

INTRODUCTION

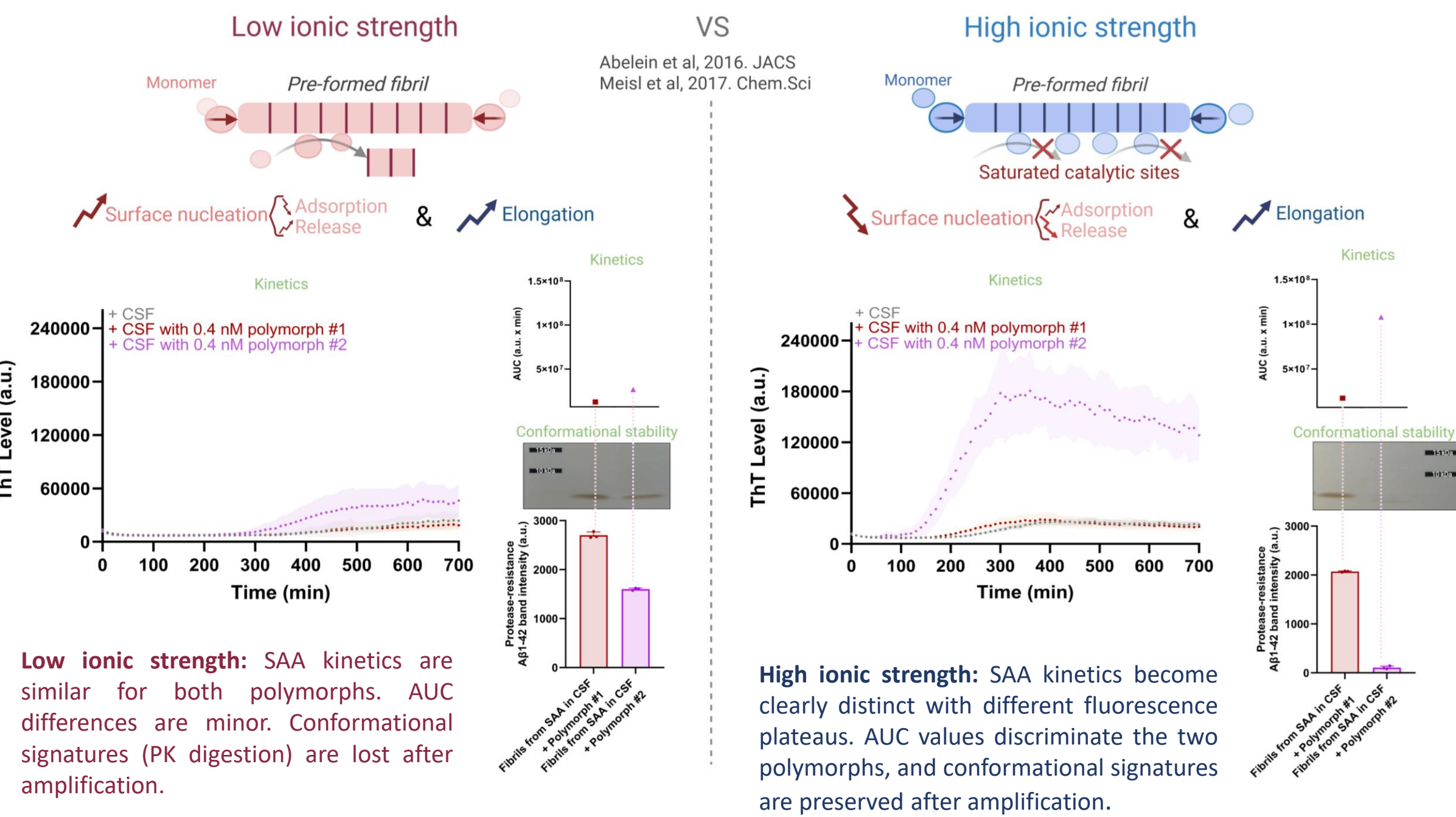
Alzheimer's disease (AD) is characterized by the aggregation of amyloid- β (A β) peptides into structurally distinct **fibril polymorphs** that might differ in toxicity, seeding properties, and clinical impact. Conformational heterogeneity may contribute to patient-specific disease progression and therapeutic response.

