

Quantitative one- and two-dimensional ^{13}C spectra of microcrystalline proteins with enhanced intensity

Rudra N. Purusottam · Geoffrey Bodenhausen · Piotr Tekely

Received: 13 May 2013 / Accepted: 23 June 2013 / Published online: 29 June 2013
© Springer Science+Business Media Dordrecht 2013

Abstract We recorded quantitative, uniformly enhanced one- and two-dimensional ^{13}C spectra of labelled microcrystalline proteins. The approach takes advantage of efficient equilibration of magnetization by low-power proton irradiation using Phase Alternated Recoupling Irradiation Schemes and benefits simultaneously from uniform sensitivity enhancement due to efficient spin exchange that can overcome $T_1(^{13}\text{C})$ constraints and the presence of heteronuclear Overhauser effects.

Keywords Solid-state NMR · PARIS *rf*-irradiation · Microcrystalline proteins · Quantitative ^{13}C spectra · Spin diffusion · Heteronuclear Overhauser enhancement

Introduction

Recording sensitive and quantitative spectra of low-gamma nuclei such as ^{13}C constitutes a long-standing challenge for solid-state NMR spectroscopy. Most ^{13}C spectra are inherently non-quantitative since cross-polarization (CP) from protons to carbons is not uniform, and single-pulse experiments are not quantitative when the delays between

successive acquisitions are too short to allow a uniform recovery of the longitudinal ^{13}C magnetization of all sites. The most pronounced distortions of intensities in CP spectra are observed between proton-carrying carbons on the one hand, and carbonyl, carboxyl, or quaternary carbons on the other, since the latter's magnetization suffers from a slow build-up during cross polarization. Additional deviations from quantitative peak intensities can also arise in CP spectra because local variations in internal mobility lead to variations in CP efficiency and/or rotating-frame relaxation rates $R_{1\rho}$.

We have recently discussed the requirements for recording quasi-quantitative single pulse or CP spectra of labelled amino acids (Herbert-Pucheta et al. 2012a). We have demonstrated that recoupling schemes such as Phase Alternated Recoupling Irradiation Schemes (PARIS) (Weingarth et al. 2009b, c) and PARIS-xy (Weingarth et al. 2010) permit one to reach similar peak amplitudes ($\pm 10\%$) for chemically different sites on time scales as short as a few hundreds of milliseconds. We have previously used these pulse schemes to record sensitive 2D correlation spectra of microcrystalline proteins (Weingarth et al. 2009c; Herbert-Pucheta et al. 2012b), amyloid fibrils (Weingarth et al. 2011b) and mixtures of crystallographic forms (Herbert-Pucheta et al. 2011), and to restore the symmetry in 2D homonuclear correlation experiments of simple amino acids (Herbert-Pucheta et al. 2012a).

In this work we show quantitative one- and two-dimensional ^{13}C spectra of microcrystalline proteins recorded after equilibration of magnetization by low-power PARIS or PARIS-xy irradiation. These spectra benefit simultaneously from a uniform sensitivity enhancement (1) due to efficient spin diffusion that allows one to overcome $T_1(^{13}\text{C})$ constraints and (2) the presence of heteronuclear Overhauser effects. Such NOE effects have first been observed in solids

R. N. Purusottam · G. Bodenhausen · P. Tekely (✉)
Département de Chimie, Ecole Normale Supérieure, 24 rue
Lhomond, 75005 Paris, France
e-mail: piotr.tekely@ens.fr

R. N. Purusottam · G. Bodenhausen · P. Tekely
Université Pierre-et-Marie Curie, Place Jussieu,
75005 Paris, France

R. N. Purusottam · G. Bodenhausen · P. Tekely
Laboratoire des Biomolécules, CNRS, UMR 7203, 24 rue
Lhomond, 75005 Paris, France

in a single crystal of L-alanine (Naito and McDowell 1986) and more recently in a microcrystalline protein (Giraud et al. 2006; Lesage et al. 2006). The nuclear Overhauser polarization (NOP) induced by DARR irradiation (Takegoshi et al.

2001) has been used for the uniform enhancement of single-pulse ^{13}C spectra of glycolisoleucine and threonine (Takegoshi and Terao 2002) and of lyophilized proteins (Katoh et al. 2004).

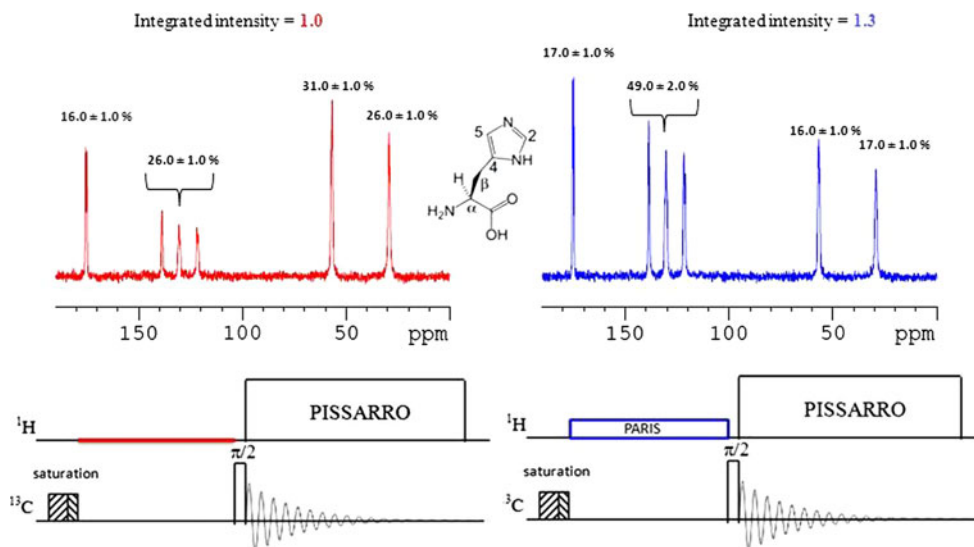
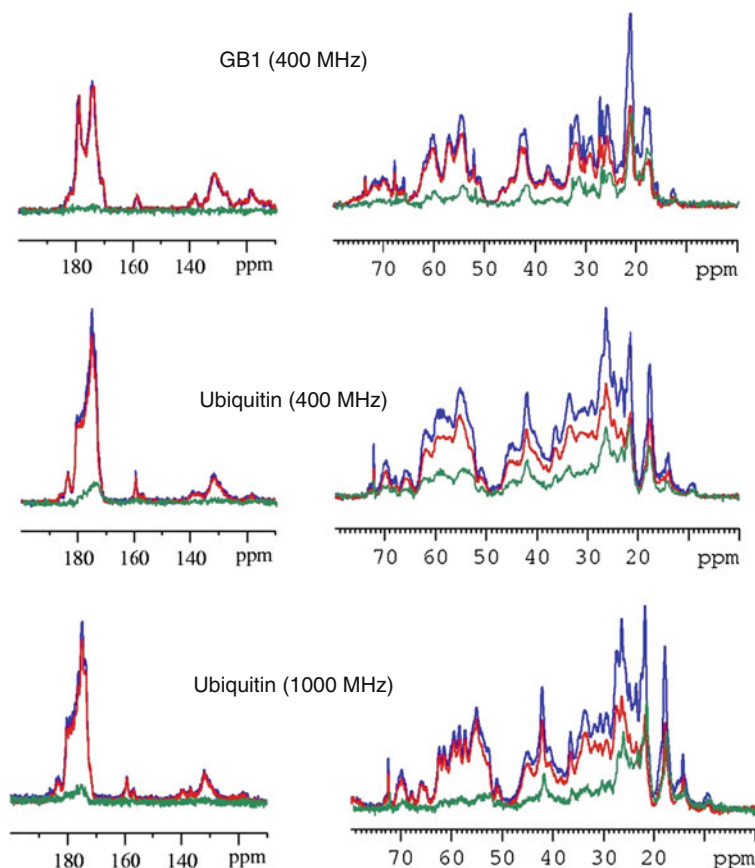


