

Two decades of progress in structural and dynamic
studies of amyloids by solid-state NMR

Christopher P. Jaroniec*

*Department of Chemistry and Biochemistry, The Ohio State University,
100 West 18th Avenue, Columbus, Ohio 43210, USA*

*E-mail: jaroniec.1@osu.edu

Abstract

In this perspective article I briefly highlight the rapid progress made over the past two decades in atomic level structural and dynamic studies of amyloids, which are representative of non-crystalline biomacromolecular assemblies, by magic-angle spinning solid-state NMR spectroscopy. Given new and continuing developments in solid-state NMR instrumentation and methodology, ongoing research in this area promises to contribute to an improved understanding of amyloid structure, polymorphism, interactions, assembly mechanisms, and biological function and toxicity.

Keywords: Solid-state NMR; Magic-angle spinning; Protein structure and dynamics; Amyloid fibrils

1. Introduction

The past two decades or so have seen incredible advances in the application of magic-angle spinning (MAS) solid-state nuclear magnetic resonance (NMR) to biological systems. Until the mid 1990's, solid-state NMR structural studies of biomolecules were largely confined to samples containing NMR-active low- γ ^{13}C and ^{15}N nuclei incorporated at specific sites, and carried out at low to moderate magnetic fields (< 500 MHz ^1H frequency) and sample spinning rates (< 10 kHz) by using radiofrequency pulse schemes designed to measure anisotropic chemical shift and/or through-space magnetic dipole-dipole interactions [1,2]. While “low throughput” and cost and labor intensive, these pioneering studies nevertheless clearly underscored the potential of solid-state NMR—which suffers from relatively few limitations related to molecular size or crystalline state—to grow into a powerful tool for the atomic level structural (and dynamic) analysis of large biomacromolecular complexes and assemblies that may contain proteins, nucleic acids, carbohydrates, lipids and/or small molecule cofactors or ligands and are not amenable to or present considerable difficulties for other, complementary high-resolution techniques including solution NMR, X-ray crystallography and cryogenic electron microscopy (cryo-EM). This motivated and paved the way for a number of critical developments in solid-state NMR instrumentation and methodology as well as in sample preparation approaches including: (i) homogeneous high-field (800-1000 MHz) magnets, with commercial >1 GHz instruments in production or final stages of development, (ii) high-quality triple- and quadruple-resonance MAS NMR probes optimized for biological samples and capable of achieving sample spinning rates exceeding 100 kHz [3-7], (iii) new isotope labeling methods in addition to the standard uniform ^{13}C and ^{15}N enrichment [8-10], (iv) general protocols for the generation of optimal solid-state NMR samples with high degree of local order that yield the highest resolution

1
2
3
4 spectra [10-12], and (v) efficient pulse schemes for spin decoupling, recoupling and
5
6 magnetization transfer facilitating backbone and side-chain assignments and distance and torsion
7
8 angle measurements in coupled multiple spin systems [13,14]. Collectively, these developments
9
10 have made possible the comprehensive solid-state NMR analysis of both structured and dynamic
11
12 domains of large complexes and supramolecular assemblies, containing protein subunits of up to
13
14 ~200-300 amino acids in size and exhibiting motions on a wide range of timescales, under
15
16 physiologically relevant conditions [15-34]. Most importantly, they have enabled fundamentally
17
18 new questions, not readily addressable by other experimental techniques, to be explored,
19
20 providing unique insights into biological function and mechanism.
21
22
23
24

25
26 In this short perspective article, I highlight the remarkable progress that has been made
27
28 over the past ~20 years in the application of MAS solid-state NMR spectroscopy toward the
29
30 understanding of structure, dynamics and interactions in amyloids, which are a representative
31
32 example of the types of systems that can be successfully investigated by this technology
33
34 [16,17,19,21,25,27,29,32]. Amyloids, which are discussed in additional detail below, are fibrillar
35
36 supramolecular peptide or protein aggregates that are particularly challenging to probe at atomic
37
38 resolution by most experimental structural biology tools given that they are highly polymorphic,
39
40 frequently lack exact long-range order, and typically contain both structured and dynamically
41
42 disordered large domains. In addition to amyloids, solid-state NMR has been utilized to
43
44 successfully investigate other classes of biomacromolecular complexes and assemblies including
45
46 but not limited to membrane proteins, enzymes, cytoskeletal and viral protein assemblies and
47
48 protein-nucleic acid complexes such as chromatin, as discussed in detail in recent reviews
49
50 [15,18,20,23-26,28,30,31,33,34].
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

2. Amyloids

The assembly of peptides and proteins into amyloids, including structural and mechanistic aspects as well as the biological consequences of amyloid formation, has been discussed in depth in a number of excellent reviews [35-41]. Briefly, amyloids are filamentous structures that are typically several nanometers in diameter and up to a few microns in length (see Figure 1 for a representative example). They may consist of one or multiple protofilaments that wind together along the fibril long axis, where the core of each protofilament is made up of peptide or protein molecules having similar conformation stacked onto one another in a cross- β architecture with the β -strand segments of successive molecules roughly perpendicular to the fibril long axis and hydrogen-bonded [35,36,40]. While many polypeptides having disparate amino acid sequences and native structures, ranging from intrinsically disordered to globular to membrane-bound, are capable of undergoing conformational conversion to the amyloid state under appropriate conditions *in vitro* (e.g., at low pH or in presence of denaturants), ~50 human proteins can do so *in vivo* under physiological conditions leading to disease with β -amyloid and tau proteins associated with Alzheimer's disease and α -synuclein associated with Parkinson's disease being among the most prominent examples [39]. Furthermore, recent studies indicate that in certain cases amyloid formation is non-pathogenic and important for function [37,38].

3. Solid-State NMR Structural Studies of Amyloids

Until the early 1990's structural characterization of amyloids was largely limited to X-ray fiber diffraction, which typically revealed ~4.8 Å meridional and ~10 Å equatorial reflections indicative of the characteristic cross- β structure with multiple β -sheets separated by ~10 Å running parallel to the fibril long axis and each β -sheet composed of hydrogen-bonded strands

1 spaced by the canonical distance of ~ 4.8 Å [42]. MAS solid-state NMR—with its ability to probe
2
3 both rigid and highly flexible segments in non-crystalline biological solids by using pulse
4
5 sequences based on dipolar coupling and J-coupling mediated magnetization transfers,
6
7 respectively (Figure 1) [27], and to accurately determine site-specific intermolecular distances up
8
9 to ~ 5 -10 Å in proteins via measurements of dipolar couplings as well as protein backbone
10
11 dynamics via measurements of nuclear dipolar couplings and spin relaxation rates [13,14,43]—is
12
13 ideally suited for providing atomic resolution information on protofilament structure and
14
15 dynamics in amyloids. Indeed, many of the fundamental principles of amyloid atomic structure
16
17 were originally established by solid-state NMR, generating major impact across the scientific
18
19 community. The structural information from solid-state NMR, when combined with additional
20
21 data available from microscopic techniques including cryo-EM, scanning transmission electron
22
23 microscopy (STEM), dark-field TEM and/or atomic force microscopy (AFM) and spectroscopic
24
25 and spectrometric techniques including electron paramagnetic resonance and
26
27 hydrogen/deuterium exchange coupled with mass spectrometry or solution NMR, may then be
28
29 used within an integrated approach to derive atomistic models for entire fibrils [19,27,29]. It is
30
31 also worth mentioning that high-resolution structures could be determined in the absence of
32
33 solid-state NMR data for amyloid fibrils formed by a number of short peptide sequences (~ 4 -12
34
35 amino acids) using X-ray and electron microcrystallography [44-48] and for several larger
36
37 peptides and proteins using cryo-EM [49-53], in spite of the fact that amyloids generally do not
38
39 exhibit exact translational symmetry.
40
41
42
43
44
45
46
47
48
49
50
51

