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Low-temperature dynamic nuclear polarization with helium-cooled samples and nitrogen-driven magic-angle spinning

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Abstract

We describe novel instrumentation for low-temperature solid state nuclear magnetic resonance (NMR) with dynamic nuclear polarization (DNP) and magic-angle spinning (MAS), focusing on aspects of this instrumentation that have not been described in detail in previous publications. We characterize the performance of an extended interaction oscillator (EIO) microwave source, operating near 264 GHz with 1.5 W output power, which we use in conjunction with a quasi-optical microwave polarizing system and a MAS NMR probe that employs liquid helium for sample cooling and nitrogen gas for sample spinning. Enhancement factors for cross-polarized ^{13}C NMR signals in the 100–200 range are demonstrated with DNP at 25 K. The dependences of signal amplitudes on sample temperature, as well as microwave power, polarization, and frequency, are presented. We show that sample temperatures below 30 K can be achieved with helium consumption rates below 1.3 l/hr. To illustrate potential applications of this instrumentation in structural studies of biochemical systems, we compare results from low-temperature DNP experiments on a calmodulin-binding peptide in its free and bound states.

Graphical abstract

Keywords

solid state NMR; dynamic nuclear polarization; low-temperature NMR; magic-angle spinning; protein structure

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Introduction

Dynamic nuclear polarization (DNP) can provide enormous improvements in the sensitivity of nuclear magnetic resonance (NMR) measurements, as demonstrated by numerous laboratories since 1953 [1–5]. The recent surge in DNP-related activity in the solid state NMR community was originally driven by results from the Griffin group, including their demonstration of large NMR signal enhancements at high fields in paramagnetically doped frozen solutions using gyrotron microwave sources [6, 7], their demonstration of efficient DNP at high fields via the cross-effect mechanism [8, 9], and their introduction of nitroxide-based biradical dopants [10]. Applications of DNP in studies of biological [11–17] and non-biological [18–20] systems are now being actively pursued in many laboratories.

Our own involvement in DNP began with the development of a magic-angle spinning (MAS) NMR probe capable of operating at sample temperatures below 30 K [21], which was motivated by our interest in structural properties of peptides, proteins, and peptide/protein complexes in frozen solution [22–27]. Our NMR probe design is unusual in that we use cold helium for sample cooling and warmer nitrogen gas for MAS bearings and drive. Although our original plan was to use this probe without DNP, taking advantage of the Curie-law dependence of nuclear spin polarizations on temperature to enhance NMR signals by factors greater than ten [21], we subsequently decided to combine this probe design with microwave irradiation in order to achieve further signal enhancements by DNP [11, 28–31].

In this article, we describe several aspects of our DNP experiments that have not been described in detail in earlier publications, including the performance characteristics of our microwave source (an extended interaction oscillator, or EIO), the dependence of DNP-enhanced solid state NMR signal amplitudes on microwave polarization and microwave power, the dependence of signal amplitudes on sample temperature, and the dependence of liquid helium consumption on sample temperature. Among other things, we show that sample temperatures below 30 K can be achieved with helium consumption rates below 1.3 liters per hour in MAS NMR experiments.

Description of the DNP system

Our DNP system operates with a 9.39 T NMR magnet, implying ^1H NMR frequencies around 400.8 MHz and microwave frequencies around 264 GHz. Our initial DNP experiments [29, 32–35] employed a low-power (30 mW) tunable microwave source obtained from Virginia Diodes, Inc. We subsequently obtained the EIO (Fig. 1A) from Communications & Power Industries, LLC. The EIO produced 0.8 W of microwave power when it was delivered in October 2012. After 770 hr of operation, it was returned to CPI for adjustments. The EIO now produces 1.5 W of microwave power, mechanically tunable from 263.5 GHz to 266.9 GHz. We have used it for a total of approximately 1100 hr, usually in 5–10 hr runs. Frequency stability is roughly ± 2 MHz over periods of seconds and ± 6 MHz over periods of hours. Power stability is better than $\pm 1\%$ over periods of hours after an initial warm-up period of 20 min.

