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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/h. 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.

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1. 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 l/h in MAS NMR experiments.

2. Description of the DNP system

Our DNP system operates with a 9.39 T NMR magnet, implying ^{1}H 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

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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 h 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 h, usually in 5–10 h runs. Frequency stability is roughly ±2 MHz over periods of seconds and ±6 MHz over periods of hours. Power stability is better than ±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° 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

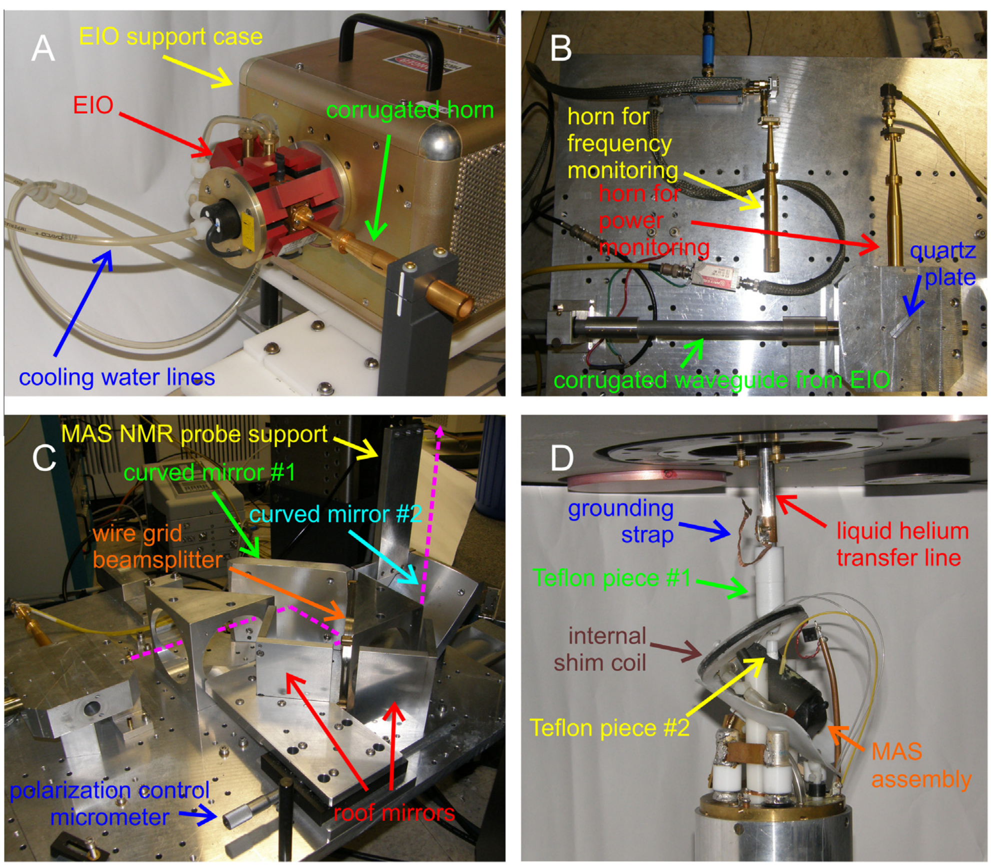

Fig. 1. Components of the DNP system. (A) Linearly polarized microwaves are supplied by an extended interaction oscillator (EIO), which provides 1.5 W of power at frequencies near 264 GHz. Microwaves exit the EIO through WR-4 waveguide and are converted to HE11 mode by the corrugated horn (12.7 mm inner 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 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.