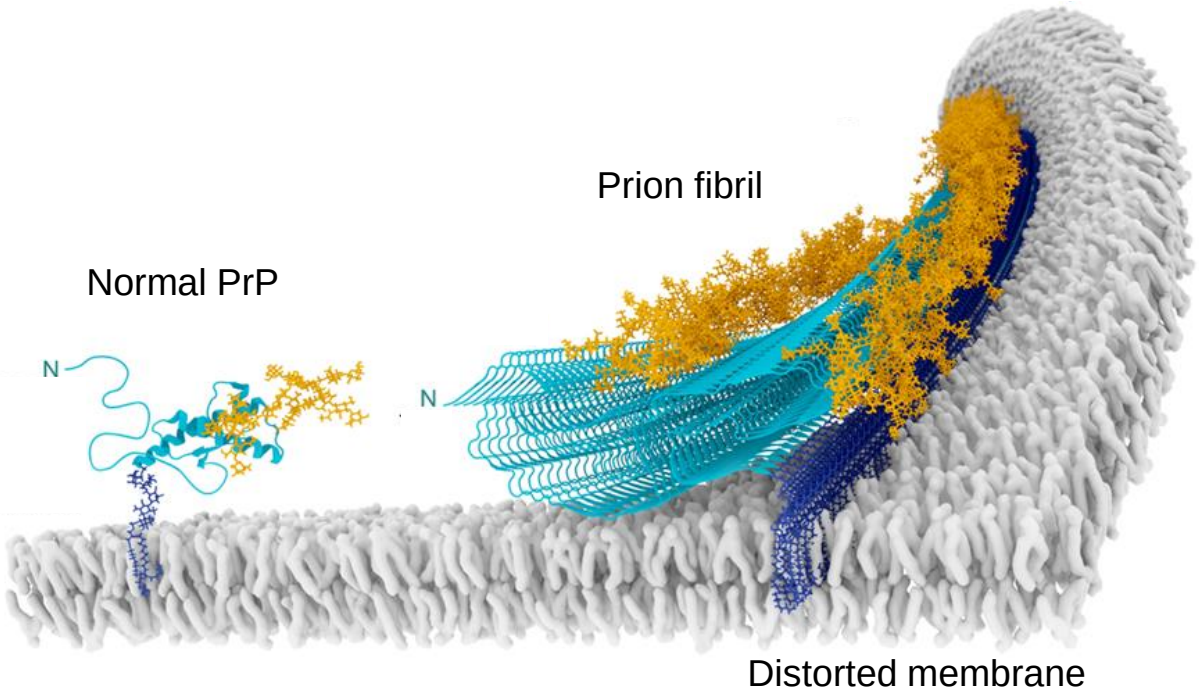
Structural biology of PrP prions

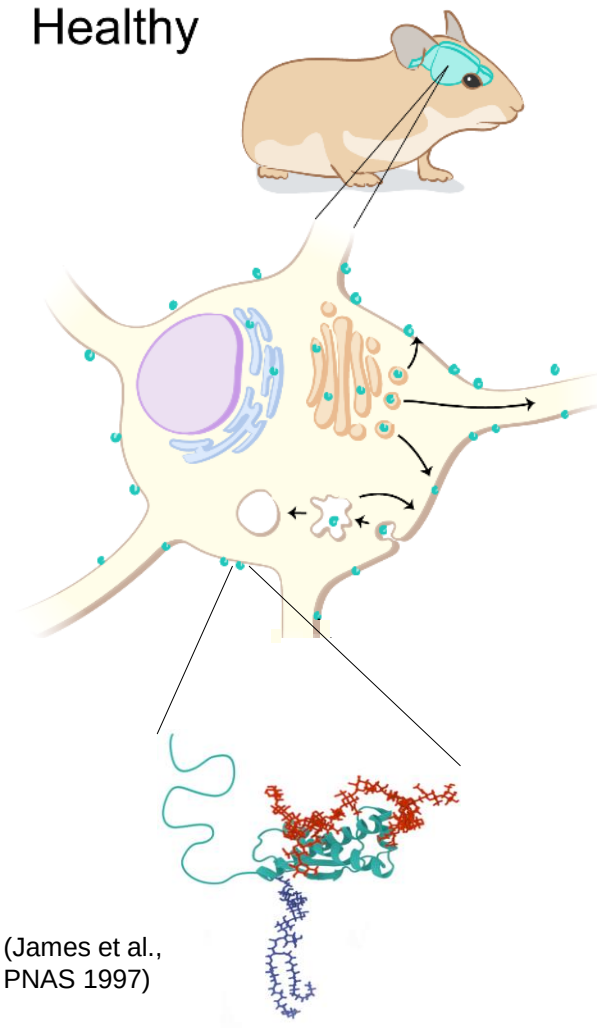


Byron Caughey
Rocky Mountain Laboratories

NIH National Institute of Allergy and Infectious Diseases

Samantha King, Francesco Silvestri, Christina Orrù, ???, Efrosini Artikis, Parvez Alam, Jakub Soukup, Andy Hughson

Molecular basis of prion diseases: refolding and aggregation of PrP



(James et al., PNAS 1997)

PrP^C

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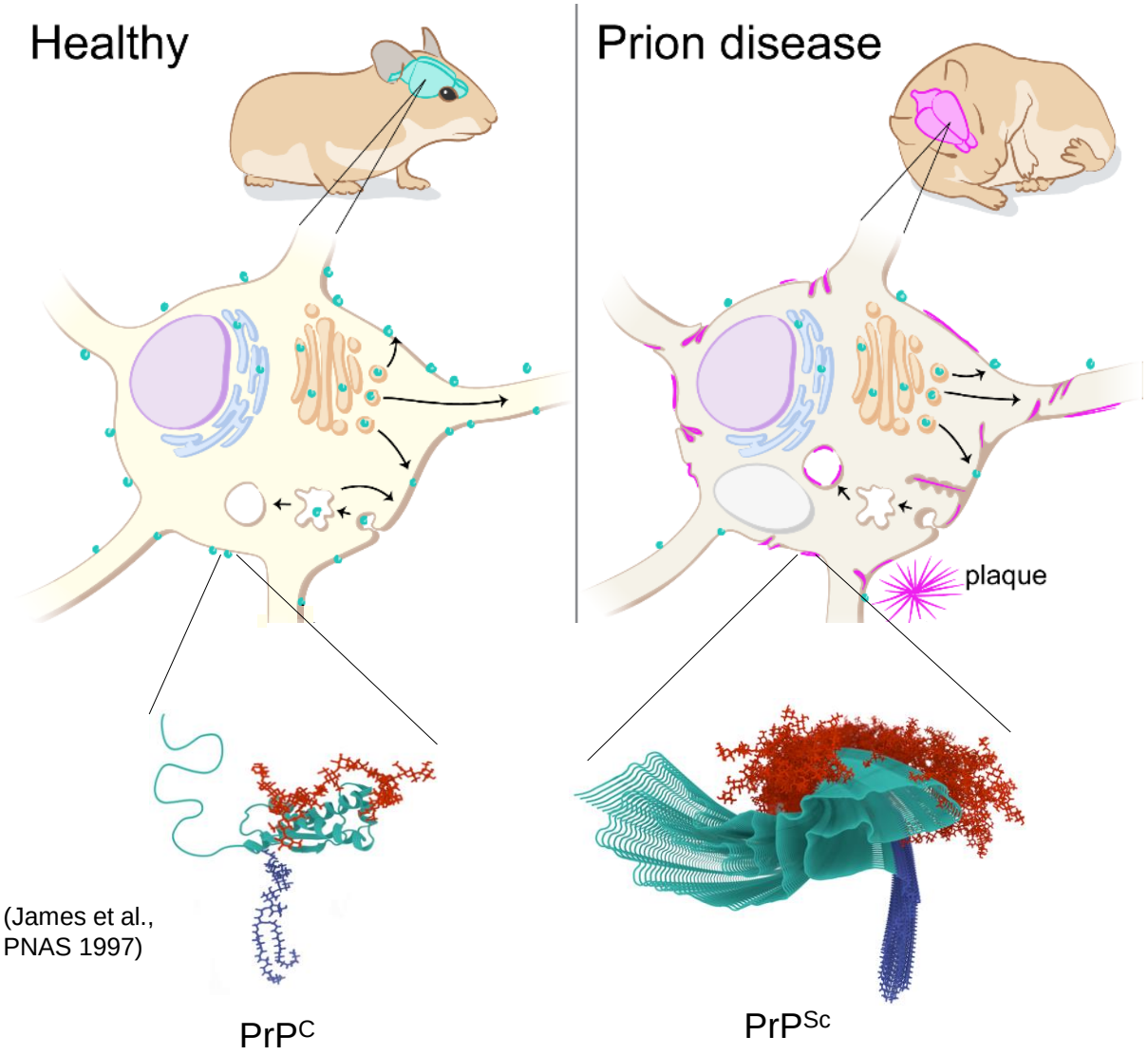

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Graphics: Rose Perry-Gottschalk, Austin Athman & Efrosini Artikis

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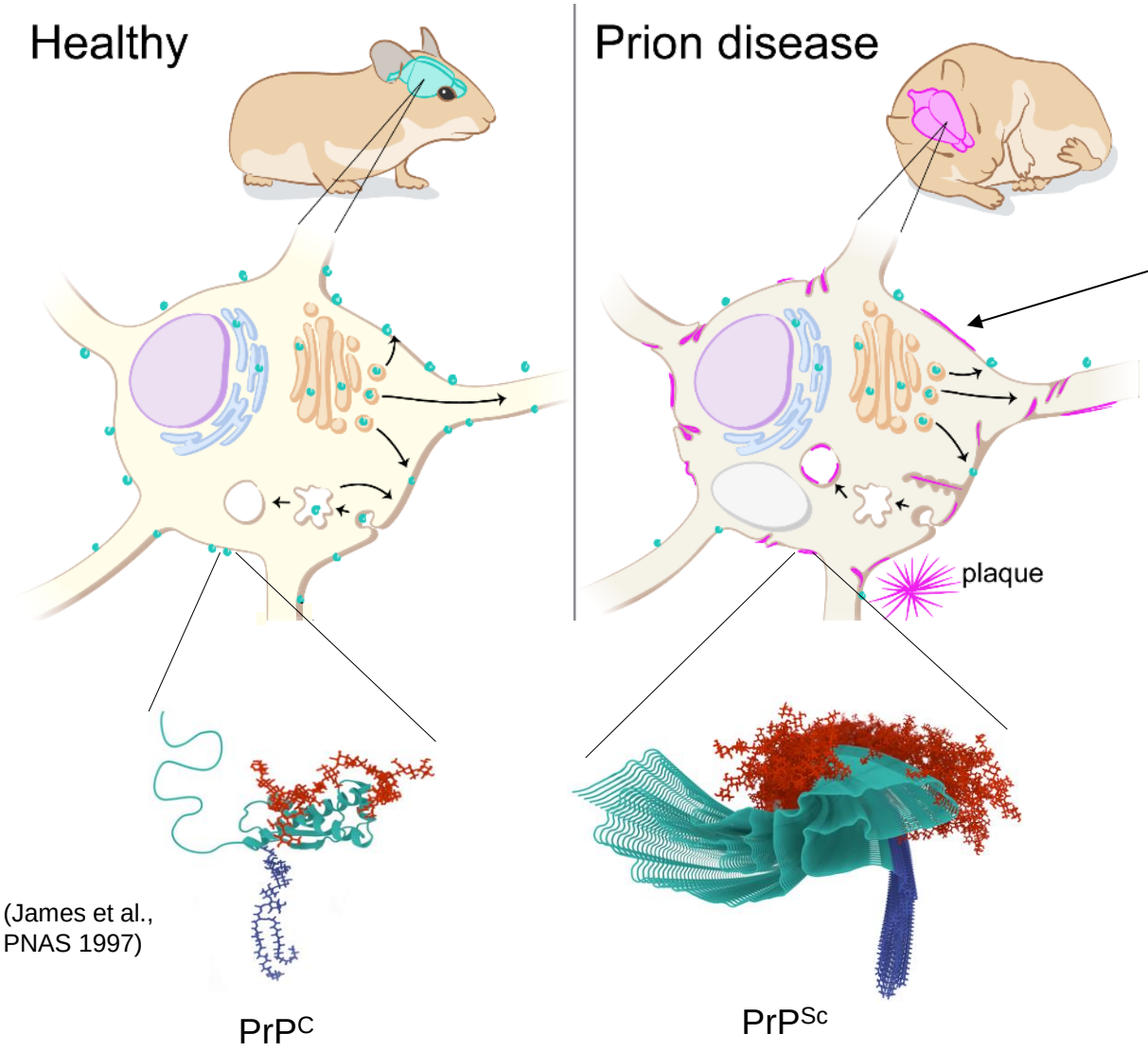

Can be fibrillar:

Wegmann et al., EJP 2008

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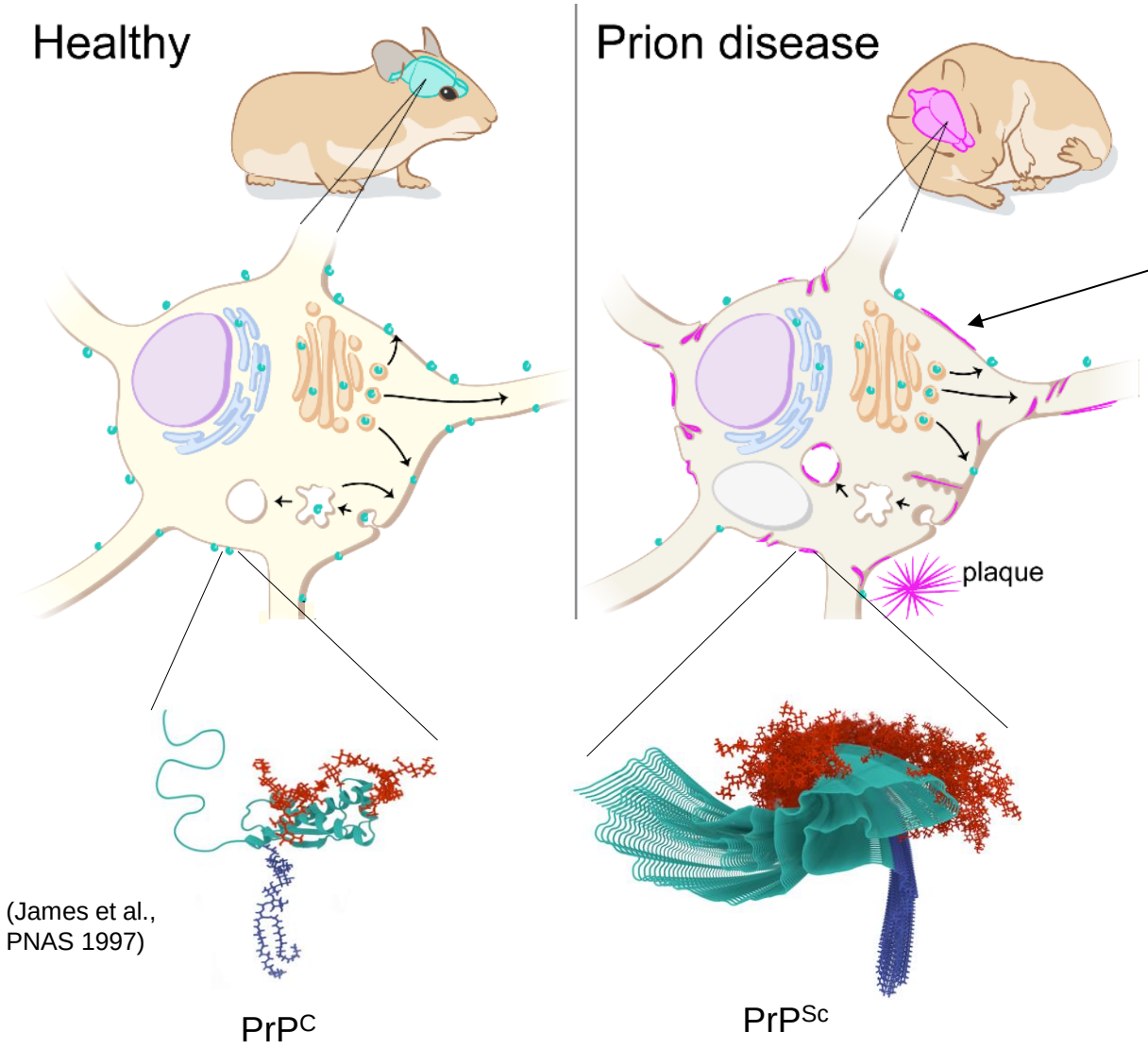

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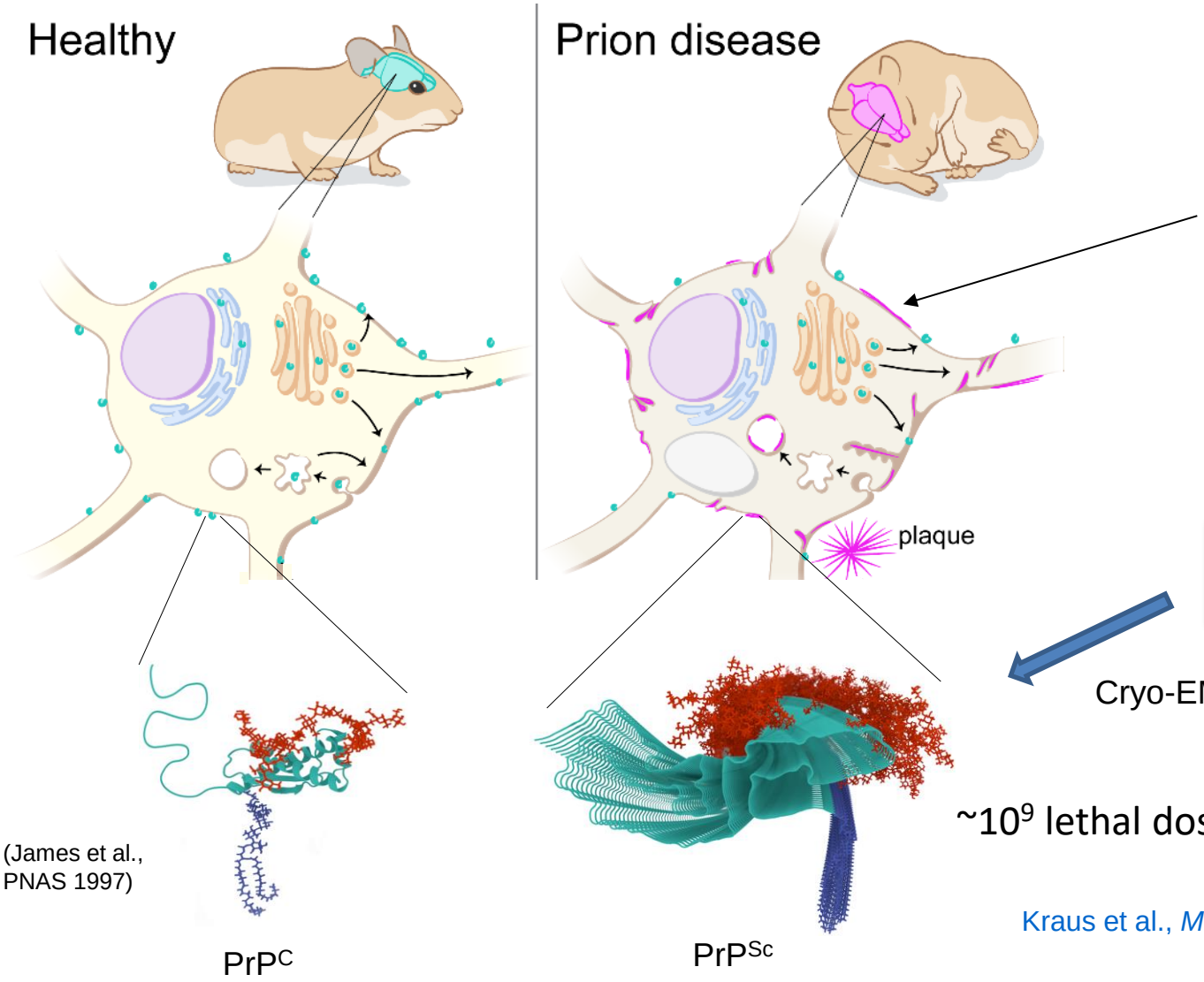

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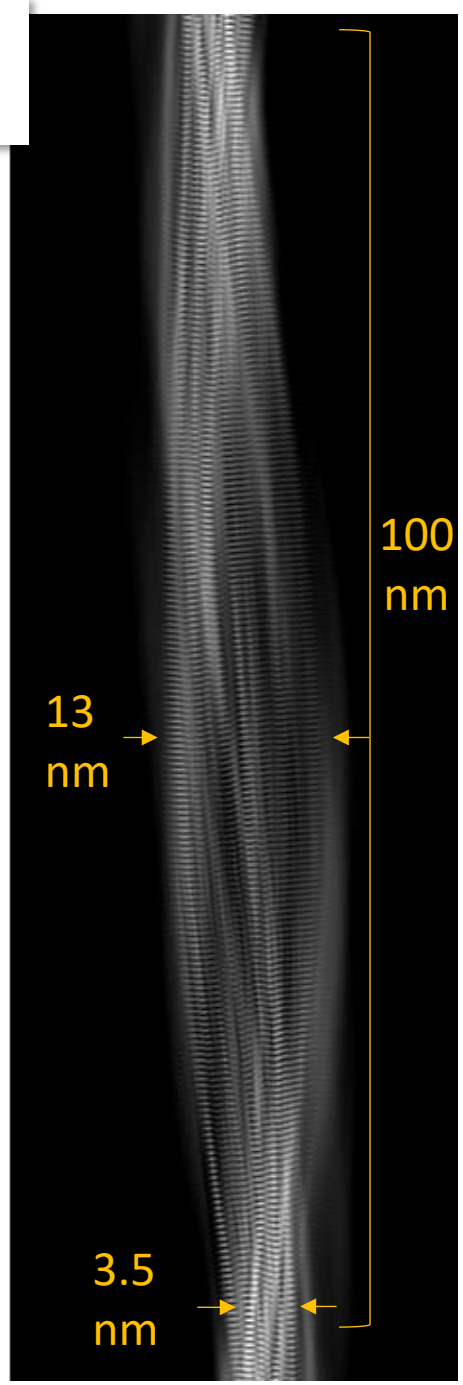
Structure(s) that allow PrP^{Sc} prions to propagate as infectious pathogens?