Linearly-polarized microwaves leave the EIO through WR-4 waveguide and are converted to HE11 mode by a corrugated horn (Thomas Keating, Ltd.) before being transmitted to the

quasi-optical system through corrugated waveguide (Fig. 1B). This waveguide was made in our laboratory by threading sections of aluminum pipe (12.6 mm inner diameter, 15.9 mm outer diameter, 20 cm length) with a M13-0.5 tap. Four sections are joined together, making a waveguide with total length of 80 cm. Microwave power loss in the waveguide is less than 0.5 db. Microwaves then pass through a directional coupler (Fig. 1B), which has a quartz microscope slide at 45 degrees that reflects a fraction of the microwaves to allow continuous monitoring of microwave power or frequency during NMR experiments [36]. Alternatively, the microscope slide can be removed and the corrugated waveguide sections pushed together to send all of the microwave power to the NMR probe. From the directional coupler, the microwaves enter the quasi-optical polarizing system (Thomas Keating, Ltd.; Fig. 1C) and, after conversion from linear to circular polarization, are directed vertically upward. Curved mirrors in the quasi-optical system refocus the microwave beam at the entrance to a corrugated waveguide within the MAS NMR probe that transmits the beam to the NMR sample, as previously described [29, 37].

Cold helium is supplied from a liquid helium tank (typically 60 l volume) through a transfer line that includes a needle valve in the supply leg (Janis Research Co., model FHT-ST The helium flow rate in our experiments is controlled both by the needle valve opening and by the liquid helium tank pressure, which we adjust with a pressure regulator (Omega Engineering, model PRG101-25) that is connected between a helium gas cylinder and the liquid helium tank. The delivery leg of the transfer line is supported within the 89 mm bore of the superconducting NMR magnet (Oxford Instruments) by a home-built “chimney” that mates with the top of the MAS NMR probe. To prevent transmission of extraneous radio-frequency (RF) signals to the probe, the transfer line is grounded to the magnet bore at two points along its length. The end of the transfer line is also grounded to the shield of the probe itself, using a piece of copper braid that is clamped to the transfer line (Fig. 1D).

Within the past year, we have reduced the liquid helium consumption of our DNP system significantly by modifying the connection between the end of the transfer line and MAS module within the NMR probe. The current configuration is shown in Fig. 1D.

NMR signal enhancements by DNP

As previously described [29], our MAS NMR probe uses zirconia rotors with 4.0 mm outer diameters and 80 μ l maximum sample volumes. MAS frequencies ν_{MAS} up to approximately 7.0 kHz are feasible at the lowest temperatures. RF field amplitudes of 90 kHz on ^1H and 45 kHz on ^{13}C channels are achieved with RF powers of 32 W and 53 W, respectively. Typical ^1H decoupling fields are 80–90 kHz.

Fig. 2 shows one-dimensional (1D) ^{13}C NMR spectra of a frozen solution containing uniformly ^{15}N , ^{13}C -labeled L-alanine and L-proline in partially deuterated glycerol/water (glycerol- d_8 , D_2O , H_2O , and $\text{DMSO-}d_6$ in 57:28:10:5 volume ratios) at pH 5, doped with 10 mM DOTOPA-Ethanol [31]. $\text{DMSO-}d_6$ is present in the sample because the DOTOPA-Ethanol dopant was dissolved in $\text{DMSO-}d_6$ prior to addition to the glycerol/water solution. DMSO was deuterated to avoid increasing the intrinsic ^1H spin-lattice relaxation rate of the frozen solution. Spectra were obtained with standard ^1H - ^{13}C cross-polarization and ^1H

