SCIENCES & BIOENGINEERING SCIENCES

The Research Group

VIB-VUB center for Structural Biology

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

Tomasz UCHANSKI

to obtain the degree of Doctor of Bio-engineering Sciences

Title of the PhD thesis:

Megabodies as next-generation tools in structural biology

Promotor:

Prof. dr. ir. Jan Steyaert

The defense will take place on

Thursday July 4 2019 at 3 pm

in Auditorium D.2.01 at the Campus Humanities, Sciences and Engineering of the Vrije Universiteit Brussel, Pleinlaan 2, 1050 Brussel, and will be followed by a reception.

Members of the jury:

Prof. dr. Serge Muyldermans (Chairman)
Prof. dr. Cecil Vincke (Secretary)
Prof. dr. Sophie Hernot
Prof. dr. Savvas Savvides (Univ. Gent, BE)
Prof. dr. Radi Aricescu (Univ. Cambridge, UK)

Curriculum vitae

Tomasz Uchański graduated as a Master of Biotechnology from the Jagiellonian University in Poland in 2013. After a one-year internship at the University of Chicago, he started his PhD at the Vrije Universiteit Brussel in 2014. His research focused on antibody engineering and their applications in cryo-EM. His PhD project led to the discovery of first in class Nanobody-based chimeric proteins called Megabodies. This research is published in three articles in peer-reviewed journals and protected in one patent application.

Abstract of the PhD research

Obtaining high resolution structural information remains a key challenge for studying biological macromolecules. X-ray crystallography is a well-established approach to reach this goal. However, many proteins do not crystallize. As an alternative, single particle cryo-EM is becoming a versatile technique for the structural analysis of macromolecular complexes, but this method also suffers from limitations. In addition to the homogeneity of a given sample, the highest achievable resolution of the 3D reconstruction is greatly dependent on the ability to iteratively refine the orientation parameters of each individual particle. Whereas large molecules are relatively easy to recognize in noisy low-dose images of frozen hydrated samples, the process of collecting and processing images of small particles is much more difficult. Preferred particle orientation also represents a recurring problem in cryo-EM.

To overcome inherent performance barriers of X-ray crystallography and cryo-EM, we designed rigid antibody chimeras, we call Megabodies. These Megabodies are built from Nanobodies that are grafted onto large scaffold proteins via two short peptide linkers to produce stable and rigid monomers. We demonstrate that Megabodies add enough mass and defined features, even to relatively small target proteins, for accurate image alignment of the vitrified particles in cryo-EM.

We successfully validated such Megabodies as structural chaperones in cryo-EM by solving several high-resolution structures of the heteropentameric type A γ-aminobutyric acid receptor ($GABA_A$), the principal mediator of inhibitory neurotransmission in the vertebrate nervous system.