EU-DOCS for SmUCS

CO-SUPERVISED SUBJECT PROPOSAL FOR A DOCTORAL CONTRACT

Title of the thesis project: Investigation of the regulation of post-translational modifications by Heparanase 1 And Membrane-type matrix metalloproteinase 1 in macrophages during Inflammatory processes (HAMI)	
La Rochelle University Research Unit: LIENSs (LIttoral ENvironnement et Sociétés)	**Partner university:** University of Calgary (UC), Canada **Cotutelle research unit:** Antoine Dufour's Lab.
Name of the LRUniv supervisor: Kévin Baranger CNRS researcher (CR CNRS - HDR)	**Name of the co-supervisor:** Antoine Dufour Associate Professor
Non-academic partner: MolDrug AI Systems SL (Valencia, Spain) - January 2026-May 2026	
Keywords *(6 max)*: Heparanase 1, MT1-MMP, therapeutic target, inflammation, drug design, bio-inspired molecules.	

Scientific description of the research project

Scientific context

Macrophages are key cells in the innate inflammatory response. Along with neutrophils, they are the first cells to arrive on site to initiate, organise and then resolve inflammatory processes. One key biological function at these different stages is the controlled polarisation of macrophages. This is essential for resolving inflammation and minimising the development of autoimmune diseases.

The molecular players involved in this activity include heparan sulphate (HS)-based proteoglycans, known as HSPGs, located in the macrophage membrane, as well as the production of an enzymatic arsenal comprising glycosidases (degradation of sugars) and proteases (cleavage and/or degradation of proteins). Among the glycosidases, heparanase 1 (HPSE1) is the only mammalian enzyme responsible for degrading HS chains and is well known for its pathological involvement in inflammatory processes and immune cell polarisation. Proteases act in concerted networks to amplify cellular signals, regulate pathophysiological aspects by activating and/or inactivating proteins through proteolysis, and are molecular effectors involved in certain macrophage immune functions. Among these proteases, membrane-type matrix metalloproteinase 1 (MT1-MMP or MMP14) appears to be an interesting candidate for macrophage cellular homeostasis through the control of HSPG metabolism, which is dysfunctional during inflammation.

However, the precise contribution of HPSE1 and MT1-MMP to macrophage polarisation through cleavage of HSPGs at the cell surface, either directly or in synergy with HPSE1, remains to be established. These cleavages have the potential to be an important therapeutic target for inflammatory diseases but also cancers.

We have recently highlighted intriguing regulatory aspects of macrophage proteases, particularly MT1-MMP, which could, in a synergistic manner, modulate the inflammatory cell signalling axis orchestrated by HPSE1, as we have previously demonstrated in cancer processes.

Several inhibitors of HPSE1 have entered clinical trials to treat cancers and most are heparan sulphate mimetics rather than small molecules. But none of these have been approved as drugs, often due to issues like poor bioavailability, side effects, and unfavourable pharmacokinetics The chemical space of HPSE1 inhibitors has been confined to relatively few chemical classes, limiting the development of diverse, drug-like compounds. This narrow focus has made it challenging to identify novel scaffolds with improved properties.
In the LIENSs laboratory, we possess a collection of unique small molecules featuring various heterocyclic structures. This chemical library is composed of pharmacomodulated bio-inspired molecules including oxindole derivatives (Bisoxindole structures isolated from marine molluscs from the Atlantic coast, La Rochelle). Oxindole moieties/cores are well-known structures already found in cancer-approved drugs such as sunitinib. Over the past few years, the chemistry team has developed an original approach to accessing poly-nitrogen and sulphur-containing molecules, specifically studying the reactivity of iminodithiazoles and dithiazolyloxindoles in alkaline and nucleophilic environments. These highly versatile synthons, after ring opening, lead to molecular diversity. Heterocyclic small molecules (N, O, S) will be prepared using sustainable chemistry methods, in unconventional media, under microwave irradiation, following the principles of atom and step economy. The laboratory has strong expertise in the synthesis and pharmacomodulation of small molecules through organocatalyzed coupling methods. While nanobodies strategy seem to be efficient to target MT1-MMP, there is no small molecule inhibitor of this enzyme. We have recently explored our chemical library and found that among our molecules, some are inhibitors of HPSE1 or MT1-MMP, and some are dual inhibitor of HPSE1 and MT1-MMP in vitro.

In this context and based on our preliminary results we wanted to 1) improve/optimize and develop new original bio-inspired compounds as dual inhibitor of HPSE1 and MT1-MMP using structure-based drug design, virtual screening and chemical synthesis and 2) decrypt HPSE1/MT1-MMP-mediated HSPGs metabolism and validate the efficacies of our molecules in vitro and in vivo using proteomic and N-terminomics approaches in activated macrophages. These aims summarise the fundamental aspects that will be addressed in the doctoral thesis proposed here.