decoupling and $\nu_{\text{MAS}} = 6.6\text{--}6.8$ kHz, both with and without microwave irradiation, at temperatures between 25 K and 88 K. MAS stability was typically ± 100 Hz over periods of minutes, without active stabilization by the MAS speed controller. With active stabilization, stability better than ± 10 Hz can be achieved. The full microwave power was used, with optimal polarization and frequency (263.9 GHz). Sample temperatures were varied by varying the liquid helium tank pressure and needle valve opening, and were determined from measurements of ^{79}Br spin-lattice relaxation in an external sample of KBr powder [38] under identical pressure and valve settings. Spectra were recorded with recycle delays set to $2T_{\text{DNP}}$, where T_{DNP} is the characteristic build-up time for cross-polarized ^{13}C signals of the alanine/proline sample under microwave irradiation, measured with a saturation-recovery technique.

Spectra in Fig. 2 show resolved signals from glycerol (natural-abundance ^{13}C), L-alanine and L-proline, with the smallest linewidths being about 3 ppm (full width at half maximum). Values of T_{DNP} and the DNP signal enhancement factor ϵ (defined as the ratio of cross-polarized ^{13}C NMR signal amplitudes with and without microwave irradiation) are indistinguishable for different lines in these spectra. The L-alanine methyl signal at 18 ppm becomes relatively weak and broad at temperatures near 80 K, but is sharper at both higher and lower temperatures. Broadening near 80 K is due to interference between time scales for methyl rotation and time scales for MAS and RF nutation [39].

Fig. 3A shows the dependences of ϵ and T_{DNP} on temperature for the alanine/proline sample. For this sample, T_{DNP} is only weakly dependent on temperature, reaching 3.8 ± 0.2 s at 25 K. Signal enhancements depend strongly on temperature, decreasing from $\epsilon = 128 \pm 20$ at 25 K to $\epsilon = 13 \pm 2$ at 88 K. Uncertainties in ϵ are primarily due to the limited signal-to-noise ratios in spectra obtained without microwave irradiation. Fig. 3B shows the dependences of the DNP-enhanced, cross-polarized ^{13}C NMR signal amplitude and the effective sensitivity on temperature, where the sensitivity is proportional to the signal amplitude and inversely proportional to $T_{\text{DNP}}^{1/2}$. The sensitivity at 25 K is approximately 17 times greater than at 88 K, implying a 300-fold reduction in measurement time to achieve the same signal-to-noise ratio.

Dependences on microwave power, polarization, and frequency

Fig. 4A shows the dependence of ϵ on microwave power, at 264.0 GHz microwave frequency and the optimal polarization. These data were obtained with a sample of isotopically labeled, synthetic melittin (PALI-melittin, see ref. [29]) in partially deuterated, ^{13}C -depleted glycerol/water at pH 7.4. The microwave power was varied by changing the electron current in the EIO, and adjusting the frequency tuning of the EIO to maintain a constant frequency. At 24 K, the NMR signal enhancement saturates above 0.6 W. At 100 K, saturation apparently occurs above 1.2 W. This difference is attributable to more rapid electron spin-lattice relaxation at the higher temperature. Interestingly, the DNP signal enhancement factor at 1.2 W is approximately 50 for the melittin sample at 100 K, but only 13 for the alanine/proline sample at 88 K (Fig. 3A). Differences among ϵ values for different samples are not fully understood, but we note that values of T_{DNP} are larger for the melittin sample, both at 24 K ($T_{\text{DNP}} = 6.3$ s) and at 100 K ($T_{\text{DNP}} = 5.1$ s).

Fig. 4B shows the dependence of the cross-polarized ^{13}C NMR signal amplitude from the alanine/proline sample on the polarization of microwaves from the quasi-optical system, at full power and optimal frequency. The polarization is varied by adjusting one of the path lengths in the quasi-optical interferometer with a micrometer (Fig. 1C). Data in Fig. 4B are periodic functions of the micrometer setting with a period of 0.57 mm, in excellent agreement with an expected period equal to half of the wavelength at 263.9 GHz.

