PROTEO
FONCTION | INGÉNIERIE APPLICATIONS DES PROTÉINES

PROGRAMME

24th Annual Symposium

May 22 and 23, 2025

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AKNOWLEDGEMENT

Sponsors

Plateformes technologiques de l'Institut de Pharmacologie de Sherbrooke

THE ORGANIZING COMMITTEE

Marylou Meyer, Pierre Luc Boudrault, Alice Gravel Trudeau, Gabriel Lemieux, Alexis Lavallée, Sébastien Chrétien, Pierre Lavigne, Martin Audet, Steve Jean et Nicolas Bisson

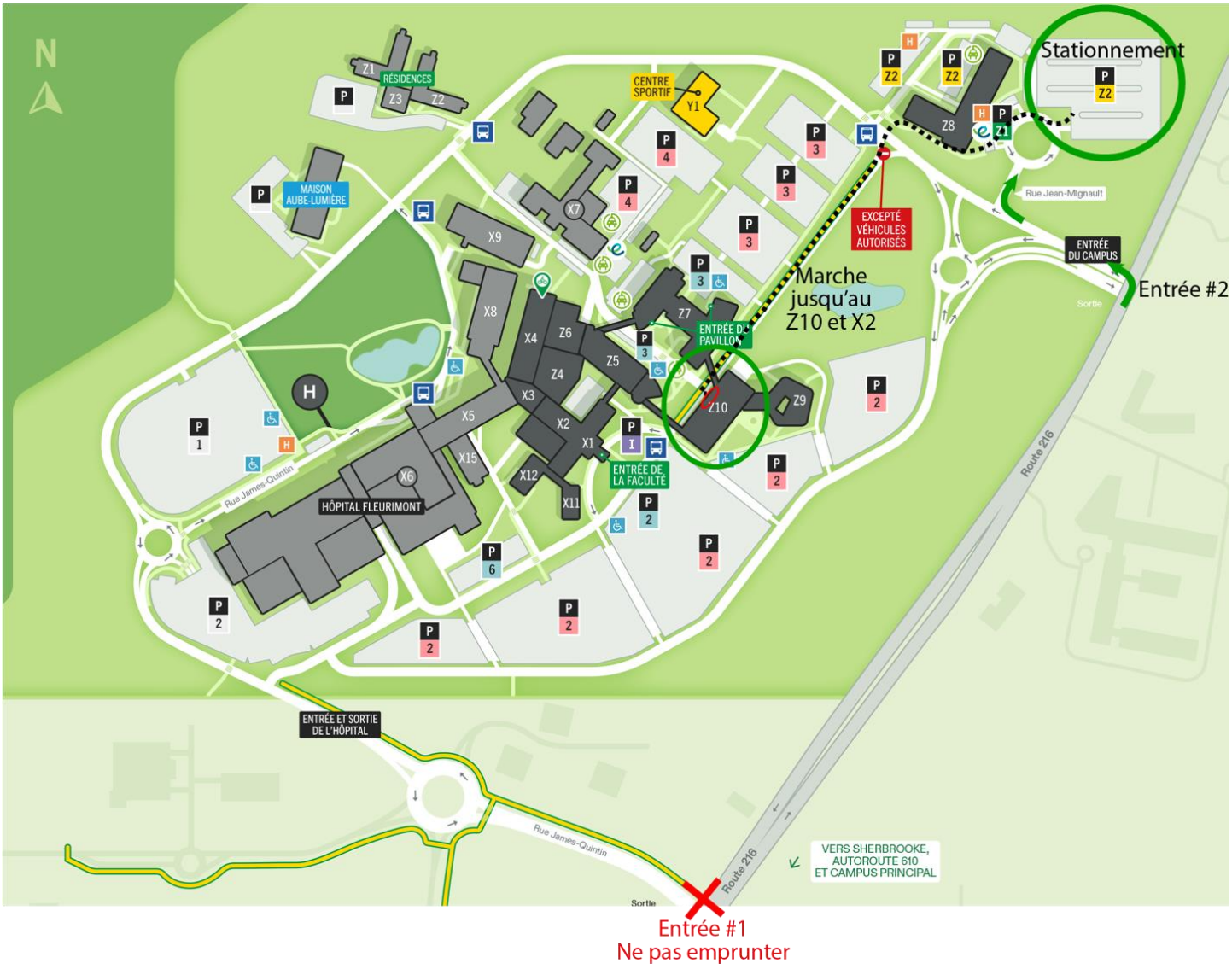
DIRECTIONS

Campus de la santé de l'Université de Sherbrooke

3001, 12e Avenue Nord, Sherbrooke (Québec) J1H 5N4

Oral presentations will take place in X2-6214.

Poster presentations will take place in Z10.



SCHEDULE

Thursday, May 22, 2025

10:00 a.m.	Registration	**Hall-Z10**
11:00 a.m.	General meeting of members (professors)	Z9-1004
11:00 a.m.	General meeting of student members	X2-6214
12:00 p.m.	Lunch	**Hall-Z10**
1:25 p.m.	Word of welcome	X2-6214
1:30 p.m.	Keynote Speaker - Professeur James Piret University of British Columbia *Process Analytical Utility of Raman Microscopy for Cell Therapy Manufacturing*	
2:30 p.m.	**Student oral presentations** 2025 Michèle Auger Award Recipient - Camille Bédard, *Systematic assessment of the conservation of drug resistance mutations across orthologs through comparative mutational analysis* Feriel Yala, *Structure-based clustering reveals functional insights into alternative proteins encoded by non-canonical open reading frames* Alex Paré, *SPASE : Une plateforme computationnelle intuitive pour la conception et l'optimisation des protéines* Camila Brisighello, *Development of multispecific VHHs for the neutralization of SARS-CoV-2 and H5N1* Louis-David Guay, *Les peptides antimicrobiens en tant qu'alternatives aux antibiotiques : synthèse et relation structure-activité du lipopeptide brévibacilline* Antoine Bouchard, *A short SUMOylation tag modulates p53 transcription factor activity*	
4:00 p.m.	Poster presentation Session #1	**Hall-Z10**
4:30 p.m.	Reception	

Friday, May 23, 2025

08:30 a.m.	Registration	**Hall-Z10**
09:00 a.m.	Keynote Speaker - Professeure Joelle Pelletier Université de Montréal *Engineering enzymes in all their dimensions*	**X2-6214**
10:00 a.m.	**Student oral presentations** Jonathan Besna, *Leveraging Experimental Datasets for Machine Learning-Based Predictions* Pascale Lemieux, *Directed exploration of protein interaction sequence space reveals the role of binding availability for interaction strength* Manon Jehoulet, *Utilisation de biosenseurs pour l'analyse fonctionnelle d'acteurs du trafic endosomal* Thomas DesCôteaux, *NRGRank: Coarse-grained structurally-informed ultra-massive virtual screening* Julian Wagner, *Structural Basis for the Pathogenicity of Parkin Catalytic Domain Mutants*	
11:30 a.m.	Poster presentation Session #2	**Hall-Z10**
12:30 p.m.	Lunch Lunch for registered students with speakers	
2:00 p.m.	**Student oral presentations** Benjamin Ouellet, *Leveraging cold-induced changes in yeast lipid composition for the production of enhanced biodiesel variants* Santa Mariela Olivera Ugarte, *Streamlining SARS-CoV-2 Vaccine Development by Targeting Linear Epitopes* François Rouleau, *Predicting antifolate resistance in an uncultivable fungal pathogen* Jean-Christophe Berger-Dancause, *Engineering a novel PET-depolymerizing enzyme from Streptomyces diastaticus to address plastic pollution* Cindy Klaus, *Distal mutations in a designed retro-aldolase alter loop dynamics to shift and accelerate the rate-limiting step*	X2-6214

3:30 p.m.	Keynote Speaker - Professeur Michel Bouvier Institut de recherche en immunologie et en cancérologie *Biais de signalisation et de localisation des récepteurs couplé aux protéines G (RCPG); Deux aspects de la sélectivité fonctionnelle dans l'action des médicaments*
5:00 p.m.	**Closing remarks and awards**

INVITED SPEAKERS

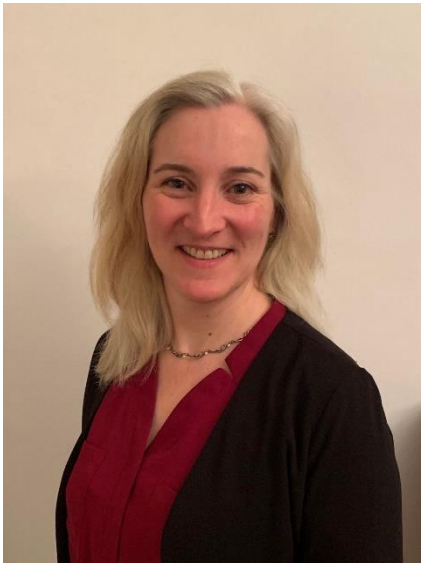
Dr. James Piret

Dr. Piret is a Professor in the Department of Chemical & Biological Engineering and the Michael Smith Laboratories at the University of British Columbia. He has a Bachelor's from Harvard in Applied Mathematics to Biochemistry and a Chemical Engineering doctorate from MIT. At UBC since 1989 his research focus has been on innovative process and device technology for therapeutics biomanufacturing. His multi-disciplinary research ranges from bioreactor engineering to molecular biology, often including collaborative work with scientists and industry. His recognitions include the Canadian Chemical Engineering RS Jane research award, the Cell Culture Engineering Award from the Engineering Conferences International and student Teaching Excellence awards. **http://www.msl.ubc.ca/faculty/piret**

Process Analytical Utility of Raman Microscopy for Cell Therapy Manufacturing

New clinical therapies based on implanting living cells into patients have the potential to cure degenerative and deadly diseases. However, populations of living cells are far more inherently variable and complex than any molecular drug. Additionally, they cannot be purified or analyzed anywhere near as stringently as drugs. Both accelerating process development and ensuring the long-term success of cell therapies will depend largely on the development of improved methods to validate both the final cell product quality and the expected critical process parameters during manufacturing. Raman spectroscopy offers a label-free approach to distinguish cell types and physiological states by analyzing cellular macromolecular composition changes. Using Raman microscopy, we have shown that spectral markers can detect early apoptotic cell death, discriminate between stem cells and differentiated progeny, and between T-cell subtypes and activation states. Overall, Raman spectroscopy offers a promising approach to provide a highly informative, and even a non-destructive means to enhance our ability to monitor, validate, and control the quality of cell therapy manufacturing. Importantly, given the variability of cell therapy manufacturing, this process analytical technology has the potential to provide feedback control of the harvest and other manufacturing process operations.

Dre. Joelle Pelletier



Ayant complété un baccalauréat en biochimie à l'Université d'Ottawa, sa ville natale (1989), un doctorat en biochimie à l'Université McGill (1995) et des stages postdoctoraux à l'Université de Montréal et à l'Université de Zürich, Joelle Pelletier est devenue professeure de chimie et professeure accréditée de biochimie à l'Université de Montréal en 1999. Depuis 2021, elle est titulaire de la Chaire de recherche du Canada en ingénierie des protéines appliquées. La professeure Pelletier a établi des méthodes avancées pour l'ingénierie automatisée et à haut débit des protéines, la biodétection appliquée et la biocatalyse, ainsi que la modélisation informatique. L'évolution de la résistance aux antibiotiques, la biodétection de diverses cibles biologiques, la génération « intelligente » de banques de protéines et la dynamique des protéines figurent parmi ses principaux sujets de recherche.

Au fil de sa carrière, elle a occupé de nombreux postes de direction et comités de sélection au Canada, aux États-Unis et en Europe, et est actuellement éditrice à ACS Catalysis. En 2015, elle a lancé la jeune pousse « Les instruments Affinité », où elle occupe le poste de vice-présidente de la recherche. Elle a reçu le prix Clara Benson 2021 de la Société canadienne de chimie, en reconnaissance de sa contribution remarquable à la chimie. La professeure Pelletier se passionne pour le mentorat et la promotion des jeunes scientifiques qui dirigent aujourd'hui la nouvelle génération de chercheurs.

L'ingénierie des enzymes dans toutes leurs dimensions

Les enzymes présentent collectivement une grande variété de propriétés catalytiques, mais sont individuellement confinées à une ou quelques tâches catalytiques spécifiques. Malgré des avancées majeures dans le domaine de l'ingénierie enzymatique, notre capacité à prédire les effets des mutations sur la fonction reste nébuleuse. Nous présentons ici les progrès réalisés dans l'ingénierie de la reconnaissance des substrats non natifs en vue de leur transformation biocatalysée en produits utiles. Nous présentons des approches visant à modifier les profils de produits d'une lipase, d'une transglutaminase et d'une oxydase à cytochrome P450, en appliquant des stratégies qui comprennent le criblage à haut débit, l'ingénierie des substrats et l'ingénierie des tunnels. Nous envisageons le potentiel des grands ensembles de données expérimentales pour former des algorithmes de conception plus intelligents pour l'ingénierie enzymatique.

Dr. Michel Bouvier

Michel Bouvier est professeur titulaire de biochimie et de médecine moléculaire et chercheur principal à l'Institut de recherche en immunologie et cancérologie (IRIC) de l'Université de Montréal (UdeM) et a été directeur général de l'IRIC de juin 2014 à mai 2024. Il a obtenu son baccalauréat (1979) en biochimie et son doctorat (1985) en sciences neurologiques. Après un stage postdoctoral dans le laboratoire de Robert J. Lefkowitz à l'Université Duke (1985-1988), il est revenu à Montréal comme professeur de biochimie à l'UdeM et est devenu directeur du département (1997-2005). Le Dr Bouvier est un expert de renommée mondiale en pharmacologie moléculaire. Ses travaux sur les récepteurs couplés aux protéines G ont mené au développement de nouveaux outils et à des changements de paradigme qui ont un impact significatif sur la découverte de médicaments. Ses travaux sur la sélectivité fonctionnelle des récepteurs couplés aux protéines G (RCPG) ont contribué à établir le concept de signalisation biaisée par le ligand qui est maintenant intégré dans de nombreux programmes de découverte de médicaments. Il a également été le pionnier du développement et de l'utilisation de méthodes basées sur le transfert d'énergie par résonance de bioluminescence (BRET) pour l'étude des interactions protéine-protéine et de l'activité de signalisation dans les cellules vivantes. Il est l'auteur de 376 publications et a donné plus de 500 conférences invitées. Ses articles ont attiré 44 481 citations (facteur h de 117). Il a supervisé les études de 37 étudiants de premier cycle, 78 étudiants de maîtrise et de doctorat et 46 boursiers postdoctoraux. Son laboratoire a également accueilli 36 étudiants invités et 7 professeurs/scientifiques invités. Ses contributions ont été reconnues par de nombreux prix, dont le prix Julius Axelrod de l'American Society for Pharmacology and Experimental Therapeutics (2017), le prix Wilder Penfield du gouvernement du Québec (2017) et le prix Killam 2021 du Conseil des arts du Canada. Il est membre de la Société royale du Canada et chevalier de l'Ordre national du Québec.

Biais de signalisation et de localisation des récepteurs couplé aux protéines G (RCPG); Deux aspects de la sélectivité fonctionnelle dans l'action des médicaments

Les récepteurs couplés aux protéines G (GPCR) représentent la plus grande famille de protéines impliquées dans la transduction du signal à travers les membranes biologiques. À ce titre, ils sont la cible de plus de 30 % des médicaments existants et restent des cibles privilégiées pour le développement de nouveaux. Il est désormais clair qu'il ne s'agit pas de commutateurs unidimensionnels qui activent ou désactivent une seule voie de signalisation. Au lieu de cela, chaque récepteur peut engager plusieurs partenaires de signalisation pouvant engager divers systèmes effecteurs en aval. Les ligands individuels peuvent avoir des efficacités différentielles envers des sous-ensembles spécifiques du répertoire d'effecteurs de signalisation

engagés par un récepteur donné. Ce phénomène, connu sous le nom de signalisation biaisée par le ligand ou sélectivité fonctionnelle, ouvre de nouvelles opportunités pour le développement de médicaments ciblant des voies thérapeutiques pertinentes tout en épargnant celles conduisant à des effets indésirables. Pourtant, cette nature pluridimensionnelle de l'efficacité de la signalisation présente un défi pour établir le profil de signalisation complet des médicaments et pour comprendre la base structurelle de la sélectivité fonctionnelle des GPCR. À l'aide d'une collection de biocapteurs et d'imagerie basés sur le transfert d'énergie par résonance de bioluminescence (BRET), nous avons caractérisé les profils de signalisation pluridimensionnels de nombreux GPCR et surveillé la propagation du signal spatio-temporel dans des compartiments intracellulaires distincts. L'analyse assistée par ordinateur des divers profils de signalisation observés permet le regroupement des composés en différents groupes permettant l'association de signatures de signalisation spécifiques avec des résultats fonctionnels distincts. Ces études ouvrent de nouvelles voies pour la conception rationnelle de ligands biaisés possédant les propriétés de signalisation souhaitées.

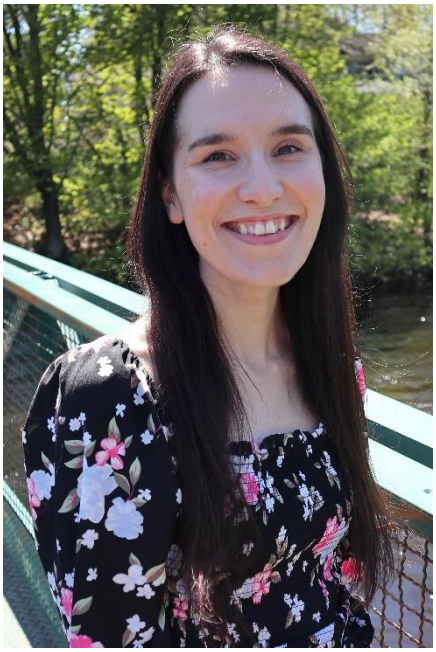
STUDENT ORAL PRESENTATIONS

WINNER OF THE MICHÈLE-AUGER PRIZE : Camille Bédard

Systematic assessment of the conservation of drug resistance mutations across orthologs through comparative mutational analysis

Camille Bédard[1,2,3,4], Isabelle Gagnon-Arsenault[1,2,3,4], Jonathan Boisvert[1,3,4] and Christian R. Landry[1,2,3,4]

[1]Institut de Biologie Intégrative et des Systèmes (IBIS), Université Laval, G1V 0A6, Canada, [2]Département de Biochimie, de Microbiologie et de Bio-informatique, Faculté des Sciences et de Génie, Université Laval, G1V 0A6, Canada, [3]PROTEO, Le regroupement québécois de recherche sur la fonction, l'ingénierie et les applications des protéines, Université Laval, G1V 0A6, Canada, [4]Centre de Recherche sur les Données Massives (CRDM), Université Laval, G1V 0A6, Canada



The structure of the proteins targeted by antimicrobials is often conserved across species, enabling the broad-spectrum activity of many drugs. One such target is the cytochrome P450 lanosterol 14-alpha-demethylase (Erg11/Cyp51), inhibited by azole antifungals. While the protein structure and function are largely conserved across species, it remains unclear whether the mutations that confer drug resistance are similarly conserved in orthologs. Such conservation would allow us to transfer what we know about resistance mechanisms from one species to another. We assembled the FungAMR database, which presents about ten resistance mutations in Erg11 that are recurrent across diverse fungal pathogens, suggesting a high level of conservation. However, whether this trend is generalisable or limited to a subset of well-studied mutations remains unknown.

To address this, we systematically mapped resistance mutations in Erg11 of two yeast species that diverged more than 200 Mya, *Candida albicans* and *Saccharomyces cerevisiae,* using deep mutational scanning. We generated a library of nearly 4,000 amino acid variants per ortholog and assessed their fitness in the presence of two clinical azoles and in the absence of a drug. The comparative analysis of the mutational effects revealed conserved and species-specific resistance mutations. Fitting the data to a global epistasis model suggests that most mutations have similar functional impacts across species, although notable shifts were observed for some variants. Ongoing work aims to elucidate the determinants underlying these differences.

This comparison of mutations across orthologs provides new insights into the molecular basis of resistance and may improve resistance prediction across fungi.

About Michèle Auger

Born in Grand-Mère, QC, Michèle Auger merited a B. Sc. in Biophysics from UQTR. She obtained a Ph. D. in Chemistry from the University of Ottawa and undertook postdoctoral reseach at the MIT, specializing in solid-state NMR applied to proteins. She pursued an outstanding scientific career in molecular biophysics at Université Laval before losing her battle to cancer at too young an age, on October 2018. She has been a model for generations of scientists, especially for women. Her engagement in the scientific community and in promoting science was notable and inspiring.

Fériel Yala

Structure-based clustering reveals functional insights into alternative proteins encoded by non-canonical open reading frames

Feriel Yala[1], Sebastien Leblanc[1], Xavier Roucou[1]

[1]University of Sherbrooke

Alternative proteins (altProts) are largely small proteins (below 100 amino acids) also termed microproteins, translated from non-canonical open reading frames. They represent a newly emerging layer of the proteome with increasing experimental evidence of translation and functional relevance. However, most of them remain uncharacterized due to their absence from standard annotations and homology-based pipelines.

As part of the OpenProt project, a resource dedicated to annotating alternative proteins, we performed a large-scale structural analysis of 540285 human altProts with AlphaFold. By comparing them to 214 million structures from UniProt using Foldseek, we generated 2.5 million structural clusters.

Of these, 1.2 million clusters include a representative protein with functional annotation (e.g., Pfam domain). 5179 altProts are present within 3594 of these annotated clusters, suggesting structural homology and function with known proteins. For instance, the altProt IP_752012 is assigned to a cluster containing a protein with the RVT_1 (reverse transcriptase) domain.

The remaining 1.3 million clusters lacked any known annotation designating them as “dark clusters”. 5898 altProts are present within 3000 dark clusters.

Ongoing analysis aims to infer the function of these dark clusters using DeepFri, a structure-based function prediction tool leveraging Gene Ontology (GO) terms, and uncover novel domain families using PfamSDB, a structural domain database derived from Pfam, which enables domain detection based on 3D structure rather than sequence.

These analyses will provide insights into the functional potential of altProts. Identifying novel folds or domains, together with conservation studies, will advance our understanding of their place within the dark proteome.

Alex Paré

SPASE : Une plateforme computationnelle intuitive pour la conception et l'optimisation des protéines

Alex Paré[1], Sacha Larda[1], Nicolas Doucet[1, 2]

[1]Centre Armand-Frappier Santé Biotechnologie, Institut national de la recherche scientifique (INRS), Université du Québec, Laval, QC, Canada., [2]PROTEO, le regroupement québécois de recherche sur la fonction, l'ingénierie et les applications des protéines, Université du Québec à Montréal, Montréal, Québec, Canada.

Les avancées récentes en apprentissage automatique transforment l'ingénierie des protéines, stimulant l'innovation en biologie synthétique et en développement thérapeutique. Cependant, un défi majeur persiste : Concevoir des variants de protéines synthétiques qui maintiennent leur intégrité structurelle tout en optimisant leur solubilité, leur stabilité et leur expression. Pour répondre à ce défi, nous avons développé SPASE (***Soluble Protein Analog Selection Engine***), une plateforme computationnelle automatisée combinant apprentissage profond et bioinformatique pour générer des séquences protéiques aux propriétés biophysiques optimisées.

À partir d'une structure protéique donnée, l'utilisateur définit des résidus critiques, et SPASE utilise ProteinMPNN pour générer 10 000 variants synthétiques qui conservent le repliement natif et les résidus spécifiés. Cette librairie est ensuite affinée à travers un processus de sélection en plusieurs étapes : 1) Protein-Sol évalue la solubilité, 2) ESMFold prédit la structure 3D et son score de confiance, et 3) Aggrescan3D identifie les variants sujets à l'agrégation. Les séquences de moindre qualité sont éliminées, aboutissant à un ensemble d'environ 25 analogues optimisés pour une validation expérimentale.

Grâce à son interface intuitive, SPASE simplifie la conception et le criblage de protéines *in silico*, réduisant la charge de travail et accélérant l'identification de protéines fonctionnellement viables. Il génère des séquences solubles et résistantes à l'agrégation, offrant ainsi un outil clé dans le domaine des biotechnologies. SPASE se distingue par son intégration sur une plateforme web intuitive, accélérant la découverte et l'optimisation de biomolécules sans nécessiter d'expertise avancée en programmation ou en conception de librairies pour l'évolution dirigée.

Development of multispecific VHHs for the neutralization of SARS-CoV-2 and H5N1

Camila Brisighello[1, 2], Zalma Sanchez[1, 2], Olatz San Miguel[1, 2], Alina Burlacu[2], Alex Pelletier[2], Brian Cass[2], Sylvie Perret[2], Debbie Callaghan[2], Jamshid Tanha[2], Kassandra Belanger[2], Gregory De Crescenzo[3], Simon Joubert[2], Yves Durocher[2]

[1]UdeM, [2]CNRC, [3]Polytechnique Montréal

The rise of H5N1, a highly pathogenic avian flu virus capable of infecting mammals, and COVID-19 highlights the need for effective viral therapies. Current antiviral treatments and vaccines often fail due to the mutation rate, leading to immune evasion. Camelidae produce functional immunoglobulin G (IgG) molecules lacking a light chain and CH1 domain, with a variable region consisting of a single domain called VHH or nanobody. Although they are smaller than full-length antibodies (15 kDa vs. 150 kDa), they can be expressed autonomously while having similar binding specificity and affinity. Their small size allows them to access and recognize antigenic sites that are inaccessible to human antibodies, have high solubility and stability, and be administered in aerosolized form. VHHs also facilitate modularity to create constructs such as biparatopic and bispecific antibodies. We fused two identical nanobodies, resulting in a bivalent dimer, which typically exhibits increased avidity compared to the individual monomers, and two different nanobodies that target non-overlapping epitopes on the same antigen. We also engineered the VHHs with the introduction of the LS mutation and Fc region, improving pharmacokinetic profiles. This study aims to develop dimeric, biparatopic, and bispecific VHHs targeting SARS-CoV-2 at firsty. The heterodimerized modality with the greatest performance in neutralization assays will be used to engineer a CHO stable pool for production. Additionally, this methodology will be applied to VHHs against the H5N1 virus, for which the immune library has already been established. The project's methodologies could advance antibody engineering and therapeutic potential, especially against H5N1.

Louis-David Guay

Les peptides antimicrobiens en tant qu'alternatives aux antibiotiques : synthèse et relation structure-activité du lipopeptide brévibacilline

Louis-David Guay[1, 2, 3], Florence Henley[1, 2, 3], Maxim Boucher[1, 3], Fayanne Nolin[1], Omar Fliss[1, 2, 4], Ismail Fliss[2, 4], Éric Biron[1, 2, 3]

[1]Faculté de pharmacie, Université Laval et Centre de recherche du CHU de Québec-Université Laval, Québec (QC), Canada, [2]Centre de recherche en infectiologie et Institut de nutrition et des aliments fonctionnels, Université Laval, Québec (QC), Canada, [3]PROTEO, Regroupement québécois de recherche sur la fonction, l'ingénierie et les applications des protéines, Canada, [4]Département des sciences des aliments, Faculté des sciences de l'agriculture et de l'alimentation, Université Laval, Québec (QC), Canada

La résistance aux antibiotiques est devenue un problème majeur dans la prévention et le traitement des infections bactériennes. Parmi les bactéries problématiques, on retrouve entre autres les *Enterococcus* résistant à la vancomycine (**ERV**), *Staphylococcus aureus* résistant à la méthicilline (**SARM**) et plusieurs Gram-négatif comme *Escherichia coli* et *Pseudomonas aeruginosa.* Face à cette situation critique, le développement de nouveaux antimicrobiens avec de nouveaux modes d'action est devenu une priorité. Parmi les composés prometteurs, le lipopeptide brévibacilline est très intéressant car il démontre une activité inhibitrice contre plusieurs bactéries d'intérêt clinique dont plusieurs multirésistantes. Dans le but de mieux comprendre son mode d'action et d'optimiser ses propriétés pharmacologiques, l'objectif était de préparer des analogues structuraux de la brévibacilline pour réaliser des études structure-activité et identifier les composantes modulant l'activité antimicrobienne, la toxicité et la stabilité. Des analogues de la brévibacilline modifiés à des points stratégiques ont d'abord été synthétisés puis leur activité antimicrobienne, cytotoxicité et stabilité gastro-intestinale évaluées in vitro. L'étude structure-activité a permis d'identifier les résidus essentiels à l'activité antimicrobienne et responsables de la cytotoxicité. L'accès au lipopeptide nous a également permis de générer des analogues plus accessibles par synthèse et de découvrir des analogues démontrant un spectre d'action élargi. L'étude réalisée démontre le grand potentiel de la brévibacilline et de ses analogues comme antimicrobiens ainsi que la grande tolérance du squelette envers les modifications pour augmenter les rendements de production et optimiser les propriétés pharmacologiques pour des applications dans les secteurs alimentaire, vétérinaire et médical.

