CRBS Centre de Recherche en Biologie Structurale

4ème Colloque Annuel du CRBS
4th Annual CRBS Symposium

7 Novembre 2022 / November 7, 2022

Nouvelle Résidence, Université McGill
McGill New Residence Hall
3625 Avenue du Parc, Montréal, Qc, H2X3P8

BRUKER

cytiva

SFR SYSTEMS FOR RESEARCH

4ème Colloque Annuel du CRBS – 7 novembre 2022
4th Annual CRBS Symposium - November 7, 2022

8h00 Ouverture du Bureau d'Inscription / Registration Desk Opens
Entrée Salle de Bal / Ballroom Lobby

8h00-9h00 Installation des Affiches / Poster Set-up
Café & Croissants avec les Exposants / Coffee & Croissants with Exhibitors
Salle de Bal / Ballroom

9h00-9h10 Mots d'Ouverture / Opening Words – Martin Schmeing, *McGill University*

Salle de Bal / Ballroom A — Séance 1 / Session 1

Sous la Présidence de / Session Chair: Rodrigo Reyes, *McGill University*

9h10-10h00 Alessandro Costa, *Francis Crick Institute, London, UK*
Activation of an origin of replication visualised by cryo-EM

10h00-10h30 Siavash Vahidi, *University of Guelph, Canada*
Mechanisms of allosteric regulation of *M. tuberculosis* proteasome function

10h30-10h45 Lauralicia Sacre, *McGill University*, Prix Annuel Excellence #1
Understanding the role of Pol II-β-clamp interactions in polymerase switching during TLS

10h45-11h15 Pause Santé avec les Exposants & Session d'Affiche / Health Break with Exhibitors & Poster Session

Salle de Bal / Ballroom A — Séance 2 / Session 2

Sous la Présidence de / Session Chair: Chris Thibodeaux, *McGill University*

11h15-12h05 Maria Spies, *Carver College of Medicine, University of Iowa, USA*
Replicate, repair or recombine? Conformational dynamics of the replication protein A

12h05-12h20 Annie Shao, *McGill University*, Prix Annuel Excellence #2
Biochemical and structural characterization of *Pseudomonas aeruginosa* cyclopropane fatty acid synthase

12h20-13h45 Diner avec les Exposants & Session d'Affiche / Lunch with Exhibitors & Poster Session

Salle de Bal / Ballroom A — Séance 3 / Session 3

Sous la Présidence de / Session Chair: Natalie Zeytuni, *McGill University*

13h45-14h35 David Schriemer, *University of Calgary, Canada*
Structural proteomics: tools for a post-AlphaFold2 world

14h35-15h05 Jérôme Waldispühl, *McGill University, Canada*
Non-canonical base pair interactions improve the scalability and accuracy of the prediction and analysis of RNA 3D structures

15h05-15h20 **Pause / Break**

Salle de Bal / Ballroom A **Séance 4 / Session 4**

Sous la Présidence de / Session Chair: Garvit Bhatt, *McGill University*

15h20-15h35 **Dominic Arpin, *McGill University***
Cooperativity of ribosome assembly GTPases in the maturation of the bacterial large subunit

15h35-15h50 **Michael Wozny, *McGill University***
Cryo-electron microscopy of poliovirus VP4

15h50-16h05 **Hossein Davarinejad, *University of Ottawa***
The histone H3.1 variant regulates TONSOKU-mediated DNA repair during replication

16h05-16h20 **Angelos Pistofidis, *McGill University***
Structural and biochemical studies of the chemical mechanism of condensation in nonribosomal peptide synthetases (NRPSs)

Salle du Parc / du Parc Room **Séance 5 / Session 5**

Sous la Présidence de / Session Chair: Avrin Ghanaeian Miandoab, *McGill University*

15h20-15h35 **Tara Shomali, *McGill University***
Characterization of small molecule inhibitors of PINK1

15h35-15h50 **Meriem Beniani, *INRS-Armand-Frappier***
Discovery of a new virulence factor in *H. pylori*

15h50-16h05 **Daniel Moses, *McGill University***
The discovery and characterization of two novel structural motifs on the C-terminal domain of kinetoplastid RNA editing ligases

16h05-16h20 **Léonie Frigon, Université de Montréal**
Structural and functional analysis of the human PARP4 catalytic domain

Salle de Bal / Ballroom A and B **Séance 6 / Session 6**

16h20-18h00 **Salon des Affiches & Salon des Exposants / Poster Pub & Exhibition Area Open**
Présentation des Prix & Remerciements / Presentation of Awards & Closing Words

Conférencier / Speaker

Salle de Bal / Ballroom A Session 1

9h10 – 10h00

Alessandro Costa

Francis Crick Institute, London, UK

***Seminar*: Activation of an origin of replication visualised by cryo-EM**

Activation of eukaryotic origins of replication occurs in temporally separated steps to ensure that chromosomes are copied only once per cell cycle. First, the MCM helicase is loaded onto duplex DNA as an inactive double hexamer. The loaded form of the MCM is selectively phosphorylated by the Dbf4-dependent kinase. This event facilitates the assembly of two Cdc45-MCM-GINS (CMG) holo-helicases that melt the double helix, exposing the single-stranded DNA template for the replicative polymerases. We used in vitro reconstitution combined with (time-resolved) cryo-EM approaches to characterise the processes of MCM loading onto DNA, CMG formation and activation. Our results explain the molecular basis for the establishment of bidirectional replication in eukaryotic cells.

***Biography*:**

Alessandro's research focuses on DNA replication and genome maintenance. The lab combines biochemical reconstitution and single-particle cryo-EM to understand complex cellular pathways. Recent work involved time-resolved imaging to establish the mechanism for loading the eukaryotic replicative helicase onto DNA and the mechanism for opening the double helix. Alessandro's lab also develops methods to optimise protein-DNA complex assembly and analysis by cryo-EM.

Alessandro Costa obtained a "Laurea" degree in Biotechnology at the University of Padua (2004) and a PhD in Structural Biology at Imperial College London (2008). He trained as a postdoc at the University of Oxford and later as an EMBO fellow at the University of California, Berkeley. In 2012 Alessandro established his own group at the London Research Institute Clare Hall Laboratories, Cancer Research UK. In 2015 he joined the Francis Crick Institute as a Group Leader and was awarded tenure in 2018.

Conférencier / Speaker

Salle de Bal / Ballroom A Session 1

10h00 – 10h30

Siavash Vahidi

University of Guelph, Canada

***Seminar*: Mechanisms of allosteric regulation of *M. tuberculosis* proteasome function**

Tuberculosis (TB) is the leading cause of mortality from a single infectious agent worldwide. The lack of an effective vaccine and the continued spread of multidrug-resistant *Mycobacterium tuberculosis* (MDR)-*Mtb* threaten to undermine the progress made against TB. *Mtb* proteasome-mediated protein degradation is essential for pathogenesis and therefore the proteasome system represents a novel therapeutic target for developing antibacterials against MDR-*Mtb*. Proteasomes are large, oligomeric complexes that selectively degrade damaged/misfolded proteins using two main components: the 20S core particle (20S CP) which carries out proteolytic function and regulatory particles (RPs) that translocate substrates into the 20S CP for destruction. Fourteen catalytic sites are sequestered within the degradation chamber of the 20S CP, and substrate access to this chamber is restricted via narrow, gated pores. The binding of RPs mediates conformational changes in the pores and enables the proteolysis of target substrates. My research program aims to discover new mechanisms of functional modulation of the *Mtb* proteasome machinery that operate based on allosteric (long-range) pathways unique to *Mtb*. During my talk, I will highlight how the combination of NMR- and mass spectrometry-based structural biology and functional assays reveals mechanisms via which conformational dynamics and allostery control the function of the *Mtb* 20S CP in response to interactions with RPs. Identifying allosteric hotspots will enable the screening of small-molecules that specifically target *Mtb* proteasome away from its active site and chart a novel path towards drug development against MDR-*Mtb*.

***Biography*:**

As a teenager in high school, my main goal was… to play basketball in the NBA! A combination of a knee injury and inadequate basketball skills made me re-evaluate my life choices. My interest in biophysical mass spectrometry lured me to the laboratory of Prof. Lars Konermann at UWO for a PhD, where I developed and applied biomolecular mass spectrometry methods to study protein folding, structure, and dynamics. After a poor start, I managed to be productive and won the Paul de Mayo award for the best PhD thesis in the department. My fascination with structural biology set me on a path for a career in academia and to a CIHR postdoctoral fellowship with Prof. Lewis Kay at the University of Toronto and the SickKids Hospital. Through my postdoctoral research, I broadened my scientific horizons and learned to use biomolecular nuclear magnetic resonance (NMR) spectroscopy on large protein system. I also worked closely with the Rubinstein group at SickKids to complement our studies with electron cryomicroscopy. In 2020, I started my own lab at the Department of Molecular and Cellular Biology, University of Guelph. My independent research program takes advantage of an integrative approach in structural biology by combining various methods, including mass spectrometry and NMR, which can provide a detailed picture of the functions and dysfunctions of mega Dalton-sized biomolecular machines in health and disease.

Prix Annuel d'Excellence du FRQ-S

Salle de Bal / Ballroom A Session 1

10h30 – 10h45

***Seminar*: Understanding the role of Pol II-β-clamp interactions in polymerase switching during TLS**

Lauralicia Sacre[1], Michelle Scotland[2], Monica Pillon[3], Mark Sutton[2], Alba Guarné[1]

[1]Department of Biochemistry and Centre de Recherche en Biologie Structurale, McGill University, Montreal, QC, Canada, [2]Department of Biochemistry, University Buffalo, NY, USA, [3]Department of Biochemistry, Baylor College of Medicine, TX, USA.

DNA damage blocks high-fidelity polymerases and stalls replication forks leading to lethal double strand breaks. Translesion DNA synthesis (TLS) bypasses DNA lesions using specialized polymerases (TLS pols). This requires a highly regulated replacement of the high-fidelity replicative pol with the TLS pol, coordinated via interactions between the polymerase and β-clamp, and between the two polymerases (Pol-Pol). Pol II, one of three TLS pols in *E. coli*, interacts with the β-clamp through a clamp-binding motif (CBM) on its C-terminus and a hydrophobic cleft present on each β-clamp protomer. Previous surface plasmon resonance (SPR) assays done by our collaborators have suggested that Pol II binds two surfaces of the β-clamp. Moreover, the β cleft/loop II regions seem to be required for proper Pol II function. To understand the molecular mechanism of polymerase switching, we aim to solve a series of high-resolution structures of the Pol II–β-clamp–DNA complex using cryo-electron microscopy (cryo-EM). We have obtained a cryo-EM map of Pol II bound to the clamp in the presence and absence of DNA. From the Pol II–β-clamp model, two contacts are observed: (1) Pol II CBM interacting with the hydrophobic cleft and (2) Pol II loop containing residues 732-736 contacting loop 2 of the β-clamp. In our Pol II–β-clamp-DNA model, Pol II maintains the interaction between the CBM and hydrophobic cleft. Additionally, we observed a novel contact between Pol II residues 671-675 and residues 296-299 in the β-clamp. We are currently validating these interactions with SPR. In the future, we will also include additional lesion bypass polymerases to further explore Pol–Pol interactions. The structural characterization of Pol II–β-clamp–Pol complexes on DNA will provide insights on how their interactions coordinate switching between high-fidelity and TLS pols during DNA damage.

Conférencier / Speaker

Salle de Bal / Ballroom A Session 2

11h15 – 12h05

Maria Spies

Carver College of Medicine, University of Iowa, USA

***Seminar*: Replicate, repair or recombine? Conformational dynamics of the replication protein A**

Replication protein A (RPA) plays a key role in most cellular DNA metabolic events. Virtually every single-strand DNA (ssDNA) intermediate in the cell is rapidly bound by RPA which then melts secondary DNA structures, activates the DNA damage response, and hands off ssDNA to appropriate downstream players. Six OB-folds commonly referred to as DNA binding domains (DBD-A, B, C, D, E and F) in RPA, allow for a dynamic protein-ssDNA interaction, whereby a macroscopically bound RPA cycles between high affinity and low affinity binding modes and can be displaced by lower affinity downstream proteins. To visualize and quantify the microscopic RPA conformational dynamics in the context of macroscopically bound protein, we constructed fluorescent versions of *Saccharomyces cerevisiae* and human RPA site specifically labeled with MB543, an environmentally sensitive dye. Using single-molecule total internal reflection fluorescence microscopy (smTIRFM) we showed that the RPA-ssDNA complexes are highly dynamic. Both DBD-A and DBD-D rapidly bind to and dissociate from ssDNA, while RPA as a whole remains bound . In the yeast system, the recombination mediator protein Rad52 selectively modulates the dynamics of DBD-D to allow access by the Rad51 recombinase (Pokhrel, Caldwell et al 2019 NSMB). Recently, using bulk FRET (Sharma et al. 2022 Blood) and smTIRFM (unpublished), we observed DNA binding, G-quadruplex melting, and conformational dynamics of the human gain-of-function RPA mutant associated with short telomere syndrome. I will discuss our working model of how hnRNPA1 and TERRA RNA regulate exchange of RPA with telomeric ssDNA binding protein POT1 through the formation of ternary RPA-DNA-hnRNPA1 complex, and how enhanced interaction between RPA DBD-A and ssDNA interferes with this exchange at human telomeres.

***Biography*:**

Maria Spies is a Professor of Biochemistry and Molecular Biology at the University of Iowa Carver College of Medicine, and has a secondary appointment at the Department of Radiation Oncology. She is also an Associate Director of the University of Iowa Center for the Bioprocessing and Biocatalysis where she heads a graduate training program in biotechnology. A graduate of the St. Petersburg Polytechnic University (MS in Physics/Biophysics, Red Diploma with Distinction, which is an equivalent of *Cum Laude*, 1996), Maria Spies received her Ph.D. in 2000 from Osaka University (Japan) and completed a postdoctoral training (2000-2005) working with Prof. Stephen Kowalczykowski (UC Davis, USA), where she was the first to reconstitute, at the single-molecule level, the first steps in bacterial homologous recombination, namely DNA end resection and recognition of a recombination hot spot by RecBCD helicase/nuclease. Professor Spies is an internationally recognized single-molecule biochemist. Her research focuses on the molecular mechanisms underlying genome stability. Her lab utilizes a broad spectrum of techniques from biochemical reconstitutions of DNA recombination, repair and replication reactions to structural and single-molecule analyses of the proteins and enzymes coordinating these reactions. The ability to understand the coordinated and dynamic nucleoprotein transactions critical for high fidelity DNA repair and replication, and the mechanisms that funnel “normal” DNA repair intermediates into “rogue” processes that destabilize the genome is translated in the Spies’ lab into development of new therapeutic interventions, which will target recombination deficient cancers, as well as cancers “addicted” to homology-directed DNA repair. Among notable

honors, Dr. Spies was a recipient of the Howard Hughes Medical Institute (HHMI) Early Career Scientist Award (2009), Margaret Oakley Dayhoff Award in Biophysics from the Biophysical Society (2010), and the University of Iowa Distinguished Scholar Award (2020); she was a Finalist for the 2015 Iowa Women of Innovation Award.

The work in the Spies' lab is supported by grants from the US National Institutes of Health, National Science Foundation and Department of Defense. Her previous funding included grants from American Cancer Society, Howard Hughes Medical Institute and NASA. She has published ~70 peer-reviewed papers, edited five books, and is serving on the editorial boards of *Journal of Biological Chemistry*, *Nucleic Acids Research*, and *eLife*. She is currently serving as a Chair of the US National Institutes of Health grant review panel in Macromolecular Structure and Function B.

Prix Annuel d'Excellence du FRQ-S

Salle de Bal / Ballroom A Session 2

12h05 – 12h20

***Seminar*: Biochemical and structural characterization of Pseudomonas aeruginosa cyclopropane fatty acid synthase**

Annie Shao[1]

[1]McGill University

Pseudomonas aeruginosa (PA) is a gram-negative opportunistic pathogen that is notorious for the acquisition of multidrug resistance (MDR). A major contributor to drug resistance in PA is the outer membrane (OM), which presents a low permeability barrier to many drugs. An accumulation of cyclopropane fatty acids (CFAs) in the bacterial envelope is often observed in gram-negative bacteria as they enter stationary phase and form biofilms. CFAs provide chemical and physical stability to the bacterial membrane and contribute to drug resistance through poorly-understood mechanisms. Cyclopropane fatty acid synthase (CFAS) is the cytosolic enzyme responsible for the biosynthesis CFAs. This enzyme installs cyclopropane rings into the unsaturated acyl chains of inner membrane (IM) phospholipids using *S*-adenosyl-l-methionine (SAM) as a methylene donor. The enzyme-catalyzed reaction is interesting in that the soluble CFAS enzyme must associate with the membrane surface where it cyclopropanates a very hydrophobic acyl chain using a very hydrophilic SAM co-substrate. To date, no biochemical or structural analysis has been performed on PA-CFAS, and protein-membrane interactions are poorly understood in general. Thus, we have initiated a project to investigate the *in vitro* activity of PA-CFAS on phospholipid vesicles and to characterize the conformational dynamics of the enzyme using hydrogen-deuterium exchange mass spectrometry (HDX-MS). The structural and functional insights into PA-CFAS gained from these studies could eventually facilitate future drug discovery efforts to treat PA-related infections.

