# Use of *in vitro* cell models for assessing alpha-synuclein aggregate morphology and other polymorphic amyloid variants using amyloid specific fluorescent ligands

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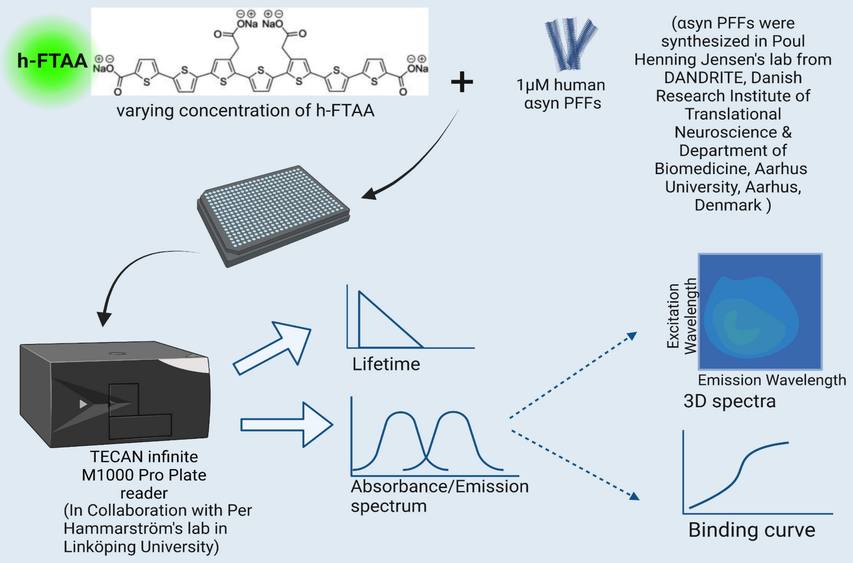
## Aims, research questions and working hypothesis:

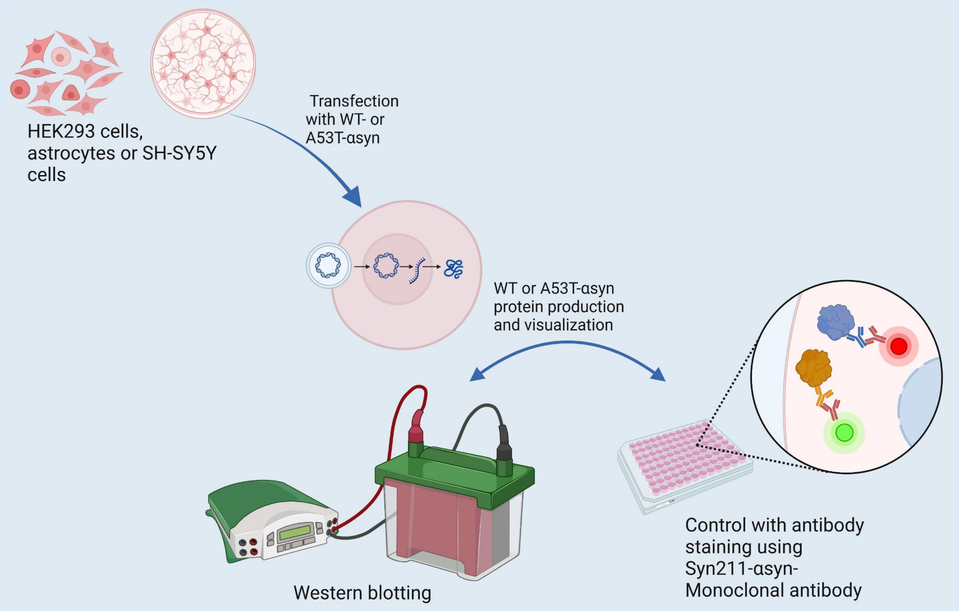
The Parkinson's Disease (PD) is the fastest growing neurodegenerative disorder affecting mainly the elderly. PD involves dysregulated molecular alterations in proteins that are crucial for the proper functioning of our brain and peripheral organs. Alpha-synuclein (αsyn) misfolding is the primary underlying protein pathology in PD. αsyn undergoes extensive structural rearrangement into different amyloidogenic morphotypes (also called strains) with variable toxicity to different cell types. αsyn strains are able to propagate bidirectionally through the autonomic connectome, affecting multiple organs. The clinical representation of PD patients is highly heterogenous at the early disease stages, complicating diagnosis. The location of the very first αsyn strain deposit may impact the structure of the amyloidogenic morphotypes formed. In our project, we hypothesize that both the disease onset site (gut or brain) and the structure of the initial αsyn strain are interdependent determinants of the clinical representation of early PD. We aim to develop a new method for the detection and structural characterization of different αsyn strains. The currently available amyloid detection methods pose a caveat in identifying different amyloidogenic morphotypes. In our part of the PD-PAM project, we employ cell models to validate new conformation-specific fluorescent ligands (LCOs) for the structural characterization of αsyn strains. The Swedish and Danish partner have further validated these ligands *in vivo* as potential early disease indicator.

