

High-resolution Structural Studies of Infectious Mammalian prions

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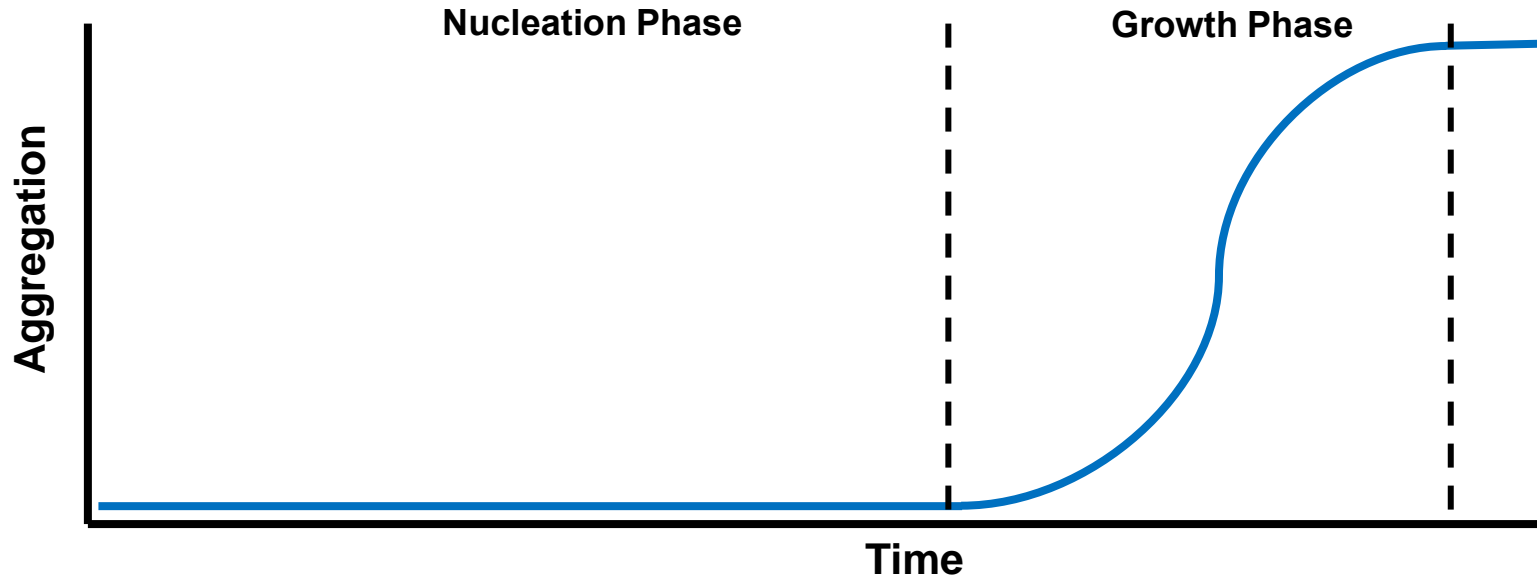
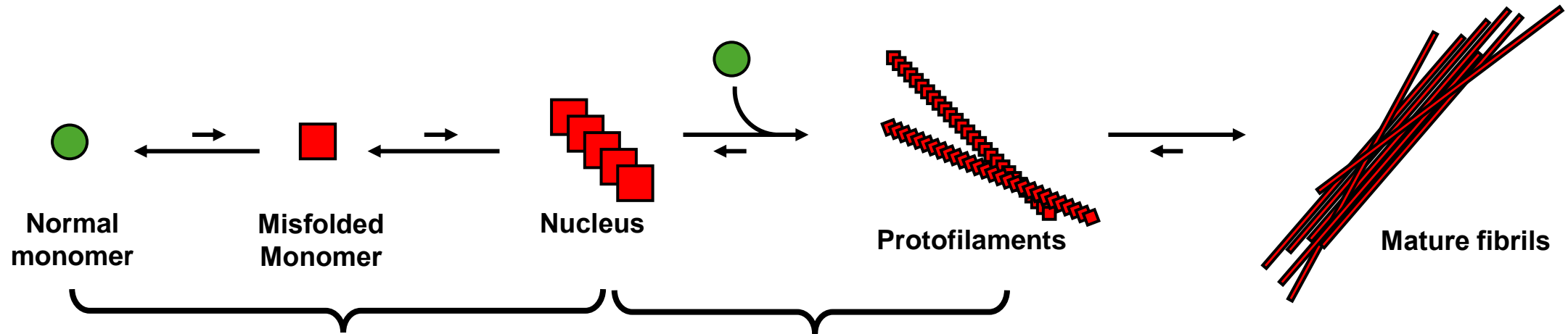
What is Prion?

- *The word prion, coined in 1982 by Stanley B. Prusiner, is derived from protein and infection, hence prion, and is short for “proteinaceous infectious particle”*
- A protein with neurotoxicity + self-propagation
- Prion rods, prion filaments, prion amyloid, etc
- Prion aggregates, prion particles, PK-sensitive prions, prion aggregates

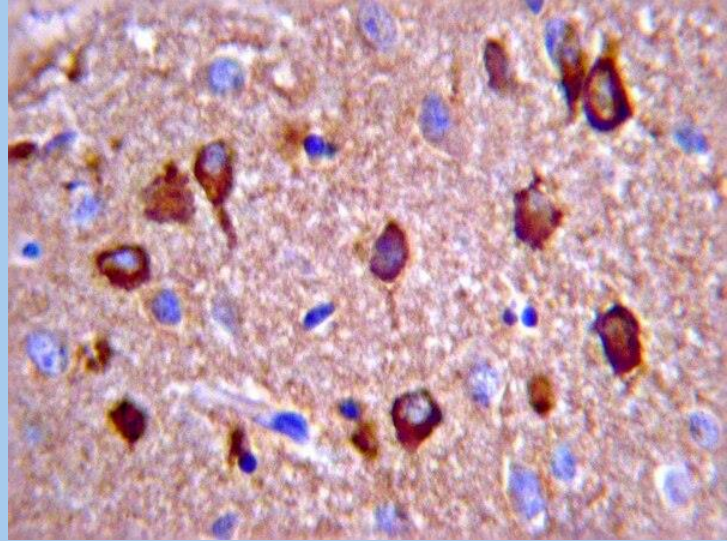
“It is very easy to answer many of these fundamental biological questions; you just look at the thing!”

