3. NMR instrumentation

3.1 Layout of a high resolution NMR spectrometer

In this section the major features of a modern NMR spectrometer are discussed. A spectrometer used for studies of biological macromolecules does not differ in its basic functionality from any other NMR instrument used for studies of compounds in solution. Fig. 8 displays a block diagram which is used in the following as a basis for a short discussion of the major components. A modern NMR spectrometer is controlled by a dedicated spectrometer computer which is instructed by the operator *via* a general purpose host computer work station. Additional processors perform specific functions under the master timing and control of the spectrometer computer. In Fig. 8 the main parts of a spectrometer are connected by arrows drawn with heavy lines.

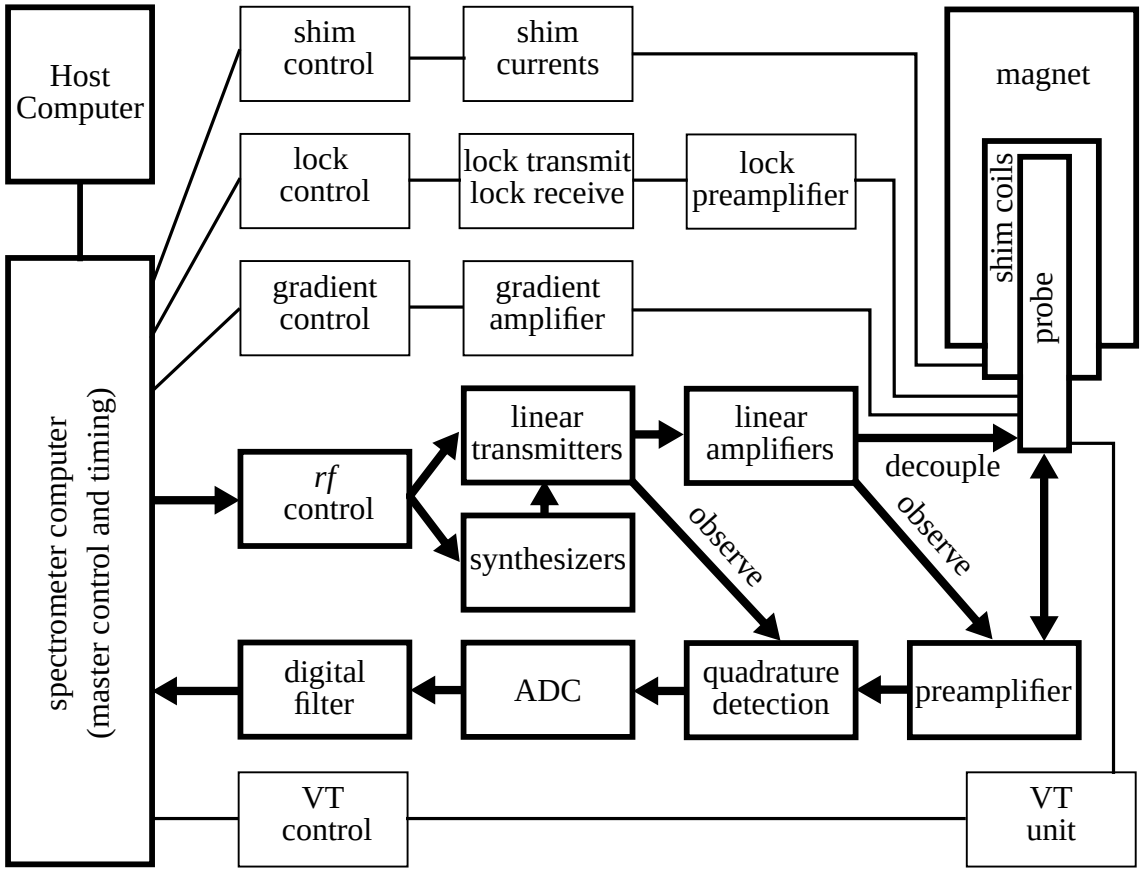

Fig. 8. Block diagram of a typical high resolution NMR spectrometer. The main parts of the spectrometer are drawn with heavy lines; the arrows indicate the pathway from the spectrometer computer to the probe and back to the computer where the NMR signal is stored. From the preamplifier both the *rf* pulses on the observe channel and the detected NMR signal travel on the same cable. For the observe channel the reference frequency for detection has to be fed into the receiving pathway. The parts drawn and connected with thin lines provide auxiliary functions of the spectrometer which are essential for a spectrometer used for applications with biological macromolecules; VT stands for variable temperature and ADC for analog-to-digital converter.

