



Two-Dimensional (2D) ^{13}C - ^{13}C Homonuclear Correlation Spectroscopy Employing a Low ^1H rf Mixing Field at an Ultrafast MAS Spinning Rate

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Introduction

As the probe technology has recently undergone revolutionary development in the solid-state NMR spectroscopy, an ultrahigh-speed of magic-angle spinning (MAS) rotation of the sample specimen up to 100 kHz has become possible.¹ Thus, it is possible to significantly increase the resolution of the MAS NMR signals in the solid state because the strength of ^1H - ^1H dipolar couplings can be significantly averaged out and ^1H -X (X = ^{13}C , ^{15}N , etc.) dipolar couplings can be easily removed by employing a low ^1H decoupling power, reducing the rf sample heating effect significantly. In addition, since the ^1H signals can be obtained very narrowly, it is also possible to collect ^1H signals directly by further increasing the signal intensity, while securing one more dimension toward the ^1H channel for obtaining more signal information. Unfortunately, these gains are obtained by sacrificing other beneficial factors: it reduces the strength of ^{13}C - ^{13}C dipolar interactions that provides useful structural information; if measurements are made under a high magnetic field strength, the difference in the chemical shifts between the pair of carbon atoms in the dipolar coupling increases significantly. Thus, all the recoupling methods made under low MAS spinning conditions become ineffective at high MAS spinning rate under an ultrahigh magnetic field strength. A new method was developed that can alleviate these drawbacks by employing an adiabatic pulse scheme during the mixing time, implementing an efficient ^{13}C - ^{13}C dipolar recoupling method under an ultrahigh MAS spinning rate while enlarging the ^{13}C offset frequency profile significantly. This method can also employ an extremely low rf pulse power during the mixing that further reduces rf sample heating effect.

Experimental

The pulse scheme we developed is shown in Figure 1A. Protein samples employed for our experiments include U- ^{13}C labeled CrgA,² Barstar,³ and wild type Transthyretin⁴ that are packed in 3.2 mm (CrgA) and 1.3 mm (Barstar and Transthyretin). Experiments were carried out at a low temperature (-20 ~ -30 °C).

Results and Discussion

Fig. 1B and 1C compares the aliphatic regions of the 2D ^{13}C - ^{13}C correlation spectra obtained on ^{13}C -labeled Barstar by using our method (C) and the CORD⁵ (B) that has been known so far as the best method when a fast MAS rate is used, employing a mixing time of 160 ms and a MAS spinning rate of $\omega_r/2\pi = 40$ kHz. It is clearly visible in our spectrum that the new ^{13}C - ^{13}C dipolar mixing scheme invented in our research provides additional cross peaks among ^{13}C sites that are not visible in the CORD spectrum. The ^1H pulse power employed during the mixing time in our method was mere ~ 13 kHz (CORD uses 40 kHz and 20 kHz of ^1H rf pulse power, respectively, for the 1/3 and 2/3 of the total mixing period). Our method works even at $\omega_r/2\pi = 50$ or 60 kHz but the conventional method, CORD, stops working at $\omega_r/2\pi > 40$ kHz.

Conclusions

The new mixing scheme employing an adiabatic pulse provides better ^{13}C - ^{13}C correlations than CORD at $\omega_r/2\pi = 40$ kHz with a very weak rf pulse power.

Acknowledgements

The NHMFL is supported by NSF/DMR-1157490/1644779 and the State of Florida.

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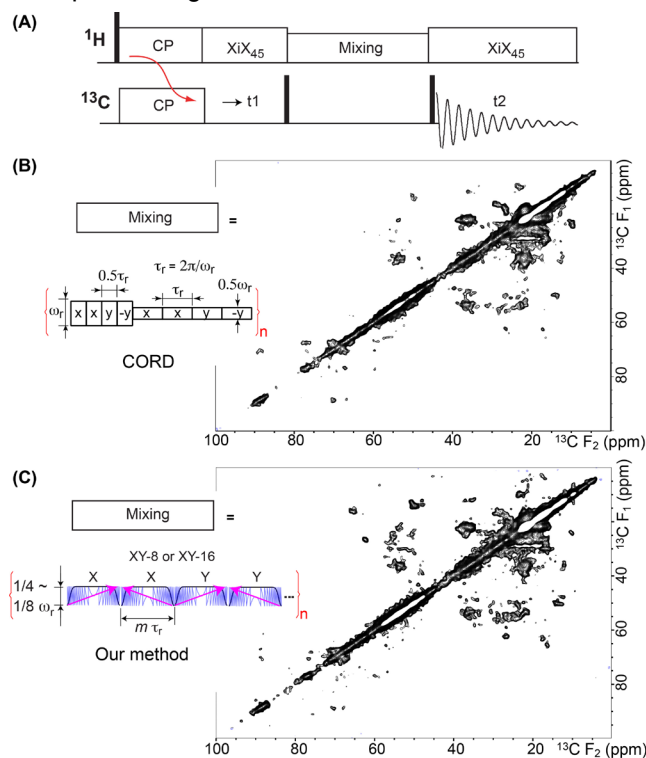


Fig.1 The pulse scheme (A) and 2D ^{13}C - ^{13}C spectra of ^{13}C -labeled Barstar at $\omega_r/2\pi = 40$ kHz by using CORD (B) and our method (C) at a mixing time of 160 ms.