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Biomolecular solid state NMR with magic-angle spinning at 25 K

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ABSTRACT

A magic-angle spinning (MAS) probe has been constructed which allows the sample to be cooled with helium, while the MAS bearing and drive gases are nitrogen. The sample can be cooled to 25 K using roughly 3 L/h of liquid helium, while the 4-mm diameter rotor spins at 6.7 kHz with good stability (±5 Hz) for many hours. Proton decoupling fields up to at least 130 kHz can be applied. This helium-cooled MAS probe enables a variety of one-dimensional and two-dimensional NMR experiments on biomolecular solids and other materials at low temperatures, with signal-to-noise proportional to $1/T$. We show examples of low-temperature ^{13}C NMR data for two biomolecular samples, namely the peptide $A\beta_{14-23}$ in the form of amyloid fibrils and the protein HP35 in frozen glycerol/water solution. Issues related to temperature calibration, spin–lattice relaxation at low temperatures, paramagnetic doping of frozen solutions, and ^{13}C MAS NMR linewidths are discussed.

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1. Introduction

Nuclear magnetic resonance (NMR) experiments are often limited by sensitivity. One very general method to increase the signal for solid state NMR is to cool the sample [1,2]. At temperatures above 1 K and in standard NMR magnets, the nuclear spin polarization at thermal equilibrium is proportional to $1/T$, where T is the temperature. Thus, if we cool the sample to 25 K from room temperature (~295 K), NMR signals can be increased by a factor greater than 10. Additional improvement in signal-to-noise can be gained by cooling the detection circuit along with the sample [3,4]. Even this smaller enhancement from cooling the detection circuit, but not the sample, was a leap forward in solution NMR technology [4]. In our solid state NMR probe, we use the signal enhancement from cooling the sample, with the future possibility of additional signal-to-noise benefit by cooling the detection circuit.

Cooling a static sample with liquid helium is relatively straightforward [5–11], but cooling with helium while using magic-angle spinning (MAS) has proven more difficult. Several groups have developed MAS probes that use helium gas for both cooling and spinning [12–16]. These designs have had various limitations, including low spinning speed [12,16], low spinning stability [13–15], and high helium consumption. For our application of solid state NMR to study biomolecular systems, we want a large sample volume, and to use two-dimensional (2D) NMR and multiple pulse recoupling techniques that have not yet been demonstrated with these all-helium MAS probes. In particular, to be useful in our studies of biomolecular structure, a low-temperature MAS probe should have high spinning stability (i.e., MAS frequency fluctuations much less than 1%), must be able to achieve high radio-frequency (rf) field strengths on two or more rf channels for periods greater than 10 ms (e.g., 50 kHz ^{13}C rf fields and 100 kHz ^{1}H fields), and must have sufficiently low helium consumption that experiments can be run for many consecutive hours at low temperatures.

In this paper, we describe a MAS probe that can cool the sample to 25 K with liquid helium. This probe is designed for experiments at moderate MAS frequencies with relatively large sample volumes. The probe uses room temperature nitrogen as the bearing and drive gas. This enables stable spinning at moderately high MAS frequencies, so that standard multiple pulse and 2D NMR experiments can be performed. Liquid helium consumption at 25 K is approximately 3 L/h, with the 4-mm diameter rotor spinning at 6.7 kHz with good stability (±5 Hz) for more than 12 consecutive hours. We have successfully applied proton decoupling fields up to 130 kHz for 5 ms during dipolar recoupling periods, and 75 kHz for 20 ms during evolution and detection periods of 2D measurements. To demonstrate the capabilities of the probe, we show examples of ^{13}C MAS NMR measurements on two samples, namely amyloid fibrils formed by residues 14–23 of the β-amyloid peptide associated with Alzheimer's disease ($A\beta_{14-23}$) [17] and the protein HP35 in frozen solution [18].

2. Materials and methods

2.1. Probe design

Fig. 1a shows a sketch of the MAS unit. The design is based on a 4 mm outer diameter rotor that is 4.57 cm long. Compared with standard MAS rotor lengths of 3.1 cm or less, the greater rotor

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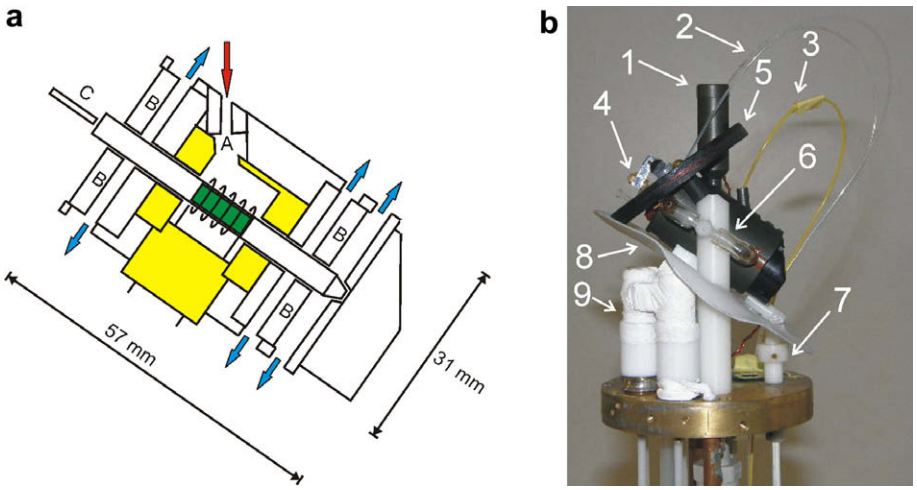