Conférencier / Speaker

Salle de Bal / Ballroom A Session 3

13h45 – 14h35

David Schriemer

University of Calgary, Canada

***Seminar*: Structural proteomics: tools for a post-AlphaFold2 world**

Structural proteomics uses protein chemistries and mass spectrometry to generate structure/function models of large multi-protein assemblies, with the goal of achieving structure analysis for proteins and assemblies of any scale. Prior to AF2, the concept involved building protein structures into larger complexes, through optimization routines guided by empirical data from several mass spectrometry methods. If these methods deliver sufficient spatial information, molecular modeling can generate accurate representations of the whole. Scaling this approach was always hobbled by an incomplete database of structures, requiring cryoEM, X-ray diffraction, SAXS etc. of the building blocks and various stages of assembly. With AI-based fold prediction technology, the potential exists for a more accurate and streamlined strategy using little more than crosslinking mass spectrometry (XL-MS) as a source of data. In this lecture I will discuss the promise (and challenges) inherent to crosslinker-based modeling with XL-MS data. Progress in the installation and detection of crosslinks will be described, illustrated with structure modeling examples, and I will suggest a new path towards accurately modeling protein assemblies at any scale.

***Biography*:**

Dave Schriemer is an academic and entrepreneur. He graduated with degrees in organic chemistry and bioanalytical chemistry and received further training in biochemistry during postdoctoral work. He was the founder of INH Technologies Inc., a biotech startup in Calgary, and served as Research Director in MDS Proteomics Inc. before joining the Department of Biochemistry & Molecular Biology at the University of Calgary in 2001. Dr. Schriemer's laboratory maintains a basic research program in integrative structural biology of complex systems, incorporating novel proteomics technologies, reagent development and computational methods. Dr. Schriemer has been a Canada Research Chair in Chemical Biology and a Senior Scholar of the Alberta Heritage Foundation for Medical Research. He served as the director of the SAMS Centre for Proteomics until 2017, is currently Chief Science Officer and Director of Nepetx LLC, and is establishing a scientific software development company.

Conférencier / Speaker

Salle de Bal / Ballroom A Session 3

14h35 – 15h05

Jérôme Waldispühl

McGill University, Canada

***Seminar*: Non-canonical base pair interactions improve the scalability and accuracy of the prediction and analysis of RNA 3D structures**

A vast and complex network of base interactions stabilizes the 3D architecture of RNAs. Beyond the canonical Watson-Crick and Wobble base pairs, each pair of nucleotides can interact in up to 12 different ways. The frequency of each type of base pair varies a lot, but in most cases their occurrence is essential to shape the local or global geometry of structured RNAs. Non-canonical base pairs are primarily found within or between unpaired regions of the secondary structure commonly refereed as loops. Their concentration in loops creates sophisticated base interaction patterns that are representative of the 3D structure supported by this network.In this talk, we show that working with a graphical model based on the set of canonical and non-canonical base pairs offers novel opportunities to develop efficient algorithms for predicting and analyzing physical and biochemical properties of RNA 3D structures. We present applications of this framework to (i) the automated discovery of structural motifs in databases, (ii) the prediction of small molecules binding RNAs, and (ii) the prediction of the 3D structure of large RNAs. These results suggest novel avenues to study of the evolution of RNAs or accelerate RNA drug discovery.

***Biography*:**

Jérôme Waldispühl is an associate professor of Computer Science at McGill University. He holds a PhD from École Polytechnique (France), and previously was an instructor in Applied Mathematics at MIT (2006-2009). Jérôme conducts research in RNA structural bioinformatics and cheminformatics. He pioneered the use of video games to engage the public in genomic research with Phylo (2010), Colony B (2016), Borderlands Science (2020) and Project Discovery Phase 3 (2020), which he presented at the White House OSTP (2013), Québec Parliament (2016) and French Academy of Science (2018). He is a recipient of the Tomlinson Scientist Award and the Fessenden Professorship in Science Innovation.

Communications Orales / Short Talks

Salle de Bal / Ballroom A Session 4

15h20-15h35 Cooperativity of ribosome assembly GTPases in the maturation of the bacterial large subunit

Dominic Arpin[1], Amal Seffouh[1], Robert Britton[2], Joaquin Ortega[1]

[1]Department of Anatomy and Cell Biology, McGill University, [2]Department of Molecular Virology and Microbiology, Baylor College of Medicine

The assembly of the bacterial ribosome is a complex process involving a broad array of factors including GTPases. RbgA, YphC and YsxC are three highly conserved essential GTPases implicated in the assembly of the large subunit. To investigate their roles, the genes were placed under the control of an inducible promoter. Large subunit assembly intermediates accumulate in depletion conditions, and characterization of the intermediates resulting from RbgA ($45S_{RbgA}$), YphC ($45S_{YphC}$) or YsxC ($44.5S_{YsxC}$) depletion revealed that they are strikingly similar. Moreover, studies in mitoribosomes have shown that the homologs of the three proteins are simultaneously bound to assembly intermediates. Using a combination of biochemical and biophysical approaches, our research aims to elucidate the molecular function and cooperativity of RbgA, YphC and YsxC during the assembly of the ribosomal large subunit. Microscale thermophoresis (MST) experiments shows that all three enzymes irrespectively bind the assembly intermediates with high affinity (K_D ~ 100 nM). Furthermore, RbgA and YphC show positive cooperativity in their binding i.e., when one enzyme is bound, the affinity and stability of the other is significantly increased. According to the results obtained by MST, a complex was formed with the $45S_{RbgA}$ with RbgA and YphC bound and analyzed by Cryo-EM. Initial results show that RbgA and YphC respectively bind in the P and E site of the functional core and that they form stabilizing interactions between each other. Thus, RbgA and YphC seem to orchestrate the maturation of the functional core as well as the recruitment and binding of other essential factors. These results provide new insight into the network of assembly factors required for proper stepwise maturation of the bacterial ribosome and allow us to propose that they are potential key targets for the rational development of a new generation of antibiotics aiming to hamper ribosomal assembly in bacteria.

15h35-15h50 Cryo-electron microscopy of poliovirus VP4

Michael Wozny[1], Mike Strauss[1]

[1]McGill University

Viral infection and entry into a cell require dedicated machinery for the hydrophilic viral genome to exit its protein shell (capsid) and pass through the hydrophobic cellular membrane. In picornaviruses, including polio virus, this process of viral genome uncoating likely depends on pore formation within the cellular membrane by a complex of viral proteins including VP4. Receptor mediated change in the viral structure causes VP4 to rearrange from the inner surface of the viral capsid and be released to partition to the cellular membrane. VP4 is known to perforate synthetic liposomes, strongly suggesting its role in pore formation. Still, the molecular mechanisms by which VP4 binds RNA and chaperones the viral RNA genome through the hydrophobic membrane remains poorly understood. Here we describe single-particle cryo-electron microscopy (cryo-EM) VP4 polymers for structural determination of VP4. We further describe cryo-electron tomography (cryo-ET) and subtomogram averaging of intact polio virus bound to single stranded RNA in complex with VP4, which we refer to as polio-on-a-stick.

15h50-16h05 The histone H3.1 variant regulates TONSOKU-mediated DNA repair during replication

Hossein Davarinejad[1], Yi-Chun Huang[2], Benoit Mermaz[2], Chantal LeBlanc[2], Axel Poulet[2], Geoffrey Thomson[2], Valentin Joly[2], Marcelo Muñoz[3], Alexis Arvanitis-Vigneault[1], Devisree Valsakumar[4], Gonzalo Villarino[2], Alex Ross[3], Benjamin Rotstein[5], Emilio Alarcon[3], Joseph Brunzelle[6], Philipp Voigt[4], Jie Dong[2], Jean-François Couture[1], Yannick Jacob[2]

[1]Ottawa Institute of Systems Biology, Department of Biochemistry, Microbiology and Immunology, Faculty of Medicine, University of Ottawa, Ottawa, Ontario K1H 8M5, Canada., [2]Department of Molecular, Cellular and Developmental Biology, Yale University, New Haven, CT 06511, USA., [3]BEaTS Research Laboratory, Division of Cardiac Surgery, University of Ottawa Heart Institute, Ottawa, Ontario K1Y 4W7, Canada., [4]Wellcome Centre for Cell Biology, School of Biological Sciences, University of Edinburgh, Edinburgh EH9 3BF, UK., [5]Department of Biochemistry, Microbiology, and Immunology, Faculty of Medicine, University of Ottawa, Ottawa, Ontario K1H 8M5, Canada., [6]Department of Molecular Pharmacology and Biological Chemistry, Feinberg School of Medicine, Northwestern University, Chicago, IL 60611, USA.

The tail of replication-dependent histone H3.1 varies from that of replication-independent H3.3 at the amino acid located at position 31 in plants and animals, but no function has been assigned to this residue to demonstrate a unique and conserved role for H3.1 during replication. We found that TONSOKU (TSK/TONSL), which rescues broken replication forks, specifically interacts with H3.1 via recognition of alanine 31 by its tetratricopeptide repeat domain. Our results indicate that genomic instability in the absence of ATXR5/ATXR6-catalyzed histone H3 lysine 27 monomethylation in plants depends on H3.1, TSK, and DNA polymerase theta (Pol q). This work reveals an H3.1-specific function during replication and a common strategy used in multicellular eukaryotes for regulating post-replicative chromatin maturation and TSK, which relies on histone monomethyltransferases and reading of the H3.1 variant.

16h05-16h20 Structural and biochemical studies of the chemical mechanism of condensation in nonribosomal peptide synthetases (NRPSs)

Angelos Pistofidis[1], T. Martin Schmeing[1]

[1]Department of Biochemistry and Centre de recherche en biologie structurale, McGill University, Montreal, QC, Canada

Non-ribosomal Peptide Synthetases (NRPSs) are a large family of megaenzymatic complexes that synthesize many pharmaceutically relevant molecules such as penicillin, cyclosporin and actinomycin D. The biosynthetic activity of NRPSs is distributed over several modules, each responsible for the incorporation of a single amino acid to the growing peptide. A canonical module consists of an adenylation (A) domain that recognizes and activates the module's cognate substrate, a peptidyl carrier protein (T) domain that transports substrates around the module, and a Condensation (C) domain that joins amino acids and peptidyl intermediates from adjacent modules. Although structural, biochemical and computational studies have contributed to our understanding of A and T domains, our knowledge of the chemistry and dynamics of C domains remains limited. A thorough understanding of the chemical mechanism of C domains will help guide future NRPS bioengineering efforts for the development of novel therapeutics. The aim of our research is to build an accurate model of the chemical mechanism of C domains, using the first two modules of linear gramicidin synthetase (LgrA). We have expressed and purified the two modules of LgrA, separately, and have modified the T domain of each with its respective non-hydrolysable substrate analogue. Using asparaginyl endopeptidase 1 (AEP1)-mediated ligation we have 'stitched' the two modules together and obtained a high-resolution crystal structure of the LgrA complex 'paused' in the condensation step. To the best of

our knowledge, this is the first high resolution dimodular structure and the first structure of an NRPS with two substrate-analogues in the C domain active site. By verifying our observations through biochemical assays and bioinformatics we are in the process of deciphering the chemical mechanism of C domains and how we could one day exploit them to synthesize novel therapeutics.

Communications Orales / Short Talks

Salle du Parc / du Parc Room Session 5

15h20-15h35 Characterization of small molecule inhibitors of PINK1

Tara Shomali[1], Shafqat Rasool[1], Nathalie Croteau[1], Luc Truong[1], Simon Veyron[1], Jean-François Trempe[1]

[1]McGill University

Parkinson's disease (PD) is a devastating neurodegenerative disease which is increasingly affecting our ageing population. The autosomal recessive juvenile form of PD is caused by loss of function mutations in proteins including the PINK1 kinase. Under physiological conditions, PINK1 is responsible for sensing mitochondrial damage and initiating mitochondrial turnover pathways. Modulation of PINK1 through small molecules could help reinstate PINK1's function and provide an important research tool in PD. Here our goal is to characterize small molecule inhibitors of PINK1 as leads for the development of tool compounds. In this project, IC50 of PINK1 inhibitors previously identified through thermal shift assays were determined. The IC50 of PRT062607, PRT060318, JNJ-7706621, CYC116 and TAK659 were determined to be 1.6 ± 1.2 mM, 2.5 ± 1.2 mM, 30.4 ± 1.2 mM, 76.7 ± 1.1 mM and 7.2 ± 1.1 mM respectively against Tribolium castaneum (Tc)PINK1. Furthermore, crystal structure of TcPINK1 with PRT062607 and with CYC116 have been obtained. Following IC50 determination, the potency of these inhibitors needed to be measured against Homo sapiens (Hs)PINK1. However, HsPINK1 cannot be purified in E. coli or yeast. This challenge was overcome by developing a surrogate humanized TcPINK1 construct, in which human-like mutations were introduced in the ATP binding domain, where the inhibitors bind. We show that the ATP binding pocket might differ between orthologues as the IC50 values obtained are 1.5 ± 1.1 mM for PRT062607 and 18.1 ± 1.3 mM for PRT060318. This work will provide the baseline for development of PRT062607 derivatives with higher potency. Furthermore, the humanized TcPINK1 construct provides a basis for HsPINK1 specific modulators, a method with has not been explored before for PINK1.

15h35-15h50 Discovery of a new virulence factor in *H. pylori*

Meriem Beniani[1], Mariem Chalbi[1], Cyrielle Martini[1], Charles Calmettes[1]

[1]INRS Armand-Frappier Santé Biotechnologie Research Centre

H. pylori is a human pathogenic bacterium that can infect the gastric mucosa of its host. Most infected people are asymptomatic, but in others, these bacteria can cause disorders such as peptic ulcer disease, mucosa-associated lymphoid tissue (MALT) lymphoma, and gastric adenocarcinoma. This organism utilizes pathogenic processes such as colonization and immune escape to thrive successfully in its host. We currently investigate a polysaccharide lyase that can potentially be a virulence factor, which, as a result, can represent a vaccine or drug target. The protein structure has been elucidated at 2.2 Å resolution revealing a typical polysaccharide lyase fold. While the sugar specificity is elusive, we investigated the degradative activity toward a variety of polysaccharides and found that our enzyme of interest is a hyaluronidase, rendering it likely to have a role in colonization or immune escape. The determination of the reduction of the substrates and the kinetic parameters were determined via the measure of the absorbance over time at 235 nm. To study the interaction of the protein with cells we will use microscopy and flow cytometry

15h50-16h05 The discovery and characterization of two novel structural motifs on the C-terminal domain of kinetoplastid RNA editing ligases

Daniel Moses[1], Vaibhav Mehta[1], Reza Salavati[1]

[1]Institute of Parasitology, McGill University, 21111 Lakeshore Road, Ste. Anne de Bellevue, H9X 3V9 Quebec, Canada

Parasitic protozoans of the *Trypanosoma* and *Leishmania* species have a uniquely organized mitochondrial genome, the kinetoplast. Most kinetoplast mRNAs are cryptic and encode multiple subunits for the electron transport chain following maturation through a uridine insertion/deletion process called RNA editing. This process is mediated in an enzyme cascade by an RNA editing complex (editosome), where the final ligation step is catalyzed by kinetoplastid RNA editing ligases, KREL1 and KREL2. While the N-terminal domain (NTD) of these proteins is highly conserved with other DNA ligases and mRNA capping enzymes, with five recognizable motifs, the functional role of their diverged C-terminal domain (CTD) has remained elusive. In this work, we performed *in vitro* functional assays using recombinant protein to unveil critical residues involved in protein-protein interaction and dsRNA ligation activity. We show that the α-helix (H)3 of KREL1 CTD interacts with the αH1 of its editosome protein partner KREPA2. Moreover, a specific KWKE motif on the αH4 of KREL1 CTD is implicated in ligase auto-adenylylation analogous to motif VI in DNA ligases. Additionally, our data suggest that the OB-fold domain and the zinc fingers on KREPA2 do not influence the RNA ligation activity of KREL1. In summary, we present KREL1 with a motif VI on its CTD for ATP catalysis and a binding motif for editosome integration via KREPA2.

