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In Situ Temperature Jump Dynamic Nuclear Polarization:

Enhanced Sensitivity in Two Dimensional ^{13}C-^{13}C Correlation Spectroscopy in Solution

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Abstract

Dynamic nuclear polarization is combined with temperature jump methods to develop a new 2D ^{13}C- ^{13}C NMR experiment that yields a factor or 100-170 increase insensitivity. The polaization step is performed at ~100 K and the sample is subsequently melted with a 10.6 mm laser pulse to yield a sample with highly polarized ^{13}C spins. ^{13}C detected 2D ^{13}C- ^{13}C spectroscopy is performed in the usual manner.

Over the last decade there has been a resurgence of interest in the application of dynamic nuclear polarization (DNP) techniques directed at increasing the sensitivity in nuclear magnetic resonance (NMR) experiments [1-7]. The interest is driven by the possibility of enhancing signal intensities in NMR spectra by factors of 10^2 - 10^3 which historical precedent suggests will open many new avenues of research. The experiments were initially directed at signal enhancement in magic angle spinning (MAS) spectra of solids[4, 8-15], and, since they were performed at magnetic fields ≥ 5T, they required development of gyrotron microwave sources operating in the range ≥140 GHz for irradiation of the EPR spectrum of the exogenous paramagnetic polarizing agent [4, 13, 16, 17]. There is also considerable interest in applying these techniques to high field solution-state NMR experiments, and, recently there have been three efforts aimed at achieving this goal. The first relied on scalar relaxation, a mechanism that is operative at high fields, but is, unfortunately, only applicable in special cases [18]. The second is the “dissolution experiment” where the solid sample is polarized at low field, dissolved with excess superheated solvent, and then transferred to a second spectrometer for observation of the NMR spectrum [19, 20]. Dissolution DNP was recently integrated with the single scan multi-dimensional NMR technique to obtain two-dimensional (2D) ^{15}N-^{1}H correlation spectra [21]. In the third approach, we demonstrated the possibility of performing the polarization and spectroscopy in the same spectrometer, the latter being achieved by melting the sample with CO_2 laser irradiation [5]. Our approach has the advantage of circumventing the polarization loss associated with shuttling the sample to another spectrometer. Thus, we refer to this experiment as *in situ* temperature jump DNP (TJ-DNP). Further, and in contrast to the dissolution experiment, the TJ-DNP experiment can be recycled and is therefore suitable for the acquisition of multiple scans of comparable intensity. Here we demonstrate that it is possible to incorporate the extensive repertoire of multidimensional solution-state NMR experiments into this scheme.

Figure 1 shows the ^{13}C NMR spectrum of an 800 mM [$^{13}C_6$,$^2H_7$,]-glucose solution obtained with and without TJ-DNP. The enhancement, $\varepsilon^\dagger$ is ~ 170 for C_1, ~140 for C_{2-5}, and ~100 for C_6. We are exploring approaches to make the intensities more quantitative, for example, by improving the rate of melting. Note also that $\varepsilon^\dagger=\varepsilon(T_{obs}/T_{\infty wave})$ includes a factor accounting for the increased Boltzmann population due to the lower temperature, where ε is the enhancement at temperature where polarization occurs, $T_{\infty wave}$ (in this case 100 K), and T_{obs} is the temperature where the liquid state spectra are recorded [5]. Incorporation of the TJ-DNP experiment into a multidimensional solution experiment is illustrated in Figure 2, and is accomplished by inserting an evolution and mixing period into the sequence following the melting step. In the case depicted here, we employ TJ-DNP together with a TOCSY mixing sequence to record a ^{13}C-^{13}C correlation spectrum [23]. The resulting 2D spectrum is shown in Figure 3, and immediately obvious is the fact that the presence of the paramagnetic polarizing agent does not lead to severe broadening of the NMR signals. This is best seen in the expansion of the $^{13}C_1$ region that clearly shows the one-bond ^{13}C-^{13}C scalar coupling constants present in the α and β forms of glucose.

