Recovering Invisible Signals by Two-Field NMR Spectroscopy

Samuel F. Cousin, Pavel Kadeřávek, Baptiste Haddou, Cyril Charlier, Thorsten Marquardsen, Jean-Max Tyburn, Pierre-Alain Bovier, Frank Engelke, Werner Maas, Geoffrey Bodenhausen, et al.

▶ **To cite this version:**

Samuel F. Cousin, Pavel Kadeřávek, Baptiste Haddou, Cyril Charlier, Thorsten Marquardsen, et al.. Recovering Invisible Signals by Two-Field NMR Spectroscopy. Angewandte Chemie International Edition, 2016, 55 (34), pp.9886 - 9889. 10.1002/anie.201602978 . hal-01387986

HAL Id: hal-01387986
https://hal.sorbonne-universite.fr/hal-01387986

Submitted on 26 Oct 2016

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Recovering Invisible Signals by Two-Field Nuclear Magnetic Resonance Spectroscopy

S. F. Cousin,[a,b,c]+ P. Kadeřávek,[a,b,c]+ B. Haddou,[a,b,d] C. Charlier,[a,b,c] T. Marquardsen,[e] J.-M. Tyburn,[f] P.-A. Bovier,[g] F. Engelke,[e] W. Maas,[h] G. Bodenhausen,[a,b,c] P. Pelupessy,[a,b,c] and F. Ferrage*[a,b,c]

[a] S. F. Cousin, P. Kadeřávek, B. Haddou, C. Charlier, G. Bodenhausen, P. Pelupessy, F. Ferrage
Department of Chemistry
Ecole Normale Supérieure - PSL Research University,
24 rue Lhomond,
75005 Paris, France
[b] *Sorbonne Universités, UPMC Univ Paris 06, LBM, 4 place Jussieu, 75005 Paris, France*
[c] CNRS, UMR 7203 LBM, 75005 Paris, France
[d] CNRS, UMR 8640 PASTEUR, 75005 Paris, France.
E-mail: Fabien.Ferrage@ens.fr
[e] T. Marquardsen, F. Engelke
Bruker BioSpin GmbH
Silberstreifen 4, D 76287 Rheinstetten, Germany.
[f] J.M. Tyburn
Bruker BioSpin
34 rue de l'Industrie BP 10002, 67166 Wissembourg Cedex, France
[g] P.-A. Bovier
Bruker BioSpin AG
Industriestrasse 26, 8117 Fällanden, Switzerland.
[h] W. Maas
Bruker BioSpin
Billerica, Massachusetts 01821, USA.
[+] These authors contributed equally to this work.

Supporting information for this article is given via a link at the end of the document.

Abstract: *Nuclear Magnetic Resonance has benefited tremendously from the steady increase of magnetic fields. Spectacular improvements in both sensitivity and resolution have allowed the investigation of molecular systems of rising complexity. At very high fields, this progress may be jeopardized by line broadening due to chemical exchange or relaxation by chemical shift anisotropy. Here, we introduce a two-field NMR spectrometer designed for both excitation and observation of nuclear spins at two distinct magnetic fields in a single experiment. NMR spectra of several small molecules as well as a protein were obtained, with two dimensions acquired at vastly different magnetic fields. We show that signals of exchanging groups broadened beyond recognition at high field can be sharpened up to narrow peaks in a low-field dimension. Two-field NMR makes it possible to measure chemical shifts at optimal fields, allows the observation of molecular systems that suffer from internal dynamics, and opens new avenues for NMR at very high magnetic fields.*

The ability of Nuclear Magnetic Resonance (NMR) to probe the chemical and physical properties of matter at atomic resolution makes it a universal spectroscopic tool for molecular chemistry, material science, structural biology and medicine. The ubiquity of NMR has greatly benefitted from the enhanced resolution and sensitivity offered by high magnetic fields and the introduction of two- and multi-dimensional NMR experiments.[1] Severe line broadening due to micro- to millisecond dynamics (known as "chemical exchange") occurs in many chemical and biological systems.[2] Such line-broadening effects are often exacerbated at high fields. Hence, chemists and biologists face a dilemma: they must choose between good sensitivity and resolution at high fields, or more favorable line-widths and relaxation rates at lower fields.

