SCIENCES & BIOENGINEERING SCIENCES

The Research Group

Structural Biology Brussels

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

Maruša Prolič-Kalinšek

to obtain the degree of Doctor of Bioengineering Sciences

Title of the PhD thesis:

Regulatory interplay between the toxin-antitoxin cluster *paaR2-paaA2-parE2* and prophage genes *ydaS-ydaT* from *Escherichia coli* O157:H7

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

The defense will take place on
Tuesday, May 16, 2023 at 17h in auditorium I.2.02

(The defense can be followed through a live stream. Contact Marusa.Prolic.Kalinsek@vub.be for more information)

Members of the jury

Prof. dr. Peter Tompa (VUB, chair)
Prof. dr. ir. Benoit Stijlemans (VUB, secretary)
Prof. dr. Janine Brünner (VUB)
Prof. dr. Frank De Proft (VUB)
Prof. dr. Sergei Strelkov (KU Leuven)
Prof. dr. ir. Julie Bouckaert (Université de Lille)

Curriculum vitae

Maruša Prolič-Kalinšek obtained her B. Sc. and M. Sc. in Biochemistry at the University of Ljubljana, Slovenia. In November of 2017 she started a doctoral study at Vrije Universiteit Brussel under supervision of Prof. Dr. ir. Remy Loris. The results from this doctoral research were published in four peer-reviewed international publications and were presented at international conferences and scientific meetings. During her PhD she supervised one master thesis student and contributed to several practical courses.

Abstract of the PhD research

Toxin-antitoxin (TA) systems are small operons encoding a toxin that interferes with a basic cell function and induces growth arrest and an antitoxin that counteracts the activity of the toxin. The goal of this PhD project is to study the regulation of the *paaR2-paaA2-parE2* and *ydaST* operons that are encoded within the prophage CP-993P in the genome of *Escherichia coli* O:157. The *paaR2-paaA2-parE2* operon is a three-component type II TA system where the toxin ParE2 and the antitoxin PaaA2 form a nontoxic complex. Unlike other type II antitoxins PaaA2 is devoid of a DNA binding domain. The third component PaaR2 therefore acts as a repressor for the operon. The *ydaST* operon is located adjacent to *paaR2-paaA2-parE2* but is encoded in the opposite direction. YdaS is a DNA binding protein able to repress transcription of *paaR2-paaA2-parE2*; the intergenic region between the operons is recognized by both YdaS and PaaR2. YdaT is another transcription factor that binds outside the intergenic region at the end the *ydaS gene* and potentially provides an alternative transcript for the TA-system.

The structures of YdaS, YdaT and PaaR2 were determined using X-ray crystallography, NMR spectroscopy and SAXS. YdaS is a partially disordered monomer in solution. The N-terminal DNA binding domain of PaaR2 on its own is monomeric as well but the full-length protein makes an octamer via its C-terminal oligomerization domain. The DNA binding domains do not dimerize in an octamer. Both YdaS and PaaR2 bind to four imperfect palindromes in the intergenic region and compete together for these sites. In both cases two monomers bind one palindrome sequence. YdaT binds adjacent to a promotor upstream of the *paaR2-paaA2-parE2* operon. YdaT is a tetramer in the crystal and in solution. The tetramer is formed via its C-terminal long alpha helix that associates into an antiparallel four helix bundle. The N-terminal domain can rotate relative to the central helix bundle of the tetramer. Upon DNA binding, this structure rigidifies.

Structurally and functionally YdaS, PaaR2 and YdaT act as functional analogues to Cro, CI and CII respectively. The results support the hypothesis that the toxin-antitoxin system *paaR-paaA-parE* highjacked the immunity region of a prophage for its transcription regulation and that it likely contributes to the stability of the cryptic prophage CP-933.