

Faculté de pharmacie

Université 
de Montréal



Chaire en Biothérapie de l'Association Canadienne du Médicament

Générique (ACMG) et Biosimilaires Canada

Davide Brambilla

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Montréal

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Biosimilars Canada
The Voice of Biosimilar Medicines in Canada



L'équipe



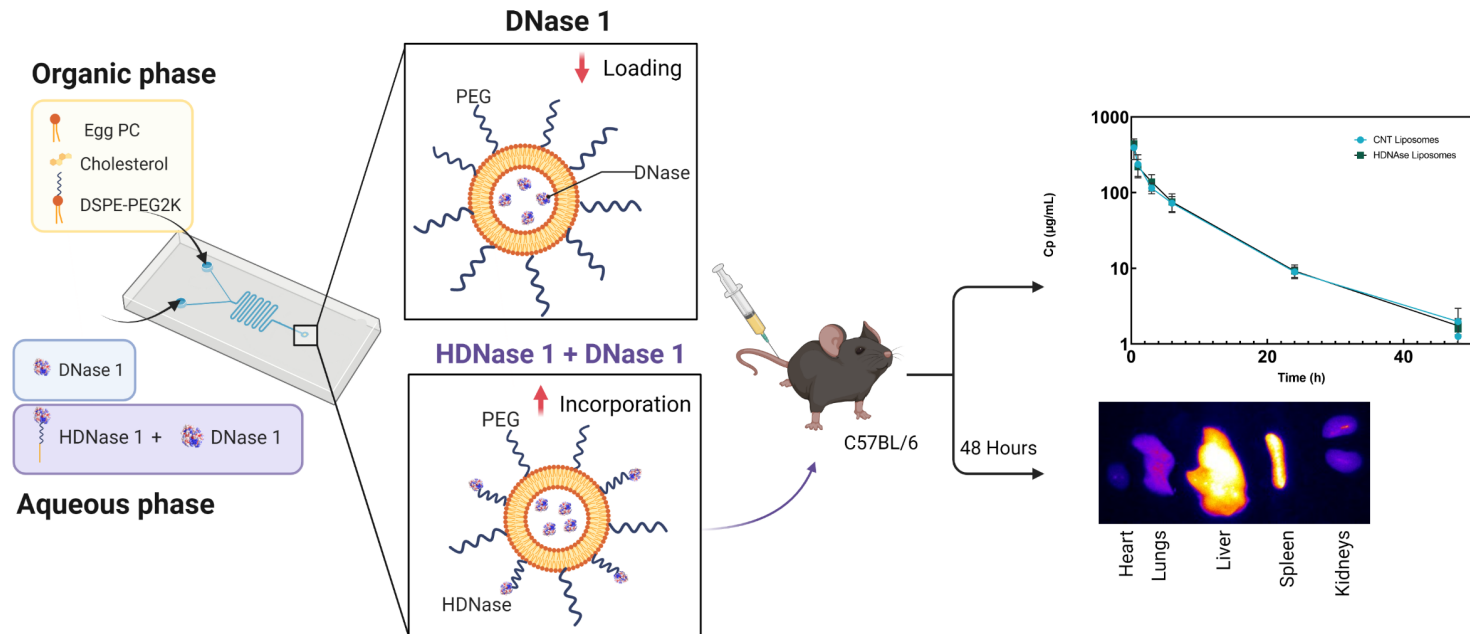
www.brambillaudem.com

Sam Babity: étudiant PhD
Philippe Delbreil: étudiant PhD
Fatma Moawad: étudiante PhD
Carolina Penalzoa: étudiant PhD
Yasmin Ruel: étudiante PhD (ULaval, Prof. Pouillot)
Nastaran Rezaei: étudiant PhD
Naghme Rezania: étudiante Maitrise
Alfonso Nieto: étudiant PhD (McGill et INRS)
Matthias Zadory: étudiant PhD (ETH Zurich)
Alessia Filippini: étudiante PhD (X. Banquy)

Stage et. internationales

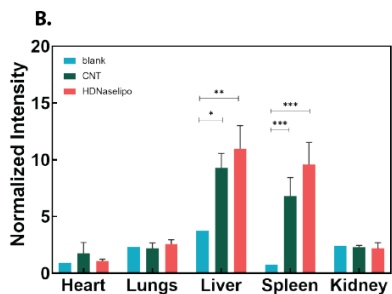
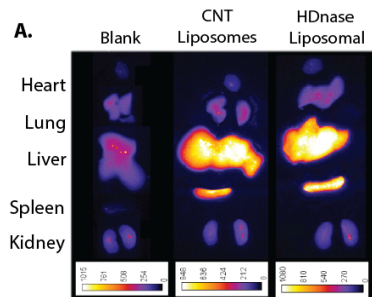
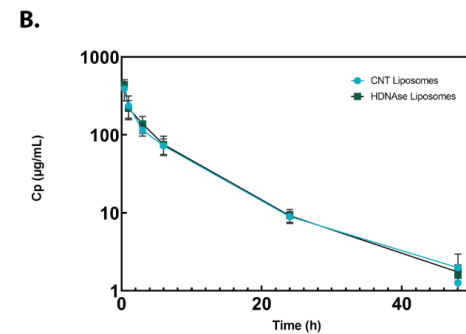
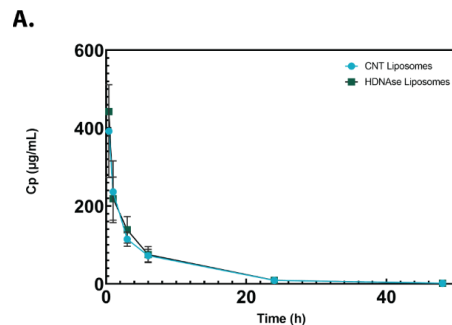
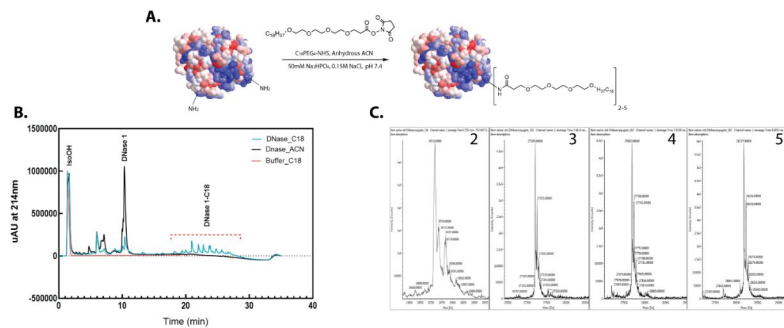
Nour Masmoudi (SUI)
Grace Amazoune (SUI)
Dalia Benhalilou (SUI)
Elliot Lopez (FR)