Although the micrometer varies the polarization continuously from right circular to left circular states (with linear polarizations in between), and although one direction of circular polarization should be incapable of driving electron spin transitions and should therefore produce no DNP, in practice the signal modulation depths in Fig. 4B are only about 38% at 25 K and 53% at 88 K. This observation is attributable to changes in polarization as microwaves pass through the RF coil around the sample and reflect from zirconia surfaces within the MAS rotor. The polarization seen by the NMR sample is not identical to the polarization that leaves the quasi-optical interferometer. Nonetheless, data in Fig. 4B show that circular polarization increases DNP-enhanced NMR signals by 19–26% under our experimental conditions, compared with linear polarization. The difference in modulation depths at 25 K and 88 K in Fig. 4B is apparently related to the difference in power dependences at 24 K and 100 K in Fig. 4A. At the lower temperature, the DNP-enhanced NMR signal amplitude is less sensitive to the effective microwave power (i.e., the power in the field with the correct direction of circular polarization).

In our MAS NMR probe, the microwave beam propagates to the sample at an angle of $\pi/2 - \theta_m$ relative to the external static magnetic field direction, where $\theta_m \approx 54.7^\circ$ is the magic angle [29]. Strictly speaking, DNP depends on the polarization state of microwave fields perpendicular to the static field, not the propagation direction. Depending on the orientation of the MAS NMR probe relative to the quasi-optical system (i.e., the angle of rotation of the probe within the bore of the superconducting NMR magnet), it may not be possible to create pure circular polarization at the sample with our quasi-optical system, even if the RF coil and MAS rotor do not affect the microwave polarization. However, the effect of the propagation direction is small. If the linearly polarized microwave magnetic field from the EIO has amplitude B_1 , the microwave field perpendicular to the static field at the sample can be expressed as a sum of two counter-rotating components with amplitudes

$$B_{\pm} = \frac{B_1}{2} \left[1 + \sin^2 \theta_m - \cos^2 \theta_m \cos \phi \sin 2\eta \pm 2 \sin \theta_m \sin \phi \right]^{1/2} \quad (1)$$

where ϕ is the variable microwave phase shift induced by the micrometer and η is an angle that specifies the orientation of the MAS NMR probe relative to the quasi-optical system. Only one of these components is absorbed by the electron spins. The maximum and minimum values of the microwave power in this “useful” component occur when the micrometer is adjusted to satisfy

$$\cos \phi = \pm \sqrt{\frac{3}{2} \frac{\sin 2\eta \sin \phi}{6}} \quad (2)$$

When Eq. (2) is satisfied, the microwave power in the useful component is

$$P_u \propto B_1^2 \left(\frac{5}{12} \pm \left(\sqrt{\frac{3 \sin^2 2\eta}{2 \cdot 72}} + \sqrt{\frac{1}{6}} \right) \sin \phi \right) \quad (3)$$

When $\sin 2\eta = 0$, minimum and maximum values of P_u , in units of B_1^2 , are approximately 0.825 and 0.009 (at $\sin \phi = \pm 1$). When $\sin 2\eta = 1$, minimum and maximum values are

approximately 0.833 and 0.001 (at $\sin \phi = \pm \sqrt{\frac{24}{25}}$). Thus, the fact that the microwave beam propagates to the sample at angle $\pi/2 - \theta_m$ to the static field reduces the maximum power in useful circularly polarization by approximately 17%, but does not affect the depth of useful power modulation significantly.

Fig. 4C shows the dependence of ε for the melittin sample on microwave frequency, at 25 K, full power, and optimal polarization. The frequency dependence in Fig. 4C has a maximum at 263.9 GHz and a minimum at 264.7 GHz that are less sharply defined than the corresponding extrema observed with a 30 mW microwave source [29]. Broadening of the frequency dependence is also attributable to saturation of the DNP signal enhancement shown in Fig. 4A.