The direct detection of ^{13}C in solution offers a valuable alternative to indirect detection via ^1H, provided that the sensitivity of ^{13}C can be enhanced, as demonstrated here. ^{13}C detected experiments dramatically shorten the duration of the pulse sequence when compared to their corresponding ^1H detected counterparts, thereby reducing losses due to transverse relaxation. ^1H solvent suppression is also unnecessary. ^{13}C detected experiments are particularly beneficial for the study of paramagnetic samples because the paramagnetic contributions to relaxation are reduced by a factor of 10 compared to ^1H. ^{13}C detected experiments also facilitate the investigation of spectral regions where ^1H detection may be difficult such as those undergoing chemical or conformational exchange. Finally, as illustrated here, ^{13}C detected experiments also enable measurements in completely deuterated samples. In total, these advantages of direct ^{13}C detection should enable new experiments for studies of larger bio-molecular systems. Other groups have appreciated these features in recent years and as a consequence there has been considerable effort devoted to developing experiments that utilize direct ^{13}C detection [24-26]. The sensitivity gain available from TJ-DNP could rapidly accelerate or improve the realization of many of these advantages.

Despite the dramatic increase in sensitivity reported here we anticipate that the experimental protocol could be improved in several respects. First, the temperature at which the polarization is performed could be lowered from 100 K to 10 K, yielding another factor of ≥10 in signal intensity as the Boltzmann factor and the polarization enhancements from DNP would both be larger. Second, we are currently using quartz rotors and most of the heat from the laser pulse is expended in bringing the rotor from 100 K to 300 K. Alternative rotor materials may allow faster and more uniform melting, reducing losses due to relaxation, and improving the line shape. Finally, it would be beneficial to move the experiments to a higher operating frequency where contemporary solution NMR experiments are currently performed. We anticipate that 2D TJDNP will be widely applicable to NMR experiments involving small molecules, for example in metabolomics, and perhaps to some proteins.

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References

1. Wind RA, Duijvestijn MJ, Vanderlugt C, Manenschijn A, Vriend J. Progress in Nuclear Magnetic Resonance Spectroscopy 1985;17:33–67.
2. Afeworki M, Mckay RA, Schaefer J. Macromolecules 1992;25(16):4084–4091.
3. Afeworki M, Schaefer J. Macromolecules 1992;25:4092–4096.
4. Becerra LR, Gerfen GJ, Temkin RJ, Singel DJ, Griffin RG. Physical Review Letters 1993;71(21): 3561–3564. [PubMed: 10055008]
5. Joo C-G, Hu K-N, Bryant JA, Griffin RG. J. Am Chem. Soc 2006;128:9428–9432. [PubMed: 16848479]
6. Maly T, Debelouchina GT, Bajaj VS, Hu K-N, Joo C-G, Mak-Jurkauskas ML, Sirigiri JR, Wel P. C. A. v. d. Herzfeld J, Temkin RJ, Griffin RG. J. Chem Physics 2008;128:052211.