Project 1.



60% loading for best formulation

Project 1.



C.

Parameter	CNT	CV%	HDNase	CV%2
Cmax (µg/ml)	412.435	33.307	414.279	13.501
Tmax (h)	0.400	0.000	0.400	0.000
t1/2 (h)	7.715	9.917	7.614	3.415
MRTt (h)	6.593	5.023	6.756	7.113
AUC 0-t (µg/ml*h)	1720.459	24.022	1713.275	19.397
CL (µg)/(µg/ml)/h	0.240	26.557	0.237	16.640
Vd (mg)/(µg/L)	2.625	15.877	2.599	16.144

DNase alone in mice (4-5 minutes)

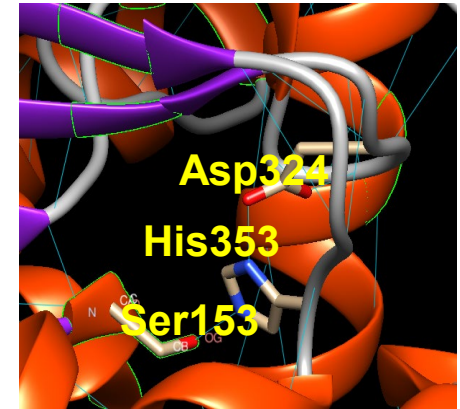
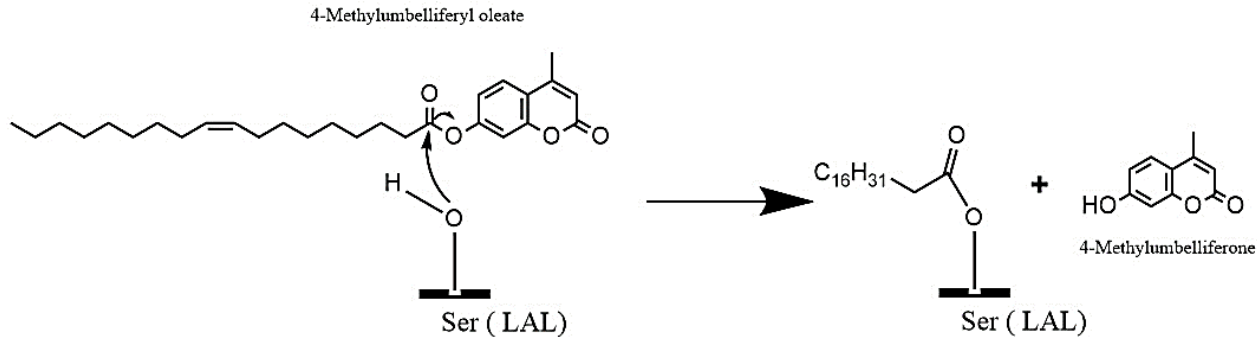
Project 2.

Lysosomal acid lipase deficiency (LAL-D)

Deficiency in the serine hydrolase lysosomal acid lipase (LAL) enzyme

Break down cholesteryl esters (Ecs) and Triglycerides

Caused by deleterious mutations in LIPA gene



Project 2.

LAL-D can affect infants and adults

Most progressive form: Wolman disease, mostly in infants

Hepatosplenomegaly

Growth and liver failure

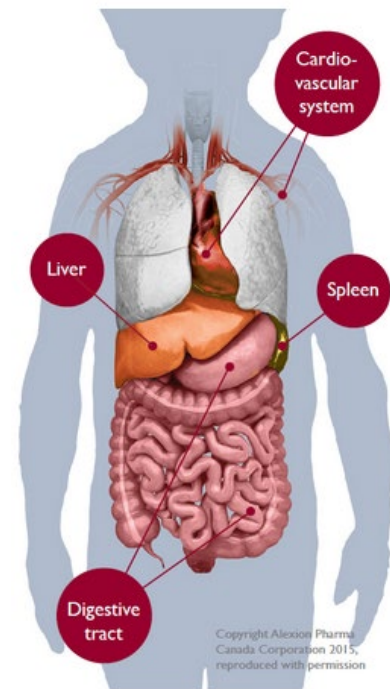
Rarely surviving beyond 6 months of age

Less progressive form: cholesteryl ester storage disease (CESD) in children and adults

Progressive liver disease (liver fibrosis, failure)

Accelerated atherosclerosis with premature mortality

Prevalence estimated at 1:40'000



Project 2.

Available treatments

Kanuma[®]: sebelipase alfa, rhLAL enzyme,

Indication: Long-term treatment of LAL for pediatric and adult patients

Weekly injections

High costs: 10'000\$/10ml vial, estimated annual costs: 890'000-4'905'100\$

Development of ADA

large inter-individual variability in the clinical responses of individual patients

Rapid clearance, extracellular



Project 2.

