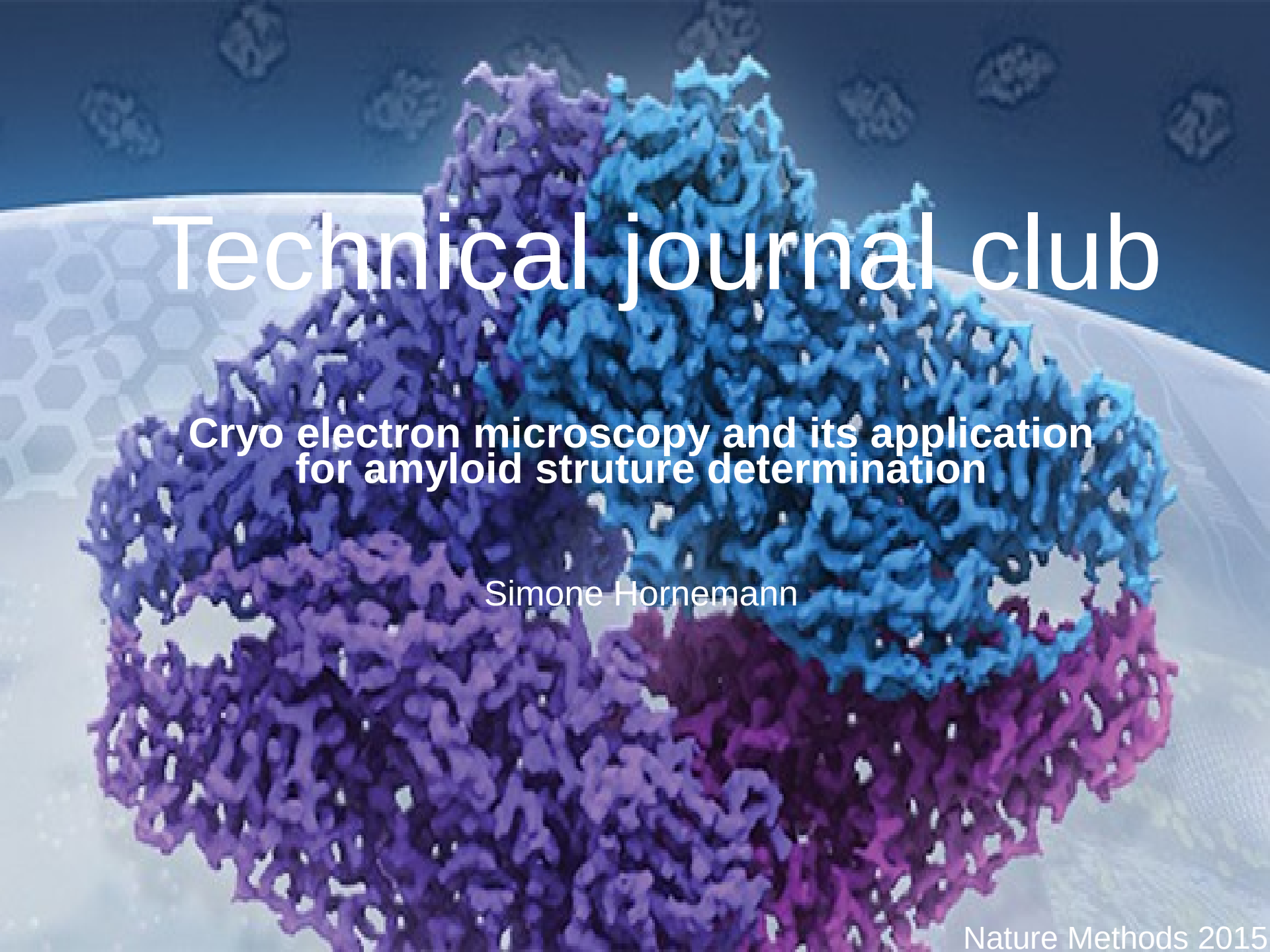


Technical journal club

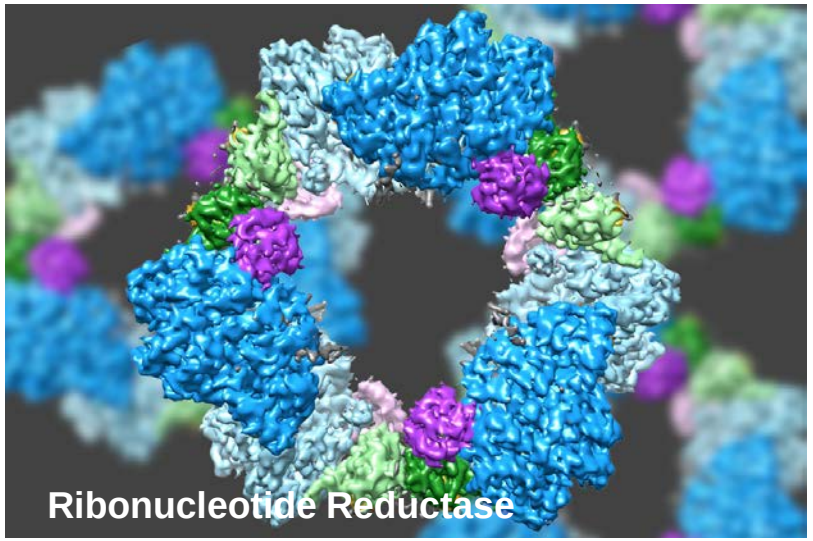
Cryo electron microscopy and its application for amyloid struture determination

Simone Hornemann

Nature Methods 2015

Why do we need to know protein structures?

- Prediction of protein function from 3D structure (active site, folding motives)
- Understanding mechanisms of function (e.g. enzymatic reactions)
- Rational design of novel diagnostic and therapeutic agents

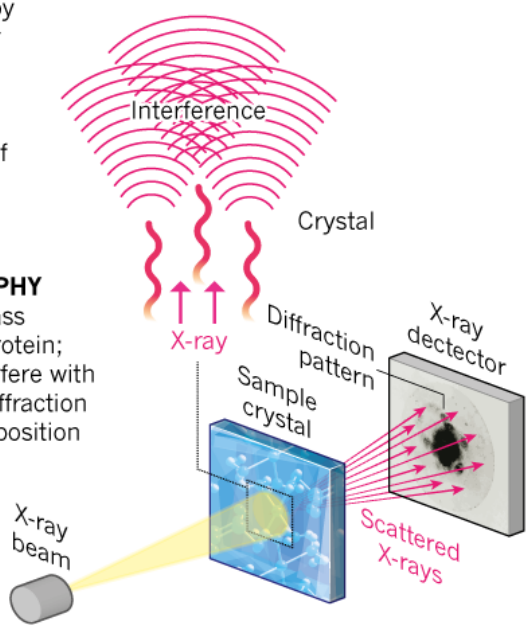


https://analyticalscience.wiley.com/do/10.1002/imaging.6243

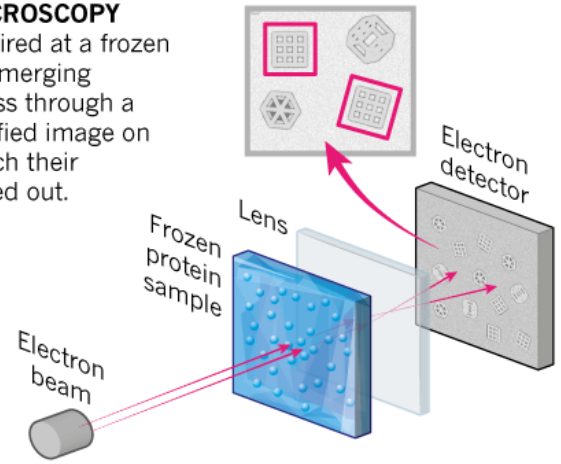
The rise of cryo-electron microscopy

Cryo-electron microscopy is taking over from X-ray crystallography as a method to deduce high-resolution protein structures, particularly of large molecules.

X-RAY CRYSTALLOGRAPHY
X-rays scatter as they pass through a crystallized protein; the resulting waves interfere with each other, creating a diffraction pattern from which the position of atoms is deduced.



