SCIENCES & BIOENGINEERING SCIENCES

The Research Group
Structural Biology Brussels

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

Babette Deckers

to obtain the degree of Doctor of Bioengineering Sciences

Title of the PhD thesis:

Deciphering the interactome of ObgE: a widely conserved GTPase acting at the crossroads of cell survival and cell death

Promotor:
Prof. dr. ir. Wim Versées (VUB)

The defence will take place on

Friday, March 1, 2024 at 16h in auditorium I.0.03

Members of the jury

Prof. dr. Joske Ruytinx (VUB, chair)
Prof. dr. Janine Brunner (VUB, secretary)
Prof. dr. Steven Ballet (VUB)
Prof. dr. Arjan Kortholt (University of Groningen, The Netherlands)
Prof. dr. Régis Hallez (Université de Namur)

Curriculum vitae

Babette Deckers obtained a degree of Master of Science in Bioengineering Sciences: Cell and Gene Biotechnology at the Vrije Universiteit Brussel in 2018. After graduating, she started a PhD, funded by an FWO fellowship, within the lab of Prof. dr. ir. Wim Versées (Structural Biology Brussels). During her PhD, Babette presented her work at different (inter)national conferences and scientific meetings. Her research resulted in a high-impact peer-reviewed paper as first author, while she also contributed to three additional publications as co-author. Finally, she guided and supervised two master thesis students while also assisting in practical courses for bachelor students.

Abstract of the PhD research

In the light of the current antibiotic crisis, with an increasing number of pathogenic bacteria displaying resistance against multiple antibiotics, it is of crucial importance to develop new ways to fight bacterial infections. The widely conserved bacterial GTPase Obg is regarded as an appealing new antibiotic target since it is essential for bacterial survival and plays a role in a wide variety of fundamental cellular processes. Moreover, Obg has also been identified as a central regulator of persistence, a phenomenon that allows bacteria to survive antibiotic treatments. Hence, Obg could serve a dual targeting purpose: while interfering with the essential function of Obg could directly kill bacteria, specifically targeting its persistence function could serve as an anti-persister therapy and thus increase the efficacy of existing antibiotics. In order to disrupt Obg's functions, more information on the exact molecular pathways it is involved in is required. Therefore, the main aim of this PhD research was to identify and characterize new interactors of the Obg GTPase in *E. coli* (ObgE).

First, we confirmed and characterized the interaction of ObgE with a selection of potential ObgE interactors, identified in an *in vivo* crosslinking experiment. ObgE's interaction with the newly identified interactor YbiB, a fairly uncharacterized DNA-binding protein, was studied in more detail. Using a variety of biophysical and structural methods, we showed that ObgE and YbiB Interact with high affinity in a characteristic biphasic manner. We demonstrated that the negatively charged and intrinsically disordered C-terminal domain of ObgE is the main driver of the interaction and binds in the positively charged grooves present on YbiB's surface. Correspondingly, ObgE efficiently inhibits the binding of DNA to YbiB. Further research into the precise function of the YbiB protein is required to unravel the consequences of this inhibition and assess the potential of targeting this interaction as an antimicrobial strategy.

In a second part of this PhD research, we studied a cytotoxic variant of ObgE (ObgE*) that induces rapid cell death when expressed. First, we biochemically characterized the ObgE* protein and identified its GTP-bound state as the toxic form of the protein. Subsequently, we demonstrated that ObgE* confers its toxicity by directly interacting with and inhibiting the activity of the LpxA enzyme. LpxA is involved in the biosynthesis of lipopolysaccharides (LPS), which are important building blocks of the outer membrane of Gram-negative bacteria. Consequently, ObgE* decreases the amount of LPS present in the Gram-negative cell envelope, finally leading to cell death. Thus, by studying the ObgE* mutant, we exposed a novel link between the Obg protein and the bacterial cell envelope, which was not reported before.

Taken together, the PhD research presented here unveiled some pieces of the complex interaction network of ObgE, bringing us one step closer to targeting the Obg protein as an antimicrobial strategy.