Magnetic-field dependent properties can be probed by a broad range of NMR techniques that explore two or more fields in a single experiment, as in fast field-cycling relaxometry,[3] zero-field NMR,[4] dynamic nuclear polarization,[5] and other methods.[6] Such systems have allowed the characterization of a host of properties for magnetic resonance imaging,[7] and particularly contrast agents,[8] materials[9] as well as macromolecules.[10] However, to the best of our knowledge, no experiment has ever been proposed that allows one to correlate chemical shifts at two different fields.

The requirement that both fields should be homogeneous has so far been a formidable obstacle. Here, we introduce a two-field NMR spectrometer and illustrate its potential for high-resolution two-field NMR spectroscopy. The benefits of high fields (sensitivity and resolution) and those of low fields (line-narrowing when chemical exchange occurs) can thus be combined. This approach can yield high-resolution chemical shift correlations between high and low fields, for example in heteronuclear (e.g. ^{1}H-^{13}C) spin systems in small molecules and proteins alike. We show that signals of methyl groups that cannot be observed at 14.1 T because of chemical exchange can be recovered by two-field correlation spectroscopy. This work is important because it shows how a wide range of molecules and biomolecules prone to excessive broadening by chemical exchange can be studied by NMR. Two-field NMR spectroscopy paves the way to a new generation of NMR spectrometers, where multiple fields can be explored in the course of a single experiment in order to achieve an optimal combination of sensitivity, resolution, and spectral information.

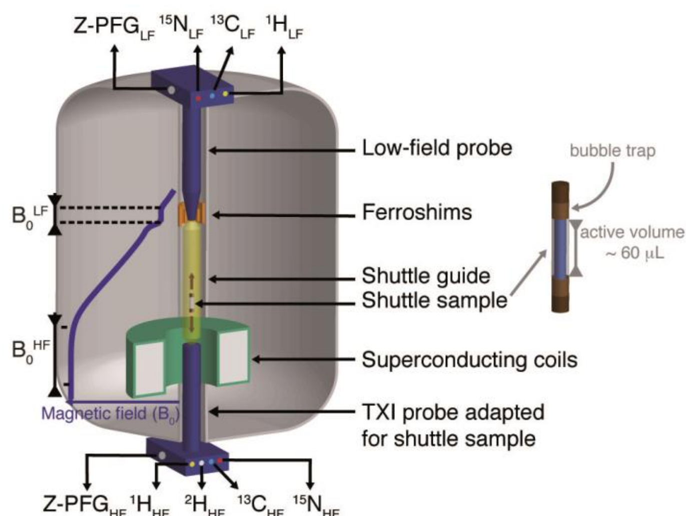

Figure 1. Schematic representation of the two-field NMR spectrometer. Ferroshims that are placed in the bore of the magnet provide a plateau at B_0^{LF} = 0.33 T. In addition to a classical probe positioned at the high field center B_0^{HF} = 14.1 T, a second probe is introduced from the top of the bore and positioned at the plateau B_0^{LF}. The sample is shuttled between the two magnetic centers by a pneumatic system

Our two-field NMR spectrometer is based on a commercial NMR spectrometer at 14.1 T (600 MHz for protons) with a series of new accessories (Fig. 1). First, a system was designed to obtain a reasonably homogeneous field “plateau” at 0.33 T in the stray field of the 14.1 T superconducting magnet, with an homogeneity of about 10 ppm over the 2 cm length of the sample (Fig. S1). A patterned structure of soft magnetic materials, known as ferroshims, was optimized.[5a, 11] A low-field triple resonance probe (^{15}N, ^{13}C, ^{1}H) equipped with a single-axis gradient was designed and combined with radio-frequency (rf) synthesizers and amplifiers operating at 1.42, 3.52, and 14.1 MHz. Finally, a pneumatically driven sample shuttle[3f] ensures fast transfer between the two magnetic centers. The transfer times between high and low fields are about 100 ms.