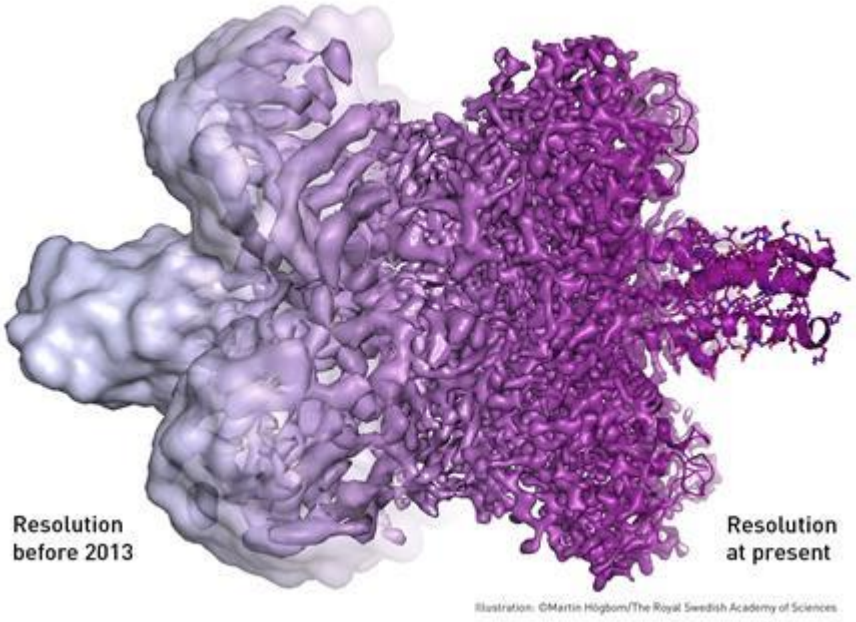
CRYO-ELECTRON MICROSCOPY
A beam of electron is fired at a frozen protein solution. The emerging scattered electrons pass through a lens to create a magnified image on the detector, from which their structure can be worked out.



Cressey and Callaway, , Nature, 2017

Cryo-EM:
The resolution evolution

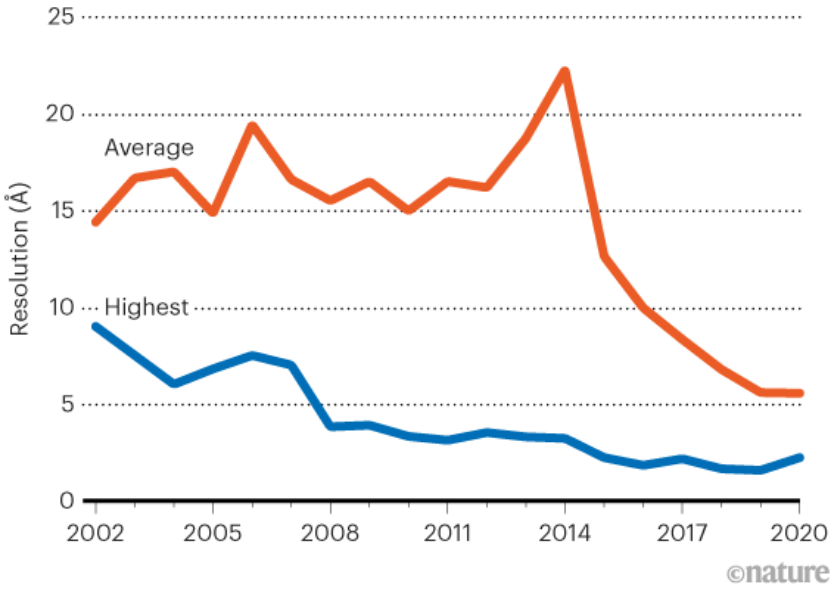
- Cryo-EM has emerged as powerful structure determination technique in recent years
- Has been awarded as **method** of the year in 2015 (Nature Method)
- Noble prize in Chemistry in 2017 had been awarded to **Jacques Dubochet, Richard Henderson and Joachim Frank** for their pioneering works in the development of cryo-EM as a structure determination technique



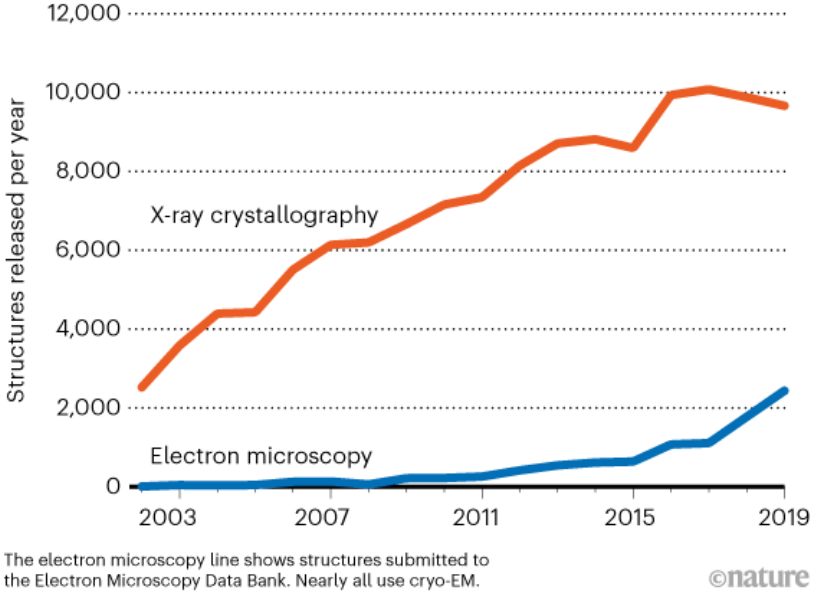
Revolution of Cryo-EM

Cryo-EM has emerged as powerful structure determination technique in recent years

Resolution



Number of published papers



Ewen Callaway, Nature 2020

Cryo-EM and related methods

- **Cryo transmission electron microscopy** (**CryoTEM**), commonly known as **cryo-EM**.
- Can be used to view and characterize a wide range of samples. With Cryo-EM it becomes possible to view molecules well over 500kDa and under optimal condition below 100kDa.

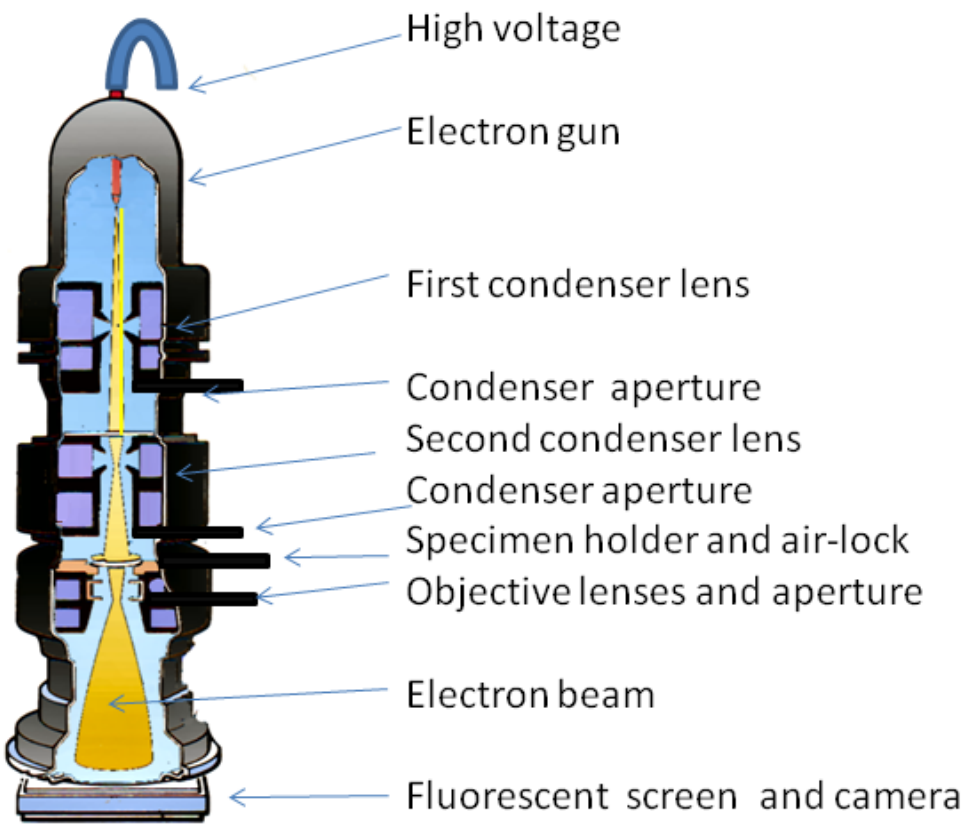
The two main methods are:

- **Single particle analysis (SPA)**, a method to determine high-resolution protein structures
- **Cryo-electron tomography (cryo-ET)** is another powerful approach that enables visualization of protein complexes in their native cellular environment.

Others are:

- **micro-electron diffraction (MicroED)**: crystallographic method for 3D structure determination based on electron diffraction.
- **cryo-scanning transmission electron microscopy (cryo-STEM):** imaging method that uses a sharply focused electron beam to raster-scan the sample.