Antoine Bouchard

A short SUMOylation tag modulates p53 transcription factor activity

Antoine Y. Bouchard[1, 2, 3], Anaïs J. I. Vivet[4], Valérie C. Cabana[1, 2, 3], Marc P. Lussier[1, 2, 3], Sylvie Mader[4, 5], Laurent Cappadocia[1, 2, 3]

[1]Département de Chimie, Université du Québec à Montréal, Montréal, Québec, Canada., [2]Centre d'Excellence en Recherche sur les Maladies Orphelines - Fondation Courtois (CERMO-FC), Faculté des Sciences, Université du Québec à Montréal, Montréal, Québec, Canada, [3]Regroupement québécois de recherche sur la fonction, l'ingénierie et les applications des protéines (PROTEO), Montréal, Québec, Canada, [4]Institut de Recherche en Immunologie et Cancérologie (IRIC), Université de Montréal, Montréal, Québec, Canada., [5]Département de Biochimie et Médecine Moléculaire, Université de Montréal, Montréal, Québec, Canada.

SUMOylation is a post-translational modification (PTM) that regulates multiple aspects of protein biology, including the activity of transcription factors such as p53. While strategies exist to decrease protein SUMOylation in a targeted manner, options are limited to increase SUMOylation on specific substrates. Here, we describe the development of a strategy for induced SUMOylation relying on fusion of a small 21 residue tag constituting the SUMO E3 module of ZNF451 to target proteins. Through *in vitro* and cell-based assays, we established that the SUMOylation tag promotes robust poly and/or multi-SUMOylation of transcription factor p53, used as a model substrate, with a strong preference for SUMO2/3 as compared with SUMO1. Induced poly/multi-SUMOylation repressed p53 transcriptional activity in luciferase reporter assays. Our results introduce a new strategy to achieve target protein SUMOylation and study the impact of this modification on protein substrates, and are compatible with the general repressive effects of SUMOylation on transcription factor activity

Jonathan Besna

Leveraging Experimental Datasets for Machine Learning-Based Predictions

Jonathan Besna[1, 2, 3], Douglas Fansher[2, 3, 4], Joelle Pelletier[1, 2, 3, 4]

[1]Université de Montréal, Département de biochimie, [2]PROTEO, [3]CCVC, Centre de Chimie Verte et Catalyse, [4]Université de Montréal, Département de Chimie

Enzyme catalysis offers a greener, more specific and tailored alternative to traditional chemical synthesis, but designing biocatalysts via directed evolution remains challenging. Machine-learning guided directed evolution (MLDE) accelerates protein engineering, enabling broader sequence exploration. Screening for desired properties is time-intensive, though high-throughput methods, such as colorimetric assays, facilitate data collection for substrate promiscuity studies. However, ensuring assay alignment with target activity is crucial. Machine learning has improved catalytic activity prediction, protein sequence encoding, and novel substitution identification. The Design-Build-Test-Learn cycle supports incremental optimization, generating data for continuous refinement.

A notable enzymatic system is the bacterial cytochrome P450 BM3 known for its oxidation capabilities, including hydroxylation of indole to form indigo, a visually identifiable dye useful for screening. Extensive engineering has allowed for diversification of both enzyme activity and substrate promiscuity, yet understanding function-structure relationships remains challenging. While effective for single-point mutations, recent studies have shown that indigo-based assays is less effective for combinatorial variant libraries.

This work focuses on generating large activity datasets for ML applications in substrate hydroxylation prediction. An initial library of 475 single variants has been screened for indigo formation, with a subset tested for aromatic hydroxylation. A combinatorial library of >30,000 multiple variants has also been generated, with over 500 screened.

Ongoing efforts aim to leverage these datasets for predictive modeling, enhancing BM3 variants through ML-guided directed evolution. This approach addresses indigo assay limitations for complex variants, paving the way for rationally designed P450 BM3 biocatalysts with tailored substrate specificities and improved hydroxylation activity.

Pascale Lemieux

Directed exploration of protein interaction sequence space reveals the role of binding availability for interaction strength

Pascale Lemieux[1, 2, 3, 4], Alexandre K. Dubé[1, 2, 3, 4], Christian R. Landry[1, 2, 3, 4]

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A challenge in synthetic biology is to transfer the knowledge acquired *in vitro* to *in vivo* applications. For instance, binding affinity between purified peptide binding domains (PBDs) and their binding peptides is usually measured in simple buffers and in the absence of the cell machinery responsible for proteostasis. Consequently, a discrepancy between their interaction quantified *in vivo* and the expected result based on *in vitro* measurements is often observed. Therefore, we aim to better understand the determinants of synthetic interaction in yeast and to identify strong binding pairs for *in vivo* applications. To this end, we designed libraries of peptides with two degenerated positions which are expected to alter binding to PBDs, based on data collected by phage-display. Using DHFR PCA, we assessed the interaction strength of 2800 PBD-peptide pairs in yeast. We found that most substitutions disrupt binding, but we also identified peptides with stronger interaction than the peptide expected to show maximal binding to its PBD according to the phage-display data. Then, we performed an assay to quantify binding availability, i.e. the amount of protein able to effectively bind other proteins in the cytosol, which could contribute to the discrepancy between the PCA and the phage-display results. We found that peptides with a stronger binding also show a higher binding availability. We demonstrated that the binding availability, which seems mainly defined by the stickiness of the peptide, is key for the design of strong synthetic interactions in yeast.

Manon Jehoulet

Utilisation de biosenseurs pour l'analyse fonctionnelle d'acteurs du trafic endosomal

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Les cellules communiquent entre elles via des protéines transmembranaires dont les niveaux et la localisation doivent être régulés. Il existe plusieurs complexes de tri impliqués dans le trafic endosomal. Ces complexes interagissent les uns avec les autres pour assurer le bon devenir des protéines transitant par cette voie. Malheureusement, la régulation des fonctions de ces complexes, retrouvés aux endosomes précoces, est encore peu décrites et les divers stimuli ou stress les modulant sont peu connus. De façon importante, des mutations dans ces complexes peuvent entrainer de nombreux dysfonctionnements cellulaires aboutissant à différentes maladies.

Notre hypothèse est que l'interaction et les fonctions des complexes de tri endosomal sont régulés par des signaux intracellulaires telles que des phosphorylations.

Mes objectifs de recherche se concentrent sur la caractérisation fonctionnelle et non biaisée des mécanismes régulant le recrutement et les interactions des complexes de tri. Pour cela, nous avons créé des biosenseurs utilisant la luciférase fragmentée comme reporteur de ces interactions. Nous avons réussi à faire ressortir, grâce aux biosenseurs, une proximité entre divers complexes impliqués dans le trafic endosomal. Des vérifications de la localisation de biosenseurs par immunofluorescence démontre également que l'ajout de la luciférase sur les protéines n'altèrent pas leurs recrutements aux endosomes précoces dans la cellule. Ces biosenseurs sont créés et testés dans le but de cribler une banque de phosphatases/kinases. Cela permettra de définir les éléments de régulation du trafic endosomal.

Finalement, cette étude caractérisera de nouvelles voies de signalisation régulant le trafic endosomal avec une nouvelle sorte de biosenseurs.

Thomas DesCoteaux

NRGRank: Coarse-grained structurally-informed ultra-massive virtual screening

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NRGRank is a coarse-grained structurally-informed software capable of performing ultra-massive virtual screening with accuracy comparable to docking-based methodologies but with 100 fold speed increase. It evaluates pairwise atom-type pseudo-energy interactions, implicitly accounting for ligand and side-chain flexibility as well as limited backbone movements. We benchmark NRGRank against Glide, AutoDock Vina, and DOCK 3.7 using enrichment factors at 1% (EF1) on the DUD-E dataset, observing broad variations in performance across targets and methods. On AlphaFold2 or apo structures, NRGRank outperforms Glide on 12-13 of 37 targets. Even on holo structures, it surpasses AutoDock Vina, DOCK 3.7, and Glide on 13, 10, and 5 targets, respectively. NRGRank identifies complementary binders to Glide, detecting hits missed by docking methods. Additionally, NRGRank's top-ranked compounds maintain high accuracy when re-scored with Glide, with an improved hit rate within the top 50 predictions. With an average evaluation time of 0.3 seconds per molecule, NRGRank can screen 1,000,000 molecules in 24 hours on an 8-core laptop, up to two orders of magnitude faster than the reported speed of DOCK 3.7, AutoDock Vina running on GPUs and Glide. NRGRank's ease of use and speed aim to make high-performance ultra-massive virtual screening accessible to all.

Julian Wagner

Structural Basis for the Pathogenicity of Parkin Catalytic Domain Mutants

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Parkinson's Disease (PD), the second most common neurodegenerative disease, is characterized by the loss of dopaminergic neurons in the midbrain. Whereas most PD cases are sporadic, approximately 10% of patients present a familial form of the disease. Pathogenic mutations in *PRKN* and *PINK1* cause early-onset PD. PINK1 and parkin mediate selective turnover of damaged mitochondria via mitophagy. PINK1 is a protein kinase that regulates the E3 ubiquitin ligase parkin, ensuring it becomes active only in response to mitochondrial damage. Parkin is a RING-between-RING (RBR) E3 enzyme and catalyzes ubiquitination through a two-step mechanism. Despite structural studies of parkin in different conformations, some pathogenic parkin mutations remain poorly understood. Here, we sought to characterize two parkin catalytic domain mutants, T415N and P437L, combining biochemical and biophysical methods with AlphaFold modeling. We demonstrate that both mutants exhibit impaired activity using autoubiquitination and ubiquitin vinyl sulfone assays. After identifying the parkin minimal ubiquitin binding fragment, we show that both mutants display impaired binding to the ubiquitin charged onto the E2 enzyme and quantify their interaction. An AlphaFold model of the complex formed by activated parkin and the ubiquitin-charged E2 enzyme consolidates our experimental findings. Interestingly, the model reveals a previously unobserved α-helix to position ubiquitin for transfer from the E2 to parkin. Our results provide insights into the structural and molecular basis for the pathogenicity of these parkin variants, advancing our understanding of their role in PD and paving the way for the development of new therapeutics.

Benjamin Ouellet

Leveraging cold-induced changes in yeast lipid composition for the production of enhanced biodiesel variants.

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Lipids are major components of cell membranes whose chemical structures are modulated with respect to their fatty acid chain length and unsaturation level to maintain, amongst others, membrane fluidity under variable temperatures. Such structural modifications can be leveraged to support the production of enhanced biodiesel variants whose performance and physicochemical properties are dictated by the structures of fatty acids from which biodiesels are made. This study aimed at developing a strategy in which the oleaginous yeast *Yarrowia lipolytica* is exposed to cold stress as a means to enhance the unsaturated fatty acid content in this yeast's oils. Changes in oil profile were studied in this yeast when grown at 4 °C compared to that when grown at 28 °C. Fatty acid analysis revealed that cold-stress exposure resulted in an increased unsaturated-to-saturated fatty acid (UFA/SFA) ratio in all tested yeast strains and cultivation media (PO1f and mutant *mfe1*Δ strains; rich and lipogenic media). Overall, linoleates constituted more than 50 $\%_{wt}$ of total fatty acids content by weight and minimal changes in overall chain length were observed. Though cold stress delayed growth, similar biomass concentrations were ultimately attained with improved lipid productivity. Biodiesels made from unsaturated-fatty-acid-rich lipids tend to perform better under cold operating conditions, since saturated-fatty acid-rich biodiesels tend to gel or crystallize at low temperatures. This study shows how a native lipid-profile adaptation, that a cell adopts to preserve its membrane fluidity under cold stress, can be leveraged for the production of enhanced oils for biodiesel suited for cold related properties.

Santa Mariela Olivera Ugarte

Streamlining SARS-CoV-2 Vaccine Development by Targeting Linear Epitopes

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The COVID-19 pandemic reshaped the vaccinology field, enabling the accelerated development of SARS-CoV-2 Spike-based vaccines able to protect from infection and reduce transmission. However, their efficacy was outpaced due to the high viral genetic variability and immune imprinting. Therefore, identifying immunogenic epitopes in SARS-CoV-2 proteins with potential to speed vaccine manufacturing is crucial for rapid outbreak response. Here, we follow this approach by exploring the antigenic potential of the SARS-CoV-2 Receptor-binding motif (RBM): an attractive target for neutralizing antibodies due to its direct interaction with the ACE2 receptor. We previously identified the RBM along with the RBD C-terminal as the main linear epitopes targeted by our SARS-CoV-2 RBD-based vaccine. To preserve its secondary structure for vaccine development, we inserted the RBM from Omicron XBB.1.5 into the scaffold protein RadA, obtaining the RadA/RBM protein. Mice were immunized twice, three weeks apart, with RadA/RBM linked to the immue-modulator PapMV or mixed with Quil-A adjuvant. A control group received the RBM (without secondary structure) linked to PapMV (PapMV-RBM). All formulations induced RBM-specific IgG antibodies. However, the candidates containing RadA/RBM failed neutralize SARS-CoV-2 in vitro. Surprisingly, only the PapMV-RBM control provided partial neutralization (40% inhibition). Supporting this result, epitope-mapping ELISA showed that PapMV-RBM uniquely targeted an RBM region enriched with critical ACE2-binding residues. These results highlight the potential of linear epitopes for developing versatile SARS-CoV-2 vaccines to fight viral outbreaks. Next, we will complement the PapMV-RBM vaccine with the highly conserved RBD C-terminal epitopes, in an attempt to boost neutralization and expand variant coverage.

François Rouleau

Predicting antifolate resistance in an uncultivable fungal pathogen

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Pneumocystis jirovecii is a fungal pathogen causing Pneumocystis pneumonia in humans, which is treated using the antifolate combination drug trimethoprim-sulfamethoxazole (TMP-SMX). In recent years, here has been an increase of treatment failure. However, experimental methods to study this pathogen are limited, as it cannot be grown *in vitro*. Most other fungi are insensitive to TMP-SMX through DHFR-independent mechanisms, preventing the use of functional complementation to study the evolution of resistance to this drug combination. In a previous study, we conducted DMS on PjDHFR to test its resistance to methotrexate, another antifolate drug. Here, by using this data, as well as computational data modeling aspects of protein function and stability in the PjDHFR-MTX complex, we train a machine learning model to predict the effect of mutations on MTX resistance. By using this model on computational data generated using the PjDHFR-TMP complex, we predict the effect of mutations on resistance to TMP. We compare the predictions from this model to PjDHFR sequenced from clinical samples and find that the model can predict the effect of mutations outside of its training dataset. We also find that the best predictors of resistance, such as distance to ligand and effect on region flexibility, are coherent with previously established models, and that experimental data about the effect of mutations on protein function is critical to optimize model performance. Our results offer insight into the determinants of TMP resistance in PjDHFR, as well as methods to predict resistance in hard-to-study organisms.

Jean-Chirstophe Berger-Dancause

Engineering a novel PET-depolymerizing enzyme from Streptomyces diastaticus to address plastic pollution

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Plastic pollution in Quebec, both on land and in the Saint-Lawrence River, is increasing at an alarming rate. Polyethylene terephthalate (PET) accounts for a substantial portion of plastic wastes in landfills and ecosystems. To address this issue, we screened local landfill-isolated bacterial strains for potential PET-hydrolyzing enzymes and identified several promising candidates. Among them, SdPETase, derived from *Streptomyces diastaticus*, exhibited the highest sequence and structure similarity to known PET hydrolases (PETases). Genome analysis of the strain also revealed the presence of multiple genes involved in the terephthalic acid metabolism including a MHETase, suggesting its ability to use PET as a carbon source. Purified SdPETase expressed in *E. coli* exhibited both esterase and lipase activities as well as calcium-dependent thermostability, which is consistent with known thermostable PETases such as LCC and TfCut2. *In vitro* assays showed that SdPETase hydrolyzes powder and film PET substrates, with optimal depolymerization at 45 °C. To enhance SdPETase efficiency, we engineered a novel variant ($SdPETase_{GICCFH}$) through structural analysis and molecular docking, introducing six targeted mutations. Notably, a stabilizing disulfide bridge was incorporated and removed the calcium dependency to significantly improved thermostability, achieving PET depolymerization at up to 70 °C. The catalytic efficiency was also greatly improved with $SdPETase_{GICCFH}$, yielding 800 % more depolymerization products than the wild-type enzyme. Ongoing protein engineering efforts aim to further optimize $SdPETase_{GICCFH}$ performance to develop a sustainable approach for PET recycling and offer a potential solution to Quebec's plastic pollution crisis.

Cindy Klaus

Distal mutations in a designed retro-aldolase alter loop dynamics to shift and accelerate the rate-limiting step

Serena E. Hunt[1], Cindy Klaus[1], Aqza E. John[2], Niayesh Zarifi[1], Alec Martinez[3], Ferran Feixas[2], Marc Garcia-Borràs[2], Michael C. Thompson[3], Roberto A. Chica[1]

[1]University of Ottawa, [2]University of Girona, [3]University of California, Merced

Amino-acid residues distant from an enzyme's active site are known to influence catalysis, but their mechanistic contributions to the catalytic cycle remain poorly understood. Here, we investigate the structural, functional, and mechanistic impacts of distal and active-site mutations discovered through directed evolution of the computationally designed retro-aldolase RA95. Active-site mutations improve catalytic efficiency by 3,600-fold, while distal mutations alone offer no improvement. When combined with active-site mutations, distal mutations further increase efficiency by 6-fold, demonstrating an epistatic effect. X-ray crystallography and molecular dynamics simulations reveal that distal mutations promote active site opening by altering loop dynamics. Kinetic solvent viscosity effects and electrostatic analysis show that distal mutations accelerate the chemical transformation by 100-fold, shifting the rate-limiting step to product release, which is further accelerated by the increased opening of the active site. These findings highlight the critical role of distal residues in shaping the active-site environment and facilitating the structural dynamics essential for progression through the catalytic cycle.

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POSTER PRESENTATIONS

1 - Enzymatic characterization of biocatalytic polymers: PEEK/CMD/Glucose oxidase assembly

Carlos Andres Martinez Bonilla[1], Joelle N. Pelletier[1], Nick Virgilio[2], Gregory Crezcenso[2], Arthur Lassus[2], Benoit Liberelle[2]

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Enzymatic characterization is used to gain a deeper understanding of enzymes and involves determining various chemical and physical properties through a series of techniques and laboratory procedures. Some properties can be examined using kinetic enzyme studies to reveal *enzymatic activity* under specific conditions such as pH range, ionic strength, temperature, or the nature of the substrate. The Michaelis-Menten constant (K_M) describes the substrate concentration ([S]) at which the enzyme reaches half of its maximal activity (V_{max}) and can be calculated toward a specific substrate using enzymatic activity assays. K_M is useful for comparing enzymatic activity (reaction rate) with different substrates or understanding how they can be affected by changes such as immobilization steps. Here, we present the enzymatic characterization and development of a biocatalytic monolith with a surface decorated with enzyme biocatalysts through covalent immobilization to increase enzyme stability and reusability. To this end, we have chosen polyether ether ketone (PEEK), a biocompatible polymer with a degradable profile and environmental compatibility. The PEEK surface will be covalently modified to anchor enzyme biocatalysts. We showed that biocatalytic PEEK increases the long-term stability and reusability of enzyme biocatalysts because of the protection offered by the polymeric matrix and superficial covalent bonding, which allows it to serve as an active solid support for enzyme immobilization.

2 - Unlocking the enzymatic versatility of CalA: from fatty acid hydrolase to plastic degrader

Mathilde Auffray[1], Claudèle Lemay-St-Denis[2], Stella Cellier-Goetghebeur[2], Lorea Alejaldre[2], Joelle N. Pelletier[2], Daniela Quaglia[1]

[1]UQAM, [2]Université de Montréal

CalA is a versatile biocatalyst that, even though less studied than famous CalB, presents a unique combination of features (high temperatures and acidic pH stability and ability to accept bulky substrates) making it an ideal target for development into an industrial lipase.

An engineering campaign of the enzyme was carried out to improve its selectivity towards the hydrolysis of fatty acid esters of different chain lengths: a sought-for quality in industry for fatty acid enrichment. While allowing for the desired selectivity, our mutational efforts unlocked a deeper understanding of the mode of action of the enzyme. We confirmed the existence of a tunnel region fundamental for substrate recognition, and, unexpectedly, we discovered that other residues in distal regions are also key for modulating substrate selectivity, giving rise to a much more complex mechanism than expected and shedding light on epistatic interactions.

This work led to the hypothesis that CalA might accept bulky substrates other than triglycerides. We, therefore, screened the libraries of CalA variants towards a new polymeric substrate of interest and discovered its potential as a plastic depolymerizing enzyme. Indeed, while the wild-type CalA showed weak plastic degrading activity, a few variants were found to have activity on the substrate. This discovery will further improve our understanding of the mechanism of CalA, and allow us to develop a new enzyme for the degradation of PET (polyethylene terephthalate) and/or PU (polyurethane).

Here, we present this discovery journey, what we learnt so far, and our latest results on this polyvalent biocatalyst.

3 - Are cutinases all the same? The quest for homologs with PET degrading capabilities.

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[1]Université du Québec à Montréal, [2]Université de Montréal, [3]CNETE

Cutinases are enzymes of the family of hydrolases that have been found to be able to depolymerize PET (polyethylene terephthalate). PET is one of the most commonly found plastics (bottles, fibers, and packaging materials all contain it) and also one of the most challenging in terms of environmental impact (i.e. microplastics). At present, when recycled, plastic is converted into inferior materials. Enzymatic degradation offers superior potential: when recovered, monomers can be used to make plastic again, but they are also appealing precursors to other chemicals to give plastic a second life in the form of valuable products (upcycling).

Although the use of enzymes for PET biodegradation has shown some potential in recent years, it is still an underexplored field. In our laboratory, we embarked in the quest of finding new PET-degrading enzymes, in particular, novel cutinases. Through metagenomic research we have individuated ten homologues of a cutinase-query that is knows for its PETase activity. Here, we show our preliminary results from our *ad-hoc* screening, and we compare the homologues. Not all present show activity even though they are close homologues: why? To answer this question, we thoroughly analyse their structure *in silico* and their expression levels experimentally (in *E.coli* vs *Pichia pastoris*).

4 - Dominant-negative mutations in antimicrobial resistance

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Understanding how mutations contribute to antimicrobial resistance (AMR) is a major objective in microbiology. In diploid fungi, AMR mutations can be in both heterozygous and homozygous genotypes, and multiple studies have shown that these mutations are often recessive compared to the wild-type allele. Loss-of-function mutations (LOF) in many genes cause AMR in haploid fungi, for instance, through the loss of activity of the drug targets. These LOFs are thought not to confer resistance in diploid fungi because LOFs are typically recessive. However, we found that, as is the case for some human diseases, some LOF mutations causing AMR exhibit degrees of dominance. Our hypothesis is that this occurs when the mutant allele 'poisons' the wild-type allele—a phenomenon referred to as a dominant-negative effect—disrupting its normal function and protein assembly.

Identifying how dominant negative mutations operate is essential for knowing how AMR arises in diploid fungi. For this purpose, we used genes involved in resistance to 5-Fluorocytosine (5-FC), an antifungal drug that must be metabolized to produce a toxic agent that disrupts DNA replication, ultimately leading to cell death. This process relies on multiple proteins, and mutations that alter any of these proteins can prevent the formation of toxic molecules from 5-FC and thus, lead to resistance. Our work focuses on Fur1, a homotetramer involved in 5-FC resistance, where a number dominant-negative mutations have been identified at the protein's interface. Our objective is to measure the fraction of LOF mutations in Fur1 that drive resistance through dominant negative effects.

6 - Deep mutational scanning of an engineered high-affinity ligand of the poly(A) binding protein MLLE domain

Ali Behvarmanesh[1], Guennadi Kozlov[1], Julian P. Wagner[1], Yu Seby Chen[2], Kalle Gehring[1]

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The MLLE domain is a peptide-binding domain found in the poly (A) binding protein (PABP) and the ubiquitin protein E3 ligase N-recognin 5 (UBR5) that recognizes a conserved motif, named PABP-interacting motif 2 (PAM2). The majority of PAM2 sequences bind to MLLE domains with low-micromolar affinity. Here, we designed a chimeric PAM2 peptide termed super PAM2 (sPAM2) by combining classical and trinucleotide repeat-containing 6 (TNRC6)-like binding modes to create a superior binder for the MLLE domain. The crystal structure of the PABPC1 MLLE-sPAM2 complex shows crucial role of conserved sPAM2 leucine, phenylalanine and tryptophan residues in the interaction. We used deep mutational scanning (DMS) coupled with isothermal titration calorimetry (ITC) to characterize the specificity profiles for PABPC1 and UBR5 MLLE. The best sPAM2 sequence binds to PABPC1 MLLE with low-nanomolar affinity and nearly 20-fold more tightly than the best natural PAM2 sequence. This suggests that the affinities of natural PAM2 sequences are tuned to control their binding to PABPC1 and UBR5. Our study will aid in the discovery of new PAM2-containing proteins (PACs) and facilitate *in vivo* studies of PAM2-mediated cellular pathways.

7 - Pichia pastoris : la levure au service des membres de PROTEO!

Catherine Sanche[1], Louis-Philip St-Yves[1], Anthony Guedon[1], Nauh'a Jean-Louis[2], Sina Rahimi[3], Nicolas Malenfant[1], Daniela Quaglia[2], Jean Sévigny[3], Jean-Francois Lemay[1]

[1]CNETE, [2]UQAM, [3]Université Laval

Pichia pastoris est une levure méthylotrophe largement utilisée pour la production de protéines recombinantes. Son succès repose sur plusieurs avantages : une croissance rapide, une capacité à atteindre de fortes densités cellulaires et un système de sécrétion efficace facilitant la purification des protéines d'intérêt. De plus, elle est capable de réaliser certaines modifications post-traductionnelles, bien que celles-ci diffèrent parfois des modifications humaines. Son système d'expression est une alternative efficace aux bactéries comme *Escherichia coli*, notamment pour des protéines nécessitant un repliement correct et des modifications spécifiques. Son potentiel d'optimisation continue d'en faire un hôte clé en biotechnologie et en production pharmaceutique.

Dans le cadre de projets collaboratifs entre le CNETE et divers membres de PROTEO, nous avons pu optimiser la production de protéines recombinantes grâce à *P. pastoris*. L'affiche présentera deux cas à succès avec des membres de PROTEO et expliquera les différentes étapes pour passer de 0-2mg /L de protéines produites à près de 0,5 à 3g/L.

