



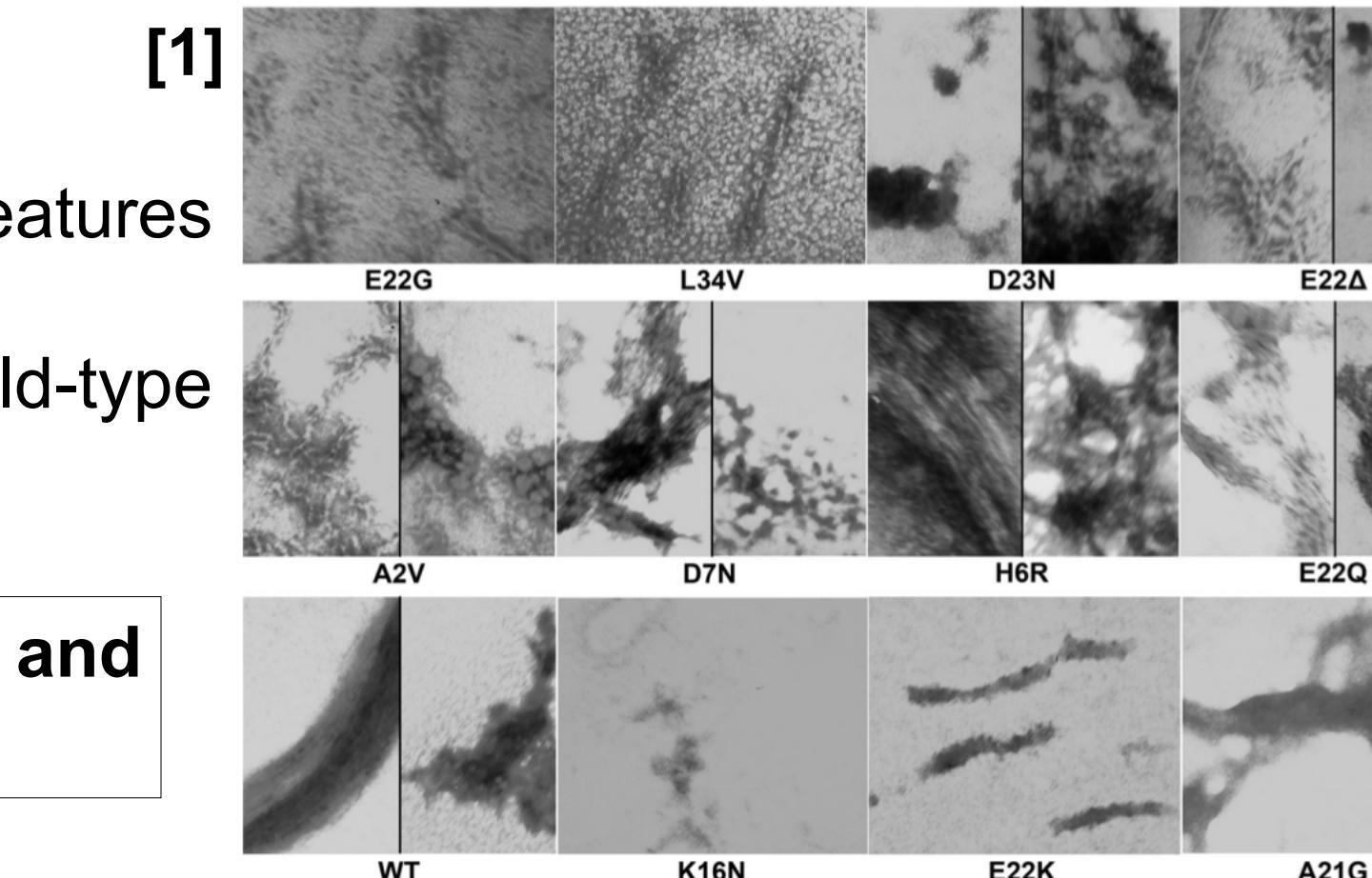
Abstract

Alzheimer's Disease affects millions of people and is becoming more prevalent. It is believed to be caused by aggregates of β -amyloid ($\text{A}\beta$) peptides. The end-points of aggregation are amyloid fibrils, though it is widely believed that soluble, oligomeric precursors of the fibrils are the more important neurotoxins. $\text{A}\beta$ is known to form highly polymorphic fibrils, with many mutant forms observed in patients. Some mutants, such as the Iowa (D23N) and the Osaka (E22 Δ), are known to have increased neurotoxicity and faster aggregation in relation to wild-type peptides. To investigate the differences between wild-type and mutant $\text{A}\beta$ -peptides, we modeled their behavior at four levels: monomer, oligomer, finite and infinite fibrils; using long all atom molecular dynamics simulations. The two wild-type fibril structures in our simulations were PDB entries 2LMN and 2LMP (having two- and three-stacks), the Osaka mutant structure was 2MVX (having two-stacks) and the Iowa mutant structures were 2LNQ and 2MPZ (having one- and three-stacks). The Osaka mutant demonstrated the most structural stability evident from its higher average β -content (that is known experimentally to be strongly correlated to stability in amyloid fibrils). We attribute this to strong inter-backbone hydrogen-bond network and a strong salt-bridge between residues E3-R28 unique to the Osaka mutant. We also observed a larger number of sodium ions accumulated on the interior pockets of the Osaka-mutant fibril. These can explain the very fast aggregation of the Osaka mutant and possibly its higher neurotoxicity. The Iowa mutant structure (2MPZ) and wild-type structure (2LMP) had similar structural stability as well as aqueous-pore-like behavior, which might disrupt cell function if inserted through a membrane. The one-stack Iowa mutant showed the least structural stability that may imply the need for more than one-stack for a strong fibril integrity.