Seeded amplification assays (SAAs) enable ultrasensitive detection of protein aggregates in biofluids. However, identification of A β 1–42 polymorphs remains challenging due to secondary nucleation, which can homogenize fibril conformations.

Objective: To optimize an A β 1–42 SAA capable of preserving and discriminating polymorph-specific signatures using synthetic fibril polymorphs spiked into non-demented CSF, and to apply the optimized assay in a pilot study for stratifying Alzheimer's disease patients

RESULTS

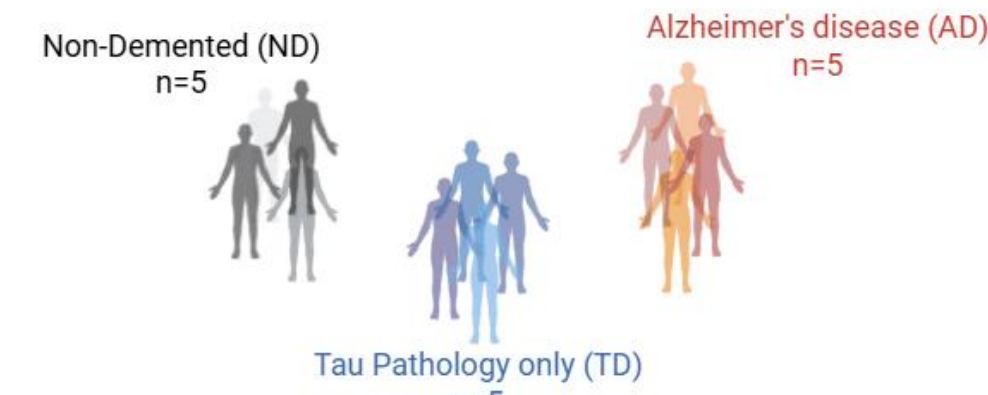
Optimized A β 1–42 SAA Enables Discrimination of Fibril Polymorphs in CSF



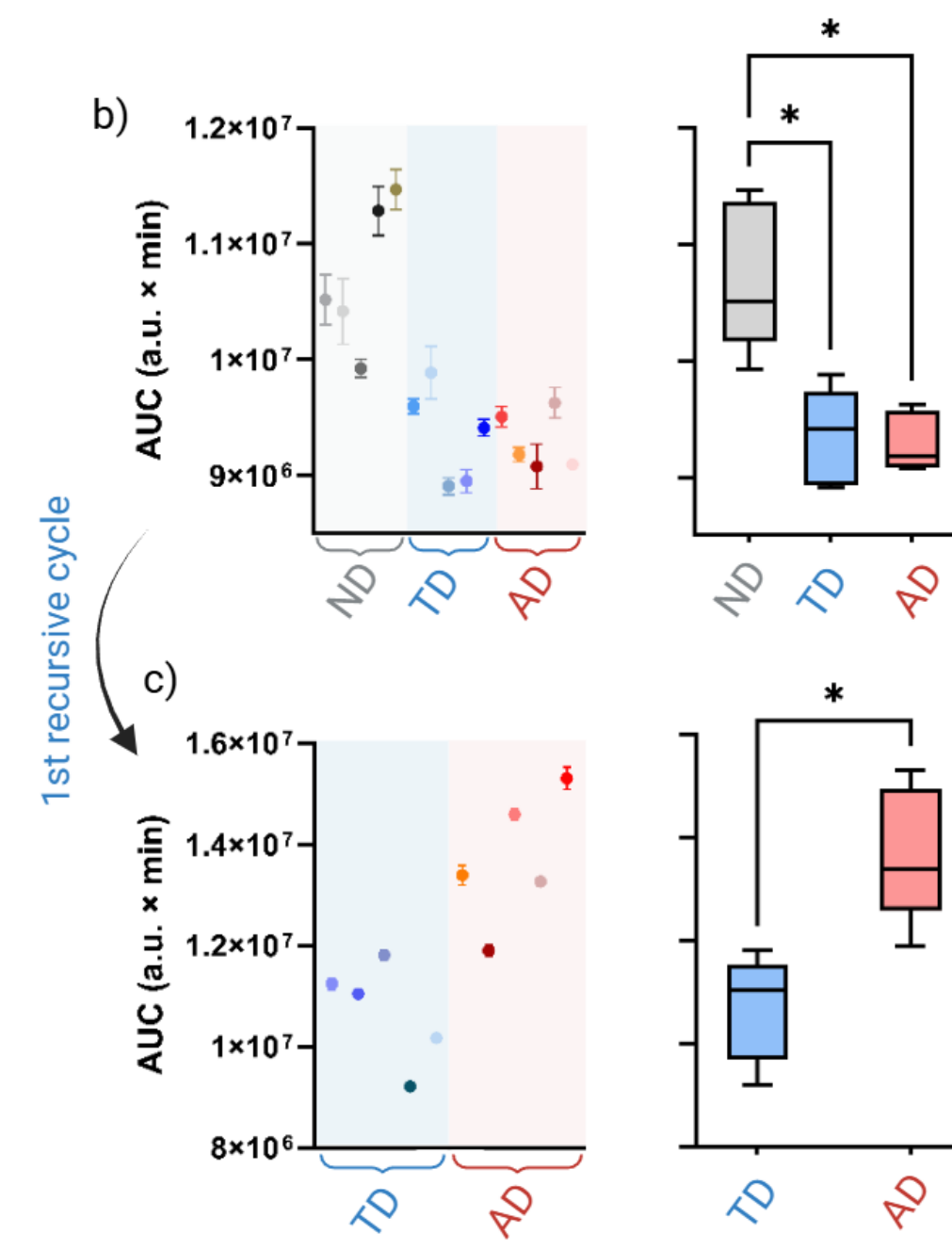
PILOT STUDY – Application to Patient CSF