Fig. 1 (Top) ^{13}C spectra of uniformly labeled L-histidine: single-pulse reference spectrum (red) and quantitative spectrum with enhanced intensity (blue). The numbers give integrated intensities of individual resonances or regions. Both spectra were recorded in a magnetic field of 9.4 T (400 MHz for ^1H) at a spinning frequency of

23 kHz. (Below) The reference spectrum was obtained after a delay of 3 s; the quantitative spectrum was recorded using PARIS ($N = 2$) irradiation with a proton r_f amplitude of 15 kHz during 3.0 s. The proton decoupling field amplitude was 96 kHz using the PISSARRO sequence (Weingarth et al. 2008, 2009a, 2011a)

Fig. 2 Single-pulse reference spectra (red), transient NOE spectra (blue), and difference spectra (green) of ubiquitin and GB1, recorded in magnetic fields of 9.4 T or 23.5 T (400 MHz or 1 GHz for protons) with spinning frequencies of 22.5 and 24 kHz respectively and a recycle delay of 10 s. The transient NOE spectra were recorded with a cross-relaxation delay $t_{cr} = 300$ ms for GB1 and 500 ms for Ubiquitin (see pulse sequence in Fig. 3). The ^1H and ^{13}C r_f amplitudes were 167 and 71 kHz, respectively. The proton decoupling field amplitude was 96 kHz using the PISSARRO sequence



When recording quantitative spectra of labelled microcrystalline proteins, we realized that, apart from efficient equilibration of magnetization and accelerated longitudinal recovery, one can also benefit from the heteronuclear Overhauser enhancement induced by low-power PARIS irradiation. This approach has several advantages over DARR irradiation. PARIS pulse sequences allow one to achieve efficient dipolar recoupling and to induce uniform exchange of magnetization with low rf field amplitudes over a very broad range of spinning frequencies and static fields (Weingarth et al. 2009b, c, 2010). PARIS sequences do not require any optimization, are immune to dipolar truncation (Weingarth et al. 2011b), and need not fulfill any rotary or rotational resonance for transferring magnetization between spectrally close or distant carbons. In contrast to DARR, the efficiency of PARIS recoupling schemes does not depend critically on the rf amplitude (Weingarth et al. 2009c) which permits one to achieve an efficient magnetization transfer with low-power rf irradiation even at high spinning frequencies. In contrast to DARR, which uses continuous-wave irradiation, both PARIS and PARIS-xy schemes are largely immune to the inherent inhomogeneity of the rf field, so that the full sample volume effectively contributes to the signal (Weingarth et al. 2009b). When recording uniformly enhanced spectra of labelled microcrystalline proteins, the numerous advantages of PARIS permit one to use much lower rf amplitudes and shorter durations of irradiation compared to DARR.

Results and discussion

Efficient spin exchange allows overcoming $T_1(^{13}\text{C})$ constraints

To record quantitative solid-state NMR spectra of low-gamma nuclei with signal intensities that are proportional to the number of nuclei, one needs to distribute the magnetization evenly between different sites. This is illustrated in Fig. 1 by comparing intensities of resonance signals of different carbons of L-histidine recorded without and with PARIS irradiation applied prior to detection. The latter case leads to a transfer of magnetization from rapidly relaxing aliphatic carbons to slowly recovering carboxyl and aromatic carbons. At the same time, efficient spin diffusion in the presence of PARIS recoupling accelerates recovery of the latter carbons (vide infra). For L-histidine, this dramatically reduces the time required to record quantitative spectra. Under the experimental conditions used to record the spectra in Fig. 1, a recovery delay $t_{\text{rd}} = 3$ s suffices, while roughly $t_{\text{rd}} = 200$ s is required to record quantitative spectra in the absence of PARIS irradiation. Similar observations show that the T_1

constraints can be overcome as has been reported recently for L-tyrosine (Hou et al. 2010) and L-histidine (Giffard et al. 2009).

Somewhat unexpectedly, in contrast to L-histidine and L-arginine, we observed a uniform enhancement of all peaks, as compared with single-pulse spectra obtained after complete relaxation of the longitudinal magnetization, when applying PARIS irradiation to record quantitative ^{13}C spectra of microcrystalline proteins. This prompted us to investigate the role of heteronuclear Overhauser enhancements that could be induced by cross-relaxation in these systems.

