SCIENCES & BIOENGINEERING SCIENCES

The Research Group
Structural Biology Brussels

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

Anke Breine

to obtain the degree of Doctor of Bioengineering Sciences

Title of the PhD thesis:

Tackling superbugs: Development of novel strategies to target the human pathogen *Acinetobacter baumannii*

Promotors:
Prof. dr. Charles Van der Henst
Prof. dr. Han Remaut

The defence will take place on

Friday, October 4, 2024 at 4 p.m. in auditorium D.0.08

Members of the jury

Prof. dr. ir. Eveline Peeters (VUB, chair)
Prof. dr. Anastassia Vorobieva (VUB, secretary)
Prof. dr. Kim Roelants (VUB)
Prof. dr. Thomas Demuyser (UAntwerpen)
Prof. dr. Paul Higgins (University of Cologne, Germany)

Curriculum vitae

Anke Breine obtained the degree of Master of Science in Bioengineering Sciences: Cell and Gene Biotechnology at the Vrije Universiteit Brussel in 2019. After graduating, she started her PhD in the VIB-VUB Center for Structural Biology with a FWO-SB fellowship. During her PhD, Anke presented her work at several national and international conferences. Her research resulted in a patent and two high-impact peer-reviewed papers as first author, while she also contributed to four additional publications as co-author. Finally, she guided and supervised three master thesis students while also assisting in practical courses for bachelor students.

Abstract of the PhD research

The rise of drug-resistant bacteria is rendering once-treatable diseases lethal again. The World Health Organization (WHO) and the Centers for Disease Control and Prevention (CDC) have assembled a list of critical pathogens for which the development of new therapeutics needs to be prioritized. Carbapenem-resistant *Acinetobacter baumannii* (CRAb) is at the top of this list. *A. baumannii* is a human pathogen that has acquired this position due to a dangerous combination of characteristics: (i) the facile acquisition of drug resistance genes and (ii) the ability to resist disinfectants and survive prolonged periods of desiccation. This combination enables *A. baumannii* to thrive in clinical settings. In addition, *A. baumannii* isolates are highly diverse, which makes it very difficult to develop new therapeutics.

The aim of this doctoral research was to discover and characterize new (bio)molecules targeting *A. baumannii* that could be developed into targeted therapeutics or into diagnostic tools. Three approaches were used to accomplish this.

First, through a repurposing screen of a chemical library we identified HDC1, a harmine-derived compound that inhibits the growth of 43 clinical *A. baumannii* strains, including 40 carbapenem-resistant isolates.

Second, we used a nanobody-based approach to target *A. baumannii*. We generated an unbiased nanobody library targeting the entire cell surface of the pathogen. Screening this library led to the discovery of NbH7. This nanobody binds *A. baumannii* and a subset of *Acinetobacter* species but not *E. coli* or *K. pneumoniae*. We discovered that NbH7 targets Omp25, a conserved outer membrane protein, which enhances the potential of NbH7 as a diagnostic tool.

Lastly, a nanobody-based peptide showed improved binding to live *A. baumannii* cells compared to the parent nanobody, confirming this peptide as the minimal binding element required for binding.

Ultimately, this doctoral research resulted in the discovery of three new (bio)molecules to target *A. baumannii*. The nanobody has great potential for development as a diagnostic or therapeutic tool. Therefore, this research provides proof-of-concept for the development of tools to potentially target other problematic pathogens.