SOLID STATE $^1$H/$^{15}$N CHEMICAL SHIFT CORRELATION EXPERIMENTS OF ALIGNED SAMPLES AT 900 MHZ

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Polarization inversion spin exchange at the magic angle (PISEMA) (1), which correlates the orientation-dependent, anisotropic $^1$H-$^{15}$N dipole-dipole couplings and $^{15}$N chemical shifts, has become a powerful tool to obtain high-resolution orientational restraints from membrane proteins in a lamellar phase lipid environment. One of the major problems in the structural determination using PISEMA is the multiple solutions associated with the orientational restraints. Generally, a peptide plane has four possible orientations with respect to $B_0$ that fulfill the same $^{15}$N chemical shift and $^{15}$N-$^1$H dipolar coupling. Therefore, the $^1$H anisotropic chemical shift restraint from the peptide plane, which is not observed in the PISEMA spectra, is believed to be an important parameter for structural characterization of aligned membrane proteins.

In this report, we use high fields to obtain high-resolution $^1$H chemical shifts of aligned samples. Fig. 1 shows the anisotropic $^1$H-$^{15}$N chemical shift correlation spectrum of a static $^{15}$N-acetyl-valine (NAV) crystal at 900 MHz recorded with and without $^{15}$N decoupling during $^1$H chemical shift evolution. Two $^{15}$N resonances were observed at this arbitrary orientation due to the existence of two inequivalent molecules per unit cell in the crystal. After considering the scaling factor in the $^1$H chemical shift dimension resulting from $^1$H homonuclear dipolar decoupling (2), the $^1$H linewidth was 1.2 ppm, while it was 2.1 ppm from the same crystal sample at 300 MHz. Without $^{15}$N decoupling, the corresponding $^1$H-$^{15}$N dipolar couplings split each of the resonances into two peaks. Therefore, such experiments allow us to obtain the $^1$H-$^{15}$N dipolar couplings, as in PISEMA experiments (1), and to obtain the additional anisotropic $^1$H chemical shift restraints, without a need to perform time-consuming three-dimensional experiments (3,4) for obtaining the $^{15}$N and $^1$H chemical shifts and their $^{15}$N-$^1$H dipolar coupling. Therefore, with better $^1$H resolution at 900 MHz, it will be possible to introduce $^1$H anisotropic chemical shift restraints into the structural determination to eliminate some of the degeneracies.

Fig. 1. $^{15}$N-$^1$H correlation spectra of a NAV crystal at 900 MHz with (Top) and without (Bottom) $^{15}$N decoupling during the $^1$H chemical shift evolution. The magic sandwich high order truncation (MSHOT) homonuclear decoupling sequence (2) was used in the $t_1$ dimension. A short cross polarization contact time of 120 μs was used to ensure that the $^{15}$N magnetization was transferred from its closest $^1$H. The $^1$H $B_1$ fields were 92 and 50 kHz during the MSHOT decoupling and cross polarization, respectively, while the $^{15}$N $B_1$ field of 50 kHz was used for cross polarization and $^{15}$N decoupling in the $t_1$ dimension. The quadrature detection in the $t_1$ dimension was achieved using the States phase cycling. In the spectra, the scaling factor has been taken into account in the $^1$H chemical shift dimension. The $^1$H chemical shift was referenced to the water signal of $\text{NH}_4\text{NO}_3$ solution at 4.7 ppm.

References