SCIENCES & BIOENGINEERING SCIENCES

The Research Group

Structural Biology Brussels

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

Joris Van Lindt

to obtain the degree of Doctor of Bioengineering Sciences

Title of the PhD thesis:
***In vitro* Modelling of Liquid Liquid Phase Separation in Amyotrophic Lateral Sclerosis**

Promotors:
Prof. dr. Peter Tompa
Prof. dr. Dominique Maes

The defense will take place on
Friday, May 12, 2023 at 17h in auditorium I.0.02

Members of the jury

Prof. dr. ir. Remy Loris (VUB, chair)
Prof. dr. ir. Wim De Malsche (VUB, secretary)
Prof. dr. ir. Jo Van Ginderachter (VUB)
Prof. dr. Frank De Proft (VUB)
Prof. dr. Ludo Van Den Bosch (KULeuven)
Prof. dr. Sandra Macedo Ribeiro (Institute for Molecular and Cellular Biology, Porto, Portugal)

Curriculum vitae

Joris Van Lindt obtained his degree of Master of Science in Biology: Molecular & Cellular Life Sciences at the Vrije Universiteit Brussel in 2018. After graduating, he obtained a FWO fellowship "aspirant fundamental research" and started his PhD in the Research Group of Structural Biology. Joris's work resulted in six international journal articles, two talks and several poster presentations at (inter)national conferences. During his PhD trajectory, he supervised one master thesis students and guided different practical courses for bachelor and master students.

Abstract of the PhD research

In addition to membrane bound organelles, cells also contain so-called membraneless organelles (MLOs), for example stress granules. These MLOs are formed through liquid liquid phase separation (LLPS). MLO misregulation often leads to devastating diseases. One of such diseases is Amyotrophic Lateral sclerosis (ALS), an uncurable neurodegenerative disease that leads to progressive paralysis and death. ALS is characterized by the presence of protein aggregates in the affected neurons. Many of the proteins involved in ALS have been shown to undergo LLPS. In addition, many functions related to LLPS, such as protein aggregation, stress response, DNA damage response and local translation are found to be impaired in ALS, highlighting the involvement of LLPS in ALS pathology.

As ALS is uncurable, better understanding of the disease pathology is needed. To this end, the LLPS-behavior of heterogeneous nuclear ribonucleoprotein A2B1 (hnRNPA2) was studied. hnRNPA2 is an abundant, LLPS-prone protein, which also tends to interact with other proteins often mutated in ALS patients. The main aim of the study was to determine the phase separation behavior of purified hNRNPA2. The influence of nucleic acids was studied as well as the phase separation behavior of hnRNPA2 in cells.

First, a novel strategy for the purification of disordered phase separating proteins was developed. This method was compared to most common methods using a combination of microscopy and biophysical assays.

MLOs formed by the disordered domain of hnRNPA2 lacking an RNA binding domain were found to recruit RNA *in vitro*. Aromatic amino acids tyrosine and phenylalanine were identified as the main drivers of this interaction.

hnRNPA2 was also identified to be involved in the osmotic stress response, where it translocates to stress granules, and to the perinuclear area inside the nucleus.

While the PhD focuses on hnRNPA2, the results can be broadened to LLPS of disordered domains in general, because the underlying mechanisms are often shared with other RNA-binding proteins.

This thesis thus leads to a better understanding of the role of disordered domains and nucleic acids in LLPS.