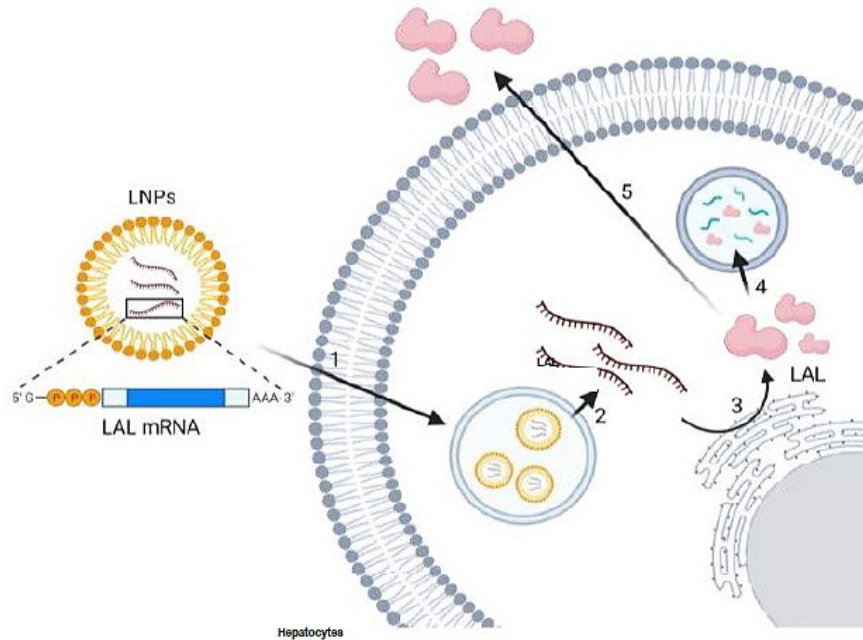
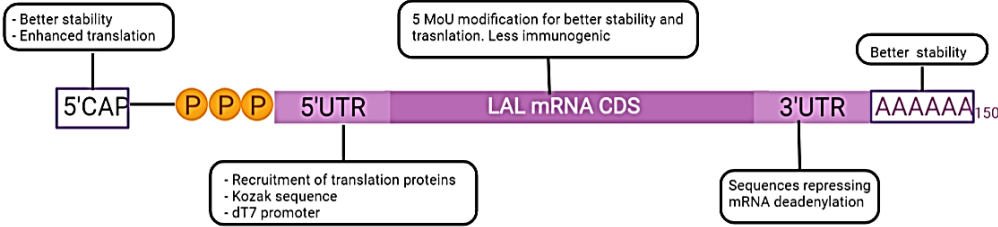


Fig 2 . Innovative approach of LAL-D treatment using LNP for the delivery of LAL mRNA to the cytosol.

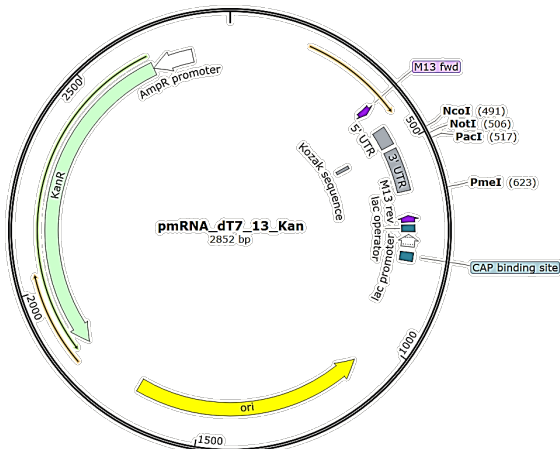
Use “Onpattro®” formulation as benchmark
Hepatocytes targeting

Extended expression
Intracellular expression

Project 2.



Wild type sequence, to start



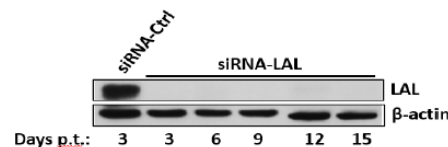
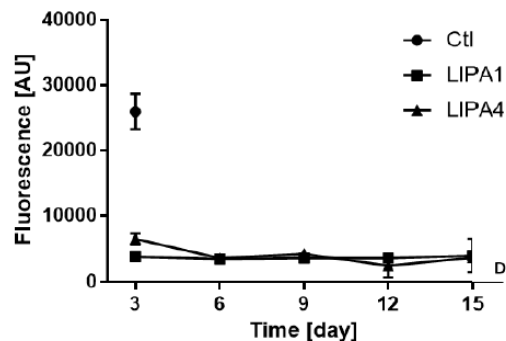
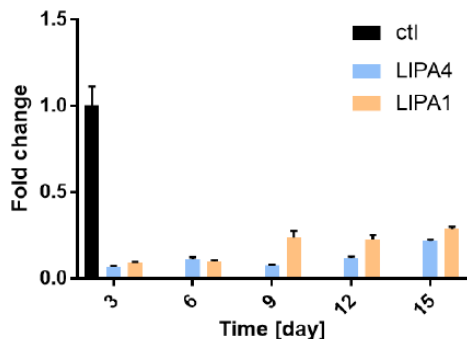
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AAG
    