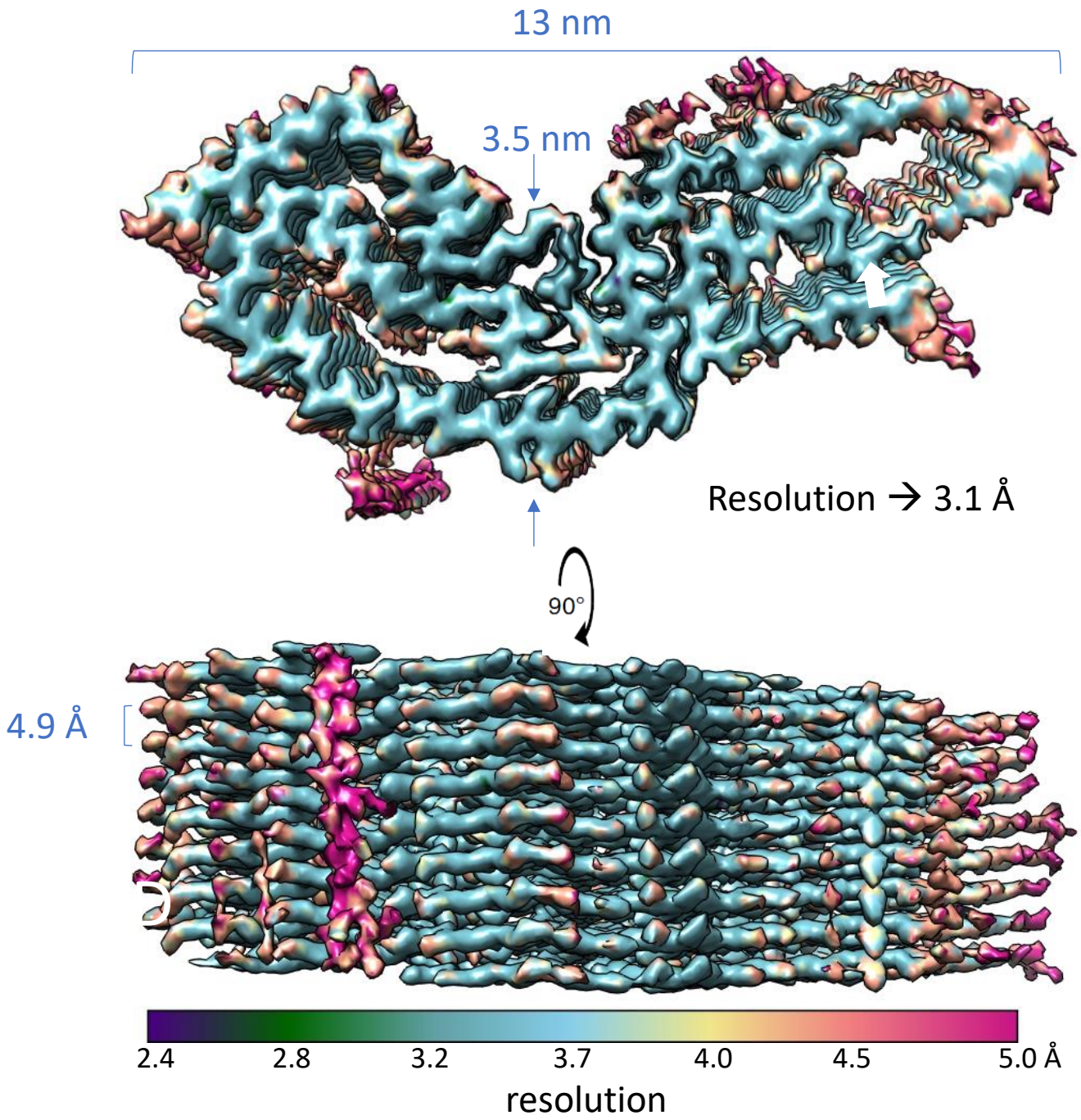
Cryo-EM

~10^9 lethal doses/mg

Kraus et al., *Mol Cell* 2021

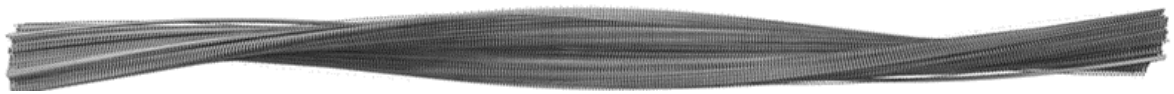
Cryo-EM density map of prion fibril



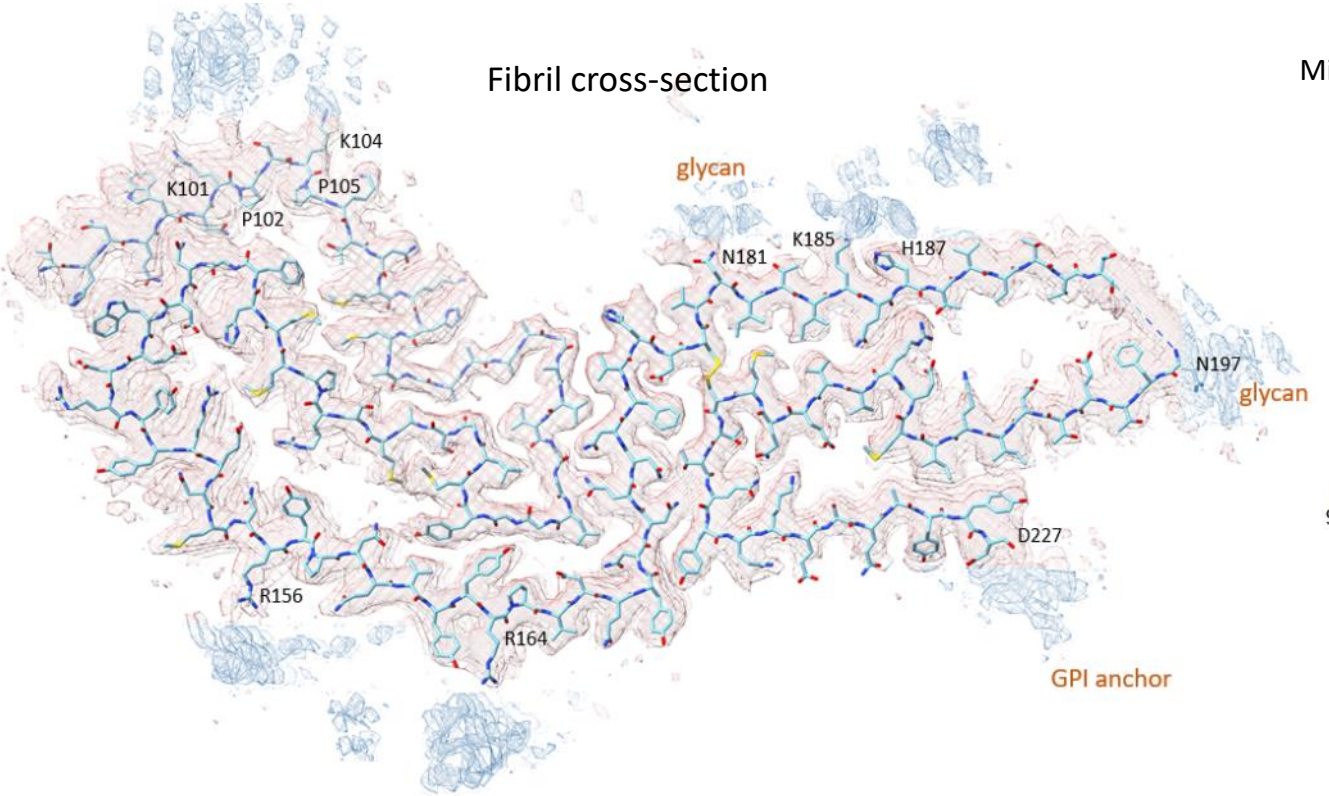


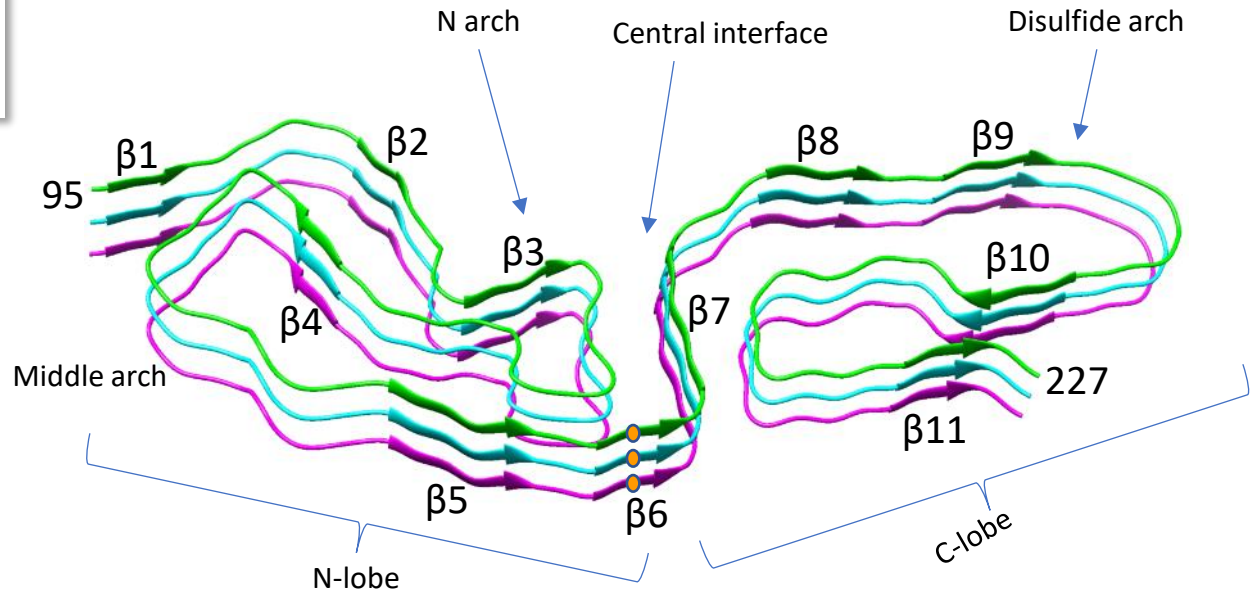
Kraus et al., *Mol Cell* 2021

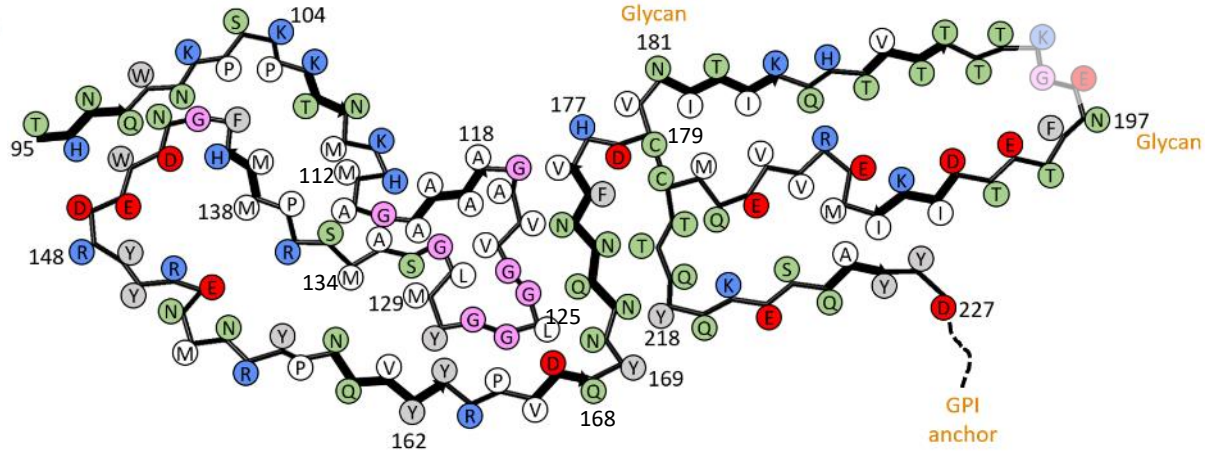
Cryo-EM-based atomic model of 263K scrapie prion fibril based on threading/fitting of PrP residues of PK-resistant core



Fibril



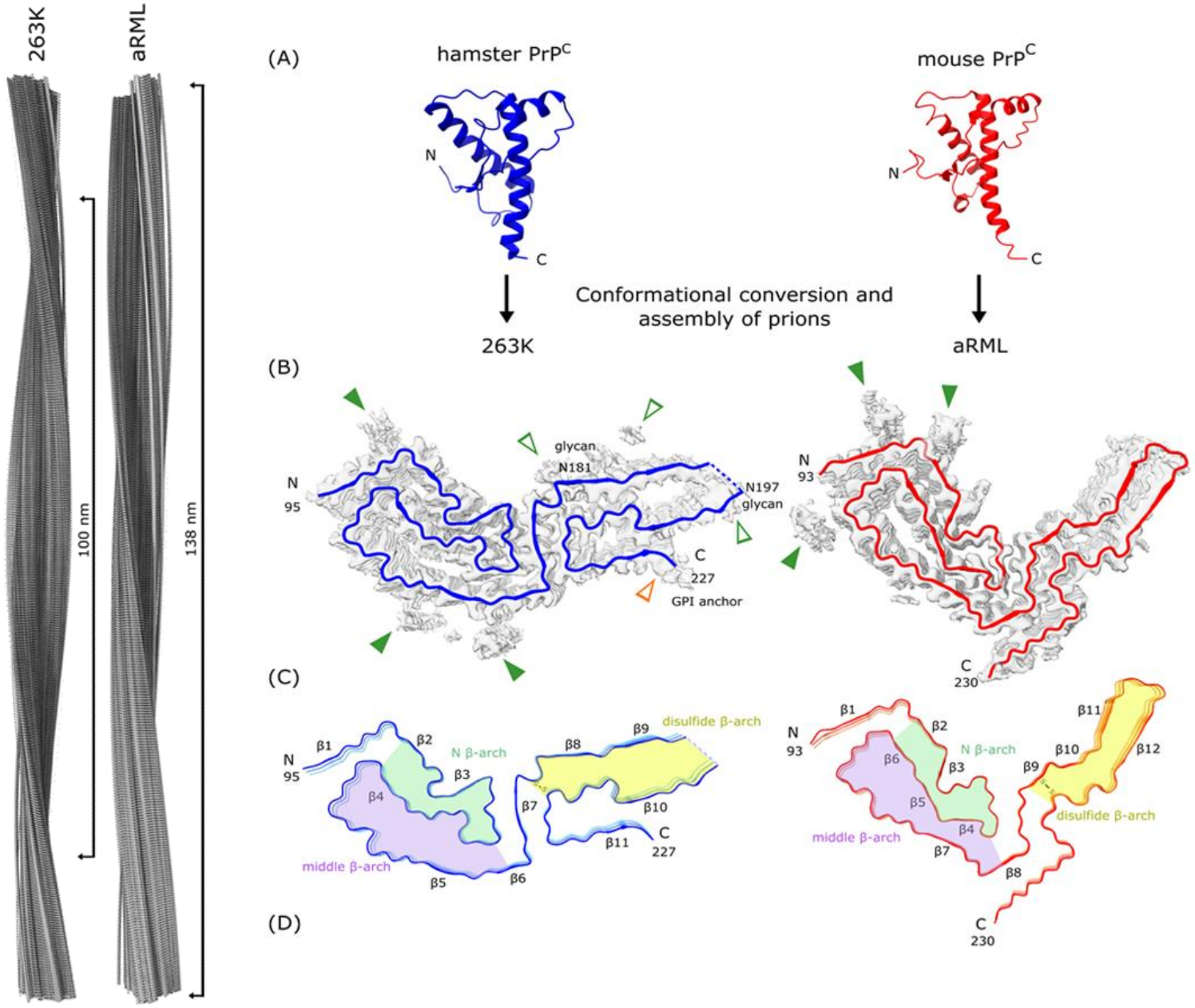




- Cross-section: single monomer with β-strands and loops
- Monomers stacked with parallel in-register intermolecular β-sheet (PIRIBS) architecture
- Glycans and GPI anchor project outward
- Cofactors on periphery?

Kraus et al., *Mol Cell* 2021

Interspecies variation: hamster 263K vs mouse aRML prion cryo-EM structures



Variations in shared motifs and sequence (at 8 residues within core region):

- distinct templates for faithful propagation of strain conformers
- bases for differential transmission (e.g. species) barriers.