16h05-16h20 Structural and functional analysis of the human PARP4 catalytic domain

Léonie Frigon[1], John M. Pascal[1]

[1]Université de Montréal

PARP4 is found in cytoplasmic vault organelles (also referred to as vault PARP), but its precise cellular role remains to be determined, and there are very few insights into PARP4 structure and mechanism of regulation. Sequence comparison with other PARP family members indicates that the PARP4 catalytic region contains a helical domain (HD). The HD is an autoregulatory domain found in PARPs 1, 2, and 3 that acts to block substrate NAD^+ binding to the catalytic site. Interaction with DNA strand breaks allosterically alters the HD structure to relieve autoinhibition and allow access to substrate NAD^+. Deletion of the HD in PARPs 1, 2, and 3 relieves this autoinhibitory mechanism and leads to constitutive synthesis of ADP-ribose modifications. We deleted the HD from the PARP4 catalytic domain and observed a modest change in catalytic output, suggesting that the HD does not perform an autoinhibitory function in PARP4. We determined the X-ray crystal structure of the PARP4 catalytic domain and confirmed the presence of an HD; however, the HD structure is distinct from that found in PARPs 1, 2, and 3. Most notably, the PARP4 HD adopts an open conformation that allows NAD^+ access to the catalytic site, providing a structural basis for the apparent lack of an HD autoinhibitory role in PARP4. The crystal structure also provides insights into the basis for the mono-ADP-ribosylation activity of PARP4. Ongoing studies are investigating the impact that other PARP4 domains might have on regulating catalytic activity, with the overall goal of providing structural and functional insights that contribute to the investigation of PARP4 cellular functions.

Affiches / Poster

Salle de Bal / Ballroom A Session 6

16h20-18h00

P1 - Structural insights into the biosynthesis and biodegradation of cyanophycin

Itai Sharon[1], Martin Schmeing[1]

[1]*McGill department of biochemistry*

Cyanophycin is a natural biopolymer consisting of a chain of poly-L-Asp residues with L-Arg residues attached to the b-carboxylate side chains by isopeptide bonds. First discovered in cyanobacteria in 1886, cyanophycin is produced by a wide range of bacteria and is important for cellular nitrogen storage. Two enzymes are known to produce cyanophycin: cyanophycin synthetase 1 (CphA1), which builds cyanophycin from the amino acids Asp and Arg by alternating between two separate reactions for backbone extension and side chain modification; and cyanophycin synthetase 2 (CphA2), which polymerizes b-Asp-Arg dipeptides. The degradation of cyanophycin into Asp and Arg happens in two steps: in the first, cyanophycinase degrades the polymer into b-Asp-Arg dipeptides; in the second, the dipeptides are degraded into amino acids by one of several enzymes with isoaspartyl dipeptidase activity. Cryo-EM and X-ray crystallography structures of CphA1, CphA2, cyanophycinase and a novel b-Asp-Arg dipeptidase shed light on the previously unknown mechanisms of cyanophycin biosynthesis and biodegradations.

P2 - Structural basis of activation of the tumor suppressor protein neurofibromin

Malik Chaker-Margot[1]

[1]*Université de Montréal*

Neurofibromin is a negative regulator of the small GTPase Ras and mutations in the NF1 gene which encodes it are the cause of a genetic disease, neurofibromatosis type I. Disease-causing mutations span the entire protein sequence, yet little was known of the function of non-catalytic regions of the protein, in part due to lack of structural information. We have determined the cryo-EM structure of full-length human neurofibromin in two conformations: an occluded, auto-inhibited conformation and an open active conformation. We further show that the equilibrium of these conformations is triggered by the presence of nucleotides, which releases auto-inhibition by interacting with residues involved in maintaining the occluded state. The addition of nucleotides was observed to activate the protein in vitro which suggests that neurofibromin may be regulated by fluctuating nucleotide concentrations in cells. Finally, we studied the impact of disease-causing mutations on neurofibromin showing that they broadly cause a decrease in protein stability. These results and contemporaneous studies in the field demonstrate the exquisite level of regulation that neurofibromin undergoes to modulate Ras pathway activation.

P3 - Bioinformatics approach to identifying new human atypical SUMO E3 Ligases

Ali Harake[1], Antoine Bouchard[1], Laurent Cappadocia[1]

[1]UQAM

SUMOylation is a post-translational modification of proteins that allows cells to adapt rapidly to metabolic or environmental changes. This reversible modification consists of the affixing of a protein called SUMO to a substrate protein via a covalent bond on lysines. SUMOylation modifies protein characteristics such as localization, stability and/or inter-protein interactions. SUMOylation is a predominantly nuclear process that involves the action of three proteins: an E1 to activate SUMO, an E2 to conjugate SUMO and an E3 to direct the E2 enzyme to specific substrates and stimulate catalysis. E3 Ligases are divided into 2 categories: typical ligases containing a RING domain and atypical ligases containing other structural motifs. A protein discovered in 2015, ZNF451, belongs to the latter category. It contains 2 SUMO-interacting motifs (SIMs) that are necessary and sufficient for affixing SUMO to proteins. Our hypothesis is that there are other proteins with SUMO E3 Ligase activity through SIM motifs or similar regions. Our approach consists of bioinformatics identification of candidate proteins followed by the purification and characterization of these proteins. Our work has already led to the identification of a first candidate, the transcription factor ZNF24. Our data seem to support the activity of ZNF24 as a SUMO E3 Ligase. Furthermore, 37 other candidates have been identified having SUMO E3 Ligases characteristics. Characterization tests are currently taking place to determine wether these candidates present an E3 activity. Overall, this study will provide a better understanding of the molecular mechanisms implemented by cells to rapidly regulate many protein properties and will have repercussions in the biomedical field as well as the plant biology field.

P4 - Cryo-electron tomography structure of the central pair in the ciliary tip

Thibault Legal[1], Khanh Huy Bui[1]

[1]*McGill University*

Cilia are essential organelles that protrude from the cell body. Primary cilia act as biological sensors while motile cilia are essential to propel cells or move fluids. Cilia are made of a cylindrical, microtubule-based structure called the axoneme that contains nine microtubule doublets at the periphery. Motile cilia also have two microtubule singlets in the centre, known as the central pair. Cilia can be divided in different regions based on their morphology. The base is where the cilium is attached to the cell, the middle region constitutes the main part of the cilium, and the tip refers to the distal end. In most types of motile cilia, the ciliary tip is distinct from the rest of the cilium as it only contains the central pair. This region is of interest as it is thought to contain information on ciliary length regulation and ciliary growth, two processes that are not well understood. Here, we used cryo-electron tomography and subtomogram averaging to obtain the structure of the central pair in the ciliary tip of the ciliate *Tetrahymena thermophila* at sub-nanometer resolution. We show that the central pair in the ciliary tip is structurally different from the central pair in the rest of the axoneme and highlight newly identified projections. By visualising the subtomogram average in tomograms, we find that the microtubules of the central pair twist significantly more in the tip. These data provide the first structure of the tip central pair and may provide clues on ciliary tip functions.

P5 - Building functional biomaterials and hydrogels the Bacillus subtilis way

Mélanie Côté-Cyr[1, 2], Steve Bourgault[1, 2]

[1]Université du Québec à Montréal, [2]The Quebec Network for Research on Protein Function, Engineering, and Applications (PROTEO)

Bacillus subtilis represents a powerful tool for recombinant expression of functional proteins and is currently used for production of enzymes with applications in the dairy and food industries. As previously shown for *Escherichia coli* biofilms, *B. subtilis* biofilms can be engineered for various applications, such as tissue regeneration. A main advantage of using *B. subtilis* over *E. coli* as an expression system is that it does not produce lipopolysaccharides, which are proinflammatory molecules and can interfere with biomedical applications. *B. subtilis* strains are generally regarded as safe, which enables their usage for the design of engineered living materials. In this study, we aim to produce genetically engineered *B. subtilis* to produce living functionalized biofilm for tissue regeneration applications. In this regard, we knocked out the epsA-O gene cluster and the sinR and tasA genes. The epsA-O cluster is responsible for production of exopolysaccharides, which allow interaction with a hydrophobic surface protein covering the biofilms. SinR acts as a repressor of TasA, which is the scaffold protein of the biofilm. Deletion of tasA will allow the expression of functionalized biofilm via the introduction of expression plasmids containing tasA genetic fusions with targeted sequences. Scarless and markerless deletions were achieved by allele replacement following transformation of *B. subtilis* with suicide plasmids in which we introduced flanking regions of the target genes for recombination. Deletion of the targeted genes results in altered morphology and/or production of the biofilms. Ultimately this modified strain could be further engineered for *in situ* production of therapeutic molecules for various applications, including tissue regeneration.

P6 - Elucidating the Mechanistic Contributions of the Hydrophobic Central Domain of Amylin in Amyloid Formation and Associated-Cytotoxicity

Vy Nguyen[1], Mathilde Fortier[1], Mélanie Côté-Cyr[1], Margaryta Babych[1], Phuong Trang Nguyen[1], Roger Gaudreault[1], Steve Bourgault[1]

[1]UQAM

Amylin, also known as the islet amyloid polypeptide (IAPP), is a 37-residue aggregation-prone peptide hormone whose deposition as insoluble fibrils in the islets of Langerhans is associated with type II diabetes. Therapeutic interventions targeting amylin aggregation, which contributes to pancreatic β-cell degeneration, remain elusive owing to the lack of understanding of the self-assembly mechanisms and of the quaternary species participating in toxicity. Numerous studies have investigated the contributions of the 20–29 amyloidogenic core in self-assembly, whereas the central region, of amylin has been less considered, notwithstanding its potential key role in oligomerization. In this study, we aimed at investigating the physicochemical and conformational properties driving amylin self-assembly and associated cytotoxicity. Computational tools and all-atom molecular dynamics simulation suggested that the hydrophobic 12–17 segment promotes peptide self-recognition and aggregation. Alanine scanning revealed that the hydrophobic side chains of Leu12, Phe15 and Val17 are critical for amyloid fibril formation. Destabilization of the α-helical folding by Pro substitution enhanced self-assembly when the pyrrolidine ring was successively introduced at positions Ala13, Asn14 and Phe15. Modulating the peptide backbone flexibility at position Leu16 through successive incorporation of Pro, Gly and α-methylalanine, inhibited amyloid formation and reduced cytotoxicity, while the isobutyl side chain of Leu16 was not critical for self-assembly and IAPP-mediated toxicity. Overall, these results highlight the importance of the 12–17 hydrophobic region of amylin for its amyloidogenicity, potentially supporting the identification of therapeutic approaches to arrest oligomerization and/or fibrillization.

P7 - Molecular self-assembly of hybrid π-conjugated peptides into optoelectronic nanomaterials

Nadjib Kihal[1], Mélanie Côté-Cyr[1], Ali Nazemi[1], Steve Bourgault[1]

[1]UQAM

The integration of nanomaterials as a bridge between the biological and electronic worlds has revolutionized understanding of how to generate functional bioelectronic devices and has opened new horizons for the future of bioelectronics. The use of optoelectronic nanomaterials as a versatile interface in bioelectronics offers many practical solutions and has recently emerged as a highly promising route to overcome technical challenges in the control and regulation of communication between biological and electronics systems. Nonetheless, the availability of functional nanomaterials with efficient conductivity, while being biocompatible remains limited. In this context, we harnessed the high self-assembling propensity of the amyloidogenic peptide to template the organization of perylenediimide (PDI) to design semiconductive nanostructures. Owing to their chemical and thermal stability as well as their unique physicochemical and optoelectronic properties, PDI was chosen as p-conjugated semiconductor to incorporate electronic properties to the resulting suprastructures. Asymmetric and symmetric amyloid p-conjugated peptides were synthesized by introducing the targeted sequence at one or both imide-positions of perylene diimide, respectively. The analyses revealed that both PDI-derivatives self-assemble into conductive, long, and linear nanofilaments with a cross-β-sheet quaternary organization. The *I-V* curves exhibited a clear signature of semiconductors for both assemblies and cellular assays revealed their cytocompatibility with moderate emission properties. Overall, this study exposes that optoelectronic and biocompatible nanostructures can be obtained from amyloid peptide building blocks, opening the possibility to exploit them in various biomedical fields.

P8 - Structural polymorphism of guanine quadruplex-containing regions in human promoters

Christopher Hennecker[1], Lynn Yamout[1], Chuyang Zhang[1], Chenzhi Zhao[1], Nicolas Moitessier[1], Anthony Mittermaier[1]

[1]*McGill University*

Intramolecular guanine quadruplexes (G4s) are non-canonical nucleic acid structures formed by four guanine (G)-rich tracts that assemble into a core of stacked planar tetrads. G4-forming DNA sequences are enriched in gene promoters and are implicated in the control of gene expression. Most G4-forming DNA contains more G residues than can simultaneously be incorporated into the core resulting in a variety of different possible G4 structures. While this kind of structural polymorphism is well recognized in the literature, there remain unanswered questions regarding possible connections between G4 polymorphism and biological function. Here we report a detailed bioinformatic survey of G4 polymorphism in human gene promoter regions. Our analysis is based on identifying G4-containing regions (G4CRs), which we define as stretches of DNA in which every residue can form part of a G4. We found that G4CRs with higher degrees of polymorphism are more tightly clustered near transcription sites and tend to contain G4s with shorter loops and bulges. Furthermore, we found that G4CRs with well-characterized biological function tended to be longer and more polymorphic than genome-wide averages. These results represent new evidence linking G4 polymorphism to biological function and provide new criteria for identifying biologically relevant G4-forming regions from genomic data.

P9 - Enhancing the mechanical and physical properties of cellulose nanofibrils with protein amyloid structures to generate novel biocomposites

Vinay Khatri[1], Maziar Jafari[1], Marc Beauregard[2], Denis Archambault[3], Mohamed Siaj[1], Eric Loranger[4], Steve Bourgault[1]

[1]Department of Chemistry, Université du Québec à Montréal, C.P. 8888, Succursale Centre-Ville, Montréal, H3C 3P8, Canada , [2]Department of Chemistry, Biochemistry and Physics, Université du Québec à Trois-Rivières, 3351 Des Forges, Trois-Rivières, Québec, Canada, [3]Department of Biological Sciences, Université du Québec à Montréal, C.P. 8888, Succursale Centre-Ville, Montréal H3C 3P8, Canada , [4]Lignocellulosic Materials Research Center, Université du Québec à Trois-Rivières, 3351 Des Forges, Trois-Rivières, Québec, Canada

Because of their sustainability, biodegradability and low cost, cellulose nanofibrils (CNFs) have been considered for several applications including nanocomposites, reinforcement agents, drug delivery systems, and tissue engineering. Despite their desirable properties, CNFs have limited mechanical properties (stiffness, strength, and toughness), which constitutes a major limitation for their use in several applications. Therefore, to improve the physical and mechanical properties of CNFs for high-strength applications, non-biodegradable polymers, graphene, carbon nanotubes, or silver nanoparticles have been evaluated as adducts. These materials, however, have toxicity and environmental concerns, which limit their usage in a sustainable manner. Amyloid fibrils are one of the stiffest filamentous biopolymers known, owing to the cross–β-sheet quaternary organization, which would improve the rigidity of the CNF films as a renewable biocompatible alternative. In this study, we incorporated self-assembled amyloid proteinaceous structure into CNFs. These novel CNF-amyloid biocomposites showed superior mechanical properties (up to 7-folds) when amyloid polypeptides were preassembled into cross-β-sheet structures prior to their inclusion into CNF films. Wettability and thermogravimetry analyses demonstrated improved hydrophobicity (97%) and thermal tolerance. Bulk spectroscopic characterization of bio-nanocomposite films by Fourier-transform infrared spectroscopy, X-ray photoelectron spectroscopy and energy-dispersive X-ray spectroscopy provided insight into protein fibrils organization. Atomic force microscopy and photo-induced force microscopy results depicted the location of amyloid protein in high resolution within the CNF films. This novel amyloid-CNF bio-nanocomposite paves the way for future use in environmentally friendly applications demanding robust mechanical and water-repelling properties and/or thermal resilience.

P10 - Molecular and structural characterization of the determinants leading to the SUMO E3-ligase SIZ1 interaction with its substrats in Arabidopsis thaliana facing environmental stresses.

Faustine Hutinet[1], Laurent Cappadocia[1]

[1]*Université du Québec à Montréal UQAM*

SUMOylation is a reversible protein post-translationnal modification characterized by the covalent attachment of a Small Ubiquitine like MOdifier protein (SUMO) on a lysine residue of a substrate. The whole process is regulated by an enzymatic cascade composed of an activating enzyme (E1), a conjugating enzyme (E2) and a ligase enzyme (E3) which associates substrates and SUMO-charged E2 within a SUMO-E2/E3 complex that facilitates SUMO tranfer to the target protein.

SUMOylation plays a crucial role in plant response to environmental stresses (drought, flooding, freezing temperature…). Although hundreds of SUMOylated substrates have been identified in *Arabidopsis thaliana* plants facing environmental stresses, the precise mechanism of substrate selection remains elusive.

Our hypothesis is that the E3 is able to recognize its substrates through the presence of different structural and molecular determinants.

Our objective is to elucidate those determinants leading to the E3-substrate interaction in *Arabidopsis thaliana.* To do so, we screened interactions between the E3 SIZ1 and 1500 transcription factors using AlphaFold. To validate certain interactions, recombinant E1, E2, E3, SUMO and substrates were obtained using heterologous protein expression in *E. coli*. The importance of specific protein interactions will soon be assessed using *in vitro* SUMOylation essays.

Given the implication of SUMOylation in plant response to environmental stresses, a better understanding of E3-Substrate interactions would pave the way to production of more resilient crops through molecular engineering or selection.

