SCIENCES & BIOENGINEERING SCIENCES

The Research Group

Structural Biology Brussels

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

Ting Luo

to obtain the degree of Doctor in Bioengineering Sciences

Title of the thesis:

Identifying protein interaction partners and cysteine oxidative modifications: case studies for peroxiredoxin 2 and STIM2

Promotors:
Prof. dr. Joris Messens (VUB)
Dr. Daria Ezeriņa (VUB)

The defense will take place on
Friday, December 16, 2022 at 16 h in auditorium D.2.01

Members of the jury

Prof. dr. Steven Ballet (VUB, chair)
Prof. dr. Janine Brunner (VUB, secretary)
Dr. Didier Vertommen (UCLouvain)
Prof. dr. Joske Ruytinx (VUB)
Prof. dr. Massimo Santoro (University of Padua)

Curriculum vitae

Ting Luo holds a bachelor's degree in Pharmacy from the Shanghai University of Traditional Chinese Medicine in China. In 2016, he obtained his master's degree in Molecular Biology at the Vrije Universiteit Brussel, Belgium, where he studied the synthesis and reactivity of spirocyclic indolones as a master thesis project under the supervision of Prof. dr. ir. Guido Verniest. He then started a PhD research project in the lab of Prof. dr. Joris Messens where he developed new cellular methods for the identification of peroxiredoxin 2 interactors and of cysteine oxidative modifications of the protein STIM2. His results have been published in four research articles. For his next career challenge, Ting Luo recently moved to Basel, Switzerland.

Abstract of the PhD research

Hydrogen peroxide (H_2O_2) acts as a signalling molecule by oxidizing specific cysteine thiols in proteins. Recent evidence has established a role for cytosolic peroxiredoxins in transmitting H_2O_2-based oxidation to a multitude of target proteins. Moreover, it is becoming clear that peroxiredoxins fulfil their function in organized microdomains, where not all interactors are covalently bound. However, previous studies aimed at identifying peroxiredoxin interactors were based on methods that only detect disulphide linked partners. Here, I applied two thiol-disulphide unbiased in-cell trapping methodological approaches for the identification of interaction partners of peroxiredoxin 2. First, I combined biotin-dependent proximity-labelling (BioID) in intact live cells with mass spectrometry. I identified 13 interactors under elevated H_2O_2 conditions, among which CSN5, subunit five of the COP9 signalosome (CSN) complex, known to play a role in protein homeostasis and in the signalling of STAT3, an established Prdx2 interactor. Second, I used a chemical crosslinking protocol followed by immunoprecipitation, where the c-Jun protooncogene was revealed to interact with Prdx2. The CSN5- and c-Jun-Prdx2 interactions were then confirmed in a proximity ligation assay. Both methods identified known and novel Prdx2 interactors involved in markedly different biological processes. Taken together, my results demonstrated that BioID and crosslinker-IP are alternative methods in the identification of interactors of peroxiredoxins.
In a second case study, I focused on the oxidative modifications of STIM2. STIM1 and STIM2 are responsible for sensing the Ca^{2+} levels in the lumen of the endoplasmic reticulum (ER) and for the gating of the ORAI channels. It has been shown that oxidative modifications of cysteines of STIM1 and OARI1 have an impact on their function and lead to modulation of Store-operated calcium entry (SOCE). Recently, through FLIM and FRET microscopy as well as MD-simulations, Cys313 was identified as the main redox sensing residue of STIM2 and it was indicated that oxidative modifications of Cys313 alter the STIM2 activation dynamics, thereby hindering STIM2-mediated gating of ORAI1. As a collaborator on this project, I revealed the oxidative status of Cys313 using recombinant STIM2-GFP expressed in a mammalian cell line using nanobody-based GFP-trapping followed by target specific mass spectrometry. I showed that Cys313 forms a disulphide with Cys302 under normal Ca^{2+} levels in the lumen of the ER. Further studies are needed to reveal the oxidative modifications of Cys313 under conditions of luminal Ca^{2+} depletion of the ER and under oxidative stress.