SCIENCES & BIOENGINEERING SCIENCES

The Research Group

Structural Biology Brussels

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

Pieter De Bruyn

to obtain the degree of Doctor of Bioengineering Sciences

Title of the PhD thesis:

Toxin-antitoxin modules: functional characterization of transcription regulation and potential use against pathogenic bacteria

Promotor:
Prof. dr. ir. Remy Loris
Co-promotor:
Em. prof. dr. Henri De Greve

The defense will take place on

Thursday, September 2, 2021 at 18h00

The defense can be followed through a live stream. Contact Pieter.De.Bruyn@vub.be for more information

Members of the jury

Prof. dr. ir. Eveline Peeters (VUB, chair)
Dr. ir. Inge Van Molle (VUB, secretary)
Prof. dr. Kim Roelants (VUB)
Prof. dr. Jan Michiels (KULeuven)
Prof. dr. Tanel Tenson (University of Taru, Estonia)

Curriculum vitae

Pieter De Bruyn (1991) obtained a B.Sc. in Biochemistry and Biotechnology at the Universiteit Antwerpen followed by a B.Sc. and M.Sc. in Bio-Engineering Sciences at the Vrije Universiteit Brussel. His doctoral research was carried out in the lab of Prof. Dr. ir. Remy Loris (VIB-VUB Center for Structural Biology). He started his PhD combined with the position as an assistant with the aim to study the function and regulation of toxin-antitoxin systems by combining biophysical methods with cloning.

Abstract of the PhD research

Bacteria have extraordinary ways to deal with stress in general and antibiotics in particular. The use and at times unnecessary overuse of antibiotics has resulted in the rapid development and spread of resistance to multiple types of antibiotics in pathogenic bacteria. Therefore, a very limited number of antibiotics, if any, remain available to fight certain multidrug resistant pathogens, such as methicillin resistant *Staphylococcus aureus* (MRSA). Aside from this inheritable antibiotic resistance, multidrug tolerance can also be linked to a non-inheritable trait by entering a dormant state called persistence. Because antibiotics predominantly work on metabolically active cells, these bacteria are capable to withstand different antibiotics during the time of treatment. Bacterial toxin-antitoxin (TA) modules may be a solution. They consist of a toxin (which has a deleterious effect on the cell) and an antitoxin (which rescues the cell from the toxin's activity) and have to be tightly regulated to prevent cell death. Deregulation of these tightly regulated systems can therefore inhibit cell growth and kill the bacteria.

My PhD work can be divided into two parts. The first part focuses on the regulation of a unique three component TA module: the *paaR2-paaA2-parE2* operon. This operon is present in cryptic prophage CP-933P from enterohemorrhagic *Escherichia coli* O157:H7 strains. Aside from the toxin (ParE2) and antitoxin (PaaA2), there is an additional regulator (PaaR2) that is a transcriptional repressor of the operon. The objective of my work was to understand how these three components interact to autoregulate the operon. Expression clones and purification protocols were established, and it has been shown via a combination of structural biology, biochemistry, biophysical experiments and DNA-binding studies that the regulation hijacks a prophage regulation pathway. The second part of my PhD deals with the validation of TA modules as potential leads for new antibiotics. This work was performed using *E. coli* and *S. aureus* as model organisms. MRSA as well as multidrug resistant *E. coli* are becoming more prevalent and pose a high risk for hospitalized patients. Exogenous toxins from TA modules were introduced into these organisms and the effect of the toxins was evaluated. This research brings us a step closer to understanding how TA modules are regulated as well as being a first stage in the use of toxins as antimicrobials.