8 - Optimizing microbial cell factories for in-vivo DNA production and assembly with enhanced homology-directed repair systems

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Recombinant DNA technologies are essential tools in molecular biology, but current cloning techniques are often time-consuming and inefficient. To address these limitations, researchers have proposed using a new technique that would make recombining DNA more efficient in vitro. By using the lambda-red recombination system, scientists are hoping to make DNA assembly methods more efficient as this new method will use *Escherichia coli*'s efficient DNA repair mechanism and will require only 50 base pairs of homology between fragments. This study will first establish a standard reference for DNA assembly efficiency using a Gibson assembly-based red-blue colorimetric gene replacement assay; a high-copy number plasmid containing a blue fluorescent protein gene will be used in a gene replacement assay. Successful assembly will result in the replacement of the blue fluorescent protein with a red fluorescent protein. It will then evaluate the effect of different homology lengths on transformation by testing homology regions of varying lengths (15, 30, 60, 120, and 240 base pairs). Finally, this study will investigate the overall efficiency of the lambda-red system in transforming DH5α cells. This will be done by comparing the efficiency of DNA assembly in DH5α cells with and without the lambda red system. Colony colours on kanamycin plates will serve as an indicator of success, with red colonies confirming successful recombination. We anticipate that the lambda red system will significantly improve DNA assembly efficiency, producing more red colonies compared to control plates lacking the system. Longer homology regions are expected to result in higher transformation efficiency.

9 - Finding and innovating : searching for new PETases and developing original solutions to increase their activity

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PET is one of the world's most widely used plastics (plastic bottles, fibers and packaging materials), and one of the most challenging in terms of environmental impact. A solution to this problem is the use of natural enzymes as catalysts for its depolymerization. PETases are enzymes that can depolymerize PET, and the development of an efficient recycling system based on their use would provide an attractive alternative to incineration or current recycling methods, while offering superior potential (upcyling of the products).

Our project starts with the discovery of new PETases through metagenomic analysis. We used AI-powered methods to identify thousands of new putative PETases from existing databases. We then selected the best 30 candidates based on their environment and organism of origin and ordered their genes in plasmids for expression in *E.coli*. *In-vivo* and *in-vitro* screenings were developed to check their esterase and PETase activity with model substrates: here we present our initial findings. The expression of the best variant will be scaled up, and they will be tested with actual plastic substrates.

It has been demonstrated that better adsorption of plastic degrading enzymes to the plastic substrate can dramatically enhance activity. For this reason, in parallel, we are also developing a platform for the expression of PETase enzymes in a living biofilm to tackle this problem: here, we also present our preliminary results with two model PETases. Our system will be applied in the future to our best newly discovered enzyme.

10 - A Method to Determine Amino Acid Insertion Free Energy in a POPC Bilayer

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Interactions between proteins and lipids are essential for the proper function of membrane proteins. However, studying these interactions remains an experimental challenge due to their atomic-scale nature, diversity, and specificity. In this work, we introduce a new, simple, and rapid theoretical approach to determine the membrane binding free energies of amino acids for membranes with defined lipid compositions. This method relies on the membrane distributions of amino acids obtained from molecular dynamics simulations, which can be performed for various membrane compositions. Preliminary results for the distribution of amino acids in a POPC bilayer highlight the potential of this promising approach for predicting protein-membrane binding.

11 - Refining the St1Cas9 CRISPR Tool to Enhance Efficiency

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For decades, researchers have pursued precise methods to correct point mutations causing monogenic diseases. Collaborative efforts in basic science, clinical research, and biotechnology led to the emergence of CRISPR technology, a groundbreaking tool for effective gene editing.

The CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) system is widely used for genome modification and epigenetic regulation. The type II CRISPR system employs the Cas9 endonuclease, guided by a single-guide RNA (sgRNA), to target specific DNA sequences. The Cas9-sgRNA complex recognizes its target sequence through a protospacer adjacent motif (PAM), a short DNA sequence located downstream of the sgRNA target that is identified by the WED (Wedge) and PI (PAM interaction) domains of Cas9.

Despite its success, in vivo genome editing with CRISPR faces challenges due to the large size of Cas9 and limitations in genome targeting caused by PAM sequence restrictions. Researchers have tackled these issues by characterizing new Cas9 orthologs and re-engineering existing ones.

In 2020, our group demonstrated how specific amino acid residues in the WED and PI domains of *Streptococcus thermophilus* Cas9 (St1Cas9), a smaller nuclease encoded by a 3.4 kb gene, interact with A-T rich PAM sequences. Additionally, we observed that engineered St1Cas9 variants, created by swapping WED and PI domains from different bacterial sub-strains, also showed interactions with non-canonical PAMs.

Building on these findings, we developed optimized St1Cas9 mutants with enhanced nuclease efficiency. These innovations expand the CRISPR toolkit, providing versatile tools for genetic editing in both in vivo and in vitro applications.

12 - Développement d'un protocole innovant basé sur le modèle des monocouches de Langmuir pour l'étude des propriétés mucoadhésives des nanoparticules d'or: vers des systèmes de libération de médicaments ophtalmiques plus efficaces

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Problématique: La majorité des médicaments ophtalmiques est administrée sous forme de gouttes oculaires. Cependant, en raison d'une faible propriété mucoadhésive, moins de 0,02 % des molécules actives atteignent leurs cibles. Dans ce contexte, les systèmes de relargage de médicaments mucoadhésifs se révèlent prometteurs pour améliorer l'efficacité de ces traitements. Mon équipe de recherche a précédemment démontré des propriétés mucoadhésives dans un groupe de nanoparticules d'or (AuNPs). Néanmoins, nous manquons d'informations sur ces propriétés d'un point de vue moléculaire.

Hypothèse et but: Le modèle des monocouches de Langmuir servira à analyser l'influence des mucines, des lipides et des paramètres environnants sur la mucoadhésion des AuNPs.

L'objectif principal de ce travail est la caractérisation globale de la mucoadhésion en mimant l'environnement de la surface cornéenne.

Méthode:
Nous explorerons le comportement des mucines en présence des lipides de la surface cornéenne en calculant leurs paramètres de liaison et en capturant des images de l'interface via la microscopie à angle de Brewster (BAM).
Nous évaluerons le comportement des AuNPs en présence des lipides, puis **analyserons l'influence des lipides sur la mucoadhésion des nanoparticules** dans un système ternaire en utilisant la tensiométrie de surface et la microscopie BAM.
Résultats et conclusions: Les mucines interagissent préférentiellement avec des lipides qui présent des chaînes acyles saturées et une tête polaire phosphoéthanolamine. La microscopie BAM révèle les différents comportements des AuNPs à l'interface selon les molécules présentes.

La caractérisation détaillée de la mucoadhésion contribuera au développement de AuNPs plus efficaces en tant que systèmes de relargage des médicaments.

13 - Optimisation de la couverture du métabolome et protéome de lait maternel par LC-MS/MS

Danko Brukner[1], Nathan Ghafari[1], Lekha Sleno[1]

[1]UQAM

Le lait maternel est un biofluide dynamique et complexe dont la composition varie en fonction de plusieurs facteurs, dont l'état de santé de la mère. Dans cette étude, nous avons testé plusieurs méthodes de préparation d'échantillons pour l'extraction de métabolites et de protéines du lait maternel afin d'optimiser la couverture des métabolites, notamment des marqueurs d'exposition aux xénobiotiques, et des protéines détectables par analyse LC-MS/MS non ciblée. La méthode optimisée sera ensuite appliquée à l'étude des différences de profils métabolomiques et protéomiques du lait maternel dans une cohorte incluant des mères atteintes de diabète gestationnel. L'objectif de ce projet final sera de mieux comprendre l'impact potentiel sur la nutrition et le développement néonatal.

14 - Multiomics of mucopolysaccharidoses (MPS II) model by LC-MS/MS

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Hunter syndrome or mucopolysaccharidosis type 2 (MPS II) is a rare disease with a prevalence of 1/100 000, mainly in boys. It is caused by a deficiency of iduronate sulfatase enzyme, resulting in the accumulation of glycosaminoglycans (GAGs), such as dermatan and heparan sulfate, in lysosomal cells. This accumulation leads to multiple symptoms, such as hydrocephalus, enlargement of the liver and hearing loss. In this study, a combination of metabolomics and proteomics were used to investigate biological perturbations linked to MPS II and its treatment. Livers were obtained from three different groups of mice (WT, MPS model and MPS treated by enzyme replacement therapy) and metabolites were extracted using a protein precipitation protocol, with resulting pellets for proteomics. All data were acquired on a liquid chromatography coupled with a quadrupole-time-of-flight system (LC-MS/MS). These methods resulted in 157 putative metabolites and 1045 proteins significantly-changing between the WT and MPS mice. Numerous metabolic pathways, including purine metabolism, associated with various liver disruptions, were identified with high confidence using both metabolites and proteins. Additionally, 35 proteins were linked to lysosomal degradation. The multiomics analysis indicates that ERT treatment does not significantly impact most of the metabolic disturbances observed in the liver of the MPS II model. Future work includes the comparison with different MPS model, such as C. elegans mutants, human sample from MPS patient and different organs from MPS mouse model.

15 - L'adaptation de protéines orthologues peut être dirigée par de légers changements de température - Un exemple chez deux bactériophages

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Lorsqu'un bactériophage infecte son hôte, celui-ci doit parvenir à répliquer son génome et à empaqueter celui-ci dans de nouvelles particules virales qui seront relâchées quand la cellule sera lysée. Dans beaucoup de cas, le génome phagique sera présent sous forme linéaire lorsqu'il sera encapsidé. Ainsi, certains phages neutralisent l'activité exonucléase de leur hôte pour protéger leur génome. Par exemple, le phage tempéré Mu de *E. coli* exprime la protéine MuGam qui lie et protège l'ADN linéaire composant son génome.

Les résultats que nous présentons se concentrent sur MuGam et sur p33, une protéine orthologue à MuGam retrouvée dans le phage virulent 2972 qui infecte *Streptococcus thermophilus*. D'abord, en utilisant des tests de retard sur gel et des tests de dégradation de l'ADN, nous démontrons que même si Mu et 2972 sont très distants, p33 et MuGam sont homologues dans leur liaison et leur protection de l'ADN. Pour comparer ces protéines dans un contexte *in vivo*, nous avons utilisé l'édition génétique ciblée CRISPR-Cas9 pour générer un 2972 mutant où p33 a été échangée pour MuGam. En calculant la valeur sélective (fitness) du mutant à 37°C et à 42°C nous démontrons que MuGam est beaucoup moins adaptée pour fonctionner à des températures élevées.

En utilisant des courbes de dénaturation par chaleur, nous démontrons que p33 est grandement stabilisée par ses interactions avec l'ADN linéaire, alors que MuGam ne l'est pas du tout. Ceci nous mène à l'hypothèse que p33 aurait évolué pour protéger l'ADN à des températures plus élevées que MuGam.

16 - Characterization of the modulation of islet amyloid polypeptide self-assembly by the chaperone BiP

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Several diseases are associated with the tissue accumulation of insoluble amyloid fibrils, including Alzheimer's disease and type II diabetes. In order to acquire this insoluble amyloid cross-beta structure, the soluble protein undergoes an infinite array of transient secondary and quaternary conformations, making the understanding and treatment of these diseases particularly challenging. Chaperones are proteins that assist in protein folding mainly by preventing misfolding and aggregation. The 70 kDa heat shock protein (HSP70) chaperone family has been identified as potential inhibitors of amyloid aggregation, in the presence or absence of adenosine triphosphate (ATP). However, the interaction site that could support the development of a therapeutic approach against amyloid aggregation remains unknown. In order to identify the domain responsible for the inhibition of amyloid aggregation without ATP, this project aims to use the islet amyloid polypeptide (IAPP), an amyloid peptide linked to type II diabetes, and the Binding immunoglobulin Protein (BiP), the HSP70 of the endoplasmic reticulum. Full-length BiP and its substrate-binding subunits SBDα and SBDβ were recombinantly expressed in *E.coli*. Biophysical assays, such as Thioflavin T fluorescence, a probe specific to amyloid fibrils, atomic force microscopy and bioinformatics analyses show that the complete structure of BiP is likely necessary for this inhibition. By characterizing the residues involved in this inhibitory interaction, this project could provide a better understanding of the interaction between HSP70 chaperones and amyloidogenic peptides, promoting the development of effective therapies against amyloid diseases.

17 - Harnessing peptide self-assembly to design fully synthetic glycoconjugate nanovaccines

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Vaccination remains the key strategy to control infectious diseases afflicting humans and animals. Owing to their biocompatibility, peptides have emerged as essential components of subunit vaccines. Over the last years, we have reported a strategy to generate synthetic nanovaccines based on a chimeric peptide comprising a self-assembling sequence and a peptide antigen, with the resulting nanoassemblies acting as an immunostimulant and a delivery system. In this study, we used this 10-mer self-assembling sequence (I10) to conceive a nanoplatform for the delivery of the tumour-associated carbohydrate antigen Thomsen-Friedenreich (TF). After solid phase synthesis, cleavage and purification, conjugation of TF was accomplished by NHS ester-activated cross-linking. Then, self-assembly was initiated by dispersing the lyophilized glycopeptides in aqueous buffer. To characterize the supramolecular architecture of the resulting nanostructures, we used transmission electron microscopy and atomic force microscopy. By circular dichroism spectroscopy, the secondary conformational transition from random coil to β-sheet was confirmed. Then, the kinetic of self-assembly was followed using the fluorogenic probe thioflavin T. Following the confirmation of the supramolecular assemblies, we evaluated their biocompatibility and their internalisation using immune cells. In addition, we studied the activation of the innate immune response with stably transfected cells expressing specific Toll-like receptors on their surface. Finally, BALB/c mice were intramuscularly immunized and high IgG titers against the glycan was observed by ELISA. The results revealed the robustness of this supramolecular platform and confirmed that fully synthetic cross-β-sheet nanoparticles can elicited strong glycoantigen-specific immune response.

18 - Negative interference between homomeric paralogs preserves genetic redundancy

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Models of gene duplication assume that loss-of-function mutations neutrally promote the return to the ancestral single-copy state. These models ignore the potential functional interference between duplicated proteins stemming from their physical interactions. Here, we use a model to estimate the rate of transitions from the initial state of redundancy towards loss-of-function states, both in the presence and absence of paralog interference. We show that for heterodimerizing paralogous proteins, selection against loss-of-function mutations in one paralog that negatively interfere with the other could contribute to maintaining gene redundancy. A critical parameter that modulates this effect is the ratio of loss-of-function mutations that are negatively interfering. We experimentally estimate this proportion to be around 6.1% (63 out of 1036 deleterious mutations) for the small homotetrameric DfrB1 protein. Such mutations generally have mild effects on protein stability and tetramer assembly. Studying their location in the tetramer structure, we observed that they cluster in the active site and the tetramerization interface, but not in the dimerization interface. Thus, their distribution reveals that protein copies harboring negative interference mutations typically sequester WT copies into catalytically inactive tetramers or dimers, depending on the site of the mutation. Overall, our work describes negative interference mutations emerge from the architecture of protein complexes and how they can contribute to the retention of redundant homomeric paralogs.

19 - NRGSuite-Qt: A PyMOL plugin for high-throughput virtual screening, molecular docking, normal-mode analysis, the study of molecular interactions and the detection of binding-site similarities

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We introduce NRGSuite-Qt, a PyMOL plugin that provides a comprehensive toolkit for protein modeling, virtual screening, normal mode analysis, and binding-site similarity calculations. Building on the original NRGSuite plugin for FlexAID, this updated version integrates five new functionalities: protein-protein and protein-ligand interaction analysis using Surfaces, ultra-massive virtual screening with NRGRank, binding-site similarity detection with IsoMIF, normal mode analysis using NRGTEN, and mutational studies through integration with the Modeller Suite. By merging these advanced tools into a cohesive platform, NRGSuite-Qt streamlines complex workflows and facilitates high-throughput computational studies within a single interface. Additionally, we benchmark a newer version of the Elastic Network Contact Model for normal mode analysis method ENCoM, utilizing the same 40 atom-type pairwise interaction matrix that is used in all other software. This version outperforms the default model in multiple benchmarking tests.**Availability:** The Installation guide and tutorial is available at https://www.biorxiv.org/content/10.1101/2025.01.23.634566v1

20 - La carbonylation affecte les propriétés structurales et fonctionnelles de la 2-Cys peroxirédoxine d'Arabidopsis thaliana

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La 2-Cys peroxirédoxine (2-CysPrx) est une peroxydase thiol chloroplastique codée chez *Arabidopsis thaliana* par deux gènes nucléaires (2-CysPrxA et 2-CysPrxB) et connue pour son rôle crucial dans le maintien de l'homéostasie redox. Cependant, son identification parmi les protéines sensibles à la carbonylation en réponse à l'ABA et au H_2O_2 a soulevé des questions sur le rôle de la carbonylation dans le mécanisme de régulation redox chez les plantes. Ainsi, nous explorons dans cette étude l'impact de la carbonylation, avec des dérivés carbonylés réactifs, 2-hexénal et acroléine, sur la conformation native, la stabilité et l'activité enzymatique des protéines 2-CysPrxA et 2-CysPrxB. Les changements conformationnels des protéines ont été analysés par spectrofluorimétrie et dichroïsme circulaire, tandis que la distribution de la taille moléculaire et l'agrégation ont été évaluées par diffusion dynamique de la lumière. L'activité enzymatique a été analysée en suivant l'oxydation du NADPH avec le système thiorédoxine/thiorédoxine réductase. Nos résultats ont montré que la carbonylation affecte significativement les propriétés structurales et fonctionnelles des protéines 2-CysPrxA et 2-CysPrxB, avec des implications potentielles pour la réponse des plantes au stress oxydatif. Ainsi, nos travaux proposent de nouvelles perspectives sur le rôle de la carbonylation dans la régulation des protéines sous stress oxydatif.

22 - Dynamic regulation of the fibrous Corona in space and time.

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The kinetochore is a large, highly conserved and proteinaceous structure that in eukaryotes assembles onto chromosomes during cell division. It forms the site of direct interaction between the dividing chromosomes and the mitotic spindle, as well as the major signalling hub during mitosis, and is thus essential for the accurate segregation of chromosomes. The outermost layerof the kinetochore, the fibrous corona, promotes attachment of the segregating chromosomes during mitosis to microtubules within the spindle that eventually pull the duplicated chromosomes apart into each of the nascent daughter cells. The corona also promotes chromosome orientation on the spindle, is a microtubule nucleation platform, and supports the spindle assembly checkpoint (SAC), the most important mechanism for maintenance of genome integrity during mitosis. The fibrous corona has recently been reported to expand significantly in size in the absence of microtubule attachments, a mechanism that is thought to increase the surface area available for microtubule capture. Little is known about the function and composition of this enigmatic and highly dynamic structure. Our global **hypothesis** is that in animals, where corona formation is prevalent, novel signalling at the kinetochore plays an important role in the formation and function of the fibrous corona and that signalling networks are significantly altered at this structure in response to microtubule attachment and in response to kinase and phosphatase signalling. Here we **propose** to use proximity proteomics and network analysis to study the composition and plasticity of the fibrous corona.

23 - Structural and Functional Characterization of N-Methyltransferases in Natural Product Biosynthesis

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Methylation is a fundamental transformation in the biosynthesis of natural products, playing an important role in their bioactivation and structural diversification. This modification profoundly influences essential properties such as bioavailability and bioactivity, ultimately shaping the functional characteristics of these molecules.

SAM-dependent natural product methyltransferases (NPMTs) catalyze the structural modification of a diverse range of natural compounds. Among them, O-methyltransferases (OMTs) are the most prevalent, while N-methyltransferases (NMTs), though less common, are found across all domains of life. In this study, we characterized the structure and function of an N-methyltransferase enzyme involved in the methylation of a specific class of natural products, demonstrating its role in modulating biological activity. By resolving the structural and mechanistic basis of N-methyltransferase activity, we identified key determinants of substrate recognition, regioselectivity, and catalytic efficiency. A comprehensive understanding of this enzyme family will provide valuable insights into the broader class of tailoring enzymes involved in natural product biosynthesis.

This knowledge may further contribute to biotechnological advancements, enabling the rational engineering of methyltransferases for the selective modification of bioactive molecules. Such efforts could facilitate the development of novel therapeutics and enhance the biosynthetic potential of engineered microbial systems for drug discovery.

24 - Édition in vivo du locus de la transferrine pour la correction systémique des maladies de stockage lysosomal

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Les maladies métaboliques rares nécessitent des solutions thérapeutiques innovantes. Mon projet explore une correction génique durable via l'intégration ciblée de transgènes par CRISPR-Cas9 (St1Cas9) et vecteurs AAV8 pour une administration systémique chez des modèles murins néonataux. Les transgènes codent des protéines de fusion thérapeutiques, comme l'α-L-iduronidase (IDUA) et l'α-glucosidase acide (GAA), pour traiter les maladies lysosomales (MPS I et maladie de Pompe).

Les protéines de fusion ciblent le locus du gène de la transferrine (intron 16), favorisant leur sécrétion et biodisponibilité via les récepteurs de la transferrine et le mannose-6-phosphate (M6P). L'insertion du transgène utilise un vecteur donneur avec signaux d'épissage, bras d'homologie et promoteur hépatique. Les enzymes conservent leur activité catalytique et traversent la barrière hémato-encéphalique chez la souris.

Les AAV8 sont injectés dans le sinus rétro-orbitaire de souris néonatales (2 jours), permettant une intégration spécifique du transgène dans les hépatocytes via HDR et NHEJ. Chez les souris Idua-/- et Gaa-/-, cette approche a entraîné une expression stable des protéines de fusion dans le sérum, le foie, le cœur et le cerveau. L'activité enzymatique cérébrale a normalisé les GAGs après 6 mois chez les souris Idua-/-, et une amélioration de la force musculaire a été observée après 8 mois chez les souris Gaa-/-.

Cette technologie combine ingénierie du génome et administration systémique pour une correction durable des défauts hépatiques et ouvre la voie à une plateforme générique pour les maladies métaboliques rares.

25 - Mutational landscape and molecular bases of echinocandin resistance

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One of the front-line drug classes used to treat invasive fungal infections is echinocandins, which target the fungal-specific beta-glucan synthase (Fks). They act as non-competitive inhibitors of the enzyme, preventing the synthesis of the essential component of the cell wall that is beta-1,3-glucan. Treatment failure due to resistance often coincides with mutations in three regions of Fks known as hotspots. The biophysical bases by which such mutations confer resistance and cross-resistance among echinocandins are largely unknown. Here, we used deep-mutational scanning to quantify the resistance level of 885 mutations in the three Fks hotspots to the three most widely used echinocandins in the clinic. Independently, we performed molecular dynamics simulations coupled with molecular docking to predict the binding of each drug. We detail the constraints acting on drug binding and explain the resistance specificity for some mutations using the drug-protein interactions from our molecular models. Our findings will enable DNA sequence-based predictions of resistance to this important drug family and the improvement of future molecules that could overcome current resistance mutations.

26 - gyōza: a Snakemake workflow for modular analysis of deep-mutational scanning data

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Deep-mutational scanning (DMS) is a powerful technique that allows the screening of large libraries of mutants at high throughput. It has been used in many applications, including to estimate the fitness impact of all single mutants of entire proteins, to catalog drug resistance mutations and even to predict protein structure. Here, we present gyōza, a Snakemake-based workflow to analyze DMS data. gyōza requires little programming knowledge and comes with comprehensive documentation to help the user go from raw sequencing data to functional impact scores. Complete with quality control and an automatically generated HTML report, this new pipeline should facilitate the analysis of any DMS experiment. gyōza is freely available on GitHub (https://github.com/durr1602/gyoza).

28 - Synthèse d'acides aminés pentafluorosulfanylés et incorporation dans des peptides d'intérêt.

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Les peptides antimicrobiens (PAM) sont d'intéressants candidats pour aborder le problème que représente la résistance aux antibiotiques. Leurs modes d'action variés et leur large spectre d'action justifient cet intérêt, mais leur faible biodisponibilité et leur sensibilité aux protéases limitent présentement l'étendue de leur utilisation. Dans ce contexte, les acides aminés fluorés (FAA) sont couramment utilisés en chimie médicinale afin de moduler les propriétés physico-chimiques et pharmacologiques de peptides en vue de leur application désirée. Le groupement pentafluorosulfanyle est un substituant fluoré émergent ayant déjà démontré un potentiel intéressant en chimie médicinale, mais dont le potentiel en synthèse peptidique demeure largement inexploré. Ainsi, ce projet est centré autour de l'élaboration de méthodes de synthèse pour mener à des acides aminés comportant un groupement pentafluorosulfanyle dans leur chaîne latérale et de leur incorporation subséquente dans des peptides d'intérêts. Nous présenterons ici nos résultats sur ce projet.

29 - SRC homology 3 domains phosphorylation. OFF switch or new SH2 binding motif?

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The SRC homology 2 (SH2) and SH3 domains are among the archetypal protein interaction modules. Many protein-protein interactions with Pro/Arg-rich effectors are nucleated around SH3 domains in response to various stimuli. However, how these signaling complexes are specifically and dynamically modulated in space and time to yield stimuli-specific responses remains unclear. Our previous findings with the SH2-SH3 adaptors GRB2 and NCK1/2 revealed that SH3 functions can be terminated by phosphorylation of an evolutionarily conserved tyrosine present at the C-terminus of most human SH3 domains (148/285).

Using peptide arrays, we now show that the kinase domains of RTKs and SRC family kinases can selectively phosphorylate SH3 domains at this position. Interestingly, we also find that SH2 domains can specifically recognize the corresponding phosphotyrosine (pTyr) peptides. Using the GRB2 adaptor protein as a model, we investigated SH3 phosphorylation and SH2 domain recruitment with recombinant proteins both in vitro and in cells. We observed that the EGFR kinase domain specifically phosphorylated GRB2-SH3.1. Furthermore, the purified pSH3.1 and pSH3.2 domains were able to bind to different subsets of SH2 domains in vitro. Notably, we found that the C-terminal SH2 domain of the regulatory subunit of PI3K (P85 SH2.C) preferentially binds to pSH3.1 with 50-fold greater affinity. Accordingly, EGFR-phosphorylated GRB2 SH3.1 specifically recruits P85 via this SH2 domain following growth factor stimulation in HEK293T cells.

Thus, we propose that SH3 domain phosphorylation provides a means to dynamically modulate the composition of protein complexes assembled around signaling adaptors via SH2-bearing protein recruitment.

30 - Caractérisation d’éléments cis-régulateurs transcrits et de leur environnement épigénétique

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L’expression des gènes est régulée par des éléments *cis*-régulateurs (CREs), qui se lient à des régulateurs de transcription, tels que les facteurs de transcription. Situés dans les régions non-codantes du génome, proches ou éloignées des gènes, ces éléments contrôlent ainsi l’accessibilité à la chromatine et participent à l’initiation et au maintien de la transcription des gènes. Leur caractérisation passe souvent par la détection de signatures épigénétiques ou de régions conservées à travers l’évolution.

Nous avons donc cherché à caractériser l’environnement régulateur du riz en utilisant trois modes de détection : 1- éléments conservés entre les espèces proches, 2- éléments détectés grâce à la méthode d’accessibilité de la chromatine, et 3- des éléments présentant une transcription bidirectionnelle naissante (PRO-seq) détectée par l’outil d’apprentissage automatique dREG.