Using this figure the essential steps in obtaining a NMR spectrum can be followed. First, the spectrometer computer instructs the *rf* control processor to set the necessary frequencies in the synthesizers and to send *rf* pulses from the transmitter to the different amplifiers according to the experimental requirements. Based on digitally stored data the transmitter prepares the pulses with the required shapes, phases, durations and power settings before sending them to the linear amplifiers. Modern NMR spectrometers are able to set all necessary parameters within a few microseconds during the execution of a pulse sequence. All stages in the transmitter and the power amplifiers have to be linear otherwise pulses with non-rectangular shapes will be distorted and cannot act in the way expected (Section 2.2.2). *Via* the linear amplifiers the *rf* pulses reach the probe which contains a coil that delivers the *rf* frequency to the sample located in the top of the probe. When no *rf* pulse is applied the amplifiers are completely blanked, so that the spins can precess free from any *rf* disturbance. Because the same coil is used for the application of *rf* pulses at the observe frequency and for receiving the very weak response from the spins the pulses at the observe frequency are routed through the preamplifier. A special safety circuitry protects the preamplifier from the very high voltage present during a *rf* pulse and directs the pulse only towards the coil. In the receive mode the NMR signal picked up by the receiving coil reaches the preamplifier stage with minimal attenuation. The signal leaves the preamplifier still at the actual NMR frequency amplified sufficiently to preserve the *S/N* in the subsequent amplification and mixing stages. The signal cannot be digitized at the NMR frequency and must be transformed into a frequency range of a few tens of kHz where digitizers with a high dynamic range exist. Reducing the frequency does not happen in one step. First all NMR frequencies are transformed to the same intermediate frequency and the two quadrature channels are created (Section 3.3.1). A further mixing step reduces the signal frequencies of the quadrature channels to lower frequencies which can be digitized in the analog-to-digital converter (ADC) which samples the signal at discrete time points and converts it into a series of numbers. Modern ADCs oversample the data [101] and digitize a frequency range that is much larger than the one desired. The final spectral range is selected using digital filters which can be designed to have a much better performance than corresponding analog filters (Section 2.4.4). If digital filters are not available the corresponding analog filters must be used before the ADC. After digitizing and filtering the signal is sent to the computer memory where different scans are summed up and the data can later be processed.

In addition to the main task of recording the NMR spectrum, a spectrometer fulfils many auxiliary functions which make modern NMR spectroscopy possible (Fig. 8). Very stable adjustable shim currents must be supplied to the shim coils which create a correcting magnetic field to obtain the required homogeneity of the main magnetic field. The homogeneity thus obtainable by far outperforms the long-term stability of the frequency sources and the magnetic field. To obtain sufficient stability the ratio of the frequencies and the magnetic field is kept constant by permanently monitoring a reference NMR signal at a specific frequency. For this locking of field and frequency usually the deuterium signal of some deuterated solvent added to the sample is observed. Any deviation of the deuterium frequency from a preset value is corrected by a very small change of the magnetic field using a special coil in the room temperature shim system. In this context the variable temperature (VT) capabilities of a spectrometer become very important. In biological NMR measurements deuterated water often serves as lock substance. However the deuterium resonance frequency of D_2O depends strongly on the temperature and without a very stable temperature in the sample the lock could not perform its task.

3.2 Spectrometer configuration for biomolecular NMR

The use of a NMR spectrometer for studies of biological macromolecules in solution defines the hardware configuration to a large extent. The importance of proton resonances with their limited spectral dispersion and the large number of resonances present in a protein sample demands for the majority of applications a proton resonance frequency of at least 600 MHz which corresponds to a magnetic field strength of 14.1 Tesla (T). In addition to protons the nuclei ^{2}H, ^{13}C, ^{15}N, ^{31}P and ^{19}F are most frequently measured (Table 1). In practice up to four of these nuclei may be correlated in one experiment requiring four radio-frequency channels in addition to the lock channel. For example, larger proteins are often triple labelled with ^{13}C, ^{15}N and ^{2}H. Even though usually only ^{1}H, ^{13}C and ^{15}N nuclei are directly correlated, decoupling of deuterons is required to reduce the linewidths of carbon and/or nitrogen resonances. In some experiments it seems very convenient to use two channels for one nuclear species as in the case of carbons where aliphatic and carbonyl carbons show a large chemical shift difference which makes these two groups of resonances behave in many aspects like heteronuclei. More and more experiments require shaped pulses and/or frequency shifted pulses for best performance. Consequently at least three *rf* channels should be equipped with pulse shaping capabilities which include the possibility to set the phase of the *rf* frequency in small increments.

Individual spectrometers will show most variability in their probes (Section 3.5) which depend rather strongly on the applications envisaged. For studies of proteins, a probe tunable to the frequencies of the nuclei ^{13}C, ^{15}N, and ^{1}H is necessary to carry out the standard experiments of proteins labelled with ^{13}C, ^{15}N and/or ^{2}H. For the decoupling of deuterium the deuterium lock coil can be used. Such a triple resonance probe is designed to detect protons. With protons being the most important nucleus when working with proteins a proton only probe may seem necessary, however, the newest probe designs offer at best only modest sensitivity advantage for a selective probe compared to a modern probe with an additional broadband channel. Such a broadband inverse detection probe provides the advantage of making a wide range of other nuclei accessible which may be of interest in some applications. In addition when working with proteins only labelled with ^{15}N this probe becomes preferable due to its better performance at the nitrogen frequency compared to a triple resonance probe. This is because in a triple resonance probe the nitrogen and the carbon frequency are transmitted by the same coil which is optimized for the carbon frequency reducing the performance for pulses on nitrogen. In addition, the broadband inverse detection probe can be used for the occasional direct detection of heteronuclei. In such applications the sensitivity is about half of that obtainable on a probe with a heteronuclear broadband detection coil. For occasional measurements of ^{3}H or ^{19}F, a coil intended for proton pulses can be tuned to excite and/or detect one of the two frequencies. However, proton decoupling with these nuclei requires a dedicated probe in combination with special filters to prevent spurious irradiation of *e.g.* fluorine frequency *via* the proton channel. A very versatile probe, a quadruple resonance probe, is simultaneously tuneable to ^{2}H, ^{13}C, ^{15}N, ^{31}P and ^{1}H and can be used to measure proteins and nucleic acids. However such probes have reduced specifications compared to a triple resonance probe and the latter should be preferred for applications where one of the heteronuclear frequencies is not required.