Helium consumption

Helium consumption is an important practical consideration in our DNP experiments, because we do not recover the helium that is used for sample cooling in our MAS NMR probe. Our previous publications [21, 29] reported liquid helium consumption rates of 3–4 l/hr at 25 K and with $\nu_{\text{MAS}} = 6\text{--}7$ kHz. Fig. 5A shows our current consumption rates as a function of the sample temperature, using either room-temperature nitrogen gas or nitrogen gas cooled to 95 ± 2 K (at the input to the probe) to supply the MAS bearings. In both cases, room-temperature nitrogen was used for the MAS drive. Within the probe, the drive and bearing gases travel through vacuum-insulated glass tubes to points that are approximately 5 cm below the sample spinning module, after which they travel through Delrin plastic posts and short Tygon tube sections (Fig. 1D). It should be noted that, even when the bearing and drive gases enter the probe at room temperature, they are cooled significantly as they travel to and enter the spinning module when the probe operates at low sample temperatures.

Helium consumption rates in Fig. 5A were determined by setting the liquid helium tank pressure and the transfer line needle valve to values that produced known sample temperatures, based on KBr spin-lattice relaxation rates. Cold helium from the transfer line (disconnected from the MAS NMR probe) was passed through a coil of copper tubing that was immersed in a bucket of water, to bring the helium to room temperature, and then through a floating-ball flowmeter (Gilmont Instruments, model GF-2300). The flowmeter was separately calibrated for helium gas flows by direct measurements of the time required to inflate a plastic bag within a cylindrical metal container of known volume (5.7 l) at various flowmeter readings. We estimate the uncertainties in helium consumption values in Fig. 5A to be approximately $\pm 10\%$, primarily due to limitations on the precise reproducibility of liquid helium tank pressures and needle valve settings. Disconnecting the MAS NMR probe from the transfer line does not affect the helium flow significantly

because the connection to the MAS NMR probe does not introduce significant back pressure in the transfer line. Helium consumption rates determined in this way are in good agreement with actual helium consumption over long DNP experiments.

Data in Fig. 5A show that sample temperatures below 30 K (with $\nu_{\text{MAS}} \approx 6.7$ kHz) can be achieved with helium consumption rates below 2.0 l/h, even with room-temperature nitrogen gas for MAS bearing and drive. When cold nitrogen gas is used for the MAS bearings, helium consumption rates can be reduced by about 0.7 l/h at the lowest temperatures, and by about 0.4 l/h at higher temperatures.

As is well known [40], cooling MAS bearing or drive gas to low temperatures can be problematic because pressurized nitrogen condenses at temperatures significantly above its ambient-pressure boiling point (77 K). At 200 kPa (29 psi), the boiling point rises to 88 K. If liquid nitrogen at 77 K is used to cool the pressurized nitrogen gas, condensation within the tubing that carries the nitrogen gas can produce large instabilities in the MAS frequency. To overcome this problem, we use the apparatus shown schematically in Fig. 5B. Nitrogen bearing gas from our MAS frequency controller (typically 140–190 kPa) first travels through a tubular heater (Omega Engineering, model AHP-7561), increasing the nitrogen gas temperature before it travels through a two-turn coil of copper tubing that is immersed in liquid nitrogen. The nitrogen gas then travels to the MAS NMR probe through a flexible, vacuum-insulated hose (Cryo Industries, model NTL-1363-B), terminating in a fitting that contains a Pt resistance temperature detector (RTD). The RTD is read by a temperature controller (Omega Engineering, model CN76022), which supplies current to the heater in order to maintain the RTD at the temperature setpoint. As long as the setpoint is above the boiling point of nitrogen at the relevant bearing pressure, condensation does not occur.

Data in Fig. 5A illustrate that our MAS NMR probe operates stably in two temperature regimes, either 22–30 K or above 60 K. Sample temperatures between 30 K and 60 K are difficult to maintain without drifting toward higher or lower values. Temperatures below 22 K often cause unstable spinning, perhaps because of nitrogen condensation within the MAS assembly.

