Supporting Information

Structural Changes associated with Transthyretin Misfolding and Amyloid Formation Revealed by Solution and Solid-State NMR

Kwang Hun Lim*[,†], Anvesh K. R. Dasari[†], Ivan Hung[‡], Zhehong Gan[‡], Jeffery W. Kelly[§], David E. Wemmer*[,⊥]

[†] Department of Chemistry, East Carolina University, Greenville, NC 27858, USA.

[‡] Center of Interdisciplinary Magnetic Resonance (CIMAR), National High Magnetic Field Laboratory (NHMFL), 1800 East, Paul Dirac Drive, Tallahassee, FL 32310. USA.

[§] Department of Molecular and Experimental Medicine and the Skaggs Institute for Chemical Biology, The Scripps Research Institute, La Jolla, CA 92037, USA.

⊥ Department of Chemistry, University of California, Berkeley, CA 94720, USA.

E-mail: limk@ecu.edu, dewemmer@berkeley.edu

Materials and Methods

Protein Expression. Wild type TTR was expressed in BL21 (DE3) *E. coli*, and was purified as previously described.[1] Uniformly ^{15}N-labeled protein was prepared using ^{15}N-NH_4Cl as a sole source of nitrogen. For the selectively ^{13}CO- and ^{13}Cα-labeled samples, M9 media supplemented with unlabeled amino acids (100 mg per liter culture) and ^{13}C-labeled amino acids (50 mg/L) were used for protein expression. In order to selectively label six amino acids, ^{15}N-NH_4Cl and ^{13}C-glucose were used in M9 media supplemented with the other fourteen unlabeled amino acids (100 mg/L).

Amyloid Formation. Amyloid samples were obtained by incubating the WT TTR (0.2 mg/mL) in 200 mM acetate buffer (100 mM KCl, 1 mM EDTA, pH 4.4) for a period of 30 days at 37 °C. The insoluble amyloid was spun down and washed twice with deionized water to remove remaining soluble tetramers and soluble aggregates. The TTR amyloid was examined by transmission electron microscopy (TEM) and thioflavin T binding assay (Supplemental Figure S1).

Under the amyloidogenic condition of pH 4.4, WT TTR tetramers are in a dynamic equilibrium with amyloidogenic TTR monomers. Previous ultracentrifugation experiments showed that

approximately 25 % of the tetramers are observed at a protein concentration of 0.2 mg/ml (ref. 1 in the main text). Thus about 20 – 25 % of tetramers might be present under our experimental conditions (0.15 mg/ml). In this report, conventional HSQC NMR spectra (non-TROSY version) were acquired using fully protonated proteins on a 500 MHz NMR spectrometer, and thus NMR peaks from the tetramers are highly unlikely to be observed in the HSQC spectra (Figure 1b) due to much broader NMR peaks and low concentration for tetrameric TTR.

As for the monomeric variant (F87M/L110M, M-TTR), M-TTR was shown to exist as mainly monomeric forms (more than 95 % at a protein concentration of up to 1.4 mg/ml, ref. 11 in the main text) by ultracentrifugation experiments. The monomeric TTR may undergo conformational exchanges between native monomers and monomeric precursor states on millisecond time scales, which lead to the disappearance of the NMR peaks from residues in DAGH β-sheet (Figure 1b). Previous relaxation dispersion NMR experiments on M-TTR showed that the monomeric precursor state is populated about 3 – 4 % at the physiological pH of 7.0 (ref. 12 in the main text). However, the relative population of the monomeric precursor state at the amyloidogenic pH of 4.4 could not be determined due to the aggregation of proteins under the experimental condition.

NMR Spectroscopy. For solid-state NMR experiments, the protein aggregates prepared from WT TTR were dried using Ar gas at 25 ^{o}C and packed into a 3.2 mm rotor. A small amount of water (5 μL) was then added for rehydration. For the native state, the WT tetrameric TTR at pH 7.3 was precipitated using 90% ammonium sulfate and dried under Ar gas. The dried native TTR was packed into the rotor and rehydrated with 5 μL water. The native WT TTR samples were not obtained under crystallization conditions, but high-resolution solid-state NMR spectra were obtained using the precipitates.