β -Amyloid Fibrils are Highly Polymorphic

Background:

- β -Amyloid fibrils are highly polymorphic.
- $\text{A}\beta$ mutants with distinct structures and features from the wild-type are observed in patients.
- Several mutants aggregate faster than wild-type $\text{A}\beta$ and many of them are more neurotoxic.



What makes mutant $\text{A}\beta$ aggregate faster and be more neurotoxic?

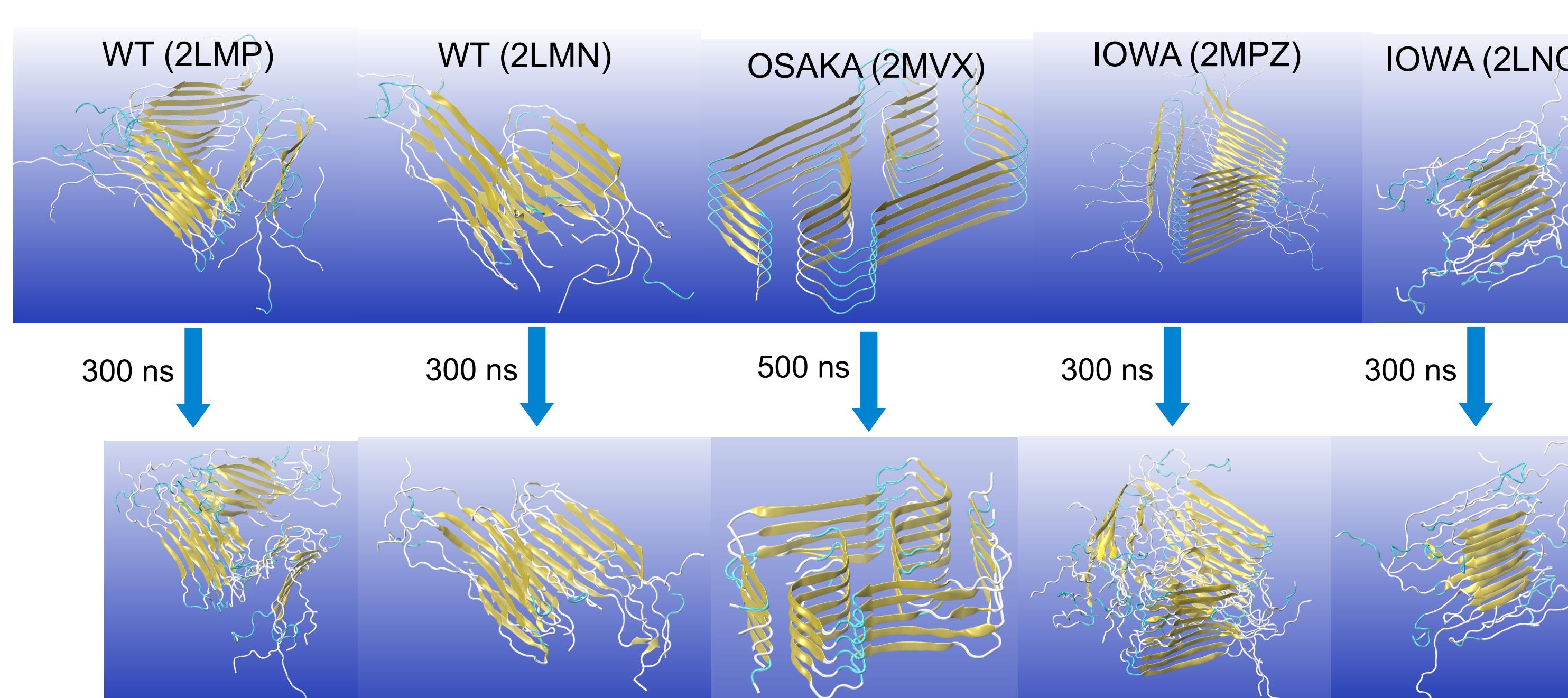
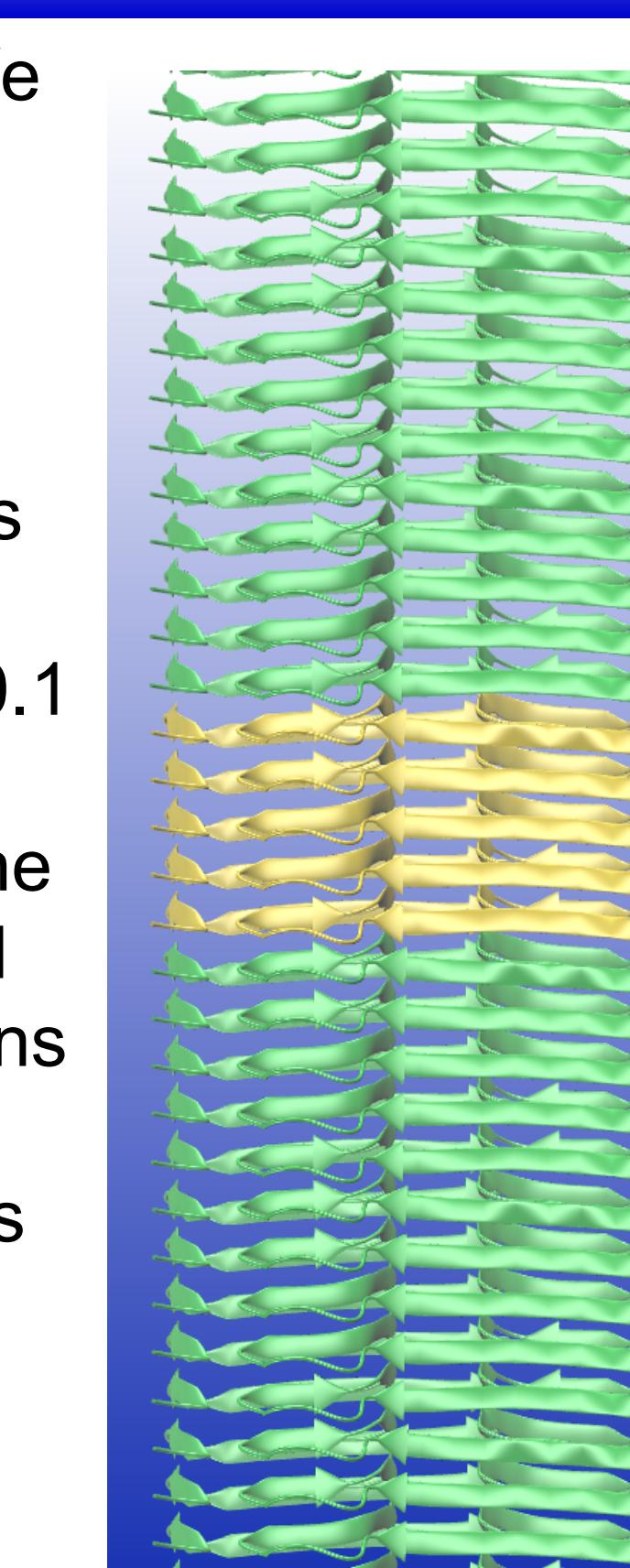
Hypothesis: Faster aggregation, and possibly the increased neurotoxicity, of the mutants might be caused in part by stronger similarity of secondary and tertiary structure between the pre-aggregation state and the end-point of aggregation, and better energetics.

