SCIENCES & BIOENGINEERING SCIENCES

The Research Group

Structural Biology Brussels

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

Anna Caccamo

to obtain the degree of Doctor of Bioengineering Sciences

Joint PhD with Université de Liège

Title of the PhD thesis:

The role of ascorbate peroxidase 2 and protein cysteine modifications in the green alga *Chlamydomonas reinhardtii*

Promotors:

Prof. dr. Joris Messens (VUB)

Prof. dr. Claire Remacle (Université de Liège)

The defense will take place on

Thursday, January 25, 2024 at 15h30 in Université de Liège in the auditorium of Botanique institute, B22, Sart Tilman

The defense can also be followed through a live stream: https://teams.microsoft.com/l/meetup-join/19%3ameeting_OGZmODVjYzktNWIzZS00YjM3LTgxZGItNGYxZjA0MzZlZGVh%40thread.v2/0?context=%7b%22Tid%22%3a%22695b7ca8-2da8-4545-a2da-42d03784e585%22%2c%22Oid%22%3a%224003c475-5c45-4529-a30e-810737cc49a3%22%7d

Members of the jury

Prof. dr. Marc Hanikenne (ULiège, chair)
Prof. dr. ir. Geert Angenon (VUB, secretary)
Prof. dr. Joske Ruytinx (VUB)
Dr. Frédéric Kerff (ULiège)
Dr. Didier Vertommen (UCLouvain)
Prof. dr. Michael Hippler (Universität Münster, Germany)

Curriculum vitae

Anna Caccamo completed her Bachelor's degree in biology at the University of Turin, Italy. In 2014, she started her Master's studies in Plant Biotechnology at the same institution. In 2016, Anna was awarded an Erasmus+ scholarship, enabling her to pursue her Master's thesis in Biology at the National University of Ireland. There, she worked in the lab of Dr. Maria Tuohy, focusing on the nutraceutical properties of algal polysaccharide-rich extracts.
Afterwards, Anna relocated to Belgium to start her Ph.D. thesis, a collaborative project involving ULiège and VUB. Her research delved into the role of the ascorbate peroxidase 2 protein and protein cysteine modifications in the green alga *Chlamydomonas reinhardtii*. Anna's research findings have been published in two research articles, and she has also made contributions to a book chapter.

Abstract of the PhD research

H_2O_2, a reactive oxygen species (ROS), is known for causing oxidative damage during stress. However, it is now recognized as a signaling molecule, particularly in modifying protein cysteine residues, leading to cysteine sulfenylation in specific proteins. Cellular signaling can be anterograde, driven by the nucleus to other organelles such us chloroplast or mitochondria in the photosynthetic organisms, or retrograde, directed backwards from the organelles to the nucleus. Organisms that undergo photosynthesis, such as cyanobacteria, algae, and plants, primarily produce H_2O_2 in the chloroplasts during photosynthesis and in the mitochondria during respiration.

In this study, we used the green alga *Chlamydomonas reinhardtii*. Specifically, our attention was directed towards the H_2O_2-scavenging enzyme ascorbate peroxidase 2 (APX2), recently classified as ascorbate peroxidase-related (APX-R), of which the function remains unclear. We investigated the cellular role of APX2 and we identified modified cysteines in their sulfenylated form (-SOH) in Chlamydomonas under oxidative stress.

With an explorative screening of mutants through molecular and genetic methods, employing 1H-NMR, activity assays, and biophysical techniques with recombinantly expressed and purified APX2 and plastocyanin proteins, and utilizing structure predictions, we discovered that APX2 is most probably localized in the lumen of the chloroplasts and has an impact on plastocyanin. APX2, distinct from typical H_2O_2 scavengers, also exhibited next to peroxidase activity copper binding in a typical metal binding MxxM sequence motif and is involved in regulating the cellular plastocyanin levels.

Through the refinement of a mass spectrometric sulfenome analysis technique applied to protein extracts from Chlamydomonas wild type with and without oxidative stress, we successfully pinpointed one sulfenylated peptide associated with three light-harvesting complexes of photosystem II and one sulfenylated peptide associated with the ATP/ADP carrier protein AAA1. This discovery serves as an initial indication that the regulation of these complexes and of the AAA1 carrier protein involves the modification of a cysteine residue.

All in all, our findings reveal a novel function for APX2 and offer insights into sulfenylation patterns in Chlamydomonas.