52 The initial structural solid-state NMR studies of amyloid fibrils were performed by
53
54 Griffin, Lansbury and co-workers for a 9-residue peptide corresponding to amino acids 34-42 of
55
56 the β -amyloid peptide (A β 34-42) [54,55]. These studies were based on rotational resonance ^{13}C -
57
58
59
60
61
62
63
64
65

¹³C distance measurements [56] in site-specifically labeled samples and culminated in the determination of a relatively low-resolution structural model [55] in which the cross-β architecture was composed of antiparallel β-sheets. The same group concurrently carried out rotational resonance solid-state NMR measurements and proposed an antiparallel β-sheet model for residues 20-29 of human islet amyloid polypeptide (IAPP) associated with type II diabetes [57]. Subsequent ¹³C-¹³C dipolar recoupling and ¹³C multiple quantum solid-state NMR measurements for longer, site-specifically ¹³C labeled fragments of the β-amyloid peptide, including Aβ10-35, Aβ1-40 and Aβ1-42, by Botto, Lynn, Meredith and co-workers and Tycko and co-workers [58-63] conclusively showed that these fibrils all adopt a parallel in-register β-sheet architecture, which, while somewhat surprising at the time in light of the data available for the shorter amyloid peptides, has since been found to be a common structural motif for most protein amyloids. Additionally, Tycko and co-workers proposed a much more detailed structural model for Aβ1-40 fibrils based on a larger set of distance and torsion angle restraints obtained from a set of fibril samples prepared with backbone ¹³CO labeling of specific residue pairs as well as uniform ¹³C, ¹⁵N labeling of consecutive or non-consecutive residues, combined with fibril mass-per-length data [64].

Around the same time, in the early 2000's, solid-state NMR methods for sequential resonance assignments and high-resolution structure determination of highly and/or uniformly ¹³C, ¹⁵N labeled peptides and small globular proteins were successfully demonstrated [8,65,66], opening up the possibilities for the application of analogous approaches to amyloids. In one such study, Griffin, Dobson and co-workers determined the high-resolution structure of an 11-residue peptide fragment of transthyretin (TTR105-115) within fibrils based on ca. 7 solid-state NMR intramolecular ¹³C-¹⁵N distance and/or dihedral angle restraints per residue (Figure 2A) [67,68];

combined with the monomer structure, additional solid-state NMR intermolecular distance measurements together with X-ray diffraction, AFM, STEM and cryo-EM data obtained by the same group permitted atomic-resolution structures to be determined for entire fibrils corresponding to three distinct TTR105-115 amyloid polymorphs containing different numbers of protofilaments [69,70]. Additional early solid-state NMR studies of this kind included the high-resolution structure determination of amyloid forming peptide fragments of the transcriptional activator human CA150 [71] and β 2-microglobulin [72], as well as HET-s(218-289) prion amyloid fibrils (Figure 2B) [73,74]. For HET-s(218-289) fibrils a large number of intra- and intermolecular restraints (>1,000) was used to constrain the ~40 structured residues of HET-s(218-289) in the β -solenoid amyloid core, and the solid-state NMR data provided key information about the protein conformation and incorporation into the amyloid scaffold, including details of the hydrophobic core interactions, salt bridges and asparagine ladders. In subsequent years, atomic-resolution structures have been determined by similar solid-state NMR methods for a number of other peptide and protein amyloid fibrils including several A β 1-40 polymorphs generated *in vitro* [75,76] and A β 1-40 seeded with brain-derived amyloid [77], two A β 1-40 mutants associated with early onset neurodegeneration [78,79], A β 1-42 (Figure 2C) [80-82], α -synuclein associated with Parkinson's disease (Figure 2D) [83], and the low-complexity domain of the FUS RNA-binding protein [84].

The above examples of successful solid-state NMR studies that have resulted in elucidation of high-resolution amyloid fibril structures, as well as the numerous investigations of other amyloidogenic peptides and proteins along these lines that are underway in multiple research groups [19,27], clearly demonstrate the importance of solid-state NMR spectroscopy for understanding amyloid structure and assembly. These endeavors will undoubtedly be further

1
2
3
4 facilitated by recent developments in solid-state NMR methodology including fast (~60-120
5
6 kHz) MAS combined with ^1H detection [6,85-90], the use of covalent paramagnetic tags,
7
8 including nitroxide spin labels and metal chelates, which enable site-resolved measurements of
9
10 multiple long-range (up to ~20 Å) structural restraints in the form of electron-nucleus distances
11
12 [91-97] and dynamic nuclear polarization (DNP) [69,98-103]. Collectively, these methodological
13
14 advances are expected to permit rapid structural analysis using smaller (sub-milligram) amyloid
15
16 samples. As one example, in Figure 3 we illustrate a recent application of paramagnetic solid-
17
18 state NMR to amyloids where a low-resolution fold for fibrils formed by residues 23-144 of
19
20 human prion protein (PrP23-144) could be determined based on a sparse set of intra- and
21
22 intermolecular paramagnetic relaxation enhancement (PRE) restraints measured in nitroxide spin
23
24 label and Cu(II)-EDTA tagged fibril samples [96].
25
26
27
28
29
30
31
32

33 **4. Beyond High-Resolution Structure Determination**

34
35 Many amyloid peptides and proteins are capable of assembling into multiple distinct,
36
37 self-propagating fibril structures. This molecular level polymorphism is believed to be
38
39 responsible for the emergence of strains and transmissibility barriers in prion diseases [104,105],
40
41 and similar phenomena appear to be operative in neurological disorders, including Alzheimer's
42
43 and Parkinson's diseases, where amyloid polymorphism may play a role in clinical manifestation
44
45 and pathogenesis [25]. In solid-state NMR spectra, information about molecular structure and
46
47 structural heterogeneity is encoded in resonance frequencies and/or linewidths. This permits
48
49 rapid fingerprinting of distinct structural conformers (including the concurrent presence of
50
51 multiple polymorphs within the same sample), without the necessity to determine high-resolution
52
53 structures. In the context of amyloids, solid-state NMR has been used to identify distinct
54
55
56
57
58
59
60
61
62
63
64
65

1
2
3
4 structural polymorphs for different peptides and proteins including A β [75-77,106,107], IAPP
5
6 [108], α -synuclein [109-111], tau [112-115] and PrP23-144 variants [116] (see Figure 4 for a
7
8 representative example), investigate the influence of amino acid mutations and/or deletions on
9
10 amyloid core structures [78,79,116-120], and assess the structural variation in A β fibrils
11
12 stemming from different regions of the brain or associated with different patients and/or disease
13
14 subtypes [77,121]. In related studies, available high-resolution amyloid structures have been
15
16 successfully used to characterize the binding of small molecule ligands to fibrils (Figure 5) [122-
17
18 126]. Investigations in the latter direction promise to contribute to the development of improved
19
20 amyloid markers and/or drug molecules.
21
22
23
24
25

26
27 Finally, as noted above (c.f., Figure 1), solid-state NMR experiments enable facile
28
29 identification of both relatively rigid amyloid core residues as well as highly dynamically
30
31 disordered domains typically located outside the core region [27]. While the functional relevance
32
33 of such dynamic domains in amyloids is generally unclear, in certain cases their flexibility
34
35 appears to be correlated with fibril toxicity [127,128]. In addition, solid-state NMR
36
37 measurements of nuclear dipolar couplings and spin relaxation rates permit the characterization
38
39 of protein backbone motions on a wide range of timescales from picoseconds to milliseconds
40
41 [43]. Interestingly, for several amyloid peptides and proteins, including A β , HET-s(218-289) and
42
43 PrP23-144 [89,129-132], such measurements have revealed that the core regions can exhibit
44
45 considerable dynamics in spite of their overall highly ordered nature.
46
47
48
49
50
51
52

53 54 **5. Concluding Remarks**

55
56 Major progress in solid-state NMR instrumentation and methodology has enabled
57
58 tremendous advances to be made over the past two decades in the investigation of molecular
59
60
61
62
63
64
65