Kraus et al., *Mol Cell* 2021
Hoyt et al., *Nature Comm* 2022
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Caughey et al., *PLoS Path*, 2022

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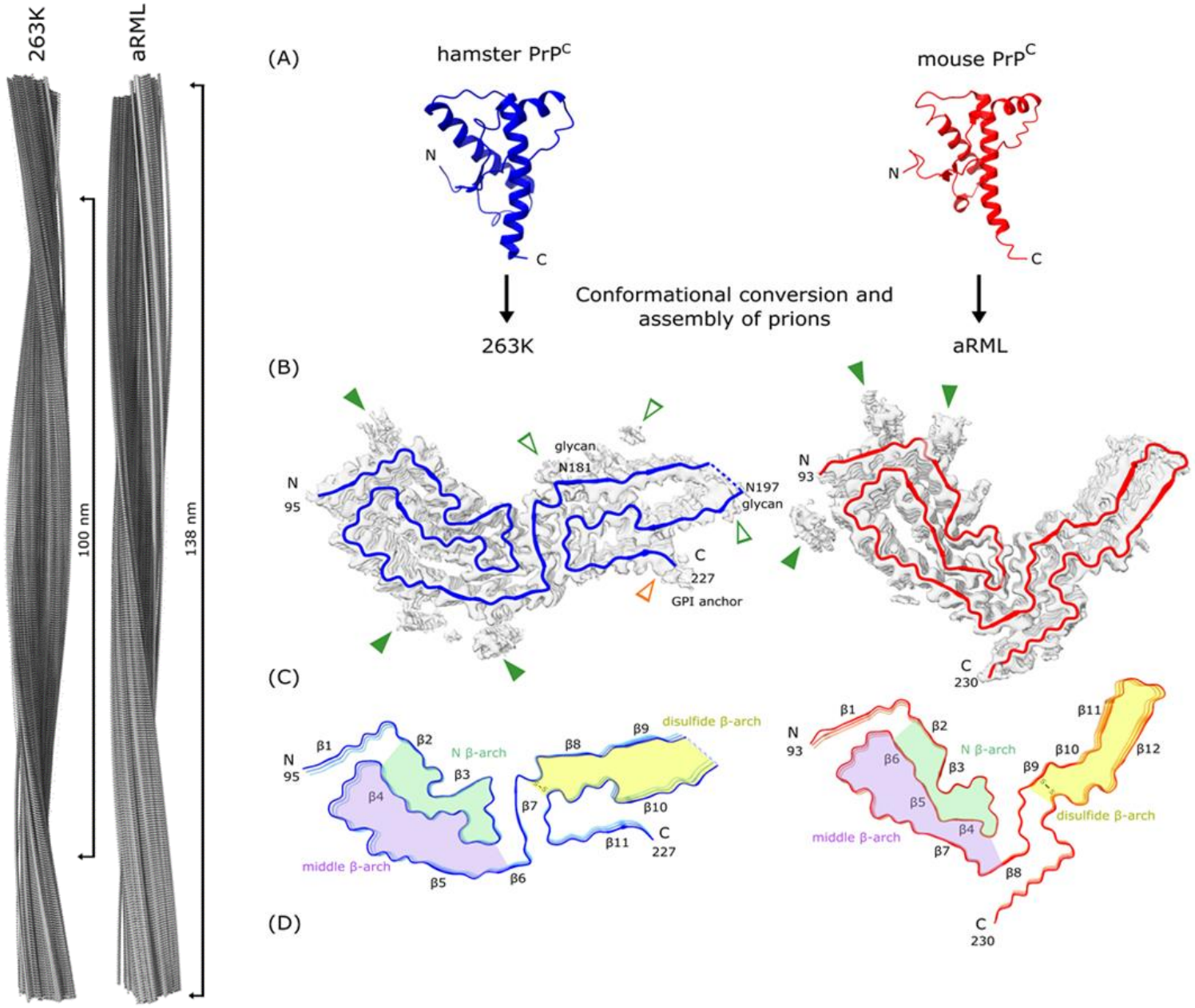

Variations in shared motifs and sequence (at 8 residues within core region):

- distinct templates for faithful propagation of strain conformers
- bases for differential transmission (e.g. species) barriers.

- Needed comparison of strains from **same host genotype** to identify purely conformational determinants of prion strain phenotypes
  - Same PrP sequence
  - Same cofactor pool

Kraus et al., *Mol Cell* 2021
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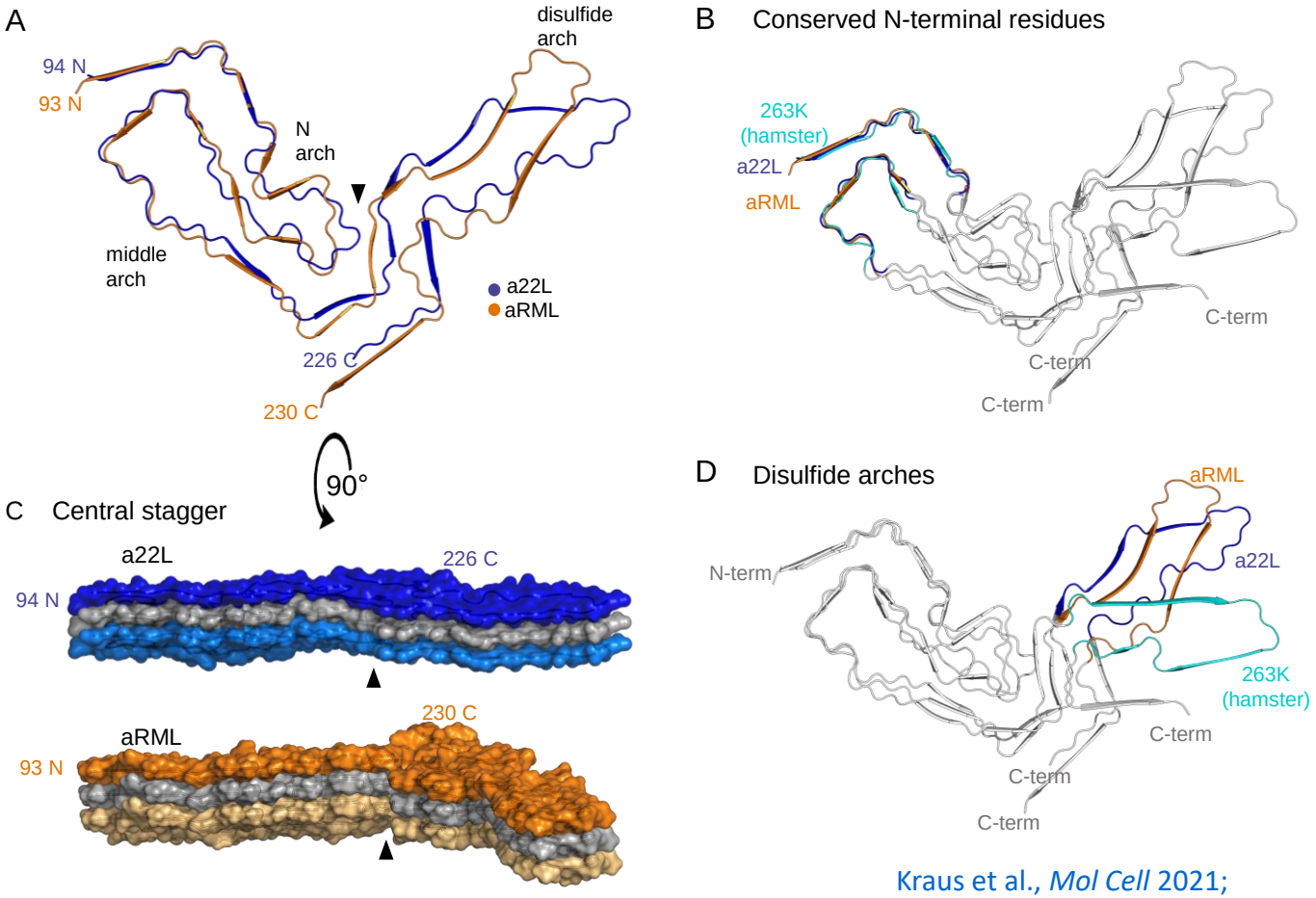
Strain variation: a22L & aRML prion strains from the same genotype of mouse



Kraus et al., *Mol Cell* 2021;
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- **Conformationally conserved regions**, especially near N-terminus
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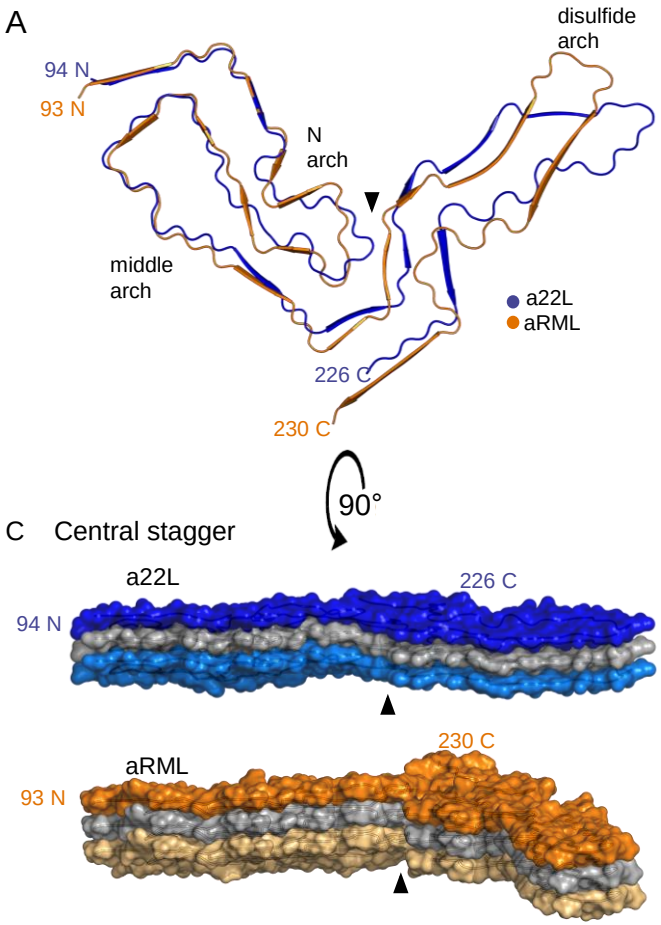

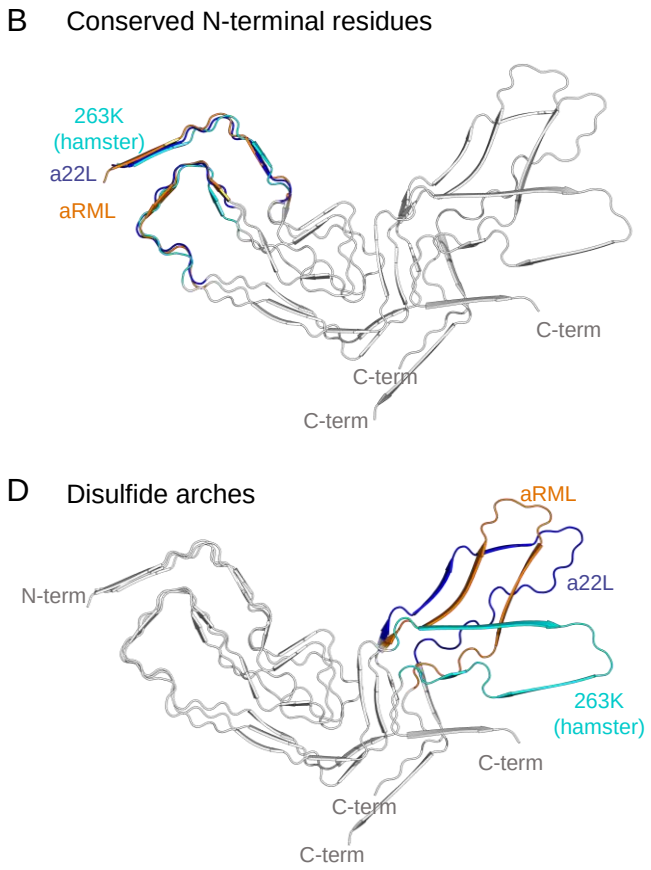

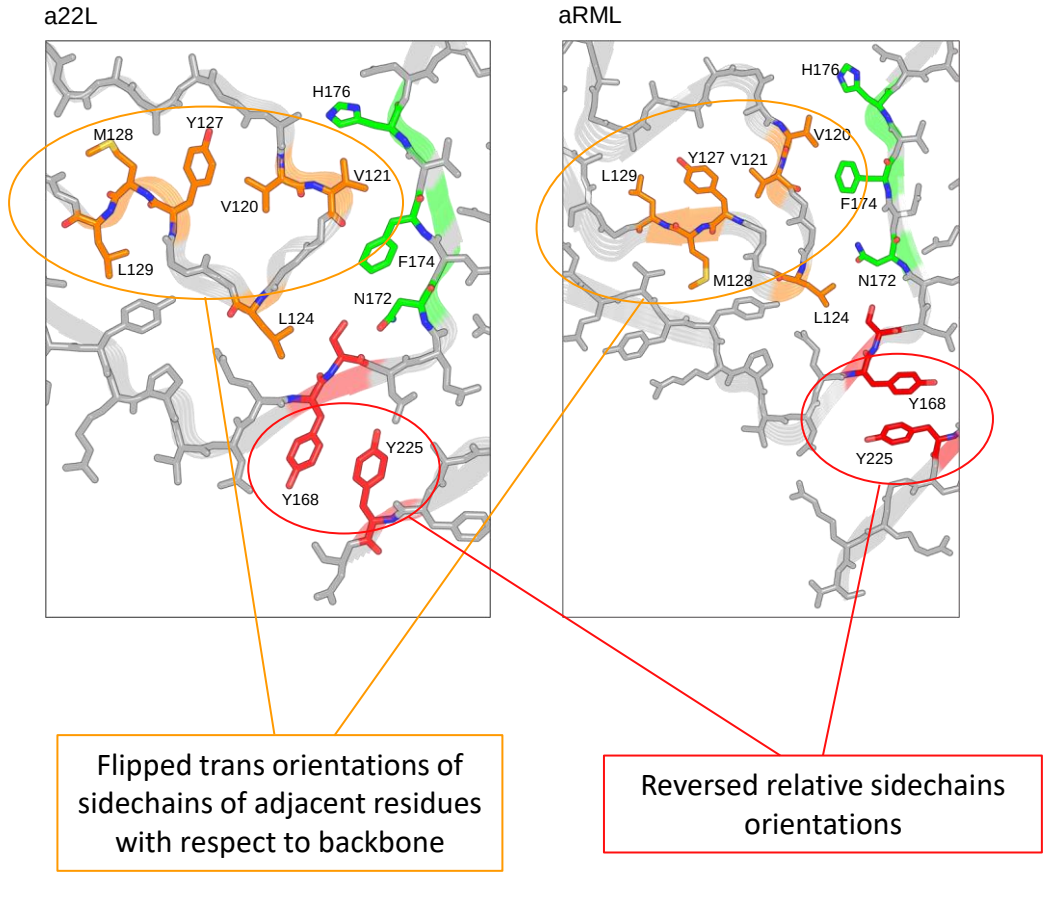

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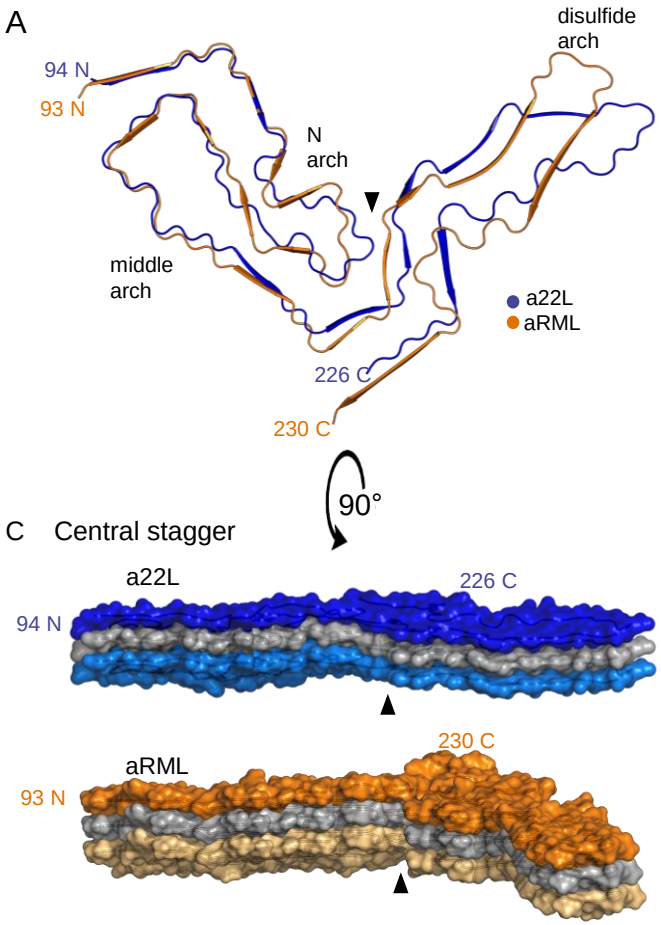

