Protons are characterized by having a natural abundance of more than 99.9%, as well as a high gyromagnetic ratio. These attributes provide much greater detection sensitivity than those of $^{13}$C or $^{15}$N, making $^1$H-detected NMR spectroscopy very attractive, not just for solution NMR.[1-6]

Soluble samples benefit from the fact that unwanted dipolar couplings are cancelled out by molecular tumbling, a feature that insoluble molecules lack. As a result, a strong network of homonuclear proton dipolar couplings dramatically broadens the linewidth of any $^1$H signal in solid-state NMR.

Two approaches especially have been shown to overcome this obstacle; sample preparation using perdeuteration-reprotonation to dilute the dense proton network[1-3], and the use of increased magic angle spinning (MAS) speeds in the order of 60 to 111 kHz (Figure 1).[3,5]

In collaboration with Guido Pintacuda and co-workers, Bruker BioSpin presents three basic $^1$H-detected pulse programs (‘pulprog’) that allow initial protein backbone assignment under fast MAS[3]:

1. hNH: 1D and 2D, Figures 1A and 3, pulprog: hNH2D.dcp
2. hCaNH: 1D-3D, Figures 1B and 4, pulprog: hCaNH3D.tcp
3. hCONH: 1D-3D, Figures 4 and 6B, pulprog: hCONH3D.tcp
Introduction

Depending on the sample properties, $^1$H dipolar couplings can be in the order of several tens of kHz. The higher the MAS rate, the more attenuated the unwanted $^1$H dipolar couplings will be (Figure 2, black), resulting in better resolved $^1$H signals and allowing for the use of polarization transfer using scalar couplings\cite{7} (Figure 2, orange).

In the past, solid-state NMR was limited to moderate MAS rates ($\leq 25$ kHz) by probe designs. Fortunately, recent developments have seen a move towards small rotor sizes – for example Bruker 1.9, 1.3 and 0.7 mm H/C/N probes – allowing the use of fast (40 to 60 kHz) or even ultra-fast (60 to 111 kHz) MAS\cite{3,5}.

Under these conditions, low power irradiation for heteronuclear decoupling and selective heteronuclear cross polarization (CP)\cite{8} is efficient, minimizing RF irradiation-induced sample heating.

Another approach to reducing $^1$H dipolar couplings targets the dilution of the dense $^1$H network\cite{1}. During sample preparation, the protein of interest is expressed in a perdeuterated growth medium. When the sample is subsequently incubated with a defined $^2$H$_2$O: $^1$H$_2$O buffer, exchangeable sites such as amide, amine and hydroxyl groups will undergo a deuteron to proton back-exchange. Depending on the $^2$H:$^1$H ratio, the $^1$H network can be strongly diluted – for example 10 to 20 % back-exchange\cite{2,4,6} – or fully reprotonated at all exchangeable sites\cite{1,3,5}. Even with a 100 % back-exchange, the sample is diluted due to deuterons at non-exchangeable sites.

Increasing $^1$H spin dilution will not only reduce unwanted dipolar couplings (Figure 2, purple), but the overall $^1$H signal-to-noise ratio. Furthermore, selective dipolar transfer pathways may not be possible, for example due to the low propensity of two protons being in close proximity. Moreover, in the regime of fast MAS, it depends on the experimental conditions whether dipolar or scalar coupling-based experiments are preferable (Figure 2, orange and green)\cite{3-6}, and so when using fast MAS rates, experimental conditions should be carefully chosen. Alternatively, the use of strong dilution combined with moderate MAS rates\cite{2,6} or full reprotonation under (ultra-)fast MAS\cite{3-4}, where the spinning is sufficient to weaken the $^1$H dipolar couplings (Figure 2, red and black), is recommended.

Experiments and Pulse Programs

For $^1$H-detected experiments, the easiest way to begin with is the heteronuclear hNH experiment, which is comparable to a solution NMR HSQC experiment, but which is based on CP. This correlates the amide proton to its nitrogen (Figure 3B, purple). Using the hCaNH sequence (Figure 3B, orange, and Figure 4A), the Ca of a residue can be correlated to the amide.
The off-resonance pulse causes phase evolution of the on-resonance spins, an effect often referred to as the ‘Bloch-Siegert shift’ in solution NMR\(^9\) (Figure 5A, purple). Furthermore, the initial evolution time, \(t_1(0)\), is greater than zero, resulting in unwanted initial chemical shift evolution for the first TD point (Figure 5A, red).

