VIP Protein Analysis

DOI: 10.1002/anie.201008244

Proton-Detected Solid-State NMR Spectroscopy of Fibrillar and Membrane Proteins**

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Structural information is key to understanding biological processes. Insoluble proteins like membrane proteins and amyloid fibrils are a large class of proteins which are underrepresented in the protein databank (PDB). As of today, only 7% of all entries in the PDB (total number of entries: 66161; http://www.rcsb.org, November 2010) refer to either a membrane protein (4994 entries) or an amyloid fibril structure (67 entries). Given the fact that many drugs target membrane proteins involved in, for example, signal transduction,[1] structural information is highly desirable to obtain a better understanding of the underlying biochemical mechanisms. In contrast to X-ray crystallography and solution-state NMR spectroscopy, solid-state NMR spectroscopy is a method that allows the investigation of membrane proteins in their native environment. Clearly, the lipid environment can have an impact on their functionality and structure.[2] However, crystallographic structures in cubic lipid phases are not easily obtained.[3] Although electron microscopy is able to provide images in the native lipid environment, the resolution is comparatively low. Although nanodisc preparations yield solution-state NMR spectra in a nativelike context,[4,5] the molecular weight of the resulting complexes is on the upper limit for conventional assignment experiments.

To date, only a few uniformly isotopically enriched membrane proteins could be structurally characterized by magic-angle spinning (MAS) solid-state NMR spectroscopy, including proteorhodopsin,[6] VDAC,[7] and phospholamban.[8,9] Fibrillar proteins, which play a crucial role in many neurological disorders such as Parkinson's or Alzheimer's disease, are not accessible using conventional structure determination methods and could to date only be analyzed indirectly, for example, by solution-state NMR spectroscopy using H/D exchange methods.[10] Recently, the structure of the prion domain of HET-s was solved.[11,12] Interestingly, while high-resolution spectra of certain systems like HET-s and the human prion protein[13] are available, spectral resolution is limited in the cases of amyloid fibrils of the Alzheimer's disease β-amyloid peptide[14,15] and α-synuclein.[16,17]

In contrast to the solution state, the coherence lifetimes in the solid state are not affected by molecular tumbling, which makes the technique amenable to proteins of higher molecular weight. Clearly, broad NMR resonances can arise from sample heterogeneity. However, preparation of biological samples becomes more and more routine, and is—after careful optimization—not the main factor determining the spectral quality. Additional contributions to the resonance linewidth arise from limitations of the applicable heteronuclear decoupling radio-frequency fields and temperature fluctuations within the sample volume or throughout the experiment. Decoupling-induced heating is significant for protonated samples, even if probes are employed which exclude electric fields in the sample volume.[18] Heating can further affect sample stability and thus spectral resolution. These drawbacks are avoided in extensively deuterated proteins.[19–21] The elimination of most of the unwanted strong $^{1}H,^{1}H$ dipolar interactions keeps only the weak couplings of interest. Potential resonance broadening arising from motion on the nano- to microsecond timescale can be accounted for by making use of TROSY-type experiments in the solid state.[22] The availability of an additional nucleus for chemical-shift dispersion increases the reliability of the assignment process[23] and allows the determination of long-range distances among weakly coupled spins, which in turn provide useful restraints for structure calculations.[21,24,25]

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[**] We are grateful to Clemens Glaubitz, Frankfurt/Main, for access to measurement time on the 850 MHz spectrometer. This research was supported by the Leibniz-Gemeinschaft and the DFG (Re1435, SFB449, SFB740). As a Kekulé fellow, R.L. received financial support from the Verband der Chemischen Industrie (VCI).

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/anie.201008244.

Since no high-power proton decoupling is required for deuterated samples, PRE (paramagnetic relaxation enhancement) can be used to achieve extremely high experimental repetition rates.[18,26] To date, high-resolution ^{1}H-detected spectra in the solid state have only been reported for microcrystalline proteins.[19,27] Herein, we show that noncrystalline, deuterated membrane proteins and amyloid fibrils yield high-resolution proton spectra with a spectral quality that is comparable to that obtained for microcrystalline proteins. The experiments are demonstrated using a fibril sample of the Alzheimer's disease β-amyloid peptide $A\beta^{1-40}$, the α-helical membrane protein bacteriorhodopsin, and the β-barrel outer membrane protein OmpG.