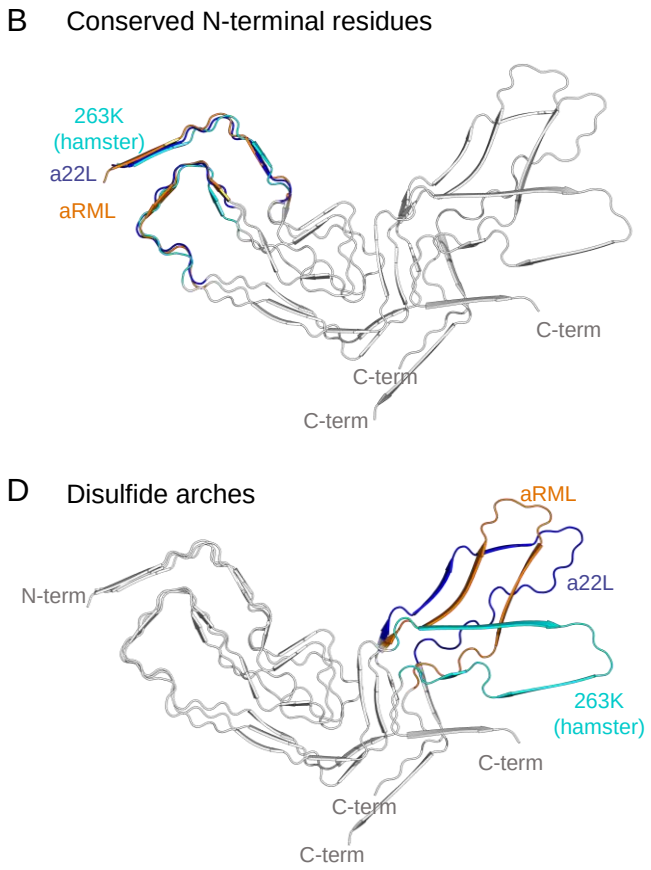

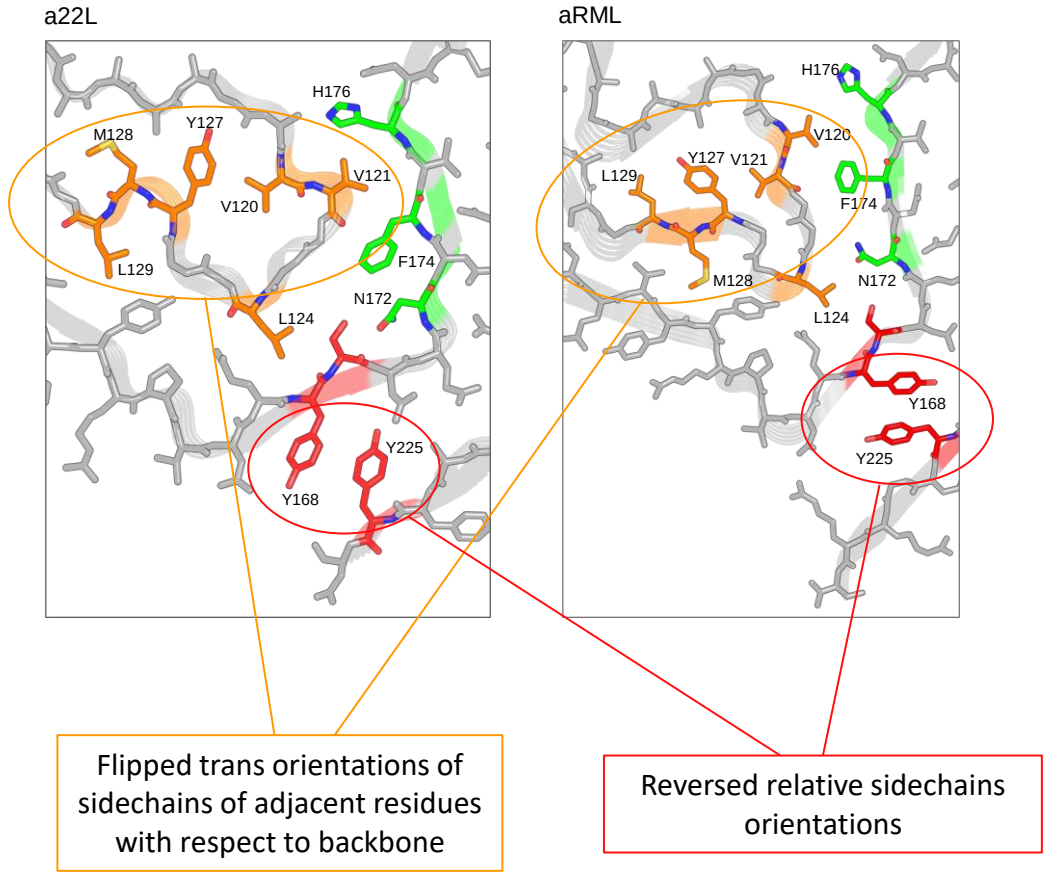

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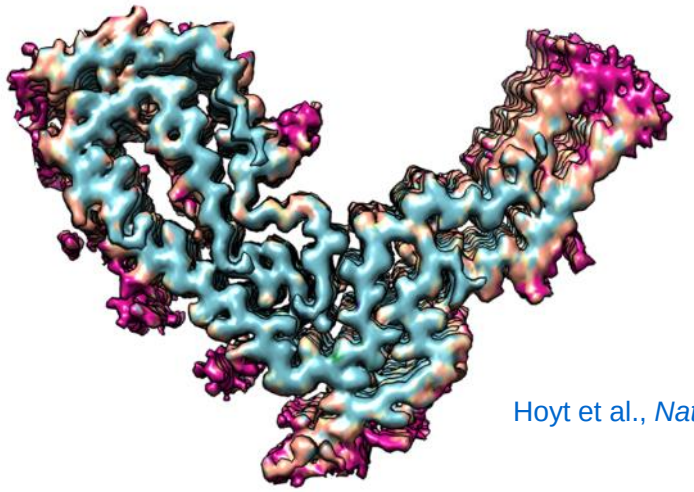
- **Conformationally conserved regions**, especially near N-terminus
- **Strain-dependent variations** within shared motifs outside of conserved region
- Similar themes from comparison of **RML & ME7** strains (Manka et al, *Nat Comm* 2022 & *Nat Chem Biol* 2023)

➡ Distinct conformational templates provide a molecular basis for the "encoding" of prion strain characteristics

Influence of GPI anchor & N-linked glycans?

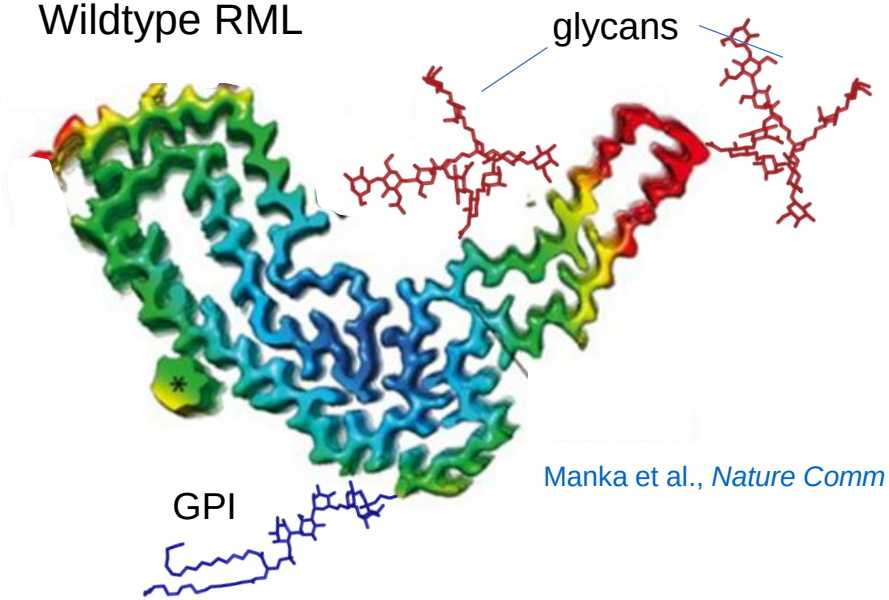
aRML (GPI-anchorless, underglycosylated)



Hoyt et al., *Nature Comm* 2022

- Glycans & GPIs have **little effect** on core conformation of RML strain

Wildtype RML



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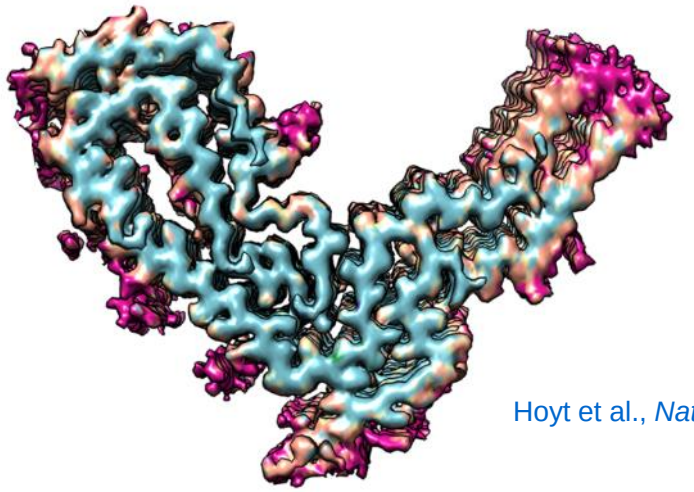

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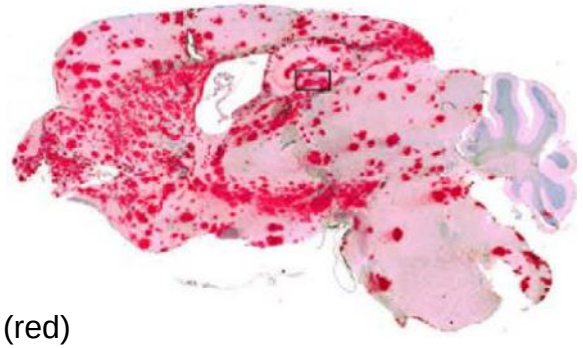
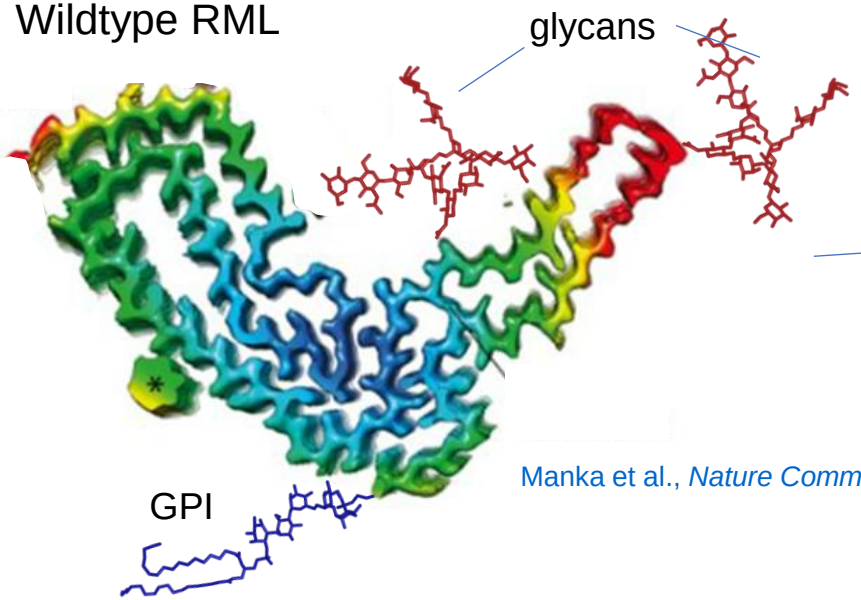


IHC: abnormal PrP (red)
@ terminal stage

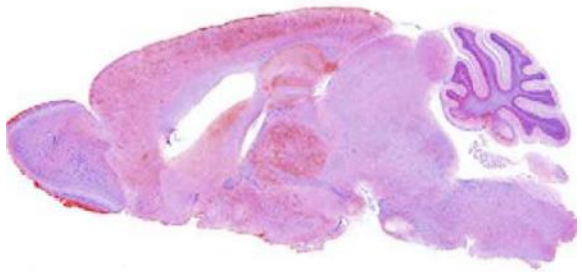
- Amyloid angiopathy
- Heavy PrP^{Sc} load

Chesebro et al., *Science* 2005
Chesebro et al., *PLoS Path* 2010

Wildtype RML



Manka et al., *Nature Comm* 2022



- Diffuse, neuropil
- Lighter PrP^{Sc} load
- Higher specific toxicity (per unit mass)?

- Nonetheless, **both** anchorless and wildtype prions are **lethal**.
  - Both GPI-*dependent* and -*independent* toxicities

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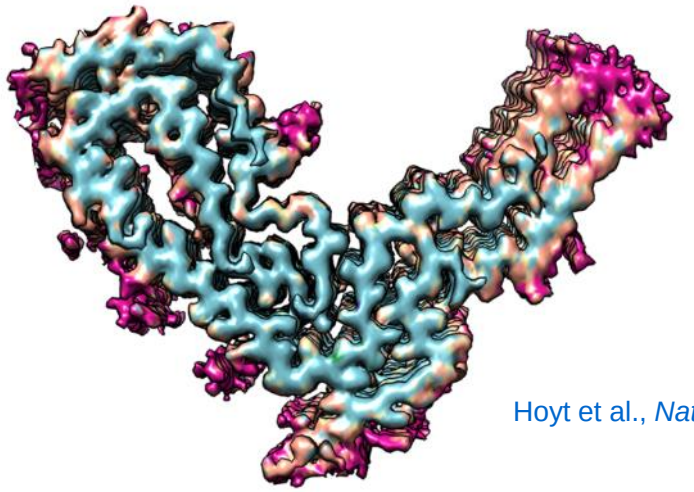

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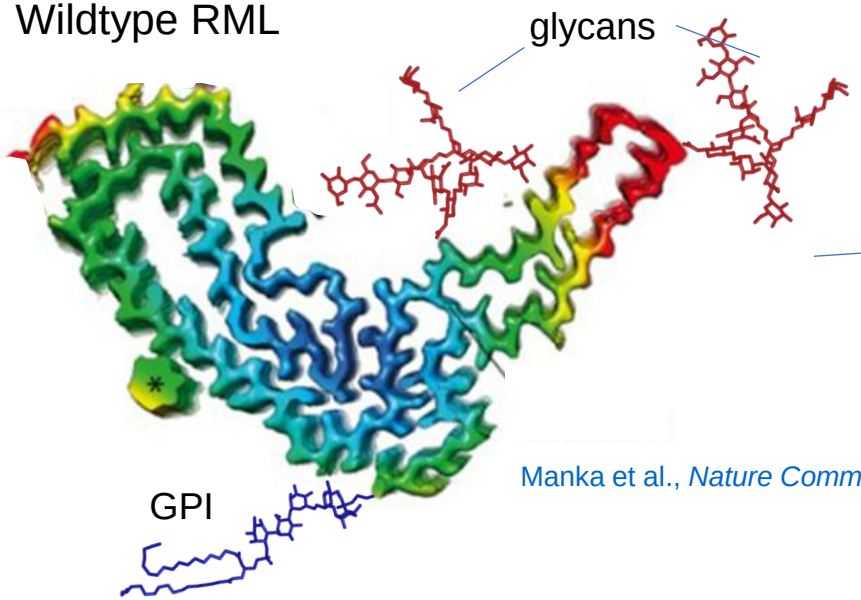

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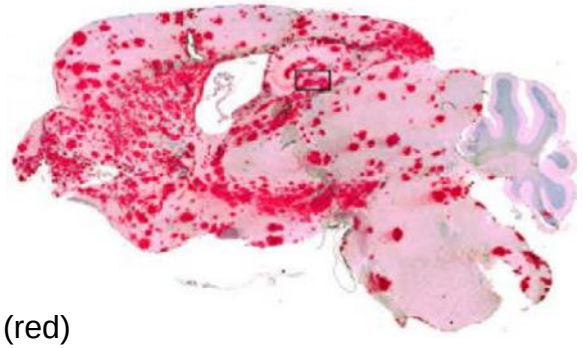

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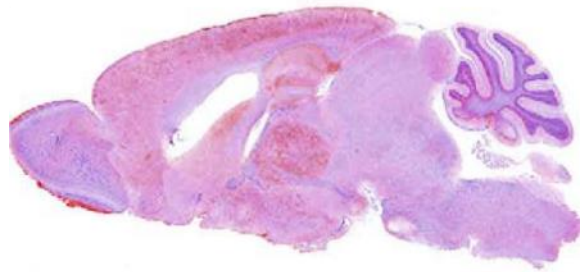

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- Nonetheless, **both** anchorless and wildtype prions are **lethal**.
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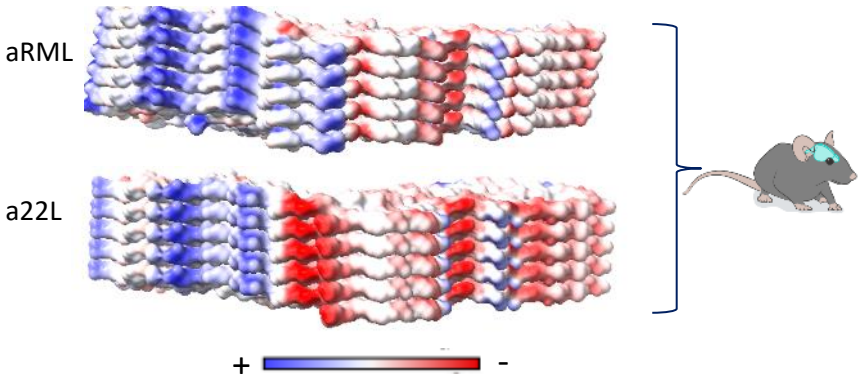
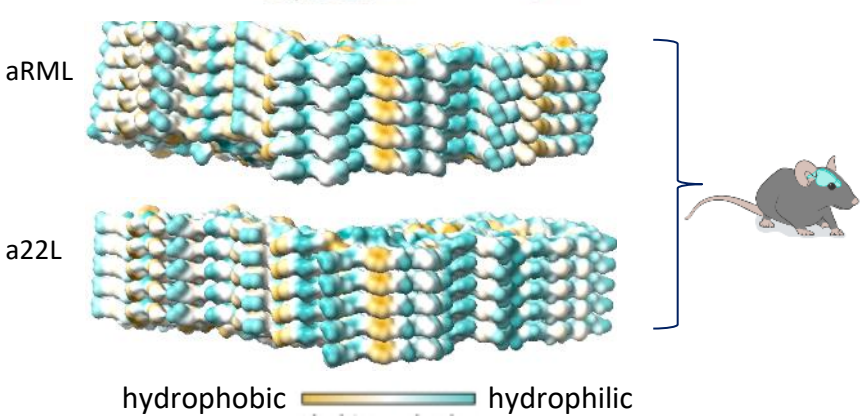
- **Amyloid form can encipher strain characteristics**

How might distinct structures mediate strain-dependent pathogenesis?

A Charge



B Hydrophobicity-hydrophilicity



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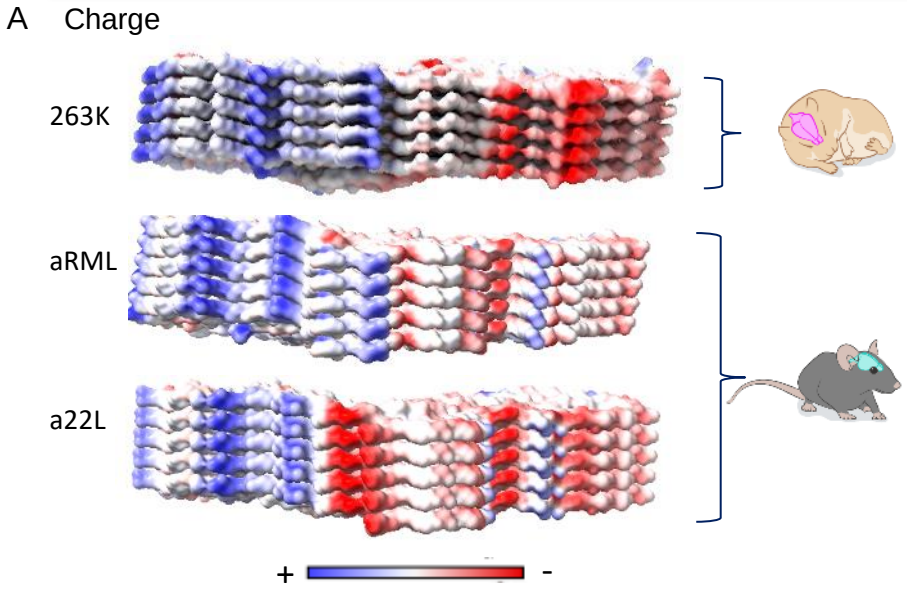

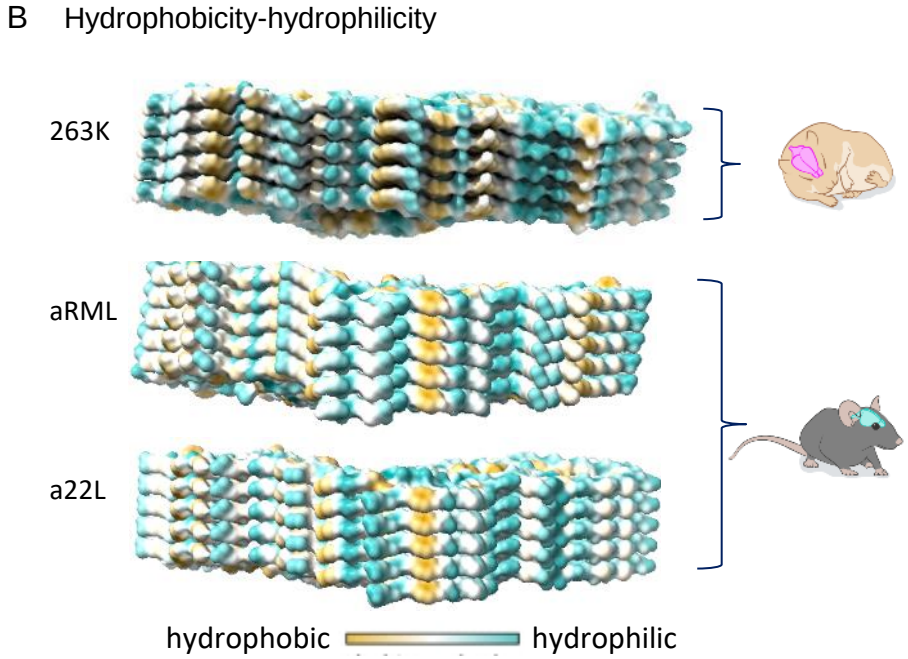

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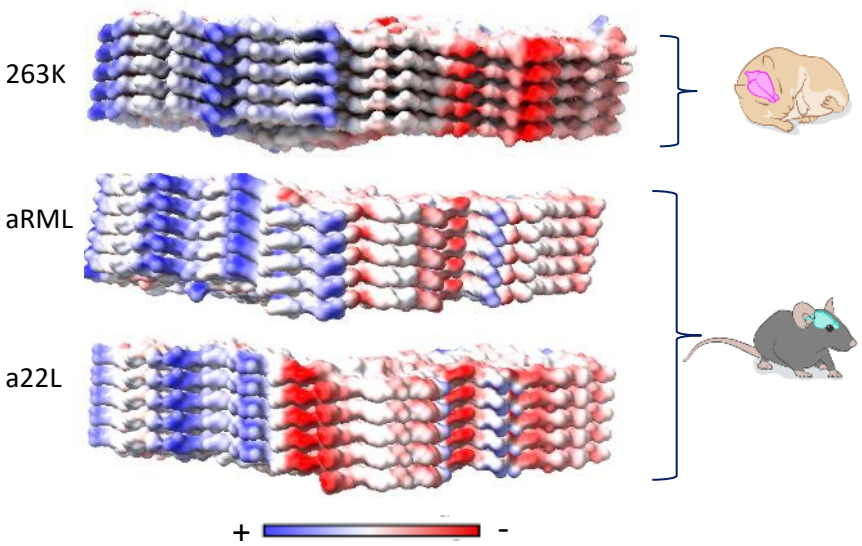