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7. van der Wel PCA, Hu K-N, Lewandoswki J, Griffin RG. J. Am Chem. Soc 2006;128:10840–10846. [PubMed: 16910679]
8. Hall DA, Maus DC, Gerfen GJ, Inati SJ, Becerra LR, Dahlquist FW, Griffin RG. Science 1997;276:930–932. [PubMed: 9139651]
9. Rosay M, Lansing JC, Haddad KC, Bachovchin WW, Herzfeld J, Temkin RJ, Griffin RG. J. Am. Chem. Soc 2003;125:13626–27. [PubMed: 14599177]
10. Rosay M, Weis V, Kreischer KE, Temkin RJ, Griffin RG. Journal of the American Chemical Society 2002;124(13):3214–3215. [PubMed: 11916398]
11. Bajaj VS, Farrar CT, Hornstein MK, Mastovsky I, Vieregg J, Bryant J, Elena B, Kreischer KE, Temkin RJ, Griffin RG. J. Mag. Res 2003;160:85–90.
12. Hu KN, Yu HH, Swager TM, Griffin RG. Journal of the American Chemical Society 2004;126(35):10844–10845. [PubMed: 15339160]
13. Bajaj VS, Hornstein MK, Kreischer KE, Sirigiri JR, Woskov PP, Mak-Jurkauskas ML, Herzfeld J, Temkin RJ, Griffin RG. Journal of Magnetic Resonance 2007;189(2):251–279. [PubMed: 17942352]
14. Hu K-N, Bajaj VS, Rosay MM, Griffin RG. J. Chem. Phys 2007;126:044512. [PubMed: 17286492]
15. Hu K-N, Song C, Yu H.-h. Swager TM, Griffin RG. J. Chem. Phys 2008;128:052321. [PubMed: 18266438]
16. Becerra LR, Gerfen GJ, Bellew BF, Bryant JA, Hall DA, Inati SJ, Weber RT, Un S, Prisner TF, McDermott AE, Fishbein KW, Kreischer KE, Temkin RJ, Singel DJ, Griffin RG. Journal of Magnetic Resonance Series A 1995;117(1):28–40.
17. Joye CD, Griffin RG, Hornstein MK, Hu K-N, Kreischer KE, Rosay M, Shapiro MA, Sirigiri JR, Temkin RJ, Woskov PP. IEEE Transactions on Plasma Science 2006;34:518–523. [PubMed: 17431442]
18. Loening NM, Rosay M, Weis V, Griffin RG. Journal of the American Chemical Society 2002;124(30):8808–8809. [PubMed: 12137529]
19. Ardenkjaer-Larsen JH, Fridlund B, Gram A, Hansson G, Hansson L, Lerche MH, Servin R, Thaning M, Golman K. Proc. of the Natl Acad. of Sci. of 2003;100(18):10158–10163.
20. Wolber J, Ellner F, Fridlund B, Gram A, Johannesson H, Hansson G, Hansson LH, Lerche MH, Mansson S, Servin R, Thaning M, Golman K, Ardenkjaer-Larsen JH. Nuclear Instruments & Methods in Physics Research Section a-Accelerators Spectrometers Detectors and Associated Equipment 2004;526(12):173–181.
21. Frydman L, Blazina D. Nature Physics 2007;3(6):415–419.
22. Song C, Hu K-N, Swager TM, Griffin RG. J. Am Chem. Soc 2006;128:11385–90. [PubMed: 16939261]
23. Eletsky A, Moreira O, Kovacs H, Pervushin K. Journal of Biomolecular Nmr 2003;26(2):167–179. [PubMed: 12766412]
24. Lee D, Vogeli B, Pervushin K. Journal of Biomolecular Nmr 2005;31(4):273–278. [PubMed: 15928994]
25. Bermel W, Bertini I, Felli IC, Matzapetakis M, Pierattelli R, Theli EC, Turano P. Journal of Magnetic Resonance 2007;188(2):301–310. [PubMed: 17719814]
26. Bermel W, Bertini I, Felli IC, Piccioli M, Pierattelli R. Progress in Nuclear Magnetic Resonance Spectroscopy 2006;48(1):25–45.