Electron microscope

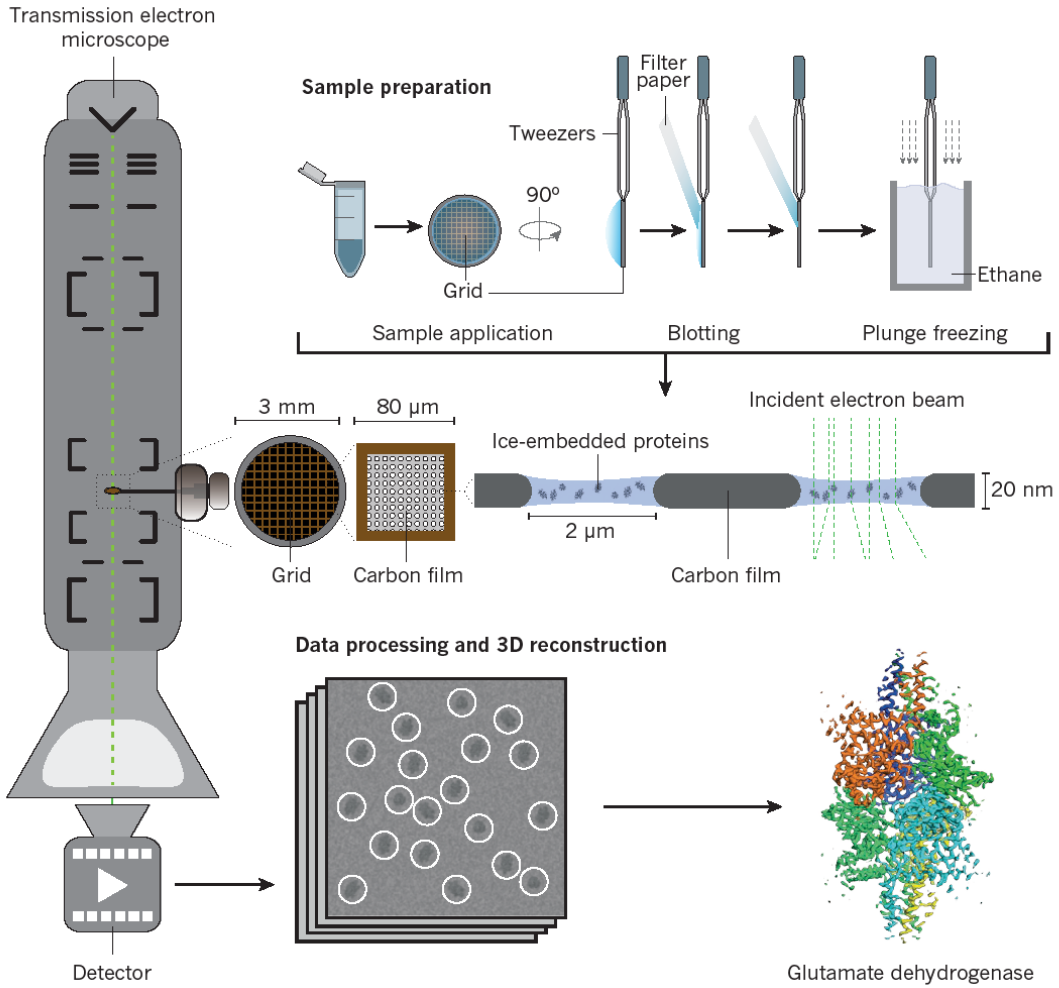


Transmission Electron Microscope

https://www.fei.com/introduction-to-electron-microscopy/tem/

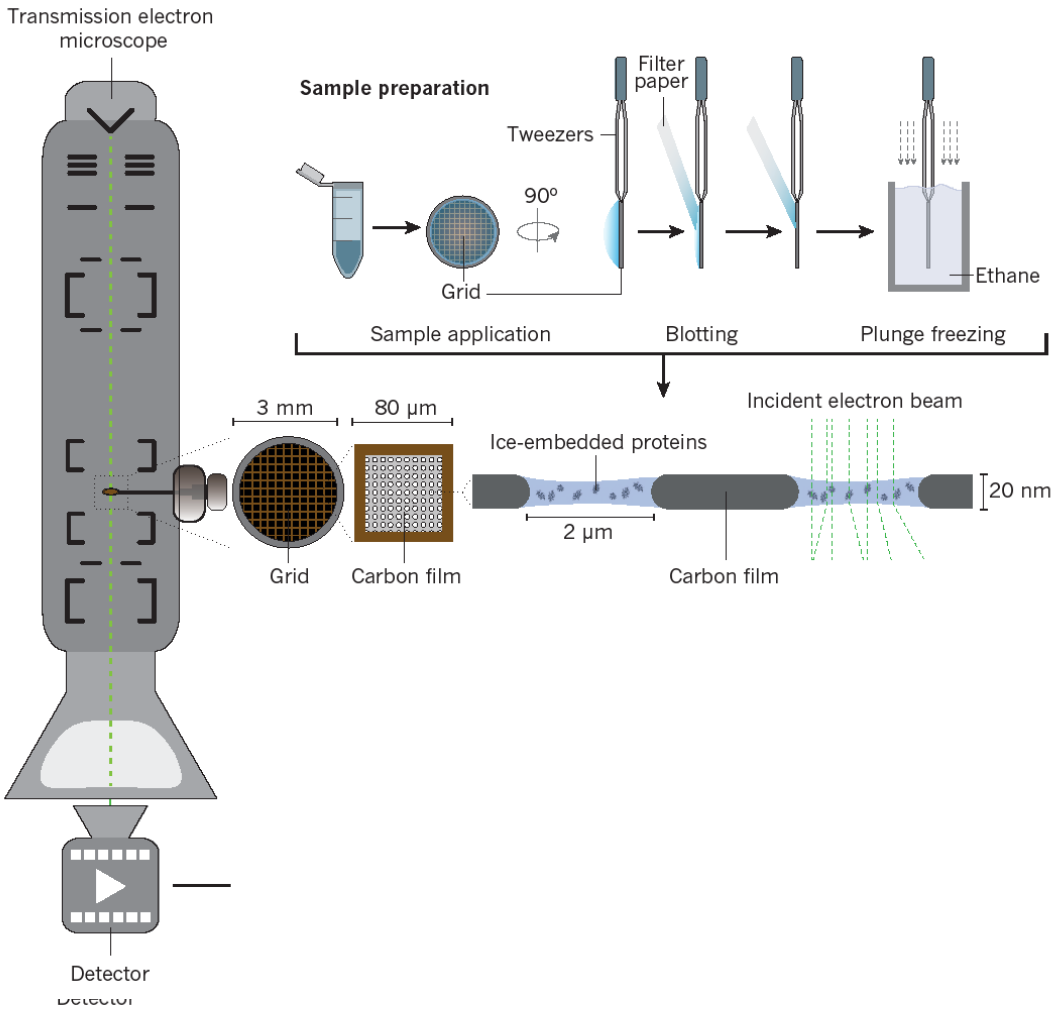
https://www.microscopemaster.com/

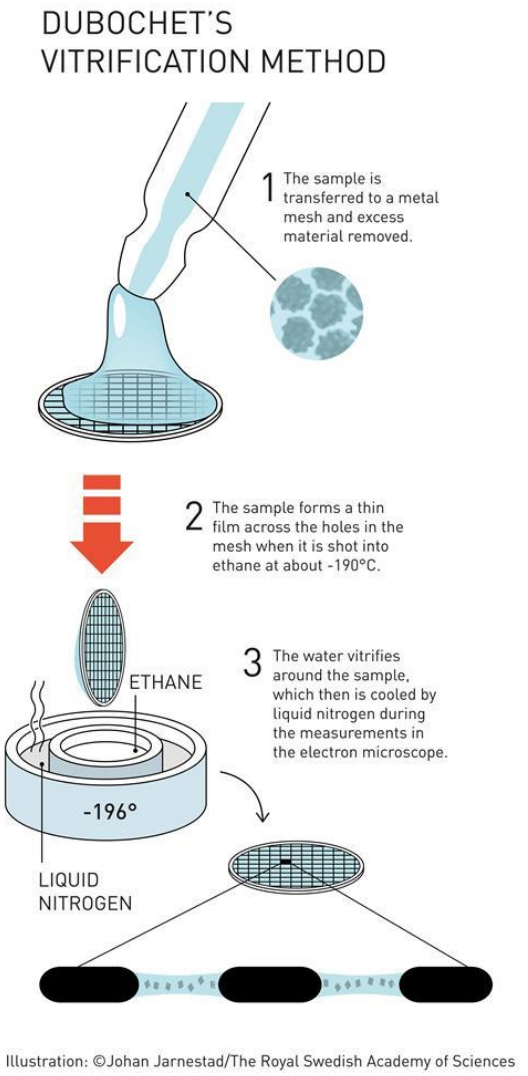
Cryo-EM workflow:



Fernandez-Leiro and Scheres, 2016.