Solid-state NMR spectra were acquired using Bruker 800 and 830 MHz spectrometers equipped with a 3.2 mm CP-MAS probe at room temperature. The two-dimensional solid-state NMR spectra for the selectively labeled samples were mainly recorded using the Bruker DRX 830 MHz spectrometer with an ultra-narrow bore (31 mm) 19.6 T magnet. A low-E MAS probe[2] developed at the NHMFL with a Revolution NMR stator and 3.2 mm pencil rotors was used for the measurements.

A linear amplitude ramp on the ^{1}H channel was used for the ^{1}H/^{13}C cross-polarization with a contact time of 1 ms. The 90° pulse-lengths for ^{1}H and ^{13}C were 3.0 μs and 5.0 μs, respectively. The two-pulse phase-modulated (TPPM) decoupling scheme was employed with a radio-frequency field strength of 80 kHz. Two-dimensional ^{13}C-^{13}C correlation NMR spectra were recorded using a proton-driven spin diffusion (PDSD) mixing scheme at spinning frequencies of 11 – 12 kHz. For the 2D PDSD spectra, complex data points of 1024 (t2) × 362 (t1) and 1024 × 145 were collected for the native and amyloid states, respectively, with acquisition delay of 2 sec, and 48 – 64 FIDs were accumulated for each t1 data point.