L'exploration de ces séquences nous révèle différentes cibles de régulation avec des rôles spécifiques. Les éléments conservés semblent favoriser une régulation complexe, tandis que les régions ouvertes de la chromatine avec une transcription bidirectionnelle naissante favorisent une régulation plus stable. Nous avons également constaté qu'un certain nombre de sites de régulation abritaient des régions impliquées dans le silençage d'éléments transposables, tandis que la présence d'autres éléments était corrélée à une augmentation de l'expression génique. Ces résultats suggèrent que nous pouvons détecter des CREs actifs et transcrits. Nous avons également identifié les interactions moléculaires entre les gènes et ces CREs à l'aide de données de contact en 3D. L’identification de ces séquences régulatrices permet ainsi de mieux comprendre les mécanismes impliqués dans la régulation génique dans le riz.

31 - Engineering orthologous Cas9 nucleases for in vivo genetic correction of inborn errors of metabolism

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Rare genetic diseases, whose severity can range from serious to life threatening, affect approximately 500 000 children in Canada alone. Moreover, because they have an impact on a relatively small subset of individuals, few adequate therapeutic options are available. Fortunately, most human pathogenic mutations can either be corrected by an A>G transition, an A>C transversion, or by a C>T transition. Furthermore, several readily available molecular tools can induce such DNA-modifying events.

For instance, base editors can convert adenine (ABE) or cytosine (CBE) nucleobases into guanine or thymine, respectively. In addition, RuvC-impaired base editors represent an interesting therapeutic option as their activity is cell cycle-independant. Besides, such base editors also limit undesired generation of insertions or deletions due to their inactived RuvC nuclease domain. Nevertheless, therapeutic base editing is currently hindered by the limited targetable sequence repertoire, as well as the restrictive nature of adeno-associated viral (AAV) vectors, which prompts the need for further innovation.

Here, we contribute to the development of novel therapeutic genome editing tools by generating rationally-designed and size-optimized base editors. By engineering orthologous Streptococcus thermophilus CRISPR-associated proteins, we repurpose St1Cas9 nucleases as AAV-compatible DNA-targeting scaffolds for adenosine or cytidine deaminases. Furthermore, we leverage our newly generated tools to mediate robust base editing at various genome-wide loci, including clinically-relevant targets harboring point mutations associated with rare genetic diseases such as hereditary tyrosinemia type I (HT-1).

32 - Analyse de la spécificité de liaison des domaines SRC Homology 2 et 3 des protéines adaptatrices NCK1 et NCK2

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Les protéines adaptatrices NCK1 et NCK2 orchestrent l'assemblage de complexes moléculaires via leurs domaines SH2 et SH3, facilitant ainsi diverses voies de signalisation. Initialement considérées comme redondantes, ces protéines se révèlent pourtant impliquées dans des fonctions cellulaires distinctes, dont les mécanismes sous-jacents restent peu compris.

Mon projet de doctorat explore l'hypothèse selon laquelle les régions interdomaines (ITD) des protéines NCK1 et NCK2 influencent leur spécificité d'interaction. Pour cela, j'ai comparé l'interactome des formes sauvages de NCK1/2 à celui de chimères où les trois ITD ont été inversées. Ces modifications entraînent des changements majeurs dans les interactions protéiques, avec notamment une inversion de spécificité pour certains partenaires.

J'ai ensuite analysé ARHGEF10L, un interacteur spécifique de NCK2 qui se lie également à la chimère NCK1 portant les ITD de NCK2. J'ai démontré que son interaction avec le domaine SH2 de NCK2 est régulée par l'ITD3, notamment via trois résidus clés qui libèrent le site de liaison.

Mes travaux mettent en évidence de nouveaux interacteurs spécifiques à NCK2 et révèlent un mécanisme inédit expliquant la spécificité du domaine SH2 des protéines NCK, soulignant ainsi le rôle crucial du contexte protéique environnant dans la reconnaissance des partenaires.

33 - Metal-catalyzed oxidation of the islet amyloid polypeptide alters self-assembly and associated-cytotoxicity

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The islet amyloid polypeptide (IAPP) is a 37-residue peptide hormone secreted by pancreatic β-cells, playing a key role in Type 2 Diabetes (T2D), a condition characterized by chronic hyperglycemia. IAPP has been implicated in β-cell dysfunction and death due to its ability to misfold and aggregate into cytotoxic oligomers and amyloid fibrils. Despite extensive research, the exact molecular mechanisms initiating IAPP aggregation and its cytotoxicity on β-cells are not fully understood. Among hypotheses, the oxidative stress prevailing in the pancreas, and resulting in an imbalance of reactive oxygen species (ROS), could be a possible contributor to protein misfolding and aggregation. This study investigates the effects of the non-enzymatic post-translational modifications induced by ROS on IAPP through metal-catalyzed oxidation (MCO). This was performed by using a biologically relevant Cu/ IAPP/ dioxygen/ ascorbate system to generate and characterize oxidized IAPP (^{ox}IAPP). The results obtained by mass spectrometry allowed us to identify the oxidation of key residues including threonine and histidine, with the latest known for its strong affinity to copper. It was also demonstrated that site-specific oxidation affects amyloid self-assembly by favoring the formation of amorphous aggregates with low thioflavin T binding, contrasting with the long cross-β-sheet-rich fibrils seen for the non-oxidized counterpart. Moreover, these aggregates were shown to be non-toxic to pancreatic β-cells, unlike the pre-fibrillar species of unmodified IAPP. Understanding how oxidative stress affects IAPP aggregation may provide new insights regarding the pathogenesis of T2D and could lead to the development of therapeutic strategies aimed at preventing amyloid formation.

34 - Exploration structure-activité en N-terminal du lipopeptide antimicrobien brévibacilline

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Problématique : Plusieurs bactéries pathogènes ont acquis des mécanismes de résistance aux antibiotiques. Ce phénomène d'antibiorésistance rend la prévention et le traitement d'infections très problématique. Face à cette situation critique, il y a un besoin criant pour de nouveaux agents antimicrobiens. Parmi les alternatives prometteuses aux antibiotiques conventionnels, le lipopeptide brévibacilline, produit par *Brevibacillus laterosporus*, a particulièrement retenu notre attention puisqu'il possède un large spectre d'action contre des pathogènes Gram-positif et Gram-négatif dont plusieurs multirésistants.

Objectif : Pour mieux comprendre son mode d'action et d'optimiser ses propriétés pharmacologiques, l'objectif était de synthétiser des analogues structuraux de la brévibacilline modifiés au niveau de la chaîne grasse et du N-terminal pour faire une étude structure-activité et identifier les composantes modulant l'activité, le spectre d'action et la cytotoxicité.

Méthodologie : Différents fragments dipeptides N-terminal ont été préparés par chimie organique en solution puis incorporés au peptide sur support solide pour générer des analogues de la brévibacilline modifiés en N-terminal. L'activité antimicrobienne a ensuite été évaluée et comparée à la brévibacilline native.

Résultats : L'étude structure-activité a permis d'identifier les chaînes grasses optimales pour l'activité antimicrobienne dans le but de simplifier la synthèse de la brévibacilline. En évaluant le spectre d'action et la toxicité des différents analogues, il a été possible d'identifier les limitations par rapport à la taille et la nature de la chaîne grasse de la brévibacilline.

Conclusion : L'étude des analogues de la brévibacilline a permis d'identifier les composantes importantes en N-terminal pour l'activité antimicrobienne, les propriétés physicochimiques et la fenêtre thérapeutique de ce lipopeptide prometteur.

35 - Investigating the role of RIF1 in early mitosis

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Accurate chromosome segregation during mitosis is crucial for genomic integrity. Errors in chromosome-spindle attachment lead to aneuploidy, a hallmark of cancer. The spindle assembly checkpoint (SAC) normally delays mitotic exit to correct these errors, highlighting the importance of balanced kinase-phosphatase signaling. SAC silencing, a dynamic process, relies on this equilibrium. Disruptions in this balance can cause mitotic errors and contribute to aneuploidy, yet the precise mechanisms remain unclear. We hypothesize that Rap1 interacting factor 1 (RIF1), primarily known for its role in DNA damage repair and as a protein phosphatase 1 (PP1) adaptor, potentially plays a novel role in regulating kinase-phosphatase signaling during early mitosis. We propose that a small pool of RIF1-PP1 complexes remains active at kinetochores, influencing chromosome-spindle attachment and SAC silencing. By investigating RIF1's function in this context, we aim to elucidate the mechanistic origins of mitotic errors and their connection to aneuploidy, thereby providing insights into potential therapeutic strategies for a spectrum of aneuploidy-related conditions.

36 - Self-assembly of a synthetic peptide into a soft nanomaterial for wound repair

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Chronic wounds represent a burden for healthcare systems around the world and critically impair quality of life of patients. It is estimated that chronic wounds have a prevalence of 2 per 1000 people. Hydrogels have emerged as promising treatment options, acting as scaffolds for cells and delivery systems for therapeutic agents. Self-assembled peptide-based hydrogels have attracted great interest owing to their biocompatibility, degradability and low immunogenicity. In this study, we aim to produce fully synthetic peptide-based hydrogels functionalized with bioactive sequences for wound healing. The synthetic I10 peptide, known to assemble into cross-β-sheet fibrils, was functionalized with cell-adhesion (PRa), cell migration-promoting (FGF2) or antimicrobial (IG19) sequences. A hydrogelating version of I10 was designed by adding a lysine residue and an acid C-terminus (K-I10-COOH) to allow higher order assembly. Functionalized I10 peptides individually self-assembled into β-sheet- and β-turn-rich fibrils, as observed by circular dichroism (CD) spectroscopy and atomic force microscopy (AFM) but did not form hydrogels. However, assembly of K-I10-COOH and its co-assembly with functionalised I10 sequences resulted in the formation of hydrogels containing both β-sheet- and β-turn-rich fibres, as confirmed by CD spectroscopy, AFM, and scanning (SEM) and transmission (TEM) electron microscopy. Resulting functionalized co-assemblies showed adhesive properties for skin cells such as keratinocytes (HaCaT) and fibroblasts (1BR3, L929), allowing their proliferation on the material. Evaluation of cell migration and antimicrobial properties is underway, and wound healing potential is being evaluated at the moment on a mouse full-thickness wound model.

37 - Engineering Novel Glycopeptide Antibiotics through One-Pot Biocatalysis Using Tailoring Enzymes

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Glycopeptide antibiotics (GPAs) are clinically important for treating serious infections caused by Gram-positive bacteria, such as *Staphylococcus aureus, Enterococcus, and Streptococcus* species, but their efficacy is increasingly threatened by resistance. The heptapeptide scaffolds of all GPAs are synthesized by nonribosomal peptide synthetases (NRPS) followed by extensive cross-linking and various decorations by a variety of tailoring enzymes. Enzymatic tailoring provides a powerful strategy to modify GPAs and generate novel derivatives with improved antibacterial properties. In this study, we employ a one-pot biocatalytic approach, utilizing methyltransferases, sulfotransferases, and glycosyltransferases to selectively modify glycopeptide scaffolds. By optimizing enzyme combinations and reaction conditions, we could generate a diverse library of GPA analogs. This enzymatic strategy enables efficient and selective modifications, offering a sustainable approach for developing next-generation glycopeptide antibiotics.

39 - Screening of oligonucleotides, natural cofactors and their analogs for inhibition of an antibiotic-resistance enzyme

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Trimethoprim (TMP) is an antibiotic used both to treat common clinical infections and to prevent bacterial infections in livestock and aquaculture. TMP inhibits microbial dihydrofolate reductase (FolA), an essential metabolic enzyme. FolA catalyzes the reduction of dihydrofolate to tetrahydrofolate, a precursor in the biosynthesis of nucleic acids and certain amino acids. However, microbial type B dihydrofolate reductases (DfrB) catalyze the same reaction as FolA without being inhibited by TMP, thereby conferring TMP resistance. In our efforts to develop DfrB inhibitors, we take inspiration from the structure of NADPH, since this dinucleotide serves as the cofactor in the DfrB reaction. Upon screening oligonucleotides against DfrB, we observed weak but sequence-dependent inhibition. Upon screening nucleotide-derived natural cofactors, we observed inhibition by FAD. In addition, using other natural cofactors, we found that biotin and pyridoxal phosphate inhibited DfrB. Furthermore, we screen natural analogs of these potent cofactors to gain insight into the structure-activity relationship. These results demonstrate the significant contribution of nucleobases to the binding of nucleotide-derived molecules to DfrB. These results could guide the design of new molecular scaffolds for the discovery of more potent DfrB inhibitors.

40 - Caractérisation de structures d'ARN en réponse au stress de haute température

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Les changements climatiques soumettent les plantes à des extrêmes de température de plus en plus fréquents. Or, tout comme les protéines, la dynamique des ARN est influencée par la température. Des régions ou modules d'ARN thermosensibles ont été identifiées chez les bactéries, et récemment *in vivo* chez les plantes, mais aucune en lien avec le stress de haute température (HT).

Notre objectif est d'étudier l'effet du stress HT sur la structure de l'ARN à l'échelle du génome et son importance dans la modulation de l'expression génique. Trois aspects des changements de structure d'ARN sont visés : la stabilité des ARN (Sous-objectif 1), la détection de structures d'ARN (Sous-objectif 2) et l'expression des protéines (Sous-objectif 3).

Le riz (*Oryza sativa*), une culture essentielle à la sécurité alimentaire mondiale, sera utilisé comme plante modèle. Des plantules en plusieurs réplicats seront exposées à un stress HT (40°C, 1h) ou maintenues à une température contrôle (28°C). Pour caractériser la stabilité des ARN à l'échelle du génome (Sous-objectif 1), nous utiliserons une approche combinant les méthodes de génomique PRO-seq (*Precision nuclear run-on sequencing*) et RNAseq. Pour cartographier les structures d'ARN à l'échelle d'un nucléotide (Sous-objectif 2), nous testerons pour la première fois la méthode nano-SHAPE-MaP *(**S**elective 2'-**h**ydroxyl **a**cylation analyzed by **p**rimer **e**xtension and mutational profiling followed by nanopore sequencing*). Enfin, le protéome des plantules sera examiné (Sous-objectif 3).

Ce projet avancera les connaissances fondamentales sur les structures d'ARN des plantes, ouvrant la voie à l'identification de cibles pour améliorer les cultures face aux extrêmes climatiques.

41 - RNF13 est un nouvel interacteur de l'enzyme iduronate 2-sulfatase permettant de modifier sa glycosylation et son routage intracellulaire

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La mucopolysaccharidose de type II, ou syndrome de Hunter, est une maladie rare causée par une défaillance de l'enzyme iduronate 2-sulfatase (IDS). Elle entraîne une accumulation pathologique de glycosaminoglycanes dans les lysosomes et un dysfonctionnement multisystémique, dont environ deux tiers des patients présentent une atteinte neurologique sévère. IDS est exprimée sous forme de protéine précurseure, dont la maturation implique plusieurs étapes de transformation essentielles à son ciblage lysosomal et à son bon fonctionnement. Cependant, son routage intracellulaire reste encore peu décrit, alors qu'une meilleure compréhension de celui-ci pourrait ouvrir de nouvelles perspectives pour le développement de stratégies thérapeutiques. Dans ce projet, l'objectif est de mieux comprendre le routage et la maturation d'IDS. Pour cela, nous avons utilisé AlphaFold pour prédire des interactions avec des protéines impliquées dans le transport intracellulaire. Cette analyse a identifié RNF13 comme un potentiel nouveau partenaire d'interaction. RNF13 est une protéine dont la fonction est, entre autres, associée au stress du réticulum endoplasmique, à l'autophagie ainsi qu'à la dégradation et localisation de certaines protéines. La caractérisation expérimentale de cette interaction a démontré que RNF13 interagit principalement avec une forme d'IDS peu glycosylée pour réguler à la fois sa glycosylation et sa maturation. De plus, nos résultats indiquent que la déglycosylation d'IDS entraîne sa dégradation rapide par le protéasome, tandis que RNF13 exerce un effet protecteur. Dans l'ensemble, notre étude révèle un nouveau double rôle de RNF13 dans la maturation et dégradation d'IDS, améliorant ainsi notre compréhension des voies régulant les fonctions moléculaire et cellulaire d'IDS.

42 - Development of covalent DfrB1 inhibitors to overcome antibiotic resistance

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Dihydrofolate reductases (DHFRs) are enzymes in the folate pathway that catalyze the reduction of dihydrofolate to tetrahydrofolate. This allows for the biosynthesis of nucleic acids and certain amino acids in bacteria, thereby ensuring their proliferation. Trimethoprim (TMP) is a bacterial DHFR inhibitor used clinically to treat urinary tract infections and as a prophylactic in the food industry. Its inappropriate use contributes to the emergence of TMP resistance in bacteria, particularly through the expression of type B dihydrofolate reductases (DfrBs). This family of enzymes, first identified in the 1970s, is structurally different from bacterial DHFRs and is insensitive to TMP. Its presence in the bacterium therefore confers a resistance mechanism. Its proximity-based catalytic function inserts dihydrofolate and nicotinamide adenosine dinucleotide phosphate (NADPH) by electrostatic pairing with lysine 32 (K32) at the entrance of the active site tunnel. The symmetrical structure of these enzymes inspired the following fragment- and structure-based design of DfrB1 inhibitors. Bis-carboxybenzimidazoles have shown potency against DfrB1 in vitro. However, the overall negative charge of these compounds at physiological pH and low aqueous solubility offers poor prospects for in-cell assays. In this project, the synthesis and evaluation of potent inhibitors with improved efficiency will be explored by means of covalent inhibition. Bis-carboxybenzimidazole analogues bearing covalent warheads capable of forming covalent bonds with the K32 of DfrB1 will be synthesized. Covalent inhibition kinetics of DfrB1 will be evaluated.

43 - Unveiling the Kinetic Tango: Exploring G-quadruplex Ligand Binding Dynamics and Transfer Mechanisms

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Guanine quadruplex (G4s)-containing genes have been implicated in oncogenesis, making them valuable targets for therapeutics. Small molecules can interact with G4s, modulating their stability and function. In this context, the binding kinetics are critical as it determines the bound lifetime of the complex. This governs how the ligand finds its in-cell target and the extent to which it becomes trapped in off-target complexes. In this study, we utilize NMR and SPR techniques to measure the binding kinetics of the cMYC G4 with the porphyrin ligand TMPyP4.

We found that the kinetics were extremely slow, to the extent that the ligand is effectively trapped onto the first G4 structure encountered. However, these rates increased dramatically as the G4 concentration increased. This revealed a mechanism in which ligands are transferred directly between G4s via collisions in solution, mimicking facilitated diffusion in protein-DNA interactions. The same direct transfer effect was also observed when dsDNA was introduced to the system. This allows ligands to rapidly reach intended G4 targets by utilizing more readily available neighbouring dsDNA. This has potential implications for the development of G4-binding ligands which can capitalize on this direct transfer for enhanced therapeutic activity.

44 - Development of a new high-resolution, high-throughput nucleic acid polymerase blockage assay

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G-quadruplexes (G4s) are non-canonical DNA structures which have been implicated in cancer biology and are attractive drug targets. G4s are typically composed of four G-tracts of three or more consecutive deoxyguanosine (dG) residues that come together to form stacked planar G-tetrads. The biological activity of G4s is related to their ability to block the progression of DNA polymerase enzymes, influencing replication and gene transcription. Current assays to detect polymerase blockage involve gel electrophoresis making them low resolution and low throughput. Thus, we currently lack the detailed kinetic information required to understand how G4 sequence, structure, dynamics, and ligand/drug binding affect their ability to block polymerases.

We developed a new high-resolution, high-throughput polymerase blockage assay that can provide critical information on the interaction of non-canonical nucleic acid structures with DNA polymerases, and how these interactions are modulated by small molecule drugs. The assay utilizes a fluorescence probe and quencher to track the progress of polymerase read through using a plate-reading fluorimeter. The assay is currently being used to explore the effects of G-quadruplex polymorphism, namely loop length and spare-tire tracts, on polymerase read-through. In addition, this platform will be used to characterize a library of naturally occurring G4 sequences together with a library of DNA-binding small molecules and the Klenow fragment of the E. coli DNA Polymerase I. The developed assay will allow us to form a comprehensive, quantitative understanding of how non-canonical DNA blocks nucleic acid polymerases and how these interactions can be modulated by potential drugs.

45 - Utilisation de la technologie des aptamères pour développer des outils d'analyse des procédés de fabrication du vecteur viral adéno-associé

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Les outils de technologie analytique de procédés (PAT) ont été récemment intégrés dans les procédés industriels de fabrication biopharmaceutique dans le but d'obtenir une performance optimale de toutes les étapes des bioprocédés et ainsi garantir une fabrication en masse de produits ayant des attributs de qualité compatible avec son utilisation chez l'humain. En raison de son efficacité et de sa tolérance chez l'homme, le vecteur viral adéno-associé (AAV) est le plus utilisé dans le domaine de la thérapie génique. Jusqu'à présent, il n'y a pas encore d'implémentation de l'approche PAT dans les procédés de fabrication des AAV, car les techniques utilisées pour caractériser les AAV sont souvent basées sur l'utilisation d'anticorps, donc très coûteuses, ce qui limite fortement l'accès à ces thérapies. Ce manque de caractérisation contribue à la présence, dans le produit final, de capsides dépourvues de matériel génétique ayant des propriétés immunogéniques et diminuant l'efficacité de traitement. Dotés d'une grande affinité et sélectivité pour se fixer sur des cibles précises, les aptamères sont de plus en plus employés pour des applications thérapeutiques et diagnostiques. Notre hypothèse de recherche est que la technologie des aptamères pourrait être pertinente pour développer de nouveaux outils PAT afin d'améliorer les procédés de biofabrication des AAV. Nous proposons dans un premier temps de cribler et d'évaluer des aptamères ciblant la capside des AAV à l'aide de la technologie d'enrichissement SELEX, puis de mettre au point des méthodes d'analyse des procédés permettant la quantification, l'enrichissement et la purification des AAV.

46 - Fortifying resistance to dairy phages by leveraging the diverse defense systems of Streptococcus thermophilus

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Anti-phage defense systems have long been exploited in the dairy industry to develop industrial strains with enhanced resistance to lytic phages, a persistent challenge in fermentation processes. In *Streptococcus thermophilus*, a key bacterium in yogurt and cheese production, CRISPR-Cas systems are ubiquitous and have been traditionally leveraged for generating phage-resistant strains. However, phages producing anti-CRISPR proteins (ACRs), which compromise CRISPR-based immunity, have emerged. We recently showed that *S. thermophilus* also frequently harbors additional defense systems, which could be harnessed to further strengthen industrial strains.

Here, we explore the industrial potential of these additional defense systems to enhance *S. thermophilus* resistance. Several systems that conferred phage resistance when tested on a low-copy plasmid were selected to assess their efficacy once chromosomally integrated, a prerequisite for industrial applications. All tested systems were integrated through natural competence and homologous recombination, and provided phage resistance, with some, such as PD-Lambda-1, exhibiting greater efficacy than their plasmid-encoded counterparts. To evaluate the benefits of stacking defense systems, we also introduced multiple systems within the same strain and assessed phage resistance levels notably against phage D5691, which encodes two ACRs that neutralize the two type II-A CRISPR-Cas systems (CR1 and CR3) of the model strain DGCC7710. Interestingly, synergistic effects were observed for certain defense system combinations depending on the MOI, particularly in the case of CRISPR-Cas and Gabija. Finally, we assessed the potential fitness cost of integrating additional defense systems in an industrial strain by monitoring bacterial growth and milk acidification rates.

47 - Développement de procédé avancé pour la production d'adénovirus recombinant avec une lignée cellulaire dérivée des A549

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La production d'adénovirus recombinants pour la création de produits thérapeutiques est en constante augmentation. Les vaccins à base d'adénovirus ont démontré leur efficacité dans la lutte contre des maladies telles que la COVID-19 en stimulant une réponse immunitaire robuste. Cependant, la production de virus recombinants défectifs repose principalement sur la lignée cellulaire HEK293, qui présente certaines limitations. Notamment, cette lignée produit une proportion non-négligeable de virus ayant récupéré leur capacité réplicative, ce qui pose des risques de sécurité.

Une des solutions possible à ces limitations réside dans l'utilisation de nouvelles lignées, construite avec des outils génétiques modernes. Par exemple, le CNRC a développé une lignée cellulaire SF-BMAdr, capable de produire des titres viraux importants, sans le problème de production de RCA. Cependant, aucun développement ni optimisation de procédé, compatible avec la production à grande échelle, n'ont encore été rapportés.

Les objectifs spécifiques de ce projet seront :

Établir un point de référence pour les travaux d'optimisation en documentant une culture du système BMAd-rAd.
Réaliser les expériences nécessaire pour développer un modèle métabolique minimal et prédictif du système BMAd-rAd par des essais à petite échelle en reliant les vitesses de réaction à la concentration des molécules pertinentes en utilisant, entre autres, la planification statistique des expériences.

Effectuer des simulations numériques afin d'identifier les paramètres optimaux d'opération.
Valider cette optimisation expérimentalement.
Les résultats obtenus permettront de conclure que la souche nouvellement développée permet de produire des titres viraux en quantité et en qualité supérieures aux méthodes actuellement utilisées pour la production pharmaceutique.

48 - Unraveling Lanthipeptide Biosynthesis: High-Resolution Insights via Nuclear Magnetic Resonance.

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HalM2 is a model class II lanthipeptide biosynthetic enzyme, catalyzing post-translational modifications of the HalA2 precursor peptide. It recognizes the *N*-terminal leader peptide portion of the precursor and facilitates the dehydration of seven serine/threonine residues and the formation of four thioether rings in the *C*-terminal core peptide with high fidelity, yielding a single poly-macrocyclic isomer. Despite extensive study, the fidelity source remains unknown, necessitating high-resolution NMR techniques. The enzyme kinetics will be studied at an atomic level via a time-resolved HSQC-NMR experiment where a ^{13}C labeled HalA2 is incubated with HalM2 and $^{13}C/^{1}H$ correlation spectra are collected over 24 hours. Changes in peak intensities will provide kinetic information on each HalA2 dehydration and cyclization event. This approach offers details about timing of the post-translational modifications and high-resolution insight into the regio- and stereochemistry of the thioether bridges. We will also employ saturation transfer difference (STD)-NMR to identify HalA2 peptide residues crucial for enzyme recognition and probe the putative extended peptide binding site using a variety of HalM2 variant enzymes. These NMR approaches will provide the first atomic-level details to the complex, multistep maturation of an antimicrobial lanthipeptide opening the door for rational manipulation of the system through targeted binding interactions.

49 - Nouvelle interaction protéine-protéine entre les familles des protéines S100 et des Annexines présentes dans les photorécepteurs

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CONTEXTE ET OBJECTIFS : Une étude protéomique récente suggère que les protéines S100A16 et Annexine A4 participent au maintien de l'intégrité membranaire dans le segment externe des photorécepteurs de l'œil. Les familles des protéines S100 et Annexine sont deux grandes familles de type main-EF, qui sont connues pour jouer un rôle dans le maintien de l'intégrité membranaire et dans la réparation membranaire, respectivement. Plusieurs protéines issues de ces deux familles forment des complexes ensemble, en présence ou en absence de calcium. Il est probable que certains complexes protéiques n'aient pas encore été découverts, notamment pour la protéine S100A16 qui est l'une des dernières protéines de la famille S100 à avoir été identifiée. De plus, la concentration calcique varie dans les bâtonnets entre le jour et la nuit et pourrait avoir un impact sur la liaison du complexe. L'objectif général consiste à étudier la capacité éventuelle des protéines S100A16 et l'Annexine A4 à former un complexe ainsi que l'influence que le calcium pourrait avoir sur leur liaison membranaire. **MATÉRIEL ET MÉTHODES** : Des mesures par polarisation de fluorescence, de thermophorèse à micro-échelle (MST), de résonance de plasmon de surface (SPR) et l'utilisation d'outils de prédiction bio-informatique ont été effectuées pour étudier le comportement des protéines d'intérêt. **RÉSULTATS** : Les protéines S100A16 et Annexine A4 ont été obtenues avec une pureté supérieure à 99%. Les résultats préliminaires suggèrent que les protéines S100A16 et Annexine A4 interagissent ensemble et formeraient un complexe sous forme d'un hétérotétramère en l'absence d'ions calcium.

