the spectral window (i.e., do not change `tof`) after setting up the WET element below. If a solvent signal is very large — as with an H_2O/D_2O mixture at 90/10 volume ratio — it may be necessary to initially decrease the non-selective pulse (i.e., `pw`) to prevent ADC overflow.

3. Join another experiment, then load and process (e.g., `wft`, `aph0` and `dc`) the previously saved data set. (One could perform the processing, etc., in the original experimental workspace, but it is convenient to access the non-suppressed spectrum without reloading the data.)

4. Adjust the vertical scale, then set the threshold to select only the peak(s) desired for suppression. Enter **`dpf`** or **`dll`** to check that only the desired resonances are detected by the peak-picking routine.

5. Enter **`wet`** to execute the automated setup macro, which performs these steps:

   (a) Executes the `wet1d` script to convert the starting s2pul pulse sequence into the wet1d sequence, a modification that incorporates the WET suppression element.

(b) Executes the `wetit` script to set up a shifted laminar pulse for selective suppression of the desired resonance(s), as identified by those peaks above the selected threshold. Each peak thus targeted for suppression is characterized by its frequency and a bandwidth, which is related to $\Delta\nu_{1/2}$.

(c) Initializes important parameters (`pw(90)`, `ss=2`, `nt=4`, `gain`, `composit='y'`).

(d) Displays the `wet1d` help entry to advise the user with setup and optimization.

(e) Displays the time-domain signal of the selective, shaped pulse thus generated for peak suppression.

6. Acquire and process a new data set to see how effectively the suppression worked. If acceptable, edit the acquisition parameters further to obtain the desired signal-to-noise ratio, etc., then acquire the final data set.

7. If the initial suppression result was not acceptable, enter **`wetopt`** to execute an optimization process — analogous to the `PSopt` optimization macro described above — to improve the suppression performance, although the benefits may be minimal or nonexistent. Note that one must run the `wet` macro starting from a spectrum with "normal" solvent peaks; that is why it was suggested to retain the original starting data in another experiment workspace.

8. Seek assistance from the NMR Facility Director early on if apparent troubles arise.

8.3.3 WATERGATE

8.4 Post-Acquisition Processing

Refer to Section 8.5 of the Varian *Getting Started* manual.

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For additional literature references, cf. pages 66 and 67 of the Varian *VNMR 6.1C User Guide: Liquids NMR*.

Appendix A

Additional Resources

Included in this section are lists of resources specializing in, or related to, magnetic resonance.

A.1 Periodicals

- *Concepts in Magnetic Resonance*
- *Journal of Magnetic Resonance*
- *Magnetic Resonance in Chemistry*
- *Solid State Nuclear Magnetic Resonance*

A.2 Periodical Reviews

- *Annual Reports on NMR Spectroscopy*
- *Progress in Nuclear Magnetic Resonance Spectroscopy*
- *A Specialist Periodical Report on Nuclear Magnetic Resonance*
- *NMR: Basic Principles and Progress*

A.3 Magnetic Resonance Monographs

- "Principles of Nuclear Magnetism," A. Abragam, Clarendon Press, 1961.
- "Principles of Magnetic Resonance," C. P. Slichter, Springer, 1990.
- "Principles of Nuclear Magnetic Resonance in One and Two Dimensions," R. R. Ernst, G. Bodenhausen and A. Wokaun, Clarendon Press, 1992.
- "Quantum Description of High-Resolution NMR in Liquids," M. Goldman, Clarendon Press, 1988.