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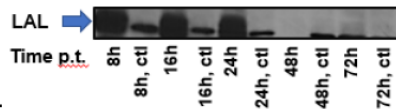
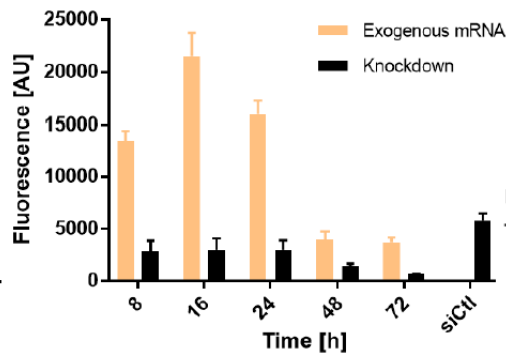
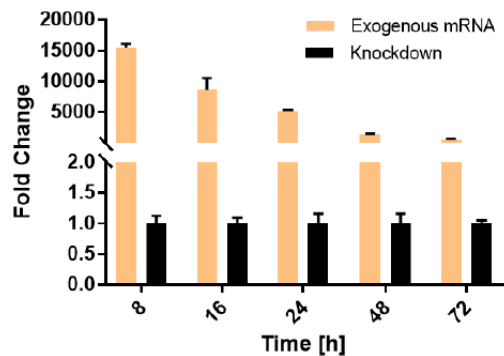
d17 Promoter: TAATACGACACACTATA
 Kozak sequence: GAAACC(ATG)
 Lip: GACTACAAAGACGATGACGACAAAG
 STOP Codon: TGA
 START Codon: ATG
 Signal peptide: AAAATGCGGTTCTTGGGGTTGGTGTCTGTTGTTCTCTGGACCCTGCATTCTGAGGGG
 Lipa SEQUENCE
 5'UTR and 3'UTR

Project 2.






Knockdown model (siRNA), HepG2 cells



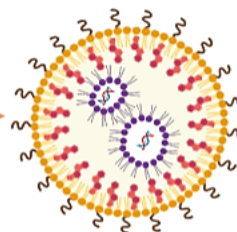
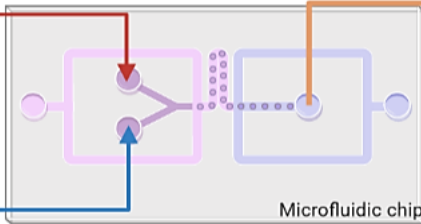
LAL rescue with mRNA and Lipofectamine©:



Project 2.

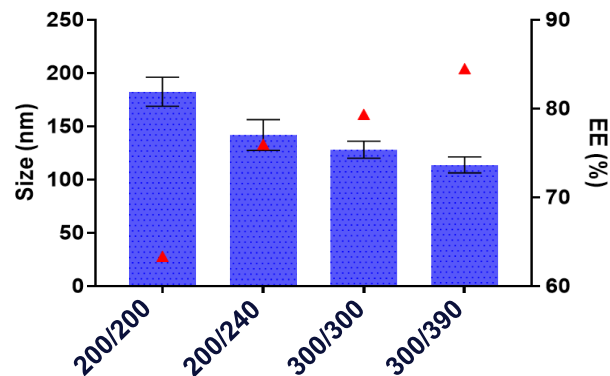
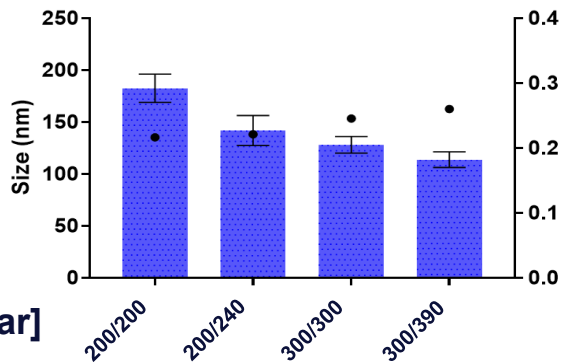
-  Ionizable lipid (DODMA, MC3, 5A2 SC8, ALC0315)
-  Helper lipid (DSPC, DOPE, DOPC, SOPC)
-  Cholesterol
-  DMG-PEG
-  mRNA

Microfluidic mix, 25 mM acetate buffer pH 4



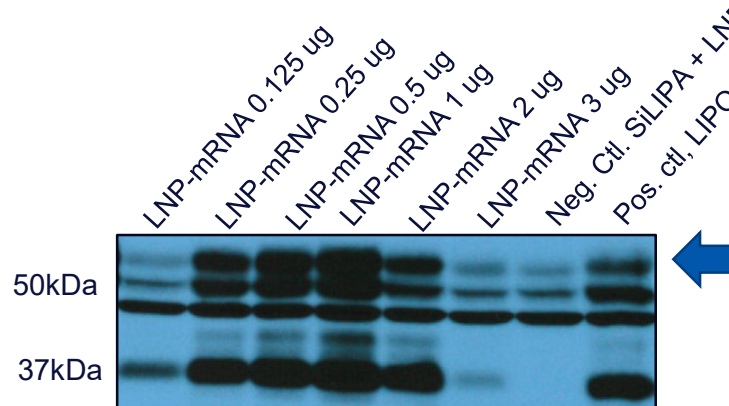
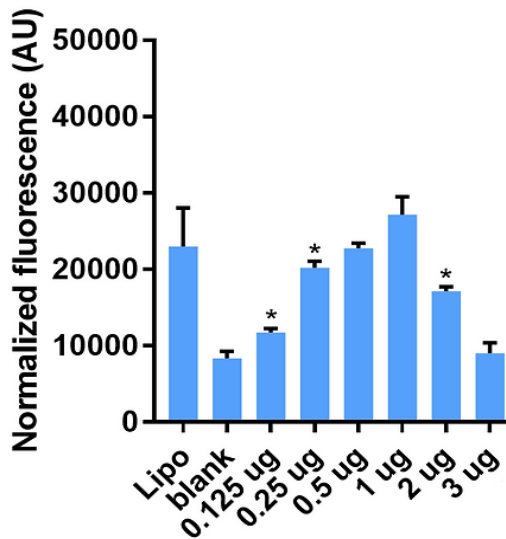
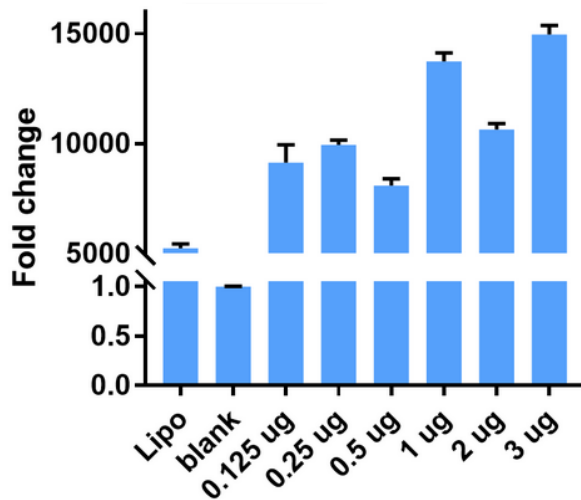
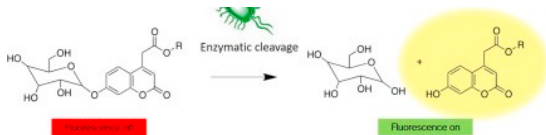
MC3 (50)
 DSPC (10)
 Chol (38,5)
 DMG-PEG (1,5)