50 - Effects of surface hydrophobicity on protein aggregation

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Polypeptide aggregation is implicated in numerous human diseases and has significant impacts on the biofabrication and formulation of biological drugs, such as monoclonal antibodies. Although adsorption to surfaces and subsequent misfolding and/or self-association of proteins are known to contribute to their aggregation, the influence of biological and non-physiological surfaces on polypeptide aggregation has been poorly studied. The objective of this study is to examine the influence of surface properties on polypeptide adsorption, aggregation and formation of amyloid fibrils. Specifically, our investigation explores the impact of hydrophobic surfaces on amyloid polypeptide fibrillation. To modulate surface properties, including hydrophobicity, different polymers such as polyethylene glycol, poly (2-hydroxyethyl methacrylate) and polystyrene were applied as thin layers on mica by spin coating. Photo-induced force microscopy (PiFM) confirmed the formation of a uniform polymer layer with homogeneous chemical distribution. Next, by using the islet amyloid polypeptide (IAPP), whose aggregation is involved in type II diabetes mellitus, we investigated its surface-induced aggregation. Atomic force microscopy (AFM) revealed the peptide fibril formation on polymer-coated surfaces, at concentrations below the critical aggregation concentration, whereas no fibrils were observed in solution. Notably, aggregation and fibrillization were more pronounced on the highly hydrophobic surface PS. Furthermore, we evaluated the capacity of the natural polyphenols TGG and corilagin, which are known inhibitors of amyloid formation in bulk solution, to prevent surface-induced aggregation and amyloid fibrillation. These observations support the development of novel strategies to inhibit the formation of amyloid fibrils associated with human pathologies and to improve the biopharmaceutical stability.

51 - Shuttle Peptide Mediates the Delivery of Phosphorodiamidate Morpholino Oligomers in Mucus Hypersecretion Models

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Context: Muco-obstructive lung diseases (MOLDs) are characterized by the obstruction of respiratory airways. Studies indicate that reducing specific gene expressions linked to mucus hypersecretion can alleviate the airway obstruction phenotype in mouse models. Phosphorodiamidate morpholino oligomers (PMOs) are short chemically modified oligonucleotides that bind targeted mRNA and downregulate protein-of-interest. PMOs hold potential for inhalation therapy development to treat MOLDs, as they can be designed to target genes associated with mucus hypersecretion. However, the clinical applications of PMOs are hindered by their low permeability across the plasma membrane. Feldan Shuttles are synthetic amphiphilic peptides that facilitate the intracellular delivery of biomolecules in various tissues and organs, including the lungs.

Hypothesis: We hypothesize that Shuttles can deliver PMOs into airway epithelial cells under conditions of mucus hypersecretion.

Objectives: Our study aims to validate PMO delivery using an *ex vivo* model of human primary bronchial epithelial cells cultured at the air-liquid interface (HBE-ALI), assess Shuttle activity and stability in pathological mucus, and evaluate the effects of Shuttle-mediated PMO delivery in mucus hypersecretion mouse models.

Results: We have demonstrated that Shuttles deliver PMOs into HBE cells and maintain stability in pathological sputum. In a PMO-reporter mouse model, we observed that Shuttle-dependent PMO activity persists six weeks. Additionally, we have shown PMO delivery to airway cells in a mouse mucus hypersecretion model.

Conclusion: Shuttles show favorable properties for the delivery of PMOs in mucus-enriched airways. We believe that Shuttle-mediated PMO delivery targeting genes involved in mucus hypersecretion could represent a promising approach for treating MOLDs.

52 - Synthèse de substituts de ponts disulfures et étude structure-activité du peptide antimicrobien ranalexine

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La propagation de la résistance aux antibiotiques est un devenue un important problème de santé publique à travers le monde. Face à cette menace croissante, le développement de nouveaux agents antimicrobiens avec des modes d'action innovants est maintenant une priorité mondiale. Avec sa forte activité contre plusieurs bactéries pathogènes problématiques et son mode d'action original, le peptide antimicrobien ranalexine produit par la grenouille Rana catesbeiana est particulièrement intéressant. Par contre, la ranalexine est difficile à produire par fermentation et son pont disulfure amène une certaine instabilité. Dans le but de produire la ranalexine par synthèse chimique et de remplacer le pont disulfure, l'objectif du projet était de réaliser la synthèse de substituts de ponts disulfures et de les incorporer au peptide durant sa synthèse sur support solide. La synthèse des substituts de pont disulfure de type cystathionine et éther diaminodiacide a été entamée grâce à diverses réactions en solution et stratégies de protections orthogonales. La synthèse peptididique sur support solide a été utilisée pour la préparation de la ranalexine et ses analogues. L'activité antimicrobienne a été évaluée par microtitration sur différentes souches bactériennes pour déterminer la concentration minimale inhibitrice. La ranalexine et ses analogues ont été synthétisés avec succès. L'analogue C-terminal amide a démontré une hausse d'activité face aux bactéries Gram-positif et Gram-négatif testées. À terme, ce projet permettra de généner des substituts de ponts disulfures qui pourront être ajoutés à des peptides antimicrobiens dans le but d'augmenter leur stabilité et d'améliorer leurs activités inhibitrices envers des pathogènes problématiques.

53 - Développement d'un système de production de plasmides

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Les plasmides sont cruciaux dans le secteur biopharmaceutique. Ils sont notamment utilisés pour des applications vaccinales (vaccins ARN) et en thérapie génique (pour la production de vecteurs adéno associé et lentivirus).

La demande de production de plasmides ne cesse de croître ; cependant, leur production rencontre plusieurs obstacles : rendements limités, préoccupations de sécurité liées aux marqueurs de sélection et qualité des plasmides réalisés. À l'heure actuelle, les entreprises produisant ces plasmides ne disposent pas des infrastructures nécessaires pour répondre à cette demande. À titre d'exemple, la production d'un seul vaccin ARN nécessiterait de mobiliser plus de la moitié de la capacité de production mondiale de plasmides.

Nous proposons de développer de nouveaux systèmes de production de plasmides, dans un premier temps, utilisés pour la fabrication des vecteurs AAV, mais utilisables pour d'autres applications. Le développement technologique portera sur la structure des plasmides, la génétique bactérienne ainsi que la composition du milieu de culture.

Ce projet permettra de mettre en place des outils facilitant la mise au point et la commercialisation de vaccins et de thérapies innovantes.

54 - Role of N-glycans in Betacoronavirus spike protein dynamics

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Betacoronaviruses are known for their high zoonotic potential and have been the cause of multiple epidemics (SARS-CoV-1, MERS) and the COVID-19 pandemic (SARS-CoV-2). Coronavirus infection of host cells is initiated by the interaction between the host cell receptor and the spike (S) glycoprotein decorating the viral surface. N-glycans are added post-translationally to many viral fusion proteins, including the Betacoronavirus S protein, to form the “glycan shield”. These glycans can hide protein epitopes and promote immune evasion, but recent studies on the SARS-CoV-2 S protein have suggested that they are also involved in controlling protein conformation. N-glycosylation sites are highly conserved between SARS-CoV-2 variants and between other viruses of the Sarbecovirus subgenus. Therefore, it is worth investigating the evolutionary conservation of the S protein’s N-glycosylation sites across the Betacoronavirus genus and identify common dynamic mechanisms. Cryo-EM was performed to observe structural changes resulting from a glycan deletion on the SARS-CoV-2 S protein. Single-particle analysis of this mutant demonstrates a lower proportion of particles with a RBD in the up conformation crucial for receptor recognition. A small population of particles were also seen to be missing domains, possibly due to increased instability and flexibility. Further refinement of the generated maps will allow for model building and structural analysis, and binding assays between the mutant spike and their primary receptors will help elucidate the functional impact of glycans in viral infectivity. Conserved structural features between Betacoronaviruses are major targets for vaccine and antiviral development and are relevant for preparing against future pandemics.

57 - Proteomic characterization of RAB-ORP populated membrane contact sites by split-proximity labeling

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Intracellular exchanges are essential for cellular homeostasis. Membrane contact sites (MCS) are close appositions of two organelle membranes that facilitate non-vesicular transport of lipids and ions. Initially observed decades ago by electron microscopy, their functional significance is now being elucidated. Dysregulation of exchanges at membrane contact sites is known to be associated with neurodegenerative and metabolic diseases.Recent studies reveal that RAB GTPases, such as RAB7 and RAB9 at late endosomes, promote MCS formation with the endoplasmic reticulum (ER). Using proximity labeling on multiple RAB GTPases, our laboratory detected interactions between specific RABs and lipid transfer proteins (ORPs), suggesting their involvement in MCS regulation. Thus, our study aimed to define the protein composition of RAB-ORP membrane contact sites to identify factors necessary for their formation and maintenance.

To achieve this, we employed a split-AirID proximity-labeling system, where one half of the biotin ligase was fused to a RAB and the other to an ORP. When these proteins are in close proximity, AirID reconstitutes, selectively biotinylating nearby proteins. This technique makes it possible to label proteins present specifically at membrane contact sites. We validated the system for five RAB-ORP pairs, confirming AirID reconstitution by Western blot and correct subcellular localization by immunofluorescence. Subsequent mass spectrometry analysis of biotinylated proteins revealed potential MCS regulators.

Thus, the split-AirID methodology is a promising technique for identifying the subcellular proximity proteome of proteins of interest, and we believe our results will lead to a better understanding of membrane contact sites populated by RAB and ORP proteins.

58 - Analysis of 3D Chromatin Architecture Dynamics in Rice Under Drought Conditions

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Background

The spatial organization of chromatin within the nucleus plays a crucial role in gene regulation and stress responses in eukaryotes. However, in plants, our understanding of how 3D chromatin architecture is remodeled upon exposure to environmental stress remains limited. In this study, we aim to investigate the structural dynamics of chromatin in rice (*Oryza sativa*) under drought conditions.

Methods

High-resolution three-dimensional (3D) chromatin contact maps of *Oryza sativa* under standard growth conditions have been previously generated using advanced chromatin conformation capture techniques, including Pore-C, Hi-C, and Micro-C. These intra-chromosomal contacts have been shown to facilitate the identification of potential regulatory interactions between cis-regulatory elements and their target genes. To detect the dynamics in architecture between standard and drought conditions, we aim to first complement our current dataset by constructing a 3D chromatin interaction map of rice under drought conditions.

We will compare the chromatin organization of drought-stressed and control growth conditions. Primary, we will focus on the structural dynamics of topologically associated domains (TADs), which are genomic regions characterized by high intra-domain interactions and play a crucial role in gene regulation. We will determine whether drought stress induces significant remodeling of chromatin architecture.

Conclusion

This study will enhance our understanding of how 3D chromatin architecture is remodeled in response to drought stress and its impact on gene regulation in rice. By identifying structural alterations in topologically associated domains and chromatin interactions under drought conditions, we aim to uncover regulatory mechanisms that facilitate stress-responsive gene expression.

59 - Ingénierie des cellules HEK293SF par des approches omiques pour optimiser la biofabrication des vecteurs lentivirus et particules pseudo-virales.

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Les cellules HEK293 sont largement employées dans la production de biothérapeutiques notamment pour la fabrication de vecteurs viraux et de particules pseudo-virales (VLP), composants essentiels au développement de thérapies géniques et vaccins novateurs. Parmi les différents types de lignées HEK293 disponibles, les lignées dites producer présentent un intérêt particulier. Ces dernières intègrent de façon stable les éléments nécessaires à la production de particules virales, permettant ainsi de s'affranchir de l'utilisation de plasmides et des biais associés à la transfection transitoire.

Cependant, de nombreuses limitations intrinsèques aux cellules productrices continuent de limiter les rendements et la qualité des particules obtenues. Parmi celles-ci, on retrouve l'expression de facteurs de restriction cellulaires qui perturbent le cycle de production viral, ainsi que la sécrétion de vésicules extracellulaires avec des caractéristiques physiques et compositionnelles similaires aux particules virales, compliquant les étapes de purification.

Afin de mieux saisir ces processus restrictifs, le projet s'appuie sur des analyses omiques afin d'identifier les voies cellulaires impliquées dans la production de lentivirus et d'évaluer l'impact de la production virale sur la sécrétion de contaminants vésiculaires. Ces données permettront de cibler des gènes d'intérêt pour des modifications génétiques spécifiques. Des approches de type *CRISPR-Cas9* seront mises en œuvre afin d'optimiser à la fois les rendements et la pureté des productions. Finalement, une validation dans un bioréacteur permettra d'évaluer le potentiel industriel des modifications effectuées.

60 - Characterisation of candidate cis-regulatory elements in the drought response of Asian rice

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With environmental conditions becoming more severe, plants need to respond to the ensuing abiotic stresses with extensive gene expression reprogramming. This leads to the creation of stress-specific gene expression profiles, resulting in global morphological adaptations. Major regulators of these processes are noncoding genomic regions called *cis*-regulatory elements (**CREs**). The most abundant class of CREs are **enhancers**, which control and increase the magnitude of spatiotemporal expression of their target genes. Active enhancers are able to display distinct characteristics, for example the transcription of enhancer RNA (**eRNA**). eRNA is a small, unstable, bidirectional, unspliced and unpolyadenylated transcript, which is used as hallmark for active enhancers in several mammalian and prokaryotic organisms. In contrast, transcribed plant eRNA has not been widely investigated, especially in the stress-response context.

My PhD project focuses on the characterisation of active CREs involved in the drought stress response of Asian rice (*Oryza sativa*). Using a systems biological approach, applying **functional genomics**, we identified transcribed, drought-specific CRE candidates, as well as candidates that display a drought-induced increase in activity. Additionally, genomic regions displaying further active enhancer characteristics, such as increased accessibility and a low methylation status, were localised. Established 3D chromatin architecture data was consulted to highlight known enhancer-target pairs under stress conditions. By combing, comparing, and filtering the identified genomic regions, sequences displaying multiple marks of active enhancer are being determined.

These sequences will build the base for further investigations of the genetic fine tuning and potential engineering sites of the drought stress response of *O. sativa*.

62 - Distal mutations enhance catalysis in designed enzymes by facilitating substrate binding and product release

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The role of amino-acid residues distant from an enzyme's active site in facilitating the complete catalytic cycle—including substrate binding, chemical transformation, and product release— remains poorly understood. Here, we investigate how distal mutations promote the catalytic cycle by engineering mutants of three de novo Kemp eliminases containing either active-site or distal mutations identified through directed evolution. Kinetic analyses, X-ray crystallography, and molecular dynamics simulations reveal that while active-site mutations create preorganized catalytic sites for efficient chemical transformation, distal mutations enhance catalysis by facilitating substrate binding and product release through tuning structural dynamics to widen the active-site entrance and reorganize surface loops. These distinct contributions work synergistically to improve overall activity, demonstrating that a well-organized active site, though necessary, is not sufficient for optimal catalysis. Our findings reveal critical roles that distal residues play in shaping the catalytic cycle to enhance efficiency, yielding valuable insights for enzyme design.

63 - ENHANCED OXIDATIVE ACTIVITY OF P450 BM3 VARIANTS

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Monooxygenases perform C-H insertion chemistry under mild conditions with a high degree of regioselectivity and enantioselectivity without the need of protecting groups. $CYP450_{BM3}$ is a self-sufficient monooxygenase that natively hydroxylates fatty acids using molecular oxygen, with NADPH as an electron source. Evolution of P450 BM3 is the topic of extensive engineering for new reactivities but screening the various substrate/reaction combinations remains a time-consuming process. We previously identified indigo-producing point-substituted variants by colorimetric colony-based screening. Here, we recombined these substitutions to generate a combinatorial variant library which contains indigo (+) and indigo (-) variants. The point-substituted and combinatorial variant libraries were screened for oxidation activity against a panel of compounds. High oxidation activity is most often observed in variants containing multiple substitutions and demonstrates that positions that enable the indigo (+) phenotype, or hotspot residues, function as a general mechanism for increasing oxidation activity. Here we describe our screening efforts towards enantioselective oxidation using this combinatorial P450 BM3 library derived from indigo (+) variants and its application in biocatalytic cascades.

64 - A high-throughput random mutagenesis approach to identify antifungal resistance mutations

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Many fungal species cause life-threatening systemic infections in immunocompromised individuals. Those infections often have mortality rates exceeding 50%, despite the availability of antifungal treatments. Our ability to treat systemic infections is further compromised by the emergence of resistance. Resistance to azoles, the most frequently used antifungal class, is often acquired through mutations in the *ERG11* gene. However, which mutations confer resistance remains unknown for many prevalent fungal pathogens, such as *Candida auris* and *Cryptococcus neoformans*. As systematic methods like Deep Mutational Scanning can be resource and time-intensive, we need more efficient approaches to uncover the mutations that lead to resistance. In this project, we investigate the applicability of high-throughput random mutagenesis to study azole resistance mutations in different fungal pathogens. We first built a variant library of *ERG11* from the pathogenic yeast *Candida albicans* (*CaERG11*) using an error-prone PCR method to introduce mutations in this gene. We generated a library of more than 1500 CaErg11 variants with a single amino acid change. The quality of the variant library was assessed with next-generation sequencing. We find that error-prone PCR is a useful method to quickly build a library with a good coverage of the mutations accessible through a single nucleotide change. Next, with orthologous expression of the library in *Saccharomyces cerevisiae*, we will identify resistant mutants by selection on solid media containing an azole. This approach could allow for rapid and low-cost identification of the mutations most likely to lead to azole resistance in fungal pathogens.

65 - Evaluation of a novel Toll-like receptor agonist for the prevention of respiratory infections

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Respiratory infections pathogens were the primary cause of pandemics in the last century and will most likely continue to be so in the future. The COVID-19 pandemic has highlighted the need to develop new therapeutic tools to fight these infections, especially at their onset while other treatments are being developed. Ideally, such a treatment should be easy to manufacture, universal against a broad range of pathogens, and administrable intranasally to protect at the site of infection. Toll-like receptor (TLR) agonists meet all these criteria. Specifically, TLR-7/8 are localized in the endosome, detect invading nucleic acids, and protect against respiratory diseases upon activation. However, the only TLR-7/8 agonist on the market, imiquimod (R837), is administrable topically due to severe adverse effects. Therefore, there is a need to find a safe way to stimulate TLR-7/8 in the lungs and investigate which cells are activated. PapMV is a rod-shaped nanoparticle made from recombinant papaya mosaic virus coat proteins assembled around a non-coding single-stranded RNA. By labeling PapMV with a fluorescent probe, we evaluated its internalization in relevant epithelial and immune cells of the respiratory tract by confocal microscopy and flow cytometry. Furthermore, its co-localisation within endosomes was confirmed with STED microscopy. Finally, its internalization *in vivo* in 9 mouse lung epithelial and immune cells will be evaluated by flow cytometry using a comprehensive 12-marker panel. Determination of which lung cells internalizes PapMV is the first step in characterizing this novel agonist that has the potential to offer universal antimicrobial stimulation against respiratory infections.

66 - Direct interaction between RAB21 and SWIP allows the regulation of the WASH complex.

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Three endosomal sorting complexes exist: the retromer, the commander, and the ESCPE complex. Their recruitment and/or function at early endosomes requires the WASH complex, which is responsible for the polymerization of endosomal actin filaments, essential for cargo sorting. Dysregulation of the complexes is associated with multiple cancers but also with neurodegenerative diseases. Proteins of the RAB GTPase family dynamically regulate early endosomes. Among those, RAB21 localizes to early endosomes and plays a role in cargo recycling. Genetic and proteomic studies identified important interconnections between RAB21, the commander complex, and the WASH complex. **Given the multiple lines of evidence linking RAB21 to the WASH and commander complexes, we aim to elucidate their functional interactions.**

Various cell lines genetically invalidated for RAB21 have been produced and validated. Immunofluorescences and immunoblotting were performed to identify the impact of RAB21's absence on WASH localization and its activity. These experiments highlighted that the lack of RAB21 significantly affected the WASH complex subunit's localization, cargo recycling, and endosomal F-actin levels. We performed rescue experiments and the reintroduction of WT-RAB21 restored the recruitment of WASH complex subunits to endosomes and endosomal F-actin levels and cargo recycling. RAB21 and WASH complex subunits were purified from bacteria and SF9 insect cells to perform biochemical studies. It demonstrated an interaction between GTP-bound RAB21 and the SWIP subunit of the complex. Our study highlights a new role of RAB21 as a regulator of the WASH complex and endosomal sorting with a potential implication for neurodevelopmental disorders.

67 - Analysis of the role of altered SARS-CoV-2 RBD conformational dynamics in promoting immune evasion

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Le SRAS-CoV-2, responsable de la COVID-19, infecte les cellules hôtes en utilisant la protéine S (« *Spike* ») présente à sa surface pour lier le récepteur ACE2 (enzyme de conversion de l'angiotensine 2) à la surface des cellules de l'hôte. C'est le domaine de liaison au récepteur (RBD) de la glycoprotéine S homotrimérique qui permet la liaison à ACE2. Le RBD est aussi la cible de plusieurs anticorps et nanocorps neutralisants. La dynamique du RBD (ouvert/fermé) et l'impact de multiples mutations apparues chez les variants facilitent l'évasion immunitaire. Ce projet vise donc à évaluer l'impact de la dynamique conformationnelle locale du RBD afin de faciliter l'évasion immunitaire. Nous avons établi un système d'expression en *Escherichia coli* pour le RBD ainsi que le nanocorps H11-H4. Nous réaliserons des expériences de résonance magnétique nucléaire (RMN) afin d'évaluer la dynamique locale du RBD et l'impact des mutations. Le nanocorps H11-H4 sera utilisé comme modèle d'anticorps liant le RBD afin d'évaluer les changements de dynamique à la suite de sa liaison. Nos résultats démontrent que le nanocorps possède une forte stabilité thermique et une interaction particulière avec le variant Delta de la protéine S. Ce projet permettra de mieux comprendre l'impact des mutations sur la dynamique du RBD et son rôle envers l'évasion immunitaire.

68 - An Enzyme-Based Electrochemical Biosensor to Detect Aqueous Pyridine

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Pyridine is a small heterocyclic amine widely used in industrial applications such as solvents, drug precursors, and steel coking. It is a systemic toxin and a group 2B carcinogen, with an estimated 250 tonnes released annually into the environment, primarily contaminating soil and water. Current field quantification of pyridine relies on liquid chromatography-mass spectrometry (LC-MS), creating a need for a rapid, reliable *in situ* detection method. To address this, we developed a biosensor based on the pyridine-degrading enzyme PyrA and the cyclic voltammetry (CV) of its cofactor, FMN. The CV parameters of FMN were first optimized for stability and quantification. Kinetic parameters K_M and V_{max} of PyrA were determined using an LC-MS based assay. An N-terminal poly-cysteine tag was added to PyrA for immobilization onto a gold electrode via gold-thiol chemistry. The biosensor was then optimized for detecting biologically relevant pyridine concentrations in aqueous solutions. This system offers a promising approach for rapid and accurate pyridine monitoring, with potential applications in environmental protection and pollution management.

69 - Optimizing microbial cell factories for in-vivo DNA production and assembly with enhanced homology-directed repair systems

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Recombinant DNA technologies are essential tools in molecular biology, but current cloning techniques are often time-consuming and inefficient. To address these limitations, researchers have proposed using a new technique that would make recombining DNA more efficient in vitro. By using the lambda-red recombination system, scientists are hoping to make DNA assembly methods more efficient as this new method will use *Escherichia coli*'s efficient DNA repair mechanism and will require only 50 base pairs of homology between fragments. This study will first establish a standard reference for DNA assembly efficiency using a Gibson assembly-based red-blue colorimetric gene replacement assay; a high-copy number plasmid containing a blue fluorescent protein gene will be used in a gene replacement assay. Successful assembly will result in the replacement of the blue fluorescent protein with a red fluorescent protein. It will then evaluate the effect of different homology lengths on transformation by testing homology regions of varying lengths (15, 30, 60, 120, and 240 base pairs). Finally, this study will investigate the overall efficiency of the lambda-red system in transforming DH5α cells. This will be done by comparing the efficiency of DNA assembly in DH5α cells with and without the lambda red system. Colony colors on kanamycin plates will serve as an indicator of success, with red colonies confirming successful recombination. We anticipate that the lambda red system will significantly improve DNA assembly efficiency, producing more red colonies compared to control plates lacking the system. Longer homology regions are expected to result in higher transformation efficiency.

70 - RAGE as a potential receptor for SARS-CoV-2 entry, leading to a pro-inflammatory response

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Understanding the molecular mechanisms underlying protein-protein interactions is essential to fully comprehend biological processes. This knowledge is also central to the development of new strategies to prevent and control future pandemics. The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is now endemic. Infections can contribute to hyperactivation of the inflammatory response, leading to severe forms of COVID-19 and long COVID. RAGE (Receptor for advanced glycation end-products) activates several pro-inflammatory cascades, including an intracellular signaling pathway inducing NF-κB. It binds various ligands and is expressed on several cell types where ACE2 (angiotensin-converting enzyme 2) is not or poorly expressed (*e.g.*, macrophages). RAGE has also been proposed to be able to bind the SARS-CoV-2 Spike (S) glycoprotein and promote viral entry. In this context, we hypothesize that RAGE promotes SARS-CoV-2 entry in cell types that do not, or weakly, express ACE2, leading to the activation of a pro-inflammatory response via NF-κB. Here, we aim to characterize the involvement of RAGE in SARS-CoV-2 entry and evaluate its role in severe and long COVID-19. Using biochemical methodologies, we produced RAGE and the SARS-CoV-2 S protein and probed their interaction. Utilizing structural biology methodologies (X-ray crystallography and cryo-EM), we will next determine how the complex is formed and confirm RAGE involvement in SARS-CoV-2 entry through the S protein in cell lines not expressing ACE2. This work will enhance our understanding of SARS-CoV-2 entry mechanisms and lead to the identification of new therapeutic targets for the prevention of severe and long COVID-19.

71 - Functional effects of swapping distal mutations between two artificial enzymes evolved from the same precursor

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Current enzyme design methods struggle to predict beneficial mutations far from the active site. Understanding the role of distal mutations in achieving optimal activity could aid in refining design strategies. Here, we investigate the catalytic impact of distal mutations discovered through directed evolution of *de novo* Kemp eliminases 1A53-2 and KE59, both designed from the same natural protein scaffold. Leveraging their shared ancestry, we exchange distal mutations between these enzymes, resulting in Swap variants. We also generate variants containing only active-site mutations (Core) or both sets of distal mutations (Sum). Circular dichroism confirms similar folds in 1A53-2 variants, but melting temperature analysis demonstrates reduced stability with additional distal mutations. Kinetic studies reveal that the Swap variant (k_{cat}/K_M 8100 $M^{-1}s^{-1}$) is less active than the Evolved enzyme containing the original set of distal mutations (k_{cat}/K_M 15500 $M^{-1}s^{-1}$) as well as the Core variant (k_{cat}/K_M 11000 $M^{-1}s^{-1}$), indicating that swapped distal mutations negatively affect the Core activity. The Sum variant displays significantly lower activity (k_{cat}/K_M 600 $M^{-1}s^{-1}$), revealing epistatic effects of both distal mutation sets. Kinetic solvent viscosity effects suggest that swapping distal mutations decreases activity by impeding substrate binding, likely due to altered loop dynamics at the active site entrance observed by molecular dynamics. Our findings underscore the importance of distal mutations in enzyme catalysis and highlight the necessity for developing improved enzyme design methods capable of predicting and incorporating these crucial elements accurately.

