Supplementary Materials

Two Distinct Pathways in Transthyretin Misfolding and Amyloid Formation

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Figure S1

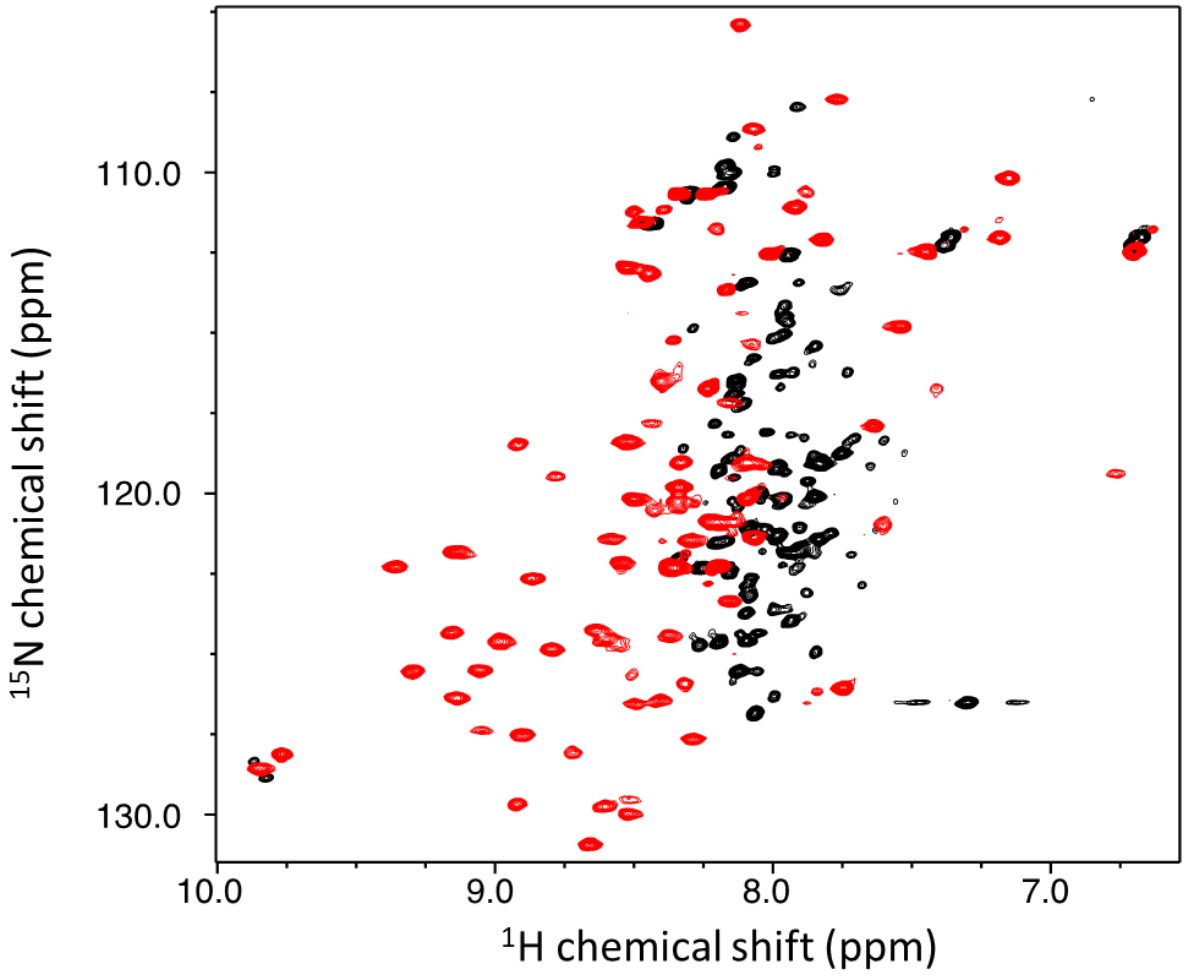

Figure S1. Overlaid 2D ^{1}H/^{15}N HSQC NMR spectra of TTR at pH 2.4 (black) and 4.4 (red).