Probes are built for a specific sample tube diameter. The most commonly used diameter is 5 mm. Probes for 3, 8 or even 10 mm are available but not so universally applicable. Typically large diameter probes do not yet reach the performance expected by extrapolating specifications of a 5 mm probe. In addition these probes suffer from more severe problems with the H_2O resonance because shimming tends to be more difficult and adverse effects like radiation damping are more

pronounced (Section 5.4.1). Nevertheless in situations were a protein cannot be dissolved at the desired concentration for a 5 mm tube a larger diameter sample tube is definitely an interesting alternative.

Precise and stable control of the sample temperature in the probe is very important for optimal performance of the spectrometer. More specifically the variable temperature (VT) control system must fulfil the following requirements. Firstly, it must be very stable and keep the temperature within 0.05 K of the set temperature. This is because the field-frequency lock is based on the deuterium resonance of water which is very temperature sensitive with a resonance shift of 0.01 ppm/K. Even small temperature changes will degrade the performance of the spectrometer by causing field corrections which are not based on a field-frequency drift. Since many protein resonances have a very small intrinsic temperature dependence these field changes can severely deteriorate the spectral quality. Secondly, easy and secure temperature stabilization is essential in the temperature range from 273 K to 323 K where most experiments are performed. For proper operation of the regulation circuitry the input temperature of air supplied to the probe should be at least 10 K lower than the temperature desired in the probe. A cooling unit which can deliver dried air (or nitrogen) at 260 K at the probe entry facilitates the VT operation in the desired temperature range.

In recent years pulsed magnetic field gradients (PFGs) have found widespread applications in experiments used for studies of biological macromolecules [71, 72]. To allow such experiments to be carried out, a *z* gradient channel which can deliver gradients up to 0.3 T/m (30 G/cm) or more should be available. A gradient channel (Fig. 8) includes the gradient control hardware and the gradient amplifier as well as a special actively shielded gradient coil in each probe. A gradient shaping unit may not be absolutely necessary but is certainly a desirable feature when working with strong gradients. Presently available triple axis gradient coils seem to reduce the performance of the *z* gradient due to interactions of the three different gradient coils. Currently most applications require only a *z* gradient and the necessity of installing triple axis gradients on a particular spectrometer must be carefully evaluated.

Modern spectrometers use a general purpose work station as host computer which does not really need a special configuration except for a fast link with the spectrometer computer. For optimal performance large memory and disk capacities are an advantage. When operated within a computer network the host computer should not require any data for its operation as a spectrometer host computer from the network to prevent spectrometer down times during network interruptions.

3.3 Radio frequency components

3.3.1 The transmitting path

The transmitting path starts at the synthesizer and ends at the *rf* coil in the probe (Fig. 8). The synthesizer delivers the basic frequency for the transmitter which sets the final offset frequency, duration and phase of the *rf* pulse. The frequency can be set in steps of at least 0.1 Hz, the phase in steps of 1^{o} or smaller and the pulse duration in steps of 50 ns or smaller. For shaped pulses the time dependent amplitude of the pulse is formed according to a shape stored in a waveform memory. Before entering the final linear power amplifier the pulse passes through an attenuation stage which sets the preset power level. The *rf* power can be attenuated from the maximum power over

a very large range, typically over 80 dB (Eq. (3.2)) or more, in steps of 0.1 dB or smaller. From the power amplifier the pulse travels to the coil and a linearly polarized, oscillating magnetic field $2B_1$ is established across the sample during the pulse. When different *rf* frequencies are applied during an experiment each channel has to be optimized by inserting a proper bandpass filter between the power amplifier and the probe. This prevents *rf* frequencies widely different from the one assigned to the channel from reaching the sample where the additional frequencies could severely affect the measurement if they happen to mach another resonance frequency. As discussed in Section 2.1.1 only one of the two circularly polarized waves into which B_1 can be decomposed interacts with the spins depending on the sign of the gyromagnetic ratio γ:

$$2B_1 \cos(i2\pi\nu_o t) = B_1 \exp(i2\pi\nu_o t) + B_1 \exp(-i2\pi\nu_o t) \tag{3.1}$$

Therefore, half of the transmitter power cannot be used to excite the spins. Typical transmitter powers for protons are 50 to 100 W. Reference to Eq. (2.18) makes clear that for small values of γ higher power amplifiers are required to prevent exceedingly long *rf* pulses with their unsatisfactory excitation profile (Section 2.2.1). Consequently so called *X* frequency amplifiers deliver 300 to 400 W. All elements in the transmitter path must be linear including the final power amplifiers otherwise the performance of shaped pulses is compromised and tedious phase adjustments may be necessary when using pulses with different power. Some shaped pulses require rather high peak powers demanding the transmitter path to be linear to high power levels. The linearity has the advantage that power levels of all the pulses can be calculated from one rectangular pulse irrespective of shape, length, power and phase. This avoids the experimental determination of these parameters which may be a rather cumbersome procedure.