Cryo-EM workflow: Sample preparation



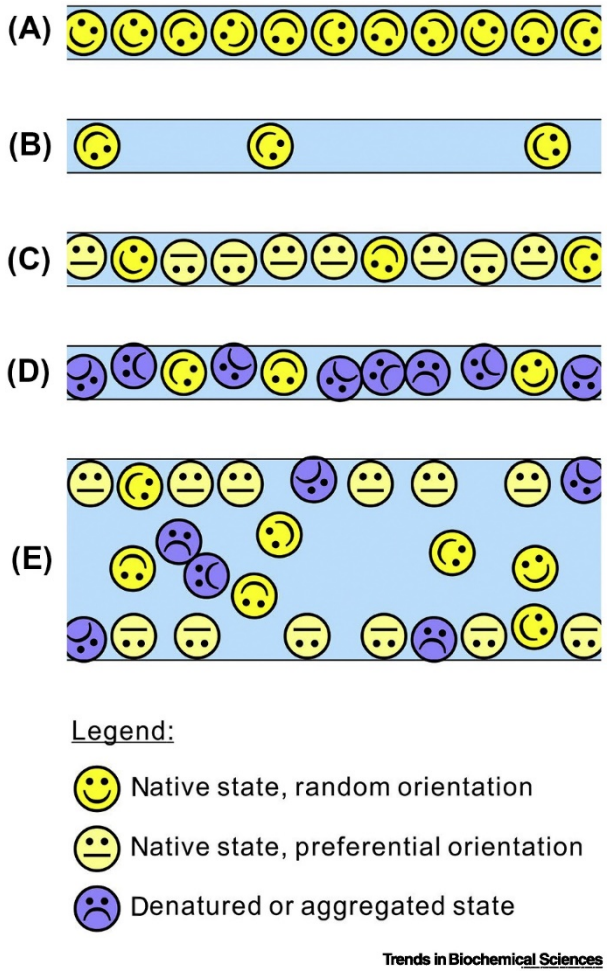


Fernandez-Leiro and Scheres, 2016.

SPA: Specimen preparation: It is not simply ‘plunge and play’ but rather ‘plunge and pray’.

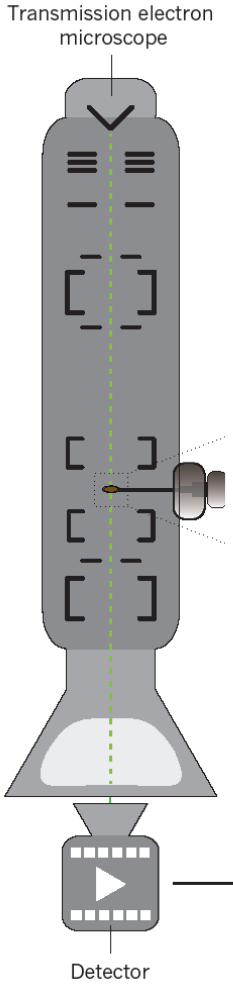
Two methods:

- Thin film: Specimen is placed on EM grid and is rapidly frozen without crystallizing it
- Vitreous Sections: Larger samples are vitrified by high pressure freezing, cut tinely and placed on EM grid.



Danev et al, 2019.

Cryo-EM workflow: data collection to 3D model



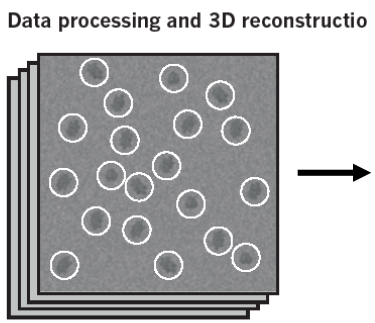
(1) The computer discriminates between the traces and the fuzzy background.

(2) Individual particles are selected and aligned for 2D class average placing similar ones in the same group.

(3) Using thousands of similar traces the computer generates a high-resolution 2D image.

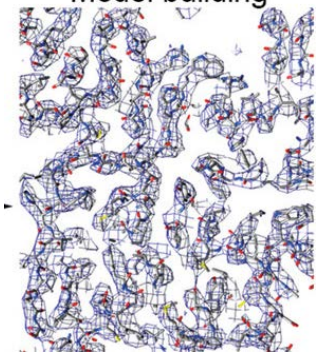
(4) the computer calculates how the different 2D images relate to each other for 3D classification.

(5) Further iterative refinement of 3D reconstruction will finally provide the high-resolution cryo-EM structure.

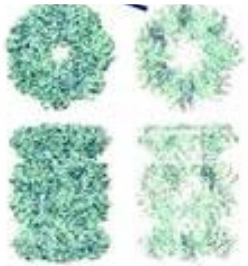


Alignment &
2D class average

3D classification



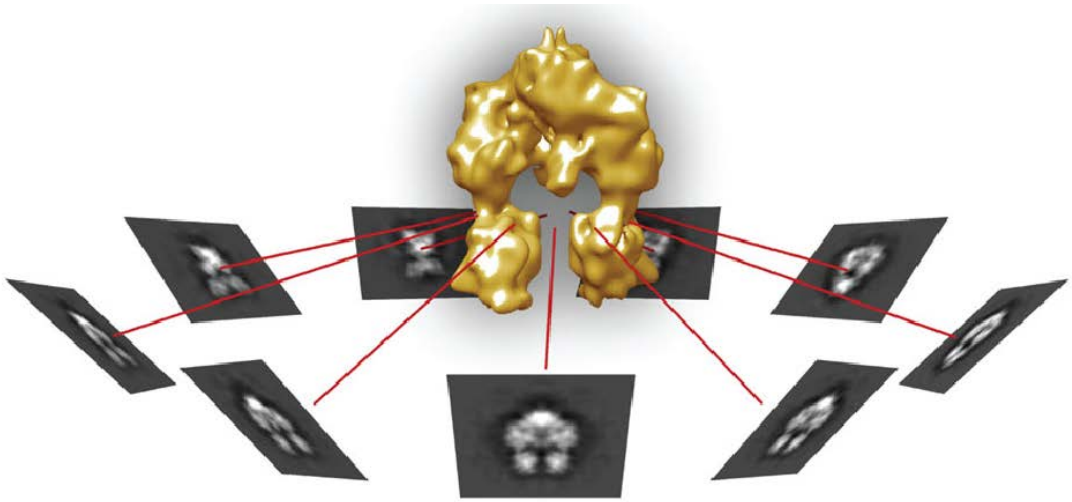
Model building

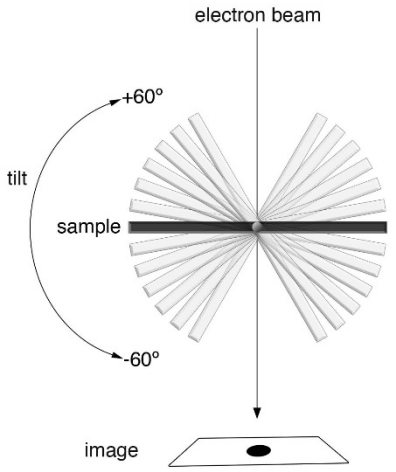


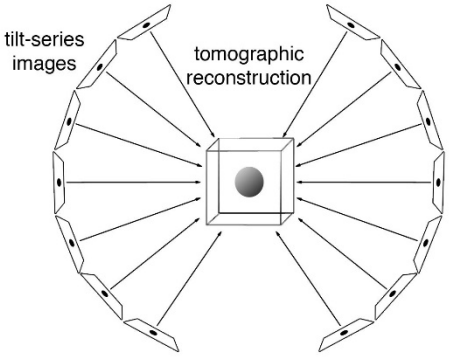
3D refinement &
final high-resolution structure
(20S proteasome)

Fernandez-Leiro and Scheres, 2016. *Dutta,* J. Indian Inst. Sci. 2018

Cryo electron tomography (CryoET)







Skiniotis and Southworth, Microscopy, 2016, 9–22

Advantages/Disadvantages of Cryo-EM

Advantages

- **Study samples in their natural, hydrated state** – Observe the structure in a biologically relevant environment, including sample and buffer concentration.
- **Suitable for larger assemblies** – Characterise molecules larger than 150 kDa, including those that are heterogeneous, metastable, have multiple sub-units or are difficult to crystallise.
- **Atomic resolution structures** – Study asymmetric side chains, hydrogen bonds and water molecules, as well as α-helices and β-sheets.
- **Controlled chemical environment** – Adjust the experimental conditions to observe molecules in various functional states.
- **No crystallisation** – Skip the long, uncertain preparation steps, for faster results.
- **Low sample amounts**: this method requires only a small amount of sample (about 0.1 mg), is more forgiven on sample purity, and does not need the protein to crystalize.

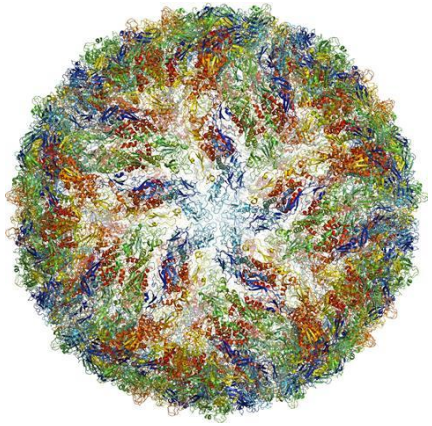
Disadvantages

- It takes time to generate suitable sample
- High levels of noise, due to the use of limited electron doses to minimize radiation damage
- Costly

What is cryo-EM used for?