Revealing the sources of the Overhauser enhancement

To confirm the presence of heteronuclear Overhauser effects and identify the sources of the intensity enhancement, while avoiding the equilibration of magnetization, we used transient Overhauser experiments that are commonly used in solution (Neuhaus and Williamson 2000). This allows one to identify the role of cross-relaxation in a

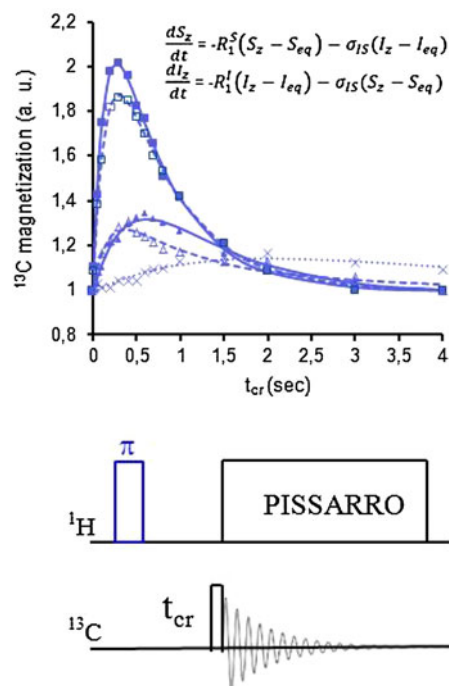


Fig. 3 (Top) Build-up curves of carbon-13 magnetization due to transient heteronuclear Overhauser effects as a function of the cross relaxation delay t_{cr} for isotropic ^{13}C signals of GB1 at 17.8 ppm (filled squares), 21.1 ppm (open squares), 42.2 ppm (open triangles), 54.4 ppm (filled triangles) and 174.0 ppm (crosses). The spectra were recorded in a magnetic field of 9.4 T with a spinning frequency of 24 kHz and a recycle delay of 10 s. The solid, dashed and dotted lines represent fits to the Solomon equations. To fit the build-up of the peak at 42.2 ppm (open triangles), the presence of two populations of CH_2 with different relaxation parameters had to be postulated. (Bottom) Pulse sequence to determine the build-up of transient heteronuclear Overhauser effects as a function of the cross relaxation delay t_{cr}

straightforward manner, and clearly reveals the mechanism underlying the intensity enhancement when comparing spectra recorded with and without a π pulse (Canet et al. 1992; Palmas et al. 1993). As shown in Fig. 2 for the microcrystalline proteins GB1 and Ubiquitin, significant enhancements of some signals are observed after cross-relaxation delays t_{cr} of a few hundreds of milliseconds. Such enhancements are not only visible for methyl groups that are expected to constitute the main reservoir of the heteronuclear Overhauser enhancement (Kato et al. 2004), but also for their nearest neighbours, and even for carbons that are spatially distant from methyl groups. This suggests that the resonance signals stemming from carbons located in the most mobile parts of the protein, especially in side-chains that are inherently mobile, also benefit to some degree from such enhancements. Figure 3 shows that different ^{13}C sites feature specific build-up behaviours of the magnetization as a function of the cross-relaxation delay t_{cr} . It is remarkable that they strictly obey the Solomon equations. This opens the way to site-specific quantitative NOE measurements that will be presented elsewhere.

Corroborative evidence that mobile side-chains can also constitute a source of heteronuclear Overhauser enhancements is provided by the transient ^{15}N spectra shown in Fig. 4. The negative enhancements are most pronounced for lysine residues, as has been observed in Crh (Giraud

et al. 2006). We also noted that fast chemical exchange involving mobile water protons or their direct interaction with a protein do not lead to any significant enhancement, at least on a time scale up to a few seconds.

Promoting uniform enhancements by low-power PARIS irradiation

In analogy to solution-state NMR, as illustrated in Fig. 5 for L-threonine, large variations of NOE enhancement factors from one carbon site to another constitute a major impediment to a quantitative utilisation of ^{13}C peak intensities in spectra enhanced by transient NOE. To record quantitative spectra, one needs to *equilibrate* the magnetization that has been enhanced by cross-relaxation. As shown in Fig. 5, this can be accomplished by PARIS irradiation that can *simultaneously* promote heteronuclear Overhauser enhancements *and* the equilibration of magnetization. This permits one to record uniformly enhanced peak intensities that are proportional to the number of nuclei.

Figure 6 shows ^{13}C spectra of the microcrystalline protein GB1 recorded without and with PARIS irradiation prior to signal detection. The observed differences demonstrate that low-power PARIS irradiation indeed allows one to record quantitative spectra of microcrystalline proteins with the combined benefits of enhanced and

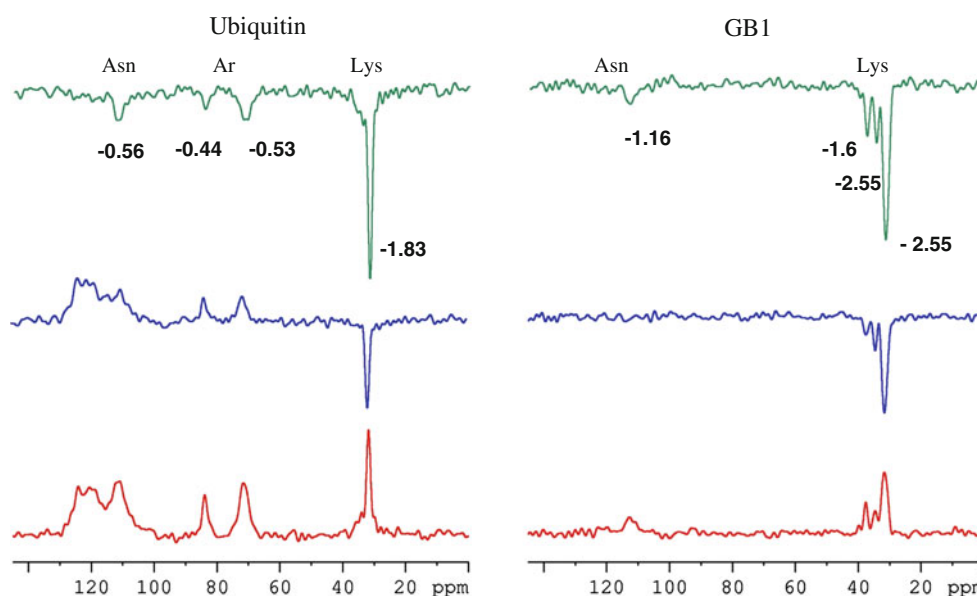


Fig. 4 Single-pulse ^{15}N reference spectra (*bottom*), spectra enhanced by transient heteronuclear Overhauser effects (*middle*), and difference spectra (*top*) of Ubiquitin (*left*) and GB1 (*right*). All spectra were recorded in a magnetic field of 9.4 T (400 MHz for ^1H) with a spinning frequency of 22.5 kHz. In both cases the transient NOE spectra were recorded with a cross-relaxation delay $t_{cr} = 500$ ms. For Ubiquitin a recycle delay of 4 s was used, while for GB1 two

saturation pulses (see Fig. 1) were applied to the ^{15}N spins before the 180° proton pulse. The numbers give the NOE enhancement factors (η) for individual resonances. The three resonance lines of lysine residues in GB1, that appear at the isotropic chemical shifts 31.7, 34.7, and 37.7 ppm, most probably result from the presence of different polymorphs and/or crystallographically different forms

