SCIENCES & BIOENGINEERING SCIENCES

The Research Group

Structural Biology Brussels

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

Gabriela Garcia Rodriguez

to obtain the degree of Doctor of Bioengineering Sciences

Title of the PhD thesis:

Regulatory mechanisms behind the activities of bacterial HEPN ribonuclease RnlA and ParE2 gyrase poison

Promotor:

Prof. Dr. ir. Remy Loris

The defense will take place on

Monday, October 5, 2020 at 16h00

The defense can be followed through a live stream. Contact gabriela.garcia.rodriguez@vub.be for more information

Members of the jury

Prof. Dr. Peter Tompa (VUB, chair)
Prof. Dr. Joske Ruytinx (VUB, secretary)
Prof. Dr. Frank De Proft (VUB)
Prof. Dr. Christine Dunham (Emory University school of Medicine, Atlanta)
Prof. Dr. Abel Garcia-Pino (ULB)

Curriculum vitae

Structural biologist skilled in protein production, biochemical and biophysical characterization, including X-ray crystallography and SAXS for the understanding of molecular mechanisms behind the regulation of enzymatic activity, protein-protein and protein-DNA interactions. Obtained her Lic. (MSc equivalent) degree in Biochemistry and Molecular Biology at the School of Biology in the University of Havana, Cuba, in 2014 (Summa Cum Laude Diploma). From 2015 she has been a Ph.D. Candidate in Bioengineering at VIB-VUB Center for Structural Biology, Brussel, Belgium.

Abstract of the PhD research

The staggering biodiversity of microbes stands as a testament to the long evolutionary history of the oldest lifeforms on Earth. Horizontal gene transfer events in prokaryotic cells, as well as the arms race between bacteriophages and their bacterial hosts, assisted in the wide spread of molecular weapons that target many vital cell processes and their counteracting entities that provide a natural defence. These prokaryotic toxins are ubiquitous and abundant in the genomes of almost all species, and yet they exist under tight regulation to protect the cell against self-harm under homeostasis. The molecular mechanisms by which prokaryotes accomplish this task take place at many levels of regulation, i.e., direct inhibition of protein activity by protein-protein interactions, transcriptional regulation by repressor binding to DNA promoter to abrogate the activity of RNA polymerase, etc. By looking at the molecular level into the activity and regulation of the *Escherichia coli* RnlA:RnlB toxin-antitoxin (TA) system I have unveiled a novel regulatory mechanism exerted on the toxin RnlA by its cognate chromosomic antitoxin RnlB. This toxin functions as an endoribonuclease first reported as the host-encoded RNase responsible for the T4 phage late gene silencing when a *dmd*⁻ mutant of the phage infects the cells, halting its propagation. Here I show that RnlA is a HEPN (Higher Eukaryotes and Prokaryotes Nucleotide-binding domain) ribonuclease with broad sequence specificity *in vitro*. The drastic conformational changes on the structure of RnlA exerted by its antitoxin RnlB and detected by X-ray crystallography and SAXS, constitute the basis for its inhibition and the first example of a mechanism involving quaternary structural changes halting the activity of a ribonuclease known to the scientific community.
The toxic entities can also exert its function by directly binding to and blocking essential targets in the cell, like topoisomerases, key players in maintaining the right balance of DNA supercoiling, which is essential for cell growth and division. ParE2 is a gyrase poison encoded in chromosome II of the human pathogen *Vibrio cholerae*, which is directly counteracted by its cognate antitoxin ParD2. Here I have determined the crystallographic structure of the ParD2:ParE2 complex, which unveiled the molecular basis for the control of this toxin's poisonous effect by direct interaction with its cognate antitoxin. Moreover, I have also mapped the operator region upstream of the *parde2* operon where this TA module binds to and represses its own operon transcription; as well as determined the stoichiometry of the ParD2:ParE2 complex by X-ray crystallography, SAXS and native mass spectrometry studies. Electrophoretic mobility shift assays point to the presence of conditional cooperativity in the transcriptional autoregulation of this system.