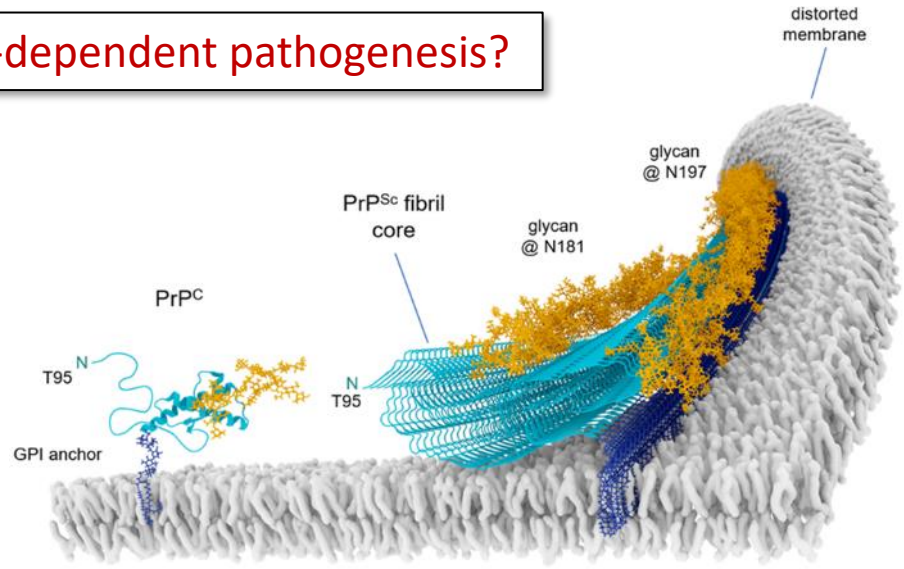

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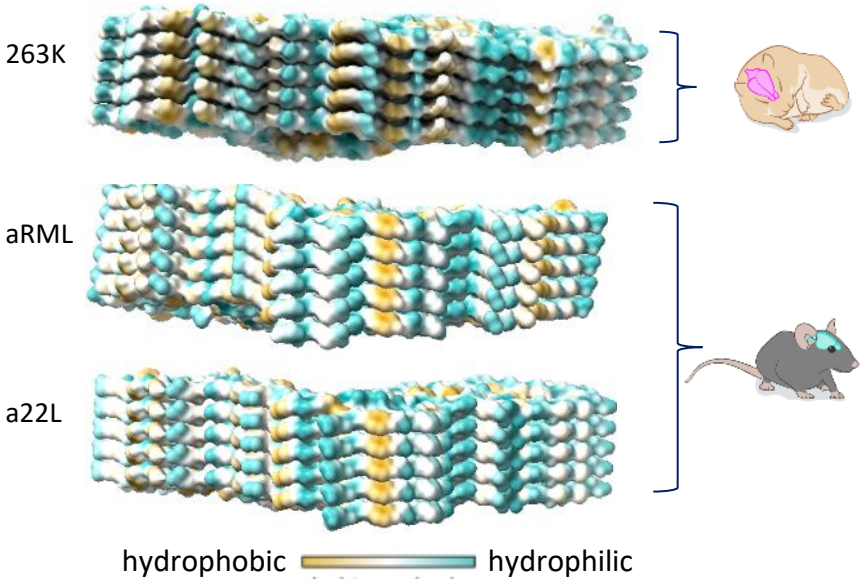

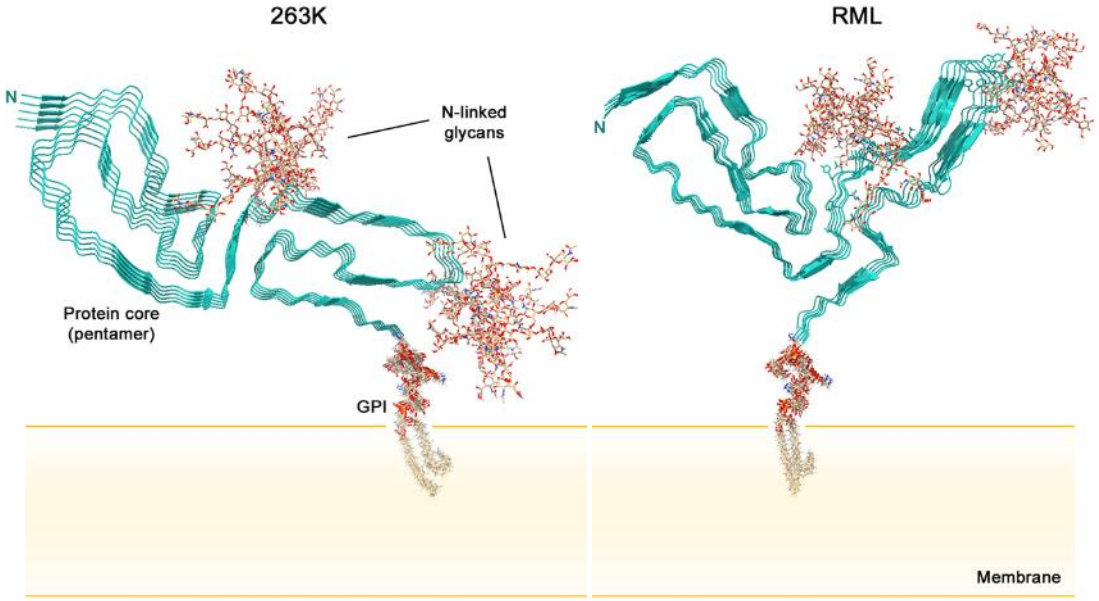

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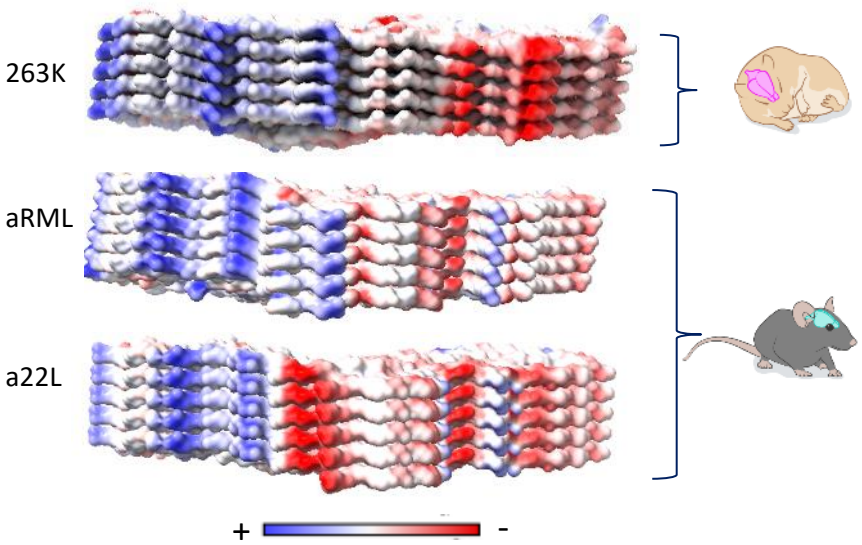

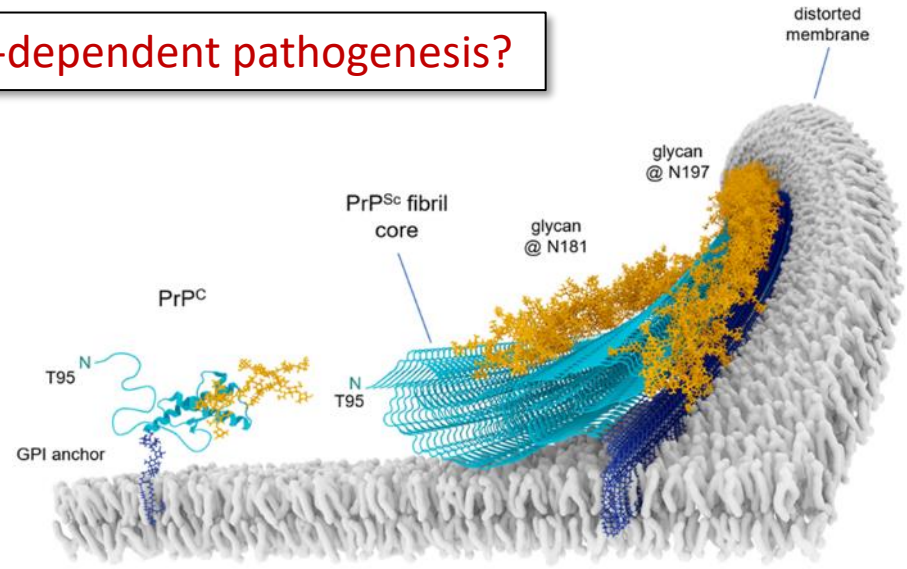

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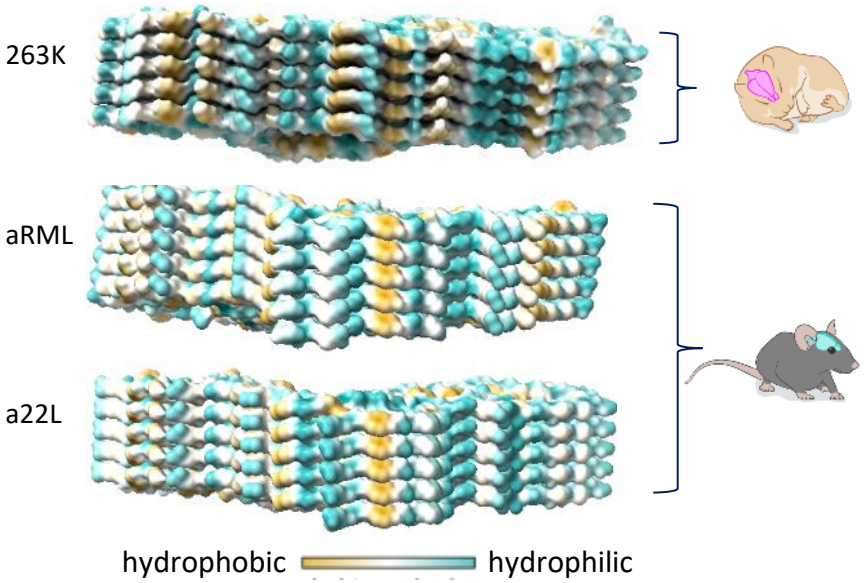
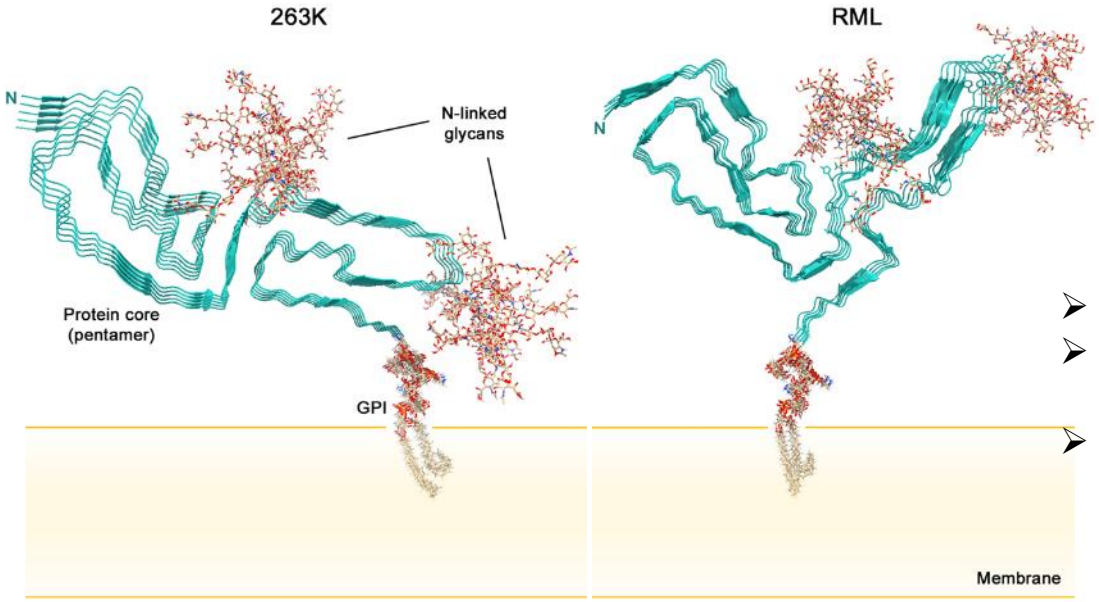




Distinct templates & lateral surfaces

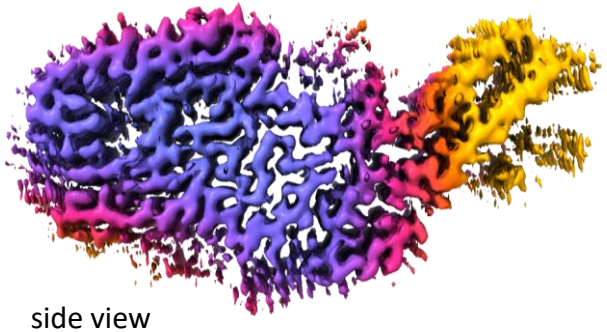
- Differential interactions with:
  - Cofactors?
  - Membrane components?
  - Extracellular matrices?
  - Innate immune & proteostatic factors?

  e.g. Hasebe et al., ***Virol*** 2012

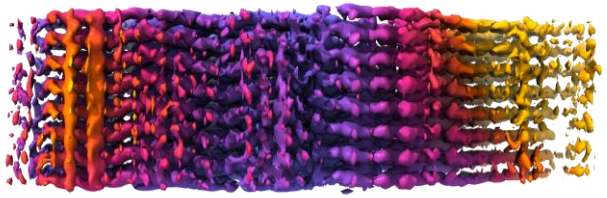
- Cell/tissue/region dependence
- **Strain-dependent patterns** of deposition & pathology in brain
- **Distinct clinical phenotypes**

Structure of a **natural** prion: **CWD** fibrils from deer (Alam, Hoyt, Artikis, et al., *Acta Neuropath* 2024)

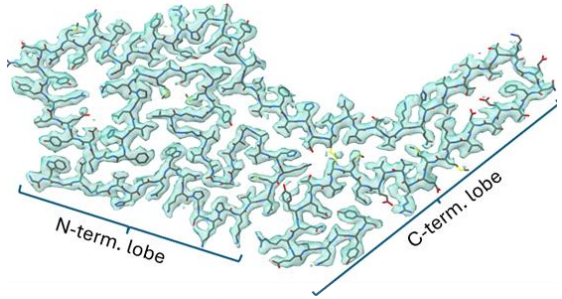
top view

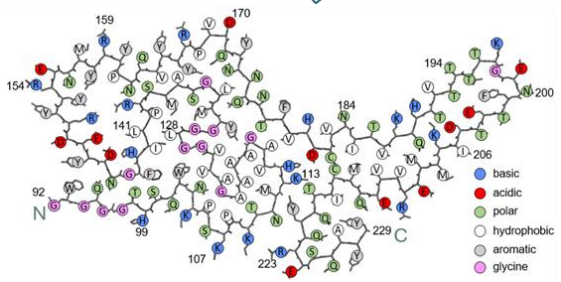


side view



Atomic model embedded in EM density map





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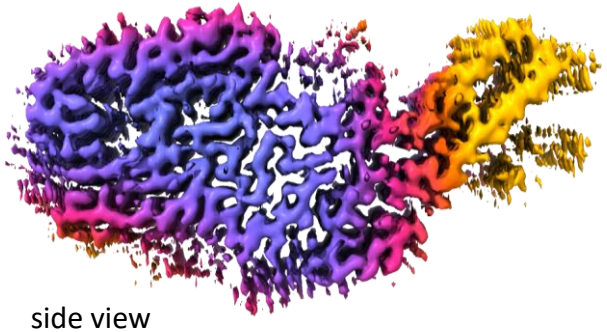
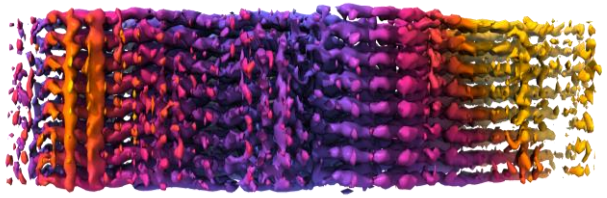


side view



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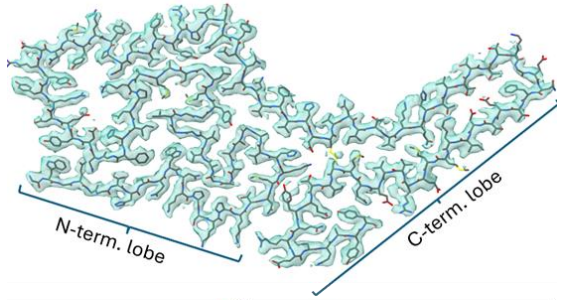

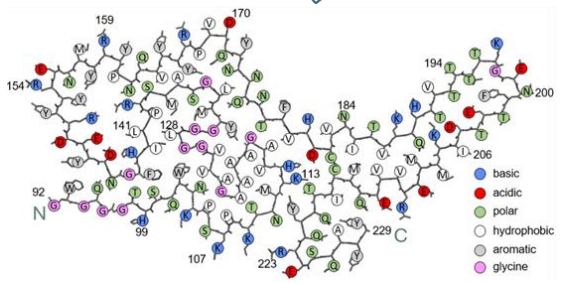

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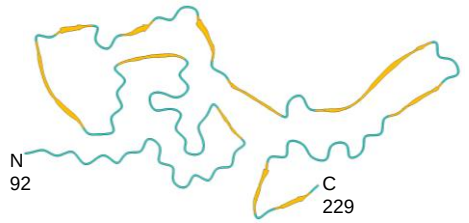
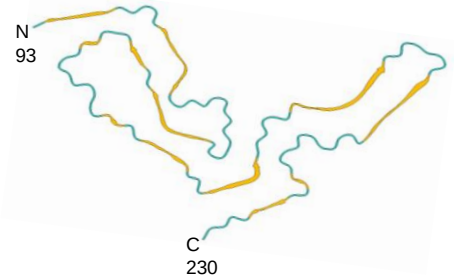


aRML (mouse)