Amyloidogenic, fibrillar proteins represent the greatest challenge for spectroscopy, as a sample may contain a large number of different structural polymorphs.[14,15,28] Conformational variations are observed even if fibril growth is seeded and buffer conditions are controlled (pH value, salt, temperature).[28] As a consequence, resonance lines can potentially be inhomogeneously broadened, which is detrimental to sensitivity. To show that the deuteration approach can be successfully implemented in noncrystalline systems, we prepared amyloid fibrils of the triply labeled Alzheimer's disease β-amyloid peptide $A\beta^{1-40}$.[29] The fibrils were grown by performing several rounds of seeding (see the Supporting Information). During preparation, the sample was constantly agitated to accelerate fibril growth. The fibrils obtained in this way are very homogeneous as judged by electron microscopy (EM) and contain mostly fibrils of twisted morphology with a crossover distance of approximately (130 ± 20) nm (Figure 1 A). A representative 2D H/N correlation is shown Figure 1 B. Typical line widths are on the order of 90–220 and 30–50 Hz for $^{1}H^{N}$ and ^{15}N, respectively. Even though the H/N correlation spectrum appears to have a lot of spectral overlap, the respective ^{13}C-edited 3D experiments are well-dispersed (Supporting Information Figure S3). The resolution in the ^{15}N,^{13}CO dimension appears to be better than the resolution that is achieved in the ^{1}H,^{15}N projection. Sequential assignments were obtained by proton-detected HNCO/HNCACO[30] and tailored HNCACB/HNCA[23] experiments (Figure 1 C). The respective pulse schemes are given in the Supporting Information. With this approach, we were able to assign residues A21to V39 in $A\beta^{1-40}$. The N terminus of the peptide (residues 1–10) has been reported to exhibit significant line broadening.[31] Also, the ^{13}C linewidths for residues 10–20 are systematically increased (by ca. 25 %) compared to C-terminal residues, which makes assignment of these residues difficult.

A second system that we tested to validate the method is the outer membrane protein G (OmpG), which is involved in the uptake of large oligosaccharides in Gram-negative bacteria.[32] In contrast to preparations of $A\beta^{1-40}$ fibrils, in which structural homogeneity is difficult to control, 2D crystals of reconstituted OmpG in its native lipid environment can be prepared with a high degree of homogeneity. This homogeneity is reflected in the resolution achievable in the electron microscopic projection reconstruction structure,[33] as well as in the ^{13}C,^{13}C correlation spectra,[34] which are of exceptional quality and comparable to the spectra of a microcrystalline protein preparation (see the Supporting Information). Perdeuterated OmpG was reconstituted in perdeuterated *E.coli* lipid bilayers using a buffer containing 70 % D_2O and 30 % H_2O. A representative H/N correlation spectrum of the sample is shown in Figure 2 A. Back-exchange of protons was achieved during refolding from a urea-denatured protein preparation (see the Supporting Information). Obviously, H/N correlations are more sensitive to subtle conformational alterations and to dynamics in comparison to 2D ^{13}C,^{13}C correlation spectra, since the apparent resolution in the latter is determined by ^{13}C,^{13}C scalar couplings. The correlation spectrum superimposes well with the solution-state spectra obtained in detergent

