Sensitivity Enhancement in Solid-State $^{13}$C NMR of Synthetic Polymers and Biopolymers by $^1$H NMR Detection with High-Speed Magic Angle Spinning

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Indirect detection of $^{13}$C and $^{14}$N nuclear magnetic resonance (NMR) spectra through $^1$H NMR signals offers large sensitivity advantages in studies of organic and biological molecules in solution and is almost universally employed. 1-3 Although sensitivity enhancement by indirect detection was first demonstrated in NMR4-6 and nuclear quadrupole resonance7,8 of solids, direct detection has generally been preferred in solid-state NMR. 9 This is because the broad $^1$H NMR lines of organic solids negate sensitivity enhancement under the most common conditions. We have recently10 that substantial sensitivity enhancements can in fact be achieved by indirect detection in one-dimensional (1D) solid-state $^{13}$C NMR spectroscopy of organic compounds and biopolymers under magic angle spinning (MAS) at speeds that greatly reduce the $^1$H NMR line widths.10 Here we demonstrate the feasibility of sensitivity enhancement in solid-state $^{13}$C NMR spectroscopy of general organic solids. We present experimental results both for the noncrystalline synthetic polymer poly(methyl methacrylate) (PMMA) and for the heptapeptide $\alpha$-acetyl-Lys-Leu-Val-Phe-Phe-Ala-Glu-NH$_2$ (A$_4$) in the form of amyloid fibrils. We report extension to $^{13}$C NMR, which forms the basis for many structural and dynamical studies in organic and biological systems, and to 2D spectroscopy significantly broadens the impact and generality of indirect detection methods in solid-state NMR.

The sensitivity enhancement factor $\xi$, defined as the ratio of frequency-domain signal-to-noise ratios for $^{13}$C-detected and $^{13}$C-detected measurements, is given by

$$\xi = \left( \frac{x_{\text{d}}}{\alpha} \right)^{1/2} \left( \frac{y_{\text{d}}}{y_{\text{c}}} \right)^{1/2} \left( \frac{W_{\text{d}}}{W_{\text{c}}} \right)^{1/2} \left( \frac{Q_{\text{e}}}{Q_{\text{c}}} \right)^{1/2} \frac{A_{\text{H}}}{A_{\text{C}}}$$

where $y$ is the magnetogyric ratio, $W$ is the effective line width, $Q$ is the quality factor of the sample coil, and $A$ subsumes properties such as coil geometry, filling factor, receiver noise

Figure 1. 2D $^{13}$C/$^1$H heteronuclear correlation spectra of PMMA powder (9 mg, unlabeled) obtained with $^{13}$C detection (a, c, e, g) and with $^1$H detection (b, d, f, h). 1D slices are shown at $^1$H shifts of 0.9 (c, d), 3.7 (e, f), and −3.5 ppm (g, h).

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15 Figure 1 compares 2D $^{13}$C/$^1$H heteronuclear correlation (HETCOR) spectra of PMMA powder obtained with conventional $^{13}$C detection and with $^1$H detection. These spectra are acquired at 17.6 T (749.5 and 188.5 MHz $^1$H and $^{13}$C NMR frequencies) and...
nuclei that do not participate in polarization transfer to $^{13}$C nuclei.

To obtain the sensitivity enhancements described above, we apply
and with $^{1}$H detection. In this case, because the $^{13}$C-detected
measurement is 1D but the $^{1}$H-detected measurement is necessarily
in the 1D$^{13}$C-detected measurement. For quaternary and proto-
dated $^{13}$C sites, $\xi$ is reduced by the factor $\alpha^{15}$. To compensate for this
reduction, $^{1}$H signals are detected with pulsed spin-locking
(PSL), i.e., $^{1}$H signals are sampled in windows between rotor
synchronized radio frequency (rf) pulses that reduce the effective
$^{1}$H line width to roughly 50 Hz. Because of the finite pulse lengths
and receiver dead time, the sampling windows comprise a fraction
$d = 0.438$ of the total acquisition time. $^{1}$H chemical shift
information is lost under PSL, but this information is also absent
in the 1D $^{13}$C-detected measurement. For quaternary and proto-
nated $^{13}$C sites, $\xi \approx 2.5$ in Figure 2. For the carbonyl site, $\xi \approx 1.5$.

