Appl Magn Reson (2012) 43:81–90
DOI 10.1007/s00723-012-0357-2

Applied
Magnetic Resonance

High-Temperature Dynamic Nuclear Polarization Enhanced Magic-Angle-Spinning NMR

Ümit Akbey · Arne H. Linden · Hartmut Oschkinat

Received: 6 February 2012 / Revised: 3 May 2012 / Published online: 23 May 2012

Abstract Dynamic nuclear polarization (DNP) transfers electron spin-polarization to nuclear spins in close proximity, increasing sensitivity by two-to-three orders of magnitude. This enables nuclear magnetic resonance (NMR) experiments on samples with low concentrations of analyte. The requirement of using cryogenic temperatures in DNP-enhanced solid-state NMR (ssNMR) experiments may impair the resolution and hence limit its broad application to biological systems. In this work, we introduce a "High-Temperature DNP" approach, which aims at increasing spectral resolution by performing experiments at temperatures of around 180 K instead of ~100 K. By utilizing the extraordinary enhancements obtained on deuterated proteins, still sufficiently large DNP enhancements of 11–18 are obtained for proton and carbon, respectively. We recorded high sensitivity 2D ^{13}C–^{13}C spectra in ~9 min with higher resolution than at 100 K, which has similar resolution to the one obtained at room temperature for some favorable residues.

1 Introduction

Sensitivity is an important issue when applying solid-state nuclear magnetic resonance (ssNMR) to biological systems such as membrane proteins and ligand-receptor complexes [1], for example. Dynamic nuclear polarization (DNP) increases the

Electronic supplementary material The online version of this article (doi:10.1007/s00723-012-0357-2) contains supplementary material, which is available to authorized users.

Ü. Akbey (✉) · A. H. Linden · H. Oschkinat (✉)
NMR Supported Structural Biology, Leibniz-Institut für Molekulare Pharmakologie (FMP), Robert Roessle Str. 10, 13125 Berlin, Germany
e-mail: akbey@fmp-berlin.de

H. Oschkinat
e-mail: oschkinat@fmp-berlin.de

sensitivity of nuclear magnetic resonance (NMR) spectroscopy by two-to-three orders of magnitude [2–4]. Applications to biological systems [5–10], polymers and materials [11–14], as well as experiments with contrast agents, such as pyruvate in medical imaging studies were reported recently [15–17]. In the last decade, DNP has been applied at magnetic fields >5 T with the aim of combining sensitivity and resolution [18, 19]. DNP relies on the transfer of the very large electron spin-polarization to nuclear spins ($\gamma_e/\gamma_{1H} \approx 660$) by irradiation of the electron transitions. Organic radicals are used as electron sources (mostly TOTAPOL at an optimized concentration) [20, 21] and a gyrotron as a high-power microwave (MW) source [22]. Furthermore, cryogenic temperatures are required for slowing relaxation processes. However, care needs to be taken to prevent resolution losses at cryogenic temperatures when investigating biological systems [23]. Even though we have demonstrated that for a specially prepared membrane receptor system high resolution can be recovered [7], a more general technique for achieving DNP enhancements while preserving resolution is of particular importance. As a possible solution, temperature variations have been exploited for this purpose [24, 25]. A temperature-jump solid-solution transition approach has been presented to increase the resolution by polarizing the sample at low temperatures ($\sim$90 K), followed by a fast laser-melting and detection at ambient temperatures. High-resolution one-dimensional (1-D) and 2-D NMR spectra were successfully obtained on glucose; however, application of rapid cooling and heating to proteins can be problematic due to sample degradation.

Here, we present an approach for increasing resolution while preserving DNP enhancements by choosing intermediate temperatures ($\sim$180 K) and using deuterated proteins [26, 27]. It is based on the fact that DNP enhancements can be increased up to a factor of $\sim$4 (for ^{1}H, $\varepsilon \sim 120$) and $\sim$19 (for ^{13}C, $\varepsilon \sim 148$) when deuterated proteins are employed in comparison to measurements on protonated proteins under similar experimental conditions [8]. It allows raising the DNP operation temperature into a range where inhomogeneous broadening is not too severe while preserving sufficient enhancement, both combined to achieve DNP at sufficient resolution.