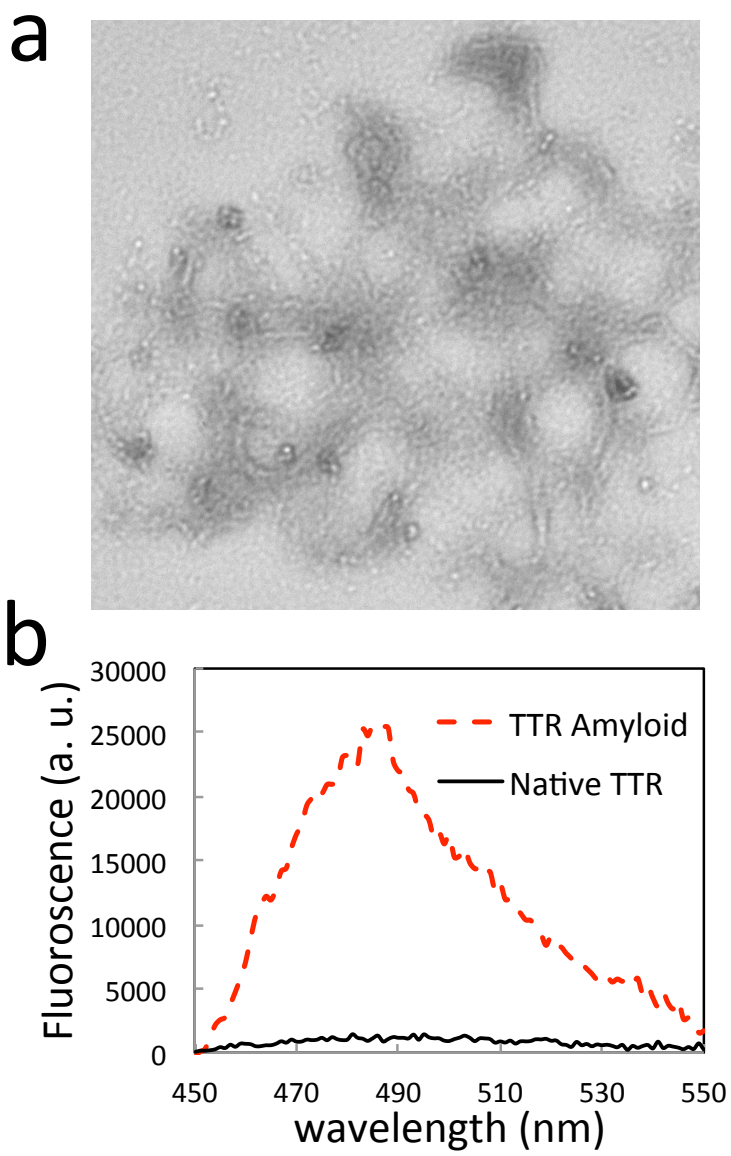

Figure S1. (a) TEM image of TTR amyloid obtained after negative staining with uranyl acetate (2 %). (b) Increases in the fluorescence intensity of ThT upon binding to TTR amyloid (0.2 mg/mL).

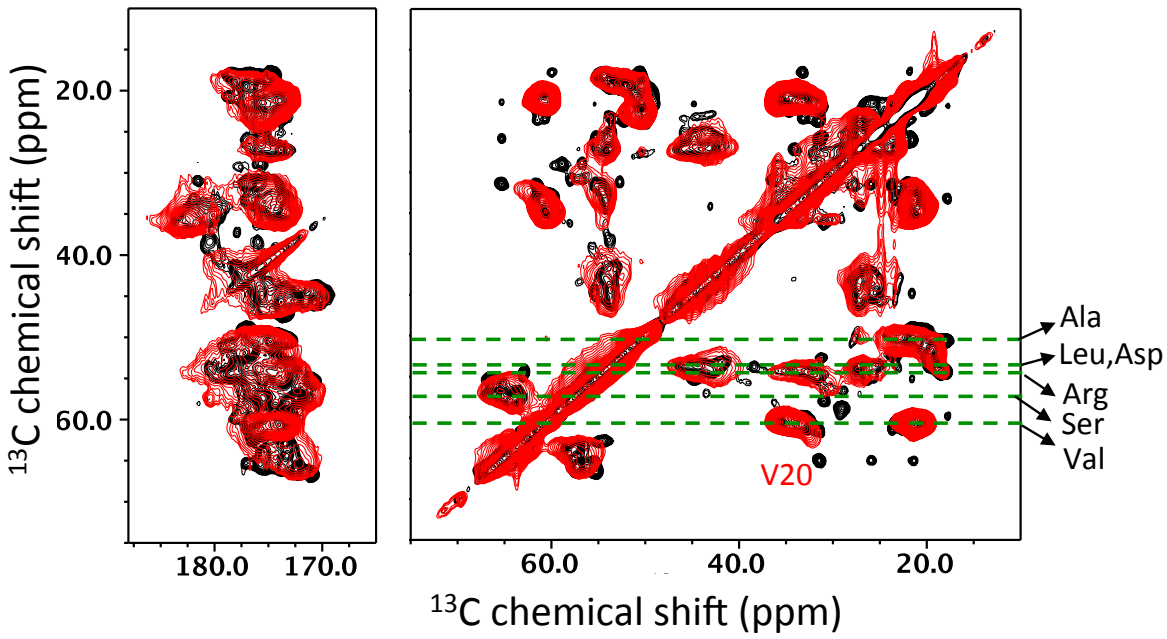

Figure S2. Overlay of cross-polarization (CP) based ^{13}C-^{13}C 2D correlation MAS NMR spectra of TTR native (black) and amyloid (red) states obtained using a PDSD mixing time of 25 ms at 800 MHz (^{1}H frequency). The green lines denote the average chemical shifts for β-strand conformations of the amino acids.[3] In order to simplify the 2D spectra, six amino acids were selectively ^{13}C/^{15}N labeled, as described in the Materials section in Supplemental Information.

