

Diversifying the tau amyloid toolkit to probe structure-function relationships

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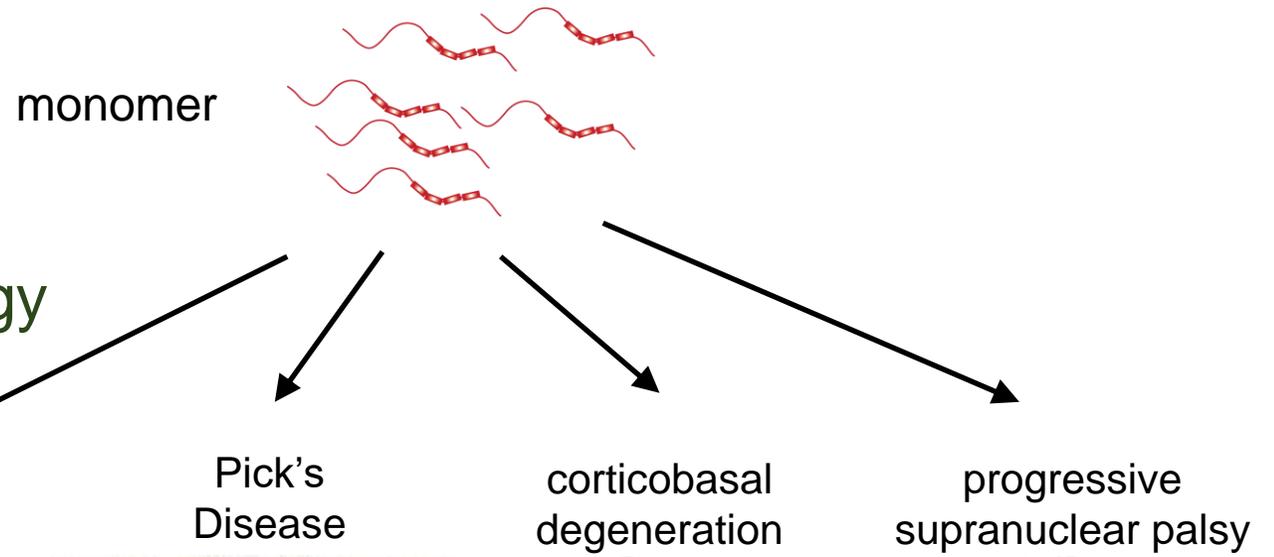
University of Alberta

Mechanisms of Neurodegeneration in Human Prion Diseases and Their
Intersection with AD/ADRD