2 Results and Discussion

To demonstrate the feasibility of DNP experiments in ssNMR at higher temperatures, we have recorded 1D ^{13}C and 2D ^{13}C–^{13}C MAS NMR spectra from $\sim$100 K up to $\sim$200 K using an [u-^{2}H,^{13}C,^{15}N] SH3 sample with 50 % proton content at the exchangeable sites, and an [u-^{13}C,^{15}N] SH3 sample. The 2D spectra of the deuterated sample were recorded in less than 9 min. We have monitored the enhancements for both ^{1}H-DNP and ^{13}C-DNP (Fig. 1a–c). The resolution in ^{13}C–^{13}C correlation spectra are compared for different temperatures and samples (protonated and deuterated; Figs. 2 and 3). The DNP samples were prepared by using a glass-forming water/glycerol (40/60 v/v %) mixture with a controlled proton/deuterium ratio ($\sim$10 and $\sim$50 % for protonated and deuterated proteins, respectively), which is used as a cryo-protectant to ensure homogeneity at low temperatures at low temperatures [28] and by using 20 mM TOTAPOL.

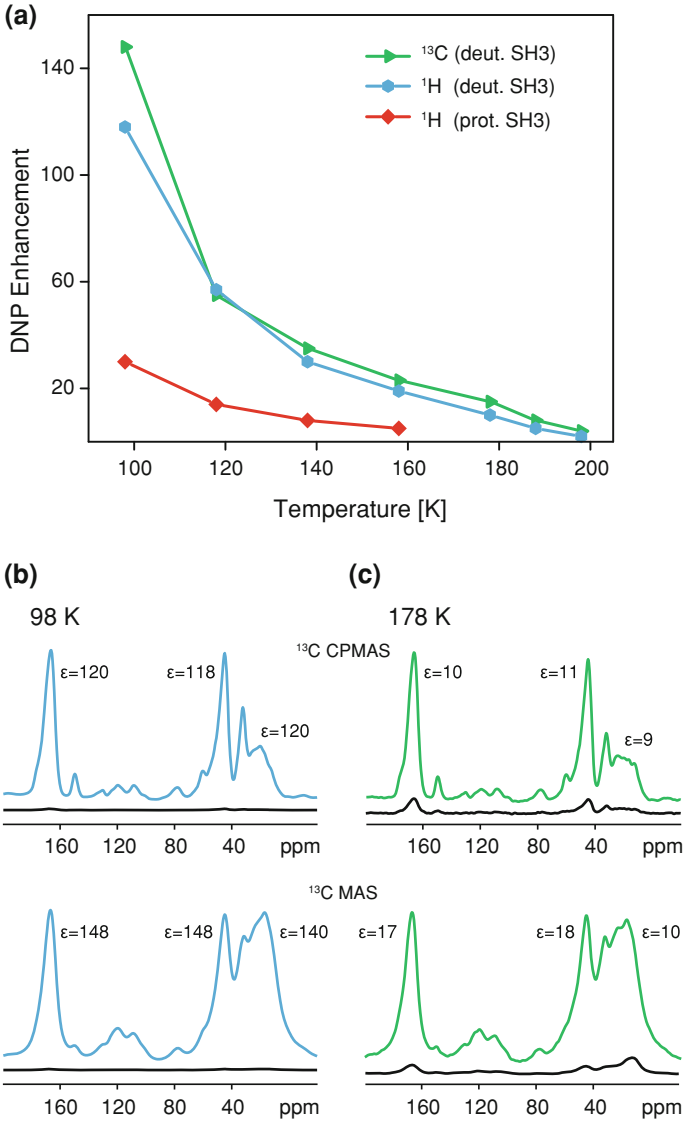

Fig. 1 **a** Dependence of the ^{1}H and ^{13}C enhancements on the experimental temperature for protonated and deuterated SH3 samples. **b** The 1D ^{13}C MAS and ^{1}H–^{13}C CPMAS spectra of the deuterated sample with and without MW irradiation at 98 K (*blue*) and **c** at 178 K (*green*). The enhancements are indicated at the measured resonance. The spectra were recorded at 400 MHz ^{1}H and 263 GHz electron Larmor frequency by using 8,889 Hz MAS and 20 mM TOTAPOL radical. All spectra were recorded with 32 scans, and plotted by fitting the MW-on spectra to the window size in **b**, **c**. The intensity ratios of the MW-on spectra to the MW-off ones are always kept. 2 and 5 s of recycle delays were used for the CP and direct-excitation NMR experiments, respectively (color figure online)