P11 - Captured snapshots of PARP1 in the active state reveal the mechanics of PARP1 allostery

Elise Rouleau-Turcotte[1], Dragomir Krastev[2], Stephen Pettitt[2], Christopher Lord[2], John Pascal[1]

[1]Université de Montréal, Montréal, Canada, [2]Institute of Cancer Research, London, UK

PARP1 rapidly detects DNA strand break damage and allosterically signals break detection to the PARP1 catalytic domain to activate poly(ADP-ribose) production from NAD+. PARP1 activation is characterized by dynamic changes in the structure of a regulatory helical domain (HD); yet, there are limited insights into the specific contributions that the HD makes to PARP1 allostery. Here, we have determined crystal structures of PARP1 in isolated active states that display specific HD conformations. These captured snapshots and biochemical analysis illustrate HD contributions to PARP1 multi-domain and high-affinity interaction with DNA damage, provide novel insights into the mechanics of PARP1 allostery, and indicate how HD active conformations correspond to alterations in the catalytic region that reveal the active site to NAD+. Our work deepens the understanding of PARP1 catalytic activation, the dynamics of the binding site of PARP inhibitor compounds, and the mechanisms regulating PARP1 retention on DNA damage.

P12 - Cell-derived vesicles to close the gap between synthetic bilayers and live cell membranes

Mathew Sebastiao[1, 2], Margaryta Babych[1, 2], Noe Quittot[1, 2], Kiran Kumar[1, 2], Alexandre Arnold[1], Isabelle Marcotte[1, 2], Steve Bourgault[1, 2]

[1]UQAM, [2]PROTEO

A vast array of peptides and proteins share the innate ability to self-assemble into long fibrillar aggregates known as amyloid. These amyloid fibrils are found as plaques in individuals with different types of pathological conditions, such as various forms of familial amyloidosis, neurodegenerative conditions such as Alzheimer's and Parkinson's diseases, and organ-specific amyloidosis. It is currently suspected that the corresponding cellular dysfunction largely comes from the process by which amyloid forms, and the pre-fibrillar species. The current literature suggests that these pre-fibrillar species act by directly disrupting the integrity of the plasma membrane, resulting in cell death. At present, however, there is a distinct lack of tools and methodologies which can be used to study the biophysics of membrane perturbation on cell membranes. The majority of model lipid bilayers are devoid of the complexity and molecular diversity which can be found in plasma membranes, and considerable literature demonstrates the sensitive nature of the amyloidogenic process which can be significantly altered in the presence of other biomolecules. To overcome this, we developed an approach to study the perturbations of membrane vesicles generated from mammalian cells via fluorescence microscopy and a high-throughput microplate assay. Both of these approaches demonstrate that plasma membrane from multiple cell types behave in a similar fashion when treated with the rapidly aggregating peptide hormone islet amyloid polypeptide (IAPP). Using a non-aggregating rat variant of IAPP, it was confirmed that both vesicle systems were robust and responded to the aggregation but not the monomeric peptide. These results demonstrate that it is possible to study the biomechanics of membrane perturbations associated with amyloid diseases using biologically relevant models, a promising development to aid in understanding the underlying mechanisms by which amyloid exerts its devastating effects.

P13 - Identification and characterization of new factors for the acid adaptation in Helicobacter pylori

Mariem Chalbi[1], Meriem Beniani[1], Charles Calmettes[1]

[1]*Institut National de la Recherche Scientifique, Centre Armand-Frappier Santé Biotechnologie*

Helicobacter pylori (H. pylori) is the only pathogen capable of colonizing the gastric niche, infecting the stomach of half of the human population worldwide. Persistent colonization by H. pylori is associated with the development of major gastric pathologies like gastritis, peptic ulcer and adenocarcinoma. These pathologies are mostly due to the acid adaptation mechanism of H. pylori allowing them to persist in the human stomach. However, not all of proteins related to this adaptation mechanism are identified and characterized. Therefore, we are interested in identifying and characterizing new factors of acid adaptation of H. pylori. These latter could be new targets for the eradication of pathogen infection. Sequences coding for new proteins of gastric specific strain was identified by analyzing phylogenetic tree of ancestral intestinal helicobacter genomes. Among them, we are focusing the research on two new secreted protein Tip-alpha like protein and dehydrogenase like protein that has no sequence homology to any other protein.

P14 - Regulating The Type 9 Secretion System and Virulence Factor Secretion

Anshu Saran[1], Natalie Zeytuni[1]

[1]*Anatomy and cell biology*

Chronic periodontitis is an inflammatory gum disease that affects ~50% of the world's population, with 7 out of 10 Canadians developing periodontitis during their lives. *Porphyromonas gingivalis* is a notorious keystone bacterial pathogen involved in periodontitis that manifests its infection primarily via the secretion of toxic proteolytic virulence factors known as gingipains. Interestingly, *P. gingivalis* and its secreted gingipains also play pivotal roles in the progression of various systemic conditions such as Alzheimer's disease, atherosclerosis, aspiration pneumonia, rheumatoid arthritis, diabetes, and cancer. The gingipains are released via the conserved Type 9 Secretion System (T9SS). Several of the essential T9SS proteins are regulated by a two-component system (TCS) comprising of proteins PorX and PorY, responsible for the generation of a functional T9SS and subsequent release of gingipains.

Here, we present the structural and biochemical characterization of the response regulator PorX – a protein central to the T9SS regulatory system. We successfully determined the crystal structure of PorX in its phosphorylated-like dimeric state. Each PorX's monomer exhibits a two-domain organization – a receiver domain and an effector domain, interconnected by a stalk region. Interestingly, the effector domain was found to adopt a fold of an alkaline phosphatase (ALP) enzymatic domain – emphasizing PorX as an atypical RR. Similar to other ALPs that coordinate two metal cations in their binding site in support of their enzymatic activity, we demonstrated that PorX can bind two zinc cations at its ALP active site. Our enzymatic assays confirmed PorX as a phosphodiesterase. Finally, we present a comprehensive analysis indicating a cross talk between phosphorylation, metal cation binding at the ALP effector domain, PorX dimerization and gingipains secretion.

P15 - A lesion-mimic mutant of Catharanthus roseus accumulates the opioid agonist, akuammicine

Fanfan Li[1], Mehran Dastmalchi[1]

[1]*McGill University*

Catharanthus roseus is a medicinal plant that produces an abundance of monoterpenoid indole alkaloids (MIAs), notably including the anticancer compounds vinblastine and vincristine. While the canonical pathway leading to these drugs has been resolved, the regulatory and catalytic mechanisms controlling many lateral branches of MIA biosynthesis remain largely unknown. Here, we describe an ethyl methanesulfonate (EMS) *C. roseus* mutant (M_2-117523) that accumulates high levels of MIAs. The mutant exhibited stunted growth, partially chlorotic leaves, with deficiencies in chlorophyll biosynthesis, and a lesion-mimic phenotype. The lesions were sporadic and spontaneous, appearing after the first true bifoliate and continuing throughout development. The lesions are also the site of high concentrations of akuammicine, a minor constituent of wild type *C. roseus* leaves. In addition to akuammicine, the lesions were enriched in 25 other MIAs, resulting, in part, from a higher metabolic flux through the pathway. The unique metabolic shift was associated with significant upregulation of biosynthetic and regulatory genes involved in the MIA pathway, including the transcription factors *WRKY1*, *CrMYC2*, and *ORCA2*, and the biosynthetic genes *STR*, *GO*, and *Redox1*. Following the lesion-mimic mutant (LMM) phenotype, the accumulation of akuammicine is jasmonate (JA)-inducible, suggesting a role in plant defence response. Akuammicine is medicinally significant, as a weak opioid agonist, with a preference for the *κ*-opioid receptor, and a potential anti-diabetic. Further study of akuammicine biosynthesis and regulation can guide plant and heterologous engineering for medicinal uses.

P16 - The avian eggshell-membrane interface revealed in 3D by X-ray and FIB-SEM tomography

Daniel Buss[1], Natalie Reznikov[1], Marc McKee[1]

[1]*McGill University*

Roughly 80% of extant vertebrates rely on eggshells for embryonic development. In birds, mineralized eggshell formation is preceded by the assembly of fibers at the outer surface of the egg "white" to form a membrane onto which shell/mineral deposition occurs. Membrane fiber components are produced by tubular gland cells lining the isthmus region of the hen's oviduct. Following fiber assembly, organic-rich cores positioned along the outermost membrane fibers (furthest from the egg white) are associated with shell mineral nucleation and then initial circumferential growth of the calcitic shell. Using X-ray micro-computed tomography and FIB-SEM serial-surface-view volume imaging – both coupled with deep-learning based segmentation and 3D reconstruction – we report here on an analysis of full-thickness eggshell membrane and its interdigitation with the mineralized eggshell. We describe the nature and extent of fiber interdigitation with individual shell mammillae in 3D, and describe a thin organic membrane present around each mamillary baseplate that seemingly delimits the occurrence and location of mineralization events. Using TEM with selected-area electron diffraction and EDS analysis and imaging on FIB-SEM-cut undecalcified lamellae, we show that the mantle zone of fibers at the membrane-shell interface are permeated with poorly crystalline mineral. Additionally, using micro-computed tomography, we describe in 3D the membrane split that produces the air sac at the blunt end of the egg, essential for providing oxygen to a hatching (pipping) chick. These findings serve to prompt novel discussion and future studies on initial mineralization events that ultimately dictate the structural competence of avian eggshells.

P17 - Elucidating the structural dynamics and modification mechanism of Haloduracinβ using Nuclear Magnetic Resonance Spectroscopy

Stephanie Peslherbe[1]

[1]*McGill University*

Lanthipeptides are a class of thioether linkage-containing RiPPs with numerous biological functions such as antimicrobial, antifungal, antiviral, etc. The class II lanthipeptide synthetase HalM2 catalyzes a total of seven dehydrations and installs four thioether rings into its HalA2 precursor peptide substrate. This highly dynamic enzyme has piqued our interest because, while it must possess relaxed substrate specificity in order to iteratively modify HalA2, the enzyme nevertheless maintains strict biosynthetic fidelity and only installs a single set of thioether rings. It has been hypothesized that a conformational sampling of the enzyme-peptide complex makes important contributions to these properties. We are interested in understanding the biophysical processes guiding the interesting functional properties of HalM2 at atomic resolution using Nuclear Magnetic Resonance Spectroscopy. Accordingly, we have performed a combination of 2D and 3D NMR experiments on isotopically labelled peptide (^{15}N, ^{13}C, ^{1}H) to fully assign the peptide. We observe significant changes in chemical shifts as the peptide is modified by the enzyme. We also performed T1-T2-NOE experiments to study the structural dynamics of the peptide in both its unmodified and modified forms. The NOE relaxation experiments suggest that the leader peptide, which is highly conserved throughout lanthipeptides, has considerable local structure which may help the enzyme to recognize, bind and therefore modify the peptide.

P18 - Investigation of GlpG rhomboid protease using 19F solution NMR

Medhani Mohottalage[1]

[1]*University of Ottawa*

Rhomboids are a family of intramembrane serine proteases that cleave other transmembrane protein substrates embedded within the lipid membrane. Rhomboids are well conserved across many organisms and the substrates they cleave play a key role in a diverse range of biological processes including cell signalling, bacterial quorum sensing and parasite invasion to name a few.

While prior X-ray crystallographic structures and functional studies have provided insights into rhomboid's mechanism of serine proteolysis, questions remain concerning how transmembrane substrates gain access into rhomboid's water-filled active site from the hydrophobic lipid membrane environment.

Currently, there exists a growing body of evidence that supports the notion that the *α*5 helix plays a major role in substrate gating and proteolysis.

A powerful method that can monitor the dynamics of rhomboid helix gating is solution state NMR. Although conventional NMR studies on proteins make use of isotropically enriched multidimensional NMR experiments, obtaining conformational dynamic data using these experiments has not been straightforward. One-dimensional ^{19}F NMR could allow for the study of rhomboid dynamics by providing a simplified spectrum with high sensitivity to changes in rhomboid dynamics. In particular, site-specific labelling of cysteine residues using sulfhydryl-reactive probes, post expression was found to give rise to well resolved spectra with promising characteristics.

Here we present preliminary results on our investigations into rhomboid dynamics around helix 5 for the GlpG rhomboid protease from *E. coli* labelled with 2-bromo-*N*-(4-(trifluoromethyl)phenyl)acetamide (BFTMA) ^{19}F probe. Labeling at a single site on helix 5 gives rise to spectra with more than one broad peak, suggesting exchange between at least two states. This encouraging result provides the basis for current investigations into the effect of substrates, inhibitors and activating mutations on this dynamic equilibrium.

P19 - Purification of Prostaglandin F2α receptor (FP) for its study in structural biology

Marine Salze[1], Madalina Macovei[1], Eric Barbeau[1], Aaron Cho[2], Tegvir Boora[2], Véronique Blais[1], Stéphane Laporte[2], Martin Audet[1, 3]

[1]Department of Pharmacology and Physiology, Faculty of Medicine and Health Sciences, Université de Sherbrooke, Sherbrooke, QC, Canada, [2]Department of Medicine, Division of Experimental Medicine and Endocrinology & Metabolism, McGill University, Montréal, QC, Canada, [3]CHUS research center, CIUSSS-Estrie-CHUS, Sherbrooke, QC, Canada

Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) is a bioactive lipid involved in many physiological and pathological conditions such as parturition, glaucoma, and brain injuries. $PGF_{2\alpha}$ binds and activates FP, a G protein-coupled receptor (GPCR) at the cellular plasma membrane, leading to the recruitment of intracellular G protein effectors such as G_q or $G_{12/13}$ to the receptor, and the subsequent activation of cellular reactions cascades. As such, FP is a target for various drugs used in clinic.

Biased activation of GPCR is a promising approach to gain clinical benefit and reduce drugs side effect. Solving the structural determinant of biased FP activation would give an essential insight to guide the development of molecular tools aiming at further understanding the role of each pathway in FP biological functions. To enable the study of FP using structural biology approaches, the receptor was optimized for X-ray crystallography and purified in complex with G_{12} and G_{13} for Cryo-EM assays.

Soluble stable domains and mutations were engineered into FP for optimization purpose. Biased mutants were also produced to study FP signaling. We expressed and purified these constructions and verified their expression and yield through flow cytometry and SEC analysis. Stability was analyzed through thermostability assays. Complexes FP-G proteins were purified, isolated by SEC and verified by western blotting. These results show promising conditions for the study of FP structure and biased signaling.

P20 - Integrated modelling of the Nexin-DRC reveals its regulation mechanism

Avrin Ghanaeian Miandoab[1]

[1]*Anatomy and cell biology department*

Cilia are microscopic hair-like structures that extend from the surface of eukaryotic cells and are responsible for cell motility and sensory functions. A cilium is formed by a bundle of nine doublet microtubules called axoneme. The doublet microtubules are connected to outer and inner dynein arms and nexins and form the nexin-dynein regulatory complex (N-DRC) that is known as a core engine of cilium motility. Functionally, The N-DRC moves the cilia by generating sliding force between doublet microtubules and is believed to restrict the sliding force and convert sliding into axonemal bending by acting as an elastic linkage connecting two adjacent doublet microtubules. Although the N-DRC has been studied extensively using genetic, biochemical, and structural approaches, the precise arrangement of the 11 (or more) N-DRC subunits remains unknown. Here, by using cryo-EM, and integrative modelling we have identified and modelled the components of N-DRC in cilia from *Tetrahymena thermophila*. Our results reveal that DRC1/2/4 forms a core complex that serves as the scaffold for the assembly of the "functional subunits," namely DRC3/5–8/11. Also, we found DRC7 as a central linker subunit that connects the distal domain to the proximal domain. Two copies of DRC8 form a subcomplex with DRC11 in the proximal lobe of the linker domain that is required for the formation of stable contacts with the neighbouring B-tubule. DRC9/10 and DRC3 are defined as regulatory hubs of Inner Dynein Arm (IDA) e and IDAg, respectively. These findings shed light on N-DRC assembly and its role in regulating flagellar beating.

P21 - Structural and functional understanding of PKS-NRPS hybrid systems

Anirudh Mantri[1], Martin Schmeing[1]

[1]*McGill University*

Natural products biosynthesized by nonribosomal peptide synthetases (NRPSs) and polyketide synthases (PKSs) are some of the most important drugs for the treatment of a wide variety of diseases. NRPSs and PKSs carry out their biosynthetic activity using similar modular assembly-line. The use of many different substrates including amino acids, aryl acids, hydroxy acids, and fatty acids in NRPSs, and the extensive and varied modification of nascent peptides by tailoring domains in PKSs allow these mega-enzymes to make products with an access to a wide spectrum of chemical space.

Hybrid enzymes have modules from both classes of mega-enzymes and therefore combine carbon-carbon bond forming capabilities from PKSs and amide bond forming capabilities from NRPSs to further expand the number of potential products that can be synthesized. Examples of hybrid natural products used therapeutically are epothilones, ikarugamycin and bleomycin as anti-cancer drugs, rapamycin as an immunosuppressant, and leinamycin as an antibiotic.