--Richard Feynman

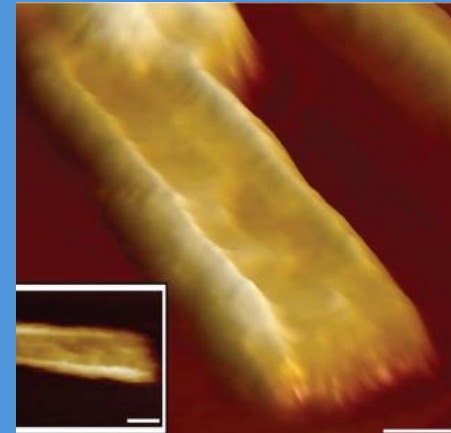
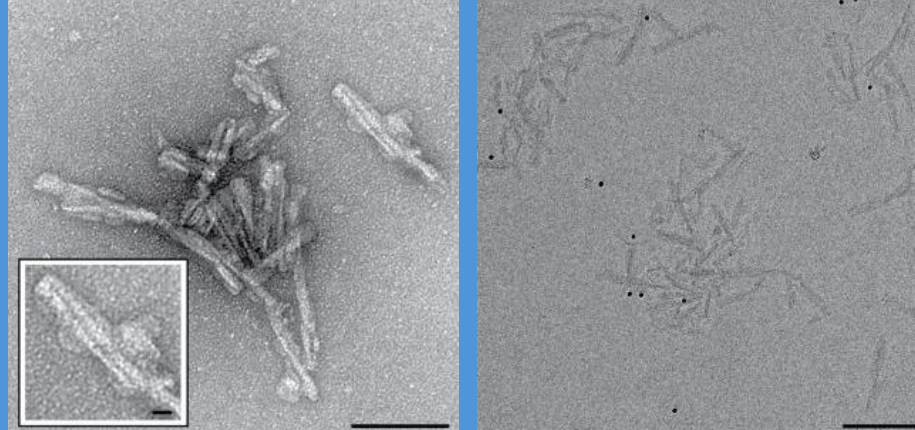
Template-based Conformation Conversion Model



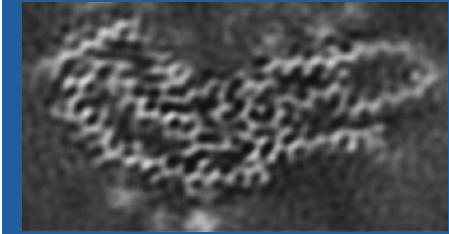
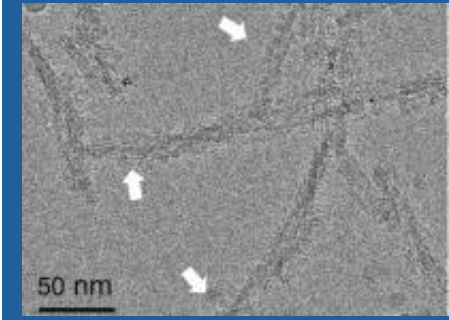
How to Look at Prions?



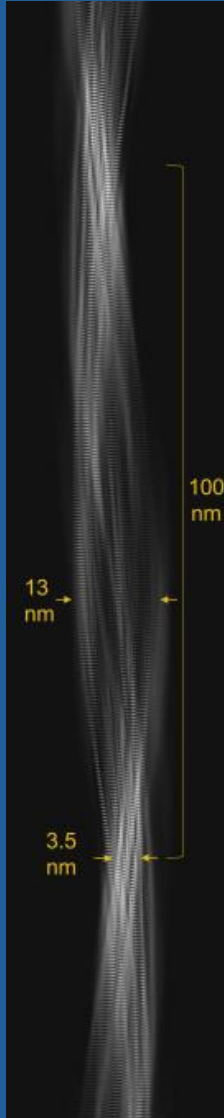
- Antibody stain
- Light microscopy



- Purification
- (optional) Stain
- Electron scanning
- Mechanical tapping



- Purification
- Imaging
- Averaging
- Reconstruction



“.....you just look at the thing!” Why?

Prion diseases:

Structure change of one single protein → neurodegeneration

- **Understanding the disease mechanism**
 - Structure differences between MM1 and MM2 sCJD prions
- **Diagnosis**
 - Structure based PET reagent design
- **Therapeutics**
 - Structure based drug design