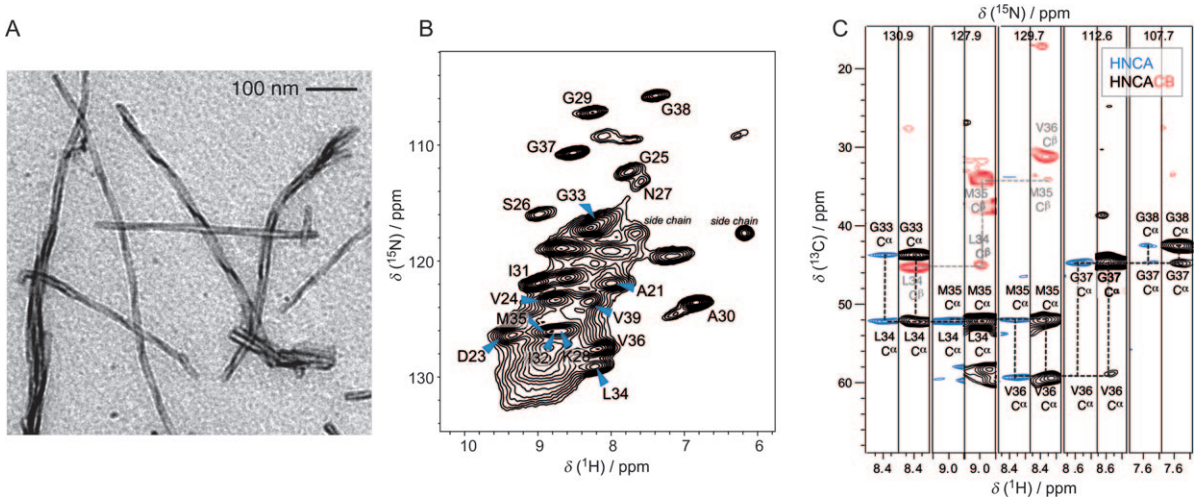

Figure 1. MAS solid-state NMR experiments recorded for a perdeuterated fibril sample of the Alzheimer's disease peptide $A\beta^{1-40}$. A) Representative EM image of the fibrils employed for the NMR spectroscopy experiments. B) 2D H/N correlation of $A\beta^{1-40}$ fibrils. C) Strip plot of a proton-detected HNCA (blue) and HNCACB experiment, showing the sequential assignments between residues L34 and G38 (black and red are used for positive and negative contour levels, respectively). All experiments were conducted at approximately 25 °C and a magnetic field strength of 14.1 T with a MAS rotation frequency of 20 kHz.

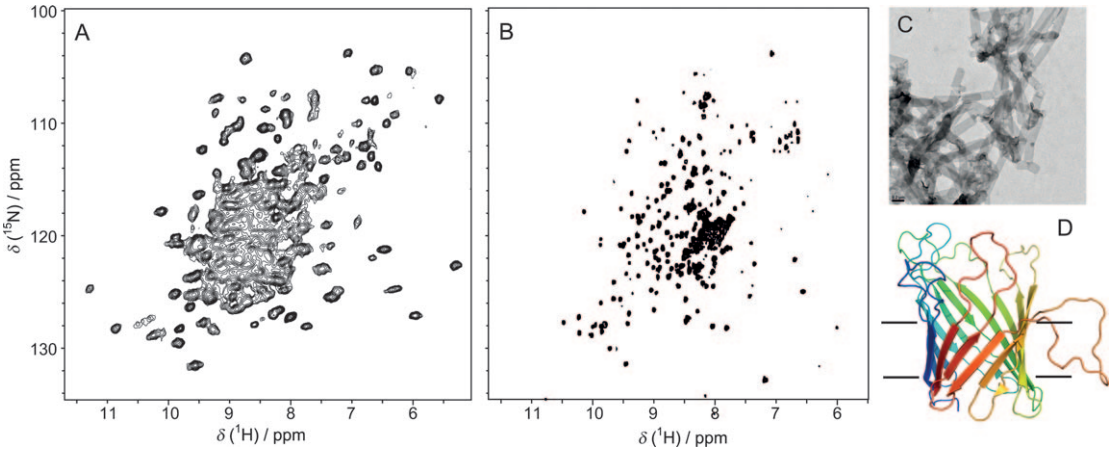

Figure 2. The β-barrel porin OmpG. Comparison of ^{1}H-detected 2D H/N correlations obtained from perdeuterated and partially proton-back-substituted samples prepared with deuterated lipids in the solid state at approximately 25 °C and a magnetic field strength of 19.97 T (A) and solution-state NMR TROSY spectra (B, at 21.14 T and 27 °C). For the solution-state sample, $^{1}H^{N}$ linewidths on the order of 15–20 Hz are observed (vs. 50–80 Hz in the solid-state spectrum). Solution-state spectra were recorded using dodecyl-β-D-maltoside-solubilized OmpG. C) Electron micrograph of 2D crystals of OmpG reconstituted in lipid bilayers. D) Structure of OmpG in detergent micelles (PDB: 2JQY).[35]

micelles[35] (see the Supporting Information). The similarity of the isotropic chemical shifts is a first hint that the structure of OmpG in the micelle-bound state and in the lipid bilayer are highly similar.

