# Transthyretin Aggregation Pathway toward the Formation of Distinct Cytotoxic Oligomers

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## Introduction

Transthyretin (TTR) is a homotetrameric protein with 127 amino acid residues in each monomer and is rich in β-sheet structure in which eight β-strands are arranged in a β-sandwich consisting of two β-sheets (strands CBEF and DAGH). Misfolding/unfolding of TTR to form beta sheet rich amyloid is associated with numerous amyloid diseases. Increasing evidence suggested that small oligomers formed at an early stage of amyloid formation are real cytotoxic species. Characterization of oligomeric intermediate states is, therefore, critical to understanding the molecular mechanism of pathogenic oligomerization process. Purpose of this study is to probe molecular mechanism of transthyretin (TTR) oligomerization process through characterization of small intermediate states of WT and a mutant form of TTR (G53A) by using 2D $^{13}C$-$^{13}C$ correlation experiments.

## Experimental

Cross-polarization (CP) based two-dimensional $^{13}C$-$^{13}C$ solid-state NMR spectra were acquired using a COmbined R2n(v)-Driven (CORD) recoupling mixing scheme with $^{1}H$ radio-frequency (rf) field strengths of 30 and 15 kHz for $R2_1^v$ and $R2_2^v$ symmetry sequences, respectively at NHMFL (Tallahassee).

## Results

The structural features of the WT oligomers were compared to those of native TTR using solid-state NMR (Figure 1). The strong NMR cross-peaks from WT oligomers (red) shown in Figure 1a suggests that the oligomers contain rigid, structured regions. It is also notable that the NMR peaks from the oligomers are overlapped well with those of native state TTR (black), indicating that the oligomeric TTR contains native-like β-structures. However, NMR resonances from the oligomers are substantially broader, and many of the cross-peaks in native TTR are not observed in the oligomer spectrum. These NMR results indicate that the WT oligomeric states are substantially more disordered than the native state, which is in good agreement with our CD spectroscopy results. The structural feature of the oligomeric state was also compared to that of the final product, amyloid and it showed that the 2D solid-state NMR spectra for the two states are also overlapped well, suggesting that the two states have similar structural features. Our previous solid-state NMR studies showed TTR amyloid state contains extensive native β-sheet conformations,[1-3] suggesting that the native-like β-sheet structures are maintained in the oligomeric and amyloid states of TTR. The 2D solid-state NMR spectrum was also acquired for G53A oligomer and compared to that of WT oligomer (Figure 1b). The similar NMR spectra suggest that the two oligomeric states possess similar structural features. However, NMR cross-peaks for the G53A oligomer are much weaker in intensity than those of WT oligomer, implying that G53A oligomer is more disordered than WT oligomer.

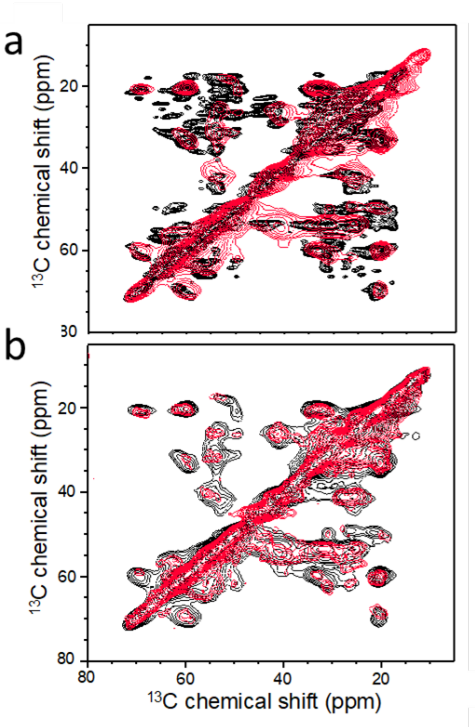


Figure 1. (a) Overlaid two-dimensional (2D) $^{13}C$-$^{13}C$ correlation solid-state NMR spectra of WT (native) and WT oligomer (red) obtained with COmbined R2n(v)-Driven(CORD) $^{38}$recoupling scheme. (b) Overlaid 2D spectra of WT (black) and G53A (red) oligomers. The CORD mixing time was 115 ms.

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## References

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