In the following a few technical definitions and relations shall be presented which are important in the context of the transmitter path. The transmitter power P is often expressed as a ratio to a reference power P_{ref} on a logarithmic scale (in dB units):

$$P\ [\mathrm{dB}] = 10 \log(P_{ref}/P) = 20 \log(V_{ref}/V) \tag{3.2}$$

where V stands for the applied voltage. The B_1 field is proportional to the voltage across the coil. Suppose, for example, that the user wishes to double the 90^o pulse length, then the voltage at the coil must be halved and the power must be cut by a factor of four. Changing the pulse length for a 90^o pulse by a factor of 2 requires a change in power by 6dB. Another scale, the dBm scale, indicates the power P based on the logarithm of its magnitude in milliwatts (mW)

$$P\ [\mathrm{dBm}] = 10 \log(P[\mathrm{mW}]) \tag{3.3}$$

On this scale 100W correspond to 50 dBm or 1 mW to 0 dBm. The power P of an amplifier can be calculated from the peak-to-peak voltage V_{pp} measured across a 50 Ohm load resistance using the equation

$$P = V_{pp}^2/400. \tag{3.4}$$

Using Eq. (2.3) and (2.18) B_1 can be expressed in units of Gauss (G) and in units of kHz establishing a useful relationship between the two units

$$B_1[\mathrm{G}] = 0.2334\ B_1[\mathrm{kHz}] \tag{3.5}$$

The strength of B_1 is often specified in units of Gauss instead of the SI unit Tesla (T) which contains 10^4 G. For example a B_1 field of 5 G corresponds to 21.42 kHz or a 90^o pulse length of 11.7 us.

Modern pulse sequences depend on phase and amplitude stability of the *rf* pulses which are under normal operating conditions set and maintained to high accuracy by the spectrometer hardware. If it is suspected that the performance may have been degraded test procedures are available to check for the pulse reproducibility on the spectrometer [129].

3.3.2 The receiving path

The receiving path starts in the *rf* coil in the probe and ends with the digitized signal in the computer memory (Fig. 8). After being picked up by the receiver coil (Section 3.5.1) as an oscillating voltage the signal is routed to the preamplifier. The typical voltage induced in the coil by the nuclear magnetization of interest is in the range of μV or smaller and transmission losses have to be minimized by placing the preamplifier as close as possible to the detection coil. Together with the receiving coil in the probe the quality of the preamplifier determines the maximal signal-to-noise ratio (*S/N*) that can be obtained with a particular spectrometer. From the preamplifier the signal is sent to the main electronic console where its frequency is converted to an intermediate frequency range which is the same for all nuclei.

The use of an intermediate frequency allows a further treatment of the signal that does not dependent on the nucleus measured. At the intermediate frequency the signal is split into two channels. This is accomplished by mixing the signal with a reference frequency which has a phase difference of 90^o for the two channels. Thus the two channels correspond to a sine and a cosine modulation of the signal, respectively, which determine the sign of the resonance frequency with respect to the carrier frequency. This quadrature detection scheme allows reduction of the bandwidth to be measured by a factor of two and concomitantly reducing the noise by $\sqrt{2}$. A further mixing step reduces the signal frequency to a few tens of kHz while maintaining a frequency range of at least 100 to 200 kHz. The signal is digitized and submitted to a digital filter which rejects all noise and possible signals outside the selected spectral range of typically a few kHz. The resulting digitized signal is passed to the computer memory. If digital filtering is not available narrow band analog filters have to be used before digitizing the signal. The benefits of digital filtering are discussed in Section 2.4.4. The digitizer must have at least 16 bit resolution so that the dynamic range is high enough to digitize signals from macromolecules dissolved in H_2O and the strong residual solvent signal simultaneously.

Some elements of the signal path discussed above need to be optimized by the operator of the NMR instrument. Following the signal path starting at the sample first the receiving coil circuitry needs optimization and has to be tuned to the proper frequency and matched to an impedance of 50 Ohms (Section 3.5.1). When heteronuclear decoupling is applied during acquisition a band-pass filter for the observe frequency must be inserted between the probe and the preamplifier to prevent noise created in the decoupling channel from entering into the detection path. Finally the voltage of the detected analog signal must not exceed the dynamic range of the ADC or of earlier amplification or mixing stages. A more detailed description of the receiving pathway lies outside the scope of this article. More details can be found in excellent reviews on this topic [127, 128].