Amyloid Structure and Prion Structure at High Resolution

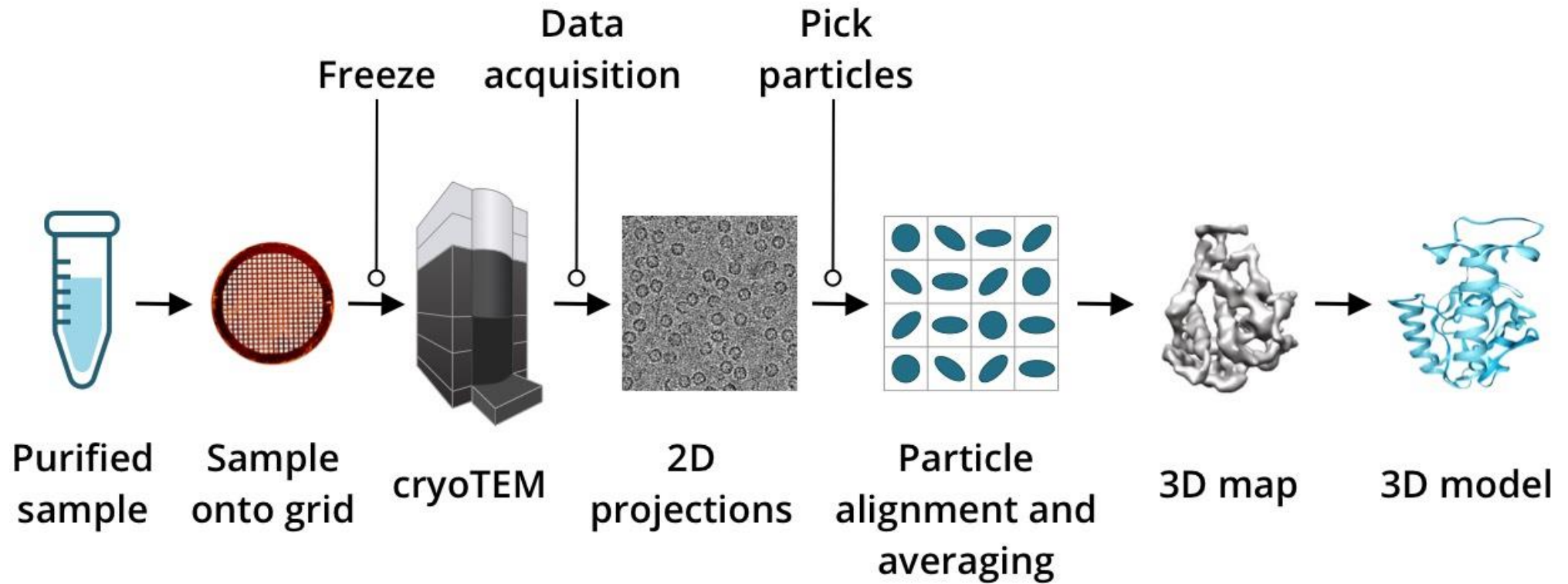
Tau filaments – the most well studied amyloid fibril

- Structures of tau filaments from multiple types of tauopathies, subtypes, individuals, and brain regions
- Identified PET tracer binding sites
- Drug design
- Post-translational modifications

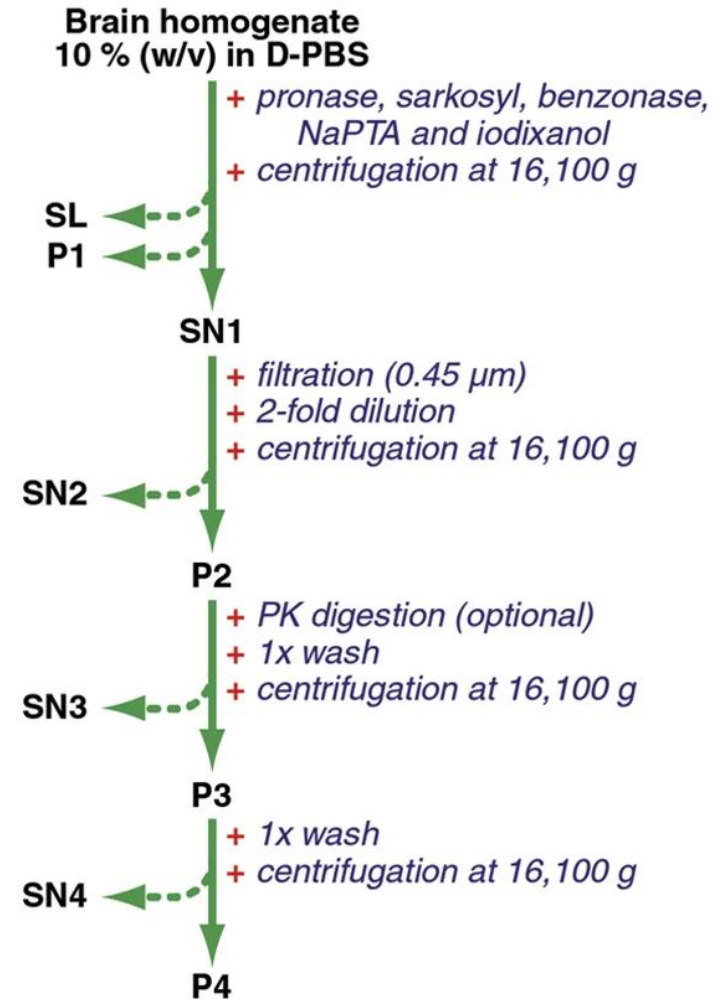
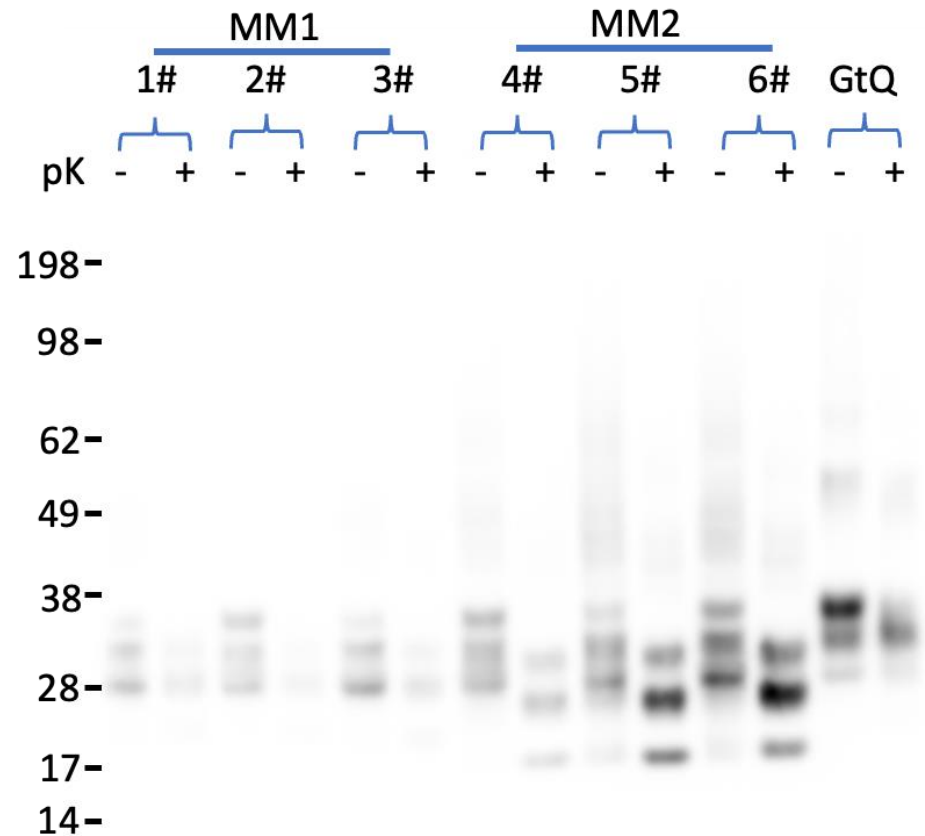
Prions - the only type of amyloid fibrils that are confirmed to cause diseases

