Exploring the interactome and composition of variants of proteasome complexes involved in NeuroDevelopmental Disorders using proteomics and MS approaches

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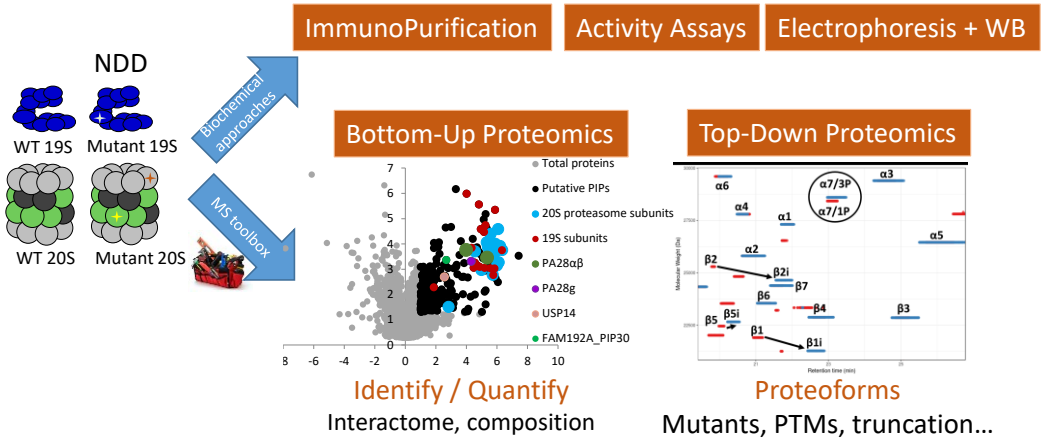
Background

Neurodevelopmental disorders (NDDs) are a major public health concern affecting 3-10% of children worldwide [1]. 10-15% of the genes involved in NDDs encode components of the ubiquitin-proteasome system (UPS) [2]. This pathway is essential for the proteostatic equilibrium of eukaryotic cells. It ensures the specific degradation of denatured proteins that have become non-functional or even toxic. The UPS has been shown essential to neuronal development and function. The recognition of UPS-related NDDs (UPS-NDDs) is fairly recent, and, although about 1,200 genes contribute to the UPS pathway, amongst which many are highly expressed in the brain, only a limited number of UPS-related NDDs (UPS-NDDs) have been identified to date. 150 pathogenic or likely pathogenic variants are associated with **NDD in 17 UPS genes** and of which **8 genes are proteasomal subunits**. Biological samples from 67 families from child and unaffected parents, have been collected by our collaborators at the CHU of Nantes, resulting in a biobank of 140 individuals. Preliminary functional studies from affected individuals T cells showed **impaired proteolytic activity and/or assembly of proteasome 26S** caused by *PSMD12*, *PSMC3*, *PSMC5*, *PSMB3* or *PSMB5* variants.



The main objective of this project is to study the consequences of the mutations in NDD-UPS genes on proteasome complexes composition (20S core subunits as well as 20S-associated regulators), proteasome assembly, but also on the proteasome interactome, as these three levels of regulation are known to affect proteasome cellular activity both in physiological and pathological situations [3].

The experiments will involve:

- The biochemical purification of endogenous 20S proteasome [4],
- The functional characterization of purified proteasome from mutant T cells and their controls,
- Their analysis using bottom-up proteomics and top-down proteomics using already established methods [4].

References

[1] Ebstein et al. MedRxiv. (2021). [2] Alvarez-Castelao et al. (2015). [3] Coux O. *et al*. (2020) Adv. Exp. Med. Biol. [4] Fabre et al. (2015) Mol. Sys. Biol. ; Zivkovic D. *et al* (2022) PNAS.

Techniques used during the internship:

- Protein analysis (SDS-PAGE, Western Blot), Protein complexes purification (immunopurification) and biochemical enrichment (Size Exclusion Chromatography),
- Proteasomal enzymatic activity measurement (Fluorescence-based kinetic assay),
- Bottom-up and top-down mass spectrometry analysis (nanoHPLC ESI-MS/MS on different types of Orbitrap mass spectrometers),
- Mass spectrometry-based differential quantification and statistical analysis.