TTR quickly forms moderately ordered amyloid at pH 4.4 (Supplemental Figure S1a), leading to the broad NMR resonances with a full-width at half-maximum of ~ 2.5 ppm. The linewidth is somewhat broader than those from previously reported homogeneous amyloid fibrils (< 1 ppm) derived from other proteins.[4-6] Although the NMR resonances from amyloid state (red) are broader than signals from the native state (black), the relative signal intensities from the side-chains in TTR amyloid are almost identical to those from its native state, as evidenced in the 1D traces for the chemical shifts along the green line corresponding to typical β-strand conformation for each amino acid (Figure S3). In addition, the chemical shifts of the Cα and Cβ carbons of the six amino acids indicate that a majority of those residues are located in β-sheet regions, implying the presence of a rigid, ordered amyloid core rich in β-sheet conformations.

The strong NMR signals of TTR amyloid in the dipolar based solid-state NMR are also comparable to or narrower than those from Aβ oligomers[7] and β-microglubulin aggregates[4] formed at pH 3.6 (2 – 4 ppm) that are shown to be still homogeneous aggregates. In addition, a single set of the cross-peaks in the extensive 2D correlation spectra described in the main text and in Supplemental Figure 4b strongly indicates that TTR amyloid used in this study is a uniform protein assembly consisting of a single monomeric core conformer. The broad NMR resonances most likely result from some inhomogeneity of interfacial interactions in subunit-subunit interfaces and/or local dynamic processes.