- Structures of scrapie derived prions from lab rodents
- Structure of GSS prions

The Method: cryo-EM

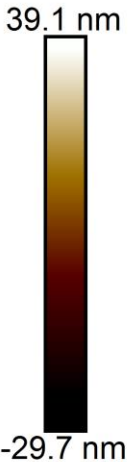
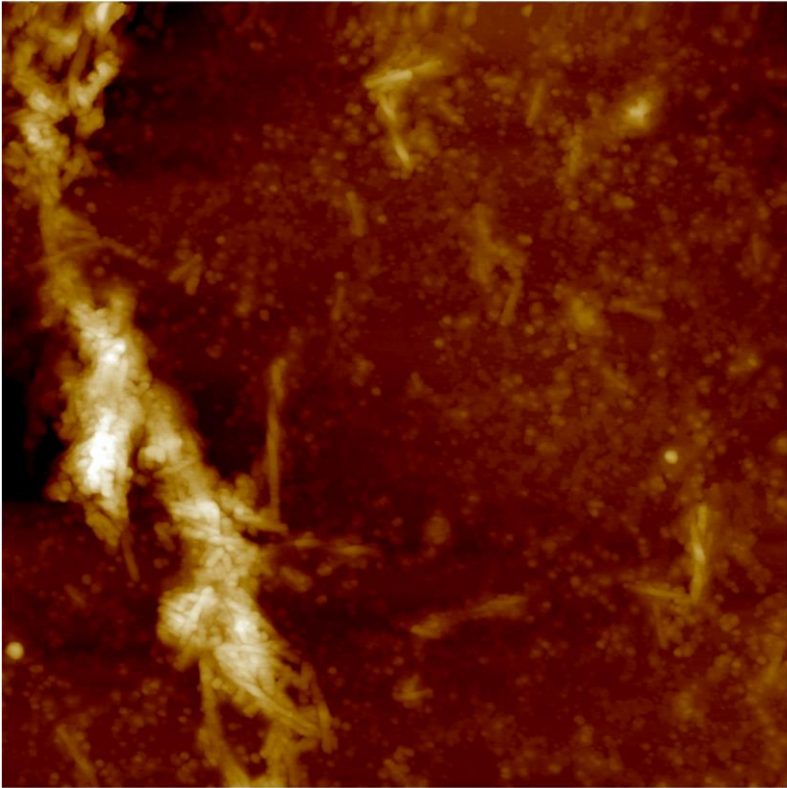


Result: Purification

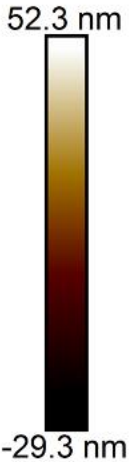
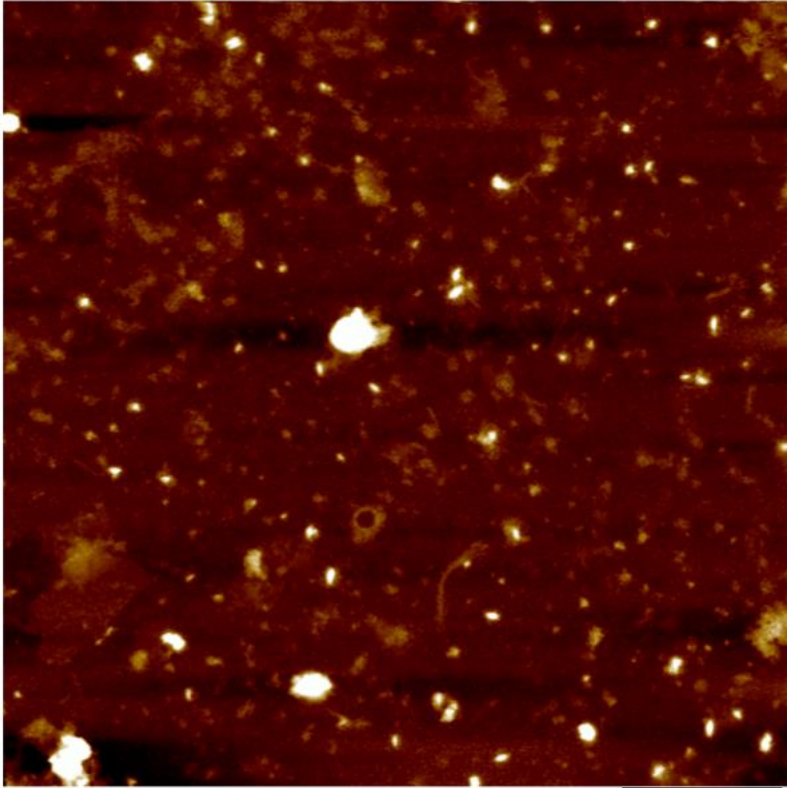