Figure S2

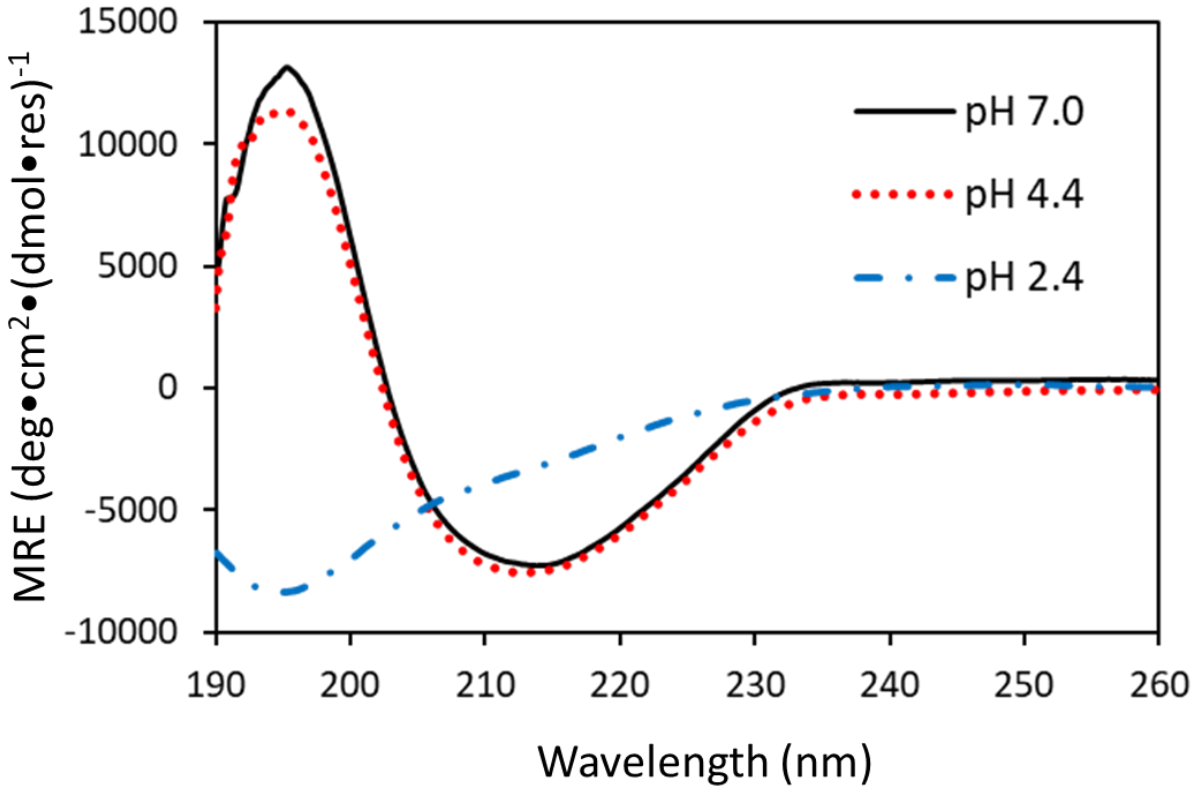

Figure S2. CD spectra of WT of TTR (0.14 mg/ml) at different pH values recorded at 20 ºC. Identical spectra were obtained at a lower protein concentration (0.02 mg/ml), suggesting that the CD signals originate mainly from monomeric precursor states of TTR. Four scans were collected and averaged for the CD spectra.