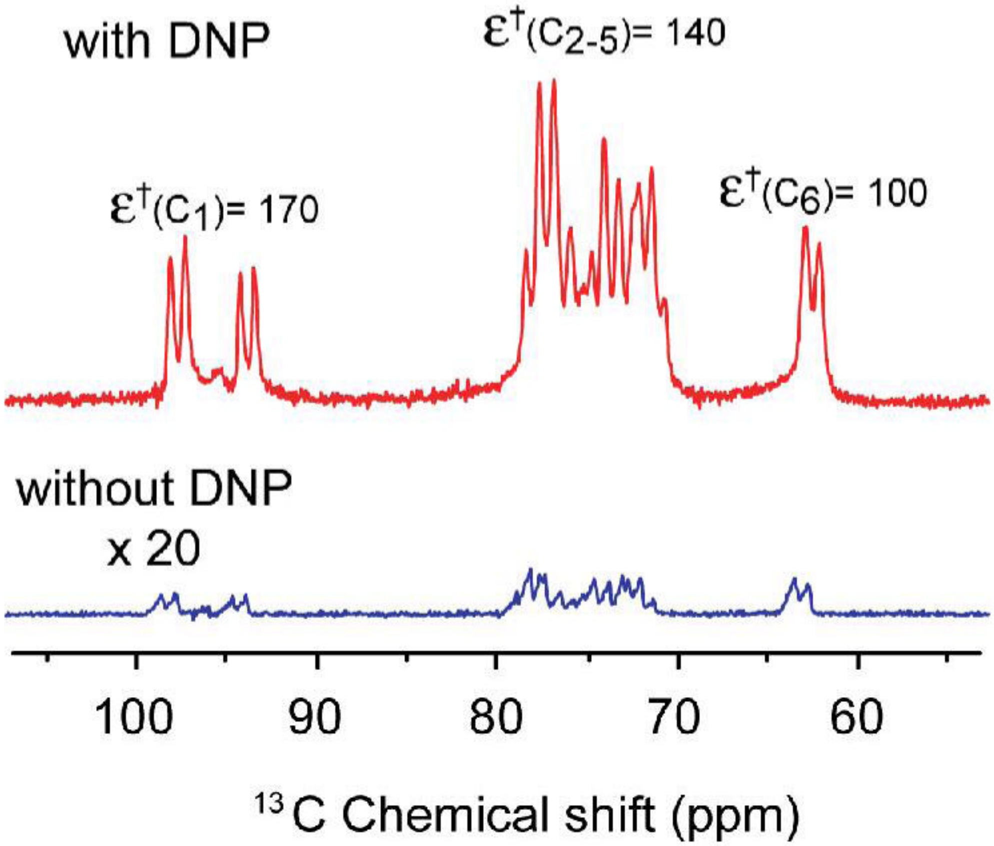

Figure 1.

^{13}C NMR spectra of 800 mM $^2H_7$, $^{13}C_6$ glucose solution prepared with $^2H_6$-DMSO/^2H_2O/ H_2O = 5/4/1 and 5 mM TOTAPOL, with (*top*) and without (*bottom*) TJ-DNP. For TJ-DNP, 140 GHz microwave irradiation was applied for 30 s at 100 K. A 0.8 s CO_2 laser pulse was used to melt the sample. The room temperature spectrum without DNP (bottom) was scaled up by 20 for ease of comparison. The enhancement was ~ 170 for C_1 (both α and β forms), ~140 for $C_{2\text{-}5}$, and ~100 for C_6. TJDNP spectrum was recorded with a single scan, and the room temperature spectrum without DNP is the result of 2048 scans. The polarizing agent TOTAPOL [1-(2,2,6,6,-Tetramethyl-1-oxyl-4-piperidinyl)oxy-3-(2,2,6,6-tetramethyl-1-oxy-4-oioeridinyl)amino-propan-2-ol] [22]] supports the cross effect mechanism [12, 14, 15]. The concentration of TOTAPOL was 5 mM. All experiments were conducted on a 5 T (211 MHz for ^1H, 53.31 MHz for ^{13}C, and 32 MHz for ^2H) magnet with a home built multi-channel DNP NMR probe. An 8 - 10 ∞L sample was placed in a 2.5 mm o.d. quartz rotor. The temperature was maintained below 100 K by circulating cold N_2 gas. Samples were spun at 200-800 Hz to rotationally average the unidirectional microwave and laser irradiation. 140 GHz microwaves were produced by a gyrotron. For a sample with 5 mM TOTAPOL, the enhanced signal is typically saturated after 30 s of microwave irradiation. The temperature jump was achieved by irradiation of 10.6 ∞m light from a 10 W CO_2 laser through a silver coated silica hollow waveguide.

