Subject: Amyloid-PAINT to study huntingtin exon 1 aggregate polymorphism

Project type: MEP

Supervision: Dr. Kristin Grussmayer, k.s.grussmayer@tudelft.nl

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Start date: as soon as possible, latest Nov-2024

Project background

Insoluble deposits of misfolded proteins that form aggregates are hallmarks of major neurodegenerative disorders such as Huntington's disease. Increasing data suggest that smaller aggregates cause a higher toxic response than filamentous aggregates (fibrils). However, the size of small aggregates has challenged their detection within biologically relevant environments. Covalent tags such as fluorescent fusion proteins have further been shown to influence molecular interaction, thereby altering aggregation kinetics and aggregate morphology. In this project, we will evaluate next-generation non-covalently binding dyes with an affinity to certain secondary or tertiary structures exhibited by the peptide (huntingtin peptide, Htt) and its aggregates involved in the formation of Huntington's disease. This research presents an exciting strategy to determine specificity of aggregate toxicity, domain relevance and post-translational modification influence within heterogeneous samples under truly physiological conditions.

Project goals and activities

The project is a new research line in the lab with initial protein production pilot experiments already conducted. It involves the advanced purification of Htt exon (HttEx1) and mutant variants of it, the evaluation of dye affinity and labelling suitability by means of confocal or TIRF and super-resolution fluorescence imaging (PAINT, dSTORM). Further, analysis of aggregation formation heterogeneity and super-resolution aggregate fingerprinting will be conducted. The project will be in close collaboration with the supervising PhD student (Moritz) and can be tailored to the student's interest.

Your profile

- student of biophyics, molecular biology, biochemistry or similar
- wet lab experience (intermediate, ideally in protein purification)
- coding experience (beginner, high-level programming languages)
- interested in optics & light microscopy
- independent, curious & organized

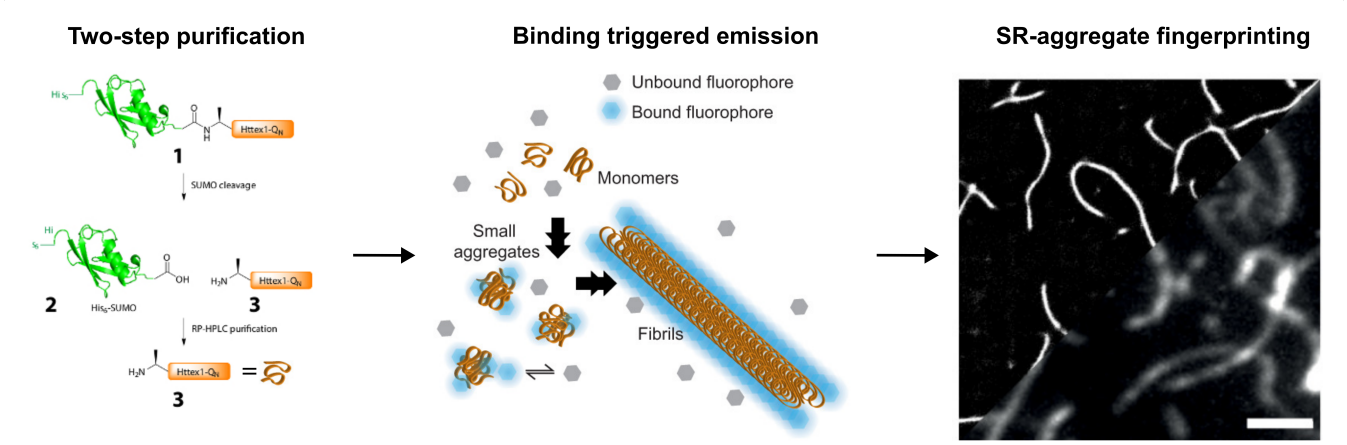

Figure 1: Project outline, including production of native untagged HttEx1 peptide via two-step His-tag and SUMO cleaving purifcation, evaluation of amyloid targeting dyes for labeling, and super-resolution aggregate fingerprinting to deduce key aspects of aggregate polymorphism. Figure adapted from Morten et al., PNAS, 2022