Structural analyses of NRPSs and PKSs on their own have proven to be useful in deciphering their biosynthetic mechanisms and will help in development of new drugs. However, there is no available structural information revealing how NRPS and PKS segments work together. Here, we have targeted 2 very interesting hybrid targets: pppD, a tri-modular stand-alone hybrid NRPS-PKS system from the *Pseudovibro* family and IkaA, a di-modular iPKS-NRPS system from the *Streptomyces* family.

We have successfully expressed and purified both of these systems in *E.coli* by affinity and size exclusion chromatography. I have confirmed the adenylation activity for substrates including valine, leucine, isoleucine and proline for pppD. I further plan to do biochemical (bioactivity assays), biophysical (to determine the oligomerization state) and, structural studies (specially the intramodular interfaces) using x-ray crystallography and cryo-EM for better understanding towards functioning of these hybrid systems.

P22 - Exploring product formation in an NRPS assembly line

Elias Kalthoff[1], Maximilian Eivaskhani[1]

[1]*McGill*

Nonribosomal peptides are a class of natural small molecules made by microbes. Among them are medicines that have been game-changers in healthcare and have saved countless lives, such as the antibiotic penicillin or the immunosuppressant cyclosporin.

The fascinating bio-machines that produce these compounds are called nonribosomal peptide synthetases (NRPSs) and resemble assembly lines made of multiple workstations. At each workstation, a building block is added to the growing natural product, until the entire assembly line has been traversed and the finished product is released.

The assembly-line nature of NRPSs immediately gave rise to the idea of genetically engineering them to re-arrange the assembly lines and thereby biosynthetically build new molecules. But so far, most of the bioengineering efforts have not yielded the desired results. The most likely reason for this is that NRPSs are not linear assembly lines but complicated 3-dimensional complexes that have evolved with sophisticated spatial interactions between the individual workstations.

Here, I present the Maxibactin pathway a three-protein assembly line that produces a putative siderophore. It consists of the three NRPS proteins BvnA, B, and C which condense salicylic acid with three cysteines that are subsequently cyclized into thiazoline rings. Interestingly, BvnB and BvnB also contain a total of three methyltransferases but initial analysis shows that the final product is only methylated twice.

The pathway is named after Maximilian Eivaskhani, a former colleague who tragically died in a bicycle accident. He was able to solve the structure of one of the three proteins and thereby shine a structural light on the integrated methyltransferase. I am now attempting to solve the product identity and sequence of biochemical steps in the pathway to further bolster his characterization of the pathway.

P23 - Cellular senescence stabilizes microtubules in intestinal epithelial cells

Siwei Chu[1], Kais Bietar[1], Khaled Skaik[1], Ursula Stochaj[1]

[1]*McGill University*

Intestinal epithelia are critical to sustain homeostasis in the gastrointestinal tract. Aging promotes intestinal dysfunction through the accumulation of senescent epithelial cells (IECs) in the intestine. To date, the properties of senescent IECs are poorly understood. Here, I developed two model systems to study IEC senescence using two physiologically relevant compounds: butyrate and lopinavir. Butyrate is a short-chain fatty acid that is produced by the gut microbiome. It can also be delivered to the intestine through diet. Lopinavir is an anti-retroviral drug which has been linked to intestinal dysfunction. IECs treated with butyrate or lopinavir developed hallmarks of cellular senescence. Specifically, the treatments reduced cell proliferation, increased lysosomal content, and caused nuclear dystrophy. Using these validated models, I examined microtubule stability by evaluating microtubule disassembly. Results revealed that microtubules become stabilized in senescent IECs. To explore the potential mechanisms, I evaluated the levels of α-tubulin acetylation on lysine-40, histone deacetylase 6, and microtubule-associated proteins. Notably, butyrate and lopinavir employed different mechanisms to stabilize microtubules. In conclusion, my study provides novel knowledge on the physiological changes associated with cellular senescence. I have demonstrated that microtubules are stabilized in senescent cells and that different molecular pathways can promote this stabilization.

P24 - Feedforward activation of parkin E3 ubiquitin ligase

Rayan Fakih[1, 2]

[1]McGill University, [2]Gehring lab

Parkinson's disease (PD) is a neurological disorder characterized by the progressive loss of dopaminergic neurons in the midbrain. The majority of early onsets of PD are caused by mutations in parkin, a cytosolic E3 ubiquitin ligase which mediates the clearance of damaged mitochondria from the cell. Parkin activity is regulated by the mitochondrial kinase PINK1 (PTEN-induced kinase 1) via a two-step cascade. PINK1 first phosphorylates ubiquitin, which binds a recruitment site on parkin to localize it to damaged mitochondria. Next, PINK1 phosphorylates parkin on its ubiquitin-like domain (Ubl) domain, which binds a regulatory site to release ubiquitin ligase activity. Here, we report an alternative feedforward mechanism that bypasses the need for parkin phosphorylation through the binding of a second phospho-ubiquitin (pUb) molecule. We also reveal a crystal structure of an active parkin with pUb bound to both the recruitment and regulatory sites. Our results demonstrate the flexibility in the process of parkin activation and provide an avenue in the development of therapeutic approaches for treating PD.

P25 - Angiogenin and galectin-7: A new molecular alliance for cancer progression?

Trung Hieu Bui[1], Myriam Létourneau[1], Ismaël Chakir[1], Yves St-Pierre[1], Nicolas Doucet[1, 2]

[1]Centre Armand-Frappier Santé Biotechnologie, Institut National de la Recherche Scientifique (INRS), Université du Québec, 531 Boul. des Prairies, Laval, QC, H7V 1B7, Canada, [2]PROTEO, The Quebec Network for Research on Protein Function, Engineering, and Applications, 1045 Avenue de la Médecine, Université Laval, Québec, QC, G1V 0A6, Canada

Angiogenin (hANG) and galectin-7 (hGal7) have been shown to play roles in tumor progression. hANG (or ribonuclease 5) is part of the human pancreatic-type ribonuclease enzyme family and plays a defining role in blood vessel formation. hGal7 is a member of the galectin family composed of proteins characterized by a common affinity for β-galactosides. Interestingly, these two proteins have been recognized as biomarkers of breast cancer and their colocalization with p53 is well documented. Furthermore, their respective structural/functional homologs, i.e., RNase 3 and galectin-10, were recently shown to interact during eosinophil granulogenesis. Based on these observations, we evaluated whether hANG and hGal7 could interact to form a biologically relevant protein complex. Purified recombinant proteins were prepared and assessment of binding affinity along with evaluation of protein-protein interactions were performed by microscale thermophoresis (MST). Surprisingly, a high-affinity binding (K_d = 0.25-2.7 µM) was observed between hANG and hGal7. This represents a stronger affinity than that of known small molecule ligands of each protein. Furthermore, the presence of both exogenous hANG and hGal7 in media showed a much higher induction of proliferation than that of each individual protein on HeLa cells. Binding assays (MST) with other structural homologs will allow us to evaluate the specificity of this interaction, while NMR titration experiments will enable mapping of binding interactions between each partner. Finally, the influence of this new interaction will be determined by performing assessment of biological activities of this protein complex in cancer progression.

P26 - Ancestral Sequence Reconstruction Dissects Structural and Functional Differences Among Eosinophil Ribonucleases

Thi Thanh Quynh Tran[1], Chitra Narayanan[2], Andrea N. Loes[3], Timothy H. Click[4], Ngoc Thu Hang Pham[5], Myriam Létourneau[6], Michael J. Harms[3], Charles Calmettes[1], Pratul K. Agarwal[4], Nicolas Doucet [1, 7]

[1]Centre Armand-Frappier Santé Biotechnologie, Institut national de la recherche scientifique (INRS), Université du Québec, Laval, Quebec, Canada, [2]Chemistry Department, New Jersey City University, Jersey, New Jersey, USA, [3]Institute of Molecular Biology and Department of Chemistry and Biochemistry, University of Oregon, Eugene, OR, 97403, USA, [4]Department of Physiological Sciences and High-Performance Computing Center, Oklahoma State University, Stillwater, Oklahoma, USA, [5]Department of Chemistry and Biomolecular Sciences, University of Ottawa, Ontario, Canada, [6]NMX Research and Solutions Inc., Laval, Quebec, Canada, [7]The Quebec Network for Research on Protein Function, Engineering, and Applications, Université Laval, Québec, Quebec, Canada.

Distinct biological activities can evolutionarily arise from a conserved structural fold. For instance, human pancreatic-type ribonucleases (RNases) share a common structural core that evolved to maintain diverse biological functions such as antibacterial, and cytotoxic activities, while also preserving ribonucleolytic activity. Ancestral Sequence Reconstruction (ASR) can reveal the relationship between sequence and function during protein evolution. In this study, an ancestral sequence (AncRNase) between two human eosinophil RNases (human RNases 2 and 3) was generated based on a phylogenetic analysis of 26 different RNase sequences. AncRNase exhibits 91% and 73% sequence identity with human RNases 2 and 3, respectively. We showed that AncRNase also shares common properties with modern RNases, including ribonucleolytic and antibacterial activities. Based on the functional properties of AncRNase and its contemporary homologous sequences, we identified four residues playing important roles in antibacterial and cytotoxic activity within this fold. Crystallographic structure elucidation shows that AncRNase preserves the typical RNase fold and exhibits distinct active-site conformations for two essential catalytic residues. Atomic-scale analysis offers insights into mutational and evolutionary pathways governing structure and function in this promiscuous fold, in addition to offering means to specifically target and modulate RNase-associated functions.

P27 - Viral evasion of IFIT1 binding by methylation and 5'UTR secondary structure

Zixian Li[1], Bhushan Nagar[1]

[1]*Department of Biochemistry and Centre de Recherche en Biologie Structurale (CRBS), McGill University*

The life-threatening nature of viral infection has been brought to the forefront by the Covid-19 pandemic, the novel coronavirus has caused over six-and-a-half million deaths globally. As the first line of host defense, the innate immune system detects pathogen-associated molecular patterns (PAMPs) upon viral invasion. PAMPs, such as viral nucleic acids, are sensed by specific pattern recognition receptors, which trigger signaling cascades resulting in the expression of virus-responsive type I interferon (IFN) and pro-inflammatory cytokines. IFNs further induce the expression of diverse IFN-stimulated genes (ISGs) that inhibit viral replication. Amongst the most potently expressed ISGs are the interferon-induced proteins with tetratricopeptide repeats (IFITs). The crystal structure of IFIT1 sequestering 5' terminal region of nonself Cap0-RNA was solved in the Nagar lab. Facing the potent viral inhibition exerted by IFITs and other anti-viral effectors, viruses are driven to counteract to survive and thrive. Using in vitro viral RNA binding assays complemented with molecular dynamics simulations, we reveal how viruses utilize methylation and secondary structure at 5'UTR of genomic or messenger RNA to circumvent IFIT1 defense.

P28 - Essais de décalage thermique en contexte cellulaire (CETSA) et évaluation du potentiel de liaison de nouveaux ligands d'une protéine RAS.

Jonathan Gagnon[1], Hala Elasmy [1], Michael Maddalena [1], Sacha Larda[2], Steven Laplante[1, 2]

[1]INRS, Centre Armand-Frappier Santé Biotechnologie. , [2]NMX Research and Solutions Inc.

Les protéines de la famille RAS dont les mutations sont très répandues dans une vaste panoplie de cancers représentent des cibles de premier plan pour le traitement de plusieurs types de cancers, incluant le cancer de la vessie. Par contre, pour des raisons structurales, cibler directement ces protéines représente un défi de taille. Elles ont longtemps été qualifiées comme non ciblables ce qui a mené au développement d'approches indirectes afin d'inhiber leurs activités. Ce n'est que récemment que des stratégies d'inhibition avec ligand direct des protéines RAS ont été développées. Grâce à l'approche de découverte de médicaments basée sur des fragments, notre laboratoire en collaboration avec l'équipe de NMX Research Solutions Inc. à découvert de nouvelles molécules ayant la capacité d'interagir avec des protéines RAS en contexte *in vitro*. Le prochain objectif est donc d'évaluer leur potentiel de liaison dans des contextes cellulaires au moyen de la technique d'essais de décalage thermique en contexte cellulaire (CETSA). Cette méthode se base sur le principe d'une modulation de la stabilité thermique des protéines lorsqu'elles interagissent avec un ligand afin d'évaluer le potentiel de liaison d'un composé. Jusqu'à présent, des résultats préliminaires pour les nouveaux ligands de RAS ont été générés dans un système *in vitro* et dans des lysats de bactéries surexprimant une protéine RAS mutante. Des essais de CETSA ont également été réalisés sur des lysats provenant d'une lignée cellulaire du cancer de la vessie exprimant de manière endogène une forme mutante de RAS. Cette technique et ces résultats seront utiles afin d'évaluer une potentielle stabilisation ou déstabilisation thermique des protéines cibles en réponse à une interaction avec les ligands en contexte cellulaire. Au long terme, l'étude de ces composés pourrait mener au développement de nouvelles thérapies hautement efficaces face aux différents cancers où des mutations des protéines RAS sont retrouvées.

P29 - Effects of lanthanide-doped upconverting nanoparticles on nuclear homeostasis

Kais Bietar[1], Siwei Chu[1], Renata Sabelli[1], Vaibhavi Shetty[1], Ursula Stochaj[1]

[1]*McGill University*

Upconverting nanoparticles (UCNPs) can generate high energy photons through the absorption of multiple photons of lower energy through a process known as photon upconversion. Lanthanide-doped UCNPs (Ln-UCNPs) are a class of nanoparticles that have unique physio-chemical properties. These features are ideal for biological and medical applications. Accordingly, Ln-UCNPs have emerged as promising tools for theranostics. However, the bio-nano interactions of Ln-UCNPs are poorly understood. This knowledge gap has limited the use of Ln-UCNPs in living cells.

Nuclear homeostasis is essential to cope with stress, such as the exposure to nanomaterials. Our research defines the impact of Ln-UCNPs on cell physiology, as it relates to nuclear biology and stress responses. To this end, we are assessing the effects of Ln-UCNPs on cell viability, the localization and abundance of key components of the nucleus, and the damage to this organelle. Our work provides a quantitative readout for stress responses, proteostasis, and cell organization in Ln-UCNP treated cells. Non-malignant fibroblasts and cancer cells serve as main model systems.

Collectively, our experiments determine the biocompatibility and subcellular interactions of Ln-UCNPs. Long-term, this research is expected to generate novel therapeutic and diagnostic tools that can be used for targeted drug delivery and biomedical imaging.

P30 - Sterol Binding and Selectivity by ABCG5/G8

Danny Farhat[1]

[1]*Department of Biochemistry, Microbiology and Immunology, Faculty of Medicine, University of Ottawa*

In the current study, we have determined the crystal structure of ABCG5/G8 in cholesterol-bound state. The structure combined with amino acid sequence analysis shows that in the proximity of the sterol-binding site, a highly conserved phenylalanine array is critical for ABCG cholesterol/sterol transporter functions. Lastly, *in silico* docking analysis of cholesterol and stigmasterol (a plant sterol) suggests sterol-binding selectivity on ABCG5/G8.

P31 - Probing the cancer mutational landscape and binding properties of Cfp1 PHD domain

Sabrina Grégoire[1], Janelle Grégoire[1], Monika Joshi[2], Sabrina Capitani[1], Jean-François Couture[3]

[1]Undergraduate Student, [2]Research Assistant, [3]Principal Investigator

COMPlex ASsociating with SET1 (COMPASS) is a histone H3K4 tri-methyltransferase predominantly linked to actively transcribed genes. COMPASS comprises many subunits, including CxxC zinc finger protein 1 (Cfp1). Cfp1 plays a crucial role in controlling H3K4 methylation, and such activity is mediated by Cfp1's functional domains, including a CXXC domain and plant homeodomain (PHD). CXXC domain binds unmethylated CpG islands and Cfp1 PHD domain directly binds histone H3 when K4 is tri-methylated, contributing to COMPASS binding to chromatin. How Cfp1 PHD domain binds H3K4me3 is still unknown. The structure of Cfp1 PHD folds as an anti-parallel b-sheet coordinating two zinc atoms using two clusters of cysteine and histidine residues. Comparative analysis shows that Cfp1 PHD domain N-terminus folds in the opposite direction compared to other PHD domains. Most cancer mutations mapping to Cfp1 PHD domain negatively impact its ability to bind H3K4me3 with minimal impact on its structure. Finally, we show that CXXC domain negatively regulates Cfp1's ability to bind H3K4me3. These results collectively suggest that the cancer-related mutations in the Cfp1 PHD domain can potentially impact epigenetic signalling and a multi-step mechanism control Cfp1 binding to chromatin.