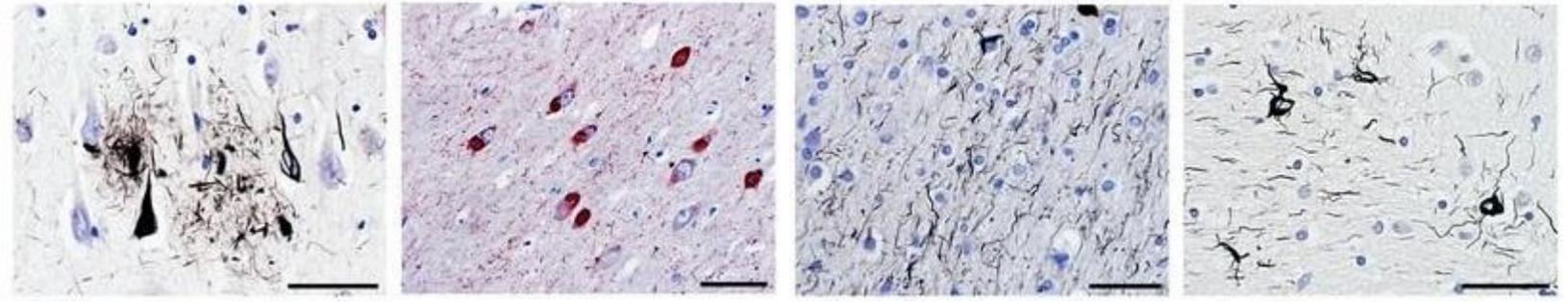
November 12, 2024

Tauopathies

- a class of neurodegenerative diseases characterized by tau aggregate pathology

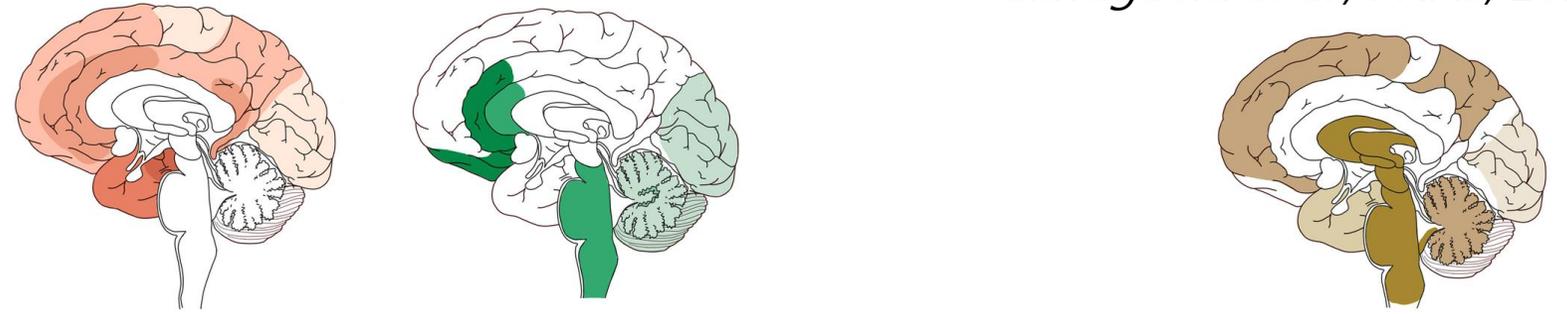


Tau aggregate pathology



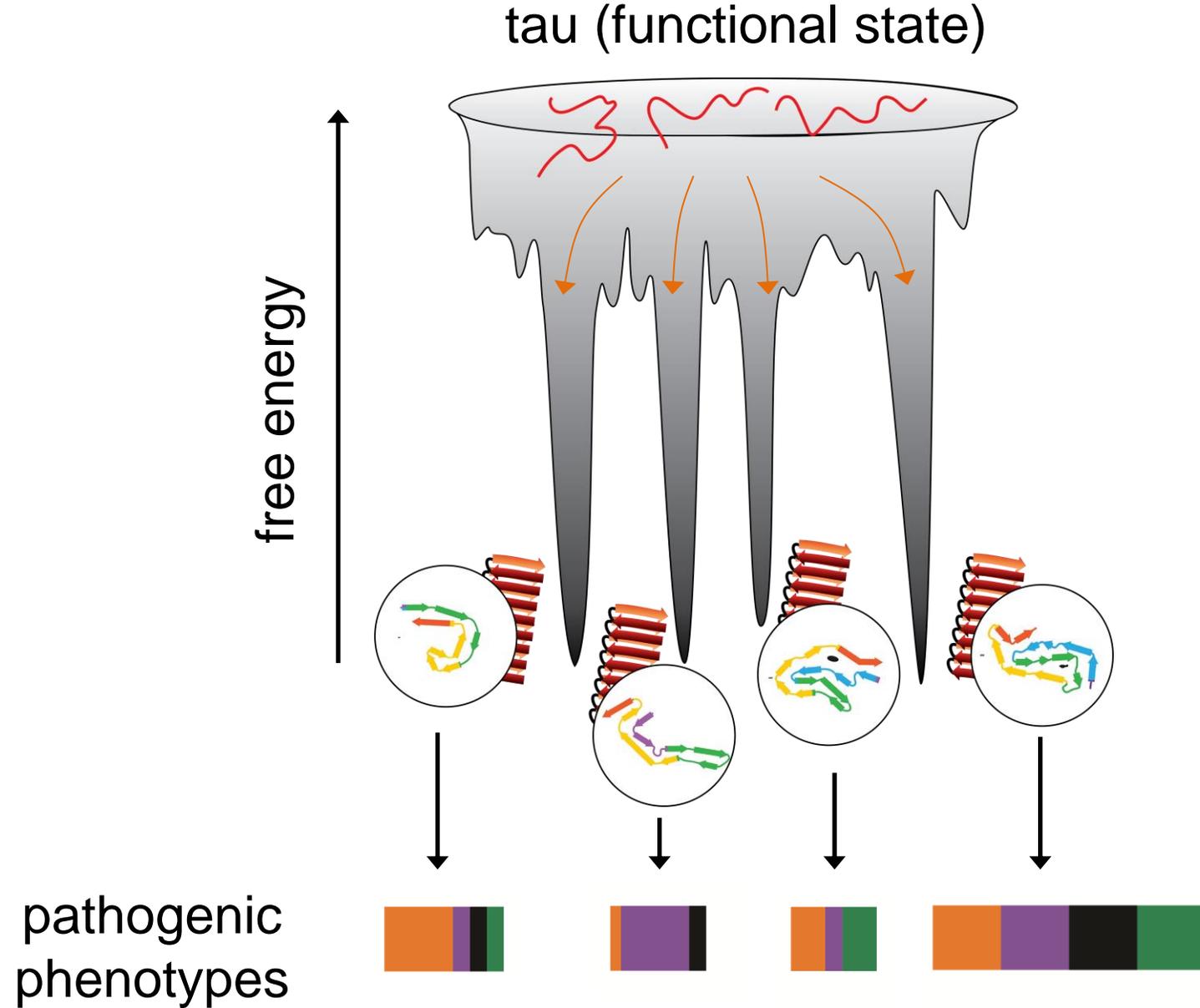
Clavaguera et al., PNAS, 2016

Pathology by cell type and affected brain regions



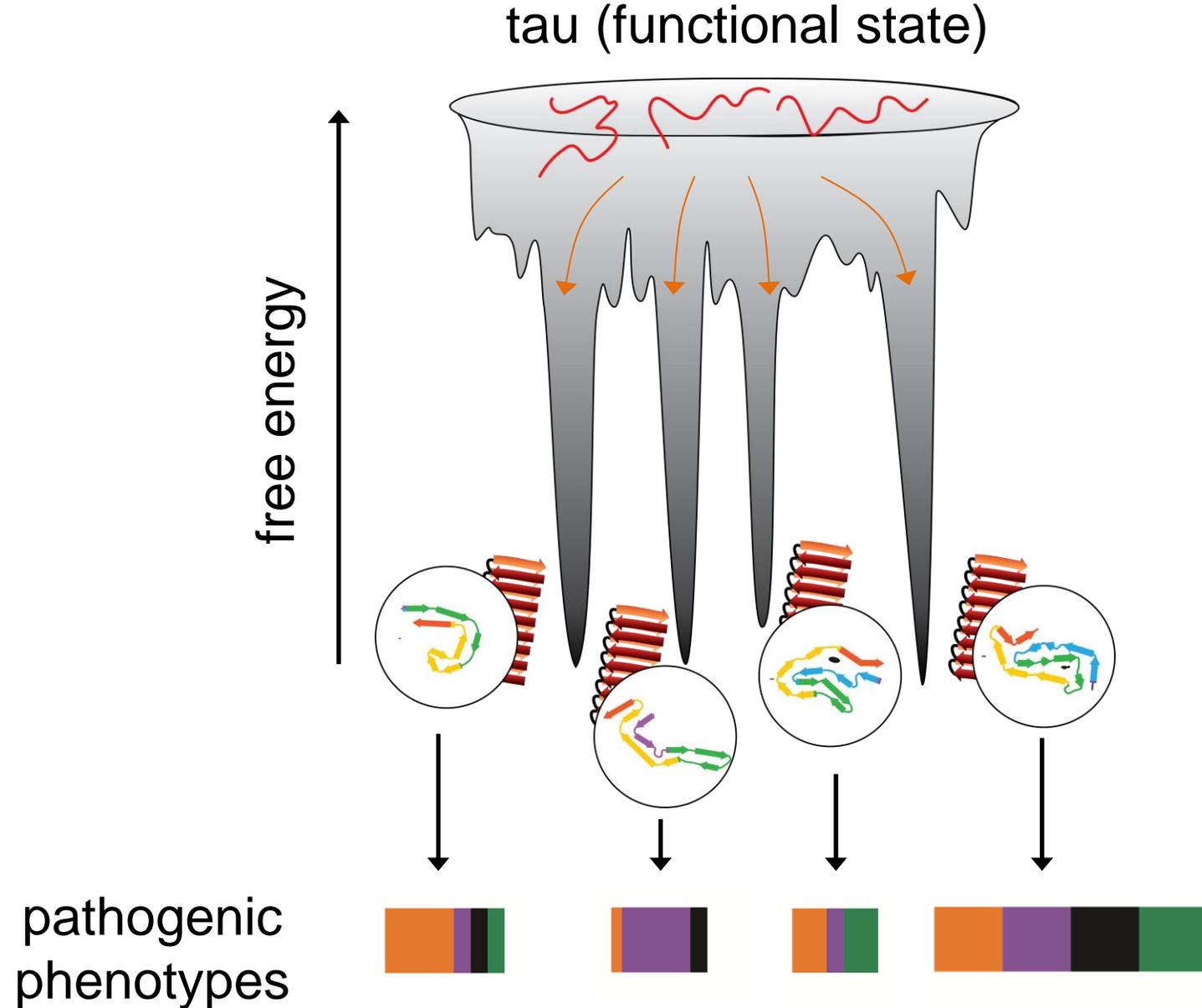
Zhang et al., Mol. Neurodeg., 2022

Tau: dissecting sequence-structure-dysfunction relationships



- One sequence, multiple amyloid conformations
- Distinct structures associate with subsets of tauopathies

Tau: dissecting sequence-structure-dysfunction relationships

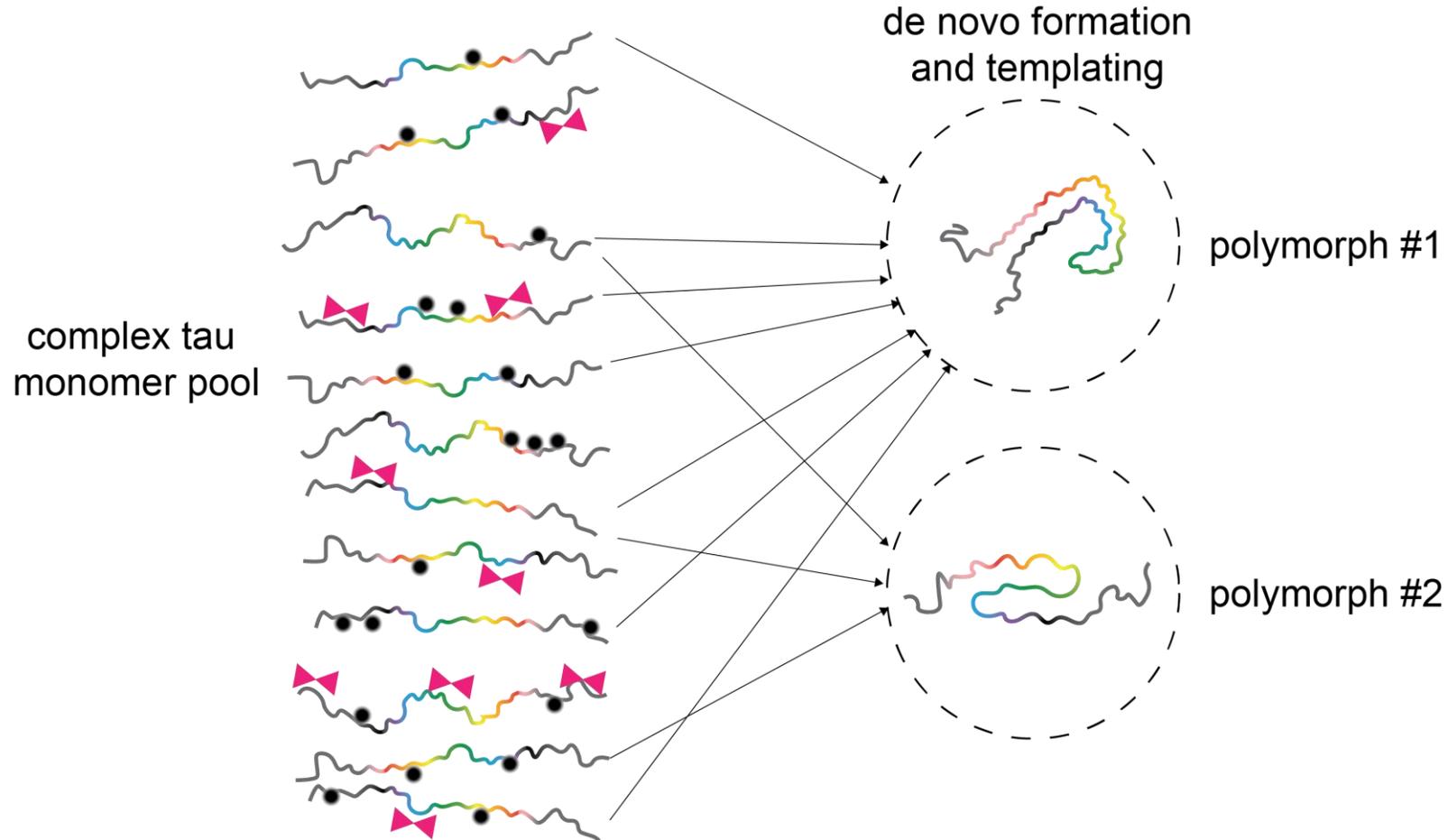


- One sequence, multiple amyloid conformations
- What are the dominant sequence and biochemical determinants regulating amyloid conformations?
- Distinct structures associate with subsets of tauopathies
- What structural elements of amyloids are responsible for dysfunction?

The complex tau biochemical landscape and protein misfolding

inducers, co-factors, protein interactors 

PTMs 



The complex tau biochemical landscape and protein misfolding

inducers, co-factors, protein interactors

PTMs

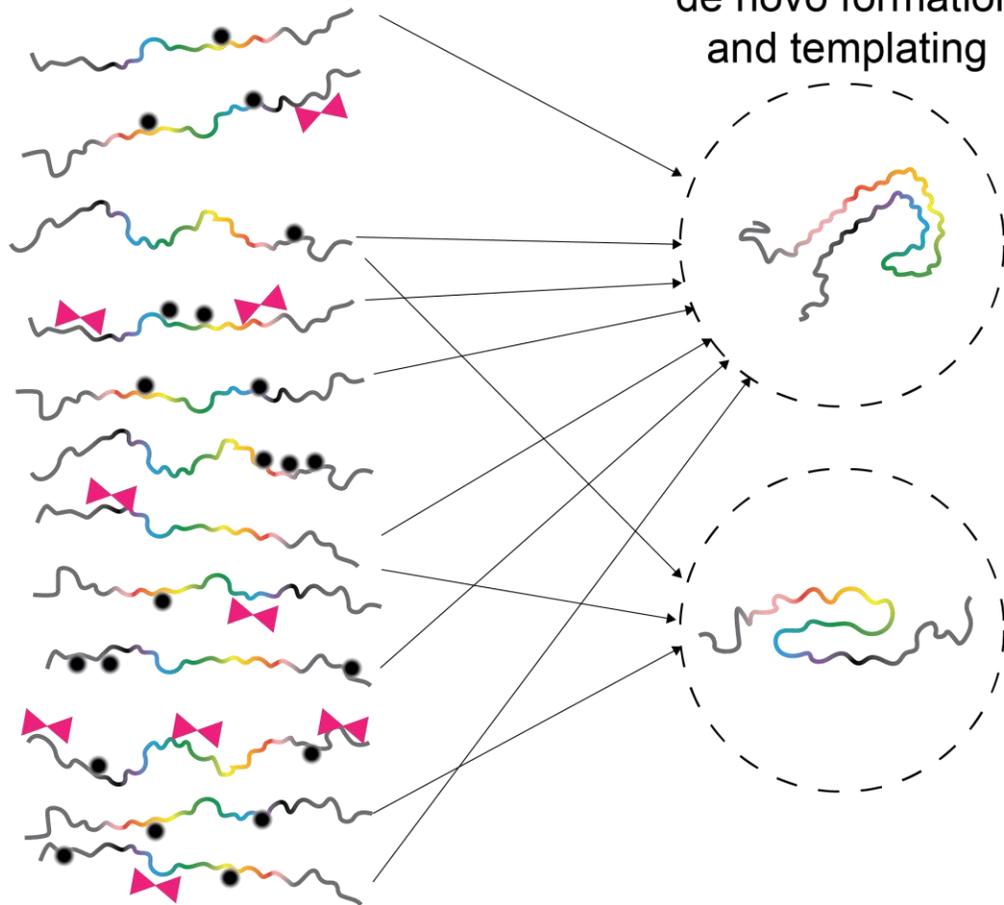
complex tau monomer pool

de novo formation and templating

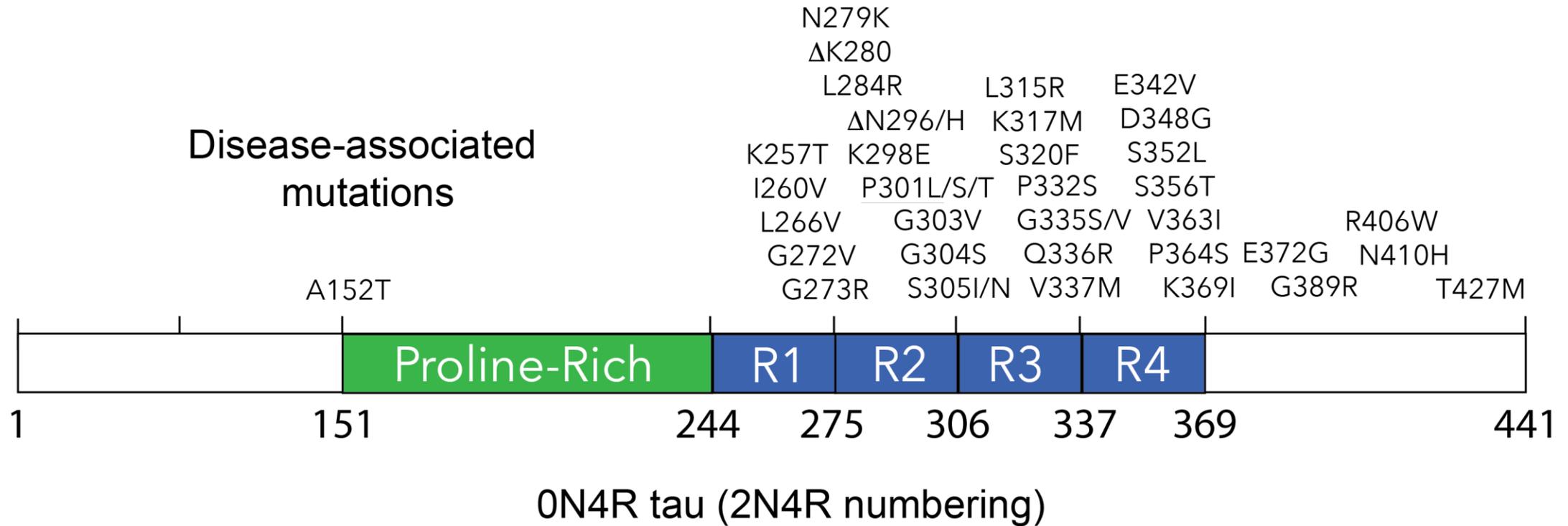
polymorph #1

polymorph #2

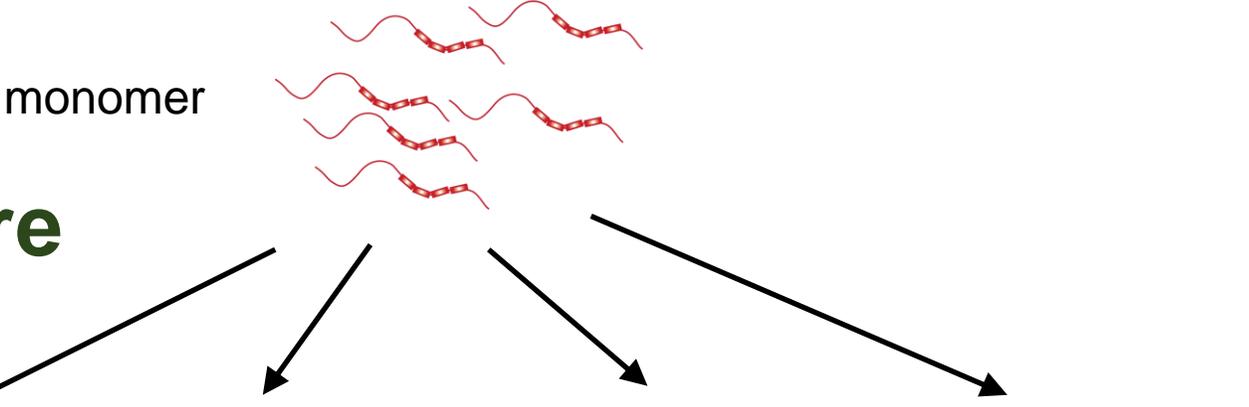
Can we dissect what biochemical features of tau that drive it towards one polymorph versus another?