Methods

Constructing Infinite Molecular Models of $\text{A}\beta$ 1-40 Fibers: We performed all atom, explicit solvent, molecular dynamics simulations based on five structural models of β -amyloid fibrils: two wild-type, one Osaka mutant, and two Iowa mutants. The missing residues for the NMR structures missing initial or end segments were added using the program Modeller. Infinite fibrils were constructed by replicating each structure along the z-axis using periodic boundary conditions. We simulate in solution of 0.1 M to 0.15 M of NaCl.

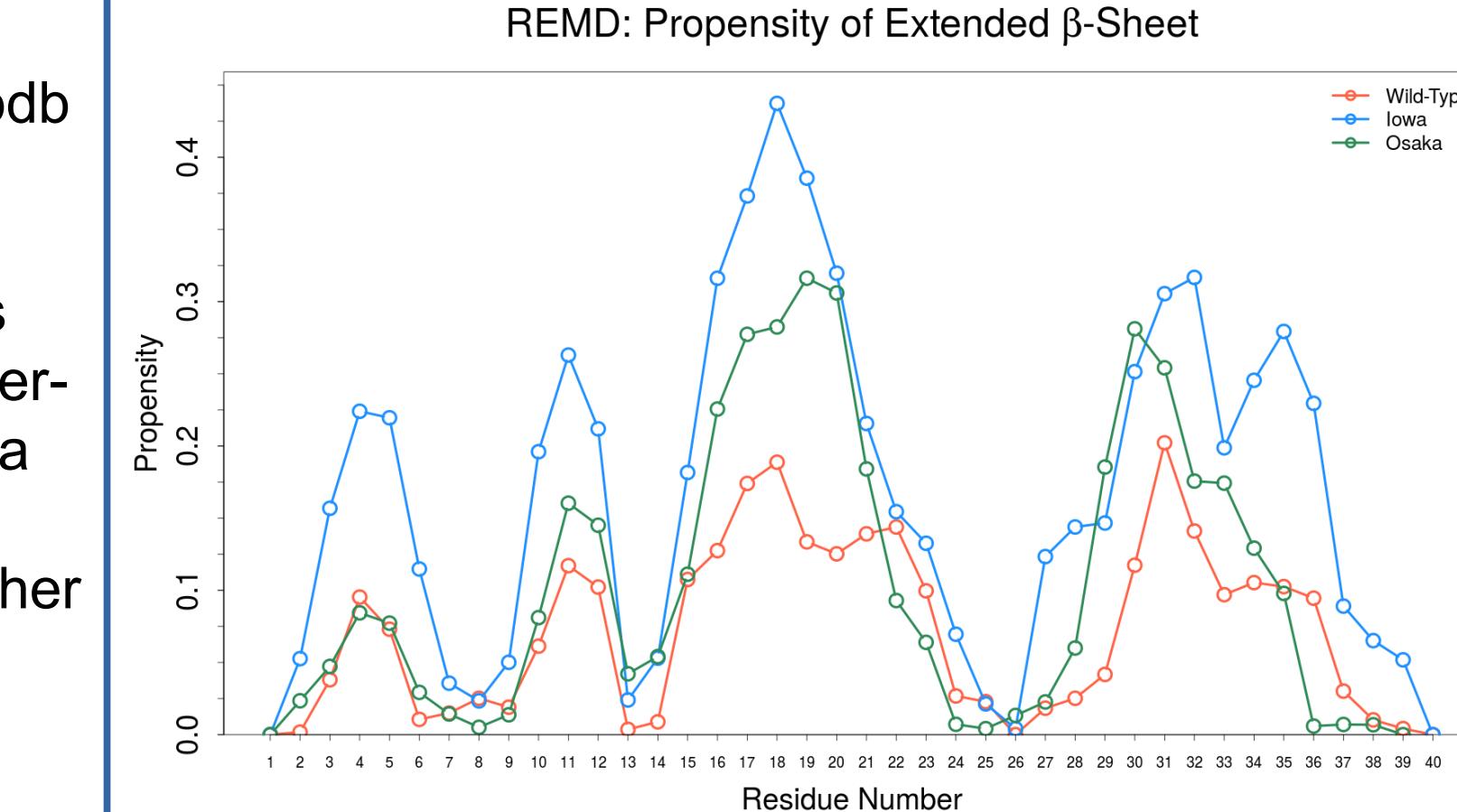
Simulation Parameters: All simulations were performed with the NAMD molecular dynamics package, explicit TIP3P solvent and the CHARMM36 force field at 300 K and 1 Bar, for at least 300 ns of simulation time.

REMD: We perform temperature replica exchange of monomers of wild-type β -amyloid, as well as the Iowa and Osaka mutants. For each we have 62 replicas across a temperature range of 300K-600K. We analyze a total of 100ns after convergence is reached.

