# The Mechanism of G335D Promoting the Aggregation and Fibrillization of TDP-43 Amyloidogenic Core Region: A Computational Study

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## Introduction

- The molecular interaction and structural transformation of TDP-$43_{311\sim360}$ during aggregation have stirred up increasing interest in recent years.
- Extensive experimental and computational investigations have shown that TDP-$43_{311\sim360}$ peptide can spontaneous aggregation and the amyotrophic lateral sclerosis (ALS) linked G335D mutation can promote the aggregation.
- However, the mechanism at the molecular level remains largely unknown.
- Here, combining molecular dynamics (MD) simulations and replica exchange with solute tempering version2 (REST2) simulations, we investigated the mechanism of dimerization (the first step of the aggregation) of WT and G335D TDP-$43_{311\sim360}$ variants.

## Materials and Methods

**TDP-$43_{311\sim360}$ amino acid sequence:**
$_{311}$MNFGAFSINP$_{320}$AMMAAAQAAL$_{330}$QSSWGMMGML$_{340}$ASQQNQSGP S$_{350}$GNNQNQGNMQ$_{360}$

**Method:** MD in NPT ensemble, two independent simulations, 310 K, 1200 ns; REST2 in NPT ensemble, two independent 18 replicas simulations and one 24 replicas simulation, effective temperature of 310~568 K for 18 replicas and 310~670 K for 24 replicas, 500 ns

**Force Field:** Amber99sb-ILDN **Water Model:** TIP3P

**Systems:** WT and G335D TDP-$43_{311\sim360}$ dimer

**Packages:** Gromacs-2018.3 and PyMOL

## Results

### ◆ G335D dimer has a stronger aggregation tendency than WT.

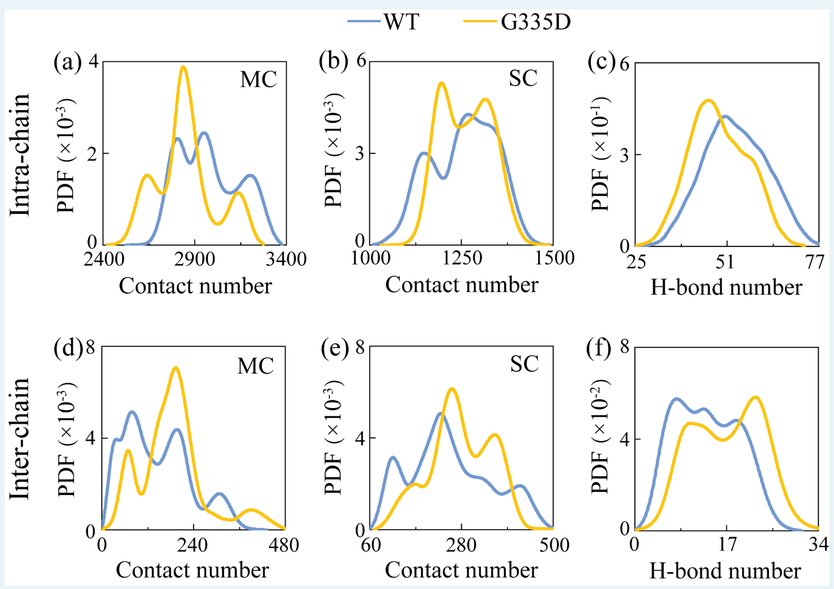


Figure 1. Conformational and physical properties of WT and G335D dimers.