72 - Résistance d'orthologues de ERG3 et ERG6 aux antifongiques

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Pour traiter les infections causées par les mycètes, les seuls outils efficaces sont les drogues antifongiques. Deux des quatre classes d'antifongiques cliniques, les azoles et les polyènes, ciblent la voie de synthèse de l'ergostérol. Les drogues de la classe des azoles inactivent la protéine Erg11, causant ainsi la production d'un stérol alternatif qui est toxique à la cellule. Quant à elles, les drogues de la classe des polyènes lient directement l'ergostérol présent dans la membrane plasmique et affectent l'étanchéité de la membrane. Les gènes *ERG3* et *ERG6* sont impliquées dans la synthèse de l'ergostérol, mais aussi dans la synthèse du stérol alternatif toxique créé par l'utilisation des azoles. Certaines mutations affectant ces deux gènes retrouvées dans la littérature peuvent conférer la résistance aux azoles et aux polyènes, mais également aux échinocandines, une classe de drogues qui cible une protéine membranaire non impliquée dans la synthèse de l'ergostérol. Pour étudier comment les variations dans ces protéines affectent la résistance, nous avons exprimé des orthologues de *ERG3* et *ERG6* dans l'organisme modèle *Saccharomyces cerevisiae.* La majorité des orthologues complémentent la fonction native, ce qui nous permet d'étudier leur capacité de résistance en utilisant le châssis de *S. cerevisiae*. Les orthologues testés ont des profils de résistance similaires aux gènes natifs. Cependant, les délétions de *ERG3* et *ERG6* dans *S. cerevisiae* peuvent soit conférer la résistance ou rendre plus sensible aux antifongiques. Par contre, dans le cas des azoles, les délétions causent un phénotype de tolérance plutôt que de résistance.

73 - Effects of flanking regions on DNA i-motif folding and stability

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i-Motifs (iMs) are four-stranded non-canonical nucleic acid secondary structures that are formed by cytosine-rich sequences. Putative iM forming sequences are concentrated in human promoter and telomeric regions, suggesting possible biological roles. However, many iMs do not readily fold at neutral pH, sparking interest in factors that may stabilize them. We performed a systematic study on how the nucleotides flanking iMs affect their stabilities and folding kinetics. We found that the mere presence of flanking nucleotides led to dramatically slower folding and lower stability, compared to isolated iMs. Conversely, complementary flanking nucleotides that comprise an inverted repeat and form a hairpin with the iM in the loop led to greater stability and faster folding than the iM on its own. A bioinformatic analysis of human promoter regions showed that the flanking regions are more likely than average to be complementary to each other, suggesting that this stabilization might be biologically relevant. We analyzed several naturally occurring iM sequences and found that complementary flanking regions substantially stabilized the structures (up to 64-fold faster folding and 17.2 ℃ more stable). Our results show that the regions of DNA flanking iMs are an important and hitherto overlooked factor in iM folding and stability.

74 - Caractérisation de gènes dérivés d'éléments transposables dans le génome du riz (Oryza sativa)

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Les éléments transposables (ET) sont des séquences d'ADN considérées comme « parasitiques » en raison de leur abondance et leur capacité à se propager dans leur génome hôte. Des études ont néanmoins démontré que les ET peuvent être une source importante de séquences pour l'émergence de nouveaux gènes. Ce processus évolutif nommé exaptation des ET a donné lieu à des gènes essentiels à la valeur sélective des animaux et des plantes. Leur ressemblance en séquence à des ET font qu'ils sont souvent masqués dans des analyses génomiques et donc moins étudiés.

Récemment, une analyse *in silico* a permis d'identifier une clade de nouveaux ETE spécifiques aux céréales (*Poales*). Ces ETE auraient une origine similaire à deux ETE conservés dans les plantes, FAR-*RED ELONGATED HYPOCOTYL3* et *FAR-RED-IMPAIRED RESPONSE1*, qui codent pour des facteurs de transcription essentiels à la perception de la lumière. Ces nouveaux ETE pourraient être importants dans le riz (*Oryza sativa*), notre modèle d'étude.

L'objectif de ce projet est de caractériser expérimentalement les gènes ETE de cette clade et de déterminer leur rôles grâce à une approche de génétique inverse.

Nous validerons l'expression et la localisation des protéines issus de ces ETE ainsi que leur rôle à l'aide du système CRISPR/Cas9 (observation des phénotypes résultants) et d'un séquençage ARN pour détecter des changements dans l'expression génique. Nous testerons les interactions protéiques potentielles de ces ETE et leur capacité de liaison à l'ADN.

Ce projet permettra de caractériser ces gènes potentiellement clés à la régulation et la perception de la lumière.

76 - Exploring the effects of protein abundance changes in a gene duplication context

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Gene duplication is an important evolutionary mechanism in evolution and played an important role in the complexification of life through the emergence of new genes. In order for genetic innovations to occur through this process, both copies of the duplicated gene need to be fixed and maintained in a population through long periods of time. Immediate changes in protein abundance have been suggested to be a mechanism conferring an immediate selective advantage to duplication, which could contribute to the fixation of the duplicates. While a previous study identified several genes for which duplication leads to an immediately higher fitness compared to the single copy state, the relationship between protein abundance and fitness remains unknown. This relationship would allow us to determine if duplication leads to the optimal dosage or if subsequent changes in abundance would confer a higher fitness. In this project, we used 100 synthetic promoters to modify the expression of several genes of *Saccharomyces cerevisiae* for which duplication was reported to lead to a fitness advantage. We then performed competition assays to estimate selection coefficients associated with each promoter. The protein abundance associated with each of these promoters can also be estimated by fluorescence-activated cell sorting, allowing to combine protein abundance and selection coefficients to establish fitness functions. Overall, our project provides an approach to better understand the mechanisms by which duplication can immediately be selected positively, as well as to identify the most probable evolutionary trajectory of dosage following gene duplication.

77 - FungAMRseer : Prediction of Antifungal Resistance

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Patients suffering from invasive fungal infections face a high mortality rate. Alarmingly, treatment of these infections can fail due to the emergence of antifungal resistance. In the presence of an antifungal drug, resistant mutants are favored and they proliferate. Currently, antifungal susceptibility testing is a long and complex process. To accelerate this procedure, it has been proposed to sequence the genome of fungal pathogens directly from patient samples. In such a case, targeted sequencing is needed to amplify the fungal DNA from the samples. However, targeting the whole genome of a fungal pathogen is ludicrous. Fortunately, we now have access to the FungAMR database which houses more than 54000 entries of mutations linked to antifungal resistance across 202 genes. This data allows us to target specific genes associated with resistance. Furthermore, this data opens the way for the design of a computational tool complementary to the wet lab approaches of antifungal susceptibility testing for the prediction of antifungal resistance. We propose FungAMRseer. This tool will include a genomic pipeline able to map the reads from targeted sequencing samples to a genome of resistance genes. This way, it will identify both the species and the genetic variants in a sample. The variants identified will be compared to the FungAMR database. FungAMRseer will use a collection of supervised machine learning models trained on structural biology data to predict antifungal resistance of unknown variants. FungAMRseer will be tested with laboratory and clinical samples.

78 - Synthèse, évaluation biologique et interactions synergiques du lipopeptide antimicrobien latérocidine

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PROBLÉMATIQUE: La propagation alarmante des bactéries résistantes aux antibiotiques impose un besoin urgent de nouveaux agents antimicrobiens avec des mécanismes d'action innovants. Grâce à leur grande diversité structurale et leurs modes d'action variés souvent différents des antibiotiques en usage, les peptides antimicrobiens constituent des alternatives prometteuses. Parmi eux, le lipopeptide latérocidine produit par *Brevibacillus laterosporus* se distingue par son activité inhibitrice contre plusieurs pathogènes Gram-négatif d'importance clinique. Malgré son énorme potentiel, les problèmes associés à sa production par fermentation limitent son étude et son utilisation. **OBJECTIF:** Ce projet vise à synthétiser la latérocidine et des analogues afin d'effectuer une étude structure-activité, d'évaluer leur impact sur les cellules eucaryotes, d'analyser leur comportement en milieux biologiques et d'explorer des effets synergiques avec d'autres antimicrobiens.
MÉTHODOLOGIE: Une combinaison de synthèse organique pour préparer les lipopeptides, de microbiologie pour évaluer leur activité antimicrobienne et de sciences pharmaceutiques pour améliorer les propriétés pharmacologiques sera utilisée. Leur cytotoxicité et stabilité face aux protéases du tractus gastro-intestinal seront ensuite évaluées. **RÉSULTATS:** La latérocidine et ses analogues ont été préparés avec succès par synthèse peptidique sur support solide. La latérocidine a démontré une forte activité contre plusieurs bactéries Gram-négatif et très peu de toxicité envers les érythrocytes et cellules Caco-2.
CONCLUSION: En plus de son activité prometteuse, notre étude a mis en évidence des effets synergiques de la latérocidine avec d'autres antimicrobiens contre des bactéries Gram-négatif. Les résultats obtenus démontrent le fort potentiel de ce lipopeptide et ses analogues pour des applications dans les secteurs alimentaire, médical et vétérinaire.

79 - Evolution of Protein Dimer-Dimer Interfaces to Acquire Catalytic Activity

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Antibiotic resistance is considered a major public health problem that is accelerated by the misuse and overuse of antibiotics. Trimethoprim (TMP), an antibiotic introduced in 1962, was synthesized to inhibit bacterial dihydrofolate reductase (FolA). That enzyme is responsible for the synthesis of a cofactor that is essential for bacterial proliferation and therefore constitutes a prime target for antibiotics. However, in the early 1970s, a type B dihydrofolate reductase (DfrB) was reported to confer microbial resistance to TMP. This enzyme catalyzes the same reaction as FolA yet has a distinct structure and is therefore insensitive to TMP.

DfrB is active only in homotetrameric form. This tetramer assembly requires the formation of specific dimer-dimer interfaces to create the central active site. Through a combination of biochemical experiments and bioinformatic analyses, we are currently investigating how dimer-dimer interfaces evolved to lead to tetramerization. DfrB homologs were previously identified by the team and organised into clusters according to their sequence identity. These sequences have been aligned, revealing patterns either in the identity of the residues or in their physicochemical properties. With the information obtained from these analyses, we will carry out directed evolution of the dimer-dimer interface by selecting for formation of active homotetramers from a (necessarily) inactive dimeric homolog. This will enhance our understanding of the mechanism of tetramerization that promoted evolution of an inactive homodimer into an active homotetrameric enzyme. In the long term, improving our understanding in this field could guide the development of antibiotic therapies targeting this dimer-dimer interface.

80 - Conception d'un récepteur membranaire synthétique chez Mesoplasma florum

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Les biothérapeutiques vivants sont considérés comme le futur de la médecine de précision. Nous avons choisi la bactérie quasi-minimale *Mesoplasma florum* pour le développement d'un récepteur membranaire synthétique. Cet organisme est doté d'une seule membrane plasmique à découvert, semblable à celle des eucaryotes. Ce projet de recherche se divise en trois partie.

En premier, nous souhaitons contrôler l'expression d'un gène rapporteur par un facteur de transcription dépendant de l'AMPc. Le facteur de transcription utilisé sera la protéine réceptrice d'AMPc retrouvé chez *E. coli*. L'AMPc pour l'induction sera produite par une adénylate cyclase photoactivée. Le système sera encodé dans des plasmides intégratifs adaptés à *M. florum* qui permettent l'insertion spécifique de nos assemblages de gènes dans le génome de *M. florum*.

Ensuite, nous confirmerons l'activité d'un système basé sur l'hybridation de l'adénylate cyclase bactérien (BACTH) dans *M. florum*. L'adénylate cyclase est divisé en deux parties pouvant être fusionnées à des protéines d'intérêt. Lorsque ces protéines interagissent ensemble, le rapprochement des deux segments de l'adénylate cyclase permet la régénération de son activité. La détection de l'AMPc peut ensuite se faire à l'aide du système inductible précédent.

Finalement, la conception du récepteur est inspirée des récepteurs hétérodimériques eucaryotes. Des anticorps mimétiques serviront de domaine de reconnaissance. Le système BACTH produira le second messager AMPc lors de la dimérisation. Différents duos d'affitins seront testés. Par sélection positive en alternance avec une sélection négative en absence de ligand, il sera possible d'identifier les combinaisons qui nous permettront d'obtenir un comportement en dose-réponse du récepteur.

81 - Customizing the Structure of a Minimal TIM Barrel to Craft a De Novo Enzyme

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The TIM barrel is the most prevalent fold in natural enzymes, supporting efficient catalysis of diverse chemical reactions. While de novo TIM barrels have been successfully designed, their minimalistic architectures lack structural elements essential for substrate binding and catalysis. Here, we present CANVAS, a computational workflow that introduces a structural lid into a minimal de novo TIM barrel to anchor catalytic residues and form an active-site pocket for enzymatic function. Starting from two de novo TIM barrels, we designed nine variants with distinct lids to form active sites for the Kemp elimination. Experimental testing identified one active variant with catalytic efficiency comparable to previously reported Kemp eliminases, and mutational analyses validated the essential role of the designed catalytic residues. Sequence optimization of this variant improved solubility and stability, enabling X-ray structure determination, which confirmed the designed lid structure. This study reports the first enzymatically active de novo TIM barrel and establishes a platform for designing enzymes from minimal protein scaffolds.

82 - Development of bispecific nanobodies targeting human galectin-7 and functional analogs using DNA nanostructures

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Purpose

Selective targeting of protein-protein interactions plays a crucial role in modulating and regulating cell apoptosis. Human galectin-7 (hGAL-7) functions as a homodimer that mediates glycan-dependent interactions between pro- and anti-apoptotic partners, ultimately influencing cell fate. Structural modifications of hGAL-7 can disrupt these interactions, leading to apoptosis resistance in various human cancer cells. This highlights hGAL-7's critical role in cell survival and underscores its potential as a therapeutic target. Our group has evolved selected camelid antibody fragments (Nanobodies, Nbs) that can specifically bind and modulate the function of hGAL-7. These Nbs presumably bind to different epitopes on hGAL-7. It is hypothesized that multiple Nbs can act cooperatively to enhance hGAL-7 inhibition. To verify this hypothesis, we will use DNA nanotechnology to build and randomize artificial bispecific nanobody architectures and test their functional effect in vitro and in vivo.

Methods

His-tagged humanized recombinant Nbs were expressed in E. coli BL21(DE3) and purified by nickel affinity chromatography. Single-stranded DNA (ssDNA) oligonucleotides with a maleimide group at one terminus will be covalently coupled to engineered selected Nbs. These ssDNA oligos will be hybridized to DNA nanostructures to prepare hybrid bispecific Nbs. The concepts of DNA nanotechnology will be applied to explore various bispecific nanobody geometries and architectures. Affinity and specificity will be assessed via ELISA, MST and fluorescence assays, while structural and biophysical properties will be analysed via NMR and X-ray crystallography. The modulatory activity of the hybrid bispecific Nbs will be tested via apoptosis assays in Jurkat T cells.

84 - The key role of bioprocess intensification in reducing organic residues and producing bioenergy, with a focus on sustainable development

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The escalating volume of organic waste necessitates the exploration and development of effective mitigation strategies. Anaerobic digestion, a cost-effective process for pollution reduction and renewable energy production, offers a promising avenue for biotransformation. Nevertheless, the presence of lignin-rich residues often imped this technology. The adoption of effective treatments is often essential to ensure successful outcomes. This research investigates a process intensification method for improving biogas production through anaerobic digestion, employing enzymatic hydrolysis treatment and thermal oxidation. A diverse range of organic waste materials, including invasive weeds, animal manure, paper mill waste and agrifood waste, were evaluated. The enzymatic hydrolysis step facilitated the breakdown of non-soluble macromolecules into smaller molecules by extracellular enzymes. This accelerated the degradation rate of complex matter, promoting efficient fermentation. Biochemical methane potential (BMP) tests confirmed effective degradation of organic matter and biogas-rich methane production. The resulting digestate exhibited favorable nutritional characteristics and was devoid of unpleasant odors, rendering it suitable for bio-fertilization applications. To improve the valorisation of the liquid digestate, hydrothermal oxidation under controlled pressure and temperature was used. This process yielded organic acids, which were subsequently recycled as inputs for anaerobic digestion process, thereby optimizing resource utilization and contributing to a more circular economy, which can also reduce green house gas emissions.

Keywords: Organic waste, Greenhouse gas emissions, Hydrolytic treatment, biomethanisation, Hydrothermal oxidation, Bioenergy

86 - CRISPR-Cas9 in Candida auris: challenging research in a challenging bug

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Candida auris is an emergent fungal pathogen of significant interest for molecular research. Yet, only a handful of CRISPR-Cas9 based allele editing tools have been optimized for *C. auris*. Nonetheless, allele editing in this species remains a significant challenge, and different systems have different advantages and disadvantages. In this work, we compare four systems to introduce the genetic elements necessary for the production of Cas9 and the guide RNA molecule in the genome of *C. auris,* replacing the *ENO1*, *LEU2* and *HIS1* loci respectively, while the fourth system makes use of an episomal plasmid. We observed that the editing efficiency of all four systems was significantly different and strain dependent. However, we did not detect correct integration of linear CRISPR cassette constructs in integration-based systems, in over 4,900 screened transformants. Still, all transformants, whether correctly edited or not, grew on selective nourseothricin media, suggesting common random ectopic integration of the CRISPR cassette. The plasmid-based system has the highest editing efficiency with 41.9% correct transformants on average despite the relatively lower yield of transformants compared to the other systems. Silencing the non-homologous end joining (NHEJ) DNA repair pathway, by deleting two main NHEJ factors, *KU70* and *LIG4*, did not improve the editing efficiency. While our research highlights important challenges in precise genome editing of *C. auris* by quantitatively evaluating the editing and targeting efficiencies of different methods, it also clearly shows the safety and usefulness of plasmid-based systems like *EPIC,* which we recommend for molecular work in this enigmatic fungal pathogen.

87 - Binding of antiparasitic acetogenins on protozoa membrane models with mucins at the air-water interface

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Introduction. Mucins are highly glycosylated proteins which are the main component of human mucus, as well as the cell membrane of parasites such as *Trypanosoma* genus protozoa. Therefore, the study of the interaction of bioactive molecules with membranes bearing mucins is of great importance for the development of new therapies against several kinds of diseases. Annonaceous Acetogenins (ACGs) are natural products with potent antitumor and antiparasitic activities and are going to be studied with these membrane models.

Objectives. This study aims to evaluate the binding parameters of three acetogenins to Langmuir films of phosphatidylethanolamine (PE) polar head lipids with mucins and evaluate the results on a physiological level.

Methodology. Langmuir films of three different PE lipids are going to be prepared by spreading the lipid solutions at the air-water interface, followed by injection of a mucin solution into the subphase of those films. After waiting for the new equilibrium, the acetogenin solutions are going to be injected. The binding parameters after the injection of the drugs are going to be compared to those before.

Results. Pure ACGs don't bind well to the films, with maximum insertion pressures (MIP) below 30 mN/m. However, the mixture of ACGs 1 and 2 showed notably greater binding, with MIP values of 50 mN/m.

Conclusions. The acetogenins studied present difficulties on binding to membranes bearing mucins on their pure form. However, the binding properties of their mixture corroborate with the previous findings that their mixture has a synergic effect that results in a greater bioactivity.

88 - Reconstruction 3D de protéines par super résolution assistée par apprentissage profond.

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La cryomicroscopie électronique (cryo-EM) est une méthode de biologie structurale qui permet d'étudier des protéines et des complexes de grande taille. Bien qu'elle permette de générer des modèles structuraux d'une précision remarquable, son utilisation repose principalement sur des microscopes électroniques à haut voltage (300 kV), qui sont rares et extrêmement coûteux, ce qui limite considérablement son accessibilité. Ce projet de recherche cible de développer une méthode innovante de traitement des images issues de microscopes électroniques à faible voltage (120 kV) afin de démocratiser l'étude structurale des protéines. En s'appuyant sur des modèles d'apprentissage profond, tels que les réseaux basés sur le principe de diffusion, nous visons à améliorer la qualité des images obtenues avec des instruments de 120 kV, plus répandus et abordables, pour atteindre une résolution atomique. Ces modèles sont entraînés sur des données issues de cryo-EM de haute qualité et optimisés grâce à des techniques d'augmentation de données, renforçant ainsi leur précision et leur capacité à s'adapter à divers types d'échantillons. L'objectif de ce projet est de rendre possible la reconstruction précise des cartes de densité électronique en utilisant des équipements facilement accessibles, permettant ainsi d'obtenir des résultats comparables à ceux obtenus avec des appareils à haut voltage, mais à moindre coût.

89 - Chromosomal translocations leads to the formation of fusion's proteins implicated in cancer.

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Chromosomal translocations can lead to the formation of fusion proteins involved in many cancers. These fusions often involve transcriptional regulators acting within chromatin.

Endometrial stromal sarcoma (ESS) is characterized by chromosomal translocations leading to the formation of fusion proteins. It is classified as low-grade (LG-ESS) and high-grade (HG-ESS). These translocations physically link two protein complexes with opposing activities: the NuA4/TIP60 complex and the Polycomb Repressive Complex 2 (PRC2). NuA4/TIP60 is an acetyltransferase complex composed of 17 subunits and acts as a major transcriptional co-activator by modifying chromatin structure at regulatory sequences. In contrast, PRC2 methylates chromatin, leading to transcriptional repression.

LG-ESS is frequently characterized by recurrent protein fusions involving PRC2 and NuA4/TIP60, such as JAZF1-SUZ12 and EPC1-PHF1. These fusions have been molecularly characterized in the Côté laboratory, revealing that they lead to the assembly of mega-complexes. Functional genomic analysis of cells expressing these fusions has shown that genes normally repressed by PRC2 become activated due to the misdirected activities of NuA4/TIP60.

Conversely, chromosomal translocations found in HG-ESS involve subunits of another Polycomb repressive complex, specifically the histone ubiquitinase PRC1 through its BCOR/BCORL1 proteins. Although fusion partners are more diverse, they have also been found to include NuA4/TIP60 subunits, as seen in LG-ESS, as well as other acetyltransferases (CBP/EP300) and methyltransferases (MLL2/KMT2B). The oncogenic molecular mechanisms leading to HG-ESS are currently not understood, and this project aims to resolve this issue.

90 - Screening the Formation of Reactive Metabolites by cytochrome P450 BM3 mutants using LC-MS

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This project aims to develop an innovative system to study the formation of reactive metabolites derived from drugs and environmental contaminants, addressing the major challenge of their inherent instability. Due to their high reactivity, these metabolites cannot be directly administered to cells or organisms, complicating the evaluation of their biological effects. To overcome this limitation, the P450 BM3 enzyme from *Bacillus megaterium* is an interesting model to use to catalyze the formation of these metabolites. P450 BM3 is a highly efficient enzymatic system, particularly due to its fused structure with a reductase domain and can be engineered through directed mutagenesis to enhance its selectivity and ability to oxidize specific substrates. The objective is to screen BM3 mutants using *in vitro* cell lysates, with and without the addition of glutathione or *N*-acetyl cysteine, to form detectable adducts analyzed by LC-MS/MS. Mutants demonstrating increased catalytic activity for the formation of reactive metabolites from acetaminophen, clozapine and estradiol will later be selected for *in vivo* experiments. These compounds will be administered directly to bacterial strains expressing the selected enzymes, and metabolite and protein extracts will be subjected to quantitative metabolomic and proteomic analyses. This approach will allow the identification of biological pathways specifically disrupted by exposure to reactive metabolites, providing a novel model to study their impact in this model system. Ultimately, this system can offer new perspectives for investigating the toxicity of reactive metabolites in complex biological systems.

91 - 2-hexénal : est-ce un inhibiteur covalent ou non covalent de la succinate semialdéhyde déshydrogénase (SSADH) chez Arabidopsis thaliana ?

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Les molécules biogéniques jouent un rôle clé dans les interactions plantes-environnement, notamment en réponse aux agressions des ravageurs et agents pathogènes. Parmi elles, le 2-Hexénal (2-Hex), un composé volatile émis par les plantes, a montré des propriétés allélopathiques en inhibant la croissance racinaire de *Arabidopsis thaliana*. Cependant, les cibles moléculaires et le mécanisme d'action de cette molécule restent mal compris. Ce projet de recherche a pour objectif d'étudier l'effet du 2-Hex sur la succinate semialdéhyde déshydrogénase (SSADH), une enzyme essentielle dont la mutation perturbe gravement la croissance des plantes.

Nous avons émis l'hypothèse que le 2-Hex inhibe l'activité enzymatique de la SSADH. Pour tester cette hypothèse, une protéine SSADH recombinante est produite et purifiée à partir d'*Escherichia coli*, permettant de réaliser des études cinétiques en présence de concentrations croissantes de 2-Hex.

Les résultats attendus permettront non seulement de mieux comprendre le mode d'action de cette molécule bioactive, mais aussi de révéler des mécanismes moléculaires impliqués dans les réponses phénotypiques des plantes soumises au 2-Hex. Les résultats contribueront au développement de nouvelles stratégies de lutte biologique contre les ravageurs et les mauvaises herbes.

92 - The shape of an enzyme's expression-fitness function makes its toxicity robust to promoter mutations

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A protein's contribution to organismal fitness arises from the product of its activity and abundance. Yet, most research mapping genotype-to-phenotype relationships has focused on amino acid substitutions within proteins, potentially overlooking the importance of expression level changes. We generated all possible single-nucleotide substitutions, insertions and deletions in the first 150 base pairs of the yeast *FCY1* promoter and assayed their effect in the presence of prodrug 5-fluorocytosine (5-FC), which gets converted into toxic 5-fluorouracil by the Fcy1 enzyme. Contrary to expectations, we show that none of the studied mutations have a measurable impact on fitness in these conditions. This could occur if the activity of the *FCY1* promoter were mutationally robust, but we report substantial expression variation among the >1000 variants. Fluorescent protein-based measurements even reveal that tens of single-nucleotide changes affect expression level by 10% or more. We investigated this disconnect between the phenotypic and molecular effects of mutations by characterizing the underlying expression-fitness relationship. We show that, across many concentrations of 5-FC, this prodrug's toxicity is largely independent from the expression level of the *FCY1* gene. As such, the shape of the fitness function ensures the robustness of the phenotype to promoter mutations. These results contrast with a previously published study of the Fcy1 protein, where one third of amino acid substitutions confer resistance to 5-FC. This discrepancy highlights how extreme phenotypes, such as antimicrobial resistance, may not always be equally accessible through promoter and coding sequence mutations.

93 - Cost-effective cloning using barcodes for deep mutational scanning of large proteins: study of a key transcription factor involved in multidrug resistance

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Drug resistance mutations significantly hinder treatment effectiveness against various pathogens, highlighting the urgent need for innovative approaches to study and counteract these mechanisms. Deep Mutational Scanning (DMS) is a powerful tool for high-throughput analysis of all possible single amino acid substitutions in a protein. While well-suited for small proteins or hotspots, its application to large proteins has been limited by complexity and high costs.

To overcome these limitations, we developed a cost-effective two-step cloning strategy using DNA barcodes to generate comprehensive plasmid libraries for DMS of large genes. Our approach ensures high replication and barcode diversity while using short-read sequencing to minimize costs. Key innovations include a protocol combining degenerate oligonucleotides and DNA barcodes, optimized Gibson and Golden Gate cloning steps, and methods to maximize mutation coverage, barcode diversity, and experimental reproducibility.

We focus on antifungal resistance, a growing issue due to the rise of invasive fungal infections and the limited treatment options. The transcription factor Pdr1 plays a key role in resistance to multiple antifungals. Mutations in Pdr1 affect the regulation of its target genes, including efflux pumps, leading to resistance. We generated a plasmid library enabling high-throughput analysis of all possible amino acid substitutions in Pdr1, linking mutations to antifungal resistance and cross-resistance.