Discrimination of Alzheimer's Disease From Other Neurodegenerative Diseases

AUC values obtained by SAA for non-demented individuals (n=5), tauopathy patients (n=5), and Alzheimer's disease patients (n=5).

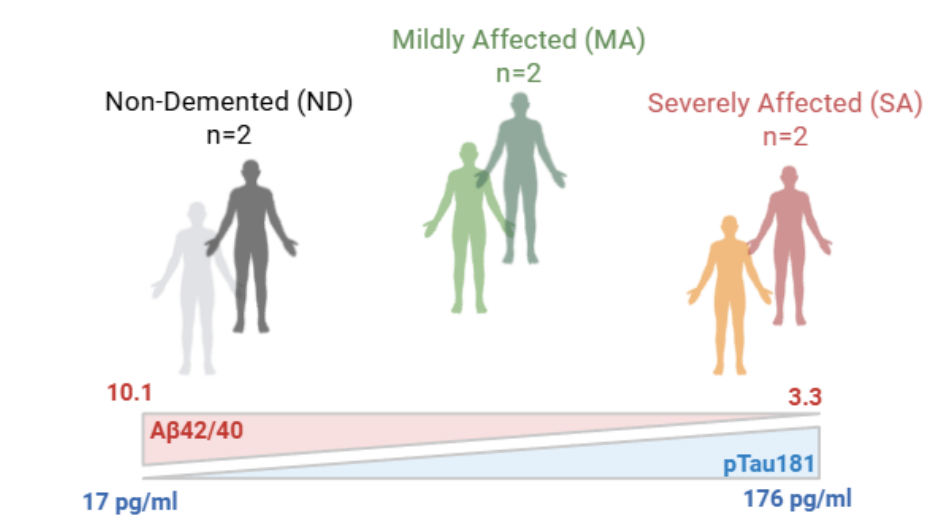


After a first amplification cycle, the resulting fibrils were re-seeded at high concentration (10%), promoting elongation-driven amplification and improving group discrimination.