Pressure [mbar]



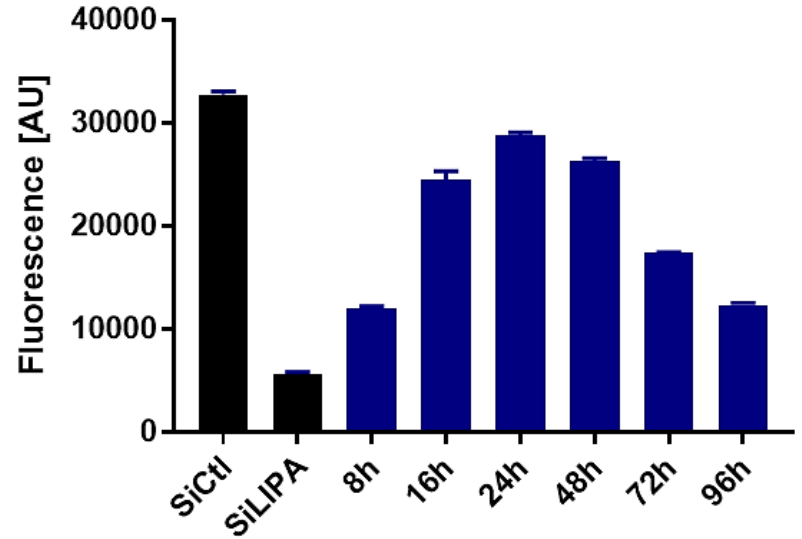
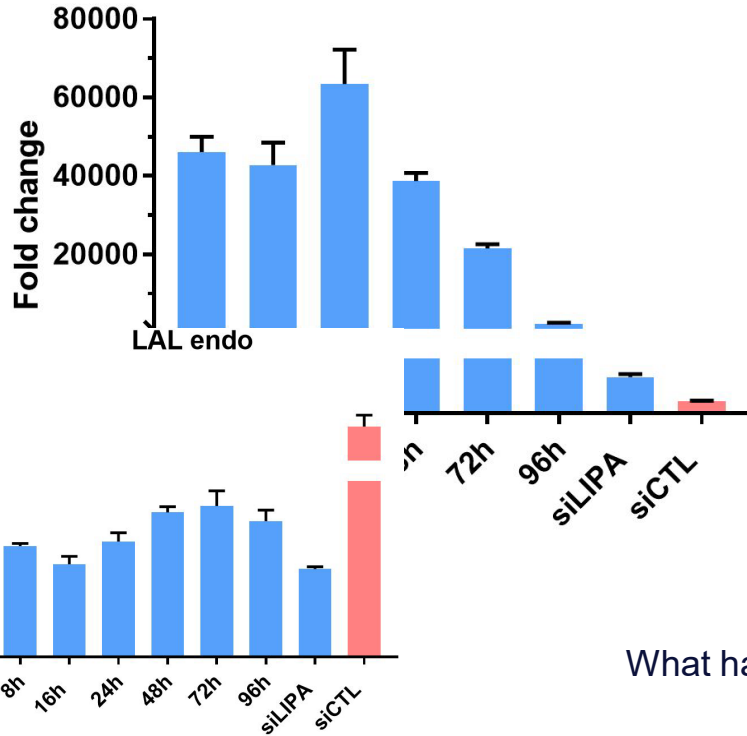
Project 2.

LAL rescue with LNP-mRNA:



Project 2.