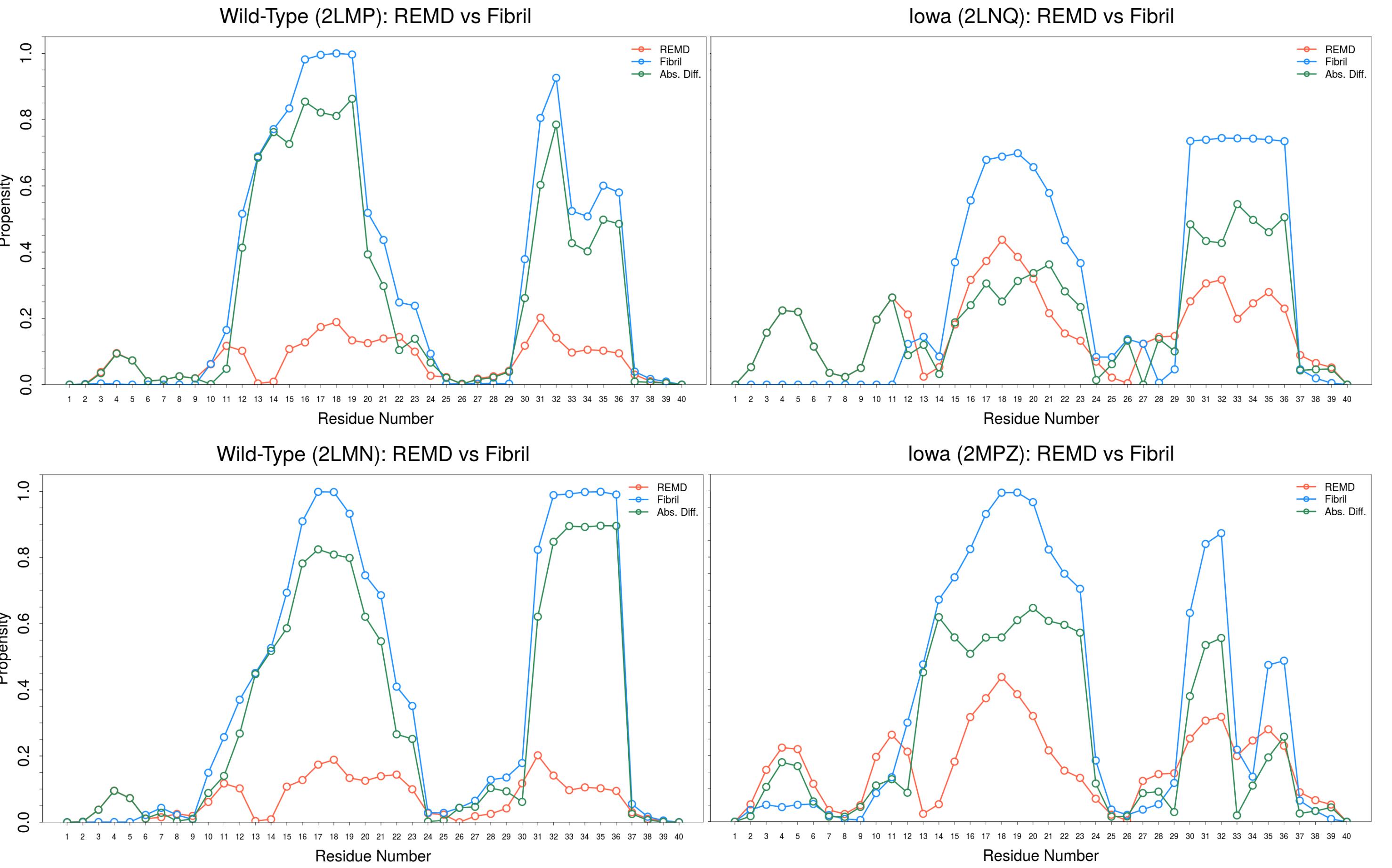


Mutant Pre-Aggregates are Structurally Closer to End-Point of Aggregation

For the REMD simulations we see that the mutants have higher propensity for β -sheet than the wild-type, with the Iowa having the most propensity. What might justify

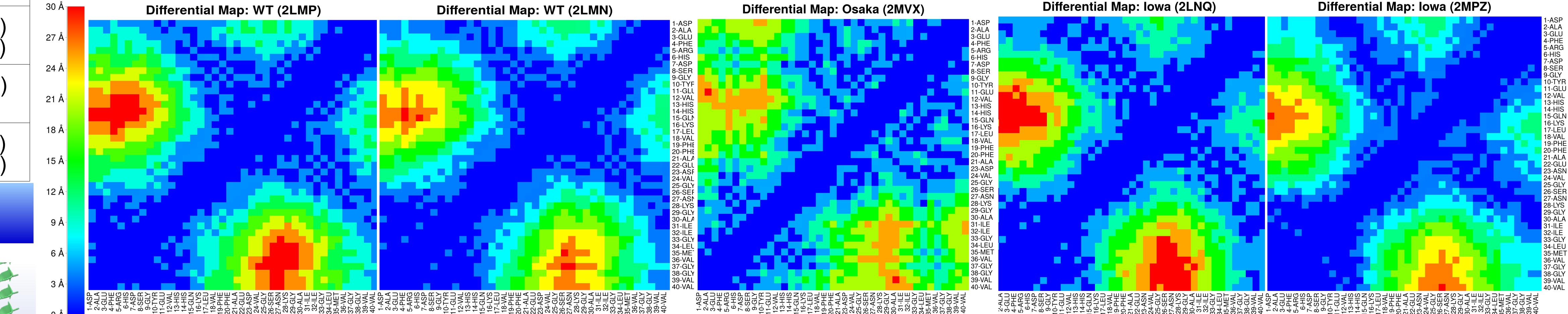


Comparing the REMD monomers propensity with the average propensity for the corresponding fibrils we see that the monomeric Iowa $\text{A}\beta$ is closer to both of its fibrils, than the Osaka and the wild-type are to their fibrils, while the wild-type is significantly further from its corresponding fibril in comparison to the similarity between the mutants and their corresponding fibrils.