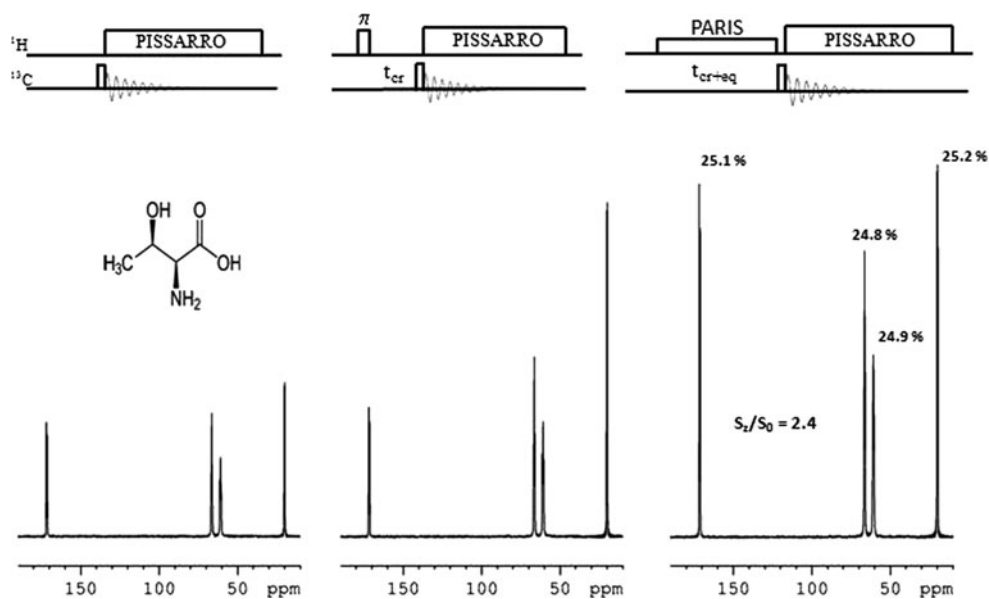


Fig. 5 (Bottom) Single-pulse ^{13}C reference spectrum (left), spectrum enhanced by transient heteronuclear Overhauser effect (middle) and quantitative spectrum with enhanced intensity (right) of uniformly labeled L-threonine. The numbers give relative integrated intensities (in %) of the individual resonances. All spectra were recorded in a magnetic field of 9.4 T (400 MHz for ^1H) at a spinning frequency of

20 kHz. (Top) The single-pulse reference spectrum was recorded with a recovery delay $t_{\text{rd}} = 4$ s, the transient NOE spectrum with $t_{\text{rd}} = 3.4$ s and $t_{\text{cr}} = 600$ ms, the quantitative spectrum with $t_{\text{rd}} = 3$ s and PARIS-xy ($m = 1$) irradiation with a proton rf amplitude of 15 kHz during 1 s

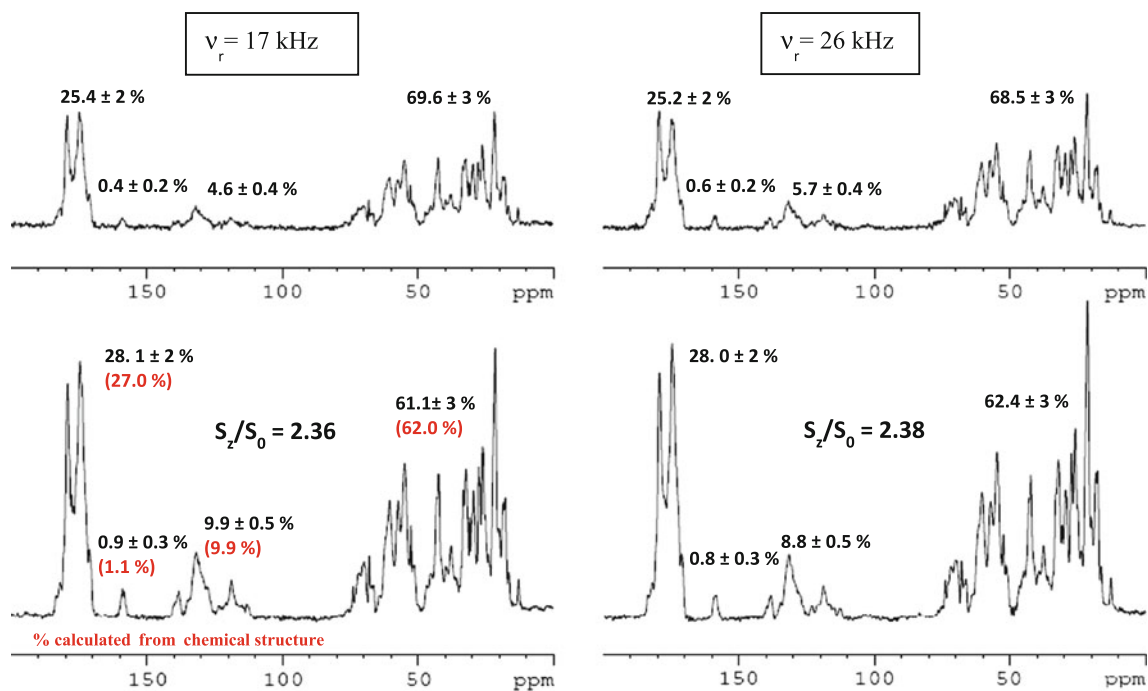


Fig. 6 Single-pulse ^{13}C reference spectra (top) and uniformly enhanced quantitative spectra (bottom) of GB1 recorded at spinning frequencies $\nu_{\text{rot}} = 17$ and 26 kHz using PARIS-xy ($m = 1$) irradiation with a proton rf amplitude of 12 kHz during 2.3 and 3.0 s, respectively. The reference spectra were recorded with a recycle delay $t_{\text{rd}} = 7.0$ s while the corresponding uniformly enhanced spectra

were recorded with $t_{\text{rd}} = 4.7$ and 4.0 s, respectively. The numbers give relative integrated intensities for different spectral regions. The overall intensity gain is $S_2/S_0 = 1 + \eta$, where η represents the nuclear Overhauser enhancement factor (referring to the S_0 value of the aliphatic carbons.). The proton decoupling field amplitude was 96 kHz using the PISSARRO sequence