P32 - Recruitment and activation of the structure-specific endonuclease Rad1-Rad10

Javier Rodriguez Gonzalez[1, 2], Alba Guarne[1, 2]

[1]McGill University, [2]Centre de Recherche en Biologie Structurale

Most DNA repair pathways rely on the activity of structure-specific endonucleases to process damaged DNA substrates. Because unregulated cuts on DNA can be highly deleterious to the cell, repair nucleases must undergo specific recruitment and activation for activity. *Saccharomyces cerevisiae* Rad1-Rad10 (XPF-ERCC1 in humans) is a major endonuclease of the DNA damage response with activity in several repair pathways, including single-strand annealing that fixes double-stranded breaks in the DNA. Recently, it was shown that the role of Rad1-Rad10 in single-strand annealing requires the Saw1 protein. However, the mechanism by which Saw1 recruits and activates Rad1-Rad10 is currently unknown. To understand this mechanism, we assembled the Rad1-Rad10-Saw1-DNA complex *in vitro* and pursued its cryo-EM structure. Imaging of the sample yielded a 4.2 Å resolution map into which we were able to build a partial model of the complex. Our model reveals how Saw1 wraps around the N-terminal region of Rad1 forming an extensive interface. We thus identified the stoichiometry of the apo-complex and the interactions that are important for complex formation, which were validated using site-directed mutagenesis. Our EM map, however, lacks density for the DNA that is likely dissociating at the air-water interface during sample preparation. To prevent this, we are currently exploring the use of chemical crosslinkers and detergents. Comparison of the apo- and DNA-bound structures will reveal the interactions that are important for the recruitment and activation of the Rad1-Rad10 nuclease to recombination intermediates. This work sheds light on the mechanism of action of DNA repair regulatory factors, a fundamental aspect of the DNA damage response and genomic stability.

P33 - Structure-guided optimization of functional tetrahedral DNA nanocages sensitive to hAGT

Tianxiao Yang[1], William Copp[2], Stacey Wetmore[3], Alba Guarné[1]

[1]McGill University, [2]Concordia University, [3]University of Lethbridge

DNA is a promising biomaterial that has been used to create nanostructures with diverse functions. Certain DNA nanostructures, such as DNA nanocages, have been employed as delivery devices and biosensors of macromolecules. We have designed tetrahedral DNA nanocages held together by alkylene linkers that can be recognized and cleaved by the human DNA repair protein O6-alkylguanine-DNA alkyltransferase (hAGT). As hAGT is overexpressed in multiple types of cancers, we hypothesize that these cages can be selectively "opened" in cancer cells to facilitate the delivery of cargo and/or act as an hAGT biosensor. Though DNA nanocages have been extensively characterized using single-molecular techniques, atomic resolution approaches have not yet been explored. We are therefore using a novel structure-guided approach by combining cryo-electron microscopy, x-ray crystallography, and molecular dynamics simulations to characterize the structure of these cages in complex with a catalytic dead variant of hAGT. Ultimately, this work will reveal how hAGT recognizes the alkylene linkers at the molecular level so that the design of the cages can be optimized to maximize their function in cancer cells.

P34 - Structural insights into the interplay between UTX-MLL4 to identify its role in epigenetic signalling and cancer

Monika Joshi[1], Jean-Francois Couture[1]

[1]*Ottawa Institute of Systems Biology, Department of Biochemistry, Microbiology and Immunology, Faculty of Medicine, University of Ottawa, 451 Smyth Road, Ottawa, Ontario K1H 8M5, Canada*

Cancer challenges global public health as one of the major causes of mortality worldwide. Epigenetic changes such as dysregulation of enzymes modifying DNA or histones are directly linked to oncogenesis. Numerous pieces of evidence show that the deregulation of Histone 3 lysine 27 (H3K27) demethylation leads to cancer progression. The histone demethylase UTX (Ubiquitously transcribed tetracopeptide repeat on chromosome X) specifically targets di- and tri-methyl groups on lysine 27 on histone H3 (H3K27me2/3). UTX function has been linked to homeotic gene expression, embryonic development, and cellular reprogramming. UTX comprises a tetracopeptide repeat (TPR) domain that interacts with the lysine methyltransferase Myeloid Lymphoma Leukemia 4 (MLL4). To better understand the biochemical determinants underlying the interactions between MLL4 and UTX, we mapped the minimal binding region of MLL4 binding to UTX^{TPR} and showed that the residues 4552-4631 of MLL4 binds UTX. We also optimized the purification of UTX^{TPR} in complex with a fragment of MLL4. In the future, we will combine mutational studies, isothermal titration calorimetry, and structural studies to solve the structure of the UTX^{TPR} /MLL4 complex. The structural and functional characterization of UTX^{TPR} /MLL4 complex will eventually unravel how these proteins are linked to epigenetic signalling and contribute to cancer development and progression.

P35 - Investigation of the unique structural elements and catalytically important dynamic regions of the Nisin biosynthetic enzyme NisC

Nuwani Weerasinghe[1], Christopher Thibodeaux[1]

[1]*McGill University*

Ribosomally synthesized and post translationally modified peptides (RiPPs) are structurally diverse natural products that often possess potent antimicrobial properties. The widely-used food preservative, Nisin, is an antimicrobial RiPP that possesses intramolecular thioether linkages which are critical for its bioactivity. Modification of the precursor peptide NisA to bioactive nisin, is catalyzed by two enzymes NisB and NisC. NisB catalyzes dehydration of serine/threonine residues in NisA and NisC catalyzes intramolecular addition of cysteine residues onto the dehydrated sites to form the thioether rings, with precisely controlled regio- and stereospecificity. Previous studies have demonstrated how the presence of thio-ether linkages increase the stability and bioactivity of RiPPs. Furthermore, the genetic encodability of RiPPs and the relaxed substrate specificity of the biosynthetic enzymes make them promising candidates for the engineering of novel drugs. However, the mechanistic details that regulate the peptide modification remain unclear. In this research, our goal is to better understand the molecular mechanism and the biophysical interactions involved in the cyclization process. Towards this goal, we have developed a mass spectrometry based assay to monitor the NisC activity under in vitro conditions. Furthermore, our hydrogen deuterium exchange mass spectrometry studies of NisC in the presence and absence of NisA revealed a potentially novel leader peptide binding motif and other interesting structural elements which will be further investigated with mutational studies.

P36 - Converting an amyloidogenic polypeptide into a potent GPCR ligand through α-helix stapling

Margaryta Babych[1, 2], Phuong Trang Nguyen[1, 2], Vy Nguyen[1, 2], Frédérique Bérubé[1, 2], Steve Bourgault[1, 2]

[1]Department of Chemistry, Université du Québec à Montréal, [2]Quebec Network for Research on Protein Function, Engineering and Applications, PROTEO

The islet amyloid polypeptide (IAPP) is a 37-residue peptide hormone co-secreted with insulin by the pancreatic β-cells, which plays a role in glucose homeostasis via the activation of G-protein-coupled receptors (GPCRs). In addition to playing a physiological role as an hormone, IAPP is particularly known as the main component of amyloid deposits found in the pancreatic islets of patients afflicted with type 2 diabetes (T2D). The adoption of different conformations of this natively disordered peptide finely modulates its physiological role, by providing high affinity to cognate GPCRs, or its pathological implication via aggregation into toxic transient oligomers and eventually cross-β-sheet amyloid fibrils. In this context, the objective of this project is to develop potent stable helical derivatives of IAPP using various chemical approaches to modulate and investigate the function/cytotoxicity duality of IAPP. To achieve this, we hypothesized that the stabilisation of transient helical secondary conformation in N-terminus could interfere with the early steps of (mis)folding of the native peptide and prevent, or delay, aggregation while preserving its affinity for its receptors. Two macrocyclisation strategies to design a library of constrained analogs were used. Stabilization of the helical conformation was carried out between two non charged residues in the central domain and located on the same face of the helix (*i, i+4*), by lactamization or azide-alkyne click chemistry to confer structural restriction. The stabilized helical structure strongly altered the propensity of IAPP to self-assemble, to perturb synthetic lipid vesicles, and to induce death of β-pancreatic cells. Overall, this study constitutes the first example of macrocyclization as a chemical tool applied to an amyloidogenic peptide. Targeting specific helical conformations could lead to a high receptor activation combined with an anti-aggregation effect on endogenous circulating IAPP.

P37 - Interrogating a cytoskeleton-associated nanoscale condensate using super-resolution microscopy

Katherine Morelli[1], Sandro M Meier[2], Madeline Willis[1], Angela Zhao[1], Yves Barral[2], Jackie Vogel[1]

[1]Department of Biology, McGill University, Montreal, Canada, [2]Institute of Biochemistry, Department of Biology, ETH Zürich, Zürich, Switzerland

Eukaryotic cells coordinate interactions between actin filaments and microtubules to serve various important cellular functions. One such function is positioning and alignment of the mitotic spindle during asymmetric cell division in budding yeast. To position the spindle, astral microtubules which are nucleated at the spindle pole bodies (SPBs, yeast centrosomes) and project into the cytoplasm interact with the polarized actin network which originates at the tip of the bud through the plus-end tracking (+TIP) Kar9. Recent work demonstrates that Kar9 and two other +TIPs, the EB1 homolog Bim1 and the CLIP170 homolog Bik1, form a liquid-like condensate which tracks the plus ends of growing and shrinking astral microtubules. However, directly observing predicted liquid-like behavior in living cells is challenging because Kar9 condensates have diameters around 200 nanometers or less in vivo. Therefore, we use lattice structured illumination microscopy super-resolution to study behaviors which emerge from material properties and determine condensate functions in vivo. Using time series with >15 frames per second, we develop a method to annotate dynamic microtubule behavior according to dynamic instability parameters using Kar9 as a plus end marker. Strikingly, shorter metaphase microtubules appear to grow and shrink along the contour of longer microtubules from the same SPB. These astral microtubules undergo rapid angular motions of astral microtubules relative to the SPB which most likely result from actin-bound myosin V pulling on Kar9 at their tips. Remarkably parallel microtubules remain linked during these motions. We conclude that microtubules are bundled by a mechanism that persists during phases of microtubule dynamic instability and rapid motion through the cytoplasm.

P38 - Huntingtin polyglutamine expansions impair the transport of signalling and degradative cargoes

Emily Prowse[1], Brooke Turkalj[1], Adam Hendricks[1]

[1]McGill University Bioengineering Department

Huntingtin scaffolds motor proteins and adaptors to vesicular cargoes. Through interactions with kinesin, dynein, and myosin-VI, huntingtin regulates the direction of transport along microtubules and switching between microtubules and actin filaments. Huntington's disease (HD) is caused by polyglutamine (polyQhtt) expansion of length >35Q at huntingtin's n-terminus, with longer repeats leading to more severe neurodegeneration. Defects in transport of brain-derived neurotrophic factor (BDNF) and lysosomes have been reported in cells expressing polyQhtt. We track signalling BDNF vesicles and degradative lysosomes in induced neurons from isogenic human stem cell lines with repeat lengths of 18, 30, 45, 65, and 81Q to understand how transport of signalling and degradative cargoes are affected in HD. Preliminary data indicate that lysosomes have lower processivity and similar run lengths with pathological polyQhtt. Interestingly, the 45Q cells have lower processivity and run length compared to all other conditions, indicating divergent mechanisms might contribute to mild and severe disease phenotypes. Lysosomes are typically transported towards the soma. However, with polyQhtt we observe increased outward motility, suggesting huntingtin's ability to regulate transport direction is impaired. To examine how polyQ huntingtin alters the activity of kinesin and dynein, we use optical tweezers to measure the forces exerted on endocytosed BDNF-coated nanodiamonds in control compared to 81Q neurons. BDNF-coated nanodiamonds (~100 nm) are readily endocytosed by neurons and enable reliable force measurements due to their high refractive index. In parallel, we developed methods to isolate BDNF-phagosomes from neurons and reconstitute their motility along microtubules. Together, live-cell imaging, optical trapping, and in vitro reconstitution reveal how HD mutations impact the transport of signalling and degradative cargoes by misregulating the recruitment and activity of kinesin and dynein.

P39 - Structural and functional characterization of a Helicobacter pylori adhesine like-protein

Paula Armoa Ortiz[1]

[1]*Institut National de Recherche Scientifique - AFSB*

Helicobacter pylori (*H. pylori*) est un bacille gram-négatif, qui colonise la muqueuse gastrique et est capable d'établir une infection chronique à vie (infections gastro-intestinales, ulcère peptique et cancer gastrique). La première étape de sa pathogénicité est l'adhésion aux cellules épithéliales gastriques.

Nous avons identifié plusieurs gènes présents dans des souches gastriques d'*H. pylori,* une protéine effectrice putative d'*H. pylori* est codée par l'un de ces gènes. Pour étudier la fonction de cette protéine effectrice/adhésine putative d'*H. pylori* nous avons résolu sa structure par cristallographie qui montre la présence de 4 domaines et des mutants rationnels ont été créés à partir de ces informations obtenues. Des études avec des modèles cellulaires gastriques et immunitaires seront menées pour étudier son effet sur la régulation de l'expression de gènes, la localisation sur les cellules et ses possibles partenaires d'interaction.

P40 - Inter-domain coupling and pharmacophores modulate ABC-transporters post-translational folding landscape

Naoto Soya[1], Haijin Xu[1], Ariel Roldan[1], Yang Zhengrong[2], Haoxin Ye[1], Aiswarya Premchandar[1], Guido Veit[1], Susan Cole[3], John Kappes[2], Tamas Hegedus[4], Gergely Lukacs[1]

[1]McGill University, [2]University of Alabama School of Medicine, [3]Queen's University Cancer Research Institute, [4]Semmelweis University

The contribution and mechanism of the obligate co- and facultative post-translational domain- folding to the conformation maturation of multi-domain membrane are not fully understood. Here, we interrogate the contribution of domain-domain interactions to the kinetically controlled conformational biogenesis of ATP-binding cassette (ABC)-transporters C subfamily, consisting minimally of two nucleotide binding (NBD1,2) and transmembrane (TMD1,2) domains. Disruption of NBD1-TMD2 or NBD1-TMD1 interfaces revealed their permissive role in the post-translational cooperative domain folding of CFTR(ABCC7), MRP1(ABCC1), and ABCC6 nascent chains. Conversely, genetic or pharmacological rigidification of NBD1 or TMDs dynamics promoted native-like conformational ensembles by rearranging interdomain allosteric networks of purified CFTR-variants, indicated by limited-proteolysis, hydrogen-deuterium-exchange mass spectrometry (HDX-MS), and molecular dynamic simulation. The long-range conformational stabilization of a folding-defective purified CFTR mutants by the VX-809 (VX-661 analogue) or VX-445 CFTR-modulator (components of the FDA-approved Trikafta drug combination for >170 CF mutations) was unraveled with the HDX-MS technique. Jointly, based on these and other published results, we propose a folding model of ABCC transporters that incorporates a critical role for the inter-domain allostery that tunes the folding-trajectory of post-translational folding-intermediates, is frustrated by diseases-causing mutations, and essential for the pharmacophore-dependent CFTR misfolding correction.

P41 - Structural basis of TnsC recruitment to TnsD binding sites in Tn7 transposition

Yao Shen[1], Michael Petassi[2], Joseph Peters[2], Alba Guarné[1]

[1]Department of Biochemistry, McGill University, Montreal (QC), Canada, [2]Department of Microbiology, Cornell University, Ithaca (NY), USA

Tn7 and CRISPR-associated Tn7 transposons (CAST) elements are unique in their ability to recognize highly specific target sites, making them ideal tools for site-directed DNA insertion. The precise targeting mechanism of Tn7 relies on the coordinated actions of multiple Tn7-encoded proteins, including a heteromeric transposase (TnsAB), an AAA+ ATPase (TnsC), and target selection proteins (TnsD/TniQ and TnsE). TnsD in the Tn7 element recognizes the *attTn7* site in a sequence-specific manner, TniQ recognizes CRISPR-effector proteins in CAST systems for RNA-guided transposition, and TnsE targets sites of replication in conjugal plasmids. In both Tn7 and CAST systems, the interplay between TnsC and the target selection proteins facilitates the insertion of Tn7 in a defined orientation at a fixed distance from the target site. Here we present the cryo-EM structures of several TnsC-TnsD complexes from the prototypical Tn7 elements. These structures explain how TnsD recruits and promotes TnsC oligomerization at a target site. These findings, combined with our previous work on TnsC, provide the mechanistic understanding of underlying targeting at the *attTn7* site and recruitment of the TnsAB transposase at a strict spacing from the target site.

P42 - Structural Characterization of Single Module Non-Ribosomal Peptide Synthetase IndC

Kamal Adhikari[1]

[1]Biochemistry

Nonribosomal peptides (NRPs) are a major group of natural product compounds, well known for their biological activities. They are clinically used for many therapeutic applications, acting as antivirals (cyclosporin A), immunosuppressants (rapamycin), antifungal (caspofungin), anticancer agents (dactinomycin), or antibiotics (penicillin, vancomycin, and daptomycin) and along with the industrial application, including polymers and dye. Indigoidine synthetase (IndC) is a single-module NRPS and is involved in the biosynthesis of a bioactive, bright purple dye called Indigoidine from two molecules of L-glutamine in an ATP-dependent reaction. Structural characterization is needed to understand the NRPSs architecture, function, and communication of the NRPS domain during the synthetic cycle of NRPS and to facilitate bioengineering to produce new chemical entities. In this present study we cloned a gene, IndC from thermophilic *Streptomyces thermodiastaticus* and heterologously expressed it in *E. coli* BAP1 cell, and then purified the recombinant proteins using standard purification protocol. Differential scattering fluorimetry (DSF) was conducted to identify buffer strength and a melting point of IndC. We have shown that adenylation activity shows more typical substrate specificity to L-glutamine for IndC. Biochemical characterization was performed to ensure IndC is active, or it was simply monitored how quickly the solution in the tube turns purple. Product formation was confirmed by using mass spectrometry. Purified IndC was crystallized using the sitting-drop vapor diffusion method in a 1:1 ratio of protein and mother liquor (0.1M HEPES pH 7.5, 22 % BSP) at room temperature. All the diffraction data were collected at Advance Photo Source (APS), USA.