The contact maps of the temperature REMD simulations of the wild-type monomer, as well as the Iowa and Osaka mutant monomers, are compared to their corresponding fibrils. The maps have a range of 30 Å, and for the fibrils we average the intra-strand contacts for each segment. Taking the average difference in distance map we get the following relation of similarity of tertiary structure:

2LNQ > 2MVX > 2MPZ > 2LMP > 2LMN



NAMD Energy Comparison

$$E = \sum_{\text{bonds}} K_b(b - b_0)^2 + \sum_{\text{angles}} K_\theta(\theta - \theta_0)^2 + \sum_{\text{Urey-Bradley}} K_{UB}(b_{1-3} - b_{1-3,0})^2 \\ + \sum_{\text{dihedrals}} K_\varphi(1 + \cos(n\varphi - \delta)) + \sum_{\text{impropers}} K_\omega(\omega - \omega_0)^2 + \sum_{\text{residues}} u_{\text{CMAP}}(\Phi, \Psi) \\ + \sum_{\text{nonb. pairs}} \varepsilon_{ij} \left[\left(\frac{r_{ij}^{\min}}{r_{ij}} \right)^2 - 2 \left(\frac{r_{ij}^{\min}}{r_{ij}} \right) \right] + \sum_{\text{nonb. pairs}} \frac{q_i q_j}{\epsilon r_{ij}}$$

Arrhenius Equation: $k = Ae^{-E_a/(k_B T)}$

- Mutants are likely more energetically favorable than the wild-type in the pre-aggregation state as well as the end-point of aggregation.
- Non-bonded energies are the main contributors to the energy difference to the wild-type in the monomeric state.
- Iowa (2MPZ) has the best energetics after 250ns of simulation, followed by the Osaka fibril.
- By Arrhenius Equation, the mutants having lower energy implies higher aggregation rates in comparison to the wild-type.

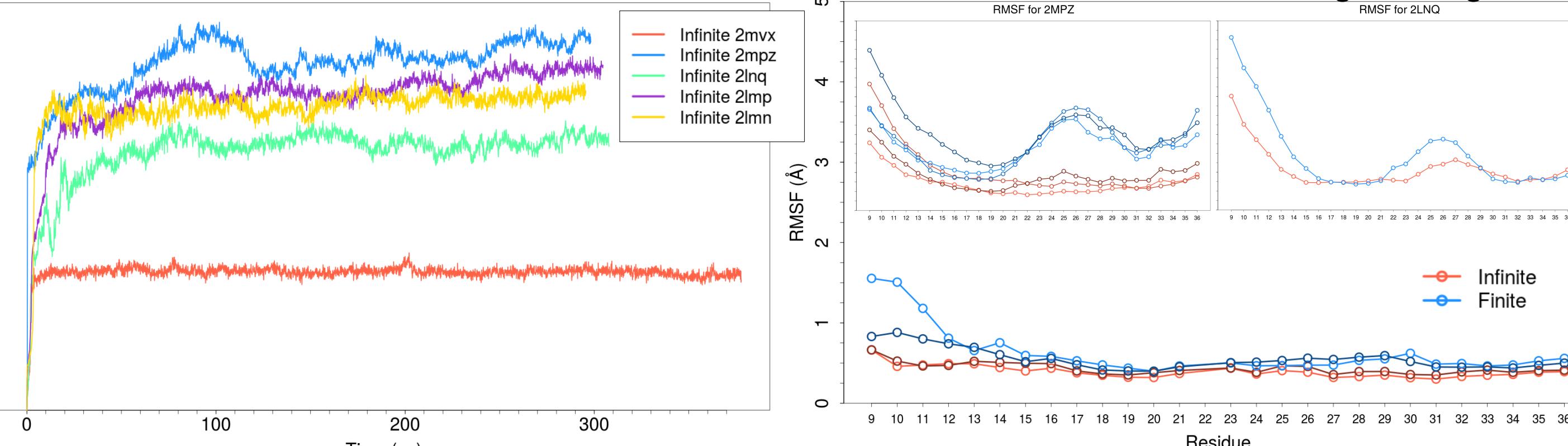
Conclusions

- The Osaka fibril is much more stable than any of the other fibrils, likely because of its unique salt-bridge (D3-K28), which might only be possible due to the deletion of residue E22, it's more robust interbackbone hydrogen network, higher β -content, and due to the possibly stabilizing contacts with sodium ions. This strong stability of the Osaka mutant might contribute to its stronger neurotoxicity.
- Both mutants show higher proximity between the monomeric state and the fibril state in relevant secondary structure and in tertiary structure, as well as smaller energy. This indicates, that in the pre-aggregation state the monomers are structurally closer to the end-point of aggregation giving a possible explanation for the faster aggregation of the mutants.
- The mutant monomers have higher propensity for β -sheet, a feature that is known to be strongly correlated to aggregation. This might provide some justification to why the mutants aggregate faster.