Morphology Characterization by Atomic Force Microscopy

MM1 case 1



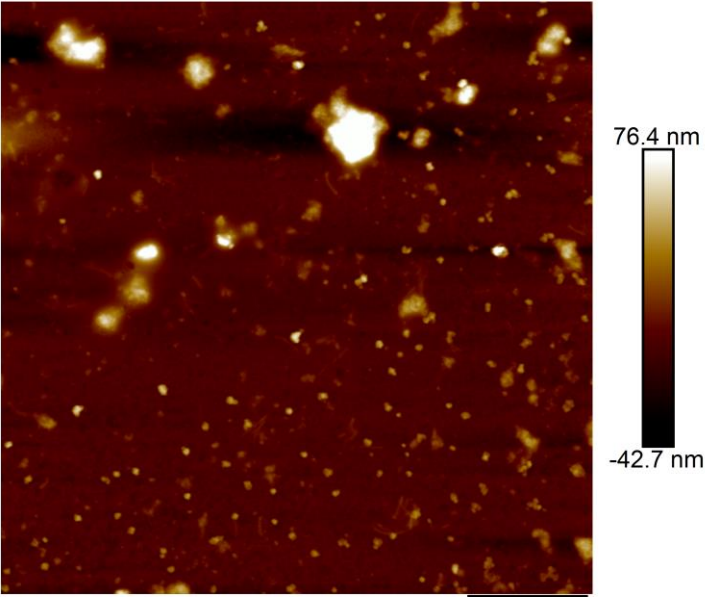
MM1 case 3



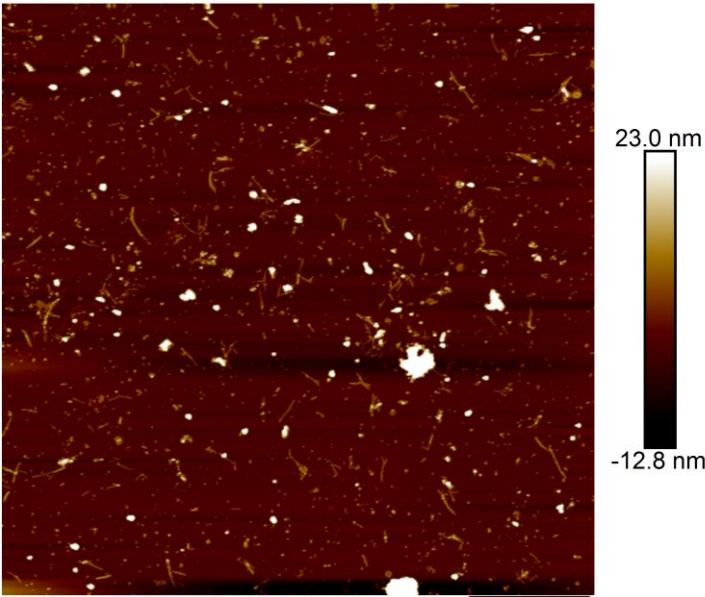
Scale bar = 2 μm

Morphology Characterization by Atomic Force Microscopy

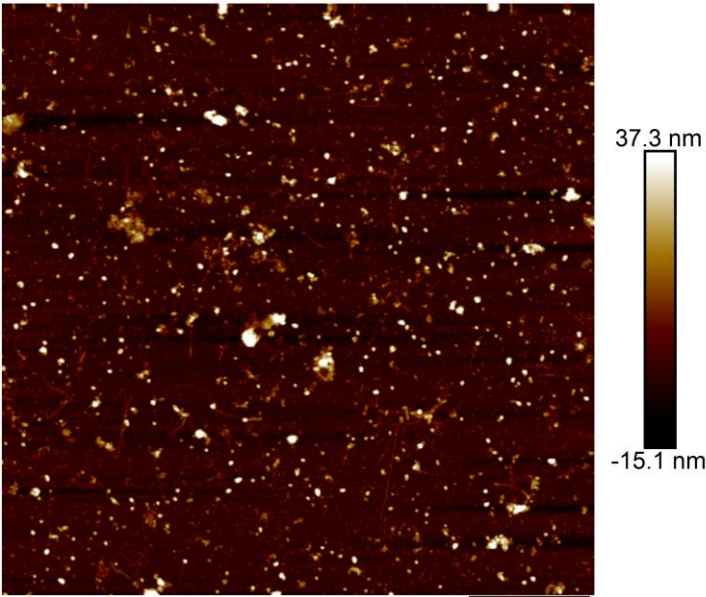
MM2 case 4



MM2 case 5



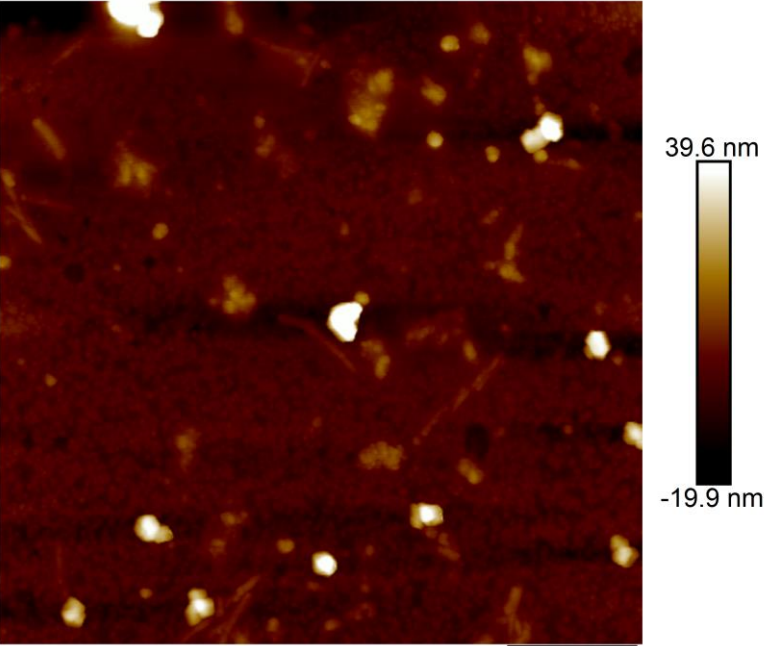
MM2 case 6



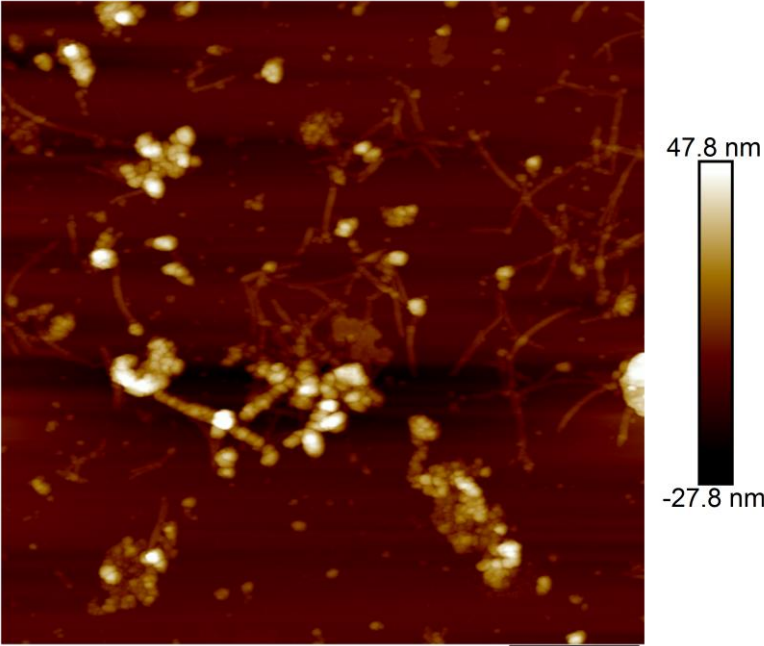
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Morphology Characterization by Atomic Force Microscopy