Figure S3

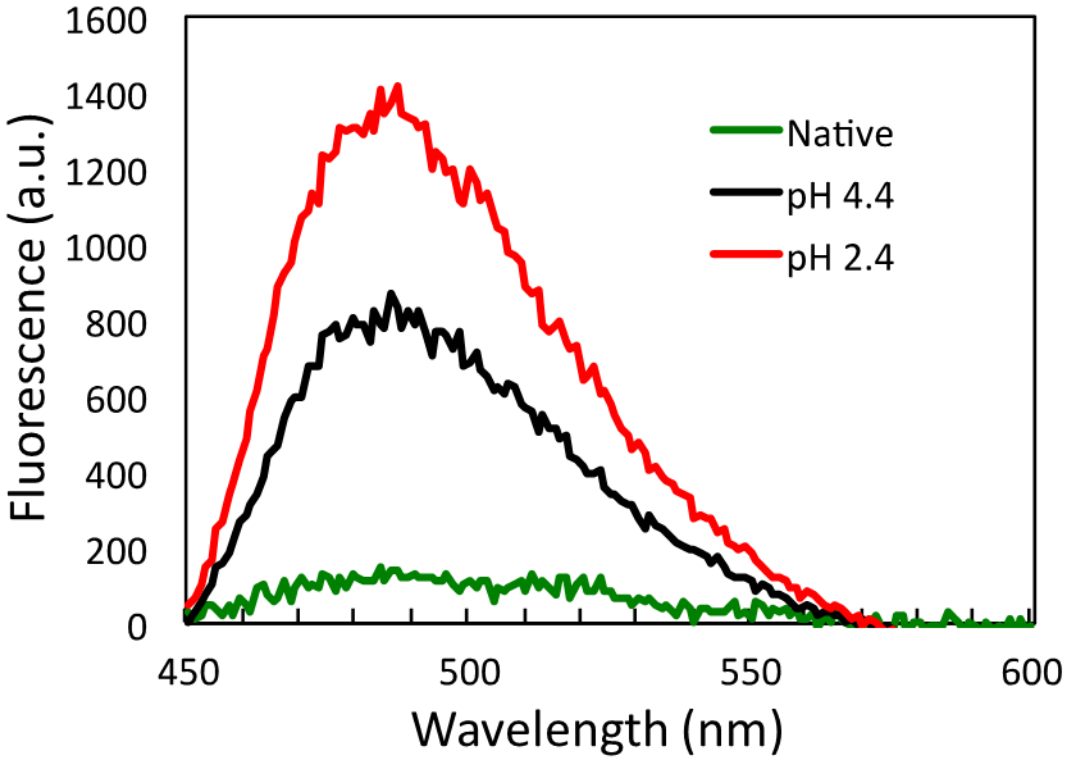

Figure S3. Thioflavin T (ThT) fluorescence emission spectra of the two TTR states. For the fluorescence measurements, 50 μL of ThT (1 mM in PBS buffer, pH 7.4) was mixed with the native and two amyloid states of TTR (0.2 mg/ml). The emission spectra were recorded with an excitation at 440 nm.

Figure S4

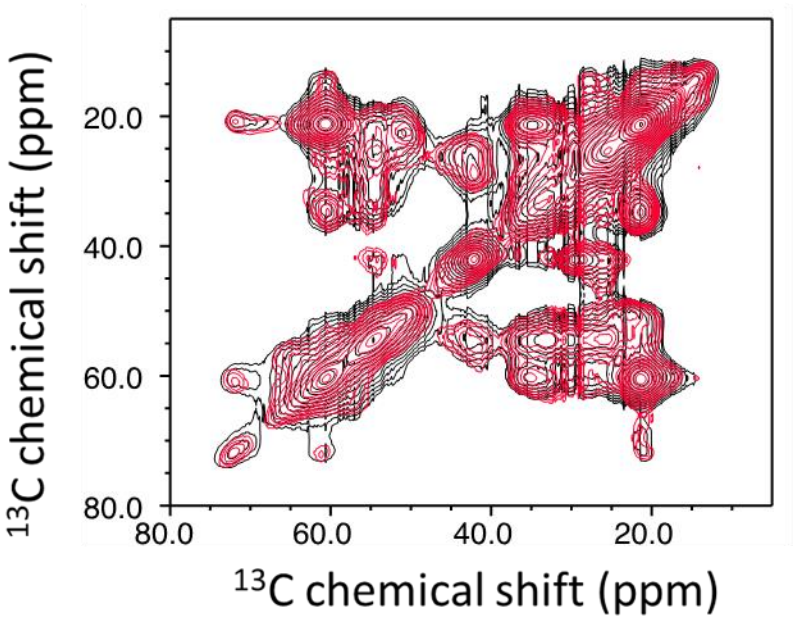

Figure S4. Overlaid two-dimensional ^{13}C-^{13}C correlation NMR spectra of the two amyloid states (2.4: black and 4.4: red) obtained using a proton-driven spin diffusion (PDSD) mixing scheme with a mixing time of 20 ms.