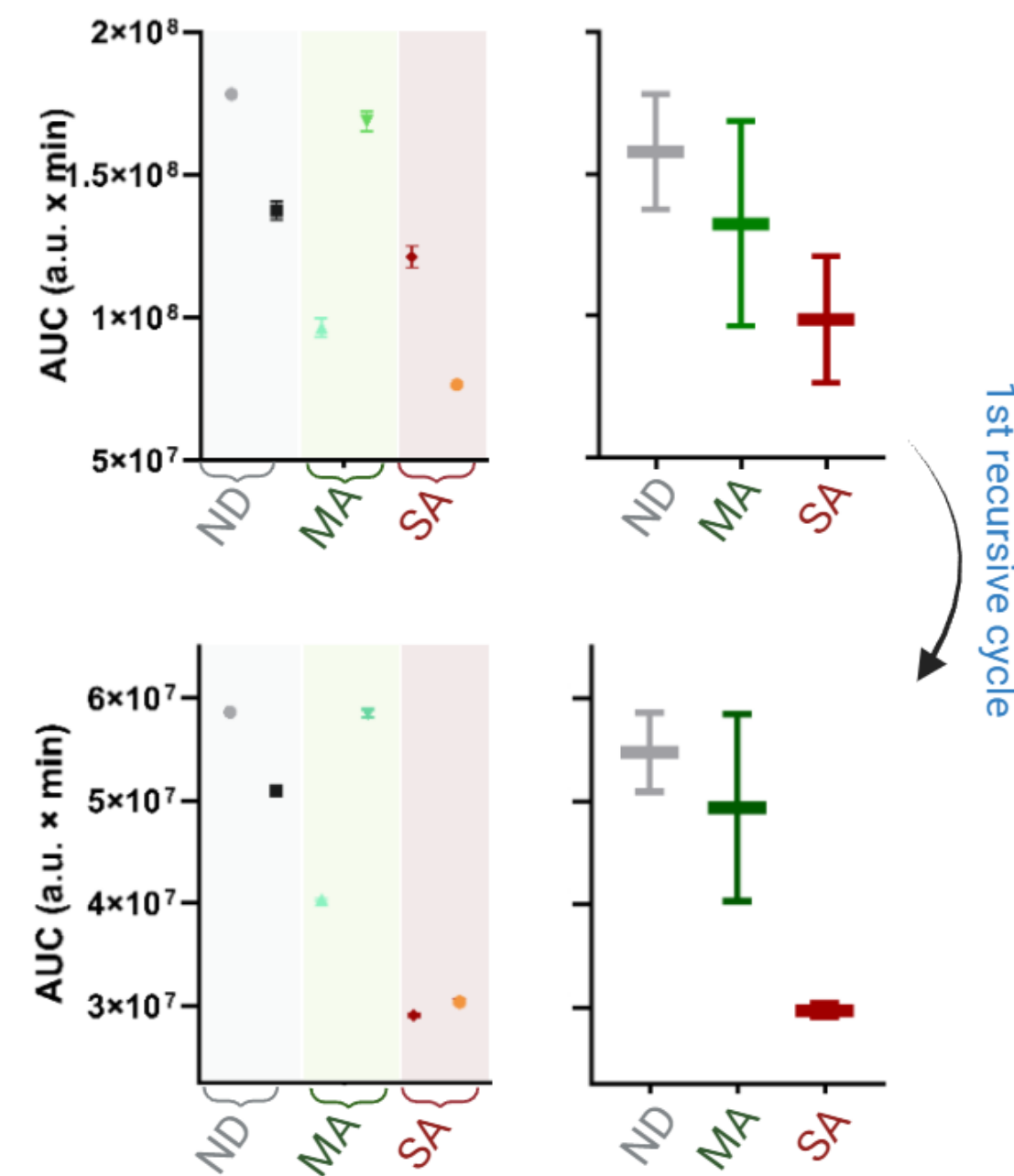


PILOT STUDY – Stratification of AD individuals

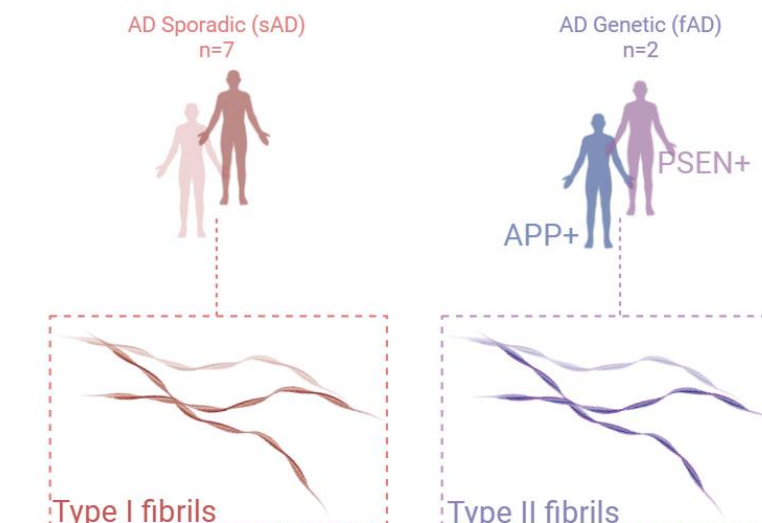
SAA Reflects Amyloid Burden



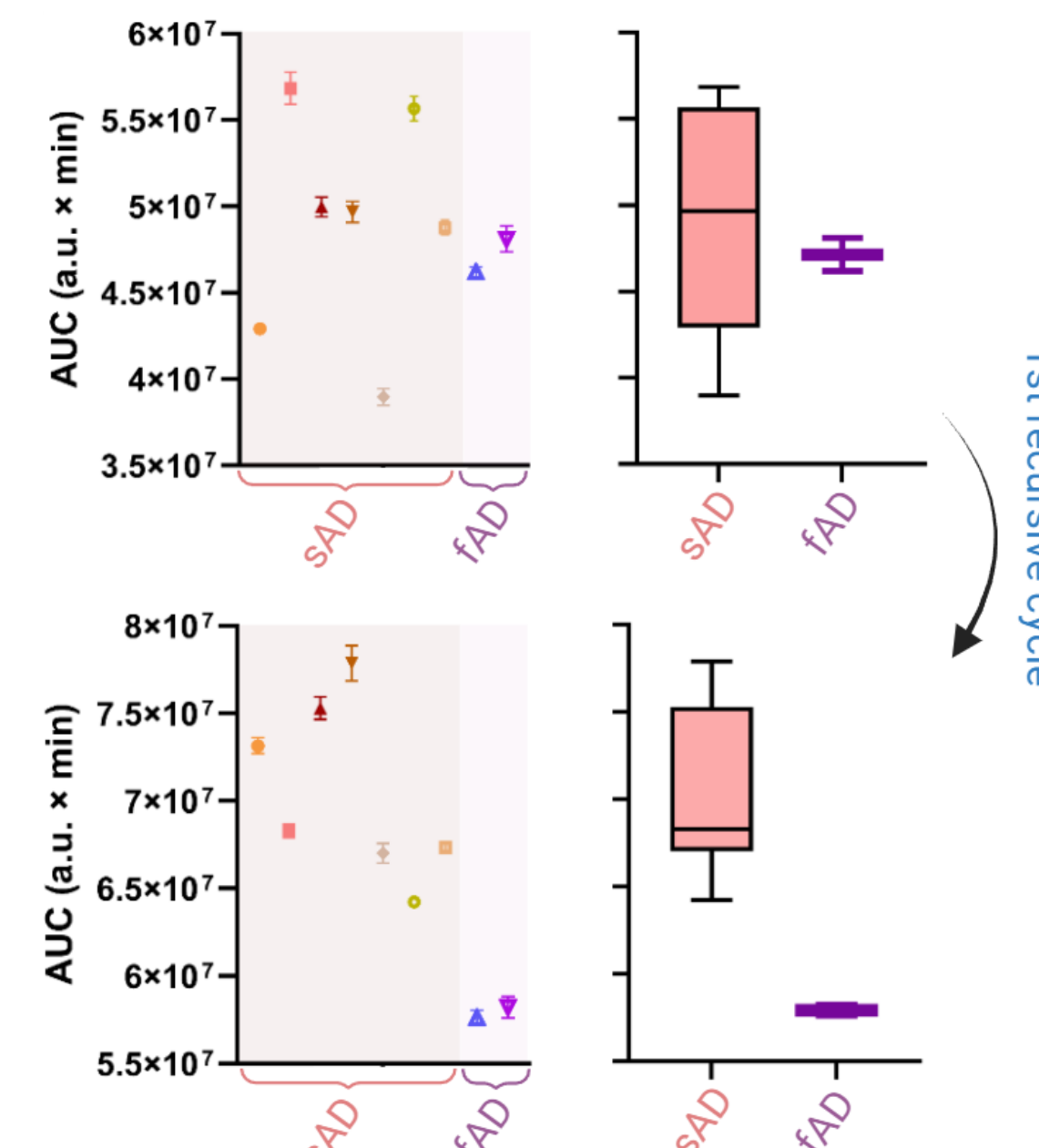
SAA enabled an initial stratification based on amyloid burden. After one recursive amplification cycle, group separation became more pronounced, allowing clear discrimination between non-demented individuals and high-burden AD patients. Individuals with intermediate burden displayed intermediate profiles. Results should be interpreted with caution given the limited sample size.