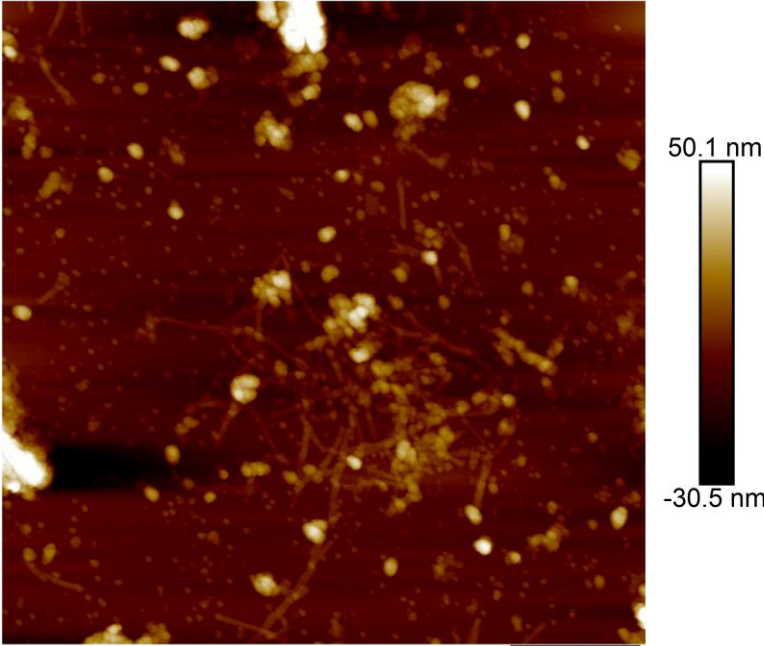
MM2 case 4



MM2 case 5



MM1 case 6



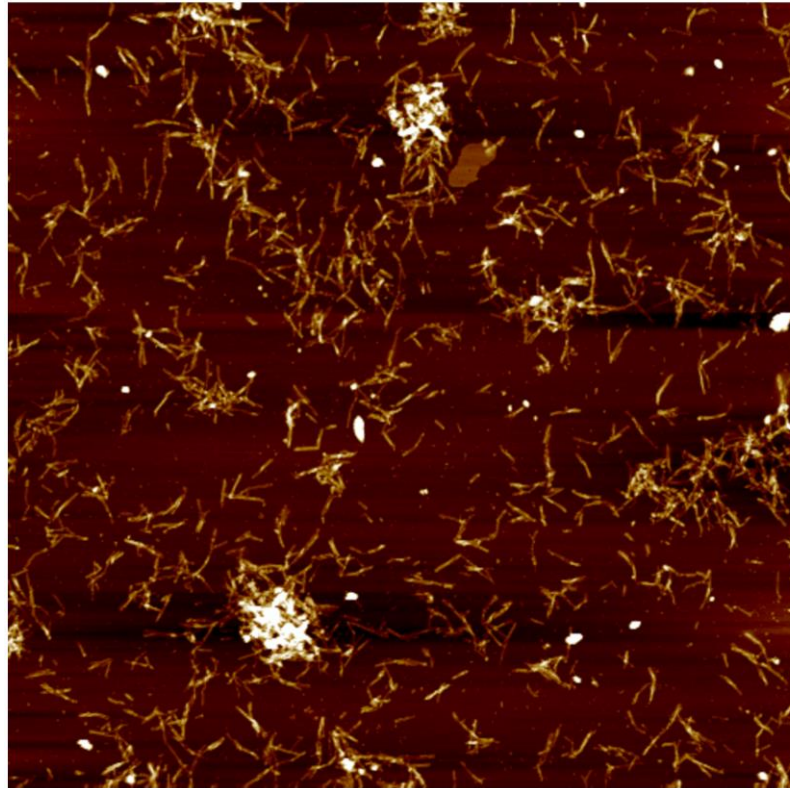
Scale bar = 0.67 μm

Structure determination of CWD Prions

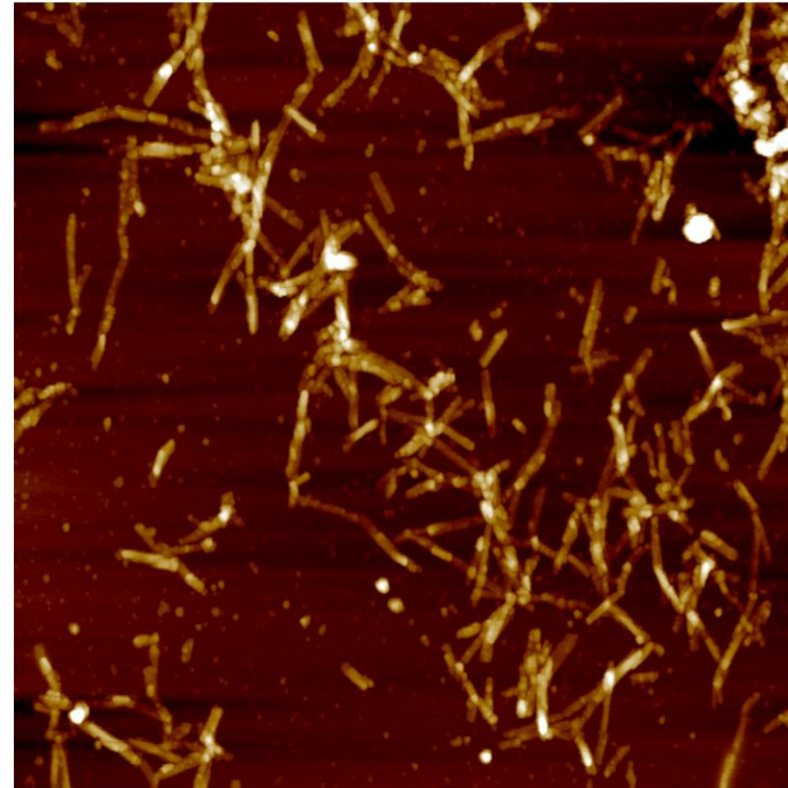
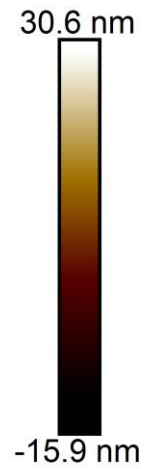
Chronic Wasting Disease (CWD)

- Affect cervids in nature
- Transmissibility to human is tested in model systems
- Controversial epidemic studies
- Direct molecular evidence is missing
- W99 prion from deer
- Transmitted in deerized mice
- Purified for cryo-EM studies