Figure S5

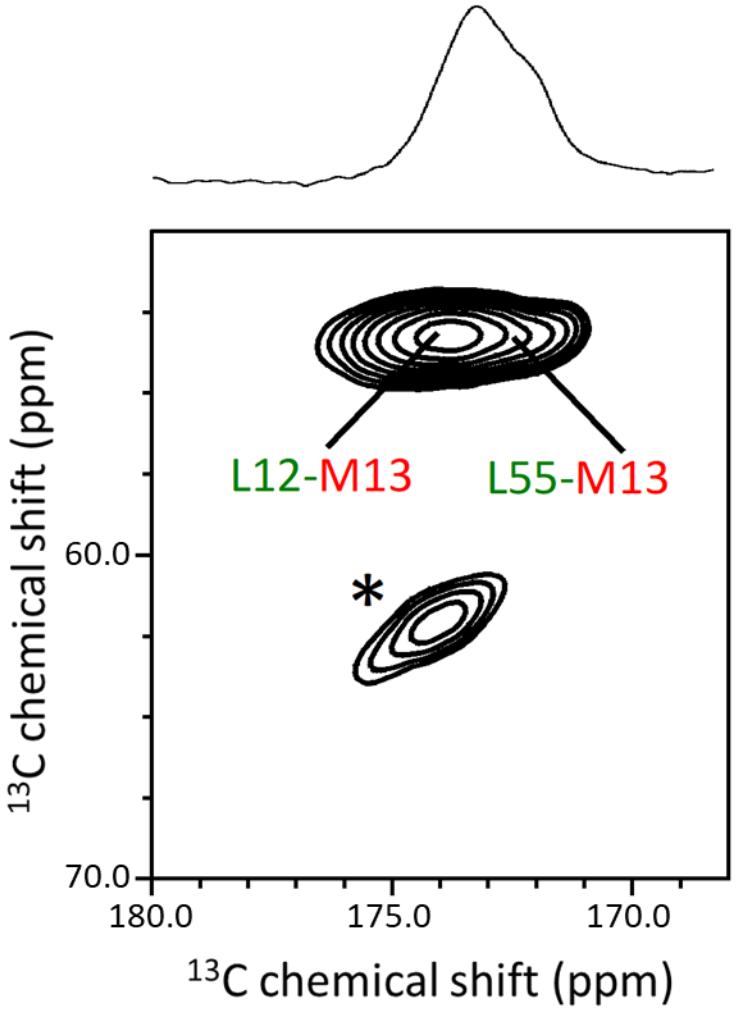

Figure S5. An enlarged 2D PDSD spectrum for the L55-M13 spin pair of the amyloid-4 state. * denotes a spinning sideband.