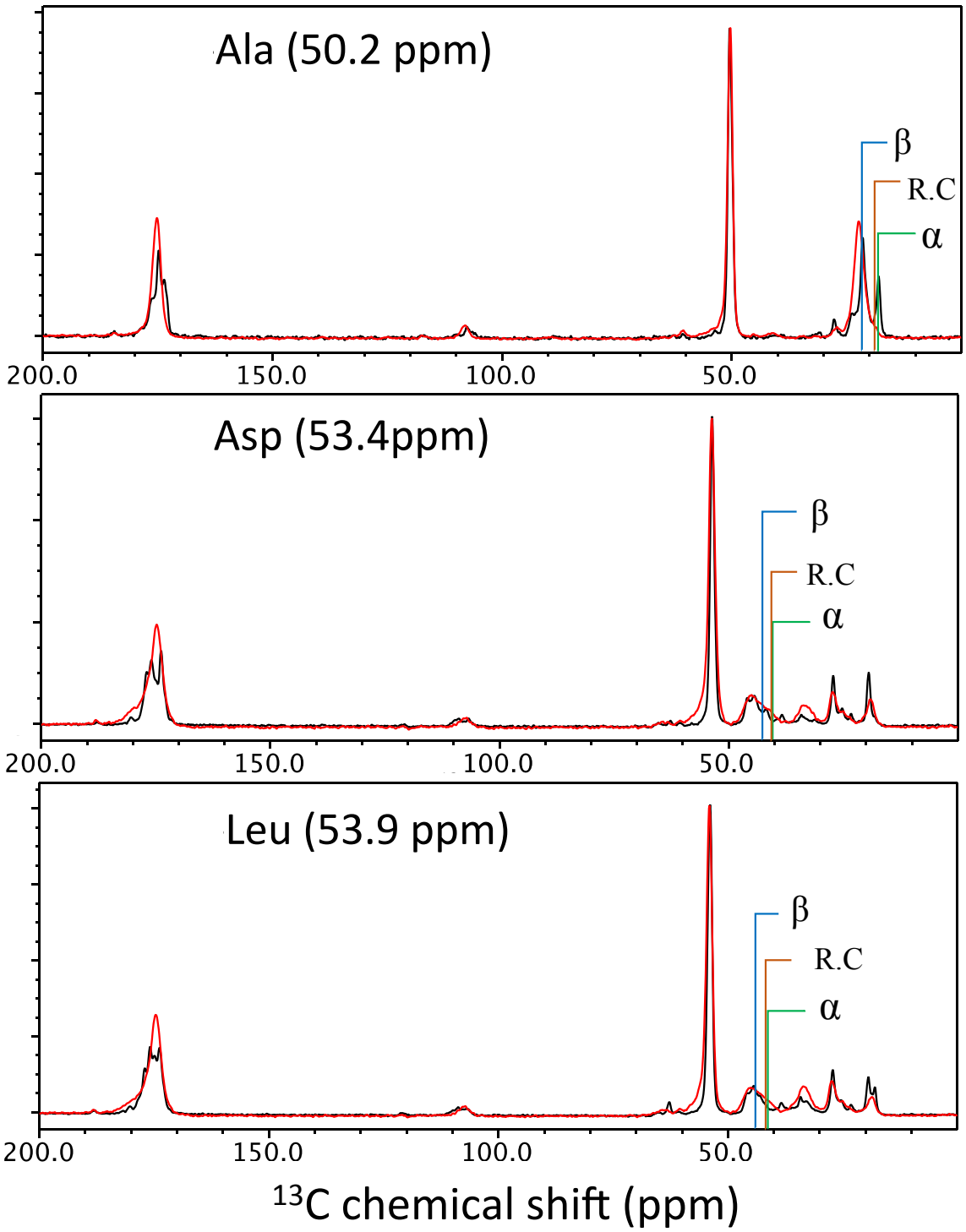

Figure S3. One-dimensional traces of the amino acids in the 2D PDSD spectra (Figure S2) for the native (black) and amyloid (red) states of TTR. Average chemical shifts of the Cβ carbons indicative of the secondary structures are denoted.