## Workplan and methodologies implemented:

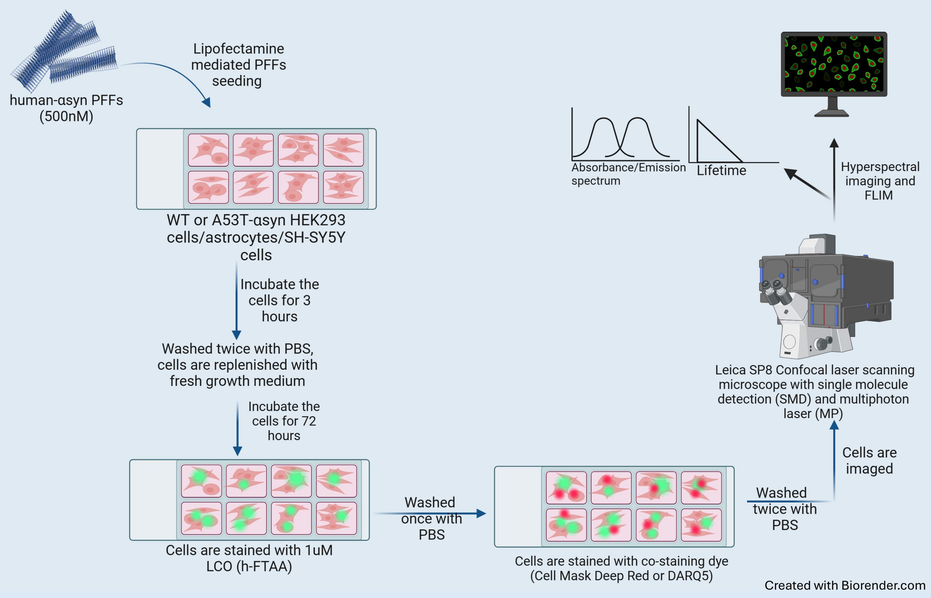
- Photophysical measurements of LCOs (h-FTAA) in different solvents and assessment of *in vitro* binding of LCOs to the αsyn pre-formed fibrils (PFFs)




- Transfection of cell models with either wildtype (WT) or A53T-mutated αsyn to induce endogenous expression of monomeric αsyn in cells

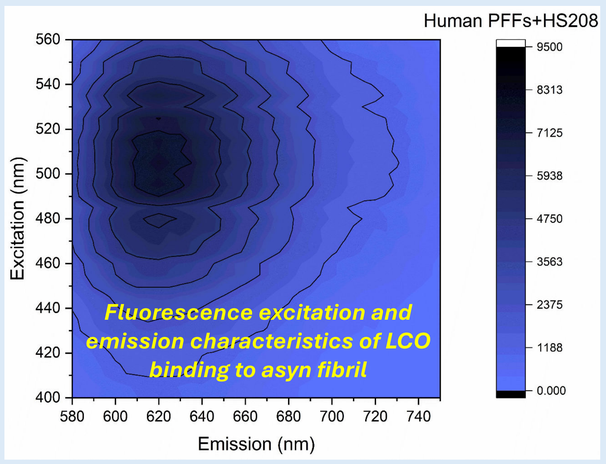


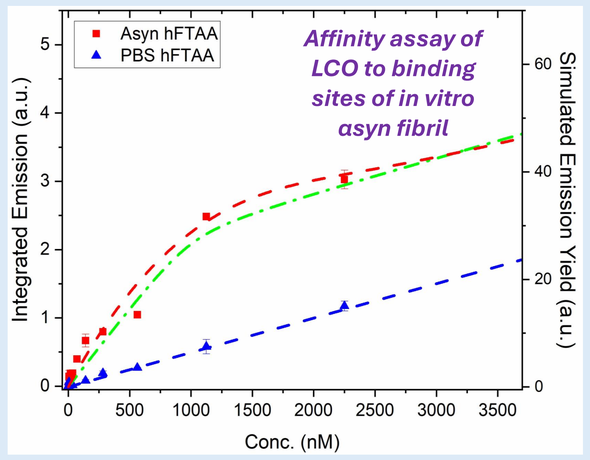

- Exposure of transfected cells to PFFs followed by staining with LCOs




## Milestones, representative key results:

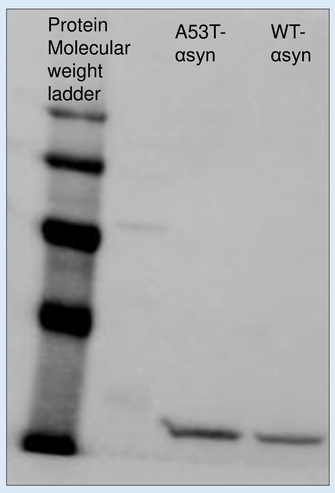
- Example: spectral assessment of h-FTAA and *in vitro* binding to αsyn pre-formed fibrils (PFFs)



