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Molecular pathology of neurodegenerative diseases by cryo-EM of amyloids

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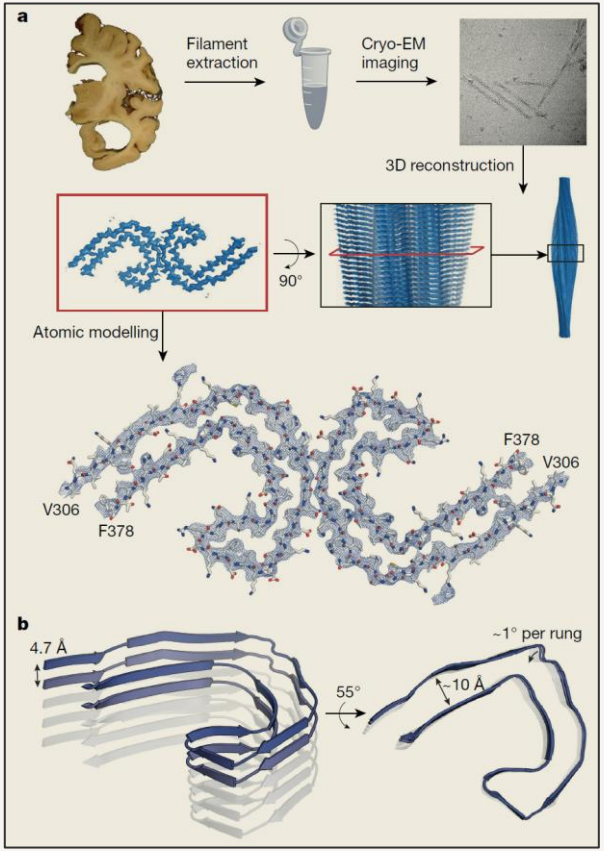
Introduction

- 100+ years ago abnormal brain inclusions were identified as defining features of neurodegenerative diseases such as Alzheimer's and Parkinson's disease.
- These inclusions were shown to contain amyloids, indicated by the presence of green–yellow birefringence under polarised light after being stained with Congo red.
- Neurodegenerative diseases are caused by mutations in the genes that encode the key filament-forming proteins: amyloid-β, tau, α-synuclein, and TDP-43.
- Traditional methods to study the molecular structures of amyloids used X-ray fibre diffraction which showed amyloid filaments have a β-sheet structure and negative-stain EM which showed the presence of paired helical filaments of tau.
- Advances in cryo-EM enabled atomic structures of amyloids to be determined.
- In 2013, electron detectors and image processing software were developed, enabling high resolution atomic structures of amyloid filaments.
- The first structures were tau filaments from a brain with Alzheimer's disease and since then multiple amyloid structures from human brains have been reported.

Abstract

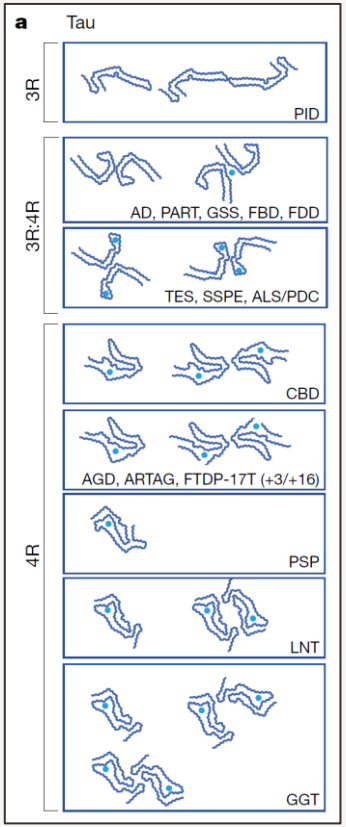
- The abnormal assembly of amyloid-β, tau, α-synuclein, and TDP-43 proteins into amyloid filaments defines most neurodegenerative diseases.
- Genetics provides a direct link between filament formation and the causes of disease.
- Cryo-EM enables the atomic structures of amyloids to be determined from postmortem brains.
- This review will cover the structures of amyloid filaments and discuss their impact on research into neurodegeneration.
- A single protein can form multiple filament structures, where specific folds are associated with specific diseases providing a basis for studying disease at an atomic level.