The third example to demonstrate the deuteration approach involves the α-helical membrane protein bacteriorhodopsin (bR).[36] bR is a bacterial proton pump found in the purple membrane of *H. salinarium*, which adopts two conformational states in the dark, bR_{555} and bR_{568}, at a ratio of 60:40.[37] In the case of bR, we find significantly better resolved spectra in the solid state (Figure 3). The $^{1}H/^{15}N$ linewidths are comparable to the linewidths found for a microcrystalline protein preparation (α-spectrin SH3). Cross sections along the ^{15}N and ^{1}H dimensions for a representative peak are depicted in Figure 3D. Instead of the expected 2 × 250 resonances, however, we observe only approximately 100 cross peaks. We assume that predominantly resonances of residues in the water-accessible channel are observed. Resonances of residues located in flexible loops are presumably not observable, as experiments using dipolar and scalar polarization transfers yield similar spectra. Unlike OmpG, exchangeable deuterons were back-substituted by incubation at pH 10 over a period of 3–4 weeks. These conditions seem insufficient to substitute all exchangeable protons in the core of the protein structure. In the future, labeling schemes in which protons are randomly distributed at non-exchangeable sites will overcome this preparative bottleneck.[21]

The solution-state NMR spectrum of detergent-solubilized bR (Figure 2B) contains incorrectly folded protein, showing H^{N} proton chemical shifts at around $\delta =$ 8.4 ppm. Clearly, these resonances are absent in the solid-state NMR spectrum, underlining the significance of the native lipid environment for structural investigations of membrane proteins.

Table 1 shows the experimental T_2 relaxation times for $^{1}H^{N}$, ^{15}N, and ^{13}CO for a fibril sample of $A\beta^{1-40}$ as well as for a microcrystalline sample of the α-spectrin SH3 domain at the same temperature. The average T_2 value in $A\beta^{1-40}$ is approximately 30% smaller. Although the high β-sheet content should, in principle, yield a favorable chemical-shift dispersion, the effective resolution of OmpG (280 amino acids) in the 2D H/N correlation spectra is limited in the solid state. This limitation is due to the experimental line width on the

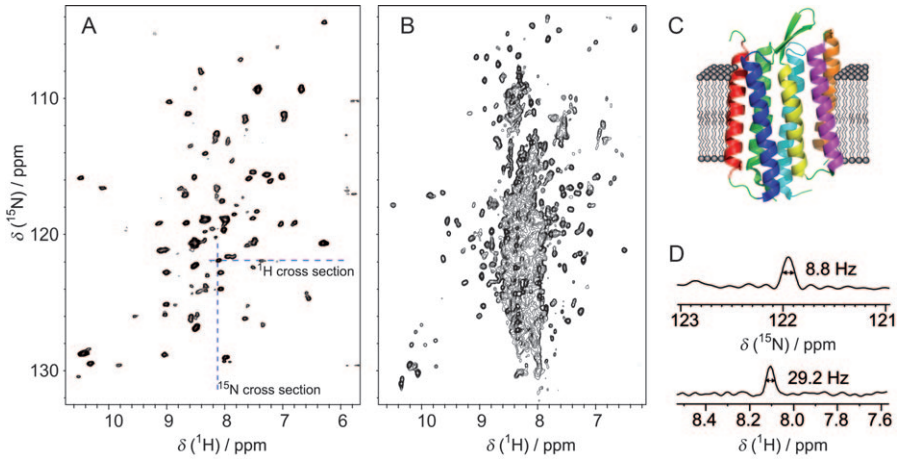

Figure 3. Bacteriorhodopsin (bR) from the purple membrane of *H. salinarium*. A) Proton-detected solid-state NMR H/N correlation of perdeuterated bR in purple membrane after back-substitution of 10% protons at exchangeable sites. The spectrum was recorded over 6 h at 16.44 T and approximately 25 °C. B) Solution-state NMR TROSY correlation of detergent-solubilized bR.[38] Solution-state spectra were recorded using dodecyl-β-D-maltoside-solubilized bR. C) Structure of bR in the lipid bilayer (PDB: 1C3W).[39] D) Cross sections extracted from (A) at the positions marked by dashed lines. The line width in the solid state can be as narrow as 9 and 30 Hz in the ^{15}N and ^{1}H dimensions, respectively.

