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Hierarchical chemical determination of amyloid polymorphs in neurodegenerative disease

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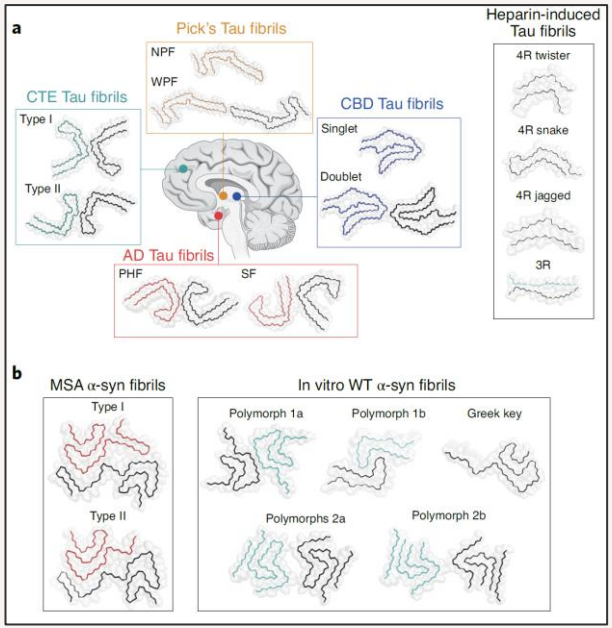
Introduction

- Neurodegenerative diseases (NDs), such as Alzheimer's and Parkinson's disease, are caused by the progressive loss and dysfunction of neurons due to amyloid aggregation of misfolded proteins (Tau, α-synuclein (α-syn), amyloid-β (Aβ), and prion).
- Amyloid aggregation occurs within or among brain cells when intrinsically disordered or misfolded proteins self-polymerise into ordered, insoluble fibrils via a liquid-solid phase transition.
- In 2005, micro-beam X-ray diffraction was used to develop the first atomic model of amyloids which depicted two β-sheets in a steric zipper structure stabilised by side-chain interactions.
- Recently, advances in cryo-electron microscopy (cryo-EM) and solid-state NMR spectroscopy (ssNMR) have led to the determination of high-resolution structures of amyloid fibrils.
- In 2017, atomic structures of Tau fibrils isolated from a brain with Alzheimer's disease were reported and since then nearly 30 high-resolution cryo-EM structures of amyloids have been reported.
- Amyloid polymorphism is when the same protein can form distinct amyloid fibril structures which are linked to different neurodegenerative diseases and environmental conditions.
- Recently, new fibril structures of proteins with different post-translational modifications (PTMs) and unidentified cofactors have been modeled highlighting their importance.

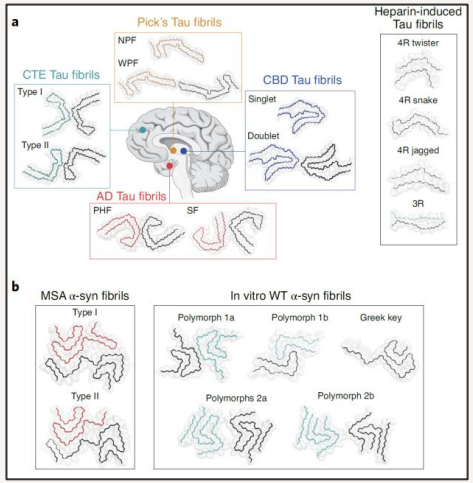
Abstract

- Amyloid aggregation disrupts protein homeostasis causing neurodegenerative diseases.
- Polymorphism, the structural diversity of amyloid fibrils, is strongly linked to ND onset, progression, and clinical phenotypes.
- Central question: How can one protein form many stable fibril structures?
- Answer: Recent atomic structural evidence suggests that chemical modifications guide polymorphic fibril formation.
- Main types of chemical modifications:
  - Covalent post-translational modifications (PTMs)
  - Noncovalent cofactor binding
- This review will highlight the determinant role of chemical modifications in amyloid assembly and pathology focusing on the latest α-syn and Tau fibril structures.