- "Density Matrix Theory," T. C. Farrar and J. E. Harriman, Farragut Press, 1998.
- "High Resolution NMR: Theory and Chemical Applications," 3rd Ed., E. D. Becker, Academic Press, 2000.
- "High-Resolution NMR Techniques in Organic Chemistry," Timothy D. W. Claridge, Elsevier, 1999.
- "Introduction to Magnetic Resonance," Carrington and McLachlan, Chapman and Hall, 1980.
- "Multidimensional NMR in Liquids: Basic Principles and Experimental Methods," F. J. M. van de Ven, VCH, 1995.
- "NMR Spectroscopy: Basic Principles, Concepts, and Applications in Chemistry," Harald Günther, Wiley, 1996.
- "Introduction to Pulse NMR Spectroscopy," T. C. Farrar, Farragut Press, 1989.
- "Experimental NMR: A Nuts and Bolts Approach," E. Fukushima and S. B. W. Roeder, Addison-Wesley, 1981.
- "Modern NMR Spectroscopy: A Guide for Chemists," J. K. M. Sanders and B. K. Hunter, Oxford University Press, 1994.
- "Modern NMR Techniques for Chemistry Research," A. E. Derome, Permagon Press, 1987.
- "The Nuclear Overhauser Effect," J. H. Noggle and R. E. Schirmer, Academic, 1971.
- "The Nuclear Overhauser Effect in Structural and Conformational Analysis," 2nd Ed., D. Neuhaus and M. Williamson, Wiley, 2000.
- "150 and More Basic NMR Experiments: A Practical Course," S. Braun, H.-O. Kalinowski and S. Berger, Wiley–VCH, 1998.
- "Structure Elucidation by Modern NMR: A Workbook," H. Duddeck, W. Dietrich and G. Tóth, Steinkopff, 1998.
- "NMR of Proteins and Nucleic Acids," K. Wüthrich, Wiley, 1986.
- "Protein NMR Spectroscopy: Principles and Practice," J. Cavanagh, W. J. Fairbrother, A. G. Palmer III and N. J. Skelton, Academic Press, 1996.

A.4 Magnetic Resonance Dictionaries and Encyclopedias

- "A Handbook of Nuclear Magnetic Resonance," R. Freeman, Addison Wesley Longman, 1997.
- "Encyclopedia of Nuclear Magnetic Resonance," D. M. Grant and R. K. Harris, Eds., Wiley, 1996.

Appendix B

Advanced Probe Configurations and Tuning Methods

This appendix provides brief descriptions of unusual or advanced probe and filter configurations and related tuning methods. Tabulated probe-configuration and tuning-related specifications are presented for select probes in the facility; the goal is for these data to provide a useful reference when configuring and tuning an instrument for non-standard experiments, e.g., for X-nuclide experiments on the HCX probe's broad-band circuit.

B.1 Varian HCX Probe

The Varian HCX probe has three circuits dedicated to a fixed nuclide (i.e., ^{1}H, ^{13}C, and ^{2}H lock), plus a fourth circuit that is continuously tunable over the range of frequencies encompassing ^{15}N on the low end (ca. 50 MHz) and ^{31}P on the high end (ca. 200 MHz). The common term for this type of widely tunable circuit is an X circuit. The HCX probe is therefore not capable of experiments involving ^{19}F, which has a resonance frequency of 470 MHz at this magnetic field strength.

The X circuit provides slightly better performance for ^{13}C than does the dedicated ^{13}C circuit;[1] for this reason, the standard configuration for ^{1}H,^{13}C 2D experiments in our lab is to tune and use the X circuit for ^{13}C, and detune the dedicated ^{13}C circuit to avoid potential and undesirable interference effects arising from two coupled resonators.

For ^{1}H,X experiments where X is not ^{13}C, the X circuit must, of course, be tuned properly for the target nuclide. Again due to design considerations, to tune across the entire 150 MHz range of the X circuit, it is necessary to manually install or remove additional circuit elements to change the overall inductance or capacitance of the circuit; refer to Figure 7.1 for a reminder of the role each element plays in such a circuit.

In addition to ^{1}H,X indirect-detection experiments, it is also possible to perform direct X-detection experiments. In both direct- and indirect-detection experiments, it is necessary to consider and use the appropriate radio-frequency (RF) filters to simultaneously (1) pass the desired RF bandwidth, and (2) reduce the introduction of extraneous noise into the data. There are two different filters to achieve these goals: One filter is a discrete device connected in-line

[1] This may seem counterintuitive, but the reason is due to design and optimization considerations and decisions.

with the RF cable between the preamplifier (J5311 connector) and the probe; the other filter is a short RF cable known as a "quarter-wavelength" ($\lambda/4$) filter that connects directly to a pair of BNC terminals on the low-band preamplifier unit. Both filters must be chosen to match the resonance frequency of the X nuclide under study. These filters are labeled according to their frequency and other properties, to identify their designated use.

In Table B.1 are shown various data related to configuration and tuning of the Varian HCX probe's X circuit. A useful tip from the "Varian Indirect Detection NMR Probe Installation" manual is that "[t]he lower the frequency of the nucleus, the more the smooth match knob needs to be turned clockwise" (as viewed from underneath the probe, looking at the end of the match knob, as if unscrewing it).

