SCIENCES & BIOENGINEERING SCIENCES

The Research Group

Organic Chemistry

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

Camille Lozada

to obtain the degree of Doctor of Sciences

Joint PhD with CY Cergy Paris Université

Title of the PhD thesis:

Alteration of the membranotropic properties of cell-penetrating and fusion peptides by means of conformationally constrained tryptophan derivatives

Promotors:
Prof. dr. Steven Ballet (VUB)
Prof. dr. Nadège Lubin-Germain (CY Cergy Paris Université)

The defense will take place on
Thursday, June 22, 2023 at 14h30 in the MIR auditorium, 1 Rue Descartes, 95000, Neuville-sur-Oise, France.

Members of the jury

Dr. Abdelghani Oukhaled (CY Cergy Paris Université, chair)
Prof. dr. Ulrich Hennecke (VUB, secretary)
Dr. Simon Gonzalez (CY Cergy Paris Université)
Dr. Timo De Groof (VUB)
Dr. Sophie Faure (Université Clermont Auvergne)
Prof. dr. José C. Martins (University of Ghent)

Curriculum vitae

Camille Lozada graduated at CY Cergy Paris Université in 2019 as a Master of Chemistry. Next, she started a joint PhD in the group of Biomolécules: Conception, Isolement, Synthèse (BioCIS) under the supervision of Prof.Dr. Nadège Lubin-Germain at CYU, in collaboration with the Research Group of Organic Chemistry led by Prof. Dr. Steven Ballet at VUB.
She published one review and submitted one scientific paper in peer-reviewed international journals as (shared) first author.
During her PhD, she guided bachelor and master students during their internships, and was involved in teaching practical sessions for bachelor students.

Abstract of the PhD research

Membranotropic peptides are generally short sequences able to interact with membranes. Among these bioactive peptides, two categories of peptides were studied: cell-penetrating peptides (CPPs) and viral fusion peptides (FPs). CPPs are commonly cationic and have been well-studied for their capacity to cross cellular membranes. Therefore, they have been much utilized to improve the cellular uptake of polar therapeutic agents. On the contrary, FPs are short hydrophobic sequences found in viral proteins. They are essential for enveloped viruses because of their capacity to insert into the host cellular membrane, to subsequently allow the viral membrane to merge during the infection process. We developed a vectorization method using FPs of hepatitis C virus (HCV) as anchors into biological membranes. However, preliminary results showed a moderate membranotropic activity, probably due to their lack of structuration, a crucial feature for membranotropic behavior. Therefore, it was suggested to introduce constrained amino acids to induce or facilitate peptide structuration. Two cyclic Trp surrogates were chosen as local constraints and synthesized as suitable building blocks to be incorporated in unstructured membranotropic peptides: a tetrahydro-β-carboline derivative **Tcc**, and an indoloazepinone scaffold **Aia**. These surrogates present constrained dihedral angles and limited side chain orientations which were hypothesized to influence the peptide's structure and/or membranotropic activity. To study the influence of the introduction of these local constraints on membrane activity and structuration, we selected four reference peptides: one CPP (**RW9**) and three viral peptides (**HCV7**, **Flav**, and **C8**). These peptides were synthesized by SPPS and Trp residues were replaced by either **Tcc** or **Aia** building blocks, varying the number of modifications and their positions, eventually resulting in a series of 34 peptides. Their membranotropic activities were studied by spectrofluorescence titration in presence of liposomes, allowing to calculate partition coefficients (K_p). Results showed that improvement of the activity by introducing **Tcc** or **Aia** was sequence dependent and influenced by the number of modifications. The most potent peptides were found to be **[Aia^4]RW9** (H-RRW-**Aia**-**Arg**-RWRR-NH_2) and **[Tcc^{1,4}]C8** (Ac-**Tcc**-ED-**Tcc**-VGWI-NH_2) which showed K_p values 3-fold higher than their native counterparts. Further studies by molecular dynamics (MD) simulations were in correlation with the trend experimentally observed. Further structural studies were performed by circular dichroism (CD). Finally, tagged versions of the peptides with fluorescein were added to culture medium of CHO-K1 cells for permeation studies using confocal microscopy. Future studies will demonstrate the capacity of the modified sequences to transport various types of bioactive cargoes into cells.