SCIENCES & BIOENGINEERING SCIENCES

The Research Group
Structural Biology Brussels

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

Yana Girardin

to obtain the degree of Doctor of Bioengineering Sciences

Title of the PhD thesis:

Exploring the interaction landscape of the *Vibrio cholerae* toxin ParE2: Gyrase inhibition and neutralization by ParD2

Supervisor:
Prof. dr. ir. Remy Loris (VUB)

The defense will take place on

Monday, August 4, 2025 at 4 p.m.

VUB Etterbeek campus, Pleinlaan 2, Elsene auditorium D.2.01

The defense can also be followed online via Microsoft Teams:
Meeting ID: 350 489 290 791 7
Passcode: Qx77Sa9d

Members of the jury

Prof. dr. ir. Eveline Peeters (VUB, chair)
Dr. ir. Inge Van Molle (VUB)
Prof. dr. Frank De Proft (VUB)
Prof. dr. ir. Yann Sterckx (UAntwerpen)
Prof. dr. Christine Dunham (Emory University, USA)

Curriculum vitae

Yana obtained a degree of Master of Science in Bioengineering Sciences at the Vrije Universiteit Brussel in 2019. After graduating, she received a fundamental research grant from the Research Foundation Flanders (FWO) to pursue a PhD within the research group of Prof. dr. ir. Remy Loris (Structural Biology Brussels).
During her doctoral research, she co-authored six scientific publications in peer-reviewed journals, three of which as first author. She supervised two master's theses and one internship and assisted in practical courses for both bachelor's and master's programs. In addition, she actively contributed to science communication by participating in outreach events aimed at engaging the general public with science.

Abstract of the PhD research

Antibiotics are powerful medicines used to treat bacterial infections. While they have saved countless lives, their overuse and misuse have accelerated the emergence of antibiotic-resistant bacteria. As a result, infections that were once easily treatable are becoming increasingly difficult, and sometimes even impossible, to cure. This rising resistance is a major global health concern which underlines the urgent need for new antibiotics to fight harmful bacteria.

One promising area of research involves toxin-antitoxin (TA) systems. These are built-in bacterial regulatory mechanisms where one component (a toxin) can halt bacterial growth or kill the cell, much like traditional antibiotics, while the other component (an antitoxin) neutralizes the toxin's effects.

This PhD research focuses on one specific TA system found in *Vibrio cholerae*, known as *parDE2*. This system encodes two proteins: the toxin *Vc*ParE2 and its corresponding antitoxin *Vc*ParD2. *Vc*ParE2 targets Gyrase, an essential bacterial enzyme involved in managing DNA structure and a well-known target of several antibiotics. Although *Vc*ParE2 just like these antibiotics inhibits Gyrase, it appears to do so through a different, yet unknown mechanism. The goal of this research is to learn more about how *Vc*ParE2 functions, how it interacts with Gyrase, and how *Vc*ParD2 can prevent it from causing damage.

A key challenge in this work was the production of sufficient quantities of active *Vc*ParE2 and *Vc*ParD2 proteins. This was particularly complicated for the toxin, which is harmful to the bacterial cells used for its production. To overcome this, a multi-level control system was developed to enable safe cloning of the toxin. Additionally, production was established through different strategies, including (i) co-expressing the toxin with its antitoxin and separating them later and (ii) using insect cells instead of bacteria to avoid toxicity. Once successfully produced, both proteins underwent extensive structural and biophysical characterization. Furthermore, this work identifies the specific region of the toxin responsible for Gyrase inhibition and explores how *Vc*ParD2 interacts directly with the toxin to stop it from doing harm.

In summary, it deepens our understanding of the *Vc*ParE2 toxin and the *parDE* TA family in general, while offering valuable methods for working with toxic bacterial proteins in the laboratory.