SCIENCES & BIOENGINEERING SCIENCES

The Research Group

Structural Biology Brussels (SBB)

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

Katrien WILLEGEMS

to obtain the degree of Doctor of Bioengineering Sciences

Title of the PhD thesis:

Structural and biochemical characterization of ryanodine receptors in lipid environment

Promotor:

Prof. dr. Rouslan Efremov

The defence will take place on

Monday September 17 2018 at16:00h

in Auditorium E.0.12 at the Campus Humanities, Sciences and Engineering of the Vrije Universiteit Brussel, Pleinlaan 2 – 1050 Elsene, and will be followed by a reception.

Members of the jury:

Prof. dr. Peter Tompa (chairman)
Dr. Inge Van Molle (secretary)
Prof. dr. Patrick Vanderheyden
Prof. dr. Luc Leyns
Prof. dr. Lucia Chavez Gutierrez (KUL)
Prof. dr. Cedric Govaerts (ULB)

Curriculum vitae

Katrien Willegems (06/05/1990, Leuven, Belgium), obtained her Master in Biology – Genetics, Cel and Developmental Biology with great distinction at the VUB in 2013. She went on to pursue a PhD in Structural Biology, investigating the structure of Ryanodine Receptors. Her PhD research has led to 1 first-author and 1 co-author publication in peer-reviewed journals and one book chapter. During her PhD, Katrien was responsible for the organization and teaching of DNA-manipulation practical courses. Her research was funded by the IWT.

Abstract of the PhD research

Ryanodine receptors (RyRs) are ion channels residing in sarcoplasmic reticulum or endoplasmic reticulum membranes. They couple action potential excitation with contraction of muscles, in the process known as excitation-contraction coupling (EC-coupling), by releasing calcium from the intracellular calcium stores. While the function of RyRs has been primarily characterized in muscle contraction, these receptors are expressed in many cell types including neuronal, smooth muscles, endothelial and pancreatic cells, where exact involvement of RyRs in calcium homeostasis is less well understood. More than 800 mutations in RyRs have been linked to human diseases, rendering RyRs important drug targets. Modern rational drug design is based on high-resolution structures of drug target proteins in complex with regulators. With recent advances in single particle cryo-EM, determination of the structures of membrane proteins has significantly accelerated. Membrane proteins evolved to be embedded and function in a lipid bilayer and the importance of lipids closely interacting with the transmembrane region for protein structure, function and stability has been demonstrated for multiple important ion channels.

The objective of the PhD project was to determine the high-resolution structure of cardiac RyR (RyR2) using single particle cryo-EM. In my PhD work, I first developed a purification strategy for RyR1, reconstituted the ion channel into lipid nanodiscs and performed biochemical and structural characterization of RyR1 in a lipid environment. The structure of reconstituted RyR1 was determined under two conditions that revealed an open channel conformation in the lipid environment. Comparison to published RyR1 structures determined in detergents combined with additional biochemical assays revealed the effect of lipid mimetics on the open probability of the channel. The purified RyR1 was used for the generation of RyR specific nanobodies that were used to develop a novel nanobody-based purification of bovine cardiac RyR isoform, RyR2, in lipid nanodiscs. This fast and reproducible purification with small amounts of starting material has allowed preparation of cryo-EM samples. The intermediate resolution structures of RyR2 were determined with and without the accessory protein FKBP12.6 using cryo-EM. These results pave the way towards structural characterization of RyR2 in complex with small-molecule modulators towards developing efficient drugs targeting the cardiac arrhythmia disorders CPVT1 and ARVD2, which are an important cause of sudden cardiac death in young people.