SCIENCES & BIOENGINEERING SCIENCES

The Research Group

Cellular and Molecular Immunology

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

Christopher Kariuki

to obtain the degree of Doctor of Bioengineering Sciences

Title of the PhD thesis:
Deciphering the structure and function of select membrane associated proteins in haemo-protozoan parasites

Promotor:
Prof. dr. ir. Stefan Magez (VUB)

The defense will take place on
Wednesday, June 22, 2022 at 16h in auditorium D.0.05

Members of the jury

Prof. dr. ir. Remy Loris (VUB, chair)
Prof. dr. ir. Benoit Stijlemans (VUB, secretary)
Dr. Els Pardon (VUB)
Prof. dr. Luc Leyns (VUB)
Prof. dr. Sebastian Zoll (Czech Academy of Sciences)
Dr. Veerle Lejon (Institut de Recherche pour le Développement)

Curriculum vitae

Christopher Kariuki obtained his Master's degree in Molecular Biology from the Vrije Universiteit Brussel (VUB) in 2014. He embarked on his doctoral research in 2014 at the Structural and Functional Immuno-Parasitology (SFIP) group under the supervision of Prof. Dr. ir. Stefan Magez. He was later enrolled as a teaching assistant in the "Interuniversity Programme Molecular Biology" (IPMB) within the Bio-engineering department (DBIT), Faculty of Science and Bio-engineering Sciences (WE), VUB. In this capacity, he assisted in designing and teaching the first-year Master of Science Molecular Biology course "Experimental Research Skills". Together with IPMB, he also initiated the "Hands-on Practical Training Workshop in Molecular Biology" course, held in Kenya (2017 & 2022), Tanzania (2018) and Uganda (2019). He has participated as a co-Principal Investigator in the VLIR-UOS funded JOINT project between the Institute of Primate Research (Kenya), Addis Ababa University (Ethiopia) and KU Leuven, entitled "Initiating a Training Network and Capacity Building to Improve Control of Foot-and-Mouth Disease in Kenya and Ethiopia (KEET)". Christopher has participated and presented his work at several international events and, via this work, published 3 scientific articles in peer-reviewed international journals. He has supervised one master's and bachelor's thesis, in addition to several laboratory trainings for ERASMUS students at CMIM.

Abstract of the PhD research

Haemoparasitic protists occupy the nutrient-rich haemolymphatic compartment of the vertebrate host. Two haemoparasitic protists belonging to the genera Trypanosoma (Kingdom Protozoa) and Plasmodium (Kingdom Chromista), are agents of vertebrate diseases of great medical and veterinary importance, that is sleeping sickness and malaria. Their cell membrane, decorated with a GPI-linked proteome together with non-covalently linked components, is a major virulence factor in these parasitic infections. The elucidation of its various components is a critical requirement for the development of diagnostic or prophylactic measures targeting these parasites.

This thesis presents the work done towards the deciphering of the structure and function of select membrane associated proteins on haemoprotozoan parasites with a focus on the trypanosomal transferrin receptor (*Tb*TfR). The *Tb*TfR is a heterodimeric complex derived from two similar genes (pESAG6 and 7) from the bloodstream expression site (BES) of *Trypanosoma brucei*. The *Tb*TfR allows the bloodstream trypanosome to acquire host-derived iron in the form of serum transferrin. In this study, recombinant pESAG6 and pESAG7 protein constructs were expressed in an *Escherichia coli* expression system, forming inclusion bodies. However, their characterization proved untenable due to the inability to extract and purify soluble, pure, and properly folded protein from the so-formed inclusion bodies.

Using several anti-pESAG6 nanobodies, this study designed a novel protocol for nanobody expression. By making simple yet biologically significant changes to the conventional bacterial expression protocol, this novel protocol could achieve up to a 9-fold increase in the yield of pure nanobody. Using indirect ELISA, binding of these anti-pESAG6 nanobodies was demonstrated to the expressed and extracted, but unfolded and impure pESAG6, pESAG7 and pESAG6/7 constructs. This was considered indicative of conserved epitopes. The anti-pESAG nanobodies, when applied on western blots of the pESAG6 construct, also established that the recognised epitopes were linear. The anti-pESAG6 nanobodies also bound to a non-VSG component of whole trypanosome lysate on the closely related *T. brucei* and *T. evansi* but not the distantly related *T. congolense*. Similarly, binding of the nanobodies to live and fixed trypanosomes was demonstrated via flow cytometric assays. However, the nanobodies did not elicit a biological phenotype on cultured *ex vivo* trypanosomes. The results hint to possibilities of using the nanobodies as a diagnostic tool, subject to an improved antigen expression, nanobody library generation and selection.