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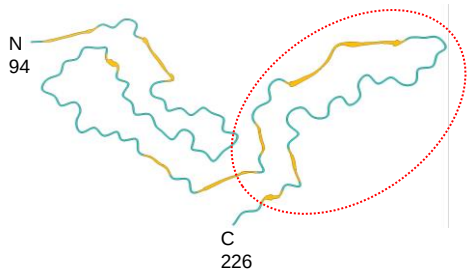
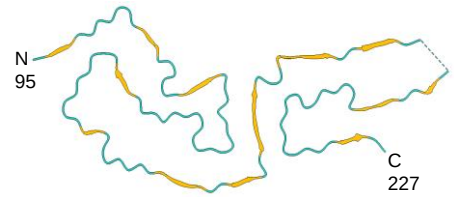


263K (hamster)



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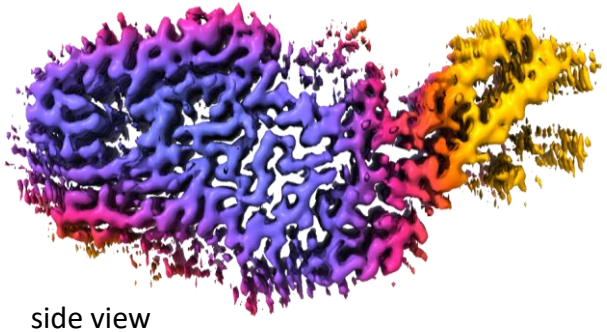

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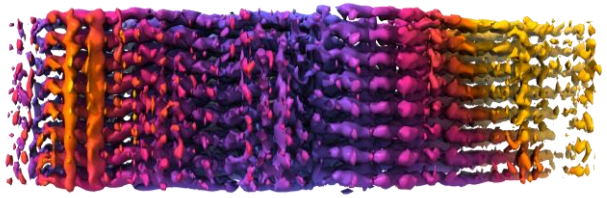

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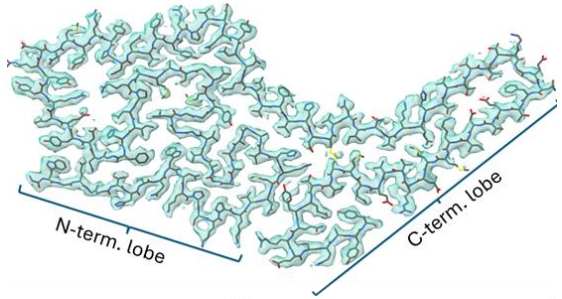

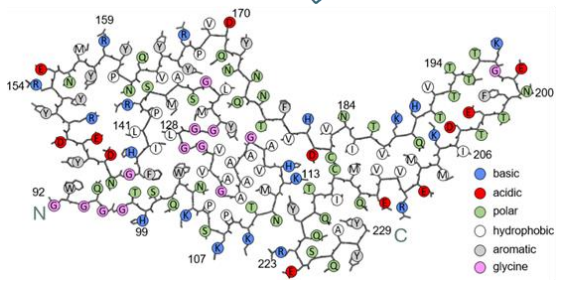

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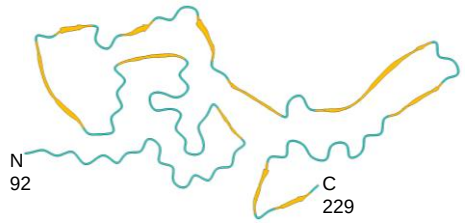

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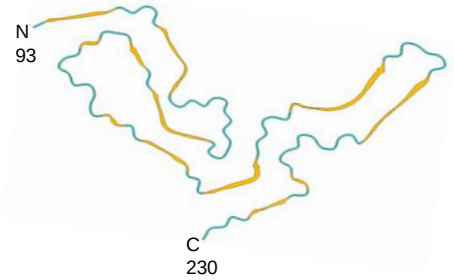

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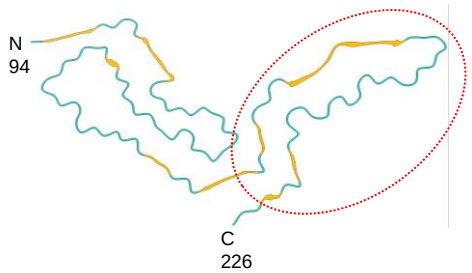

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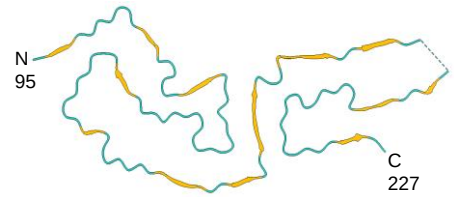

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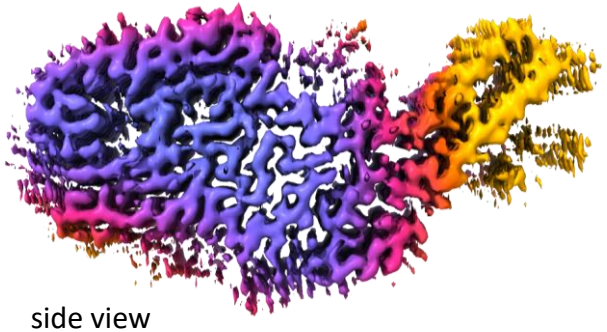

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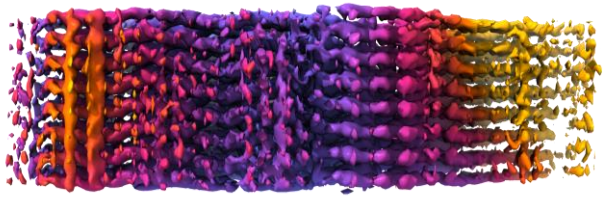

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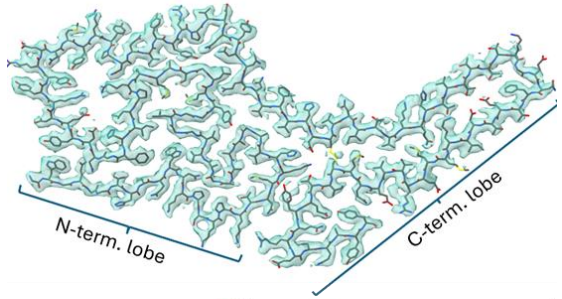

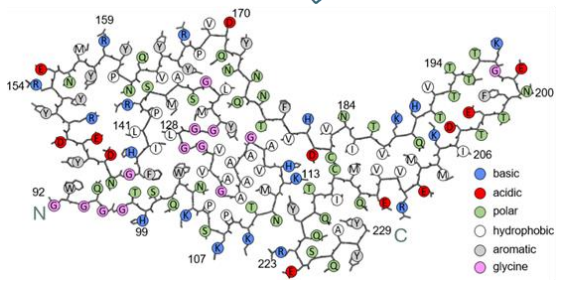

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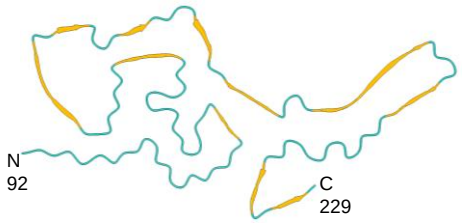

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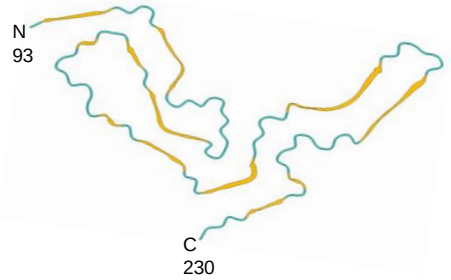

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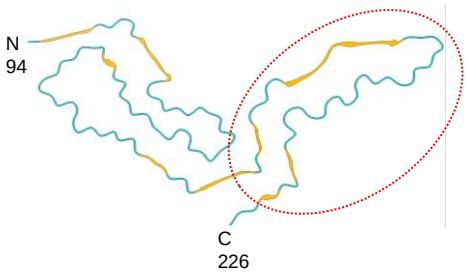

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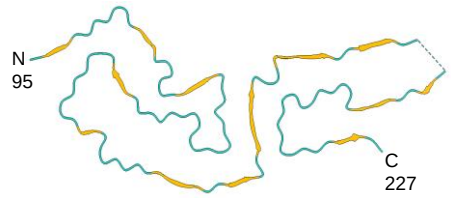

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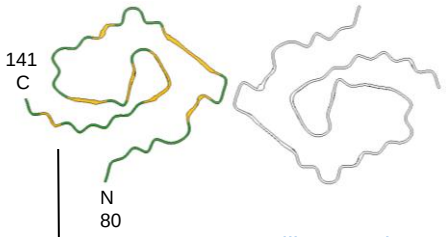

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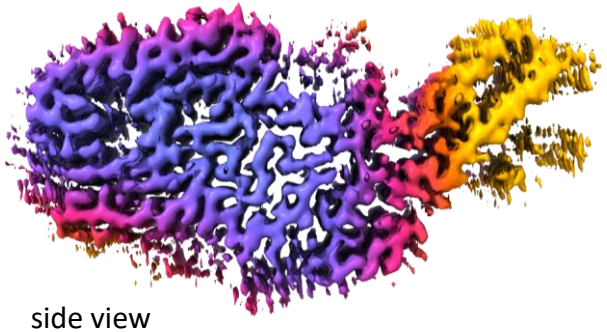

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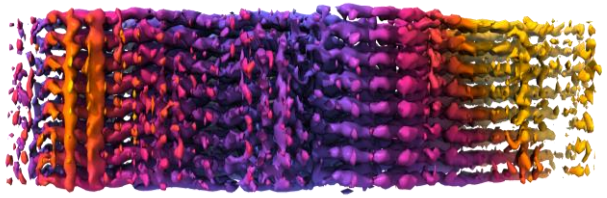

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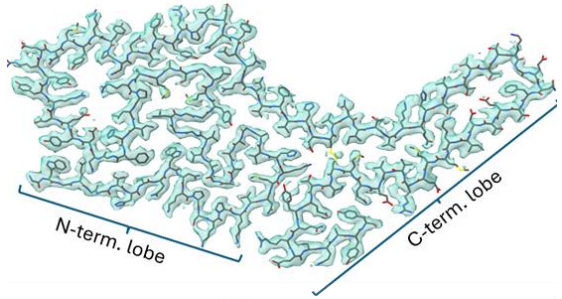

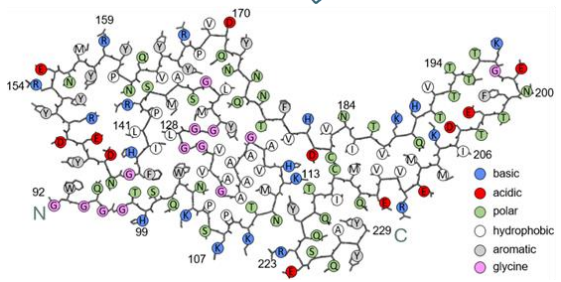

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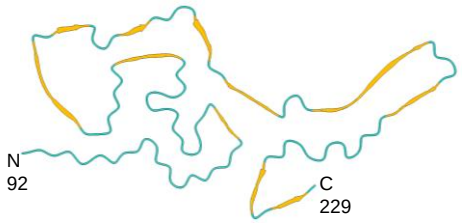

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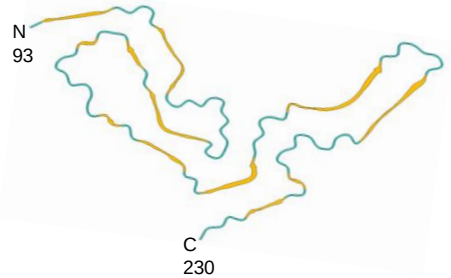

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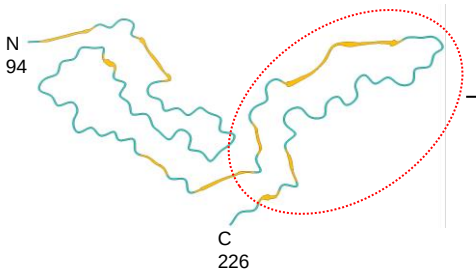

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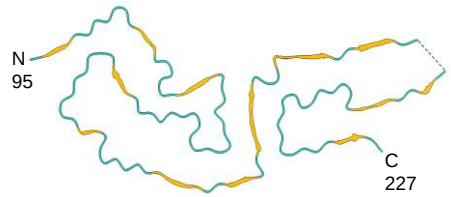

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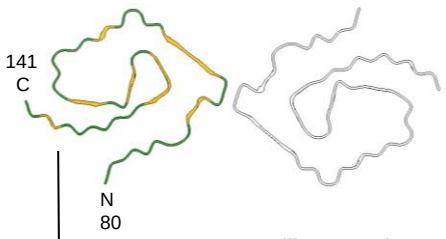

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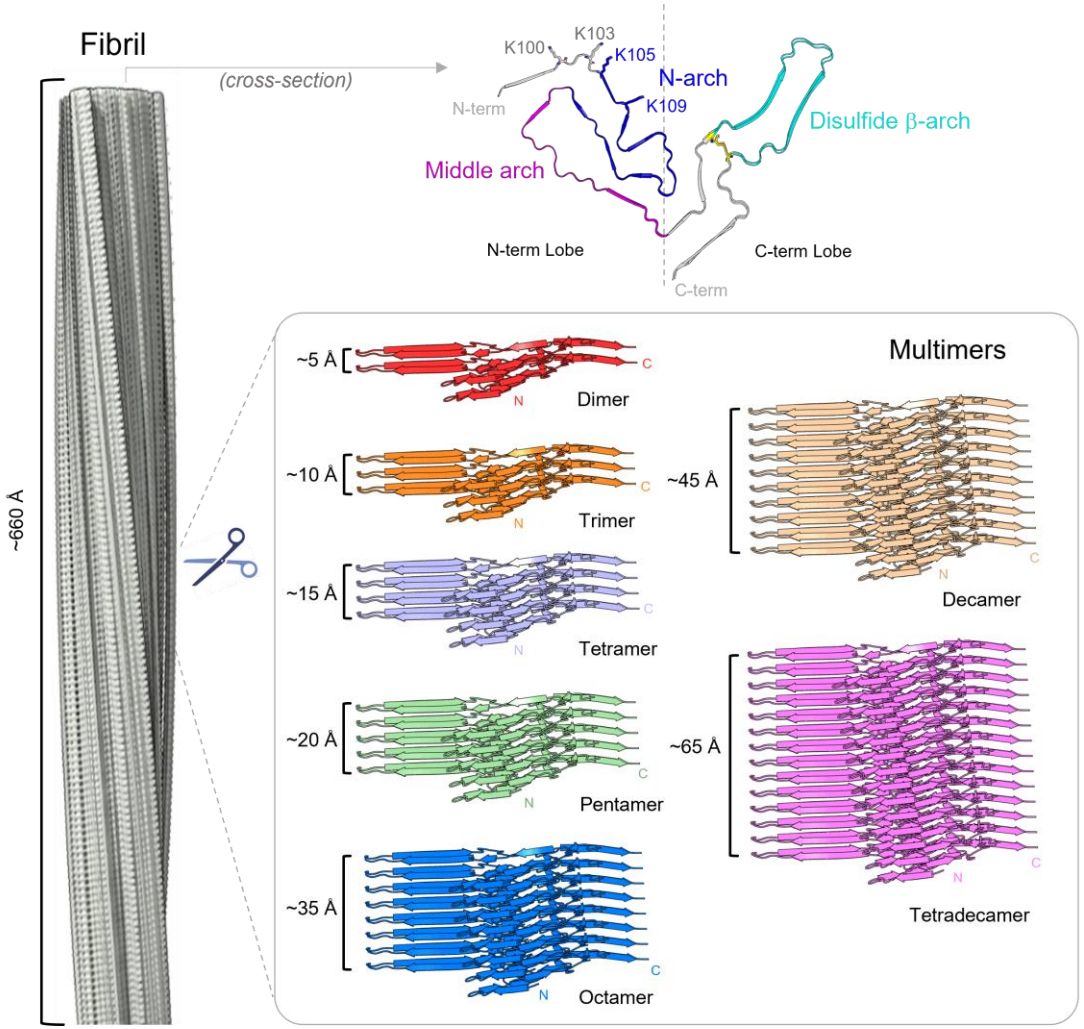
- Synthetic PrP amyloid fibrils with *only* C-lobe cores are **barely** or **non-infectious**

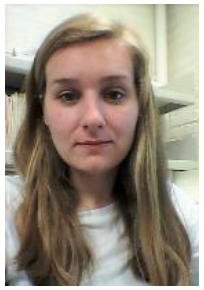
- But, **small conformational differences** can make huge differences in infectivity

Wang et al, PLoS Path 2017

➡ Implications in assessing potential infectivity of other types of amyloids

Minimal stable size of the infectious prion core: Molecular dynamics simulations of *dimeric* (n=2) to *tetradecameric* (n=14) fragments