To compensate for both these effects, an on-resonance \(\pi\) pulse needs to precede \(t_1\) (Figure 5A, orange). For complete refocusing (Figure 5B), a symmetry construct (Figure 4, yellow) centered around this pulse has to be created, resulting in a second off-resonance \(\pi\) pulse and a symmetry delay (\(\Delta\)) which compensates for \(t_1(0)\). Both are applied consecutive to the initial \(1H\) to \(13C\) CP.

Any other TD point is acquired with a \(t_1\) larger than \(\Delta\), leading to desired additional chemical shift evolution, which is unaffected by refocusing (Figure 5C, cyan).

For heteronuclear decoupling (Figure 4, white pulses), a \(15N\) \(\pi\) hard pulse and \(1H\) low power decoupling are applied during \(t_1\), followed by a SPECIFIC \(13C\) \(\alpha\) or \(13CO\) to \(15N\) CP \([10,11]\) transfer (Figure 4, purple pulses).

Afterwards, both sequences equal the ‘\(hNH2D.dcp\)’ (Figure 3A), where \(15N\) evolution (\(t_1\) or \(t_2\)) is attended by heteronuclear decoupling using a \(13C\) \(\pi\) hard pulse (white pulse) and \(1H\) low power decoupling.

To suppress the water signal, continuous wave (cw, dark and light gray) pulses are applied alternately along \(X\) and \(Y\), on-resonance with the water peak.

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To suppress the water signal, continuous wave (cw, dark and light gray) pulses are applied alternately along \(X\) and \(Y\), on-resonance with the water peak.
During this so-called MISSISSIPPI $H_2O$ suppression$^{[12]}$, $^{15}N$ magnetization is stored along $Z$ by a $\pi/2$ hard pulse. With a second $\pi/2$ hard pulse, $^{15}N$ magnetization is brought back into the $XY$ plane to transfer polarization to $^1H$ in a final $^{15}N$ to $^1H$ CP (dark blue pulses), followed by $^1H$-detection under low power heteronuclear decoupling on $^{15}N$ and $^{13}C$.

**Which Experimental Conditions to Choose?**

The following recommendations have been tested on a $u$-$[^{13}C, ^{15}N]$-labeled, perdeuterated and 100% $^1H$ back-exchanged ubiquitin sample at 60 kHz MAS using a Bruker 1.3 mm rotor and a Bruker Avance III HD 600 MHz Wide Bore system.

The sample temperature of approximately 294 K was regulated with a BCU II, using a N$_2$ gas flow of ~1,100 l/h to reach a set temperature of 257 K. Because faster MAS produces more frictional heating, the ideal sample temperature and the N$_2$ gas flow required should be adjusted beforehand using, for example, $^{79}$Br$^{[13]}$. Nevertheless, a gas flow of more than 1300 l/h should be avoided to not cause rotor imbalances at high spinning speeds. Additionally, when using temperature regulation, frame cooling should be used.

As known from ‘conventional’ solid-state NMR, the Hartmann-Hahn-condition $n\times v_R = v_I \pm v_S$ must be fulfilled to achieve CP transfer, with $n$ as an integer (usually $\pm 1$ or $\pm 2$), $v_R$ the MAS rate and $v_I$ and $v_S$ the RF field strengths acting on the I and S spins respectively. Addition of $v_I$ and $v_S$ is known as a double-quantum (DQ) transition, and subtraction as a zero-quantum (ZQ) transition. Under (ultra-)fast MAS, selective CP is efficient already at low power irradiation, resulting in higher sensitivity for the DQ CP condition compared to ZQ (Figure 6A).

For all initial and final CP steps ($v_R = 60$ kHz), RF field strengths of 50 kHz applied on $^1H$ and only 10 kHz on $^{15}N$ or $^{13}C$, respectively, have been proven well.

SPECIFIC $^{13}C$ to $^{15}N$ CP gave best results using 35 kHz applied on $^{13}C$ and 25 kHz on $^{15}N$. Here, the 50/ 10 kHz condition results in poor transfer efficiency and is thus not suited (Figure 6B). Nevertheless, on unknown samples it is always recommended to test different Hartmann-Hahn conditions.