Systematic characterization of resistance genes like PDR1 is key to understanding resistance mechanisms and generating data required for rapid antifungal diagnostics. This work demonstrates DMS feasibility for large proteins, offering a step toward genomics-based diagnostic tools and advancing the fight against antimicrobial resistance.

94 - Characterization of Computationally Designed Protein Nanotubes

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Protein nanotubes—homo-oligomeric alpha-helical assemblies—offer exciting potential for biotechnological applications such as drug delivery, catalysis and sensing. In this study, we developed a computational pipeline integrating ProteinMPNN, AlphaFold, and our in-house method Volumizer to design de novo protein nanotubes with defined length and internal diameter. Selected designs comprised peptides of approximately 60 residues forming a single helix, predicted to assemble as homodecamers. These were expressed in *E. coli*, purified via immobilized metal affinity chromatography, and further characterized by size exclusion chromatography (SEC) and circular dichroism (CD) to assess oligomerization, folding and stability. We found that protein yield and purity were highly dependent on the position of the His-tag, with C-terminal tags outperforming N-terminal ones. SEC revealed a concentration-dependent distribution of oligomeric states, while CD indicated partial unfolding at low concentrations—supporting the hypothesis that oligomerization promotes alpha-helical folding. Guided by these results, we are now exploring new designs featuring helix-turn-helix motifs that assemble as 15-mers to enhance structural stability and promote the formation of more homogeneous oligomers.

95 - Étude structurale de la reconnaissance des nucléosomes par les complexe histones acétyltransférases de la famille MYST

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Les nucléosomes sont centraux pour orchestrer les différentes fonctions qu'accomplissent la chromatine. Les modifications post-traductionnelles des queues d'histones recrutent des facteurs qui accomplissent des fonctions spécifiques à des régions précises du génome. Malgré leur lien phylogénétique rapproché, les histones acétyltransférases de la famille MYST catalysent l'acétylation de différentes histones à des positions variées. La dérégulation de leur activité enzymatique et leur délocalisation sont impliquées dans la progression de plusieurs maladies telle que les cancers, les rendant des cibles thérapeutiques intéressantes. Or, l'information structurale et architecturale de plusieurs de ces complexes est limitée et incomplète. Également, les mécanismes de reconnaissances des nucléosomes qu'emploient ces complexes multiprotéiques demeurent inconnus. Nous montrons biochimiquement une affinité d'association de ces complexes pour un nucléosome triméthylé sur la lysine K4 de l'histone H3 qui est localisée au promoteur de gènes exprimés. Surprenament, la région acide du nucléosome, la plus importante zone de liaison de facteurs chromatiniens à l'octamère d'histones, n'est pas requise pour l'association des acétyltransférases de la famille MYST aux nucléosomes. En revanche, elle est cruciale à l'acétylation des queues d'histones par ces complexes. Cette étude permet une meilleure compréhension de certains domaines « lecteurs » de la chromatine partagées à travers la famille MYST des histones acétyltransférases. Nous espérons que nos découvertes encourageront le développement de molécules thérapeutiques ciblant ces enzymes.

96 - Optimizing bacterial r-protein bioproduction process through advanced operational strategies

Barbara Mejia Bohorquez[1], Alain Garnier[1]

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Since the outbreak of COVID-19, interest in mRNA vaccines has grown exponentially. These vaccines are produced via *in vitro* transcription catalyzed by the T7 RNA polymerase enzyme, typically produced in *E. coli* using fed-batch fermentation. This operation mode increases biomass yields while reducing byproducts accumulation that could inhibit bacterial growth. However, in a pandemic response context, the industry continues to seek ways to optimize yields and improve production efficiency. Cell recycle fermentation offers an advantage in this regard, as it increases biomass yields by removing inhibitory byproducts. Combining fed-batch with cell recycle presents an opportunity to enhance biomass and recombinant protein yields in a single cultivation cycle.

An in-depth characterization of *E. coli* growth and expression kinetics is being performed under various chemical compositions. Kinetic growth studies in different media led to the selection of a chemically defined medium. Further investigation into acetate inhibition revealed that bacterial growth is inhibited at acetate concentrations above 60 mM.

In a fully-automated 3L bioreactor, fed batch cultures using an exponential feeding strategy achieved a biomass concentration of 70 gDW/L of bacterial. The addition of a cell recycle fed-batch approach, incorporating recirculating loop equipped with a tangential filtration module, increased the biomass yield to 100 g/L. Key parameters, including specific rates, yields, k_La, and the maintenance coefficient were determined. The inducible expression of a r-protein is currently being studied in this system under varying parameters to support the development of a mathematical model capable of representing and optimizing the system across different operational modes.

97 - Conception in silico de nanocorps liant le RBD de SRAS-CoV-2

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Le SRAS-CoV-2, responsable de la COVID-19, infecte les cellules hôtes en exploitant la protéine de surface Spike (S), dont le domaine de liaison au récepteur (RBD) interagit avec le récepteur de l'enzyme de conversion de l'angiotensine 2 (ACE2). C'est d'ailleurs à ce domaine que plusieurs anticorps et nanocorps neutralisant se lie. Toutefois, l'apparition de mutations dans la protéine S empêche leur liaison et contribue à l'évasion immunitaire. Ce projet vise à concevoir des nanocorps pour mieux répondre à l'apparition de mutations dans le RBD de la protéine S de SRAS-CoV-2. En premier lieu, des expériences *in silico* de *Deep mutational scanning* (DMS) sur le nanocorps H11-H4 permettront d'améliorer son affinité pour différents variants. En second lieu, des expériences impliquant d'autres variants permettront de mieux identifier l'impact des mutations prédites et observées sur la protéine S. Finalement, nous testerons l'affinité des meilleurs nanocorps conçus afin de comparer ceux-ci à la protéine native. Ce projet permettra de mieux comprendre comment concevoir un nanocorps en fonction des diverses mutations du RBD observées chez les variants du SRAS-CoV-2.

99 - Design and Testing of Uncompetitive Inhibitors

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There is a pressing need for new molecular targets to help combat Antimicrobial Resistance. Core metabolic enzymes that catalyze transformations of building blocks in bacteria are often overlooked because most developed inhibitors are competitive in nature. Upon inhibition, they lead to accumulation of the natural substrate and are easily displaced, a phenomenon known as metabolic resistance. Uncompetitive inhibitors structurally differ from the natural substrate and bind to the enzyme-substrate complex; hence, they are not outcompeted by the accumulation of natural substrate and drive the inhibition even further. Despite these advantages, there are no methodologies to rationally design uncompetitive inhibitors. We aim to develop a computational and experimental methodology to develop uncompetitive inhibitors. Diaminopimelate decarboxylase (DAPDC), a core metabolic enzyme that catalyzes the final step of lysine biosynthesis in bacteria, will be used as a proof-of-concept enzyme for the rational design of uncompetitive inhibitors. Lysine is an essential component for protein production and bacterial cell wall biosynthesis, making it an attractive target for developing new antimicrobial drugs. By performing Molecular Dynamics of DAPDC with its substrate, followed by docking simulations of commercially available compounds, we aim to identify potential molecular candidates to be tested. Promising compounds will be tested by an optimized isothermal titration calorimetry assay, allowing the determination of their mode of inhibition and binding affinity in a single experiment. The usage of DAPDC will serve as a proof-of-concept target to rationally generate uncompetitive inhibitors and establish a broad methodology that can be applied to other metabolic targets.

100 - Affinity-Based Approach for Profiling Protein Carbonylation in Plant Proteomes

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Protein carbonylation is a key oxidative modification involved in plant stress responses. Initially, protein labeling relied on Cy5.5 and Cy7.5 hydrazide probes to capture the carbonylated proteins. However, these probes presented some limitations, including their broad reactivity to other organic groups and increased background fluorescence. Moreover, they are challenging to achieve precise quantification of carbonylated proteins.To overcome these issues, we have optimized the methodology by introducing m-aminophenylacetylene (m-APA) as a labeling reagent with a different chemical reactivity. Unlike previous dyes, m-APA offers higher reactivity and selectivity towards carbonylated proteins due to its enhanced nucleophilicity. It operates optimally at acidic pH (5-6), promoting efficient labeling while preventing unwanted imine formation through cyanoborohydride reduction. After labeling, proteins undergo methanol precipitation to remove excess reagents, followed by a click chemistry reaction to facilitate detection. This refined approach improves the accuracy and efficiency of protein carbonylation analysis, providing deeper insights into oxidative modifications in plant proteomes. The enhanced detection method paves the way for a better understanding of plant stress responses and their biochemical regulation, contributing to advancements in sustainable agriculture.

102 - Metabolomic and proteomic analysis of Hirschsprung's disease in three mouse models

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Hirschsprung's disease is a rare congenital disorder characterized by colonic obstruction due to the absence of nerve cells in the distal colon. It affects approximately 1 in 5,000 births, with a higher prevalence in boys and newborns with trisomy 21. In some cases, children may develop severe infections, leading to life-threatening complications. To better understand this disease, three mouse models—*Holstein, TashT,* and *Piebald*—have been developed. This study aims to investigate differences in protein and metabolite levels in colon tissue and fecal samples, and compare the biological pathways affected.

Three groups of mice were used for this study, including wild-type (healthy) mice, the disease model, and the latter treated with glial cell-derived neurotrophic factor (GDNF). Proteins from colon and fecal samples from the proximal and distal regions were precipitated, with the supernatant being used for metabolomic analysis, and protein pellets used for quantitative proteomics, both using LC-HRMS/MS. Proteins and metabolites of interest were established based on significant differences between groups of mice. This presentation will highlight the multi-omic workflow and comparative analysis across the three HSCR models, providing insight on disease-associated molecular signatures and the potential impact of GDNF treatment.

103 - Optimisation du récepteur de la prostaglandine F2α avec des mutations ponctuelles et domaines solubles protéiques pour son étude structurale

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Le récepteur de la prostaglandine $F_{2\alpha}$ (FP) est un récepteur couplé aux protéines G (RCPG) qui joue un rôle important dans plusieurs conditions physiologiques, telles que la parturition, le maintien de la pression sanguine et l'inflammation. FP active les voies de signalisation intracellulaires G_q et $G_{12/13}$, ce qui entraîne une cascade de signalisation intracellulaire conduisant à des effets physiologiques. L'objectif de ce projet est de mieux comprendre comment FP active ces voies de signalisation au niveau atomique en utilisant des approches de biologie structurale, telles que la cristallographie aux rayons X et la cryo-EM. FP est instable *in vitro* et ces approches nécessitent une purification à grande échelle du récepteur. La stabilité et le rendement de la purification doivent donc être optimisés. À cette fin, des mutations et des domaines solubles protéiques ont été insérés dans la séquence de FP. Les protéines de fusion ainsi obtenues ont été exprimées et purifiées. Puis, elles ont été analysées par chromatographie d'exclusion stérique et par essai de thermodénaturation pour déterminer leur état d'agrégation, leur rendement de purification et leur stabilité. En utilisant cette stratégie, nous avons pu augmenter de manière significative la stabilité et le rendement de purification du récepteur. À ce titre, une double insertion utilisant les domaines solubles rubredoxine et bRIL, combinée à la mutation S127A, confère à FP un rendement de purification et une stabilité thermique les plus élevés. Finalement, les résultats montrent que la stabilité et l'expression de FP sont suffisamment élevées pour permettre son étude en biologie structurale.

104 - Development of a targeted LC-MRM method for S100 and annexin protein quantitation applied to the analysis of human tears and plasma samples

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S100 and annexin proteins represent two important classes of calcium-binding proteins involved in various physiological processes and are known to play a central role in the development of several human pathologies, including cancer, neurodegenerative diseases, inflammatory disorders, and certain ocular diseases. Given the importance of these proteins, accurate quantification of these proteins could provide essential information on various diseases.

We developed a targeted LC-MRM method, on a triple quadrupole platform, to quantify 17 S100 proteins and 10 annexins. Standard proteins derived from bacterial expression were used to optimize the method for monitoring unique tryptic peptides for each protein of interest. The targeted method was finally applied to analyze peptides derived from trypsin digested tear and plasma samples. An initial screening of tears from patients with either eye or liver diseases was performed, followed up by a second analysis from tears and plasma of liver disease patients. Our preliminary data shows that several of these proteins are found to be significantly impacted in the liver disease group of patients.

105 - Purification of human prostaglandin F2α receptor in complex with different heterotrimeric G proteins for cryoEM structural study.

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Prostaglandin $F_{2\alpha}$ receptor (FP) is a G protein-coupled receptor (GPCR) involved in many physiological and pathological conditions, such as parturition, glaucoma, and brain injuries. Prostaglandin $F_{2\alpha}$ binds and activates FP, leading to recruitment of intracellular $G_{q/11}$ or $G_{12/13}$ protein effectors, and subsequent activation of signaling cascades. These effectors contribution to the physiological role of FP and the atomic details of G protein coupling is not fully known. Solving the structural determinants of FP-G protein coupling would give an important insight to develop molecular tools to decipher the role of each pathway in FP biological functions. The recent publication of the CryoEM structure of FP in a complex with a Gs/i/q protein chimera has shed light on the molecular events at the atomic level leading to FP activation toward G_q pathway. Here we present the stabilization, purification and characterization of the complex between FP and its heterotrimeric G proteins. Our vitrified samples will serve as materials for CryoEM data collection and the resolution of FP-G_q and FP-$G_{12/13}$ signaling complexes.

107 - Computational analysis of AT1 receptors ligands activity

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The renin-angiotensin-aldosterone system is a hormonal system involved in the blood pressure regulation through electrolytic balance, vascular resistance and the action of key components, notably the renin enzyme, aldosterone, and angiotensin II receptors (AT1R, AT2R) that are G protein coupled receptors. AT1R is often the target of clinical approaches surrounding heart problems such as congestive heart failure and hypertension. This study aims to deepen our understanding of AT1R activation mechanisms and to contribute to the development of new antihypertensive drugs.

Two active conformations of the AT1R receptor analysed and processed. The structure of the complex will be obtained using molecular docking to model their interactions with a library of 286 molecules and small peptides for which G-protein and β-arrestin (Barr) signaling responses were previously measured using BRET-based assays (Emax and EC50). A per-residue binding energy decomposition will be performed to identify residues whose contact impacts activation for each pathway. Subsequently, a dynamical signature for each receptor-ligand complex will be computed using normal mode analysis to assess conformational flexibility and identify residues associated with functional outcomes.

The next steps involve the development of a machine learning model capable of identifying ascending levels of complexity in patterns within the energy decomposition profiles and dynamical signatures, in relation to the known activity of the ligands. This model will be applied to enhance the understanding of the AT1R activation mechanism and to predict the activity of new ligands.

108 - Phosphorylation-dependent regulation of Src homology modular domains protein interactions

Bohdan Zhuravel[1, 2, 3], François Chartier[1, 2, 3], Nicolas Bisson[1, 2, 3, 4]

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Intercellular communication is often mediated by signaling networks acting downstream of transmembrane receptors, such as receptor tyrosine kinases (RTKs). The most common signaling effectors for RTK signaling are adaptor proteins composed of SRC Homology (SH) 2 and SH3 modular domains, such as NCK1/2 and GRB2. Our lab previously discovered that SH3 Tyr phosphorylation inactivates their canonical protein binding function and results in signal termination, dismantling RTK-initiated signaling networks.

We hypothesize that RTK-mediated SH3 domain phosphorylation also creates new pTyr binding motifs to rewire cell signaling networks via gain-of-function properties due to binding of effector proteins with pTyr-reader domains (e.g. SH2).

First, we purified recombinant WT and in vitro-phosphorylated SH3 (pSH3) domains from GRB2, along with a library of human recombinant SH2 domains, to perform high-throughput pull-down assays. We identified SH2 associating with individual pSH3s (but not WT SH3s) using mass spectrometry. Second, we validated associations identified by re-testing positive pSH3-SH2 pairs individually via protein pull-down. We found that each pSH3 of GRB2 can be selectively recruited by different signaling effectors via their SH2 domains in vitro. Among such, we identified proto-oncogenes: PI3KR, YES and FYN. We validated a subset of these associations in cells. We have developed cell lines expressing non-phosphorylatable GRB2 Y/F mutants to assess this phosphorylation's functional effects.

Our work contributes to uncover how SH3 domain Tyr phosphorylation rewires cell signaling pathways. Understanding this novel mechanism within traditional RTK-mediated signaling could reshape our understanding of cell signaling regulation, driving innovation in drug discovery and biomedicine.

109 - Identifying and enriching high-expressing CHO cells to increase pool productivity

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[1]University of Montreal, [2]National Research Council Canada

Rapid and scalable production of biotherapeutics and vaccine antigens is critical for responding to public health emergencies. Traditional Chinese Hamster Ovary (CHO) cell line development (CLD) is a lengthy process, taking 8-10 months to establish monoclonal production lines. To expedite early-stage clinical material generation, CHO stable pools offer an alternative, reducing this timeline to 5-8 weeks. However, their productivity remains significantly lower due to random integration of gene-of-interest expression cassette, leading to heterogeneous expression profiles, with some cells being high producers, some moderate and others non-producing. Fishing out high producers from a heterogenous pool would thus improve the overall pool productivity. However, assessing expression levels of cells in a stable pool is challenging as most of the recombinant protein product is secreted in the culture medium. Here, we describe a novel enrichment strategy leveraging glycosylphosphatidylinositol (GPI)-anchored surface marker and hemagglutinin-based detection system to identify and selectively enhance high-producing cells within a stable pool. Further, we characterize and compare this approach with an established methodology called cold capture, wherein under cold conditions, the secreted product is transiently retained at the CHO cell surface. Our results show that cell surface expression of GPI-anchored surrogate marker correlates with target protein expression. This enabled us to identify and sort for high-expressing subpopulations, which showed higher transgene copy numbers, mRNA expression levels, and cell specific productivity, leading to a 2-fold increase in the volumetric titers. Overall, this strategy represents a significant advancement in CLD workflows, accelerating biomanufacturing timelines to support global health initiatives.

110 - Neutral variation in a protein interaction network limits the predictability of protein evolution

Soham Dibyachintan[1], Alexandre Dubé[1], David Bradley[1], Pascale Lemieux[1], Ugo Dionne[1], Christian Landry[1]

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The evolutionary fate of a mutation is dependent on its phenotypic effects. In recent years, multiple evolutionary models have been developed that use variation in natural sequences to predict the impact of a mutation in any given protein. However, many proteins display multiple phenotypes, most mediated by specific protein domains. Furthermore, many proteins originate from gene duplication and share most of their evolutionary history. How such factors affect the predictability of evolution is still being determined owing to the lack of comprehensive experimental data on such proteins. Our study uses the endocytosis protein interaction network in yeast involving a pair of duplicated myosins as a model system to test the limits of such predictability in evolution.

We combined genome editing and high-throughput phenotyping assays to quantify the impact of all single-amino acid substitutions in two functionally redundant paralogous Src Homology 3 (SH3) domains on the interaction with their cognate interaction partners in yeast. We observed that the effect of many mutations was not conserved across interactions or between the paralogs. A comparison of our experiments with evolutionary models revealed that these models do not capture most differences in the effect of mutations between paralogs for these phenotypes. Moreover, certain evolutionary models are better at capturing the effect of mutations on some interactions than others, while other models are equivalent across interactions.

Broadly, our results illustrate that neutral sequence variation over time in the components of a protein interaction network limits our ability to predict protein evolution accurately using existing methods.

111 - Optimisation des conditions de cristallisation du transporteur de prostaglandines

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Les prostaglandines sont des lipides bioactifs qui jouent un rôle essentiel dans de nombreux processus physiologiques, tels que la parturition, l'inflammation et le cancer. Le transporteur de prostaglandines (PGT) transporte de manière passive les prostaglandines du milieu extracellulaire vers le cytoplasme, où elles sont inactivées. L'objectif de cette étude est de comprendre, par cristallographie aux rayons X, le mécanisme d'activation et de régulation du transporteur à l'échelle atomique. Nous présentons ici l'obtention des cristaux initiaux du transporteur et l'optimisation de leur croissance. Les cristaux initiaux sont apparus dans une des 3840 conditions de cristallisation testées, d'une taille moyenne de 5 × 1 µm. Après optimisation, nous avons obtenu des cristaux de 150 × 15 µm dans quatre conditions et nous avons également identifié plusieurs autres conditions produisant des cristaux de taille inférieure. La prochaine étape consistera à récolter ces cristaux et à évaluer leur diffraction au synchrotron. Les cristaux offrant la meilleure diffraction seront utilisés pour acquérir un ensemble de données servant à résoudre la structure à l'échelle atomique du transporteur de prostaglandines.

112 - Caractérisation du rôle du complexe FIRRM-FIGNL1 dans la réponse au stress réplicatif

Marie-Anne Bérubé[1, 2], Julien Dessapt[1, 2], Élise G. Lavoie[1], Andréanne Blondeau[1], Raquel Cuella-Martin[3], Amélie Fradet-Turcotte[1, 2]

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Lors de la division cellulaire, la réplication de l'ADN est essentielle pour transmettre l'information génétique. Lorsque cette réplication est perturbée, l'arrêt prématuré de la machinerie de réplication peut conduire à une instabilité génomique appelé stress réplicatif. Les pontages interbrins d'ADN (PIBs), causés par des agents thérapeutiques tels que la cisplatine, lient de manière covalente deux brins d'ADN, induisant ce type de stress. Pour assurer leur survie, les cellules ont développé des mécanismes complexes permettant de détecter, signaler et réparer ces lésions.

Grâce à un criblage CRISPR-Cas9, notre laboratoire a identifié FIRRM comme un acteur clé de la réponse aux PIBs. FIRRM forme un hétérodimère obligatoire avec l'ATPase FIGNL1, et ce complexe est nécessaire à la réparation des cassures d'ADN causés par la cisplatine (induction de PIBs) et à la survie des cellules dans ces conditions. Malgré le fait que la stabilité de FIRRM et FIGNL1 est interdépendante, nous avons découvert que la surexpression d'un mutant de séparation de fonction de FIRRM permet de restaurer la dynamique la réparation et la survie cellulaire en réponse à la cisplatine et ce, indépendamment de FIGNL1. Afin de carctériser les rôles respectifs de FIRRM et FIGNL1 en réponse au PIBs, un criblage par édition de base sera utilisé. La outils cellulaires et moléculaires créés et la stratégie choisie pour réaliser ce criblage seront présentés.

L'identification de mutations spécifiques affectant la fonction de FIRRM et/ou FIGNL1 ouvrira la voie à une meilleure compréhension de leur rôle dans la réponse au stress réplicatif.

113 - Enzyme Gene Expression in Microbes for Sustainable Biofertilizer Development

Ziyed Sahlia[1], Jean-Christophe Berger Dancause[1], Pauline Fortin[1], Stéphanie Bertrand[1], Habib Horchani[1], Émilie Bédard[2], Salma Taktek[1]

[1]Cégep de Rivière-Du-Loup, [2]Polytechnique Montréal

Arianne Phosphate, a significant mining company, is engaged in a sustainable and innovative initiative to integrate the investigation of gene expression through microbial mechanisms involved in phosphate solubilisation and nitrogen fixation.The objective of this study is to explore the potential of indigenous micro-organisms to boost nutrient bioavailability by associating phosphate apatite with organic residues. The project focus on the interaction between these micro-organisms and plant rhizobia, through advanced biomolecular analyses. More specifically, the expression of genes encoding enzymes implicated in nutrient cycling was assessed by extracting genomic DNA from various residual biomasses. Outsourced sequencing provided complete genomic information from each DNA extract. Quantitative PCR (qPCR) was used to evaluate the expression levels of functional genes linked to phosphate solubilisation, in particular those associated with Penicillium bilaiae. Bioinformatics analysis using the Galaxy platform was used to identify genes such as phoD (alkaline phosphatase), nif (nitrogen fixation) key enzymatic functions in nutrient cycling. Particular attention was also paid to the presence and expression of genes encoding enzymes such as glucoxidases (pqq/gcd) and phosphatases, known for their roles in mineral solubilization and phosphorus release.Preliminary analysis has highlighted a correlation between the origin of the residual matter and the expression of genes associated with plant biostimulation. Assessment of the biofertilisers, based on gene expression profiling and germination tests on lettuce and watercress, identified the most promising formulations. These results open up new prospects for optimising functional combinations of microbial genes to improve nutrient uptake and support sustainable agriculture.

114 - Enzyme Gene Expression in Microbes for Sustainable Biofertilizer Development

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115 - Bridging the Gap: An Automated Biomanufacturing Platform for Point-of-Care Production of Patient-Specific Tissue Grafts

Matthew Adams[1], Adrien Perrin[1], Patrick Vermette[1]

[1]Université de Sherbrooke

The field of tissue engineering faces challenges in the fabrication of thick, functional tissues and scaling up production for clinical applications. Current methods struggle with maintaining cell viability and functionality within large, bioengineered tissue constructs due to insufficient vascularization and oxygen/nutrient diffusion. Moreover, manual processes dominate tissue manufacturing, leading to high costs, contamination risks, and limited scalability. To address these issues, we are developing a modular platform for 3D scaffold-based mammalian cell culture and an automated biomanufacturing unit for point-of-care tissue graft production.

Our proprietary 3D culture platform enables the creation of physiologically relevant structures with integrated macro-vascular networks, promoting cell survival and functionality. Using a composite approach, we achieve both mechanical integrity and enhanced cellular responses. This modular platform can seamlessly adapt to diverse geometries, sizes, and tissue types. Our first application focuses on autologous liver grafts, utilizing innovative tissue dissociation techniques to preserve native cellular populations and extracellular matrix properties, enhancing graft functionality and minimizing rejection risks.

The automated biomanufacturing unit integrates a robotic arm and a custom-designed perfusion bioreactor. The robotic arm (in-development) automates critical steps such as scaffold assembly, hydrogel injection, and graft handling, minimizing human intervention and contamination risks. The perfusion bioreactor ensures precise control of culture parameters (temperature, pH, oxygenation) while providing dynamic perfusion to enhance nutrient and oxygen delivery.

This technology holds transformative potential in regenerative medicine by enabling scalable, cost-effective and point-of-care production of personalized grafts. Ultimately, it aims to democratize access to life-saving tissue therapies while reducing reliance on donor organs.

116 - Investigation chimique des champignons du genre Lactarius du Nord du Québec

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Historiquement, les champignons ont été une source cruciale de composés bioactifs, dont les propriétés ont eu un impact direct sur certains aspects de la médecine. En effet, les champignons renferment diverses classes de molécules présentant un large éventail d'activités pharmacologiques. Or, malgré ce potentiel prometteur, la biodiversité des champignons du Canada est méconnue, de même que leur composition en métabolites spécialisés. Toutefois, il est connu que les champignons du genre *Lactarius* sont caractérisés par la production d'un liquide laiteux lorsqu'ils sont coupés, et ce liquide renferme des métabolites spécialisés diversifiés jouant un rôle défensif contre les micro-organismes, les insectes et les animaux. D'ailleurs, certaines substances bioactives ont été isolés des lactaires. Cependant, les lactaires de régions nordiques n'ont jamais fait l'objet d'investigations chimiques. Nous formulons l'hypothèse que les lactaires du Québec, en particulier des écosystèmes nordiques, produisent des métabolites secondaires inédits en raison des stress uniques auxquels ils sont exposés. Les travaux faits jusqu'à maintenant ont permis de valider une méthode d'extraction, de purification et de caractérisation des métabolites spécialisés des champignons à l'aide de *Lactarius repraesentaneus* recueilli à Kuujjuarapik comme modèle. Des extraits bruts ont été obtenus par macération en gradient de polarité, leur profil global évalué par HPLC-DAD/ELSD, HPLC-MS et GC-MS. Les composants ont été purifiés par MPLC et leur structure élucidée par différentes techniques spectrométriques et spectroscopiques. Ces travaux permettront de créer une banque de substances naturelles inédites pouvant être testées sur une myriade de cibles thérapeutiques à l'étude dans les divers laboratoires des membres de PROTEO.