qPCR kinetics



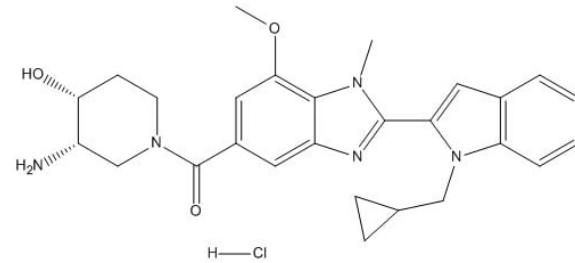
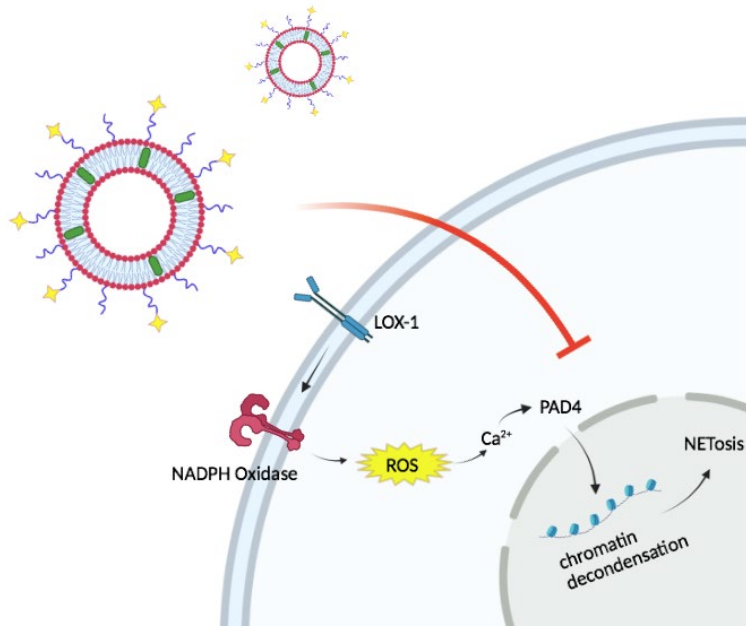
What happens to the enzyme? Half life? Degradation pathway?

Project 2.

- Kinetics experiment with LNP-mRNA formulation
- Formulation screening in in vitro models (PRC)
- **Explore strategies for extended mRNA translation and protein expression (ASO, Inhibitors of Caspase, PROTAC inverted, self-replicating mRNA)**
- Planning of in vivo PK studies (Austria)

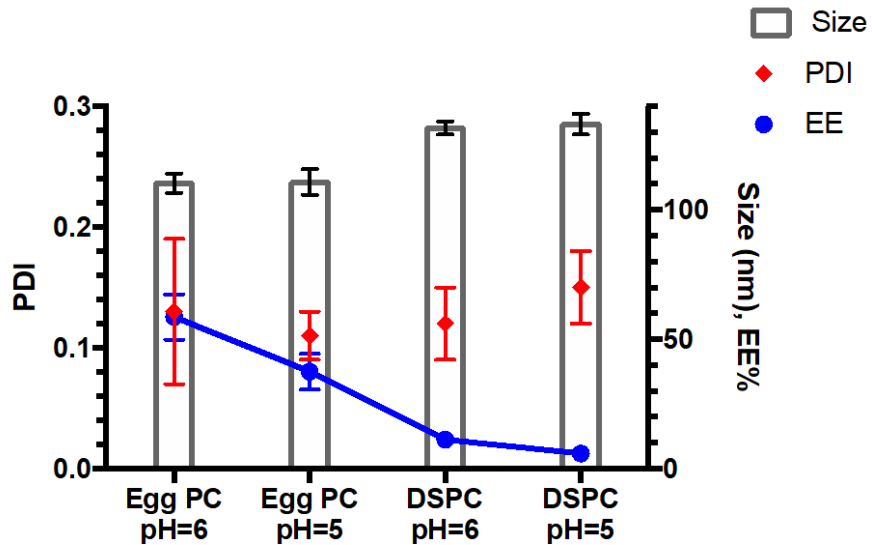
Project 3.

Design of Low-Density Granulocytes Targeting Nanoparticles: A New Potential Approach for the Management of Systemic Lupus Erythematosus

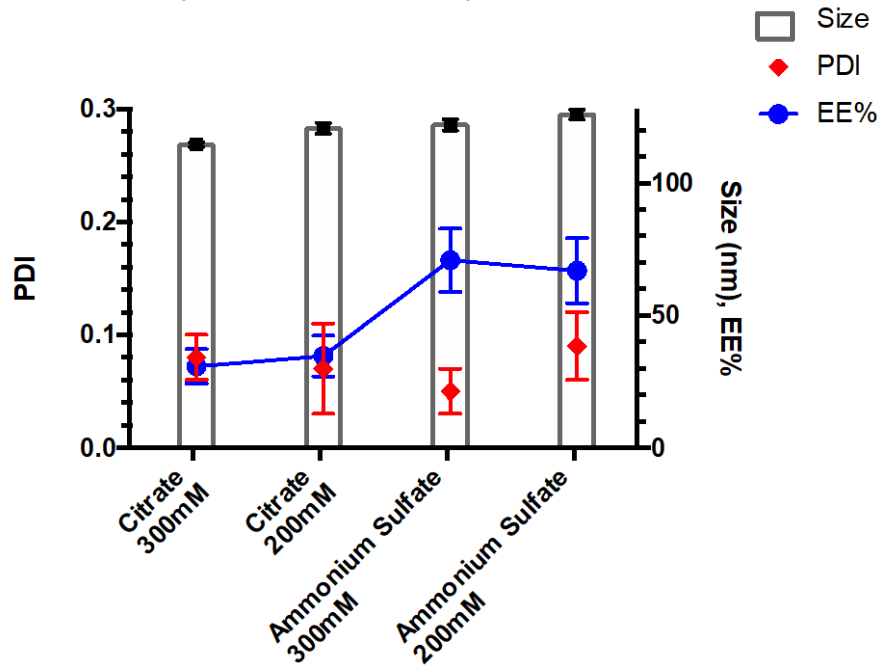


Project 3.