High-resolution zero-quantum spectra can be obtained in heteronuclear systems, provided the effective chemical shift evolution is carefully tailored.[12] Here, we introduce a two-field heteronuclear zero-quantum correlation experiment (2F-HZQC), where a zero-quantum ^{13}C-^{1}H coherence evolves under the difference of the offsets of ^{13}C and ^{1}H with respect to the relevant carrier frequencies at low field, while single-quantum ^{1}H coherences are detected at high field. The experiment is based on a simple sequence for heteronuclear multiple quantum coherence (HMQC)[13] and related to methyl HZQC experiments[14] with the following modifications (Fig. 2a): a two-spin order $2H_zC_z$ is generated before each transfer between the high- and low-field centers; a zero-quantum ^{13}C-^{1}H coherence is selected by phase cycling at the lowest field;[15] and a 180º pulse is used to scale the ^{1}H offset down by the ratio of gyromagnetic ratios γ_C/γ_H = 0.2514 in the indirect dimension. The evolution of the coherence at low field is thus determined by a combination of the ^{13}C and ^{1}H chemical shifts: $\Omega_{LF}^{eff} = \Omega_{LF}(^{13}C) - \gamma_C/\gamma_H\ \Omega_{LF}(^{1}H)$. This combination is immune to line-broadening due to magnetic field inhomogeneities.

The contribution of magnetic field inhomogeneities to the line width in the indirect dimension is significantly reduced by the tailored zero-quantum sequence of Figure 2a in a model sample (Figure 2b). In the natural-abundance high-resolution [^{1}H,^{13}C] 2F-HZQC spectrum of 0.5 M *tert*-butanol and 0.5 M acetone in D_2O (Figure 2b), the methyl signals of both butanol and acetone have full line-widths at half-height below 1 ppm. The single-quantum ^{13}C chemical shifts at low field can be obtained after a shearing transformation of the spectrum, safely assuming that the proton chemical shifts do not depend on the magnetic field. The spectrum has a similar appearance as a conventional high-field heteronuclear single-quantum coherence (HSQC) correlation[16] spectrum (Fig. S3).

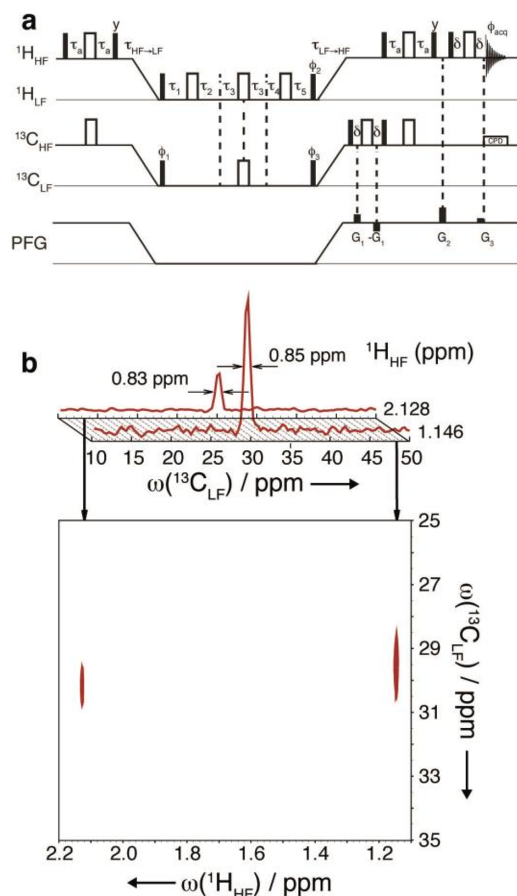

Figure 2. Two-field heteronuclear correlation spectrum. (a) Pulse sequence for 2F-HZQC. Full details are given in Supporting Information. Narrow black and wide open rectangles represent 90° and 180° pulses, respectively. All pulses are applied along the x-axis of the rotating frame unless otherwise indicated. The phases are cycled as follows: ϕ_1 = x, -x; ϕ_2 = 4{x}, 4{y}; ϕ_3 = 2{x}, 2{-x}, 2{y}, 2{-y}; with the receiver phase ϕ_{acq} = x, -x, -x, x. The phase ϕ_3 was incremented following the TPPI scheme.[17] The delays were $\tau_a = 1/(4J_{CH})$ with J_{CH} = 125 Hz, δ comprises of the length of the gradient and the gradient recovery delay, $\tau_1 = (\tau_0 + n_1\Delta t_1)c$, $\tau_2 = (\tau_0 + n_1\Delta t_1)(1\text{-}c)$, $\tau_3 = \tau_0(2c\text{-}1) + n_1\Delta t_1\ (c\text{-}0.5)$, $\tau_4 = \tau_0 c$, and $\tau_5 = \tau_0(1\text{-}c)$; Δt_1 is the time increment in the indirect dimension; and n_1 is the index of the time increment. The constant $c = (\gamma_C/\gamma_H+1)/2 = 0.6257$. Gradients G_1, G_2, and G_3 of 0.9 ms duration were applied along the z-axis with respective amplitudes 10.2 G.cm^{-1}, 15.3 G.cm^{-1}, $G_3=2(\gamma_C/\gamma_H)G_1$. GARP composite pulse decoupling[18] was applied on the ^{13}C channel during detection with $\gamma B_{1GARP}/2\pi$ = 2.08 kHz. (b) Natural abundance {^{13}C$_{LF}$, ^{1}H$_{HF}$} 2F-HZQC spectrum of a sample of 0.5 M acetone and 0.5 M tert-butyl alcohol. Low-frequency one-dimensional ^{13}C spectra (vertical cross-sections) extracted from the 2F-HZQC spectrum at δ(^{1}H$_{HF}$) = 1.146 ppm (*tert*-butyl alcohol) and δ(^{1}H$_{HF}$) = 2.128 ppm (acetone). A shearing transformation was used to obtain the ^{13}C$_{LF}$ chemical shift in the indirect dimension.