Discussion

The above results demonstrate some of the features and potential advantages of performing DNP-enhanced solid state NMR measurements at sample temperatures below 30 K. Compared with the more conventional operation above 80 K, lower-temperature operation allows very large NMR signals to be obtained with relatively low microwave powers. As shown in Fig. 3, absolute sensitivity (for the same microwave power) can be increased by a factor of 10, corresponding to a 100-fold reduction in measurement time. Additional degradation of spectral resolution in measurements on frozen solutions is not observed (or expected) as temperatures decrease below 100 K. ^{13}C NMR signals from certain sites, such as methyl groups, may broaden at intermediate temperatures due to the slowing of molecular motions, but such signals sharpen again below 30 K where motion effectively ceases, as shown in Fig. 2.

Our choice of an EIO to produce microwaves was motivated in part by its compactness. The EIO itself is approximately 15 cm in diameter, and requires only a power supply (similar in size to a typical high-power RF amplifier) and a water chiller. Together with the quasi-optical interferometer, the entire microwave system is approximately $0.8\text{ m} \times 1.5\text{ m}$, easily fitting in the space below our superconducting NMR magnet. The laboratory room that houses our DNP system could not accommodate a gyrotron, for example. As shown in Fig. 4A, microwave powers above 1.0 W provide only small benefits at 25 K under our experimental conditions. The 1.5 W EIO output is also sufficient to generate signal enhancements in the 10–50 range at temperatures above 80 K, depending on details of the samples.

To our knowledge, EIO sources with similar power levels have not yet been produced at higher microwave frequencies. Thus, EIO sources may not be a viable option for higher-field DNP experiments.

In our experiments using the EIO, values of the DNP enhancement factor ϵ are typically in the 50–200 range below 30 K. Although we do not fully understand sample-to-sample variations in ϵ , factors that may be important include solubility of the paramagnetic dopant [31], binding of the dopant to the solutes of interest, glassiness of the frozen solution, and the intrinsic ^1H spin-lattice relaxation rate of the solute. The intrinsic spin-lattice relaxation rate (i.e., in the absence of paramagnetic doping) is affected by the aggregation state of the solute and its content of methyl groups [11], and can be easily be 0.2 s^{-1} or greater below 30 K [21]. It should also be noted that, under MAS at low temperatures, ϵ typically overestimates the enhancement of nuclear spin polarizations relative to their true thermal equilibrium values, largely due to MAS-induced depolarization in the absence of microwave irradiation [30].

A potential drawback of DNP-enhanced MAS NMR experiments below 80 K is the requirement that helium be used for sample cooling. Data in Fig. 5 show that helium consumption rates under experimentally relevant conditions can be below 1.3 l/hr. This makes MAS NMR experiments below 30 K practical for us from pragmatic and economic standpoints, even when extensive signal averaging or higher-dimensional spectroscopy is required. Operation costs could potentially be reduced if helium gas were recovered from the probe, separated from nitrogen gas, and recycled. We currently do not have the necessary infrastructure for helium recycling. Other laboratories are developing low-temperature MAS NMR systems that incorporate helium recycling [41–44].

In conclusion, numerous applications to molecular structural problems in biochemical systems are possible. One such application is illustrated in Fig. 6, which shows preliminary results from a study of the binding of the peptide M13 (residues 577–602 of skeletal muscle myosin light chain kinase) to human calmodulin in the presence of Ca^{2+} [45, 46]. For these experiments, M13 was synthesized with uniform ^{15}N , ^{13}C -labeling of residues 584–587 (sequence FIAV) and calmodulin was expressed in *E. coli* in unlabeled form. 1D ^{13}C NMR spectra of the calmodulin-bound (Fig. 6A) and unbound (Fig. 6B) peptide in frozen glycerol/water at 25 K show large signal enhancements from DNP and large differences in linewidths and spectral features. Two-dimensional (2D) spectra (Figs. 6C–6F) show a pronounced

sharpening of ^{13}C - ^{13}C crosspeak signals in the calmodulin-bound state, as well as a shift of the signals toward chemical shifts that are typical of α -helices (e.g., C_α chemical shifts near 64 ppm for isoleucine and valine). Thus, the DNP-enhanced spectra provide strong evidence for conformational disorder of M13 in the unbound state and α -helical formation in the bound state.