During CP, the use of a shaped pulse on one of the two nuclei involved increases transfer efficiency dramatically because several Hartmann-Hahn conditions can be passed and $B_1$ inhomogeneity compensated (Figure 6A).$^{[14]}$ A shape can be applied on either of the two nuclei, while the other nucleus experiences a rectangular pulse in the form of, for example, a ‘square.1000’ shape.

Here, linearly ramped up and ramped down shapes were applied on $^1H$ for all initial and final CP steps respectively. Theoretically, a flat ramp from 90 to 100 % (e.g. ‘ramp90100.1000’/’ramp10090.1000’) should perform well at high MAS rates, ensuring that magnetization is transferred during most of the contact time. However, sometimes, a steeper ramp from 70 to 100 % (e.g. ‘ramp70100.1000’/’ramp10070.1000’) can be more efficient, as in the presented data.

For SPECIFIC $^{13}C$-$^{15}N$ CP, a tangential amplitude modulated shape, for example ‘tacn80’, applied on $^{15}N$ is most efficient.

According to solution NMR, Gaussian pulse cascades$^{[15]}$ (e.g. ‘Q3.2000’) can be applied for 256 and 350 $\mu$s for selective $^{13}C\alpha$ and $^{13}CO$ $\pi$ pulses. Note that a longer pulse is more selective. When setting the constants ‘cnst21’ and ‘cnst22’ as frequency offsets for $^{13}CO$ and $^{13}C\alpha$ in ppm, respectively, all necessary frequency changes are calculated and set automatically in the pulprog.
During MISSISSIPPI, the $H_2O$ saturation time ($t_{sat}$ in Figures 3 and 4, ‘d19’ in pulprogs), should be optimized between 100 and 300 ms. CW decoupling programs (cpdprg) are called ‘cwX_13nofq’ and ‘cwY_13nofq’. As the names imply, parameter ‘plw13’ is the corresponding power level, which should be set to ¼ of the MAS rate, for example 15 kHz at 60 kHz MAS.

Note: The suffix ‘nofq’ reveals that no frequency switch is taking place with the cpdprg itself.

Likewise, heteronuclear decoupling of only 15 kHz on $^1H$ is sufficient during $^{13}C$/$^{15}N$ evolution times using ‘sltppm_12nofq’ at power level ‘plw12’.[16]