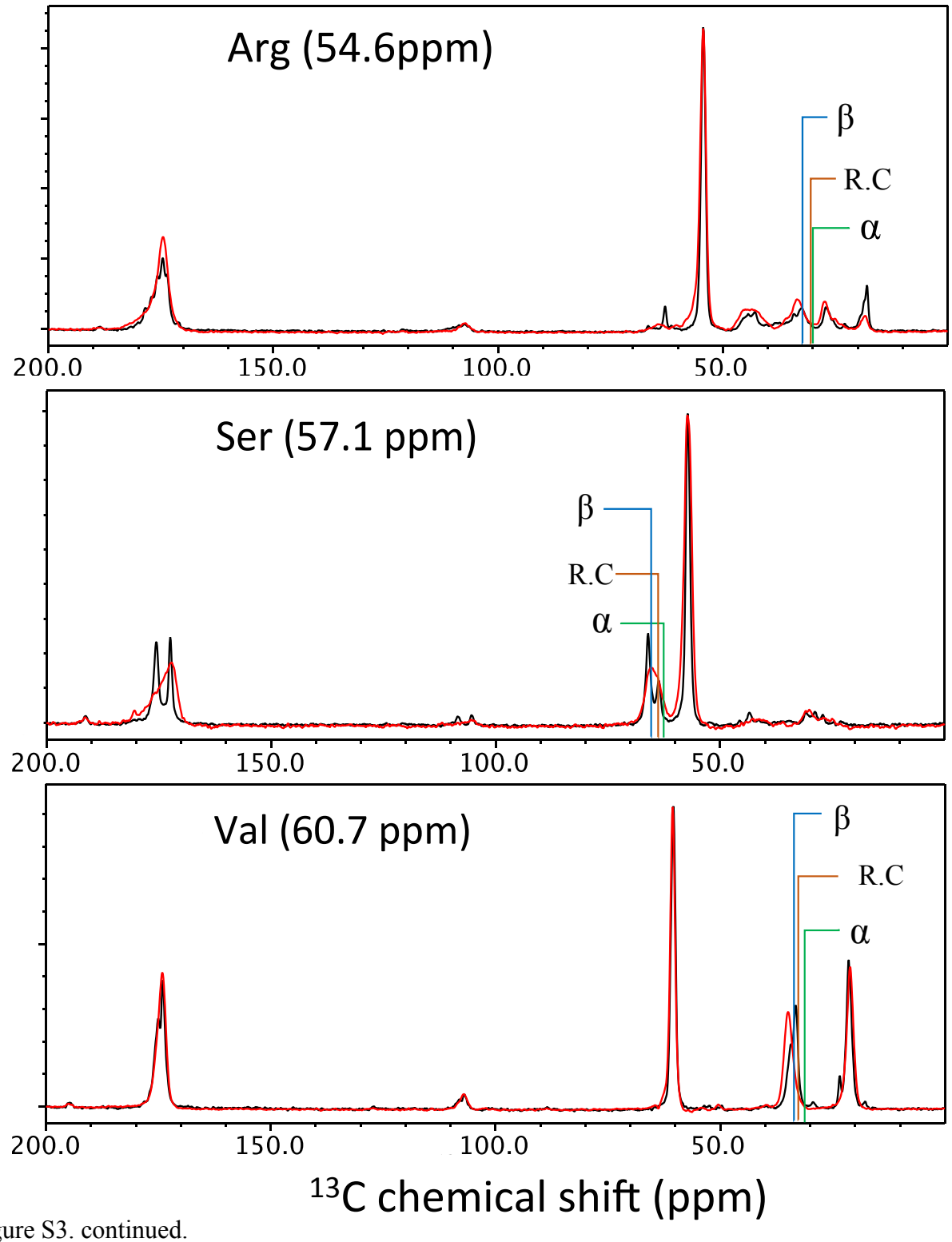

Figure S3. continued.