Profile and skills required: Master's degree in chemistry and biochemistry, and who possesses technical know-how and a real interest in the fields of drug-design, mass spectrometry, proteomics, bioinformatics and cell biology. These skills will be an undeniable asset for successfully completing this project.

Scientific objectives

The objectives are to develop new original dual inhibitor against HPSE1 and MT1-MMP based on bio-inspired marine molecule as anti-inflammatory drug and validate them to control HPSE1/MT1-MMP-mediated HSPGs metabolism in macrophage reactivity. The strategy proposed here could lead to molecules as alternative to expensive immunotherapies to treat inflammatory diseases.
In this PhD thesis, and in a perfect continuation of the ongoing collaboration between Kévin Baranger (La Rochelle, France) et Antoine Dufour (Calgary, Canada), we gather the private partner MolDrug (Valencia, Spain). Together, and with our complementary skills, we will lead this project and evaluate the yet unknown synergy between HPSE1 and MT1-MMP in the management of HSPGs and the consequences for macrophage polarisation and inflammatory processes using an original strategy based on a dual inhibitor of these enzymes.
Based on our already available chemical structures, our non-academic partner MolDrug, will use computed structure-based drug design to guide La Rochelle partner for the chemical synthesis and optimization of the compounds targeting HPSE1 and MT1-MMP, considering not only the bioactivity of the candidates but also their drug-like profile (e. g. considering their pharmacokinetics and potential toxicity and side effects). Molecular docking of the lead compounds already identified, will allow access to potentially more active compounds. In parallel of the synthesis of compounds and their validation in vitro, we will study the impact of cleavage of HSPG HS chains by HPSE1 and the management of HS-free HSPGs by MT1-MMP on active proinflammatory macrophages. This will be possible via 1) the various cell models available in the two laboratories (La Rochelle and Calgary), 2) the use of commercial inhibitors of HPSE1 and MT1-MMP and original bio-inspired synthetic inhibitors The three partners have and use state-of-the-art equipment and approaches such as TAILS/N-terminomics and proteomic analyses (Calgary), chemical synthesis (La Rochelle) and drug design (MolDrug). Together, they will identify, for the first time, the substrates shared by HPSE1 and MT1-MMP implicated in macrophages polarisation.

This collaborative project aims to fill the data gap concerning the synergy of HPSE1 and MT1-MMP activities on HSPGs and their impact on the initiation and/or resolution of inflammatory processes, by identifying all MT1-MMP substrates, with a focus on HSPGs and develop a new original strategy targeting both enzymes as potent anti-inflammatory drug with low-cost production.

Scientific challenges

The main scientific challenges of the HAMI project are 1) to develop, using computed structure-based drug design, a dual specific inhibitor of HPSE1 and MT1-MMP and 2) to successfully synthesized it. To validate its specificities for both enzymes, we will used enzymes, as negative control with closed activities, such as glucuronidase (for HPSE1) and MT5-MMP (for MT1-MMP). The size of our molecules is <400 Da which meets the Lipinski's rules of 5 for a good pharmacokinetic and pharmacodynamic properties. In addition to the high-added value of such inhibitor, we will decrypt the basic synergy between both enzymes on HSPGs metabolism.

Methods

- MolDrug: Structure-based design, molecular docking, QSAR, virtual chemistry, hit-to-lead approaches.
- LRU: Bio-inspired molecules, chemistry, preliminary encouraging results, enzymatic tests, in vitro, in cellulo assays using plasmids.
- UCalgary: mass spectrometry, N-terminomics/TAILS, proteomics, bioinformatics, and metabolomics, computer-aided proteomic and metabolomic analyses.

MolDrug will form and help the PhD candidate to successfully design potent dual inhibitors using computer-aided structure-based design by molecular docking. LRU will give the structures of the "hits" already identified as dual inhibitors to be optimized.

LRU: According to the results obtained with MolDrug, the PhD candidate will chemically synthesize molecules with Pr. Valérie Thiéry, head of the chemistry team. All the tools are available in the lab (or in closed collaboration) to control each step of the synthesis and the purity of the compounds (13C NMR, 1H NMR, MS, IRTF, flash chromatography, etc..).
After synthesis, efficacy of the compounds will be evaluated in vitro using enzymatic assays and *in cellulo* using plasmids encoding either HPSE1 or MT1-MMP and syndecan 1 (SDC1), all with appropriate tags These plasmids will be transfected in HEK293 cells, and the metabolism of SDC1 followed by WB using specific tags present in the constructions.