P43 - Synthesis and evaluation of inositol phosphate analogues as therapeutics against Clostridioides difficile toxin B

Rebecca Cummer[1], Dr. Raphaël Bolteau[1], Lauren Finn[2], Dr. Bettina Keller[2], Dr. Bastien Castagner[1]

[1]McGill, [2]Freie Universität Berlin

Clostridioides difficile (*C. diff*) is a bacterium that causes the most prevalent form of hospital-acquired infection. This infection can cause symptoms varying from diarrhea to life-threatening inflammation of the colon. *C. diff* is considered one of the 5 most urgent antibiotic resistant pathogens by the U.S Center for Disease Control and Prevention. The most prevalent treatments for *C. diff* infections are antibiotics. However, antibiotics kill bacteria in the intestine that prevent *C. diff* from colonizing, resulting in high rates of disease relapse. As a result, there is a need for new treatments against *C. diff* infections. One treatment strategy would be to target toxins released by *C. diff*. The toxins make an ideal drug target as they are the primary cause of symptoms. At present there are no small molecule drugs that target these toxins, neither on the market nor in clinical testing. We propose to develop a small molecule drug that disassembles the toxins in the gut lumen, rendering the toxins inactive, before they can provoke disease.

The toxins have four functional domains: a cysteine protease domain (CPD), a glucosyltransferase domain (GTD), a receptor binding domain, and a transmembrane domain. Inositol hexakisphosphate (IP6), found in eukaryotic cells, can bind to the CPD, triggering an auto-proteolysis event. This event liberates the GTD inside the cell so that it causes pathogenesis. Our lab has previously shown the efficacy of IP6 analogues at pre-emptively inducing auto-proteolysis, which renders the toxin ineffective. We are currently optimizing the IP6 analogues by probing their structure activity relationship with the toxin. We have found one lead compound which is more effective at cleaving the toxin than IP6, due to its improved affinty for the CPD. This drug design tactic could provide a much-needed alternative to antibiotics for treating *C. diff* infections.

P44 - HIV-1 Maturation Inhibitors and Retroviral Inositol Phosphate Packaging

Justin Meneses[1], Bastien Castagner[1]

[1]*McGill*

Inositol hexakisphosphate (IP6) is an important co-factor for multiple proteins, protein-protein interactions, exogenous proteins such as bacterial toxins and effectors, as well as viral proteins. Recently, it was shown that IP6 facilitates the assembly of the immature HIV-1 Gag polyprotein lattice and is essential to HIV-1 maturation. Moreover, the new class of HIV-1 antiretrovirals termed maturation inhibitors (MIs), which inhibit protease mediated cleavage of Gag, have been shown to bind directly below the IP6 binding site. This suggests that IP6 coordinating residues and the IP6 binding site may be exploited by novel MIs that extend into the IP6 cavity. We hypothesize that simultaneous inhibition of Gag cleavage and IP6 packaging by these extended MIs could provide dual inhibitory mechanisms and lead to more potent MIs that are less susceptible to the development of resistance. We aim to design and synthesize extended MIs to evaluate their structure-activity relationship to inhibiting HIV-1 maturation, by quantifying their ability to prevent Gag cleavage and inhibit IP6 packaging. Most HIV-1 MIs are based on the naturally occurring pentacyclic triterpenoid betulinic acid. IP analogs bearing a succinate linker moiety on the axial hydroxyl have been prepared and coupled to betulinic acid's C-3 hydroxyl group. A variety of groups able to interact with IP coordinating lysine residues have also been coupled to betulinic acid's C-3 hydroxyl group, which is believed to be pointing towards the IP6 binding cavity in the HIV-1 immature Gag hexamer. These molecules will be assessed on their ability to maintain Gag cleavage inhibition, hinder IP packaging, and ultimately prevent viral maturation. This work expands upon our synthetic methodology to prepare novel IP analogs and investigates the relationship between HIV-1 maturation inhibitors and packaged inositol phosphates, potentially revealing novel therapeutic strategies.

P45 - Native doublet microtubules from Tetrahymena thermophila reveal the importance of outer junction proteins

Corbin Black[1], Shintaroh Shintaroh Kubo[1], Ewa Joachimiak[2], Shun Kai Yang[1], Thibault Legal[1], Katya Peri[1], Ahmad Khalifa[1], Avrin Ghanaeian[1], Melissa Valente-Paterno[1], Chelsea De Bellis[1], Phuong Huynh[1], Zhe Fan[1], Dorota Wloga[2], Khanh Huy Bui[1]

[1]McGill University, [2]Nencki Institute of Experimental Biology of Polish Academy of Sciences

Cilia are hair-like organelles responsible for cellular motility and sensory functions. The cytoskeletal core of the cilium consists of nine hollow doublet microtubules arranged in a ring around two central singlets. It was recently discovered through cryogenic electron microscopy that there exist many different microtubule inner proteins (MIPs) inside each doublet microtubule. These MIPs form a meshwork of luminal proteins weaving into the tubulin lattice. Knockout of MIPs such as FAP52 and FAP45 destabilizes the doublet microtubule and causes a reduced swimming phenotype observed in both *Tetrahymena thermophila* and *Chlamydomonas reinhardtii*. The interaction of MIPs with the tubulin lattice suggests that some MIPs are essential for assembly and stability of the doublet microtubule. However, the individual functions of MIPs are largely unknown. I identified and modeled MIPs that are potentially responsible for the assembly and stability of the *Tetrahymena*doublet microtubule in the outer junction region. Gene knockout of the conserved outer junction protein CFAP77 reduces swimming and ciliary beating in *Tetrahymena*. Cryogenic electron tomography of cilia from the CFAP77 knockout strain reveals damage to doublet microtubules compared to wild type. These results suggest that CFAP77 has an important role in outer junction stability and ciliary beating.

P46 - How does γ-tubulin phosphoregulation control microtubule identity during spindle assembly?

Shannon Sim[1], Katherine Morelli[1], Angela Zhao[1], Jackie Vogel[1]

[1]*Department of Biology, McGill University*

The mitotic spindle ensures the accurate partitioning of the chromosomes during mitosis. It is composed of microtubules (MTs) and of proteins that influence its organization and function. The spindle MTs carry out one of two essential functions. The kinetochore MTs attach to the chromosomes and promote the separation of the sister chromatids during anaphase. The interpolar MTs (ipMTs) form antiparallel pairs with MTs nucleated by the opposite spindle pole. In budding yeast, spindle assembly begins when the ipMT precursors are formed by Cin8 (kinesin-5) crosslinking. Following bipolar spindle formation, these antiparallel overlaps are increased and become the ipMTs that form the spindle midzone. The study of spindle MT organization has been primarily limited to the interactions between MTs and chromosomes. However, the control of MT pairing is equally important, as the ipMTs stabilize the spindle and promote chromosome capture. Surprisingly, the regulation of γ-tubulin, which nucleates MTs, may control ipMT number. The phosphomimetic Y445D mutation in γ-tubulin triggers the spindle assembly checkpoint, disrupts the spindle midzone, and causes spindle instability. The ipMT crosslinker Ase1, essential in the absence of Cin8, is essential in Y445D cells. We also showed that the Y445D γ-tubulin C-terminus explores unique extended conformations. Using live-cell fluorescence microscopy, I find that Cin8 is enriched at the spindle poles and decreased at the midzone. Ase1 is found at the midzone of Y445D spindles, as in the wild type, but in larger amounts. Y445D spindles are more stable in the absence of the mitotic cyclin Clb2 and when the phosphorylation of Ase1 is inhibited, but the localization of Cin8 remains abnormal. I propose that the phosphorylation of Y445, which alters the conformations of the γ-tubulin C-terminus, sequesters Cin8 at the spindle poles, inhibiting the formation of the ipMTs, and that Ase1 partially compensates for Cin8's absence at the midzone.

P47 - Structural study of CNNM extracellular domain

Ashkan Shahsavan[1]

[1]*Biochmeistry department, McGill University*

Cystathionine-β-synthase (CBS)-pair domain divalent metal cation transport mediators (CNNMs) are a highly evolutionarily conserved family of integral membrane proteins that are implicated in magnesium homeostasis. Out of the four members of CNNMs in mammals, CNNM2 and CNNM4 posses Mg^{2+} efflux activity. Mutations in CNNM2 and CNNM4 are associated with hereditary diseases such as hypomagnesemia and Jalili syndrome, respectively. The conserved core of CNNMs consist of transmembrane and CBS-pair domains, while eukaryotic CNNMs contain an additional extracellular domain. It has been shown that the N-glycosylation of the extracellular domain of CNNM2 is essential for its stabilization on the plasma membrane. Recently, ADP-ribosylation factor-like GTPase 15 (ARL15) has been identified as CNNM binding partner and it is suggested that ARL15 affects CNNM transport activity through modification of N-glycosylation at the extracellular domain. Using X-ray crystallography, we solved the structure of the extracellular domain of an ortholog of CNNM4 from *Echinococcus granulosus* (dog tapeworm) at 2.7 Å resolution. Despite the low sequence identity of EgCNNM4 extracellular domain to that of human CNNM4 (27%), the crystal structure of EgCNNM4 is similar to hCNNM4 structure predicted by Alphafold as they both have Ig-like fold. Analytical ultracentrifugation (AUC) showed that both EgCNNM4 and hCNNM4 extracellular domains form dimers in solution. Electron density of glycans is observed at Asn-51 and Asn-63 in the crystal structure of EgCNNM4 and glycosylation of hCNNM4 is confirmed by mass spectrometry. In this study, we solved the crystal structure of the extracellular domain of a eukaryotic CNNM4 ortholog for the first time that will be therapeutically insightful given the disease-associated mutations on this domain.

P48 - Identification and characterization of a novel adaptive factor of Helicobacter pylori

Cyrielle Martini[1], Charles Calmettes[1]

[1]*Institut National de la Recherche Scientifique, Centre Armand-Frappier Santé Biotechnologie, Laval, QC.*

Helicobacter pylori, a gram-negative bacteria, is the only pathogen capable of colonizing the gastric niche in human. Meta-analysis shows that almost half of the worldwide population is infected with *H. pylori*. Even if most people infected have no symptoms, persistent infections can lead to gastric pathologies like peptic ulcer, gastritis, and adenocarcinoma, most frequently because *H. pylori* can adhere to the stomach lining and adapt to the acid environment by unknown processes.

Our phylogenetic study of different strain of *Helicobacter*, allowed us to identify a putative adhesin potentially involved in this mechanism. As this protein has no homologous known, we first determine its structure by X-ray crystallography. After optimization of crystallisation condition, an X-ray data set was collected to 3 Ångström resolution and the structure was determined by molecular replacement.

The structure reveals the presence of 2 unstructured loops located at the same place on the protein. The first one contains a potential sialic acid motif (KRTIQK) while the second loop contains several hydrophobic residues suggesting their participation in an interaction.

In vitro analyzes will be carried out to determine the ability of our protein of interest to bind to gastric cells (AGS cells) as well as to determine its interaction partners.

The characterization of this protein will allow us to better understand colonization mechanism of *Helicobacter pylori* and identify potential new therapeutic targets.

P49 - Structural and Functional Studies of Integral Membrane Nonribosomal Peptide Synthetases

Timothy Ho[1], Martin Schmeing[1]

[1]*McGill University*

Poly-aminoacyl synthetases (PASs) are integral membrane nonribosomal peptide synthetases (NRPSs) found in many bacteria and fungi that produce special amino acid homopolymers. These homopolymers, such as ε-poly-lysine (ε-PL), are currently used in many countries as natural food preservatives due to their unique antimicrobial, water-soluble, thermostable, biodegradable and non-toxic properties. Very little is known about the structures and mechanisms of PASs. Our current understanding of these enzymes is that they consist of an adenylation and thiolation domain commonly found in other canonical NRPSs plus a final unique, membrane embedded domain. Since membrane proteins pose as challenging subjects to work with, the goal of this study was to develop a medium-throughput strategy by screening various PASs in different detergents to assess their stability and integrity for future structural and biochemical experiments. Here I will present expression trials for a ε-PL PAS as a recombinant protein in E. coli with a GFP tag followed by detergent screening by fluorescence size exclusion chromatography. My findings show high aggregation of membrane protein in various detergents and hence call for a new effective approach by heterologous expression in a different bacterial host. To also understand the functional aspect of these PASs specifically the substrates capable of being adenylated by these enzymes, I will present the purification of the soluble domains of this ε-PL PAS followed by an adenylation activity assay and crystallization screen. My results demonstrate high adenylation specificity for L-lysine, as would be expected, and successful protein crystals which require further optimization. The study of these PASs will lead to greater insight into the structures and functions of members of this unusual class of integral membrane NRPSs as a whole and inform bioengineering approaches for overexpression of useful natural and new-to-nature homo-amino acid polymers.

P50 - Méthodologie de criblage par AlphaFold pour la prédiction d'interacteur : application à la protéine RNF13

Antoine Bouchard[1], Valérie Cabana[1], Audrey Sénécal[1], Marc Lussier[1], Laurent Cappadocia[1]

[1]*Université du Québec à Montréal*

La protéine à doigts de zinc de type RING 13 (RNF13) est une ubiquitine E3 ligase principalement localisée aux endosomes tardifs et aux lysosomes. Lors de nos recherches, nous nous sommes intéressés à deux mutations de la protéine, L311S et L312P, connues pour causer l'encéphalopathie épileptique et développementale 73 (DEE73), une maladie génétique rare. Ces mutations causent une perte de la localisation cellulaire de RNF13 aux lysosomes sans toutefois perturber celle aux endosomes tardifs. Pour expliquer ces phénotypes, nous avons étudié l'interaction entre RNF13 et le complexe protéique de la protéine adaptatrice-3 (AP-3), connu pour le transport protéique du Golgi vers les endosomes tardifs et les lysosomes. Grâce à *AlphaFold*, nous avons montré que le motif dileucine (LL) de RNF13 est important pour l'interaction entre RNF13 et la sous-unité σ1 d'AP-3. Pour justifier le maintien de la localisation de RNF13 aux endosomes malgré les mutations, nous avons suspecté une interaction entre RNF13 et la protéine adaptatrice-1 (AP-1), connue pour le transport bidirectionnel entre le Golgi et les endosomes. Nous avons confirmé que le routage aux endosomes des variants L311S et L312P est AP-1-dépendente contrairement à la protéine sauvage qui utilise les voies AP-1 et AP-3. Nos données *AlphaFold* montrent ici que l'interaction entre les deux protéines implique la sous-unité µ1 d'AP1 et un motif YxxΦ inverse atypique de RNF13. Ce motif qui n'avait jamais été répertorié a ainsi permis de comprendre l'interaction entre RNF13 et AP1µ1. Afin d'identifier plus facilement de potentiels substrats interagissant avec une protéine d'intérêt, nous avons depuis mis sur pied une méthodologie de criblage à large échelle des protéines permettant de prédire les interactions entre une protéine et ses interacteurs potentiels et d'identifier les résidus impliqués dans ces interactions. Cette méthodologie est applicable à une grande variété de substrat tant en recherche fondamentale que clinique.

P51 - Novel insights into the role of the YsxC GTPase in the assembly of the 50S ribosomal subunit revealed by Cryo-EM

Amal Seffouh[1]

[1]*Department of Anatomy and Cell Biology, McGill University, Montreal, Quebec H3A 0C7, Canada.*

The assembly of the ribosome is assisted by biogenesis factors that are not part of the ribosome's final structure. One of them is YsxC, an essential GTPase in most pathogenic bacteria that facilitates the assembly of the large subunit. Homologous proteins to YsxC have been described for the mitochondrial and eukaryotic ribosomes. Depletion of YsxC causes a slow growth phenotype and accumulation of immature ribosomal 50S particles called $44.5S_{YsxC}$ particles. These particles show disordered functional rRNA helices and are severely depleted of late binding ribosomal proteins (r-protein). To explore YsxC function in the 50S assembly process, we determined the high-resolution Cryo-EM structure of the $44.5S_{YsxC}$ particle in complex with the YsxC protein. Unexpectedly, YsxC occupies the binding site of the core r-protein uL2. Microscale thermophoresis shows that it binds with high affinity to the ribosomal subunit (Kd ∼107 ± 36 nM). Analysis of this complex allowed us to examine the conformational changes that occur during the interaction with the ribosomal subunit and the structural elements involved in nucleotide and ribosome binding. YsxC active site shows that the spatial arrangement of residues surrounding the β and γ-phosphate groups is similar to that observed in related GTPase. However, the resolution of available structures does not provide enough detail of the water molecules in the immediate environment of the γ-phosphate group of the GTP-analogue. Our high-resolution Cryo-EM model allows the identification of the position in the structure of the attacking nucleophilic water molecule and the catalytic residue, which together with the divalent cation ion, are thought to play a major role in the catalysis by YsxC (unpublished data). This work provides new insights into the molecular mechanisms of GTP hydrolysis by YsxC.