Figure S6

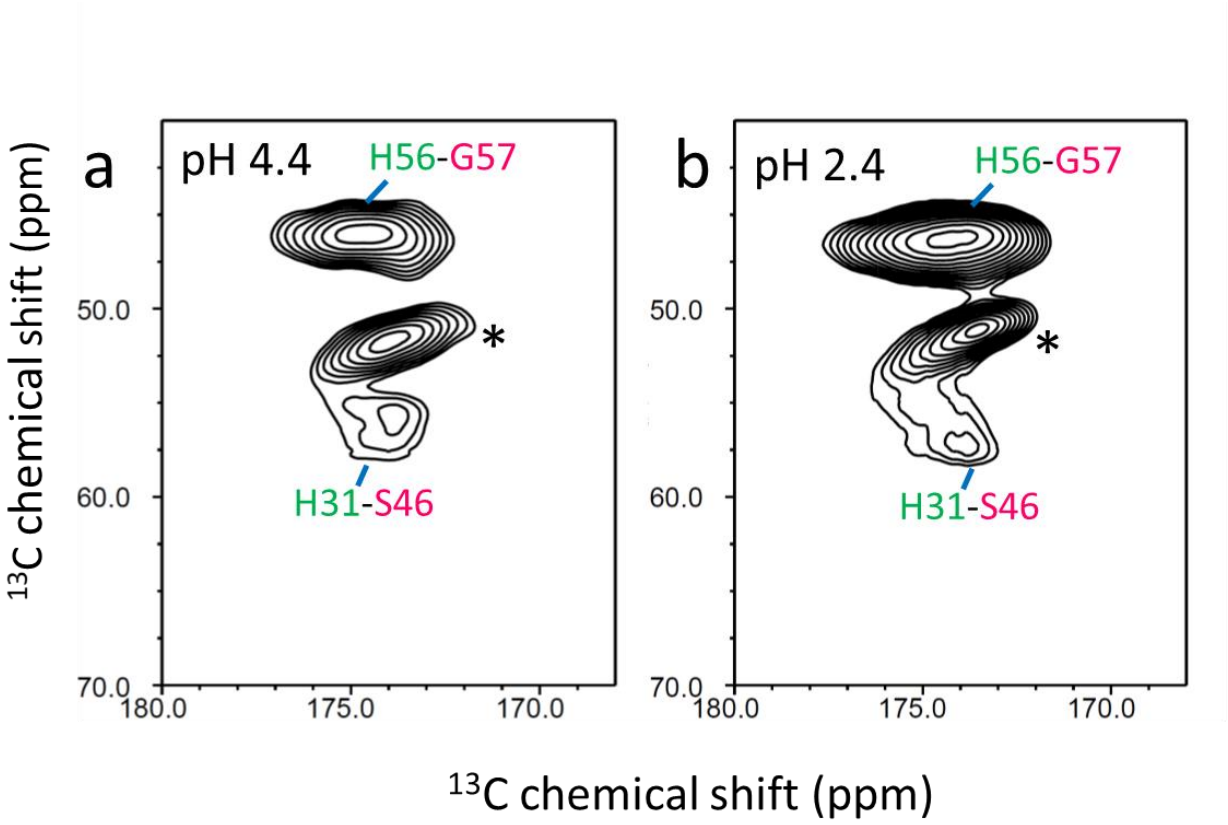

Figure S6. 2D PDSD spectra of the two amyloid states with ^{13}CO-His/^{13}Cα-Gly/^{13}Cα-Ser labeled on CB strands.

Figure S7

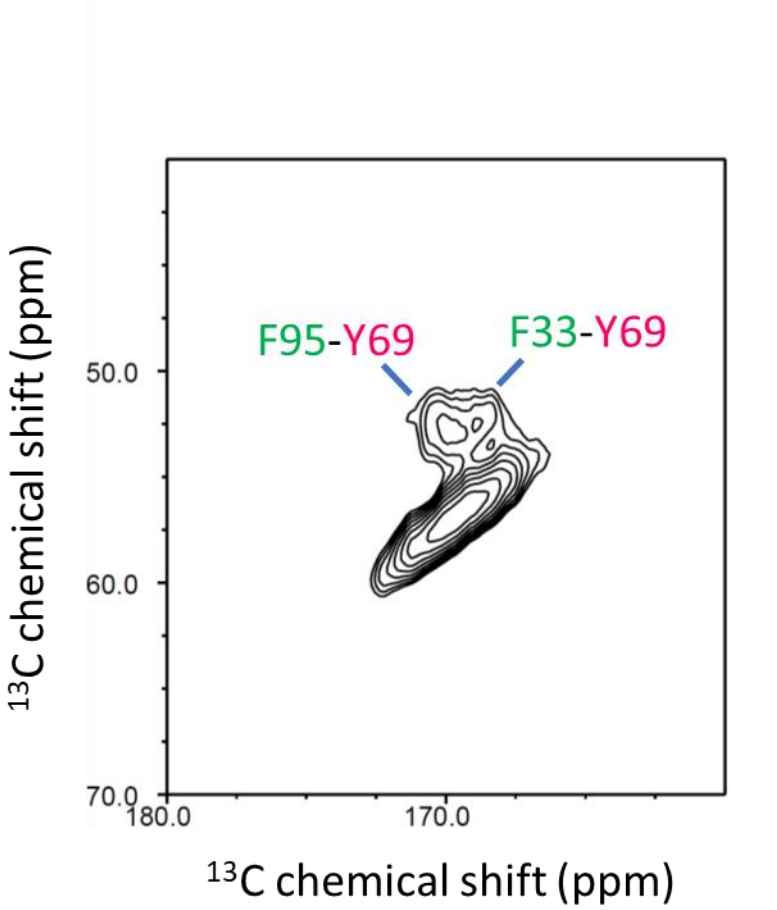

Figure S7. 2D PDSD spectrum of the amyloid-2 with ^{13}CO-Phe/^{13}Cα-Tyr labeled on BEF strands.