The ^{1}H and ^{13}C DNP enhancements determined from the 1-D spectra are decreasing rapidly with increasing temperatures. An increase of 20 K results in a 30–40 % decrease of the observed DNP enhancement. Hence, nearly no DNP

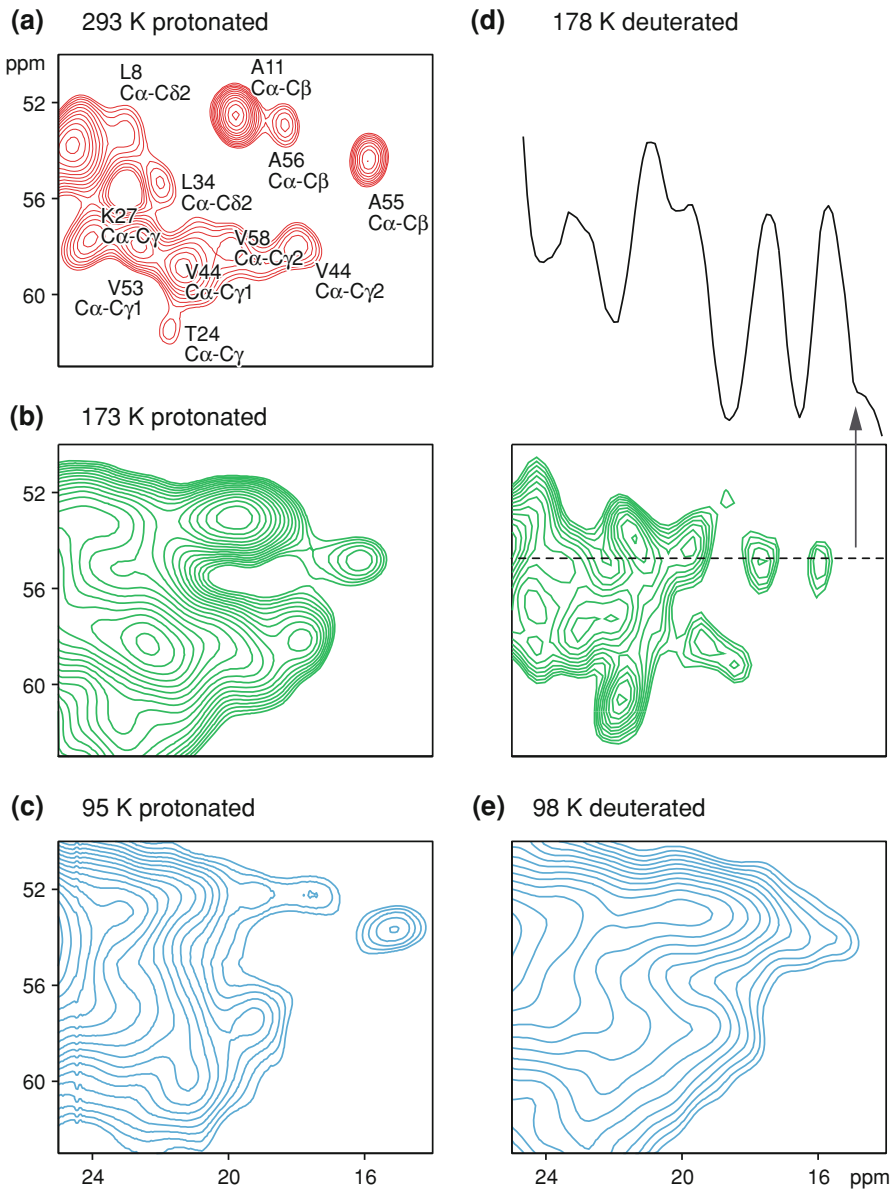

Fig. 2 2-D ^{13}C–^{13}C spectra of the protonated (**a**–**c**) and deuterated SH3 sample (**d, e**) at 293 K (*red*), 173 K (*green*), and 95 K at the alanine C_α–C_β region. A 1-D slice out of the 2-D spectrum at the indicated position (*dotted line*) is shown in **d**, additionally. The spectra of the deuterated sample were recorded under MW irradiation using DONER mixing [31] with 50 ms mixing time and 20 mM TOTAPOL. For the protonated samples no radical or glycerol was used and the spectra were recorded at 293 and 173 K without MW by using a PDSD mixing period of 10 ms. The 95 and 98 K spectra were recorded with 1 scan by using 256 increments for the indirect dimension (∼5 ms), the 178 K spectrum of the deuterated samples was recorded with 4 scans by using 128 increments (∼2.5 ms), and the 173 K spectrum of the protonated sample was recorded with 8 scans and by using 512 increments (∼5 ms, due to smaller increments). All spectra were recorded at 8,889 Hz MAS and processed with a Gauss–Lorentz function (broadening of 20 Hz, Gauss maximum of 0.08) in the direct dimension and a mixed sine/cosine function (phase shift of 2/π) in the indirect dimension (color figure online)

enhancement remains for protonated samples at ~150 K and for deuterated samples at ~200 K, due to unfavorably increased relaxation times of the respective nuclei and electrons. The 1-D ^{13}C spectra recorded at 178 K show proton and carbon DNP enhancements up to ~11 and 18, respectively. These DNP enhancements are still significant and correspond to a remarkable ~120–300 fold decrease in experiment time, even though a reduced CP efficiency is expectred when using the deuterated proteins [8, 29]. The chosen temperature is still below the "glass transition" of proteins, which occurs around 200–240 K [30]. Since we did not observe a sudden change in the spectra at any temperature and the hydration shell was described to freeze at around 170 K [31], we assume the increased mobility of side chains to be the reason for the better resolution we observed at 178 K. The 2-D spectra recorded at 98 and 178 K for the protonated and deuterated sample are shown in Figs. 2, 3. The 275 K spectrum of the protonated SH3 and its comparison to the 178 K spectrum are shown in Figure SI 1, 2.