1
2
3
4 structure, dynamics and interactions of amyloids and other large biomacromolecular complexes.
5
6 Importantly, these studies, frequently in combination with experimental and computational data
7
8 available from complementary techniques, are collectively yielding unprecedented insights into
9
10 biological function and mechanism. In the coming years, ongoing developments in solid-state
11
12 NMR technology, including rapid sample spinning coupled with proton detection, dynamic
13
14 nuclear polarization and paramagnetism-based approaches, promise to further increase the
15
16 throughput and information content of solid-state NMR studies for amyloids and other
17
18 assemblies of biological macromolecules.
19
20
21
22
23
24
25

26 **Acknowledgments**

27
28 I acknowledge support from the National Institutes of Health (R01GM094357,
29
30 R01GM123743, RF1AG061797), the National Science Foundation (MCB-1715174), and the
31
32 Camille and Henry Dreyfus Foundation (Camille Dreyfus Teacher-Scholar Award). I also thank
33
34 all the current and former members of my research group for stimulating discussions.
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

References

- [1] L.M. McDowell, J. Schaefer, *Curr. Opin. Struct. Biol.* 6 (1996) 624-629.
- [2] R.G. Griffin, *Nat. Struct. Biol.* 5 (1998) 508-512.
- [3] R.W. Martin, E.K. Paulson, K.W. Zilm, *Rev. Sci. Instrum.* 74 (2003) 3045-3061.
- [4] J.A. Stringer, C.E. Bronnimann, C.G. Mullen, D.H.H. Zhou, S.A. Stellfox, Y. Li, E.H. Williams, C.M. Rienstra, *J. Magn. Reson.* 173 (2005) 40-48.
- [5] P.L. Gor'kov, E.Y. Chekmenev, C. Li, M. Cotten, J.J. Buffy, N.J. Traaseth, G. Veglia, W.W. Brey, *J. Magn. Reson.* 185 (2007) 77-93.
- [6] J. Struppe, C.M. Quinn, M. Lu, M. Wang, G. Hou, X. Lu, J. Kraus, L.B. Andreas, J. Stanek, D. Lalli, A. Lesage, G. Pintacuda, W. Maas, A.M. Gronenborn, T. Polenova, *Solid State Nucl. Magn. Reson.* 87 (2017) 117-125.
- [7] S. Penzel, A. Oss, M.L. Org, A. Samoson, A. Bockmann, M. Ernst, B.H. Meier, *J. Biomol. NMR* 73 (2019) 19-29.
- [8] F. Castellani, B. van Rossum, A. Diehl, M. Schubert, K. Rehbein, H. Oschkinat, *Nature* 420 (2002) 98-102.
- [9] D. Lacabanne, B.H. Meier, A. Bockmann, *J. Biomol. NMR* 71 (2018) 141-150.
- [10] R.W. Martin, J.E. Kelly, J.I. Kelz, *J. Struct. Biol.* 206 (2019) 73-89.
- [11] R.W. Martin, K.W. Zilm, *J. Magn. Reson.* 165 (2003) 162-174.
- [12] D. Lacabanne, M.L. Fogeron, T. Wiegand, R. Cadalbert, B.H. Meier, A. Bockmann, *Prog. Nucl. Magn. Reson. Spectrosc.* 110 (2019) 20-33.
- [13] M. Baldus, *Prog. Nucl. Magn. Reson. Spect.* 41 (2002) 1-47.
- [14] C.M. Rienstra, Magic-angle spinning recoupling techniques for distance determinations among spin-1/2 nuclei in solid peptides and proteins, in: A. Ramamoorthy (Ed.), *NMR Spectroscopy of Biological Solids*, CRC Press, Boca Raton, FL, 2006, pp. 1-38.
- [15] A.E. McDermott, *Curr. Opin. Struct. Biol.* 14 (2004) 554-561.
- [16] R. Tycko, *Q. Rev. Biophys.* 39 (2006) 1-55.
- [17] H. Heise, *Chembiochem* 9 (2008) 179-189.
- [18] A. McDermott, *Annu. Rev. Biophys.* 38 (2009) 385-403.
- [19] R. Tycko, *Annu. Rev. Phys. Chem.* 62 (2011) 279-299.
- [20] A. Loquet, B. Habenstein, A. Lange, *Acc. Chem. Res.* 46 (2013) 2070-2079.
- [21] G. Comellas, C.M. Rienstra, *Annu. Rev. Biophys.* 42 (2013) 515-536.
- [22] M. Tang, G. Comellas, C.M. Rienstra, *Acc. Chem. Res.* 46 (2013) 2080-2088.
- [23] S. Wang, V. Ladizhansky, *Prog. Nucl. Magn. Reson. Spectrosc.* 82 (2014) 1-26.
- [24] C.M. Quinn, M. Lu, C.L. Suiter, G. Hou, H. Zhang, T. Polenova, *Prog. Nucl. Magn. Reson. Spectrosc.* 86-87 (2015) 21-40.
- [25] R. Tycko, *Neuron* 86 (2015) 632-45.
- [26] M. Kaplan, C. Pinto, K. Houben, M. Baldus, *Q. Rev. Biophys.* 49 (2016) e15.
- [27] P.C.A. van der Wel, *Solid State Nucl. Magn. Reson.* 88 (2017) 1-14.
- [28] C.M. Quinn, T. Polenova, *Quarterly Reviews of Biophysics* 50 (2017) e1.
- [29] B.H. Meier, R. Riek, A. Bockmann, *Trends Biochem. Sci.* 42 (2017) 777-787.
- [30] P.C.A. van der Wel, *Emerg. Top. Life Sci.* 2 (2018) 57-67.
- [31] C.M. Quinn, M. Wang, T. Polenova, *Methods Mol. Biol.* 1688 (2018) 1-35.
- [32] A. Loquet, N. El Mammeri, J. Stanek, M. Berbon, B. Bardiaux, G. Pintacuda, B. Habenstein, *Methods* 138-139 (2018) 26-38.
- [33] C.L. van Emmerik, H. van Ingen, *Prog. Nucl. Magn. Reson. Spectrosc.* 110 (2019) 1-19.