Figure S8

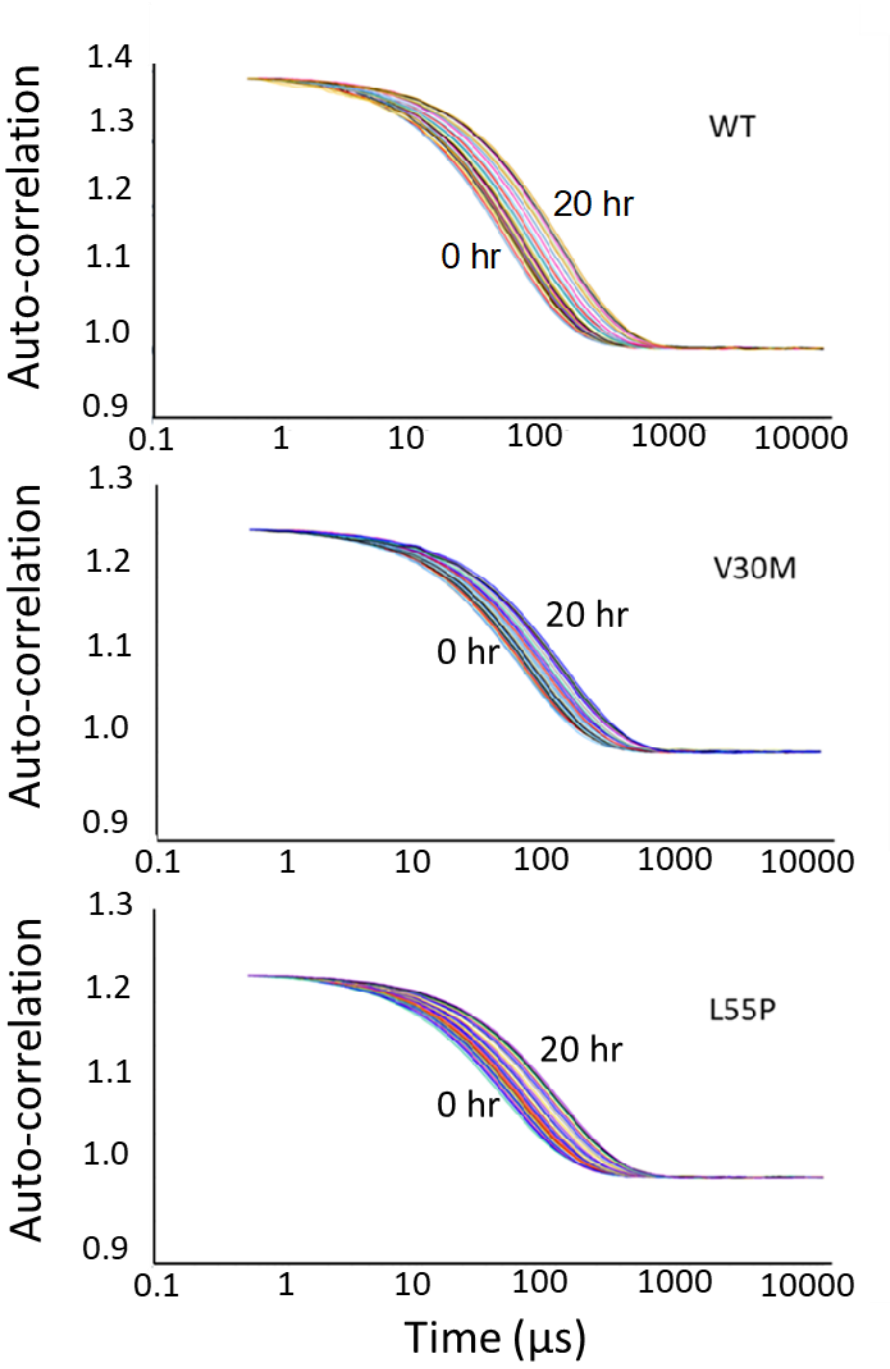

Figure S8. Changes in autocorrelation function from dynamic light scattering over an incubation period of 20 hours. The autocorrelation function intensity gradually increases at longer incubation times, indicating increases in the particle size.