The 2-D ^{13}C–^{13}C spectra of the protonated and deuterated samples recorded at 95/98 K (Figs. 2c, e and 3c, e) shows very broad resonances, exemplified by the alanine C_α–C_β cross-peaks, which makes the assignment very difficult. In addition, several expected cross-peaks do not appear [23]. According to our high-temperature approach, we recorded 2-D spectra at 173 and 178 K (Figs. 2b–d and 3b–d) and much better resolution is obtained. Their appearance is more similar to the room temperature spectrum. The resolution in the 2-D spectrum of the deuterated protein at 178 K is comparable to the room temperature spectrum, when comparing alanine and threonine residues. However, some other resonances appear with broader linewidth compared to the spectrum recorded at room temperature, see Fig. 2a, d. Inhomogeneous broadening is not severe at this intermediate temperature of 178 K. Summarizing the content of the spectra, we can categorize three types of resonances in the spectrum recorded at 178 K: (i) the resonances which appear at similar chemical shifts as in the room temperature spectrum, such as T24 C_α–C_γ and A55 C_α–C_β (Fig. 2d). (ii) the resonances which are not observed in the spectrum, such as T32 and T37 C_α–C_γ (Figure SI2). (iii) the resonances which are present but slightly shifted in the spectrum, such as A11 and A56 C_α–C_β (Fig. 2). Unfavorable dynamics of certain carbon sites results in disappearing cross-peaks. Some of these missing peaks in 2-D spectra might be re-obtained at lower temperatures, however, as split or broadened ones. The observed chemical shift changes with temperature occur most likely due to small structural changes or as a result of differential dynamics of protein, solvent and hydration shells. To clarify this, we compared the 278 K 2D spectrum of the protonated samples with the 178 and 98 K ones of the deuterated sample.

The alanine and threonine C_α–C_β cross-peak regions of the 2-D ^{13}C–^{13}C spectra of protonated and deuterated SH3 recorded at different temperatures are shown in Figs. 2, 3. Due to different sample nature (protonation/deuteration), different behaviors are observed with temperature changes. Most spectral differences between protonated and deuterated SH3 at lower temperature are due to different dynamics of their respective residues, and are systematic chemical shift changes. For example, the three threonine signals (T24, T32 and T37) are always detectable with their C_β–CO, C_β–C_α and C_β–C_γ cross-peaks for the protonated sample, even