- **3D Structure visualisation of:**
  - Single particles such as the Ribosome
  - Viruses (e.g. Zika virus)
  - Proteins
  - Macromolecules, Lipid vesicles
  - Membrane proteins
- **Macromolecular dynamics** (rotor shaped V-ATPase; proton pump)
- **Amyloid fibrils** (associated with many fatal degenerative diseases)

Zika virus



https://www.chemistryworld.com/news/explainer-what-is-cryo-electron-microscopy/3008091.article

Progress in amyloid structure determination

Limitations

- Amyloids are highly polymorphic, forming many different types of fibrils.
- Amyloid structures are large, they have been difficult to study by traditional structure determination methods
- Only limited amounts of fibrils are available

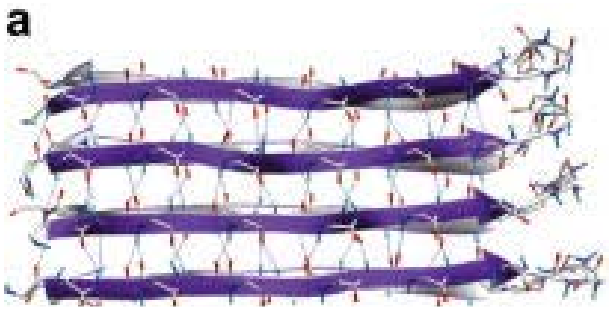
Advancement

- Cryo-EM can now achieve a resolution necessary for de novo structure determination
- The ability to assemble fibrils in vitro from synthetic peptides and naturally occuring fibrils has fueled developments in each structural technique.
- These techniques have thus reduced work with animal-derived protein version used in earlier years in such study in compliance with the **3R** for animal welfare

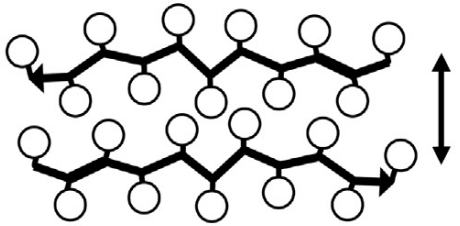
Replacement
Reduction
Refinement

https://www.eurekalert.org/pub_releases/2019-09/ou-tso090119.php

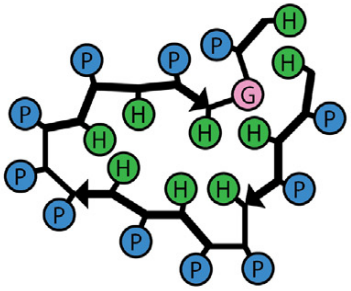
Organisation of amyloid fibils

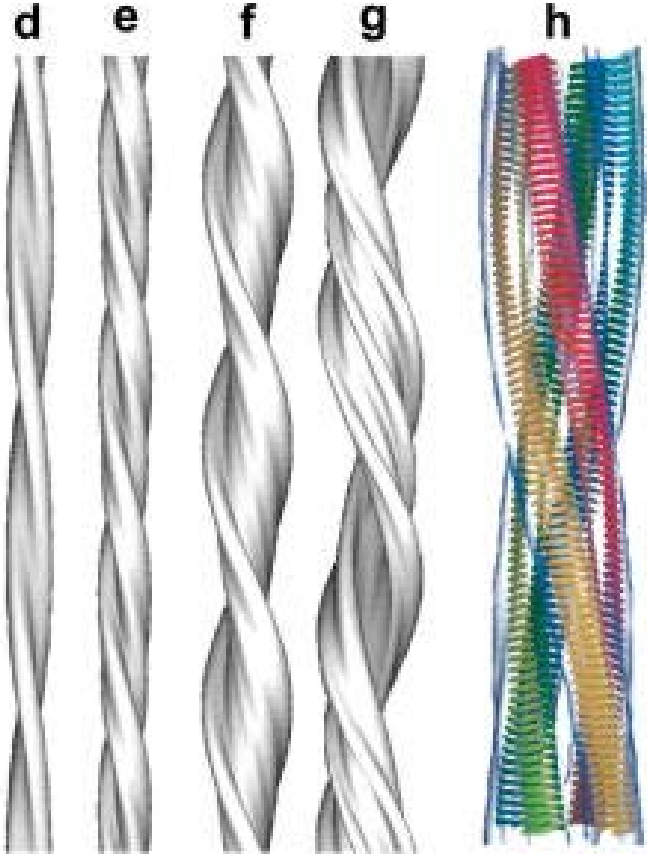


β-sheet



Cross β-helix





Jahn & Radford Archives of Biochemistry and Biophysics 469(1):100-17 2008

Novel tau filament fold in chronic traumatic encephalopathy encloses hydrophobic molecules

Benjamin Falcon[1], Jasenko Zivanov[1], Wenjuan Zhang[1], Alexey G. Murzin[1], Holly J. Garringer[2], Ruben Vidal[2], R. Anthony Crowther[1], Kathy L. Newell[3], Bernardino Ghetti[2], Michel Goedert[1,4]* & Sjors H. W. Scheres[1,4]*

Nature, 2019.

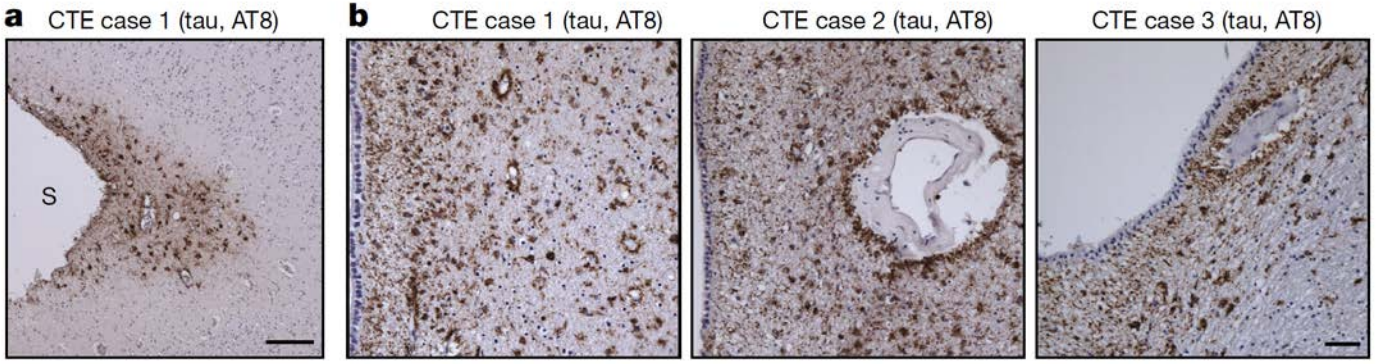
Chronic traumatic encephalopathy (CTE)

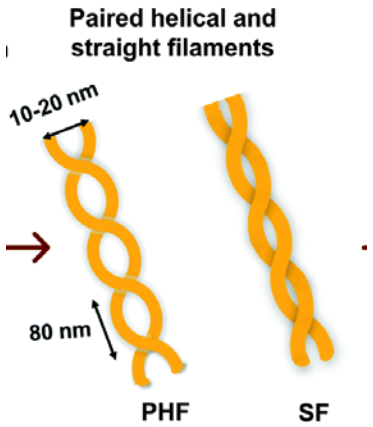
- **CTE is a neurodegenerative tauopathy that is associated with repetitive head impacts or exposure to blast waves. First described as punch-drunk syndrome and dementia pugilistica in retired boxers**
- **CTE has since been identified in former participants of other contact sports, ex-military personnel and after physical abuse.**
- **No disease-modifying therapies currently exist, and diagnosis requires an autopsy.**
- **CTE is defined by an abundance of hyperphosphorylated tau protein in neurons, astrocytes and cell processes around blood vessels**
- **This, together with the accumulation of tau inclusions in cortical layers II and III, distinguishes CTE from Alzheimer's disease and other tauopathies.**
- **However, the morphologies of tau filaments in CTE and the mechanisms by which brain trauma can lead to their formation are unknown.**

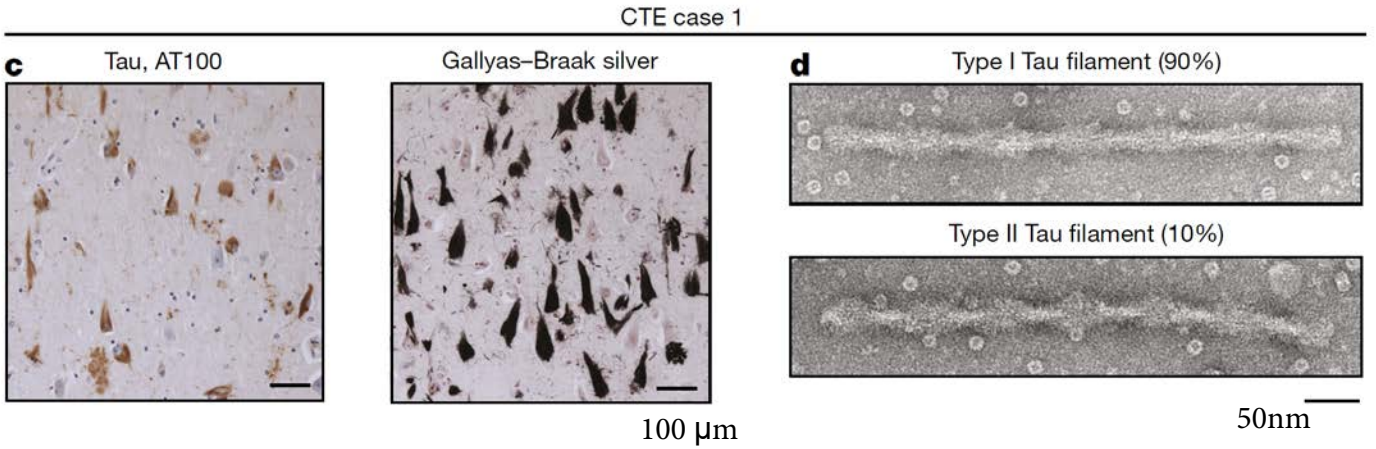
Filamentous tau pathology of CTE.

