DNP-Enhanced MAS NMR Spectroscopy at 16.4 T and 30 K –Instrumentation and Applications–

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Precise information on 3D structure of bio-macromolecules is valuable for elucidating their functions. Magic-angle sample spinning solid-state NMR (MAS SSNMR) spectroscopy is suited to study atomic-resolution structure and dynamics of insoluble and/or non-crystalline molecular systems such as membrane proteins and amyloid fibrils, but suffers from its inherently low sensitivity.

Dynamic nuclear polarization (DNP) is a method that enhances the NMR sensitivity by transferring three order of magnitude larger electron polarization to the nuclei of interest through a high-power microwave irradiation. Recently, DNP-enhanced SSNMR is becoming increasingly popular at moderate external field conditions (B_0 < 9.4 T) and temperatures (T > 90 K), but less accepted at higher field conditions. High-field condition is crucial for gaining spectral resolution and basic NMR sensitivity, but decreases the efficiency of the Cross Effect-based DNP, progressively diminishing the merit of high field DNP.

In coping with this issue for biological applications, advanced hardware development should play a key role. Here, we describe two innovations we have recently made in instrumentation for DNP-enhanced SSNMR at B_0 = 16.4 T (700MHz for ^{1}H, 460 GHz for electron spin resonance):

1. Closed-cycle helium-cooling MAS probe system[1]. The low sample temperature (~30K) improves both the DNP efficiency and the Boltzmann nuclear polarization, recovering the sensitivity gain at high fields. This system cools the compressed spinner gases with Gifford-McMahon cryo-coolers, spins the rotor while cooling, and re-compresses the return gas, for the first time, in a completely closed gas circulation path. Thus, the system does not consume any helium to sustain stable MAS (4-12 kHz) at cryogenic temperatures (35-120 K) for an extended period of time (e.g. >2 weeks) with low running cost (~$3/hr for mostly electricity expense). The long-term stability has enabled routine use of high-dimensional spectroscopy at cryogenic temperatures, strongly promoting application of high-field DNP to very complex and/or high-molecular weight chemical/ biological systems. With the present system, we have so far obtained the sensitivity gain of over 1000 from DNP and the temperature effect combined at 30 K.

2. Double-frequency sub-millimeter wave (SMMW) irradiation system[2]. Many exciting possibilities will open up for high-field DNP NMR if the spectrometer is able to handle two SMMWs in different frequencies. The system involves two 460 GHz frequency-tunable and frequency-agile gyrotrons as SMMW sources, quasi-optical transmission system, universal polarizer, and a custom designed beam combiner. Recently, we have confirmed in a simple experiment the benefit of using double-gyrotron irradiation.

In the presentation, we present a brief overview on the high-field DNP techniques (methods and instruments), as well as the latest experimental DNP data we obtained with the above setups.

References: 1. Y. Matsuki et al., *JMR* **259**, 76- (2015) [Open Access]
2. Y. Matsuki et al., *JMR* **264**, 107- (2016)