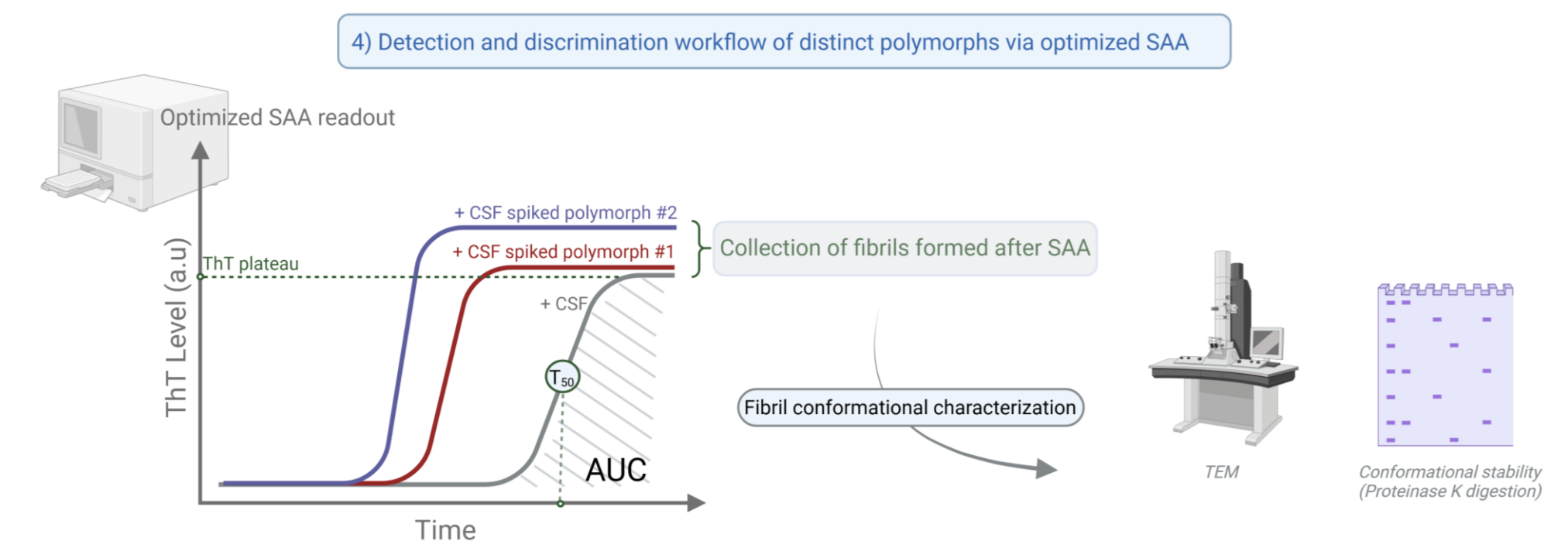
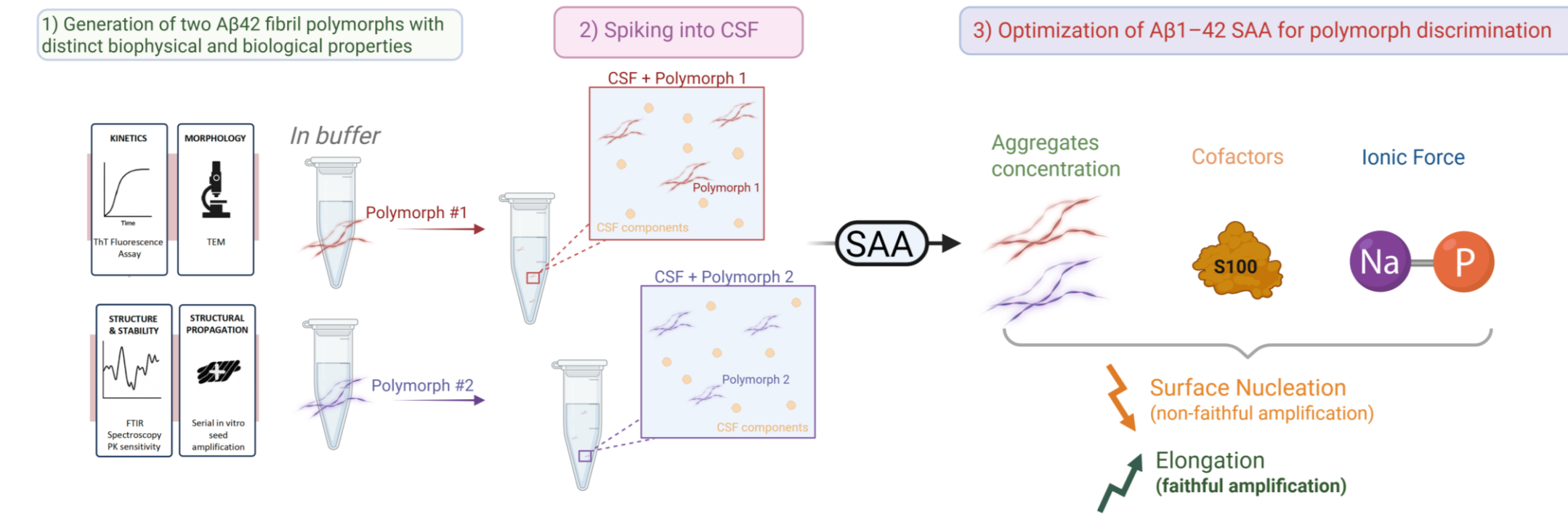
Stratification of Alzheimer's Disease Patients Based on Amyloid Polymorphs: Comparison of Sporadic and Genetic Cases