UCalgary: We will profile and characterize MT1-MMP substrates using proteomics and N-terminomics/TAILS approaches that are routinely performed in Antoine Dufour's laboratory. Briefly, we will compare WT, HPSE1-/- (generated by Kévin Baranger) and MT1-MMP-/- human monocytic THP-1 cells (obtained by CRISPR/Cas9) activated with PMA (Phorbol 12-myristate 13-acetate) and interferon γ (IFNγ) to generate pro-inflammatory macrophages. We will prepare lysates for proteomics and isotopically label them with heavy/deuterated (WT) or light (HPSE1-/-, MT1-MMP-/-) formaldehyde. After trypsin digestion, negative selection against the unlabelled α-amines of the trypsinized peptides will be carried out by incubating the samples with the TAILS polymer. Unbound peptides containing the cleavage sites generated in vivo will be separated from the polymer-bound peptides by filtration. After analysis by liquid chromatography-tandem mass spectrometry (LC-MS/MS), the data will be analysed using MaxQuant with a false-positive rate of 1%, followed by TopFIND).
The new MT1-MMP (and/or HPSE1) substrates identified will be validated in Kévin Baranger's team using plasmids and biochemical approaches such as SDS-PAGE gel, silver staining and Western blots as described above. We will prioritize all HSPGs identified in our proteomic data. All this research will also be carried out with commercial inhibitors of MT1-MMP and HPSE1 from the chemical library (La Rochelle) of MT1-MMP, HPSE1 and dual targeting MT1-MMP andHPSE1.

LRU/UC: The pathophysiological consequences of the MT1-MMP/HPSE1 synergy will be analysed mainly by ELISA through the measurement of the inflammatory mediators TNFa, IL-1b and IL-6. Macrophage polarisation will be assessed by RT-qPCR and cytometry and the following markers will be evaluated: IL-1 b, TNFa, IL-6, CD86 for M1 proinflammatory polarisation and IL-10, TGF-b, Arg1, CD206, CD163, VEGF for M2 anti-inflammatory polarisation. Finally, the change in phagocytosis capacity linked to polarisation will be assessed using an immunocytochemical model of phagocytosis of dextran-Rhodamine bead.

The cleavage fragments identified will be cloned into plasmids and/or lentiviruses for overexpression in THP-1 cells and conditioned supernatants. Using RT-qPCR, WB and ELISA approaches, we will establish macrophage polarisation and inflammatory mediator production. In addition, using conditioned supernatants, we will process naïve THP-1 cultures to assess the ability of these identified fragments to modify macrophage polarization as described above.
All the protocols and techniques are perfectly mastered by the two partner laboratories.

Expected results

- Selection of several hits in our chemical library (anti-HPSE1 and anti-MT1-MMP drug).
- Molecular docking and hit-to-lead design.
- Drug design: pharmacomodulation using chemical synthesis.
- Validation *in vitro* and *in cellulo* at each step.
- Identification of HPSE1 and MT1-MMP shared substrates; comprehension of their metabolism in macrophage polarisation.
- Efficacy of the drugs on macrophages polarisation using mass spectrometry, WB, ELISA, qPCR, cytometry.

Perspective

The PhD work will lead to 1) basic data on HSPGs metabolism in macrophages inflammatory processes and 2) the production of an original dual inhibitor of HPSE1 and MT1-MMP; its efficacy as anti-inflammatory drug will be assessed *in cellulo*. According to the results, this compound could be used *in vivo* in zebrafish after cutting the tail fin, a model of macrophages recruitment but also in imiquimod-mouse model of inflammatory skin disease; both models are available through collaborators of LRU lab. We could also use mice subjected to a peritonitis model in which MT1-MMP 1 and/or HPSE1 expression is specifically silenced in macrophages (Baranger, in production). Primary peritoneal macrophages of WTor *Mmp14* and *Hpse1* cKO mice will be characterized using proteomics and the difference in substrates cleavage will be evaluated using N-terminomics/TAILS analysis.

PhD candidate speciality at the end of the PhD: molecular docking, drug-design, chemistry, bio-informatic, cell culture.

Organisation of the doctoral research programme

October - December 2025: La Rochelle University. (3 months)
Registration and doctoral training. Bibliography/state of the art. Introduction to chemical synthesis: synthesis of HIT structures. Introduction to enzymatic assay, cell biology and biochemistry techniques (transfection, qPCR, WB, ELISA).

January - May 2026: MolDrug, Spain. (5 months)
Training in molecular docking, structure-based drug design and establish drug-like profile (e. g. considering their pharmacokinetics and potential toxicity and side effects). Autonomy in different bio-informatic approaches. Proposal for pharmacomodulation of HITs to become LEAD structures.

June - December 2026: La Rochelle University. (7 months)
Production of LEAD molecules, purification, enzymatic assay in vitro against MT1 and HPSE1, cytotoxicity, in cell efficacy using HPSE1, MT1, SDC1 encoding plasmids and transfections in HEK293 cells. Readouts: WB and ELISA.