Structures of amyloid filaments from human brains

Tau

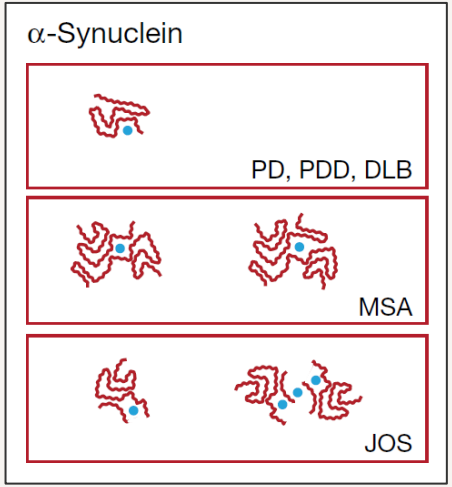
- Tau helps stabilise microtubules in brain cells.
- Tau has 6 isoforms:
  - 3 isoforms have all four microtubule-binding repeats (4R tau)
  - 3 isoforms are missing R2, leaving 3 repeats (3R tau)
- Tauopathies are diseases caused by abnormal tau filament formation.
- Cryo-EM shows disease-specific aggregation, for example, in Alzheimer's all isoforms aggregate, in Pick's only 3R tau aggregate, and in PSP only 4R tau isoforms aggregate to form filaments.
- Cryo-EM shows that tau filaments have a rigid, C-shaped core, while the remaining disordered regions of the protein, called the fuzzy coat, are less well-defined due to intrinsic properties.
- Each disease has its own unique core tau fold shape; some diseases share similar folds which suggests they have similar causes.



Structures of amyloid filaments from human brains

α-Synuclein

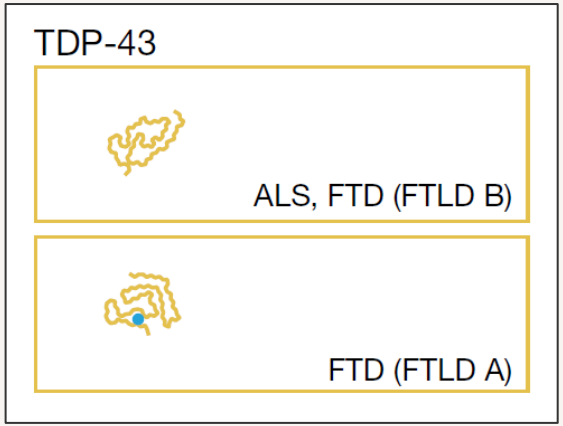
- α-Synuclein is a protein that binds to lipid membranes, especially in nerve cells. It has a structured core, and a fuzzy coat.
- It aggregates into filaments in neurodegenerative disorders such as Parkinson's disease (PD), Dementia with Lewy bodies (DLB), and Multiple system atrophy (MSA).
- Specific filament folds of assembled α-synuclein define different diseases, for example:
  - MSA - two distinct protofilament folds per filament
  - Lewy pathology (PD/DLB) – single "Lewy fold," even in non-twisting filaments
  - Juvenile-onset synucleinopathy (JOS) - unique fold due to a 7-amino-acid insertion mutation
- Mutations in the gene encoding α-synuclein are linked to PD and DLB, but not MSA, thus suggesting that MSA may involve non-genetic, possibly environmental, triggers.



Structures of amyloid filaments from human brains

TDP-43

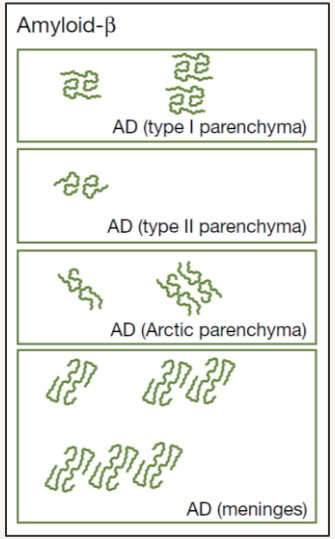
- TDP-43 is a protein involved in mRNA processing, which plays a critical role in regulating gene expression, particularly in the nervous system.
- TDP-43 filaments have a fuzzy coat.
- Cryo-EM indicates that specific diseases have distinct filament folds:
  - ALS & FTLD B - double spiral-shaped fold
  - FTLD A - chevron-shaped fold with extra N-terminal residues
- FTLD C and D filament structures remain unknown, further research is required.