- [34] A. Marchanka, T. Carlomagno, *Methods Enzymol.* 615 (2019) 333-371.
- [35] C.M. Dobson, *Trends Biochem. Sci.* 24 (1999)
- [36] F. Chiti, C.M. Dobson, *Annu. Rev. Biochem.* 75 (2006) 333-366.
- [37] D.M. Fowler, A.V. Koulov, W.E. Balch, J.W. Kelly, *Trends Biochem. Sci.* 32 (2007) 217-224.
- [38] J. Greenwald, R. Riek, *Structure* 18 (2010) 1244-1260.
- [39] T.P. Knowles, M. Vendruscolo, C.M. Dobson, *Nat. Rev. Mol. Cell. Biol.* 15 (2014) 384-96.
- [40] R. Riek, D.S. Eisenberg, *Nature* 539 (2016) 227-235.
- [41] C.M. Dobson, T.P.J. Knowles, M. Vendruscolo, *Cold Spring Harb. Perspect. Biol.* (2019) doi: 10.1101/cshperspect.a033878.
- [42] L.C. Serpell, P.E. Fraser, M. Sunde, *Methods Enzymol.* 309 (1999) 526-536.
- [43] P. Schanda, M. Ernst, *Prog. Nucl. Magn. Reson. Spect.* 96 (2016) 1-46.
- [44] O.S. Makin, E. Atkins, P. Sikorski, J. Johansson, L.C. Serpell, *Proc. Natl. Acad. Sci. USA* 102 (2005) 315-320.
- [45] R. Nelson, M.R. Sawaya, M. Balbirnie, A.O. Madsen, C. Riek, R. Grothe, D. Eisenberg, *Nature* 435 (2005) 773-778.
- [46] M.R. Sawaya, S. Sambashivan, R. Nelson, M.I. Ivanova, S.A. Sievers, M.I. Apostol, M.J. Thompson, M. Balbirnie, J.J. Wiltzius, H.T. McFarlane, A.Ø. Madsen, C. Riek, D. Eisenberg, *Nature* 447 (2007) 453-457.
- [47] M.R. Sawaya, J. Rodriguez, D. Cascio, M.J. Collazo, D. Shi, F.E. Reyes, J. Hattne, T. Gonen, D.S. Eisenberg, *Proc. Natl. Acad. Sci. USA* 113 (2016) 11232-11236.
- [48] L. Saelices, S.A. Sievers, M.R. Sawaya, D.S. Eisenberg, *Protein Sci.* 27 (2018) 1295-1303.
- [49] L. Gremer, D. Scholzel, C. Schenk, E. Reinartz, J. Labahn, R.B.G. Ravelli, M. Tusche, C. Lopez-Iglesias, W. Hoyer, H. Heise, D. Willbold, G.F. Schroder, *Science* 358 (2017) 116-119.
- [50] A.W.P. Fitzpatrick, B. Falcon, S. He, A.G. Murzin, G. Murshudov, H.J. Garringer, R.A. Crowther, B. Ghetti, M. Goedert, S.H.W. Scheres, *Nature* 547 (2017) 185-190.
- [51] R. Guerrero-Ferreira, N.M. Taylor, D. Mona, P. Ringler, M.E. Lauer, R. Riek, M. Britschgi, H. Stahlberg, *Elife* 7 (2018) e36402.
- [52] Y. Li, C. Zhao, F. Luo, Z. Liu, X. Gui, Z. Luo, X. Zhang, D. Li, C. Liu, X. Li, *Cell Res.* 28 (2018) 897-903.
- [53] B. Li, P. Ge, K.A. Murray, P. Sheth, M. Zhang, G. Nair, M.R. Sawaya, W.S. Shin, D.R. Boyer, S. Ye, D.S. Eisenberg, Z.H. Zhou, L. Jiang, *Nat. Commun.* 9 (2018) 3609.
- [54] R.G.S. Spencer, K.J. Halverson, M. Auger, A.E. McDermott, R.G. Griffin, P.T. Lansbury, *Biochemistry* 30 (1991) 10382-10387.
- [55] P.T. Lansbury, P.R. Costa, J.M. Griffiths, E.J. Simon, M. Auger, K.J. Halverson, D.A. Kocisko, Z.S. Hendsch, T.T. Ashburn, R.G.S. Spencer, B. Tidor, R.G. Griffin, *Nat. Struct. Biol.* 2 (1995) 990-998.
- [56] D.P. Raleigh, M.H. Levitt, R.G. Griffin, *Chem. Phys. Lett.* 146 (1988) 71-76.
- [57] J.M. Griffiths, T.T. Ashburn, M. Auger, P.R. Costa, R.G. Griffin, P.T. Lansbury, *J. Am. Chem. Soc.* 117 (1995) 3539-3546.
- [58] T.L.S. Benzinger, D.M. Gregory, T.S. Burkoth, H. Miller-Auer, D.G. Lynn, R.E. Botto, S.C. Meredith, *Proc. Natl. Acad. Sci. USA* 95 (1998) 13407-13412.

- [59] D.M. Gregory, T.L.S. Benzinger, T.S. Burkoth, H. Miller-Auer, D.G. Lynn, S.C. Meredith, R.E. Botto, *Solid State Nucl. Magn. Reson.* 13 (1998) 149-166.
- [60] T.S. Burkoth, T.L.S. Benzinger, V. Urban, D.M. Morgan, D.M. Gregory, P. Thiagarajan, R.E. Botto, S.C. Meredith, D.G. Lynn, *J. Am. Chem. Soc.* 122 (2000) 7883-7889.
- [61] O.N. Antzutkin, J.J. Balbach, R.D. Leapman, N.W. Rizzo, J. Reed, R. Tycko, *Proc. Natl. Acad. Sci. USA* 97 (2000) 13045-13050.
- [62] O.N. Antzutkin, R.D. Leapman, J.J. Balbach, R. Tycko, *Biochemistry* 41 (2002) 15436-50.
- [63] J.J. Balbach, A.T. Petkova, N.A. Oyler, O.N. Antzutkin, D.J. Gordon, S.C. Meredith, R. Tycko, *Biophys J.* 83 (2002) 1205-1216.
- [64] A.T. Petkova, Y. Ishii, J.J. Balbach, O.N. Antzutkin, R.D. Leapman, F. Delaglio, R. Tycko, *Proc. Natl. Acad. Sci. USA* 99 (2002) 16742-16747.
- [65] A. McDermott, T. Polenova, A. Bockmann, K.W. Zilm, E.K. Paulsen, R.W. Martin, G.T. Montelione, *J. Biomol. NMR* 16 (2000) 209-219.
- [66] C.M. Rienstra, L. Tucker-Kellogg, C.P. Jaroniec, M. Hohwy, B. Reif, T. Lozano-Perez, B. Tidor, R.G. Griffin, *Proc. Natl. Acad. Sci. USA* 99 (2002) 10260-10265.
- [67] C.P. Jaroniec, C.E. MacPhee, N.S. Astrof, C.M. Dobson, R.G. Griffin, *Proc. Natl. Acad. Sci. USA* 99 (2002) 16748-16753.
- [68] C.P. Jaroniec, C.E. MacPhee, V.S. Bajaj, M.T. McMahon, C.M. Dobson, R.G. Griffin, *Proc. Natl. Acad. Sci. USA* 101 (2004) 711-716.
- [69] G.T. Debelouchina, M.J. Bayro, A.W. Fitzpatrick, V. Ladizhansky, M.T. Colvin, M.A. Caporini, C.P. Jaroniec, V.S. Bajaj, M. Rosay, C.E. MacPhee, M. Vendruscolo, W.E. Maas, C.M. Dobson, R.G. Griffin, *J. Am. Chem. Soc.* 135 (2013) 19237-19247.
- [70] A.W. Fitzpatrick, G.T. Debelouchina, M.J. Bayro, D.K. Clare, M.A. Caporini, V.S. Bajaj, C.P. Jaroniec, L. Wang, V. Ladizhansky, S.A. Muller, C.E. MacPhee, C.A. Waudby, H.R. Mott, A. De Simone, T.P. Knowles, H.R. Saibil, M. Vendruscolo, E.V. Orlova, R.G. Griffin, C.M. Dobson, *Proc. Natl. Acad. Sci. USA* 110 (2013) 5468-5473.
- [71] N. Ferguson, J. Becker, H. Tidow, S. Tremmel, T.D. Sharpe, G. Krause, J. Flinders, M. Petrovich, J. Berriman, H. Oschkinat, A.R. Fersht, *Proc. Natl. Acad. Sci. USA* 103 (2006) 16248-16253.
- [72] K. Iwata, T. Fujiwara, Y. Matsuki, H. Akutsu, S. Takahashi, H. Naiki, Y. Goto, *Proc. Nat. Acad. Sci. USA* 103 (2006) 18119-18124.
- [73] C. Wasmer, A. Lange, H. Van Melckebeke, A.B. Siemer, R. Riek, B.H. Meier, *Science* 319 (2008) 1523-1526.
- [74] H. Van Melckebeke, C. Wasmer, A. Lange, E. AB, A. Loquet, A. Böckmann, B.H. Meier, *J. Am. Chem. Soc.* 132 (2010) 13765-13775.
- [75] A.K. Paravastu, R.D. Leapman, W.M. Yau, R. Tycko, *Proc. Natl. Acad. Sci. USA* 105 (2008) 18349-18354.
- [76] I. Bertini, L. Gonnelli, C. Luchinat, J. Mao, A. Nesi, *J. Am. Chem. Soc.* 133 (2011) 16013-16022.
- [77] J.X. Lu, W. Qiang, W.M. Yau, C.D. Schwieters, S.C. Meredith, R. Tycko, *Cell* 154 (2013) 1257-1268.
- [78] W. Qiang, W.M. Yau, Y. Luo, M.P. Mattson, R. Tycko, *Proc. Natl. Acad. Sci. USA* 109 (2012) 4443-4448.