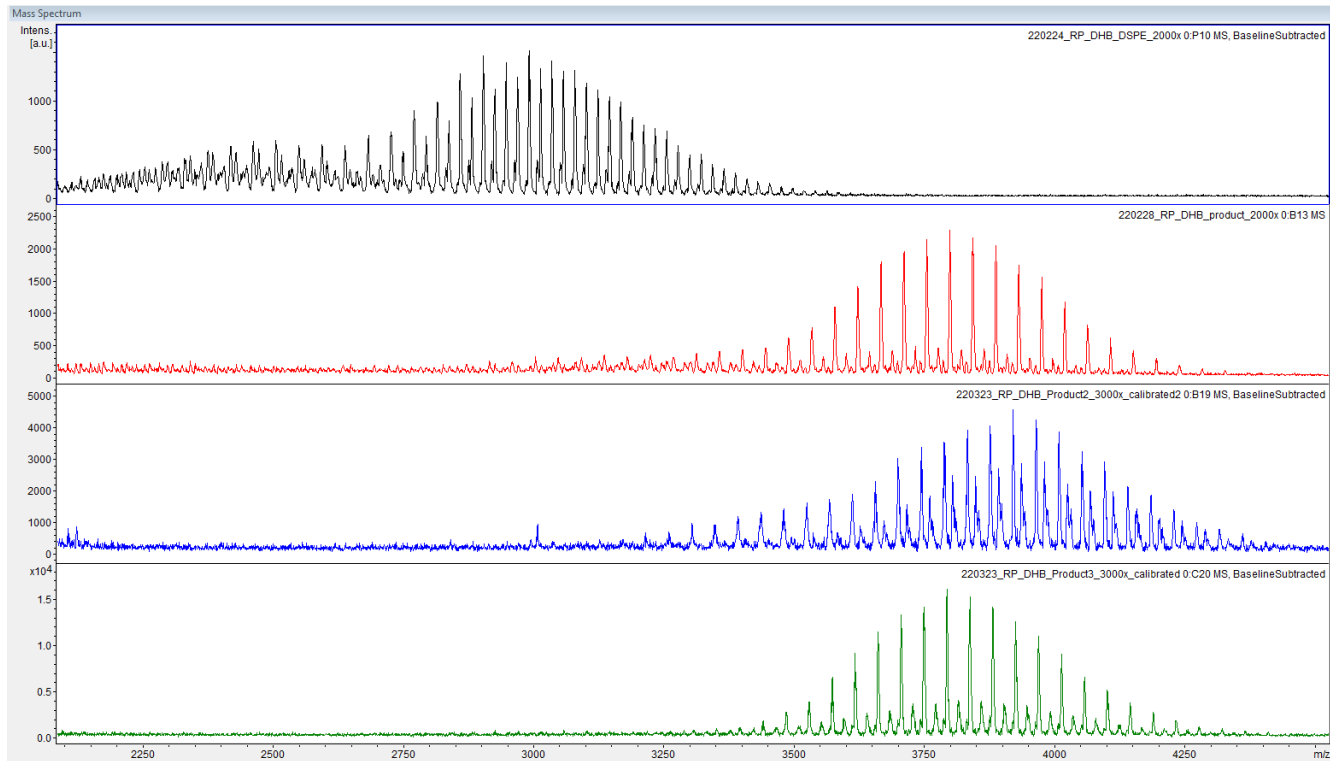
Passive Loading



Active Loading (DSPC) (Dox properties)



Project 3.



Maleimide-DSPE-PEG

S-Peptide

Project 3.

- Preparation of liposome-encapsulated PAD4 inhibitor has been favorably obtained.
- The optimized formulation demonstrates appropriate physico-chemical properties.
- Based on hydrophobic nature of the drug, suitable encapsulation efficiency has been acquired.
- The conjugation of DSPE-PEG with the targeting peptide is successfully achieved.

Ongoing steps

- Synthesis of targeted Liposome formulation
- Investigation of peptide targeting capacity to LOX-1 receptor by a suitable *in vitro* model (cells and *in vitro*)
- Evaluation of the anti-NETs functionality of the formulation on the desired cell line

Financement

1. CIHR (UBC), co-applicant (450 k\$) (120 k\$)
2. New Frontiers Exploration, Co-PI (INRS, IRIC) (200 k\$)
3. Canadian Institutes of Health Research /Canada Cancer Society, co-PI, (150 k\$)
Natural Sciences and Engineering Research Council, Collaborative Research and Training Experience program (CREATE), co-PI (11 PIs), (1'650 k\$)
4. Phospholipid Research Center, PI (Germany, 220 k\$)
5. Leo Fondation, co-PI, (Denmark, 670 k\$)
6. Fond Servier Faculté de Pharmacie, UdeM, PI, (30 k\$)

PhD:

Alexander Graham Bell NSERC PhD scholarship (Samuel Babity)

TransMedTech (x3)