Experiments in Fig. 6 used 120 nmoles (0.36 mg) of M13, with data acquisition times less than 1 hr for the 2D spectra. These data indicate that 2D spectroscopy on frozen solutions with concentrations below 0.5 mM is quite feasible. In future experiments, we will attempt to examine intermediate states in protein recognition processes (such as the binding of M13 to calmodulin) and protein folding processes [23, 25–27], by combining rapid mixing and freeze-quenching methods with DNP-enhanced solid state NMR.

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Highlights

- Cross-polarized ^{13}C signal enhancements of 100–200 with 1.5 W microwaves at 24–30 K
- Liquid helium consumption below 1.3 l/hr at temperatures below 30 K and 6.7 kHz MAS
- Initial DNP-enhanced ^{13}C NMR data for a peptide/calmodulin complex

Figure 1.

Components of the DNP system. (A) Linearly polarized microwaves are supplied by an extended interaction oscillator (EIO), which provides 1.5 W of power at frequencies near 264 GHz. Microwaves exit the EIO through WR-4 waveguide and are converted to HE11 mode by the corrugated horn (12.7 mm inner diameter; Thomas Keating, Ltd.). (B) Microwaves travel from the EIO through corrugated waveguide to a directional coupler, within which roughly 8% of the microwave power reflects from a quartz plate (1.0 mm thick) to a corrugated horn for monitoring power or frequency. For power monitoring, the horn terminates in a diode detector (Pacific Millimeter Products, model YD), the output of which goes to a voltmeter. For frequency monitoring, the horn terminates in a harmonic mixer (Pacific Millimeter Products, model HMO), which mixes the microwaves with a 14th subharmonic reference signal from a signal generator (Agilent, model N5183A). The mixer output is amplified by an RF preamplifier (MITEQ, model AU-1114), filtered, and Fourier-transformed with a digital oscilloscope (Tektronix, model TDS680B). (C) After the directional coupler, the microwave beam (dashed magenta lines) enters a quasi-optical interferometer (Thomas Keating, Ltd.). After reflecting from curved mirror #1, the beam is split into two beams with orthogonal linear polarizations by a wire grid beamsplitter, with wires oriented at 45° to the incoming polarization. The two beams then reflect from two roof mirrors (which rotate their polarizations by 90°) and are recombined by the same beamsplitter. The net polarization of the recombined beam can be varied by adjusting the distance to one of the roof mirrors with a micrometer. The beam then reflects upward to the MAS NMR probe from curved mirror #2. The MAS NMR probe attaches to the probe

support to maintain the alignment of the microwave beam with a corrugated waveguide within the MAS NMR probe [29], which transmits microwaves to the sample. (D) Head of the MAS NMR probe with its outer cover removed, viewed below the 9.39 T, 89 mm bore superconducting NMR magnet. The final 5 cm of the liquid helium transfer line is contained within Teflon piece #1, which provides thermal insulation and mates with Teflon piece #2, which that plugs snugly into the MAS assembly. Cold helium passes through Teflon piece #2 into the NMR sample space, where it cools the central portion of the MAS rotor.

Figure 2.