3.3.3 The lock system

With modern NMR spectrometers a full width of a proton signal at half height of 0.3 Hz at 800 MHz can be obtained which requires a stability of better than 10^{-9}. The stabilization of both the magnetic field and the *rf* frequency to at least the same order of magnitude is not possible. Fortunately, only the ratio of these two factors must be stable to 10^{-9}. The desired experimental conditions can thus be achieved by permanently measuring the NMR signal of a specific nucleus and compensating the field for any deviation of this signal from its resonance position. This coupling of the magnetic field to a resonance frequency, referred to as a field-frequency lock, typically uses the deuterium resonance. Deuterium can be incorporated in many solvents replacing protons. The lock circuitry detects the dispersion mode signal of deuterium (Fig. 9). This signal

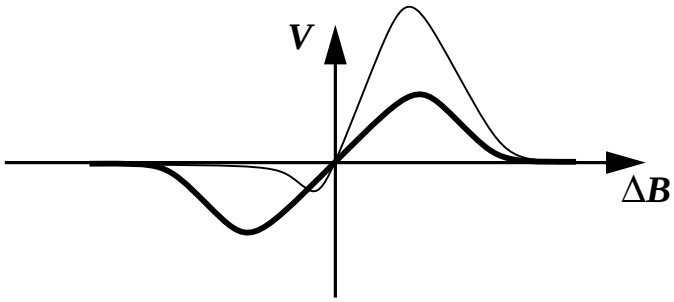

Fig. 9. Plot of the dispersion signal (voltage *V*) versus the deviation of the main magnetic field ΔB for the deuterium signal which is continuously recorded with the field-frequency lock activated (thick line). The thin line represents the lock signal with a wrongly adjusted phase setting.

has zero voltage precisely at the resonance of the reference compound at $\Delta B = 0$. The lock circuitry detects any departure from the resonance position and converts it into a correcting current which is fed into a z_o coil in the room temperature shim system. The z_o coil adds a small homogeneous magnetic field to the main magnet field to re-establish the original field-frequency lock condition. The lock system requires the exact dispersion line which can be obtained by proper adjustment of the phase of the detected signal. A wrongly adjusted phase makes the lock response non-linear and generates a correction that deviates from the actually required field setting. A situation that is indicated in Fig. 9 by a resonance that deviates from a dispersion line.

The power of the lock channel is usually set to be just under saturation. Changing the power setting influences the phase of the detected lock signal and requires a readjustment of the phase. For measurements in H_2O adding 5 to 10% of D_2O to the sample suffices to establish a good field-frequency lock. In addition to the dispersion line used to maintain the field-frequency lock, the absorption line can be used by the operator to assess field homogeneity during shimming. When the system is not locked the magnetic field on the z_o coil is swept over a small range which facilitates finding the lock frequency. This field sweep must be switched off in special applications where a spectrum without lock must be measured.

When working with large proteins uniform double labelling with ^{15}N and ^{13}C may no longer be sufficient for a complete structure determination and ^{2}H labelling may be used in addition [130]. Since the lock signal is derived from a deuterium resonance, every probe has a coil that is tuned

to deuterium which can also be used to decouple deuterium [131, 132] and to apply full power deuterium pulses [133]. In full analogy to the receiving channel the deuterium preamplifier has to be protected during the application of deuterium pulses and the lock regulation has to be set on hold. However since the deuterium channel was originally not intended for these uses the necessary protection circuitry is not present on all spectrometers.

3.4 The Magnet

The magnetic field B_o removes the degeneracy of the nuclear spin energy levels. Higher fields B_o result in a better chemical shift dispersion in the spectrum and in a higher sensitivity which depends on ${B_o}^{3/2}$ (Eq. (4.2)). After the initial installation of the magnet system the magnet requires very little attention aside from the regular fillings of the cryostat with liquid nitrogen and liquid helium to keep the solenoid in a superconducting state. On the other hand the magnet usually sets the most stringent boundary conditions for the selection of the installation site of an NMR spectrometer. Superconducting magnets have noticeable fringe fields which extend from the magnet by several meters depending on the field strength. Disturbances in the fringe field interfere with the homogeneity of the field in the magnetic center of the solenoid where the NMR experiment is performed. Control over the stray field area is therefore a prerequisite for stable long term operation. Whereas movement of small ferrous items such as small tools in an area where the stray field is smaller than 1 mT do not interfere noticeably with the performance, large moving ferrous objects (cars, elevators) should circulate only in areas where the stray field is smaller than 0.1 mT. For safety reasons the 0.5 mT stray field should be contained within the NMR laboratory. Newly developed actively shielded magnet systems permit to greatly reduce the stray field and consequently its adverse effects. A 500 MHz actively shielded magnet produces a field of 0.5 mT in a maximal horizontal distance of 1.3 m which compares favourably with the 3.0 m for a conventional magnet. Actively shielded magnets promise to greatly reduced interference from the environment and will probably replace conventional magnets in the future except for the highest field magnets which are running at the technical limits. In addition to magnetic interferences the performance of the spectrometer is very sensitive to vibrations. Vibrations are transmitted *via* the magnet stand or *via* vibrating ferromagnetic objects which result in small fluctuations of the magnetic field producing sidebands in the NMR spectrum.