Perivascular staining of tau inclusions in the depth of a sulcus (S) in the frontal cortex

Perivascular staining of tau inclusions in the subependymal regions







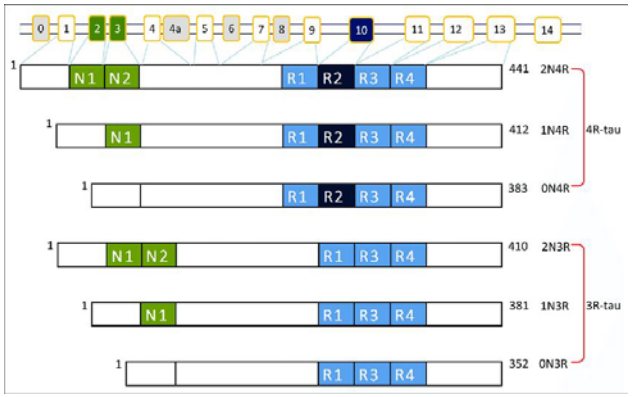
superficial cortical layers

Type I: 20–25 nm width and crossover spacings of 65–80 nm
Type II: twisted; widths of 15–30 nm (PHF)

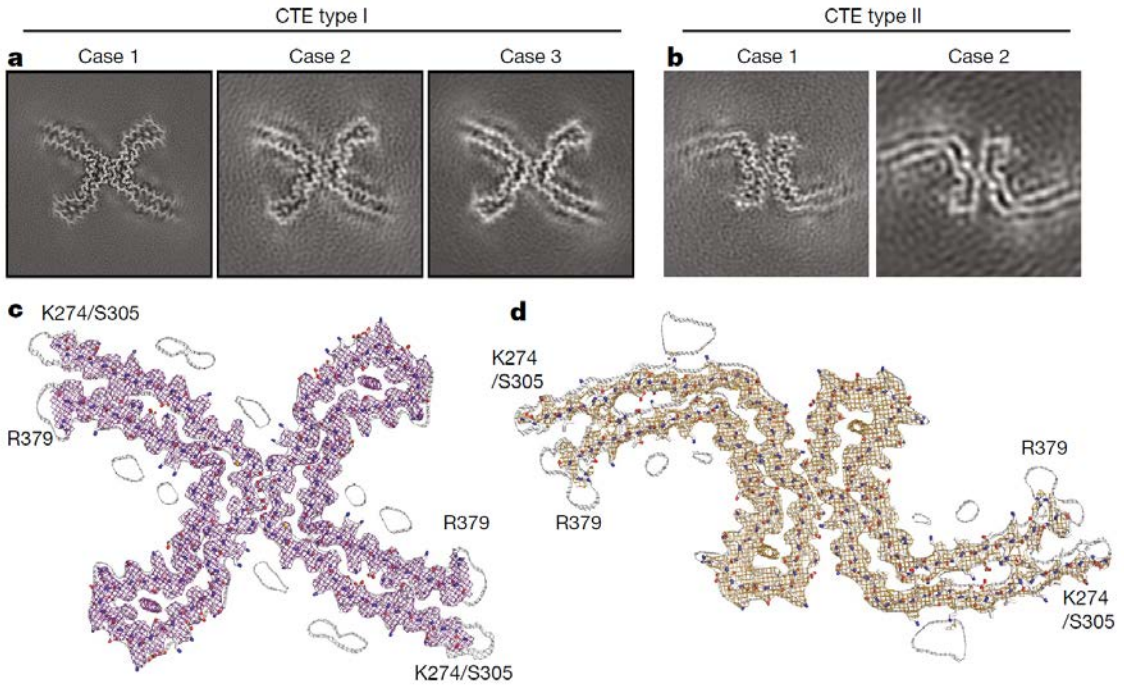
Cryo-EM structures of CTE type I and type II tau filaments

- filaments from the temporal cortex

Organisation of tau.



Wu et al. Chinese medical journal 130(24) 2017



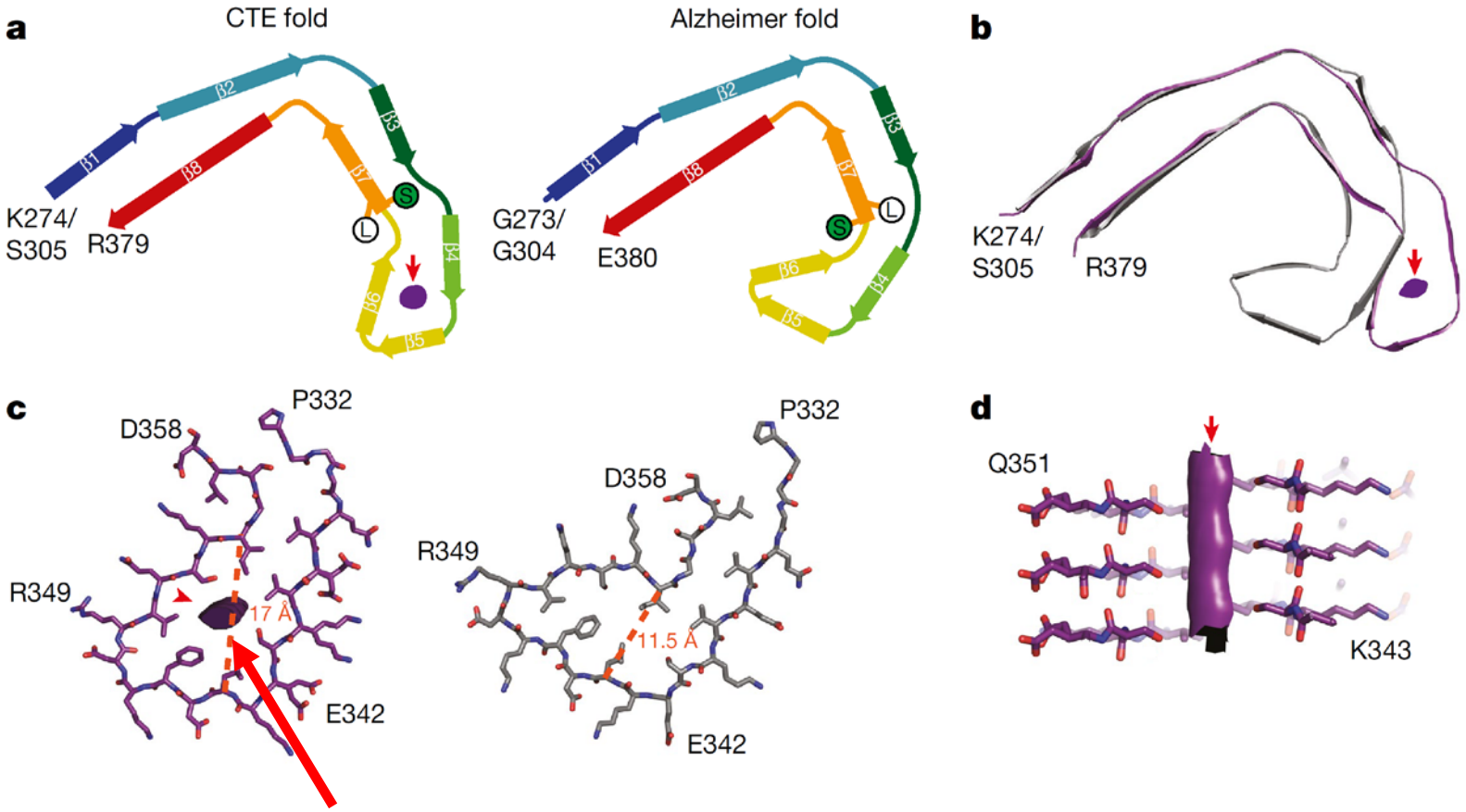
2.3 Å-resolution map

3.4 Å-resolution map

- K274–R379 of three-repeat tau and S305–R379 of four-repeat tau form the ordered core of two identical C-shaped protofilaments.