The generality of two-field NMR was further demonstrated on a protein in solution (Figure 3). A high-resolution 2F-HZQC spectrum was obtained on a sample of human ubiquitin (concentration 1.5 mM, pH 4.5) with specific isotope labeling on the δ1 ^{13}C-^{1}H$_3$ methyl groups of its seven isoleucine residues within a ^{2}H,^{12}C background.[19] The narrow line-widths in the indirect dimension are likely due to relaxation interference effects, in the manner of transverse optimized relaxation spectroscopy (TROSY**)** [20] The sensitivity is sufficient to obtain such a spectrum in 9 hours. Weak artifacts appear 2.7 ppm away from the most intense peaks in the indirect dimension. The details of this spectrum will be the subject of a forthcoming study.

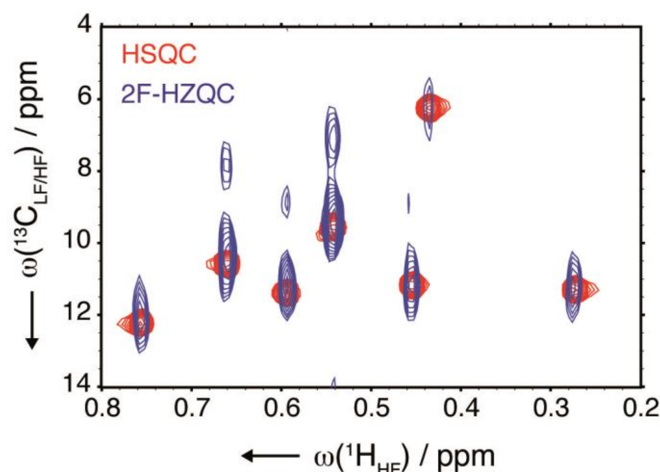

Figure 3. Two-dimension ^{1}H, ^{13}C correlation spectra obtained on human ubiquitin specifically labeled with $^{13}C^{1}H_3$ groups on isoleucine 1 positions. An HSQC spectrum recorded at 14.1 T (red) is overlaid to a two-field HZQC spectrum recorded on the two-field spectrometer (blue) with the sequence of figure 2a (additional pulsed field gradients flank all 180º pulses at both high and low field).

Most chemical and biological systems are subject to exchange broadening. In many organic molecules and biomolecules, this effect can be subtle and merely gives rise to slight perturbations of NMR spectra. In other instances, e.g., when labile protons exchange with a solvent, or in so-called molten globules,[21] chemical exchange may lead to dramatic broadening of spectra that can rule out any NMR measurements. The line broadening R_{ex} strongly depends on the magnetic field B_0 and reducing the field may have dramatic effects on the line widths.