Environment-sensitive amyloid formation

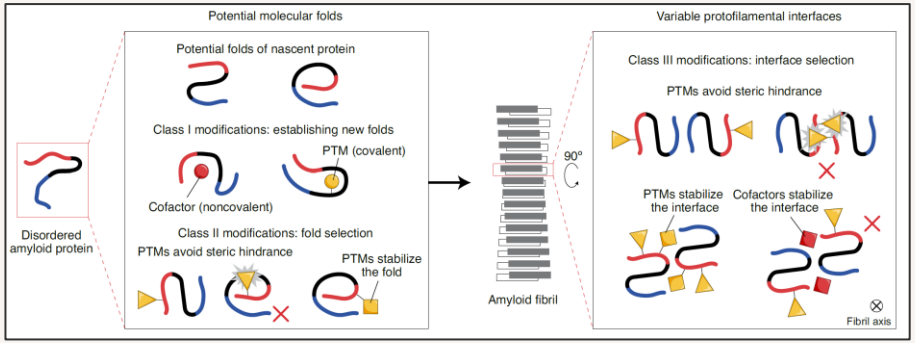
- From a single protein, Tau, α-syn, Aβ, and mutated huntingtin (HTT) can form multiple fibril structures that correspond to unique NDs, they also have self-replication and propagation properties.
- Brain regions and cell types yield different amyloid fibril structures which cause distinct NDs and clinical representations:
    - α-Synuclein: Different fibril structures in MSA and PD/Lewy body dementia
    - Tau: Cryo-EM has shown that there are structurally unique Tau fibril folds in AD, Pick's disease, and CTE, this is linked to regional brain damage
    - Aβ: Structural variations between AD subtypes was confirmed by ssNMR
- Amyloid structure is highly sensitive to environmental conditions:
    - *In vitro* - varying factors such as protein concentration, pH, salt, and temperature yield different polymorphs
    - *In vivo* - more complex as PTMs, cofactors, and cell type dictate fibril structure
- *In vivo* fibril structures are not replicable *in vitro*; this indicates how sensitive amyloid fibril assembly is to the environment.



- The *in vitro* fibrils are missing PTMs and cofactors resulting in smaller, less complex cores.
- Further research is needed for both *in vitro* and *in vivo* amyloid fibrils

Hierarchical regulation by chemical modifications

- Diversity in fibril structures is due to genetic mutations, PTMs, cofactors, and their environment.
- PTMs affect protein structure, stability, localisation, and activity via covalent bonding, they include phosphorylation, ubiquitination, glycosylation, acetylation, SUMOylation, acylation, methylation, nitration, and truncation.
- Molecular cofactors regulate amyloid protein aggregation via noncovalent interactions, they include metal ions, lipids, and organic small molecules.
- A hierarchical regulation of PTMs and cofactors in amyloid fibril formation is proposed:
  - Class I: within the interior of the fibril core, defines protein folding in the initiating step of fibril assembly
  - Class II and III: on the exterior surface of the fibril core, selects the fibril polymorphs during molecular folding and protofilament packing respectively
  - Class IV: absent from the fibril core, occurs on residues that are not in the fibril core, uncertain influence on the fibril assembly, evidence indicates it could affect conformation selection or helical elongation

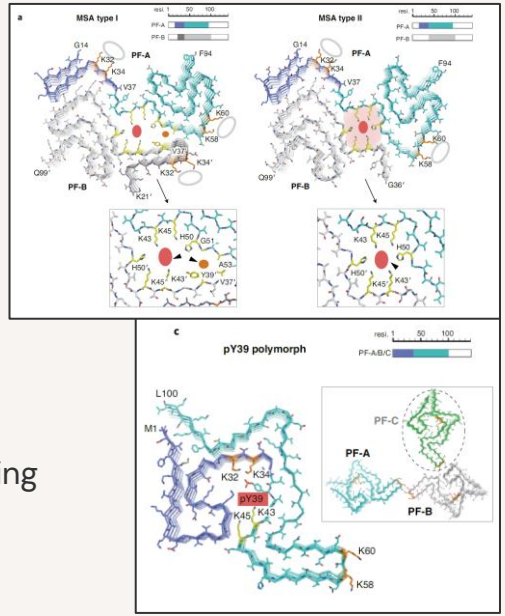


Class I modifications - new fibril conformations

- Class I modifications occur within the fibril core and fundamentally alter protein folds

α-Syn in MSA and PD

- Cryo-EM shows that there are two α-syn fibril types, MSA type I and II, they both contain unidentified cofactors in the core which likely have a negative charge as they interact with lysines and histidines (K43, K45, H50), these give order to disordered N-terminal regions as well as enlarge the fibril core and define the polymorphic structure.
- MSA types differ in their cofactor cavity size and N-terminal stability.
- Identifying the cofactors is important to assess their role in NDs.