117 - CHO cell production of a single enveloped VLP vaccine targeting SARS-CoV-2, Influenza A and RSV

Zalma Sanchez-Martinez[1], Yves Durocher[1], Matthew Stuible[2], Brian Cass[2], Simon Lord-Dufour[2], Rohan Mahimkar[1], Sabahudin Hrapovic[2], Anh Tran[2], Gregory De Crescenzo[3]

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The overlapping circulation of SARS-CoV-2, Influenza A virus (IAV), and respiratory syncytial virus (RSV) continues to strain global healthcare systems, particularly among vulnerable populations. A single vaccine targeting all three pathogens could streamline immunization efforts and enhance protection, while reducing manufacturing costs. We previously showed that CHO cell-derived enveloped virus-like particles (eVLPs) formed through expression of full-length SARS-CoV-2 spike (S) protein not only exhibit high S density and strong immunogenicity but also serve as a platform to co-display heterologous antigens such as IAV hemagglutinin (H1) and neuraminidase (N1). Here, we extend this approach by producing a trivalent eVLP candidate that simultaneously displays SARS-CoV-2 S, IAV H1, and RSV pre-fusogenic fusion (F) protein. Trivalent S/H1/F eVLPs were successfully produced in CHO cells using both transient and stable gene expression systems and purified via affinity chromatography. The presence of all three antigens on the same particles was confirmed by Western blot and immuno-electron microscopy. Their immunogenicity is currently being evaluated in vivo to assess their potential as a single vaccine against SARS-CoV-2, IAV, and RSV.

118 - The role of the apelinergic system in the treatment of muscular dystrophies

Soukayna Lekhel[1], Pierre-Luc Boudreault[1], Florian Bentzinger[1], Philippe Sarret[1]

[1]Université de Sherbrooke

Muscular dystrophies (MD) are a group of heterogenetic diseases, with no cure or treatment, that cause the severe degeneration of muscle tissues. The heterogeneity of the mutations causing the disease make the development of a genome targeted therapy difficult. The development of a mutation-independent treatment is therefore an interesting approach that could be applicable to a broader range of MD patients. Recently, it was discovered that the defects in the muscle tissue microvasculature are a common denominator in three preclinical models of MD. It was also observed that Apelin, an angiogenic peptide, can restore the microvasculature of these MD models and ameliorate skeletal muscle regeneration. Apelin-13 has a short in vivo half-life, therefore, different apelin analogs with better pharmacokinetics and pharmacodynamics have been screened based on endothelial cells (EC) proliferation. Two lead candidates have been identified; the linear peptide AM02-123 with a high affinity for the APJ receptor of 0.02 nM and an improved half-life of 50 minutes and the macrocycle KT03-69 with a lower affinity of 396 nM but with a significant effect probably due to its higher stability. To further confirm the efficacy of the analogs, in vitro assays on myofiber proliferation will be done and in vivo assays on MD mice will assess whether better PK/PD lead to a better treatment compared to apelin. Finally, BRET assays on EC will help us understand what signalling pathways are activated by apelin and its analogs to generate the proliferative effect that stimulates muscle fiber regeneration.

119 - Complete reaction coordinate multistate design of a Michaelase

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Designing enzymes for complex, multistep reactions remain a significant challenge in the field of enzymology. Traditional computational approaches often focus on stabilizing a single transition state on fixed-backbone structures, neglecting to account for all limiting steps in the reaction coordinate. To address this, we adopt a multistate design approach to design enzymes for the C-C bond-forming Michael addition. First, we used density functional theory (DFT) to propose a detailed mechanism construct theozymes - residues arranged optimally to stabilize transition states. An initial round of design integrated two such theozymes into crystallographically refined ensembles of natural aldolases, yielding variants with improved substrate binding. A second round of design focuses on incorporating the remainder of the constructed theozymes into the design cycle, with the aim of proportionally stabilizing the entire reaction coordinate. Beyond the generation of novel Michaelases, this work aims to establish a robust workflow for designing multi-step biocatalysts.

120 - Mise au point d'un biosenseur BRET pour l'étude de l'activité du transporteur aux prostaglandines

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Les prostaglandines sont des lipides bioactifs importants dans les processus physiologiques et pathophysiologiques comme l'inflammation, l'hypertension et le glaucome. Le transporteur aux prostaglandines (PGT) est un transporteur passif membre de la famille des Solute Carrier Transporters (SLC) responsable de la recapture des prostaglandines vers le cytoplasme pour leur dégradation. Le mécanisme moléculaire de transport des prostaglandines par PGT est peu connu et peu d'essais de transport sont disponibles pour aider à l'élucider. Ce projet vise à développer un nouvel essai pour mesurer l'activité de PGT en utilisant le transfert d'énergie de bioluminescence par résonance (BRET). À cette fin, la renilla luciferase (Rluc) et la protéine fluorescente verte (GFP) ont été insérées dans différents domaines de PGT. Vingt-quatre constructions chimériques ont été exprimées dans les cellules Sf9 et un signal BRET a été mesuré. Ce dernier a pu être modulé de manière dose-dépendante en présence de substrats et d'inhibiteurs de PGT. Il a été aussi possible de mesurer une variation BRET en insérant des mutations prédites pour stabiliser différentes conformations actives de PGT. Ensemble, ces données suggèrent que le BRET est une approche permettant le développement d'un biosenseur pour la mesure cellulaire de l'activité de PGT.

121 - Inhibition des interactions protéine-protéine : une nouvelle approche thérapeutique

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Les interactions protéine-protéine (IPP) jouent un rôle essentiel dans de nombreux processus biologiques, notamment la signalisation cellulaire et les réponses immunitaires, ce qui en fait des cibles de choix pour le développement de traitements pour diverses applications thérapeutiques. Avec environ 650 000 IPP identifiées contre seulement 20 000 gènes codants, leur modulation représente un enjeu thérapeutique majeur. Cependant, la nature souvent plane et dépourvue de cavités des interfaces d'IPP complique la conception de modulateurs efficaces. L'identification de "hot spots" - résidus contribuant de manière significative à l'énergie de liaison entre deux protéines - ouvre de nouvelles perspectives pour le développement de modulateurs d'IPP, offrant une alternative prometteuse aux chimiothèques classiques et pouvant améliorer significativement le taux de succès dans l'identification de modulateurs d'IPP.

Ce projet vise à concevoir une chimiothèque enrichie en "hot spots" afin d'accroître les chances d'identifier des inhibiteurs d'IPP. Notre approche repose sur trois axes principaux : (1) la conception et la synthèse de petites molécules, (2) le développement de macrocycles peptidiques, et (3) l'évaluation biochimique des composés. Les synthèses sont réalisées sur phase solide et l'évaluation de l'activité repose sur la technologie de bioluminescence NanoBit, permettant un suivi en temps réel des interactions protéiques dans des cellules vivantes.

Les premiers résultats obtenus avec la chimiothèque de petites molécules révèlent une grande diversité structurale et une correspondance des descripteurs moléculaires avec ceux d'inhibiteurs d'IPP répertoriés dans la littérature, confirmant ainsi le potentiel de cette approche pour les criblages à haut débit.

122 - Synonymous and single nucleotide changes facilitate the adaptation of a horizontally transferred gene

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Horizontal gene transfer events have been important in dictating species' evolutionary trajectory. Given that a horizontally acquired gene could come from another species, there are several reasons why the newly acquired gene may not be functional in the new host. To understand how and what could restore the functionality of such a gene, we mimic a horizontal gene transfer event in which the gene (AmtA), which is an ammonia transporter from the amoeba *D. discoideum*, is shifted to the yeast *S. cerevisiae*. We identify that AmtA in *S. cerevisiae* suffers from an inability to localize in the cell membrane, and hence, is non-functional in the new host. Screening of ~500 AmtA mutants leads to identifying 9 AmtA variants that restore low ammonia growth. Interestingly, the functionality is restored by acquiring only one to three SNPs in several gene parts. For the isolated mutants, we then quantify the evolvability and the pleiotropic and collateral effects. Our results show that (a) a single SNP can solve protein localization problems in horizontally transferred genes, (b) the success of a horizontal gene transfer event depends on the fitness landscape of the gene in the new host, and (c) a horizontally transferred gene could be more beneficial to a host than its native gene. With this, we provide insights into the mechanisms and effects of adaptation of horizontally transferred genes.

123 - Identification of novel Proteins by the integration of ribosome profiling data into a transcriptomic language model for deeper mass spectrometry analysis

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Background - The human transcriptome contains millions of open reading frames (ORFeome) potentially coding for currently unknown or unannotated proteins. Typical mass spectrometry (MS)-based proteomics pipelines use protein databases to identify known proteins in a biological sample. The detection of proteins encoded in the human ORFeome would require a customized database including millions of proteins. However, proteomics analyses cannot be performed with millions of proteins predicted from the human ORFeome because of unacceptable high false detection rates caused by large protein databases.

Goal - Identify the functional human ORFeome by excluding random ORFs to get a deeper detection of the proteome by detecting unannotated proteins in addition to previously annotated proteins.

Method - 78 Ribosome profiling (Ribo-seq) studies from 47 unique tissue or cell lines samples were reanalysed to curate a set of ORFs showing ribosomal activity. This set was added to the training set of TIS transformer, a transcriptomic language model used to predict functional ORF across the human transcriptome.

Results - The retraining and inference of the TIS transformer model allowed us to obtain a database containing 45 000 new unique protein sequences. In a preliminary experiment, our reanalysis of the 'Deep HeLa proteome' confirmed the expression of previously undetected proteins.

Conclusion - Combining Ribo-seq with a transcriptomic language model can sort out relevant ORFs for the discovery of unannotated proteins. The reanalysis of a bigger set of MS studies will be able to identify proteins with a high potential to be biologically relevant.

124 - Développement de petites molécules agonistes du récepteur de l'apéline

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Le récepteur APJ, un récepteur couplé aux protéines G (classe A), et ses ligands endogènes (apéline, Elabela) sont impliqués dans plusieurs conditions physiopathologiques pour lesquelles les besoins médicaux ne sont pas satisfaits (douleur, maladies cardiovasculaires, complications liées au diabète). Toutefois, les faibles biodisponibilités et demi-vies (*in vivo*, $t_{1/2}$ < 5 min) des ligands endogènes ainsi que leur effet hypotenseur limitent leur usage thérapeutique. Notre étude consiste à développer des petites molécules agonistes du récepteur APJ avec des propriétés pharmacologiques améliorées, telles que la biodisponibilité et la stabilité, afin de favoriser une administration par voie orale.

L'étude de la relation structure-activité réalisée a permis d'identifier des petites molécules innovantes se distinguant par une forte affinité pour le récepteur APJ (K_i 50 nM), un faible recrutement des β-arrestines (424 nM), et par conséquent une absence d'effet hypotenseur, une stabilité accrue ($t_{1/2}$ microsomale > 60 min) et une bonne biodisponibilité (70%). De plus, certains composés se sont avérés efficaces pour le traitement de la douleur aiguë, comme démontré par un test au formol effectué chez le rat. Cette série de petites molécules apélinergiques agonistes du récepteur APJ se démarquent donc par leur profil pharmacologique améliorée favorisant un traitement par voie orale et représentant une nouvelle avenue thérapeutique prometteuse.

125 - Transmission of trimethoprim resistance in pathogenic bacteria by type B dihydrofolate reductases (DfrB)

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Type B dihydrofolate reductases (DfrB) are potentially related to trimethoprim resistance in DfrB-expressing bacteria. Such bacteria have been found in contexts associated with human activity, such as agriculture and wastewater. However, because their prevalence is not regularly monitored, *dfrB* genes have only rarely been reported in human pathogens. Their contribution to the modern resistome is therefore difficult to estimate. Recent genomic studies by the Pelletier laboratory have identified 61 unique genomic sequences of *dfrB* genes, some of which are found in multi-resistant cassettes. Most of these were associated with pathogenic bacteria, in the vicinity of mobile genetic elements (MGEs).

Here, we aim to identify the events that have mobilized *dfrB* genes in clinically relevant pathogens. It is not yet understood whether *dfrB* genes diversified before or after their integration via MGEs in pathogenic bacteria. To address this question, we are conducting a comprehensive search of genomic and metagenomic databases for clinically significant DfrBs. In addition, we identify MGEs and other resistance genes in close proximity to *dfrB* genes as a key component of the research. The GC content of *dfrB* genes will be compared to that of their genomic environment to detect horizontal gene transfer events responsible for their integration into the resistome. This work will provide information on the mobilization of these enzymes in the resistome and will help assess their impact on trimethoprim efficacy, to better manage its use in clinical and veterinary settings.

126 - Synergie infectieuse entre SRAS-CoV-2 et Rhizopus oryzae

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Au cours de la pandémie de SRAS-CoV-2, une augmentation des cas de mucormycose a été observée. La mucormycose est une infection fongique sévère causée par des moisissures de la famille des mucorales. Les protéines CotH retrouvées à la surface du fungi permettent l'attachement et l'invasion de cellules humaine par une interaction avec la protéine BIP (GRP78). Cette chaperonne, résidente du réticulum endoplasmique, peut sous condition de stress être relocalisé à la surface cellulaire et permettre l'entrée de virus (ex : MERS, Ebola). BIP a aussi été suggéré comme pouvant lier le domaine de liaison au récepteur (RBD) de la protéine Spike (S) à la surface du SRAS-CoV-2 et permettre l'entrée de façon dépendante et indépendante au récepteur primaire ACE2. Le projet a été entamé à l'été 2023. Depuis nous avons établi un système d'expression de purification pour la protéine HaBiP et nous tentons d'établir un système d'expression pour la protéine CotH3. Par la suite, nous cristalliserons la protéine CotH dans le but de créer une structure des complexes, car ceux existants possèdent moins de 20 % d'identité. Pour finir, si l'ensemble des expériences fonctionne, nous aimerions mesurer les interactions entre les protéines : CotH-Spike et CotH-BiP. En somme, ce projet est d'une grande pertinence et est directement lié avec les thématiques scientifiques de PROTEO soit l'étude fondamentale du fonctionnement des protéines.

127 - RNF13 influence la voie endolysosomale par son interaction avec la petite GTPase Arl8B

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La protéine à domaine à doigt de zinc de type RING 13 (RNF13) est une ubiquitine E3 ligase transmembranaire, connue pour se localiser au réticulum endoplasmique, aux endosomes tardifs et aux lysosomes. Plusieurs évidences suggèrent qu'elle joue un rôle dans la dégradation, le trafic et la localisation des protéines, mais ses fonctions spécifiques et les mécanismes moléculaires sous-jacents restent mal compris. Les interactions protéine-protéine étant essentielles à la dynamique des processus cellulaire, notamment la signalisation cellulaire et le transport vésiculaire, il est essentiel d'identifier les partenaires d'interaction de RNF13 pour mieux comprendre ses fonctions. Dans ce contexte, nous avons cherché à identifier et à caractériser les complexes protéiques et les voies de signalisation associées à RNF13. À cette fin, Alphafold a été utilisé pour prédire des interactions possibles entre RNF13 et plus de 500 protéines candidates. Parmi elles, la petite GTPase Arl8B, appartenant à la famille des Arf (ADP-ribosylation factor), a été prédite pour interagir fortement avec RNF13. Expérimentalement, l'interaction entre RNF13 et Arl8B a été validée par co-immunoprécipitation. Par mutagénèse dirigée et co-immunoprécipitation, les résidus d'interaction critiques ont été caractérisés. La localisation intracellulaire des protéines a été validée par co-marquage immunofluorescent. Nos analyses fonctionnelles et essais d'imagerie en temps réel suggèrent que l'interaction dynamique entre RNF13 et Arl8B joue un rôle clé dans la régulation du trafic endolysosomal. En conclusion, cette étude met en évidence l'implication de RNF13 dans la fonction endolysosomale via son interaction avec Arl8B, apportant ainsi des informations clés sur la régulation de cette voie.

128 - Synthèse totale de dibenzofuranes naturels isolés du lichen nordique Stereocaulon paschale

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Le Nunavik, un vaste territoire situé dans le nord du Québec, demeure marginalement étudié phytochimiquement en dépit de ses conditions climatiques singulières. Celles-ci imposent un stress important aux organismes s'y développant, les poussant ainsi à élaborer des voies métaboliques uniques pour se défendre. Il y a donc un potentiel énorme de découvrir des substances naturelles inédites ayant des propriétés thérapeutiques intéressantes fabriquées par les organismes du Nunavik.

Notre groupe s'est récemment engagé dans l'étude phytochimique du lichen *Stereocaulon paschale* récolté à Umiuaq, au Nunavik. Cette étude a conduit à la découverte de 13 métabolites lichéniques, dont deux dibenzofuranes totalement inédits. Bien que ces composés aient généré d'intéressants résultats lors d'études biologiques préliminaires, leur très faible abondance dans la matrice naturelle limite grandement l'approfondissement de leurs propriétés.

Afin de faciliter l'accès à ces nouveaux produits naturels, ainsi qu'à certains analogues, nous développons actuellement une voie de synthèse permettant leur préparation. Notre stratégie implique deux étapes clés. Premièrement, le couplage de Suzuki-Miyaura est exploité pour la préparation du motif 2-arylphénol. Un bromure phénolique et un acide boronique sont ainsi joints par une liaison $C(sp^2)$-$C(sp^2)$. Ensuite, le motif tricyclique dibenzofurane est obtenu via une *O*-arylation intramoléculaire par activation C-H sélective. Après cette réaction de cycloéthérification, les composés ciblés seront finalement générés via une séquence réactionnelle de déprotection. Le potentiel des composés générés dans ce projetsera testé pour plusieurs cibles thérapeutiques, grâce à diverses collaborations. Cette présentation couvrira les concepts clés de ce projet de recherche, ainsi que les plus récents résultats obtenus.

130 - The MARCHF5 gene is dual-coding

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The annotation of human proteins on their parent nucleotide sequences started with the Human Genome Project (1990–2003). Initially, only a single protein was considered per mRNA transcript. More recently, ribosome profiling (Ribo-Seq) identified multiple active sites of translation's initiation and elongation per RNA molecule. Those newly identified open reading frames (ORFs) potentially give rise to polypeptide products distinct from the canonical protein in their amino acids' sequence. However, only a fraction of them is detected when searching publicly available mass spectra using a library that includes those polypeptides.The *MARCHF5* gene is transcribed into a mRNA containing an ORF coding for a canonical protein called MARCHF5 (membrane associated ring-CH-type finger 5), an E3 ubiquitin ligase of the mitochondrial outer membrane. Upstream and overlapping this ORF (but in a different frame) is another ORF translating to an unannotated protein that will be called uoMARCHF5. Although uoMARCHF5's expression is evident in Ribo-Seq at the translation level, evidence is poor in mass spectrometry at the protein level. This project aims to characterize uoMARCHF5: demonstrate its expression along with MARCHF5, determine its cellular localization, identify its interaction partners and its function.MARCHF5 and uoMARCHF5 are co-expressed on western blot and immunofluorescence in cells lines transiently transfected with a dual-coding construct (derived from the wild transcript with the addition of a different peptide tag for each protein). Tagged uoMARCHF5 localizes to the nucleus. The affinity purification-mass spectrometry (AP-MS) of tagged uoMARCHF5 identifies the enrichment of several proteins (mainly nuclear ones) compared to controls.

131 - Understanding Biased Signaling in C-Terminally Modified AngII Analogs through an Extensive SAR Approach: From Structural Insights to In Vivo Cardiovascular Effects

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The angiotensin II type 1 (AT1R) receptor belongs to the large family of G protein-coupled receptors (GPCRs), and plays a crucial role in regulating cardiovascular homeostasis by interacting with distinct intracellular effectors. The signaling pathways can be selectively modulated by AngII analogs, leading to preferred effector engagements and biased responses. Therefore, biased AT1R ligands represent a promising therapeutic strategy, although the molecular determinants of functional selectivity are poorly defined, and translating these effects in vivo remains challenging.

The project aims to design and synthesize C-terminal AngII analogs, using selected natural or unnatural amino acids, to unravel the relationship between structure, signaling bias and biological activity. Forty-eight novel analogs with substitution at Phe^8, a pharmacophore essential for AT1R signaling bias, were synthesized and evaluated via BRET assays for their ability to engage Gαq and β-arrestin2. The analogs were compared to the β-arrestin-biased TRV027 ([Sar^1, D-Ala^8]AngII), which does not engage $G\alpha_q$ and AngII.Remarkably, two structurally related analogs, **12** ([Tyr(Bzl)^8]AngII) and **11** ([Tyr(Me)^8]AngII), demonstrated similar β-arrestin2 recruitment, however showed very distinct effects on $G\alpha_q$. **12** has a partial agonist activity on the $G\alpha_q$ pathway, whereas **11** showed full activity on both. **12** minimally increased blood pressure in normotensive rats compared to AngII and **11**, additionally, **12** was the only analog that increased left ventricular ejection fractions.These findings demonstrate the potential of C-terminal modifications to differentially engage $G\alpha_q$ and β-arrestin and offer insight for translating cellular signaling into in vivo responses for leveraging next-gen AT1R therapies.

133 - Synthèse totale de l'Anhydrosakisacaulon A et de ses dérivés isolés du lichen nordique Stereocaulon pascale

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Le Nunavik constitue un territoire encore largement inexploré en ce qui concerne ses produits naturels d'origine végétale, bien qu'il présente un fort potentiel pour la découverte de molécules bioactives. Les conditions environnementales extrêmes du climat nordique — froid intense, rayonnement UV élevé, prédation — favorisent l'adaptation biochimique des organismes qui y résident, menant à la production de métabolites spécialisés uniques. Dans le cadre d'une étude phytochimique menée sur un échantillon de lichen *Stereocaulon paschale* provenant du Nunavik, treize métabolites spécialisés ont été isolés et caractérisés. Parmi eux figurent le pseudodépsidone Anhydrosakisacaulon A (**1**) et trois de ses dérivés naturels : Sakisacaulon A (**2**), sa forme méthylée (**3**) et sa forme estérifiée (**4**). Anhydrosakisacaulon A a démontré une activité antibactérienne notable vis-à-vis de *Porphyromonas gingivalis* et de *Streptococcus mutans*, en plus d'inhiber significativement l'activation du facteur NF-κB et la sécrétion de TNF-α. La faible abondance naturelle de ce métabolite dans *Stereocaulon paschale* limite toutefois l'étude approfondie de ses propriétés biologiques, nécessitant le recours à la synthèse chimique pour en produire en quantité suffisante. Le présent projet rapporte nos avancées vers la première synthèse totale de l'Anhydrosakisacaulon A et de ses dérivés. La stratégie repose sur un couplage de Sonogashira, suivi d'une cyclisation intramoléculaire et d'un couplage d'Ullmann pour la formation du squelette pseudodépsidone de l'Anhydrosakisacaulon A. L'Anhydrosakisacaulon A servira ensuite de précurseur à la préparation de ses trois analogues (**2**-**4**). Au-delà de ses applications potentielles en pharmacologie, ces travaux mettent en lumière la richesse moléculaire encore largement inexploitée des plantes, lichens et champignons des écosystèmes arctiques.

134 - Analyse protéomique de l'impacte du déficite autophagique dans l'intestin de drosophile.

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Introduction. L'intestin, essentiel pour l'absorption des nutriments et la protection contre les pathogènes, voit son homéostasie perturbée dans les Maladies Intestinales Chroniques Inflammatoires (MICIs), comme la maladie de Crohn. La mutation T300A du gène Atg16, fréquente chez ces patients, perturbe le flux autophagique, et est responsable de la mauvaise prise en charge des protéines et bactéries à éliminer. Les données préliminaires du laboratoire montrent que la perte d'Atg16, dans les entérocytes de drosophiles, affecte l'homéostasie intestinale. Notre hypothèse est que la perte d'Atg16 dérégule des mécanismes impliqués dans le maintien de l'homéostasie intestinale. **Objectifs.** Dans cette étude, deux objectifs sont explorés : 1) l'analyse du protéome intestinal des mouches sauvages et mutantes (*KO* et *Atg16 T300A*) pour identifier les protéines dont l'expression est dérégulée, et 2) la caractérisation du phénotype des mouches *Atg16 T300A* en conditions normales et de stress. **Méthodes et résultats.** Des extraits protéiques intestinaux de drosophiles sauvages ou mutantes âgées de 5, 15 et 30 jours ont été analysés par spectrométrie de masse. Les résultats suggèrent que la dérégulation de l'expression protéique augmente avec l'âge et que certaines voies métaboliques semblent être affectées. Les individus KO et Atg16 T300A présentent un flux autophagique réduit comparé aux sauvages, confirmé également par l'observation de l'accumulation de la protéine p62/Ref(2)p par immunobuvardage. **Conclusion.** L'étude de cette mutation chez la drosophile permettra de mieux comprendre son impact sur l'homéostasie intestinale. De plus la possibilité d'appliquer des stress aux mouches fait de cet organisme un bon modèle pour l'étude des MICIs.

135 - Development of an avian H5N1 flu vaccine based on recombinant H5 antigen expressed in CHO cells

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H5N1 is a highly pathogenic avian influenza virus that is responsible of the highly infectious respiratory illness called “bird flu”. Since 1996, the virus has expanded across the globe spilling into different animal groups such as poultry, wild and domestic mammals, as well as over 900 humans including one case in BC, Canada (November 2024). Since 2022 an increase of the outbreak has been reported, causing the death of millions of birds and leading to the death of approximately 20 million hens as of January 2025 and one human death in The United States.

The virus is constantly evolving, and it has diversified and accumulated mutations throughout the years. Despite no transmission between humans having been reported yet, there are concerns that the virus is only a few mutations away from being able to transmit between humans, leading to a new potential pandemic.

Most of the current available H5N1 vaccines are manufactured through a lengthy egg-based production process and the protection they provide is often based on outdated H5N1 strains. An alternative option for flu vaccines is the use of recombinantly produced antigens, such as hemagglutinin. Since CHO is the industry’s most used cell factory to produce r-proteins with a glycosylation similar to humans, their use is more and more considered for vaccine manufacturing. In my presentation, I will detail our strategy to produce a H5N1 recombinant hemagglutinin (H5) antigen in CHO cells that is able to generate immunogenicity in vivo, as well as improving its manufacturability.

136 - Coupling Proximity Biotinylation with Genomic Targeting to Characterize Locus-Specific Changes in Chromatin Environments

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Regulating gene expression involves significant changes in the chromatin environment at the locus level, especially at regulatory sequences. However, their modulation following pharmacological treatments or pathological conditions remain mostly undetermined. Here, we report versatile locus-specific proteomics tools to address this knowledge gap, which combine the targeting ability of the CRISPR/Cas9 system and the protein-labeling capability of the highly reactive biotin ligases TurboID (in CasTurbo) and UltraID (in CasUltra). CasTurbo and CasUltra enabled rapid chromatin protein labeling at repetitive sequences like centromeres and telomeres, as well as nonamplified genes. We applied CasUltra to A375 melanoma cell lines to decipher the protein environment of the *MYC* promoter and characterize the molecular effects of the bromodomain inhibitor JQ1, which targets bromodomain and extra-terminal (BET) proteins that regulate *MYC* expression. We quantified the consequences of BET protein displacement from the *MYC* promoter and found that it was associated with a considerable reorganization of the chromatin composition. Additionally, BET protein retention at the *MYC* promoter was consistent with a model of increased JQ1 resistance. Thus, through the combination of proximity biotinylation and CRISPR/Cas9 genomic targeting, CasTurbo and CasUltra have successfully demonstrated their utility in profiling the proteome associated with a genomic locus in living cells.