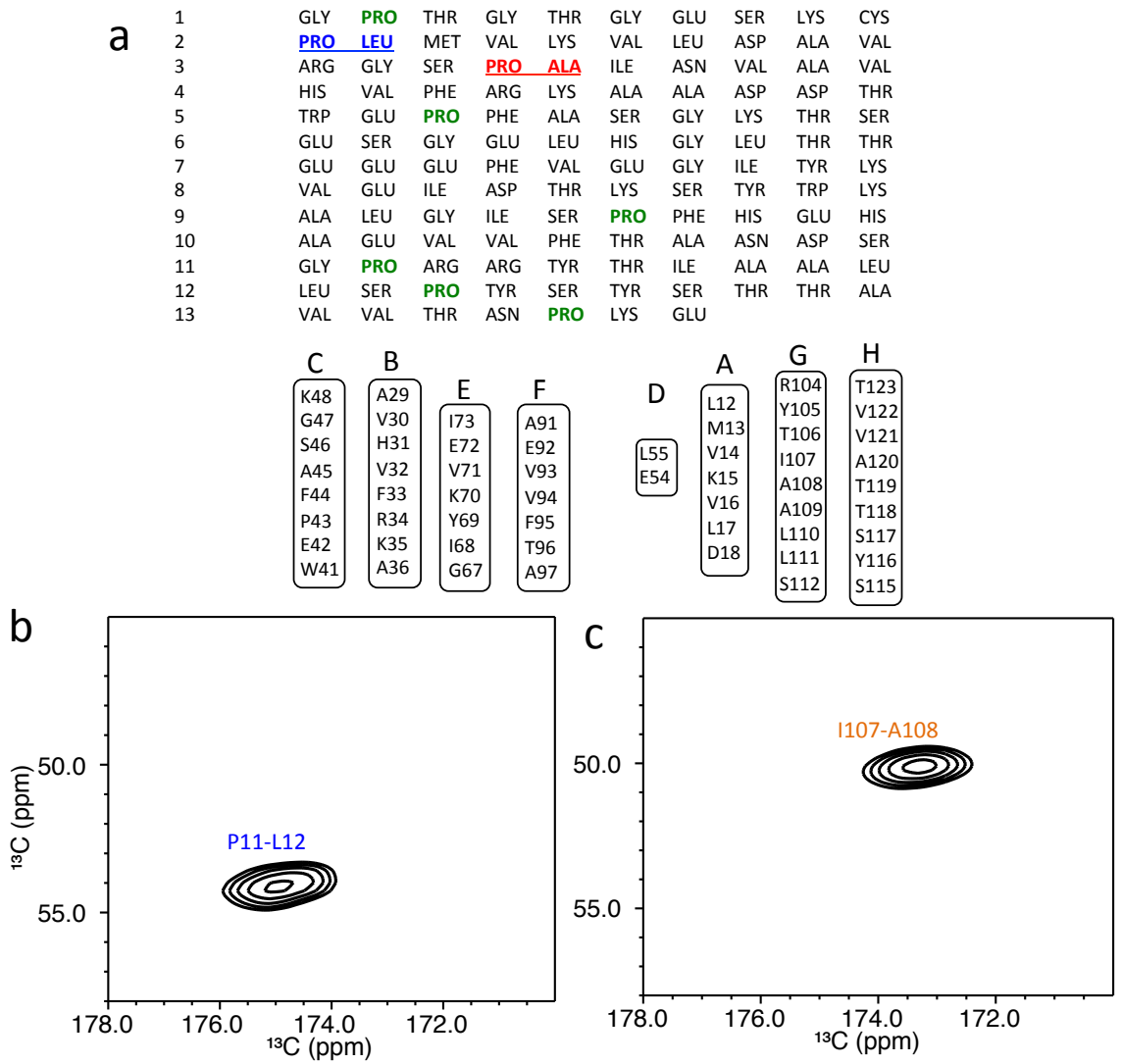

Figure S4. (a) Amino acid sequence of the TTR. (b) A 2D PDSD NMR spectrum of amyloid state of ^{13}CO-Pro, ^{13}Cα-Leu, and ^{13}Cα-Ala labeled TTR. (c) A 2D PDSD NMR spectrum of amyloid state of ^{13}CO-Ile and ^{13}Cα-Ala labeled TTR. A mixing time of 500 ms was used.

In the 2D PDSD spectrum for the amyloid state of the ^{13}CO-Pro, ^{13}Cα-Leu, and ^{13}Cα-Ala labeled TTR (Figure S4b), a cross-peak for the strongly coupled P24-A25 (2.4 Å) spin pair was not observed. Under the same experimental condition, a strong cross-peak for the other dipolar-coupled spin pair (I107-A108, 2.4 Å) was clearly observed in Figure S4c, indicating that the AB loop region is disordered in amyloid state.

In addition, the residue V20 in a tight turn in the native state (65 ppm, black) is not detected in amyloid state spectrum (red, Supplemental Figure S2). Relative intensities of the cross-peaks in amyloid state spectrum are also identical to those in the native state spectrum (Supplemental Figure S2 and S3), which rules out overlaps of the chemical shift for the AB loop regions with other NMR peaks due to minor structural changes. These NMR results strongly suggest that the AB loop regions that interact with strand A become unfolded and disordered, exposing strand A that can be available for intermolecular associations.

References

(1) Lai, Z. H., Colon, W., and Kelly, J. W. (1996) *Biochemistry. 35*, 6470-6482.

(2) McNeill, S. A., Gor'kov, P. L., Shetty, K., Brey, W. W., and Long, J. R. (2009) *J. Magn. Reson. 197*, 135-144.

(3) Wang, Y., and Jardetzky, O. (2002) *Protein Sci. 11*, 852-861.

(4) Debelouchina, G. T., Platt, G. W., Bayro, M. J., Radford, S. E., and Griffin, R. G. (2010) *J. Am. Chem. Soc. 132*, 10414-10423.

(5) Helmus, J. J., Surewicz, K., Surewicz, W. K., and Jaroniec, C. P. (2010) *J. Am. Chem. Soc. 132*, 2393-2403.

(6) Daebel, V., Chinnathambi, S., Biernat, J., Schwalbe, M., Habenstein, B., Loquet, A., Akoury, E., Tepper, K., Muller, H., Baldus, M., Griesinger, C., Zweckstetter, M., Mandelkow, E., Vijayan, V., and Lange, A. (2012) *J. Am. Chem. Soc. 134*, 13982-13989.

(7) Parthasarathy, S., Inoue, M., Xiao, Y., Matsumura, Y., Nabeshima, Y., Hoshi, M., and Ishii, Y. (2015) *J. Am. Chem. Soc. 137*, 6480-6483.