Fig. 1. (a) Cross-section of MAS unit of helium-cooled low temperature solid state NMR probe. Cold helium (red arrow) enters the MAS unit through the tube labeled A. The sample (green) sits in the sample space region defined by two Teflon pieces (yellow), which fit together to hold the NMR coil and separate the helium-cooled sample space from the nitrogen gas. Helium gas exits the sample space in the small gap around the circumference of the rotor. Both the nitrogen gas, used for the air bearings (B) and for spinning, and the helium gas can vent from the MAS unit on either side of each of the bearings (blue arrows). For stable spinning, the rotor requires a "pointer" (C) which acts to dampen vibration of the rotor. (b) Photograph of the probe head, with outer aluminum can removed. Arrows indicate the helium entry tube (1), optical fibers for MAS tachometer (2), fiber optic temperature sensor (3), hinged pointer for stabilizing spinning (4), shim coil (5), bearing gas supply tubes (6), MAS angle adjust rod (7), Teflon baffle (8), tuning and matching capacitors for ^{13}C channel (9). (For interpretation of color mentioned in this figure the reader is referred to the web version of the article.)

length provides increased separation between the helium-cooled sample region and the warmer nitrogen bearing and spinning gas. The rotor drive tip, brass stator, and ceramic bearings are from a Varian 4 mm MAS unit. Liquid helium is fed from a pressurized helium dewar (7–8 psi for low temperatures) through a vacuum-insulated metal transfer line with a needle valve to control helium flow (Janis Research). The transfer tube feeds into a short (5 cm long) segment of Torlon at the top of the sample region. This short section of plastic helps to eliminate excess noise pickup from the helium transfer line, in combination with grounding of the bottom end of the transfer line to the probe body. Cold helium then enters the MAS unit through the hole labeled A in Fig. 1a.

To avoid electrical discharge arcing in the helium gas atmosphere [11], the coil is made with Teflon-coated wire (Alpha Wire Co., 18 gauge wire with 0.25 mm of Teflon insulation, 5 turns, inner diameter 4.7 mm), and the sample space is surrounded by a Teflon insert (shown in yellow in Fig. 1a). This Teflon insert also defines the sample space cooled by the helium. The helium-cooled sample space is mostly closed, but the helium gas can vent around the outer surface of the rotor into the two bearing areas. Then, both the helium gas and the nitrogen gas vent out of the MAS unit on both sides of each bearing (indicated by blue arrows on Fig. 1a). It is important to have a vent for the nitrogen bearing gas on the sample side of the bearings, to avoid warming the sample space with the nitrogen gas. Currently, we are using room temperature nitrogen gas for both the bearing and drive gases. In a future design, cooling of the bearing and drive gas may enable lower sample temperatures or lower helium consumption. At room temperature, this MAS unit achieves MAS frequencies up to ν_{MAS} = 10 kHz. At low temperatures, we have performed most experiments at ν_{MAS} = 6.7 kHz. At 9.4 T, this MAS frequency is high enough for a wide variety of solid state NMR techniques, and is high enough to prevent overlap of MAS sideband signals from natural-abundance ^{13}C of glycerol with other ^{13}C signals (see below). Because room temperature nitrogen gas is used for the bearings and spinning, there is a tradeoff between increasing spinning speed and achieving low temperatures.

Fig. 1b shows a photograph of the probe head. Commercial variable capacitors (Polyflon) are used for tuning and matching of the ^{13}C channel and for matching of the ^{1}H channel, and a home-built cylindrical coaxial capacitor that can be adjusted by moving a Teflon dielectric is used for ^{1}H tuning. Although the current probe design also includes a pair of capacitors for a ^{15}N channel, this third channel was not connected in experiments described below. During experiments, nitrogen purge gas flows continuously into the area of the capacitors, and also into the lower probe body. The outer diameter of the probe body is 88 mm, necessitating removal of the room temperature shims from our 89-mm bore superconducting magnet before the probe can be raised into the magnet. A single internal shim coil was therefore included in the probe to enable shimming. The shim coil consists of 15 turns of 22 gauge wire on a 6 cm diameter form, mounted roughly 2 cm above the center of the rotor, and tilted at a 35° angle to avoid other probe parts. A shim current of roughly 0.65 A was used in the experiments described below, provided by a DC power supply (HP 6236B or GW PPT-3615). RF noise from the shim coil was avoided by using a coaxial lead with the outer conductor grounded to the probe body, and capacitors and RF chokes for filtering. At low temperatures, the shim current was adjusted to minimize the ^{79}Br NMR linewidth for KBr powder (140 Hz) under MAS. The sample volume available (green area in Fig. 1a) is 48 or 82 μl depending on the wall thickness of the rotor. All experiments reported below used a thick-wall (48 μl) rotor except for the data of Fig. 9, which were taken in a thin-wall (82 μl) rotor.

MAS frequencies were monitored with a fiber optic tachometer. A fiber optic temperature sensor (Neoptix model *T*1) in contact with the MAS unit was used to monitor liquid helium flow and as a rough indicator of sample temperature. Sample temperatures were determined more precisely as described below.

2.2. Sample preparation

An $A\beta_{14-23}$ peptide sample (amino acid sequence HQKLVFFAED) with uniform ^{15}N and ^{13}C labeling of V18 and A21 was synthesized and fibrillized as previously described [17]. For measurements described below, 2.7 mg of fibrils were mixed into 25 μl of glycerol/water (3:2 ratio by volume) in the MAS rotor, with 160 μM DyEDTA. A similar sample was also prepared without DyEDTA. Previous solid state NMR measurements have shown that $A\beta_{14-23}$ fibrils have a highly ordered antiparallel β-sheet structure, with intermolecular hydrogen bonding of V18 and A21 within the β-sheets [17].

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