Table 1: ^{1}H,^{15}N T_2 relaxation times and theoretical and experimental line widths for a fibril sample of perdeuterated $A\beta^{1-40}$, the outer membrane β-barrel protein OmpG, and the microcrystalline α-spectrin SH3.[a]

	$^{1}H^{N}$ T_2	^{1}H LW		^{15}N T_2	^{15}N LW	
	[ms]	calc [Hz]	exp [Hz]	[ms]	calc [Hz]	exp [Hz]
OmpG in						
protonated lipids[b]	6.8 ± 0.5	46.8 ± 3.4	121 ± 42	10.0 ± 1.0	31.8 ± 3.2	60 ± 34
deuterated lipids[b]	9.2 ± 1.0	34.6 ± 3.8	101 ± 31	11.1 ± 2.3	28.7 ± 6.2	53 ± 23
$A\beta^{1-40}$[c]	8.2 ± 0.2	38.8 ± 1.0	170 ± 47	13.5 ± 0.2	23.6 ± 0.3	38 ± 10
α-spectrin SH3[c]	12.4 ± 0.9	25.7 ± 1.8	25 ± 5	21.8 ± 3.3	14.6 ± 2.2	15 ± 3

[a] T_2 was measured in an echo experiment with one 180° pulse in the center of the incremented delay for the H^{N} bulk signal. In all cases, T_2 values were determined setting the MAS rotation frequency to 20 kHz. The SH3 domain and the $A\beta^{1-40}$ fibril sample were prepared in the presence of 75 mM [Cu(edta)], whereas no metal chelate was added in the OmpG preparations. All relaxation values were determined setting the effective temperature to 25 °C. [b] Relaxation times for OmpG samples containing 30% protons at exchangeable sites were determined in the absence of [Cu(edta)]. As shown previously,[18] paramagnetic relaxation enhancement only has an effect on longitudinal relaxation, but leaves transversal relaxation and line width unaffected. [c] Relaxation times for $A\beta^{1-40}$ and α-spectrin SH3 refer to a sample containing 25% protons at exchangeable sites. These samples were fibrillized in the presence of 75 mM [Cu(edta)].

order of (101 ± 31) and (53 ± 23) Hz for ^{1}H and ^{15}N, respectively. We do not find large differences in resolution for OmpG samples reconstituted in deuterated versus protonated lipids. For comparison, the linewidths of a microcrystalline preparation of the α-spectrin SH3 domain are on the order of 25 and 15 Hz for ^{1}H and ^{15}N, respectively.[40] The experimental homogeneous linewidths for OmpG are on the order of 30 Hz for both ^{1}H and ^{15}N (Table 1). Under these circumstances, H/N dipolar-based magnetization transfers perform significantly better than scalar-based schemes. However, a smaller homogeneous linewidth (with respect to the experimental one) indicates some degree of conformational heterogeneity. Taking into account that the proton density is comparable to the SH3 preparation, we assume that the difference between the homogeneous linewidths for OmpG and SH3 is due to dynamics. The residual $^{1}H/^{15}N$ linewidth in the case of OmpG is thus determined by a slow uniaxial rotation in the lipid bilayer. In this case, a rotational correlation time on the microsecond timescale might be expected.[41,42]

Similar to the situation for OmpG, we find for $A\beta^{1-40}$ that the echo T_2 transversal relaxation times are systematically longer than what would be expected from the observed linewidth (Table 1). Sample heterogeneity accounts for the greater part of this discrepancy. Interestingly, the apparent resolution in the heteronuclear dimension such as ^{13}CO and ^{15}N seems better than that achieved in the ^{1}H dimension (Supporting Information Figure S3). This finding might be due to the large gyromagnetic ratio of protons and the fact that protons sense structural heterogeneity with greater sensitivity. Furthermore, echo T_2 times are reduced in comparison to the relaxation times measured for the α-spectrin SH3 domain. As the spin density for the two samples is comparable, we assume that dynamic processes can in part account for the lower T_2 relaxation times. We do not find any differential relaxation for the spin states ^{15}N-Hα and ^{15}N-Hβ in $A\beta^{1-40}$ fibrils at 20 °C (Supporting Information, Figure S5). This result indicates that processes on the nano- to microsecond timescale are not the origin of the observed line broadening. Exchange dynamics between soluble and insoluble aggregation states, however, has been observed previously.[43–45] We therefore speculate that chemical exchange can account for the observed differences of the echo T_2 transversal relaxation times between $A\beta^{1-40}$ fibrils and microcrystalline proteins.