Magnetic fields are measured in Tesla but in NMR often the proton resonance frequency in MHz at the corresponding field is used instead, for example 17.63 T correspond to 750 MHz. NMR systems above 100 MHz require superconducting magnets which are operated at the temperature of liquid helium which is 4.2 K at standard pressure and for the highest available fields even at 2 K. The magnet coils are constructed using niobium alloy wires embedded in a copper wire which allows the winding of a solenoid. The niobium alloys used become superconducting at low temperatures unlike copper. In the case of a sudden, unintentional collapse of the superconductivity, known as a quench, much of the current may flow through the copper. In addition copper guarantees efficient heat dissipation which helps to prevent damage to the superconducting wire. However most of the energy is dissipated by protective resistors which are placed in parallel to the main coil. After a quench the solenoid can usually be recharged with no loss in performance. At a critical current density and magnetic field strength superconductivity is lost and for presently used materials these two factors are the principle limitations in the design of ever higher field magnets. The stability needed for high resolution NMR magnets requires operation in a persistent mode, *i.e.* with no connection to a power supply after the initial charging. Consequently the joints which connect different superconducting wires must have an extremely small resistance to mini-

mize the gradual reduction of the magnetic field. This drift of the field is very small indeed and even at 750 MHz the proton resonance frequency typically shifts only by 5 Hz in an hour. With this drift rate it would take 23 years before the field is reduced by only 1 MHz (0.024T).

In addition to the main coil, a superconducting magnet contains additional superconducting coils, shim coils, which produce specific magnetic field gradients that can be used to improve the basic homogeneity of the main coil. Still the homogeneity required for high resolution NMR experiments can only be reached by additional non superconducting shim coils which are mounted in the room temperature bore of the superconducting magnet. Typically 30 different room temperature shims are available making possible lineshapes which have a full width of 0.4 Hz at 50% of the peak maximum, 5 Hz at 0.55% and 8 Hz at 0.11% with a non-spinning sample of 1% chloroform in d_6-acetone. Spinning the sample can improve the lineshape but at the same time introduces instabilities which may compromise the performance of multidimensional NMR experiments. These are consequently run without spinning the sample (Section 3.5.1). Shimming directly on the rather broad D_2O resonance of a protein sample can be rather cumbersome because it is not so sensitive and responsive to changes as for example the deuterium resonance of d_6-acetone. In order to obtain optimal homogeneity it seems helpful to have a range of samples for shimming which have different filling heights and a susceptibility matched to that of water. For example, a mixture of 3% chloroform, 84% $CBrCl_3$ and 13% d_6-acetone fulfils this criteria. Modern spectrometer offer gradient shimming procedures [134] which are most effectively used for solutions in H_2O. With such gradient shimming procedures starting from some standard shim values a good homogeneity on protein samples can routinely be obtained in a few minutes without interaction of the operator.

3.5 The probe

3.5.1 The radio-frequency coil

The probe contains resonance circuitry with a coil that acts as antenna which transmits the radio-frequency pulses to the sample and subsequently receives the response of the spins *via* the precessing magnetization which induces a voltage across the coil. The use of two different coils for transmitting and receiving would have some conceptional advantages but designs with two coils at the same frequency suffer from interference effects between the coils and result in inferior performance. The coil produces a linearly polarized oscillating magnetic field $2B_1$ perpendicular to the main static magnetic field (Section 2.1.1). The coil is mounted in the probe with some solid material and possibly glue. Special care is taken in the choice of materials to limit the background NMR signal from such material at the detection frequency.

One coil can be tuned simultaneously to at most three different frequencies or can be designed to be tunable over a whole range of frequencies with one additional fixed frequency. Best performance can be obtained for coils which are optimized for one specific frequency only. A probe contains one or two coils with different frequency ranges. The receiving coil requires mounting as close as possible to the sample and is usually optimized for one receiving frequency and in addition tuned to the lock frequency. If more frequencies are needed the probe contains one additional coil outside of the receiving coil. This coil finds application for decoupling or polarization transfer experiments. With current technology only one of all the possible frequencies can be made tunable over an extensive range, for example from the ^{15}N to the ^{31}P resonance frequency, the

other tuning ranges must be narrow and allow only the adjustment for one nuclear species.

One of the parameters characterizing the coil is the quality factor Q which describes the damping in the coil circuitry. In general, the higher the Q the greater the sensitivity and the larger the *rf* field that can be obtained with a given *rf* power. Since Q characterizes the damping, it describes also the lag time for any changes in the *rf* power or phase. The lag time in nanoseconds is approximately equal to the value of Q. Typical Q values lie in the range from 300 to 400. With superconducting coils much higher Q values can be reached [135] but the low tolerance to salt and the multinuclear capabilities of superconducting probes limit their applicability for measurements with biological macromolecules. The response of the resonance circuitry of the probe depends on its electric and magnetic properties. Using sample spinning these properties can be modulated by a non-cylindrical shape of the sample tube or small imbalances in the spinning rate. Such modulation produce small, varying sidebands in the NMR spectrum which often lead to artifacts in multidimensional NMR experiments. Hence, these spectra are measured without sample spinning. A high Q is only one of many requirements for good overall performance of a probe. For sensitivity reasons the coil must be mounted close to the sample. This spatial proximity can cause problems when the coil and the surrounding medium, usually air or nitrogen used for the temperature stabilization, do not have the same magnetic susceptibility. In this situation the presence of the coil may distort the main static magnetic field in the sample which can render shimming very difficult. Coils can be produced which match the slightly different susceptibilities of nitrogen or air and should be used with the corresponding gas.