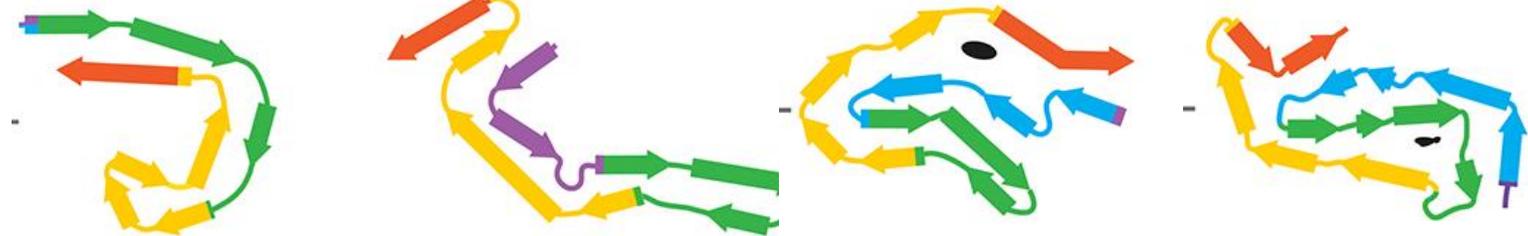
How sensitive is the tau misfolding landscape to single missense mutations?



Simple biochemical methods can also be used to identify changes in aggregate structure

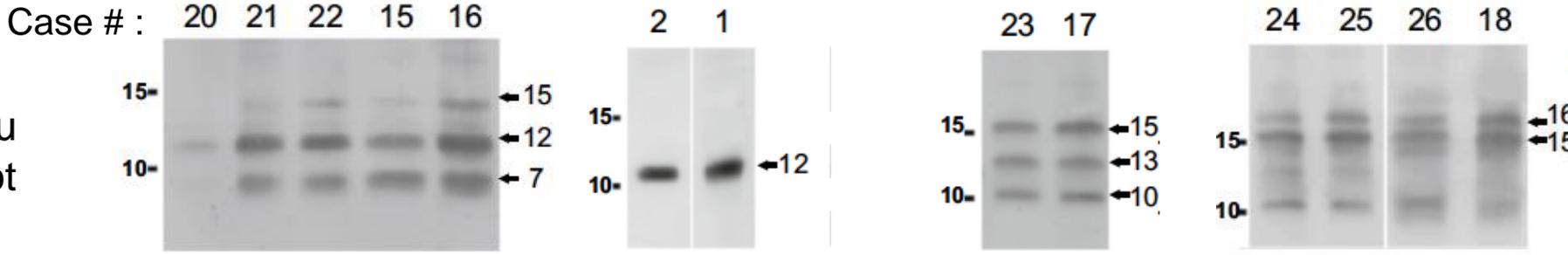


Cryo-EM structures from patient samples



Shi et al., Nature, 2022

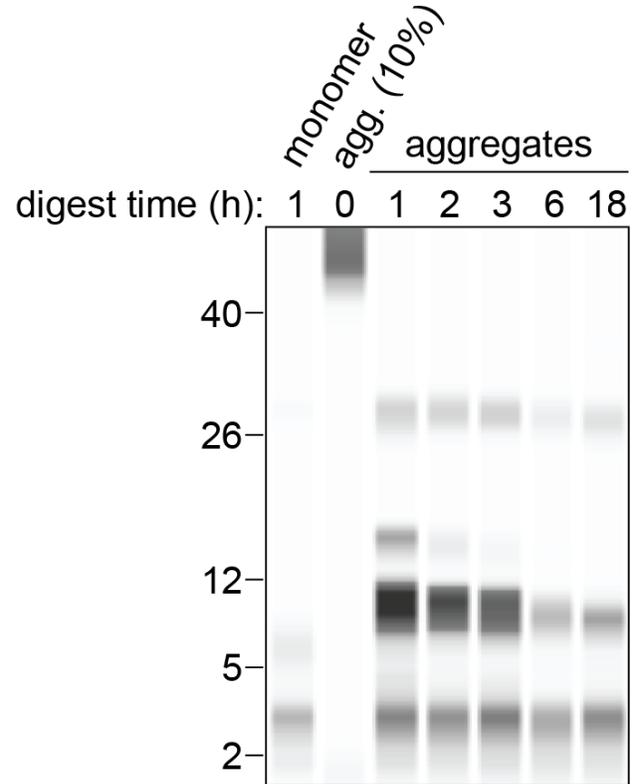
Trypsin-digested patient tau fibrils visualized by immunoblot



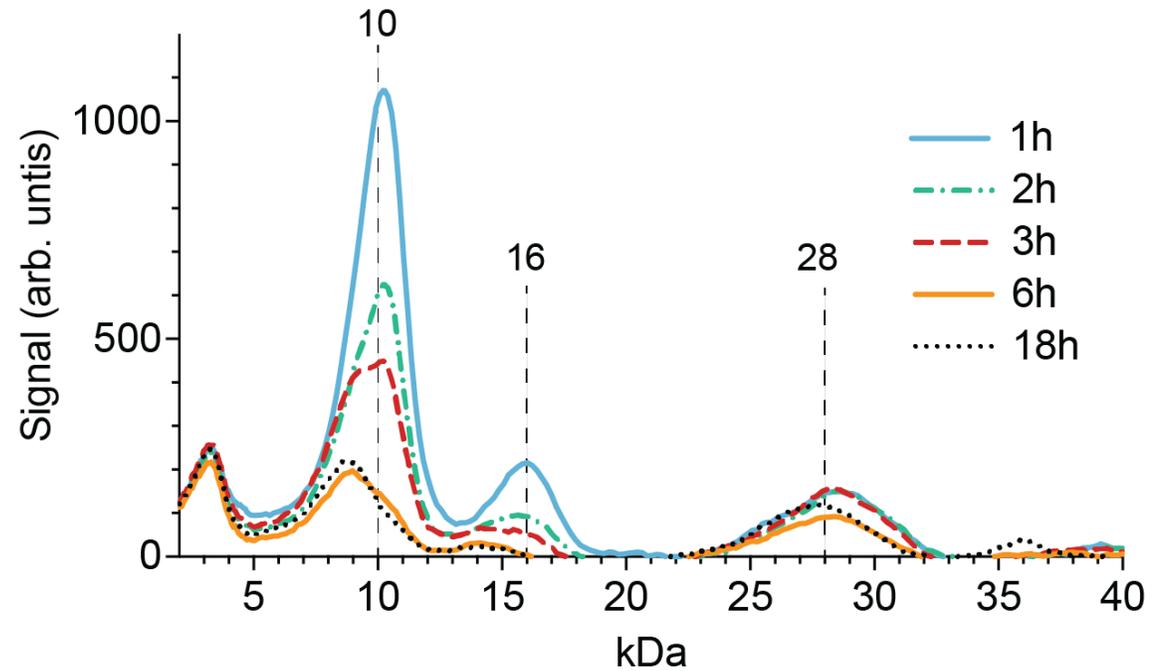
Taniguchi-Watanabe et al., Acta Neuropathol. 2016

Defining the trypsin-resistant core of recombinant tau aggregates

WT 0N4R tau + heparin



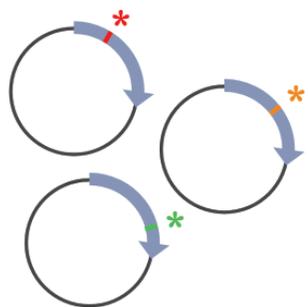
Lane view



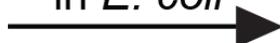
Chromatogram view

High-throughput biochemical platforms to study tau variants, kinetics and aggregate structures

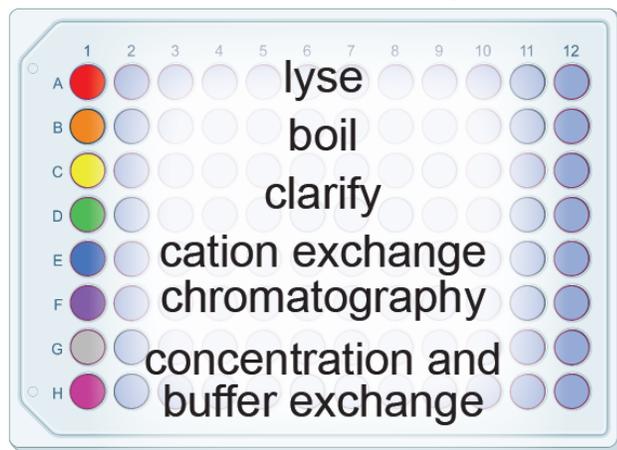
tau missense mutants



small-scale expression in *E. coli*

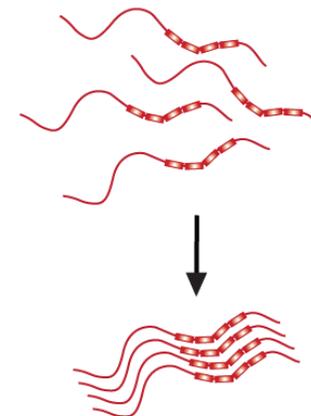


small-scale, in-well, tau purification



one tau variant per well
200 μ g purified tau per well

trigger aggregation



high-throughput assays:

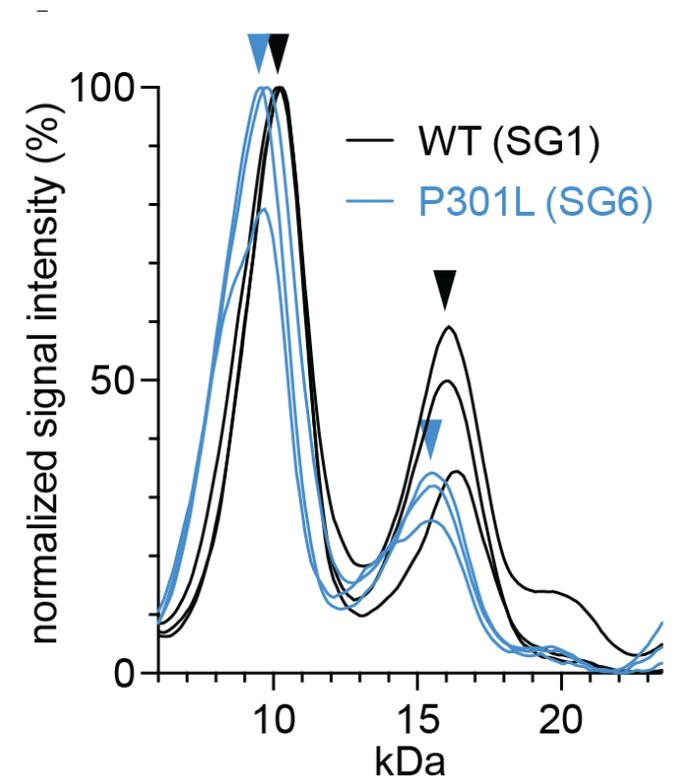
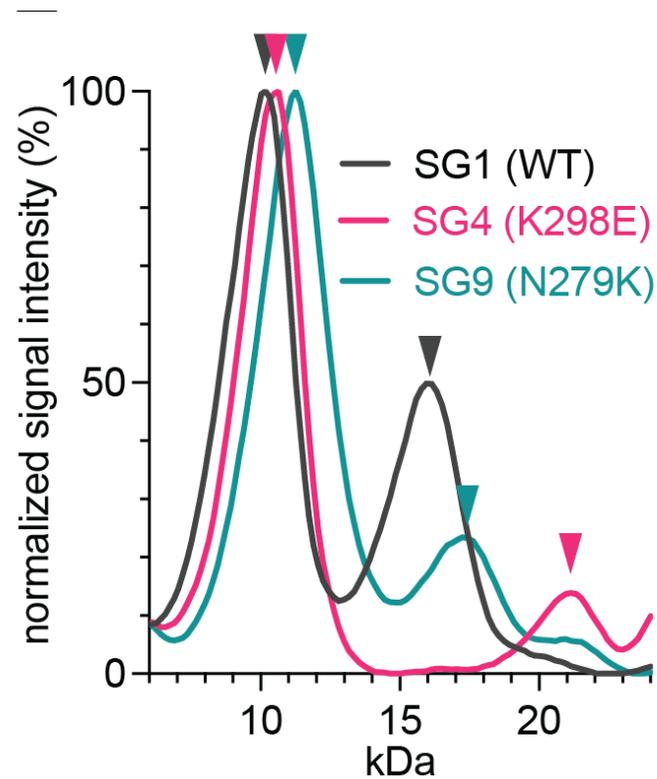
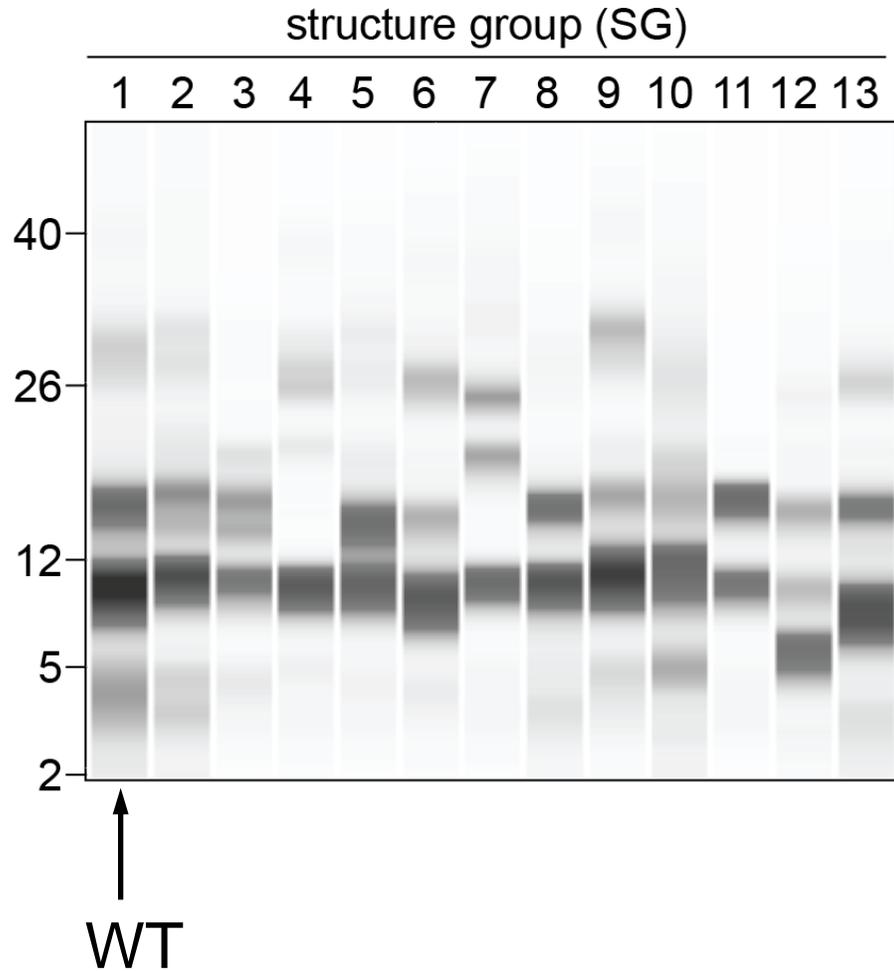
- kinetics (ThT)
- aggregate conformation
- protease fragmentation
- antibody reactivity

WT and 36 disease associated mutants for human 0N4R tau

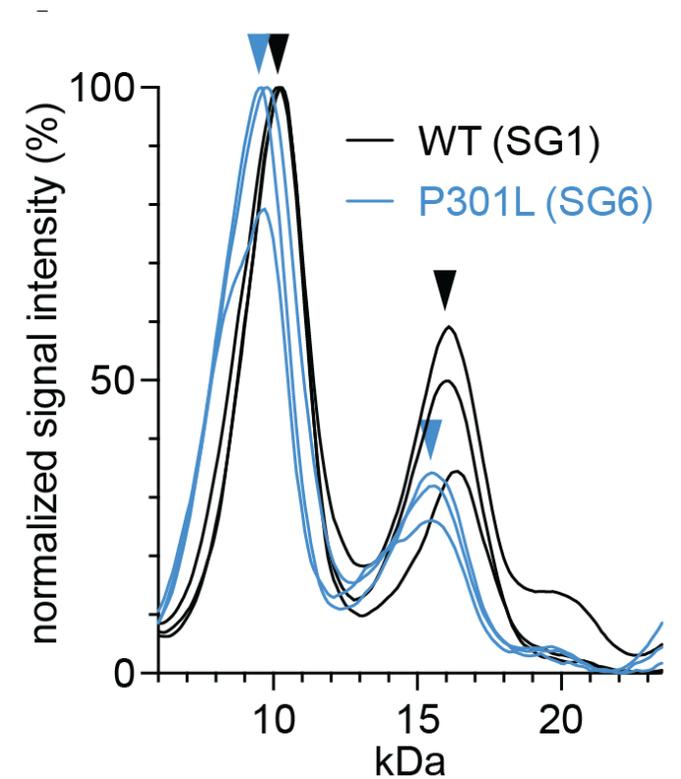
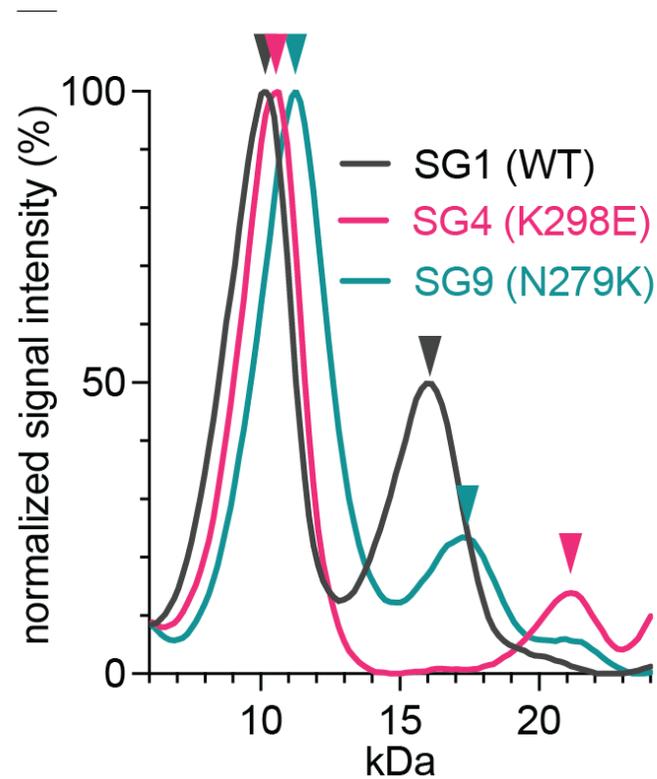
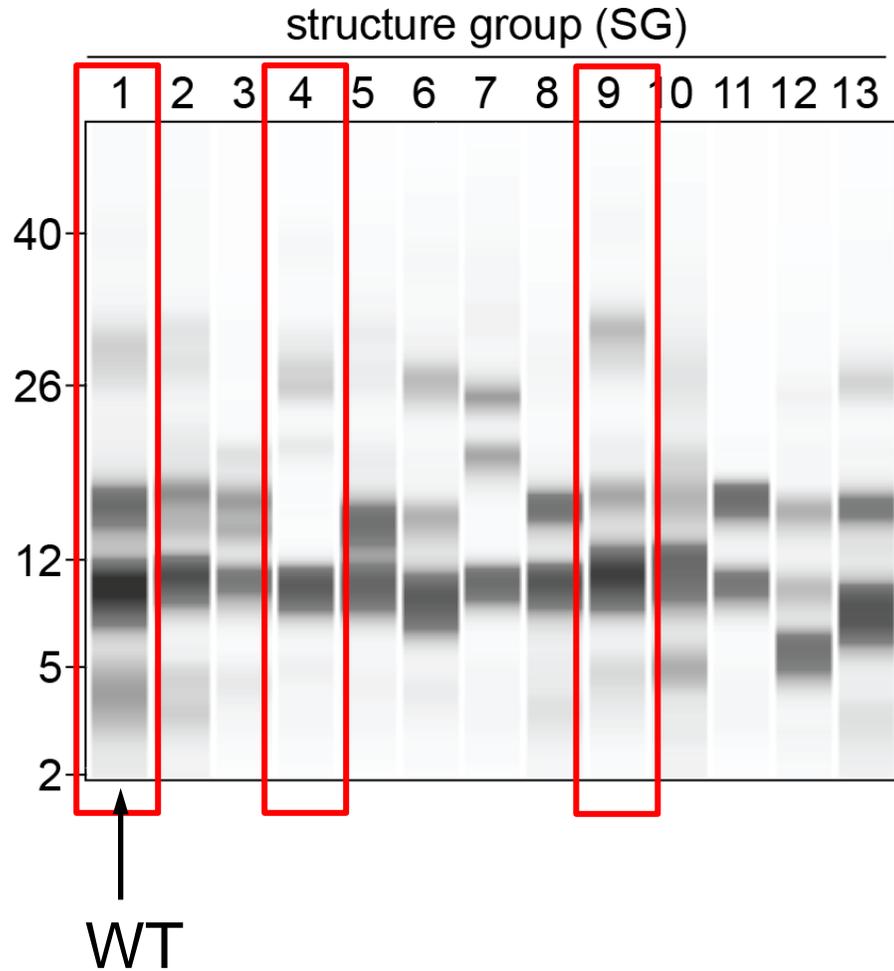


Tark Patel, Allan Yarahmady, Kerry Sun

Disease-associated tau mutants generate aggregate structures distinct from WT 0N4R tau