In summary, we have shown that perdeuteration and partial back-substitution of exchangeable protons can be successfully implemented for the investigation of membrane proteins and amyloid fibrils in the solid state. The labeling scheme presented provides high sensitivity and enables proton detection and correlation experiments involving ^{1}H, ^{15}N, and ^{13}C resonances. The inclusion of proton chemical shifts yields more reliable assignments for protein backbone resonances and enables the application of a large suite of experiments for characterization of structure and dynamics for a large range of pharmacologically important proteins.

Received: December 29, 2010
Published online: April 14, 2011

Keywords: amyloid fibrils · lipid membrane · membrane proteins · outer membrane protein G · solid-state NMR spectroscopy

[1] J. Drews, *Science* **2000**, *287*, 1960.
[2] S. Dracheva, S. Bose, R. W. Hendler, *FEBS Lett.* **1996**, *382*, 209.
[3] E. M. Landau, J. P. Rosenbusch, *Proc. Natl. Acad. Sci. USA* **1996**, *93*, 14532.
[4] J. M. Gluck, M. Wittlich, S. Feuerstein, S. Hoffmann, D. Willbold, B. W. Koenig, *J. Am. Chem. Soc.* **2009**, *131*, 12060.
[5] T. Raschle, S. Hiller, T.-Y. Yu, A. J. Rice, T. Walz, G. Wagner, *J. Am. Chem. Soc.* **2009**, *131*, 17777.
[6] L. Shi, E. M. R. Lake, M. A. M. Ahmed, L. S. Brown, V. Ladizhansky, *Biochim. Biophys. Acta Biomembr.* **2009**, *1788*, 2563–2574.
[7] R. Schneider, M. Etzkorn, K. Giller, V. Daebel, J. Eisfeld, M. Zweckstetter, C. Griesinger, S. Becker, A. Lange, *Angew. Chem.* **2010**, *122*, 1926; *Angew. Chem. Int. Ed.* **2010**, *49*, 1882.
[8] K. Seidel, O. C. Andronesi, J. Krebs, C. Griesinger, H. S. Young, S. Becker, M. Baldus, *Biochemistry* **2008**, *47*, 4369.
[9] N. J. Traaseth, L. Shi, R. Verardi, D. G. Mullen, G. Barany, G. Veglia, *Proc. Natl. Acad. Sci. USA* **2009**, *106*, 10165.
[10] N. A. Whittemore, R. Mishra, I. Kheterpal, A. D. Williams, R. Wetzel, E. H. Serpersu, *Biochemistry* **2005**, *44*, 4434.
[11] C. Wasmer, A. Lange, H. Van Melckebeke, A. B. Siemer, R. Riek, B. H. Meier, *Science* **2008**, *319*, 1523.