Structures of amyloid filaments from human brains

Amyloid-β (Aβ)

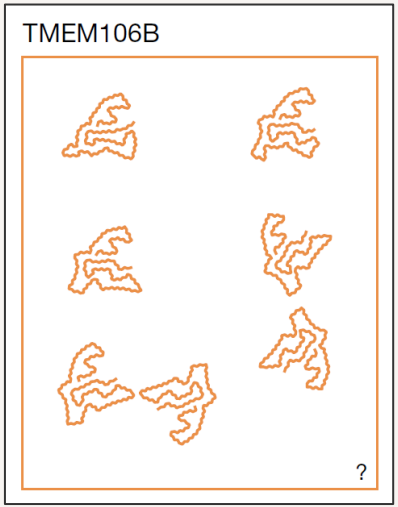
- Aβ is generated from APP and cleaved into 40 (Aβ40) or 42 (Aβ42) amino acid chains.
- Aβ aggregates outside cells, unlike other filaments which aggregate inside cells.
- Different Aβ filament folds all result in Alzheimer's disease. This means fold variation in Aβ does not correspond to different diseases.
- Aβ40 filaments are found mainly in blood vessels (cerebral amyloid angiopathy)
    - Made of two identical protofilaments
    - All 40 amino acids are structured - no fuzzy coat
    - Variants with 4 or 6 protofilaments exist, but all share the same fold
- Aβ42 filaments are found mainly in brain tissue (parenchyma)
    - Type I - dominant in sporadic Alzheimer's
    - Type II - dominant in familial Alzheimer's
    - Both have S-shaped protofilaments, but differ in amino acid orientation and packing
- Arctic mutation (E693G):
    - Alters Aβ42 - distinct filament structures with two non-identical protofilaments



Structures of amyloid filaments from human brains

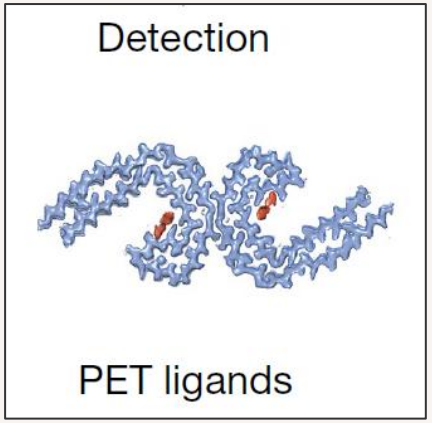
TMEM106B

- Cryo-EM revealed that TMEM106B forms amyloid filaments in human brains. It has been found in both healthy individuals and those with neurodegenerative diseases, filament levels increase with age.
- Three different filament folds have been discovered, but only one per brain.
- TMEM106B filament folds do not correlate with specific diseases.
- TMEM106B filaments are less harmful than other amyloid filaments, this could be because they lack a fuzzy coat, which could influence toxicity.
- Future research is needed to determine how the how/if the formation of TMEM106B filaments influences neurodegenerative diseases, and if TMEM106B aggregation protects or harms cells.



Amyloid detection

- Detecting amyloid filaments *in vivo* is important for early diagnosis, clinical trial recruitment, and monitoring the effects of drugs.
- Current tools for detection, Pittsburgh Compound B (PiB) and florbetapir, are used to visualise amyloid-β plaques via positron emission tomography (PET) imaging but fail in cases involving the Arctic mutation Alzheimer's disease.
- Tau PET ligands, such as flortaucipir, are tools to visualise tau aggregates in brains with Alzheimer’s disease.
- PET ligands for 3R/4R tauopathies, α-synuclein, and TDP-43 detection are still needed.
- This structural insight will be essential for developing more effective diagnostic tools and potential treatments for neurodegenerative diseases.

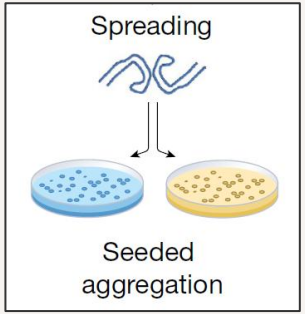


Amyloid formation

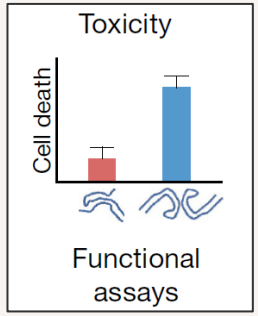
- A better understanding of how amyloids form in the brain is crucial for their prevention.
- Recently, structures of *in vitro* assembled filaments of tau, α-synuclein, amyloid-β, and TDP-43 have been obtained via cryo-EM.
- None of these structures are the same as those extracted from postmortem human brains.
- Studies have shown that this difference is likely not caused by extraction methods, but rather cellular environment, post-translational modifications, and other unknown cofactors.
- Only tau filaments have been replicated *in vitro*, the next steps are to replicate the structures of amyloid-β, α-synuclein, and TDP-43.
- Once assembly conditions that replicate structures of a given disease have been identified, cryo-EM and NMR experiments at different stages of the assembly process may provide further insights into their formation mechanisms.

