SCIENCES & BIOENGINEERING SCIENCES

The Research Group of

Organic Chemistry (ORGC)

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

Kevin Van holsbeeck

to obtain the degree of Doctor of Sciences

Joint PhD with Ghent University

Title of the PhD thesis:

Downsizing the Downsized:
Nanobody Complementarity-Determining Region Peptidomimicry

Promotors:
Prof. Dr. Steven Ballet (VUB)
Prof. Dr. José C. Martins (UGent)

The defense will take place on
Tuesday, May 16, 2023 at 5 PM in auditorium D.2.01

Members of the jury

Prof. Dr. Sophie Hernot (VUB, chair)
Prof. Dr. Ulrich Hennecke (VUB, secretary)
Prof. Dr. Freija De Vleeschouwer (VUB)
Prof. Dr. Annemieke Madder (UGent)
Prof. Dr. Christian Gruber (Medical University of Vienna)
Prof. Dr. Wim De Borggraeve (KU Leuven)

Curriculum vitae

Kevin Van holsbeeck graduated at the Vrije Universiteit Brussel in 2018 as a Master of Science in Chemistry with greatest distinction, for which he obtained two awards. After this, he obtained a strategic basic research fellowship of the Research Foundation Flanders (FWO) to perform a PhD at the Research Group of Organic Chemistry (ORGC) led by Prof. Dr. Steven Ballet at VUB, in collaboration with the NMR and Structure Analysis Research Group (NMRSTR) of Prof. Dr. José C. Martins at Ghent University. In addition, he performed a short research stay with Dr. Nicolas Floquet at the Institut des Biomolécules Max Mousseron in Montpellier (France), funded by a travel grant of the FWO. He published one review and co-authored six scientific papers in peer-reviewed international journals, amongst which three as (shared) first author, and presented his work at multiple (inter)national conferences. Over the course of his PhD, he supervised and guided three master students and one internship student, and was involved in teaching practical sessions for bachelor and master students.

Abstract of the PhD research

Antibodies represent an important therapeutic drug family thanks to their high affinity and specific binding capabilities. However, they suffer from several drawbacks, such as a high production cost and limited cellular access, due to their overall molecular architecture and high molecular weight. For these reasons, downsized functional antibody fragments were developed, among which the popular single variable heavy domains named nanobodies (Nbs). Despite the tenfold reduction in size, as compared to classical antibodies, nanobodies can reach similar and thus favorable high affinities and specificities for antigens.

Whereas the antigen-binding regions of a classical antibody are centered around six different complementarity-determining regions (CDRs), a nanobody has only three CDRs available for interaction with an antigen, resulting in a more compact interaction surface, in comparison with classical antibodies. Therefore, the possibility was considered to downsize these nanobodies even further by preparing low molecular weight peptides based on the CDR sequences. Peptides might present more cost-effective templates as therapeutics due to lowered production costs and immunogenic effects, combined with an increased amenability for intracellular access.

To mimic the biological outcomes by peptides structurally derived from nanobody CDR sequences, nine nanobodies interacting with five different target types were considered: a protein-protein interaction, a G protein-coupled receptor, a protein kinase, bacteria and betacoronaviruses. In all attempts, peptides representing the CDR1-3 sequences were synthesized and evaluated in biochemical and/or *in vitro* assays to assess their potential interaction with the native nanobody antigens. In addition, cyclic peptides were considered to constrain the flexibility of linear peptides, and (potentially) structurally mimic the nanobody CDR loops. In two cases, (partial) functional mimicry was observed: (i) Nb14 CDR3 mimetics were able to enhance the nucleotide exchange activity of RAS proteins through interaction with SOS1:RAS in a similar mode of action as the native Nb14, next to structurally mimicking its CDR3, and (ii) NbD4 CDR3-presenting peptides were able to interact with the membrane of living *A. baumannii* bacteria, in contrast to the native nanobody which required cell fixation. The first case study especially benefitted from a nanobody interacting with a single dominant loop, binding into a clearly delineated binding pocket. Altogether, it was demonstrated that downsizing a nanobody towards smaller synthetic peptides based on its CDRs is feasible in particular cases, although significant decreases in binding affinity or potency are generally observed in initial series. Future endeavors will unravel up to which potency such mimetics can be developed by structural fine-tuning.