SCIENCES & BIOENGINEERING SCIENCES

The Research Group

Structural Biology Brussels (SBB)

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

Cesyen Augusto CEDENO MOGOLLON

to obtain the degree of Doctor of Bio-engineering Sciences

Title of the PhD thesis:

Structure and function analysis of two Early Response to Dehydration Proteins, intrinsically disordered proteins preventing the collapse of the proteostasis network during abiotic stress in plants

Promotors:

Prof. dr. Peter Tompa
Prof. dr. Gustavo J. Gutierrez

The defence will take place on

Friday September 7 **2018 at 13:30h**

in Auditorium D.0.02 at the Campus Humanities, Sciences and Engineering of the Vrije Universiteit Brussel, Pleinlaan 2 - 1050 Elsene, and will be followed by a reception.

Members of the jury:

Prof. dr. Geert Angenon (chairman)
Prof. dr. Patrick Vanderheyden (secretary)
Prof. dr. Jean-Pierre Hernalsteens
Prof. dr. Caroline Smet-Nocca (Lille, France)
Prof. dr. Emilia Pedone (University of Naples, Italy)

Curriculum vitae

Cesyen Cedeno graduated as a Chemist from the Universidad de Los Andes, Mérida, Venezuela in 2010. He then joined the Research Structural Biology group at the VIB-VUB with the help of a Marie Curie fellowship (IDPbyNMR program). In the context of his PhD, Cesyen attended several international (European) conferences and courses. Cesyen authored 4 publications during his time in Venezuela and 7 publications during his time in Belgium as PhD student. Cesyen has contributed in the supervision of a handful of Bachelor and Master students at the VUB.

Abstract of the PhD research

The early response to dehydration (ERD) plant proteins ERD10 and ERD14 are intrinsically disordered chaperones. Understanding their biologically relevant structural and functional behaviours inside living plant cells is only possible through the combination of advanced structural and cell biology methodologies.

Current state-of-the-art structural biology techniques, however, are mostly applied to molecules which are isolated from their native environment. The major goal of this PhD thesis, was therefore to perform *in-cell* nuclear magnetic resonance (NMR) using ERD10 and 14 proteins, i.e., to analyse the structure-function relationship of ERDs under truly natural *in vivo* conditions. This approach requires delivery of labelled proteins into cells under physiological conditions. Using a multidisciplinary approach that included recombinant protein production, Confocal fluorescence microscopy, NMR spectroscopy and different intracellular protein delivery strategies, the possibility to develop *in-cell* NMR studies in living plant cells was exhaustively explored. While a comprehensive framework to set-up such a system was established and it is herein provided, the efficient intracellular introduction of isotope-labelled proteins still remained a bottleneck difficult to circumvent. Future directions should envisage the use of cell-penetrating peptides possibly facilitating the delivery of proteins inside plant cells.

Here, we also report the complete chemical shift assignment of the disordered protein ERD10, to serve as a starting point for more detailed structural studies in the future.

In addition, we have unveiled a novel post-translational modification (PTM) of ERDs. We have found that ERD10 and 14 can be SUMO(*small Ubiquitin-like modifier protein*)-ylated. Interestingly, SUMOylated ERD14 efficiently binds to the *Arabidopsis thaliana* NAC transcription factor ATAF1. The functional *in vivo* significance of this interaction remains nevertheless to be further understood.

Finally, to directly assess the anti-aggregation activity of ERD14, its effect on the amyloid-forming *Escherichia coli* CsgA protein was tested. Whereas we observe signs of a chaperone-like effect, we conclude that the chaperone activity of ERD14 should be checked on naturally-occurring aggregating plant proteins.

Altogether, our research sheds some interesting light on the structural and functional features of the enigmatic ERD proteins *in vitro* and *in vivo*, opening the way for subsequent dedicated *in vivo* studies addressing molecular and cellular details of the structure and function of intrinsically disordered chaperones in a more general context.