SCIENCES & BIOENGINEERING SCIENCES

The Research Group

Structural Biology Brussels

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

Piotr Kolata

to obtain the degree of Doctor of Bioengineering Sciences

Title of the PhD thesis:
The structure of *Escherichia coli* respiratory complex I

Promotor:
Prof. dr. Rouslan Efremov (VUB)

The defense will take place on
Friday, February 25, 2022 at 16:00 in auditorium D.0.08

Due to COVID-19 measures, the capacity to physically assist the event at the Campus of Humanities, Sciences and Engineering of the Vrije Universiteit Brussel, Pleinlaan 2, 1050 Elsene, will be limited. The defense can also be followed through a live stream: https://bit.ly/3uwbaCA

Members of the jury

Prof. dr. Han Remaut (VUB, chair)
Prof. dr. Janine Brunner (VUB, secretary)
Prof. dr. Sophie de Buyl (VUB)
Prof. dr. Steven Ballet (VUB)
Prof. dr. Cédric Govaerts (ULB)
Prof. dr. Edmund Kunji (University of Cambridge)

Curriculum vitae

Piotr Kolata studied Molecular Biotechnology and Technical Biochemistry at the Lodz University of Technology in Poland. He did his master thesis work entitled "Molecular effects of a novel mutation in Rab33b associated with a muscular disease" at the Lille 1 University – Science and Technology in France. In 2016, he started his PhD in the laboratory of Rouslan Efremov at the VIB Center for Structural Biology/VUB Structural Biology Brussels. In his work, he used a rapidly developing structural biology technique – single-particle electron cryogenic microscopy (cryo-EM) to study the molecular basis of energy conversion in cells. During his PhD, he supervised two master thesis students and an Erasmus intern. Piotr gave presentations at international conferences and the CryoEM school of Belgian Biophysical Society. The results of his PhD work were published in the eLife journal.

Abstract of the PhD research

The ability to capture energy from the environment to drive cellular processes is fundamental for life. Heterotrophic organisms, including humans, consume nutrients to synthesize adenosine triphosphate (ATP), the main energy currency of the cell. The most effective process to achieve this is respiration. There, macromolecules such as fats, proteins, and sugars are broken down into simpler molecules with the concomitant release of energy. This energy is conserved as an electrochemical gradient that among other processes is used for ATP synthesis. In aerobic organisms, including humans and many bacteria, most of the ATP is synthesized by the electron transport chain that consists of a set of enzymes residing in the inner mitochondrial membrane of eukaryotes, or the cytoplasmic membrane of bacteria.

Respiratory complex I is the first member of the electron transport chain, creating 40 % of the electrochemical gradient for ATP synthesis during respiration. It is a multi-subunit membrane protein complex that reversibly couples NADH oxidation and ubiquinone reduction with proton translocation against transmembrane potential with nearly 100% efficiency. The molecular mechanism of this large protein complex has been puzzling scientists for more than 30 years.

Complex I from *Escherichia coli* is the simplest and among the best functionally characterized known complex I homologs. Thus, it is an attractive target for mechanistic studies. However, such research has been hindered by the lack of the complete structure of *E. coli* complex I. Historically, structural analysis of this protein has been prevented for two main reasons. First, the fragility of *E. coli* complex I made its purification in the intact form difficult. Second, the high flexibility of the complex has made the actual structural studies very challenging.

In the scope of this PhD thesis, a novel method for efficient expression and purification of intact *E. coli* complex I have been developed. It allows obtaining a catalytically active protein in conditions compatible with structural studies by single-particle electron cryogenic microscopy. The entire *E. coli* complex I structure has been solved to high resolution. It represents the first complete reconstruction of complex I from a mesophilic bacterium. It has revealed the architecture of the complex in atomic detail and explained the reasons for its high flexibility. The complex is stabilized by the unique terminal extensions and an insertion loop in the peripheral arm. An unusual conformation of the conserved interface between the peripheral and membrane domains suggests that the purified complex adopts an uncoupled conformation. Based on the structural data, a new, hypothetical coupling mechanism for the complex I has been proposed.