[12] H. Van Melckebeke, C. Wasmer, A. Lange, A. B. Eiso, A. Loquet, A. Bockmann, B. H. Meier, *J. Am. Chem. Soc.* **2010**, *132*, 13765 – 13775.
[13] J. J. Helmus, K. Surewicz, P. S. Nadaud, W. K. Surewicz, C. P. Jaroniec, *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 6284 – 6289.
[14] A. T. Petkova, R. D. Leapman, Z. H. Guo, W. M. Yau, M. P. Mattson, R. Tycko, *Science* **2005**, *307*, 262.
[15] A. K. Paravastu, R. D. Leapman, W.-M. Yau, R. Tycko, *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 18349.
[16] H. Heise, W. Hoyer, S. Becker, O. C. Andronesi, D. Riedel, M. Baldus, *Proc. Natl. Acad. Sci. USA* **2005**, *102*, 15871.
[17] K. D. Kloepper, K. L. Hartman, D. T. Ladror, C. M. Rienstra, *J. Phys. Chem. B* **2007**, *111*, 13353.
[18] R. Linser, V. Chevelkov, A. Diehl, B. Reif, *J. Magn. Reson.* **2007**, *189*, 209.
[19] V. Chevelkov, K. Rehbein, A. Diehl, B. Reif, *Angew. Chem.* **2006**, *118*, 3963; *Angew. Chem. Int. Ed.* **2006**, *45*, 3878.
[20] V. Agarwal, B. Reif, *J. Magn. Reson.* **2008**, *194*, 16.
[21] S. Asami, P. Schmieder, B. Reif, *J. Am. Chem. Soc.* **2010**, *132*, 15133 – 15135.
[22] R. Linser, U. Fink, B. Reif, *J. Am. Chem. Soc.* **2010**, *132*, 8891 – 8893.
[23] R. Linser, U. Fink, B. Reif, *J. Magn. Reson.* **2008**, *193*, 89 – 93.
[24] D. H. Zhou, J. J. Shea, A. J. Nieuwkoop, W. T. Franks, B. J. Wylie, C. Mullen, D. Sandoz, C. M. Rienstra, *Angew. Chem.* **2007**, *119*, 8532; *Angew. Chem. Int. Ed.* **2007**, *46*, 8380.
[25] R. Linser, B. Bardiaux, V. Higman, U. Fink, B. Reif, *J. Am. Chem. Soc.* **2011**, DOI: 10.1021/ja110222h.
[26] N. P. Wickramasinghe, M. Kotecha, A. Samoson, J. Past, Y. Ishii, *J. Magn. Reson.* **2007**, *184*, 350.
[27] P. Schanda, M. Huber, R. Verel, M. Ernst, B. H. Meier, *Angew. Chem.* **2009**, *121*, 9486; *Angew. Chem. Int. Ed.* **2009**, *48*, 9322.
[28] K. Klement, K. Wieligmann, J. Meinhardt, P. Hortschansky, W. Richter, M. Fandrich, *J. Mol. Biol.* **2007**, *373*, 1321.
[29] M. Dasari, A. Espargaro, R. Sabate, J. M. Lopez del Amo, U. Fink, G. Grelle, J. Bieschke, S. Ventura, B. Reif, *ChemBioChem* **2011**, *12*, 407 – 423.
[30] R. Linser, U. Fink, B. Reif, *J. Biomol. NMR* **2010**, *47*, 1.
[31] A. T. Petkova, Y. Ishii, J. J. Balbach, O. N. Antzutkin, R. D. Leapman, F. Delaglio, R. Tycko, *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 16742.
[32] D. A. Fajardo, J. Cheung, C. Ito, E. Sugawara, H. Nikaido, R. Misra, *J. Bacteriol.* **1998**, *180*, 4452.
[33] M. Behlau, D. J. Mills, H. Quader, W. Kuhlbrandt, J. Vonck, *J. Mol. Biol.* **2001**, *305*, 71.
[34] M. Hiller, L. Krabben, K. R. Vinothkumar, F. Castellani, B. Van Rossum, W. Kühlbrandt, H. Oschkinat, *ChemBioChem* **2005**, *6*, 1679.
[35] B. Liang, L. K. Tamm, *Proc. Natl. Acad. Sci. USA* **2007**, *104*, 16140.
[36] D. Oesterhelt, W. Stoeckenius, *Nature* **1971**, *233*, 149.
[37] M. L. Mak-Jurkauskas, V. S. Bajaj, M. K. Hornstein, M. Belenky, R. G. Griffin, J. Herzfeld, *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 883.
[38] M. Schubert, M. Kolbe, B. Kessler, D. Oesterhelt, P. Schmieder, *ChemBioChem* **2002**, *3*, 1019.
[39] H. Luecke, B. Schobert, H. T. Richter, J. P. Cartailler, J. K. Lanyi, *J. Mol. Biol.* **1999**, *291*, 899.
[40] Ü. Akbey, S. Lange, T. W. Franks, R. Linser, A. Diehl, B. J. van Rossum, B. Reif, H. Oschkinat, *J. Biomol. NMR* **2010**, *46*, 67 – 73.
[41] P. G. Saffman, M. Delbruck, *Proc. Natl. Acad. Sci. USA* **1975**, *72*, 3111.
[42] R. H. Austin, S. S. Chan, T. M. Jovin, *Proc. Natl. Acad. Sci. USA* **1979**, *76*, 5650.
[43] S. Narayanan, B. Reif, *Biochemistry* **2005**, *44*, 1444.
[44] N. Carulla, G. L. Caddy, D. R. Hall, J. Zurdo, M. Gairi, M. Feliz, E. Giralt, C. V. Robinson, C. M. Dobson, *Nature* **2005**, *436*, 554.
[45] J. Bieschke, J. Russ, R. P. Friedrich, D. E. Ehrnhoefer, H. Wobst, K. Neugebauer, E. E. Wanker, *Proc. Natl. Acad. Sci. USA* **2010**, *107*, 7710.