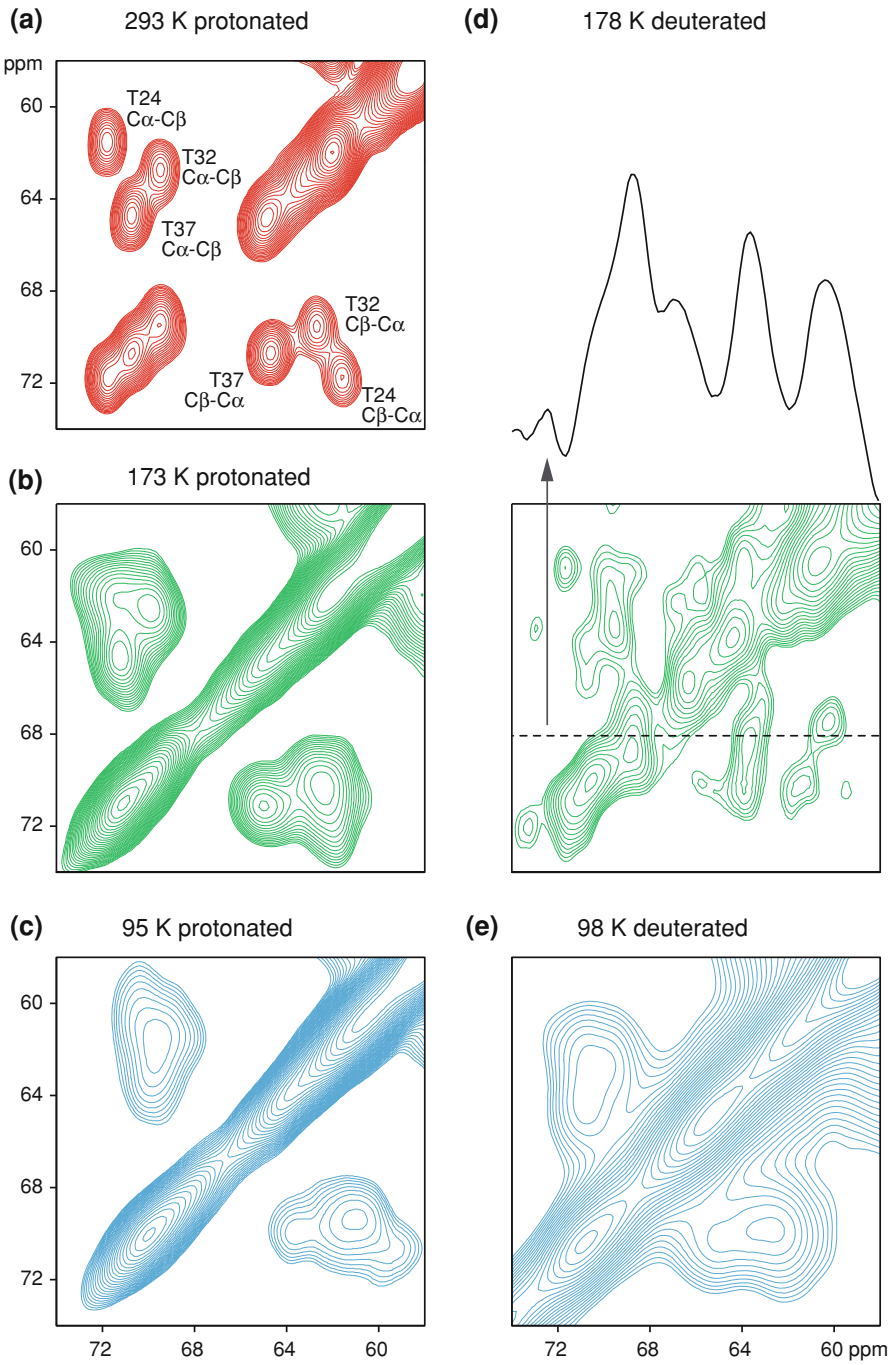

Fig. 3 2-D ^{13}C–^{13}C spectra of the protonated (**a**–**c**) and deuterated SH3 sample (**d, e**) at 293 K (*red*), 173 K (*green*), and 95 K at the threonine C_β–C_α region. A 1-D slice out of the 2-D spectrum at the indicated position (*dotted line*) is shown in **d**, additionally. The spectra were recorded and processed as in Fig. 2

though their C_β–C_γ cross-peaks are unresolved below 213 K. On the other hand, the C_α–C_γ peak of the T24 is well resolved at 178 K for the deuterated sample, which is again unresolved at 98 K.