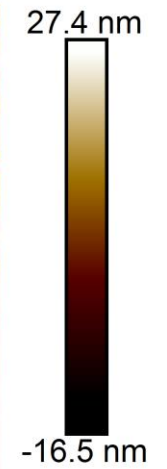
Imaging CWD Prions



2 μm

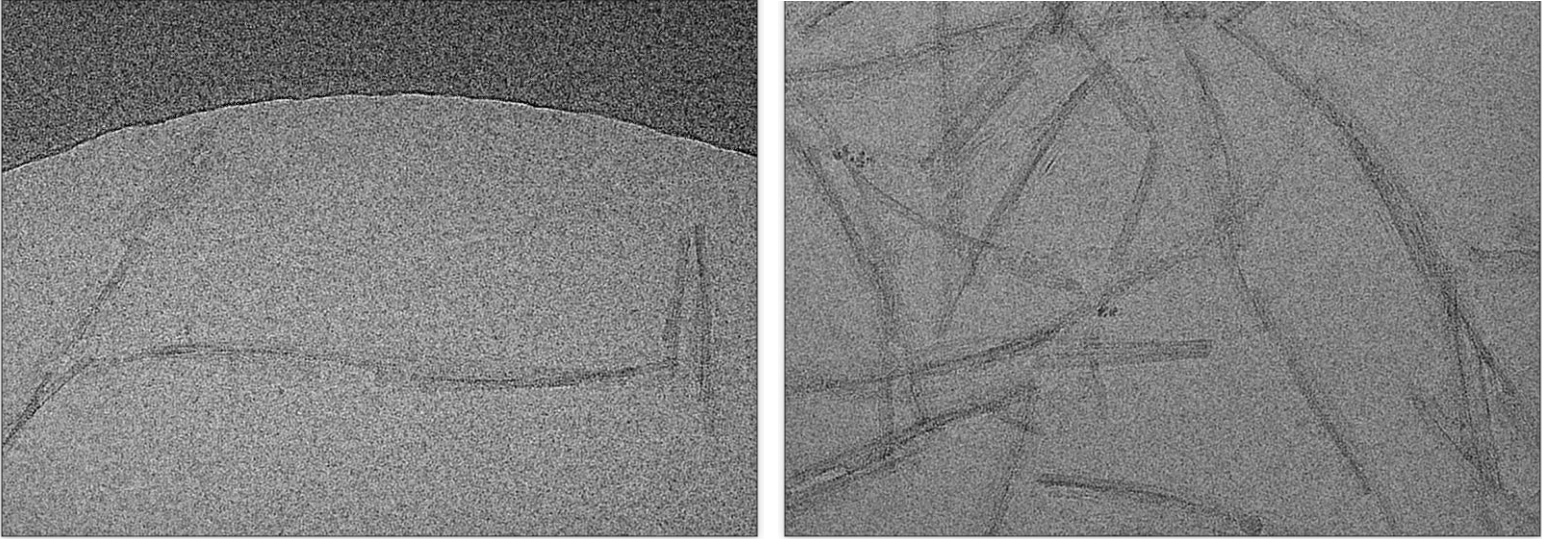


0.67 μm

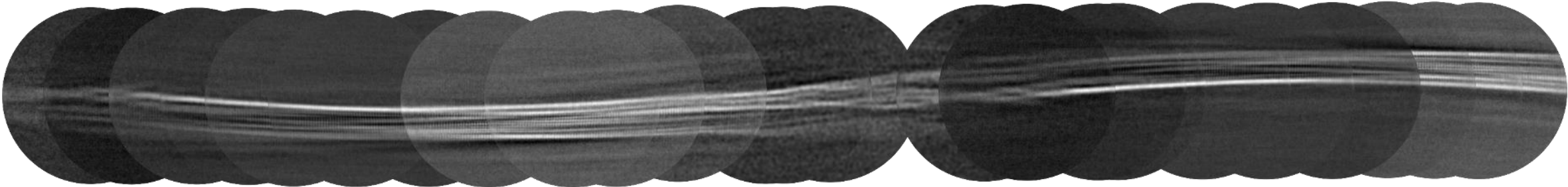


Sample Preparation and 2D Processing

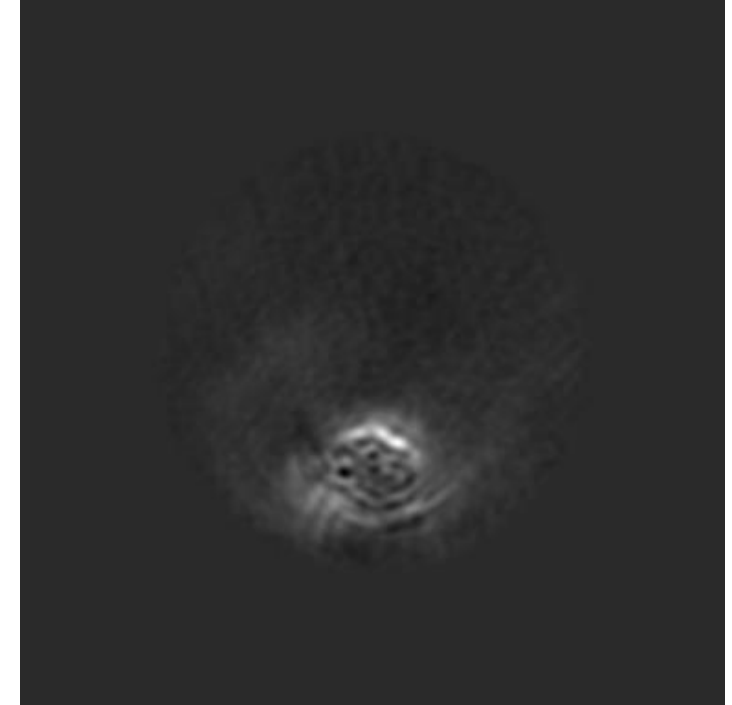
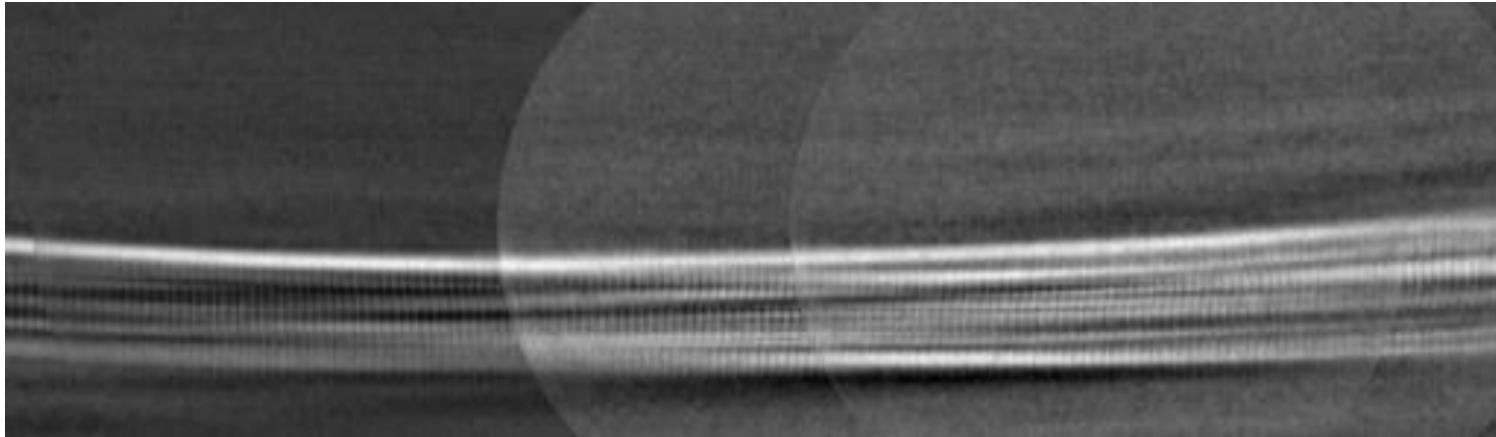
Cryo-EM imaging, filament tracing , particle averaging



10 nm



3D Reconstruction



10 nm

- Parallel in-register β -sheet architecture
- Very 'slow' twist
- Off-centered cross-section

A Solenoid Shaped Filament



GSS prion



RML prion



CWD prion

Summary

- Brains of sCJD patients contain prion protein filaments (prion rods)
- Prion rods originating from different species adopts different molecular structures
- Non-fibrillar PrP aggregates do exist and sometimes account for the majority of purified prions
- The morphology and structure of prion rods may explain the mechanism of neurodegeneration in prion diseases
- Understanding the molecular structure of prion rods will facilitate future development of diagnosis and therapeutics

Acknowledgement

- **Surewicz Lab**

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Thanks! Questions?