The spatial homogeneity of the radio frequency field B_1 applied during the pulses constitutes a further important property of the coil [136]. With an inhomogeneous B_1 field not all the spins in the sample experience the same nominal pulse length. Whereas for a single 90^o pulse this is hardly a problem, complicated pulse sequences with numerous *rf* pulses may suffer from substantial signal loss and from increased number of artifacts. A common measure of the *rf* homogeneity uses the ratio of the signals obtained after a 810^o pulse and after a 90^o pulse. Theoretically this should result in the same signal amplitude, in practice signal loss occurs due to *rf* inhomogeneity. A good probe produces a ratio larger than 75%. A further problem which is partly related to the B_1 inhomogeneity comes from the electric field caused by the *rf* pulse. In aqueous protein solutions the electric field leads to a substantial sample heating during the application of long pulse trains, for example during decoupling or TOCSY sequences. Due to the inhomogeneity of the electric field the heating is not uniform and the temperature increase in some parts of the sample may be significantly higher than the average temperature rise observed. Generally the average temperature in the sample depends on the experiment performed and can be different for the same settings of the variable temperature control system. When the sample volume is limited to the coil size or slightly smaller, the B_1 homogeneity can be improved and the heating reduced [127, 137]. This can be accomplished by limiting the sample volume to the appropriate region using a sample plug inserted into special NMR tubes. The plugs have a magnetic susceptibility that is matched to the solution. None the less some more tedious shimming may be required.

A probe must fulfil very stringent requirements. The optimal performance of a coil can easily be compromised by choosing the wrong operating conditions. The best sensitivity is obtained when the coil is tuned exactly to the resonance frequency of the individual nuclear species for every sample. In addition the coil has to be matched to the impedance of the transmitter and receiver path, usually 50 Ohms, which is required for optimum transmission of the *rf* pulses and the induced NMR signal *via* the coaxial cables. A filter inserted in the transmission path directly before the probe forms part of the circuitry and should not be removed for tuning and matching.

The insertion or removal of a filter should not lengthen the duration of a 90^o pulse by more than 5% under optimized conditions.

3.5.2 The magnetic field gradient coil

Modern probes not only contain *rf* coils but in addition actively shielded coils for the application of magnetic field gradients in one or three dimensions. To obtain a gradient strength of 0.5 T/m (50 G/cm) these coils are driven with currents in the range of 10 A. When switching such large currents on and off, for example at the start and end of a rectangular pulse, the gradient coil tends to vibrate and in addition eddy currents are induced in the surrounding metals used to build the probe. These effects interfere with the requirement of a very fast recovery of the basic, very homogeneous magnetic field after the application of a gradient pulse. Modern probes equipped with a *z* gradient reach full recovery of the homogeneity within 100 μs after a 2 ms rectangular gradient pulse with a strength of 0.5 T/m. Even shorter recovery times are obtained for smoothly changing pulse amplitudes such as sine or elongated sine square shapes [68]. For triple axis magnetic field gradient coils the interference between the different gradient coils in general increases the individual recovery times. On the other hand triple axis gradients offer much more flexibility and less hassle in preventing the accidental refocusing of undesired magnetization during a pulse sequence. Furthermore efficient automatic gradient shimming can be used for all shims. With only a *z* gradient the room temperature shim coils may be used for gradient shimming of non-axial shims but this procedure is less efficient and tends to be less reliable.

For many applications the absolute gradient strength does not need to be known very precisely and a simple calibration procedure can be used to obtain the approximate gradient strength. If the gradient is applied during the acquisition of the water signal in a H_2O sample the width of the resonance line $\Delta\nu$ in the absolute value spectrum is related to the approximate gradient strength G_i, with i representing the *x*, *y* or *z* axis, by

$$G_i = 2\pi\Delta\nu/(\gamma d) \tag{3.6}$$

where *d* stands for the dimension of the sample in the direction of the applied gradient. Along the *z* axis the sample is generally longer than the *rf* coil and *d* is set to the length of the *rf* coil. A more precise calibration can be obtained by measuring the diffusion of a compound with known diffusion constant [68]. Although the absolute strength of gradients rarely need to be known very accurately, the relative strength must be set very precisely in experiments using gradients for the selection of coherence pathways (Section 4.4) otherwise severe signal loss will occur. For example, a gradient with strength 0.3 T/m and a duration of 2 ms is applied to proton magnetization which should be refocused after a 180^o pulse by an identical gradient. To refocus 95% of the original signal the strength 0.3 T/m of the refocusing gradient must be set to a precision better than $\pm 4 \cdot 10^{-4}$ T/m (Eq. (2.31)).