The PMMA samples in Figures 1 and 2 are not $^{13}$C-labeled. A potential pitfall in $^{1}$H-detected $^{13}$C NMR measurements, especially
at natural abundance, is the large “$t_1$ noise”$^{14}$ contributed by $^{1}$H
nuclei that do not participate in polarization transfer to $^{13}$C nuclei.
To obtain the sensitivity enhancements described above, we apply
two 400 $\mu$s rf pulses at the $^{1}$H NMR frequency, with phases $x$ and $y$ and with amplitudes set to $t_y/2$ for rotary resonance recoupling$^{20}$
during the $^{13}$C dephasing period $t_d$ (see Figure 1 caption). These pulses destroy $^{1}$H magnetization that would otherwise generate $t_1$ noise.

Figure 3 compares $^{13}$C-detected and $^{1}$H-detected $^{13}$C/$^{1}$H HET-
COR spectra of $\beta_{16-22}$ fibrils obtained at $\tau_R = 31250 \pm 5$ Hz
and 17.6 T. Ten percent of $\beta_{16-22}$ molecules are $^{13}$C-labeled at
all carbon sites in the central five hydrophobic residues.$^{13}$ $\xi$ values are up to 2.4 for protonated and 1.8 for nonprotonated $^{13}$C signals.
Although the sharper $^{13}$C lines in $\beta_{16-22}$ fibrils lead to smaller $\xi$
values than in Figure 1, these results still indicate a reduction of data acquisition time by a factor of 10.

The spectrum in Figure 3b provides new constraints on the structure of $\beta_{16-22}$ amyloid fibrils.$^{1}$ $^{13}$C chemical shift assign-
ments, initially determined from $^{13}$C/$^{13}$C 2D exchange spectra,$^{13}$
are confirmed by the present data. Additionally, $^{1}$H chemical shifts
determined from Figure 3b (5.1, 4.7, 5.1 ppm $^{1}$H shifts for Leu17,
Val18, and Ala21, respectively, $\pm 0.3$ ppm precision; 1.2 and 0.8
$^{1}$H ppm $^{1}$H shifts for Leu17 and Ala21) support a $\beta$-strand backbone
conformation for the labeled residues.$^{21}$ $^{1}$H$_{\beta}$ (5.8 ppm) and $^{1}$H$_{Y}$
(1.6 and 3.2 ppm) shifts for Phe residues and the $^{1}$H$_{Y}$ (1.2 ppm)
shift for Val18 are anomalous,$^{21}$ possibly indicating intermolecular
contacts of Phe residues and intermolecular or intramolecular
contacts between Phe and Val residues in a laminated $\beta$-sheet structure.$^{13}$

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Supporting Information Available: Table of chemical shifts from
Figure 3 and expansion of Figure 3b with assignments (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.
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Figure 2. 1D $^{13}$C NMR spectra of PMMA powder (4.5 mg, unlabeled)
obtained with $^{13}$C detection (a) and with $^{1}$H detection (b). Total of 344
scans for each spectrum. Spectrum a is obtained in a 1D manner with
CP and decoupling conditions as in Figure 1. Spectrum b is a single slice
of a 2D spectrum obtained with the conditions in Figure 1b, but with a
pulse spin locking (PSL) train applied in the $t_{1H}$ period and with $t_d = 4$
ms. The PSL train consists of one 6 $\mu$s $\pi/2$ pulse with phase $x$ per sample
rotation period. Complex $^{1}$H signal points are sampled every 0.5 $\mu$s during
14 $\mu$s windows between PSL pulses.

Figure 3. 2D $^{13}$C/$^{1}$H heteronuclear correlation spectra of amyloid fibrils
formed by the heptapeptide $\beta_{16-22}$ (2 mg, lyophilized powder; peptides
uniformly $^{13}$C-labeled in the central five amino acid residues are diluted to
10% in unlabeled peptides) obtained with $^{13}$C detection (a, c, e, g) and with $^{1}$H detection (b, d, f, h). 1D slices are shown at $^{1}$H shifts of 0.7
(c, d), 6.9 (e, f), and 13.0 ppm (g, h, vertical scale increased to show
noise level). Experimental conditions are the same as in Figure 1 but $t_d$
= 6.5 ms, maximum $t_c$ and $t_{1H}$ (or $t_{1C}$ and $t_{1H}$) values are 1.50 and 0.75
ms, and 9726 total scans per spectrum. Lorentzian broadening of 335 Hz
in the $^{13}$C dimension and Gaussian broadening of 675 Hz in the $^{1}$H
dimension are applied.