Internship Proposal

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Project Title:
Nanobodies as tools to find new therapies for Machado-Joseph disease: Structural studies

Level:

Master Student

Project Summary:

Machado-Joseph disease (MJD) is a rare neurodegenerative disorder caused by the abnormal expansion of a glutamine tract (polyQ) in the protein Ataxin-3 (Atx3). PolyQ expansion elicits a pathogenic cascade culminating in the appearance of cellular inclusions enriched in the mutant protein. Alterations in functional intermolecular protein interactions and self-assembly into toxic oligomers, likely acting in concert, have been proposed to contribute to initial pathogenesis. Despite intensive research in the last two decades, the mechanistic understanding of Atx3 function in health and the downstream neurotoxicity routes in disease is still limited, impairing the much-needed progress in therapy development.
The project aims to exploit a collection of Atx3-specific nanobodies available at the host lab, which outcompete Atx3 toxic, amyloid-promoting interactions. These molecules will constitute a toolbox with the ability to enable innovative strategies to approach this multifaceted disease.

Work to be developed by the student:

Protein engineering; Protein expression and purification in E. coli; Biochemical and biophysical characterization of Atx3 interaction with nanobodies (Thioflavin-T assays; binding kinetics determined by isothermal titration calorimetry); Bioinformatic analysis of Atx3 interaction with the nanobodies; and Structural determination by macromolecular X-ray crystallography and electron microscopy.

References:

1) A Robust Assay to Monitor Ataxin-3 Amyloid Fibril Assembly. Figueiredo F, Lopes-Marques M, Almeida B, Matscheko N, Martins PM, Silva A, Macedo-Ribeiro S. Cells. 2022 Jun 19;11(12):1969. doi: 10.3390/cells11121969.

2) MIRRAGGE - Minimum Information Required for Reproducible AGGregation

Experiments.

Martins PM et al., and Macedo-Ribeiro S. Front Mol Neurosci. 2020 Nov 27;13:582488. doi: 10.3389/fnmol.2020.582488. eCollection 2020.

3) Distribution of Amyloid-Like and Oligomeric Species from Protein Aggregation Kinetics.

Silva A, Almeida B, Fraga JS, Taboada P, Martins PM, Macedo-Ribeiro S. Angew Chem Int Ed Engl. 2017 Nov 6;56(45):14042-14045. doi: 10.1002/anie.201707345.

4) Polyglutamine expansion diseases: More than simple repeats.

Silva A, de Almeida AV, Macedo-Ribeiro S. J Struct Biol. 2018 Feb;201(2):139-154. doi: 10.1016/j.jsb.2017.09.006.

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