SCIENCES & BIOENGINEERING SCIENCES

The research group
Structural Biology Brussels

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

# Giacomo Pedrelli

to obtain the degree of Doctor of Bioengineering Sciences

Title of the PhD thesis:

**Biophysical investigation of folding and assembly in *de novo* designed transmembrane ß-barrels**

Supervisor:
**Prof. dr. Anastassia Vorobieva (VUB)**
Co-supervisor:
**Prof. dr. Han Remaut (VUB)**

The defence will take place on
**Friday, September 25, 2026 at 4 p.m.**

VUB Etterbeek campus, Pleinlaan 2, Elsene, Auditorium D.0.08

## Members of the jury

Prof. dr. Joris Messens (VUB, chair)
Prof. dr. Charlotte Martin (VUB)
Prof. dr. Peter Tompa (VUB)
Prof. dr. Charles Van der Henst (VUB)
Dr. Chloé Martens (ULB)
Dr. Robert Jefferson (King's College London. UK)

## Curriculum vitae

After studying Molecular and Industrial Biotechnology at the University of Bologna, with a Master's thesis in Protein Design at the University of Bristol, Giacomo started his PhD in Bioengineering Sciences at the VIB-VUB Center for Structural Biology. His research focuses on studying the folding and assembly of *de novo* designed transmembrane ß-barrel proteins. His work led to a first-author publication in *PNAS* and was presented at the Gordon Research Conference in Barcelona. He also supervised a Master's student during his PhD.

## Abstract of the PhD research

Transmembrane ß-barrel proteins (TMBs) are an important class of membrane proteins with significant biological and biotechnological relevance. However, the molecular principles governing their folding and membrane assembly remain only partially understood. This is also true for *de novo* designed TMBs, whose successful assembly is difficult to predict from sequence alone. A major limitation is the lack of experimental methods that are both reliable for evaluating native-like folding and scalable across multiple variants.
This thesis addressed these challenges by investigating TMB folding in membrane-mimicking environments and developing experimental strategies for its characterization. First, a workflow combining complementary biochemical and biophysical assays was established to characterize TMBs in detergent micelles and liposomes. The results showed that no single assay is sufficient to define native-like folding in all cases and identified 1H NMR as a particularly informative method for rapid screening. An exploratory split-GFP fluorescence assay was also evaluated as a potential high-throughput readout of membrane assembly.
Cell-free expression in the presence of lipid vesicles was then used to investigate how sequence features influence TMB assembly. Designs optimized primarily for native-state stability were found to fail through misfolding or aggregation before productive membrane insertion. Local destabilization of ß-strands through negative design significantly improved folding and assembly. This principle was further explored in the *de novo* designed TMB2.17, where selected outward-facing polar substitutions were compatible with the ß-barrel fold and, in some cases, improved NMR spectral quality.
Overall, this thesis shows that successful assembly of *de novo* TMBs depends not only on native-state stability but also on kinetic factors including aggregation, off-pathway intermediate formation, and membrane insertion. By establishing an experimental framework for TMB characterization and identifying negative design and position-dependent surface chemistry as determinants of folding, this work provides insights into the sequence-structure relationships governing TMB assembly and a basis for more reliable computational design.