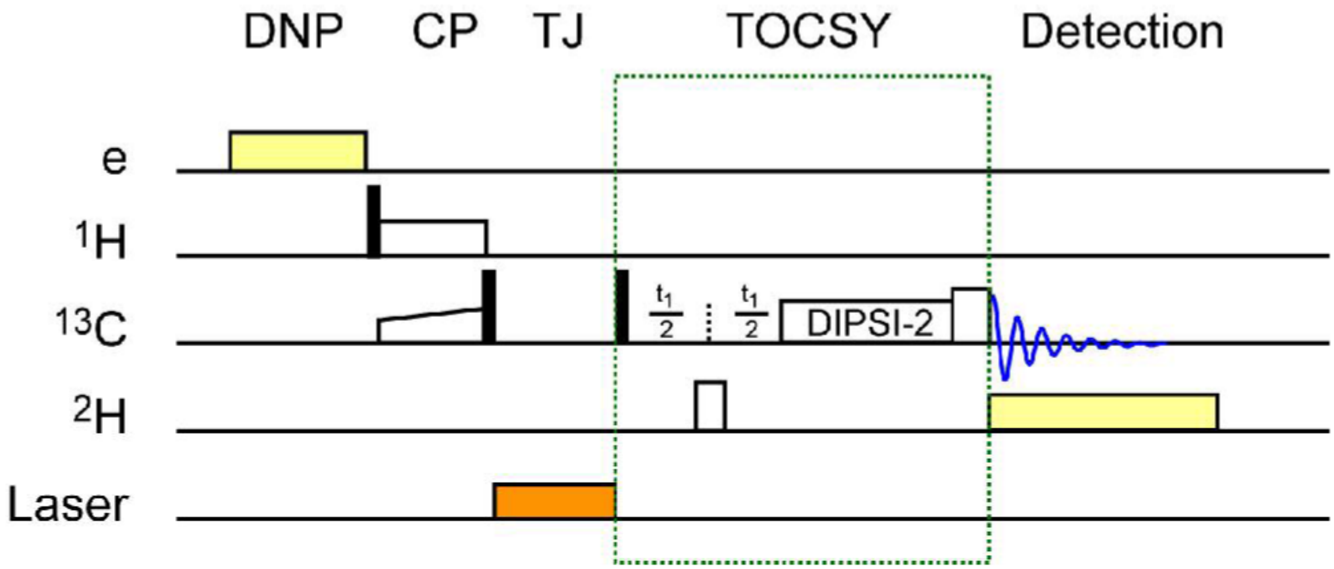

Figure 2.
Experimental scheme for 2D TJ-DNP experiment. The sample was irradiated with 140 GHz microwaves at 100 K, followed by ^{1}H-^{13}C cross polarization. The magnetization on ^{13}C was stored along the z-axis to minimize polarization loss by relaxation during melting. Immediately following melting, a 2D NMR pulse sequence was initiated. For TOCSY, a 180° ^{2}H pulse was applied in the middle of t_1 evolution for decoupling. DIPSI-2 spin locking was on for 4.5 ms followed by 1 ms trim pulse to reduce anti-phase line shape components. The ^{13}C signal was detected with WALTZ ^{2}H decoupling.

