

Impact of Magnetic Field Strength on Resolution and Sensitivity of Proton Resonances in Biological Solids

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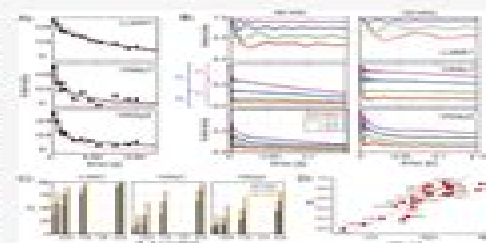


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ABSTRACT: Sensitivity and resolution together determine the quality of NMR spectra in biological solids. Higher magic angle spinning frequencies yield a more efficient suppression of the coupling network and enable atomic-level investigations of protonated protein samples. On the other hand, truncation effects induced by higher magnetic fields have an impact on the achievable sensitivity and resolution. In this work, we address the question of how the proton dipolar coupling network affects the magnetic field strength-dependent gains in sensitivity and resolution. We find that—beyond the canonical $B_0^{3/2}$ dependence—an additional factor of 2 in sensitivity can be achieved for residues embedded in the core of the protein, when the static magnetic field induces a transition from the strong- to the weak-coupling limit. The experiments are carried out using a selectively methyl-protonated ($^{13}\text{CH}_3$) α -spectrin SH3 sample, at magnetic field strengths of 11.75 T (^1H Larmor frequency of 500 MHz) and 23.5 T (^1H Larmor frequency of 1 GHz).



■ INTRODUCTION

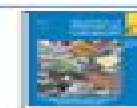
Structure determination of protonated proteins using proton-detected solid-state NMR experiments, acquired at high magnetic fields (1 GHz) and fast (100 kHz) magic angle spinning (MAS), was first demonstrated in 2016.¹ Since then, fast MAS has revolutionized biological solid-state NMR.^{2–5} Fast sample spinning at the magic angle is a prerequisite for proton-detected high-resolution solid-state NMR.⁶ Faster sample spinning averages anisotropic interactions more efficiently, which results in better sensitivity in correlation spectra.⁷ The effect of the MAS rotation frequency on the resolution of amide and methyl proton spectra has been studied recently.^{8,10–12} It has been shown that T_2 of amide protons increases proportionally with the inverse of the rotor period for most residues in a model protein.¹³ As the effective dipole–dipole interaction experienced by methyl protons is much larger than that for any other type of protons in a protein, methyl protons yield the largest line widths, even though the intramethyl dipolar couplings are scaled because of the fast rotation of the methyl group.¹⁴ For a selectively methyl-protonated sample in an otherwise deuterated background, MAS frequencies above 300 kHz are necessary to yield 80% of the maximum attainable signal intensity.¹⁵ For MAS frequencies below 70 kHz, $^{13}\text{CHD}_2$ methyl group labeling is necessary to obtain optimal spectral quality. Above an MAS frequency of 70 kHz, $^{13}\text{CH}_3$ isotopomers^{4,15–17} yield the best sensitivity depending on the density of the proton spin system.¹²

The maximum achievable rotation frequency of an MAS rotor is limited by the speed of sound on the rotor surface.¹⁸ Higher rotation frequencies can therefore only be obtained for ever smaller diameter rotors. Lower sample mass is thus traded for faster MAS rates. At first sight, this seems to come at the cost of sensitivity. A 0.7 mm MAS rotor that spins as fast as 110 kHz accommodates effectively less than a milligram of the sample.¹⁹ As the length of an MAS rotor scales approximately linearly with its diameter, the amount of sample in a fast spinning rotor decreases proportionally with r .⁵ On the other hand, the quality factor of the coil and the efficiency of detection increase with smaller coil diameters proportional to $1/r$.²⁰ The apparent coherence decay time T_2^* and thus the signal intensity during proton detection increase with higher MAS frequencies. Longer T_2^* times contribute to the overall intensity linearly with $1/r$.^{4,13,21} Even though the Hartmann–Hahn matching conditions become more selective at high MAS rotation frequencies,^{13,22} $^1\text{H}-T_1$ and T_{ρ} relaxation times increase at faster MAS frequencies which facilitate multi-dimensional solid-state NMR experiments with multiple magnetization transfer steps.⁷ Assuming that polarization transfer contributes another factor proportional to $1/r$ to the

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