## Rule of thumb

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Nuclei</th>
<th>Parameter names in pulprog</th>
<th>Rule of thumb</th>
</tr>
</thead>
<tbody>
<tr>
<td>$90^\circ$ (180°) hard pulses</td>
<td>$^1H$</td>
<td>$p3$ (p4) @ plw2</td>
<td>$-100$ kHz (2.5 $\mu$s)</td>
</tr>
<tr>
<td></td>
<td>$^{15}N$</td>
<td>$p21$ (p22) @ plw21</td>
<td>$-42$ kHz (6 $\mu$s), optimize in $hNH2D.dcp$: flag ‘–DN90’ (zero crossing)</td>
</tr>
<tr>
<td></td>
<td>$^{13}C$</td>
<td>$p1$ (p2) @ plw1</td>
<td>$-72$ kHz (3.5 $\mu$s), optimize in $hCNXH3D.tcp$: flag ‘–DC90’ (zero crossing)</td>
</tr>
<tr>
<td>$180^\circ$ selective pulses</td>
<td>$^{13}Ca$</td>
<td>$p24^*$ @ spw24</td>
<td>$-350$ us @ shape: ‘Q3.2000’; open shape in ‘shapetool&gt; stdisp&gt; Analysis&gt; Integrate Shape’ and enter: ‘length of p24, ‘180.0’, length of p1’, result tells by how much pldb1 needs to be changed for spdb24</td>
</tr>
<tr>
<td></td>
<td>$^{12}CO$</td>
<td>$p23^*$ @ spw23</td>
<td>$-275$ us @ shape: ‘Q3.2000’; open shape in ‘shapetool&gt; stdisp&gt; Analysis&gt; Integrate Shape’ and enter: ‘length of p23, ‘180.0’, length of p1’, result tells by how much pldb1 needs to be changed for spdb23</td>
</tr>
<tr>
<td>CP transfers</td>
<td>H-N</td>
<td>$p25$ @ spw42 (H) &amp; spw43 (N)</td>
<td>1-3 ms @ 50 kHz &amp; 10 kHz, shapes: ‘ramp90100.1000’ &amp; ‘square.1000’</td>
</tr>
<tr>
<td></td>
<td>H-$Ca$</td>
<td>$p18$ @ spw38 (H) &amp; spw52 (C)</td>
<td>2-6 ms @ 50 kHz &amp; 10 kHz, shapes: ‘ramp90100.1000’ &amp; ‘square.1000’</td>
</tr>
<tr>
<td></td>
<td>H-$CO$</td>
<td>$p19$ @ spw39 (H) &amp; spw53 (C)</td>
<td>2-6 ms @ 50 kHz &amp; 10 kHz, shapes: ‘ramp90100.1000’ &amp; ‘square.1000’</td>
</tr>
<tr>
<td></td>
<td>Ca-N</td>
<td>$p16$ @ spw5 (N) &amp; spw50 (C)</td>
<td>3-10 ms @ 25 kHz &amp; 35 kHz, shapes: ‘tacn80’ &amp; ‘square.1000’</td>
</tr>
<tr>
<td></td>
<td>CO-N</td>
<td>$p17$ @ spw6 (N) &amp; spw51 (C)</td>
<td>3-10 ms @ 25 kHz &amp; 35 kHz, shapes: ‘tacn80’ &amp; ‘square.1000’</td>
</tr>
<tr>
<td></td>
<td>N-H</td>
<td>$p45$ @ spw47 (N) &amp; spw46 (H)</td>
<td>400-800 $\mu$s @ 50 kHz &amp; 10 kHz, shapes: ‘square.1000’ &amp; ‘ramp10090.1000’</td>
</tr>
<tr>
<td>Decoupling</td>
<td>$^1H$</td>
<td>cpdprg1 @ pcpd1 &amp; plw12</td>
<td>‘sltppm_12nofq’ @ 33.33 $\mu$s &amp; ¼ * $\nu_z$ (15 kHz)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>cpdprg4/5 @ d19 &amp; plw13</td>
<td>‘cwX_13nofq’ / ‘cwY_13nofq’ @ 100-300 ms &amp; 15 kHz (=MISSISSIPPI)</td>
</tr>
<tr>
<td></td>
<td>$^{15}N$</td>
<td>cpdprg2 @ pcpd2 &amp; plw16</td>
<td>‘waltz16_18nofq’ @ 25 $\mu$s &amp; 10 kHz</td>
</tr>
<tr>
<td></td>
<td>$^{13}C$</td>
<td>cpdprg3 @ pcpd3 &amp; plw18</td>
<td>‘waltz16_18nofq’ @ 25 $\mu$s &amp; 10 kHz</td>
</tr>
<tr>
<td>Offsets</td>
<td>$^1H$</td>
<td>o1</td>
<td>on resonance with the water peak (~5 ppm)</td>
</tr>
<tr>
<td></td>
<td>$^{15}N$</td>
<td>o2</td>
<td>center of $^{15}N$ signal (~119 ppm)</td>
</tr>
<tr>
<td></td>
<td>$^{13}C$</td>
<td>o3 (hCaNH3D.tcp)</td>
<td>on resonance with Ca region (~54 ppm)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>o3 (hCONH3D.tcp)</td>
<td>on resonance with CO region (~174 ppm)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>cnst21</td>
<td>frequency offset for CO (~174 ppm)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>cnst22</td>
<td>frequency offset for Ca (~54 ppm)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>cnst26</td>
<td>frequency offset for CO-Ca (~114 ppm)</td>
</tr>
</tbody>
</table>

* The pulse length is linearly proportional to 1/$B_0$. 

During proton acquisition, 10 kHz of heteronuclear decoupling is applied on $^{15}N$ and $^{13}C$, using ‘waltz16_18nofq’ and ‘waltz16_18nofq’ at power levels ‘plw16’ and ‘plw18’ respectively.[17] An overview of all necessary parameters, including recommendations, can be found in Table 1.

### Outlook

In collaboration with leading scientists in the field of solid-state NMR spectroscopy, Bruker continues to implement state-of-the-art $^1H$-detected experiments for ultra-fast MAS. Further application notes based on this introductory one will follow in the near future.
References