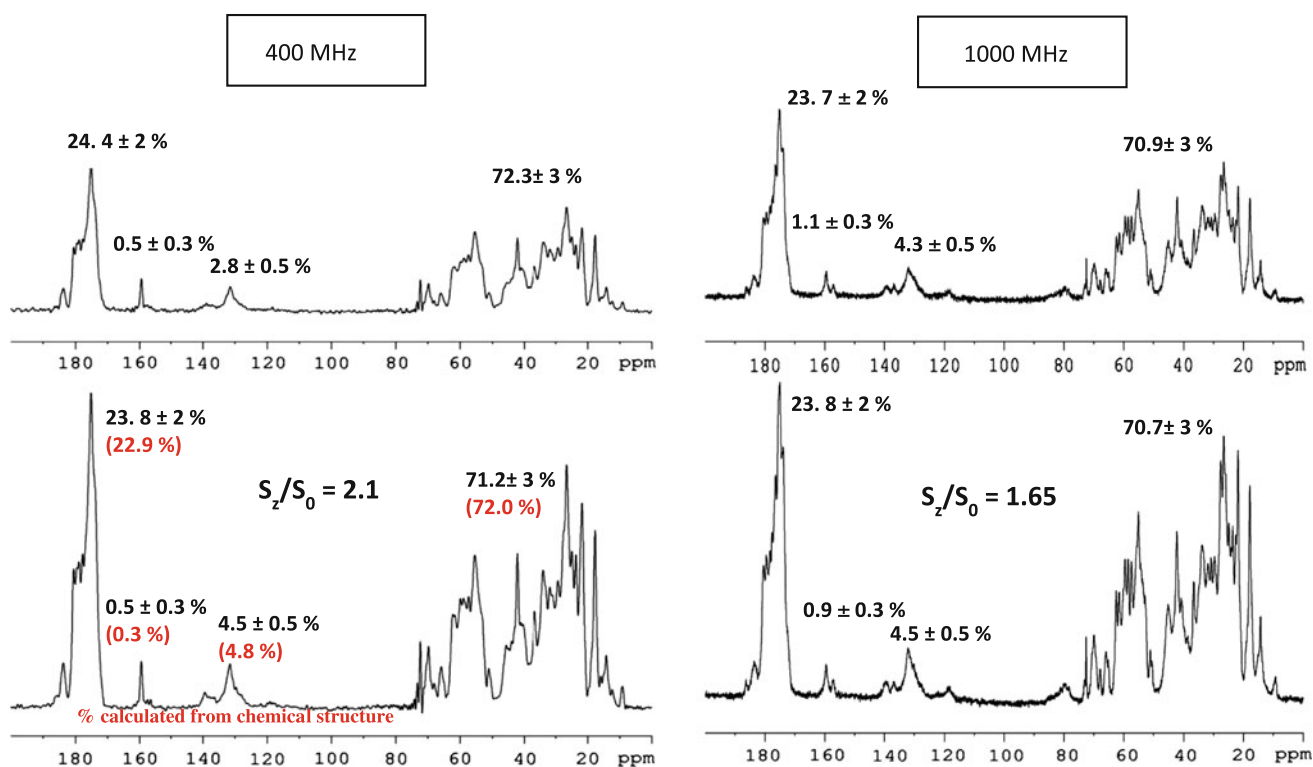


Fig. 7 Single-pulse ^{13}C reference spectra (top) and uniformly enhanced spectra (bottom) of Ubiquitin recorded at a spinning frequency of 24 kHz at two different static fields. The reference spectra were recorded with recycle delays $t_{\text{rd}} = 6$ s (at 400 MHz) and 13 s (at 1000 MHz) while the corresponding uniformly enhanced spectra were recorded with $t_{\text{rd}} = 3$ and 10 s using PARIS-xy ($m = 1$)

or PARIS ($N = 1/2$) irradiation during 3 s with proton rf amplitudes of 15 and 20 kHz applied prior to the observation pulse. The numbers give the experimental (in black) and theoretical (in red) values of the integrated intensities for different spectral regions. The overall intensity gain S_z/S_0 , refers to the S_0 value of the aliphatic carbons. The proton decoupling field was 96 kHz using PISSARRO

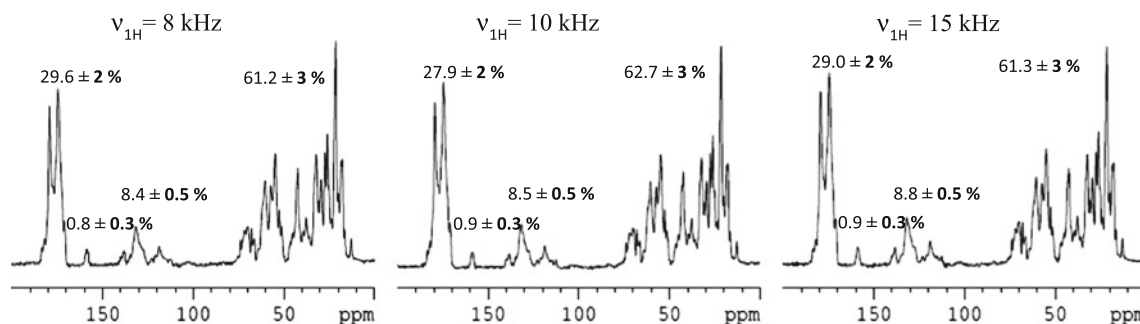


Fig. 8 Quantitative spectra of GB1 recorded at a spinning frequency of 26 kHz using PARIS-xy ($m = 1$) irradiation during 3 s with proton rf amplitudes $\nu_{1\text{H}} = 8, 10$ and 15 kHz applied prior to the observation

pulse. The numbers give relative integrated intensities for different spectral regions

uniformly equilibrated magnetization. As shown in Fig. 7 for microcrystalline Ubiquitin, quantitative spectra with enhanced intensity can be also recorded in a very high static field of 23.5 T using the same duration of low-power PARIS irradiation. It is also worth recalling that the efficiency of PARIS equilibration does not depend critically on the rf amplitude, which need not be matched with the spinning frequency. As shown in Fig. 8, this allows one to record quantitative spectra of hydrated microcrystalline proteins with very modest rf fields, while DARR at the

same spinning frequency would require a roughly seven-fold higher rf power to fulfill the rotary resonance condition. Finally, one could also try to achieve enhancements by equilibrating the magnetization after cross-polarization. However, as shown in Fig. 9, the observed enhancements appear to be modest and are hardly worth the effort required to optimize cross-polarization in microcrystalline proteins. It is also worth remembering that, because of the low proton/carbon ratio N_I/N_S in uniformly ^{13}C -labeled proteins, the theoretical maximum CP gain is reduced to