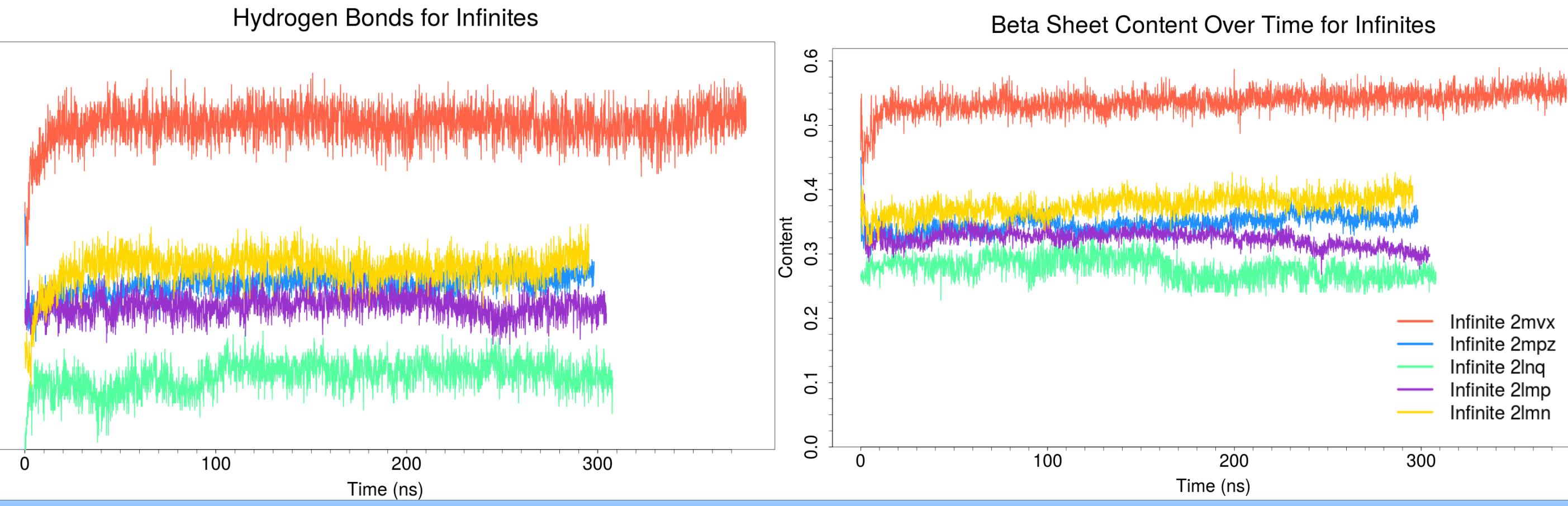
Osaka Fibril is Highly Structurally Stable

The Root Mean Square Deviation of a protein measures change in 3D structure.

Osaka has smallest and most stable RMSD

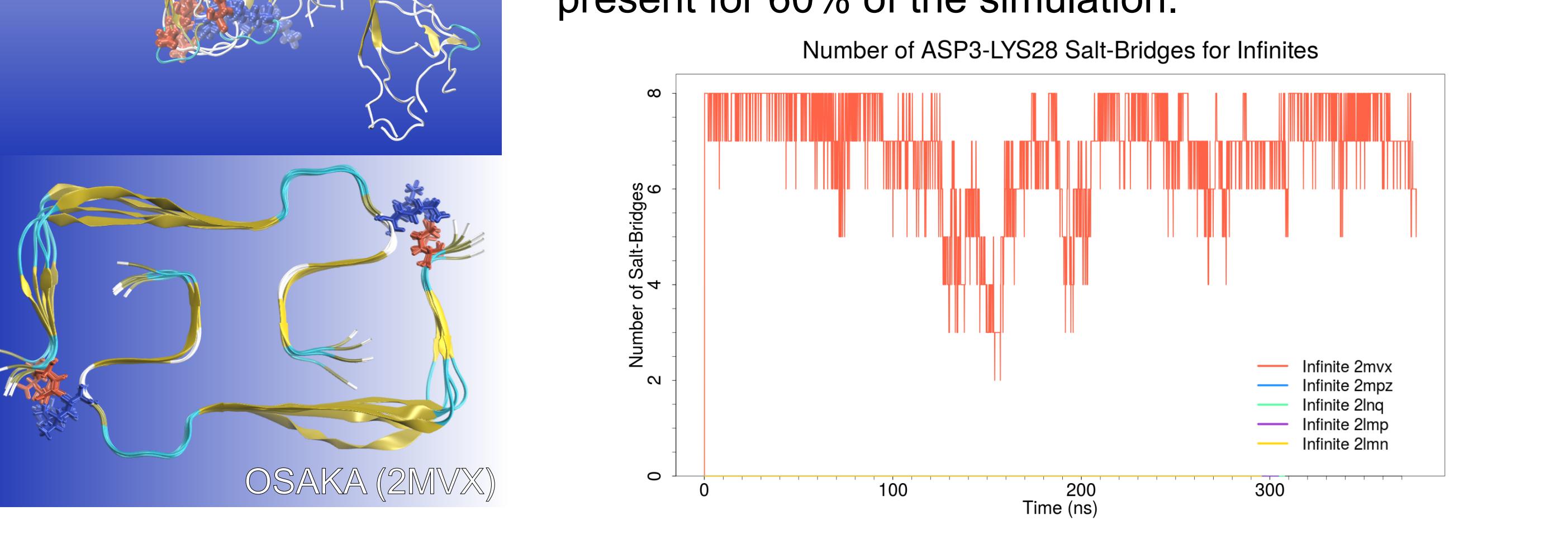


- Hydrogen bonds are averaged by stack and normalized to have data comparable to having 10 layers.
- β -sheet content is known to be strongly correlated to stability of amyloids. β -amyloid β -sheet content is heavily determined by interbackbone hydrogen bonds network.
- β -sheet content and interbackbone hydrogen bonds are correlated quantities. Higher β -sheet content yields higher Hydrogen bonding, and vice-versa.