Efrosini Artikis

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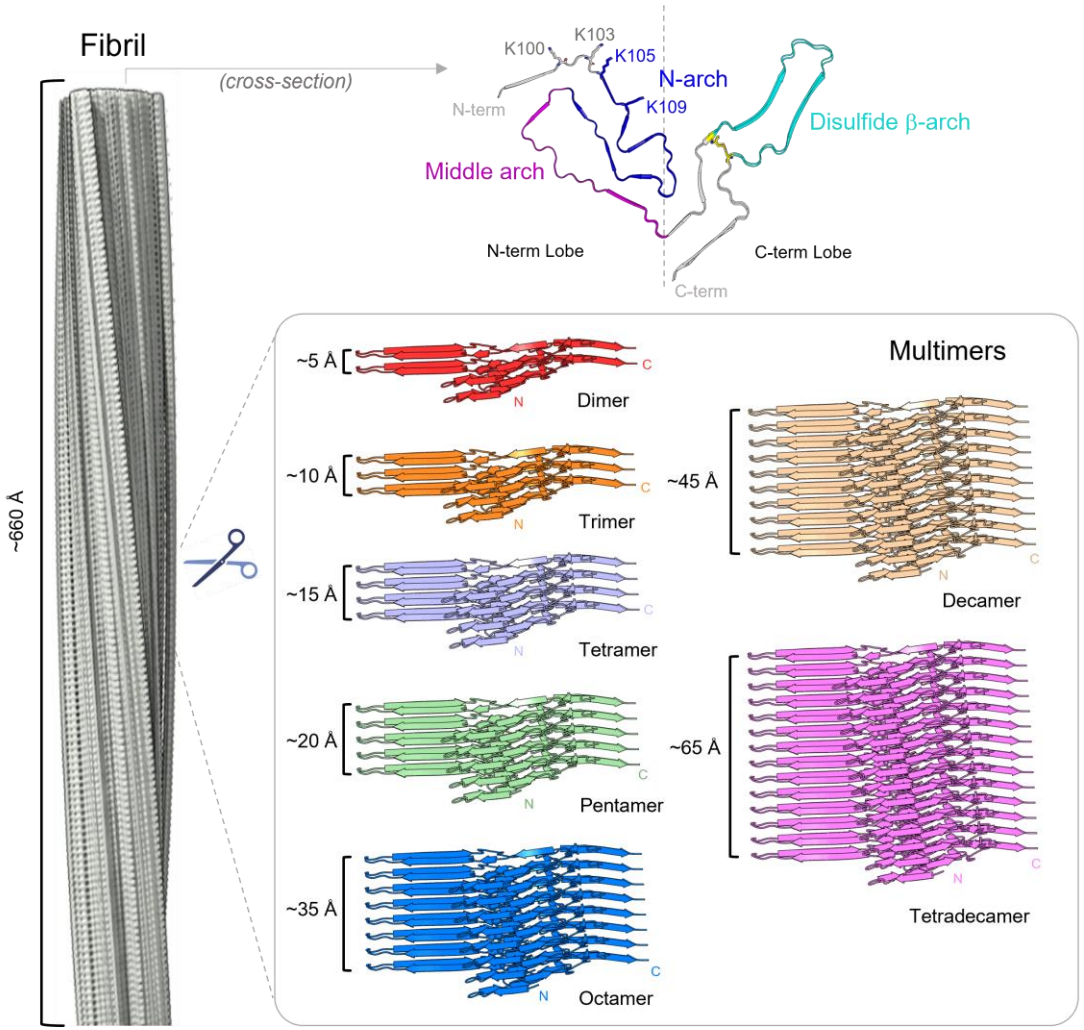
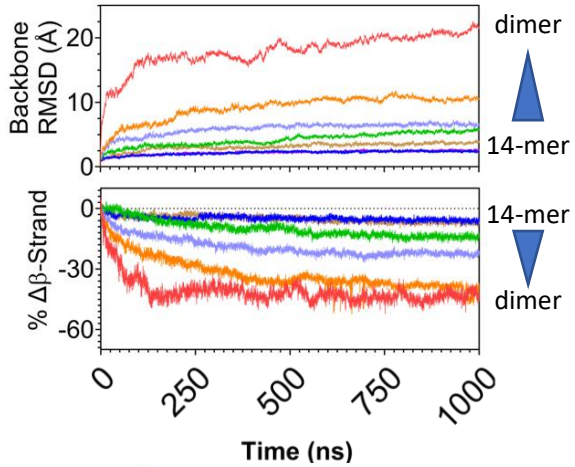
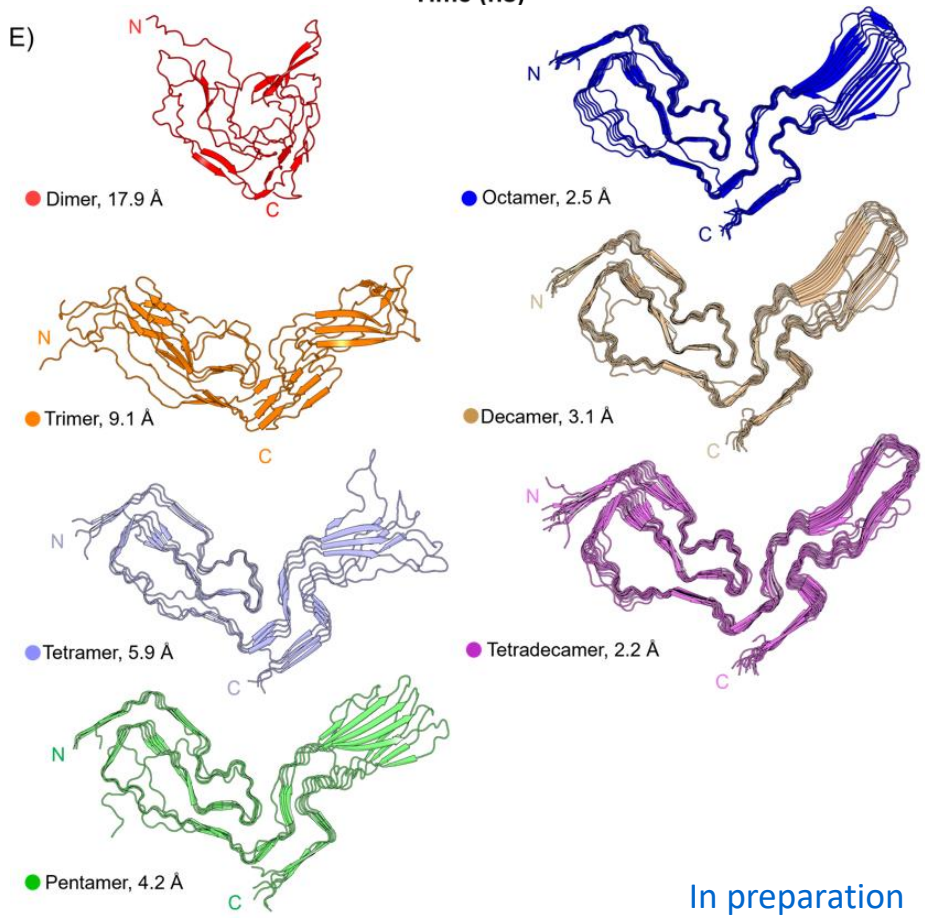


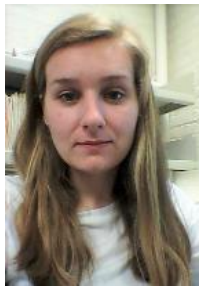
MD

~1 µsec





In preparation



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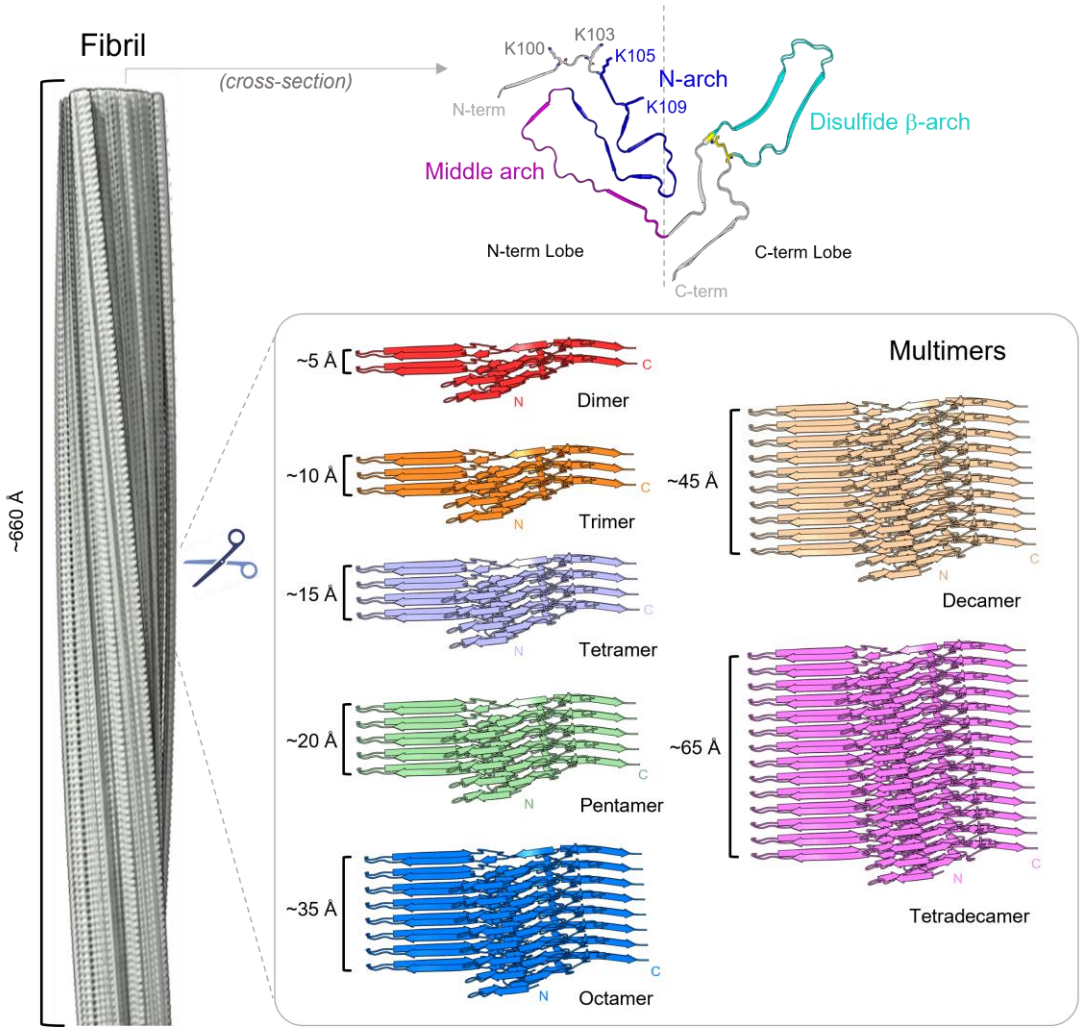

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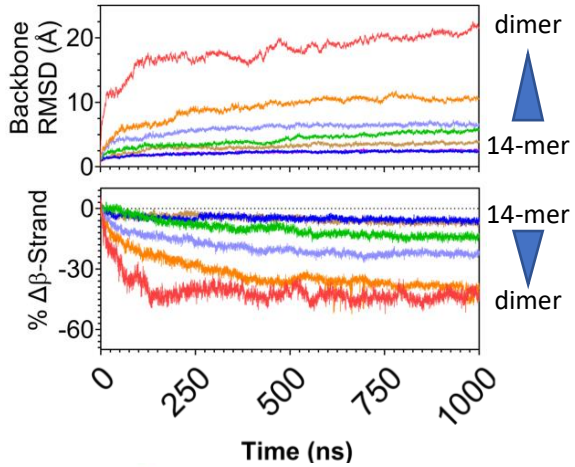
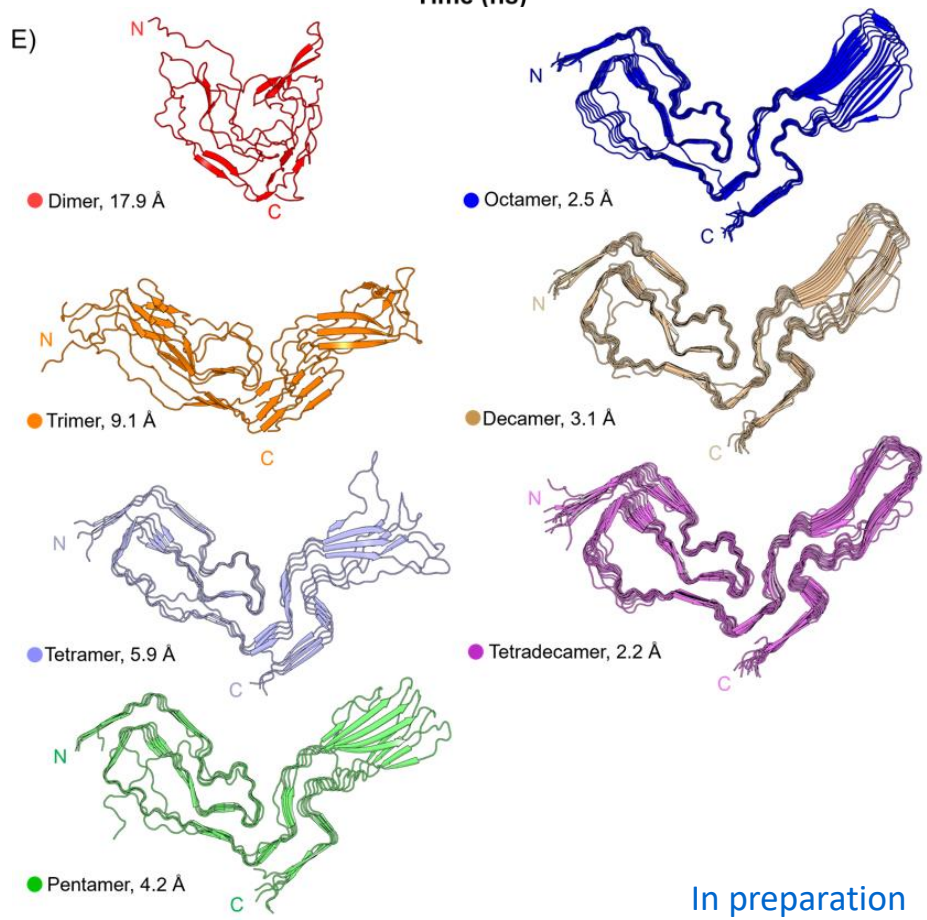
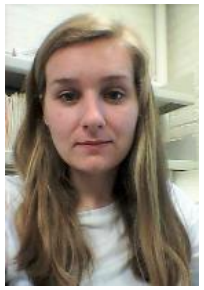




- Cross-β core oligomers appear **stable as small as a tetramer**
  (and do not have to look or behave like fibrils!)
- **No signs of fragmentation** of these oligomers (or 25-mers) within this timeframe
  (even at temperatures up to 127°C)



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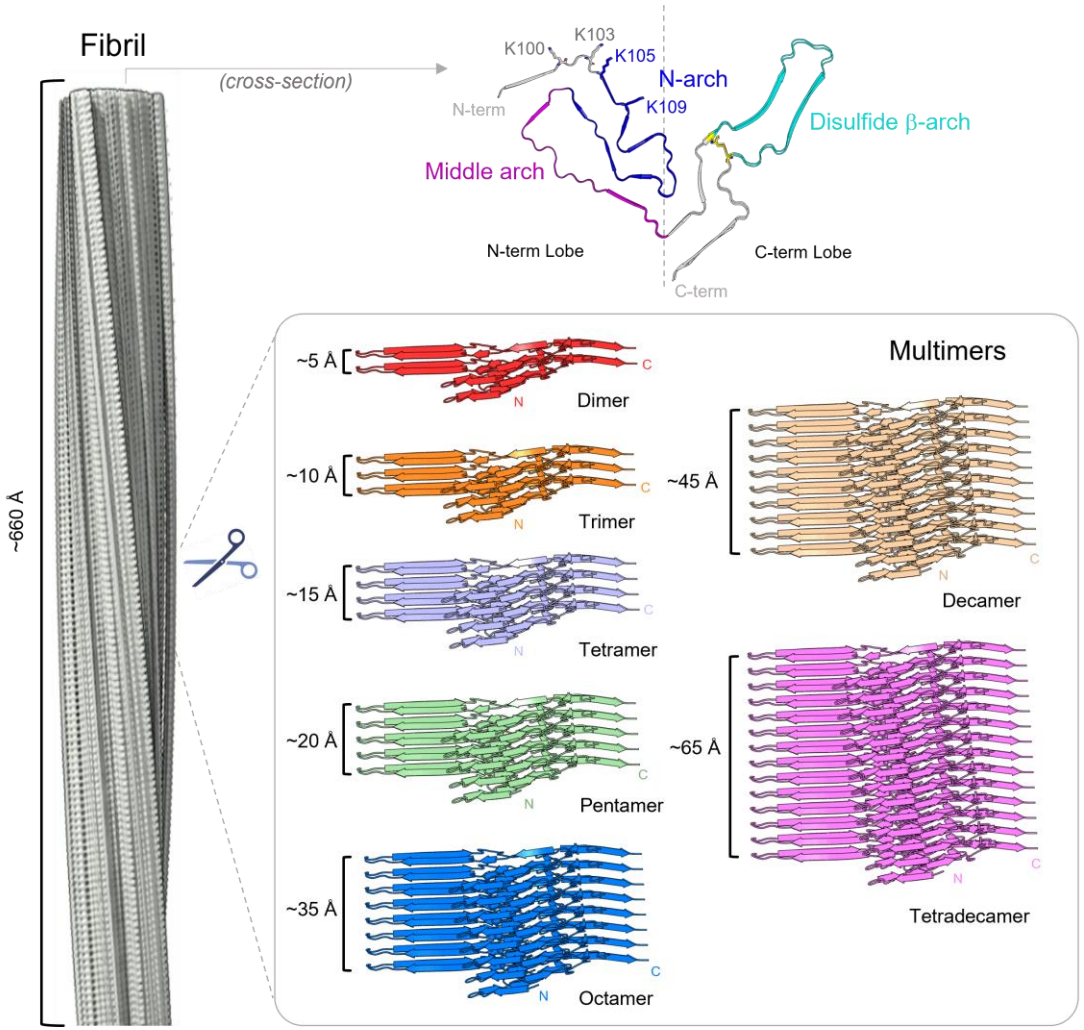

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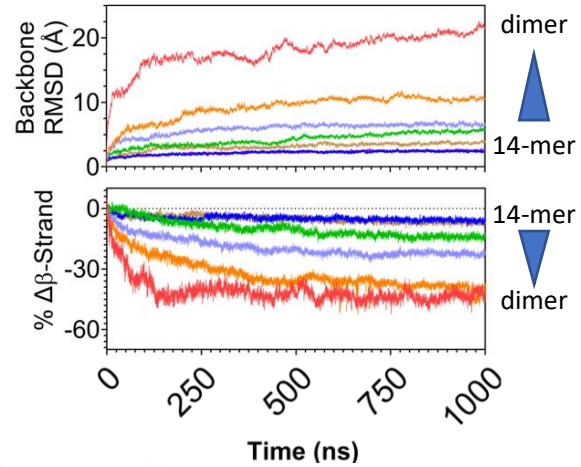

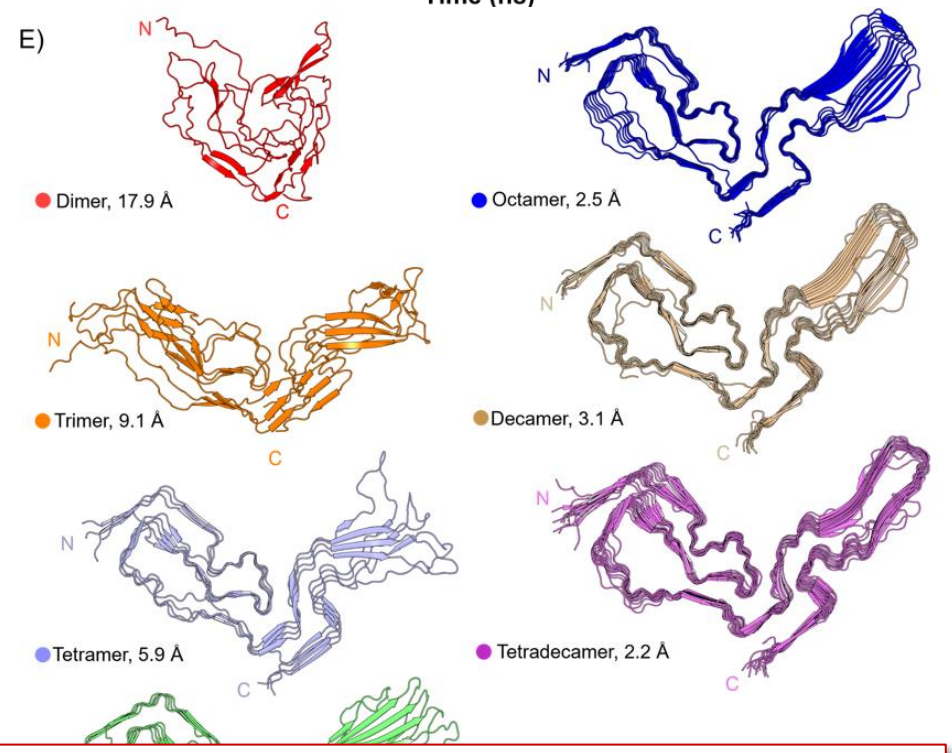

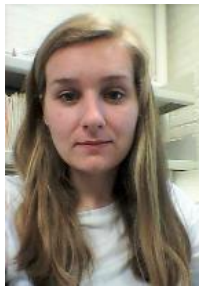

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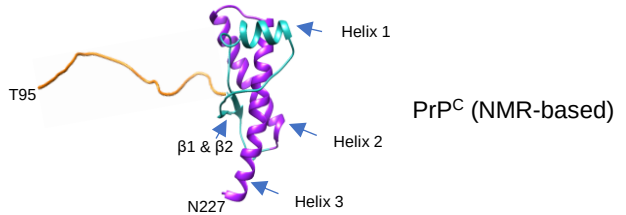
Also, biochemically: no sign of disassembly of PrP^{Sc} aggregates into small oligomers under non-inactivating conditions (in our hands)

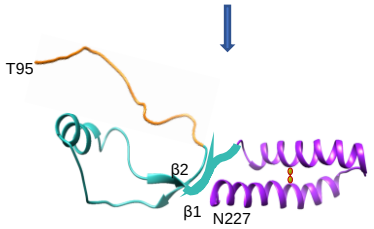
➢ Prion fragmentation **in vivo** may require physiological assistance

Summary

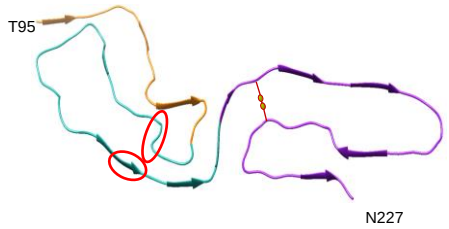
- **High-resolution structures of brain-derived PrP fibrils:**
  - **Experimentally rodent-adapted scrapie** (263K, RML, aRML, 22L, ME7)
  - **Natural CWD** from naturally infected white-tailed deer
  - Human **GSS F198S**
- **PIRIBS-based architectures**
  - ➢ All brain-derived or synthetic PrP **fibrils** with unambiguously determined architectures to date are PIRIBS-based (n>13)
- **Complete refolding of PrP^C is required.**
- **Glycans and GPI anchors** of wildtype prions project outwards but have little effect on core structure (RML)
- **Distinct templating surfaces** on ends of fibrils **encipher strains**
- **Transmission barrier mechanisms**?
  - 263K hamster → mice (Kraus et al., *Mol Cell* 2021)
  - CWD → humans (Alam et al., *Acta Neuropath* 2024)
- **Non-infectious PrP fibrils** have smaller ordered cores
- **aRML core structure** is mostly stable when as small as a **tetramer** (*in silico*)
  - ➢ PIRIBS prions do not have to look like fibrils!
- We've seen **no evidence efficient spontaneous disassembly** of PrPSc aggregates into small oligomers
- Many more prion structures (fibrillar & non-fibrillar?) remain to be determined…



PrP^C (NMR-based)



Intermediate?