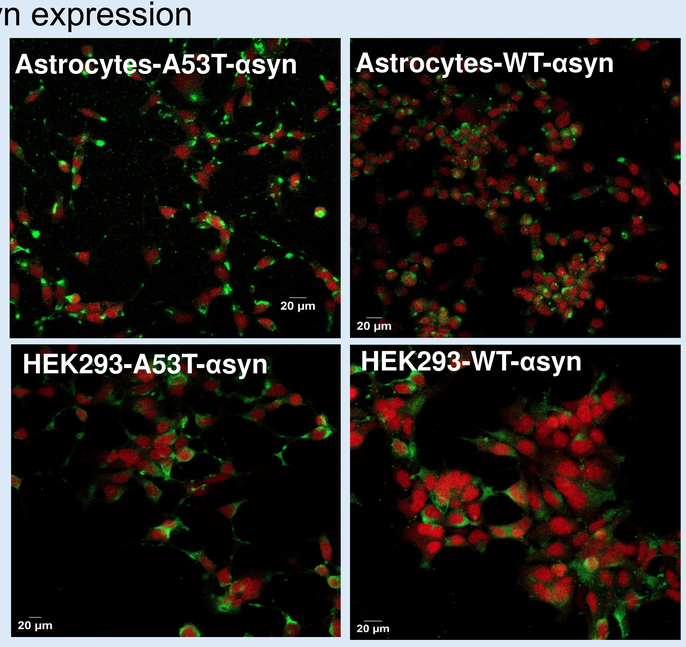




**For more details, see: P. Swaminathan et al. Int. J. Mol. Sci. 2024, 25(22), 12458.**

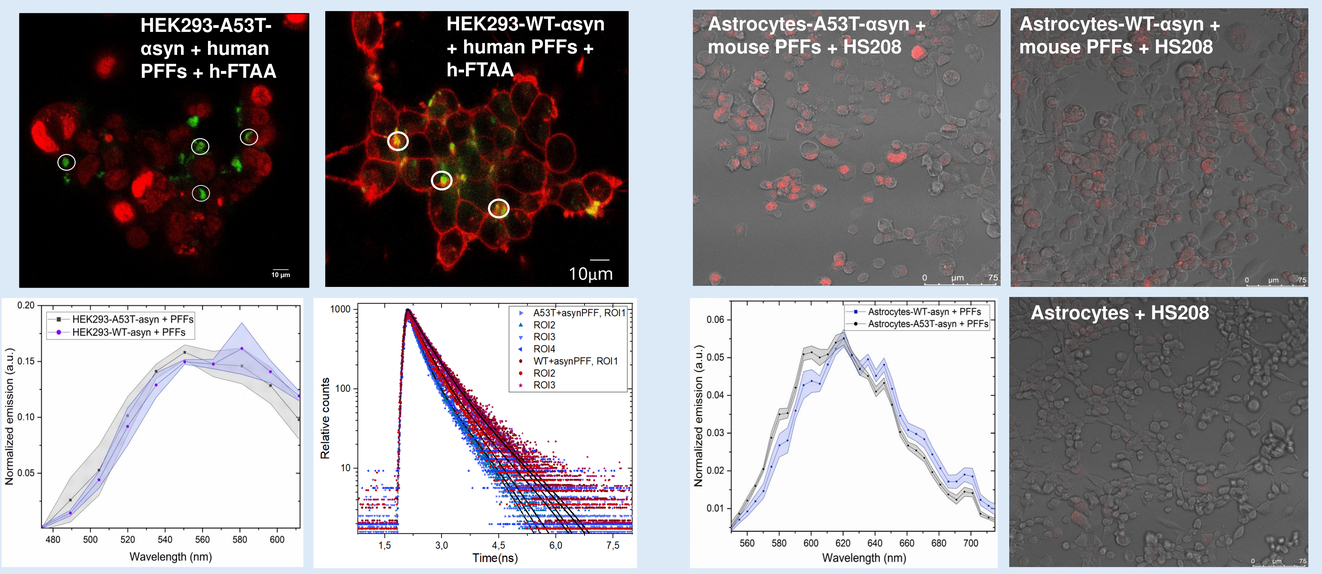
- HEK293 cells and astrocytes transiently transfected with wildtype-αsyn (WT) or A53T-mutated αsyn show endogenous αsyn expression




Representative western blot image showing αsyn expression in cells expressing WT-αsyn or A53T-αsyn




- Hyperspectral imaging and Fluorescence Life-Time Imaging (FLIM) of αsyn aggregate morphology in cells transfected with A53T-αsyn and WT-αsyn




h-FTAA $\Lambda_{ex}$= 475nm

HS208 $\Lambda_{ex}$= 530nm

## Significance and impact of the work on the field:

The ability of LCOs to detect and bind to distinct morphotypes of αsyn and other amyloid aggregates in cell-culture may allow stratification of subtype-specific conformations of αsyn in future. This might allow subtyping PD based on the structural variations observed between distinct αsyn morphotypes which could possibly create a large therapeutic window for early intervention in the prodromal phase.

## Future challenges:

This approach will also be extended further for *in vitro* evaluation of other polymorphic amyloid variants, such as, chiral insulin amyloid, lysozyme, amyloidβ by combining the use of different conformation-specific LCOs to understand structural changes of amyloids in cellular environment.