Figure S9

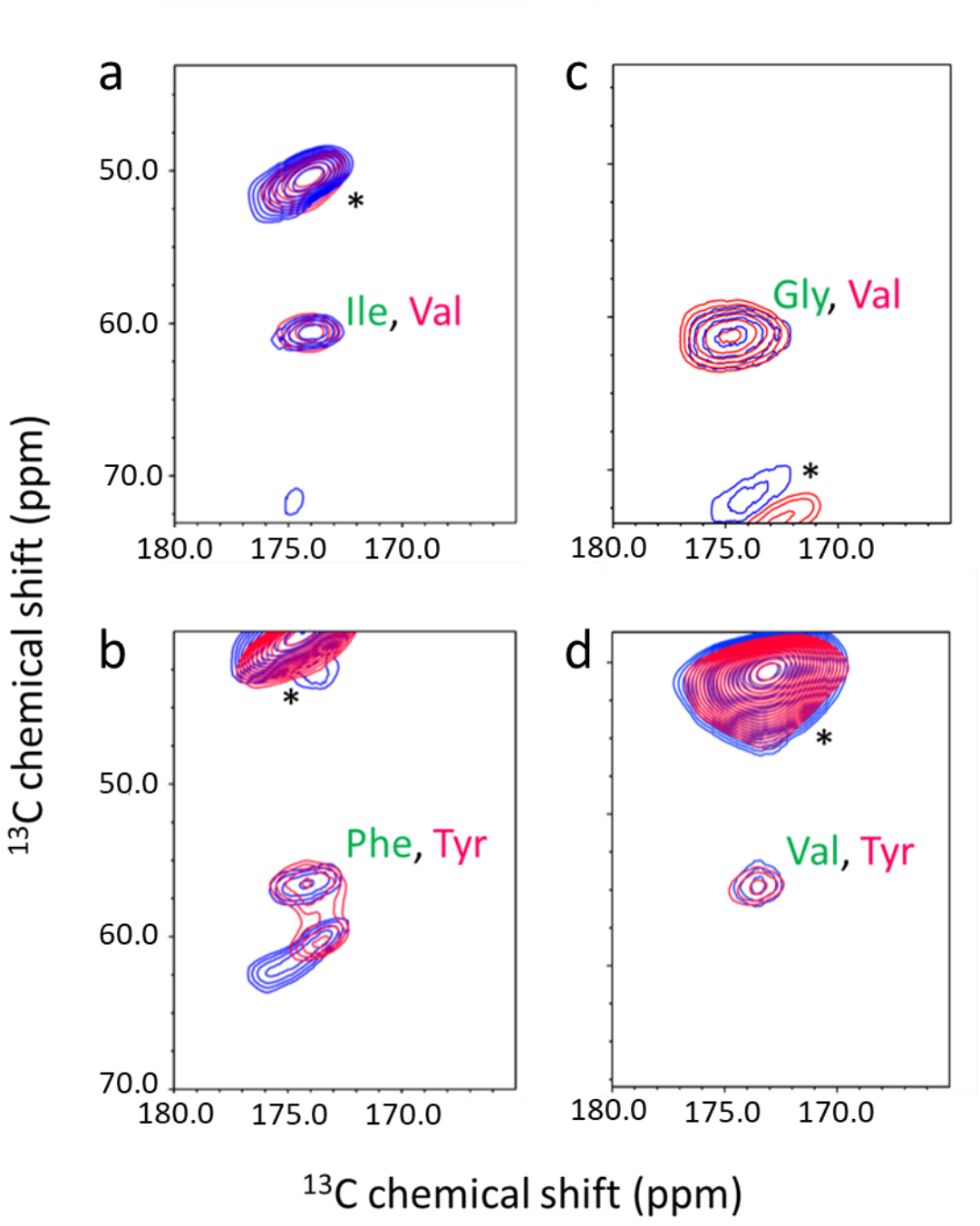

Figure S9. Duplicated 2D PDSD spectra of the amyloid-4 with various labeling schemes that cover CBEF and DAGH β-sheet in Figure 2. The NMR spectra were reproduced well for the amyloid samples. * denotes spinning sidebands.