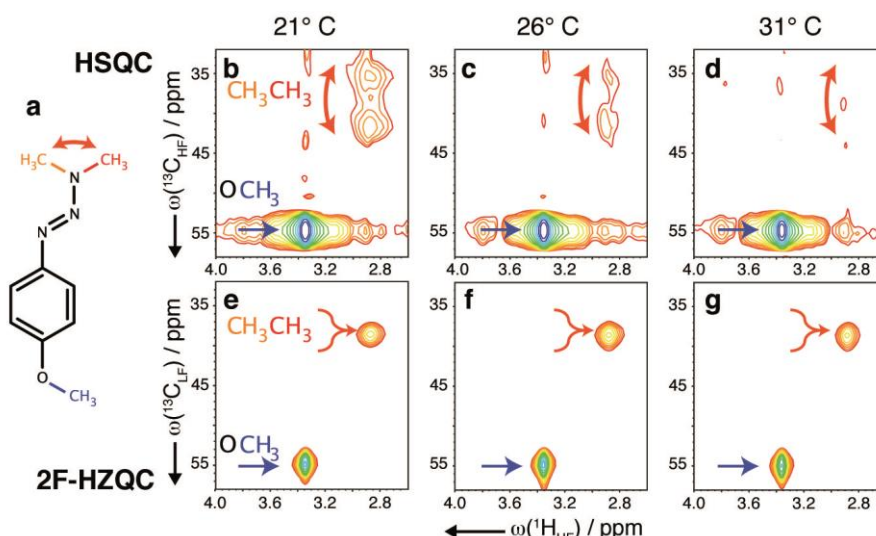

Figure 4. (a) The triazene compound under study. The two exchanging methyl groups shown in orange and red swap their positions on a sub-millisecond timescale at 31º C. The non- exchanging methoxy group shown in blue provides a reference signal. Upper boxes (b-d): conventional high-field HSQC spectra featuring increasingly broad lines at 21, 26 and 31° C. The lowest contour corresponds to 1/403 of the intensity of the methoxy signal at 31° C to show the weak signal of exchanging methyl groups. Lower boxes (e-g) two-field HZQC spectra with sharp lines regardless of temperature. The lowest contour corresponds to 1/8.3 of the intensity of the methoxy signal at 31° C.

Here, we combine excellent sensitivity and resolution at high fields with a dramatic reduction of exchange-induced line broadening at low fields. At 14.1 T, the carbon-13 signals of the two methyl groups of the dimethyl triazene compound[22] of Figure 3a are in slow exchange at 21º C. Line-shape analysis based on Markov-chain Monte-Carlo (MCMC)[23] provides k_{ex}(21º C) ~ 1700 s^{-1} (Fig. 4b and Fig. S5). The system approaches coalescence at 26º C (Fig. 4c). At 31º C, the signals of the exchanging methyl groups are broadened beyond detection (Fig. 4d). For comparison, the peak height of the signal of the non-exchanging methoxy group is at least 1000 times higher (Fig. S6). The decay rate of the carbon-13 coherence is estimated to be about 1000 s^{-1} at 14.1 T for an estimated k_{ex}(31º C) ~ 3400 s^{-1}. At 0.33 T, the exchange contribution is predicted to be dramatically reduced to 1.9 s^{-1} for

k_{ex}(31° C) ~ 3400 s^{-1}, corresponding to a line narrowing by a factor 500. Remarkably, the signal stemming from the exchanging methyl groups is readily observable and barely affected over the whole range of temperatures in the two-field correlation spectra (Fig. 4e-g). These were recorded with a suitable 2F-HZQC sequence (see Fig. S2).

As very high fields (1 GHz and above) become available, possible drawbacks of higher fields will be hard to predict. Chemical exchange can lead to severe line broadening. Carbon-13 relaxation due to chemical shift anisotropy (CSA) can become a major limitation. Many multi-dimensional NMR experiments include delays for evolution under chemical shifts that may not be optimal at one and the same field for all nuclei. In some cases, the best fields may be extremely high, (e.g., to reduce effects of second-order quadrupolar couplings[24]). For single-quantum evolution of carbonyl ^{13}C nuclei in large proteins, the optimal magnetic field lies below 14.1 T.[25] The transverse ^{13}C relaxation rates in proteins are predicted to be minimal between ca. 2 and 5 T.[26] On the other hand, the optimal field may be close to 1 GHz for TROSY of amide ^{15}N-^{1}H pairs.[27] Two-field NMR spectroscopy offers the possibility of manipulating spins such as ^{15}N, ^{13}C or ^{31}P at a field that is most appropriate for them, and detect signals of other nuclei such as ^{1}H at a higher field where the best resolution and sensitivity can be achieved. The effects of field inhomogeneities can be greatly reduced by exploiting zero-quantum coherences. The superb resolution and sensitivity that can be achieved at high fields can thus be combined with favorable properties offered by lower fields. Most high-field NMR systems may be turned into a two-field spectrometer with a plateau at 0.33 T. The design of the ferroshims requires a simple optimization for each type of superconducting magnet. Two-field systems with a low-field center above 0.5 T should rely on other technologies, which are currently available,[28] to obtain a second plateau of magnetic field. This study marks the beginning of a new generation of two-field NMR experiments. Our work opens the way to the characterization of a wide variety of systems.