Comparison of the CTE and Alzheimer tau filament folds

cross-β and β-helix fold



non-polar sterols and sterol derivatives, as well as fatty acids? (damaged axons, compromised BBB)?

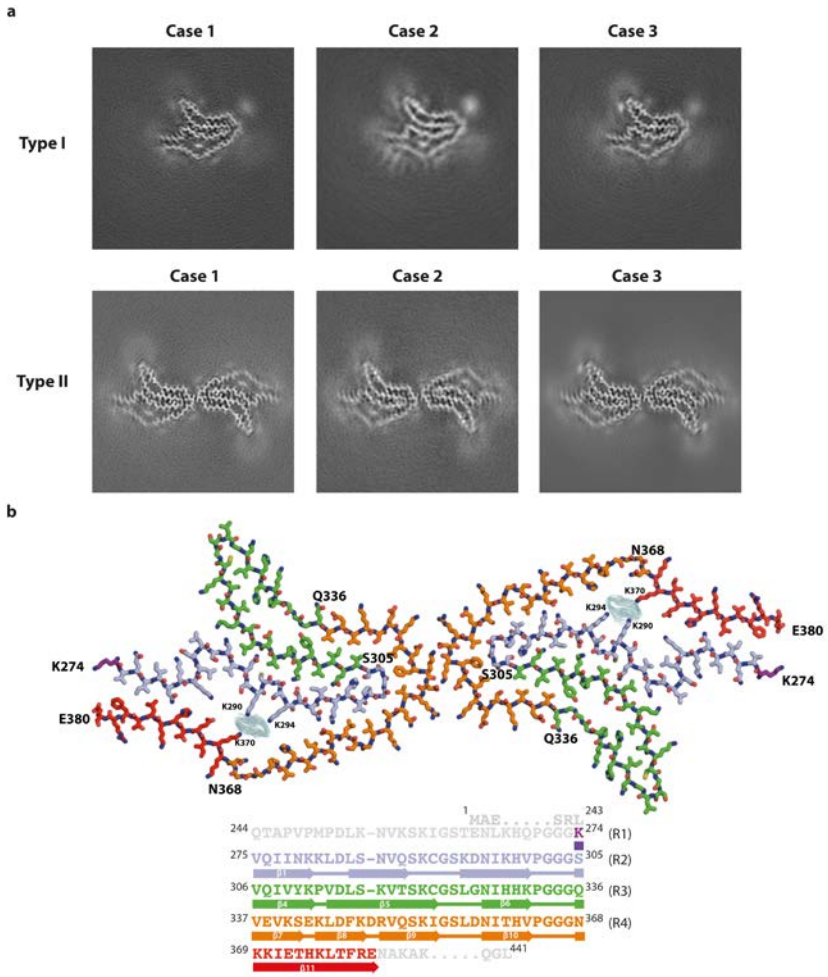
Summary

- The authors show that filament structures are identical in the three cases but are distinct from those of Alzheimer's and Pick's diseases, and from those formed in vitro
- Similar to Alzheimer's disease, all six brain tau isoforms assemble into filaments in CTE, and residues K274–R379 of three-repeat tau and S305–R379 of four-repeat tau form the ordered core of two identical C-shaped protofilaments.
- A different conformation of the β-helix region creates a hydrophobic cavity that is absent in tau filaments from the brains of patients with Alzheimer's disease. This cavity encloses an additional density that is not connected to tau, which suggests that the incorporation of cofactors may have a role in tau aggregation in CTE.
- Filaments in CTE have distinct protofilament interfaces to those of Alzheimer's disease. Structures provide a unifying neuropathological criterion for CTE, and support the hypothesis that the formation and propagation of distinct conformers of assembled tau underlie different neurodegenerative diseases.

Novel tau filament fold in corticobasal degeneration

Wenjuan Zhang, Airi Tarutani, Kathy L. Newell, Alexey G. Murzin, Tomoyasu Matsubara, Benjamin Falcon, Ruben Vidal, Holly J. Garringer, Yang Shi, Takeshi Ikeuchi, Shigeo Murayama, Bernardino Ghetti, Masato Hasegawa, Michel Goedert & Sjors H. W. Scheres

Science, 2020



- Corticobasal degeneration (CBD) is a neurodegenerative tauopathy that is characterised by motor and cognitive disturbances.
- The structures of tau filaments extracted from the brains of three individuals with CBD using electron cryomicroscopy (cryo-EM). They were identical between cases, but **distinct from those of Alzheimer's disease, Pick's disease and CTE**.
- Determination of the CBD fold opens **4R tauopathies** to structural analysis. It supports the hypothesis that distinct conformers of filamentous tau define different tauopathies.
- Tau filaments from Alzheimer's disease, Pick's disease and CTE adopt different folds.

Article

Structures of α-synuclein filaments from multiple system atrophy

https://doi.org/10.1038/s41586-020-2317-6

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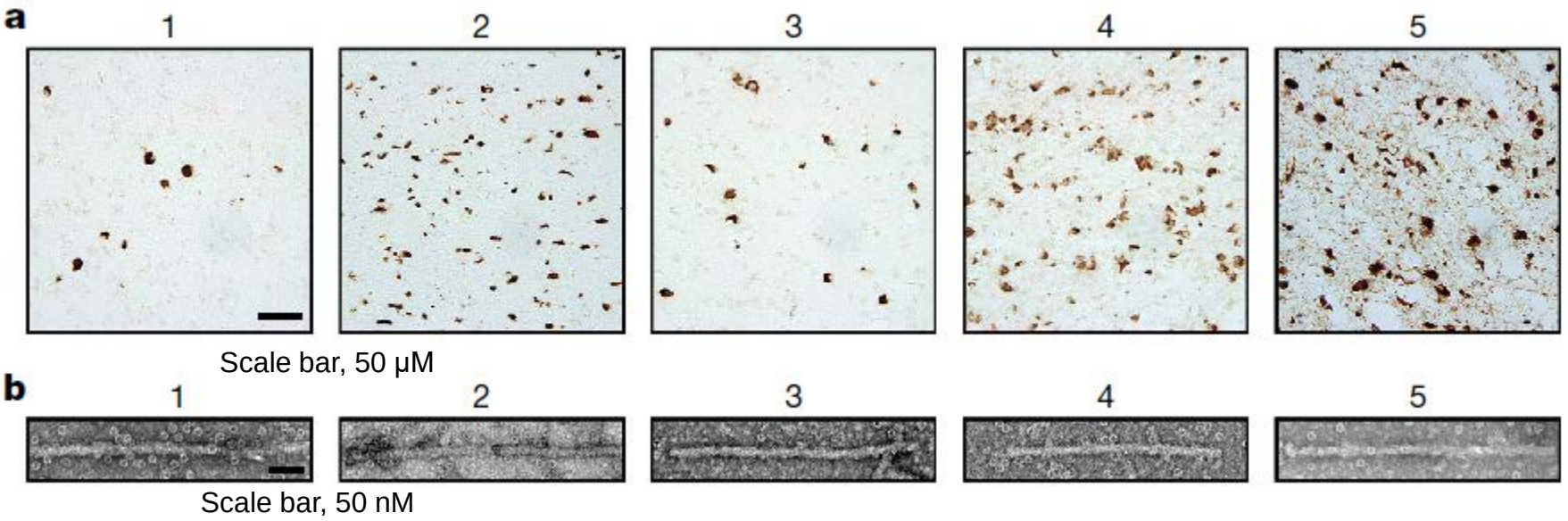
Manuel Schweighauser[1,9], Yang Shi[1,9], Airi Tarutani[2,3], Fuyuki Kametani[2], Alexey G. Murzin[1], Bernardino Ghetti[4], Tomoyasu Matsubara[5], Taisuke Tomita[3], Takashi Ando[6], Kazuko Hasegawa[7], Shigeo Murayama[5], Mari Yoshida[8], Masato Hasegawa[2], Sjors H. W. Scheres[1,10]✉ & Michel Goedert[1,10]✉