The alanine cross-peaks nicely show the favorable effects of the high-temperature DNP and the deuteration. The cross-peaks from at least two alanine residues are still resolved at 178 K for the deuterated sample, while in the protonated sample A11 and A56 merge into one peak below 253 K. Additionally, the splitting into several peaks observed at 98 K for each protonated residue can be avoided by the use of higher temperature. Resolved signals can be observed for the deuterated SH3 especially at 178 K.

The dynamics of a specific residue and of the hydration-shell molecules changes individually with temperature and between protonated and deuterated samples. This may lead to the observation of different chemical shift values at different temperatures and peak-splitting at low temperatures as observed in previous studies [23]. Additionally, the protonated sample was prepared by using water as solvent; however, the deuterated sample for DNP was prepared by using a water–glycerol mixture, which can as well cause observed differences in the linewidths of the two samples.

To be able to compare the resolution more quantitatively, we analyzed the apparent linewidths of T37 C_β–C_α and A55 C_α–C_β cross-peaks (Table 1) obtained for different samples and at different temperatures. For the room temperature spectrum of protonated SH3 a different processing was performed to express better linewidth and T_2^*, and the resulting narrowing linewidths are given in parenthesis in Table 1.

At room temperature, the protonated SH3 sample shows reasonable linewidths of $\sim$1.1–1.3 ppm with the described processing parameters. At 98 K, the

Table 1 The DNP enhancement values and observed linewidths in protonated and deuterated SH3 proteins for different nuclei and at different temperatures

Sample and temperature	ε (^{1}H)	ε (^{13}C)	FWHM (ppm)	
			T37 C_β–C_α	A55 C_α–C_β
98 K (Protonated, No-DNP)	–	–	4.6	3.2
98 K (Deuterated)	120	148	4.2	>4–5
173 K (Protonated, No-DNP)	–	–	3.4	3.2
178 K (Deuterated)	9–11	10–18	2.8	1.2
278 K (Protonated, No-DNP)	–	–	1.3 (0.6)	1.1 (0.7)

The data are recorded at 400 MHz ^{1}H Larmor frequency, 98, 178 and 278 K of sample temperature and by using 3.2 mm zirconium-oxide NMR rotor with $\sim$9 kHz of MAS. The values for 98 and 178 K are recorded by using samples, which are in Glycerol–H_2O–D_2O solvent, with 20 mM of TOTAPOL radical, and the data for the 278 K is recorded by using a protonated SH3 protein without cryo-protectant and radical. The linewidths are obtained from the 2D spectra, which were recorded with 10 and 5 ms in the direct and indirect dimensions. The direct dimensions were processed with a Gauss–Lorentz function with a broadening of 20 Hz and a Gauss maximum of 0.08, whereas the indirect dimensions were processed with a mixed sine/cosine function with a phase shift of $\pi/2$. The linewidth values in the parenthesis represent the values obtained, with acquisition times of 25 and 10 ms for the direct and indirect dimensions without any line broadening

resonances are broadened up to 4–5 ppm for both protonated and deuterated samples and hence resolvable only in rare cases. In contrast, at 178 and 173 K the signals are narrower, and carbon linewidths of 1.2–2.8 and 3.2–3.4 ppm were obtained for deuterated and protonated samples, respectively. For some of the residues such as A55, the linewidth obtained at $\sim$178 K becomes comparable to the room temperature situation (see C_α–C_β cross-peak), keeping in mind that the lines appear broad since the spectra were processed such that S/N is enhanced. This proves the current approach's feasibility and importance by showing that the resonances, which are unresolved at 98 K can be resolved with a spectral quality similar to room temperature, which will especially be attractive for 3-D NMR techniques. Even though the data represents the feasibility of the approach, accumulation of more transients and more indirect data points for the 2-D spectrum recorded at 178 K could have allowed us to process the data in such a way that narrower linewidhts might be observed. The experimental success can be appreciated more when thinking that the DNP-enhanced 2-D spectrum of deuterated SH3 recorded at 178 K was obtained only in less than 9 min with 4 scans and 256 increments in the indirect dimension. 3D experiments may thus be recorded in reasonable experimental times at 178 K via DNP with sufficient resolution to facilitate unambiguous assignments of biological systems.