P52 - A Series of Controlled Movements Mediate Enzyme Activity: Conformational Dynamics in Cytochrome P450 Reductase

Alexia Piercey[1], Karine Auclair[1], Christopher Thibodeaux [1]

[1]McGill University

Cytochrome P450 enzymes (CYPs) and their redox partner, cytochrome P450 reductase (CPR), play important roles in the metabolism of small molecule medicines. CPR is a diflavin reductase that mediates electron transfer from nicotinamide adenine dinucleotide phosphate (NADPH) to the heme prosthetic group of CYPs via two tightly bound flavin co-factors. Proper heme reduction is required for CYP function, making CPR activity essential for metabolism. The past few decades have led to major advances towards understanding the mechanistic features of CYPs; however, less attention has been given to CPR. It is known that proper CPR function is absolutely necessary for CYP activity. Moreover, mutations that negatively impact CPR function are thought to have serious consequences on human health. Currently, it is well known that CPR reduction involves structural changes that alter CPR activity and influence its interactions with CYPs. It is suspected that many of these clinically relevant mutations alter CPR function by altering CPR structural dynamics. However, before being able to study mutational effects on the structural dynamics of CPR, we must first develop a concrete understanding of the dynamics of the wild type enzyme. Our lab uses a technique known as hydrogen-deuterium exchange mass spectrometry (HDX-MS) to investigate local changes in enzyme structure. Using HDX-MS, we are currently working towards developing a fundamental understanding of the dynamics involved in CPR function. This includes investigating effects of co-factor binding, electron transfer, and CYP-CPR interactions. This presentation will summarize our most recent findings.

P53 - TamA POTRA domains interact with native lipid membrane

Abdelkader Mellouk[1]

[1]*PhD student*

Gram-negative bacteria outer membrane (OM) is an essential organelle, composed predominantly of outer membrane proteins (OMPs). The OMPs play a crucial role in cell mobility, signaling, survival, virulence, pathogenicity, etc. The assembly and the insertion of these OMPs within the OM is a fundamental process which requires the contribution of the translocation and assembly module (TAM). The catalytic unit TamA of the TAM complex consists of a C-terminal β-barrel domain anchored to the OM and a N-terminal periplasmic domain, POTRA. Despite the efforts to characterize the TAM complex, the role of TamA in absence of its protein partner TamB remained enigmatic. Here we characterize the TamA catalytic unit of *Pseudomonas aeruginosa* PAO1 by combining X-ray crystallography, molecular dynamic simulation and biomolecular interaction methodologies to assess the putative interaction between the POTRA domain and the inner surface of the OM. Our finding reveals that the POTRA domains interact with native bilayer membranes composed mainly of phosphatidylethanolamine, phosphatidylglycerol and cardiolipin with high affinity for the POTRA-1 domain. Moreover, the study of this interaction using a monolayer model shows that the POTRA domain interacts with different type of phospholipids with a preference for phosphatidylglycerol. According to the molecular dynamic simulation, seven polar amino acids were found with high residency toward the outer-membrane. Mutation of these amino acids alter the affinity to our membrane model confirming their importance in maintaining interactions between the POTRA and the bacterial membrane at the internal sheet of the OM. Therefore, if the interaction POTRA/membrane appears essential to the correct function of TAM machinery, the mutated amino acids would be relevant therapeutic targets.

P54 - Structure based domain annotation

Poorya Mirzavand Borujeni[1], Reza Salavati[1, 2]

[1]Institute of parasitology McGill, [2]Department of Biochemistry, McGill University

The most widely used tools for functional annotation rely on domain annotation. This is done by using tools that annotate domains and the curated association tables which show the functions of the domains. Pfam, one of the most widely used domain annotation tools, has a list of well-known protein domain sequences called seed sequences. For each domain, Pfam draws the sequence signature of the seed sequences, which can be used for querying protein sequences and annotating domains. Even though helpful, in some cases, Pfam falls off predicting domains in proteins with low similarity to the seed sequences.

From a biological point of view, structures are more conserved than sequences. So, we expect to annotate more domains in phylogenetically remote proteins if we consider the structural similarity. It wasn't possible on a large scale until recently, as the structure of a limited number of proteins had been resolved experimentally, and computational approaches weren't accurate enough. Besides, there were no efficient tools for finding structurally similar proteins quickly.

In this study, we took advantage of two state-of-the-art programs, Alphafold2, the game-changer program for predicting protein structures with an accuracy comparable to experiments, and Foldseek, the ultra-fast program for finding structurally similar proteins, to annotate domains of proteins. We aimed to annotate the functional domains of proteins based on the structural similarity of the proteins to the Pfam Seed structures. We trained a model for estimating the confidence score based on alignment output. And finally, we used the model for predicting the domains of the proteome of *Trypanosoma brucei*, an early-diverged eukaryotic parasite. Over 2000 new domains in *T. brucei* are annotated, which increases the coverage of proteins with Pfam domains up to 30%.

P55 - Study of the microtubule acetylation effects on the mechanical properties of the cell.

Magda G. Sanchez Sanchez[1], Adam Hendricks[1]

[1]*McGill Bioengineering Department*

The cytoskeleton is a network of polymers whose mechanical properties and organization determine the shape, structure, and mechanics of the cell. It consists of three types of filaments: microtubules, actin, and intermediate filaments. Several studies of cytoskeleton dynamics have been focused on actin polymers; however, microtubules have been recently shown to play a key role in cell dynamics as well. The dynamic assembly of microtubules is regulated by several post translational modifications of their tubulin subunits. One of these, the tubulin acetylation, is known to produce long-lived and stable microtubules. Acetylation also makes microtubules more flexible. Microtubule acetylation controls cytoskeletal organization and regulates traction forces in the cell. In cardiac muscle, tubulin post-translational modifications control contractility and mechanics. This project aims to characterize the mechanical changes produced by tubulin acetylation using optical tweezer-based active microrheology to measure the complex shear modulus, which describes the elastic and viscous behavior contribution of a material. With this method we will compare the modulus associated to normal cells and cells overexpresssing the tubulin acetyltransferase to study the effects this PTM has on the cell mechanical properties.

P56 - Stabilization of the prostaglandin transporter for structural study

Éric Barbeau[1], Sébastien Chrétien[1], Marine Salze[1], Véronique Blais[1], Martin Audet[1, 2]

[1]Department of Pharmacology and Physiology, Faculty of Medicine and Health Sciences, Université de Sherbrooke, Sherbrooke, QC, Canada, [2]CHUS research center, CIUSSS-Estrie-CHUS, Sherbrooke, QC, Canada

Prostaglandins are bioactive lipids that play important roles in physiological and pathophysiological processes such as parturition, inflammation, and cancer. The prostaglandin transporter (PGT) is a member of the SLC family of passive transporter and has a crucial role in their regulation by transporting the prostaglandins from the extracellular medium to the cytoplasm for their degradation. However, little is known about the PGT structural mechanism of activation and regulation. Our goal is to understand the atomic details of PGT functions using X-ray crystallography. Here, we present the stabilization of PGT. Over a hundred mutants of PGT were generated and, with a CPM-based assay, we were able to see that 2 mutants improved the thermostability by 4°C and 4 mutants improved it by around 1°C compared to the wild-type. Furthermore, 2 ligands improve the thermostability by 3°C. The increasing stability of PGT allows the crystallization assay to begin. With crystal, we will be able to solve its molecular structure using X-ray crystallography, allowing us to understand prostaglandin transmembrane regulation at the atomic level.

P57 - Neuronal ribosomes are specialized to be affected by puromycin antibiotic

Jingyu Sun[1], Mina Anadolu[1], Wayne Sossin[1], Joaquin Ortega[1]

[1]*McGill University*

Neuronal ribosomes are considered natively stalled and form ribosome clusters called RNA granules, which is related to neurodevelopmental disorders. Ribopuromycylation is a classic technique for the localization of ribosomes containing nascent peptides, using emetine to block translocation and thus, trap the nascent peptide bound with a puromycin-linked marker on the ribosome. The ribopuromycylation without emetine results in the release of the puromycin-bound nascent peptides from normal ribosomes. However, it has recently been shown that in neurons, the translocation inhibitor emetine is not required for ribopuromycylation to retain the nascent peptides, suggesting the neuronal ribosomes are specialized. It has been argued these puromycin-bound nascent peptides may not be retained in ribosomes but are trapped inside the RNA granules clusters. In this study, we examine using both biochemical techniques and cryo-EM the puromycylated peptides status of the neuronal ribosomes. The results suggest that the puromycylated peptides remain inside the neuronal ribosomes without the presence of emetine. Specifically, the puromycin molecule replaces the CAA end of the 3' A/P tRNA and connects the nascent polypeptide with the large ribosomal subunit on the functional P site.

P58 - Impact de la SUMOylation sur les protéines de chocs thermiques (Hsfs) chez Arabidopsis thaliana.

Laxiga Sinnathurai[1], Laurent Cappadocia[1]

[1]*Université du Québec à Montréal*

La SUMOylation est une modification post-traductionnelle des protéines qui permet aux plantes de répondre de rapidement à des stress abiotiques comme la déshydratation ou l'exposition à des température extrêmes. La SUMOylation des protéines est typiquement dépendante de la présence de sites de SUMOylation (motif YKxE où Y est un résidu hydrophobe, K la lysine ciblée par la SUMOylation, x un résidu quelconque et E un glutamate) sur ces protéines. Du fait de leur très petite taille, les sites de SUMOylation peuvent rapidement apparaître ou disparaître au cours de l'évolution sous l'effet de simples mutations de substitutions. Notre hypothèse est que des changements au niveau de l'abondance et de l'emplacement des sites de SUMOylation, bien qu'ils puissent contribuer à court terme à des instabilités des réseaux protéiques, contribuent à plus long terme à une adaptation rapide des plantes à de nouveaux environnements. Notre objectif est ainsi de fournir une perspective évolutive quant aux rôles de ces mutations. Plus précisément, nous déterminerons les conséquences fonctionnelles d'une augmentation ou d'une diminution du nombre de sites de SUMOylation chez les protéines Hsfs impliquées dans la réponse aux chocs thermiques chez Arabidopsis thaliana, la plante modèle en biologie. Ceci se fera au moyen d'approches bio-informatiques, biochimiques et via la caractérisation de plantes mutantes.

P59 - Structural and functional investigation of the Nisin biosynthesis machinery

Gyana Prakash Mahapatra[1], Nuwani Weerasinghe[1], Christopher J. Thibodeaux[1], Natalie Zeytuni[1]

[1]*McGill University*

Many human pharmaceuticals are derived from natural products and their semi-synthetic analogs. The ribosomally synthesized and post-translationally modified peptides, also known as Lanthipeptides, are a structurally diverse class of natural products with potent antimicrobial activities. One of the most extensively studied lanthipeptides is Nisin, produced by the Gram- positive bacterium *Lactococcus lactis* that have attracted considerable interest as an alternative to existing antibiotics. Like all lanthipeptides, Nisin possesses thioether linkages that are critical for its biological activity. These thioether linkages are formed in a multistep enzymatic pathway involving dehydration of serine/threonine residues in precursor peptide (NisA), followed by intramolecular addition of cysteine thiols onto the nascent dehydration sites to form (methyl)lanthionine macrocycles. The dehydration and cyclization reactions are governed by the enzymes NisB and NisC, respectively. The NisT ATP-binding cassette (ABC) transporter is then required for the secretion of modified NisA across the bacterial membrane which is then proteolyzed by NisP to yield mature Nisin. Previous studies on the Nisin biosynthetic system have shown that NisA/B/C/T form a biosynthetic complex (stoichiometry of NisAB2CT2), but despite these studies, successful isolation of the complete NisAB2CT2 complex has yet to be reported. While high-resolution structures are available for NisC and the NisA:NisB complex, no structures of NisT or the entire NisAB2CT2 complex exist, which limits our investigation of the molecular mechanism of NisA modification, transport and maturation. The structural elucidation of the exporter NisT and the entire modification complex will reveal the key interactions during the assembly process of the Nisin biosynthesis machinery.

Here, we present the extensive optimization of NisT purification by screening a wide range of detergents. Following our successful NisT purification, we confirmed its ATPase activity in the absence of its substrate (NisA). Lastly, we present our recent advances toward the high- resolution structure determination of NisT using cryo-electron microscopy.

P60 - Deep Mutagenesis of Protein-Protein Interactions

Ali Behvarmanesh[1]

[1]*CRBS, Department of Biochemistry, McGill University*

Deep Mutational Scanning (DMS) is an emerging technology that makes it possible to study many variants in a single experiment. This versatile technology addresses the unrevealed aspects of protein structure and function by interrogating the impact of multiple mutations in hundreds of thousands of variants in an unbiased fashion. To carry out a successful DMS experiment, a library of variants, as well as an assay that links the variants' phenotype to their genotype, are required. A library of variants is synthesized and subjected to phage display (PD) screening. Through multiple rounds of PD selection, the library is narrowed down in favor of the variants with the desired activity. Next, high throughput sequencing (HTS) uses millions of reads to identify the DNA sequence of selected mutants. Finally, a heat map is generated to compare the frequency of each amino acid in the initial library and after PD selection to reveal mutations with desirable characteristics. We have applied DMS to study the selectivity of poly(A) binding protein (PABP) for binding a conserved amino acid motif called PAM2. The PABP MLLE domain recognizes roughly 20 amino acids in PAM2 motifs in proteins that bind PABP. The results from two DMS experiments were in excellent agreement with previous analyses of sequence conservation and structural studies. DMS confirmed four positions in PAM2 that are highly selective for MLLE binding. Despite the presence of all amino acids in the input library, after selection, only a small number of amino acids were found at each position. In a complementary experiment, we mutated four positions that were predicted to be variable based on their lack of conservation among PABP binding proteins. We are currently conducting additional screens and isothermal titration calorimetry affinity measurements to complete our understanding of MLLE/PAM2 interaction and its role in the mRNA translation machinery.

P61 - A Medicinal Chemistry approach on Hit to Lead in Fragment Based Drug Discovery

Eleonore Delaire[1], Saba Alapour[1], Steven R. LaPlante[1]

[1]*INRS - Centre Armand Frappier*

Fragment-Based Drug Design (FBDD) has been considered as an effective strategy in drug discovery for several decades now. It is a fast and reliable method which aims to develop drugs from initial low molecular weight fragment hits. These fragments are the starting points for designing more potent compounds and screening them allows medicinal chemists to ascertain and quantify the affinity between the fragments and proteins of interest. After identifying and validating the hits, the challenge lies in making these fragments more drug-like by modifying their molecular structure, which includes different fragment-based optimization strategies like fragment linking, merging and growing. Herein, this poster highlights these different medicinal chemistry strategies by using several examples, emphasizing in the importance of choosing the most suitable approach.

P62 - Structural Biology Platform - Université de Montréal

Normand Cyr[1]

[1]*Université de Montréal*

The creation of the Structural Biology Platform at the Université de Montréal was completed in 2018 following substantial funding from the Canadian Foundation for Innovation. It now supplies state-of-the-art research equipment to answer structural biology questions for the scientific community. The platform is equipped with three high-field NMR spectrometers with Bruker NEO consoles, including a 500 MHz instrument, a 600 MHz magnet equipped with a cryoprobe and a new 700 MHz instrument.

The platform also includes a biological small-angle X-ray scattering (SAXS) system designed by SAXSLAB/Xenocs and coupled to a Excillum Metaljet X-ray source. This SAXS system uses liquid handling robotics and automated sample loading to allow rapid and high-throughput evaluation of macromolecular shapes and dynamics through the analysis of X-ray scattering in solution. It can also be coupled to a size-exclusion chromatography system for SEC-SAXS capabilities and in-line SAXS measurements.

A structural bioinformatics platform is also in place to enable users to efficiently analyze their data and accomplish resource-intensive computational tasks including structure calculations from NMR, X-ray crystallography and SAXS data, molecular dynamics and *ab initio* structure prediction.

Other biophysical tools complement the instruments offered through the structural biology platform. These include a size exclusion chromatography system combined with a multi-angle light scattering detector (SEC-MALS) for absolute molar mass determination, robots for preparing crystal trays, an isothermal titration calorimetry (ITC) system for thermodynamics studies and a spectrofluorometer for fluorescence spectroscopy.

MERCI DE VOTRE SOUTIEN!
THANK YOU FOR YOUR SUPPORT!

BRUKER

cytiva

cytiva cytiva cytiva

SFR
SYSTEMS FOR RESEARCH

csb.med@mcgill.ca

https://www.crbsmcgill.ca