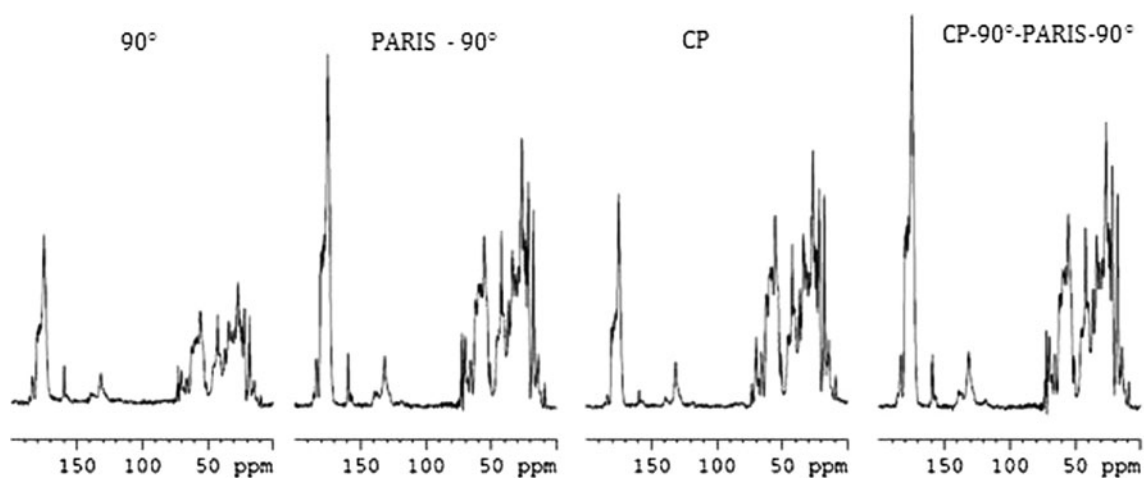


Fig. 9 (From left to right) Single-pulse ^{13}C reference spectrum, uniformly enhanced spectrum, standard cross-polarization (CP) spectrum (using a contact time of 1.5 ms that is an optimal for aliphatic carbons) and CP-PARIS spectrum of Ubiquitin, all recorded at 400 MHz. The uniformly enhanced spectra were recorded using

PARIS-xy ($m = 1$) irradiation with a proton rf amplitude of 15 kHz applied during 3.0 s prior to the observation pulse. All spectra were recorded at a spinning frequency of 24 kHz with the same number of scans and the same recovery delays after the initial saturation pulses

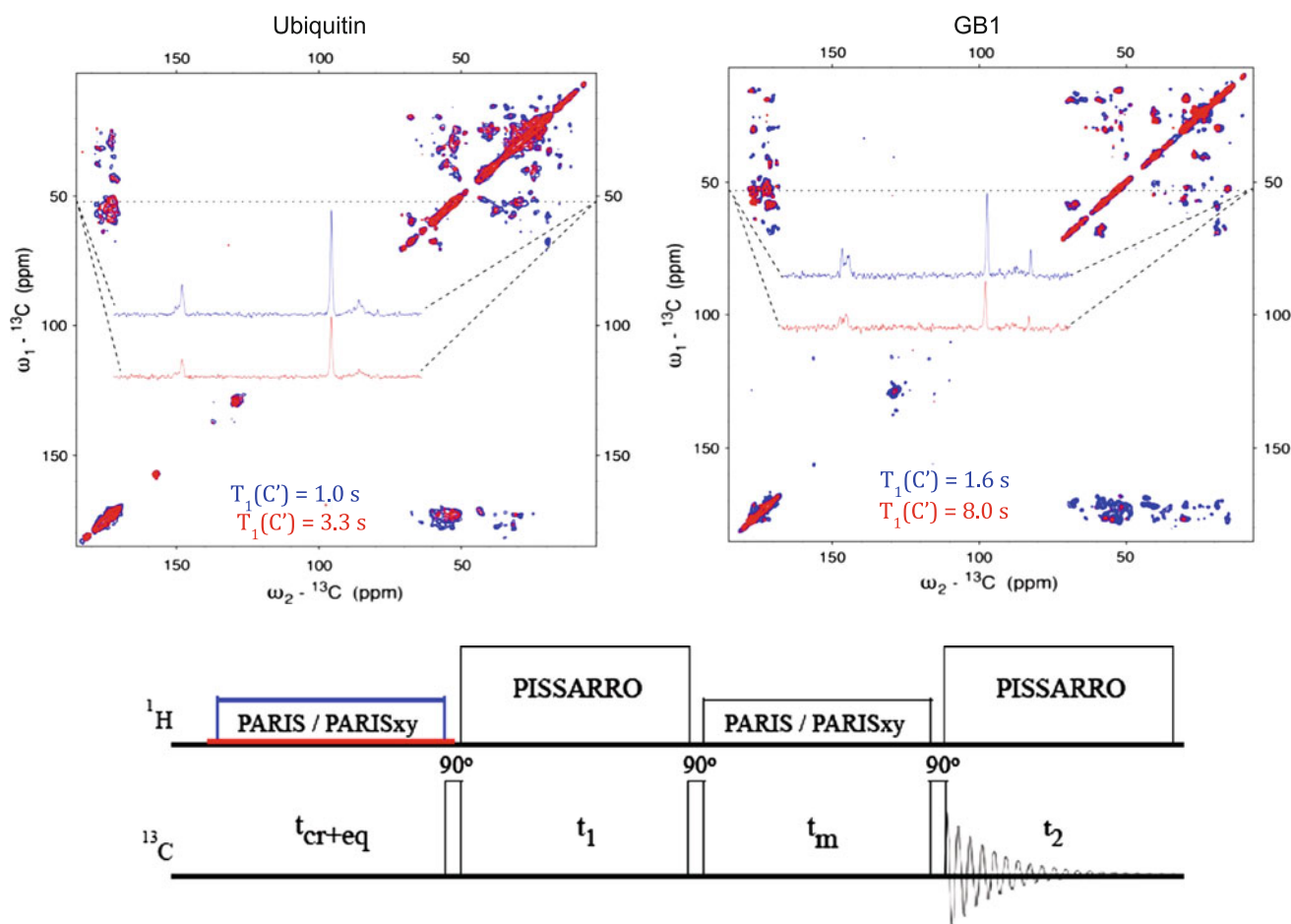


Fig. 10 (Top) 2D ^{13}C - ^{13}C correlation spectra of Ubiquitin (left) and GB1 (right) recorded at 400 MHz. Each figure shows two overlaid spectra recorded either without (red) or with (blue) PARIS-xy ($m = 1$) irradiation during 1.7 s with an rf amplitude $\nu_{1\text{H}} = 15$ kHz and recycle times 6 and 4.3 s, respectively. Both spectra were plotted with the same