- [79] A.K. Schutz, T. Vagt, M. Huber, O.Y. Ovchinnikova, R. Cadalbert, J. Wall, P. Guntert, A. Bockmann, R. Glockshuber, B.H. Meier, *Angew. Chem. Int. Ed.* 54 (2015) 331-335.
- [80] Y. Xiao, B. Ma, D. McElheny, S. Parthasarathy, F. Long, M. Hoshi, R. Nussinov, Y. Ishii, *Nat. Struct. Mol. Biol.* 22 (2015) 499-505.
- [81] M.T. Colvin, R. Silvers, Q.Z. Ni, T.V. Can, I. Sergeyev, M. Rosay, K.J. Donovan, B. Michael, J. Wall, S. Linse, R.G. Griffin, *J. Am. Chem. Soc.* 138 (2016) 9663-9674.
- [82] M.A. Walti, F. Ravotti, H. Arai, C.G. Glabe, J.S. Wall, A. Bockmann, P. Guntert, B.H. Meier, R. Riek, *Proc. Natl. Acad. Sci. USA* 113 (2016) E4976-4984.
- [83] M.D. Tuttle, G. Comellas, A.J. Nieuwkoop, D.J. Covell, D.A. Berthold, K.D. Kloepper, J.M. Courtney, J.K. Kim, A.M. Barclay, A. Kendall, W. Wan, G. Stubbs, C.D. Schwieters, V.M. Lee, J.M. George, C.M. Rienstra, *Nat. Struct. Mol. Biol.* 23 (2016) 409-415.
- [84] D.T. Murray, M. Kato, Y. Lin, K.R. Thurber, I. Hung, S.L. McKnight, R. Tycko, *Cell* 171 (2017) 615-627.
- [85] R. Linser, M. Dasari, M. Hiller, V. Higman, U. Fink, J.M. Lopez del Amo, S. Markovic, L. Handel, B. Kessler, P. Schmieder, D. Oesterhelt, H. Oschkinat, B. Reif, *Angew. Chem. Int. Ed.* 50 (2011) 4508-4512.
- [86] J. Stanek, L.B. Andreas, K. Jaudzems, D. Cala, D. Lalli, A. Bertarello, T. Schubeis, I. Akopjana, S. Kotelovica, K. Tars, A. Pica, S. Leone, D. Picone, Z.Q. Xu, N.E. Dixon, D. Martinez, M. Berbon, N. El Mammeri, A. Noubhani, S. Saupe, B. Habenstein, A. Loquet, G. Pintacuda, *Angew. Chem. Int. Ed.* 55 (2016) 15504-15509.
- [87] A.A. Smith, F. Ravotti, E. Testori, R. Cadalbert, M. Ernst, A. Bockmann, B.H. Meier, J. Biomol. NMR 67 (2017) 109-119.
- [88] J. Tolchard, M.K. Pandey, M. Berbon, A. Noubhani, S.J. Saupe, Y. Nishiyama, B. Habenstein, A. Loquet, *J. Biomol. NMR* 70 (2018) 177-185.
- [89] M.D. Shannon, T. Theint, D. Mukhopadhyay, K. Surewicz, W.K. Surewicz, D. Marion, P. Schanda, C.P. Jaroniec, *Chemphyschem* 20 (2019) 311-317.
- [90] L.B. Andreas, T. Le Marchand, K. Jaudzems, G. Pintacuda, *J. Magn. Reson.* 253 (2015) 36-49.
- [91] G. Pintacuda, G. Kervern, *Top. Curr. Chem.* 335 (2012) 157-200.
- [92] C.P. Jaroniec, *J. Magn. Reson.* 253 (2015) 50-59.
- [93] P.S. Nadaud, J.J. Helmus, N. Höfer, C.P. Jaroniec, *J. Am. Chem. Soc.* 129 (2007) 7502-7503.
- [94] I. Sengupta, P.S. Nadaud, J.J. Helmus, C.D. Schwieters, C.P. Jaroniec, *Nat. Chem.* 4 (2012) 410-417.
- [95] D. Mukhopadhyay, P.S. Nadaud, M.D. Shannon, C.P. Jaroniec, *J. Phys. Chem. Lett.* 8 (2017) 5871-5877.
- [96] T. Theint, Y. Xia, P.S. Nadaud, D. Mukhopadhyay, C.D. Schwieters, K. Surewicz, W.K. Surewicz, C.P. Jaroniec, *J. Am. Chem. Soc.* 140 (2018) 13161-13166.
- [97] A. Perez, K. Gaalswyk, C.P. Jaroniec, J.L. MacCallum, *Angew. Chem. Int. Ed.* 58 (2019) 6564-6568.
- [98] A.B. Barnes, G.D. Paëpe, P.C. van der Wel, K.N. Hu, C.G. Joo, V.S. Bajaj, M.L. Mak-Jurkauskas, J.R. Sirigiri, J. Herzfeld, R.J. Temkin, R.G. Griffin, *Appl. Magn. Reson.* 34 (2008) 237-263.

- [99] 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.* (2011) in press, doi: 10.1021/ja203756x.
- [100] J.M. Lopez del Amo, D. Schneider, A. Loquet, A. Lange, B. Reif, *J. Biomol. NMR* 56 (2013) 359-63.
- [101] A. Potapov, W.M. Yau, R. Ghirlando, K.R. Thurber, R. Tycko, *J. Am. Chem. Soc.* 137 (2015) 8294-307.
- [102] M. Nagaraj, T.W. Franks, S. Saeidpour, T. Schubeis, H. Oschkinat, C. Ritter, B.J. van Rossum, *Chembiochem* 17 (2016) 1308-11.
- [103] A. Konig, D. Scholzel, B. Uluca, T. Viennet, U. Akbey, H. Heise, *Solid State Nucl. Magn. Reson.* 98 (2019) 1-11.
- [104] J. Collinge, A.R. Clarke, *Science* 318 (2007) 930-936.
- [105] A. Aguzzi, C. Sigurdson, M. Heikenwaelder, *Annu. Rev. Pathol.* 3 (2008) 11-40.
- [106] A.T. Petkova, R.D. Leapman, Z.H. Guo, W.M. Yau, M.P. Mattson, R. Tycko, *Science* 307 (2005) 262-265.
- [107] J.M. Lopez del Amo, M. Schmidt, U. Fink, M. Dasari, M. Fandrich, B. Reif, *Angew. Chem. Int. Ed.* 51 (2012) 6136-9.
- [108] S. Luca, W.M. Yau, R. Leapman, R. Tycko, *Biochemistry* 46 (2007) 13505-13522.
- [109] H. Heise, W. Hoyer, S. Becker, O.C. Andronesi, D. Riedel, M. Baldus, *Proc. Natl. Acad. Sci. USA* 102 (2005) 15871-15876.
- [110] G. Comellas, L.R. Lemkau, A.J. Nieuwkoop, K.D. Kloepper, D.T. Lador, R. Ebisu, W.S. Woods, A.S. Lipton, J.M. George, C.M. Rienstra, *J. Mol. Biol.* 411 (2011) 881-895.
- [111] L. Bousset, L. Pieri, G. Ruiz-Arlandis, J. Gath, P.H. Jensen, B. Habenstein, K. Madiona, V. Olieric, A. Bockmann, B.H. Meier, R. Melki, *Nat. Commun.* 4 (2013) 2575.
- [112] O.C. Andronesi, M. von Bergen, J. Biernat, K. Seidel, C. Griesinger, E. Mandelkow, M. Baldus, *J. Am. Chem. Soc.* 130 (2008) 5922-5928.
- [113] V. Daebel, S. Chinnathambi, J. Biernat, M. Schwalbe, B. Habenstein, A. Loquet, E. Akoury, K. Tepper, H. Muller, M. Baldus, C. Griesinger, M. Zweckstetter, E. Mandelkow, V. Vijayan, A. Lange, *J. Am. Chem. Soc.* 134 (2012) 13982-9.
- [114] S. Xiang, J. Biernat, E. Mandelkow, S. Becker, R. Linser, *Chem. Commun.* 52 (2016) 4002-4005.
- [115] S. Xiang, N. Kulminkaya, B. Habenstein, J. Biernat, K. Tepper, M. Paulat, C. Griesinger, S. Becker, A. Lange, E. Mandelkow, R. Linser, *J. Am. Chem. Soc.* 139 (2017) 2639-2646.
- [116] T. Theint, P.S. Nadaud, D. Aucoin, J.J. Helmus, S.P. Pondaven, K. Surewicz, W.K. Surewicz, C.P. Jaronec, *Nat. Commun.* 8 (2017) 753.
- [117] M.R. Elkins, T. Wang, M. Nick, H. Jo, T. Lemmin, S.B. Prusiner, W.F. DeGrado, J. Stohr, M. Hong, *J. Am. Chem. Soc.* 138 (2016) 9840-9852.
- [118] H. Heise, M.S. Celej, S. Becker, D. Riedel, A. Pelah, A. Kumar, T.M. Jovin, M. Baldus, *J. Mol. Biol.* 380 (2008) 444-450.
- [119] E.M. Jones, B. Wu, K. Surewicz, P.S. Nadaud, J.J. Helmus, S. Chen, C.P. Jaronec, W.K. Surewicz, *J. Biol. Chem.* 286 (2011) 42777-42784.
- [120] L.R. Lemkau, G. Comellas, K.D. Kloepper, W.S. Woods, J.M. George, C.M. Rienstra, *J. Biol. Chem.* 287 (2012) 11526-11532.
- [121] W. Qiang, W.M. Yau, J.X. Lu, J. Collinge, R. Tycko, *Nature* 541 (2017) 217-221.