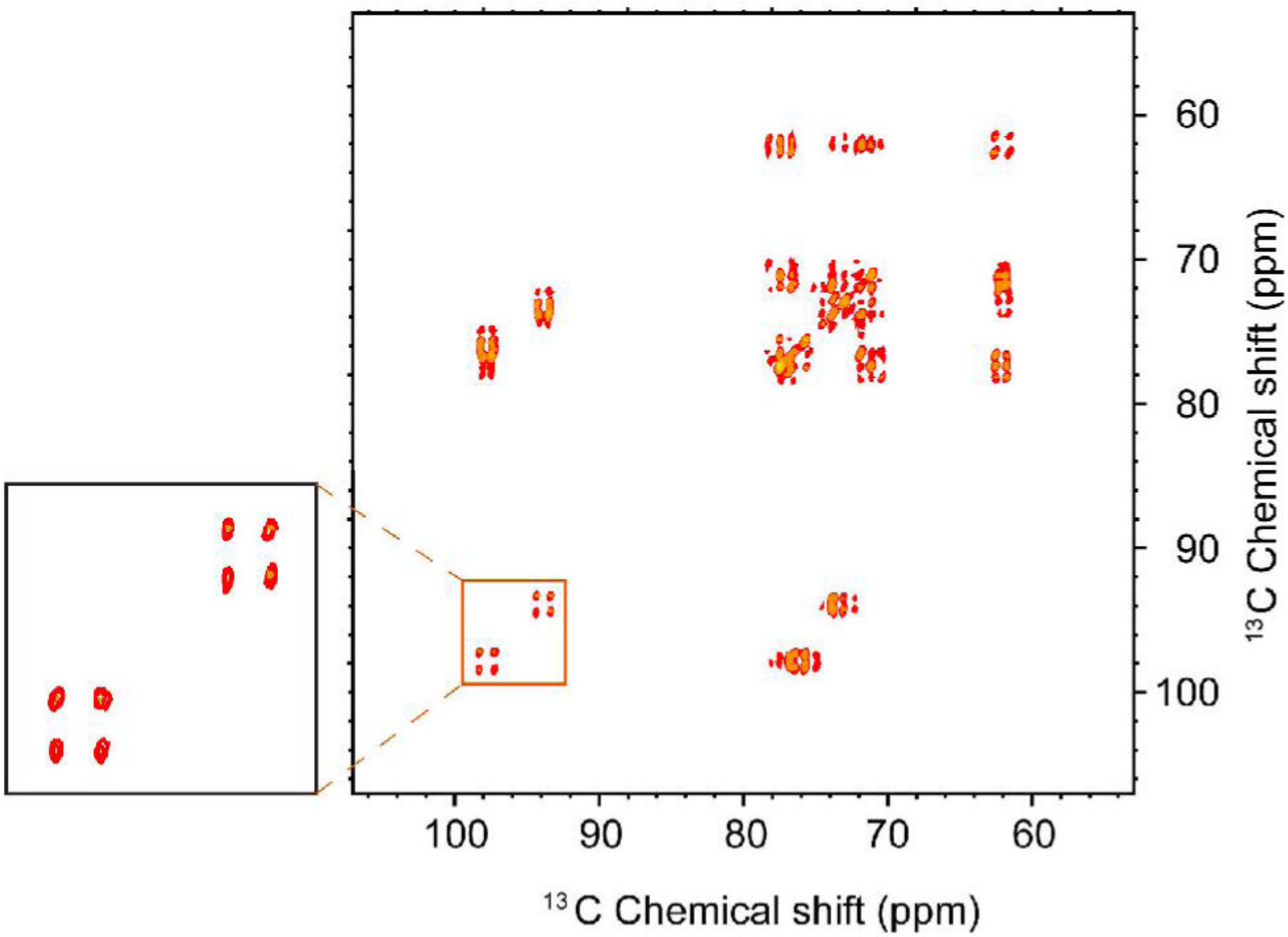

Figure 3.
^{13}C-^{13}C TOCSY NMR spectrum of [$^{2}H_{7}$, $^{13}C_{6}$]-glucose solution, with DNP (10 s)-TJ (0.8 s) and 40 s recycle delay. 1 transient of 1024 data points was accumulated for 96 t_1 increments. 4.5 ms of DIPSI-2 mixing with $\omega_1/2\pi$ = 29 kHz was used for TOCSY. The 2D data matrix was acquired with 2 * 96 * 1024 points, with a single transient per data file and a recycle time of 40 s required by the refreezing. ^{2}H decoupling was applied in both dimensions.