SCIENCES & BIOENGINEERING SCIENCES

The Research Group
Structural Biology Brussels

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

Phebe De Keyser

to obtain the degree of Doctor of Bioengineering Sciences

Title of the PhD thesis:

Nanobody-based automation and downscaling of protein purification and biopanning

Supervisor:
Prof. dr. ir. Jan Steyaert (VUB)
Co-promotor:
Prof. dr. ir. Gert Desmet (VUB)

The defense will take place on

Wednesday, August 20, 2025 at 5 p.m.

VUB Etterbeek campus, Pleinlaan 2, Elsene
Learning Theatre, Learning & Innovation Center (LIC)

Members of the jury

Prof. dr. Dominique Maes (VUB, chair)
Prof. dr. Rouslan Efremov (VUB)
Prof. dr. Nick Devoogdt (VUB)
Dr. ir. Mireille Dumoulin (ULiège)
Dr. Bruno Dombrecht (VIB)
Prof. dr. ir. Séverine Le Gac (Universiteit Twente, NL)

Curriculum vitae

Phebe De Keyser holds a Master's degree in Bioscience Engineering from the KULeuven (2015-2020), with a major in Bionanotechnology and a minor in Cell and Gene Technology. Following graduation, she pursued a PhD in the labs of Jan Steyaert and Gert Desmet at the VUB. During her doctoral research she actively contributed to science outreach initiatives and co-founded the department's Ecoteam. In early 2025 she completed an internship in coral restoration with Reef Renewal Bonaire, before joining Lima Europe as R&D and innovation manager.

Abstract of the PhD research

The biotechnology industry has witnessed a significant **trend towards miniaturization and automation** in recent years. This shift has been driven by the need for more efficient, cost-effective, and high-throughput methods to manipulate and analyze biological samples. One key technology that has emerged as a result is the lab-on-a-chip device, which integrates multiple laboratory functions onto a single **microfluidic chip**, where liquid flows in micrometer-scale channels. The use of such small volumes entails **low reagent and sample consumption**, precise liquid handling and efficient biochemical reactions.

Many **proteins**, especially membrane proteins and protein complexes, are challenging to study because they are **not expressed in high amounts** or **difficult to purify** in their native state without disrupting the interactions with their partners. Hence, the Steyaert group developed a **novel purification technique** employing Nanobodies® (Nbs), the variable domains of heavy-chain-only antibodies of camelids. This technique, called **Na**nobody **ex**change chromatography (**NANEX**), utilizes an immobilized low-affinity Nb to capture the target protein, which is subsequently eluted - along with its interaction partners - by introducing a high-affinity Nb. **However, NANEX still consumes considerable amounts of sample and most biopanning routines to discover target-specific Nbs are operator-dependent, labor-intensive and material- and time-consuming.** Here we discuss two novel Nanobody-based tools to address these issues.

First, this research **miniaturized NANEX in a microfluidic chip** (µNANEX). Along with the µNANEX chip, we validated a digital twin model, which can predict optimal experimental conditions. The µNANEX chips are integrated into a setup that enables fully automated and reproducible purifications. The effectiveness of the method is demonstrated with Nbs binding to the green fluorescent protein (GFP), allowing streamlined purification of any GFP-tagged protein from biological samples for applications in structural biology or proteomics.

Second, we developed an **improved biopanning strategy** that uses biosensors to present the antigen to phage-displayed Nbs in a well. The use of automated Octet sensors (Sartorius) enables high throughput and precise control over each step. Additionally, by downscaling and immobilizing anti-GFP Nbs on the sensor, minimal amounts of unpurified GFP-tagged antigen are required. In conclusion, both Nanobody-based methods are automated, precisely controlled and consume minimal amounts of protein.