^{13}C NMR spectra of a solution containing uniformly ^{15}N , ^{13}C -labeled L-alanine and L-proline (50 mM each) and triradical DOTOPA-Ethanol dopant (10 mM) in partially deuterated glycerol/water at the indicated temperatures. Spectra were obtained with ^1H - ^{13}C cross-polarization, ^1H decoupling, and MAS at 6.6–6.8 kHz. At each temperature, spectra recorded with microwaves on and off are compared on the same vertical scale (although with different numbers of scans). Signals from glycerol (glyc), L-alanine (Ala), and L-proline (Pro) are indicated on the 25 K spectrum. ^{13}C chemical shifts are referenced to the glycerol line at 72.7 ppm.

Figure 3.

Temperature dependence of ^{13}C NMR signals. (A) Signal enhancement factor ε and DNP-enhanced signal build-up time T_{DNP} . Data are for the sample in Fig. 2. ε is defined to be the ratio of cross-polarized ^{13}C NMR signal amplitudes (measured as the signal area between 12.2 to 79.1 ppm, and 171.5 to 184.3 ppm) with and without microwave irradiation, with otherwise identical measurement conditions. (B) Absolute DNP-enhanced signal amplitude and sensitivity, normalized to the values at 25 K. Sensitivity is proportional to the signal amplitude and inversely proportional to $T_{\text{DNP}}^{1/2}$.

Figure 4.

(A) Power dependences of cross-polarized ^{13}C NMR signal enhancements at 24 K and 100 K. Data are from measurements on isotopically labeled melittin [29] in partially deuterated glycerol/water with 10 mM DOTOPA-Ethanol. (B) Dependence of DNP-enhanced NMR signal amplitudes on microwave polarization, varied by adjusting the micrometer shown in Fig. 1C, at 25 K (blue circles) and 88 K (red triangles). Data are from the sample in Fig. 2. Signal amplitudes are normalized to their largest values. (C) Dependence of signal enhancements on microwave frequency at 24 K. Data are from the melittin sample.

Figure 5.

(A) Dependence of the liquid helium consumption rate on sample temperature, using nitrogen gas at room-temperature (blue) or 95 K (red) for MAS bearings. (B) Apparatus for cooling pressurized nitrogen gas for MAS bearings while avoiding undesirable nitrogen condensation. The heater raises the temperature of the nitrogen gas before it enters the liquid nitrogen bucket. The temperature controller varies the electrical current to the heater to maintain the Pt RTD temperature above the boiling point of the pressurized nitrogen.

Figure 6.

DNP-enhanced ^{13}C NMR measurements on a peptide/protein complex. (A) 1D spectra of a 80 μl glycerol/water solution containing 1.5 mM M13 (uniformly ^{15}N , ^{13}C -labeled in its FIAV segment), 2.0 mM calmodulin (unlabeled), 10 mM DOTOPA-4OH [31], and 15 mM CaCl_2 at 25 K and with MAS at 6.3 kHz. For this sample, $\epsilon \approx 210$. The solvent contained D_2O , 80 mM Tris-HCl-buffered H_2O at pH 7.0, ^{13}C -depleted glycerol- d_8 , and perdeuterated dimethylsulfoxide in 29:11:49:2.6 volume ratios. Recycle delay was 5.0 s. (B) 1D spectra of an identical solution, but without calmodulin, at 25 K with MAS at 6.5 kHz. For this sample, $\epsilon \approx 100$. (C) Aliphatic region of a 2D spectrum of M13 in the calmodulin-containing sample at 25 K and with 6.3 kHz MAS, recorded in 35 min with two scans per t_1 point, a 30 μs t_1 increment, 130 complex t_1 points and a 4.0 s recycle delay. A 25 ms spin diffusion period was used for ^{13}C - ^{13}C polarization transfers between t_1 and t_2 . 80 Hz Gaussian apodization is applied in both dimensions. Contour levels increase by successive factors of 1.4. (D) 2D spectrum of M13 in the sample without calmodulin at 25 K and with 6.5 kHz MAS, recorded and processed with identical conditions. (E,F) 1D slices from the 2D spectra.