Amyloid templated seeding hypothesis

- Main assumptions:
  - The presence of seeds (small amounts of amyloids) accelerate the formation of more amyloids.
  - Newly formed amyloids adopt the same structures as the seeds.
- Supporting evidence:
  - Sequential spread in brain networks.
  - Specific amyloid structures define diseases, these structures are consistent across brain regions.
- Models for templated seeding may need more research. The simplest model is primary nucleation, where growth occurs at filament ends, this accounts for the structure but is too slow for disease kinetics. The current dominating model, secondary nucleation model, where growth occurs along filament sides explains the exponential spread of disease, but it is unknown how the sides template core folds.
- Future research:
  - How amyloid structures propagate in vivo.
  - How filaments template structure during secondary nucleation.
  - Develop target seeding interfaces with structure-based inhibitors.



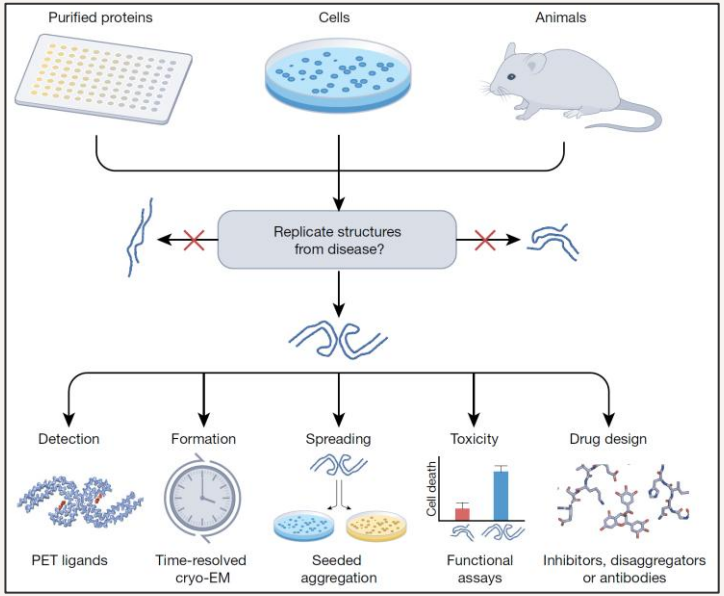
Amyloid toxicity



- While amyloid filaments define many neurodegenerative diseases, how they cause toxicity remains unclear. The main question is: What causes neurodegeneration?
- Filament hypothesis - large, insoluble aggregates physically disrupt cells.
- Oligomer hypothesis - small, soluble oligomers are more toxic, however their structures in human brains remain unknown.
- New evidence suggests that these oligomers in brain extracts may be short filaments, which are highly seed-competent, linking toxicity to propagation.
- Studies involving mice have been unsuccessful, no animal models have replicated human filament structures.
- Future research:
    - Better models in humans or animals to conduct further research on amyloid structures is needed.
    - More research on filament folds, their structure, and how they effect toxicity and spread.
    - Develop strain-specific inhibitors for the prevention of tau/α-synuclein diseases.

Author’s Conclusion

- Typically, a protein's amino acid sequence determines its structure and function, however, cryo-EM of filaments from postmortem brains indicates that a single protein can adopt multiple amyloid structures, each linked to a distinct disease.
- Although genetics confirms a direct link between filament formation and neurodegeneration, how filaments cause brain cell death for different diseases remains unclear.
- The structures of amyloid filaments provide a basis for studying the molecular mechanisms of neurodegenerative diseases.
- New experimental models that replicate disease-specific filament structures will be key for gaining a better understanding and enabling the development of safe, effective treatments.



Evaluation and conclusion

Feedback

+ Comprehensive and effective review of amyloid filament structures providing examples including major neurodegenerative diseases.
+ Discusses the limitations of current model systems and emphasises the need for future research into models that replicate disease-specific structure.
^ If possible, more quantitative data could have been included, such as biophysical properties or prevalence of filament types, to better support claims about toxicity, seeding, and disease specificity. More discussion on therapeutic applications, specifically on the development of targeted treatments and inhibitors.

- Overall, the research paper provides a clear and detailed review of how cryo-EM has advanced our understanding of amyloid structures in neurodegenerative diseases, while highlighting the importance of structural specificity for further research in diagnosis, modeling, and therapeutics.

References

Scheres, S.H.W., Ryskeldi-Falcon, B., Goedert, M. (2023). *Molecular pathology of neurodegenerative diseases by cryo-EM of amyloids*. Published: September 27, 2023. Accessed: July 18, 2025.

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Thank you for listening, questions are welcome

If you have any further questions, please contact me via

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