PrP^{Sc} monomeric unit (cryo-EM)

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Sam Treece

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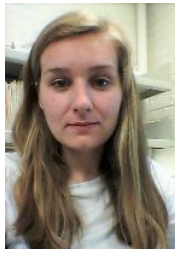
Sabiha Parveen


Samantha King


Efrosini Artikis


Parvez Alam


Jakub Soukup

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(contact me if interested)

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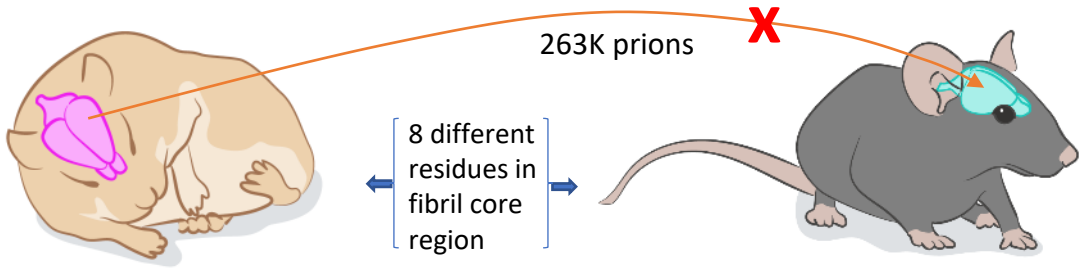
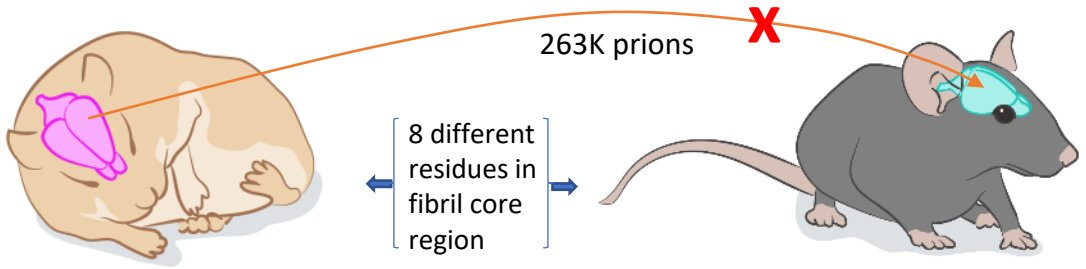
- NIH Biowulf supercomputing cluster

Transmission barrier mechanism?



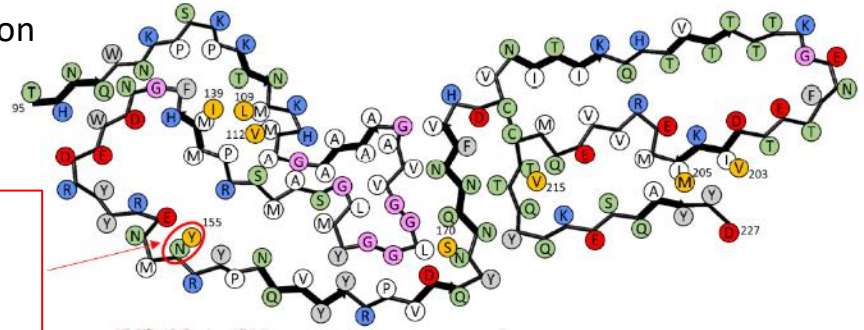
Efrosini Artikis in
Kraus et al., ***Mol Cell***, 2021

Transmission barrier mechanism?



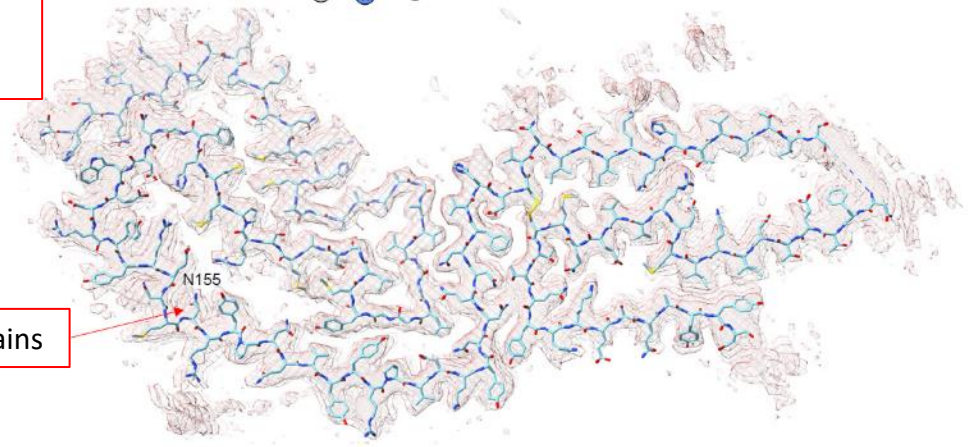
Mouse residues superimposed on hamster sequence

Hamster 263K prion cross-section



Mouse residue (Y) inhibits 263K-seeded conversion

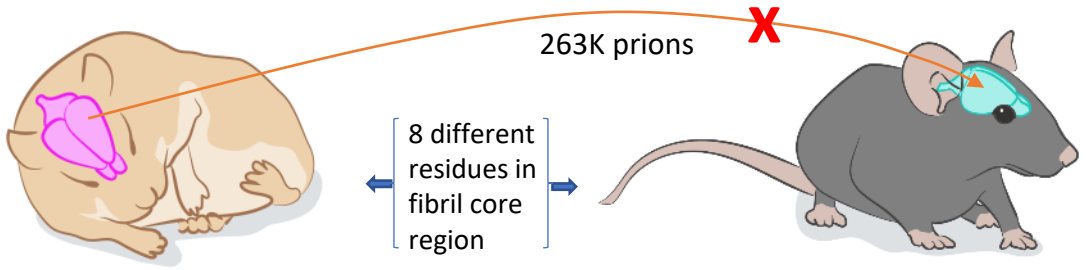
(Scott et al., *Cell* 1993; Priola et al., *J Virol* 2001)



Tightly packed sidechains

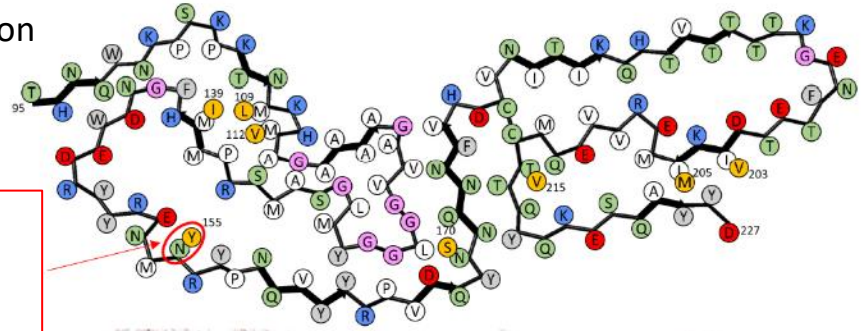
Efrosini Artikis in
Kraus et al., ***Mol Cell***, 2021

Transmission barrier mechanism?



Mouse residues superimposed on hamster sequence

Hamster 263K prion cross-sections



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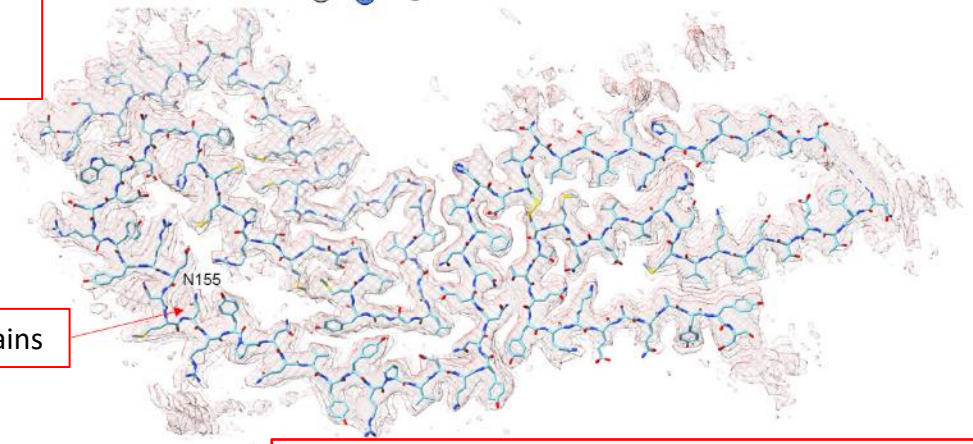
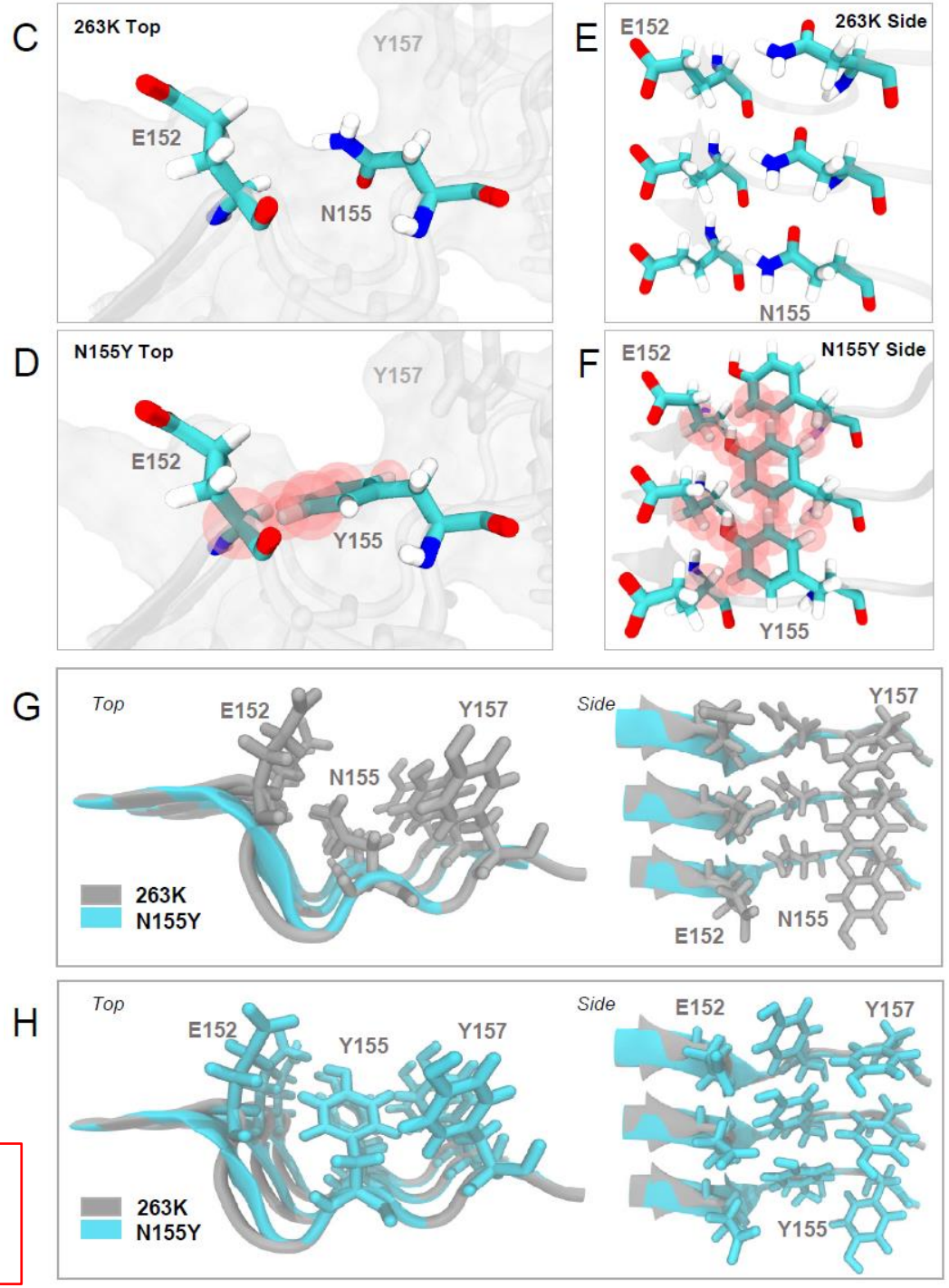


Tightly packed sidechains

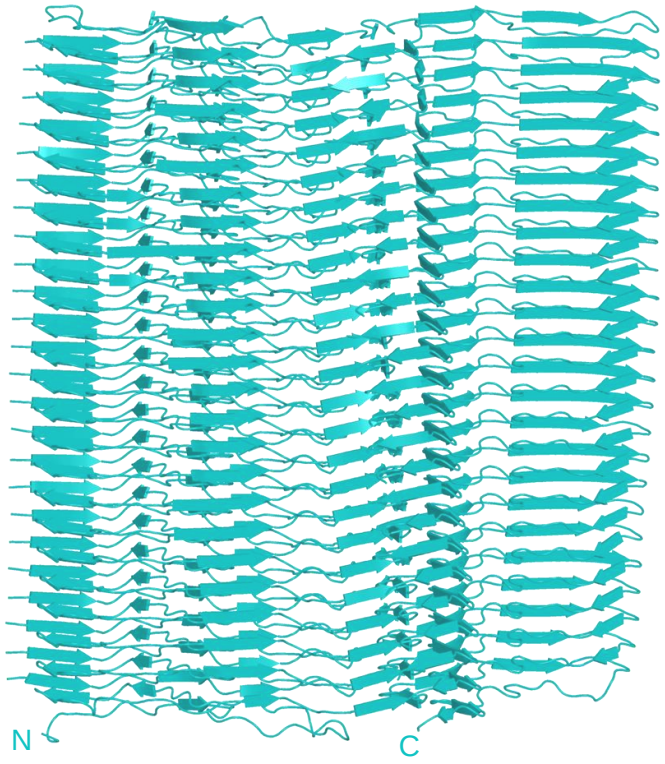
Steric clashes and required changes in H-bonding and backbone conformation likely slow 263K-templated conversion of mouse PrP^C

Efrosini Artikis in Kraus et al., ***Mol Cell***, 2021



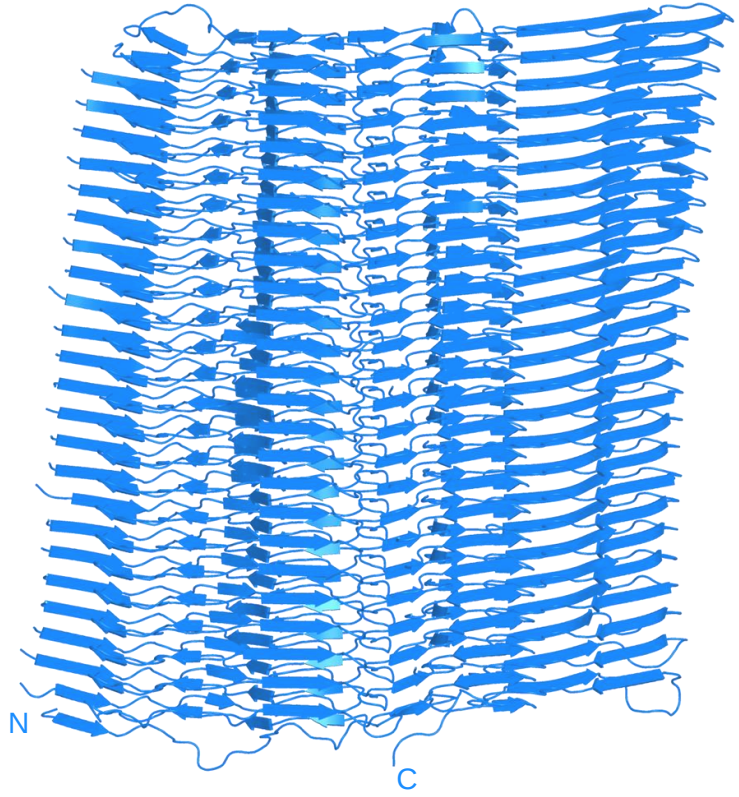
Temperature 300K

a22L PrP^{Sc}

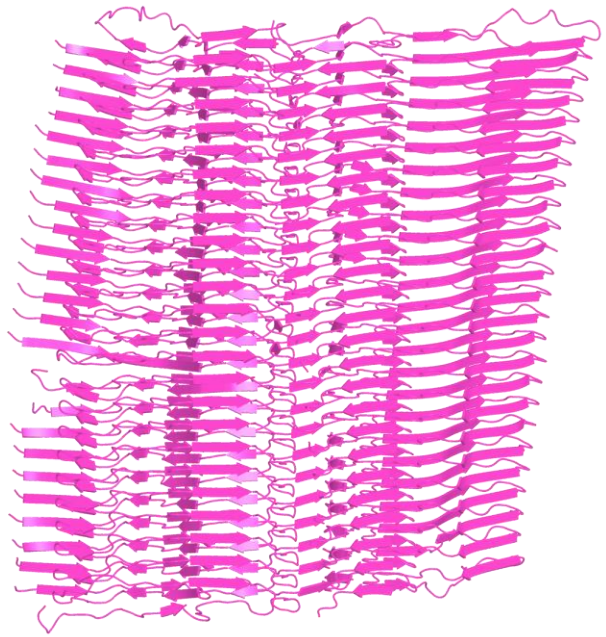


263K PrP^{Sc}

aRML PrP^{Sc}



Last frame of 500ns at 300K – cubic water box, 150mM NaCl



aRML at 400K showing a transient kink, but runs need more sampling time