3.5.3 The variable temperature operation

The sample temperature must be controlled very precisely to prevent temperature drift. This is of particular relevance to studies in aqueous solution with D_2O as the lock substance. Water exhibits a large temperature dependent resonance shift of 0.01 ppm/K. With an unstable temperature the lock system will try to compensate the shift of the water resonance which results in a shift for all

other resonances with a different temperature dependence. In multidimensional NMR experiments individual resonances will no longer be completely aligned when evolution times are incremented leading to noise bands in the spectrum (t_1 noise) which run perpendicular to the axis of the direct dimension. In addition, temperature instability contributes to increased subtraction artifacts for phase cycles and in difference spectroscopy.

For temperature regulation air flows around the sample tube after passing an electrical heating element, and a feedback system regulates the temperature of the air by controlling the power fed to the heater. For a good performance of the temperature control, the input temperature of the gas flow when entering the probe should be at least 10 K lower than the temperature required at the sample position. In addition, the flow rate must be high enough to efficiently cool the coil and sample during periods of extensive *rf* irradiation, for example during decoupling or TOCSY mixing periods. In experiments containing such sequences the probe temperature is usually set lower than the actual sample temperature required, in this way a compensation can be obtained for the average heating effect in the sample. This lower temperature can be determined experimentally by comparing the shift of an isolated resonance in a 1D spectrum, for example a methyl resonance, using only one excitation pulse eliminating the specific heating and in a spectrum with the actual sequence. Good cooling capacity requires high flow rates which may interfere with the probe performance if the flow induces vibrations of the coil or even movements of the sample. Vibration sidebands of the coil typically occur at frequencies of several thousand Hertz, are rather broad and can severely deteriorate the spectral quality. These sidebands are most easily detected around a strong solvent line and can be identified by their sensitivity to the air flow rate.

When the temperature regulation fails, built in security circuits and properly chosen regulation parameters should prevent any damage to the sample in the probe. For example, a heater failure may freeze or overheat the sample depending on the false heater current set by the system and depending on the temperature of the gas used for regulation. On the other hand, when the gas flow stops, the regulation may set the heater current to high values in a attempt to stabilize the temperature. With the normal gas flow re-established a heat wave will reach the sample which may denature the protein. These scenarios should be kept in mind when choosing the parameters for the regulation system.

3.6 Stability of the system

Instabilities during the measurement of a multidimensional NMR experiment introduce noise bands in the spectrum which run perpendicular to the frequency axis of the direct dimension and may severely distort the spectral quality. Such noise bands are generally referred to as t_1 noise since they were first observed in 2D spectra running parallel to the axis of the indirect dimension [16]. Any incoherent changes of the signals forming the FID in the indirect dimension may cause t_1 noise. Modern NMR spectrometers possess a remarkable internal stability and reproducibility. In many cases it is not an inherent instability of the NMR spectrometer which limits the performance but the interaction with an unstable environment. For optimal performance of a high field NMR spectrometer the NMR room should be far enough from any outside electromagnetic and mechanic source of interference and the temperature of the room should be regulated to within one degree. Mechanical vibrations of the magnet system in the range of a few Hertz are difficult to compensate for but may severely interfere with NMR difference techniques as for example used in some hydration measurements (Section 5.3). Special care has to be taken to prevent oscillating disturbances which potentially have more influence on the performance than a correspond-

ing random fluctuation [138]. Even in a very stable environment exceptional circumstances may still affect the measurement. In multidimensional NMR experiments with biological macromolecules, data sets are typically acquired over a period of several hours to several days and the results obtained critically depend on a high stability of the system during the entire recording time. Even a short, transient instability in the magnetic field homogeneity or a transient temperature variation can severely distort a data set. For this reason some key experimental parameters of the overall performance of the spectrometer should continuously be monitored and stored in a file [138].

The lock level, sample temperature and room temperature are particularly sensitive parameters for the detection of any malfunctioning or external disturbance. If the user detects any disturbance when inspecting the recording file he can restart the experiment at the point of interference or repeat the measurement of the corrupted FIDs before removing the sample from the magnet or changing any measurement parameter. These FIDs can be introduced into the multidimensional data set and a complete repetition of the measurement becomes unnecessary. The recordings are not only useful for the evaluation of individual experiments, but they also serve as a database for the design of improved laboratory facilities. The permanently stored recordings enable the instrument supervisor to inspect the instrument performance for repeated interferences, which can provide a lead to the cause of the malfunctioning. If the recordings are stored digitally on a computer remote control of long-term measurements becomes possible from any computer inside or outside of the laboratory. Although it appears that the recording of the lock level, the sample temperature and the room temperature monitors most disturbances that lead to spectral distortions an even more rigorous surveillance could be envisaged, possibly including the current sent to the z_0 coil by the lock system, the pressure at the helium outlet, the air flow for the temperature regulation and an indicator for the *rf* power at the amplifier output.