### ◆ G335D mutation increases inter-chain interactions of TDP-$43_{311\sim360}$ dimer.

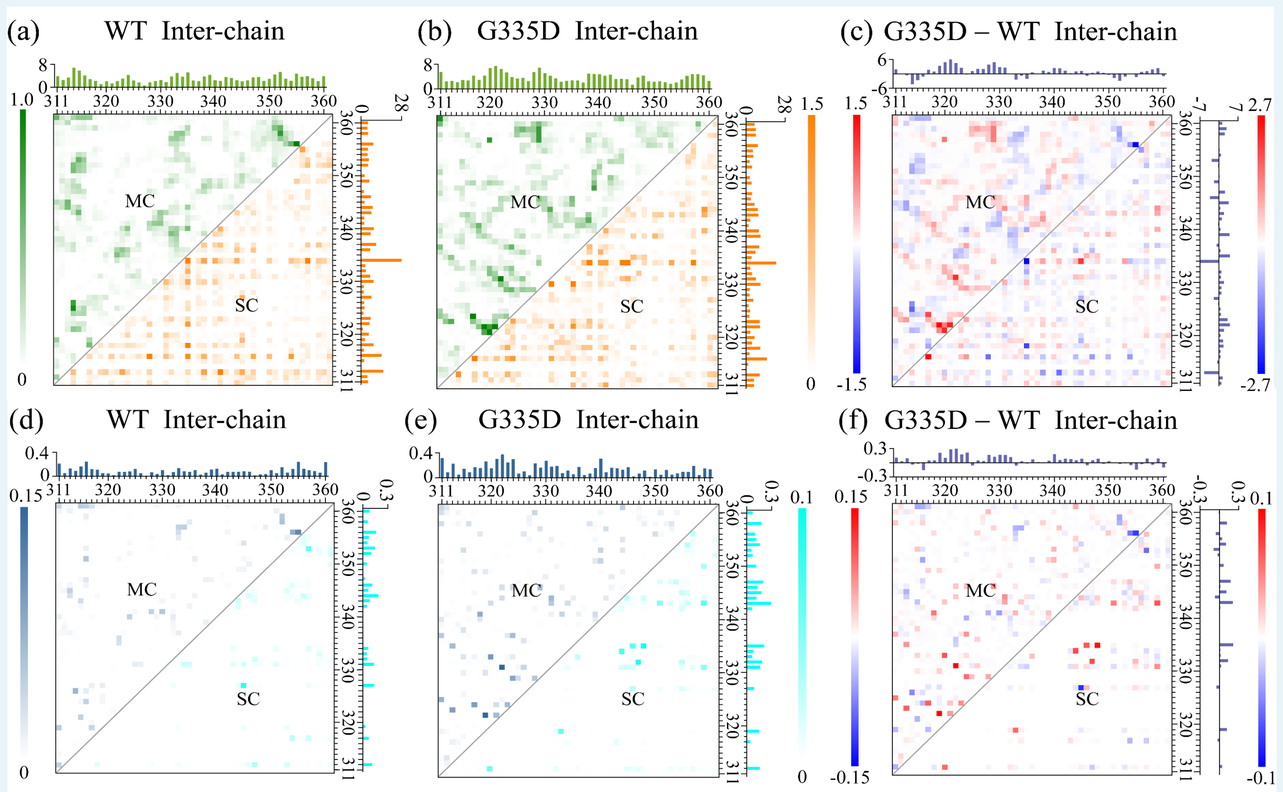


Figure 2. The effect of the G335D mutation on the interaction patterns of TDP-$43_{311\sim360}$ dimers.

### ◆ Aggregation dynamics of WT and G335D TDP-$43_{311\sim360}$ dimer.

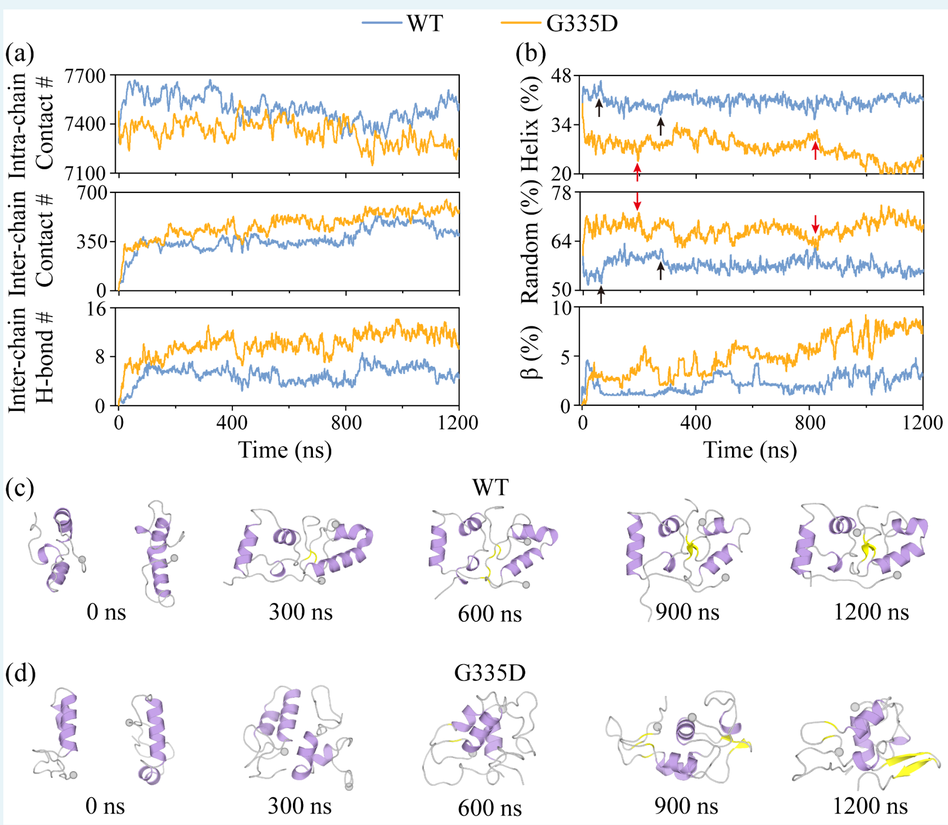


Figure 4. Analyses of the aggregation dynamics of WT and G335D dimers. Number is abbreviated as #.