contour levels. During the mixing time ($t_m = 42$ and 100 ms for ubiquitin and GB1, respectively), PARIS-xy ($m = 1$) was applied with the same rf amplitude. The 1.3 mm rotors were spun at 24 and 22.5 kHz for ubiquitin and GB1. The proton decoupling field was 96 kHz with PISSARRO. (Bottom) The pulse sequence used to record the 2D spectra

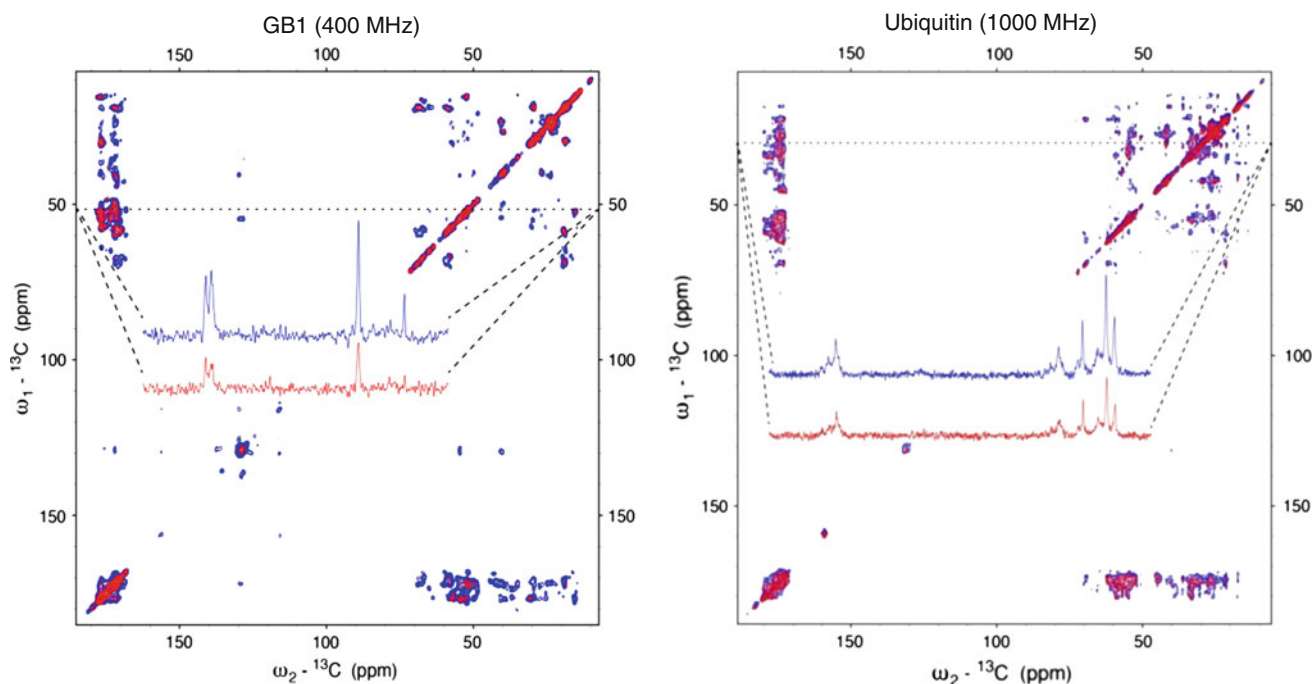


Fig. 11 2D ^{13}C – ^{13}C correlation spectra of GB1 (*left*) and Ubiquitin (*right*) recorded at 9.4 and 23.5 T respectively either without (*red*) or with (*blue*) enhancement and equilibration. For GB1, PARIS ($N = 2$) irradiation with an *rf* amplitude of 15 kHz was applied during 3.0 s for both enhancement and equilibration, and during the mixing period $\tau_m = 200$ ms. For Ubiquitin, PARIS ($N = 1/2$) irradiation with an *rf*

amplitude of 20 kHz was applied during 3.0 s for enhancement and equilibration, and during the mixing period $\tau_m = 200$ ms. The recycle delays were the same as in Figs. 7 and 10. The 1.3 mm rotors were spun at 24 kHz. The proton decoupling field amplitude was 96 kHz using PISSARRO. The same pulse sequences were used as in Fig. 10

about 2.4 (Katoh et al. 2004). In practice the CP enhancement in uniformly labeled microcrystalline proteins is found to be between 1.0 and 2.0. Consequently, as shown in Fig. 9, the heteronuclear Overhauser enhancement can be better than CP.

As mentioned in the introduction, the equilibration of magnetization using PARIS irradiation also permits one to remove commonly encountered asymmetries in ^{13}C – ^{13}C correlation spectra (Herbert-Pucheta et al. 2012a). Such asymmetries arise from a non-uniform preparation of the magnetization when cross-polarization is used or when the delay between successive acquisitions is too short to allow a uniform recovery of the longitudinal magnetization of all sites. This is illustrated in Figs. 10 and 11 where, in the absence of PARIS irradiation, the asymmetry is especially pronounced for GB1 because of the slow recovery of the longitudinal magnetization of the carboxyl carbons. The rate of recovery is significantly enhanced by PARIS irradiation since it promotes efficient spin diffusion. As we have previously demonstrated, restoring the symmetry of 2D spectra is crucial to extract reliable rate constants that allow one to deduce internuclear distances and hence structural information (Herbert-Pucheta et al. 2012a). Equally importantly, when recording symmetric 2D correlation spectra as shown in Figs. 10 and 11, one simultaneously benefits from

heteronuclear Overhauser effects, leading *in fine* to uniformly enhanced intensities of all diagonal and cross-peaks. As shown in Fig. 11, the efficient restoration of symmetry over the whole spectral range along with a uniform enhancement of intensities can be also achieved at very high static fields despite a modest *rf* amplitude. However, to record quantitative spectra at very high spinning frequencies it will be necessary to use longer irradiation periods. Heating by *rf* irradiation could be limited by using so-called E-free probes. Very recently, we have observed that low-power PARIS irradiation can also significantly enhance and equilibrate the magnetization of ^{13}C nuclei in membrane proteins.