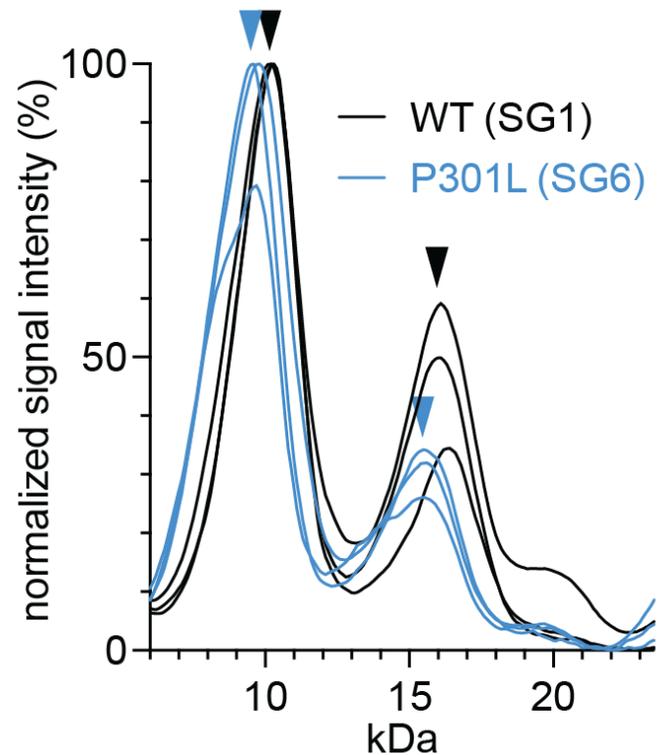


Disease-associated tau mutants generate aggregate structures distinct from WT 0N4R tau

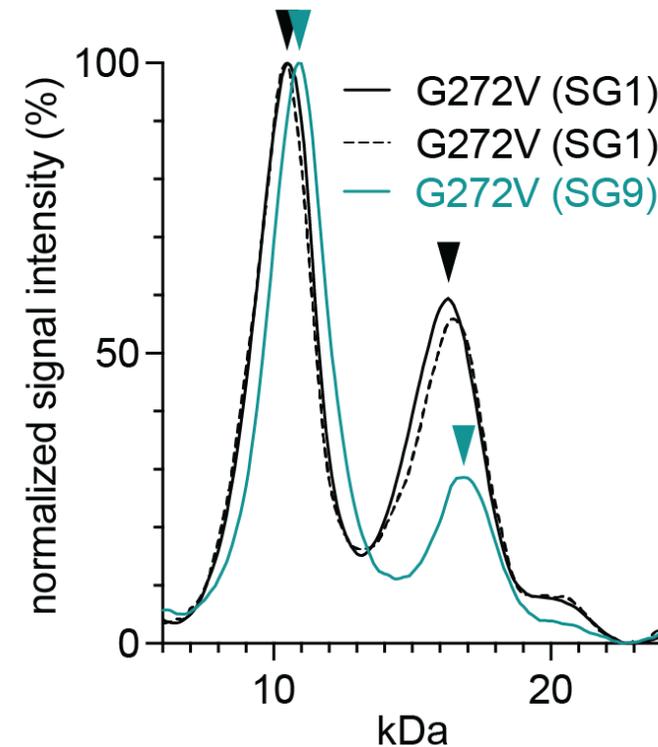


A subset of tau mutants show evidence of promoting more than one major aggregate misfolding pathway

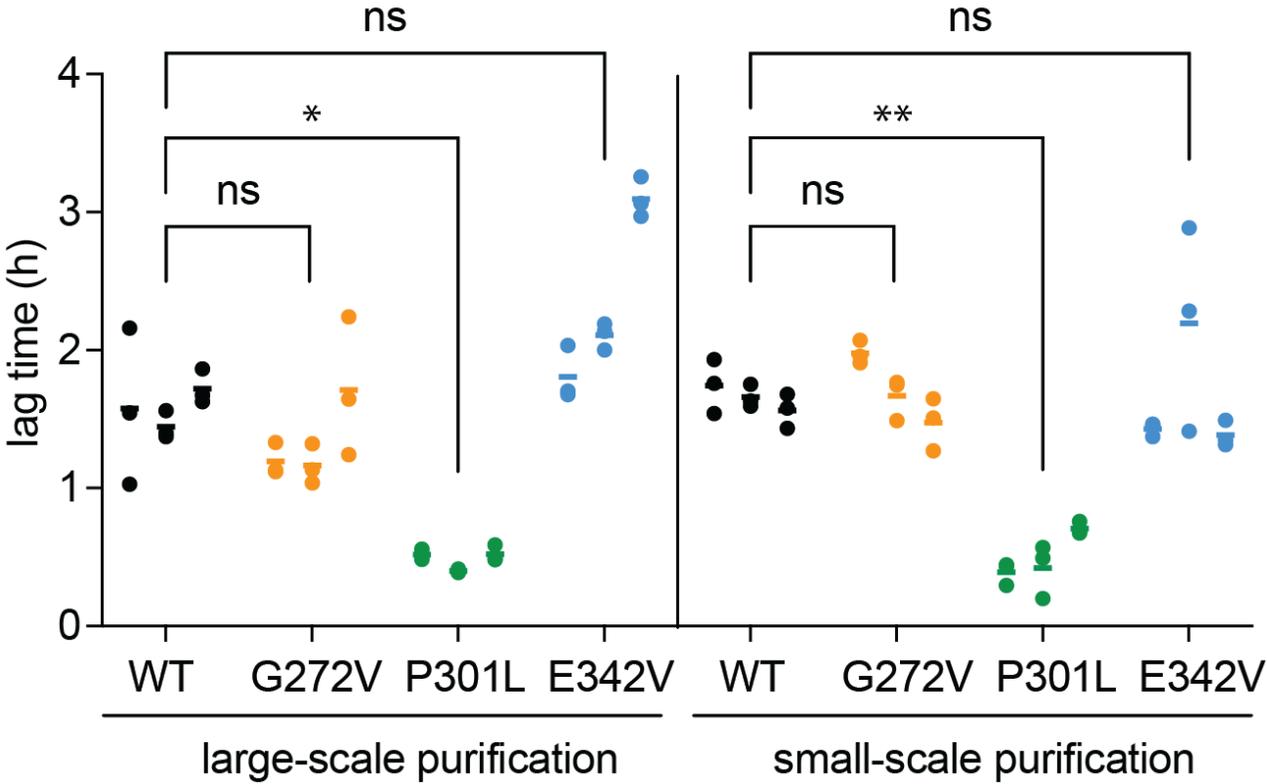
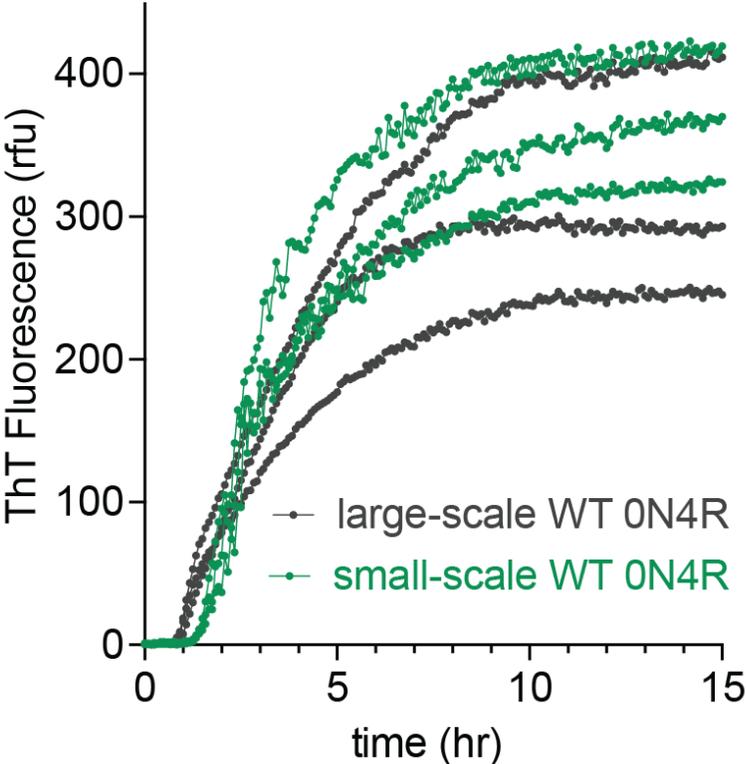
- 29/37 mutants tested generate a **consistent** trypsin digest profile



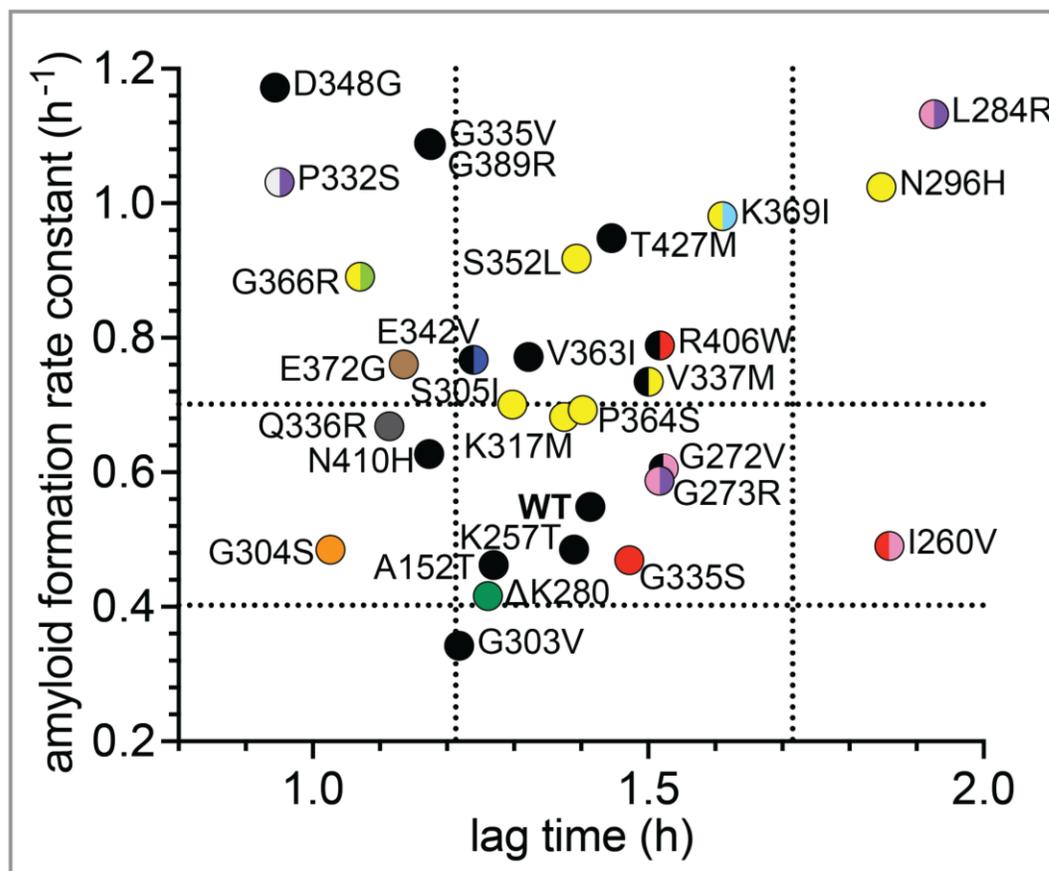
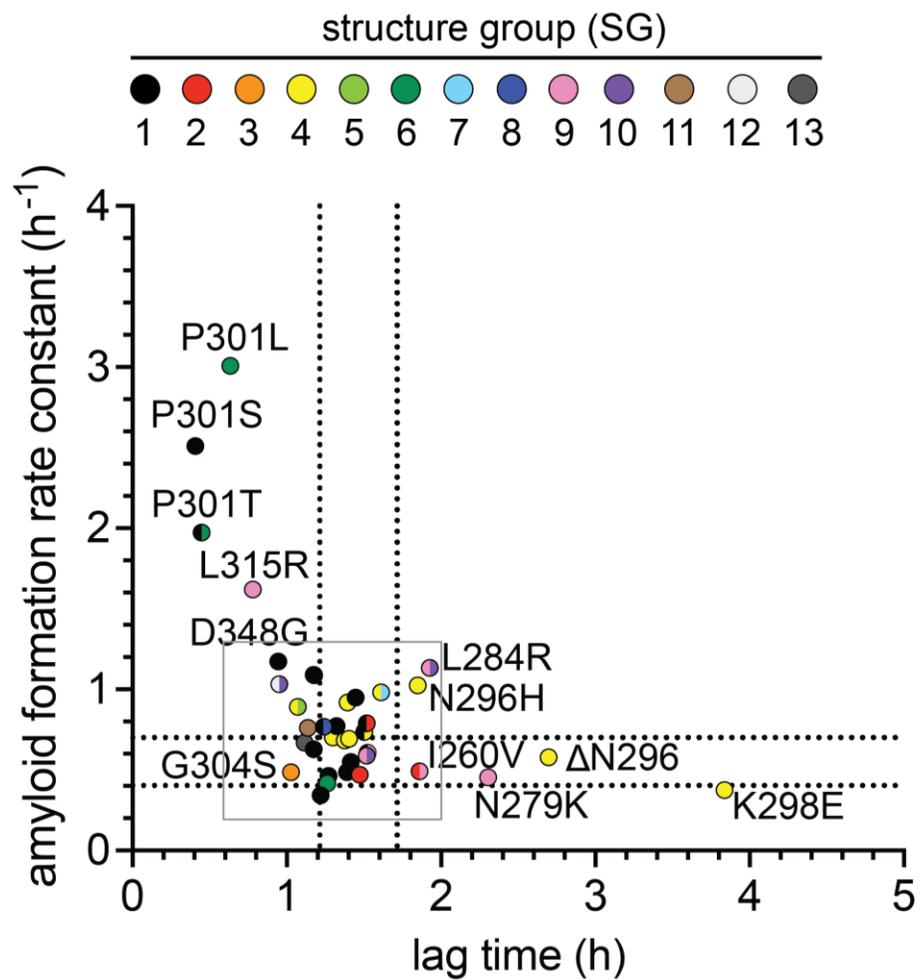
- 8/37 mutants generate **variable** digest fragment profiles between independent experiments