The storage of the biological DNP samples for long times might result in spectral changes. We observed such a behavior for our microcrystalline deuterated SH3 sample, which was stored at -20 °C for more than a year in the course of the measurement period (Figure SI2). The spectra recorded with a fresh sample resulted in the mentioned DNP enhancements of $\sim$9–18, and the spectra shown in Figure SI2 and 2. The DNP experiments performed later on the same sample (6–12 months later) had lower DNP enhancements by a factor of up to 2, and some spectral differences (Figure SI2). Cross-peak intensity changes and chemical shift differences were observed for the sample, which was stored for long time. The reason is not clear now, but a possible change in the nature of the hydration shell of the protein (different water–glycerol content by time) may cause such spectral changes. Further experiments are needed along these lines. As a result, sample storage conditions and period should be carefully considered for reproducible data of biological systems.

3 Conclusions

In summary, we have shown that by utilization of deuterated protein DNP-enhanced magic-angle-spinning NMR experiments can be performed at high temperatures, i.e. $\sim$180 K with sufficient signal enhancement. We have demonstrated that at 178 K good spectral resolution is obtained (similar to room temperature for certain sites) which is two-fold better compared to 98 K linewidths. The resolution obtained for the deuterated sample containing radical and cryo-protectant is better than for the protonated sample prepared without cryo-protectant and radical. As a result, many cross-peaks could be assigned unambiguously. We still have observed disappearing resonances, chemical-shift changes and peak-splitting at 178 K but to

a greatly reduced extend compared to 98 K. These changes are attributed to different conformations or dynamics, and the presence of several frozen conformations simultaneously in the sample. We have observed changes in the nature of the deuterated SH3 sample and in the recorded spectra after long sample storage times. This resulted in cross-peak losses and shifts, which will require further experiments to be clarified. The use of sapphire rotors may further increase the DNP operation temperatures due to the larger enhancements obtained with them and may allow to obtain considerable enhancement at even higher temperatures, and hence improve spectral resolution. This new technique, high-temperature DNP, represents both sufficient sensitivity and resolution, and can enable an unambiguous assignment of biological systems.

Acknowledgments We acknowledge Sascha Lange, Trent W. Franks and Barth-Jan van Rossum for helpful discussions. Anne Diehl and Kristina Rehbein are also acknowledged for the preparation of the SH3 samples. This work was funded by the Deutsche Forschungsgemeinschaft (grant 05106/12-1 of the DIP program) and the European Union Seventh Framework programs (FP7/2007-2013) under the grant agreements 261863 (Bio-NMR).

References

1. P.J. Judge, A. Watts, Curr. Opin. Chem. Biol. **15**, 690–695 (2011)
2. A.W. Overhauser, Phys. Rev. **92**, 411–415 (1953)
3. T.R. Carver, C.P. Slichter, Phys. Rev. **92**, 212–213 (1953)
4. T.R. Carver, C.P. Slichter, Phys. Rev. **102**, 975–980 (1956)
5. M. Rosay, J.C. Lansing, K.C. Haddad, W.W. Bachovchin, J. Herzfeld, R.J. Temkin, R.G. Griffin, J. Am. Chem. Soc. **125**, 13626–13627 (2003)
6. V.S. Bajaj, M.L. Mak-Jurkauskas, M. Belenky, J. Herzfeld, R.G. Griffin, Proc. Natl. Acad. Sci. USA **106**, 9244–9249 (2009)
7. A.H. Linden, S. Lange, W.T. Franks, U. Akbey, E. Specker, B.J. van Rossum, H. Oschkinat, J. Am. Chem. Soc. **133**, 19266–19269 (2011)
8. U. Akbey, W.T. Franks, A. Linden, S. Lange, R.G. Griffin, B.J. van Rossum, H. Oschkinat, Angew. Chem. Int. Ed. Engl. **49**, 7803–7806 (2010)
9. M.J. Bayro, G.T. Debelouchina, M.T. Eddy, N.R. Birkett, C.E. MacPhee, M. Rosay, W.E. Maas, C.M. Dobson, R.G. Griffin, J. Am. Chem. Soc. **133**, 13967–13974 (2011)
10. G.T. Debelouchina, M.J. Bayro, P.C. van der Wel, M.A. Caporini, A.B. Barnes, M. Rosay, W.E. Maas, R.G. Griffin, Phys. Chem. Chem. Phys. **12**, 5911–5919 (2010)
11. M. Afeworki, R.A. Mckay, J. Schaefer, Mater. Sci. Eng. A **162**, 221–228 (1993)
12. V. Vitzthum, F. Borcard, S. Jannin, M. Morin, P. Mieville, M.A. Caporini, A. Sienkiewicz, S. Gerber-Lemaire, G. Bodenhausen, ChemPhysChem **12**, 2929–2932 (2011)
13. A. Lesage, M. Lelli, D. Gajan, M.A. Caporini, V. Vitzthum, P. Mieville, J. Alauzun, A. Roussey, C. Thieuleux, A. Mehdi, G. Bodenhausen, C. Coperet, L. Emsley, J. Am. Chem. Soc. **132**, 15459–15461 (2010)
14. M. Lelli, D. Gajan, A. Lesage, M.A. Caporini, V. Vitzthum, P. Mieville, F. Heroguel, F. Rascon, A. Roussey, C. Thieuleux, M. Boualleg, L. Veyre, G. Bodenhausen, C. Coperet, L. Emsley, J. Am. Chem. Soc. **133**, 2104–2107 (2011)
15. K. Golman, R. in't Zandt, M. Thaning, Proc. Nat. Acad. Sci. USA **103**, 11270–11275 (2006)
16. S.J. Kohler, Y. Yen, J. Wolber, A.P. Chen, M.J. Albers, R. Bok, V. Zhang, J. Tropp, S. Nelson, D.B. Vigneron, J. Kurhanewicz, R.E. Hurd, Magn. Reson. Med. **58**, 65–69 (2007)
17. M. Marjanska, I. Iltis, A.A. Shestov, D.K. Deelchand, C. Nelson, K. Ugurbil, P.G. Henry, J. Magn. Reson. **206**, 210–218 (2010)
18. R.G. Griffin, T.F. Prisner, Phys. Chem. Chem. Phys. **12**, 5737–5740 (2010)