Publications

1. *Therapeutic nanotechnologies for Alzheimer's disease: a critical analysis of recent trends and findings.* P Delbreil, JM Rabanel, X Banquy, D Brambilla. *Advanced Drug Delivery Review*. Accepted. **2022**
2. *A biomimetic strategy to enhance epithelial sealing of percutaneous biomaterials.* A Saad, C Penaloza, M Wang, O Alkashty, Davide Brambilla, F Tamimi, M Cerruti. *Acta Biomaterialia*. **2022**
3. *Selectively triggered cell detachment from PNIPAM microgel functionalized substrates.* Guerron, A., Phan, H.T., Peñaloza-Arias, C., Brambilla, D., Roullin, V.G., Giasson, S. *Journal of Colloid and Interface Science*. **2021**.
4. *Optimization of a DNase-1 liposomal formulation with extended circulating half-life.* Peñaloza-Arias LC, Brambilla D. *Molecular Pharmaceutics*, **2022**.
5. *A naked eye-invisible ratiometric fluorescent microneedle tattoo for real-time monitoring of inflammatory skin conditions.* S Babity, EVR Campos, F Couture, M Bonmarin, D Fehr, S Hedtrich, and D. Brambilla. *Advanced Healthcare Materials*. **2021**. *Development of a diffusion-weighted mathematical model for intradermal drainage quantification.* C. Kirsch, D. Fehr, S. Babity, A. Polomska, M. Detmar, M. Bonmarin, D. Brambilla. *Pharmaceutical Research*. **2022**.
6. *Super-swelling microneedle arrays for dermal interstitial fluid (prote)omics.* Elise Laszlo, Gregory De Crescenzo, Alfonso Nieto-Argüello, Xavier Banquy and Davide Brambilla*. *Advanced Functional Materials*. 31 (46), **2021**.
7. *Lymphatic drug delivery in cardiovascular medicine.* N Tessier, F Moawad, N Amri, D Brambilla*, C Martel *. *Pharmaceutics*. **2021**. *Pharmaceutics*. Accepted.
8. *Polymer-based microneedles for decentralized diagnostics, monitoring, and screening: concepts, potentials, and challenges.* S Babity, E Laszlo, and D Brambilla*. *Chemistry of Materials*. **2021**. Accepted.
9. *A quantitative UHPLC-1 MS/MS method for the growth hormone releasing peptide-6 determination in complex biological matrices and transdermal formulations.* CL Esposito, A Garcia, E Laszlo, SV Duy, C Michaud, S Sauv  b, H Ong, S Marleau, X Banquy, D Brambilla. *Talanta*. **2021**. Accepted.
10. *Digital light 3D printing of customized bioresorbable airway stents with elastomeric properties.* N Paunovi  , Y Bao, FB Coulter, K Masania, AK Geks, K Klein, A Rafsanjani, J Cadalbert, PW Kronen, N Kleger, A Karol, Z Luo, F R  ber, D Brambilla, B von Rechenberg, D Franzen, AR Studart, JC. Leroux. *Sciences Advance*. 7, 6, eabe9499. **2021**.
11. Patent application with UdeM (ratiometric microtattoos for physiology monitoring)

Collaborations établis

Main collaboration with other researchers

Collaboration in Canada

Prof. Marta Cerruti, McGill University. Ongoing collaboration focusing on the evaluation of the biocompatibility and toxicity profile of new materials for surgical implants. Two joint funding (RQRM, CQMF).

Prof. Roxane Pouliot, Université Laval. Ongoing collaboration focusing on the design and testing of new transdermal formulations for psoriasis management. One joint funding (CIHR). Two co-supervised PhD students.

Prof. Simon Pierre Gravel, Université de Montreal. Ongoing collaboration on the design and testing of new nanoparticles-based gene delivery for the management of rare disease. One joint funding (Servier UdeM). One co-supervised PhD student.

Profs. Jinyang Liang (INRS), Fiorenzo Vetrono (INRS) and Sylvian Meloche. Ongoing collaboration on the design of an early-stage melanoma detection noninvasive procedure. Two joint fundings (Spark, New Frontiers). One co-supervised PhD student.

Profs. Jan Dutz, Manish Sadarangani, The University of British Columbia. Ongoing collaboration on the investigation of a new microneedles based universal vaccine. One joint funding (CIHR).

Prof. Xavier Banquy, Université de Montreal and Prof. Gregory Decrescenzo, Ecole Polytechnique. Ongoing collaboration on the design of superswelling microneedles for noninvasive biological fluids sampling. One co-supervised MSc student.

International Collaboration

Prof. Sarah Hedtrich, Charité Research Center, Berlin, Germany. Ongoing collaboration on the design and testing of new skin gene editing approaches.

Prof. Mathias Bonmarin, Zurich School of Engineering, Switzerland. Ongoing collaboration on the design and testing of transdermal diagnostic tools.

Prof. Sara Pellegrino, University of Milano. Ongoing collaboration on the design of a new neutrophil targeted nanoparticles formulation for the management of lupus erythematosus. One co-supervise MSc student.

Prof. Dagmar Kratky, Medical University of Graz, Austria. Ongoing collaboration on the design and testing of new nanoparticles-based gene delivery for the management of rare disease (with Prof. Gravel). In vivo transgenic model laboratory.

Autres activités 2021

Editorial activity: Assistant Editor of Journal of Controlled Release (IF: 9.9)

Peer-revision: Revision of over 30 original articles and reviews for international journals (J. Control Release, Small, Advanced Materials, Advanced Materials Technologies, Nanoscale Research Letters, *etc.*)

Presentations: 15 presentations at local, national and international simposia (8 orals, 7 posters), 2 awards

Awards: Prix contribution exceptionnelle, Faculté de Pharmacie, UdeM. 2022 Young Investigator Awards Transdermal delivery Focus Group CRS

Committee member:

- a. Pharmaceutical Science CIHR committee member
- b. Expert reviewer for Natural Sciences and Engineering Research Council of Canada, Strategic Partnership Grants competition.
- c. Expert reviewer for Canada Foundation for Innovation, John R. Evans Leaders Funds.
- d. Expert reviewer for Natural Sciences and Engineering Research Council of Canada, Discovery grant competition.
- e. Expert reviewer, Agence Nationale de la Recherche France.
- f. Expert reviewer, presentations of Controlled Release Society annual meeting.
- g. Selection committee member MSc scholarship Natural Sciences and Engineering Research Council of Canada.
- h. Selection committee member PhD scholarship Fonds de Recherche du Quebec Nature et Technologies.
- i. Expert reviewer, CQDM.
- j. Organiser and selection committee member CC-CRS travel grants.
- k. Expert reviewer for ETH Zurich Research Commission Grants

Memberships:

President of the Canada Chapter of the Controlled Release Society and Member of the International Representative Committee of the Controlled Release Society;
Treasurer du Transdermal delivery Focus Group CRS

Classes:

Chemistry Lab (sbp2015), re-organization for Covid
Pharmacokinetics (PharmD)
Production of biologics (BSBP)
Nano and micro medicine (DESS, Graduate students)

Faculté de pharmacie

Université 
de Montréal
et du monde.