Small-scale purified tau protein can be used to measure aggregation kinetics

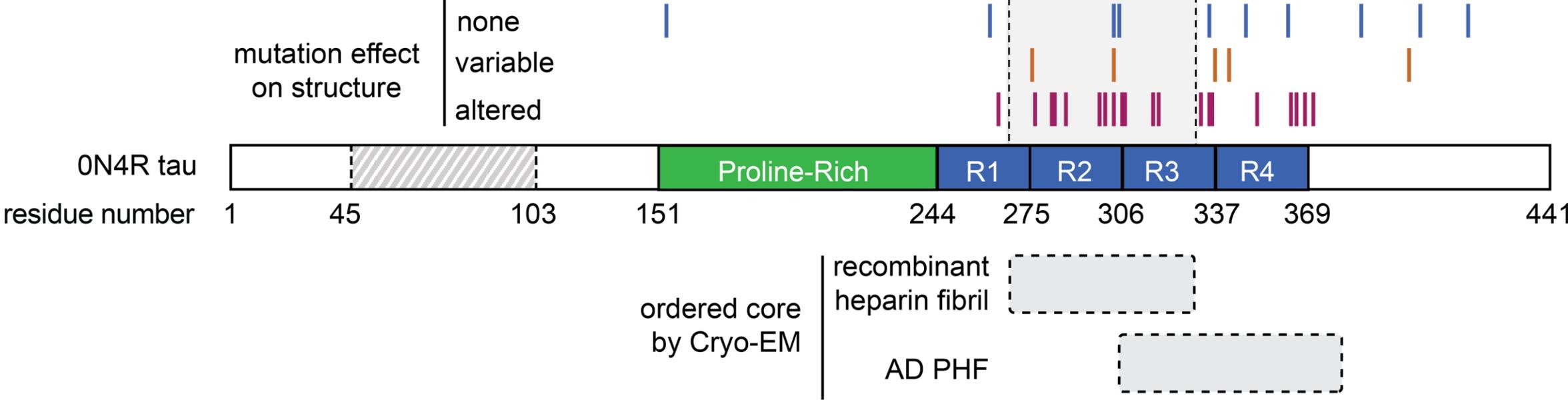


Tau aggregation kinetics and aggregate structure changes are decoupled

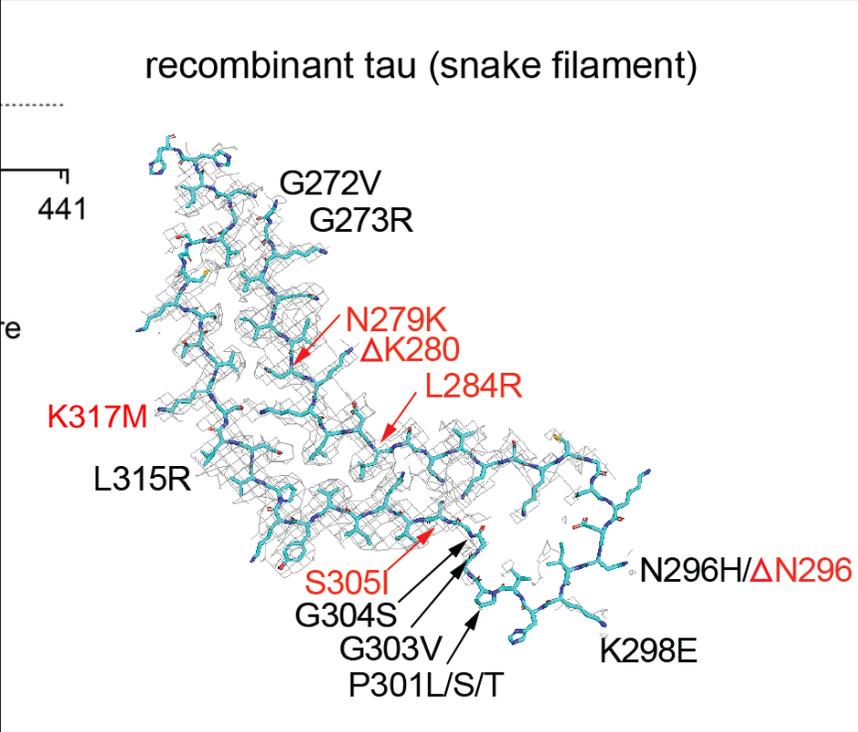
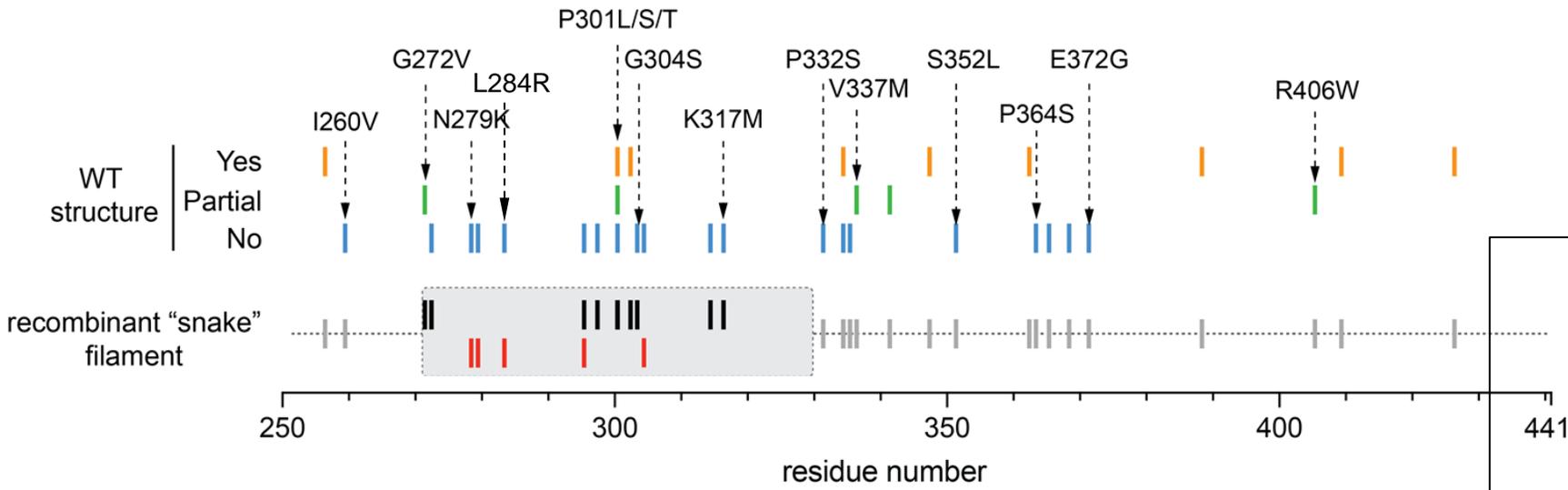


faster aggregation ← → slower aggregation

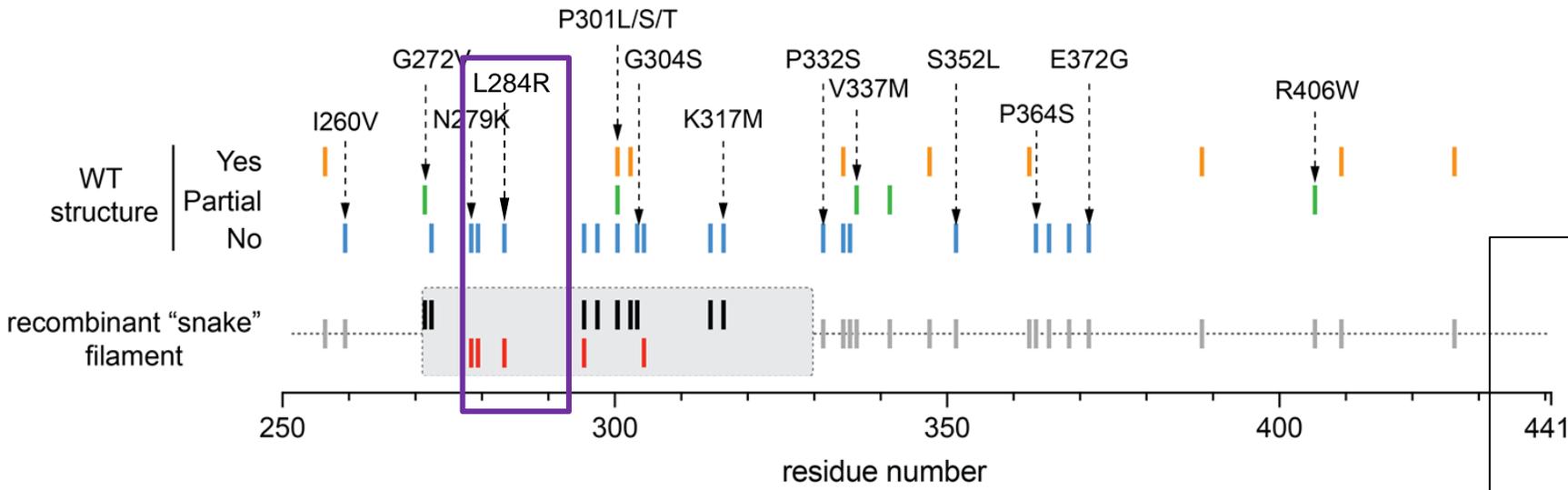
Trypsin digest assays can complement other techniques to better understand factors modulating aggregate structure