Nature, 2020

Introduction

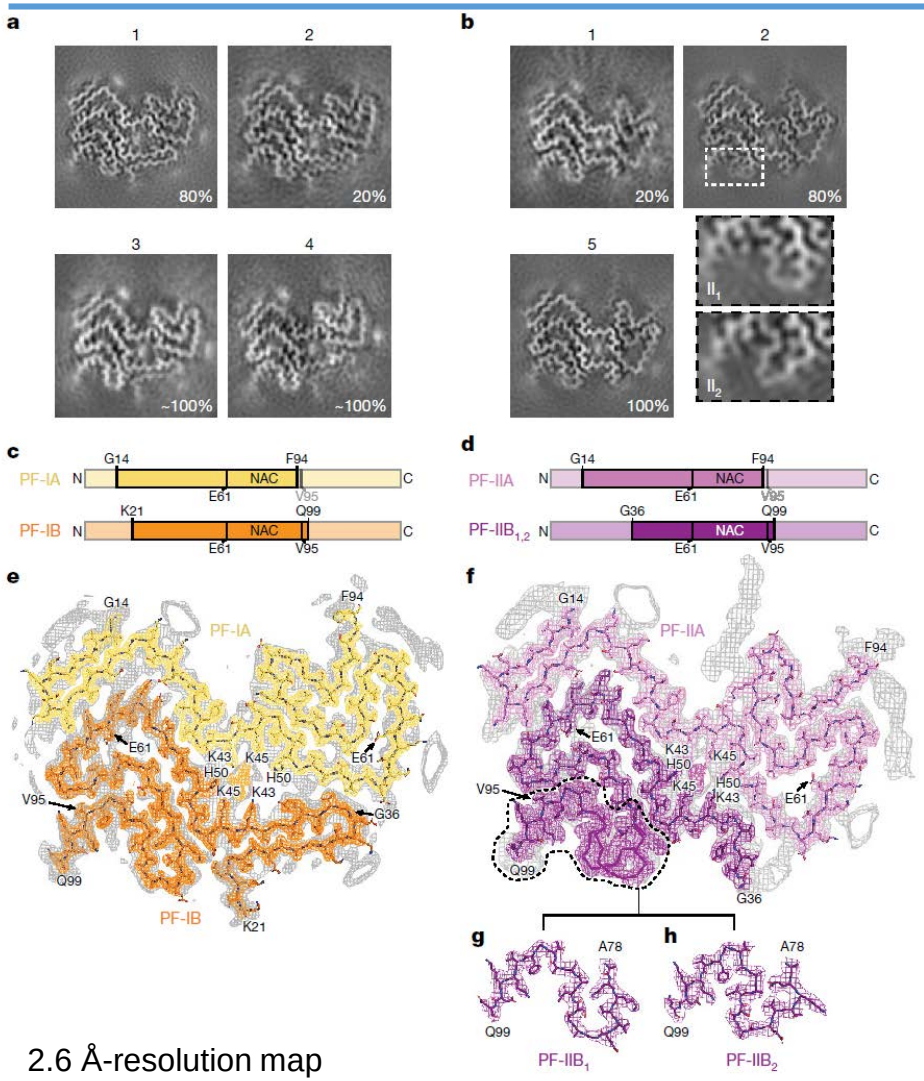
- Synucleinopathies are neurodegenerative diseases that are associated with the misfolding and aggregation of α-synuclein, including Parkinson's disease, dementia with Lewy bodies and multiple system atrophy (MSA).
- Clinically, it is challenging to differentiate Parkinson's disease and MSA, especially at the early stages of disease.
- Aggregates of α-synuclein in distinct synucleinopathies have been proposed represent different conformational strains of α-synuclein that can self propagate and spread from cell to cell and determine the disease phenotype.
- In this study, authors have determined the structure of brain-derived α-synuclein fibrils by cryo-EM.

Filamentous α-synuclein pathology of MSA



- majority of twisted filaments, which had a diameter of 10 nm and a periodicity of 80–100 nm

Cryo-EM maps and atomic models of type I and type II filaments of α-synuclein from MSA.



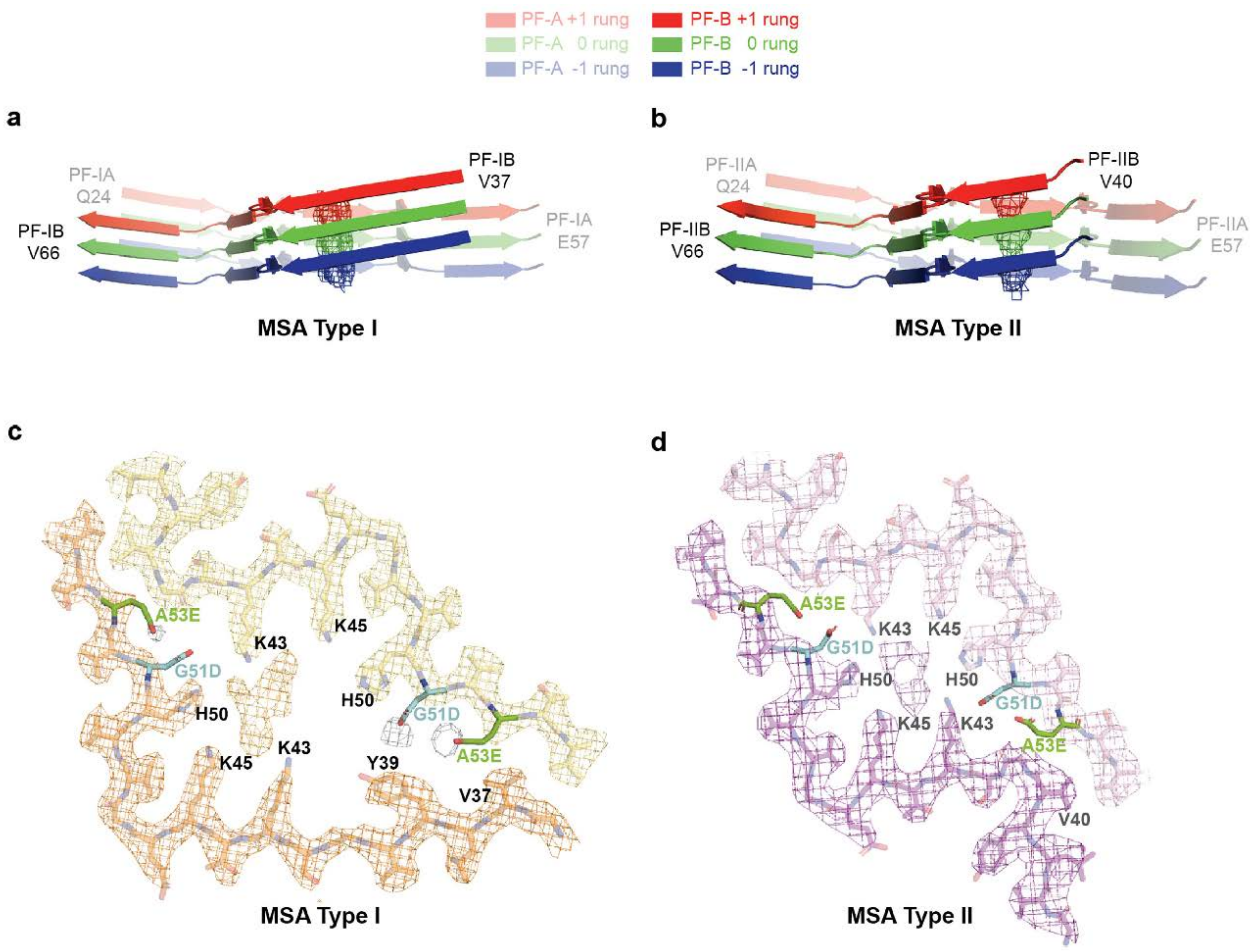
2.6 Å-resolution map

3.1 Å-resolution map

- PFs have 12 β-strands
- PFIIb is 15 amino acid shorter as PHIb and has 9 β-strands.

- Interestingly, ratio of the fibrils correlated with how long a patient had the disease.

The inter-protofilament interfaces of MSA type I and type II α-synuclein filaments



Each monomer layer attaches three layers of the opposing β-sheets.

Familial mutations (PD) in the interface may affect binding of small moleculeand thus affect disease.

Summary/conclusion

- α-synuclein fibrils present an assembly that looks quite distinct from the core protofibrils found in tau neurofibrillary tangles, amyloid-β plaques and recombinant fibrils.
- Two different protofibrils make up the core of α-synuclein filaments, rendering it asymmetric.
- Data suggest that the duration of MSA may correlate with the ratio of filament types in putamen, but additional cases of disease are required to establish this more firmly.
- Cavity is formed by the fibrils that also may include a small molecule.
- Scientists hope this information will finally help them score compounds that will make good ligands for α-synuclein PET imaging, which have thus far eluded them.

Take home message

- Cryo-EM and tomography are useful tools to study large proteins and complexes, including amyloid fibrils
- Recent technical advances have made it possible to use these methods to study the 3D structures of proteins and complexes at near atomic resolution (upto 1.5 A)
- In the upcoming years, this technique will most properly further evolve and lead to even higher resolution structures of smaller molecules
- These technical advances have made it possible to studyalso structures of amyloid fibrils in very detail
- This will help to understand the disease related to these fibrils in more detail and help to identify novel diagnostic molecules and treatments for these fatal diseases.
- In addition, the presented studies add to a growing body of evidence supporting the one polymorph, one disease' hypothesis, which states that different structural forms (polymorphs) of the same aggregated protein can cause distinct pathologies and symptoms.

Thank you for your attention!