- [122] A.K. Schutz, A. Soragni, S. Hornemann, A. Aguzzi, M. Ernst, A. Bockmann, B.H. Meier, *Angew. Chem. Int. Ed.* 50 (2011) 5956-5960.
- [123] U.S. Herrmann, A.K. Schutz, H. Shirani, D. Huang, D. Saban, M. Nuvolone, B. Li, B. Ballmer, A.K. Aslund, J.J. Mason, E. Rushing, H. Budka, S. Nystrom, P. Hammarstrom, A. Bockmann, A. Caflisch, B.H. Meier, K.P. Nilsson, S. Hornemann, A. Aguzzi, *Sci. Transl. Med.* 7 (2015) 299ra123.
- [124] E. Prade, H.J. Bittner, R. Sarkar, J.M. Lopez Del Amo, G. Althoff-Ospelt, G. Multhaup, P.W. Hildebrand, B. Reif, *J. Biol. Chem.* 290 (2015) 28737-28745.
- [125] C. Gowda, G. Zandomenoghi, H. Zimmermann, A.K. Schutz, A. Bockmann, M. Ernst, B.H. Meier, *J. Biomol. NMR* 69 (2017) 207-213.
- [126] A.K. Schutz, S. Hornemann, M.A. Walti, L. Greuter, C. Tiberi, R. Cadalbert, M. Gantner, R. Riek, P. Hammarstrom, K.P.R. Nilsson, A. Bockmann, A. Aguzzi, B.H. Meier, *ACS Chem. Neurosci.* 9 (2018) 475-481.
- [127] Y. Nekooki-Machida, M. Kurosawa, N. Nukina, K. Ito, T. Oda, M. Tanaka, *Proc. Natl. Acad. Sci. USA* 106 (2009) 9679-9684.
- [128] H.K. Lin, J.C. Boatz, I.E. Krabbendam, R. Kodali, Z. Hou, R. Wetzels, A.M. Dolga, M.A. Poirier, P.C.A. van der Wel, *Nat. Commun.* 8 (2017) 15462.
- [129] J.J. Helmus, K. Surewicz, W.K. Surewicz, C.P. Jaroniec, *J. Am. Chem. Soc.* 132 (2010) 2393-2403.
- [130] H.A. Scheidt, I. Morgado, S. Rothmund, D. Huster, *J. Biol. Chem.* 287 (2012) 2017-2021.
- [131] A.A. Smith, E. Testori, R. Cadalbert, B.H. Meier, M. Ernst, *J. Biomol. NMR* 65 (2016) 171-191.
- [132] A.A. Smith, M. Ernst, S. Riniker, B.H. Meier, *Angew. Chem. Int. Ed.* (2019) doi: 10.1002/anie.201901929.
- [133] T. Theint, P.S. Nadaud, K. Surewicz, W.K. Surewicz, C.P. Jaroniec, *Biomol. NMR Assign.* 11 (2017) 75-80.
- [134] J.J. Helmus, K. Surewicz, P.S. Nadaud, W.K. Surewicz, C.P. Jaroniec, *Proc. Natl. Acad. Sci. USA* 105 (2008) 6284-6289.

Figure Captions

Fig. 1. (A) Amino acid sequence of human PrP23-144. Relatively rigid amyloid core residues observable in cross-polarization based solid-state NMR spectra (panel C) are shown in red font and dynamically disordered residues observable in J-coupling based solid-state NMR spectra (panel D) are shown in green font. (B) Representative AFM image of human PrP23-144 amyloid fibrils. The scale bar corresponds to 1 μm . Adapted from Ref. [133]. (C) 2D 500 MHz cross-polarization based ^{15}N - $^{13}\text{C}\alpha$ spectra of human PrP23-144 amyloid fibrils recorded at MAS rate of 11.111 kHz and temperatures of ca. 0 $^{\circ}\text{C}$ (red contours) and -30 $^{\circ}\text{C}$ (blue contours). At 0 $^{\circ}\text{C}$ signals corresponding to only the most rigid amyloid core residues are detected, while at -30 $^{\circ}\text{C}$ signals from all residues are detected, including those which are conformationally flexible at 0 $^{\circ}\text{C}$. Adapted from Ref. [134]. (D) 2D 500 MHz J-coupling based ^1H - ^{13}C spectra of human PrP23-144 amyloid fibrils recorded at MAS rate of 11.111 kHz and temperature of 30 $^{\circ}\text{C}$, containing signals corresponding to only the dynamically disordered residues. Adapted from Ref. [129].

Fig. 2. Representative high-resolution structures of amyloid peptides and proteins determined by solid-state NMR. (A) TTR105-115. Adapted from Refs. [68] and [70]. (B) HET-s(218-289). Adapted from Ref. [73]. (C) A β 1-42. Adapted from Ref. [81]. (D) α -synuclein. Adapted from Ref. [83].

Fig. 3. Ensemble of ten low-energy backbone structures for the core region of human PrP23-144 amyloid fibrils (residues 109-144) corresponding to one layer of the two protofilament assembly. The structural model was derived by using sparse long-range intra- and intermolecular

paramagnetic relaxation enhancement restraints measured using fibril samples tagged with nitroxide spin labels or Cu(II)-EDTA side-chains at positions indicated by the red spheres.

Adapted from Ref. [96].

Fig. 4. 2D 800 MHz cross-polarization based ^{15}N - $^{13}\text{C}\alpha$ spectra of two structural strains of Syrian hamster PrP23-144 amyloid, generated at 25 °C under quiescent conditions (q25) and at 37 °C with continuous slow rotation (r37). Adapted from Ref. [116].

Fig. 5. Structural model of a polythiophene compound (LIN5001) bound to amyloid fibrils formed by the E265K mutant of HET-s(218-289). LIN5001, which contains four carboxylate moieties, interacts with the side-chains of lysine residues 229 and 265 (highlighted in cyan and blue) that are located in adjacent protein layers and form an extended positively charged region. Adapted from Ref. [123].