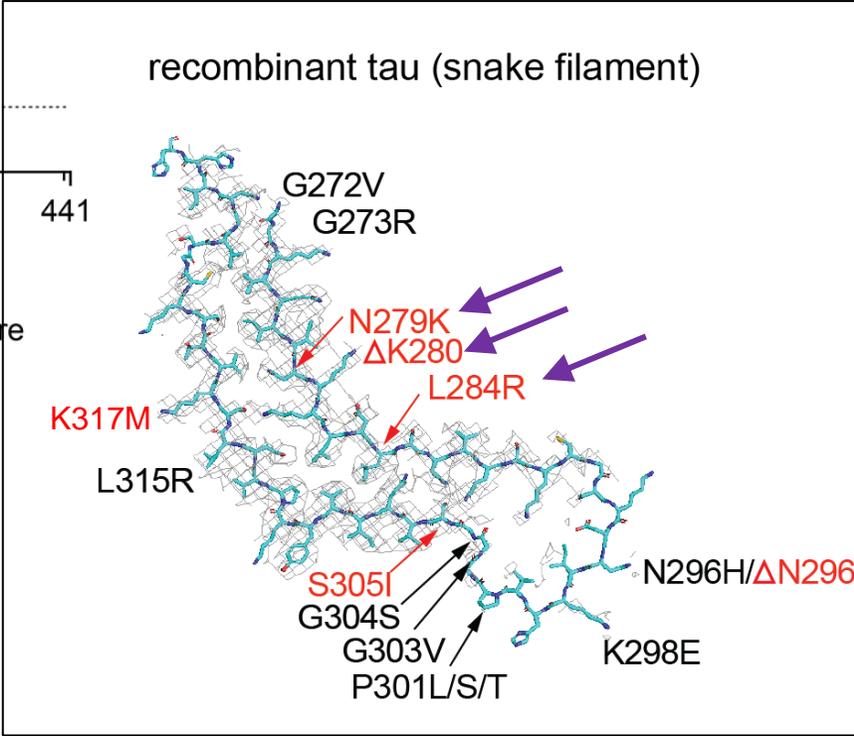
Not all mutations behave as predicted by analyzing known structures



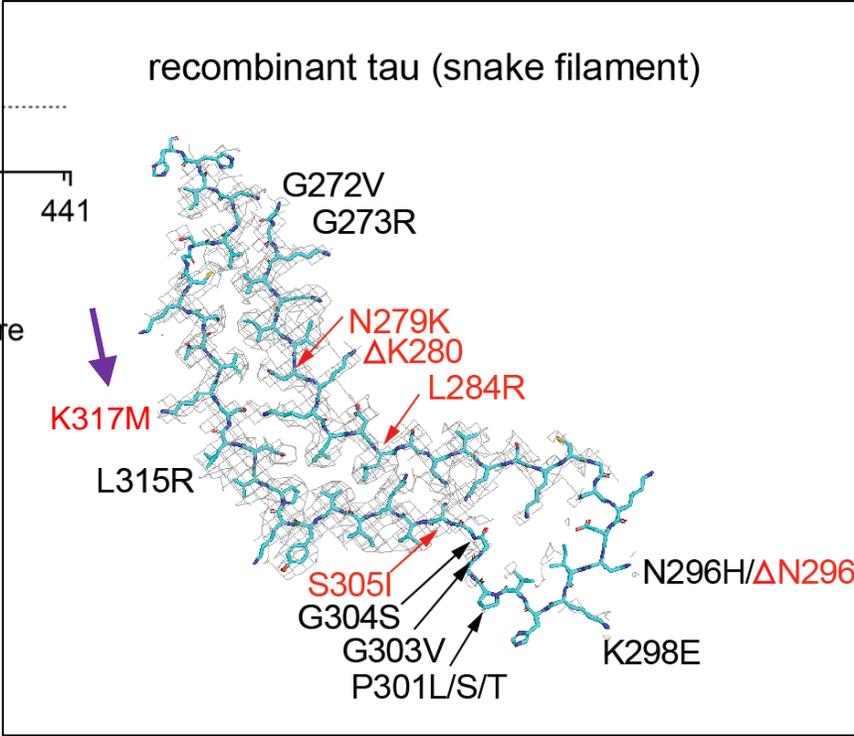
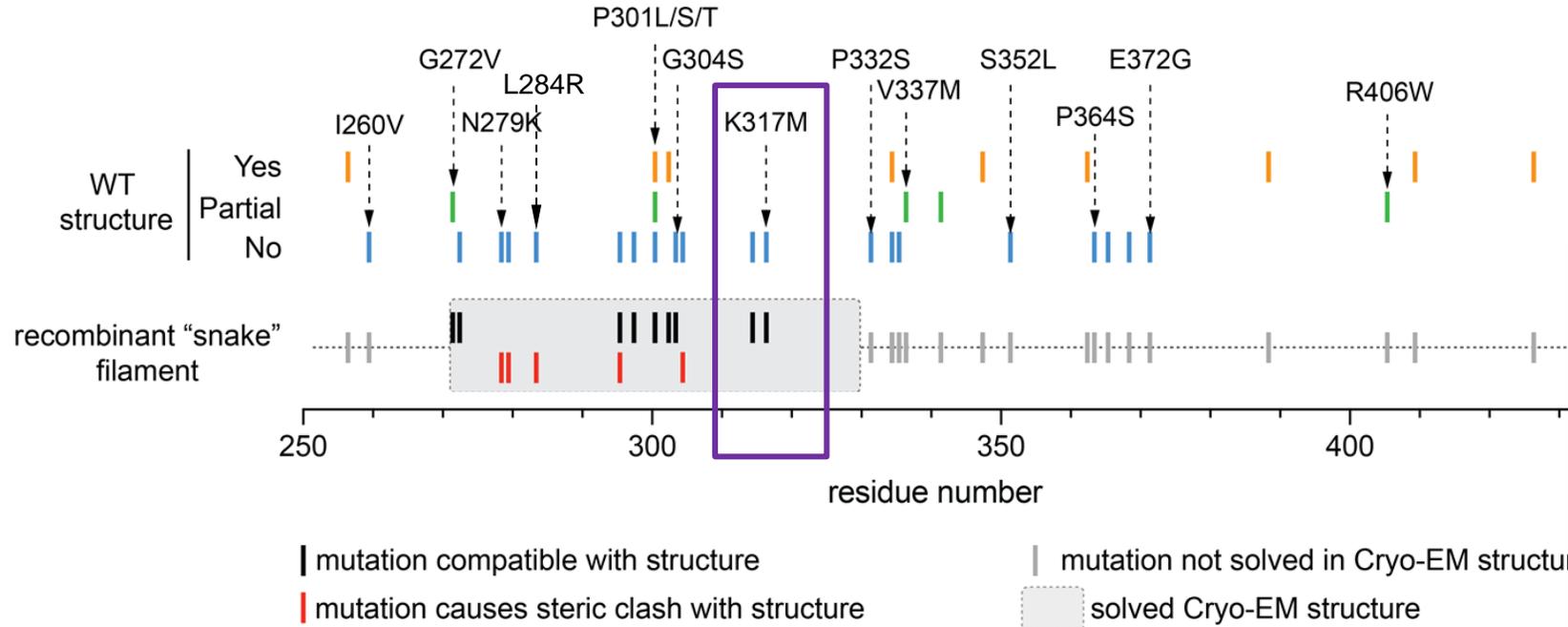
Not all mutations behave as predicted by analyzing known structures



| mutation compatible with structure
| mutation not solved in Cryo-EM structure
| mutation causes steric clash with structure
 solved Cryo-EM structure

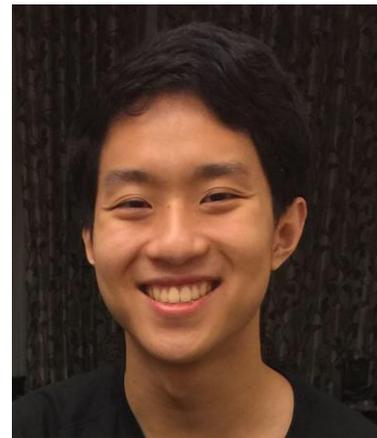
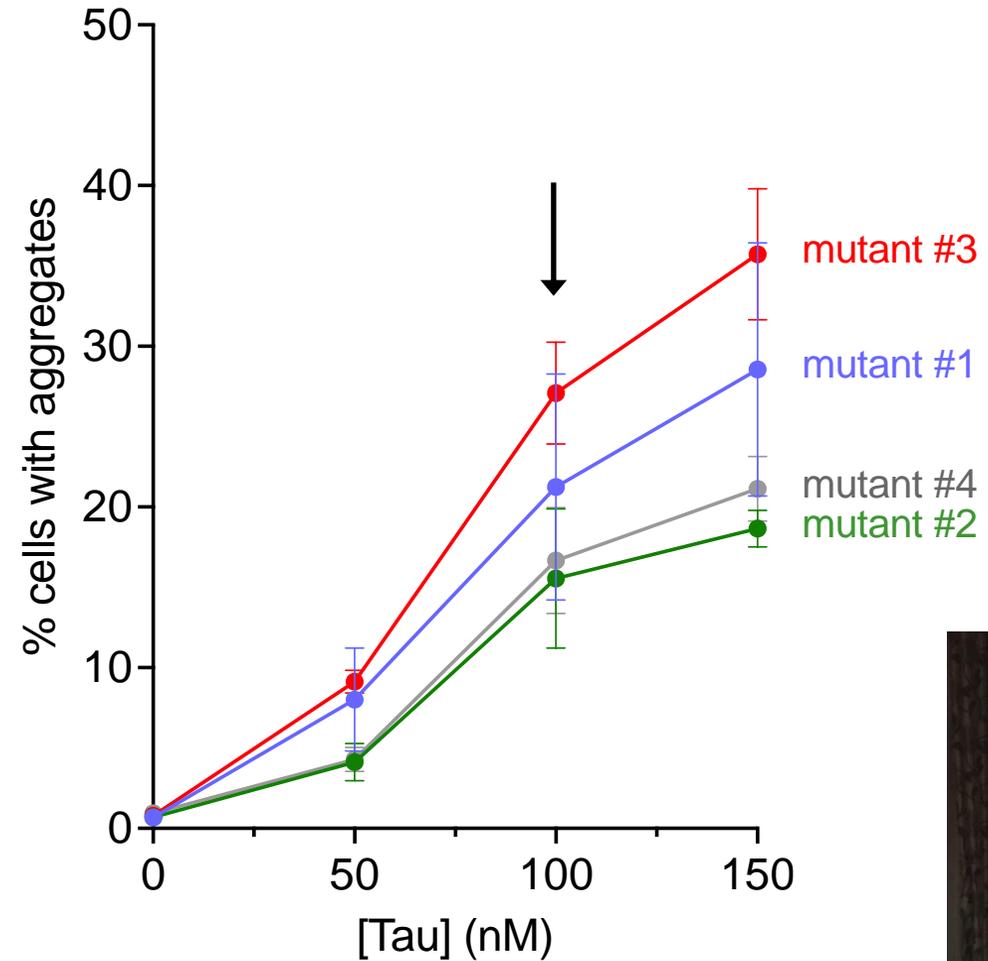
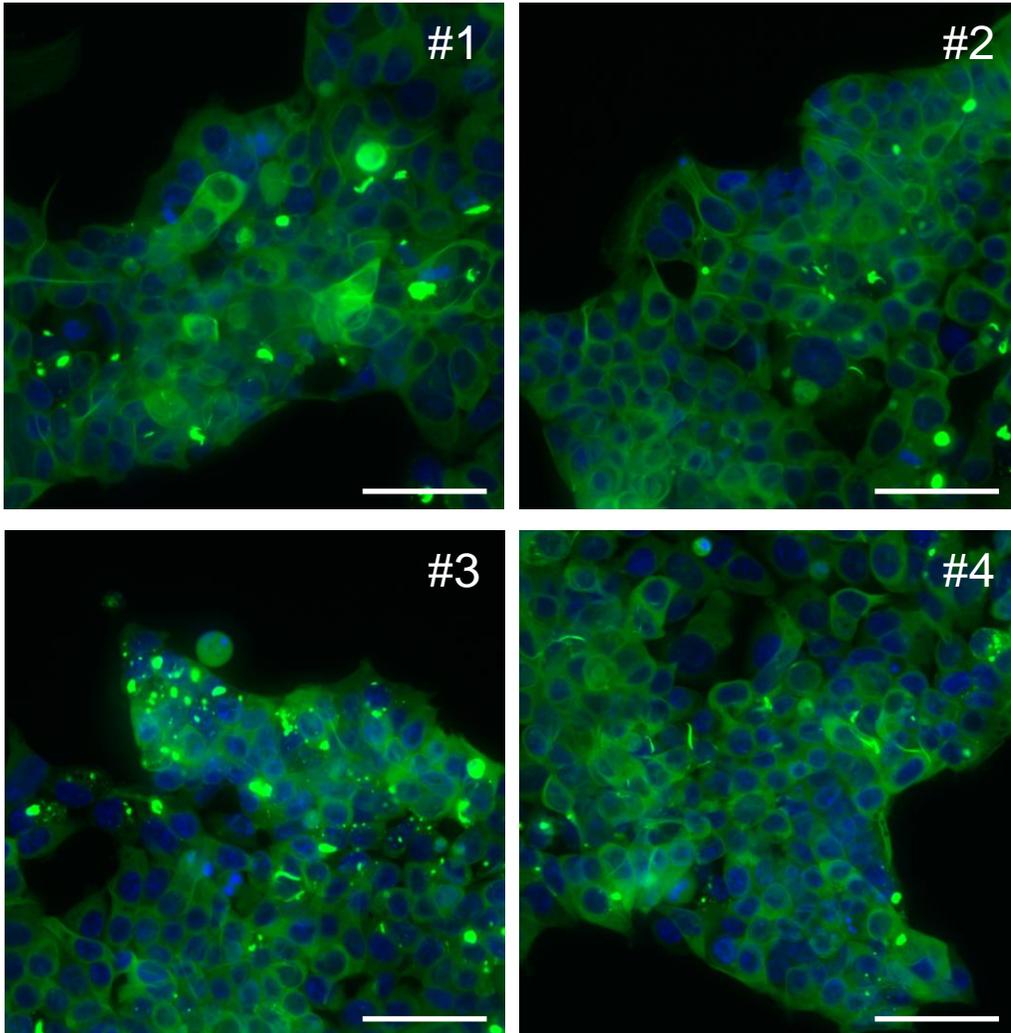


