SCIENCES & BIOENGINEERING SCIENCES

The Research Group
Structural Biology Brussels

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

Chenyao Li

to obtain the degree of Doctor of Bioengineering Sciences

Title of the PhD thesis:

Cryo-EM studies of ryanodine receptors: rapid purification and regulation of gating

Promotor:
Prof. dr. Rouslan Efremov

The defense will take place on

Tuesday, December 17, 2024 at 4 p.m. in auditorium D.0.08

Members of the jury

Prof. dr. Eveline Peeters (VUB, chair)
Prof. dr. Janine Brunner (VUB, secretary)
Prof. dr. Wen-Juan Ma (VUB)
Prof. dr. Savvas Savvides (UGent)
Prof. dr. Chris Ulens (KULeuven)

Curriculum vitae

Chenyao Li obtained the B.S. of Pharmacy at NanChang University, Nanchang, China in 2016. obtained the M.S. of pharmacy at Shanghai Institute of Materia Medica (SIMM), Chinese Academy of Sciences (CAS), Shanghai, China in 2019. She started PhD in 2020 in VIB-VUB center for structural biology. Her research resulted in two papers as first author, one havs been published another is under review.

Abstract of the PhD research

Ryanodine receptors (RyRs) are ion channels with a molecular weight of 2.2 MDa, located in the sarcoplasmic reticulum (SR) membrane. They play a crucial role in excitation-contraction coupling and calcium homeostasis. Dysfunction of RyRs is associated with a spectrum of diseases, including malignant hyperthermia (MH) and heart failure (HF), both of which can be life-threatening.

Due to their large size and SR membrane location, RyRs are difficult to express in heterologous systems. Consequently, RyRs are typically purified from native sources, with traditional methods requiring substantial amounts of starting material. These limitations have hindered structural studies of RyRs and slowed the development of RyR-targeted drugs. A more efficient purification method is required to accelerate structural analysis and facilitate the rational design of therapeutics targeting RyRs.

The gating of RyRs is precisely regulated by both modulators and the membrane environment. Even subtle changes can lead to dysfunction and disease, so understanding how individual factors, including membrane mimetics, finetune the open probability of RyRs is critical.

In my PhD thesis, I developed a rapid, small-scale nanobody-assisted purification method for ryanodine receptors optimized for cryo-EM. This method requires only 5 mg of total protein as starting material. The entire purification process, starting from isolated membranes, can be completed in just 4 hours at the bench, yielding protein suitable for cryo-EM analysis. I validated this method by solving the structures of rabbit RyR1, mouse and bovine RyR2. The nanobody binding site was localized by cryo-EM and its analysis shows that it is highly conserved across all three mammalian isoforms. This suggests that this method can be applied to purify any RyR isoform of interest, including human RyR1 and RyR3, whose structures have not yet been solved. This approach can also be applied for screening small molecules that target RyRs for structural pharmacology.

Further, the developed RyR purification method was applied to study the influence of lipid mimetics on RyR1 gating. Using the rapid and small scale nanobody-based purification method RyR1 was purified in various lipid mimicking environments, including nanodiscs, micelles with different lipid concentrations, and liposomes. I found that nanodiscs constrained channel from opening, while increasing lipid concentration in micelles (from 0.001% to 0.05%) raised the open probability from 16% to 84%. RyR1 in liposomes showed an open probability of 62%. These findings show that open probability of RyR1 is very sensitive to lipid mimetics and can be finetuned for structural studies of RyR1 gating.