Vrije Universiteit Brussel

Faculty of Sciences and Bioengineering Sciences

The Research Group of

**Structural Biology Brussels (SBB)**

has the honour to invite you to the public defence of the PhD thesis of

# ir. Alexandra VANDERVELDE

to obtain the degree of Doctor in Bio-engineering Sciences

Title of the PhD thesis:

## Mechanisms of transcription regulation in prokaryotic toxin-antitoxin modules

Promotors:

**Prof. Dr. ir. R. Loris**

**Prof. Dr. J. Danckaert**

**Prof. Dr. H. De Greve**

The defence will take place on

**September 26, 2016 at 17.00 h**

in U-Residence, green room. at the Campus Etterbeek of the Vrije Universiteit Brussel, Pleinlaan 2 in 1050 Elsene, and will be followed by a reception

## Members of the jury

Prof. Dr. S. Muyldermans (VUB, chairman)
Prof. Dr. ir. E. Peeters (VUB, secretary)
Prof. Dr. L. Leyns (VUB)
Prof. Dr. A. Garcia-Pino (ULB)
Prof. Dr. N. Kaldalu (Univ. of Tartu, Estonia)

## Curriculum vitae

Alexandra Vandervelde (born on February 17th 1989 in Bonheiden, Belgium) obtained her BSc and MSc in Bioengineering Sciences at VUB with highest distinction. During her PhD, started in October 2012, Alexandra (co-)authored a publication in a peer-reviewed journal and a book chapter. She presented research posters at a Gordon conference, an EMBO symposium and several national conferences and was a visiting researcher at MIT (MA, USA). She also supervised a Master thesis project and participated in two outreach programs. Her PhD was financially supported by a predoctoral fellowship of the FWO (Research Foundation Flanders).

## Abstract of the PhD research

In several chronic infectious diseases, persister cells play a pivotal role. Persisters are cells in a bacterial population that are tolerant to several antibiotics because they are in a dormant, non-dividing state. Toxin-antitoxin modules are small genetic elements on the genomes of bacteria, which are important factors in the generation of these persister cells. Toxin-antitoxin modules code for an intracellular toxin and its cognate antitoxin. As the toxins can be lethal to the cell, toxin-antitoxin modules are tightly transcriptionally regulated. In many typical toxin-antitoxin modules, this regulation is ensured by a mechanism named conditional cooperativity, in which the antitoxin binds the DNA and the toxin functions as a corepressor or a derepressor, depending on the toxin:antitoxin ratio.

During this PhD, the molecular mechanisms behind conditional cooperativity in the *ccdAB* operon on the F plasmid of *Escherichia coli* were elucidated. Interaction studies revealed that the low specificity and affinity of CcdA for its operator DNA can be greatly enhanced by the toxin CcdB at low toxin:antitoxin ratios. The $CcdB_2$-$CcdA_2$-$CcdB_2$ non-repressing complex is a dynamic V-shaped hexamer, while the repressing complex consists of a chain of alternating $CcdA_2$ and $CcdB_2$ dimers which spiral around the DNA, as shown by structural biology methods. A thermodynamic analysis of the interactions in the *ccdAB* autoregulation shows that the increase in affinity of the antitoxin CcdA for the DNA in the presence of the toxin CcdB is caused by the coordinated binding of multiple CcdA antitoxins, or in other words by avidity effects.

Additionally, a general framework was developed to model the dynamics of toxin-antitoxin modules. Simulations based upon these models show that an increase in the number of binding sites on the operator serves to reduce the metabolic burden of the cell by reducing the total amounts of proteins produced. Finally, in very rare cases, the dynamics of toxin-antitoxin module regulation allows a spike in the free toxin level. By including growth rate modulation in function of the free toxin level, these spikes can be linked to the generation of persister cells.