Table B.1 UI-500: Varian HCX (hcx4765) probe configuration data

Nuclide	sfrq (MHz)	Counter	Insert	Tune Range[a]	$\lambda/4$ Cable[b]	Band-pass Filter
^{15}N	50.642	76	28pF	47–55	48–65	BE 53–15–8BB
^{17}O	67.745	[c]	14pF	60–70	65–90	BE 72–12–8BB
^{2}H	76.712	[c]	9pF	70–87	65–90	BE 72–12–8BB
^{29}Si	99.282	49	none	78–133	85–120	BE 109–22–8BB
^{13}C	125.669	06	none	78–133	120–170	BE 135–15–8BB
^{23}Na	132.194	[d]	4T	100–160	120–170	BE 135–15–8BB
^{11}B	160.328	56	8T	130–210	120–170	BE 151–40–8BB
^{119}Sn	186.368	31	8T	130–210	170–250	BE 175–60–8BB
^{7}Li	195.203	24	8T	130–210	170–250	BE 175–60–8BB
^{31}P	202.289	16	8T	130–210	170–250	BE 175–60–8BB

[a] This is the nominal tuning range, in MHz, for the corresponding insert.
[b] This is the nominal working range, in MHz, for the corresponding cable. Each cable is labeled with its working range and optimal frequency.
[c] We have neither the indicated capacitor insert nor the indicated band-pass filter for this configuration.
[d] We do not have the indicated inductor insert for this configuration.

B.2 Probe Tuning via *VNMR* Q-tune

The *VNMR Q-tune* feature[2] provides a graphical representation of reflected power as a function of radio frequency. This method greatly facilitates probe tuning for probes with unusual coil configurations (e.g., simultaneously tuned ^{1}H/^{19}F and ^{13}C/^{31}P circuit pairs on a quad-nucleus probe) or in situations where the initial tuning is very far from optimal. Only a brief description and a few operational notes are given here; for detailed information refer to the Varian *Getting Started* (pages 113–126) and *VNMR Command and Parameter Reference* (page 455) manuals.

[2] Why is it called Q-tune? What is the significance of Q? Refer to Equation 6.2 and Table 6.1 for clues.

The general syntax is

```
qtune<(gain<,power>)>
```

where `gain` is typically between 20 and 50, and `power` is typically 60 to 70. Default values for `gain` and `power` are 50 and 60, respectively; optimal values depend upon spectrometer and probe details, and thus can vary significantly between instrument–probe configurations.

To configure the cable for `qtune`, connect the RF cable from the desired probe circuit to the TUNE port on the preamplifier housing TUNE INTERFACE, but leave the channel selector (CHAN) set at zero. I repeat: do not change the channel selector from its off setting of zero! Once the cable configuration is completed, start the `qtune` functionality by entering **`qtune`**, with non-default `gain` and `power` values specified as necessary.

The `tugain` parameter sets the receiver gain used by `qtune`. If the value is too large (the default is 50), the `qtune` signal may saturate, causing the display to appear as a relatively flat line. If it is necessary to change the `tugain` parameter value, first exit `qtune`, then change `tugain` and restart `qtune`.

Table B.2 illustrates a few sets of empirically determined parameters for the UI-500 with the Varian HCX probe.

Table B.2 UI-500 Q-tune parameters for the Varian HCX probe

Nuclide	Settings	
^{1}H	`tugain=25`	`qtune(20,65)`
^{7}Li	`tugain=`	`qtune(20,50)?`
^{11}B	`tugain=`	`qtune(20,50)?`
^{13}C	`tugain=`	`qtune(20,50)?`
^{15}N	`tugain=`	`qtune(20,50)?`
^{31}P	`tugain=`	`qtune(20,50)?`

Table B.3 similarly lists empirically determined parameters for the UI-500 with the Nalorac QN probe. This information is for the benefit of NMR Facility staff, as only staff are permitted to tune the QN probe. Further note that it is possible, for exceptional circumstances, to tune the ^{31}P circuit downward in frequency for ^{7}Li experiments; doing so requires detailed knowledge, and is largely unnecessary these days, since we have ready access to ^{7}Li via the Bruker AV-400 spectrometer.

Table B.3 UI-500 Q-tune parameters for the Nalorac QN probe

Nuclide	**Settings**	
^{1}H	`tugain=25`	`qtune(20,65)`
^{19}F	`tugain=`	`qtune(20,50)?`
^{13}C	`tugain=`	`qtune(20,50)?`
^{31}P	`tugain=`	`qtune(20,50)?`
^{7}Li	`tugain=`	`qtune(20,50)?`