January - December 2027: University of Calgary. (12 months)
Introduction to proteomics and N-terminomics techniques and computer analyses. Experiments on primary THP-1 and primary macrophages deficient for HPSE1 and MT1-MMP and wild type treated with our dual inhibitors (LEAD structures).

January - September 2028: La Rochelle University. (9 months)
Validation of substrates identified in Calgary by N-terminomics using plasmids and HEK293 cells. Analysis of proteomic studies carried out on 1) IMQ-treated wild-type mice (skin inflammation) +/- our dual inhibitors; and 2) mice with macrophages specifically depleted in HPSE1-/- and MT1-MMP-/-.

Publications: 2 articles + 1 patent or 1 article

1. Review article on small inhibitor of HPSE1 and MT1-MMP
2. Patent or Article on Chemical design of a dual inhibitor against HPSE1 and MT1-MMP
3. Research article: Identification of shared substrates of HPSE1 and MT1-MMP and their implication in inflammatory processes. Validation of a dual inhibitor to inhibit inflammatory processes.

Scientific alignment with EU-DOCs for SmUCS objectives

This PhD project aims at studying marine bio-inspired products and their valorisation as the seed for new original treatment in inflammatory diseases.

The HAMI project directly aligns with the scientific priorities of La Rochelle Université and the EU-SmUCS programme through its dynamic research in the field of Health and Biodiversity. It is in adequation with the LIENSs research dynamic "One Health", which aims to develop research into the development of the natural resources of our coastline and Nature-based solutions approaches, oriented towards the identification of new molecules useful as anti-inflammatory strategies. By contributing to the development of next-generation anti-inflammatory molecules with green chemistry and innovative methodologies, this research directly supports the EU-DOCs for SmUCS objectives, with significant scientific and societal impact. It will drive scientific novelty and create new potential intellectual properties to drive innovation. This project will also create new avenues of exploration and will impact subsequent collaborations with biotechnology and pharmaceutical companies in addition to generate preliminary data for grant applications.

Societal and economic challenges and contributions

Developing new therapeutics is challenging but are always welcome to heal patients. Here, we proposed the development of new compounds, cheaper than classical immunotherapy, to treat inflammatory chronic disease such as psoriasis or Crohn's disease. Such project will pave the way for further development on new strategies against these two targets and could also be used in cancer field.

Partnership context

BARANGER: He is a CNRS researcher in the BCBS team in LIENSs lab UMR7266 CNRS/LRU, leads the group "inflammation and natural molecules" (https://lienss.univ-larochelle.fr/theme-transversal-2-Mecanismes-anti-inflammatoires-des-substances-naturelles). He is biochemist, specialist of proteolytic activities and inflammation. His research unveiled the pathological contribution of MT1- and MT5-MMP in Alzheimer's disease, the implication of proteolytic and non-proteolytic events governing amyloidogenesis; and identified Hpa1/MT1-MMP/MMP-2 axis in cancer. He will manage and supervise the overall progress of the project at 100% of his time together with Antoine Dufour. He will be helped by Prof. V. Thiéry (chemist), Dr. C. Barthélémy (molecular biology) and B. Musnier (cell culture and cytometry, LRU engineer).
This doctoral thesis project is the continuation of the on-going collaboration between his group in La Rochelle (France) and Antoine Dufour's team in Calgary (Canada), which began in 2023 with the Mourou-Strickland mobility prize that he obtained, entitled 'Role of HPSE1 and MT1-MMP in inflammatory macrophages'.

DUFOUR: He is an Associate Professor and the Scientific Director of the mass spectrometry core facility (SAMS) at the University of Calgary. He leads an internationally recognized research program that uses cutting-edge proteomics and N-terminomics techniques to develop new protease inhibitors to treat chronic inflammatory diseases and cancer. With 88 publications in top journals over 4,300 citations and an h-index of 31, he is an international leader in protease biology. He will manage and supervise the overall progress of the project at 100% during the student's time in Calgary and will continue to have monthly meetings with the team when the student will be in France or Spain. He will be helped by Lab manager Daniel Young (proteomics expert) and Dr. Laurent Brechenmacher (proteomics expert) in the day-to-day supervision of the project and technical skills needed for the completion of this thesis work.

MolDrug: Dr Gozalbes has a background in drug-development. He has worked in academia and in industry, managing drug design projects, computational management of molecule datasets, and development of computational models for the generation of targeted libraries (GPCRs, CNS, kinases) and HPSE1 inhibitors.

Extensive experience in computational management of datasets of chemicals, development of models for the prediction of ADME-T properties. He has 2 current PhD students with 3 completions. Dr. Gozalbes is also the Founder and CEO of the company ProtoQSAR 2000 SL (www.protoqsar.com).

Because of the potential of this subject and the generation of breaking-barriers and high-added values molecules, consortium will pay attention of Intellectual Property. A consortium agreement between all the stakeholders of interest will be drawn up if the subject and a candidate are selected before the start of the project.