Conclusions

We have recorded quantitative, one- and two-dimensional ^{13}C spectra of microcrystalline proteins with an added bonus of enhanced sensitivity. Promoting the equilibration of magnetization by low-power PARIS irradiation leads simultaneously to (1) efficient spin diffusion that allows one to overcome $T_1(^{13}\text{C})$ constraints and (2) a heteronuclear Overhauser enhancement. This has permitted us to record quantitative, uniformly enhanced one- and

two-dimensional ^{13}C spectra of microcrystalline proteins both at moderate and the highest available static fields. Transient heteronuclear experiments have allowed us to identify unambiguously the sources of the Overhauser enhancement. Uniformly enhanced quantitative spectra of ^{13}C -labeled microcrystalline proteins, recorded without cross-polarization, show higher intensity gains than standard cross-polarization spectra that are intrinsically non-quantitative. This will benefit a wide range of solid-state NMR experiments on microcrystalline and membrane proteins.

Acknowledgments We thank Philippe Pelupessy for stimulating discussions. Financial support of the *Agence Nationale de la Recherche* (ANR-09-BLAN-0111-01), the *Ecole Doctorale* ED388 (Université Pierre-et-Marie Curie, UPMC) and the *Fédération de Recherche* (FR 3050) *Très Grands Equipements de Résonance Magnétique Nucléaire à Très Hauts Champs* (TGE RMN THC) of the CNRS is gratefully acknowledged.

References

- Canet D, Mahieu N, Tekely P (1992) Heteronuclear Overhauser effect measurements in surfactant systems. 1. The direct estimation of the distance between water and the micellar surface. *J Am Chem Soc* 114:6190–6194
- Giffard M, Bardet M, Bersch B, Covès J, Hediger S (2009) Impact of selective excitation on carbon longitudinal relaxation: towards fast solid-state NMR techniques. *J Magn Reson* 200:153–160
- Giraud N, Sein J, Pintacuda G, Böckmann A, Lesage A, Blackledge M, Emsley L (2006) Observation of heteronuclear Overhauser effects confirms the ^{15}N - ^1H dipolar relaxation mechanism in a crystalline protein. *J Am Chem Soc* 128:12398–12399
- Herbert-Pucheta JE, Colaux H, Bodenhausen G, Tekely P (2011) Disentangling crystallographic inequivalence and crystallographic forms of L-arginine by one- and two-dimensional solid-state NMR spectroscopy. *J Phys Chem B* 115:15415–15421
- Herbert-Pucheta JE, Pelupessy P, Bodenhausen G, Tekely P (2012a) Restoring symmetry in two-dimensional solid-state NMR correlation spectra. *Chem Phys Lett* 539–540:245–251
- Herbert-Pucheta JE, Chan-Huot M, Duma L, Abergel D, Bodenhausen G, Assairi L, Blouquity Y, Charbonnier JB, Tekely P (2012b) Probing structural and motional features of the C-terminal part of the human centrin 2/P17-XPC microcrystalline complex by solid-state NMR spectroscopy. *J Phys Chem B* 116:14581–14591
- Hou G, Ding S, Zhang L, Deng F (2010) Breaking the T_1 constraint for quantitative measurement in magic-angle spinning solid-state NMR spectroscopy. *J Am Chem Soc* 132:5538–5539
- Katoh E, Takegoshi K, Terao T (2004) ^{13}C nuclear Overhauser polarization-magic-angle spinning nuclear magnetic resonance spectroscopy in uniformly ^{13}C -labeled solid proteins. *J Am Chem Soc* 126:3653–3657
- Lesage A, Emsley L, Penin F, Böckmann A (2006) Investigation of dipolar-mediated water-protein interactions in microcrystalline Crh by solid-state NMR spectroscopy. *J Am Chem Soc* 128:8246–8255
- Naito A, McDowell CA (1986) Anisotropic behavior of the ^{13}C nuclear spin dynamics in a single crystal of L-alanine. *J Chem Phys* 84:4181–4185
- Neuhaus D, Williamson MP (2000) The nuclear Overhauser effect in structural and conformational analysis. Wiley-VCH, New York
- Palmas P, Tekely P, Mutzenhardt P, Canet D (1993) Heteronuclear Overhauser effect measurements in surfactant systems. IV. Direct and remote correlations within alkyl chains. *J Chem Phys* 99:4775–4785
- Takegoshi K, Terao T (2002) ^{13}C nuclear Overhauser polarization nuclear magnetic resonance in rotating solids: replacement of cross polarization in uniformly ^{13}C labeled molecules with methyl groups. *J Chem Phys* 117:1700–1707
- Takegoshi K, Nakamura S, Terao T (2001) ^{13}C - ^1H dipolar-assisted rotational resonance in magic-angle spinning NMR. *Chem Phys Lett* 344:631–637
- Weingarth M, Tekely P, Bodenhausen G (2008) Efficient heteronuclear decoupling by quenching rotary resonance in solid-state NMR. *Chem Phys Lett* 466:247–251
- Weingarth M, Bodenhausen G, Tekely P (2009a) Low-power decoupling at high spinning frequencies in high static fields. *J Magn Reson* 199:238–241
- Weingarth M, Demco DE, Bodenhausen G, Tekely P (2009b) Improved magnetization transfer in solid-state NMR with fast magic angle spinning. *Chem Phys Lett* 469:342–348
- Weingarth M, Bodenhausen G, Tekely P (2009c) Broadband carbon-13 correlation spectra of microcrystalline proteins in very high magnetic fields. *J Am Chem Soc* 131:13937–13939
- Weingarth M, Bodenhausen G, Tekely P (2010) Broadband magnetization transfer using moderate radio-frequency fields for NMR with very high static fields and spinning speeds. *Chem Phys Lett* 488:10–16
- Weingarth M, Bodenhausen G, Tekely P (2011a) Probing the quenching of rotary resonance by PISSARRO decoupling. *Chem Phys Lett* 502:259–265
- Weingarth M, Masuda Y, Takegoshi K, Bodenhausen G, Tekely P (2011b) Sensitive ^{13}C - ^{13}C correlation spectra of amyloid fibrils at very high spinning frequencies and magnetic fields. *J Biomol NMR* 50:129–136