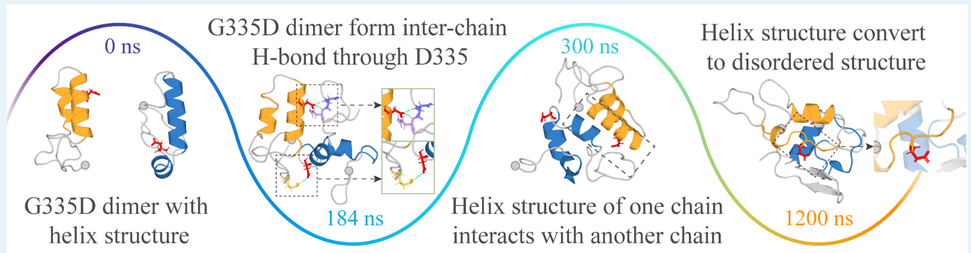


Figure 5. Schematic illustration of G335D promoting inter-chain interactions and secondary structure transformation of TDP-43 dimer.

### ◆ G335D mutation increases the fibrilization propensity of TDP-$43_{311\sim360}$ dimer.

| System \ Probability | Helix structure | β structure | Coil | Bend | Turn |
|---|---|---|---|---|---|
| WT | 22.9±1.0% | 9.5±0.2% | 35.1±1.0% | 16.6±0.8% | 15.9±0.9% |
| G335D | 17.1±0.7% | 15.0±0.4% | 36.7±0.9% | 17.6±1.5% | 13.6±1.3% |

Table 1. Secondary structure probability of WT and G335D dimers averaged over all residues.

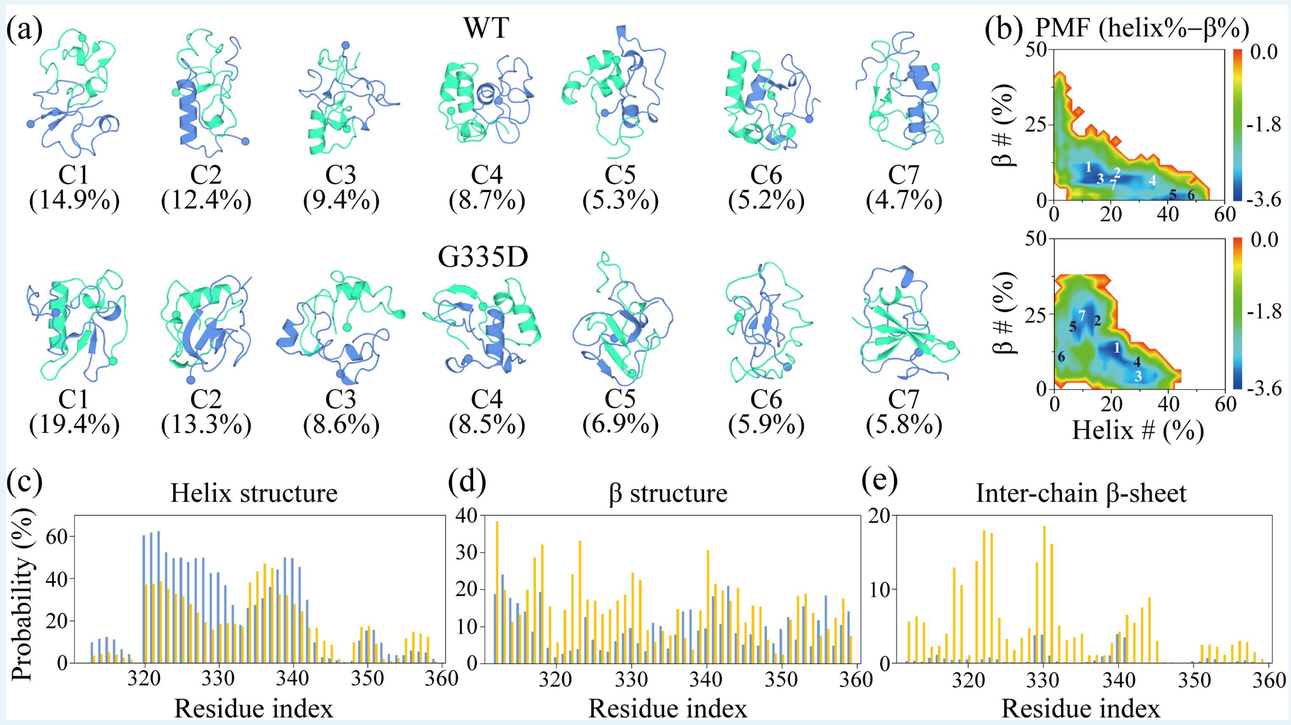


Figure 3. Conformational ensemble and secondary structure analyses of WT and G335D dimers. Probability is abbreviated as #.

## Conclusions

- G335D dimer has reduced intra-chain contacts and increased inter-chain contacts compared to WT dimer, suggesting that G335D enhances the aggregation tendency of TDP-$43_{311\sim360}$ peptides.
- G335D dimer has fewer helix structures and more β structures with longer β-sheet length and more diverse morphologies than their WT counterpart, indicative of the enhanced fibrillization tendency of G335D peptides.
- The helix-to-disordered conversion and the disordered-to-beta conversion are more prominent in the G335D system than those in WT system.
- D335 in G335D dimer plays a leading role in initiating inter-chain hydrogen-bonding interactions, which leads to strong binding between TDP-$43_{311\sim360}$ peptides, especially in the initial helix region. The enhanced inter-chain interactions promote the helix-to-disordered conversion and further facilitate the disordered-to-β conversion.