Acknowledgements

We thank Kaushik Dutta (New York Structural Biology Center) for the ubiquitin plasmid as well as Rafael Brüschweiler and Lei Brüschweiler-Li (Ohio State University) for the purification protocol of ubiquitin. This work was funded by the European Research Council (ERC) under the European Community Seventh Framework Program (FP7/2007–2013), ERC Grant Agreement 279519 (2F4BIODYN).

Keywords: NMR Spectroscopy • Kinetics • Chemical Exchange • high-field NMR • low-field NMR

[1] R. R. Ernst, G. Bodenhausen, A. Wokaun, *Principles of Magnetic Resonance in One and Two Dimensions*, Clarendon Press, Oxford, **1987**.
[2] A. G. Palmer, *Chem. Rev.* **2004**, *104*, 3623-3640.
[3] aR. Kimmich, E. Anoardo, *Prog. Nucl. Magn. Reson. Spectrosc.* **2004**, *44*, 257-320; bS. Wagner, T. R. J. Dinesen, T. Rayner, R. G. Bryant, *J. Magn. Reson.* **1999**, *140*, 172-178; cA. N. Pravdivtsev, A. V. Yurkovskaya, H.-M. Vieth, K. L. Ivanov, *J. Chem. Phys.* **2014**, *141*; dA. G. Redfield, *J. Biomol. NMR* **2012**, *52*, 159-177; eC. Y. Chou, M. L. Chu, C. F. Chang, T. H. Huang, *J. Magn. Reson.* **2012**, *214*, 302-308; fC. Charlier, S. N. Khan, T. Marquardsen, P. Pelupessy, V. Reiss, D. Sakellariou, G. Bodenhausen, F. Engelke, F. Ferrage, *J Am Chem Soc* **2013**, *135*, 18665-18672; gA. S. Kiryutin, A. N. Pravdivtsev, K. L. Ivanov, Y. A. Grishin, H.-M. Vieth, A. V. Yurkovskaya, *J. Magn. Reson.* **2016**, *263*, 79-91.
[4] D. P. Weitekamp, A. Bielecki, D. Zax, K. Zilm, A. Pines, *Phys. Rev. Lett.* **1983**, *50*, 1807-1810.
[5] aM. Reese, M. T. Turke, I. Tkach, G. Parigi, C. Luchinat, T. Marquardsen, A. Tavernier, P. Hofer, F. Engelke, C. Griesinger, M. Bennati, *J. Am. Chem. Soc.* **2009**, *131*, 15086-+; bJ. Leggett, R. Hunter, J. Granwehr, R. Panek, A. J. Perez-Linde, A. J. Horsewill, J. McMaster, G. Smith, W. Koeckenberger, *PCCP* **2010**, *12*, 5883-5892; cA. Bornet, S. Jannin, G. Bodenhausen, *Chem. Phys. Lett.* **2011**, *512*, 151-154.
[6] R. Hecht, A. G. Redfield, *Physical Review* **1963**, *132*, 972-&.
[7] S. H. Koenig, R. D. Brown, *Prog. Nucl. Magn. Reson. Spectrosc.* **1990**, *22*, 487-567.
[8] aS. Laurent, L. V. Elst, R. N. Muller, *Contrast Media & Molecular Imaging* **2006**, *1*, 128-137; bS. Aime, M. Botta, S. G. Crich, G. B. Giovenzana, R. Pagliarin, M. Piccinini, M. Sisti, E. Terreno, *J. Biol. Inorg. Chem.* **1997**, *2*, 470-479; cD. H. Powell, O. M. NiDhubhghaill, D. Pubanz, L. Helm, Y. S. Lebedev, W. Schlaepfer, A. E. Merbach, *J. Am. Chem. Soc.* **1996**, *118*, 9333-9346; dS. R. Banerjee, E. J. Ngen, M. W. Rotz, S. Kakkad, A. Lisok, R. Pracitto, M. Pullambhatla, Z. P. Chen, T. Shah, D. Artemov, T. J. Meade, Z. M. Bhujwalla, M. G. Pomper, *Angew. Chem.-Int. Edit.* **2015**, *54*, 10778-10782; eY. Gossuin, Z. Serhan, L. Sandiford, D. Henrard, T. Marquardsen, R. T. M. Rosales, D. Sakellariou, F. Ferrage, *Appl. Magn. Reson.* **2016**, *47*, 237-246.
[9] aJ. P. Korb, M. WhaleyHodges, R. G. Bryant, *Physical Review E* **1997**, *56*, 1934-1945; bS. Stapf, R. Kimmich, R. O. Seitter, *Phys. Rev. Lett.* **1995**, *75*, 2855-2858.
[10] aR. Kimmich, N. Fatkullin, *Nmr - 3d Analysis - Photopolymerization* **2004**, *170*, 1-113; bE. Persson, B. Halle, *J. Am. Chem. Soc.* **2008**, *130*, 1774-1787; cM. F. Roberts, Q. Z. Cui, C. J. Turner, D. A. Case, A. G. Redfield, *Biochemistry* **2004**, *43*, 3637-3650; dC. Luchinat, G. Parigi, *J. Am. Chem. Soc.* **2007**, *129*, 1055-