Distinct fibril polymorphs (Type I and Type II) were previously identified by cryo-EM in sporadic versus genetic Alzheimer's disease (3). Here, SAA-based AUC analysis enabled discrimination between these two cases, representing a proof-of-concept for A β 1–42 polymorph stratification in CSF. Validation in larger cohorts is required.



METHODS



Two distinct A β 1–42 polymorphs were generated and spiked into CSF from non-demented donors. Physicochemical parameters (ionic strength, seed concentration, cofactors) were optimized to favor elongation over secondary nucleation. Amplified fibrils were further characterized by TEM and conformational stability assays (PK digestion). Finally, the optimized conditions for polymorph discrimination were applied to patient CSF in a pilot study.

CONCLUSION

- 1) After SAA optimization (notably ionic strength), both synthetic A β 1–42 polymorphs were discriminated in CSF in **< 24 h**, based on AUC kinetic readouts extracted from fluorescence curves.
- 2) The assay stratified patient cohorts across the AD continuum and discriminated **sporadic vs. genetic AD** — which are known to harbor distinct A β fibril polymorph profiles.
- 3) This opens the path to **SAA-based patient stratification** driven by amyloid conformational polymorphs, suspected to influence clinical phenotype and therapeutic response.
- 4) A polymorph-sensitive SAA could represent a valuable tool for the **development and monitoring of targeted therapeutic strategies**.

REFERENCES

- 1) Ionic Strength Modulation of the Free Energy Landscape of A β ₄₀ Peptide Fibril Formation. Abelein et al, 2016. JACS
- 2) Modulation of electrostatic interactions to reveal a reaction network unifying the aggregation behavior of the A β 42 peptide and its variant. Meisl et al, 2017. Chem.Sci
- 3) Cryo-EM structures of amyloid- β 42 filaments from human brains. Yang et al, 2022. Science

CONTACTS

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