Phosphorylated α-Syn (pY39)

- pY39 α-syn fibrils formed synthetically have electrostatic interactions with lysines (K32/K34/K43/K45), incorporating the full N-terminus into the core, the wild-type α-syn cannot adopt this structure thus confirming its PTM-dependence

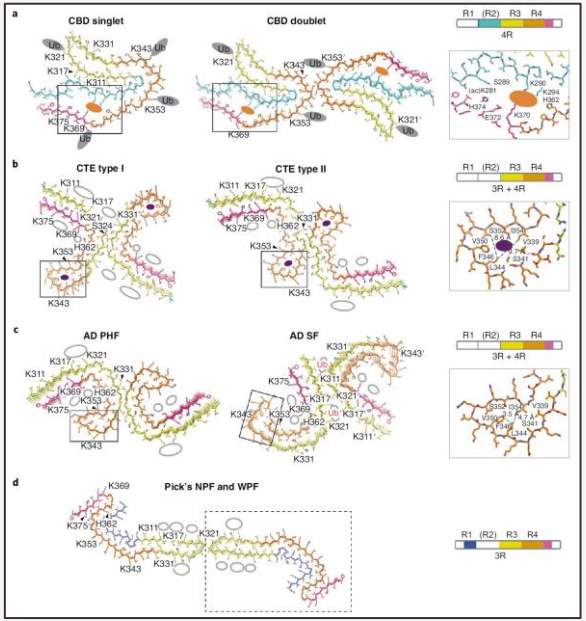
Class I modifications - new fibril conformations

Aβ Fibrils with pS8

- Aβ fibrils from different AD subtypes show high structural variability.
- Phosphorylation at S8 (pS8) form intramolecular interactions stabilising the dynamic N-terminus by folding it in towards the core, enlarging the core and creating a more rigid and stable polymorph which is distinct from unmodified Aβ fibrils.

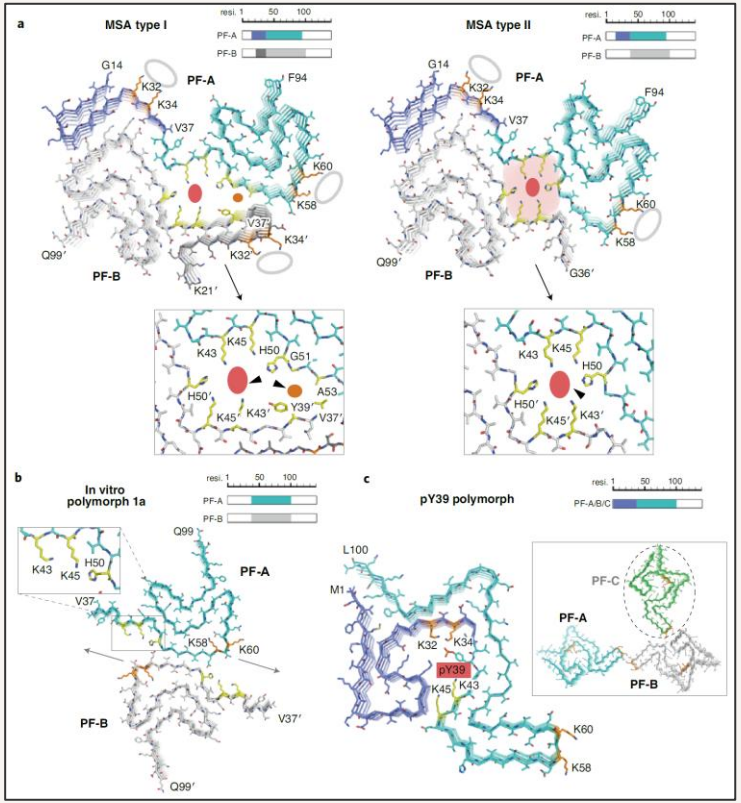
Tau Fibrils and Disease-Specific Cofactors

- CBD fibrils contain an unidentified acidic cofactor in a cavity within the core located at R2 of 4R Tau.
- CTE fibrils contain an unidentified, but distinct, hydrophobic cofactor in a β-helix motif - likely a fatty acid or sterol.
- AD fibrils have the same β-helix motif as CTE, but more tightly packed preventing the binding of additional molecules.
- Pick's disease fibrils are formed from 3R Tau and thus have a different fold that excludes CBD/CTE cofactors.