1064; eG. Diakova, Y. A. Goddard, J.-P. Korb, R. G. Bryant, *Biophys. J.* **2010**, *98*, 138-146; fM. W. Clarkson, M. Lei, E. Z. Eisenmesser, W. Labeikovsky, A. Redfield, D. Kern, *J. Biomol. NMR* **2009**, *45*, 217-225.
[11] P. A. Bovier, R. Schauwecker, D. Eckert*, Vol. US2010171495 (A1)* (Ed.: E. P. Office), **2010**.
[12] S. L. Duce, L. D. Hall, T. J. Norwood, *J. Magn. Reson.* **1990**, *89*, 273-286.
[13] L. Muller, *J. Am. Chem. Soc.* **1979**, *101*, 4481-4484.
[14] V. Tugarinov, R. Sprangers, L. E. Kay, *J. Am. Chem. Soc.* **2004**, *126*, 4921-4925.
[15] G. Bodenhausen, H. Kogler, R. R. Ernst, *J. Magn. Reson.* **1984**, *58*, 370-388.
[16] G. Bodenhausen, D. J. Ruben, *Chem. Phys. Lett.* **1980**, *69*, 185-188.
[17] D. Marion, K. Wuthrich, *Biochem. Biophys. Res. Commun.* **1983**, *113*, 967-974.
[18] A. J. Shaka, P. B. Barker, R. Freeman, *J. Magn. Reson.* **1985**, *64*, 547-552.
[19] V. Tugarinov, V. Kanelis, L. E. Kay, *Nature Protocols* **2006**, *1*, 749-754.
[20] V. Tugarinov, P. M. Hwang, J. E. Ollerenshaw, L. E. Kay, *J. Am. Chem. Soc.* **2003**, *125*, 10420-10428.
[21] aB. A. Schulman, P. S. Kim, C. M. Dobson, C. Redfield, *Nat. Struct. Biol.* **1997**, *4*, 630-634; bP. A. Jennings, P. E. Wright, *Science* **1993**, *262*, 892-896.
[22] C. S. Rondestvedt, S. J. Davis, *J. Org. Chem.* **1957**, *22*, 200-203.
[23] H. Haario, M. Laine, A. Mira, E. Saksman, *Statistics and Computing* **2006**, *16*, 339-354.
[24] Z. H. Gan, P. Gor'kov, T. A. Cross, A. Samoson, D. Massiot, *J. Am. Chem. Soc.* **2002**, *124*, 5634-5635.
[25] V. Tugarinov, R. Muhandiram, A. Ayed, L. E. Kay, *J. Am. Chem. Soc.* **2002**, *124*, 10025-10035.
[26] C. Charlier, S. F. Cousin, F. Ferrage, *Chem. Soc. Rev.* **2016**, *45*, 2410-2422.
[27] K. Pervushin, R. Riek, G. Wider, K. Wüthrich, *Proc. Natl. Acad. Sci. USA* **1997**, *94*, 12366-12371.
[28] C.-Y. Chou, F. Ferrage, G. Aubert, D. Sakellariou, *Scientific Reports* **2015**, *5*, 12200.