19. T. Maly, G.T. Debelouchina, V.S. Bajaj, K.N. Hu, C.G. Joo, M.L. Mak-Jurkauskas, J.R. Sirigiri, P.C. van der Wel, J. Herzfeld, R.J. Temkin, R.G. Griffin, J. Chem. Phys. **128**, 052211 (2008)
20. C. Song, K.N. Hu, C.G. Joo, T.M. Swager, R.G.J. Griffin, Am. Chem. Soc. **128**, 11385–11390 (2006)
21. S. Lange, A.H. Linden, U. Akbey, W. Trent Franks, N.M. Loening, B.J.v. Rossum, H. Oschkinat. J. Magn. Reson. **216**, 209–212 (2012)
22. L.R. Becerra, G.J. Gerfen, B.F. Bellew, J.A. Bryant, D.A. Hall, S.J. Inati, R.T. Weber, S. Un, T.F. Prisner, A.E. Mcdermott, K.W. Fishbein, K.E. Kreischer, R.J. Temkin, D.J. Singel, R.G. Griffin, J. Magn. Reson., Ser. A **117**, 28–40 (1995)
23. A.H. Linden, W.T. Franks, U. Akbey, S. Lange, B.J. van Rossum, H. Oschkinat, J. Biomol. NMR **51**, 283–292 (2011)
24. C.G. Joo, K.N. Hu, J.A. Bryant, R.G. Griffin, J. Am. Chem. Soc. **128**, 9428–9432 (2006)
25. C.G. Joo, A. Casey, C.J. Turner, R.G. Griffin, J. Am. Chem. Soc. **131**, 12–13 (2009)
26. V. Chevelkov, K. Rehbein, A. Diehl, B. Reif, Angew. Chem. Int. Ed. **45**, 3878–3881 (2006)
27. U. Akbey, S. Lange, W.T. Franks, R. Linser, K. Rehbein, A. Diehl, B.J. van Rossum, B. Reif, H. Oschkinat, J. Biomol. NMR **46**, 67–73 (2010)
28. T. Iijima, Cryobiology **36**, 165–173 (1998)
29. U. Akbey, F. Camponeschi, B.J. van Rossum, H. Oschkinat, ChemPhysChem **12**, 2092–2096 (2011)
30. W. Doster, Biochim. Biophys. Acta **1804**, 3–14 (2010)
31. K. Tompa, P. Bínki, M. Bokor, P. Kamasa, G.R. Lasanda, P. Tompa, Biophys. J. **96**, 2789–2798 (2009)