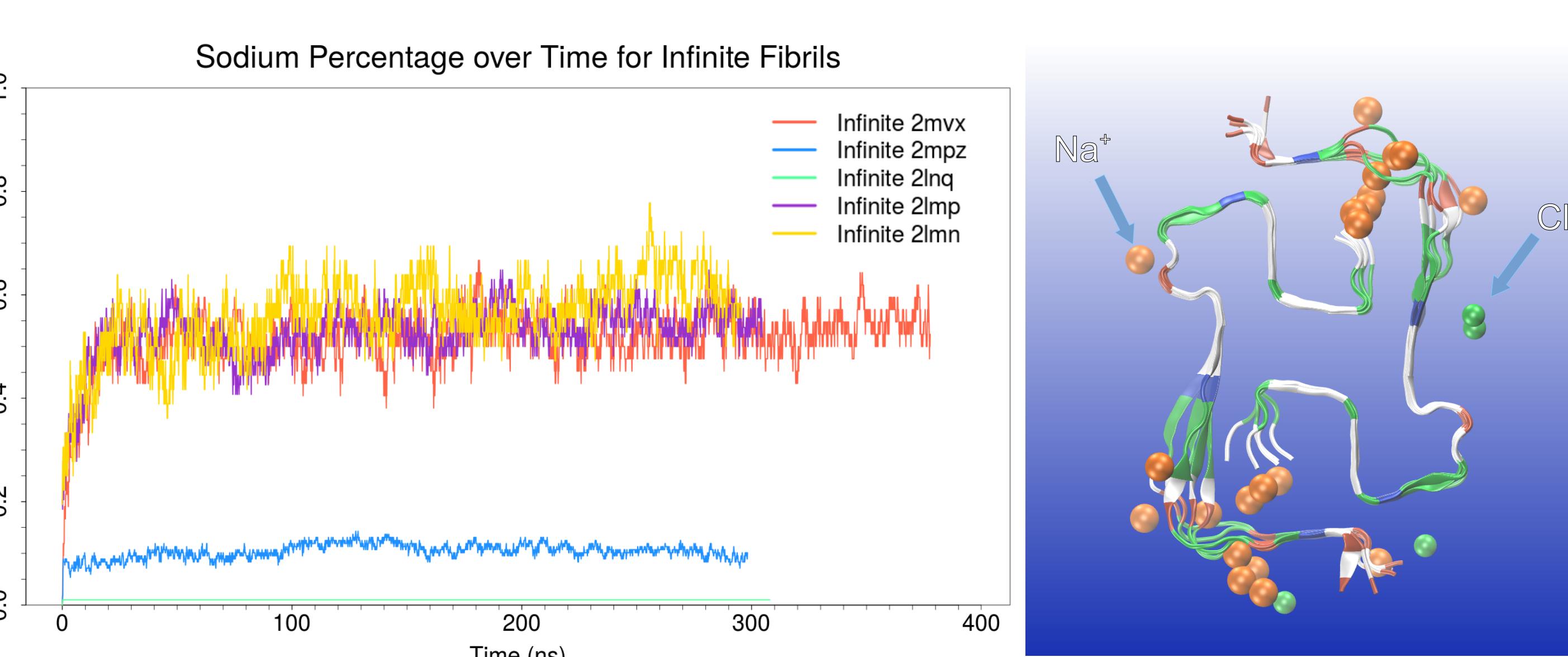


Osaka Fibril has Unique Salt-Bridge and Ion Contacts

- The strongest salt-bridge in the Osaka Mutant Fibril is D3-K28.
- Likely locks the quaternary structure of the fibril and prevents the fibril from opening on the sides. A phenomenon observed in 2LMP.
- Salt-bridges are counted if contact is within 3.7 Å and present for 60% of the simulation.



- We only count ions that stay for at least half of the simulation time within 3 Å of the fibril.
- Sodium is likely attracted to the fibrils because of their negative net charge.
- Osaka shows the highest sodium percentage among the mutants, and comparable percentages to the wild-type.



References:

- [1] Hatami, Asa, Sanaz Monjazeb, Saskia Milton, and Charles G. Glabe. "Familial Alzheimer's Disease Mutations within the Amyloid Precursor Protein Alter the Aggregation and Conformation of the Amyloid- β Peptide." *Journal of Biological Chemistry* 292, no. 8 (2017): 3172-185. doi:10.1074/jbc.m116.755264.