Class I modifications - new fibril conformations

- Cofactors are present within the fibril core and form multiple interactions with the amyloid protein. This indicates that cofactors are a part of fibril assembly at an early stage as well as during the subsequent seeded propagation. Therefore, these cofactors are promising therapeutic targets for the prevention of neurodegenerative diseases.
- *In vitro* fibril models are missing cofactors and thus have simpler cores due to simplified conditions.
- More research is needed to be able to accurately model *in vivo* models *in vitro*.



Class II & III modifications - conformation selection

- Cryo-EM reconstructions of postmortem brain amyloid fibrils show fuzzy densities on the surface of fibril cores for α-syn fibrils of MSA type I and type II as well as in various Tau fibrils.
- Many of these densities are unidentified, but they are PTMs and cofactors that bind with the fibril
- Surface modifications have a key role in conformation selection:
    - Class II: PTMs stabilise and destabilise local folds showing different patterns of PTMs and folding
    - Class III: PTMs and cofactors modulate protofilament packing interactions
- Examples from Tau fibril structures:
    - K311 and K317: In CBD fibrils, they form internal interactions with R2, contributing to structural stability. In AD, CTE, and Pick's, they're exposed to solvents and modified (ubiquitinated).
    - K331: Unmodified in some structures including CTE type II and AD-PHF, stabilising the protofilament interface. Modified in other polymorphs, preventing that interface from forming.
    - K343: Ubiquitinated in CBD singlet, but not in CBD doublet.
    - S324: Found phosphorylated in AD, it may explain why CTE type I's interface is absent in AD fibrils.
- Distinct PTM and cofactor profiles on the fibril surface stabilise specific folds, favour certain conformations, and overall influence which distinct NDs develop
- Surface modifications offer targets for strain-specific therapeutics

Class IV modifications - indirect influences

- Class IV modifications occur on residues outside of the fibril core that influence fibril behavior and modulate, but do not define, fibril structure.
- These modifications can influence fibril elongation and seeding by altering terminal region stability, modify fibril morphology (twist and straight shapes), affect aggregation speed, and often are dependent on environmental factors such as pH and ion concentration.
- Examples of class IV modifications:
    - S129 α-syn phosphorylation - alters fibril morphology
    - N-terminal acetylation - correlates with the formation of specific fibril strains
    - C-terminal truncations - preserves core structure but alters terminal dynamics and aggregation kinetics

Author's conclusion and future directions

- Different genetic backgrounds and aging lead to different PTM patterns which vary the kinetics and structures of amyloid aggregation and NDs.
- Cofactors and PTMs have a key role in shaping fibril structures, but understanding is still limited.
- Cryo-EM studies show potential for structure-based drug design, for example, EGCG, a natural compound known to inhibit amyloid formation, is able to bind to Tau fibrils.

Future research:

  - Understanding the mechanism of *in vivo* amyloid aggregation and neurotoxicity.
  - Comparing PTMs under normal conditions may provide a new strategy for diagnosis and treatment of NDs.
  - Determine the atomic structure of modified amyloid fibrils for a better understanding and early diagnosis as well as to design compounds that inhibit disease-related cofactor binding in order to prevent NDs.
  - Determining the chemical nature of PTMs and cofactors is crucial so they can be included in *in vitro* fibril models, this would better model *in vivo* fibrils and reveal unknown structures.
  - New chemical technology such as fast-flow automated protein synthesis could be used to prepare amyloid proteins with varied PTMs.

- Targeting metabolic abnormalities and restoring cofactor and PTM balance may be a new therapeutic route compared to the unsuccessful approaches targeting amyloid proteins directly.

Evaluation and conclusion

Feedback

+ Clearly explains how PTMs and cofactors contribute to amyloid polymorphism and ND heterogeneity.
+ Organises a complex topic through a hierarchical classification system (Class I–IV), which helps in understanding how different chemical modifications influence fibril structure.
^ This paper could benefit from clearer chemical identification of cofactors and using the visual aids more effectively, linking modification classes to disease-specific fibril structures.

- Overall, the research paper provides an effective and well-structured overview of how chemical modifications regulate amyloid fibril polymorphism. It highlights the critical role of PTMs and cofactors in neurodegenerative disease pathology and presents the idea that chemical biology can be used as a tool for future therapeutic development and ND prevention. Furthermore, it notes key areas within amyloid research that require further investigation.

References

Li, D., Liu, C. (2024). *Hierarchical chemical determination of amyloid polymorphs in neurodegenerative disease*. Published: February 7, 2024. Accessed: July 26, 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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