Figure 1
[Click here to download high resolution image](#)

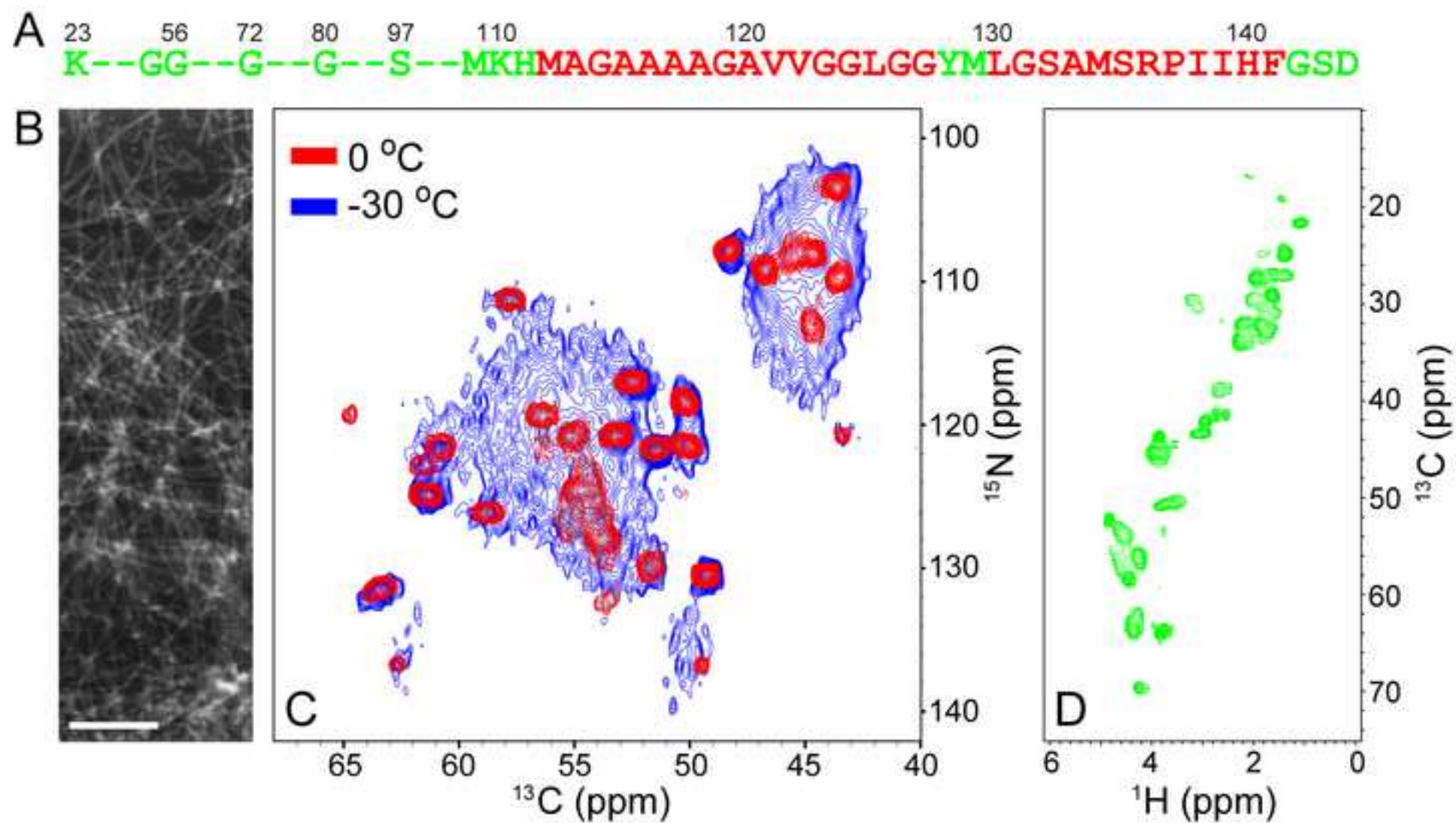


Figure 2
[Click here to download high resolution image](#)

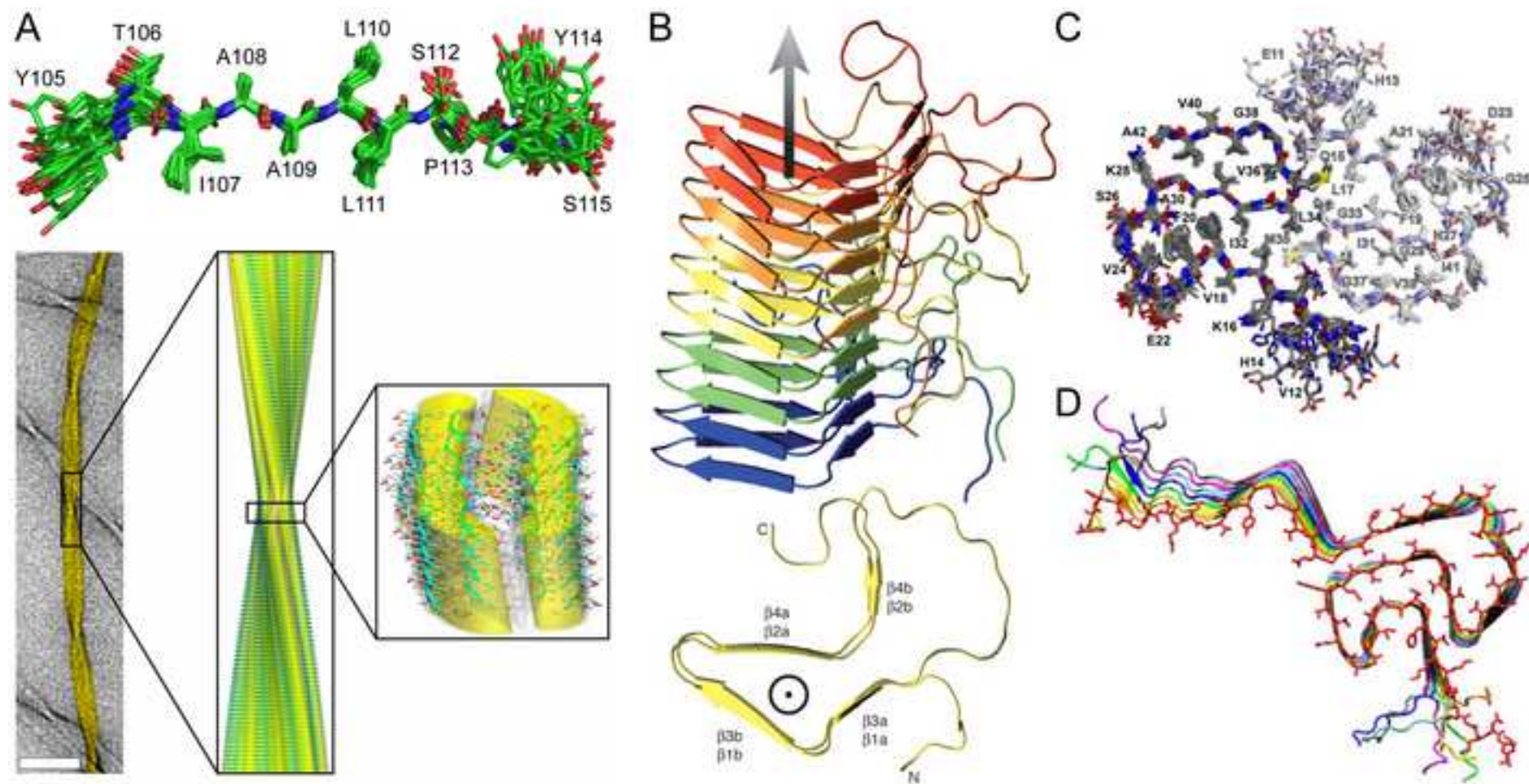


Figure 3
[Click here to download high resolution image](#)

