SCIENCES & BIOENGINEERING SCIENCES

The Research Group
**Structural Biology Brussels**

has the honor to invite you to the public defence of the PhD thesis of

# Kevin Muwonge

to obtain the degree of Doctor of Bioengineering Sciences

Title of the PhD thesis:

**Deciphering the role of ubiquitination in phase transitions in Amyotrophic lateral sclerosis and Frontotemporal dementia**

Promotors:
**Prof. dr. Remy Loris**
**Prof. dr. Peter Tompa**

The defence will take place on

**Friday, November 22, 2024 at 4 p.m. in auditorium I2.0.2, Building I, VUB Campus, Etterbeek.**

**Members of the jury**

Prof. dr. Eveline Peeters (VUB, chair)
Prof. dr. Luc Leyns (VUB, secretary)
Prof. dr. Stefan Magez (VUB)
Prof. dr. Sandrine Da Cruz (KULeuven)
Prof. dr. Patrik Verstreken (KULeuven)

## Curriculum vitae

Kevin Muwonge earned his BSc in Biomedical Laboratory Technology from Makerere University (Uganda) and worked as a Research Assistant before pursuing an MSc in Molecular Biology at Vrije Universiteit Brussel (VUB). In 2018, he began his PhD at VUB, focusing on ubiquitin-mediated interactions in neurodegenerative diseases, particularly ALS/FTD. His PhD entailed authoring publications, developing innovative strategies for expressing and purifying challenging disordered proteins, and designing a microplate assay to screen Tau condensate modulators. During his PhD, Kevin also supervised a master's student.

## Abstract of the PhD research


Amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) are two closely related neurodegenerative diseases characterized by the presence of ubiquitinated protein inclusions in affected neurons. Traditionally, ubiquitination has been viewed as a marker of the cell's inability to clear misfolded or aggregated proteins. However, this thesis hypothesizes that at earlier stages of the disease, ubiquitination may facilitate multivalent interactions that modulate the liquid-liquid phase separation (LLPS) and aggregation of inclusion body proteins. A systematic analysis of ALS inclusion proteins revealed an enrichment of protein-protein interactions and ubiquitin-binding proteins within the inclusion proteome, suggesting that ubiquitination could play a broader role in inclusion body dynamics beyond its canonical role in protein degradation.

This PhD research presents the first evidence that Tau protein has an intrinsic ability to interact with ubiquitin. Advanced biophysical techniques were used to determine the binding affinity and map specific interaction sites on both Tau and ubiquitin, supporting the hypothesis that ubiquitin-binding proteins and ubiquitin-mediated interactions are integral to the phase transitions underlying ALS/FTD inclusion body formation. These findings offer novel insights into the molecular mechanisms of Tau pathology, indicating that non-covalent interactions with ubiquitin could modulate LLPS and aggregation, processes central to the pathology of neurodegenerative diseases such as ALS, FTD, and Alzheimer's disease (AD).

Notably, this thesis demonstrates that ubiquitin exerts dual effects on Tau's behavior. While ubiquitin exhibits an inhibitory effect on Tau LLPS, it paradoxically enhances the aggregation of the FTD-associated Tau-P301L mutant. This dual role suggests a complex regulatory mechanism whereby ubiquitin-mediated interactions could slow down phase separation under certain conditions but promote aggregation in pathological contexts. Such dual functionality sheds light on how ubiquitination might regulate biomolecular condensate formation, potentially contributing to inclusion body formation during ALS/FTD progression.

To complement these findings, a novel microplate reader assay was developed to confirm ubiquitin's inhibitory effect on Tau LLPS. This assay also provided a platform for screening other potential modulators of Tau LLPS, enabling high-throughput identification of therapeutic candidates. Additionally, technical challenges inherent to studying disordered proteins were addressed in this thesis through the development of a tandem tag purification strategy and optimization of the expression of aggregation-prone recombinant proteins. These methodological advances offer valuable tools for future research on protein phase separation and aggregation, particularly relevant to neurodegenerative diseases like ALS/FTD, AD, and others.