Not all mutations behave as predicted by analyzing known structures



Tau mutant amyloids have different seeding capacities

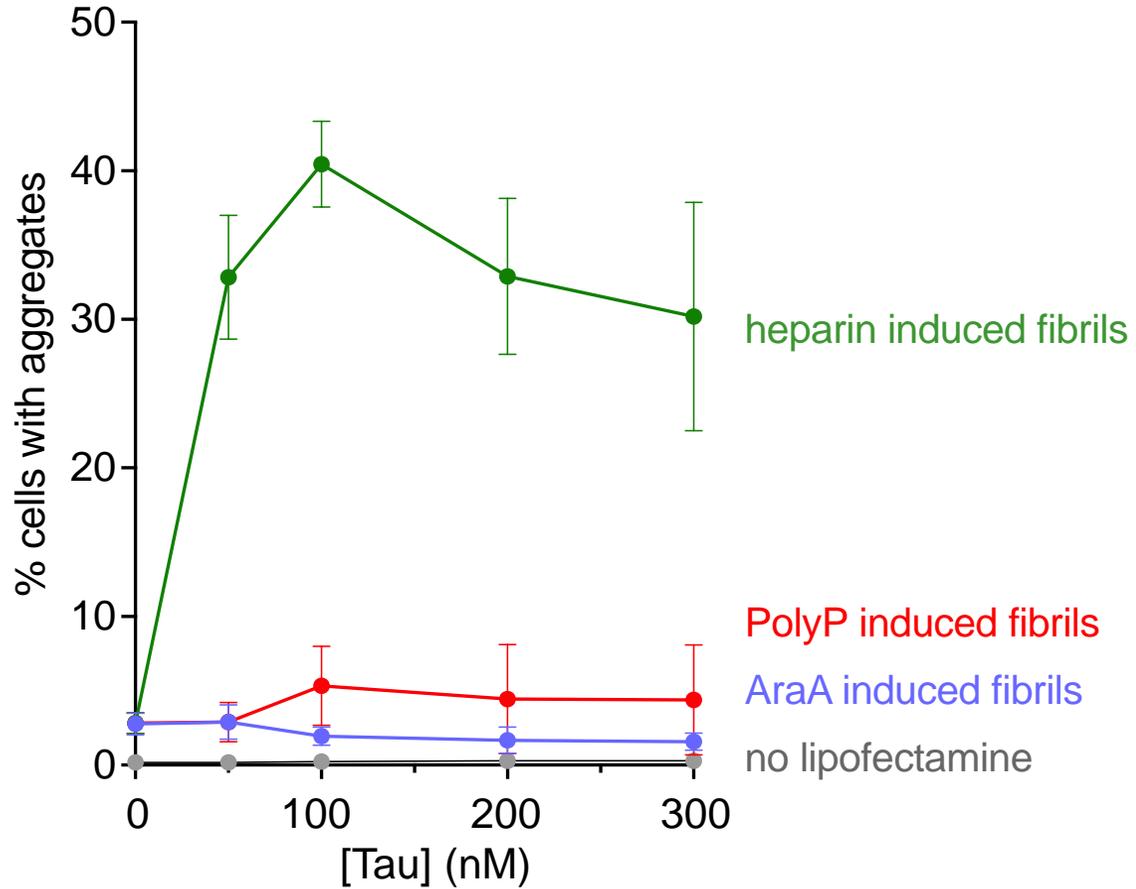
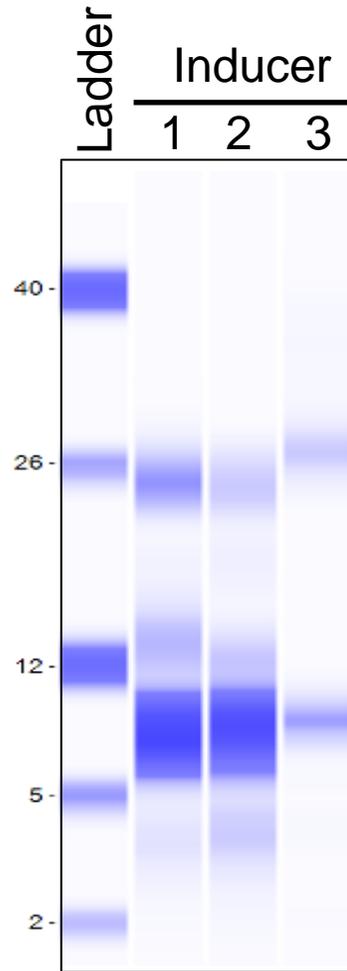
HEK2993 line expressing GFP-0N4R tau (P301L)



Kerry Sun

Tau aggregation inducers can also modulate aggregate structure and seeding capacity

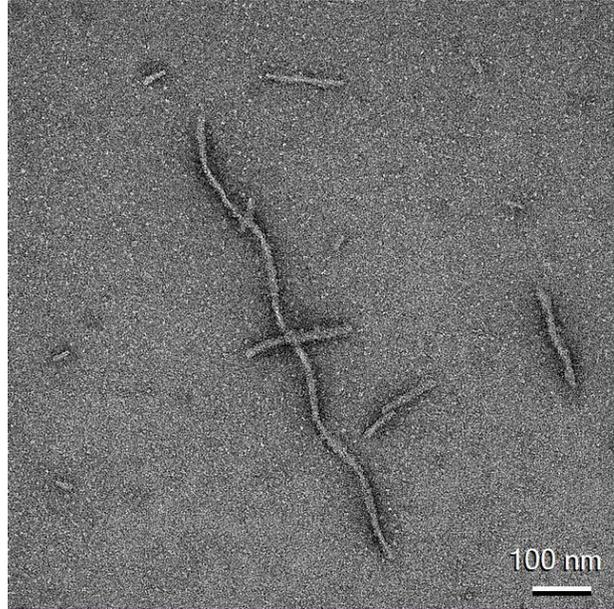
1=heparin
2=polyphosphate
3=arachidonic acid



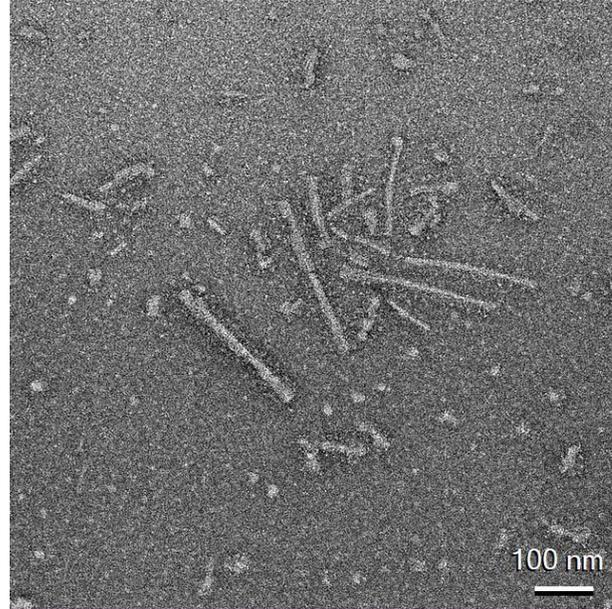
Emily McNamara

Tau fibril morphology by negative stain EM

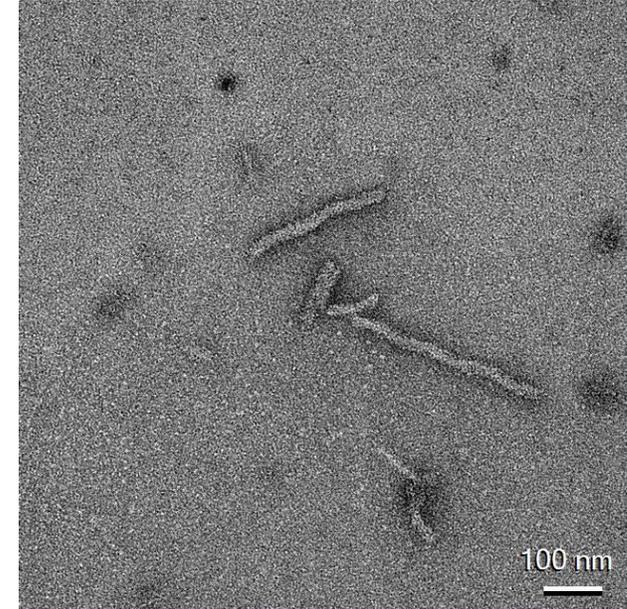
P301L heparin



P301L AraA



P301L PolyP



Take home messages

- Tau's aggregation misfolding pathway is sensitive to even small single biochemical changes such as mutations
 - Mutated tau may generate more pathogenic conformations
 - We have only scratched the surface of tau's complex biochemical landscape
- Tau aggregation kinetics and structure may be independent variables contributing to pathogenicity
- Molecular amyloid toolkits can help dissect structure-dysfunction relationships in cells and *in vivo*

Acknowledgements



Lab members

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Angela Lebrudo

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Haresh Sureshkumar
Ria Ratra
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Collaborators

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Satyabrata Kar (U. of Alberta)

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Model for generation of dominant amyloid polymorphs