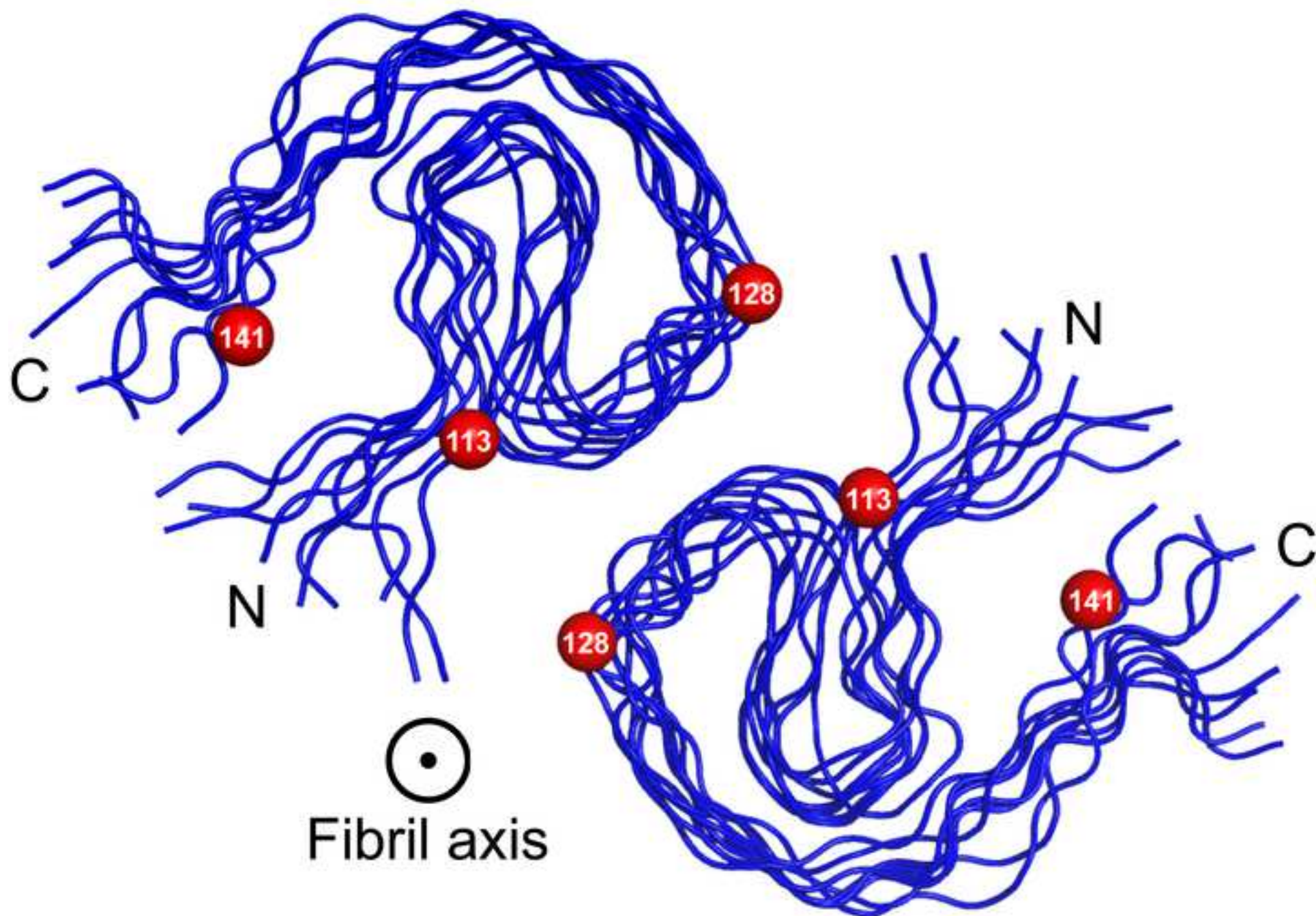


Figure 4
[Click here to download high resolution image](#)

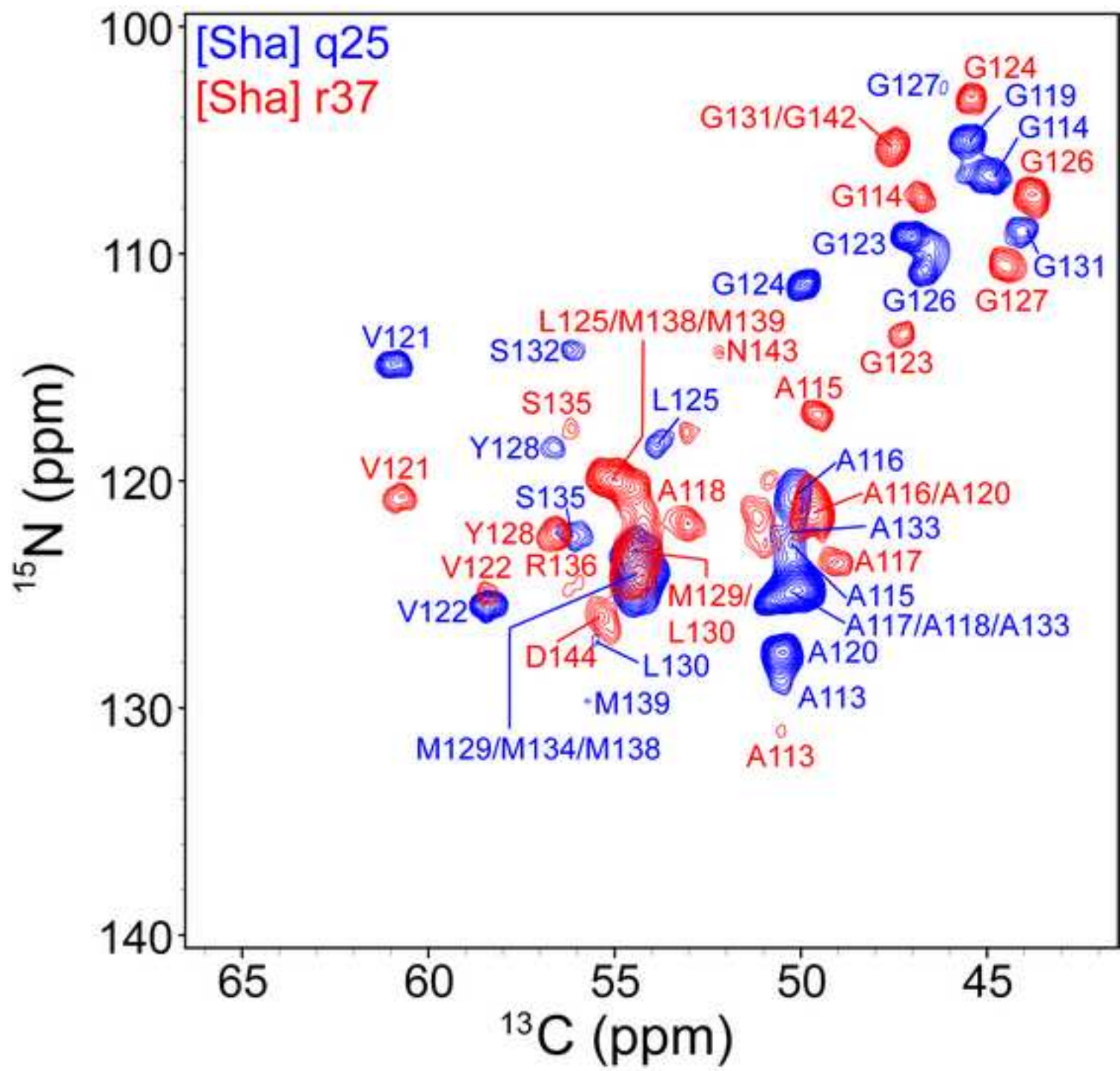


Figure 5
[Click here to download high resolution image](#)

